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SYMPOSIUM

APPLIED SCIENCE & ENGINEERING

BASIC SCIENCE

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RESEARCH NOTES

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Letters

Values of Lignin . . . Incorrect?

We would like to point out to the readers of JFS that Childs and Abajian (1976) have reported incorrect values for the lignin content of peanut hulls. Their measurement of lignin includes the cutin fraction resulting in a value for lignin that is approximately twice the actual amount present.

The procedure Childs and Abajian followed, Van Soest (1973), was designed for forages which are low in cutin. This procedure (Van Soest, 1973) defines lignin as the fraction of acid detergent fiber which is insoluble in 72% sulfuric acid. In an earlier paper, Van Soest (1969) pointed out that cutin ["a polymer of aliphatic hydroxy fatty acids (long chain saturated), long chain fatty alcohols, aldehydes and ketones, with a mixture of paraffin hydrocarbons of odd-numbered chain length up to 29 carbons"] is a contaminant when measuring lignin as the residue after treating acid detergent fiber with 72% sulfuric acid. Van Soest (1969) reported the cutin content of peanut hulls to be 22% and the lignin content as 17% as determined by the permanganate procedure of Van Soest and Wine (1968).

We have recently pointed out (Heller et al., 1977) that the particle size of the sample greatly influences the value found for cutin and lignin when analyzing peanut hull by the permanganate method. Until standard methods of fiber analysis are accepted by researchers working with plant fibers, confusing data will continue to be generated.

—*Steven N. Heller* and *L. Ross Hackler,* Dept. of Food Science & Technology, New York State Agricultural Experiment Station, Geneva, NY 14456

A Matter of Definition

The thesis of the above letter is that our measurement of the lignin content of peanut hulls is in error because this fraction contains cutin as a contaminant. The writers cite a 1969 paper by Van Soest to support their position. In a 1973 paper by Van Soest and McQueen, the following paragraph is found:

> "Lignin is generally regarded as the main noncarbohydrate fraction in plant cell walls and the source of much resistance to microbial degradation. The treatment of vegetable foods and forages with 640 ml H_2SO_4/liter tends to isolate much more than the substituted phenylpropane polymer. Cutin, protein-tannin adducts and products of the browning reactions are also isolated and may occasionally be the dominant components. Provided they are not generated as artifacts in the analytical procedure, they may be regarded as legitimate constituents of crude lignin as they all have the common property of being relatively indigestible."

Because (a) Van Soest developed the detergent analysis system and (b) contrary to the assertions in the above letter his recent writings define cutin as a portion of the lignin fraction, our paper did not attempt to differentiate between the lignin and cutin polymers in the lignin fraction.

There was also an experimental reason for not making the differentiation. At the time this work was performed there was no technique available which was accurate and precise, in our hands, for measuring the two components. In their letter, I note that Heller, Rivers and Hackler have a paper in press which they suggest will provide such a method. I look forward to reading the paper upon its publication.

—*Ernest A. Childs,* Dederich Corporation, Box 7, Hubertus, WI 53033

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ERRATUM NOTICE

● J. Food Sci. 41(5): 1222–1224 (1976), S. Leonard, G.L. Marsh, G.K. York, J.R. Heil, T. Wolcott and S. Coggins: "Determination of a Flame Sterilization Process for Apricot Halves in 303 x 406 cans."

On page 1223, line 6 under Results & Discussion: change $D_{100} = 0.69$ to read $D_{100} = 0.569$.

On page 1224, the wrong copy of Figure 1 was printed. The corrected figure is printed herewith. The caption is correct as printed on page 1224.

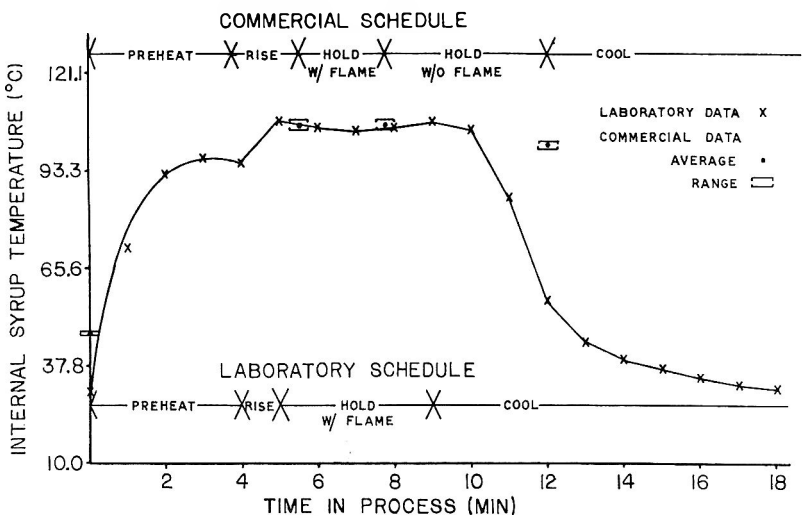

Fig. 1—A comparison of internal can temperature data taken in the commercial Stériflamme cooker-cooler to a typical heat penetration curve taken in the laboratory model Stériflamme.

SYMPOSIUM: The Basis of Quality in Muscle Foods

THIS SYMPOSIUM, sponsored by the Muscle Foods Division, comprised four papers presented at the 35th Annual Meeting of The Institute of Food Technologists in Chicago, June 8–12, 1975. The first paper in the series concerning Molecular Architecture and Biochemical Properties as Bases of Quality in Muscle Foods by D.E. Goll, R.M. Robson, and M.H. Stromer of Iowa State University is not available for publication at this time. Rather than delay publication any longer, the three papers on the basis of color, tenderness and flavor in muscle foods are being published in their entirety while the first paper by Goll et al., appears below only in abstract form.

MOLECULAR ARCHITECTURE AND BIOCHEMICAL PROPERTIES AS BASES OF QUALITY IN MUSCLE FOODS. D.E. GOLL, R.M. ROBSON & M.H. STROMER, Muscle Biology Group, Iowa State Univ., Ames, IA 50010.

Vertebrate skeletal muscles that are commonly used for food have remarkably similar structural and chemical properties when examined at the molecular level. Skeletal muscles from fish, poultry, or bovine, ovine, or porcine animals all contain approximately 15–20% of their weight as protein, 60–85% water, highly variably amounts (1–12%) of lipid, and small amounts (1–2%) of carbohydrates and soluble organic compounds. It is now clear that the proteins in skeletal muscle are responsible for most of the variation encountered in textural and processing characteristics of muscle when used as a food, whereas the lipid, carbohydrate, and soluble organic fractions may be involved along with the protein fraction in the flavor and appearance of muscle used as food. The protein fraction in vertebrate skeletal muscles can be subdivided into three classes that differ in solubility:

(1) the sarcoplasmic proteins (30–50%);
(2) the myofibrillar proteins (50–60%); and
(3) the stroma proteins (15–20%).

The myofibrillar and stroma proteins are directly involved in textural properties of muscle used as a food, but the myofibrillar proteins alone are responsible for 90–95% of the variation in water-holding capacity and emulsifying properties of muscle. Sausage products that are ostensibly normal in texture and water-holding abilities have been made from fractions of purified myofibrillar proteins. The myofibrillar protein fraction in vertebrate skeletal muscle cells consists of eight or nine different proteins arranged in a very specific and unique three-dimensional array. Molluscan muscles (lobster, clams, etc.) contain an additional myofibrillar protein, paramyosin, that is not found in vertebrate muscle, but myofibrillar proteins in molluscan muscle are arranged in the form of fibrils that are similar but not identical to the fibrils of vertebrate skeletal muscle. Relationship of the myofibrillar proteins and their unique architecture to the variation that is observed in meat quality was discussed.

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SYMPOSIUM: The Basis of Quality in Muscle Foods
THE BASIS OF COLOR IN MUSCLE FOODS

ABSTRACT

The basis of color in heme-pigmented muscle food of mammalian, avian and marine origin resides in the physiocochemical/electromagnetic properties of the oxygen-carrying heme protein, myoglobin. This paper is a discussion of literature pertaining to the underlying nature and properties of myoglobin and its derivatives that are responsible for the color of fresh, cured and cooked 'red' meat. Factors governing fresh and cured meat color stability and the nature and causes of undesirable changes are also explored. In doing so an attempt is made to interrelate structure, including electronic, with properties.

INTRODUCTION

THE SUBJECT of this paper is the 'red' muscle hemeprotein pigment, myoglobin; specifically its physicochemical/color-imparting properties in the context of muscle food color quality and stability. The interested reader is referred to related earlier reviews by Fox (1966, 1968), Solberg (1968, 1970), Govindarajan (1973), Giddings (1974) and Hamm (1975), and to recent books containing discussions of myoglobin from the standpoint of meat color (Price and Schweigert, 1971; Lawrie, 1974) and meat color measurement (Francis and Clydesdale, 1975), and from the physiological viewpoint (Antonini and Brunori, 1971; Kagen, 1973).

Apomyoglobin (globin) in colorless, the color imparting property residing in the prosthetic heme/hemin. The free heme group does not form an oxygen adduct but, rather, is rapidly oxidized by same. In order to reversibly bind oxygen an effective donor fifth ligand must first be coordinated to the iron at one axial site, yielding a square pyramidal complex. In general, the basicity of the fifth ligand in large measure determines the equilibrium and kinetic characteristics of sixth ligand binding to iron. The globin-histidine imidazole is an especially effective fifth ligand (particularly from the physiological standpoint) being a good π donor. As such it is more effective than pyridines, according to Basolo et al. (1975) and Chang and Traylor (1973), at enhancing the ability of ferrous heme iron to form an electron-sharing adduct with oxygen (Rifkind, 1973). The precise geometric orientation of the proximal imidazole vis-a-vis the heme group, and its basicity, are very important in regard to complex stabilization and are among the factors influenced by globin conformation, especially in the vicinity of the heme pocket. Peisach (1975) suggests that the hydrophobicity of the heme pocket governs the state of protonation/deprotonation of this imidazole, thus influencing electron density at the heme iron, and thereby providing the basis for electronic control in heme proteins. A less effective base than imidazole would increase the possibility of oxidation instead of oxygenation, and a stronger donor base would render oxygen binding more irreversible which, while undesirable from the respiratory standpoint, would enhance fresh red meat color stability. In this regard, however, Wagner and Kassner (1974) question the essentially of an imidazole-type base as an axial ligand for oxygen binding.

Next in importance to the proximal histidine in the apoprotein globin primary structure would probably be the so-called distal (E7) histidine (globin residue #64). It is generally believed to stabilize the heme-oxygen complex, once formed, by H-bonding between imidazole N and oxygen, rather than enhancing the formation of the complex per se since it, unlike the proximal histidine, is not an absolute requirement for oxygenation. In fact, Antonini and Brunori (1971) play down the importance of the distal histidine in stabilizing the heme iron-oxygen complex, attributing stability to a number of structural factors whose relative roles are difficult to assess precisely. They cite the hydrophobic environment about the heme and an imidazole fifth ligand as probably essential. Not to be overlooked as a factor is the detailed structure of protoporphyrin IX, the porphyrin of oxygen binding heme proteins. The influence of porphyrin structural features, notably substituents at the ring periphery, on sixth ligand binding and complex stability is discussed in some detail by Caughey (1973) in terms of steric effects and, especially, electronic (inductive) effects. Protoporphyrin IX was, of course, 'designed' to fulfill the relatively weak reversible O_2 binding requirement of respiration (Bayer et al., 1973) and not the strong, irreversible O_2 binding that would best serve fresh red meat color stability. Since in the context of meat color one must 'take myoglobin as it comes,' one must therefore seek to optimize environmental conditions within practical limits to achieve maximum color quality and stability. While the empirical approach has been quite successful in this regard, ultimately a thorough understanding of the underlying nature of the problem is what is desired.

Myoglobin Oxygenation and Autoxidation

UPON EXPOSURE of cut surfaces of fresh red muscle to the atmosphere myoglobin in the vicinity of the surface, because of its great affinity for the dioxygen biradical, rapidly becomes oxygenated to a depth of a fraction of a centimeter below the cut surface. The resulting bright, cherry red appearance is recognized as being *the* paramount, overriding point-of-purchase quality attribute although texture and flavor, and not color, are what really counts at the 'moment of truth,' namely mealtime. Nevertheless the shopper associates this bright red color in the supermarket display case with the expectation of wholesome eating enjoyment, and this particular ipso facto has proven to be 'chiseled into the granite' of consumer attitudes.

Gaseous oxygen enters and diffuses through the aqueous environment surrounding each myoglobin molecule, (in effect becoming hydrated), and then into the hydrophobic heme cleft to occupy the vacant sixth coordination site of the iron. Taking quite different approaches Austin et al. (1975) and Lapidot and Irving (1974) present a very rigorous examination of this process. The key structural features of the heme-oxygen complex of oxyhemoproteins are rather well established, due in large measure to recent success with some ingenious reversibly oxygen binding synthetic model metalloporphyrin

systems (e.g., Basolo et al., 1975; Chang and Traylor, 1975; Collman et al., 1975; and the classical work of Wang, 1970). The overwhelming weight of recent evidence from such studies, plus studies on the hemeproteins themselves (e.g., Maxwell et al., 1974, Gupta et al., 1975), and theoretical considerations (Rohmer et al., 1975; Trautwein et al., 1974) favors the bent end-on configuration with an $Fe-O_2$ bond angle of around 135° (similarly for heme adducts such as NO and azide, and cobalt-porphyrin adducts), and with the outer dioxygen atom hydrogen bonded to, or the inner oxygen atom electrophilically attracting the distal histidine imidazole (Fig. 1a). The proximal histidine imidazole may be hydrogen bonded to an adjacent globin group, thereby increasing its basicity and the oxygen bonding strength of the iron (Chang and Traylor, 1975). Along with the geometry of the heme-oxygen complex (bent end-on vs straight end-on vs the Griffith symmetrical model) the other major controversial feature, electronic distribution or covalent vs ionic iron-oxygen complex, appears to also have been recently resolved. Caughey et al. (1975; also see Maxwell et al., 1974) whom Peisach (1975) cites as having "proven" the Weiss (1964) ferric superoxide structure of oxymyoglobin and oxyhemoglobin present com pelling evidence and arguments in support of the middle resonance form in Fig. 1b, which they refer to as "dioxygen iron." It would appear, then, that the single best electronic representation of the iron-oxygen complex 'lies between' the completely covalent and completely ionic extremes, and that the "dioxygen iron" model favored by Caughey et al. (1975), with substantial, but less than complete charge transfer to oxygen, is most representative. Lapidot and Irving (1974) present a detailed analysis of the electronic structure of metal-oxygen complexes, including oxyhemeproteins, as well as events taking place during formation of such complexes and factors contributing to their stability. Both they and Bayer et al. (1973) emphasize, however, that understanding of same is by no means complete since many complex factors are involved. In short, the coming together of myoglobin (and even moreso, hemoglobin) and oxygen to form the adduct is a prohibitively complex and intricate process when viewed in depth at the molecular, atomic and subatomic levels.

It has long been known that as the oxymyoglobin heme iron undergoes a univalent oxidation to the ferric (III or 3^+) oxidation state fresh red muscle loses its attractive, bright cherry red surface appearance and becomes brown. Ferrimyoglobin is unable to form an oxygen adduct, probably because the ferric heme iron is a poorer π donor to sixth ligands than is its ferrous counterpart (Rifkind, 1973). This is compensated for in ferrimyoglobin complexes with sixth ligands (CN^-, NO, N_3^-) that are superior donors. As such they form low spin complexes with electronic properties and therefore optical spectra and appearance similar to those of low spin ferrous complexes such as oxymyoglobin. The univalent oxidation of oxymyoglobin is commonly referred to as "autoxidation," implying a nonenzymic, spontaneous oxidation by free oxygen. Although not stated explicitly (the fate of the bound oxygen usually being ignored), in the strictest sense the expression autoxidation implies that the oxygenated complex separates into ferrimyoglobin and a free superoxide anion (O_2^-), the latter carrying away an electron from the heme iron. The possibility of such a process was considered years ago (George and Stratmann, 1954; Kikuchi et al., 1955), and was actually proposed some years earlier by the theoretician (Weiss, 1935, cited and discussed by George and Stratmann, 1954) who first proposed the ferric superoxide structure for oxyhemeproteins (Weiss, 1964; see also Pauling and Weiss, 1964). There have been a few recent reports of experimental evidence for superoxide dissociation during oxyhemeprotein autoxidation (Misra and Fridovich, 1972; Wever et al., 1973; Brunori et al., 1975; Winterbourn et al., 1976), of superoxide generation coupled to hemoprotein oxidation (Goldberg and Stern, 1975, 1976), and of both ferro- and ferrihemoglobin possessing superoxide dismutase activity (Kovacs and Matkovics, 1975). However, the experimental results were not unequivocal for superoxide anion dissociation during autoxidation (e.g., see Younes and Weser, 1976) which is unlikely to occur from both the mechanistic and energetics standpoints (e.g., George and Stratmann, 1954; Wallace et al., 1974). A far more plausible interpretation of their findings, and one that better explains the observed effect of proton concentration (i.e., pH) is that of hydroperoxy radical dissociation from oxyhemeproteins during autoxidation. While the point as to whether the superoxide anion (O_2^-) or its conjugate acid, the hydroperoxy radical (HO_2, pKa 4.88) is able to dissociate during autoxiation may at first seem moot, mechanistically and energetically the distinction makes a great deal of difference. It is generally conceded on both mechanistic and thermodynamic grounds that bound oxygen cannot dissociate as superoxide (at least in the absence of thermal perturbation), and recent experimental evidence (Giddings and Markakis, 1973) employing cytochrome c as well as "tiron," which is believed to be specific for the superoxide anion (Weser and Voelcker, 1972; Greenstock and Miller, 1975) supports this position. There is a growing body of opinion that despite unfavorable energetics of univalent oxygen reduction hydroperoxy radical dissociation is not only possible, but that it is one of two major pathways of oxyhemeprotein autoxidation (Castro, 1971). The other major pathway involves a two equivalent reduction of bound oxygen to the peroxide level, iron donating one electron and a one-equivalent reducing agent donating the second one.

In regard to the univalent reduction of oxygen and hydroperoxy radical dissociation the obvious mechanistic require-

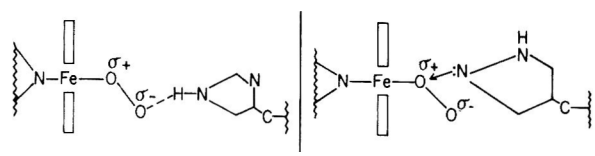


Fig. 1a.—Two models for the interaction of bound oxygen with the globin distal histidine imidazole. Left: hydrogen bonding, which requires that the outer imidazole N be protonated. Right: nucleophili-electrophile interaction between inner O atom and unprotonated N.

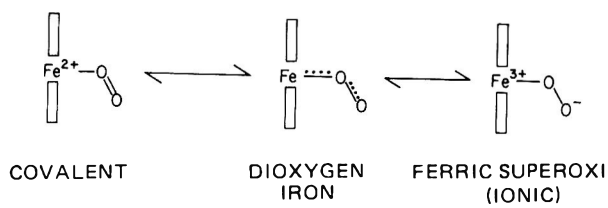


Fig. 1b.—Three resonance forms of heme iron – oxygen bonding in oxyhemeproteins. According to Caughey et al. (1975) the middle structure (dioxygen iron) is most representative. The covalent form is probably virtually nonexistent. A temperature-dependent fraction of the total population of oxymyoglobin in a given system is probably in the completely ionic (ferric superoxide) configuration, in thermal equilibrium with the dioxygen iron-type of intermediate configuration. Increasing temperature should tend to shift the equilibrium to the ionic side.

ment is the protonation of the outer atom of the bound dioxygen together with complete transfer of an electron from iron to oxygen. Such a process appears consistent with a recently proposed "proton-assisted nucleophilic displacement" mechanism (Wallace et al., 1974; Wallace and Caughey, 1975) and the findings of Wang (1970). The outer oxygen atom would appear from the foregoing to invariably carry a net negative charge, and, at thermal equilibrium some fraction of bound oxygens in an oxygenated myoglobin/hemoglobin system (e.g., a fresh red meat surface) would be expected to exhibit essentially complete charge transfer (i.e., ferric superoxide state) and therefore possess especially good proton acceptor properties. The energy differential between dioxygen-iron and ferric superoxide states should be much less than the ~20 kcal/mole between the completely covalent and completely ionic states which, according to Williams (1970) probably lie very close to one another. This is consistent with the known temperature effect on autoxidation rate (Gotoh and Shikama, 1974), as it is reasonable to assume that increasing the temperature of such a system would increase the equilibrium fraction of the population of the complex that exhibits essentially complete charge transfer from iron to oxygen. Engagement of a proton by the bound dioxygen (superoxide) would be expected to further weaken the iron-oxygen bond resulting in dissociation of the hydroperoxy radical from the now formally ferric hemeprotein. A mechanistic autoxidation scheme has recently been presented (Giddings and Markakis, 1973) that is based upon this concept plus experimental observations, and on the oxygen uptake/evolution stoichiometry (0.25 mole/0.75 mole) reported by Brown and Mebine (1969). The scheme, while undoubtedly an oversimplification, is consistent with kinetics information of George and Stratmann (1952, 1954) and others with respect to the initial dissociation.

While hydroperoxy radical dissociation can at least partly explain the well documented pH effect on autoxidation rate, increasing the proton (hydronium ion) concentration of an aqueous oxyhemoprotein system would also be expected to (a) influence globin conformation in such a way as to render it less effective in stabilizing the heme-oxygen complex (e.g., Chang and Traylor, 1975, and the ionizable heme-linked group suggestion of George and Stratmann, 1954) and (b) increase the rate of association/dissociation of heme from globin (no doubt due in part at least to conformational change). The latter is known to result in an increased autoxidation rate since oxygen rapidly oxidizes the ferrous ion of globin-free heme. According to Antonini and Brunori (1971) the heme-globin system may be considered to be in a state of equilibrium (i.e., globin + heme $\underset{k_2}{\overset{k_1}{\rightleftharpoons}}$ myoglobin) even at neutral pH, where the Keq (k_1/k_2) is estimated to be on the order of 10^{12} to 10^{15} M^{-1}, underscoring the very high affinity of globin for heme. Thus it is perhaps reasonable to expect that, while at physiological pH (ca. 7.4) the heme-globin association/dissociation rate is too low to give rise to an appreciable autoxidation rate, at typical post-rigor muscle pH (ca 5.6) both rates become significant, and heme dissociation together with bound oxygen protonation account for the pH effect. This, of course, omits the temperature effect which can operate in concert with the pH (and oxygen partial pressure or, more precisely, dissolved oxygen concentration) effect (MacDougall and Taylor, 1975). Gotoh and Shikama (1974) estimate the autoxidation half-life of bovine oxymyoglobin in solutions buffered at pH 5 to be 2.8 hr at 25°C compared with 5 days at 0°C. At pH 9 the values are 7 days and 1 yr respectively, underscoring the known importance of pH and temperature for meat color stability. They report an activation energy of 26.5 kcal/mole and a Q_{10} of 5.3, which is in agreement with data of Brown and Mebine (1969) and of George and Stratmann (1952, 1954) for saturating oxygen partial pressure (PO_2). The latter authors found a significantly lower activation energy (ca 19 kcal/mole) at well below saturating PO_2 (i.e., at 4 torr) which brings up the second major oxyhemoprotein oxidation mechanism advanced by Castro (1971) and other (e.g., Wang, 1970; Williams, 1970), namely two equivalent reduction of bound oxygen.

There is a growing body of evidence in support of the theory formalized by Castro (1971) that complete electron transfer from heme iron to bound oxygen "only ensues upon approach of a second metal ion or a proton," the latter having been discussed in the foregoing. Two equivalent reduction, of course, requires either the availability of reductant in intimate proximity to the liganded oxygen in the heme cleft (an axial or inner sphere process as per Castro et al., 1975), or, electron tunneling (Blumenfeld and Chernavskii, 1973) from a redox reaction site on the globin remote from the heme, or on the periphery of the heme itself (a peripheral or outer sphere process as per Castro, 1971, and Castro et al., 1975). George and Stratmann (1952) reported (since confirmed by others) that, while autooxidation at saturating PO_2 followed simple first order (in unoxidized myoglobin) kinetics, at well below saturation the observed rate constants showed a complex second order variation with oxygen pressure according to the product of the concentrations of Mb^{2+} and MbO_2. This finding strongly suggests a two equivalent reduction of bound oxygen to the peroxide level during autoxidation such that the oxygenated heme iron contributes one electron and the uncomplexed (five coordinated) iron of a deoxyferrous myoglobin contributes the second, perhaps via an outer sphere-type mechanism (i.e., prophyrin-porphyrin bridging and/or electron tunneling). Such a mechanism would completely account for the well documented oxygen partial pressure – autoxidation rate relationship since one would expect a rate maximum at the half-saturating PO_2 (ca 1–2 torr for myoglobin) where hemeprotein autoxidation is indeed maximal. As first proposed by Snyder (1963) and later supported by Banerjee and Stetzkowski (1970) this oxygen partial pressure relationship is consistent with a mechanism involving heme dissociation from globin, but probably only at acidic pH (Fronticelli and Bucci, 1963; Antonini and Brunori, 1971), and, only if the process involves a two equivalent reduction of oxyheme oxygen via interaction with dissociated deoxyferrous heme. Heme dissociation need not be a requirement if an outer sphere electron transfer from deoxyferrous to oxyferrous hemoprotein, and ultimately to the bound oxygen, takes place. Steric constraints probably rule out a direct redox interaction between deoxyferrous heme iron and oxyferrous oxygen (i.e., an axial inner sphere mechanism) with the hemes associated to globins within the cleft. However, heme-heme interaction appears to be the case with electron transfer between cytochromes, and could conceivably apply to myoglobin, especially if the globin assumes a more open, less heme-shielding configuration under favorable conditions (Castro, 1971, Chance, 1974; Stellwagen and Cass, 1975; O'Keefe et al., 1975). The proposed mechanism of direct electron exchange between cytochromes via interaction at exposed heme edges (Chance, 1974) or via mediation of aromatic amino acid side chains (Dickerson et al., 1971; Takano et al., 1973; Lichtin et al., 1974), or a combination of the two (Miller and Cusanovich, 1975; Satterlee and LaMar, 1976), coupled with recent evidence of direct electron exchange between ferrous myoglobin/hemoglobin and ferricytochrome c, and also between ferrylmoglobin and both ferrous cytochrome c and ferrous myoglobin (Wu et al., 1972; Giddings and Markakis, 1973) lends support to the possibility of a two equivalent autoxidation mechanism involving oxy- and deoxymyoglobin. The process would be expected to involve the "thermally populated" fraction of MbO_2 in the ferric superoxide configuration. This, like the proton-assisted univalent autoxidation process, should therefore show a direct rate-temperature relationship. This again underscores the known improvement of fresh red meat color stability brought about by lowering above-freezing temperature.

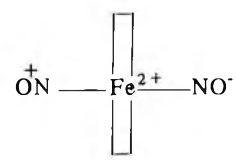
OXYMOGLOBIN PHOTOOXIDATION

ALTHOUGH APPARENTLY no more than an occasional minor problem, it is recognized that incident light (e.g., in supermarket display cases) can be a contributing factor in red meat surface discoloration. This is particularly so of frozen meat and meat extracts and the extent of the effect is dependent upon such factors as wavelength and intensity of the light, temperature and oxygen pressure under which the meat is held, meat pH, length of holding, and, in the case of pigment extracts and probably frozen meat, electrolyte concentration and presence of free transition metal ions (Owen et al., 1976; Hunt et al., 1975; Rifkind, 1974; Satterlee and Hansmeyer, 1974; Zachariah and Satterlee, 1973; Setser et al., 1973; Assaf et al., 1971, and literature cited therein). Based upon their experimental results and cited earlier reports Solberg and Franke (1971) hypothesized that a photoenergized molecule such as riboflavin may interact with and oxidize oxygenated heme pigments. More recently Lynch et al. (1976) suggested the same for the semiquinone of riboflavin generated by photoreduction. While a direct interaction between pigment and primary photoexcited species is possible, in oxygenated systems singlet oxygen ($^1O_2$) would be likely to serve as the energy-carrying intermediary and direct oxidant (e.g., see Tada et al., 1971; Aurand et al., 1976). Singlet oxygen, a high energy oxidant with a finite lifetime, is generated by energy transfer interactions between ground state triplet O_2 and any of a number of photoenergized "sensitizer" molecules (Kearns, 1971; Bland, 1976). Singlet oxygen can be generated chemically by mechanisms not involving photoactivation, and there is some controversy as to whether or not singlet O_2 is generated during superoxide dismutation viz $2HO_2 \longrightarrow H_2O_2 + {}^1O_2$ (Pederson and Aust, 1973; Khan, 1970; Nilsson and Kearns, 1974; Poupko and Rosenthal, 1973; Koppenol, 1976). What is clear, however, is that singlet O_2, which is much more reactive than ground state triplet O_2, is a potent oxidant toward proteins as well as lipids and other food constituents. It is quite conceivable that sufficient singlet oxygen generated at oxygenated meat surfaces (or in oxygenated myoglobin/hemoglobin extracts) is interacting in such a way as to cause the modest increase in autoxidation over and above that observed with comparable systems held in parallel, but in the dark. The possibility that more than one photooxidation mechanism proceed simultaneously cannot be overlooked. The apparent complexity and low efficiency of the process not doubt at least in part explains why photocatalyzed autoxidation is not a serious problem with fresh red meat colorwise. The fact that NO is a much better quencher than O_2 and has a higher electronic affinity (Kearns, 1971) may explain why cured meat color is apparently more susceptible to light-induced discoloration than is fresh meat color.

MYOGLOBIN NITROSYLATION AND CURED MEAT COLOR STABILITY

IT IS WELL established that the sixth position ligand of cured red meat myoglobin is nitric oxide (NO) which binds to the heme iron via the nitrogen atom to form an extremely stable paramagnetic adduct having the characteristic optical properties of such complexes, which gave rise to a reddish color (e.g., the α and β absorption bands of equine MbO_2 and MbNO are at 580 and 542 nm, and, 575 and 543 nm respectively). It has long been known that NO combines with both ferric and ferrous iron of unhindered hemeproteins, hemi/hemochromagens (i.e., nonprotein six-coordinate heme complexes normally having at least one nitrogenous base axial ligand), and, under certain conditions, free heme/hematin. Ferric myoglobin and hemoglobin complexes of nitric oxide are known to gradually autoreduce and assume a partial ferrous nitrosyl ($Fe^{2+} - \overset{+}{N}O$) configuration; that is, partial transfer of the odd nitrogen electron to iron (Ehrenberg and Szczepkowski, *1960*; Chien, 1969a; Yonetani et al., 1972). That is, low spin nitrosylferrohemoglobin is the sole product of both the ferrihemoglobin and ferrohemoglobin reactions with NO. The general coordination chemistry of nitric oxide was recently reviewed by Eisenberg and Meyer (1975). Antonini and Brunori (1971) point out the following with respect to NO complexes of Mb and Hb. The affinity and "on" velocity constant in both cases is exceptionally high, over 100-fold greater than for CO which, in turn, has a much greater affinity than O_2. Once formed the Mb and Hb complexes of NO are very stable in the absence of oxygen. In the presence of O_2, which rapidly oxidizes free NO to NO_2, the stability of the complexes is limited by the rate of NO dissociation since O_2 does not react directly with the bound NO. This dissociation rate is very low. Nitric oxide complexes are photodissociable however, although the quantum yield is very low (0.001 for both Hb and Mb complexes) compared with a perfect 1.0 for CO complexes, and 0.03 for sperm whale oxymyoblobin (Brunori et al., 1973). Due largely to the unpaired electron on the nitrogen atom, NO heme complexes possess certain properties not exhibited by O_2 and CO complexes in addition to exceptional stability and the nonessentiality of a trans donor axial ligand (see also Moore and Gibson, 1976). Nevertheless optical properties of complexes of all three ligands in the visible and Soret regions are quite similar and cured meat is not unlike fresh colorwise.

As pointed out by Binkerd and Kolari (1975) in a historical review the use of nitrate/nitrite salts in flesh food has it origins in antiquity, whereas an understanding of the chemistry of the reactions that lead to cured meat color has developed only recently. Tarladgis (1962b) was perhaps the first to discuss cured meat pigment chemistry in quantum mechanical terms. He concluded on the basis of optical and EPR spectroscopy coupled with theoretical considerations that the pigment of cooked-cured meat is a heme compound ("nitric oxide myochrome") containing NO groups at both axial coordination sites rather than the widely accepted denatured globin-NO hemochrome description. The possible existence of this proposed structure received recent support from Wayland and Olson (1974) who provided evidence of a di-NO complex of the model ferrous porphyrin compound "tetraphenylporphyriniron," the following structure for which was suggested:



The authors point out that the $Fe^{2+} - NO^-$ unit is isoelectronic with the FeO_2 of oxyhemeproteins. While such a complex could at least in part account for cooked-cured meat color, the presence of a similar complex having a nitrogenous base trans to the NO ligand is quite possible, and, as indicated by Rifkind (1973), tends to be more stable. In fact, as previously mentioned, a stable five-coordinated heme-NO complex can exist. In the presence of pyridine this species has been shown to exhibit an EPR spectrum and rhombic symmetry which resembles closely that of MbNO (Dickinson and Chien, 1971). Tarladgis (1962b) advanced the view that fading of cured meat color by lipid oxidation or photocatalyzed dissociation of NO from heme involves withdrawl of electron density from iron to porphyrin thus weakening the Fe-NO bond. The NO dissociates leaving the iron susceptible to oxidation by "electronegative groups present in the medium" He viewed green discoloration as being caused by electrophilic and nucleophilic attacks on areas of high and low electron density in the porphyrin ring π electron cloud causing ring opening. It is

well known that additions to (e.g., sulfmyoglobin), and rupture of the porphyrin ring can produce green chromophores. Strong reducing conditions, excess nitrite and absence of light exposure were cited by Tarladgis (1962b) as measures to avoid color fading. As demonstrated earlier by Ramsbottom et al. (1951), and explained by Fox (1966) elimination of oxygen essentially eliminates photoinduced color fading as a problem by avoidance of O_2 oxidation of dissociating NO. Singlet oxygen generation by photoexcited heme-NO is, of course, also avoided in anoxia. Tarladgis went on to recommend replacing NO-based curing salts with compounds possessing strong electron-donor power such as purines, pyrimidines and other nitrogenous bases having desired ligand-electronic properties. Subsequently, with impetus generated in part by the nitrosamine question, researchers have tested a number of possible substitutes for NO as heme ligand with patents having been issued in some cases (e.g., see MacNeil and Mast, 1973; Brown, 1973, Kelly, 1974; Kemp, 1974; Knowles et al., 1974; Fox et al., 1974, 1975b; Dymicky et al., 1975). While certain of the com pounds (e.g., imidazole, substituted pyridines, nicotinic acid) proved somewhat effective in providing reasonably good color quality and stability, this author agrees with the view expressed by Brown (1973) that it is highly unlikely that any substiture will match the all-around efficacy and effectiveness of nitrite/NO. Direct application of NO gas, while highly effective colorwise in cured meat 'emulsions' especially, will probably remain prohibitively impractical and hazardous. In addition to a possibly futile search for substitutes it is advisable, it seems to this reviewer, to also seek ways of realizing the advantages of the nitrite/NO approach to meat curing while minimizing the risk of a realistic nitrosamine hazard (e.g., see Fiddler et al., 1973; Wierbicki and Heiligman, 1974; Fox and Nicholas, 1974; Cassens et al., 1974, Fujimaki et al., 1975; Mottram et al., 1975).

While the MbNO structure for uncooked-cured meat pigment is now firmly established, uncertainty still surrounds the mechanism(s) by which ferri/ferro myoglobin and nitrite give rise to this pigment, especially the role of endogenous and added reductants (e.g., see MacDougall et al., 1975). Fox et al. (1975a) correctly point out tha the role of reductants in heme pigment chemistry in general is ambiguous since, as pointed out earlier, they can promote oxidation and even porphyrin ring rupture under certain conditions. A good case-in-point is ascorbic acid which together with its isomer, erythrobic acid, and their salts constitute probably the most effective added reductants in cured meat systems, apparently counteracting photoinduced discoloration as well. This subject has been reported on and discussed in several recent papers (e.g., see Reith and Szakaly, 1967a,b; Fox and Ackerman, 1968; Koizumi and Brown, 1971; Mohler, 1974; Ando, 1974; Cassens et al., 1974; Cheah, 1974; and Walters et al., 1975). The picture that seems to emerge may be summarized as follows. To begin with, as previously pointed out both ferrous and ferric myoglobin will combine with NO to yield the same pigment. The latter is believed to 'autoreduce' with time via internal electronic rearrangement. Further, ferrous myoglobin can reduce NO_2 to generate NO which will combine with either oxidation state of the pigment. Endogenous or added reductants such as ascorbate, sulfhydryl compounds and NADH-flavins accelerate the process by either reducing ferrimyoglobin which can then reduce NO_2, or, reducing NO_2 to NO, or both, in addition to perhaps accelerating reduction of ferri Mb-NO. Ferrihemeprotein – NO_2 has been suggested as a possible transitory intermediate in meat systems, and there is evidence of such an iron-NO_2 complex occurring with hemoglobin (Uchida and Klapper, 1970).

Mitochondrial and cytochrome c involvement in cured meat color development has been extensively studied by Walters and co-workers (Walters et al., 1975) who in this latest paper (as of this writing) indicate that functional mitochondria appear to survive the bacon curing process and may be able to mediate the transfer of NO from nitrosylferricytochrome c to ferrimyoglobin to form NbNO in same. They note that ascorbate and some sulfhydryl compounds, as well as NAD(P)H-flavins, can also accomplish this. They and Cheah (1974) also pointed out that nitrite is an inhibitor of mitochondrial respiration, acting as an inefficient electron acceptor from the cytochrome oxidase. In a study of nitrogen fixing bacteria Cox et al. (1971) obtained EPR evidence of direct participation of c-type cytochromes in the reduction of NO_2 to NO, and of a cytochrome heme-NO intermediate. The actual NO_2 reducing system was an NADH-requiring flavoprotein, and the authors concluded that the great affinity of cytochrome c for the so-generated NO causes a shift of the redox equilibrium to the NO side. Whether or not a similar mechanism of cytochrome c-connected MbNO generation (e.g., involving an NADH-dehydrogenase such as the lactate-LDH system suggested by work of Cheah, 1976) occurs in cured meat is one of a number of yet unanswered questions having to do with this most complex color developing process.

COOKED MEAT COLOR

TAPPEL (1957a, b), who produced some of the important earlier work on cured meat pigment, described the brown color of cooked beef as a mixture of denatured globin nicotinamide hemichromes which can be reduced to pink hemochromes (e.g., by irradiation-generated e^-_{aq} in anoxia). The hemichrome nature of cooked and cooked-cured meat gained additional support in a subsequent report (Tappel, 1961). Tarladgis (1962a) interpreted the optical spectra of cooked meat pigments in quantum mechanical terms and concluded that the color imparting compound of cooked meats is a high spin ferric heme having a carboxylate ion of denatured globin, and water as axial ligands. He referred to this complex as "metmyochromagen" and argued that nicotinamide, a strong donor base, as an axial ligand would render the complex low spin. However, another strong donor base, imidazole, is fifth ligand of high spin ferrimyoglobin which is believed to have a water molecule as sixth ligand. In other words, spin state is largely determined by the sixth ligand and the high spin nature of the cooked meat pigment does not rule out a denatured globin hemichrome having a nitorgenous base and water as axial ligands. Tarladgis and Din (1965) subsequently reported that ionizing radiation reduced the ferric iron of "metmyochromagen" to ferrous with associated development of the familiar pink color of irradiated-cooked red meat. In two recent publications (Ledward, 1971; 1974) Ledward concluded on the basis of optical and EPR spectroscopy results that the pigment of cooked red meat consists of diimidazole complexes of ferric hematin. That is, histidyl imidazoles of an array of denatured sarcoplasmic proteins occupy both axial coordination sites, thus explaining the low spin ferric nature that they found for the pigments at −196°C. They pointed out that the hematin of thermally denatured myoglobin and hemoglobin is readily transferred to other proteins (e.g., gelatin, bovine serum albumin) in-vitro, and that the same may hold true in cooked meat. The cooked meat pigment was essentially 100% low spin at −196°C, but apparently has some high spin character at room temperature. Although ferrimyoglobin can coordinate an imidazole group (Vickery et al., 1976), and heat denatured myoglobin can do likewise (Koizumi and Nonaka, 1975), and free ferrous heme can complex with an imidazole at each axial coordination site, Wagner and Kassner (1975) indicate that ferrous heme can coordinate only one *sterically hindered* imidazole. Since mere methylation places the imidazole group in the latter category in this context it is unlikely that two denatured protein-bound imidazoles can occupy the two axial heme coordination sites as proposed by Ledward.

While uncertainty continues to surround the exact nature of the cooked meat pigment(s), it appears certain that it is a reducible ferric complex, the sixth ligand of which can be replaced by such strong field ligands as NO, CO and CN^- to yield adducts with optical spectra and appearance similar to that of the native hemeprotein complexes. This would suggest a strong donor ligand (i.e., a nitrogenous base) at one axial coordination site and a readily displaceable ligand (e.g., H_2O) at the other.

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SYMPOSIUM The Basis of Quality in Muscle Foods

THE BASIS OF TENDERNESS IN MUSCLE FOODS

ABSTRACT

Two muscle components, collagen and the contractile apparatus, determine tenderness. The collagen contribution to toughness is due to the presence of intermolecular crosslinks which, with increasing animal age, become more thermally resistant and thus less readily broken during cooking. By contrast, toughness due to the contractile proteins is determined by conditions during the first few postmortem hours, when rapid chilling causes "cold shortening" of the muscles and a consequent very appreciable toughening. Beef and lamb are particularly prone to this process-induced defect; toughness development in these species can be largely controlled, however, by simple cooling-rate adjustment during rigor onset.

TWO STRUCTURAL COMPONENTS determine the tenderness of meat. The collagen of connective tissue has long been recognized as an important influence on quality, and indeed until very recently was regarded as the sole cause of tenderness variation. The contractile apparatus, by contrast, was first established as a potent contributor to toughness only in the early 1960's and its full significance to meat quality is still not universally appreciated. This does not mean that connective tissue has been relegated to play merely a minor role as a toughening agent; as we shall see, in some circumstances toughness is due almost entirely to collagen, whereas in others it is caused almost exclusively by the contractile machinery. Only by a fuller understanding of both these constituents can we hope to comprehend toughness and control tenderness.

Collagen

Each unit of muscle organization – fiber, fiber bundle, and entire muscle – is surrounded by a sheath or sleeve of connective tissue, rather like the insulation enwrapping every wire, each bundle of wires, and the exterior surface of a co-axial cable. The connective tissue serves many vital purposes in life: it supports the soft muscle substance within its boundaries, provides a bed for blood vessels and nerves, and protects the contractile structure from damage by over-extension. It consists of a variety of cells, a ground substance, and fibrous proteins, the principal one of which, in both quantity and tenderness significance, is collagen.

The basic structural unit of collagen is tropocollagen, a not unusually large (mol wt 300,000) protein with a high axial ratio (length:width about 200). Within this molecule are three peptide chains, wound rope-like around each other in helical form and bound to each other by intramolecular crosslinks. If these internal bridges were the only linkages present in collagen, no toughening problems would arise, for the length of the molecule, about 280 nm, would be totally unnoticed by the meat consumer no matter how resistant the structure might be to cleavage. Such a protein, however, would serve virtually no useful purpose in the living animal, for it would lack entirely the very properties of strength and inextensibility which are its *raison d'être*. Nature has endowed it with these indispensable qualities by introducing two modifications: longitudinal molecular overlap and latitudinal intermolecular crosslinkage. We now know that tropocollagen units assemble in a so-called "quarter-stagger" formation, each one overlying its neighbor by about three-fourth of its length and being bonded to it at intervals to prevent sliding or slipping under tension in the living tissue. A multi-layered wall of very long, thin and narrow bricks would be a reasonable analogy.

Clearly, these intermolecular linkages are quite adequate to bind the tropocollagen units together at all stages of life; young bridges are as effective as old in cementing the molecules in strong and relatively massive structures in vivo. In postmortem tissue, however, major differences become apparent. "Youthful" or recently formed crosslinks are relatively unstable when exposed to denaturing conditions, and as a result the collagen of young animals breaks down quite readily when heated. As the bridges grow older in the maturing animal, however – and collagen has a long "life" in the sense that its metabolic turnover is slow – they stabilize to a much more heat-resistant form; cooking thus causes far less disintegration. This age-dependent strengthening of bonds goes a long way toward explaining some of the conflicting results of past studies, for it suggests that the collagen of young animals should make a much smaller contribution to toughness than that of their more mature brethren. Numerous tenderness investigations support this view. It has long been known, for instance, that veal contains more collagen than beef despite its greater tenderness, and that the percentage of intramuscular collagen solubilized by enzymic or hot-water treatment is several times greater in veal than in mature steers. These and similar earlier observations, inexplicable though they were when first made, now fit in precisely with modern concepts of the fine structure of collagen.

The principal protein of connective tissue thus remains a major determinant of meat tenderness, but in a way which was not realized until quite recently. It is now clear that collagen quantity is of much less significance than its "quality:" the ease with which the intermolecular crosslinks are broken during postmortem enzymic treatment, acidification or cooking. Although this conclusion does not suggest any immediate or early practical remedy for the problem of connective-tissue toughness, it does open another major route for further exploration. Sc long as we considered only the amount of collagen as a matter of concern, little advance could be expected, for a significant reduction of this vital component in the living animal would probably be incompatible with life. Modification of collagen in vivo, however, as distinct from its removal or diminution, may be a much more readily attainable goal. The complex synthesis of collagen within the organism is now fairly well understood, and with this comprehension has come the knowledge that collagen structure can, in fact, be modified at certain critical stages of its biomanufacture by appropriate treatment. It is certainly within the bounds of possibility that future advances along these lines might indicate ways to extend the life of the "youthful" form of collagen crosslinks into maturity, to inhibit bridge formation by use of feed additives, or to retard intermolecular linkage by introducing a controlled nutritional deficiency.

The contractile proteins

Despite the new avenues revealed by these recent advances in connective-tissue science, it must be admitted that there would be little hope of any early practical improvement in meat tenderness if toughness were solely a function of collagen quantity or quality. Fortunately, this is not the case. It is now quite clear that collagen is only one of two causes of toughness, and the other – the contractile protein complex – is much more amenable to modification and control. It is largely due to R.H. Locker that our knowledge of this latter influence on meat quality is so far advanced; his investigations during the years 1959–63 (partly in collaboration with C.J. Hagyard) are now regarded as classics in the field.

The conclusions drawn from these pioneer studies can be stated very simply. Visibly red muscles (including all bovine and ovine muscles so far examined) are stimulated to shorten if exposed to chiller temperatures while still in a pre-rigor state, and, if not physically prevented, will contract by half or more of their initial lengths. This "cold shortening" is accompanied by a very appreciable toughening which is not necessarily prevented by normal skeletal attachments, since many muscles of the normally hanging carcass are slack and thus free to shorten if provoked to do so.

Later investigations have repeatedly confirmed these initial observations. It is now clear that, in terms of the force required to bite through the meat, cold shortening can cause as much as a four- or fivefold increase, rendering the tissue absolutely unchewable. It is equally apparent that this "processing toughness" is of fairly recent origin; the practical means to chill meat rapidly and early postmortem and the incentives to do so – quicker processing, smaller weight loss, reduced bacterial spread – have not been with us for many years. These studies have also shown why it is quite impossible to give a simple answer to a straightforward question; is it connective tissue or cold shortening which is the more important toughener? Clearly, if early postmortem conditions have discouraged shortening, then connective tissue will be virtually the sole contributor to shearing resistance. On the other hand, if the imposed environment has prompted an appreciable length change in the musculature, then the connective-tissue contribution will be of almost negligible significance since the meat will have been made almost inedible by the shortened actomyosin component.

The ability of a muscle to cold shorten declines with time postmortem, and if the primary filaments are given long enough to "lock" into rigor before the tissue is cooled below about 10°C, only a small and relatively innocuous "rigor shortening" occurs. Once this cross-bridging has taken place, chilling or freezing can cause no shortening and toughening at all, no matter how steep the applied temperature gradient. This behavior suggests an immediate solution to the problem of cold-induced toughening: leave the carcass at a temperature above, say, 10°C until incipient rigor bonds have locked the contractile structure into a relaxed configuration, and only then transfer it to a cold environment. Such a procedure has been in full commmercial operation in the New Zealand export lamb trade for the past 9 yr, during which time many millions of carcasses have been treated successfully for the North American market. Effective though it is, however, this conditioning treatment is wasteful of time, space and labor. Furthermore, direct application of the process to beef is not practical without extensive modiification, since the much bigger carcass would maintain a higher internal temperature for a longer time, so encouraging deep spoilage and the possible development of pathogens. For these reasons, other methods of reducing or totally eliminating the cold-shortening effect are being sought in several laboratories at this time.

One promising alternative stems from the original studies of Locker, who noted that "various muscles of the ox enter rigor mortis in differing stages of contraction, related to the strains present in the muscles of the hung carcass," and suggested that "it should be possible to improve the quality by hanging the carcass in such a way that the muscle is stretched and prevented from shortening." This prediction was tested and verified by the Wisconsin group, and was later developed by Texas researchers, whose simple hip-hanging procedure causes the carcass or side to take up a more natural posture in which muscles maintain themselves at lengths approximating those in the living relaxed state. In an alternative procedure developed by Cornell workers, an extendible rod is applied along the back of the side or carcass soon after slaughter; the "extenderizer" opposes any cold-shortening tendency in the tissues, and is removed when the rigor bonds have locked the musculature into a tender, unshortened configuration.

A different approach is seen in recent attempts to accelerate the postmortem changes of glycolysis. If rigor onset could be hastened, only a brief prechilling delay would be required to satisfy both the toughness-sensitive consumer and the bacteria-sensitive meat hygienist. Temperature elevation is ruled out, of course, since it would greatly accelerate bacterial proliferation, particularly of potentially pathogenic mesophiles. It has been found in Australia that a brief application of high pressure to the carcass causes very rapid glycolysis and rigor onset, the meat being very significantly tenderized despite immediate post-pressure chilling. In New Zealand, very early postmortem electrical stimulation of the lamb carcass has been shown to rush the musculature into rigor at a very high rate – in some cases within an hour of slaughter – and the process has been tested successfully in an otherwise normal commercial operation.

There are thus several possible ways already in which the serious detrimental effects of cold shortening can be minimized or prevented. Nevertheless, a substantial case can be made for continued study of the phenomenon and of its consequences, for its better understanding could well yield a better control. Furthermore, the effect is of considerable interest at a more basic level, since it bears a strong resemblance in many ways to the contraction of living muscle. We know, for instance, that both processes are brought about by a sliding of the thick and thin filaments over each other, and that both are triggered by a release of calcium ions: rapid in the physiological length change, slow in the cold-induced postmortem shortening. In one respect, however, the two effects differ greatly, for whereas both visibly red and visibly white muscles are obviously capable of vigorous shortening in life, only the former display cold shortening; the length of a rabbit psoas, for instance, is virtually unaffected by exposure to chiller temperatures early postmortem.

This observation has been used to support the view that, as in a living contraction, it is from the sarcoplasmic reticulum (SR) that calcium ions are released. It is well established that the SR is much more developed in white muscles than in red, and it is argued that the enhanced calcium-binding ability of the "white" reticulum explains the reluctance of these paler tissues to cold shorten. This explanation is not wholly satisfying, however, for it supposes that the SR of white muscle, besides being more extensive than that of the red, is also more able to retain its calcium at low temperatures. A recent alternative hypothesis suggests that the free calcium initiating cold shortening comes from the mitochondria responding to postmortem anoxia, and not from the SR reacting to cold. If this concept is coupled with the well known reluctance of the SR to sequester calcium at low temperatures, the difference in cold-shortening ability between red and white muscles can be explained quite readily, for only in the red tissue are there enough mitochondria to release sufficient calcium to trigger a length change. Although this and similar lines of investigation do not immediately suggest alternative practical procedures to eliminate cold shortening and its accompanying toughness, it is quite within the bounds of possibility that the better under-

standing they generate may lead ultimately to an improved solution.

The basis of tenderness in muscle foods has thus become much clearer in recent years. Recognition that there are two – and, almost certainly, only two – major contributors to toughness has all but eliminated the contradictory results of earlier studies, and it is now possible to study either of the prime causes without the confusing and often unknown intervention of the other. This ability to separate toughness due to collagen from that due to the contractile apparatus has led to rapid progress in both areas. Already it is practical, by appropriate postmortem treatment, to avoid the potentially great toughening caused by shortening, and modification of the collagen component during life may soon become a reality.

Additional information and specific references will be found in the reviews and other relevant papers included in the Bibliography.

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SYMPOSIUM: The Basis of Quality in Muscle Foods
RECENT DEVELOPMENTS IN THE FLAVOR OF MEAT

ABSTRACT

Techniques involved in the study of volatile flavor compounds of cooked meat, including isolation of the volatile flavor compounds, fractionation of the isolated volatiles by gas chromatography and identification of the pure gas chromatographic fractions by infrared and mass spectrometry are reviewed. The volatile compounds identified to date, as components of cooked meat, such as boiled beef and roast beef, are reported. The importance of heterocyclic compounds containing oxygen, nitrogen and sulfur in the ring and pyrazine compounds to the flavor of meat are discussed with their possible mechanisms of formation. The possible use of high pressure liquid chromatography for the fractionation of the less volatile flavor compounds in cooked meat and the many difficulties of this technique are reviewed. The deterioration of flavor during storage and processing as caused by the qualitative and quantitative changes of flavor compounds are discussed with detailed information on the change of flavor compounds during the retorting of beef stew.

INTRODUCTION

THE FLAVOR of meat is of great academic, as well as practical importance, but we are still unable to completely understand this important sensory quality of muscle foods. With the advancement of modern instrumentation, we have made a giant step forward in understanding the volatile compounds responsible for meat flavor during the last 15 yr. However, we still do not have a complete spectrum of these compounds. Consequently, the quantitative composition of meat flavor volatiles and the mechanism of their formation are also not conclusive. (For a review of meat flavor research up to 1970, see Herz and Chang, 1970; up to 1975, see Dwivedi, 1175.)

In the earlier research on meat flavor components, the compounds identified included a few simple sulfides, mercaptans, carbonyl compounds, alcohols, carboxylic acids, ammonia, hydrocarbons, esters, and aromatic compounds (Bender, 1961; Bender and Ballance, 1961; Hornstein and Crowe, 1960; Kramlich and Pearson, 1960; Sanderson, et al., 1966; Yueh and Strong, 1960). At that time a hypothesis was formulated by some researchers that we had already identified enough compounds for meat flavor, what we were lacking was simply the relative concentration of the compounds which had been identified. They claimed that if the appropriate relative concentrations were known, one could duplicate the characteristic flavor of different meats using the few simple compounds which had been identified by that time. As research continued, an increasing number of important meat flavor components were identified, including heterocyclic sulfur and nitrogen containing components. Without such compounds, meat flavor could not possibly be reconstituted, clearly demonstrating that the earlier hypothesis was an oversimplification of a complex problem.

Another hypothesis was put forth that the characteristic flavor of different meats is not produced by the lean muscle during cooking, but rather produced by the lipids during cooking (Hornstein and Crowe, 1960). Indeed, the fatty tissue may serve as the solvent for the precursors of meat flavor but lipids themselves can, in no way, produce some of the sulfur- and nitrogen-containing heterocyclic compounds which have been identified as components of meat flavor since then. In addition, the fat portion of the meat from different animals, after extraction and refining, does not produce characteristic meat flavor of different animals when it is cooked.

At present we do have a large number of compounds which have been isolated and identified in the volatiles of meat. However, we still cannot combine them to obtain a genuine flavor characteristic of meat, such as beef, pork or chicken. We probably have not yet identified the "star performers" in meat flavor which are predominantly responsible for the characteristic flavor which makes beef flavor different from that of pork or lamb.

Certainly, the aroma of a food is the total contribution of a large number of volatile flavor components. Nevertheless, most flavors do contain "star performers" or "key compounds" which play the major role in imparting a specific flavor to the food. Examples are 3-isobutyl-2-methoxypyrazine in bell pepper flavor and 4-hydroxy-3-methoxybenzaldehyde (Vanillin) in vanilla flavor.

CLASSES OF COMPOUNDS IDENTIFIED

LET US NOW briefly review the types of compounds that have been identified as components of meat flavor.

The types of compounds which, we believe, are not primary contributors to meat flavor are listed in Table 1. Carbonyl compounds have been listed here, even though some researchers believe carbonyl compounds are extremely important to the flavor of meats. They state that the only difference between the flavor of different meats is essentially due to the qualitative and quantitative differences in the content of carbonyl compounds (Hornstein and Crowe, 1963; Jacobson and Koehler, 1963; Sanderson et al., 1966). Certainly, carbonyl compounds are powerful odor components, which undoubtedly contribute to the total flavor of meat. Furthermore, it is well established that the carbonyl compounds are mostly autoxidative or oxidative decomposition products of lipids. Since the lipids which constitute the fat of different animals are composed of different fatty acids, cooking of fat-containing meat of different animals should produce qualitative and quantitative differences of carbonyl compounds. However, in our rather extensive experience in studying the autoxidative and oxidative decomposition products of refined animal fats and oils, we never noticed any odor or flavor reminiscent of meat. In addition, when lean beef, carefully trimmed of fat was boiled, a delicious boiled beef flavor was produced. Identification of volatile compounds in this boiled beef flavor showed a limited number of carbonyl compounds and none of them had meaty notes (Hirai et al., 1973).

The classes of compounds which probably are important

Table 1—Classes of compounds which may not be primary contributors to meat flavor

Aliphatic hydrocarbons
Aromatic hydrocarbons
Saturated alcohols
Carboxylic acids
Esters
Ethers
Carbonyl compounds (Aldehydes and Ketones)

Table 2—Classes of compounds which may be important contributors to meat flavor

Lactones
Acyclic sulfur containing compounds
e.g., mercaptans, sulfides
Nonaromatic heterocyclic compounds containing S, N, O
e.g., hydrofuranoids
Aromatic heterocyclic compounds containing S, N, O
e.g., pyrazines, thiophenes

contributors to meat flavor are listed in Table 2. Let us consider some examples of each of these more important classes of compounds which have been identified in meat volatiles.

The lactones that have been found both in roast beef drippings and boiled beef (Hirai et al., 1973; Liebich et al., 1972) are listed in Table 3. The lactones have very interesting flavor characteristics and can be used to improve the butter flavor of margarine and the deep-fat fried flavor in snacks (Chang and May, 1973). They certainly could contribute to the total flavor of cooked meat.

Furanoid compounds have been isolated and identified in cooked beef. Table 4 lists some of these compounds. The 2-pentylfuran and 2-thiomethylfurfural have been reported by this laboratory, and the 2-dihydrofuranones by Tonsbeek and co-workers at Unilever (Hirai et al., 1973; Tonsbeek et al., 1968). Patents have been issued on the use of the furanones for the production of imitation meat flavor.

A considerable number of various sulfur-containing compounds have been identified in cooked meat as the examples in Table 5 indicate. The 3,5-dimethyl-1,2,4-trithiolane identified by this laboratory was isolated from boiled beef and is an interesting compound having three sulfur atoms in a ring. It was initially believed to be of prime importance to the beef flavor. Unfortunately, after synthesizing it, it was found not to possess an odor which could be described as beef-like. Thiazoles have also been identified in canned beef products, both in this laboratory and by von Sydow and co-workers in Sweden (Peterson et al., 1975; Perrson and von Sydow, 1973).

The pyrazine compounds are a most important group of flavor compounds. They generally have a roasted or nutty aroma. This laboratory, as well as others, have found a number of them in boiled, roasted and shallow fried beef. Some of these compounds are listed in Table 6 (Mussinan et al., 1973; Watanabe and Sato, 1971). We are now engaged in an extensive study of the basic compounds isolated from roast beef and we hope to identify more of the important pyrazine compounds.

Even though the identification of the flavor compounds in meat is not yet completed and their mechanisms of formation are not clear, many patents have been issued on the production of imitation meat flavors by heating together a wide range of compounds as exemplified in Table 7. Such imitation meat flavors do have an odor reminiscent of meat, even though their final chemical constitution and the reactions taking place are not known. However, we must realize that the imitation meat flavors do not have the unique, characteristic flavor of meat, such as beef, lamb, or pork, which can be distinguished from one another.

ISOLATION and IDENTIFICATION OF FLAVOR COMPOUNDS

IN ORDER TO STUDY the volatile compounds which are responsible for the flavor of muscle foods, it is necessary to first isolate the volatile flavor compounds without creating artifacts which might interfere. The criterion used in this laboratory is that the isolated volatiles must have an odor which is identical to the characteristic odor of the original sample, as judged by an experienced organoleptic evaluation panel. As an example, the volatiles isolated from roast beef must be judged by the panel as having a typical roast beef aroma before the flavor sample is subjected to further analysis.

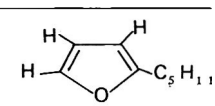
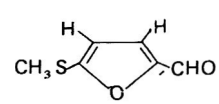
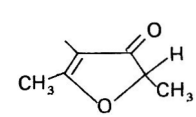
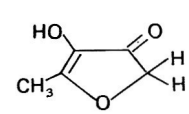
An apparatus designed in this laboratory for this purpose is shown in Figure 1 (Herz and Chang, 1966). Cooked meat was made into a water slurry which was kept under agitation at the proper temperature in the reservoir flask. The sample slurry was pumped continuously at a constant flow rate into the evaporator by a positive displacement rotating and reciprocating pump. The reservoir side of this pump is under atmospheric pressure while the evaporator side of the pump is under a vacuum of approximately 0.01 mm Hg. The water slurry of the cooked meat, when pumped into the evaporator, was at a

Table 3—Some reported lactones in cooked beef

Lactone	Source
γButyrolactone[1]	Roast beef drippings
γ-Hexalactone[1]	Roast beef drippings
γ-Heptalactone[1]	Roast beef drippings
γ-Valerolactone[2]	Boiled beef

[1] Liebich et al., 1972
[2] Hirai et al., 1973

Table 4—Some reported furanoid compounds in cooked beef

Furans	Structure
2-Pentylfuran (Hirai et al., 1973)	
5-Thiomethylfurfural (Hirai et al., 1973)	
4-Hydroxy-2,5-dimethyl-2-dihydrofuran-3-one (Tonsbeek et al., 1968)	
4-Hydroxy-5-methyl-2-dihydrofuran-3-one (Tonsbeek et al., 1968)	

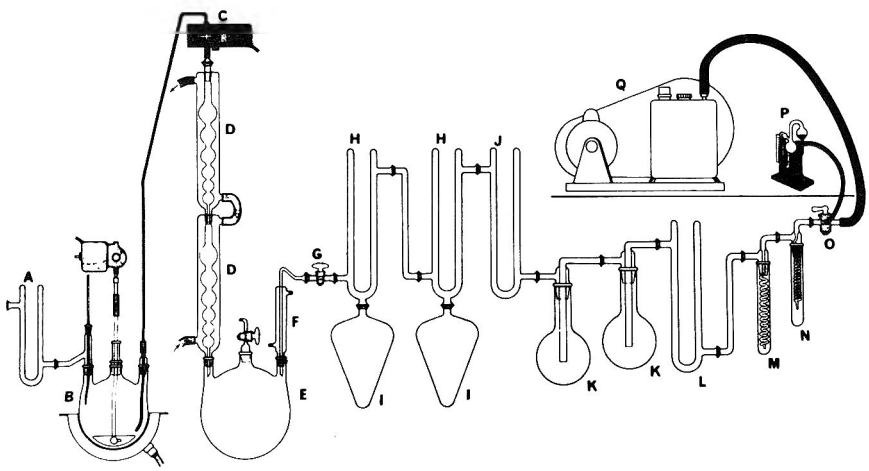


Fig. 1—Apparatus used for the isolation of volatile flavor compounds from cooked meat.

temperature near its boiling point and was suddenly released to high vacuum. Consequently, a flash evaporation would take place. The residue then flowed down the evaporator as a thin film. Heat was supplied to the film by circulating heated glycerine through the outer jackets of the evaporator. The volatile compounds in the cooked meat were therefore under the best conditions for vaporization, namely, a thin film under vacuum with heat supplied from outside. The volatile compounds and water thus evaporated were condensed in a series of traps which were cooled by either dry ice or liquid nitrogen.

The volatiles that collected in the cold traps were then extracted into ethyl ether, and the ether solution of the meat flavor components was then concentrated with a spinning band still to a volume suitable for gas chromatographic analysis.

The conditions used in this apparatus are relatively mild, However, the volatiles isolated still have some odors due to artifacts in addition to the true sample odor. In order to isolate the volatiles from cooked meat under milder conditions, the modified gas washing bottle shown in Figure 2, was designed. It is simply a long bottle with a gas inlet tube extending to the bottom. The end of the gas inlet tube is blown into a large disk with pinholes spaced equally along its circumference. The bottle is filled with a water slurry of the cooked meat and pure nitrogen gas is bubbled through the sample continuously. The exit gas is passed through a series of traps cooled by dry ice in order to condense the volatile flavor compounds together with water vapor.

The amount of volatiles isolated by this method is less than the amount obtained by the previously described method. However, the aroma of the volatiles isolated by this method more closely resembles the aroma of the original cooked meat.

The volatiles isolated from boiled beef, when gas chromatographed, yielded the curve shown in Figure 3. That of roast beef is shown in Figure 3A. Due to the complexity of the beef flavor, none of the peaks shown in this figure represents a pure compound. Therefore, each peak was accumulatively collected as an individual fraction. Each of the fracions was rechromatographed with a different stationary phase into subfractions. Each of the subfractions was again collected and chromatographed, if necessary, to yield sub-subfractions. This was continued until pure gas chromatographic fractions were obtained. The pure fractions were then identified by the use of IR and mass spectrometry. This also allowed an organoleptic evaluation panel to smell each of the trapped pure fractions in order to describe its odor characteristics.

Whenever possible, this flavor research technique is simplified by smelling the exit gas from the gas chromatograph. Only

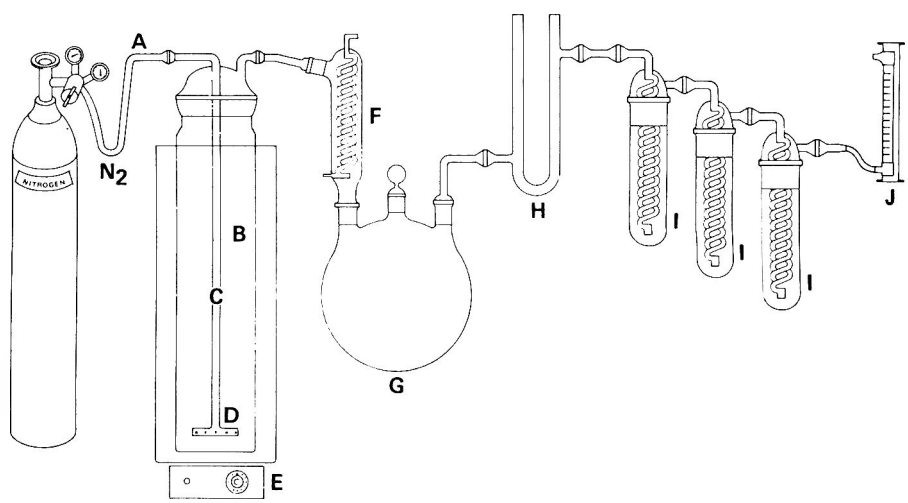


Fig. 2—Specially designed apparatus used for the isolation of volatile flavor compounds from cooked meat.

those peaks with an interesting odor, or an odor relevant to the aroma of the original sample, are collected and identified.

The method this laboratory has adopted is certainly tedious and time-consuming. However, it offers a better chance to identify those compounds which we are looking for, that is, compounds with unusual chemical structures, which may have the unique odor characteristics of interest.

Table 5—Some sulfur-containing compounds identified in cooked meat

Mercaptans	**Thiazoles**
Methylmercaptan	2-Methylthiazole
Ethylmercaptan	Benzothiazole
Sulfides	**Polysulfur heterocycles**
Methylsulfide	3,5,Dimethyl-1,2,4-trithiolane
Methyldisulfide	5,6-Dihydro-2,4,6-trimethyl-1,3,5-dithiazine
Thiophenes	2,4,6-Trimethyl-5-thrithiane
2-Methylthiophene	
Tetrahydrothiophene-3-one	

Table 6—Some reported pyrazines in cooked beef

Pyrazine	Source
2-Methylpyrazine	Shallow fried beef
2,3-Dimethylpyrazine	Boiled beef
2,5-Dimethylpyrazine	Shallow fried beef
2,6-Dimethylpyrazine	Shallow fried beef
2,3,5-Trimethylpyrazine	Shallow fried beef
	Pressure cooked beef
2,3,5,6-Tetramethylpyrazine	Shallow fried beef
2-Ethyl pyrazine	Shallow fried beef
2-Ethyl-5-methylpyrazine	Shallow fried beef
2,5-Dimethyl-3-ethylpyrazine	Shallow fried beef
	Pressure cooked beef

Table 7—Major components of patented "imitation meat flavor"

Amino acids and proteins	**Sugars**
Glycine	Ribose
Valine	Glucose
Proline	Xylose
Carnosine	**Carboxylic acids, lactones**
Albumin	α-Ketobutyric acid
Glycoprotein	Lactic acid
Sulfur contributors	γ-Lactones
Cysteine	**Oils and fats**
Cystine	Beef tallow
Glutathione	Lard
Nucleotides	Chicken fat
Adenosine-5'-monophosphate	**Phosphoric acid, phosphates**
Inosine-5'-monophosphate	
Guanosine-5'-monophosphate	

Table 8—Volatile compounds identified in boiled beef

Class of compounds	Compounds identified	Peak size[b]
Hydrocarbons	n-Hexane	Small
	n-Dodecane	Small
	n-Pentadecane	Medium
	n-Hexadecane	Small
	n-Octadecane	Small
	l-Undecene	Large
	l-Pentadecene	Small
Alcohols	Ethanol	Large
	1-Propanol	Large
	2-Methyl-1-propanol	Small
	1-Butanol	Small
	3-Methyl-1-butanol	Extra small
	1-Pentanol	Medium
	1-Octanol	Medium
	1-Penten-3-ol	Small
	2-Hexen-1-ol[a]	Small
	1-Octen-3-ol	Extra large
Ester	Ethyl acetate	Extra large
Lactone	γ-Valerolactone	Extra small
Aldehydes	3-Methyl-1-butanal	Small
	Pentanal	Large
	Hexanal	Extra large
	Heptanal	Large
	Octanal	Large
	Nonanal	Large
	Hexadecanal	Medium
	2-Octenal	Small
	6-Methyl-2-heptenal[a]	Medium
Ketones	4-Octanone	Small
	3-Nonanone	Small
	3-Dodecanone	Extra small
	Diacetyl	Large
	Acetoin	Extra large
Acids	Propionic acid	Medium
	Butanoic acid	Medium
	Hexanoic acid	Small
Sulfides	Methyl propyl sulfide[a]	Extra small
	Methyl allyl sulfide[a]	Extra small
	Dimethyl disulfide[a]	Extra small
	Diallyl sulfide[a]	Extra small
Aromatic compounds	Benzene	Large
	Toluene	Small
	Propylbenzene	Small
	1,4-Dichlorobenzene	Medium
	Benzaldehyde	Extra large
	3-Methylbenzaldehyde[a]	Small
	2,6-Di-tert.-butyl-p-hydroxytoluene	Medium
Heterocyclic compounds	5-Thiomethylfurfural[a]	Small
	2-Pentylfuran	Medium
	Thiophen-2-carboxaldehyde	Extra small
	2,5-Dimethyl-1,3,4-trithiolane	Large
	2,4,5-Trimethyl-Δ^3-oxazoline	Extra large
	2,5-Dimethylpyrazine	–
	2-Ethyl-3,6-dimethyl-pyrazine	–

[a] Tentatively identified

[b] Peak size was considered to be "extra small" when the peak area was < 50; "small" between 50 and 300; "medium" between 300 and 1,000; "large" between 1,000 and 2,000; and "extra large" > 2,000.

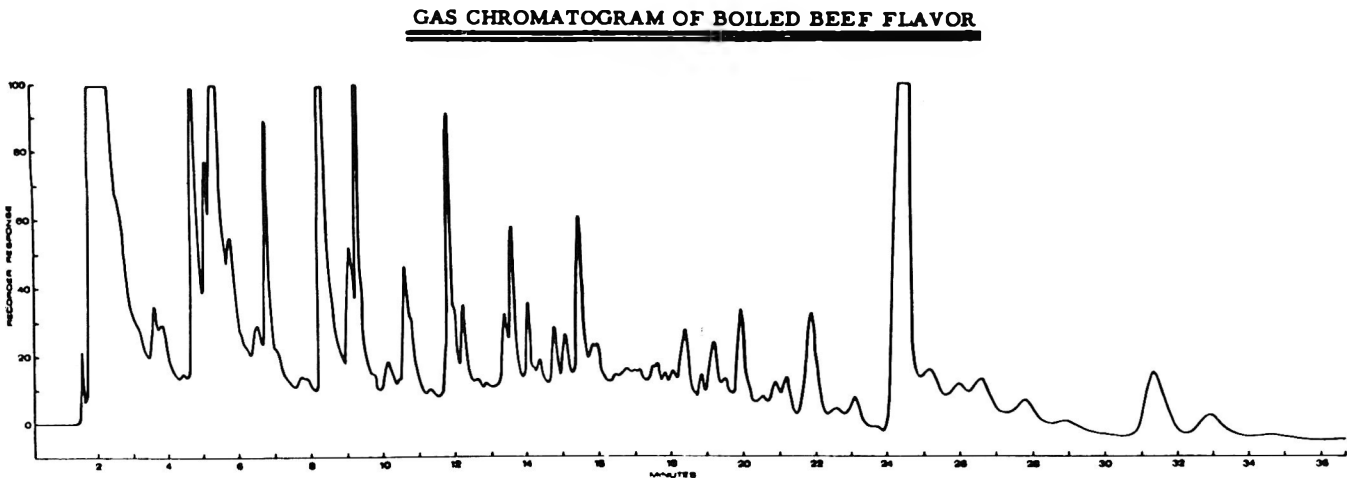


Fig. 3—A typical preliminary gas chromatogram of the volatiles isolated from boiled beef. [20 ft X 1/8 in. SS column packed with 15% Carbowax 20M on 60/70 mesh Anakrom ABS. Temperature programmed from 65 to 215° C at a rate of 4° C/min for the first 2 min, 8° C/min for another 2 min, and 12° C/min thereafter. The flow rate was 35 ml/min. FID.]

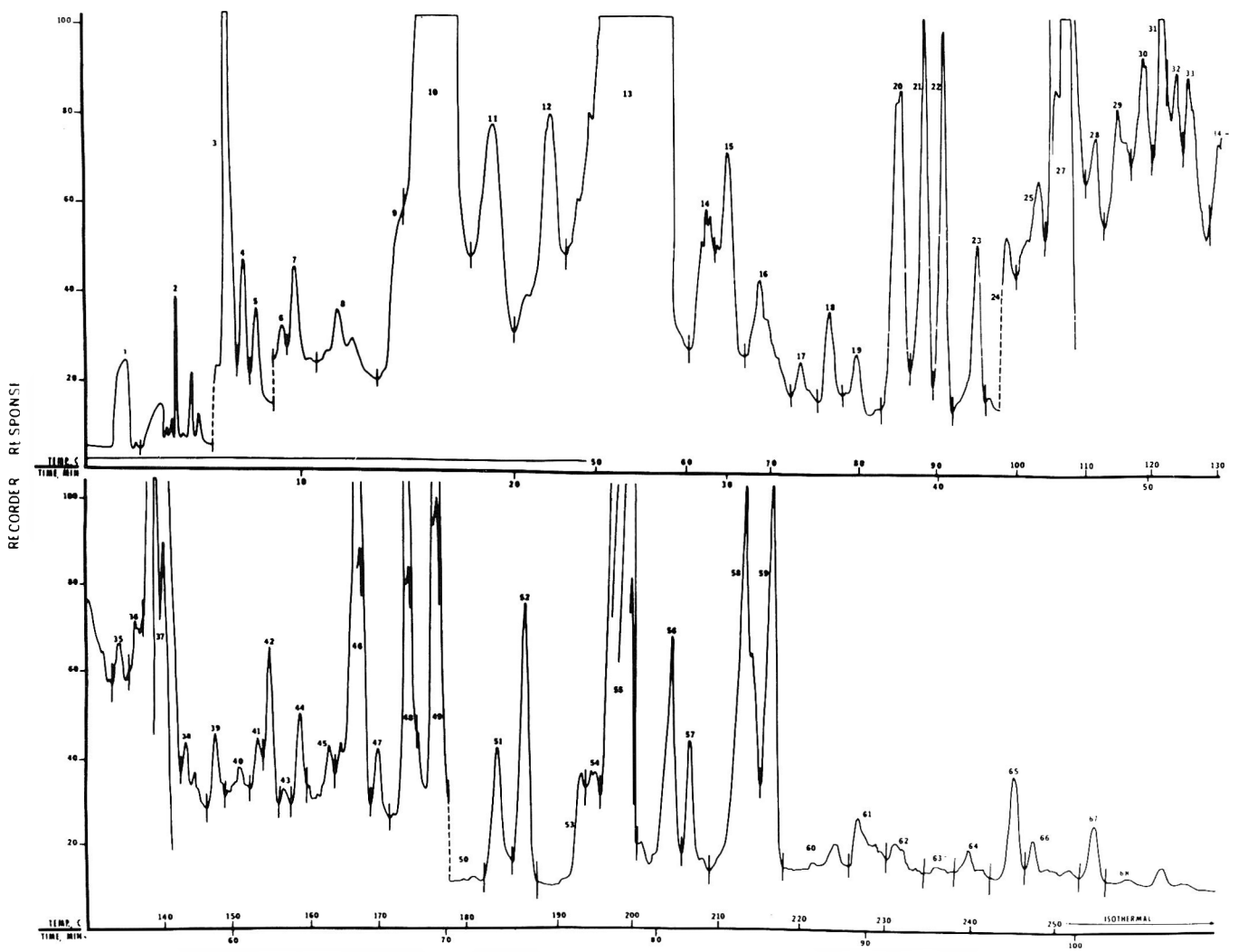


Fig. 3A—Preliminary gas chromatogram of the neutral volatile compounds isolated from roast beef. [10 ft X 1/8 in. SS column packed with 10% OV-101. Temperature programmed from 50° C (held 20 min) to 250° C (held 20 min) with program rate of 2.75° C/min. FID.]

COMPOUNDS IDENTIFIED IN BOILED BEEF AND CANNED BEEF STEW

THE VOLATILE flavor compounds identified by this laboratory from boiled beef are listed in Table 8. Among the many compounds identified, those with oxygen, sulfur, or nitrogen in a ring are probably of the most interest. Our group was the first to identify 2,4,5-trimethyl-3-oxazoline and was optimistic in regard to its importance in boiled beef flavor. Unfortunately, as with the trithiolane previously mentioned, when the compound was synthesized, it did not possess a characteristic beef aroma.

After establishing that the flavor methodology we used was quantitatively reproducible to ±5%, it was employed to compare the volatiles isolated from a fresh, boiled beef sample and a sample made from the same lot of beef, but which was freeze dried and stored under nitrogen at ambient temperature for 6 months. Organoleptic evaluation of the two samples showed that the flavor of the sample which had been freeze dried and stored was inferior to the flavor of the fresh sample. Figure 4 indicates that the deterioration of flavor resulting from freeze drying and storage is not due to the formation or elimination of a single compound, but rather, to the change in the relative concentration of a number of compounds. As can be seen in the figure, fractions 1, 2 and 5 greatly increased as a result of freeze drying and storage, while fractions 6 and 10 were greatly reduced. It is interesting to note that fraction 6 contained the oxazoline, and fraction 10 contained the trithiolane.

The techniques developed have also been applied to the study of the flavor deterioration due to retorting of beef stew. The flavor of canned beef stew is, in general, inferior to the flavor of freshly prepared beef stew. This is due to the development of a "retort flavor" during the sterilization process.

The gas chromatograms of the volatiles isolated from

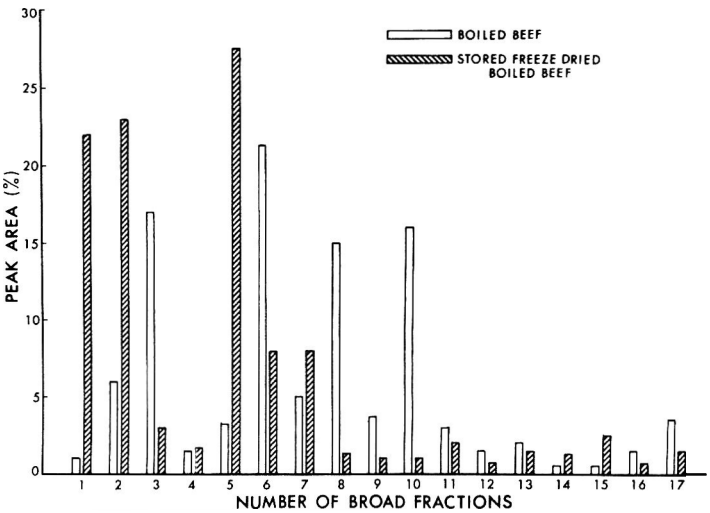


Fig. 4—Quantitative comparison of the broad gas chromatographic fractions of volatiles isolated from fresh boiled beef and boiled freeze dried and stored beef.

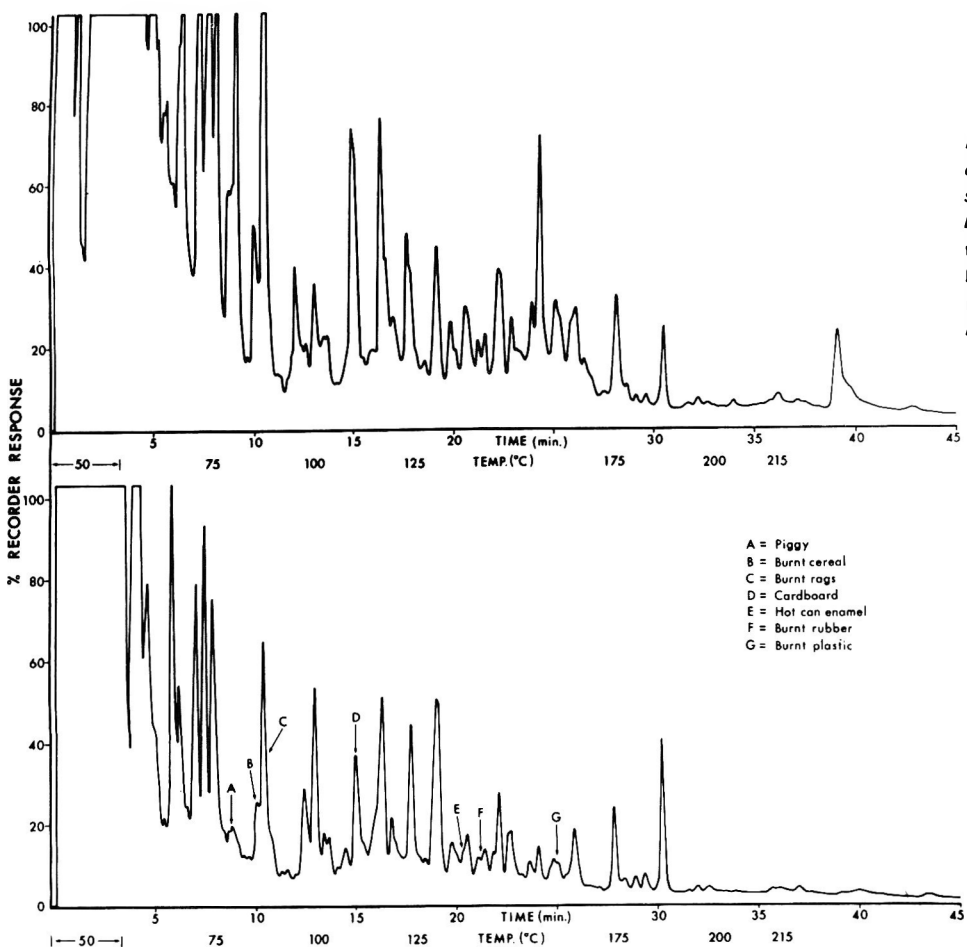


Fig. 5—Comparison of the gas chromatograms of volatiles isolated from fresh and canned beef stew (Top — fresh beef stew; Bottom — canned beef stew). [6 ft X 1/8 in. SS column packed with 5% OV-101 on 80/100 mesh Chromosorb W-HP. Temperature programmed from 50°C (held for 3.2 min) at a rate of 5 to 215°C/min. Helium flow rate was 33 ml/min. FID.]

freshly prepared beef stew and canned beef stew are shown in Figure 5. The chromatogram of the canned beef stew volatiles was recorded at twice the sensitivity of the freshly prepared beef stew volatiles. Thus, significant differences, both qualitative and quantitative, are clearly demonstrated. These differences are considered the end result of the chemical reactions which take place during retorting.

The 102 compounds identified in the volatiles isolated from canned beef stew are listed in Table 9. Both of the acylpyrroles identified have rather unpleasant, heated plastic odors when evaluated as gas chromatographic effluents. These compounds may, therefore, play an important role in the objectionable "retort flavor" developed in the canned beef stew. As mentioned, the oxazoline had been identified previously in

Table 9—Volatile compounds identified in canned beef stew

Class of compounds	Compounds identified	Peak size[a]
Saturated aliphatic hydrocarbons	2-Methylpentane	Medium
	3-Methylpentane	Large
	Heptane	Medium
	Octane	Small
	2-Methyloctane	Small
	3-Methyloctane	Small
	3-methylnonane	Extra small
	3-Methylundecane	Small
	Dodecane	Medium
	2-Methyldodecane	Small
	Tridecane	Medium
	2-Methyltridecane	Small
	3-Methyltridecane	Small
	4-Methyltridecane	Extra small
	Tetradecane	Small
	4-Ethyltetradecane	Extra small
	Pentadecane	Medium
	2-Methylpentadecane	Small
	Hexadecane	Small
	Heptadecane	Small
	Octadecane	Small
Unsaturated aliphatic hydrocarbons	1-Tridecene	Small
	1-Heptadecene	Extra small
Alicyclic hydrocarbons	Cyclohexane	Medium
	Methylcyclohexane	Large
	Ethylcyclohexane	Small
	Trimethylcyclohexane	Small
	α-Phellandrene	Medium
	γ-Terpinene	Small
	β-Caryophyllene	Medium
Aromatic hydrocarbons	Benzene	Large
	Methylbenzene	Small
	Ethylbenzene	Medium
	1,3-Dimethylbenzene	Medium
	3-Ethylmethylbenzene	Small
	Trimethylbenzene	Small
	4-Isopropylmethylbenzene	Small
	Dimethylstyrene	Small
	C_4-Alkylbenzene	Extra small
	C_5-Alkylbenzene	Small
	C_6-Alkylbenzene	Small
	Naphthalene	Medium
Alcohols	Ethanol	Large
	1-Butanol	Small
	1-Pentanol	Large
	1-Hexanol	Large
	4-Methyl-2-hexanol	Medium
	6-Methyl-1-heptanol	Medium
	1-Pentene-3-ol	Medium
	1-Octene-3-ol	Medium
	Benzyl alcohol	Extra small
	Phenol	Small
	2-Ethoxyethanol	Large
	2-Butoxyethanol	Medium
	1-Chloro-2-propanol	Small
Aldehydes	Butanal	Medium
	3-Methylbutanal	Small
	2-Ethylbutanal	Medium
	Hexananal	Large
	Heptanal	Medium
	Nonanal	Large
	Pentadecanal	Medium
	Benzaldehyde	Large
	Ethylbenzaldehyde	Small
Ketones	Acetone	Large
	3-Methyl-2-hexanone	Medium
	5-Methyl-2-hexanone	Medium
	6-Methyl-2-heptanone	Small
	2-Octanone	Medium
	2-Decanone	Medium
	2-Pentadecanone	Small
	Acetoin	Small
	Diacetyl	Small
	Methylacetophenone	Small
	β-Ionone	Small
Keto-aldehyde	3-Methyl-4-oxopentanal	Medium
Esters and lactones	Ethyl formate	Large
	Ethyl acetate	Large
	Terpinyl acetate	Small
	Diethyl phthalate	Extra small
	Dibutyl phthalate	Medium
	γ-Caprolactone	Small
Acid	Acetic acid	Medium
Furan compounds	2-Pentylfuran	Small
	Furfuryl alcohol	Medium
	2-Furfuraldehyde	Large
	5-Methyl-2-furfuraldehyde	Medium
	2-Acetylfuran	Large
	5-Methyl-2-acetylfuran	Small
Nitrogen compounds	Pyridine	Medium
	Methylpyrazine	Medium
	2,5-Dimethylpyrazine	Small
	2-Formylpyrrole	Medium
	2-Acetylpyrrole	Medium
	2,4,5-Trimethyl-Δ^3-oxazoline	Large
	2,4,5-Trimethyloxazole	Medium
Sulfur compounds	2-Acetylthiazole	Medium
	Benzothiazole	Small
	Thiophene-2-carboxaldehyde	Medium
Miscellaneous compounds	1,1-Diethoxyethane	Large
	Trichloromethane	Small
	1,4-Dichlorobenzene	Extra small

[a] Peak size was considered to be "extra small" when peak area was > 25 units; "small" when it was between 25 and 200 units; "medium" when it was between 200 and 500 units; and "large" when it was > 500 units.

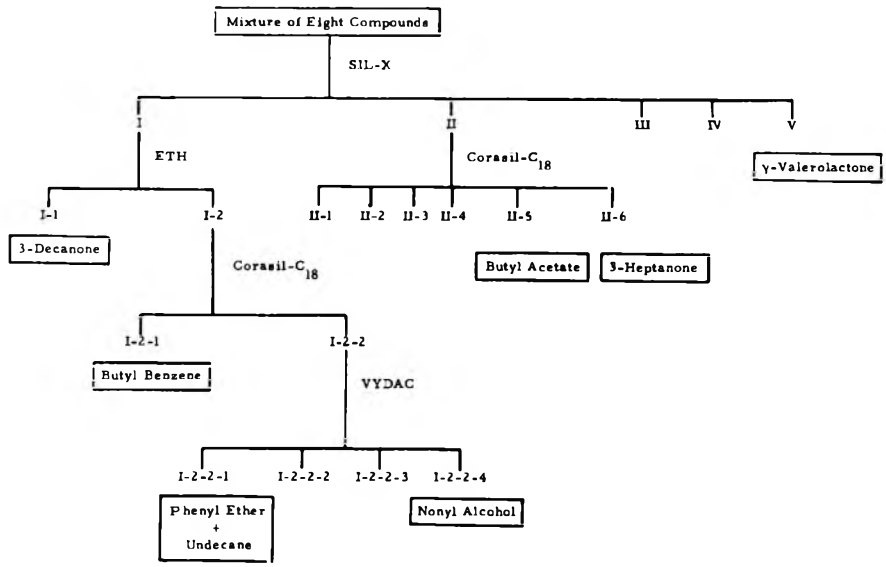


Fig. 6—Fractionation of a mixture of eight known components of boiled beef by high pressure liquid chromatography.

this laboratory in boiled beef. The completely unsaturated analogue of this compound, 2,4,5-trimethyloxazole, was now identified. This was the first time that this compound was identified as a component of the volatile flavor compounds of foods. The two thiazole compounds found had previously been only tentatively identified in pressure cooked beef by Wilson et al. (1973). Their presence in canned beef stew was confirmed by IR and mass spectra in this laboratory. Benzothiazole has a heated, rubber-like odor and may be of considerable importance in the undesirable odor notes of canned beef stew. On the other hand, the 2-acetylthiazole has a pleasant popcorn-like aroma with a strong nutty, roasted character.

A NEW APPROACH TO FLAVOR RESEARCH

DURING the gas chromatography of the volatiles isolated from cooked beef, it became obvious that not all the odoriferous compounds could be eluted from the gas chromatographic column. Some components were either decomposed by heat or were retained by the column. It may have been that such compounds were those having the important meat flavor. Therefore, we started to investigate the possibility of using high pressure, liquid column chromatography to fractionate the less volatile flavor compounds at ambient temperatures.

Figure 6 indicates that a mixture of eight compounds, which were previously identified in the volatiles of boiled beef, could be successfully separated by high pressure liquid column chromatography, if different packings and repeated chromatography were adopted.

In this fractionation, only packings available a few years ago were used. With the development of new and more effective adsorbants, such as the 5μ diameter silicic acid with 40,000 theoretical plates per meter, the use of high pressure liquid column chromatography becomes a very attractive means of fractionation for the less volatile components of meat flavor. We propose that the total volatile flavor compounds isolated from a cooked meat should be first separated into a more volatile and a less volatile fraction. The former should be fractionated by gas chromatography and the latter by high pressure liquid chromatography.

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COMPOSITION AND PALATABILITY OF MECHANICALLY DEBONED MEAT AND MECHANICALLY SEPARATED TISSUE

ABSTRACT

Four groups of Good and Choice grade carcasses were hand boned. Flat bones from the right sides were mechanically deboned using a Beehive machine with 0.46 mm holes in the cylinder and designated mechanically deboned meat (MDM). Flat bones from the left sides were hand-cleaned of all visible muscle and fat and then run through the mechanical deboner and designated mechanically separated tissue (MST). No significant differences in moisture, fat or protein between tissue from the right and left sides were present, but MDM had less ash than did MST. Higher ash content in the MST was paralleled by higher values ($P < 0.05$) for iron, calcium, magnesium, collagen and pH. Greater amounts of isoleucine ($P < 0.05$) were present in MDM than in MST. Bologna was formulated to contain 30% MDM or 30% MST. No differences in processing quality of the bolognas were noted. Independent triangle tests for flavor and grittiness showed that panel members could detect differences ($P < 0.01$) in bologna containing MDM or MST when compared to control bologna.

INTRODUCTION

INCREASED animal protein production with the use of mechanical deboners has been outlined by Field (1976). With increasing use of mechanically deboned red meat (MDM) as a human food source, the composition of tissue in deboned meat which is not skeletal muscle is of interest. Tissue other than skeletal muscle is designated as mechanically separated tissue (MST) in this study and is thought to be primarily bone marrow.

Considerable research has been done on the composition and yield of MDM (Grunden et al., 1972; Noble, 1973; Field et al., 1974a, b; Field and Riley, 1972). Amino acid composition of deboned turkey racks has been determined by Essary and Ritchey (1968). Emulsion stability and flavor aspects of deboned meat have also been investigated (Vadehra and Baker, 1970; Maxon and Marion, 1970; Kunsman and Field, 1973). However, very little data have been published on the protein composition of tissue other than muscle which is found in MDM.

Understanding the composition of mechanically separated tissue can help explain how the composition of MDM differs from the composition of hand boned lean. Characterizing MST may also lead to a way of determining how much protein other than muscle is present in MDM from different sources.

EXPERIMENTAL

FOUR GROUPS of Good and Choice grade beef carcasses were hand boned. Flat bones from the right sides of each group were ground through a 9.5 mm plate of a bone cutter and mechanically deboned using a Beehive machine with 0.46 mm openings in the cylinder head. Flat bones from the left sides were hand-cleaned of all visible muscle and fat, ground through a 9.5 mm plate, and mechanically deboned using the same Beehive machine.

MDM from the right sides and MST from the left sides were frozen at −25°C immediately after deboning. Samples were frozen 1−3 wk prior to analysis. The samples were analyzed for fat, protein, moisture and ash by standard AOAC methods (1970). Calcium, magnesium and iron were determined by atomic absorption spectrophotometry as outlined by the Perkin-Elmer Corp. (1964). Hydroxyproline analysis was conducted according to the methods of Woessner (1961) and hydroxyproline was converted to collagen using a factor of 7.25. A Corning model 12 pH meter, equipped with a surface electrode, was utilized in determining tissue pH values. Total pigments were obtained from the procedures outlined by Broumand et al. (1958). Ascorbic acid content of MDM and MST was conducted according to the modified colorimetric method of Roe and Kuether as outlined by Oser (1965). Total amino acids were determined using a modification of the Moore and Stein (1954) procedure with the aid of an Auto Amino Acid Analyzer.

Triangle difference tests were conducted to compare bologna made with MST or bologna made with MDM to a standard bologna formula made with hand boned meat. Thirty percent of the meat ingredient was replaced by MDM or MST in the bologna formulas. Seventy-four untrained staff members and students from the College of Agriculture were used as panel members for the triangle test. Panel members were asked to choose the different bologna (control vs MDM or MST) from the viewpoint of flavor. A separate triangle test for differences in grittiness of bologna containing MDM or MST vs control bologna was conducted after the flavor comparisons were completed. Panel members were also asked to indicate which samples they preferred.

Data were analyzed by least squares analysis of variance (Steel and Torrie, 1960). For the statistical analysis of the triangle test, the number of correct identifications of the odd sample was compared to a probability table to determine the statistical significance of differences (Roessler et al., 1948).

RESULTS & DISCUSSION

COMPOSITION OF MDM and of lean free MST are found in Table 1. The lower percentage figures for moisture and fat in MST when compared to MDM are due to the presence of more bone particles in MST. Lean and fat scraped from the flat bones of the left sides made up 34.3% of the total bone weight. It is assumed flat bones from the right sides also had 34% lean and fat at the time of mechanical deboning. The reason for more bone on a percentage basis in MST is the dilution of bone with lean in the MDM product (Field, 1976). Yield of MDM from the right sides (bones with lean and fat attached) averaged 30.1% while yield of MST from the left sides (bones with no lean or fat attached) averaged 19.5%. An attempt to determine the moisture and fat content of bone free MDM and of bone free MST was made by using a conversion factor of 5.0 to convert calcium content to dry-fat-free bone (Field et al., 1974c). The results are shown in parenthesis in Table 1. No differences in moisture and fat between MDM and MST are present when the data are expressed on a bone free basis.

Protein and collagen content of MDM were lower than for

MST. These differences are partially due to the presence of a higher bone content in MST. Early work by Eastoe and Eastoe (1954) stated that the marrow free epiphyses of ox femora contained 18.6% collagen, 0% fat and 8.18% water. More recently Brown et al. (1972) and Brown and Hacker (1974) have shown that collagen concentration in bone increases with maturation and that the collagen concentration is altered by many different factors. Brown et al. (1972) suggested that there was a unique chemical development pattern for each bone. Some of the pig long bones which they studied contained over 30% collagen when the pigs were 16–24 wk of age. In view of these data, it is obvious that an increased bone content will result in an increased collagen and protein content in MDM and MST. Collagen and protein percentages in bone are closely associated, since collagen comprises over 90% of the organic phase of marrow free bone (Posner, 1969). In addition to collagen from bone, some collagen from connective tissue closely associated with the bone undoubtedly became part of the MST and to a lesser extent, part of the MDM. Data reviewed by Field (1976) show that when products like lamb breasts or whole chicken necks are mechanically deboned, collagen is removed by mechanical deboning. The data in Table 1 make it clear that the amount of collagen in MDM is related not only to bone content of MDM but to the amount of lean on the bone prior to deboning.

Ash and calcium increases in MST as compared to MDM are a result of increased bone content. Percentage ash in dehydrated bone is relatively constant at 68.5% (McCance and Dickerson, 1961) and the percentage of calcium in bone ash is relatively constant at 37% (Field et al., 1974c). The increase in magnesium is also due to higher bone content since marrow free bone contains 0.4–0.7% magnesium (Eastoe, 1961).

Since bone contains only trace amounts of iron, the figures for iron in MDM and in MST can be expressed on a bone free basis using the same procedure described for moisture and fat. According to Eastoe (1961) dried rabbit bone contains 0.008% iron while rat bone contains 0.0027% iron. Increased levels of iron in mechanically separated tissue when compared to mechanically deboned meat were expected since bone marrow contains much higher levels of iron than does muscle. According to Garcia (1957), rat bone marrow from 150 and 250-day old rats contains 8.8 and 11.0 mg of iron per 100g of marrow respectively. The figure of 8.8 mg of iron per 100g of rat marrow is similar to the 8.16 mg of iron per 100g listed in Table 1 for mechanically separated bone free tissue. Watt and Merrill (1963) state that prime beef containing 41% fat (fat content similar to 42.84% in mechanically separated tissue) has 2.0 mg of iron per 100g of meat. It is possible that the increased iron content of fat-free mechanically deboned meat could be used as an indicator of the amount of red marrow present. Higher values for iron in MST were paralleled by increased total pigments present when compared to MDM.

The ascorbic acid content was studied since fresh bone marrow found in MDM and MST is high in this vitamin (Table 1). About 24 mg of ascorbic acid were found per 100g of tissue in rat bone marrow (Lutwak-Mann, 1952) and 13–15 mg per 100 cells were observed in human bone marrow (Cox et al., 1960). The ascorbic acid content of mechanically deboned meat and mechanically separated tissue in Table 1 is likely low because ascorbic acid is easily oxidized in the presence of air. Oxidation of ascorbic acid could have occurred as the split carcasses hung in the cooler and later during mechanical deboning.

The figure of 7.7 for pH of mechanically separated lean free tissue was higher than expected (Table 1). While no pH values for bone marrow were found in the literature, bone marrow undoubtedly made the major contribution to the high pH readings. The possibility exists that calcium phosphate from bone also contributed to this high pH value. Anderson and Gillett (1974) have observed that the pH of marrow extracts were more than a full unit higher than muscle extracts from the same animal. They also found that adding marrow to muscle increased the pH of the muscle. The proportion of marrow to muscle present in MDM varies widely depending upon the amount of muscle on the bones at the time of mechanical deboning. High levels of marrow present in MDM help make conditions for growth of bacteria ideal. The importance of chilling MDM rapidly and using it after short holding periods

Table 1—Characteristics of mechanically deboned meat (MDM) and mechanically separated tissue (MST)[a]

Variable	MDM	MST	Standard error
Moisture, %	37.05 (40.5)	32.97 (39.8)	1.34
Fat, %	45.66 (49.9)	42.84 (51.7)	2.03
Protein, %	11.83	12.79	0.50
Collagen, %	2.63b	6.72c	0.34
Ash, %	4.33b	8.72c	0.53
Calcium, %	1.72b	3.44c	0.23
Magnesium, μg/100g	30.00b	53.00c	3.07
Iron, μg/100g	5.25 (5.75)	6.75 (8.16)	0.56
pH	7.23b	7.70c	0.11
Total pigments, mM/ml	10.16	11.21	1.58
Ascorbic acid, μg/100g	1.37	1.28	0.24

[a] Figures in parenthesis are on a bone free basis using a conversion factor of 5.0 to convert calcium in flat bones of 12–24 month old cattle to dry-fat-free bone (Field et al., 1974c). Means on the same line bearing different letters differ significantly ($P < 0.05$).

Table 2—Amino acid composition of mechanically deboned meat (MDM) and mechanically separated tissue (MST)[a]

Amino acid	MDM	MST
Threonine	4.2	3.7
Valine	6.8	6.4
Methionine	1.7	1.6
Isoleucine	4.0b	2.7c
Leucine	7.2	6.5
Phenylalanine	4.0	4.0
Lysine	7.4	7.2
Histidine	3.1	3.0
Arginine	6.7	6.8
Tyrosine	2.9	2.5
Aspartic acid	8.5	8.2
Serine	3.8	3.8
Glutamic acid	12.5	12.4
Proline	6.2	8.3
Glycine	7.2	8.4
Alanine	6.5	7.1

[a] All values are expressed as the percentage in the crude protein (N X 6.25) and are the average of triplicate analysis on each of four different lots of MDM and MST. Means on the same line bearing different letters differ significantly ($P < 0.05$).

Table 3—Triangle test results for bologna made with hand-boned beef and mechanically deboned meat (MDM)

	No. of judgments	No. correct	No. preferring	
			Hand-boned	MDM
Flavor	41	19	12	7
Grittiness	41	29[a]	23	6

a Significant difference ($P < 0.01$)

Table 4—Triangle test results for bologna made with hand-boned beef and mechanically separated tissue (MST)

	No. of judgments	No. correct	No. preferring	
			Hand-boned	MST
Flavor	33	28[a]	15	13
Grittiness	33	26[a]	18	8

[a] Significant difference ($P < 0.01$)

at 0–3°C is obvious, if acceptable microbial levels are to be maintained. Anderson and Gillett (1974) have suggested that higher pH values in MDM increase the extractability of protein. In the present study, handmixing of the fresh MDM or MST at 0°C soon formed a sticky, rubbery-type product. This was undoubtedly a result of the high pH. Of interest to the sausage maker is the protein:moisture ratio for MDM and MST (Table 1). The values are similar to those for many hand-boned sausage ingredients and they are lower than those reported by Grunden et al. (1972) for mechanically deboned poultry.

The amino acid contents of mechanically deboned meat and mechanically separated tissue are given in Table 2. Isoleucine was the only amino acid that was statistically different ($P < 0.05$) and it was higher in mechanically deboned meat. The greater amount of collagen in mechanically separated tissue may be partially responsible for this difference since collagen is low in isoleucine (Mello et al., 1975). However, collagen is low in several other essential amino acids which did not show a statistical ($P < 0.05$) difference. Isoleucine may be a limiting essential amino acid in MST (marrow) as it is in blood (Dill, 1975).

There appear to be no differences between the nonessential amino acids found in MDM and in MST. However, glycine, proline and alanine are all slightly higher in MST. These amino acids are also higher in collagen than in muscle (Field, 1976).

Bolognas were formulated to contain 30% MDM or 30% MST Moisture, fat and protein content of the bolognas were within 1% of each other. However, higher ash percentages were noted in the bologna with MST added. This is due to the higher ash content of the MST (Table 1). Weight loss for the two bologna formulations during heating, smoking and chilling was 6.28 and 6.87% for the MDM and MST, respectively. These differences were not statistically significant. Triangle difference tests were conducted to determine whether differences in flavor and grittiness were present in bologna made from 30% MDM or 30% MST and a control bologna made with hand-boned meat. Tables 3 and 4 summarize the findings of the tests. No difference in flavor could be detected by the panel for the control bologna compared to the bologna made with 30% MDM; however, a significant difference for grittiness was found ($P < 0.01$). Of the 29 correct answers for grittiness, 23 preferred the control with only 6 preferring the MDM bologna. In the test involving the control bologna and the bologna made with MST, both flavor and grittiness were significantly different ($P < 0.01$). Of the 28 correct answers for flavor, 15 preferred the control while 13 preferred the MST bologna. These tests were conducted with panel members who had not eaten bologna made with MDM. Previous tests in our laboratory, utilizing experienced panel members, indicated that 30% levels of MDM high in calcium could be detected in bologna. The finding that approximately half of the panel members preferred the flavor of bologna with MST was surprising in light of the statement by Carpenter (1976) who associated excess iron from marrow in mechanically deboned meat with an off-taste described as a "liver" taste. It is probable that experienced panel members would have preferred the control bologna because the flavor was more typical of traditional bologna. Perhaps larger consumer tests comparing control bologna and bologna containing MDM should be conducted to ascertain if the change in flavor is objectionable to consumers as a whole.

Overall, this study has shown that with the exception of iron (pigments), pH and isoleucine, there is very little chemical difference between bone free MST and bone free MDM. The chemical composition of these products are also similar to those of muscle (Rice, 1971). The calcium content of MDM must be kept within limits to avoid grittiness in the finished product. The amount of MDM which can be added and still avoid grittiness depends upon the calcium content of the MDM and upon the size of the individual bone particles. This study has raised some question about the acceptability of flavor of MST and the findings infer that a flavor different from the traditional bologna flavor may not be objectionable to the consumer.

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MICROWAVE AND CONVENTIONAL PRECOOKING OF HOT AND COLD PROCESSED PORK LOINS

ABSTRACT

20 barrows were used to study the effects of hot and cold processing of pork loin chops and roasts precooked by conventional or microwave methods. Cooking time, losses, palatability and histological characteristics were determined. Precooking of hot processed chops appeared unfeasible due to decreased tenderness related to muscle contraction. Precooking of hot processed roasts by conventional roasting appeared feasible because of lower cooking losses and acceptable tenderness as compared to that of cold processed roasts. Microwave precooking of chops and roasts was less desirable than conventional precooking because of increased cooking losses and decreased tenderness. Sarcomere length and fiber diameter were related to muscle tenderness.

INTRODUCTION

HOT PROCESSING of pork carcasses is economically desirable because of reduced chilling costs when compared to chilling entire carcasses. The utilization of hot processed pork loins has been studied by several workers. Weiner et al. (1966) found that pork loins frozen within 1 hr postmortem had significantly higher shear and expressible moisture values than conventionally processed loins. Loins removed from the carcass within 1 hr postmortem and cooled in a 0–3°C cooler for 48 hr were significantly more tender than loins which were chilled while still attached to the carcass. Moore et al. (1966) observed that fresh loins could be cut from the prerigor carcass but that the loins required chilling in the conventional manner. They found differences ($P < 0.01$) in response due to animal variation for percent thaw loss, cooking loss, moisture loss, residual moisture and shear value. Hinnergardt et al. (1973) studied the accelerated chilling of pork loins and observed that pork loin chops chilled at −45 and −29°C were less tender than chops chilled more slowly.

Precooking of loins immediately after the hog is slaughtered offers one possible solution to the problem of decreased tenderness associated with hot processing of pork loins. Several studies have shown that muscle cooked soon after the animal is slaughtered is more tender than muscle cooked soon after the development of rigor mortis (Ramsbottom and Strandine, 1949; Paul et al., 1952; deFremery and Pool, 1963; Weidemann et al., 1967).

The objectives of this study were to determine the cooking yield and tenderness of pork loin roasts and chops from hot and cold processed pork carcass sides, to determine the effects of microwave and conventional cooking of hot and cold processed pork, and to compare the histological characteristics of reheated pork muscle initially cooked prerigor with that initially cooked postrigor.

EXPERIMENTAL

A 20 x 2 x 2 factorial experiment was conducted. The main effects were animals, processing method (hot and cold) and cooking method (conventional and microwave). The 20 barrows in the study were obtained from the Texas Tech University swine herd. They averaged 92 kg live weight and were produced in a three-breed rotational breeding program using Duroc, Hampshire and Yorkshire boars.

On the day before slaughter, the hogs were weighed, transported to the Texas Tech University Meat Laboratory and randomly assigned to a kill sequence. They were given access to water but not to feed. After an overnight fast, the hogs were weighed and moved to the kill floor where they were shackled and exsanguinated with minimal excitement. They were not stunned. This procedure was followed in an attempt to prevent an unusually rapid rate of postmortem glycolysis (Overstreet et al., 1975). The hogs were skinned, beginning within 5 min of exsanguination. The body was eviscerated and the carcass was split into sides. The sides were alternately assigned to either hot or cold processing so that each treatment contained 10 left and 10 right sides. The side assigned to cold processing was chilled overnight at 1°C. Chilling time ranged from 18–22 hr. The side assigned to hot processing was immediately broken into wholesale cuts in a 7°C cutting room. The entire procedure from exsanguination to processing or beginning of chilling required about 30 min.

Cutting of loin chops and roasts

After removal from either the chilled or unchilled side, the loin was trimmed of excess subcutaneous fat and cut into chops and roasts which were assigned to either microwave or conventional cooking methods. The intact loin was divided into anterior and posterior portions between the 10th and 11th costae. The anterior portion was cut on a band saw into three chops 2 cm in thickness beginning at the 10th costa. Two additional chops were cut from the cold processed loin. The three most anterior chops from the cold processed loin served as control chops. The posterior loin portion was cut into six chops, 2 cm in thickness, beginning with the 11th costa. Two roasts 12 cm in length were cut from the remaining posterior portion of the loin. The loin section containing the ilium was not used in the study. All of the chops were obtained from the thoracic vertebrae region of the loin, and in most sides all chops were cut from the section including the 6th through the 13th thoracic vertebrae. All except the control chops were alternately assigned to either conventional or microwave cooking treatments. The roasts also were alternately assigned to the two cooking treatments. Subcutaneous fat in excess of 0.6 cm was trimmed from all chops and roasts after they were cut from the loin.

Cooking

The precooking procedure was the same for both the unchilled and chilled chops and roasts. Chops were cooked on racks to an internal temperature of 63°C in a microwave oven (General Electric Model JET 80002, 120V, 11.2 amp., 1320 W) or were broiled in a conventional electric oven. The average rise in internal temperature was 2°C after the chops were removed from either oven. Roasts were placed in elastic netting and roasted on racks to an internal temperature of 63°C in either a 121°C conventional electric oven or microwave oven. The average rise in internal temperature after removal from the oven was 1°C for the conventional oven and 4°C for the microwave oven. Internal temperature of the conventionally cooked chops and roasts was measured with a mercury type, glass column thermometer. The temperature of chops and roasts cooked in the microwave oven was measured with a Taylor spirit thermometer. The chops from hot processed loins were placed in the ovens about 1 hr postmortem and the roasts about 1.5 hr postmortem. Regardless of the processing treatment, the chops were cooked before the roasts. After precooking, the chops and roasts were wrapped in freezer paper, frozen at −23°C, and stored for up to 6 mo.

The chops, which had been precooked by both methods, were thawed overnight at 4°C and then reheated to an internal temperature

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of 68°C by broiling in a conventional electric oven. None was reheated in the microwave oven. Three chops from each treatment were broiled per pan to determine reheating time and evaporation, drip and total cooking losses. After reheating, the chops were allowed to cool to room temperature and three 1.2-cm cores were taken from the longissimus muscle in each chop. Each core was sheared three times with a Warner-Bratzler (W-B) shear device. The average of these 27 shears was used as the shear value for each treatment.

Evaluation of chops

The same chops were used for both W-B shear and sensory panel evaluations. Sensory evaluation was performed by a 6-member experienced panel composed of 3 males and 3 females. Cores 2.5-cm in diameter were removed from each reheated chop. Each sample was identified according to anatomical location within each chop and the corresponding sample from each of the four treatments was served on warmed plates to the same panel member at each evaluation session. All chops except the control chops were evaluated at one panel session. The control chops were evaluated after all of the treatment chops had been evaluated. The samples were scored on a 9-point Hedonic scale (9 = Like extremely, 1 = Dislike extremely), and the average panel score was used in the statistical analyses. Chew count was determined on the 2.5-cm cores.

The precooked roasts were reheated from the frozen state in a 155°C electric oven to an internal temperature of 77°C. After the roasts were removed from the oven, the longissimus muscle was excised, cut in half, and four 1.2-cm cores were taken from each of the halves. Three W-B shears per core were recorded and the average of the 24 shears was used for the treatment value. A sensory panel evaluation was not performed on the roasts.

Histological studies

To determine the extent of muscular contraction, sarcomere length and muscle fiber diameter were measured on 0.5-cm cubes from the lateral side of the longissimus muscle in cooked chops from the 10th and 11th costae. These samples were fixed in 10% buffered formalin and held at room temperature until measured. The fixed samples were blended for 30 sec in physiological saline in a blender which had the cutting blades reversed to facilitate tearing rather than cutting of the muscle fibers. A drop of the supernatant was placed on a glass slide and a cover slip was applied. Sarcomere length was measured with a microscope and an ocular micrometer at 1000x by determining the distance between 25 A bands on 20 fibers. The average length of the sarcomeres was used in the statistical analyses. Muscle fiber diameter was determined with an ocular micrometer at 100x magnification. The average diameter of 100 fibers was used in the statistical analyses.

The data were analyzed by the method of least squares (Harvey, 1960.) Duncan's New Multiple Range Test was used to test the differences between means when the analysis of variance revealed a significant effect in the main factor.

RESULTS

Uncooked chop weights

Table 1 contains least squares means for variables measured on the loin chops. The uncooked control chops were heaviest ($P < 0.05$), averaging 596.2g, and the hot processed chops were heavier than the cold processed chops. The greater mean weight of the control chops probably was due to the difference in anatomical location. Control chops were obtained from a location anterior to the treatment chops and they contained additional muscles (spinalis thoracis and trapezius pars thoracia). This additional musculature accounts, in part, for the differences in initial weight. The weight differences between hot and cold processed chops were expected because of the shrinkage which occurs in carcasses during chilling.

Precooking time and losses of chops

The time required per kilogram to precook the chops by either conventional broiling or microwave methods to an internal temperature of 63°C, which is a rare doneness, varied greatly between cooking methods. The microwave oven cooked the hot processed chops 63% more quickly and the cold processed chops 53% more quickly than did the conventional broiler. However, differences in cooking time/kg between hot and cold processed chops within cooking method were not different ($P > 0.05$). The control chops, which were conventionally broiled only one time to 68°C (medium doneness), were intermediate in cooking time (52.0 min/kg) between the chops precooked by the two methods. These control chop data are included because their cooking procedure was one which is commonly practiced.

The precooking losses were affected ($P < 0.05$) by both processing and cooking methods. The control chops, which were broiled to a 5°C higher temperature than the precooked treatment chops, had the highest drip (10.4%), evaporative (19.2%) and total (29.6%) cooking losses. A processing x cooking method interaction ($P < 0.01$) existed for percent drippings. Microwave precooked, hot processed chops had 2.3% less drippings than conventionally broiled, hot processed chops; however, chops which had been cold processed dripped 3.0% less if they had been conventionally broiled. Within both processing methods, microwave cooking produced about 55% as much evaporative loss as conventional broiling. Hot processed chops shrank 2.2% less than cold processed chops when both were broiled.

Total precooking losses were less for the microwave cooking method within both processing methods. An interaction ($P < 0.01$) existed between processing and cooking methods. The difference in total losses between the two precooking methods within hot processing was over twice as large as between the cooking methods within cold processing. The reason(s) for this interaction is not known. Weight of the chops should not be a factor because mean weights within processing method did not differ ($P < 0.05$). Likewise, anatomical location on the loin should not be a causative factor because the assignment of chops to treatment was alternated between anatomical locations from hog to hog and within each loin. Animals were a significant source of variance ($P < 0.05$) in the precooking data only for evaporation loss.

Reheating time and losses of chops

All of the treatment chops were reheated to 68°C by broiling in a conventional oven. The control chop data were used for comparison purposes (Table 1). Average reheating time/kg varied from 46.0 to 53.2 min among treatments. However, the only significant difference was between the hot processed, microwave precooked chops and the cold processed, conventionally broiled chops with the latter time being greater. Reheating drippings loss as a percentage of precooked weight was decidedly ($P < 0.05$) lower for the chops which had been precooked by broiling. Processing method differences were not apparent. Evaporative losses varied less than drip losses and were lower ($P < 0.05$) for hot processed chops than for cold processed, conventionally precooked chops.

Total reheating losses were less for the hot processed, conventionally cooked chops (15.8%) than for the microwave precooked chops. None of the interactions between processing and cooking methods was significant ($P < 0.05$). Animals were a significant source of variance for drip loss ($P < 0.01$), evaporation loss ($P < 0.05$) and reheating time/kg ($P < 0.01$).

Total cooking time and losses of chops

The total cooking time/kg and cooking losses are the sum of precooking and reheating values expressed as a proportion of the uncooked weight of the chops. They are not a sum of the precooking and reheating values previously discussed because reheating values were expressed as a proportion of the precooked weight.

Although the control and the conventionally cooked chops were broiled, the control chops required less than one-half as much time per unit weight to cook as did either the hot or cold processed chops. This shortened cooking time was the result of only one cooking instead of the precooking and reheating received by the treatment chops. The longer cooking time and increased energy usage for reheating of the hot processed chops would offset, at least partially, energy savings associated with the reduction of refrigeration needed for hot

processing. Microwave precooking produced substantially ($P < 0.05$) shorter total cooking times/kg than conventional broiling. However, differences between processing methods within cooking method were not significant. Animals were a significant ($P < 0.01$) source of variance.

The percent of drippings differed ($P < 0.05$) only for cold processed, microwave cooked chops, which shrank more than the other treatments. Animals were a source of variance for both drippings and evaporation ($P < 0.01$). Evaporative losses varied much more than drippings among treatments. Chops cooked in the microwave oven evaporated much less (15.3 and 16.9%) than broiled treatment chops (20.5 and 24.1%) or control chops (19.2%). All of these differences among treatments were significant except for the difference between the control and hot processed, conventionally broiled chops. A similar pattern among treatments was found in total cooking losses. The control chops were intermediate in total losses (29.5%) among the treatments and did not differ significantly from the hot processed, conventionally broiled or cold processed, microwave cooked chops. Hot processed, microwave cooked chops had the lowest losses (26.0%), and cold processed conventionally broiled chops had the greatest losses (33.9%). A significant ($P < 0.01$) interaction was found between processing and cooking methods. Greater differences between cooking methods existed within hot processing than within cold processing.

Palatability and W-B shear of chops

Much less variability was found in mean sensory panel juiciness scores than in tenderness scores. Control chops were rated most juicy (6.9) and cold processed, microwave precooked chops were rated least juicy (5.3). Other differences among treatments were not significant ($P < 0.05$). A 5 rating was described as "neither juicy or dry" and a 6 rating as "slightly juicy." Thus, the consumer acceptability of the juiciness of all but the control chops would be questionable. Cooking the treatment chops twice surely contributed to the lowering of their juiciness scores.

Flavor scores varied less among treatments than did juiciness scores. Control chops were scored highest (7.2) and hot processed, microwave precooked chops were scored lowest

Table 1—Least squares means for the control chops and the method of processing and cooking on cooking time, losses and palatability of pork loin chops

	Treatment[e]				
		Hot processed		Cold processed	
Characteristic	Control	Conventional	Microwave	Conventional	Microwave
No. of loins	20	20	20	20	20
Uncooked wt,[a] g	596.2e	512.8f	519.8f	481.0g	481.5g
Precooking time/kg, min	52.0e	78.9f	29.0g	80.0f	37.3g
Precooking losses,[b] %					
Drippings	10.4e	6.8f	4.5g	6.5f	9.5e
Evaporative	19.2e	11.6f	6.2g	13.8h	7.5g
Total	29.6e	18.4f	10.7g	20.3h	17.0f
Reheating time/kg, min					
Reheating losses,[c] %					
Drippings	10.3e	4.6f	7.1g	4.1f	6.1g
Evaporative	19.2e	11.2f	11.0f	12.9g	12.1f,g
Total	26.5e	15.8f	18.1f	17.0f,g	18.2g
Total cooking time/kg, min					
Total cooking losses,[d] %					
Drippings	10.3e	10.5e	10.7e	9.8e	14.4f
Evaporative	19.2e	20.5e	15.3f	24.1g	16.9g
Total	29.5e	31.0e	26.0f	33.9g	31.3e
W-B shear force, kg/1.3-cm core	3.7e	6.2f	5.7g	4.5h	4.9i
Sensory panel scores					
Juiciness	6.9e	5.8f	5.8f	5.9f	5.3f
Flavor	7.2e	6.4f	6.0g	6.6f	6.5f
Tenderness	7.4e	4.4f	4.7f	6.1g	6.1g
Chew count	33.8e	50.3f	46.7f	36.7g	37.3g
Overall acceptability	7.2e	5.2f	5.2f	6.2g	6.0g
Muscle fiber characteristics (N = 5/treatment)					
Sarcomere length, μ	1.6e	1.2f	1.0f	1.5e	1.5e
Diameter, μ	58.2e	81.2f	82.5f	61.8e	64.2e

[a] Total weight of three chops
[b] Percent of the uncooked chop weight. Control chops were cooked only one time. However, the control chop data are compared to precooking, reheating and total cooking time and losses of the treatment chops.
[c] Percent of the precooked chop weight
[d] Sum of precooking and reheating losses as a percent of the uncooked chop weight
[e] Means on a line with different letters differ significantly ($P < 0.05$).

(6.0). A flavor score of 6 is described as "like slightly." The acceptability of pork with mean flavor scores in this range is questionable.

Tenderness scores showed that the control chops were most tender (7.4) while the hot processed chops cooked by either broiling (4.4) or microwave (4.7) methods were least tender. Differences between precooking methods within processing method and the interaction between processing and precooking methods were not significant ($P < 0.05$). Animals were a significant ($P < 0.01$) source of variance.

Chew count or the number of chews necessary to prepare a sample for swallowing followed the same tenderness-indicating pattern among treatments as tenderness scores. These two measures of tenderness appeared to be measuring the same attribute among treatments. However, the intraclass simple correlation coefficient was only −0.73, indicating that 47% of the variance in tenderness score was not associated with chew count. A similar relationship ($r = -0.66$) was found between W-B shear value and sensory panel tenderness score.

W-B shear force values revealed great differences among treatments in their influence on tenderness of the longissimus muscle. Shear force was least ($P < 0.05$) for the control chops (3.7 kg), indicating the greatest tenderness. Hot processed chops had the greatest shear resistance, but the microwave precooked chops had a lower average shear (5.7 kg) than the broiled chops (6.2 kg). Differences within cold processing likewise were significant, but in this treatment the microwave cooking method produced less shear resistance (4.9 kg) than broiling (4.5 kg). However, the interaction between processing and cooking methods was not significant. Animals were a source of variance ($P < 0.01$) in shear value.

The mean overall acceptability score followed the same pattern among treatments as the various tenderness measurements. No difference ($P < 0.05$) was found in acceptability within processing method, but hot processed chops were least acceptable, and the control chops most acceptable. Animals were a significant source of variance in all of the palatability traits, indicating the variability which apparently exists in the hog population.

Histological evaluation of chops

To investigate the effects of treatments on sarcomere length and diameter of the muscle fibers, longissimus muscle samples were taken from the cooked loin chops of five animals selected at random. Every treatment was represented on a within animal basis. Hot processed chops had much shorter sarcomeres than either the control or cold processed chops. Fiber diameter showed an inverse pattern among treatments; those with shorter mean sarcomere lengths tended to have the larger diameters ($r = -0.69$). Differences among treatments were large, with the means varying from 1.0–1.6μ for sarcomere length and from 58.2–82.5μ for fiber diameter.

With one exception, mean sarcomere length was closely related to mean W-B shear value. The exception was the shortest sarcomere length (1.0μ) for the hot processed, microwave precooked chops but a slightly lower shear value than for the hot processed, conventionally broiled chops.

Muscle fiber diameter was positively related ($r = 0.55$) to W-B shear value, indicating that samples with larger fiber diameters tended to be less tender. Animals were a significant ($P < 0.05$) source of variance for fiber diameter but not for sarcomere length.

Precooking time and losses of roasts

The raw roast weights (Table 2) did not differ between

Table 2—Least squares means for the effects of method of processing and cooking on cooking time and losses, shear force and fiber characteristics of pork loin roasts.

Characteristic	Processing method		Cooking method	
	Hot	Cold	Conventional	Microwave
No. of loins	20	20	20	20
Uncooked wt, g	1180.5	1116.2	1159.9	1136.8
Precooking time/kg, min	47.2**	62.9	96.3**	13.8
Precooking losses,[a] %				
Drippings	3.8**	5.4	2.4**	6.7
Evaporative	6.5**	9.2	6.1**	9.6
Total	10.3**	14.6	8.5**	16.3
Reheating time/kg, min	201.1*	217.8	209.6	209.2
Reheating losses,[b] %				
Drippings	5.8	6.2	6.9**	5.1
Evaporative	14.4	14.2	16.2**	12.4
Total	20.2	20.4	23.1**	17.5
Total cooking time/kg, min	221.4*	242.7	282.9**	181.3
Total cooking losses,[c] %				
Drippings	9.0**	10.6	8.7**	10.9
Evaporative	19.0**	21.2	20.8*	19.4
Total	28.0**	31.8	29.5	30.3
W-B shear force, kg/1.3-cm core	3.8*	3.2	3.0**	4.0
Muscle fiber characteristics (N = 6/treatment)				
Sarcomere length, μ	1.3**	1.5	1.5**	1.3
Diameter, μ	74.4*	66.5	69.5	71.5

[a] Percent of the uncooked roast weight
[b] Percent of the precooked roast weight
[c] Sum of precooking and reheating losses as a percent of the uncooked roast weight
*$P < 0.05$; **$P < 0.01$. Significantly different from the mean of the other treatment within either processing or cooking method.

processing or cooking methods or among animals ($P < 0.05$). However, precooking time/kg was much lower ($P < 0.01$) for the hot processed roasts and for the microwave precooked roasts. The interaction between method of processing and method of precooking was significant ($P < 0.01$). The average precooking times/kg for the treatments were: hot processed, conventionally roasted, 83.1 min; hot processed, microwave precooked, 11.2 min; cold processed, conventionally roasted, 109.5 min; and cold processed, microwave precooked, 16.4 min. Thus, the hot processed roasts precooked 7.4 times faster to a 63°C internal temperature in the microwave than in the conventional oven. The cold processed roasts cooked 6.7 times faster in the microwave oven. Hot processing allowed 1.3 times faster cooking in the microwave oven and 1.5 times faster cooking in the conventional oven than did cold processing.

All three measures of cooking losses favored hot over cold processing and conventional over microwave roasting. Total precooking losses were 4.3% higher for cold processing over hot processing and microwave roasting losses were almost twice as great as conventional roasting losses. Significant interactions between the methods of processing and cooking were noted for evaporation ($P < 0.01$) and total losses ($P < 0.05$). Both interactions were the result of the excessive losses of the cold processed, microwave precooked roasts. The evaporation losses for the treatment groups were 5.8% for the hot processed, conventionally precooked; 7.2% for the hot processed, microwave precooked; 6.4% for the cold processed, conventionally precooked; and 12.1% for the cold processed, microwave precooked roasts. Total losses reflected similar differences in the treatments, producing the interaction between processing and cooking methods.

Reheating time and losses of roasts

The time/kg required to reheat the roasts to 77°C in a conventional oven was 16.7 min less ($P < 0.05$) for hot processed than cold processed roasts. The hot processed roasts averaged 64.5g heavier in weight when raw and 105.8g heavier in weight after precooking. Differences in reheating time between roasts precooked by the two methods did not differ ($P < 0.05$). However, reheating time of the roasts differed ($P < 0.05$) among animals.

All three measures of reheating losses showed that animals and processing method were not significant sources of variance. However, these losses differed ($P < 0.01$) between methods of precooking with microwave precooked roasts having lower percentages. Thus, the roasts which shrank most during precooking shrank the least during reheating by conventional roasting. Significant interactions between processing and precooking methods for evaporative ($P < 0.01$) and total losses ($P < 0.05$) indicated an inconsistent response to the treatments.

Total cooking time and losses of roasts

Total cooking time/kg of raw roast differed among animals ($P < 0.01$) and between processing ($P < 0.05$) and cooking methods ($P < 0.01$). The hot processed roasts (221.4 min/kg) had a 9% advantage in cooking time over the cold processed roasts (242.7 min/kg). The microwave precooked roasts had a decided 36% advantage in cooking time over the conventionally roasted cuts. An interaction ($P < 0.05$) between processing and cooking methods indicated an inconsistent response of the roasts to treatments.

Total cooking losses showed significant processing treatment effects for drippings ($P < 0.05$), evaporation ($P < 0.01$) and total losses ($P < 0.01$). In each measure, hot processing produced lower losses, expressed as a percent of the raw roast weight, than did cold processing. Those roasts which were conventionally precooked had 2.2% lower drippings and 1.4% higher evaporation than roasts which were microwave precooked. However, total cooking losses between cooking methods were similar (29.5 vs. 30.3%).

W-B shear values of roasts

Animals were not a significant source of variance in shear value of the roasts. Processing and cooking treatments had much less effect on muscle in the roasts than in the chops. The mean W-B shear values for all treatments of the roasts (Table 2) were near or below the mean value of 3.7 kg for the reference chops (Table 1). However, treatment differences were evident ($P < 0.01$). Cold processed roasts were 0.6 kg/1.3-cm core more tender than hot processed roasts. A 1.0-kg advantage in tenderness was found for conventional over microwave precooking.

A significant ($P < 0.01$) interaction was found between processing and precooking method. Within hot processing, the roasts precooked in the conventional oven had 0.9 kg lower W-B shear values than the roasts precooked in the microwave oven. However, within cold processing, this cooking method difference was only 0.4 kg.

Histological evaluation of roasts

To evaluate the effects of the treatments on sarcomere length and fiber diameter of the longissimus muscle in the roasts, samples from six hogs were randomly selected with every treatment being represented on a within animal basis. Mean sarcomere length was 0.2μ less ($P < 0.01$) and mean fiber diameter was 7.9μ greater ($P < 0.05$) for muscle in roasts from hot processed sides than from cold processed sides. Microwave precooking produced 0.2μ shorter sarcomeres ($P < 0.01$) than conventional roasting, but the 2.0-μ difference in mean fiber diameter was not significant.

W-B shear value was related to sarcomere length ($r = -0.57$) and fiber diameter ($r = 0.21$). Sarcomere length and fiber diameter showed a lower correlation ($r = -0.51$) than in the chops.

DISCUSSION

COOKING LOSS results from the loin chops in this study are not in agreement with the work of Bollman et al. (1948), Phillips et al. (1960), Headley and Jacobson (1960) and Ruyack and Paul (1972). These researchers found that cooking losses were greater for microwave cooking than for conventional cooking. In the present study, microwave cooking did not result in greater precooking losses for the chops, possibly because the meat was precooked to lower temperatures than in the earlier studies. However, the precooking roast data generally agree with the earlier reports. Moore et al. (1966) observed that 10.2-cm pork loin samples cooked in a microwave oven had a greater cooking loss (26.1%) than similar samples cooked in a conventional oven (23.6%).

The decreased tenderness of the chops cooked prerigor as compared to those cooked postrigor is in general agreement with the report by Weiner et al. (1966). They found that 2.54–cm loin chops, which were frozen prerigor, were less tender than conventionally chilled chops. Although this reported toughness was a result of cold shortening, contraction of the prerigor muscle also influenced the tenderness of the hot processed chops in the present study.

Paul et al. (1952) observed that beef steaks cooked immediately after slaughter were more tender than steaks cooked 24 hr after slaughter. Heat rigor was observed in the muscles. Ramsbottom and Strandine (1949) found that beef was more tender 2 hr following slaughter than at any time thereafter for the next 2–6 days. Weidemann et al. (1967) evaluated muscle cooked prerigor and found that muscles always were tender even when cooked without restraint. In the present study, the reasonable assumption was that the increased toughness of the hot processed chops was caused by heat stimulated contractions of heat rigor during precooking.

Usually, the greater the state of muscle contraction at rigor, the greater the toughness of the muscle. Indeed, several workers have shown that muscle is tougher if it enters rigor in the

contracted state rather than in the relaxed state (Locker, 1960; Herring et al., 1965; Howard and Judge, 1968; Welbourn et al., 1968; Smith et al., 1969; McCrae et al., 1971). In the present study, hot processed chops precooked in the microwave oven had shorter sarcomeres (1.0μ) than chops precooked by conventional broiling (1.2μ). However, the chops with shorter sarcomeres had lower W-B shear values. The reason for this paradoxical tenderizing has been described by Marsh et al. (1974). They observed that, when muscle shortened to 55% of its initial excised length, a number of supercontracted sarcomeres fractured along the internodal zones. This fracturing process accounts for the slight increase in tenderness of very highly shortened muscles.

In this study hot processed, microwave precooked roasts were considerably less tender than conventionally precooked roasts. These results do not totally agree with results reported by Ramsbottom and Strandine (1949), Paul et al. (1952), deFremery and Pool (1963) and Weidemann et al. (1967). These researchers observed that muscle cooked soon after slaughter was more tender than muscle cooked soon after the development of rigor mortis. However, the time lapse of about an hour in the present study between slaughter of the hog and the beginning of cooking may have affected the results.

The general histological examination of the treatment and reference chop muscle tissues revealed much of the microscopic morphology described in the literature. Heat rigor nodes, as described by Paul et al. (1952), were observed in all of the cooked, hot processed tissues but were more frequently observed in the microwave heated tissues. The observation of Weidemann et al. (1967), that cooking destroyed many details of the muscle's fine structure with only the outlines remaining, was especially noticeable. Another point, relative to the sarcomere measurements in this study, concerns the maximum amount of muscle contraction possible. Sarcomere lengths of less than 1.5μ have been reported (Locker, 1960; Marsh et al., 1974; Okubanjo et al., 1975). However, many researchers have discounted these very short sarcomere lengths because the myosin filaments are reported to be 1.5μ in length. Any sarcomere shorter than 1.5μ could occur only if the Z lines were penetrated by the myosin filaments. In fact, this phenomenon occurs in supercontracted muscle tissue and has been documented by the work of Hagopian (1970) and Marsh et al. (1974).

The precooking of hot processed loin chops, using either microwave or conventional methods, does not appear feasible because of the increased toughness produced by the contraction of the muscle during precooking. However, the conventional precooking of pork loin roasts appears feasible because precooking and reheating produced a product which was very acceptable in tenderness. Additionally, the lower precooking losses favored this method of processing. The microwave precooking of pork loin roasts appeared less desirable than conventional roasting because microwave cooking increased toughness and precooking losses. However, microwave roasting should produce significant energy savings over conventional roasting.

The results of this study also indicate the need for research on the optimum temperatures for precooking both hot and cold processed pork. In addition, research investigating the ultrastructure of the precooked product is needed to determine optimum precooking conditions and endpoints.

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LONG-DISTANCE TRANSOCEANIC SHIPMENTS OF FRESH BEEF

ABSTRACT

Fresh beef cuts and quarters were shipped from Ellensburg, Wash. to Yokohama, Japan in refrigerated vans with either normal (ambient air) or modified (60% CO_2, 25% O_2, 15% N_2) atmospheres. Packaging treatments involved use of polyvinyl chloride film, calcium alginate coating, cotton stockinettes, polyethylene-lined boxes and vacuum packages for rounds, chucks, ribs, loins and/or quarters. Use of a 0.02% solution of sodium hypochlorite (NaOCl) did not affect the terminal condition of beef cuts or quarters. Use of modified atmosphere was associated with significant improvements in overall appearance and desirability of beef cuts and quarters. The best method for protecting fresh beef cuts during long-distance shipments was vacuum packaging; vacuum packaged cuts were adequately protected from deterioration and weight loss during the 20–21 day shipment. The best system for protecting fresh beef quarters during long-distance, transoceanic shipments consisted of wrapping with PVC film and transport in a modified atmosphere van; however, quarters treated in this manner were not adequately protected from deterioration and received relatively low appearance ratings.

INTRODUCTION

RECENT ADVANCES in transportation equipment and reduced transit times have increased interest in transoceanic shipments of fresh meats. The United States has an advantageous global position for producing and distributing high quality beef. Demand for fresh beef by foreign consumers has recently increased as highly industrialized or oil-rich countries have developed higher standards of living. Correspondingly, the potential for sales of United States beef to foreign markets has increased dramatically and there is a need to identify technology capable of providing adequate product protection during transport.

Inter-continental shipments via ocean-going vessels are less expensive than air shipments, but require longer transit times. The time required to transport beef from the United States to the Orient in an ocean-going vessel has been reduced to approximately 14 days. Since distribution of fresh meat within the United States involves an average of 4 days from origin to destination and the product sustains approximately 0.5% shrinkage per day (Weatherly et al., 1968), the prolonged periods of time in-transit characteristic of long-distance shipments increase shrinkage, magnify the stress placed on the product and increase the probability of deteriorative changes. Some research on beef transportation has been conducted (Johnson et al., 1959; Rea et al., 1972; Marriott et al., 1977), but none of these studies included shipments of the duration required for long-distance transoceanic shipments.

This study investigated the effects of two atmospheres, six methods of product protection and use of a chlorine rinse on the condition, appearance and shrinkage of fresh beef following a long-distance transoceanic shipment.

EXPERIMENTAL

TWO REFRIGERATED vans adjusted to maintain temperatures of 0 to −1°C were loaded with beef and monitored from shipment origin (Ellensburg, Wash.) to destination (Yokohama, Japan). One van contained a normal (ambient air) atmosphere; whereas, the other van was charged with a modified atmosphere commonly used for such shipments under commercial license which contained 60% CO_2, 25% O_2 and 15% N_2. Each van contained fresh beef quarters and subprimal cuts. Half of the beef in each van was rinsed with a solution of 0.02% sodium hypochlorite (NaOCl) prior to packaging; the other half of the beef was not treated with NaOCl.

Some of the quarters in each van were not protected (NA), others were wrapped with polyvinyl chloride (PVC) film, spray-coated (to add approx 2% of quarter weight) with calcium alginate (CA), or covered with cotton stockinettes (CS). Beef cuts (boneless rounds, trimmed full loins, wholesale ribs and bone-in blade chucks) in each van were wrapped with polyvinyl chloride (PVC) film, spray-coated with calcium alginate (CA), vacuum packaged (VP) or placed in polyethylene (PE) lined cardboard boxes. Nominal permeability characteristics for VP and PVC films were as follows: oxygen transmission rates (OTR = cc/m² /24 hr/23.9°C/50% RH) were 35 (VP) and 8370 (PVC); moisture vapor transmission rates (MVTR = g/m² /24 hr/37.7°C/70% RH) were 10 (VP) and 228 (PVC). There were 60 quarters and 64 cuts in the normal atmosphere van and 48 quarters and 64 cuts in the modified atmosphere van.

Beef cuts from each treatment were weighed, swabbed by use of a 12.9 cm² aluminum template (to obtain bacterial samples) and subjectively evaluated at both origin and destination by a trained pair of evaluators. Rating scales used for evaluating beef quarters and cuts were as follows: an 8-point scale for muscle color (8 = very bright cherry red; 1 = very dark red); a 7-point scale for surface discoloration (7 = no surface discoloration; 1 = total surface discoloration); a 6-point scale for freshness of subcutaneous fat (6 = very fresh; 1 = extensive deterioration); a 7-point scale for visual microbial damage (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony formation or mold growth); an 8-point scale for overall appearance (8 = extremely desirable; 1 = extremely undesirable); and a 3-point scale for degree of vacuum (3 = high vacuum; 1 = total loss of vacuum).

The two test shipments were prepared and loaded (within 24–32 hr following slaughter of the cattle) in Ellensburg, Wash., pulled overland to the harbor in Seattle, Wash., loaded on an ocean-going vessel and shipped to Yokohama, Japan. Upon arrival in Japan, the vans were pulled overland to two retail outlets where they were unloaded. Cuts were evaluated at the retail outlets 20–21 days following initial packaging. Losses associated with moisture evaporation and purge were determined by weight differentials.

Temperature data were collected during fabrication, loading, transit and unloading by use of Ryan (models D and F) recording thermometers positioned at various locations within each van. Additional temperature data were obtained from 24 thermocouples strategically located in each van to measure air and product temperature by use of a potentiometer.

Bacterial samples were stored in sterile 0.1% peptone broth, carefully packed with ice, placed in a styrofoam container and shipped by air (time intransit was 28 hr) to the Texas A&M University Meat Laboratory. Samples were diluted to appropriate concentrations in peptone broth, plated on standard plate count agar, incubated at 25°C for 3 days and subsequently counted.

Data were analyzed by use of analysis of variance (Snedecor and Cochran, 1967). Mean separation techniques of Duncan (1955) and Kramer (1956) were utilized for comparison of mean values when analysis of variance indicated that the main effects were statistically significant at the 0.05 probability level. In a number of instances, time pres-

sures associated with monitoring actual shipments precluded collection of complete data from cuts and/or quarters. In such instances, partial data are reported (e.g., bacterial counts, Table 10) or incomplete sets of data are not presented in tabular form (e.g., visual microbial scores for modified atmosphere cuts, Table 4).

RESULTS & DISCUSSION

PRELIMINARY ANALYSES of all of the data in the present study revealed that use of a 0.02% solution of sodium hypochlorite (NaOCl) rinse did not ($P > 0.05$) affect any of the traits in the present study. Correspondingly, the data for rinsed and unrinsed beef were combined for all subsequent analyses. The ineffectiveness of the chlorine rinse was attributed to the prolonged duration of the shipment (20–21 days).

Data from recording thermometers and thermocouples used during loading, transit and unloading revealed that the average temperature during shipment was 0°C for the normal atmosphere van and −2°C for the modified atmosphere van. Temperature of both vans remained very close to 0°C until the last 36 hr prior to shipment termination at which time (on the dock in Yokohama) the thermostat on the modified atmosphere van was accidentally or inadvertantly turned down.

Muscle color scores for cuts upon arrival in Japan, 20–21 days after fabrication, packaging and loading are presented in Table 1. Vacuum packaged cuts shipped in the normal atmosphere van sustained the least ($P < 0.05$) degradation of muscle color, while those which were spray-coated with calcium alginate and shipped in a modified atmosphere van had the greatest ($P < 0.05$) deterioration of muscle color. Muscle color did not differ ($P > 0.05$) among beef cuts shipped in the normal atmosphere van which were protected by calcium alginate, PVC film or polyethylene-lined boxes. When the shipment atmosphere was modified, muscle color was brightest ($P < 0.05$) for vacuum packaged cuts, intermediate for cuts wrapped with PVC film or shipped in polyethylene-lined boxes, and least desirable ($P < 0.05$) for cuts sprayed with calcium alginate. There data suggest that vacuum packaging increases color stability; whereas, use of modified atmosphere has a negative effect on muscle color. The latter effect was attributed to discoloration of muscle surfaces from maintenance of high concentrations of CO_2 and/or N_2 around fresh meat cuts (Ledward, 1970; Simmons, 1974).

Vacuum packaged cuts, irrespective of van atmosphere, sustained less ($P < 0.05$) surface discoloration than cuts in the other packaging treatments (Table 2). Surface discoloration did not differ ($P > 0.05$) among beef cuts shipped in the normal atmosphere van which were protected by calcium alginate, PVC film or polyethylene-lined boxes. In the modified atmosphere van, vacuum packaged cuts had the least ($P < 0.05$) surface discoloration followed by cuts wrapped with PVC film, cuts shipped in polyethylene-lined boxes and cuts sprayed with calcium alginate. These data suggest that vacuum packaging will reduce surface discoloration of cuts during long-distance shipments.

Scores for freshness of the external fat covering on beef cuts upon arrival in Japan 20–21 days after packaging and shipment are presented in Table 3. Van atmosphere had more effect than packaging method on subcutaneous fat freshness. Cuts protected with aerobic packages (CA, PVC and PE) which were shipped in the modified atmosphere van exhibited greater ($P < 0.05$) freshness than comparable cuts in the normal atmosphere van. These data suggest that vacuum packaging (irrespective of van atmosphere) or use of modified atmosphere will enhance freshness and improve condition of beef cuts shipped 20–21 days.

Visual microbial damage scores for beef cuts shipped in the normal atmosphere van are presented in Table 4. Very limited data for this trait were collected from cuts in the modified atmosphere van and these are not presented in tabular form. Vacuum packaged cuts (Table 4) had the least ($P < 0.05$)

Table 1—Mean muscle color scores[a] for beef cuts following transportation for 20–21 days

	Normal atmosphere				Modified atmosphere			
	Packaging method[b]				Packaging method[b]			
Item	CA	PVC	VP	PE	CA	PVC	VP	PE
Blade chucks	5.4	5.1	–	5.0	4.3	5.5	5.8	5.3
Wholesale ribs	5.9	5.8	5.6	5.5	4.3	5.4	6.0	3.4
Trimmed loins	5.3	4.6	6.6	6.0	3.4	2.2	5.4	4.3
Boneless rounds	4.9	5.0	7.0	4.6	2.3	3.8	4.5	3.4
Combined cuts[c]	5.4e	5.1e	6.4d	5.3e	3.5g	4.2f	5.4e	4.1f

[a] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red).
[b] CA = calcium alginate spray coating, PVC = polyvinyl chloride film wrapping, VP = vacuum packaging, PE = unwrapped cuts in polyethylene-lined boxes.
[c] Means for chucks, ribs, loins and rounds combined. Means on the same line bearing a common letter are not different ($P > 0.05$). Only means for combined cuts were tested for statistical significance.

Table 2—Mean surface discoloration scores[a] for beef cuts following transportation for 20–21 days

	Normal atmosphere				*Modified* atmosphere			
	Packaging method[b]				Packaging method[b]			
Item	CA	PVC	VP	PE	CA	PVC	VP	PE
Blade chucks	3.5	3.3	–	2.4	2.3	2.9	4.4	2.0
Wholesale ribs	3.4	3.9	3.1	3.0	2.3	4.5	5.1	2.4
Trimmed loins	2.8	1.6	5.0	3.0	1.8	2.6	4.3	2.5
Boneless rounds	2.8	2.1	6.0	2.4	1.9	2.6	4.5	2.3
Combined cuts[c]	3.1e	2.7e	4.7d	2.7e	2.1f	3.2e	4.6d	2.6ef

[a] Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).
[b] CA = calcium alginate spray coating, PVC = polyvinyl chloride film wrapping, VP = vacuum packaging, PE = unwrapped cuts in polyethylene-lined boxes.
[c] Means for chucks, ribs, loins and rounds combined. Means on the same line bearing a common letter are not different ($P > 0.05$). Only means for combined cuts were tested for statistical significance.

Table 3—Mean subcutaneous fat freshness scores[a] for beef cuts following transportation for 20–21 days

	Normal atmosphere				Modified atmosphere			
	Packaging method[b]				Packaging method[b]			
Item	CA	PVC	VP	PE	CA	PVC	VP	PE
Blade chucks	2.3	2.1	–	2.0	3.3	3.4	3.3	3.0
Wholesale ribs	2.1	3.3	3.8	1.6	4.3	5.1	4.3	4.4
Trimmed loins	2.9	2.6	3.4	3.0	3.5	4.0	4.0	3.8
Boneless rounds	2.3	3.3	3.5	2.0	3.0	3.3	3.3	3.6
Combined cuts[c]	2.4e	2.8e	3.6d	2.2e	3.5d	4.0d	3.7d	3.7d

[a] Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration).
[b] CA = calcium alginate spray coating, PVC = polyvinyl chloride film wrapping, VP = vacuum packaging, PE = unwrapped cuts in polyethylene-lined boxes.
[c] Means for chucks, ribs, loins and rounds combined. Means on the same line bearing a common letter are not different ($P > 0.05$). Only means for combined cuts were tested for statistical significance.

visible damage from microbes; cuts packaged in aerobic materials (PVC, CA and PE) evidenced serious damage from microbial proliferation and metabolic activity. Vacuum packaged cuts suffered less damage than cuts in other packaging treatments because the anaerobic conditions maintained under vacuum precludes or greatly limits growth of slime-producing bacteria on meat surfaces. Data from trimmed loins wrapped with PVC film (mean visual microbial damage score = 2.0) and blade chucks which were vacuum packaged (mean visual microbial damage score = 4.4) in the modified atmosphere van generally support these conclusions.

Mean overall appearance scores for beef cuts following transporation for 20–21 days are presented in Table 5. Vacuum packaged cuts, irrespective of van atmosphere, were most desirable ($P < 0.05$) in overall appearance while those shipped in polyethylene-lined boxes were least desirable ($P < 0.05$) in appearance. Beef cuts wrapped in PVC film and shipped in a modified atmosphere van were more desirable ($P < 0.05$) than comparable cuts from the normal atmosphere van. The latter advantage for use of modified atmosphere was also apparent for cuts sprayed with calcium alginate and for those shipped in polyethylene-lined boxes. These data suggest that vacuum packaging is the most appropriate method for protecting beef cuts during long-distance transoceanic shipments.

Beef cuts shipped in the modified atmosphere van had lower ($P < 0.05$) bacterial counts than those shipped under normal atmospheric conditions, but packaging method was not associated with differences in bacterial count (Table 6). Reduced microbial proliferation on cuts shipped in the modified atmosphere van further verifies that carbon dioxide retards bacterial growth and agrees with results reported by Ogilvy and Ayres (1951), Hoke (1967), Clark and Lentz (1969), Pohja et al. (1969), Smith et al. (1974) and Huffman et al. (1975).

Mean weight losses for beef cuts following transportation for 20–21 days are presented in Table 7. Percentage weight loss for cuts coated with calcium alginate were somewhat exaggerated since a large proportion of the weight loss could be attributed to moisture loss from the liquid coating which had insufficient time (following application and prior to initial

Table 4—Mean visual microbial damage scores[a] for beef cuts following transportation for 20–21 days

	Normal atmosphere			
	Packaging method[b]			
Item	CA	PVC	VP	PE
Blade chucks	1.5	1.9	–	1.4
Wholesale ribs	1.5	1.8	5.5	1.9
Trimmed loins	2.5	2.8	5.5	2.1
Boneless rounds	1.4	2.4	5.4	1.0
Combined cuts[c]	1.7e	2.2e	5.5d	1.6e

[a] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth).
[b] CA = calcium alginate spray coating, PVC = polyvinyl chloride film wrapping, VP = vacuum packaging, PE = unwrapped cuts in polyethylene-lined boxes.
[c] Means for chucks, ribs, loins and rounds combined. Means on the same line bearing a common letter are not different ($P > 0.05$). Only means for combined cuts were tested for statistical significance.

Table 5—Mean overall appearance scores[a] for beef cuts following transportation for 20–21 days

	Normal atmosphere				Modified atmosphere			
	Packaging method[b]				Packaging method[b]			
Item	CA	PVC	VP	PE	CA	PVC	VP	PE
Blade chucks	2.0	2.3	–	1.6	2.4	3.1	4.9	1.5
Wholesale ribs	2.1	2.6	4.3	1.9	3.3	4.9	5.4	3.4
Trimmed loins	2.6	2.4	5.1	2.4	2.8	3.1	5.0	3.5
Boneless rounds	1.8	2.1	6.3	1.4	1.9	2.9	4.8	2.6
Combined cuts[c]	2.1g	2.4fg	5.2d	1.8h	2.6f	3.5e	5.0d	2.8f

[a] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[b] CA = calcium alginate spray coating, PVC = polyvinyl chloride film wrapping, VP = vacuum packaging, PE = unwrapped cuts in polyethylene-lined boxes.
[c] Means for chucks, ribs, loins and rounds combined. Means on the same line bearing a common letter are not different ($P > 0.05$). Only means for combined cuts were tested for statistical significance.

Table 6—Mean bacterial counts[a] for beef cuts following transportation for 20–21 days

	Normal atmosphere				Modified atmosphere			
	Packaging method[b]				Packaging method[b]			
Item	CA	PVC	VP	PE	CA	PVC	VP	PE
Blade chucks	8.8	9.1	6.5	10.3	6.5	7.4	8.1	8.0
Wholesale ribs	9.0	9.4	9.5	8.9	6.4	5.8	7.3	6.9
Trimmed loins	9.5	8.6	8.1	8.5	6.3	6.2	6.5	6.5
Boneless rounds	8.8	9.3	8.6	8.5	5.9	6.6	5.9	6.5
Combined cuts[c]	9.0e	9.1e	8.2e	9.1e	6.3d	6.5d	6.9d	7.0d

[a] Bacterial counts are reported as plate counts ($\log_{10}$) per 6.45 cm^2 of muscle surface area.
[b] CA = calcium alginate spray coating, PVC = polyvinyl chloride film wrapping, VP = vacuum packaging, PE = unwrapped cuts in polyethylene-lined boxes.
[c] Means for chucks, ribs, loins and rounds combined. Means on the same line bearing a common letter are not different ($P > 0.05$). Only means for combined cuts were tested for statistical significance.

Table 7—Mean weight loss percentages[a] of beef cuts following transportation for 20–21 days

	Normal atmosphere				Modified atmosphere			
	Packaging method[b]				Packaging method[b]			
Item	CA	PVC	VP	PE	CA	PVC	VP	PE
Blade chucks	1.0	0.5	–	0.6	0.8	0.5	0.3	0.2
Wholesale ribs	2.3	0.5	0.9	0.2	2.2	0.8	1.2	0.9
Trimmed loins	1.2	0.7	0.8	0.5	0.8	0.7	0.4	1.0
Boneless rounds	2.5	1.2	0.6	2.0	1.9	0.6	0.7	1.3
Combined cuts[c]	1.8d	0.7e	0.7e	0.8e	1.4d	0.6e	0.6e	0.8e

[a] Weight loss expressed as percentages of initial cut weight.
[b] CA = calcium alginate spray coating, PVC = polyvinyl chloride film wrapping, VP = vacuum packaging, PE = unwrapped cuts in polyethylene-lined boxes.
[c] Means for chucks, ribs, loins and rounds combined. Means on the same line bearing a common letter are not different ($P > 0.05$). Only means for combined cuts were tested for statistical significance.

weighing) to lose the water associated with CA application. As a result, the real effect of calcium alginate coating on meat tissue shrinkage was not determined. Weight losses did not differ ($P > 0.05$) among cuts in PVC, VP or PE treatments. Van atmosphere did not affect ($P > 0.05$) shrinkage.

Table 8—Traits and measures for combined beef cuts[a] and combined atmospheric conditions[b] following transportation for 20–21 days

Item	Packaging method[c]			
	CA	PVC	VP	PE
Muscle color[d]	4.5l	4.7l	5.9k	4.7l
Surface discoloration[e]	2.6l	3.0l	4.7k	2.7l
Subcutaneous fat freshness[f]	3.0k	3.4k	3.7k	3.0k
Visual microbial damage[g]	1.7l	2.1l	5.0k	1.6l
Overall appearance[h]	2.4m	3.0l	5.1k	2.3m
Bacterial count[i]	7.7k	7.8k	7.6k	8.1k
Weight loss (%)[j]	1.6k	0.7l	0.7l	0.8l

[a] Means for chucks, ribs, loins and rounds combined. Means on the same line bearing a common letter are not different ($P > 0.05$).
[b] Means for normal atmosphere and modified atmosphere combined.
[c] CA = calcium alginate spray coating, PVC = polyvinyl chloride film, VP = vacuum packaging, PE = unwrapped cuts in polyethylene-lined boxes.
[d] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red).
[e] Means based on a 7-point scale (7 = no surface discoloration; 1 = total surface discoloration).
[f] Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration).
[g] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth).
[h] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[i] Mean plate counts ($\log_{10}$) per 6.45 cm^2 of muscle surface area.
[j] Weight loss expressed as percentage of initial cut weight.

An evaluation of packaging method, disregarding the effect of van atmosphere, on certain traits and measures for beef cuts is presented in Table 8. Vacuum packaged cuts had ($P < 0.05$) the brightest muscle color, the least surface discoloration, the least visual microbial damage, the highest overall appearance scores and among the lowest weight losses in the present study. Neither calcium alginate coating nor shipment in polyethylene-lined boxes satisfactorily maintained condition of beef cuts during 20–21 days intransit. PVC film wrapping was the most desirable of the three aerobic packaging systems but still would not be satisfactory for shipments of this duration.

Failure to maintain the integrity of vacuum packages during transport and storage has been shown by Marriott et al. (1977) to profoundly influence the appearance and condition of vacuum packaged cuts. In Tables 1 through 8, comparisons were made between beef cuts packaged by use of four methods without accounting for the fact that some of the vacuum packages were not intact at the destination. 75% of the vacuum packaged boneless rounds, 37.5% of the wholesale loins, 25% of the wholesale ribs and 25% of the blade chucks had maintained high levels of vacuum upon evaluation in Japan; 0, 37.5, 12.5 and 0% of the specified cuts, respectively, had completely lost their vacuum. Mean appearance scores, bacterial counts and weight losses for combined subprimal cuts evaluated 20–21 days after packaging are presented in Table 9. Terminal vacuum level was not associated with scores for muscle color, subcutaneous fat freshness or visual microbial damage and did not significantly ($P > 0.05$) affect bacterial counts or weight losses during transit and storage. Beef cuts which maintained high vs intermediate levels of vacuum during transit-storage did not differ ($P > 0.05$) in surface discoloration or overall appearance; but those with high terminal vacuum levels sustained less ($P < 0.05$) surface discoloration and had higher ($P < 0.05$) overall appearance scores than cuts which were leakers at the shipment destination. These data reiterate the importance of maintaining the integrity of vacuum packages during long-term shipments of fresh beef.

Effects of van atmosphere and packaging method on traits and measures for beef quarters are summarized in Table 10. Irrespective of van atmosphere, quarters wrapped with PVC

Table 9—Mean appearance scores, bacterial counts and weight losses for combined subprimal cuts evaluated 20–21 days after vacuum packaging[g]

Trait	Terminal vacuum level		
	High	Intermediate	None
Muscle color[a]	6.3g	5.8g	5.8g
Surface discoloration[b]	5.3g	5.3g	3.5h
Subcutaneous fat freshness[c]	4.3g	3.5g	3.2g
Visual microbial damage[d]	7.0g	7.0g	7.0g
Overall appearance[e]	5.7g	5.3gh	4.3h
Bacterial count ($\log_{10}$)[f]	7.3g	7.1g	7.4g
Weight loss (%)	1.0g	0.6g	0.7g

[a] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red).
[b] Means based on a 7-point scale (7 = no discolcration; 1 = total discoloration).
[c] Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration).
[d] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth).
[e] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[f] Reported as the number of microorganisms per 6.45 cm^2 of surface area.
[g] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

Table 10—Effects of van atmosphere and packaging method on traits and measures for beef quarters following transportation for 20–21 days[f]

Item	Normal atmosphere				Modified atmosphere			
	Packaging method[a]				Packaging method[a]			
	CA	PVC	CS	NA	CA	PVC	CS	NA
Surface discoloration[b]	1.6g	2.9f	2.2g	2.2g	1.2g	2.8f	1.4g	1.2g
Overall appearance[c]	2.4h	3.1f	2.7g	2.8g	2.0h	3.5f	2.0h	2.0h
Bacterial count[d]	–	–	4.7g	–	6.6f	6.3f	5.5f	6.6f
Weight loss (%)[e]	2.6f	0.6h	1.4g	1.6g	–	–	–	–

[a] CA = calcium alginate spray coating, PVC = polyvinyl chloride film wrapping, CS = covering with a cotton stockinette, NA = unwrapped cuts.
[b] Means based cn a 7-point scale (7 = no surface discoloration, 1 = total surface discolcration).
[c] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).
[d] Mean plate counts ($\log_{10}$) per 6.45 cm^2 of subcutaneous fat surface area.
[e] Weight loss expressed as percentages of initial quarter weight.
[f] Means on the same line bearing a common letter are not different ($P > 0.05$).

film had less ($P < 0.05$) surface discoloration and were more desirable ($P < 0.05$) in appearance than unprotected quarters and quarters protected with calcium alginate or cotton stockinettes. Contrary to suggestions by Rea et al. (1972), quarters wrapped with PVC film did not have excessively high bacterial counts. The latter result undoubtedly was associated with use of a modified atmosphere in transit. Quarters wrapped in PVC film sustained less shrinkage than quarters in the CA, CS or NA treatments. Although there were significant differences in all of the traits among packaging methods, they are inconsequential since none of the quarters was acceptable in appearance at the destination. Of the methods presently available for protecting fresh beef quarters during long-distance transoceanic shipments, PVC film wrapping and use of modified atmospheres appears to be the best.

The following conclusions were drawn from the data of the present study: (1) Use of a 0.02% solution of sodium hypochlorite (NaOCl) did not affect the terminal condition of beef quarters or cuts. (2) Use of modified atmosphere was associated with significant improvements in subcutaneous fat freshness and overall appearance of beef cuts and quarters. (3) The best method for protecting beef cuts during long-distance shipments was vacuum packaging. (4) The best system for protecting fresh beef quarters against deterioration during long-distance shipments consisted of wrapping with PVC film and transportation in a modified atmosphere van, but quarters treated in this manner were not acceptable in appearance or condition upon arrival at the destination.

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SHORT-TERM TRANSOCEANIC SHIPMENTS OF FRESH BEEF

ABSTRACT

Two refrigerated (0 to −1°C) vans containing polyvinyl chloride film (PVC) wrapped or vacuum packaged (VP) beef cuts were monitored from Richmond, Calif. to Honolulu, Hawaii. One van had a normal (ambient air) atmosphere; the other van had a modified (60% CO_2, 25% O_2, 15% N_2) atmosphere. Neither packaging system nor van atmosphere affected ($P > 0.05$) appearance of cuts evaluated 5–6 days after packaging. However, VP cuts evaluated 7–9 days after packaging were superior ($P < 0.05$) in appearance traits to PVC cuts in 6 of 10 comparisons of cuts in normal atmosphere vans and in only 1 of 10 comparisons of cuts in modified atmosphere vans. Use of a modified atmosphere decreased ($P < 0.05$) microbial growth on cuts shipped and/or stored for 7–9 days. Neither packaging method nor van atmosphere affected ($P > 0.05$) weight losses during transit and storage. Cuts with high levels of terminal vacuum were superior ($P < 0.05$) to those wrapped with PVC film in: freedom from surface discoloration (5–9 days), overall appearance (5–9 days), bacterial counts (7–9 days), and trim losses (7–9 days). Vacuum packaged cuts which were leakers (no terminal vacuum) did not differ from those wrapped with PVC film in any appearance trait (5–9 days) but sustained increased ($P < 0.05$) purge loss after 5–6 days of transit-storage. Short interval (5–6 days) transoceanic beef shipments can be consummated with acceptable product condition in normal atmosphere vans and wrapped in PVC film; if longer transit-storage periods (7–9 days) prior to cutting are anticipated, cuts should either be vacuum packaged or wrapped with PVC film and shipped in modified atmosphere vans.

INTRODUCTION

FRESH BEEF shipments involving 5 days for transit and 2–4 days for subsequent storage prior to retail cutting are encountered when beef is shipped great distances within this country or when beef is shipped from the United States to other countries. Selection of appropriate packaging materials and storage conditions are essential to prevent shrinkage and to protect the product from spoilage.

Hoke (1967) and Pohja et al. (1969) have identified methods for decreasing microbial growth and extending the storage-life of fresh meat by use of modified atmospheric conditions. Hoke (1967) reviewed studies of the effect of atmospheric conditions on meat quality and concluded that meat stability during transit and storage could be enhanced by modifying the atmosphere to contain a higher percentage of carbon dioxide. Pohja et al. (1969) found that meat stability was improved by use of atmospheres with high concentrations of carbon dioxide, whereas increased nitrogen concentration in the atmosphere did not significantly improve product condition. Ledward (1970) reported that carbon dioxide atmospheres did not significantly affect the oxidation rate of myoglobin or ultimate discoloration of meat.

The efficacy of vacuum packaging for protection of fresh beef is well documented (Baran et al., 1969; Hodges et al., 1974; Minks and Stringer, 1972; Ordal, 1962; Seideman et al., 1976a); however, vacuum packaging is expensive. Rea et al. (1972), Berry et al. (1971) and Simmons (1974) have demonstrated very satisfactory preservation of beef for periods of approximately 7 days via use of polyvinyl chloride (PVC) film. Films like PVC are much less expensive than vacuum packages.

The present study determined the effects of two atmospheres (normal and modified) and two packaging systems (vacuum packaging and PVC film wrapping) on the condition of fresh beef following short-term transoceanic shipment and subsequent storage.

EXPERIMENTAL

TWO REFRIGERATED VANS, adjusted to maintain temperatures of −1 to 0°C, were loaded with meat and monitored from shipment origin (Richmond, Calif.) to destination (Honolulu, Hawaii). One van had a normal (ambient air) atmosphere, the other van was charged with a modified atmosphere which contained 60% CO_2, 25% O_2, and 15% N_2. Twenty of each beef cut (bone-in blade chucks, semi-boneless rounds and boneless sirloin tips) from each van were evaluated. One-half of the cuts of each kind were vacuum packaged in bags comprised of co-extruded EVA-Saran (ethyl vinyl acetate and polyvinyl chloride-polyvinylidene copolymer); the remaining cuts were wrapped in PVC film. Nominal permeability characteristics for VP and PVC films were as follows: oxygen transmission rates (OTR = cc/m²/24 hr/23.9°C/50% RH) were 35 (VP) and 8370 (PVC); moisture vapor transmission rates (MVTR = g/m²/24 hr/37.7°C/70% RH) were 10 (VP) and 228 (PVC). Each refrigerated van contained 60 test cuts as part of a mixed meat load composed of fresh beef, pork and poultry as well as processed meats destined for a retail outlet.

All cuts in each treatment were weighed, muscle surfaces were swabbed by use of a 12.9 sq cm aluminum template (to obtain bacterial samples) and subjectively evaluated at both origin and destination by a trained pair of evaluators. Rating scales used for evaluating beef cuts were as follows: an 8-point scale for muscle color (8 = very bright cherry red; 1 = very dark red); a 7-point scale for surface discoloration (7 = no surface discoloration; 1 = total surface discoloration); a 6-point scale for freshness of subcutaneous fat (6 = very fresh; 1 = extensive deterioration); a 7-point scale for visual microbial damage (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony formation or mold growth); an 8-point scale for overall appearance (8 = extremely desirable; 1 = extremely undesirable); and a 3-point scale for degree of vacuum (3 = high vacuum; 1 = total loss of vacuum).

The two test vans were loaded in Richmond, Calif., pulled overland to the harbor in Oakland, Calif., loaded on an ocean-going vessel and shipped to Honolulu, Hawaii. Upon arrival at the destination, the meat vans were pulled overland to two retail outlets where they were unloaded. Cuts were monitored in the retail outlets over a period of 5–9 days following packaging. Losses associated with moisture evaporation, purge and surface trimming were determined during this period.

Temperature data were collected during cutting, loading, transit and unloading. Ryan (models D and F) recording thermometers were positioned at various locations within each van. Additional temperature data were obtained from 24 thermocouples strategically located in each van to measure air and product temperature by use of a potentiometer.

Bacterial samples were stored in sterile 0.1% peptone broth, carefully packed with ice, placed in a plastic foam container and shipped by air (time intransit was 20 hr) to the Texas A & M University Meat Laboratory, diluted to appropriate concentrations in 0.1% peptone broth, plated on standard plate count agar, incubated at 25°C for 3 days and subsequently counted.

Data were analyzed by analysis of variance (Snedecor and Cochran, 1967). Mean separation techniques of Duncan (1955) and Kramer (1956) were used to compare mean values when analysis of variance indicated that the main effects were significant at the 0.05 probability level.

RESULTS & DISCUSSION

DATA from recording thermometers and thermocouples used during loading, transit and unloading revealed that the average temperature during shipment was −1.1°C for the normal atmosphere van and 0.6°C for the modified atmosphere van. Temperature within the vans during shipment ranged from −3.9 to +5.0°C which was less variable than that reported by Johnson et al. (1959) and closely paralleled results reported by Rea et al. (1972).

Neither shipment atmosphere nor packaging method (Table 1) significantly affected the appearance of blade chucks evaluated 5 days after cutting and packaging. Based on these data, the least expensive protection system (PVC film and normal atmosphere) was adequate for protecting chucks during transoceanic shipments of 5 days duration. More rigid temperature control could potentially increase transit and storage time beyond 5 days.

Mean appearance scores for blade chucks evaluated 7 days after fabrication and packaging are presented in Table 2. Muscle color was not significantly affected by either packaging method or van atmosphere. Transit atmosphere had no significant effect on any appearance trait. In four of eight comparisons, vacuum packaged cuts had significantly ($P < 0.05$) less surface discoloration, subcutaneous fat deterioration and visual microbial damage as well as higher overall appearance scores than those wrapped in PVC film. These data suggest that for transit and storage periods of 7 days, vacuum packaging provided increased product protection; whereas, use of a modified atmosphere did not significantly improve product appearance.

Neither shipment atmosphere nor packaging method (Table 3) significantly affected the appearance of semi-boneless rounds evaluated 6 days after cutting and packaging. These results support use of PVC film wrapping and normal atmospheric conditions to protect rounds for transoceanic shipments which involve 6 days of transit and storage.

Mean appearance scores for semi-boneless rounds evaluated 8 days after fabrication and packaging are presented in Table 4. Muscle color and subcutaneous fat freshness were not significantly affected by either packaging method or van atmosphere. Transit atmosphere did not significantly affect any of the appearance traits except visual microbial damage. Use of a modified atmosphere was associated with reduced visual microbial damage (Table 4) and lower bacterial counts (Table 5). Vacuum packaged cuts shipped in normal atmosphere vans had less ($P < 0.05$) surface discoloration and visual microbial damage and were more desirable in overall appearance than PVC wrapped cuts.

Collectively, these data suggest no benefit for use of modified atmospheric conditions for PVC wrapped cuts transported and stored for 5 or 6 days (Tables 1 and 3). However, PVC wrapped cuts maintained in the modified atmosphere during transportation (for 5 days) and subsequently stored in normal atmosphere coolers for 2–3 days (Tables 2 and 4) had numerically higher scores in nine of ten comparisons of appearance traits. Maintenance of high concentrations of CO_2 and/or N_2 around fresh meat cuts causes discoloration of the muscle surface (Ledward, 1970; Simmons, 1974) either because of anoxia or because CO_2 causes a change in muscle pH (Ledward, 1970). Cuts evaluated within 1 hr following opening of the van were discolored (Table 1) to a greater extent than cuts from the normal atmosphere van; cuts evaluated 24 hr following opening of the van still had more surface discoloration than cuts from the normal atmosphere van (Table 3); cuts evaluated 48–72 hr following opening of the van (and concomitant loss of the modified atmosphere) were less discolored and more desirable in overall appearance than were PVC wrapped cuts shipped in normal atmosphere vans (Tables 2 and 4).

Mean bacterial counts for combined beef subprimals evaluated 5–9 days after fabrication and packaging are presented in Table 5. Increased concentrations of CO_2 are known to inhibit the growth of aerobic meat-spoilage microorganisms (Clark and Lentz, 1969; Pohja et al., 1969). Data of the present study

Table 1—Mean appearance scores for blade chucks evaluated 5 days after packaging

Trait	Normal atmosphere[f]		Modified atmosphere[f]	
	Vacuum packaged	PVC film wrapped	Vacuum packaged	PVC film wrapped
Muscle color[a]	6.0f	6.3f	6.0f	6.2f
Surface discoloration[b]	4.1f	5.2f	4.8f	3.9f
Subcutaneous fat freshness[c]	4.4f	4.3f	5.3f	4.0f
Visual microbial damage[d]	5.6f	6.0f	5.9f	5.2f
Overall appearance[e]	4.4f	5.2f	5.0f	3.8f

[a] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red)
[b] Means based on a 7-point scale (7 = no discoloration; 1 = total discoloration)
[c] Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration)
[d] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth)
[e] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable)
[f] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

Table 2—Mean appearance scores for blade chucks evaluated 7 days after packaging

Trait	Normal atmosphere[f]		Modified atmosphere[f]	
	Vacuum packaged	PVC film wrapped	Vacuum packaged	PVC film wrapped
Muscle color[a]	6.6f	6.1f	6.7f	6.8f
Surface discoloration[b]	4.5f	3.0g	3.7fg	3.4g
Subcutaneous fat freshness[c]	4.5f	3.0g	5.4f	3.7fg
Visual microbial damage[d]	5.8g	5.3g	6.9f	5.8g
Overall appearance[e]	4.6f	2.8g	4.1f	3.1fg

[a] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red)
[b] Means based on a 7-point scale (7 = no discoloration; 1 = total discoloration)
[c] Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration)
[d] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth)
[e] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable)
[f] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

Table 3—Mean appearance scores for semi-boneless rounds evaluated 6 days after packaging

Trait	Normal atmosphere[f]		Modified atmosphere[f]	
	Vacuum packaged	PVC film wrapped	Vacuum packaged	PVC film wrapped
Muscle color[a]	5.8f	5.8f	6.2f	6.7f
Surface discoloration[b]	5.3f	4.6f	3.5f	3.6f
Subcutaneous fat freshness[c]	3.6f	4.5f	3.7f	4.4f
Visual microbial damage[d]	6.2f	5.9f	5.7f	6.0f
Overall appearance[e]	5.2f	4.7f	3.5f	3.9f

[a] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red)
[b] Means based on a 7-point scale (7 = no discoloration; 1 = total discoloration)
[c] Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration)
[d] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth)
[e] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable)
[f] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

Table 4—Mean appearance scores for semi-boneless rounds evaluated 8 days after packaging

Trait	Normal atmosphere[f]		Modified atmosphere[f]	
	Vacuum packaged	PVC film wrapped	Vacuum packaged	PVC film wrapped
Muscle color[a]	7.0f	6.3f	6.8f	6.6f
Surface discoloration[b]	4.6f	1.8g	2.5fg	2.5fg
Subcutaneous fat freshness[c]	2.3f	3.2f	3.4f	3.2f
Visual microbial damage[d]	5.0f	4.0g	5.4f	5.0f
Overall appearance[e]	4.6f	1.7g	2.5g	2.6g

[a] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red)
[b] Means based on a 7-point scale (7 = no discoloration; 1 = total discoloration)
[c] Means based on a 6-point scale (6 = very fresh, 1 = extensive deterioration)
[d] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth)
[e] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable)
[f] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

(Table 5) support previous findings in this regard although differences were not always consistent enough for statistical significance. Nevertheless, bacterial counts were lower ($P < 0.05$) for PVC wrapped cuts shipped in vans equipped with a modified atmosphere and evaluated 7–9 days after packaging (Table 5) than were counts for PVC wrapped cuts shipped in normal atmosphere vans. These data suggest that use of a modified atmosphere containing up to 60% CO_2 had an inhibitory effect on microbial growth which was especially evident for cuts evaluated 7–9 days after fabrication and packaging.

As has been reported previously (Smith et al., 1974) bacterial counts from vacuum packaged cuts are not always lower than those for cuts wrapped with oxygen permeable films. However, it is important that the genera of bacteria which predominate may be different (*Lactobacillus* in anaerobically packaged cuts and *Pseudomonas* in aerobically packaged cuts, Seideman et al., 1976b). Use of modified atmosphere conditions decreased ($P < 0.05$) visual microbial damage for PVC wrapped semi-boneless rounds evaluated 8 days after packaging (Table 4) and for vacuum packaged blade chucks evaluated 7 days after packaging (Table 2). Although gaseous atmospheric conditions would not normally be expected to affect microbial counts on vacuum packaged cuts, the incidence of vacuum package failure (almost 42% of these cuts were "leakers" in the present study) was such that statistically significant effects of atmosphere on visual microbial damage (Table 2) and overall appearance (Table 4) were observed in the present study.

Failure to maintain the integrity of vacuum packages during transport and storage had a profound influence on data of the present study. In Tables 1 through 5, comparisons are made between vacuum packaged and PVC wrapped cuts without accounting for the fact that many of the vacuum packages were not intact at the destination. Such data can be misleading. Correspondingly, data presented in Tables 6 through 9 compare cuts wrapped with PVC film with those in vacuum packages segmented into three groups on the basis of terminal vacuum levels (at the time of evaluation at the destination). Among semi-boneless rounds, blade chucks and sirloin tips, 23.6, 20.0 and 35.4%, respectively, of the cuts had high terminal vacuum levels; while 52.8, 50.0 and 22.9%, respectively, had suffered complete loss of vacuum at shipment destination.

A comparison of appearance scores for combined cuts evaluated 5–6 days after fabrication and packaging is presented in Table 6. Vacuum packaged cuts with high or intermediate levels of vacuum sustained less ($P < 0.05$) surface discoloration and had higher overall appearance scores than vacuum packaged cuts which sustained total loss of vacuum or those cuts wrapped in PVC film. There were no significant differences in muscle color, subcutaneous fat freshness or visual microbial damage which were attributed to packaging treatment or vacuum level.

Bacterial counts and weight losses for combined subprimal cuts evaluated 5–6 days after packaging are presented in Table 7. Neither packaging treatment nor vacuum level significantly affected bacterial counts. Cuts packaged in PVC film had less purge loss than did vacuum packaged cuts which were leakers, but terminal vacuum level did not significantly affect purge loss among vacuum packaged cuts. At transport-storage intervals of 5–6 days neither vacuum level nor packaging method significantly affected weight losses or bacterial counts.

A comparison of appearance scores for subprimal cuts evaluated 7–9 days after packaging is presented in Table 8. Vacuum packaged cuts with high or intermediate levels of vacuum sustained less ($P < 0.05$) surface discoloration and had higher

Table 5—Bacterial counts[a] for beef cuts

Storage interval (days)	Normal atmosphere[b]		Modified atmosphere[b]	
	Vacuum packaged	PVC film wrapped	Vacuum packaged	PVC film wrapped
5–6	8.4b	8.4b	7.3c	8.0bc
7–9	6.9c	8.4b	6.5c	6.8c

[a] Bacterial counts are reported as the number ($\log_{10}$) of microorganisms per 6.45 cm^2 of surface area.
[b] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

Table 6—Mean appearance scores for combined subprimal cuts evaluated 5–6 days after packaging

Trait	Packaging method[f]			
		Vacuum packaging		
	PVC film wrapped	Terminal vacuum level		
		High	Intermediate	None
Muscle color[a]	6.1f	6.8f	6.8f	6.5f
Surface discoloration[b]	3.3g	5.7f	5.7f	3.2g
Subcutaneous fat freshness[c]	3.2f	4.2f	4.0f	3.8f
Visual microbial damage[d]	5.7f	6.0f	6.2f	6.0f
Overall appearance[e]	3.2g	5.8f	5.5f	3.2g

[a] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red)
[b] Means based on a 7-point scale (7 = no discoloration; 1 = total discoloration)
[c] Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration)
[d] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth)
[e] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable)
[f] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

overall appearance scores than those cuts with total vacuum loss (leakers) or those cuts wrapped in PVC film. No significant differences in muscle color, subcutaneous fat freshness or visual microbial damage were associated with either packaging method or terminal vacuum level. Vacuum packaging provides superior product protection in comparison to use of PVC film if the vacuum packages remain intact; however, 41.9% of the packages in this test had sustained total vacuum loss by 7–9 days after packaging. These data suggest that packages which sustain total loss of vacuum do not provide improved protection in comparison to use of PVC film. Since vacuum packaging costs more than five times as much as PVC film wrapping (Smith et al., 1976) the economic consequences of using a packaging system with this kind of failure rate is self-evident.

Weight losses and purge losses were not significantly affected by packaging method (Table 9). Bacterial counts did not differ ($P > 0.05$) among vacuum packaged cuts; however, cuts wrapped in PVC film had higher ($P < 0.05$) counts than any of the cuts which were vacuum packaged. Subprimals which had high or intermediate levels of vacuum at destination had less ($P < 0.05$) trim loss than those which were leakers or those which were wrapped in PVC film. Although cuts wrapped in PVC film and shipped in a modified atmosphere had fewer ($P < 0.05$) bacteria to cause discoloration, CO_2 from the modified atmosphere appeared to be partially responsible for muscle surface discoloration; therefore, trim loss was attributed to chemical discoloration. Discoloration associated with use of the modified atmosphere apparently negated benefits of reduced microbial load.

To establish relationships between visual traits associated with product stability and actual percentages of trim loss, simple correlation analyses were performed and results are presented in Table 10. Higher scores for appearance traits (indicative of greater product stability) were associated ($P < 0.05$) with decreased trim loss. Overall appearance scores were most closely associated with amount of trim loss required 7–9 days after packaging, indicating that this trait was most indicative of deterioration of the product during transit and storage. The fact that bacterial count was not indicative of trim loss further supports the contention that the type of bacteria rather than

Table 7—Bacterial counts, weight losses and purge losses for combined subprimal cuts evaluated 5–6 days after packaging

Trait	Packaging method[b]			
		Vacuum packaging		
	PVC film wrapped	Terminal vacuum level		
		High	Intermediate	None
Bacterial count ($\log_{10}$)[a]	8.4b	8.1b	8.1b	8.2b
Weight loss (%)	0.33b	0.40b	0.20b	0.26b
Purge loss (%)	0.02c	0.10bc	0.10bc	0.31b

[a] Reported as the number ($\log_{10}$) of microorganisms per 6.45 cm^2 of surface area
[b] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

Table 8—Mean appearance scores for combined subprimal cuts evaluated 7–9 days after packaging

Trait	Packaging method[f]			
		Vacuum packaging		
	PVC film wrapped	Terminal vacuum level		
		High	Intermediate	None
Muscle color[a]	6.3f	7.0f	7.5f	6.5f
Surface discoloration[b]	1.8g	5.5f	6.0f	1.5g
Subcutaneous fat freshness[c]	3.2f	2.0f	2.5f	2.5f
Visual microbial damage[d]	4.0f	5.0f	5.0f	5.0f
Overall appearance[e]	1.7g	5.5f	6.0f	1.5g

[a] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red)
[b] Means based on a 7-point scale (7 = no discoloration; 1 = total discoloration)
[c] Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration)
[d] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth)
[e] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable)
[f] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

Table 9—Bacterial counts, weight losses, purge losses and trim losses for combined subprimal cuts evaluated 7–9 days after packaging

Trait	Packaging method[b]			
		Vacuum packaging		
	PVC film wrapped	Terminal vacuum level		
		High	Intermediate	None
Bacterial count ($\log_{10}$)[a]	8.4b	7.0c	6.8c	7.1c
Weight loss (%)	0.64b	0.45b	0.20b	0.41b
Purge loss (%)	0.50b	0.40b	0.20b	0.21b
Trim loss (%)	2.75b	0.50c	0.50c	2.68b

[a] Reported as the number of microorganisms per 6.45 cm^2 of surface area.
[b] Means on the same line bearing a common letter are not significantly ($P > 0.05$) different.

Table 10—Correlation of appearance scores and bacterial counts with trim losses from combined subprimal cuts evaluated 7–9 days after packaging

Trait	Trim loss (%) Correlation coefficient
Muscle color[a]	−0.50**
Surface discoloration[b]	−0.68**
Subcutaneous fat freshness[c]	−0.58**
Visual microbial damage[d]	−0.60**
Overall appearance[e]	−0.78**
Bacterial count (log_{10})[f]	0.01

[a] Means based on an 8-point scale (8 = very bright cherry red; 1 = very dark red)
[b] Means based on a 7-point scale (7 = no discoloration; 1 = total discoloration)
[c] Means based on a 6-point scale (6 = very fresh; 1 = extensive deterioration)
[d] Means based on a 7-point scale (7 = dry, no slime formation; 1 = wet, moist or dry; extensive colony or mold growth)
[e] Means based on an 8-point scale (8 = extremely desirable; 1 = extremely undesirable)
[f] Reported as the number of microorganisms per 6.45 cm^2 of surface area
** $P < 0.01$.

the number of bacteria is critical in determining the rate and extent of product deterioration during storage.

The following conclusions were drawn from these data: (1) Since neither packaging nor atmosphere significantly ($P > 0.05$) affected appearance of cuts evaluated 5–6 days after packaging, economic considerations favor use of PVC film and a normal atmosphere for transit-storage intervals of this duration. (2) Cuts in intact vacuum packages which were evaluated 7–9 days after packaging were superior in appearance to those with total vacuum loss and those wrapped with PVC film. (3) Use of a modified atmosphere significantly ($P < 0.05$) decreased microbial growth on PVC wrapped cuts shipped and/or stored for 7–9 days but no differences were found with shorter transit-storage periods. (4) Use of a modified atmosphere decreased appearance of PVC wrapped cuts which were evaluated within 24 hr after removal from the van, but improved the appearance of these cuts when they were evaluated 48–72 hr after removal from the modified atmosphere van. (5) Neither packaging method nor van atmosphere affected ($P > 0.05$) weight losses during transit and storage. (6) Transoceanic shipments of fresh beef can be conducted with acceptable results: (a) by use of PVC film if the subprimal cuts are fabricated into retail cuts within 6 days after packaging; or (b) as intact vacuum packages if longer transit and storage periods are necessary.

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EFFICACIES OF THREE SANITIZERS UNDER SIX CONDITIONS OF APPLICATION TO SURFACES OF BEEF

ABSTRACT

Efficacies and optimum application conditions of three sanitizers (chlorine, 200–250 ppm; acetic acid, 4.0%; and quaternary ammonium, 3.78 g/liter) were determined. Variables were three rates of flow of sanitizer (1.7, 3.4 and 6.8 liters/min), washed and unwashed meat, warm (38°C) and cold meat (3.3°C), and two pressures of sanitizer application (3.5 and 14.0 kg/cm^2). Results were averaged over other variables providing the following data for individual variables. Acetic acid gave a high initial average decrease in counts (−1.47 log), and counts decreased (−1.79 log) up to 48 hr after sanitization. Chlorinated solution caused an initial mean difference of −0.31 log and a difference after 48 hr of 0.53. The quaternary ammonium compound produced differences of −0.79 and −0.03 log in immediate and 48-hr counts. Acetic acid and hypochlorite were nonselective, but the quaternary sanitizer allowed more oxidase positive, nonfermentative bacteria to survive.

INTRODUCTION

SANITIZERS are used in the food industry to destroy microorganisms on food contact surfaces. These same sanitizers can kill microorganisms on surfaces of solid foods such as meat.

Chlorinated water applied to lamb carcasses was beneficial at concentrations of 15–350 mg/liter, and effectiveness increased with concentration (Bailey, 1971). Patterson (1968) reported less than a one-log decrease in bacterial counts on dressed chickens when 5–20 mg/liter of free residual chlorine were used. Kotula et al. (1974) observed average log decreases in counts of 1.52 and 2.31 in two tests in which samples were collected with swabs from beef carcasses 45 min after use of a chlorinated wash. Average log decreases were 2.39 and 3.07 for the same tests when samples were collected 24 hr after the chlorinated wash. They noted that the effect of high-pressure washing overshadowed the effects of pH and wash water temperature.

Mountney and O'Malley (1965) observed that acetic, adipic and succinic acids were the most inhibitory to bacteria on poultry of 10 organic acids tested. They considered acetic acid unacceptable because of its pungent odor and effect on the skin.

Thomson et al. (1967) found that chlorine, antibiotics, β-propiolactone, and citric and succinic acids reduced the populations of organisms on fryer breast skin. Acetic acid (pH 1.5 and 2.0) reduced counts on pork carcasses by 10^4 according to Biemuller et al. (1973).

Although decontamination of meat carcasses with various sanitizers has been studied by many researchers, little information is available as to optimum application procedures.

The present study was undertaken to determine the efficacies of three sanitizers, optimum conditions for applying them, and whether they selectively eliminated portions of the microflora.

MATERIALS & EQUIPMENT

Materials

Strips of fresh plate meat (beef), approximately 20 × 30 × 1.5 cm, were sanitized with sodium hypochlorite (200–250 mg/liter); acetic acid (4.0%); and a quaternary ammonium compound. SRV-M conc 2622 (3.78 g/liter) supplied by Dr. B. Singh, Graduate School of Public Health, University of Pittsburg, Pittsburg, Pa. All solutions were prepared with tap water and were sprayed at temperatures of 12.8–15.6°C. The pH of the sodium hypochlorite was adjusted to 6.0 with acetic acid.

Table 1—Differences in log counts of microorganisms as affected by variables studied in application of acetic acid

Flow rate (liters/min)	Pressure (kg/cm^2)	Application rate (ml/cm^2)	Nozzle no.	Mean droplet size (μm)	Differences in log counts[a] Time of sampling after sanitization (hr) 0	1	24	48
1.7	3.5	0.09	5004	600	−1.16^b	−1.33^b	−1.48^{bc}	−1.70^c
1.7	14.0	0.09	5002	350	−1.09^b	−1.01^b	−1.30^b	−0.77^b
3.4	3.5	0.18	5008	740	−1.39^{bc}	−1.53^b	−1.92^{bc}	−1.96^{cd}
3.4	14.0	0.18	5004	450	−1.53^c	−1.62^b	−1.52^{bc}	−1.96^{cd}
6.8	3.5	0.36	5015	980	−1.75^c	−1.62^b	−1.61^{bc}	−1.86^{cd}
6.8	14.0	0.36	5018	590	−1.82^c	−1.67^b	−2.04^c	−2.41^d

[a] Means of 12 values averaged over temperature of meat and washing treatment.
[b,c,d] Values with the same superscripts in each column are not significantly different ($P < 0.05$).

Equipment

Meat was washed in the meat cleaning unit and the water was supplied by a high pressure pumping unit as previously described (Anderson et al., 1975). Wash water was sprayed with nozzle 5050. Sanitizers were sprayed in the same equipment with nozzle 5002, 5004, 5008, or 5015 which were obtained from Spraying Systems Co., Wheaton, Ill. Effective width of the spray pattern was 30.5 cm. Mean droplet size of sanitizing solution applied to the meat surface as a function of flow rate and pressure of application is given in Table 1.

PROCEDURE

THE EXPERIMENT was a 3 × 2 × 2 × 2 factorial in a randomized complete block design with three replications. Seventy-two pieces of meat were used for each sanitizer. Conditions of the study were three flow rates of sanitizing solution (1.7, 3.4 and 6.8 liters/min); washed and unwashed meat (confounded in rep 1 – sections 1 and 2); warm (38°C) and cold (3.3°C) meat (confounded in rep 2 – sections 3 and 4); and two pressures (3.5 and 14.0 kg/cm^2, confounded in rep 3 – sections 5 and 6). Nozzles were changed to obtain the correct volume for the different pressures. Meat moved past the sanitizing nozzle at the rate of 10 cm/sec.

Each day 12 pieces of meat in individual plastic bags were obtained. Samples used to simulate freshly slaughtered meat were preheated in the bags to 38°C and were tempered for 5 min at that temperature (referred to as warm meat).

When washing was required, a piece of warm or cold meat was removed from its bag, placed on a frame and washed under the following conditions before initial sampling: pressure, 14.0 kg/cm^2; flow rate, 25.4 liters/min; nozzle distance from meat, 40 cm; speed of meat past nozzle, 10 cm/sec. Thus, the rate of application was 1.4 ml/cm^2. After being washed and before being sanitized, warm and cold meat were placed on trays at 38 and 3.3°C, respectively, until they were sampled (within 20 min).

Samples, composed of 4 cores from the top surface (each 2.54 cm in diameter by 3 mm thickness), were taken from each piece of meat (Fig. 1) after meat was washed (before it was sanitized) and immediately and 1, 24, and 48 hr after it was sanitized. Initial bacterial counts on the meat ranged from $10^{3.42}$ to $10^{8.86}$/cm^2, and the mean count was $10^{7.71}$. Between times of sampling, meat was stored on open trays in a walk-in refrigerator (3.3°C, relative humidity 86 ± 5).

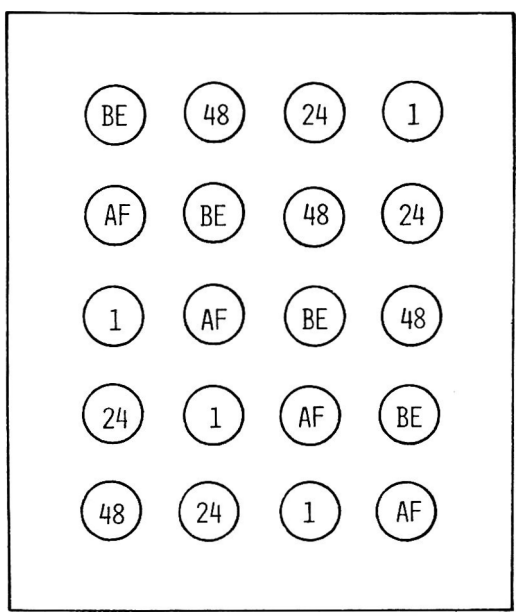

Fig. 1—Pattern for removing core samples from meat before sanitization (BE), immediately after sanitization (AF), 1 hr after sanitization (1), 24 hr after sanitization (24) and 48 hr after sanitization (48).

Samples were blended in 100 ml of sterile buffered dilution water for 1 min. Appropriate dilutions were made. Plates were poured with plate count agar (Difco) and incubated three days at 28°C before colonies were counted. Methods of plating and counting were according to standard methods (Hausler, 1972). A log ratio transformation was used to normalize the data. Before analysis the normalization of data was verified by the test of Kolmogorov, Smirnov and Lilliefors, and data were analyzed by use of ANOVA programs in the Statistical Analysis Systems (SAS) package (Service, 1972).

Three colonies were picked from each series of plates made before and 48 hr after meat was sanitized. A template with three randomly placed dots was used to select colonies. Plates having 30–300 colonies, or the nearest thereto, were placed on the template, and the three colonies closest to the dots were transferred to slants of triple sugar iron (TSI) agar. After incubation at 25°C for 48 hr, tubes were observed for type of fermentation and for evidence of gas and hydrogen sulfide.

Tests for catalase and cytochrome oxidase were performed on colonies from the TSI slants. The catalase test was positive when gas bubbles were produced by cells emulsified in a few drops of 3% hydrogen peroxide. Cells positive to the oxidase test produced a blue color on Pathotec CO test strips (General Diagnostics Div., Warner-Chilcott Laboratories, Morris Plaines, N.J.).

Numbers of isolates examined for each type of sanitizer were as follows: acetic acid, 210; hypochlorite, 216; and quaternary ammonium compound, 144. Numbers were composed equally of isolates taken before and after meat was sanitized.

To determine whether the microflora after sanitization differed from that initially present, the number of isolates which failed to produce catalase or oxidase or to ferment glucose, lactose, or sucrose was determined. Groupings were made among isolates picked before and after sanitization, and the grouped data were subjected to Chi-square analysis.

RESULTS & DISCUSSION

CONCENTRATIONS of sanitizers were selected based on findings of Kotula et al. (1974), who used hypochlorite, and of Bali (1970), who did preliminary work with acetic acid, and on recommendations of the manufacturer of the quaternary ammonium compound.

Differences in log counts of microorganisms on meat for different times of sampling after sanitization with acetic acid, hypochlorite, and a quaternary ammonium compound are shown in Figure 2. Difference in log counts = $\log_{10}$ (number of microorganisms after sanitization) – $\log_{10}$ (number of microorganisms before sanitization). Thus, negative values indicate a decrease in the microbial population and positive values an increase.

Acetic acid gave a large initial decrease in counts, averaged over other variables (–1.47 log), and counts decreased up to 48 hr after sanitization when the difference was –1.79.

Table 2—Differences in log counts of microorganisms on prewashed and unwashed meat as affected by pressure and volume of sanitizer for samples collected 48 hr after sanitization with acetic acid

Pressure (kg/cm^2)	Type of meat	Differences in log counts[a] Flow rate of acetic acid (liters/min) 1.7	3.4	6.8
3.5	unwashed	–1.78^{cd}	–1.86^{cd}	–1.58^{cd}
3.5	washed	–1.62^{cd}	–2.05^{cd}	–2.14^{cd}
14.0	unwashed	–1.46^{c}	–1.43^{c}	–2.68^{d}
14.0	washed	–0.09^{b}	–2.50^{cd}	–2.14^{cd}

[a] Means of six values.
b,c,d Values with the same superscript are not significantly different ($P < 0.05$).

Table 3—Differences in log counts of microorganisms as affected by pressure of application of hypochlorite (200–250 mg/liter) and time of sampling after sanitization

Pressure (kg/cm²)	Differences in log counts[a]			
	Time of sampling after sanitization (hr)			
	0	1	24	48
3.5	-0.15^{b}_{x}	0.01^{b}_{x}	0.28^{b}_{x}	0.90^{b}_{y}
14.0	-0.47^{c}_{x}	0.41^{c}_{x}	0.20^{c}_{x}	0.17^{c}_{x}

[a] Means of 36 values.
[b,c] Values with the same superscript in each column are not significantly different ($P < 0.05$).
[x,y] Values with the same subscript in each row are not significantly different ($P < 0.05$).

Table 4—Differences in log counts of microorganisms on warm and cold meat as affected by pressure of application of quaternary ammonium compound and time of sampling

Pressure (kg/cm²)	Temperature (°C)	Differences in log counts[a]			
		Time of sampling after sanitization (hr)			
		0	1	24	48
3.5	3.3	-0.75^{bc}	-0.50^{b}	-0.13^{c}	0.29^{b}
3.5	38	-0.79^{bc}	-0.70^{bc}	-0.34^{c}	-0.18^{bc}
14.0	3.3	-1.11^{c}	-1.05^{c}	-0.55^{c}	-0.33^{c}
14.0	38	-0.52^{b}	-0.70^{bc}	-0.14^{c}	0.09^{bc}

[a] Means of 18 values each.
[b,c] Values with the same superscript in each column are not significantly different ($P < 0.05$).

Whether microbial death resulted from low pH or as a direct effect of the acid molecule was not determined.

Effects of flow rate, pressure and time of sampling after sanitizing with acetic acid are presented in Table 1. Application rate, nozzle number and mean droplet size are presented as additional information though they were not considered in the analysis of variance. Increasing the rate of flow of acetic acid sanitizer from 1.7 to 3.4 to 6.8 liters/min produced significant ($P < 0.05$) decreases in log counts for each sampling time except 1 hr. The greatest decrease occurred 48 hr after sanitizing at the highest rate of flow and pressure, 6.8 liters/min and 14.0 kg/cm^2, respectively. Besides increased washing of the surface, the high volume of solution undoubtedly produced a lower pH and provided more unionized acetic acid in the microenvironment of the bacteria than did the other five conditions of application. However, no effect of pressure of application was shown when data were averaged over all other variables.

A significant interaction of rate of flow and pressure was observed in counts taken 48 hr after sanitization. At the flow rate of 1.7 liters/min the lower pressure, 3.5 kg/cm^2, was more effective, whereas at the highest flow rate, 6.8 liters/min, the higher pressure, 14 kg/cm^2, was more effective. The interaction was insignificant at the other times of sampling. The lesser effectiveness of acetic acid at high pressure and low flow rate may be explained as follows: as diameter of the nozzle orifice was decreased and pressure was increased, smaller droplets formed. Mean droplet size under above conditions was 350 μm compared with 600 μm when the pressure was 3.5 kg/cm^2. Many of these small droplets did not impact on the meat but were carried away by air currents. This reduced the concentration of acetic acid on the surface.

Differences in log counts (48 hr) of unwashed and washed meat as affected by pressure and flow rate of acetic acid are shown in Table 2. The mean difference in log counts was only −0.09 on washed samples sprayed at 14.0 kg/cm^2 and 1.7 liters/min. Since the log difference for unwashed samples, −1.46, was significantly greater, it is suggested that moisture absorbed at the surfaces of washed samples diluted the already low concentration of acid.

Application of hypochlorite to meat caused an initial mean difference of −0.31 log (Fig. 2). The observed increase in bacterial counts (0.53 log) over the next 48 hr indicated no residual effect of the chlorine. This is to be expected because chlo-

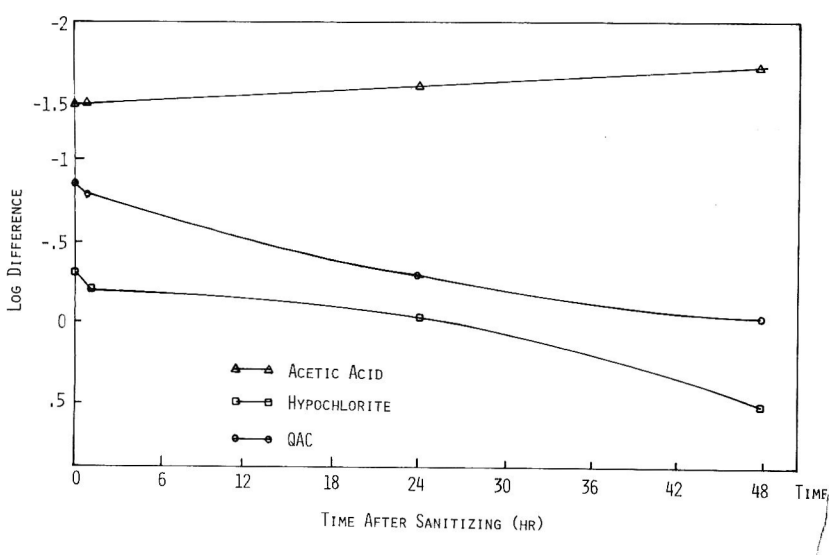

Fig. 2—Log differences of microorganisms on meat surfaces for different times of sampling after sanitization with acetic acid, hypochlorite and a quaternary ammonium compound.

Table 5—Percentages of isolates, taken before and after sanitization, positive to selected biochemical tests

Sanitizer	No. of samples	Catalase		Oxidase		Glucose fermentation		Sucrose or lactose fermentation	
		Before	After	Before	After	Before	After	Before	After
Acetic acid	105	84.8	91.4	69.5	79.0	3.8	8.6	20.0	12.4
Hypochlorite	108	94.4	92.6	92.0	83.3	16.7	12.0	17.6	23.1
Quaternary ammonium	71	95.8	100	87.3	100	7.0	4.2	15.5	1.4
Averages		91.7	94.7	82.9	87.4	9.2	8.3	17.7	12.3

rine can react with organic components of meat and become inactive.

Pressure of application and time of sampling were significant variables in experiments with sodium hypochlorite (Table 3). Counts were lowest immediately after sanitization and increased with time of storage. The higher pressure caused greater reductions in counts than did the lower pressure. The data suggest that during the 48 hr at 3.3°C residual cells reproduced three to four and two to three generations on samples sprayed at 3.5 and 14.0 kg/cm^2, respectively.

Counts (not shown in table) taken 48 hr after sanitization with hypochlorite indicated that less growth occurred ($P < 0.05$) on unwashed than on washed meat (0.27 vs 0.80 log increase, respectively). The chlorinated solution was probably diluted below an inhibitory concentration by water which had penetrated the meat during washing. The higher water activity in the washed samples probably caused the faster growth. No significant effects were noted for volume of solution or warm vs cold meat.

There were no significant main effects of volume, pressure, temperature of meat or washing of meat in experiments with quaternary ammonium sanitizer. However, an interaction of pressure and temperature of meat was observed for the various times of sampling (Table 4). The greatest difference in log counts produced by the quaternary ammonium sanitizer was −1.11, which was observed with cold meat sprayed at 14.0 kg/cm^2 and sampled immediately. This treatment tended to have the greatest differences in counts at all times of sampling. Differences averaged over all treatments were −0.79 and −0.03 (log) in immediate and 48-hr counts, respectively.

Neither acetic acid nor hypochlorite had a detectable effect on type of microflora as measured by biochemical activities of the ioslates (Table 5). However, Chi-Square analysis indicated that the quaternary ammonium sanitizer selected among the microflora of the meat and favored survival of catalase and oxidase positive, nonfermentative bacteria. These were the dominant flora of the meat. More than 90% of the isolates from the fresh samples were catalase positive and 83% produced cytochrome oxidase. Nine percent fermented glucose and nearly 18% fermented either sucrose or lactose. No culture produced gas in lactose broth, and only four of 570 isolates produced gas in TSI agar. None of these produced hydrogen sulfide.

Tests performed were those most important in identifying gram negative bacilli. Although gram stains were done sparingly, results of biochemical tests and observations of colonial morphology strongly suggested that the primary microflora was composed of the genera *Pseudomonas* and *Alcaligenes.*

Based on the findings of the study reported here, acetic acid was the best sanitizer, and it was most effective when applied at the highest rate of flow and line pressure that were used, viz., 6.8 liters/min and 14.0 kg/cm^2.

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EFFECT OF BLADE TENDERIZATION ON STORAGE LIFE RETAIL CASELIFE AND PALATABILITY OF BEEF

ABSTRACT

Boneless strip loins (n = 90) and inside rounds (n = 90) from Heavy-Choice, Heavy-Good and Light-Good carcasses were randomly assigned to one of six treatments representing combinations of storage interval (7 or 14 days), blade tenderization (prior to or following storage) and packaging (vacuum packages or polyethylene bags). Vacuum packaging was much more satisfactory than polyethylene packaging for maintaining appearance of subprimal cuts following storage and for assuring desirable overall appearance of steaks during retail display. Subprimal cuts should be blade tenderized after, rather than prior to, storage to minimize weight losses of subprimals during storage, but time of blade tenderization did not affect retail caselife or palatability traits. Light-Good beef was not generally inferior to that from Heavy-Choice or Heavy-Good carcasses in storage-life, retail caselife or palatability if subprimal cuts were stored in vacuum packages. However, Light-Good strip loins which were blade tenderized prior to storage and stored in polyethylene bags were discolored and unattractive following storage and produced steaks which had very limited retail caselife. Blade tenderization increased tenderness above that achieved by aging alone but did not otherwise affect palatability.

INTRODUCTION

THREE of the most important trends in the meat industry during the 1970's included: increased impetus toward centralized cutting and fabrication of red meats (Partch, 1976), revitalized interest in slaughter of forage-finished and short-fed cattle (Cross and Smith, 1975) and increased use of blade or needle tenderizing machines to improve the palatability of retail cuts (Bowes, 1975).

Centralization of beef warehousing, breaking and cutting operations has occurred because of needs to increase labor efficiency and to prolong storage-life. Breaking, cutting and trimming to form subprimal cuts increases the meat surface area exposed with resultant increases in shrinkage and spoilage. Vacuum packaging is effective in reducing shrinkage and in retarding spoilage of red meat, during transit, distribution and storage (Minks and Stringer, 1972; Hodges et al., 1974; Seideman et al., 1976a, b). Unfortunately, vacuum packaging is expensive and may not be economically feasible when compared to less expensive wrapping materials for storage intervals of less than 7 days (Smith et al., 1976). Other packaging systems for protecting beef cuts during transit and storage have been identified (Smith and Carpenter, 1973; Berry et al., 1971; Rea et al., 1972).

High prices for grain, unfavorable cattle-feeding margins and increased availability of lean, light-weight beef have created a dilemma for retailers. Considerable uncertainty prevails regarding the eating qualities and storage stability of Light-Good beef. Technology for increasing tenderness exists in the form of mechanical tenderization techniques developed and used extensively in the HRI meat trade. Blade or needle tenderization can be used to improve the tenderness of beef (Schwartz and Mandigo, 1974; Glover et al., 1975).

Major unanswered questions at present include: the proper time to blade tenderize beef (prior to or following shipment to retail stores); the effect of weight and grade of beef on shrinkage, retail caselife and palatability; and the effects of packaging and bacterial decontamination on the storage-life of beef. The present study investigated the incorporation of blade tenderization into systems for prefabrication, packaging and storage of beef cuts.

EXPERIMENTAL

BONELESS STRIP LOINS and inside rounds were obtained from 45 beef sides. Fifteen sides were U.S. Choice, yield grades 2 or 3 and weighed 125–150 kg (Heavy-Choice); 15 sides were U.S. Good, yield grades 2 or 3 and weighed 125–150 kg (Heavy-Good); and, 15 sides were U.S. Good, yield grades 1 or 2 and weighed 75–100 kg (Light-Good). The Heavy-Choice and Heavy-Good sides (selected from animals slaughtered at the Meats Laboratory, Texas A & M University) were aged for 2–4 days prior to cutting; the Light-Good carcasses (selected from a commercial firm) were aged 5–7 days prior to cutting. Each wholesale cut was divided into two subprimals immediately preceding treatment allocation.

Experimental design for supprimal cuts.

Boneless strip loins and inside rounds were randomly assigned to one of six treatments representing various combinations of packaging system (vacuum packaging, VP, or packaging in a polyethylene bag, PEP) storage interval at 2°C (7 or 14 days), blade tenderization (BT) with a reciprocating blade-type (Ross, model TC-700) mechanical tenderizer (before or after storage) and use of 0.02% sodium hypochlorite rinse. The six specific treatments (Table 1) were as follows: (A) Vacuum packaging and storage for 14 days; (B) Blade tenderization, vacuum packaging and storage for 14 days; (C) vacuum packaging, storage for 14 days and blade tenderization; (D) Polyethylene packaging and storage for 7 days; (E) Blade tenderization, polyethylene packaging and storage for 7 days; and (F) Polyethylene packaging, storage for 7 days and blade tenderization. Half of the cuts in treatments B and C were rinsed with a solution of 0.02% sodium hypochlorite.

All subprimal cuts in treatments A, B and C were vacuum packaged in a chamber-type packaging machine using a chamber vacuum of 29 in. of Hg and Conofresh B (barrier) bags of the type described by Seideman et al. (1976b).

Evaluation of subprimal cuts

Upon completion of storage for specified intervals, appropriate weights of subprimal cuts were obtained (to facilitate determinations of evaporative and purge loss) and each cut was subjectively evaluated by a three-member trained evaluation panel for overall appearance according to an 8-point scale (8 = extremely desirable; 1 = extremely undesirable).

Bacteriological evaluation

Bacterial counts were obtained by sampling the surfaces of subprimal cuts with a damp, sterile 5 × 5 × 1.3 cm cellulose sponge, with the analyst's hand covered with a sterile, plastic glove (Silliker and Gabis, 1975). Bacteria samples were obtained by swabbing a 129 cm^2 area of the subcutaneous fat cover and of the longissimus or semimembranosus muscles. Psychrotrophic counts were obtained by use of standard plate count agar and incubation at 7°C for 10 days.

Table 1—Experimental design for subprimal cuts (n = 180) and retail cuts (n = 360)

Treatment code	Number of subprimal cuts[a]	Treatment before storage[b]	Packaging[c]	Storage interval (days)	Treatment after storage[b]	Number of retail cuts	Retail display interval (days)
0	90	None	None	None	None	90	None
A	60	None	VP	14	None	60	4
B	60	BT	VP	14	None	60	4
C	60	None	VP	14	BT	60	4
D	30	None	PEP	7	None	30	4
E	30	BT	PEP	7	None	30	4
F	30	None	PEP	7	BT	30	4

[a] Retail cuts for treatments A and C were obtained from the same subprimal cuts; retail cuts for treatments D and F were obtained from the same subprimal cuts. Subprimal cuts were stored for 14 or 7 days, respectively, retail cuts were removed (for treatments A and D), the subprimal cuts were blade tenderized and additional retail cuts were obtained (for treatments C and F).
[b] BT = blade tenderized.
[c] VP = vacuum packaged; PEP = packaged in polyethylene bags.

Experimental design for retail steaks

Retail steaks (n = 360) were removed from the 90 subprimal cuts at four stages. One steak (2.5 cm thick) was removed from each of the 90 subprimal cuts prior to packaging, blade tenderization or storage (treatment 0). A second steak was removed from each subprimal cut assigned to treatments A and D, following storage but prior to blade tenderization. A third steak was removed from each subprimal cut in treatments A and D following blade tenderization and designated as treatments C and F. A second steak was removed from each subprimal cut assigned to treatments B and E following storage.

The 90 control steaks (treatment 0) were immediately double-wrapped in polyethylene-coated freezer paper and stored at −10°C for subsequent palatability determination. Each of the other 270 steaks (treatments A-F) was weighed (to facilitate determinations of weight loss during retail display) placed in a plastic foam tray, over-wrapped with polyvinyl chloride film (MVTR = 260; OTR = 12,000) under simulated retail caselife conditions (1–3°C with 968 lux of incandescent light).

Evaluation of retail steaks during retail display

Retail cuts were evaluated by a three-member trained panel at 24-intervals during four days of retail display. Each retail cut was evaluated for overall appearance (a composite evaluation of muscle color, freshness of fat, surface discoloration and peripheral discoloration) and surface discoloration. Following the four-day retail display period each of the 270 steaks was weighed to determine weight loss during retail display and immediately frozen and stored at −10°C for subsequent palatability tests.

Palatability evaluation

Steaks for palatability determinations were stored at −10°C for periods which did not exceed 60 days. Steaks were subsequently removed from frozen storage, thawed at room temperature (to an internal temperature of 22°C), weighed, oven-broiled in a 180°C preheated electric oven to an internal temperature of 75°C, copper-constantan thermocouples were used to monitor oven and meat temperature. A six-member trained sensory panel evaluated each steak for tenderness, juiciness, flavor and overall palatability according to 8-point rating scales (8 = extremely tender, extremely juicy, extremely flavorful and extremely desirable in palatability, respectively). Mealiness was rated on a 7-point scale (7 = no mealiness; 1 = extremely mealy). Panel members all had 1–4 yr of sensory panel experience and were trained for 3 wk prior to actual testing using the open-discussion method.

Statistical methods

Data were subjected to analysis of variance as outlined by Snedecor and Cochran (1967). Mean separation analyses were performed using the Kramer (1956) modification of the Duncan (1955) Multiple Range Test.

RESULTS & DISCUSSION

PSYCHROTROPHIC BACTERIAL COUNTS (Table 2) subprimal cuts prior to packaging and following storage did not differ ($P > 0.05$) among weight-grade classes of beef which is in agreement with Hodges et al. (1974). Subprimal cuts packaged in polyethylene bags and stored for 7 days (Treatments E and F) had slightly higher psychrotrophic counts following storage than did vacuum packaged cuts stored for 14 days (Treatments B and C). Rea et al. (1972) postulated that polyethylene bags reduced surface evaporation and thereby maintained a favorable water activity (A_w) for bacterial growth.

Data from the present study but not presented here in tabular form indicated that retail cuts removed from subprimal cuts which had been packaged in polyethylene bags possessed higher counts than those removed from vacuum packaged cuts. Pierson et al. (1970) arrived at a similar conclusion using top round steaks from PEP vs VP cuts. Subprimal cuts (Table 2) which were blade tenderized prior to packaging had higher numerical counts in 19 of 27 comparisons with cuts which were tenderized following storage. Rinsing of subprimal cuts with a 0.02% sodium hypochlorite solution did not significantly ($P > 0.05$) affect bacterial counts.

Overall appearance ratings for boneless strip loins and inside rounds are presented in Table 3. Blade tenderization prior to storage rather than following storage did not ($P > 0.05$) decrease overall appearance of strip loins in either packaging material, but did ($P < 0.05$) in 3 of 6 comparisons) decrease overall appearance ratings fo inside rounds.

Strip loins and inside rounds which were vacuum packaged and stored prior to blade tenderization (Treatment C) were more desirable ($P < 0.05$) in overall appearance than those stored in polyethylene bags prior to blade tenderization (Treatment F) in 5 of 6 comparisons. Subprimal cuts in treatment B were more desirable ($P < 0.05$) in overall appearance than those in treatment E in 5 of 6 comparisons (Table 2). Light-Good strip loins were decidedly ($P < 0.05$) inferior in overall appearance to those from heavy beef.

Effects of blade tenderization on weight losses during storage, retail display and cooking are presented in Tables 3 and 4. Weight losses during storage were increased by blade tenderization prior to, rather than following, storage. Blade tenderization prior to storage increased weight losses for vacuum packaged strip loins and inside rounds (irrespective of weight-grade) by 1.55% and 2.25%, respectively, and increased weight losses for polyethylene packaged strip loins and inside rounds by 0.97% and 2.13%, respectively (Table 3).

Retail cuts from strip loins and inside rounds which were blade tenderized prior to, or following, storage lost more weight ($P < 0.05$) during retail display than retail cuts from wholesale cuts which were not blade tenderized in only 2 of

Table 2—Mean psychrotrophic bacterial counts for subprimal cuts stratified according to treatment

Trait	Weight and grade of beef[a]	Psychrotrophic bacteria counts (log_{10}) Treatment B: BT, VP, stored[b]	Treatment B: BT and NaOCl, VP, stored[b]	Treatment C: VP, stored, BT[b]	Treatment C: VP stored, BT and NaOCl[b]	Treatment E: BT, PEP, stored[b]	Treatment F: PEP, stored, BT[b]
Count prior to packaging	1	2.4	2.2	2.3	2.0	2.3	1.5
	2	1.5	1.6	1.2	2.8	2.7	3.1
	3	1.9	3.6	3.7	2.8	2.9	2.7
Count following storage	1	5.3	5.3	4.8	4.3	5.6	5.1
	2	5.6	4.6	4.2	4.0	5.8	5.7
	3	4.9	5.1	4.9	5.7	6.3	6.1
Increase in count during storage	1	2.9	3.0	2.4	2.3	3.3	3.6
	2	4.1	2.9	3.0	1.2	3.1	2.6
	3	3.0	1.5	1.1	3.0	3.4	3.4
	1,2,3	3.4[c]	2.5[c]	2.2[c]	2.2[c]	3.3[c]	3.2[c]

[a] 1 = Heavy-Choice, 2 = Heavy-Good and 3 = Light-Good.
[b] BT = blade tenderized, VP = vacuum packaged, PEP = packaged in polyethylene bags, stored = 7 or 14 days storage as wholesale cuts, and NaOCl = rinsed with a solution of 0.02% sodium hypochlorite.
[c] Means in the same row bearing a common superscript letter are not different ($P > 0.05$).

Table 3—Mean values for weight loss during storage and overall appearance ratings for boneless strip loins and inside rounds

Weight and grade of beef[a]	Packaging treatment[b]: Vacuum packaged cuts stored 14 days, Treatment B: BT, VP, stored	Vacuum packaged cuts stored 14 days, Treatment C: VP, stored, BT	Polyethylene packaged cuts stored 7 days, Treatment E: BT, PEP, stored	Polyethylene packaged cuts stored 7 days, Treatment F: PEP, stored, BT	Rank order (highest to lowest) across treatments[c]
			Weight loss for strip loins during storage (%)		
1	4.0[d]	1.9[d]	2.4[d]	1.2[d]	B E C F
2	3.4[d]	2.2[d]	2.2[d]	1.3[d]	B C E F
3	3.6[d]	2.2[d]	1.7[d]	0.9[d]	B C E F
			Weight loss for inside rounds during storage (%)		
1	5.3[d]	2.4[d]	3.9[d]	2.0[d]	B E C F
2	4.4[d]	2.8[d]	3.8[d]	2.1[d]	B E C F
3	4.0[d]	2.3[d]	3.8[d]	1.0[e]	B E C F
			Overall appearance ratings for strip loins		
1	7.1[d]	7.4[d]	6.1[d]	6.9[d]	C B F E
2	7.1[d]	7.3[d]	5.7[d]	5.7[d]	C B E F
3	6.8[d]	7.0[d]	3.3[e]	3.8[e]	C B F E
			Overall appearance ratings for inside rounds		
1	5.7[d]	7.4[d]	3.1[d]	5.6[d]	C B F E
2	5.6[d]	6.1[e]	2.9[d]	3.8[e]	C B F E
3	5.7[d]	7.2[d]	3.7[d]	4.6[de]	C B F E

[a] 1 = Heavy-Choice, 2 = Heavy-Good and 3 = Light-Good.
[b] Ratings for overall appearance are based on an 8-point scoring system (8 = extremely desirable; 1 = extremely undesirable). There was a significant first-order interaction between weight-grade of beef and treatment for overall appearance ratings of strip loins. BT = blade tenderized, VP = vacuum packaged, PEP = packaged in polyethylene bags, and stored = 7 or 14 days storage as wholesale cuts.
[c] Means in the same row, underscored by a common line, do not differ ($P > 0.05$).
[d,e] Means in the same column, for a given cut and for each trait, bearing a common superscript letter are not different ($P > 0.05$).

24 comparisons and lost more weight ($P < 0.05$) during cooking than retail cuts from loins or rounds which were not blade tenderized in only 3 of 48 comparisons (Table 4). Blade tenderization prior to storage rather than following storage: (a) did not affect ($P > 0.05$) weight losses during retail display in 6 of 6 comparisons of steaks from vacuum packaged cuts and in 4 of 6 comparisons of steaks from polyethylene packaged cuts, and (b) did not affect ($P > 0.05$) weight losses during cooking in 5 of 6 comparisons of steaks from polyethylene packaged cuts (Table 4).

Total weight losses (during storage, retail display and cooking) for all beef tenderized prior to storage, all beef tenderized after storage and all beef which was not tenderized were 30.9, 30.3 and 29.4%, respectively, for strip loins and 36.3, 36.8 and 35.0%, respectively, for inside rounds. The combined losses are similar; however, the weight lost at particular stages in the system differed among treatments. Subprimal cuts which were tenderized prior to storage sustained more of their ultimate weight loss during storage, whereas beef which was tenderized after storage incurred more of its ultimate weight loss during cooking. Davis et al. (1975) reported that mechanically tenderized beef had significantly higher cooking losses than nontenderized beef and attributed this increase to the loss of moisture through the holes created by the tenderizer. However, other researchers have determined that cooking losses are not affected by blade or needle tenderization (Goldner et al., 1974; Schwartz and Mandigo, 1974; Bowling et al., 1976).

Comparisons of shrinkage among the three weight-grade classes of beef indicated nonsignificant ($P > 0.05$) differences in: storage loss, 7 of 12 comparisons; retail display loss, 11 of 12 comparisons; and, cooking loss, 14 of 14 comparisons (Tables 3 and 4). Hodges et al. (1974) reported that beef of the higher grades shrank less than beef of the lower grades because cuts from higher grades had less moisture and more extensive fat covering than cuts from lower grades.

Strip loin steaks removed from subprimal cuts packaged in polyethylene bags sustained more ($P < 0.05$) surface discoloration (day 1, 9 of 9 comparisons; day 4, 3 of 9 comparisons) and were less desirable ($P < 0.05$) in overall appearance (day 1, 9 of 9 comparisons; day 4, 6 of 9 comparisons) than steaks from vacuum packaged strip loins (Table 5). Round steaks removed from subprimal cuts packaged in polyethylene bags sustained more ($P < 0.05$) surface discoloration (day 1, 8 of 9 comparisons; day 4, 0 of 9 comparisons) and had lower ($P < 0.05$) overall appearance scores (day 1, 9 of 9 comparisons; day 4, 1 of 9 comparisons) than steaks from vacuum packaged inside rounds (Table 6).

Blade tenderization prior to, rather than following, storage did not affect surface discoloration ($P > 0.05$ in 12 of 12 comparisons) or overall appearance ($P > 0.05$ in 11 of 12 comparisons) of strip steaks (Table 5), and did not affect surface discoloration ($P > 0.05$ in 10 of 12 comparisons) or overall appearance ($P > 0.05$ in 10 of 12 comparisons) of inside round steaks (Table 6).

Strip loin steaks and inside round steaks from Heavy-Choice carcasses sustained less ($P < 0.05$) surface discoloration than cuts from Light-Good carcasses in 6 of 12 (Table 5) and 2 of 12 (Table 6) comparisons, respectively, and were more desir-

Table 4—Mean values for weight loss during retail display or during cooking of strip loin steaks and inside round steaks

Weight and grade of beef[a]	Packaging treatment[b]						Rank order (highest to lowest) across treatments[c]
	Vacuum packaged cuts stored 14 days			Polyethylene packaged cuts stored 7 days			
	Treatment 0 Control	Treatment A VP, stored, displayed	Treatment B BT, VP, stored, displayed	Treatment C VP, stored, BT, displayed	Treatment D PEP, stored, displayed	Treatment E BT, PEP, stored, displayed	Treatment F PEP, stored, BT, displayed	
				Weight loss for strip steaks during display (%)				
1	–	1.5[d]	0.9[d]	1.7[d]	0.4[d]	0.2[d]	1.6[d]	C F A B D E
2	–	1.7[d]	1.6[d]	1.7[d]	0.4[d]	2.9[d]	2.0[d]	E F A C B D
3	–	2.4[d]	2.3[d]	2.0[d]	0.4[d]	0.1[d]	1.9[d]	A B C F D E
				Weight loss for inside round steaks during display (%)				
1	–	2.1[d]	1.1[d]	1.9[d]	1.5[d]	1.6[d]	1.8[de]	A C F E D B
2	–	3.7[d]	1.1[d]	2.4[d]	2.0[d]	1.5[d]	2.7[d]	A F C D E B
3	–	1.9[d]	1.4[d]	0.8[d]	1.2[d]	1.0[d]	1.1[e]	A B D F E C
				Weight loss for strip steaks during cooking (%)				
1	28.2[d]	24.7[d]	25.0[d]	26.0[d]	27.4[d]	25.8[d]	27.9[d]	O F D C E B A
2	30.3[d]	25.7[d]	26.8[d]	27.3[d]	26.5[d]	28.6[d]	27.8[d]	O E F C B D A
3	29.7[d]	25.0[d]	26.8[d]	27.0[d]	30.7[d]	27.0[d]	26.0[d]	D O C E B F A
				Weight loss for inside round steaks during cooking (%)				
1	35.1[d]	29.0[d]	30.7[d]	32.5[d]	30.2[d]	31.5[d]	32.7[d]	O F C E B D A
2	37.2[d]	30.5[d]	30.7[d]	31.1[d]	31.7[d]	30.6[d]	33.8[d]	O F D C B E A
3	35.4[d]	31.2[d]	30.7[d]	32.6[d]	31.9[d]	30.1[d]	34.7[d]	O F C D A B E

[a] 1 = Heavy-Choice, 2 = Heavy-Good and 3 = Light-Good.

[b] There was a significant first-order interaction between weight-grade of beef and treatment for weight loss during display for strip steaks. BT = blade tenderized, VP = vacuum packaged, PEP = packaged in polyethylene bags, stored = 7 or 14 days storage as wholesale cuts, and displayed = 4 days display of steaks in a retail case.

[c] Means in the same row, underscored by a common line, do not differ ($P > 0.05$)

[d,e] Means in the same column, for a given cut and for each trait, bearing a common superscript letter are not different ($P > 0.05$).

Table 5—Mean ratings for overall appearance and surface discoloration of strip loin steaks

Weight and grade of beef[a]	Day of retail display	Packaging treatment[b]: Vacuum packaged cuts stored 14 days — Treatment A: VP, stored, displayed	Treatment B: BT, VP, stored, displayed	Treatment C: VP, stored, BT, displayed	Polyethylene packaged cuts stored 7 days — Treatment D: PEP, stored, displayed	Treatment E: BT, PEP, stored, displayed	Treatment F: PEP, stored, BT, displayed	Rank order (highest to lowest) across treatment[c]
				Overall appearance rating[d]				
1	1	7.7[f]	7.5[f]	7.4[f]	6.5[f]	6.0[f]	6.4[f]	A B C D F E
2	1	7.6[f]	7.5[f]	7.5[f]	5.9[f]	5.2[fg]	5.7[g]	A B C D F E
3	1	7.1[g]	7.1[f]	7.0[g]	4.5[g]	4.3[g]	4.7[h]	A B C F D E
1	4	6.8[f]	6.6[f]	6.1[f]	6.1[f]	5.0[f]	6.1[f]	A B C D F E
2	4	6.8[f]	5.6[f]	6.5[f]	5.3[f]	4.9[f]	5.1[g]	A C B D F E
3	4	5.6[g]	5.4[f]	5.9[f]	3.2[g]	3.5[g]	3.6[h]	C A B F E D
				Surface discoloration rating[e]				
1	1	7.0[f]	7.0[f]	6.9[f]	6.3[f]	6.0[f]	6.2[f]	A B C D F E
2	1	7.0[f]	6.9[f]	6.9[f]	6.0[f]	5.7[f]	6.1[f]	A B C F D E
3	1	6.9[f]	6.9[f]	6.8[f]	5.2[g]	5.3[f]	5.5[g]	A B C F E D
1	4	6.4[f]	6.2[f]	6.0[f]	6.1[f]	5.8[f]	6.1[f]	B A D F C E
2	4	6.4[f]	5.6[f]	6.2[f]	5.8[f]	5.6[f]	5.6[f]	A C D B E F
3	4	5.6[g]	5.4[f]	6.0[f]	3.9[g]	4.4[g]	4.7[g]	C A B F E D

[a] 1 = Heavy-Choice, 2 = Heavy-Good and 3 = Light-Good.
[b] There were significant first-order interactions between weight-grade of beef and treatment for overall appearance (day 1 and 4) and surface discolration (day 1 and 4). BT = blade tenderized, VP = vacuum packaged, PEP = packaged in polyethylene bags, stored = 7 or 14 days storage as wholesale cuts, and displayed = 4 days display of steaks in a retail case.
[c] Means in the same row, underscored by a common line, do not differ ($P > 0.05$).
[d] Means based on an 8-point rating scale (8 = extremely desirable; 1 = extremely undesirable).
[e] Means based on a 7-point rating scale (7 = no surface discoloration; 1 = complete surface discoloration).
[f,g,h] Means in the same column, for the same day of retail display and for each trait, bearing a common superscript letter are not different ($P > 0.05$).

Table 6—Mean ratings for overall appearance and surface discoloration of inside round steaks

Weight and grade of beef[a]	Day of retail display	Packaging treatment[b]: Vacuum packaged cuts stored 14 days — Treatment A: VP, stored, displayed	Treatment B: BT, VP, stored, displayed	Treatment C: VP, stored, BT, displayed	Polyethylene packaged cuts stored 7 days — Treatment D: PEP, stored, displayed	Treatment E: BT, PEP, stored, displayed	Treatment F: PEP, stored, BT, displayed	Rank order (highest to lowest) across treatments[c]
				Overall appearance ratings[d]				
1	1	7.1[f]	6.5[f]	7.0[f]	5.2[f]	4.7[f]	5.3[f]	A C B F D E
2	1	6.8[f]	6.7[f]	6.7[f]	5.0[f]	5.0[f]	5.0[f]	A B C D E F
3	1	6.6[f]	6.4[f]	6.7[f]	4.3[f]	3.9[g]	4.4[f]	C A B F D E
1	4	5.9[f]	4.6[f]	6.0[f]	4.5[f]	3.8[f]	4.8[f]	C A F B D E
2	4	4.9[f]	4.9[f]	4.8[g]	4.7[f]	3.9[f]	4.7[f]	A B C D F E
3	4	4.8[f]	4.0[f]	4.7[g]	3.9[f]	2.7[f]	3.8[f]	A C B D F E
				Surface discoloration rating[e]				
1	1	6.8[f]	6.2[f]	7.0[f]	5.8[f]	5.6[f]	6.2[f]	C A B F D E
2	1	6.5[f]	6.6[f]	6.6[f]	5.9[f]	5.7[f]	5.8[fg]	B C A D F E
3	1	6.4[f]	6.2[f]	6.6[f]	5.3[g]	5.3[f]	5.4[g]	C A B F D E
1	4	5.7[f]	4.7[f]	5.8[f]	5.4[f]	4.8[f]	5.4[f]	C A D F E B
2	4	5.1[f]	5.1[f]	5.1[f]	4.9[f]	4.6[f]	4.9[f]	A B C D F E
3	4	5.2[f]	4.4[f]	5.2[f]	4.7[f]	4.1[f]	4.9[f]	A C F D B E

[a] 1 = Heavy-Choice, 2 = Heavy-Good and 3 = Light-Good.
[b] BT = blade tenderized, VP = vacuum packaged, PEP = packaged in polyethylene bags, stored = 7 or 14 days storage as wholesale cuts, and displayed = 4 days display of steaks in a retail case.
[c] Means in the same row, underscored by a common line, do not differ ($P > 0.05$).
[d] Means based on an 8-point rating scale (8 = extremely desirable; 1 = extremely undesirable).
[e] Means based on a 7-point rating scale (7 = no surface discoloration; 1 = complete surface discoloration).
[f,g] Means in the same column, for the same day of retail display and for each trait, bearing a common superscript letter are not different ($P > 0.05$).

Table 7—Expected retail caselife for strip loin steaks and inside round steaks

Treatment		Retail	Expected retail caselife (days)[b]					
			Heavy-Choice		Heavy-Good		Light-Good	
Code	Description[a]	cuts	Overall appearance	Surface discoloration	Overall appearance	Surface discoloration	Overall appearance	Surface discoloration
A	VP, stored, displayed	Strips	4	4	4	4	4	4
		Insides	4	4	4	4	4	4
B	BT, VP, stored, displayed	Strips	4	4	4	4	4	4
		Insides	4	3	4	4	3	3
C	VP, stored, BT, displayed	Strips	4	4	4	4	4	4
		Insides	4	4	4	4	4	4
D	PEP, stored, displayed	Strips	4	4	4	4	1	1
		Insides	4	4	4	3	2	3
E	BT, PEP, stored, displayed	Strips	4	4	4	4	<1	2
		Insides	2	3	3	3	<1	1
F	PEP, stored, BT, displayed	Strips	4	4	4	4	2	2
		Insides	4	4	4	3	2	3

[a] BT = blade tenderized, VP = vacuum packaged, PEP = packaged in polyethylene bags, stored = 7 or 14 days storage as wholesale cuts, and displayed = 4 days display of steaks in a retail case.
[b] Expected retail caselife was defined as the last day (of a 4-day retail display period) at which overall appearance was "slightly desirable" (a score of 4.5 or higher) or at which surface discoloration was less than 50% (a score of 5 or higher).

Table 8—Mean palatability ratings for steaks from boneless strip loins stored 7 or 14 days

Weight and grade of beef[a]	Packaging treatment[b]							
		Vacuum packaged cuts stored 14 days			Polyethylene packaged cuts stored 7 days			
	Treatment O Control	Treatment A VP, stored, displayed	Treatment B BT, VP, stored, displayed	Treatment C VP, stored, BT, displayed	Treatment D PEP, stored, displayed	Treatment E BT, PEP, stored, displayed	Treatment F PEP, stored, BT, displayed	Rank order (highest to lowest) across treatments[c]
				Tenderness rating[d]				
1	4.4[f]	5.2[f]	5.4[f]	5.9[f]	5.3[f]	5.5[f]	5.9[f]	C F E B D A O
2	4.4[f]	5.0[f]	5.6[f]	6.2[f]	5.1[f]	5.9[f]	6.2[f]	C F E B D A O
3	4.6[f]	5.1[f]	5.9[f]	6.2[f]	4.4[f]	5.8[f]	5.7[f]	C B E F A O D
				Juiciness rating[d]				
1	4.9[f]	5.1[f]	5.2[f]	5.2[f]	5.0[f]	4.9[f]	4.9[f]	B C A D O E F
2	4.6[f]	5.3[f]	4.7[f]	5.0[f]	4.9[f]	4.9[f]	4.2[f]	A C D E B O F
3	5.2[f]	5.1[f]	4.5[f]	4.7[f]	5.1[f]	4.8[f]	5.0[f]	O A D F E C B
				Flavor desirability rating[d]				
1	4.6[f]	5.2[f]	5.3[f]	5.3[f]	5.4[f]	5.2[f]	5.4[f]	D F B C A E O
2	4.7[f]	5.0[f]	4.9[f]	5.7[f]	5.0[f]	5.2[f]	5.5[f]	C F E A D B O
3	5.0[f]	5.2[f]	5.3[f]	5.2[f]	5.3[f]	5.0[f]	5.1[f]	B D A C F O E
				Mealiness rating[e]				
1	4.9[f]	4.8[f]	4.3[f]	4.4[f]	5.2[f]	4.7[f]	4.5[f]	D O A E F C B
2	4.5[f]	5.0[f]	4.0[f]	4.5[f]	4.6[f]	4.1[f]	4.3[f]	A D O C F E B
3	5.0[f]	4.6[f]	4.4[f]	4.2[f]	5.6[f]	4.5[f]	4.4[f]	D O A E B F C
				Overall palatability rating[d]				
1	4.3[f]	4.9[f]	5.2[f]	5.3[f]	5.2[f]	5.1[f]	5.0[f]	C B D E F A O
2	4.2[f]	4.7[f]	4.7[f]	5.5[f]	4.8[f]	5.1[f]	5.3[f]	C F E D B A O
3	4.7[g]	4.9[f]	5.2[f]	5.0[f]	4.7[f]	5.0[f]	5.0[f]	B C E F A O D

[a] 1 = Heavy-Choice, 2 = Heavy-Good and 3 = Light-Good.
[b] BT = blade tenderized, VP = vacuum packaged, PEP = packaged in polyethylene bags, stored = 7 or 14 days storage as wholesale cuts, and displayed = 4 days display of steaks in a retail case.
[c] Means in the same row, underscored by a common line, do not differ ($P > 0.05$).
[d] Means for tenderness, juiciness, flavor and overall palatability are based on 8-point rating scales (8 = extremely tender, extremely juicy, extremely flavorful and extremely desirable in palatability, respectively).
[e] Means based on a 7-point rating scale (7 = no mealiness; 1 = extremely mealy).
[f,g] Means in the same column, for each trait, bearing a common superscript letter are not different ($P > 0.05$).

able ($P < 0.05$) in overall appearance than cuts from Light-Good carcasses in 9 of 12 (Table 5) and 2 of 12 (Table 6) comparisons, respectively. Steaks from Heavy-Choice carcasses were not different ($P > 0.05$) from those from Heavy-Good carcasses in surface discoloration or overall appearance in 46 of 48 comparisons (Tables 5 and 6).

For Heavy-Choice and Heavy-Good beef, the retail caselife (Table 7) of strip steaks was 4 days and that of round steaks was 3 days, irrespective of packaging system or time of blade tenderization. Vacuum packaging appears to be necessary (Table 6) for Light-Good beef to maintain reasonable caselife if it is stored for 7–14 days prior to merchandising. Berry et al. (1971) reported that steaks from vacuum packaged ribs had approximately 1 day of additional retail caselife when compared to steaks from ribs protected by polyvinyl chloride film.

Comparisons of untreated and blade tenderized strip loin steaks and inside round steaks (Tables 8 and 9) revealed that blade tenderization did not significantly ($P > 0.05$) affect juiciness (4 of 6 comparisons), flavor desirability (4 of 6 comparisons), mealiness (6 of 6 comparisons) or overall palatability (5 of 6 comparisons). Glover et al. (1975) and Davis et al. (1975) have previously reported that blade tenderization did not significantly influence flavor or juiciness of beef. In the present study, blade tenderization increased ($P < 0.05$) tenderness above that achieved by aging alone in 10 of 12 comparisons of strip loin steaks (Table 8) and in 4 of 12 comparisons of inside round steaks (Table 9).

Steaks from strip loins and inside rounds which were blade tenderized prior to storage were not significantly different ($P > 0.05$) from those from subprimal cuts tenderized after storage, in tenderness (10 of 12 comparisons), juiciness (12 of 12 comparisons), flavor desirability (11 of 12 comparisons), mealiness (11 of 12 comparisons), or overall palatability (12 of 12 comparisons). Comparisons of palatability attributes of strip loin steaks and inside round steaks revealed no major differences among the three kinds of beef utilized in the present study. Hodges et al. (1974) reported that difference in grade was not a significant source of the variation in palatability of steaks from beef that had been aged for 15 days.

SUMMARY & CONCLUSIONS

THE FOLLOWING CONCLUSIONS were drawn from the data of the present study:

(1) Subprimal cuts which were packaged in polyethylene bags generally had higher numerical psychrotrophic counts and had lower overall appearance ratings following storage, but sustained less shrinkage during storage than vacuum packaged

Table 9—Mean palatability ratings for steaks from boneless inside rounds stored 7 or 14 days.

Weight and grade of beef[a]	Packaging treatment[b]							
	Vacuum packaged cuts stored 14 days			Polyethylene packaged cuts stored 7 days				
	Treatment 0	Treatment A	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F	Rank order (highest to lowest) across treatments[c]
	Control	VP, stored, displayed	BT, VP, stored, displayed	VP, stored, BT, displayed	PEP, stored, displayed	BT, PEP, stored, displayed	PEP, stored, BT, displayed	
				Tenderness rating[d]				
1	3.8[f]	4.3[f]	4.3[f]	5.1[f]	4.2[f]	5.0[f]	4.2[f]	C E B A D F O
2	3.8[f]	4.7[fg]	5.1[g]	5.2[f]	3.9[f]	4.8[f]	4.9[fg]	C B F E A D O
3	4.6[g]	5.0[g]	5.2[g]	5.6[f]	4.9[g]	5.0[f]	5.7[g]	F C B A E D O
				Juiciness rating[d]				
1	4.5[f]	5.4[f]	4.4[f]	4.8[f]	4.5[f]	4.9[f]	4.4[f]	A E C O D B F
2	4.0[f]	5.0[f]	4.7[f]	4.7[f]	4.1[f]	4.8[f]	4.3[f]	A E B C F D O
3	4.3[f]	4.8[f]	4.6[f]	4.7[f]	4.8[f]	4.8[f]	4.5[f]	A D E C B F O
				Flavor desirability rating[d]				
1	4.0[f]	4.8[f]	4.9[f]	5.0[f]	5.6[f]	5.0[f]	5.0[f]	D C E F B A O
2	4.4[fg]	5.1[f]	4.9[f]	5.0[f]	4.7[f]	5.1[f]	5.1[f]	A E F C B D O
3	4.7[g]	4.9[f]	5.0[f]	5.0[f]	4.8[f]	5.2[f]	5.5[f]	F E B C A D O
				Mealiness rating[e]				
1	4.6[f]	5.1[f]	4.6[f]	4.0[f]	4.8[f]	3.9[f]	4.5[f]	A D O B F C E
2	4.4[f]	4.7[f]	4.6[f]	4.0[f]	5.4[f]	4.7[f]	4.0[f]	D A E B O C F
3	4.3[f]	4.4[f]	4.1[f]	3.7[f]	4.6[f]	4.5[f]	3.6[f]	D E A O B C F
				Overall palatability rating[d]				
1	3.8[f]	4.4[f]	4.2[f]	4.4[f]	4.4[f]	4.4[f]	4.4[f]	A C D E F B O
2	3.7[f]	4.6[f]	4.6[f]	4.5[f]	3.7[f]	4.7[f]	4.5[f]	E B A C F O D
3	4.3[g]	4.4[f]	4.5[f]	4.6[f]	4.6[f]	4.5[f]	4.9[f]	F C D B E A O

[a] 1 = Heavy-Choice, 2 = Heavy-Good and 3 = Light-Good.
[b] BT = blade tenderized, VP = vacuum packaged, PEP = packaged in polyethylene bags, stored = 7 or 14 days storage as wholesale cuts, and displayed = 4 days display of steaks in a retail case.
[c] Means in the same row, underscored by a common line, do not differ ($P > 0.05$).
[d] Means for tenderness, juiciness, flavor and overall palatability are based on 8-point rating scales (8 = extremely tender, extremely juicy, extremely flavorful and extremely desirable in palatability, respectively).
[e] Means based on a 7-point rating scale (7 = no mealiness; 1 = extremely mealy).
[f,g] Means in the same column, for each trait, bearing a common superscript letter are not different ($P > 0.05$).

cuts. Steaks from polyethylene packaged subprimal cuts sustained greater surface discoloration during retail display and were less desirable in overall appearance than steaks from vacuum packaged cuts, but packaging system did not affect weight loss during retail display, weight loss during cooking, or palatability characteristics.

(2) Blade tenderization prior to, rather than following, storage decreased overall appearance of subprimal inside rounds and increased weight losses of subprimal cuts during storage, but had little effect on weight losses during retail display, weight losses during cooking, surface discoloration and overall appearance of steaks during retail display, or flavor, juiciness, tenderness, mealiness and overall palatability of cooked steaks.

(3) There were very few differences between subprimal cuts and retail cuts from Heavy-Choice vs Heavy-Good carcasses in bacterial counts, surface discoloration, appearance, weight losses, cooking losses, retail caselife or palatability traits. When subprimal cuts from Light-Good carcasses were vacuum packaged they were not different from those of heavy beef in weight losses during storage or overall appearance. Retail cuts from vacuum packaged Light-Good subprimals were not generally different from those from Heavy-Choice or Heavy-Good subprimals in weight losses during retail display, cooking losses, retail caselife or palatability traits. However, subprimal strip loins from Light-Good carcasses which were stored in polyethylene bags were less desirable in overall appearance following storage and produced steaks which sustained greater surface discoloration, had lower overall appearance ratings following retail display and had less retail caselife than strip loins from Heavy-Choice and Heavy-Good carcasses.

(4) Blade tenderization increased tenderness above that achieved by aging alone for strip loin steaks but did not generally affect flavor, juiciness or overall palatability ratings.

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VARIABLES AFFECTING THE ACCEPTABILITY OF RADAPPERTIZED GROUND BEEF PRODUCTS.

Effects of Food Grade Phosphates, NaCl, Fat Level, and Grinding Methods

ABSTRACT

A series of experiments were conducted to determine the effect of different variables on the quality of an irradiated ground beef product. Factors studied included: different food-grade phosphates; NaCl content; fat content; and size of grind. The influence of these variables on the cooking loss (moisture retention), shear press values and sensory scores was studied. The addition of phosphates and NaCl was desirable in controlling cooking losses. The most effective phosphate was tetrasodium pyrophosphate. The addition of NaCl decreased the shear press force required to penetrate the beef patty, i.e., it tenderized the product. Phosphate addition did not affect the shear press force. Increased fat content increased the cooking losses, but did not affect the shear press force. Irradiation with sterilizing doses had a marked effect on decreasing the shear press force.

INTRODUCTION

THE EFFECTS of the addition of NaCl and phosphates have been studied by many investigators (Grau et al., 1153; Hamm, 1960; Wierbicki et al., 1963; Swift and Ellis, 1956; Sherman, 1961). The role of the NaCl and phosphate additions in pH changes, increased moisture retention and meat swelling has been adequately documented.

Shults et al. (1972) and Shults and Wierbicki (1972) showed that the addition of salt and phosphate to ground meat increased the moisture retention and bound the ground meat into a product of desirable texture and appearance.

The results of research reported from taste panel studies that attempted to define the most desirable fat content for ground beef have shown great variability. Several researchers, using consumer taste panels, found that the ground beef products containing 15–20% fat were more acceptable than products higher (25–35%) in fat content (Glover, 1964; Law et al., 1965; Carpenter and King, 1969; Mize, 1972). Conversely, Cole et al. (1960) reported that both laboratory and family taste panels rated ground beef containing 15% fat to be less palatable than ground beef containing 25, 35 or 45% fat.

Kendall et al. (1974) reported that ground beef containing 10–20% fat had less cooking loss than ground beef that contained 25–30% fat. Also ground beef with 9–12% fat tended to be less juicy, more mealy in texture, and less desirable in flavor than products with a higher fat content.

The investigations in this study were conducted to determine the factors affecting the quality of irradiated ground beef patties. Development of an irradiated product was dependent upon the effects of NaCl, phosphate addition and fat level.

MATERIALS & METHODS

Raw materials

The raw material used in these studies was USDA Choice grade beef rounds, consisting primarily of the semimembranosus, semitendinosus and biceps femorus muscles.

The phosphates studied were sodium tripolyphosphate (TPP), $Na_5P_3O_{10}$; tetrasodium pyrophosphate (PP), $Na_4P_2O_7$; hexametaphosphate (HMP), $(NaPO_3)_N$; Foodfos, a commercial preparation of HMP; Curafos 11-2 and Curafos 22-4, commercial combinations of HMP and TPP; and Kena, commercial combination of PP, TPP, and sodium acid pyrophosphate. These phosphates were obtained from the Merck Chemical Co., Pittsburgh, Pa.

Processing

The beef rounds were trimmed to approx 5% fat and ground through either a 13 or 16 mm grinding plate with added fat to approximate the desired fat levels. The ground meat was then mixed with the additives and 3% crushed ice in a mixer (Hobart Model No. H-600=D) for 3 min at moderate speed. The meat was then formed into patties of 100 ± 1g wt, 13 ± 1 mm thickness for enzyme inactivation.

The patties were placed in flexible laminated pouches, unsealed, and chilled to 4 ± 1°C. Enzyme inactivation was achieved by placing the pouches in a controlled water bath at 82 ± 2°C for 7 minutes. The internal temperature of the meat was 70 ± 2°C.

Packaging

After the enzyme inactivation, the patties were removed from the pouches and the cooking loss was measured on 18 patties by weighing the drained moisture. The patties were then placed into new flexible pouches. These pouches were composed of medium density polyethylene as the food contactant layer, aluminum foil and a Mylar exterior layer and sealed at a pressure of 3 ± 1 kPa. The sealed pouches were then frozen to −40 ± 2°C and held at that temperature until irradiated.

Irradiation processing

Irradiation was performed with a Cobalt^{60} (gamma) source at a dose rate of 14 J$(kg^{-1})(sec^{-1})$. Irradiation conditions were: dose of 3.7 – 4.3 × 10^4 J/kg; irradiation temperature of −30 + 10°C. After irradiation, the samples were thawed and held at 21 ± 2°C until evaluation. The nonirradiated sample controls were held at −20 ± 2°C.

Sensory evaluation

The samples were evaluated by technological panels (12 members per panel) for the sensory characteristics of odor, flavor, color, texture and appearance using the defined intensity scale of Table 4.

Texture evaluation

Shear press analyses were performed on all samples within 3 days after irradiation using a Kramer Shear Press. This method is described by Cohen and Rice (1974), with the following modification: A single blade shear cell was used with the 136 kg ring and the "300" (100%) scale. The beef patty was cut so that the blade penetrated a cross section 13 ± 1 mm deep by 24 ± 1 mm wide. Sixteen replications were performed on each sample and results are reported in Newtons. Samples were analyzed at a temperature of + 4 + 1°C.

Statistical analysis

Statistical analyses of the data were performed using analysis of variance and least significant difference (lsd) methods (Steel and Torrie, 1960).

Chemical analysis

Proximate and other analyses were done in duplicate on the same sample using standard AOAC (1970) methods.

Table 1—Effect of food grade phosphates on cooking loss and Kramer shear press values

Phosphate 0.375% addition	% Cooking loss		Kramer shear press (Newtons)				Statistical analyses
			Irradiated		Nonirradiated		
	Salt level		Salt level				
	0	0.75%	0	0.75%	0	0.75%	
							Cooking loss
Tetrasodium pyrophosphate (PP)	20.7	9.5	59.8	48.6	53.2	48.2	All samples: F = 46 (1% sign.) lsd (0.05) = 1.6; lsd (0.01) = 2.4
Sodium tripolyphosphate (TPP)	21.3	10.5	47.5	45.9	59.4	61.9	Samples with no salt: F = 11.1 (1% sign.) lsd (0.05) = 2.3; lsd (0.01) = 3.1
Curafos 11-2[a]	23.5	14.0	51.2	48.6	54.8	52.2	Samples with salt: F = 44 (1% sign.) lsd (0.05) = 2.0; lsd (0.01) = 2.8
							Shear Press: (Nonirradiated)
Curafos 22-4[a]	20.3	13.3	51.9	52.8	62.6	56.8	All samples: F = 2.2 (1% sign.) lsd (0.05) = 1.2; lsd (0.01) = 1.6
Kena[b]	23.1	13.9	58.0	51.5	59.2	48.6	Samples with no salt: F = 2.9 (1% sign.) lsd (0.05) = 4.7; lsd (0.01) = 6.4
Hexametaphosphate (HMP)	19.4	17.2	45.9	42.3	71.5	73.2	Samples with salt: F = 4.1 (1% sign.) lsd (0.05) = 13.8; lsd (0.01) = 18.7
							Shear press: (Irradiated)
Foodfos[c]	25.0	20.9	55.0	46.2	68.7	60.0	All samples: F = 7.6 (1% sign.) lsd (0.05) = 8.5; lsd (0.01) = 11.5
Control (no phosphate)	24.7	18.3	74.8	49.8	71.2	58.4	Samples with no salt: F = 1.6 NSD Samples with salt: F = 0.9 NSD

[a] Commercial blends of TPP and HMP
[b] Commercial blend of PP, TPP and sodium pyrophosphates
[c] Commerical preparation of HMP

RESULTS & DISCUSSION

Effect of different food grade phosphates

Table 1 lists the different food-grade phosphates evaluated at 0.375% addition level. The effects on the percent cooking loss and shear press values of irradiated and nonirradiated beef patties when used with and without 0.75% NaCl are shown.

The most effective phosphate for moisture retention, particularly in the presence of NaCl, was PP, followed by TPP. Both PP and TPP were significantly more effective than the other phosphates. Cooking losses were reduced from 24% with no additives to 9.5% with 0.75% NaCl and 0.375% PP. The use of PP without NaCl resulted in only a 4% reduction in cooking loss. The use of TPP with 0.75% NaCl gave similar results as

Table 2—Chemical analyses for samples listed in Table 1[a]

% NaCl added	% Phosphate[b] added	% NaCl analyzed	% Phosphate[c] analyzed	Type of phosphate
0	0.375	0.23	0.210	PP (Tetrasodium pyrophosphate)
0.75	0.375	0.85	0.243	PP (Tetrasodium pyrophosphate)
0	0.375	0.25	0.309	TPP (Sodium tripolyphosphate)
0.75	0.375	0.71	0.321	TPP (Sodium tripolyphosphate)
0	0.375	0.37	0.285	Curafos 11-2
0.75	0.375	0.80	0.311	Curafos 11-2
0	0.375	0.13	0.318	Curafos 22-4
0.75	0.375	0.82	0.302	Curafos 22-4
0	0.375	0.41	0.303	Kena
0.75	0.375	0.68	0.309	Kena
0	0.375	0.23	0.317	HMP (Hexametaphosphate)
0.75	0.375	0.81	0.332	HMP (Hexametaphosphate)
0	0.375	0.23	0.341	Foodfos
0.75	0.375	0.76	0.338	Foodfos
0	0	0.22	0.200	none
0.75	0	0.73	0.254	none

[a] Proximate analysis, raw meat without the additives: % H_2O = 71.54; % Protein = 21.01; % Fat = 5.06; % Ash = 1.05
[b] As sodium salts
[c] As elemental P

Table 3—Effect of salt level, phosphate level and grind on cooking loss and Kramer shear press values[a]

% TPP	% PP	Grind	% Cooking loss			Kramer shear press (Newtons)					
						Irradiated			Nonirradiated		
			Salt level			Salt level			Salt level		
			0	0.5	1.0	0	0.5	1.0	0	0.5	1.0
0	0	6 mm	24.4	22.3	21.1	25.3	26.5	35.9	32.3	38.2	40.5
0.25	0	6 mm	21.8	23.1	16.7	32.1	35.3	31.6	40.0	33.1	37.7
0.50	0	6 mm	16.5	13.3	4.8	36.7	33.4	33.1	34.9	43.5	36.9
0	0.25	6 mm	19.1	18.7	6.8	25.5	33.4	33.0	37.9	38.8	36.3
0	0.50	6 mm	14.6	10.3	2.6	29.3	33.5	30.1	33.9	36.8	41.4
0	0	13 mm	25.8	27.0	22.6	37.4	37.7	33.6	48.1	58.5	53.9
0.25	0	13 mm	16.6	17.4	13.1	32.6	30.7	37.1	50.0	48.9	38.6
0.50	0	13 mm	16.9	12.3	11.1	36.3	37.5	36.0	36.6	40.8	40.5
0	0.25	13 mm	16.3	14.4	7.8	39.9	30.1	30.2	39.6	41.1	41.9
0	0.50	13 mm	15.5	9.5	1.3	40.2	38.9	35.4	41.8	35.7	37.8

[a] Statistical analyses
Cooking loss: For 6 mm grind: F = 105 (1% sign.); lsd (0.05) = 2.0; lsd (0.01) = 2.7; For 13 mm grind: F = 124 (1% sign.); lsd (0.05) = 1.9; lsd (0.01) = 2.5.
Kramer shear press: For 6 mm grind, irradiated: F = 4.0 (1% sign.); lsd (0.05) = 5.7; lsd (0.01) = 7.7; For 6 mm grind, unirradiated F = 1.7 (5% sign.); lsd (0.05) = 7.5; lsd (0.01) = 10.2; For 13 mm grind, irradiated: F = 2.2 (1% sign.); lsd (0.05) = 10.1; lsd (0.01) = 13.6; For 13 mm grind, unirradiated: F = 7.1 (1% sign.); lsd (0.05) = 8.0; lsd (0.01) = 10.8.

PP. These results demonstrate the synergistic effects of phosphate and NaCl on water retention as reported by Mahon (1961).

In this experiment, and all other experiments in this study, irradiation of samples decreased the shear press values when compared to the corresponding nonirradiated samples. This was also demonstrated by Cohen and Rice (1974).

The addition of NaCl decreased the cooking losses of all the samples. It also decreased the shear press values of most of the samples.

Sensory tests were not done with this experiment. Earlier work by Shults (1971) on other beef products, demonstrated no sensory differences in the use of these phosphates.

Table 2 lists the NaCl and phosphate content and the proximate analysis for the samples in this experiment.

Despite evidence shown in the literature (Shults et al., 1972; Neraal and Hamm, 1973; Yasui et al., 1964), and that demonstrated in this experiment, that PP is the preferred phosphate to use, it was not used in all experiments as TPP and HMP are the only food-grade phosphates currently approved

Table 4—Effect of salt level, phosphate level and grind on sensory scores[a,b]

% TPP	% PP	% Salt	Grind	Color	Odor	Flavor	Texture	Appearance
0	0	0.5	6 mm	6.9 ± 0.8	5.5 ± 1.5	5.3 ± 2.0	6.3 ± 1.5	6.3 ± 2.1
0.25	0	0.5	6 mm	6.6 ± 0.7	5.8 ± 2.0	6.1 ± 1.2	6.8 ± 1.5	6.8 ± 1.3
0.50	0	0.5	6 mm	6.8 ± 0.6	6.5 ± 0.8	5.9 ± 1.8	6.8 ± 0.7	6.8 ± 0.8
0	0.25	0.5	6 mm	6.8 ± 0.7	6.5 ± 1.3	6.3 ± 1.2	6.8 ± 0.8	7.1 ± 1.1
0	0.50	0.5	6 mm	6.8 ± 1.0	6.0 ± 1.7	6.1 ± 1.6	6.7 ± 1.4	6.8 ± 0.8
0	0	1.0	6 mm	6.7 ± 1.0	6.2 ± 0.8	5.8 ± 1.5	6.1 ± 1.8	6.8 ± 0.9
0.25	0	1.0	6 mm	6.1 ± 1.6	5.7 ± 1.4	5.6 ± 1.4	6.1 ± 2.0	6.1 ± 1.7
0.50	0	1.0	6 mm	6.1 ± 1.9	6.2 ± 2.0	6.2 ± 2.0	6.1 ± 1.7	5.8 ± 2.0
0	0.25	1.0	6 mm	6.2 ± 1.5	6.3 ± 1.1	5.8 ± 1.5	5.3 ± 1.4	5.9 ± 1.7
0	0.50	1.0	6 mm	6.3 ± 1.3	6.2 ± 1.3	6.2 ± 1.9	6.2 ± 1.8	6.1 ± 1.6
0	0	0.5	13 mm	6.3 ± 1.2	6.1 ± 1.2	6.2 ± 1.6	6.2 ± 1.2	6.3 ± 1.3
0.25	0	0.5	13 mm	6.4 ± 1.0	6.3 ± 1.2	5.9 ± 1.6	6.6 ± 0.8	6.6 ± 0.9
0.50	0	0.5	13 mm	5.9 ± 1.2	5.8 ± 1.6	5.3 ± 1.3	6.1 ± 1.3	6.5 ± 0.8
0	0.25	0.5	13 mm	6.0 ± 1.0	5.7 ± 1.0	5.7 ± 2.0	6.3 ± 1.1	6.1 ± 1.0
0	0.50	0.5	13 mm	6.2 ± 1.2	6.3 ± 1.2	6.5 ± 1.0	6.6 ± 0.7	6.2 ± 0.5
0	0	1.0	13 mm	5.9 ± 1.0	5.3 ± 1.4	5.1 ± 1.3	5.6 ± 1.4	5.9 ± 1.2
0.25	0	1.0	13 mm	5.8 ± 1.4	5.6 ± 1.3	5.5 ± 1.3	5.7 ± 1.9	5.7 ± 1.6
0.50	0	1.0	13 mm	5.6 ± 0.9	5.6 ± 1.2	5.3 ± 1.5	5.9 ± 0.9	5.8 ± 1.3
0	0.25	1.0	13 mm	6.0 ± 0.8	5.9 ± 1.0	5.3 ± 1.7	6.2 ± 0.8	6.2 ± 1.3
0	0.50	1.0	13 mm	5.8 ± 1.3	5.8 ± 1.1	5.5 ± 1.6	5.5 ± 1.9	6.0 ± 1.3

[a] Intensity scale points: 1—extremely poor; 2—very poor; 3—poor; 4—below fair, above poor; 5—fair; 6—below good, above fair; 7—good; 8—very good; 9—excellent.
[b] Statistical analysis: No significant differences on any test. Samples grouped together were tested at the same time. All samples irradiated.

Table 5—Chemical analyses for samples listed on Table 3 and 4[a]

% NaCl added	% TPP[b] added	% PP[b] added	% NaCl analyzed	% Phosphorous[c] analyzed
0	0	0	0.12	0.161
0.5	0	0	0.60	0.163
1.0	0	0	1.00	0.160
0	0.25	0	0.13	0.224
0.5	0.25	0	0.57	0.229
1.0	0.25	0	1.05	0.235
0	0.50	0	0.15	0.295
0.5	0.50	0	0.58	0.299
1.0	0.50	0	0.54	0.279
0	0	0.25	0.08	0.225
0.5	0	0.25	0.57	0.227
1.0	0	0.25	0.99	0.228
0	0	0.50	0.11	0.277
0.5	0	0.50	0.53	0.291
1.0	0	0.50	1.03	0.282

[a] Proximate analysis, cooked meat, without additives: % H_2O = 60.93; % Protein = 17.08; % F = 20.11; % Ash = 0.84.
[b] As sodium salts
[c] As elemental P

Table 6—Chemical analyses for samples listed Tables 3 and 4[a]

% NaCl added	% TPP[b] added	% PP[b] added	% NaCl analyzed	% Phosphorous[c] analyzed
0	0	0	0.02	0.178
0.5	0	0	0.45	0.201
1.0	0	0	0.54	0.256
0	0.25	0	0.03	0.263
0.5	0.25	0	1.16	0.234
1.0	0.25	0	1.05	0.256
0	0.50	0	0.13	0.327
0.5	0.50	0	0.61	0.385
1.0	0.50	0	1.06	0.302
0	0	0.25	0.03	0.257
0.5	0	0.25	0.58	0.261
1.0	0	0.25	1.09	0.241
0	0	0.50	0.08	0.311
0.5	0	0.50	0.50	0.319
1.0	0	0.50	0.94	0.305

[a] Proximate analysis, raw meat without the additives: % H_2O = 66.21; % Protein = 18.38; % Fat = 13.40; % Ash = 0.95.
[b] As sodium salts
[c] As elemental P

by the USDA for beef products (Meat Inspection Regulations, Title 9, Chapter 2, USDA, APHIS). TPP has been shown to be the only approved phosphate which is beneficial in ground beef (Shults et al., 1972). Neraal and Hamm (1973) reported that TPP was rapidly hydrolyzed in meats to PP and orthophosphate and it was the PP moiety of TPP which was mainly responsible for the increase in the water-holding capacity of meats.

Effect of salt levels, phosphate levels and grind

The effects on shear press values and cooking losses of three levels of TPP and PP (0, 0.25 and 0.50%), three levels of NaCl (0, 0.50 and 1.0%) and two particle sizes of grind (6 and 13 mm) were studied using irradiated and nonirradiated samples (Table 3).

The addition of TPP to the ground beef patties resulted in a significantly higher cooking loss than with the addition of PP. With PP addition, in combination with 1% NaCl addition, cooking losses were reduced from 21% to 7%, with 0.25% PP, to 2.6% with 0.5% PP when a 6 mm grind was used. Similar results were obtained with PP additions with 13 mm grind. Increasing the addition of TPP and PP from 0.25 to 0.5% resulted in signficant decreases in the cooking losses when used with 0.5 or 1.0% NaCl.

Increasing NaCl content also decreased the cooking losses. Significant reductions were found when used with phosphate additons.

The size of the grind had no significant effects on the cooking losses. The 13 mm grind had a tendency to give greater shear press values than the 6 mm grind, indicating a tougher product. There was no discernible trend for the shear press value as a function of the additive or the additive level used.

Table 4 shows sensory scores for this test. In this, and with all other sensory tests, no significant differences appeared for any samples tested at the same time. It appears that the use of NaCl alone (0.5–1.0%) results in a product of acceptable quality. However, the use of NaCl with phosphates improves the product's water retention qualitites, Table 3, thus making

Table 7—Effect of salt, phosphate and fat content on cooking loss and Kramer shear press values[a]

% Salt	% TPP	Fat	% Cooking loss	Kramer shear value, (Newtons)		
				Irradiated	Nonirradiated	Grind
0	0	25.8	30.3	51.4	46.5	double, 5 mm
0.75	0	25.8	35.4	23.9	24.8	double, 5 mm
0	0.3	25.8	34.2	35.3	47.9	double, 5 mm
0.75	0.3	25.8	29.5	31.8	41.0	double, 5 mm
0	0	7.8	28.4	43.6	55.9	double, 5 mm
0.75	0	7.8	29.8	36.4	61.6	double, 5 mm
0	0.3	7.8	30.4	40.3	60.9	double, 5 mm
0.75	0.3	7.8	24.8	25.8	49.0	double, 5 mm

[a] Statistical analyses:
Kramer shear values: Irradiated: F = 7.9 (1% sign.); lsd (0.05) = 12.4; lsd (0.01) = 11.1. Nonirradiated: F = 5.3 (1% sign.); lsd (0.05) = 11.5; lsd (0.01) = 10.2.
% Cooking loss: F = 11.1 (% sign.); lsd (0.05) = 3.6; lsd (0.01) = 4.8.

Table 8—Effect of fat, salt and TPP levels on sensory scores[a,b]

% Fat	% NaCl	% TPP	Color	Odor	Flavor	Texture	Appearance	Irradiated
25.9	0	0	6.8 ± 1.1	6.4 ± 0.9	5.4 ± 1.3	6.2 ± 1.3	6.8 ± 0.9	yes
25.9	0.75	0	6.8 ± 1.0	6.3 ± 1.1	6.8 ± 0.8	6.8 ± 1.1	7.1 ± 0.5	yes
25.9	0	0.3	6.7 ± 0.8	6.3 ± 0.5	6.1 ± 1.2	6.4 ± 0.8	6.7 ± 0.7	yes
25.9	0.75	0.3	6.2 ± 1.2	6.3 ± 0.9	5.8 ± 1.5	6.3 ± 1.5	6.8 ± 0.6	yes
7.8	0	0	6.5 ± 1.2	6.1 ± 1.2	4.5 ± 1.9	6.1 ± 1.3	6.6 ± 1.2	yes
7.8	0.75	0	6.3 ± 1.4	6.0 ± 1.6	5.1 ± 1.9	6.4 ± 1.2	6.4 ± 1.1	yes
7.8	0	0.3	6.3 ± 0.9	6.0 ± 0.9	5.6 ± 1.4	6.1 ± 1.2	6.1 ± 1.2	yes
7.8	0.75	0.3	6.7 ± 1.1	6.2 ± 1.0	5.5 ± 1.4	6.2 ± 1.1	6.4 ± 0.9	yes
25.8	0	0	6.7 ± 0.5	6.8 ± 0.9	6.1 ± 1.4	6.3 ± 1.1	6.7 ± 1.0	no
25.8	0.75	0	7.1 ± 0.8	6.9 ± 0.5	7.2 ± 1.1	7.0 ± 0.9	7.3 ± 0.9	no
25.8	0	0.3	7.0 ± 0.7	6.9 ± 0.9	6.4 ± 1.4	6.6 ± 1.2	6.8 ± 0.8	no
25.8	0.75	0.3	7.3 ± 0.5	7.1 ± 0.5	6.8 ± 0.8	7.0 ± 0.6	7.3 ± 0.7	no
7.8	0	0	6.7 ± 0.8	6.7 ± 0.8	6.3 ± 1.0	6.2 ± 1.3	6.6 ± 0.8	no
7.8	0.75	0	6.6 ± 0.9	6.8 ± 1.2	6.5 ± 1.7	6.9 ± 0.9	6.6 ± 1.1	no
7.8	0	0.3	6.8 ± 0.8	6.8 ± 0.8	6.1 ± 1.0	6.6 ± 0.9	6.8 ± 0.8	no
7.8	0.75	0.3	7.1 ± 0.5	6.8 ± 1.1	6.1 ± 2.1	7.1 ± 0.5	7.3 ± 0.8	no

[a] Samples grouped together were tested at the same time.
[b] Statistical analysis: No significant differences for any test. All samples double ground, 5 mm plate.

Table 9—Chemical analyses for samples listed Table 7 and 8

Sample	% H_2O	% Protein	% Fat	% Ash	% Phos.	% NaCl	Cooked
1	57.26	15.99	25.52	0.85	0.154	0.20	no
2	57.26	15.99	25.52	1.38	0.161	0.85	no
3	57.26	15.99	25.52	1.11	0.245	0.12	no
4	57.26	15.99	25.52	1.73	0.243	0.78	no
5	70.78	19.91	6.84	1.01	0.204	0.09	no
6	70.78	19.91	6.84	1.65	0.194	0.89	no
7	70.78	19.91	6.84	1.34	0.267	0.05	no
8	70.78	19.91	6.84	1.82	0.258	0.85	no
1	51.76	20.47	25.80	0.90	0.174	0.17	yes
2	51.76	20.47	25.80	1.42	0.167	0.89	yes
3	51.76	20.47	25.80	1.22	0.253	0.16	yes
4	51.76	20.47	25.80	1.86	0.243	0.87	yes
5	63.64	25.32	7.79	1.04	0.196	0.07	yes
6	63.64	25.32	7.79	1.62	0.204	0.82	yes
7	63.64	25.32	7.79	1.28	0.275	0.09	yes
8	63.64	25.32	7.79	1.85	0.273	0.88	yes

it more juicy if increased juiciness is synonymous with water-holding capacity. In previous experiments, it was shown that the beef patties made without NaCl, or without NaCl and phosphate, tended to fall apart after irradiation and were judged inferior in quality due to the poor textural characteristic.

Table 5 lists the NaCl and phosphate content and the proximate analysis of the cooked samples listed on Tables 3 and 4. Table 6 lists the NaCl and phosphate content and the proximate analyses of the raw meat samples listed on Tables 3 and 4.

Effect of NaCl, phosphate and fat content

Two levels of TPP (0 and 0.3%) and two levels of NaCl (0 and 0.75%) were used to test the effects on shear press values and cooking loss of fat levels (25.8 and 7.8%) in irradiated and nonirradiated beef patties. Table 7 lists the levels of NaCl, phosphate and fat used in this experiment and their effect on the cooking loss and shear press values. Fat, NaCl, and phosphate showed no differences for cooking losses. Salt decreased the shear press values; increased fat did the same for the nonirradiated product but not for the irradiated product; phosphate had no effect on these values.

Table 8 shows the sensory scores for this experiment. Again, no differences were demonstrated for the samples tested.

Table 9 lists the NaCl and phosphate content and the proximate analysis for the samples shown in Table 7.

Results of these studies have demonstrated that acceptable irradiated ground beef patties can be produced. It can be concluded that fat level and degree of grind does not affect product quality. The use of NaCl and phosphates is beneficial in reducing cooking losses and in textural improvements.

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The use of trade names does not represent an endorsement of the product by the Department of Defense to the exclusion of others not mentioned.

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EFFECT OF CHEMICAL AND PHYSICAL TREATMENTS ON RANCIDITY DEVELOPMENT OF FROZEN MULLET (Mugil cephalus) FILLETS

ABSTRACT

Approximately 30 million pounds of mullet are harvested annually in Florida. However, mullet has only limited economic value compared to other commercially valuable fish species which in part is due to the poor stability of the flesh during storage. The instability of mullet flesh during storage has been attributed mainly to the development of oxidative rancidity. The purpose of this study was to retard rancidity development in the stored mullet fillets by chemical and physical treatments. The mullet fillets were dipped for 1 min in the following antioxidant solution, mono-tertiarybutylhydroquinone (TBHQ), disodium ethylenediaminetetraacetate (Na_2 EDTA) and ascorbic acid, singularly or in various combinations. Each antioxidant and their combinations retarded rancidity development in the stored mullet fillets. The chemcial tests (peroxide value and TBA number) indicated that ascorbic acid alone or in combination with TBHQ or Na_2 EDTA was more effective than other antioxidant treatments. Vacuum packaging in combination with antioxidants improved rancidity control over antioxidant treatments alone. Based on the chemical measurements rancidity development is retarded best by treatment with the antioxidants ascorbic acid and/or TBHQ in combination with vacuum-packaging.

INTRODUCTION

APPROXIMATELY 30 million pounds of mullet (*Mugil cephalus*) are harvested annually, accounting for more than one-fifth of the finfish landed on Florida coasts (USDC, 1973; Pierce, 1975). However, mullet has only limited economic value compared to other commercially valuable fish species which, in part, is due to poor stability of the flesh during storage. Processing techniques such as pickling, smoking, salting and drying generally are very effective for preserving marine products, but consumers demand convenience type products, with the characteristics of fresh marine foods (Weinstein, 1969). Freezing technology has provided consumers with products that have a reasonably good acceptance. However, frozen mullet fillets have been of low quality and rancid flavor has been considered the major factor responsible for the quality loss (Saenz and Dubrow, 1959; Beaumariage et al., 1969). The quick onset of oxidative rancidity of mullet flesh lipid during storage is believed to be due to the heme pigment present in the large lateral band of the fillets. The pigment acts as a catalyst for the oxidation of the highly unsaturated fatty acids in the flesh (Zipser and Watts, 1961; Fischer and Deng, 1976). Studies to control oxidative rancidity in frozen mullet fillets have been reported by previous workers (Saenz and Dubrow, 1959; Beaumariage et al., 1969). None of the various treatments reported could extend the shelf life of mullet fillets harvested in October beyond 5 months of frozen storage. The treatments were more effective on the relatively lean mullet harvested in April, during the nonspawning season. However, the major harvesting period for mullet is during the spawning season, the other seasons account for only a small portion of landings (USDC, 1973).

Thus, the experiment reported here was designed to investigate the effect of a combination of vacuum packaging and antioxidants on extending the storage stability of mullet harvested during the spawning season (December). Most mullet harvested during peak period (spawning season) are frozen before shipping or further processing (Cato, 1976). Thus, mullet in the round frozen at −29°C for one month were used as the raw material.

EXPERIMENTAL

FRESH CAUGHT MULLET were obtained from commercial fishermen, shipped in ice to Gainesville, Fla., immediately frozen and stored at −29°C until used. The thawed mullet were freed of bone, viscera and belly flaps. Unless otherwise specified, whole boneless fillets with skin were used in the study. The average mullet size was approximately 2.5 lb and total fat content of fillets was about 5%. All experiments in this study were conducted in duplicate. For the yield study of mullet fillets, dark and light color flesh, five mullet harvested in June (nonspawning season) and five mullet in December (spawning season) were used.

Antioxidant treatment

Only aqueous antioxidant solutions were employed. The antioxidants used were ascorbic acid (Dubois, 1949; Greig, 1967), Na_2 EDTA (Farragut, 1972) and TBHQ (Eastman Products, 1973; Sweet, 1973; Matthews et al., 1975). Mullet fillets were dipped for 1 min in various antioxidant solutions individually or in different combinations before they were frozen at −18°C. The solutions used were: (1) 2% ascorbic acid, (2) 0.025% TBHQ, (3) 0.5% Na_2EDTA, (4) 2% ascorbic acid + 0.025% TBHQ, (5) 2% ascorbic acid + 0.5% Na_2 EDTA and (6) 0.025% TBHQ + 0.5% Na_2 EDTA.

Combination treatment of antioxidant and vacuum packaging

Mullet fillets were dipped in one of the following antioxidant solutions for 1 min: (1) 0.5% Na_2 EDTA, (2) 2% ascorbic acid, (3) 0.025% TBHQ and (4) 2% ascorbic acid + 0.025% TBHQ, then vacuum packed in Cryovac barrier bags and frozen at −18°C.

Antioxidant determination

Mullet fillets were dipped in 0.5% Na_2 EDTA and 0.025% TBHQ for periods of 15, 30, 60 and 90 sec. A colorimetric determination (Sinclair and Power, 1968) was used to determine the content of Na_2 EDTA in mullet fillets after dipping. The TBHQ dipped mullet fillets were freeze dried and then analyzed for TBHQ using a colorimetric method (Eastman Products, 1974).

A standard curve was developed for each compound by using the procedure above except that undipped fish was used and known concentrations of the respective compounds were added to the ground mullet.

Chemical tests

The rancidity development was determined by thiobarbituric acid (TBA) test and peroxide value. TBA numbers were measured by the method of Yu and Sinnhuber (1967) except in preparing fish samples the frozen fillet was sliced and blended with 2 vol of cold deionized water for 30 sec. Duplicate aliquots of homogenate were used for determination of TBA number which is expressed as mg of malonaldehyde per kg of sample. Peroxide values were determined by the AOCS method (1970), following the Bligh and Dyer (1959) lipid extraction. The peroxide value is defined as mg equivalent of peroxide per kg oil. Data were subjected to analysis of variance procedure (Dixon and Massey, 1969).

Organoleptic evaluation

Sensory evaluation of the quality of frozen stored fillets were carried out by panels composed of 10 or more students and staff from the Food Science Dept. The same panelists participated each time. For evaluation of undesirable rancid flavor, the frozen fillets were thawed, cut into approximately 1/2 by 1 in. segments, placed in a dish covered with aluminum foil, heated in an oven at 177°C for 25 min, and allowed to cool to room temperature before presenting to the panelists. The scores of undesirable rancid flavor were on a 9-point hedonic scale with 1 designating none and 9 extremely intense.

RESULTS & DISCUSSION

MULLET FILLETS with and without skin were stored in a freezer at −18°C to examine rancidity development. Figure 1 shows the rancidity change in mullet fillets during storage determined by the TBA method. The TBA number of the skinless fillets increased sharply during frozen storage. For the mullet fillets with skin, the TAB number steadily increased but at a slower rate. When the skin was removed, the skinside dark muscle containing larger amounts of heme protein and nonheme iron (Fischer and Deng, 1976) was exposed and oxygen could readily contact the dark flesh, and heme or other catalysts accelerated the oxidation of mullet lipid at a higher rate. When the skin was retained on the fillets, oxygen permeability on the skinside was limited and thus the oxidation rate was reduced. The dark muscle on the skinless mullet fillets not only increases the rate of lipid oxidation but according to other workers also decreases the acceptability of fillets when they are processed into fish sticks. The appearance of mullet sticks made of skinless mullet fillets is completely different from the traditional white fish sticks made of cod or pollack (Connell, 1976). Because of the large amount of dark muscle in the meat this product is less acceptable to the consumer (Camber, 1955). If the light colored flesh is separated from the dark muscle, it could be used to prepare mullet sticks with improved consumer acceptability. However, the yield of light color flesh is only 2/3 to 3/4 of the boneless fillet during the spawning season (Table 1) and dark muscle is very difficult and uneconomical to separate completely from the light color muscle. Therefore, boneless mullet fillets with skin intact to retard oxidation were used to study the long term storage stability. Since skin-on fillets are processed into blocks and marketed (Connell, 1976), it is hoped that frozen blocks could be prepared from mullet fillets with skin and upon thawing the individual fillets would be utilized.

The concentration of TBHQ and Na_2EDTA in mullet fillets after dipping for either 15, 30 or 60 sec (Table 2) was below the legal allowance (200 ppm TBHQ/g of lipid, Eastman, 1974; Na_2EDTA approved for use in some foods up to 500 ppm, FDC, 1970). Thus, 1 min dipping in various antioxidant solutions was used.

The effect of the various antioxidant treatments on the storage stability of mullet fillets indicated by peroxide value is shown in Figure 2. Mullet fillets without any treatment showed a steady increase in peroxide value during frozen storage and no induction period was observed. The peroxide value increased sharply from 3 months up to 6 months in frozen storage and then started to decrease. The decrease in peroxide value implies further decomposition of the peroxide. The peroxide value decreased from 6 to 9 months storage and then leveled off. This corresponds with the general peroxide production which peaks and then tapers off as the lipid oxidation proceeds (Labuza, 1971). Na_2EDTA, at the concentration used in the study, was not effective in controlling the peroxidation of mullet fillets since the pattern of peroxide production was similar to the fillets without any treatment. Ascorbic acid, TBHQ and three different combinations were more effective ($P < 0.05$) than Na_2EDTA in retarding peroxidation of the mullet fillets. There was not a significant difference ($P <$

Table 1—Yield of mullet fillets, dark and light muscle during spawning (December) and nonspawning (June) seasons

Month	Length (cm)	Fish in the round (g)	Fillets yield[a] (%)	Light flesh yield[b] (%)	Dark flesh yield[b] (%)
June	38.3 ± 1.0	582.5 ± 20.2	31.8 ± 2.4	86.0 ± 2.5	14.0 ± 1.5
Dec.	46.5 ± 1.5	1403.0 ± 51.3	28.0 ± 3.0	71.0 ± 3.3	29.0 ± 2.0

[a] Calculation based on fish weight in the round
[b] Calculation based on weight of the fillet

Table 2—Concentration[a] of TBHQ and Na_2 EDTA in mullet fillets after dipping in 0.025% TBHQ and 0.5% Na_2 EDTA solution respectively for different periods of time

Dipping time (sec)	TBHQ (ppm/g lipid)[b]	Na_2 EDTA (ppm/g muscle)
15	80	270
30	94	380
60	190	492
90	286	561

[a] Values are the average of two experiments
[b] Calculation based on 5% oil in mullet fillets

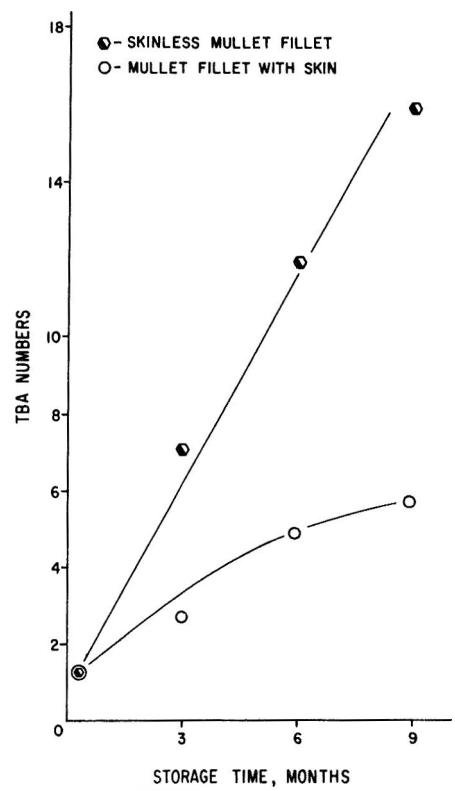

Fig. 1—Comparison of rancidity development measured by TBA numbers in skinless mullet fillets and mullet fillets with skin stored at −18° C.

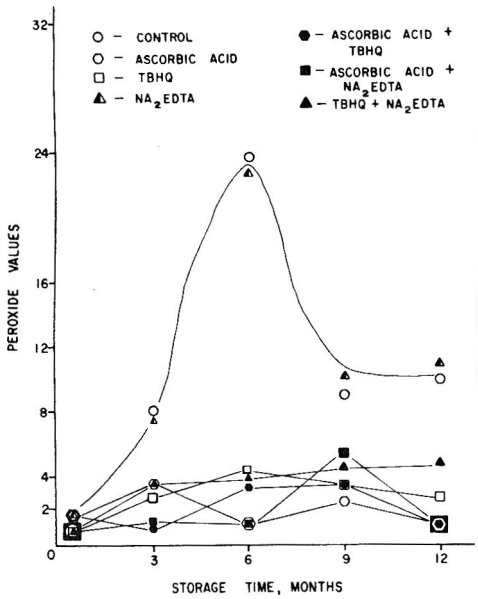

Fig. 2–Effect of various antioxidants on peroxide values of mullet fillets stored at −18° C.

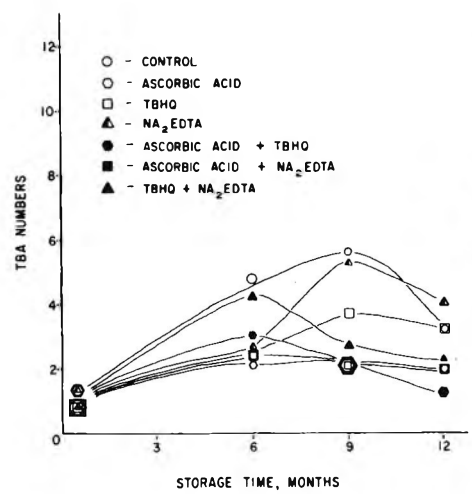

Fig. 3–Effect of various antioxidants on TBA numbers of mullet fillets stored at −18° C.

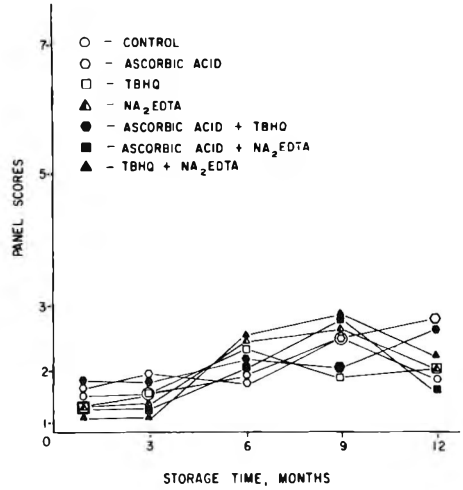

Fig. 4–Effect of various antioxidants on rancid flavor of mullet fillets stored at −18° C.

0.05) among the five antioxidant treatments, ascorbic acid, TBHQ and three different combinations.

When TBA number was used to indicate the effect of various antioxidants on the storage stability of mullet fillets (Fig. 3), it was observed that the TBA number of mullet fillets with no treatment increased from about 1.5 to 6.0 during 9 months of storage. This pattern is different from that for the peroxide value. With the exception of Na_2EDTA and/or TBHQ, all antioxidant treatments were lower ($P < 0.05$) than control in the TBA number. The fillets treated with Na_2EDTA or TBHQ alone or in combination had a higher ($P < 0.05$) TBA number than those with other antioxidant treatments.

The TBA numbers and peroxide values indicated that ascorbic acid alone or in combination with the antioxidants TBHQ or Na_2EDTA were more effective than other antioxidant treatments. Generally, it was observed that when the peroxides started to decrease, the TBA number increased further, reaching the highest around 9 months storage and then decreasing. The further increase of TBA number, as peroxides declined, is similar to the pattern after the bimolecular period of lipid oxidation in a model system (Labuza, 1971). Possible explanations for the decrease of TBA numbers after 9 months could be that lipid hydrolysis products, fatty acids, were high enough to depress the oxidation of mullet lipids (Castell et al.,

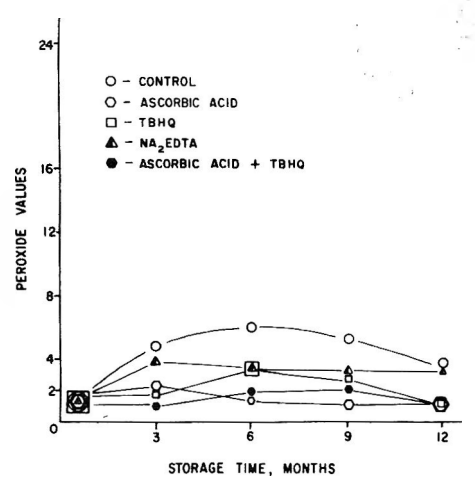

Fig. 5–Effect of various antioxidants in combination with vacuum packaging on peroxide values of mullet fillets stored at −18° C.

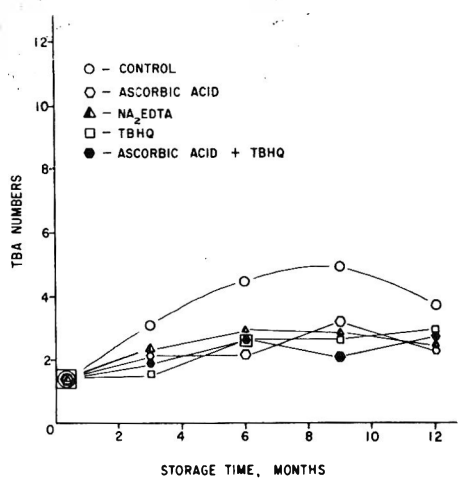

Fig. 6–Effect of various antioxidants in combination with vacuum packaging on TBA numbers of mullet fillets stored at −18° C.

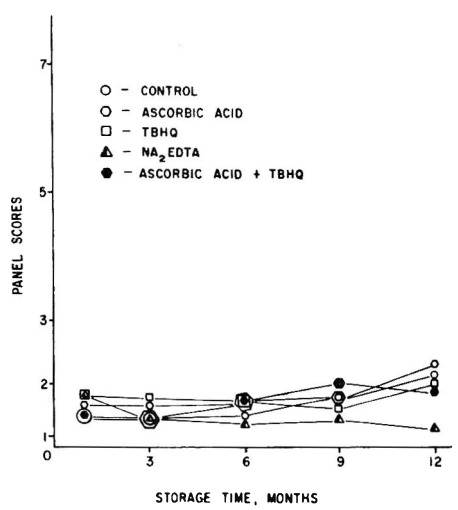

Fig. 7–Effect of various antioxidants in combination with vacuum packaging on rancid flavor of mullet fillets stored at −18° C.

1966) and decomposition products of protein, amino acids, interacted with malonaldehydes or other aldehydes (Kwon et al., 1965).

Figure 4 shows the effect of various antioxidant treatments on the rancid flavor of mullet fillets evaluated by the panelists. The trend of overall average scores of undesirable rancid flavor for various antioxidant treated fillets at different periods were in general agreement with the chemical changes measured by TBA method during 12 months storage (correlation coefficient $\gamma = 0.96$). However, panel scores indicating undesirable rancid flavor of mullet fillets with different treatments did not necessarily correlate with the TBA number. The skin retained on the fillets retarded oxidation and the TBA numbers were below 6 (Fig. 3). This might be partially responsible for the inability of the panelists to detect the rancidity and distinguish the difference between treated and untreated samples.

The effect of the combination of antioxidant treatment and vacuum packaging on the rancidity development of mullet fillets based on the determination of peroxide value is shown in Figure 5. Mullet fillets treated with vacuum packaging alone had the highest peroxide production after 6 months storage, which was similar to the nonvacuum packed mullet fillets without any treatment (Fig. 2). However, the packaged product was much lower ($P < 0.05$) than the unpackaged product in peroxide production. Similarly the mullet fillets treated with vacuum-packaging in combination with ascorbic acid, TBHQ and TBHQ + ascorbic acid had lower ($P < 0.05$) peroxide values than the unpackaged products. The fillets vacuum packaged in combination with Na_2EDTA had higher ($P < 0.05$) peroxide values than with the other combinations, indicating the Na_2EDTA was less effective than TBHQ or ascorbic acid in combination with vacuum packaging.

The effect of combination treatments of vacuum packaging and antioxidants was also interpreted by TBA number as shown in Figure 6. The TBA numbers slowly increased during 9 months storage. Generally, the TBA numbers were lower ($P < 0.05$) in mullet fillets treated with various antioxidants in combination with vacuum packaging than with vacuum packaging alone. After 9 months storage, the TBA number of mullet fillets with different treatments also decreases slightly.

As to the effect of the combination treatment of vacuum packaging and antioxidants on the undesirable rancid flavor of mullet fillets, the panels were unable to detect significant rancid flavor in the products evaluated periodically over a storage time of 12 months (Fig. 7).

In summary, the skin-on mullet fillets treated with the antioxidants, ascorbic acid and/or TBHQ, in combination with vacuum packaging had the least rancidity development as measured by chemical tests and organoleptic evaluation. Thus, skin-on mullet fillets preserved as frozen blocks potentially offer a stable mullet product with little rancid flavor and also provide a convenient source of mullet fillets for the consumer, instead of the traditional mullet in the round.

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EFFECT OF FREEZING AND FROZEN STORAGE ON SALT PENETRATION INTO FISH MUSCLE IMMERSED IN BRINE

ABSTRACT

Salting is a processing treatment used either to provide a salty flavor or to impart storage stability by decreasing water activity. Optimum salt content can enhance overall flavor acceptability and is a major factor in safe preservation of smoked fish. In this study, rate of salt penetration into fresh or frozen and then thawed fish muscle was studied by dipping mullet fillets in brine. Salt penetration curves resemble a first order change [$X = C(1 - e^{-kt})$]. Initial salt penetration rate (g salt/g sample/min) and rate constant (min^{-1}) increased respectively from 0.006 and 0.018 for fresh fish to 0.014 and 0.029 after freezing (frozen for 1 wk), then decreased to 0.011 and 0.025 after 3 wk of frozen storage, and leveled off at 0.009 and 0.018 from 5–9 wk of storage. The change in salt penetration rates closely followed changes in extractable actomyosin in muscle, indicating a dependence of the change on the degree of denaturation of fish muscle proteins. The effects of brine concentration and frozen storage on water transfer were also studied. Water migrated from the brine into the flesh if fresh mullet fillets were dipped in brine at concentrations up to 15%. When the brine concentration was 20% or higher, the water migrated from fish muscle to the brine. However, after 2 months frozen storage, fish muscle gained water if dipped in brine of 20% or below and lost water when the brine was 25% or higher.

INTRODUCTION

SALTING is one of the oldest methods for preserving fish which has been used in products such as dried fish and smoked fish. Traditionally, high levels of salt in smoked fish decreased water activity and preserved the product. Today, however, because refrigeration is widely available, salt and smoke are employed primarily for flavor acceptability rather than as primary preservatives (Cutting, 1962, 1965; Burgess et al., 1967; Deng et al., 1974; Chan et al., 1975). The heavy salting process has been shifted to a light salting operation in the production of smoked fish. It has been reported that proper amounts of salt will affect the overall acceptability of smoked fish (Burgess et al., 1967; Deng et al., 1974) and the required smoking process (temperature and time) for smoked fish (FDA, 1970; Chan et al., 1975). Thus, the degree of salt penetration in the brining process becomes a very important factor in smoked fish quality.

There are numerous reports concerning the salting process, many of which deal with a solid salt curing process (Beatty and Fougé re, 1957; McPhail, 1957; Crean, 1961; Voskresensky, 1965; van Klaveren and Legendre, 1965; Burgess et al., 1967). Only a few reports of the rate of salt penetration into muscle immersed in brine are available (Torry Research Station, 1961, 1962 and 1963; Del Valle and Nickerson, 1967a, b).

The preliminary work indicated that different periods of frozen storage would affect the degree of salt penetration into fish muscle and the overall acceptability of smoked fish. And since frozen storage is currently a common practice for storing fish, this study was designed to investigate the effects of freezing and frozen storage on the salt penetration into fish muscle, using mullet (*Mugil cephalus*) fillets as the experimental samples.

EXPERIMENTAL

Materials

Mullet were harvested in Placida, Fla., in Aug., 1975. The freshly caught mullet were kept in ice, shipped to Gainesville, Fla., and immediately filleted (skinless and boneless) or frozen in the round at −18°C. Mullet in the round were removed from the freezer at different periods of time, thawed at room temperature for 3 hr and filleted before brining.

Salt determinations

The mullet fillets were immersed in 25% brine for 2, 4, 6, 10, 20, 30 and 40 min without agitation. In order to obtain a maximum muscle salt concentration in equilibrium with brine, mullet fillets were allowed to equilibrate in brine solution for 48 hr. The salt (NaCl) content of mullet muscle after dipping was determined by AOAC procedure (1970).

Protein denaturation

Extractable actomyosin of mullet muscle was used as an indication of protein denaturation. The following extraction procedure for actomyosin is a slight modification of the method of Deng et al. (1976). All extraction steps were carried out at 4°C. The ground muscle was blended with 0.6M KCl containing 0.01M $NaHCO_3$ (pH 8.0) (6 ml/g meat) for 30 sec. The homogenate was then centrifuged at 16,000 × G for 1 hr. The supernatant was mixed with 0.2 mM $NaHCO_3$ until the ionic strength decreased to 0.2 and the mixture was centrifuged at 9,750 × G for 20 min. The precipitate was dissolved in 1M KCl with slow stirring and centrifuged at 16,000 × G for 1 hr. The actomyosin in the solution was determined according to the biuret method (Gornall et al., 1949).

Water transfer

Water transfer in mullet muscle immersed in various concentrations of brine was determined according to the method of Fougére (1952). The fillets were weighed before dipping and weighed again after dipping, allowing excess water to drain from the fillet's surface by dripping for 40 min.

RESULTS & DISCUSSION

THE EFFECT of freezing on the salt penetration of mullet muscle in the early stages of brining (25%) is shown in Figure 1A. A sharp increase of salt penetration was observed in the mullet muscle obtained from 1 wk frozen storage and then thawed mullet when compared to fresh mullet fillets. The sharp increase may be due to cell wall damage of mullet flesh after freezing, resulting in a higher rate of diffusion when thawed muscle was immersed in 25% brine. The salt penetration curves follow first order kinetics. Figure 1B shows a semilogarithmic plot of the difference between the maximum concentration (after 48 hr equilibrium in brine solution) and the concentration at any time in the brining, as a function of the

dipping time. The datum point in the early stages of brining fits the equation $X = C(1 - e^{-kt})$. The rate constant (min^{-1}) increased from 0.018 for fresh muscle to 0.029 after 1 wk frozen storage.

Figure 2 indicates the effect of various periods of frozen storage on salt penetration into thawed mullet muscle in the early stages of brining (25% brine). The salt penetration into mullet muscle decreased gradually with 1–5 wk storage. However, after 5 wk storage, there was no further decrease in salt penetration in the early stages of brining. The rate constants were 0.025, 0.018 and 0.018 respectively for 3, 5 and 9 wk frozen stored fish. If the initial rate of each salt penetration (the slopes at time 0) representing the product of the rate constant K and the equilibrium concentration C is plotted against different periods of storage time, a decrease pattern is observed (Fig. 3). The dotted line between 0 time to 1 wk is a predicted possible initial salt penetration rate. The decrease of initial salt penetration rate proved to be highly related to the protein denaturation in mullet muscle during storage (Fig. 3). The extractable actomyosin, an indication of protein denaturation, decreased gradually during the first 5 wk and then levelled off, with no observable further decrease after 5 wk storage. There is a high correlation between the decrease of initial salt penetration rate and extractable actomyosin of mullet flesh during frozen storage. The correlation coefficient is estimated to be 0.99 (Dixon and Massey, 1969). Thus, protein denaturation is the major factor responsible for the decrease of initial salt penetration rate into mullet muscle. The denaturation of muscle protein during frozen storage will lead to protein aggregation (Connell, 1962) resulting in an increased bonding or network between the myofibrillar proteins (Bramsnaes, 1969) in fish muscle which may reduce the initial salt penetration rate. On the other hand, protein–protein interaction will decrease available binding sites for water and partially cause dripping loss after fish is thawed. This dripping loss will decrease the water content and increase the salt concentration in the water phase of fish muscle which will decrease the difference of osmotic pressure and may be partially responsible for the decrease of initial salt penetration rate.

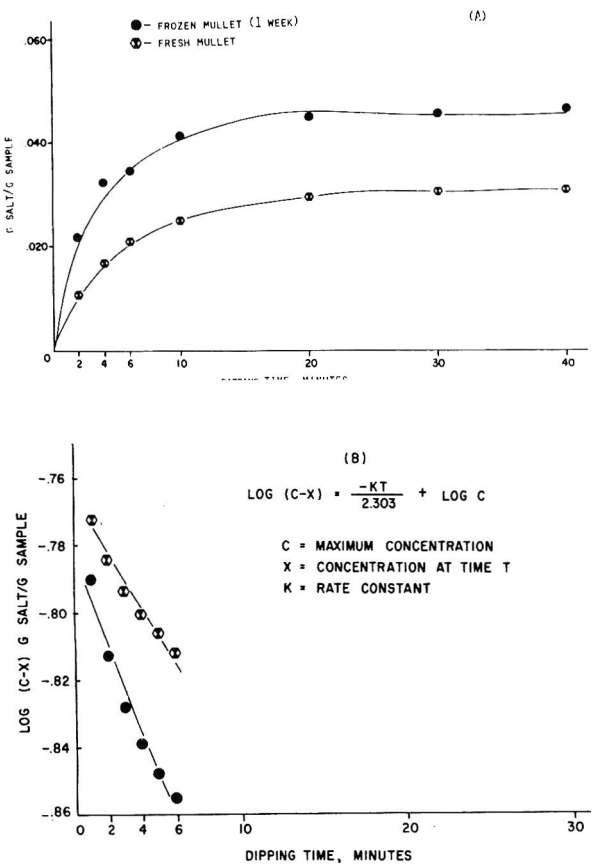

Fig. 1–Effect of freezing on salt penetration into mullet muscle immersed in 25% brine. (A) Plotted in rectangular coordinates; (B) Semi-logarithmic plot.

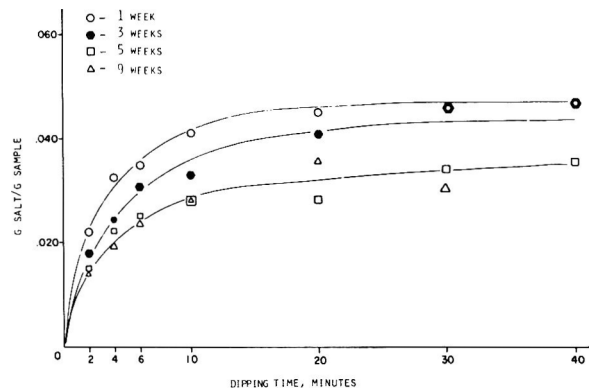

Fig. 2–Effect of various periods of frozen storage prior to dipping on salt penetration into mullet muscle immersed in 25% brine.

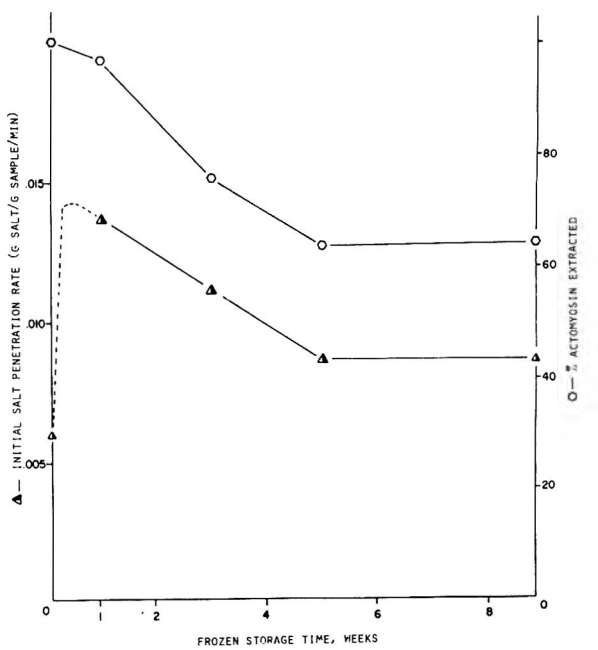

Fig. 3–Effect of various periods of frozen storage on extractable actomyosins and initial salt penetration rate (in 25% brine) of mullet muscle.

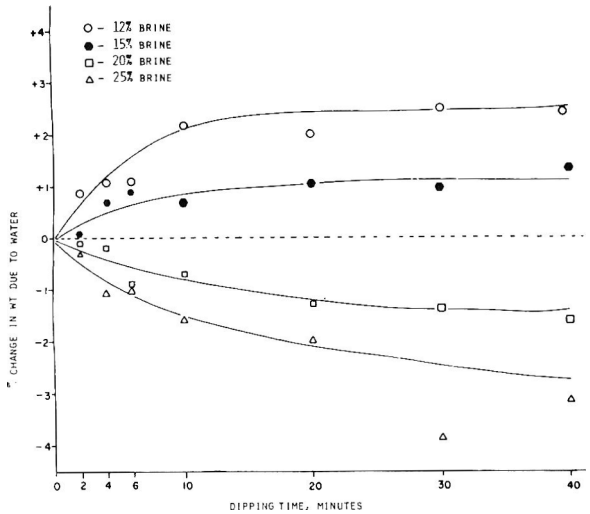

Fig. 4—Water transfer in fresh mullet muscle immersed in brine with various salt concentration.

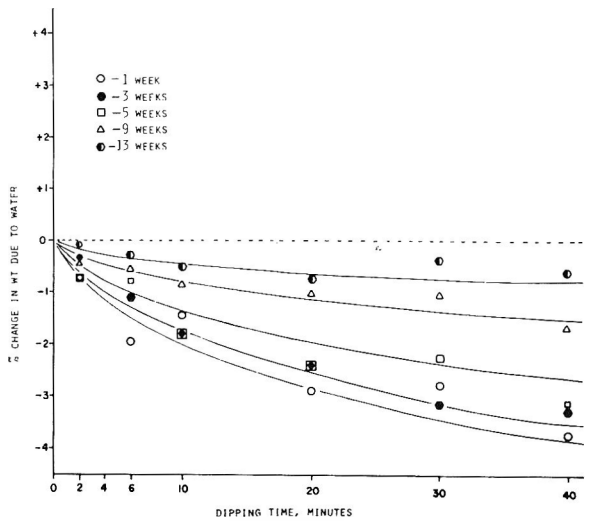

Fig. 5—Effect of frozen storage on the water transfer in mullet muscle immersed in 25% brine.

Water transfer in fresh mullet fillets immersed in brine with various salt concentrations is shown in Figure 4. Water transfer is indicated as percent change in weight of mullet due to water. A positive value indicates water gain; a negative value indicates water loss. When fresh mullet fillets were dipped in 12 and 15% brine, they gained water. When fresh mullet fillets were dipped in 20 and 25% brine, they lost water. Fougére (1952) found that cod flesh would gain water when dipped in 14% or higher brine. The possible reason for the difference of water transfer between mullet flesh and cod flesh when dipped in brine with various salt concentrations is that the mullet flesh used in this study had higher total lipid content (approx 6% on a wet basis). Although Fougére did not specify the total lipid content of cod flesh used in his study, Ackman (1967) reported that the total lipid content of cod flesh ranged from 0.5–1.0%. Thus, the higher total lipid content in mullet flesh may proportionally decrease the water content resulting in a higher natural salt concentration in the water phase of mullet flesh. This might explain why the brine with higher salt concentrations was required to transfer water from mullet flesh to the brine. This result corresponds with the report (Torry Research Station, 1963) that increasing fat content of the fish reduced the uptake of salt.

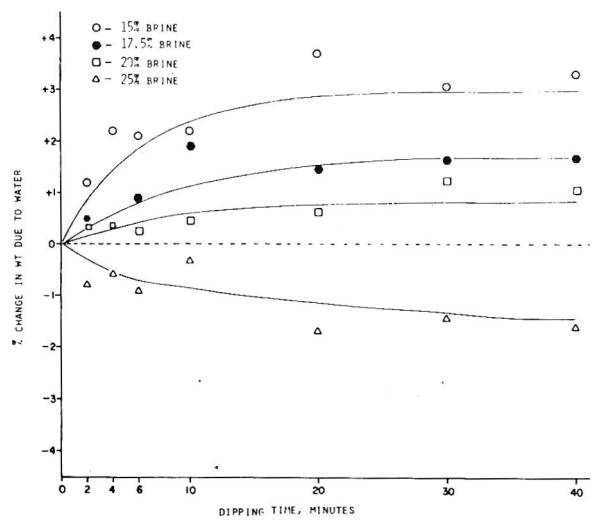

Fig. 6—Effect of frozen storage on the water transfer in mullet muscle immersed in brine with various salt concentration.

When thawed mullet muscle from different periods of frozen storage was dipped in 25% brine, the longer the storage time of the mullet the less water transferred from mullet flesh to the 25% brine (Fig. 5). This might be due to a greater extent of protein denaturation of muscle protein with increase of storage time, resulting in more water loss and a higher natural salt content in the water phase of mullet flesh.

When mullet flesh frozen for 2 months was immersed in various brine concentrations, the critical concentration for gaining water or losing water from the mullet flesh was between 20 and 25% brine (Fig. 6). This is different from fresh mullet flesh (Fig. 4) whose critical concentration was between 15 and 20% brine solution. The reason for the difference of critical concentration for water transfer between fresh and stored frozen mullet flesh is also attributed to the extent of protein denaturation.

In summary, freezing and frozen storage had a major effect on the initial salt penetration rate into mullet when they were dipped in 25% brine solution. Immediately following freezing, the initial salt penetration rate was increased and on subsequent frozen storage the initial salt penetration rate was decreased. Since the resultant salt content will affect the acceptability and required processing conditions for smoked fish, further study is needed to define guidelines of the brining process for mullet harvested in various seasons and frozen at different temperatures. The information thus obtained would be of importance to processors who may freeze fish in season, and process smoked fish from frozen fish in the off-season.

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QUALITY EVALUATION OF FROZEN STORED CHANNEL CATFISH GROWN BY TANK CULTURE: EFFECTS OF DIETARY FAT, FREEZING METHOD AND STORAGE TEMPERATURE

ABSTRACT

Channel catfish (*Ictalurus punctatus*) were grown in tank culture on diets containing either tallow or menhaden oil as 10% of diet. Processed fish were frozen in air at −18°C or in nitrogen spray at −77°C, sealed in polyethylene and stored at either −18 or −35°C. Sensory evaluations of fresh fish revealed no differences in flavor, texture, or aroma. Fish reared on tallow were rated slightly superior for flavor after 94 days storage and for aroma and flavor after 312 days. Fish frozen in air were rated slightly superior for aroma after 94 days and for texture and flavor after 312 days. Fish stored at −18°C were rated slightly superior in texture after 312 days. Initial microbial counts were approx 900 per 10 cm^2 of flesh surface; these values decreased by 65% after storage for 102 days and by 95% or more after storage for 365 days.

INTRODUCTION

FISH and fishery products are particularly susceptible to post-harvest deterioration and consequently numerous studies have been made of factors affecting post-harvest changes in physical, chemical and organoleptic properties. These studies have included the effects of treatment of fish prior to and during processing, bacterial populations, type of packaging, method of freezing, and temperature and duration of frozen storage. Although most attention has been given to marine fish, a number of studies have been made of aquatic species as well, including channel catfish (*Ictalurus punctatus*) (Dupree, 1969; Boggess et al., 1971, 1973; Lovell, 1972a, b; Heaton et al., 1971, 1972; Ammerman et al., 1973; Beuchat et al., 1973, 1975; Maligalig et al., 1973, 1975a, b).

Most farm raised catfish are grown in earthern ponds and raceways. Under such conditions, only limited control over water quality, diet and other environmental factors is achieved. Catfish readily absorb malodorous compounds from environmental waters (Lovell, 1972a; Maligalig et al., 1975a, b) and from the diet (Dupree, 1969; Lovell, 1972b; Maligalig et al., 1973) and consequently off-flavor and aroma are recurring problems in catfish production. These off-flavors may come from the numerous aquatic micro- and macroorganisms present in ponds and raceways as well as from other sources (Lovell and Ammerman, 1974). The influence of diet on flavor was demonstrated by Maligalig et al. (1973) who observed a chicken-like flavor in fish fed turkey livers and by Dupree (1969) and Lovell (1972b) who observed an objectionable fishy flavor in catfish fed diets containing marine fish oil. Lovell (1972b) reported that the initial fishy flavor increased in intensity with time during storage at −18°C and was accompanied by a large increase in thiobarbituric acid (TBA) values, indicating extensive oxidation of polyenoic acids during frozen storage.

The long term growth and success of the catfish farming industry will depend on the production of a product that is uniformly high in quality and competitve in price with similar food products. As demonstrated by Andrews et al. (1971), a system of tank culture providing for systematic water replacement is an attractive alternative to the production of fish in static or recycled waters of ponds and raceways. This method has several decided advantages from the standpoint of improved product quality. Water quality and diet can be rigorously controlled thereby eliminating malodorous compounds from these sources. Also, fecal matter, metabolic by-products, and uningested feed are systematically removed, thereby minimizing microbial populations and associated sanitation problems during processing. Because the production of catfish by aquacultural techniques is seasonal with the bulk of the harvest occurring in late fall and early winter, successful frozen storage should help to insure a uniform supply of fish throughout the year.

The purpose of this study was to examine sensory characteristics and microbial populations of frozen stored channel catfish that were grown by tank culture on diets containing either tallow or menhaden oil. Fish were frozen by conventional methods at −18°C or ultra-rapidly in nitrogen spray at −77°C and stored up to 365 days at either −18 or −35°C.

MATERIALS & METHODS

Experimental diets and cultural method

The channel catfish evaluated in this study were grown by tank culture at the Skidaway Institute of Oceanography, Savannah, Ga. The fish were maintained on experimental diets for 3½ mo at which time they weighed 350–450g each. The experimental diets were identical in every respect except for the source of fat: one diet contained 10% beef tallow and the other 10% menhaden oil. Both diets contained 40,000 International Units of Vitamin E and 113g of ethoxyguin per ton of feed. Feed was withheld 1 day prior to transporting the fish live to the Dept. of Food Science at Experiment, Ga., where they were kept overnight in aerated containers prior to slaughter.

Processing

The fish were stunned as described by Boggess et al. (1973), beheaded, eviscerated, skinned, washed in tap water, and packed in ice overnight. The following day sensory characteristics of both sets (beef tallow and menhaden oil diets) of fresh, unfrozen fish were evaluated. The remaining fish were divided into two subsets per diet. One subset from each diet was placed in an Ultra-Freeze Simulation Freezer (National Cylinder Gas, Chicago, Ill.) and frozen in nitrogen spray to an internal temperature of −15°C in a period of approx 6 min. Fish were individually packaged in polyethylene bags, sealed, and stored at −18 and −35°C. The second subsets from each diet were placed in trays and frozen at −18°C in a walk-in freezer. After 3½ hr, internal temperatures had dropped to −2°C; and after approximately 10 hr. fish were packaged as described above and stored at −18 and −35°C, thus making a total of eight groups of fish (Table 1).

Microbial enumeration

Bacterial populations on catfish were determined by swabbing 10 cm^2 areas in the anterio-dorsal regions of each fish (Beuchat et al., 1973). Swabs were taken from one side of five fresh fish that had been

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iced overnight and from six fish that had been stored for 102 days (three at −35 and three at −18°C). Four fish that had been stored 365 days (two each at −35 and at −18°C) were sampled by swabbing areas on both sides of each fish. Prior to swabbing, all frozen fish were thawed overnight at 4°C. Swabs were deposited in 0.1% peptone diluent, washed, and serial dilutions were plated in standard methods agar (BBL) using the pour-plate technique. Incubation was at 21°C and counts were made after 4 days.

Organoleptic evaluations

Prior to cooking, the frozen fish were thawed overnight at 4°C. The taste panel evaluations were conducted on fish sections that had been baked in aluminum foil at 205°C for 17 min. At each session panelists judged cooked fish sections for aroma, texture, and flavor using a 9-point scale ranging from 1, extremely poor, to 9, extremely good. Ten experienced panelists were used in all of the sessions. For the evaluation of fresh fish, panelists were presented with fillet halves. Because different portions of fish differ in composition and therefore might be expected to respond differently to freezing methods and storage temperatures, fish that had been frozen were evaluated by presenting panelists with portions of fillets so that each individual panelist evaluated comparable sections of fillets from the different treatments. Sensory data were subjected to analysis of variance to determine significance of treatment effects.

RESULTS & DISCUSSION

Microbial enumeration

Initial microbial counts revealed approximately 870 organisms per 10 cm^2 of external fish area. Similar counts were noted on fish from both tallow and menhaden diets. After frozen storage for 102 days, populations decreased by 65% or more. The fish were not examined after 312 days, but after 365 days the counts had decreased by 95% or more.

The initial counts agree quite well with those reported by Beuchat et al. (1973) for fish grown by tank culture. Low levels of micro-organisms would be of particular importance in fish to be ice-packed and sold as fresh fish. Heaton et al. (1972) found that fish grown by tank culture could be packed in ice and held for up to 12 days without significant deterioration in quality. Data for catfish grown in ponds and raceways are not available for comparison but one could reasonably expect much higher microbial populations on such fish and consequently a shorter shelf-life for ice-pack fish.

Organoleptic evaluation

The mean taste panel scores for aroma, texture and flavor are summarized in Table 2. Small but occasionally statistically significant differences were noted in response to diet, freezing method, and storage temperature. No differences in flavor, aroma, or texture were detected in the fresh fish in response to dietary fat but after frozen storage those fish given tallow were rated slightly superior in both aroma and flavor. These differences were small but statistically significant for flavor at 94 days and for aroma and flavor at 312 days. After 94 days of storage the marshy or musty flavor often detected in baked, unseasoned catfish was judged to be slightly more intense in those fish that had received menhaden oil. Surprisingly, the fishy flavor and aroma observed in catfish (Dupree, 1969; Lovell, 1972b) and other species (Crawford et al., 1975) in response to dietary marine fish oils, were not observed in this study until 312 days frozen storage. The malodorous compounds responsible for this flavor characteristic are by-products of polyenoic fatty acid oxidation (Meijboom and Stroink, 1972) and presumably may be derived either from partially oxidized oil in the diet or from fatty acids that are oxidized during fish storage. Dietary vitamin E effectively reduces the fishy aromas and flavors in meat products derived from animals fed highly unsaturated fish oils (Dupree, 1969; Crawford et al., 1975). The observation of these compounds (Lovell, 1972b) in catfish that were fed diets containing levels of α-tocopherol (Worthington and Lovell, 1973) similar to those used in this study suggests that the development of this flavor profile is dependent upon an interaction between levels of α-tocopherol and extent of oxidation of dietary fish oil. In addition, water is systematically replaced in tank culture, thereby limiting the accumulation of malodorous compounds and their consequent uptake and concentration in catfish.

Rapid freezing with nitrogen was not beneficial with regard to preserving catfish quality. In fact, in some instances, slightly lower mean scores for aroma, texture and flavor were noted for fish frozen with nitrogen. These differences were statistically significant for aroma after 94 days of storage and for texture and flavor after storage for 312 days. The differences

Table 1—Groups of catfish placed in frozen storage

Dietary lipid source	Freezing method	Storage temp (°C)
Menhaden oil	Nitrogen	−35
Menhaden oil	Nitrogen	−18
Menhaden oil	Air	−35
Menhaden oil	Air	−18
Tallow	Nitrogen	−35
Tallow	Nitrogen	−18
Tallow	Air	−35
Tallow	Air	−18

Table 2—Mean taste panel scores according to treatment and time in frozen storage

Storage time (days)	Treatments compared	Mean taste panel scores		
		Aroma	Texture	Flavor
	Diets			
0 (Fresh)	Menhaden oil	6.9	6.1	6.0
	Tallow	6.9	5.8	6.0
94	Menhaden oil	6.4	6.9	5.6
	Tallow	6.5	6.8	6.4*
220	Menhaden oil	5.9	6.1	5.2
	Tallow	6.0	6.4	5.4
312	Menhaden oil	5.4	5.8	5.3
	Tallow	5.9*	6.1	5.9**
	Freezing methods			
94	Nitrogen	6.1	6.7	5.8
	Air	6.8*	7.0	6.3
220	Nitrogen	5.8	6.4	5.3
	Air	6.1	6.1	5.3
312	Nitrogen	5.6	5.7	5.4
	Air	5.7	6.1*	5.9*
	Storage temperatures (°C)			
94	−35	6.5	6.9	6.2
	−18	6.4	6.8	5.8
220	−35	5.9	6.2	5.2
	−18	6.0	6.3	5.5
312	−35	5.5	5.8	5.5
	−18	5.7	6.1*	5.7

* Significant treatment effect, $P \leq 0.05$
** Significant treatment effect, $P \leq 0.01$

associated with freezing method were slight and panelists' comments were insufficient to indicate the nature of the differences.

Differences associated with storage temperature were small and statistically significant in only one instance. At 312 days fish stored at −18°C were judged slightly superior in texture. Catfish have a soft texture, and the higher levels of free fatty acids present in fish stored at the higher temperature (Gibson and Worthington, 1977) may have caused a slight firming in texture by interacting with muscle protein. The accumulation of free fatty acids has been associated with toughening in lean fish and in fish wherein the fat is localized (Olley and Duncan, 1965; Anderson and Ravesi, 1961; Dyer, 1966). The presence of large amounts of neutral fat dispersed throughout the muscle tissues of catfish perhaps acts as a "sink" for free fatty acids, thus preventing sufficient localized accumulation of these acids to adversely affect texture.

CONCLUSION

THESE EXPERIMENTS have shown that little if any advantage is to be gained by freezing catfish ultra-rapidly with nitrogen spray when compared with freezing in air at −18°C or by storage at −35°C when compared with storage at −18°C. Although catfish receiving dietary menhaden oil accumulated high levels of polyenoic acids in tissue lipids (Gibson and Worthington, 1977), there was no evidence of significant oxidation of these acids during frozen storage at −18 or −35°C as determined by organoleptic or instrumental techniques (Gibson and Worthington, 1977). Even though menhaden oil did not seriously affect any of the quality parameters of the fish in this study, other reports in the literature indicate that in some instances fish oil may seriously impair both fresh flavor and storage stability of catfish and caution should therefore be exercised in the use of these oils in catfish diets. It is likely that physiological requirements of catfish for polyenoic acids can be met by the inclusion of low-fat fish meal in dietary formulations thereby minimizing adverse effects on organoleptic quality.

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LIPID CHANGES IN FROZEN STORED CHANNEL CATFISH GROWN BY TANK CULTURE: EFFECTS OF DIETARY FAT, FREEZING METHOD, AND STORAGE TEMPERATURE

ABSTRACT

Channel catfish (*Ictalurus punctatus*) were grown in tank culture on diets containing either tallow or menhaden oil at 10% of diet. Processed fish were frozen in air at −18°C or in nitrogen spray at −77°C, sealed in polyethylene, and stored at either −18 or −35°C. Levels and fatty acid profiles were determined for total lipid, triglyceride, phospholipid, and free fatty acids in fresh fish that had been held on ice for 2 days and in frozen fish at 0, 94, 220, and 312 days. Dietary lipids strongly affected fatty acid profiles of all lipid fractions. Free fatty acid production occurred only in fish held on ice and in fish stored at −18°C. Changes in free fatty acid profiles and decreases in phospholipid levels indicated phospholipase activity. After 312 days storage at −18°C free fatty acid levels reached approx 0.93%. Measurable oxidative deterioration did not occur under either condition of frozen storage.

INTRODUCTION

CHANNEL CATFISH (*Ictalurus punctatus*) are particularly well adapted to warm waters and in southern areas of the U.S. are grown commercially by aquacultural techniques. Production is seasonal and frozen storage is therefore necessary to maintain a uniform supply throughout the year.

A number of changes have been observed to occur in fish during frozen storage and several of these are associated with changes in tissue lipids. These changes include oxidative deterioration, particularly in fatty fish (Banks and Hardy, 1965), and toughening of fish muscle in lean species of fish (Anderson and Ravesi, 1969). Autoxidation of polyenoic fatty acids produces fishy flavors (Meijboom and Stroink, 1972) and other undesirable flavors and aromas, and in addition may affect the nutritional and textural quality of fish as a result of the involvement of protein in the sequence of autoxidative reactions (Braddock and Dugan, 1973). Textural changes are most likely to occur however as a consequence of the denaturation and toughening of fish protein by free fatty acids (FFA) during frozen storage (Anderson and Ravesi, 1969). FFA are produced by phospholipase in lean fish and by both phospholipase and lipase activity in fatty fish (Olley et al., 1962). Neutral cellular lipid appears to mitigate the effects of FFA and toughening occurs primarily during storage of lean fish or in fish containing localized body fat (Dyer, 1966).

In a manner characteristic of monogastric animals, catfish accumulate dietary fatty acids (Stickney and Andrews, 1971; Worthington and Lovell, 1973) and as a consequence, catfish that have received fish oil high in polyenoic acids may undergo oxidative deterioration. Dupree (1969) and Lovell (1972) observed an objectionable fishy flavor and aroma in fresh fish that were grown in diets containing fish oil and Lovell (1972) reported an intensification of this flavor profile and an increase in TBA (2-thiobarbituric acid) values during storage at −18°C. Fat usually comprises 20–30% of the dry weight of channel catfish. In addition to considerable localized deposition, the fat appears to be distributed throughout the muscle tissues, although to our knowledge this has not been demonstrated histochemically.

Post-harvest changes in the lipids of channel catfish have not been investigated and the objective of this study was to determine the influence of dietary fat, freezing method, and storage temperature on changes occurring during frozen storage.

MATERIALS & METHODS

Fish

Channel catfish were grown at the Skidaway Institute of Oceanography on diets containing either tallow or menhaden oil. Processed fish were frozen either in air at −18°C or with nitrogen spray at −77°C, sealed in polyethylene and stored at −18°C or −35°C as described by Gibson et al. (1977). Fish to be examined in the fresh, unfrozen state were kept on ice for 48 hr prior to analysis.

Total lipid analysis

All lipid extractions and subsequent analyses were made on subsamples drawn from composite samples consisting of three fish (350–450g each). Frozen fish were thawed overnight at 4°C, bones and fins removed, and initial mixing accomplished by grinding the fish together in a Toledo food chopper. The tissues were frozen, lyophilized, thoroughly mixed, and stored at −35°C until time of lipid extraction (Worthington and Lovell, 1973).

Duplicate 2-g samples were rehydrated with 8.0 ml of 0.1M KCl and homogenized in a chloroform:methanol:water (0.1M KCl) ternary monophasic system of Bligh and Dyer (1959) except that 0.1M KCl was substituted for water to prevent formation of stable emulsions. Lipid extracts were reduced in volume under vacuum, transferred to 10 ml volumetric flasks, made to volume with chloroform, and aliquots withdrawn for the quantitative determination of total lipid (TL), phospholipid (PL), triglyceride (TG), and free fatty acids (FFA). Total lipid value was determined gravimetrically after evaporation of solvent under a stream of nitrogen.

Phospholipids

Phospholipids were separated from neutral lipids by column chromatography on acid washed Florisil (Carroll, 1963). Approx 8.6-g quantities of 60/80 mesh heat activated (110°C) Florisil were added to 1.0 × 30 cm glass columns containing chloroform. After addition of lipid in chloroform, the columns were washed with 200 ml chloroform to remove neutral lipids and with 200 ml methanol to remove phospholipids. The methanol eluate was reduced in volume under vacuum on a rotary evaporator at 40°C, transferred to a tared vial and the remaining solvent removed with a stream of nitrogen. After removal of traces of solvent under high vacuum, the phospholipids were quantitatively determined by weighing and then stored under nitrogen at −35°C until time of FA analysis.

Triglycerides and free fatty acids

Triglycerides and FFA were separated from TL by preparative thin-layer chromatography (TLC) on silica gel G plates with petroleum ether:diethyl ether:acetic acid (85:15:1). Prior to TLC, known quan-

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tities of triheptadecanoin and heptadecanoic acid (17:0) were added as internal standards. After spraying with the color reagent of Jones et al. (1966), the TG and FFA bands were removed and retained for FA analysis by gas-liquid chromatography (GLC).

Methyl ester analyses

The TL, PL, TG, and FFA obtained from fresh fish and from frozen fish at 0, 94, 220 and 312 days were analyzed for fatty acid content. Methyl esters were prepared essentially as described by Worthington et al. (1972). Samples to be esterified were placed in Carius combustion tubes with 5 ml of a 3:1 mixture of methanol:benzene containing 3% H_2SO_4. The tubes were closed, placed in a horizontal position in a 90°C water bath and shaken continuously for 2 hr. Upon cooling, 2 ml of 0.1M KCl were added to effect phase separation and the organic (benzene) phase was removed and filtered through anhydrous sodium sulfate. The remaining aqueous phase was washed twice with 2 ml aliquots of redistilled petroleum ether which were likewese filtered through the anhydrous sodium sulfate, and combined with the benzene extract. The methyl ester solutions were then concentrated with a stream of nitrogen and stored under nitrogen at −35°C prior to analysis by GLC.

Individual methyl esters were determined on a MicroTek 220 gas chromatograph essentially as described previously (Worthington et al. 1972; Worthington and Lovell, 1973). Peak identifications were established by comparing the retention times and equivalent chain length (ECL) values of sample methyl esters with those of standards obtained on four liquid phases – OV 101, OV 225, DEGS, EGS – differing widely in polarity (McReynolds, 1970). The ECL of unavailable methyl esters were obtained from published data (Jamieson, 1970). As additional evidence, ECL values were calculated by the method of Jamieson and Reid (1969) using data obtained with the four liquid phases.

All quantitative determinations were made with 1.8m × 4mm glass columns packed with 15% w/w EGS on 80/100 mesh Chromosorb W(AW) (DMCS) that had been coated with 3% phosphoric acid (w/w). Fatty acids within each lipid class were calculated as percent of total fatty acids (percent distribution). Additionally, the percent TG and FFA were determined by relating the integrator counts for all fatty acids within these samples to that obtained for the known amount of 17:0 added as an internal standard. These values are expressed as g/100g full-fat dry tissue.

Analysis of variance was performed on values for the levels of the major identified FFA and on values for the different lipid components obtained at 94, 220 and 312 days storage.

RESULTS & DISCUSSION

Total lipid

Levels of total extractable lipid were not significantly influenced by diet, method of freezing or by temperature or length of storage. Average total lipid values by diet, obtained from all determinations made throughout the course of the study, were 30.5% (g fat/100g lyophilized tissue) for those fish receiving menhaden oil and 29.6% for those receiving tallow.

The influence of dietary fat on the fatty acid distribution of total lipid (Fig. 1) is in agreement with previous studies with catfish (Stickney and Andrews, 1971; Worthington and Lovell, 1973) and other monogastric animals (Carroll, 1965). Levels of polyenoic acids were approximately three-fold greater in fish receiving menhaden oil than in those receiving tallow. These observations demonstrate clearly that the degree of unsaturation in the fat of catfish can be appreciably modified by dietary means.

The ratios of unsaturated to saturated fatty acids in total lipids from fish receiving menhaden oil and tallow were 2.4 and 2.6, respectively, and did not change measurable throughout the course of the study, thus indicating that significant oxidative deterioration did not occur. Some of the products of lipid oxidation have low taste thresholds and can be detected organoleptically before decreases in polyenoic acids can be detected by GLC. For example, Meijboom and Stroink (1972) assessed the fishy flavor of 2-trans, 4-cis, 7-cis-decatrienal at a concentration of 0.001%. The fishy flavor profile did not appear in this study until late in the storage period and then at low intensity (Gibson et al., 1977). Shono and Toyomizu

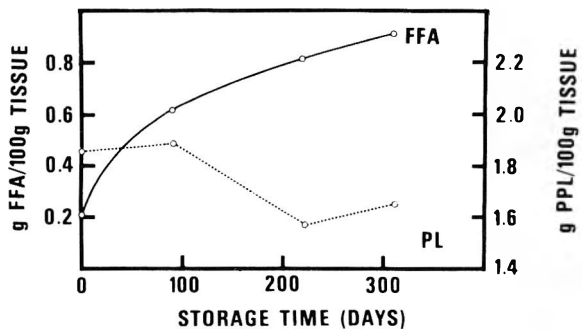

Fig. 1—Changes in free fatty acid (FFA) and phospholipid (PL) during storage at −18° C.

(1971) observed large changes in the ratio of 22:6/16:0 in fish held for 3–5 days at 5°C and suggested that the extent of oxidative deterioration be expressed as percent decrease in this ratio. Similarly Worthington (1973) observed large (25–50%) decreases in levels of 22:6 in processed catfish grown in raceways on a diet high in polyenoic acids (Heaton et al., 1973) and subsequently stored for 60 days at −18°C. In an evaluation of catfish grown in open-air concrete ponds on diets containing fats from several sources (Worthington and Lovell, 1973), Lovell (1972) observed an objectionable fishy odor and flavor in fish receiving menhaden oil, and reported that an increase in this characteristic during frozen storage at −18°C was paralleled by an increase in TBA (thiobarbituric acid) values. Clearly the results of the present study are at variance with these earlier observations.

The reasons for these differences in stability of catfish during frozen storage may be related to differences in degree of oxidation of dietary menhaden oil at the time of ingestion, a variable that was not measured in either of the studies, or it may be related to differences in cultural methods. Tank culture, the method used in the production of fish evaluated in this study, provides for systematic replacement of water and removal of extraneous feed, fecal matter, and other metabolic end products (Andrews et al., 1971), thus, malodorous compounds derived from oxidized feed lipid were not allowed to accumulate in environmental waters. Oxygen stress is not a significant factor in the culture of fish by this method (Andrews et al., 1971). By contrast, excess feed, excrement, and metabolic end products accumulate and serve as substrates for numerous micro and macroorganisms in the static waters of pond culture and in the recycled waters of raceways. In addition oxygen stress may also occur in pond waters. The actual relationship of these cultural factors to the quality and storage stability of catfish is unknown and merits further study.

Triglycerides

Approximately 77% of TL were TG and consequently the fatty acid spectrum of this fraction was similar to that of total lipids. Average TG values were slightly higher (24%) for those fish frozen with nitrogen spray than for those frozen at −18°C (22%) and an analysis of variance showed this difference to be significant at the 5% level of probability. The difference in triglyceride levels associated with freezing method might be attributed to the slower rate of freezing permitting enhanced lipase activity; however, corresponding differences due to freezing method were not observed in levels of total free fatty acids which averaged 0.48% in fish frozen by each method. Furthermore levels of TG which averaged 23% during the course of the study did not change significantly during frozen storage. Although lipase activity has been noted in other species of fatty fish (Olley et al., 1962), the difference in TG

Table 1—Influence of dietary fat on fatty acid distribution of phospholipids (PL), and free fatty acids (FFA) and of frozen storage at −18°C on the fatty acid distribution and levels of free fatty acids in catfish

	Dietary fat											
	Menhaden oil						Tallow					
	0 days[a]				312 days		0 days[a]				312 days	
Fatty acid	TG (%)[b]	PL (%)[b]	FFA (%)[b]	FFA (Mg/100g)[c]	FFA (%)[b]	FFA (mg/100g)[c]	TG (%)[b]	PL (%)[b]	FFA (%)[b]	FFA (mg/100g)[c]	FFA (%)[b]	FFA (mg/100g)[c]
14:0	7.0	2.7	5.0	12	3.9	36	2.3	1.2	2.1	5	1.3	12
16:0	18.9	22.8	18.3	4	14.2	131	19.1	21.8	16.2	39	13.1	124
16:1	10.0	4.6	9.6	23	9.3	86	4.5	3.5	4.6	11	5.1	48
18:0	3.9	7.4	5.8	14	5.4	49	6.4	7.5	6.7	16	6.1	58
18:1	29.9	18.5	29.6	71	26.1	240	51.7	30.4	46.7	112	41.5	394
18:2	8.8	4.6	9.2	22	8.4	77	8.0	8.4	9.2	22	11.4	108
18:3	2.7	1.3	2.5	6	3.0	28	2.0	1.4	1.7	4	2.1	20
20:5	7.3	10.2	9.2	22	11.3	104	0.7	2.4	1.7	4	2.3	22
22:5[d]	2.9	4.4	4.2	10	4.8	44	0.3	1.4	2.5	6	3.6	34
22:6	3.6	13.0	4.2	10	6.7	62	0.56	6.6	1.7	4	4.2	40
Other	5.0	10.5	2.4	6	6.9	63	4.5	15.4	6.9	17	9.3	88
Total	100.0	100.0	100.0	240	100.0	920	100.0	100.0	100.0	240	100.0	950

[a] Post-freezing
[b] Percent distribution
[c] mg/100g lyophilized tissue
[d] Includes ω3 and ω6 isomers

values between different freezing methods found to be significant in this study appears to have been due to sources of variance other than those included in the analysis of variance or to chance because of the random variability inherent in the testing for significance. Catfish typically show large fish-to-fish variations in total lipid – and therefore triglyceride – content.

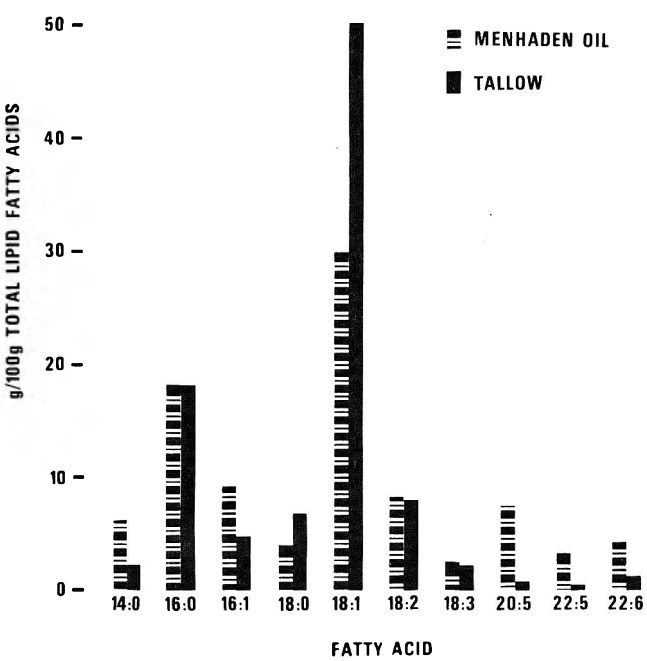

Fig. 2—Weight percentages of fatty acids from total lipids of catfish according to source of dietary lipid.

Phospholipids

The distribution of fatty acids in PL was also influenced by dietary lipids but not to the extent as was observed in TG (Table 1). Phospholipids were richer in polyenoic acids having 20 or more carbons and less rich in 14:0, 16:1, and 18:1. Those fish receiving menhaden oil accumulated considerable quantities of the polyenoic acids in the TG fraction; therefore, the greatest difference in PL and TG fatty acid makeup was observed in those fish that had received tallow (Table 1).

The level of extractable phospholipids exhibited considerable change with time ($P \leq 0.01$) and was influenced by storage temperature. In those fish stored at −35°C, the extractable PL values increased from an initial average value of 1.85% to 3.5% at 220 days storage and declined thereafter to 2.75% at 312 days. Comparable values for fish stored at −18°C were 1.85%, 1.50% and 1.63% after 0, 220 and 312 days storage (Fig. 1). Although the percent extractable PL decreased with time at −18°C, the distribution of fatty acids within this fraction did not change.

As is discussed below, the level of FFA increased with storage at −18°C, and this coupled with the increase in proportion of polyenoic acids in the FFA fraction and concurrent decrease in PL (Fig. 1) is strong evidence of phospholipase activity during frozen storage. Considerable scatter was observed in the PL data and therefore measured changes in levels of PL were not as good an indicator of lipolysis as were measured levels of FFA. Experimental error was probably the greastest contributor to the scatter of data: Florisil column bleed was noted near the end of the study and in addition Palmer (1971) has reported that some of the phospholipids may be readsorbed on sample tissues during extraction by the Bligh and Dyer technique. The catfish used in this study were not segregated according to sex and this may also have contributed to scatter of PL data. Ackman et al. (1969) reported sexual differences in levels of PL in capelin (*Mallotus villosus*).

Free fatty acids

Diet had a significant effect on the distribution pattern of

fatty acids within the FFA fraction (Table 1) and this effect was statistically significant for all fatty acids except the 22:5 isomers although levels of 18:2 were identical in unfrozen fish. Changes in the amount of FFA were also determined to be significantly related to temperature of storage. Minimal change occurred in fish stored at −35°C whereas a large increase occurred in fish stored at −18°C particularly during the initial 94 days of storage. During this period the level of FFA more than doubled from a post freezing level of 0.25 g/100g freeze-dried tissue to a level of 0.56g (Fig. 1). A substantial increase in FFA also occurred in the fish that had been held on ice for 2 days for analysis in the unfrozen state; these fish contained approx 80% (0.45g/100g) as much FFA as fish that had been stored for 94 days at −18°C. The FFA of the iced fish were not as rich in some of the polyenoic acids as were the FFA of fish stored for 94 days, and in some cases were lower than post freezing values. These differences suggest that the FFA in the iced fish were derived in part from lipase activity. Levels of total FFA continued to increase during storage at −18°C but at a decreasing rate until a level of 0.93% was reached at 312 days.

Although the mean percentage increase in the amount of total FFA was approximately 290%, the increase for individual acids was somewhat greater as shown by data in Table 1. The individual fatty acids increasing in concentration the most for both diets were the polyenoic acids 22:6ω3 and 20:5ω3, but the differences in lipid unsaturation (Fig. 2) did not have a measurable effect on the extent of overall FFA production (Table 1). Thus, it appears that the FFA formed during frozen storage were derived from phospholipids or from other lipids high in polyenoic acids.

On a fresh weight basis, the FFA content of fish stored at −18°C reached a final level of approximately 230 mg per 100g of tissue and would probably not have increased much more since FFA production appeared to be reaching a constant level at the end of the storage period (Fig. 2). The final levels of FFA were similar to those observed in other frozen fish stored at approximately the same temperature and for approximately the same length of time (Olley et al., 1969) but did not have a deleterious effect on organoliptic properties (Gibson et al., 1977). In fact those fish maintained at −18°C for 312 days were rated slightly superior ($P \leq 0.05$) in texture (Gibson et al., 1977) when compared with fish maintained at −35°C.

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BACTERIOLOGICAL SURVEY OF THE CHANNEL CATFISH (Ictalurus punctatus) AT THE RETAIL LEVEL

ABSTRACT

Using standard procedures, aerobic plate counts (APC), total and fecal coliform most probable number (MPN) determinations, and analyses for *Salmonella, Arizona, Shigella* and *Edwardsiella tarda* were performed on 335 fresh and 342 frozen sample units of channel catfish. APC values for 93.0% of the fresh and 94.5% of the frozen samples were $\leq 10^7$ organisms per g. Fecal coliform MPN values for 70.7% of the fresh and 92.4% of the frozen samples were ≤400 organisms per g. The occurrence of *Salmonella* in channel catfish was subject to seasonal variation. *Salmonella*-positive samples yielded 15 serotypes from 8 somatic groups. *Edwardsiella tarda* was isolated from two samples; *Arizona* and *Shigella* were not detected.

INTRODUCTION

THE RECENT RISE in cost of red meats and poultry has pressured consumers to seek other less expensive sources of protein. One of these has been the channel catfish, *Ictalurus punctatus*. These catfish are bottom-feeding scavengers, able to efficiently convert consumed food into edible body protein over a relatively short period of time. In addition to being caught from fresh-water ponds and lakes, primarily in Florida, Alabama, Mississippi, Louisiana, Arkansas and Texas, these fish are cultured or farmed in large open, shallow ponds. Catfish farming has become a successful commercial enterprise in the mid-South, with 8.6 million pounds of farm-raised catfish processed during the first 6 months of 1974 (NOAA/NMFS, 1974).

Although fish rarely, and catfish never, have been definitely incriminated in human outbreaks of salmonellosis and related enteric foodborne illnesses, a number of published reports document the possibility of such an occurrence. Meyer and Bullock (1973) isolated Gram-negative, motile, rod-shaped bacteria from the lesions of diseased channel catfish in an outbreak of what was called "emphysematous putrefactive disease of catfish." The causative organism was subsequently identified as the human enteric pathogen, *Edwardsiella tarda*. Working with the brown bullhead catfish (*I. nebulosus*, Le Sueur), Troast (1975) demonstrated high serum titers to *Escherichia coli* and *Enterobacter cloacae*. Several human enteric pathogens were isolated from the Crucian carp, another type of bottom-feeding scavenger with feeding habits similar to those of channel catfish (Lee, 1972). Based on the foregoing reports and on the continuing large supply of fresh-water catfish entering interstate commerce, the Food & Drug Administration (FDA) conducted a bacteriological survey of fresh-water catfish at the retail level. The objectives of this survey were to determine what percentage of marketed catfish at the retail level would meet proposed quality standards, and to determine the incidence of human enteric pathogens in this commodity.

EXPERIMENTAL

Collection of samples

Samples were collected by FDA inspectors in the Atlanta, Orlando, Nashville, Dallas and Houston areas because of the availability and economic importance of catfish in these geographical regions. To determine seasonal variation in the occurrence of pathogenic enteric bacteria in catfish, sampling was conducted during two different seasonal periods (Jan. 2–March 31, 1975 and July 1–Sept. 30, 1975). During each sampling period, each inspector collected 50–75 units of fresh (iced), fresh-water catfish and a similar number of units of frozen, fresh-water catfish. Each unit was a retail package of the finished product, weighing 1–5 lb, as offered for sale by the processor. The inspectors were asked to select samples from processors within each district, representing as wide a geographical area as possible. Additionally, they were asked to evenly space the collection of samples within each seasonal period. Finally, the processors were asked to identify, whenever possible, the exact source of their catfish in order that the relative incidence of pathogenic enteric bacteria in farm-reared and commercially caught catfish could be established.

All samples were sent air express to the Division of Microbiology laboratories in Washington, D.C., for bacteriological analysis. Fresh samples were packed with wet ice or with a dry refrigerant, while frozen samples were packed with dry ice (solidified CO_2). Fresh samples were given priority for bacteriological analysis and were analyzed immediately or within 48 hr of their receipt. Frozen samples were either analyzed immediately or transferred to a freezer until they could be examined. In general, no more than 2 wk lapsed between collection and initiation of analysis of the frozen samples. A total of 335 units of fresh catfish from 41 processors and 342 units of frozen catfish from 23 processors were examined during the survey. The majority of the processors were located in Florida (13), Mississippi (8), Alabama (7) and Texas (5). Other processors from which samples were collected were located in Georgia (3), Arkansas (3), Tennessee (1) and North Carolina (1).

Bacteriological analysis

Aerobic plate counts (APC), as well as total and fecal coliform most probable number (MPN) determinations, were performed on each unit by the official method for the examination of frozen, chilled, precooked, or prepared foods (AOAC, 1970). Additionally, the units were analyzed for *Salmonella, Arizona* and *Shigella* according to procedures in the *Bacteriological Analytical Manual for Foods* (BAM) (FDA, 1972). Additionally, the samples were analyzed for the presence of *Edwardsiella tarda* by the cultural procedures as outlined for the isolation of members of the genus *Salmonella*.

From each sampling unit a 100-g portion of the edible flesh was taken as the analytical unit. Four hundred ml of Butterfield's phosphate buffer (pH 6.8–7.0) was added as the diluent and the mixture was blended for 60–120 sec at 14,000 rev/min in a commercial blender. To maximize the possibility of detecting *Salmonella, Arizona* and *Edwardsiella*, both the lactose preenrichment and the direct selective enrichment procedures of BAM (FDA, 1972) were used. Three 50-ml aliquots of the catfish homogenate were pipetted into 500-ml Erlenmeyer flasks containing 250 ml of 0.5% lactose broth, selenite cystine (SC) broth, or tetrathionate (TT) broth with 10 mg added brilliant green dye per liter. Unless stated otherwise, all dehydrated culture media used in this study were obtained from Difco. The flasks were swirled thoroughly to completely mix the contents and the pH was adjusted to 6.8 ± 0.2; they were then placed in a walk-in incubator (35°C) for 24 ± 2 hr. A 1-ml portion of the incubated lactose broth mixture was pipetted into tubes containing 9 ml of fresh, 35°C-tempered SC or TT broth. After an additional 24 hr of incubation at 35°C, the selective enrichment broths, including those from the lactose preenriched samples as well as those from the directly enriched samples,

were streaked onto plates of brilliant green 24-hr aged bismuth sulfite (BS) and *Salmonella-Shigella* agar. All agar plates were incubated (35°C) and examined at 24 ± 2 hr. The BS agar plates were incubated an additional 24 hr and re-examined. When present, at least two colonies suspicious for *Salmonella, Arizona*, or *Edwardsiella* were picked from each plate to tubes of triple sugar iron (TSI) agar and lysine iron agar. Cultures giving reactions in the differential agars that were suspicious for these bacterial pathogens were submitted to biochemical screening and definitive serotyping (Edwards and Ewing, 1972).

Similarly, the analysis of the sample units for *Shigella* was in accordance with procedures detailed in *BAM* (FDA, 1972). A 50-ml aliquot of the catfish homogenate was pipetted into 500-ml Erlenmeyer flasks containing 250 ml of Gram-negative (GN) broth. The flasks were swirled thoroughly and the pH of the contents was adjusted to 6.8 ± 0.2. After incubation (35°C) for 16 ± 2 hr, the GN broth and the SC broth (also used in the direct enrichment procedure for *Salmonella, Arizona*, and *Edwardsiella*) were streaked to plates of xylose lysine decarboxylase, Levine's eosin methylene blue, and desoxycholate citrate agars. After incubation (35°C) for 24 hr, the plates were examined and colonies typical for *Shigella* were picked to TSI agar. Suspicious *Shigella* cultures were biochemically screened and, if necessary, serotyped. All *Salmonella* serotypes not isolated previously by this laboratory and all enteric pathogens other than *Salmonella* were sent to the Enterobacteriology Branch, Center for Disease Control, Atlanta, Ga. for confirmation of identification.

RESULTS & DISCUSSION

TABLE 1 summarizes the results of the bacteriological analyses of 335 units of fresh catfish collected from 41 processors

Table 1—Comparison of bacteriological analysis of fresh catfish from various processors

Processor	Area	No. of units examined[a]	Aerobic plate count/g (median)	Total coliform MPN/g (median)[b]	Fecal coliform MPN/g (median)[b]	No. of units containing pathogens
1	FL[c]	21	1.2×10^6	1.5×10^3	7.3	
2	FL	15	9.3×10^4	4.6×10^2	9.3	
3	FL	15	3.8×10^6	2.3×10^3	6.4×10^2	
4	FL	12	7.5×10^4	7.5×10^1	<3	
5	FL	8	1.6×10^7	2.8×10^4	1.2×10^3	2 *S*[d]
6	FL	7	1.9×10^8	2.4×10^6	2.4×10^3	
7	FL	6	2.7×10^5	3.6×10^3	1.2×10^2	
8	FL	6	7.0×10^3	2.1×10^2	3.0	
9	FL	6	2.4×10^6	5.3×10^3	1.6×10^2	1 *S*
10	FL	5	8.2×10^5	3.6×10^3	1.9×10^1	
11	FL	5	3.8×10^6	5.3×10^3	2.9×10^1	
14	MS	19	3.9×10^6	7.5×10^3	3.4×10^1	4 *S*
15	MS	18	1.8×10^6	7.5×10^1	2.8	1 *S*
16	MS	10	2.3×10^6	3.9×10^3	1.6×10^1	1 *S*
17	MS	10	6.9×10^3	<3	<3	
18	MS	8	6.7×10^5	1.9×10^3	<3	
19	MS	6	7.4×10^5	9.5×10^1	1.1	
20	MS	5	2.6×10^6	6.4×10^3	2.1×10^2	4 *S*
21	MS	5	9.8×10^5	3.9×10^3	1.5×10^2	
22	AL	10	2.7×10^6	4.6×10^3	1.6×10^2	
23	AL	5	5.9×10^5	9.5×10^2	<3	
24	AL	5	1.7×10^6	6.4×10^3	6.0	
25	AL	5	7.6×10^5	3.9×10^3	1.6×10^2	
26	AL	5	1.4×10^7	1.2×10^3	1.2×10^2	
27	AL	5	3.7×10^6	5.3×10^3	4.6×10^2	
28	AL	5	2.7×10^6	2.9×10^3	7.5×10^1	
29	LA	7	4.1×10^7	6.4×10^3	4.6×10^2	
30	LA	7	4.1×10^6	4.3×10^3	<3	
31	LA	6	1.9×10^6	9.3×10^3	1.1×10^1	
32	LA	6	7.4×10^5	2.9×10^3	2.1	
36	TX	10	4.1×10^5	4.2×10^3	2.4	
37	TX	5	2.4×10^5	1.2×10^2	1.1	
38	TX	5	1.9×10^5	1.5×10^2	<3	
41	GA	8	3.4×10^5	4.3×10^3	1.6×10^1	
42	GA	8	7.4×10^7	6.4×10^3	1.5×10^2	
43	GA	6	1.9×10^8	2.1×10^4	1.2×10^2	
44	AR	10	8.1×10^5	7.5×10^3	6.4×10^1	1 *S*; 1 *E*[d]
45	AR	5	6.0×10^5	7.5×10^3	1.5	
46	AR	5	1.5×10^6	2.3×10^3	<3	1 *S*
47	TN	10	2.6×10^6	4.6×10^3	4.6×10^2	
48	NC	10	4.3×10^6	1.1×10^4	4.2×10^1	1 *E*

[a] A unit was a 1–5 lb retail package.
[b] MPN: Most Probable Number.
[c] FL, Florida; MS, Mississippi; AL, Alabama; LA, Louisiana; TX, Texas; GA, Georgia; AR, Arkansas; TN, Tennessee; NC, North Carolina.
[d] S, Salmonella; E. Edwardsiella.

representing 9 states. When compared according to three bacteriological indices, wide ranges in median values were observed among the processors of fresh catfish: APC values (6.9×10^3 to 1.9×10^8 per g), total coliform MPN values (<3 to 2.4×10^6 per g) and fecal coliform MPN values (<3 to 2.4×10^3 per g). *Salmonella* was isolated from 15 units (4.5%) and *Edwardsiella tarda* from two units (0.6%). APC values, total coliform MPN values, and fecal coliform MPN values for individual units containing *Salmonella* and/or *Edwardsiella* ranged from 1.5×10^5 to 7.4×10^7 g, from 4.3×10^3 to 1.1×10^5 per g, and from <3 to 4.3×10^3 per g, respectively.

In Table 2 are listed the results of the bacteriological analyses of 342 units of frozen catfish. As with the fresh catfish, wide ranges in median values of three bacteriological indices were observed among the processors of frozen catfish: APC values (4.2×10^3 to 1.7×10^8 per g), total coliform MPN values (<3 to 9.3×10^3 per g), and fecal coliform MPN values (<3–230 per g). Although *E. tarda* was not recovered from any of the frozen units, *Salmonella* species were recovered from five samples (1.5%). APC values and total and fecal coliform MPN values for the individual units of frozen catfish containing *Salmonella* ranged from 2.2×10^5 to 8.3×10^7 per g, 9.3 to 2.3×10^4 per g, and <3 to 4.3×10^3 per g, respectively.

Comparative APC values of fresh and frozen catfish sample units are given in Table 3. The choice of the various category levels was arbitrary and, overall, there was no great difference in counts between sample units of fresh and frozen catfish. The International Commission on Microbiological Standards for Foods (ICMSF, 1974), an internationally recognized body, has established standards for certain categories of fish and fish products. It was thought to be of interest to compare the microbiological data obtained from the examination of catfish to those standards published by ICMSF for other types of fresh water fish. Basically, up to three of five samples examined per lot of fish may have APC values between 10^6 and 10^7 organisms per g, but an APC value in excess of 10^7 by even one sample would result in the whole lot being unacceptable. The ICMSF procedure recommends the performance of plate counts at 25°C rather than at 35°C as used in this study. In spite of this differential, it is interesting to compare the results of this study with the recommended ICMSF standards. 7% of the fresh and 5.5% of the frozen samples had APC values in excess of 10^7 organisms per g. Considering that all samples had either been chilled or frozen, it should be realized that psychrophilic organisms would constitute a significant portion of the total microflora for this product. Had the lower incubation temperature been used in this study, it is likely that higher APC values would have been obtained, resulting in larger percentages of both fresh and frozen catfish not being able to meet the recommended ICMSF standard.

The comparative distribution of total and fecal coliform MPN values for fresh and frozen catfish is given in Table 4. The choice of MPN categories was patterened according to the recommended fecal coliform limits for fresh and frozen fish by ICMSF (1974). Limits for total coliform MPN values have not been established. Up to three of five samples per lot may have fecal coliform MPN values between 4 and 4×10^2 organisms per g, with no value exceeding 4×10^2 fecal coliforms per g. Under this standard, 29.3% of the fresh sample units and 7.6% of the frozen sample units would have been judged unacceptable. Unlike the similarity in APC values of the fresh and frozen catfish, there was a significant difference in both the total and fecal coliform MPN values of the fresh and frozen

Table 2—Comparison of bacteriological analysis of frozen catfish from various processors

Processor	Area	No. of units examined[a]	Aerobic plate count/g (median)	Total coliform MPN/g (median)[b]	Fecal coliform MPN/g (median)[b]	No. of units containing pathogens
1	FL[c]	10	1.4×10^6	5.3×10^1	<3	
4	FL	27	8.0×10^4	7.5×10^1	<3	
5	FL	10	2.5×10^7	2.1×10^3	2.3×10^2	1 *S*[d]
6	FL	35	1.5×10^6	9.3×10^1	<3	1 *S*
7	FL	8	3.0×10^5	2.9×10^2	2.1×10^1	
10	FL	10	5.4×10^5	2.9×10^1	2.8	
12	FL	15	2.0×10^5	4.3×10^1	1.1	
13	FL	10	3.4×10^5	9.3×10^1	<3	
14	MS	36	5.2×10^6	9.3×10^1	3.0	1 *S*
15	MS	39	7.5×10^5	4.3×10^1	<3	1 *S*
16	MS	13	2.6×10^6	7.5×10^1	3.0	
29	LA	20	3.7×10^6	7.5×10^2	4.3×10^1	
31	LA	18	6.0×10^5	2.1×10^1	<3	
33	LA	10	4.2×10^3	4.3×10^1	6	
34	LA	10	8.0×10^5	7.5×10^2	4.3×10^1	
35	LA	10	1.7×10^8	9.3×10^3	1.5×10^2	
37	TX	5	4.5×10^6	7.5×10^1	<3	
39	TX	15	2.8×10^6	7.2×10^2	2.1	1 *S*
40	TX	5	4.0×10^5	<3	<3	
41	GA	5	2.0×10^5	2.1×10^3	1.1	
44	AR	5	4.7×10^5	7.5×10^1	1.5	
45	AR	15	6.0×10^5	2.1×10^1	<3	
47	TN	11	2.3×10^6	4.3×10^2	1.2×10^2	

[a] A unit was a 1–5 lb retail package.
[b] MPN: Most Probable Number.
[c] FL, Florida; MS, Mississippi; LA, Louisiana; GA, Georgia; AR, Arkansas; TN, Tennessee.
[d] S. Salmonella.

catfish sample units. The survival of coliform organisms at sub-freezing temperatures has been the subject of a number of investigations. Kereluk and Gunderson (1959) reported that sterile gravy samples inoculated with equal numbers (5.6 x 10^6 organisms per ml) of *Escherichia coli* and *Enterobacter aerogenes* demonstrated an MPN count of 66 organisms per ml after 481 days of storage at −6°F. In comparing the viability of *E. coli* with fecal streptococci in frozen green beans (1955a) and in frozen orange concentrate (1955b), Larkin et al. found that MPN values of *E. coli* decreased significantly during prolonged storaged (217 days at 0°F and 147 days at −10°F, respectively), whereas the level of streptococci remained relatively constant. In frozen vegetables held at 0°F for 2 yr, Hucker (1954) found that the predominating organisms were members of the genera *Flavobacterium* and *Achromobacter*. A relatively large percentage of psychrophilic microflora indigenous to the catfish surveyed in this study would explain the lack of disparity in the APC values of the fresh and frozen catfish sample units (Table 3). Even though the overall incidence of *Salmonella* in catfish was low, there was a threefold higher incidence of this pathogen in fresh (4.5%) as compared to frozen (1.5%) catfish (Tables 1 and 2). It is logical to expect the lack of survival of *Salmonella* at freezing or sub-freezing temperatures to be similar to that of *E. coli*. In studying the survival of *Salmonella* in butter at ≤40°F, Sims et al. (1969) found that the longer the sample was held at this temperature, the fewer *Salmonella* organisms survived. McCleskey and Christopher (1941) reported a significant reduction in the levels of four *Salmonella* serotypes inoculated into sliced strawberries and frozen at −0.4°F for up to 8 months.

Table 5 compares the incidence of the bacterial enteric pathogens in catfish on the bases of season and source (whether caught commercially or farm-reared). Overall, more *Salmonella* isolates were recovered from farm-reared than commercially caught catfish. When compared on a percentage basis, however, there was no significant difference in the incidence of *Salmonella* in commercially caught and farm-reared catfish. Both the actual number of *Salmonella* isolates and the percentage of *Salmonella*-positive units was greater during the July-Sept. sampling period. These results are in agreement with those of Meyer and Bullock (1973), who reported that disease outbreaks in catfish caused by *E. tarda* occurred only during the period of July-Oct. when water temperatures were above 30°C.

Table 6 lists the 15 *Salmonella* serotypes isolated from catfish in this survey. Fifteen units contained a single serotype of *Salmonella*, three units contained two serotypes, and one unit was contamined with *S. newport, S. litchfield* and *S. 6,7:1,5:−*. One of the two *E. tarda*-postive units also contained *S. anatum*. No members of the related genera, *Arizona* and *Shigella*, were isolated.

Most of the procedures in *BAM* (1972) used in the analysis of foods for the presence of *Salmonella* require preenrichment of the sample in 0.5% lactose broth, a practice based on the findings of North (1961). In foods where gross contamination is suspected (e.g., red meats and poultry) the lactose preenrichment step may be by-passed so as to minimize the possibility of over-growth of the *Salmonella* organisms by the competing lactose-fermenting organisms. Unfamiliarity with the history of this product was the basis for the use of both the lactose preenrichment and the direct selective enrichment procedures for *Salmonella* analysis in this survey. Twelve samples were exclusively positive for *Salmonella* by the lactose preenrichment procedure, one sample was positive exclusively by the direct enrichment procedure and seven samples were positive by both procedures. Thus, the use of the lactose preenrichment broth

Table 3—Distribution of aerobic plate counts of fresh and frozen catfish

Aerobic plate count/g	Percent of samples	
	Fresh	Frozen
$>10^8$	2.9	2.6
$>10^7 - 10^8$	4.1	2.9
$>10^6 - 10^7$	39.1	35.1
$>10^5 - 10^6$	30.4	37.1
$>10^4 - 10^5$	19.8	19.9
$0 - 10^4$	3.7	2.4

Table 4—Distribution of total coliform and fecal coliform MPN values of fresh and frozen catfish

MPN value/g	Percent of samples			
	Total coliforms		Fecal coliforms	
	Fresh	Frozen	Fresh	Frozen
>1000	54.0	7.6	6.9	4.4
401–1000	14.9	5.0	22.4	3.2
101–400	10.1	12.3	17.0	4.7
31–100	9.0	41.2	5.1	7.0
4.1–30	9.2	30.7	29.6	7.9
<3.0–4.0	2.8	3.2	19.0	72.8

Table 5—Incidence of Salmonella and Edwardsiella in farm-reared and commercially caught catfish during two different seasons

	Samples positive for enteric pathogens							
	Jan.–March				July–Sept.			
	Commercially caught		Farm-reared		Commercially caught		Farm-reared	
Pathogen	No.	%[a]	No.	%[b]	No.	%[c]	No.	%
Salmonella	1	0.8	2	0.9	6	4.4	11	5.7
Edwardsiella	0	0	0	0	1	0.7	1	0.5

[a,b,c,d] Expressed as percentage of 128, 220, 137 and 192 samples, respectively.

Table 6—Salmonella serotypes isolated from catfish

Serotype	Somatic group	No. of samples positive for this serotype
S. newport	C_2	5
S. anatum	E_1	2
S. infantis	C_1	2
S. inverness	38	2
S. montevideo	C_1	2
S. saint-paul	B	2
S. typhimurium	B	2
S. daytona	C_1	1
S. heidelberg	B	1
S. javiana	D	1
S. litchfield	C_2	1
S. oranienburg	C_1	1
S. poona	G_1	1
S. rubislaw	F	1
S. 6,7:1,5:−	C_1	1

proved to be an essential step for the maximal recovery of *Salmonella* from this product.

The low incidence of bacterial enteric pathogens in channel catfish may be due to the sanitizing procedures followed during the processing of this commodity. Typically, the fish are received by the processor in wire baskets. After organoleptic examination and weighing, the baskets of fish are washed in a chlorine dip (300 ppm) solution. The fish are immediately eviscerated with care taken not to rupture the gastrointestinal tract. Afterwards, the fish are individually washed and then skinned with pliers. The skinned fish are spray-washed and are decapitated with a hand saw. Workers' hands and utensils are sanitized in an iodine dip (25 ppm) solution between each of the above operations for each individual fish. The eviscerated, skinned, decapitated, fish are inspected and trimmed, if necessary, before packaging. The final packaged product is either refrigerated or frozen until sale at the retail outlet. Other factors contributing to minimizing the incidence of pathogens in channel catfish could be the biochemical incompatibility of the catfish's skin, flesh and/or gastorintestinal tract as a suitable substrate for enteric pathogens; inhibition of *Salmonella* by competing microflora both in the aquatic environment and within the gut of the catfish; and incorporation of antibiotics and other selectively bactericidal agents into the feeds. Additional epidemiological investigations are needed, however, to substantiate any of these possibilities.

Considering the overall low incidences of enteric pathogens in catfish and the terminal heating of the fish before they are consumed, it seems reasonable to conclude that the microbiological hazard potential of this particular type of domestic catfish is low. Recently, however, domestic catfish farming has felt increased competition from a large influx of less expensive catfish imported primarily from Brazil and Mexico. Freshwater catfish imports for the first 6 months of 1974 reached 4.6 million pounds, an increase of 44% over the same period for 1973 (NOAA/NMFS, 1974). Our limited experience to date with imported catfish suggests that the incidence of *Salmonella* may be somewhat higher than in domestic catfish.

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COMPARATIVE PROTEIN STUDIES (KJELDAHL, DYE BINDING, AMINO ACID ANALYSIS) OF NINE STRAINS OF *Agaricus bisporus* (Lange) IMBACH MUSHROOMS

ABSTRACT

Although it is difficult to ascertain the true protein content of mushrooms because of their variable nonprotein nitrogen content and protein heterogeneity, even among strains within a species, some knowledge about a mushroom's protein is essential for nutritional, processing and economic considerations. Protein similarities and differences in cultivated mushrooms are shown with the aid of three analytical methods. Protein data based on amino acid analyses are preferred but too expensive to obtain. Amido black dye binding is the next preferable method. Its correlation with amino acid protein was higher (D.74) than that between Kjeldahl protein and amino acid protein (D.44).

INTRODUCTION

EARLIER RESEARCH on mushroom protein has resulted in the identification of a number of ninhydrin-reacting compounds including amino acids and amino acid derivatives (Block et al., 1953; Hughes et al., 1958; Renard and Casimir, 1953). Altamura et al. (1967) isolated 53 ninhydrin-reacting compounds from mushrooms. All these studies on the isolation and identification of amino compounds involved various species of mushrooms. The findings may have been of interest at the time, but today, with more modern analytical tools available, it has become desirable for not only those interested in mushrooms to apply these tools and to learn more about the nature of protein in this food product.

The primary mushroom of commerce in North America and Europe is *Agaricus bisporus*, the culture of this mushroom having begun in France in the early eighteenth century. During the past 200 yr a large number of isolates, selections, or strains has been developed. They can be grouped conveniently into four varieties commonly referred to as white, off white or golden white, light cream, and brown. Generally speaking, scaliness, average size, specific gravity, shelf life and certain other characteristics vary directly as natural pigmentation increases. Little is known, however, of the protein composition within this species.

This study was undertaken to examine nine strains of the species *Agaricus bisporus* (Lange) Imbach with the aid of the Kjeldahl method, amido black dye binding procedure, and amino acid analysis. The protein percentages reported here are not true values. They are meant to illustrate only differences among strains within a species.

EXPERIMENTAL

Mushrooms

In these studies, nine strains were used representative of the 4 varieties within the species. All mushrooms were produced from grain spawn, in the same crop, in the same growing room, and under nearly identical cultural conditions of nutrition, environment, and general management. All strains used are deposited in the Mushroom Culture Collection of The Pennsylvania State University as follows: whites 310, 318; off whites D26, 341, 348; light creams 322, 324; and browns 321, 340. Furthermore, these cultures were obtained from each of the four common sources: single spores, multispores, mycilial transfer, and tissue.

All mushrooms analyzed were taken from the first flush or break of the crop. It is possible that corresponding tissue from other flushes would give different analyses.

Methods

The freshly harvested mushrooms were diced, kept a few days in frozen storage, then freeze-dried and subsequently further dried in a 100°C oven at 25 in. of vacuum for 24 hr. This procedure also supplied the total solids (dry matter) data. The samples were then pulverized in a Foss-Let Reactor (A/S N. Foss Electric, Hillerod, Denmark) to be further analyzed.

Organic nitrogen determinations were carried out according to the micro-Kjeldahl method (AOAC, 1975). A conversion factor of 6.25 was chosen to convert to protein percentages. This factor is obviously inaccurate and too high for mushrooms because of their variable and relatively high concentration of nonprotein nitrogen (mainly chitin). Nevertheless, in a comparison any factor is suitable.

Dye binding analyses were made with a Pro-Milk Mk II instrument (A/S N. Foss Electric, Hillerod, Denmark). However, first the samples, weighing 0.1 g, were mixed with 20 ml amido black dye solution for 15 min on a Multireactor (A/S N. Foss Electric, Hillerod, Denmark). For each sample a dye binding value was obtained from the lower scale of the instrument's galvanometer unit. These were converted to protein values with a standard curve based on bovine serum albumin (Nutritional Biochemicals Corp., Cleveland, Ohio). Any other protein would have served this purpose, since only comparative results, rather than absolute protein percentages, were expected.

Amino acids were determined with a Beckman 120 C Amino Acid Analyzer. The dried mushroom material was weighed into a 10-ml ampule (Wheaton Scientific Co., Millville, N.J.). The 0.01-g sample weight was based on a 100% protein weight as calculated from Kjeldahl nitrogen. Before the ampules were heat-sealed, 5 micromole norleucine, an internal standard, and 5 ml 6N HCl were added. The samples were hydrolyzed in an oven at 110°C for 24 hr. The hydrolysates were then passed through a glass filter and evaporated to dryness in a rotary evaporator at 40°C. The residue was redissolved in distilled water and again evaporated to dryness. Then 50 ml distilled water was added and the samples frozen until used. One-ml samples were applied to the long and short columns of the analyzer. Data for tryptophan are not shown because they cannot be obtained through acid hydrolysis.

Data processing and statistical analyses were carried out with the aid of an IBM computer at the University's Computation Center. An analysis of variance was performed on the data followed by Duncan's modified (Bayesian) least significant difference test (Duncan, 1965) for the mean separations.

RESULTS & DISCUSSION

A SUMMARY of the amino acid data from the nine strains of mushrooms is contained in Table 1. In general, the overall pattern is similar among the strains. There was no significant difference among the means of the basic amino acids lysine, histidine and arginine as denoted by a common code letter. These are the ones involved in dye binding. Table 1 also contains the mean separation data for the amino acids. Glutamic

Table 1—Amino acid composition of nine strains of Agaricus bisporus (g/100g protein)[a]

Amino acid	Strain of Agaricus bisporus								
	D26	324	310	340	318	348	322	321	341
Lysine**	12.1a	10.8a	8.1a	8.4a	7.7a	9.8a	10.9a	11.2a	10.9a
Histidine	2.2a	2.5a	1.6a	2.0a	1.7a	2.2a	2.2a	2.3a	2.3a
Arginine	5.3a	6.0a	4.1a	5.8a	4.3a	5.5a	5.0a	6.5a	6.0a
Aspartic	11.2a	10.9ab	11.2a	11.2a	10.2a	10.5ab	10.5ab	10.3ab	10.1ab
Threonine**	5.0ab	4.7bc	5.6a	5.2ab	5.1ab	4.7bc	4.8bc	4.7bc	4.3c
Serine	5.1ab	4.8ab	5.8a	5.3ab	5.4ab	5.3ab	5.1ab	5.6ab	4.6b
Glutamic	14.3b	18.5a	16.3c	18.1ab	14.5d	17.1ab	18.3ab	17.0bc	19.1a
Proline	5.6bc	5.4bc	4.8c	5.6bc	10.4a	6.1b	5.4bc	5.7bc	5.4b
Cysteine	*	*	*	*	*	*	*	*	*
Glycine	4.9ab	4.9ab	5.7a	5.3ab	5.0ab	5.0ab	4.6b	5.0ab	5.0ab
Alanine	9.7bc	8.6d	10.7ab	9.1cd	10.7ab	9.5c	10.9c	8.8cd	9.1cd
Valine**	5.5ab	4.8b	5.9a	5.1ab	5.8ab	5.3ab	5.3ab	5.1ab	5.2ab
Methionine**	*	*	*	*	*	*	*	*	*
Isoleucine**	4.7a	4.3ab	4.6ab	4.3ab	4.3ab	4.1b	4.0b	3.9b	4.0b
Leucine**	7.2ab	7.0ab	7.9a	7.5a	7.7a	7.1ab	6.7b	7.3ab	6.7b
Tyrosine	2.6ab	2.5ab	2.9a	2.3bc	2.5ab	2.3abc	2.1c	2.2bc	2.1bc
Phenulalanine**	4.6ab	4.4ab	4.7a	4.5ab	4.7ab	4.3ab	4.2b	4.3ab	4.1ab

[a] Amino acid values in each row followed by the same letter are not significantly different, $P > 0.05$).
* Trace only
** Essential amino acids

acid and alanine varied the most among strains. The most unusual variation is that of proline in Strain 318 which is almost twice that of any other strain.

Table 2 shows the data for the various protein measurements made on the samples. All of the strains were significantly different from each other when analyzed for protein by the Kjeldahl method except for strains D26 and 340. When using amido black dye for the protein measurement, in this case compared to bovine serum albumin as a standard, the variation among strains was not as much as found with the Kjeldahl method; the ranges were 17.6–25% and 19.4–38.8%, respectively. The mean separation of the Kjeldahl protein values resulted in eight groupings of samples, while the mean separation for dye binding protein values resulted in five groupings. Each grouping in the tables is identified by a common code letter: a,b,c, etc. The protein values calculated from the weights of the amino acids found with the amino acid analyzer ranged from 19.8–30.6% protein. A mean separation of the nine means resulted in four groupings. The wide variation in protein percentage in mushroom strains is in agreement with a study involving four strains of *Agaricus bisporus* in which the biuret method was used to ascertain protein quantity (Beelman et al., 1976).

It should be pointed out that no accurate method for mushroom protein content is presently available and that no standard method has been proposed. The infeasibility most certainly lies in the variable nonprotein nitrogen content of mushroom as well as in the heterogeneity of mushroom protein, as demonstrated in this paper. Protein data based on amino acid analysis are most likely preferable over those based on other methods. However, the cost is excessive. Amido black dye binding is the next preferable method, because its correlation with amino acid protein was shown to be higher (0.74) than the correlation between Kjeldahl protein and amino acid protein (0.44).

The dry matter content of the various strains is shown in Table 3. The average dry matter content was 10.5% and the range 7.45–13.66%, for strains 324 and 321, respectively. This is a considerable variation within a species of mushroom. It may be of practical importance since there is a direct relationship between dry matter content of mushroom raw product and canned product yield (Parrish et al., 1974; Dommel, 1964). Table 3 also shows the comparative protein percentages for the nine strains when calculated on a wet basis. It is obvious that because of variable water (dry matter) concentrations, these protein percentages don't agree with those shown in Table 2.

A comparison of the amino acids of *Agaricus bisporus* and those of mushrooms used in another study (Kalberer and Künsch, 1974) is contained in Table 4. In general, the two sets

Table 2—Protein values for nine mushroom strains[a]

Method of analysis	Strains								
	D26	341	340	318	324	322	348	321	310
Kjeldahl	30.2d	26.0e	29.9d	38.8a	25.6f	37.1b	24.8g	33.2c	19.4h
Dye binding	25.0a	22.0d	25.5a	23.0c	24.0b	21.5de	22.0d	21.1e	17.6f
Amino acid analysis	30.6a	30.6a	30.2ab	29.7ab	29.2abc	28.1bc	27.4bc	26.1c	19.8d

[a] Protein values in each row followed by the same letter are not significantly different, $P > 0.05$.

Table 3—Dry matter of nine mushroom strains and wet basis protein content (Kieldahl)

	Strain								
Dry matter	D26	341	340	318	324	322	348	321	310
(% of fresh wt)	11.38	9.98	10.75	10.58	7.45	9.41	11.53	13.66	10.00
% Protein (wet basis)	3.44	2.59	3.21	4.11	1.91	3.49	2.33	4.54	1.94

of data for *Agaricus bisporus* are very similar. The amino acids varying the most between the two sets are arginine, glutamic acid and proline. When comparing the differences between the two species, only lysine stands out as the most varying amino acid. The lysine average for *Agaricus bisporus* was almost twice that of *Pleurotus ostreatus*, 10.0 vs 5.2 g/100g.

In summary, as shown in Table 2, there were significant differences between the various amino acids in the nine strains of *Agaricus bisporus* investigated. It is suggested that this fact be considered in selecting strains to be used commercially. There were significant differences in the amino acid composition of the protein when investigating the proteins only. A much larger source of variation was introduced when the levels of protein in the various strains were examined on a dry weight basis. Further variation is introduced when protein or amino acid data are computed on a wet basis. The wide variation should be noted by processors, since the yield of canned mushroom products is related to the protein content of the raw product (McArdle and Curwen, 1962). Further investigations into the possible effects of various amino acids and their levels on mushroom yield should be conducted. The authors are planning to examine about 17 strains of *Agaricus bisporus* for protein differences by disc gel electrophoresis in an attempt to further differentiate the strains chemically and physiologically.

Table 4—Comparison of amino acid composition of Agaricus bisporus and Pleurotus Ostreatus (g/100g protein)

	Agaricus bisp. (avg 9 strains)	Agaricus bisp.***	Pleurotus*** ostr. (gray type)	Pleurotus*** ostr. (Florida type)
Lysine	10.0	8.0	5.2	4.8
Histidine	2.2	2.4	1.9	1.7
Arginine	5.5	10.6	6.0	5.9
Aspartic acid	10.7	8.1	10.2	10.9
Threonine	4.9	4.9	5.2	5.1
Serine	5.2	4.1	5.5	5.3
Glutamic acid	17.2	12.7	18.7	17.2
Proline	6.1	9.2	5.2	5.2
Cystine	*	1.0	0.5	0.6
Glycine	5.1	4.6	5.1	5.3
Alanine	9.6	8.3	7.2	8.7
Valine	5.3	4.6	5.9	6.0
Methionine	*	0.8	1.7	1.7
Isoleucine	4.3	4.0	4.8	5.1
Leucine	7.2	6.6	7.7	7.5
Tyrosine	2.2	3.5	3.4	3.6
Phenylalanine	4.4	3.7	4.2	4.2
Tryptophan	**	1.8	1.6	1.2

* Trace amount of amino acid present
** Tryptophan not analyzed
*** Adapted from Kalberer and Künsch, 1974.

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BEAN PROTEIN SEPARATIONS USING LABORATORY AND CONTINUOUS DECANTER CENTRIFUGES

ABSTRACT

California Small White bean meal was given three acidic extractions at low pH followed by three at high pH by laboratory and pilot plant procedures under similar conditions. Extracts and residues were neutralized to pH 6.5 and lyophilized. Acid extracted bean solids were 31% of the original bean meal by both laboratory and pilot plant procedures. The basic extracted solids were 6% by the two procedures. The protein content in the acid extract was 14% of the original meal by each procedure while the protein in the basic extract was 3% by the two procedures. The decanter-centrifuge was effective for the separation if the ground bean was not too fine.

INTRODUCTION

THE SEEDS from the genus Phaseolus in the Leguminosae family are an excellent source of protein. Phaseolus are grown world wide to the extent of 12+ million tons and thus supply millions of people with a large portion of their daily protein and calories. In the USA the yield is about one million tons, most of which is from *Phaseolus vulgaris*.

Research indicates that the potential of beans and other legumes has not yet been fully exploited and that multidisciplinary research will show ways to increase considerably the average yields with a higher content of protein of better nutritional quality (Bressani, 1973). At the present time, the protein in beans is low in quality due, primarily, to low levels of methionine. If bean protein is supplemented with this amino acid, or if beans are used in combination with other foods, mainly the cereals, the resulting protein mixture has a much higher nutritional value. Each food supplies an extra amount of an amino acid which is limiting in the other food (Bressani, 1973).

In order to determine the properties of bean protein it is necessary to extract it from the bean. Physical, chemical and nutritional data are needed to determine where genetic improvement can be made and how the overall value of the bean can be increased. Such testing and evaluation often require amounts of protein fractions larger than can be conveniently prepared in the laboratory. This report gives the results of laboratory and pilot plant experiments in producing protein-rich fractions from California Small White beans.

EXPERIMENTAL

CALIFORNIA Small White (CSW) beans harvested in 1974 were used as the raw material for these experiments. They were sorted, washed in cold water 1–2 min, and redried (16 hr, forced air, 25°C). The beans were ground at the coarsest setting in a Hobart coffee mill and then recycled through the mill three times at the finest setting. This produced a fine meal with a Coleman sieve analysis of 65% retained on #48 mesh, 11% retained on #80, 4% retained on #150 and 20% through #150. The whole meal contained 10.4% moisture and 21.9% protein (Kjeldahl N x 6.25).

The flow diagram for separation of protein-rich fractions from the meal is shown in Figure 1. Small samples of various extracts and dried solids were analyzed for Kjeldahl nitrogen and total solids. Distilled H_2O was used and the 1N HCl and 1N NaOH were prepared from ACS reagent grade chemicals.

Laboratory procedure

70g of the CSW bean meal were mixed with 560 ml of distilled H_2O in a Waring Blendor at minimum speed. The pH of 6.2 was lowered to pH 2.0 with 1N HCl. After mixing for 30 min, the slurry was centrifuged at 3000 × G for 3 min. The supernatant was decanted and neutralized to pH 6.2 with 1N NaOH. The cake was mixed with 530 ml H_2O and the pH again lowered to 2.0. After 20 min of mixing, the second extract was centrifuged as before. A third extract was made in the same manner except that the pH was not adjusted. The second and third extracts were combined and neutralized to pH 6.2. The cake was extracted the fourth time by adding 530 ml of H_2O, adjusting the pH to 11.6 with 1N NaOH, stirring for 20 min and centrifuging. A fifth extraction was made in the same manner. A sixth extraction was made with distilled H_2O. The fifth and sixth extracts were combined and neutralized. The final residue was diluted with H_2O and neutralized. All fractions were frozen and lyophilized.

Pilot plant procedure

A Sharpies P-660 Super-D-Canter centrifuge was used in this work. Horizontal decanter centrifuges of this type are commonly used in a wide variety of industries including food processing of edible animal fat, vegetable oil, and soy protein. Capacities of such centrifuges, using different models, range up to 40,000 lb of solids/hr with liquid feed rates up to 300 gal/min. This type of scroll discharge centrifugal settler is described by Ambler (1969).

Such centrifuges are so arranged as to introduce the slurry into the center of a rotating horizontal cylindrical bowl. Solids that collect on the walls of the bowl are extruded by the scroll discharge screw that forces them to one end of the bowl. The liquid portion overflows from holes at the other end of the bowl.

CSW meal (8.0 kg) was rapidly mixed with 64 kg of distilled water and the pH lowered from 6.5 to 2.0 with 1N HCl. The slurry, while still being stirred, was pumped with a positive displacement pump (Moyno)

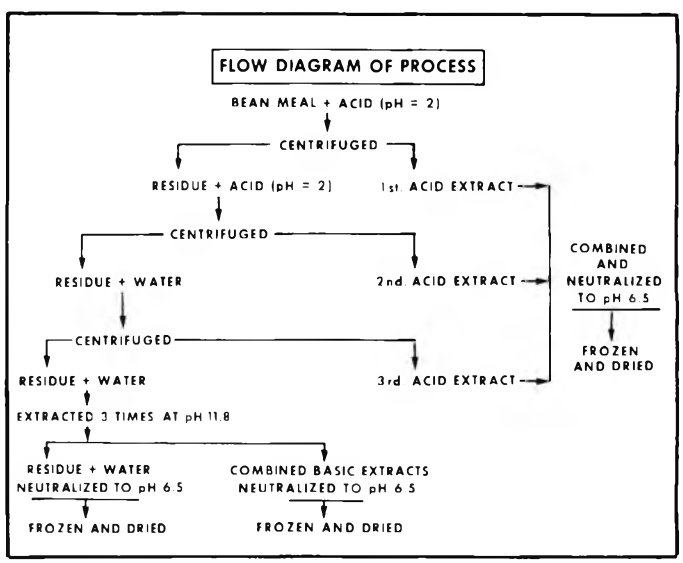

Fig. 1—Flow diagram for acid-base extraction of protein-rich fractions from CSW bean meal.

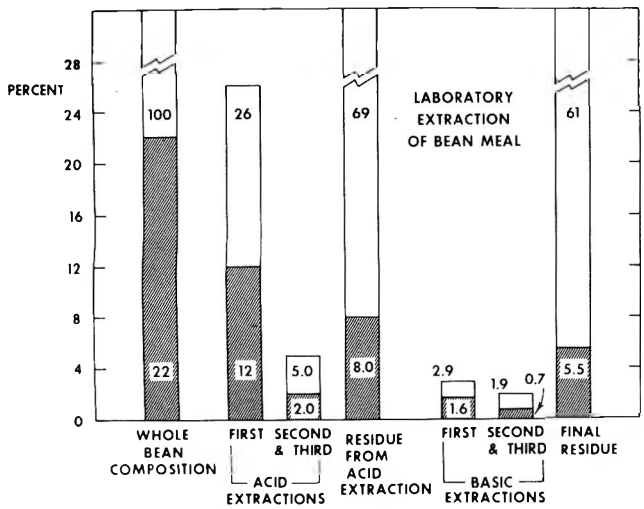

Fig. 2—Laboratory extraction of bean meal. Percentage of bean solids in each extract (upper figure) is shown by the total bar. Percent protein (lower figure) is shown by the shaded portion. Percentages based on original dry bean and the actual yields obtained (corrected for NaCl and moisture contents).

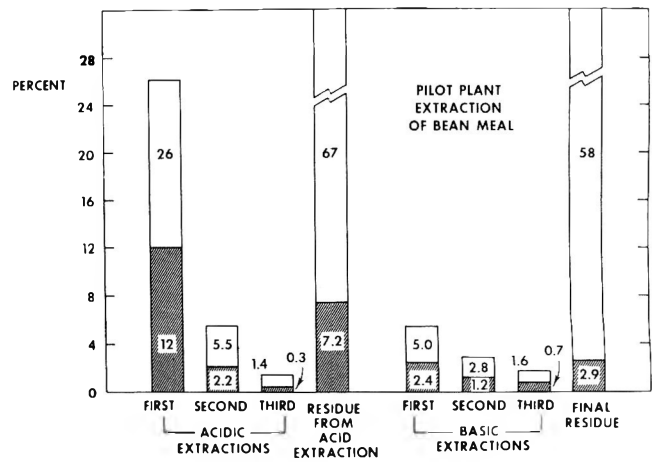

Fig. 3—Pilot plant extraction of bean meal. Percentage of bean solids in each extract (upper figure) is shown by the total bar. Percent protein (lower figure) is shown by the shaded portion. Percentages based on the original dry bean and the analysis of each aliquot (corrected for NaCl and moisture contents).

at 3.78 kg/min into the centrifuge. It was operated at 5900 rpm (3000 × G) with the scroll discharge screw at 2600 rpm; at this screw rate, the centrifuged solids are discharged at a medium rate. The liquid extract flowed out of the left end of the centrifuge and the mushlike residue was conveyed to the right end; each was collected separately.

When all the slurry had been centrifuged, the extract container was replaced and the centrifuge rinsed with 14 kg of water which was then combined with the residue. Samples of the extract were analyzed for nitrogen and total solids. The residue plus the rinse water was made up to the weight of the original slurry and reacidified (pH 2.4 lowered to 2.0) with 1N HCl. The second extraction, rinsing, sampling, etc., was made in the same manner. The third extraction was made in the same manner except that the pH was not adjusted. The three extracts were combined, sampled, and neutralized with 1N NaOH to pH 6.5 and again sampled. The solids were obtained by lyophilization.

The residue from the third acid extraction was diluted to 55.5 kg (1 part of remaining bean solid to 9 of water) and the pH increased to 11.7 with 1N NaOH. The slurry was stirred and then centrifuged in the manner previously outlined. The residue was given a second basic extraction at the high pH by adding water and additional 1N NaOH to pH 11.9. A third extraction was made with water. The three basic extracts were sampled, combined, sampled, neutralized to pH 6.5 with 1N HCl, again sampled, and lyophilized. Each of the six extractions took about 45 min. The final residue was diluted with water, neutralized to pH 6.5 and lyophilized.

RESULTS & DISCUSSION

HANG et al. (1970) reported that from 75–95% of the protein could be extracted from bean meal with dilute acid or dilute base. These researchers used mung beans (*Phaseolus aureus*), pea beans (*Phaseolus vulgaris*), and red kidney beans (*Phaseolus vulgaris*) in a comparative study. Their work did show some differences in the solubilizing of the protein from these beans at different pH's.

Preliminary work in our laboratory with CSW beans indicated that about 70–80% protein was extracted at pH 2; when the pH was increased to 11–12, a somewhat greater amount of protein was extracted from this particular lot of beans. Other work at our laboratory required fractionating sizable amounts of CSW beans into two protein rich fractions—one part that was easily extracted with dilute acid (pH 2.0), and the second part that was extracted with dilute base (pH 11.6+). Since these protein-rich concentrates were to be fed for nutritional studies, HCl and NaOH were used since the NaCl formed on neutralization to pH 6.5 could easily be removed by 70% alcohol extraction or by dialysis; any NaCl remaining would be innocuous.

In the laboratory extractions an attempt was made to duplicate what the pilot plant conditions would be with respect to centrifuge G's, times, etc. The results based on actual yields (NaCl and H_2O-free) are shown in Figure 2. The first acidic extract contained 26% solids and 12% protein based on the weight of the original bean meal. This extract therefore contained 55% of the 22% protein in the bean meal. The combined second and third acid extraction yielded 9% of the protein, and the three basic extracts yielded 10.5%. The final residue contained 25% of the total original protein. Note that the first basic extract contains nearly as much protein as both the second and third acid extracts contained.

Figure 3 shows pilot plant results when 8 kg of bean meal were extracted in a similar manner. These results are based on analyses of aliquots of each liquid extract. The first acid extract contained 55% of the total original protein, the same amount as in the laboratory run. The second and third acid extracts contained 11%. The basic extracts yielded 19.5%, leaving 13% of the protein in the residue.

The CSW beans used for the data presented in Figure 3 had been coffee-milled to a fine meal. Previously a fine bean flour obtained by turbo-milling had been used successfully in a laboratory experiment to separate protein-rich fractions. This flour had a Coleman sieve analysis of 2% retained on #48 mesh, 7% retained on #80, 7% retained on #150 and 84% through the #150. It was correctly assumed that the fine flour would give a better separation of protein. In the laboratory the separations were achieved by centrifugation at 27,000 × G for 30 min. These conditions were unobtainable in the decanter-centrifuge. The decanter-centrifuge did not give an effective separation of the liquid extract from the insoluble solids because both the centrifuge retention time (0.5 min) and the G's (3000) were too low for this fine flour. Even when the feed rate of the bean flour suspension had been lowered from 3.78 kg/min to only ¼ as much (increasing the retention time to 2 min) the

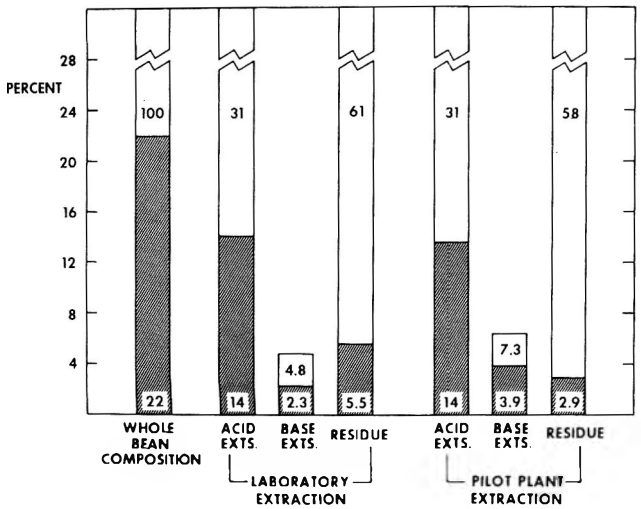

Fig. 4—Comparison of laboratory and pilot plant runs. All acid extracts combined and all basic extracts combined. Based on actual yields obtained. Percentages calculated as in Fig. 2.

separation was still very poor. When a sample of the extract from the decanter-centrifuge was recentrifuged at 27,000 X G in a laboratory centrifuge, a large cake of solids was obtained. On the other hand, the six extracts from the coffee-milled beans (Fig. 3) produced smaller cakes containing only about 10% of the total solids of the extracts. This was considered acceptable since that amount would not dilute out the protein excessively in the dried solids.

Figure 4 gives a comparison (based on actual yields calculated on a salt and moisture free basis) of the laboratory and the pilot plant runs. Data in this figure are essentially the same as that obtained from the analysis of small aliquots of the individual extractions shown in Figure 3. Similar yields were obtained in both runs although in these examples the residue from the pilot plant run contained somewhat less protein than the residue from the laboratory run.

The neutralized acid extract, it should be noted, contains not only the solubilized protein but also NaCl, sugars, amino acids, etc. amounting to more than 40% of the extract. These can be removed by 70% alcohol extraction or by dialysis, leaving a product richer in protein.

Calculations based on the weight of acid extracts and the liquid left in the decanter cake indicated that very little solubilizing of protein and nonprotein material may have occurred after the first extraction. The second and third extractions merely removed by dilution the solubilized material from the decanter cake. On the other hand, in the basic extractions similar calculations indicated that additional bean protein and nonprotein material were being extracted in the second and third basic extractions.

All of the fractionation procedures have been repeated several times in the laboratory and in the pilot plant with a repeatability of ±5%.

The results obtained with CSW bean meal show that a decanter-centrifuge can be used for protein separation if the particle size of the ground bean is not too fine.

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TEMPERATURE DEPENDENCE OF THE COOKING RATE OF DRY LEGUMES

ABSTRACT

It is well known that high temperatures reduce the cooking time of dry beans and peas. However, little quantitative information on the temperature dependence of the cooking time can be found in the literature. The temperature dependence of the cooking rate was obtained by cooking samples of dry legumes for various times at 98, 116, and 127°C and measuring the maximum force on a Kramer TP-1 shear press with a CS-1 cell containing 100g samples. The time-temperature combinations which gave the same texture were plotted to obtain z-values for softening of the beans. The following z-values for softening due to cooking were found: Black beans: 19°C; brown beans: 18°C; soybeans: 16°C; and Alaska dried peas: 16°C. The results reported can be used for more adequate design of cooking processes of beans with special regards to energy savings since beans are frequently cooked for 5 hr at 100°C.

INTRODUCTION

DRY BEANS (*Phaseolus vulgaris*) of various colors are a very popular food in Brazil and other countries. They are the main source of protein of many populations. One of the main problems of dry beans is that they require long cooking times to render them "eating-soft."

Dry beans and peas are usually hydrated for 12–16 hours prior to cooking (Campbell, 1950). The effect of hydration rates on the quality of the product was studied by Rockland (1964). Rockland's group (Rockland, 1966; Rockland et al., 1967, 1970) studied various processes of preparation of quick-cooking dry beans. Binder and Rockland (1964) used the shear press to measure the degree of cooking of beans. They distinguished the effect of the seedcoat and of the cotyledons and found that the maximum shear force decreased linearly with cooking time. Most of the work was done with Lima beans (*Plaseolus lunatus*). Morris (1964) studied the effect of moisture content and temperature during storage on the cooking time of Pinto, Sanilac and Lima beans. He found that cooking time increased with storage time, especially at moisture contents above 10%. At 13% moisture content the cooking time after 12 months was three times the initial cooking time. At 10% the cooking time after 12 months was only slightly longer than the initial time. Cooking time also increased with storage temperature, especially at high moisture contents.

Although it is well known that the cooking time of dry beans can be decreased considerably by higher temperatures such as those obtained in pressure cookers, very little quantitative information on this can be found in the literature. The main objective of this work was to determine the temperature coefficient (z-value, activation energy or Q_{10}) of the cooking rate of various beans and peas.

MATERIALS & METHODS

Products

The following dry legumes were studied: Black beans (*Phaseolus vulgaris*); Brown beans, "Carioca" variety (*Phaseolus vulgaris*); Soybeans , Santa Rosa variety (*Glycine max*); and Alaska dried peas (*Pisum sativum*).

The dimensions of the grains are summarized on Table 1.

Cooking rate

150g of dry legume were added to a flask containing 0.5 liters of tap water. Hydration was done over a period of 12–16 hr at 22°C. The grains were drained and transferred to a 1-gal pressure cooker. For atmospheric pressure cooking (98°C) 1 liter of water was added since the losses due to evaporation were large. For higher temperatures only 0.5 liter of water was added. After closing the pressure cooker, the product was heated as fast as possible to reduce the effect of the temperature lag. 3.5 min were required to reach 98°C and an additional 3.5 min to reach 127°C. For the short times required at 127°C the cooking effect of the come-up time cannot be neglected. Its effect was estimated by graphical integration with z = 20°C as 1 min at 127°C and this time was added to the actual cooking time at 127°C. The pressure cooker was well deaerated and the temperature was controlled by an adjustable safety valve. After the desired cooking time the pressure cooker was cooled rapidly under running water and the beans drained and cooled for shear press measurements at room temperature.

Shear press measurements

The degree of cooking of the samples was measured on a Kramer shear press, Food Technology Corp. model TP-1 with a TG-3000 ring and TR-1 recorder. A CS-1 shear cell (Fig. 1) containing 100g of the drained product was used. This cell has ten 1/8-in. blades that move through the sample. The maximum shear force was obtained from the recorder strip and divided by the amount of sample. The results were expressed as lbf/g.

RESULTS & DISCUSSION

Cooking rate

A typical shear press recorder output is shown on Figure 2. It was attempted to correlate the area under the curves and the

Table 1—Size of the legumes studied

Product	Avg wt of grain (g)	Dimensions (mm)
Black beans (*Phaseolus vulgaris*)	0.175	4.1 X 6.1 X 9.6
Brown beans, Carioca variety (*Phaseolus vulgaris*)	0.212	4.6 X 6.4 X 9.3
Soybeans, Santa Rosa variety (*Glycine max*)	0.134	5.2 X 6.1 X 6.5
Alaska dried peas, whole (*Pisum sativum*)	0.150	5.5 X 6.2 X 6.2
Alaska dried peas, halves, without seed coat (*Pisum sativum*)	0.069	2.8 X 5.9 X 5.9

slopes of the curves with the cooking time. However, the best results were obtained for the maximum shear force.

The maximum shear force (lbf/g) was plotted against cooking time for each of the cooking temperatures on semilogarithmic paper. Similarly shaped curves were obtained for all products. The results for black beans are shown on Figure 3. On a linear plot (not shown) the maximum shear force decreased rapidly at high shear forces and slower at lower shear forces. Binder and Rockland (1964) found a linear variation of maximum shear force with cooking time at 100°C for Lima beans.

From plots similar to Figure 3 the cooking times required to reach certain degrees of cooking at each of the temperatures were obtained. For black beans the following degress of cooking were chosen: 5.0, 4.0, 3.0, and 2.5 lbf/g. The results for black beans are shown on Figure 4. This plot is similar to the plot of D-values or F-values vs. temperature for death of microorganisms. Although only the 2.5 lbf/g beans were "eating-soft," the other degrees of cooking can be of interest for precooking processes, just as partial sterilization is used in some processes of thermal destruction of microorganisms.

The straight lines on Figure 4 represent the time-temperature combinations that give the same degrees of cooking. Thus, 9 min at 127°C has the same effect as 260 min at 98°C. The slope of the curve is related to the z-value. In the case of black beans, z = 19°C, indicating that the cooking time is decreased ten times for each increase of 19°C in the temperature.

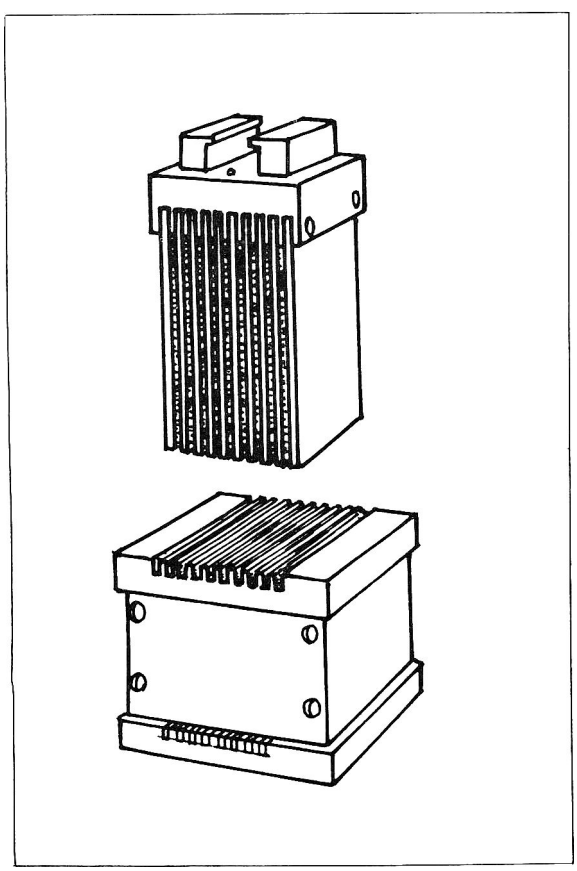

Fig. 1—Type CS-1 cell for Kramer shear press model TP-1.

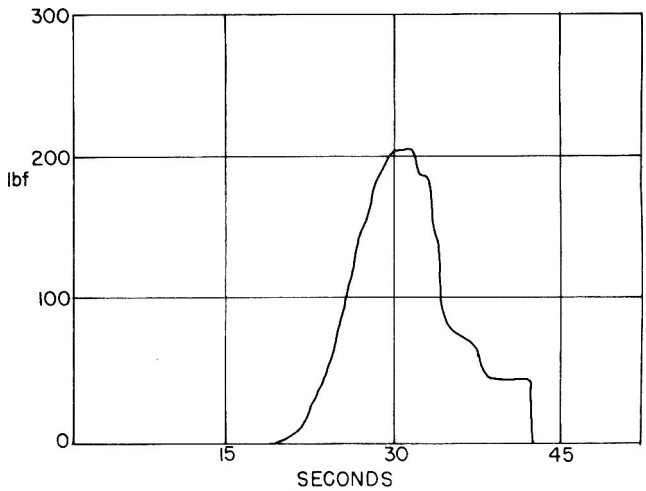

Fig. 2—Typical response curve of cooked black beans in shear press.

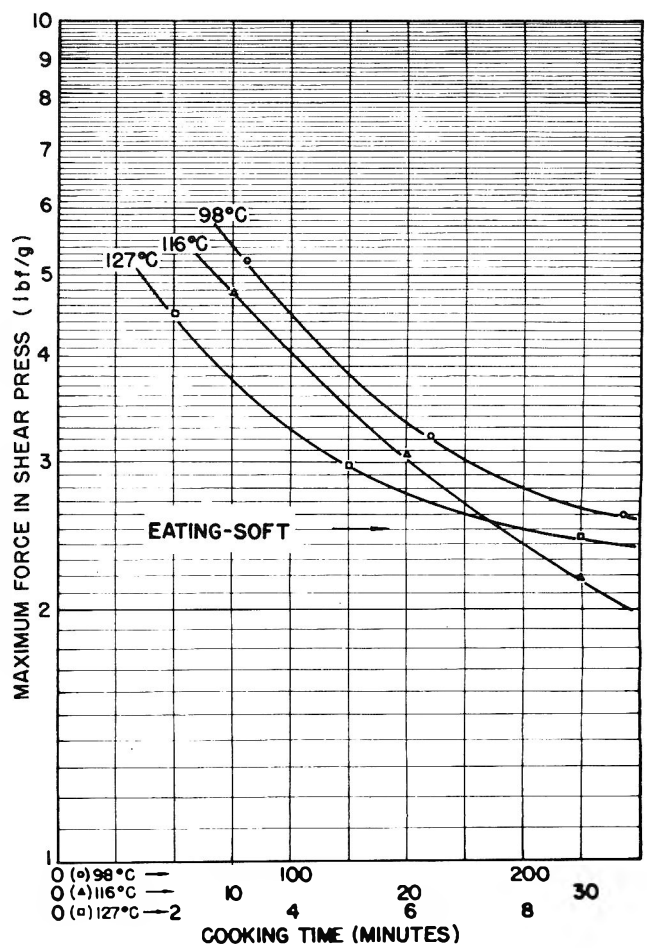

Fig. 3—Degree of cooking of black beans as a function of time and temperature.

Similar calculations were made for brown beans, soy beans and Alaska dried peas. The results are shown on Figures 5, 6 and 7. It can be seen that in all cases the straight line correlation on semilogarithmic paper is excellent. The z-values for the same product were identical for different degrees of cooking.

The temperature dependence of the cooking rate can also be represented in other forms such as the apparent activation energy (E) or the Q_{10} coefficient. These values can readily be calculated (Quast, 1976):

$$z = \frac{4.6\ T^2}{E} \quad \text{and} \quad \log_{10} Q_{10} = \frac{10}{z}$$

where T is the absolute temperature (°K), z is in °C or °K and E is cal/gmole. The results for the products studied are summarized in Table 2. It can be seen that for black beans an increase of 10°C in the cooking temperature causes a 3.36-fold reduction in the cooking time, for the same degree of cooking.

The temperature dependence of the cooking rate of dry legumes was found to be smaller (z-value larger) than the temperature dependence of the death of microbial spores (typically z = 10°C = 18°F). Therefore, very high processing temperatures could result in a commercially sterile product which is inadequately cooked. For example, black beans were adequately cooked (degree of cooking = 2.5 lbf/g) after 18 min at 121°C (Fig. 4). The sterilization value of F_{121} = 18 min can also be considered adequate for commercial sterility. A treatment of 1.8 min at 131°C would have the same sterilization value of F_{121} = 18 min for spores with z = 10°C. This treatment, however, would leave the beans incompletely cooked, at a degree between 4.0 and 5.0 lbf/g (Fig. 4). Clearly, the results can be used for the precise design of cooking operations involving the legumes studied.

With the aid of these data on the kinetics of cooking, the economic advantage of pressure-cooking can be evaluated more precisely. The required cooking time of beans increases with the age of the beans and is also a function of storage

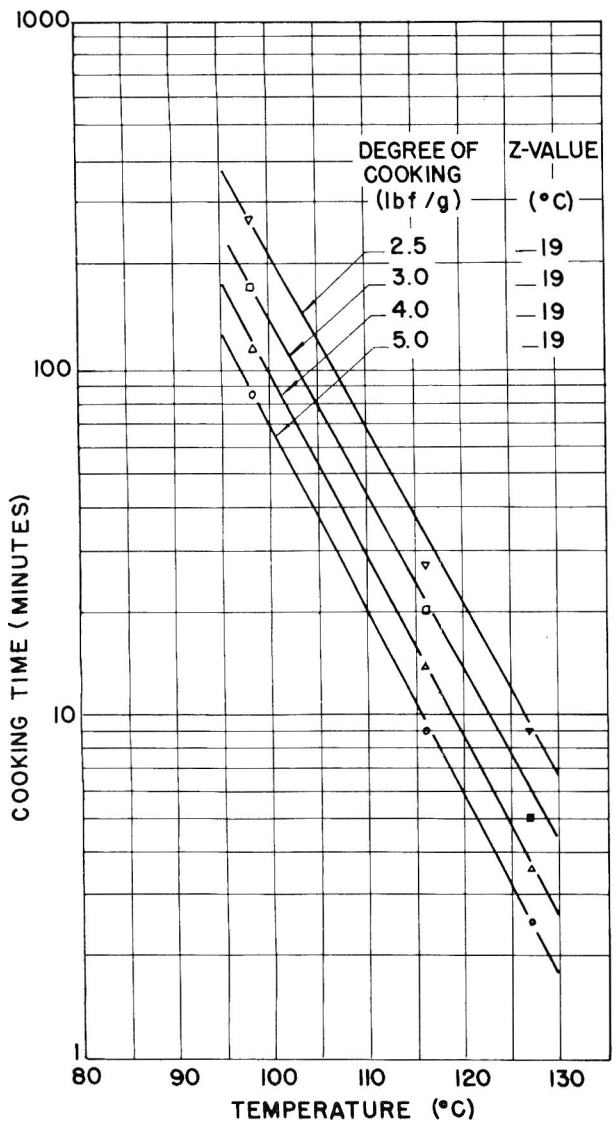

Fig. 4—Effect of temperature on the cooking time of black beans.

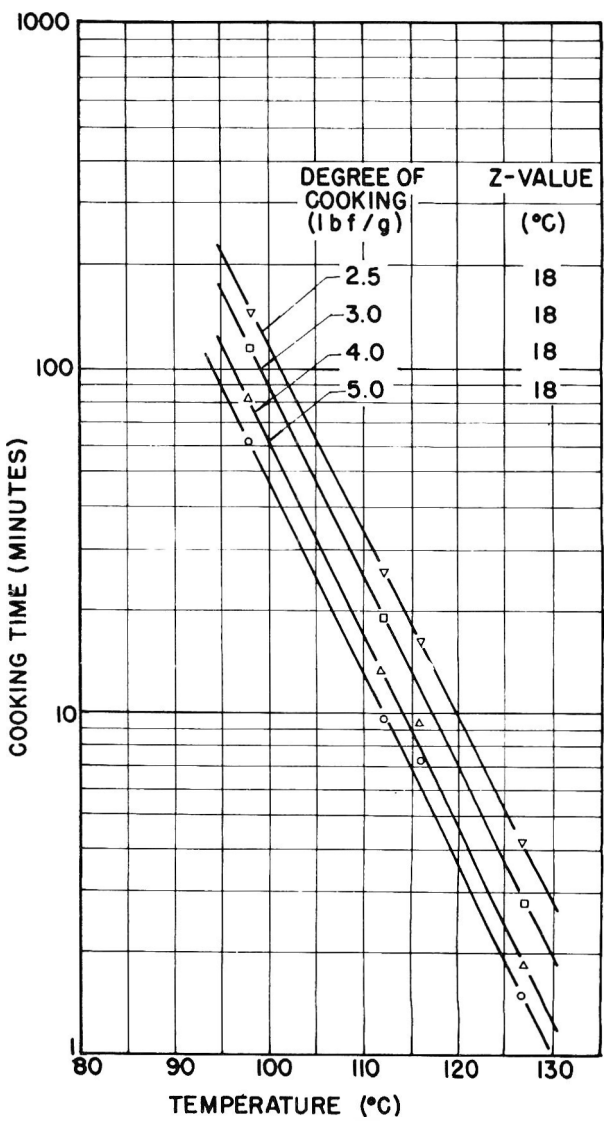

Fig. 5—Effect of temperature on the cooking time of brown beans.

conditions. However, it is not expected that the temperature coefficient (z) will vary considerably with these conditions. Rarely the beans are cooked for less than 2.5 hr at 98°C.

Since

$$t_2/t_1 = 10^{[(T_1 - T_2)/z]}$$

where t_1, T_1 and t_2, T_2 represent time-temperature combinations which give the same cooking effect, then the cooking time at 121°C for z = 19 can be calculated:

$$t_2 = 150 \times 10^{[98 - 121)/19]}$$

$$t_2 \cong 10 \text{ min}$$

Table 2—Temperature dependence of the cooking rate of various dry legumes

Legume	z (°C)	E (cal/gmole) at 110°C	Q_{10}
Black beans (*Phaseolus vulgaris*)	19	35,500	3.36
Brown beans, Carioca variety (*Phaseolus vulgaris*)	18	37,500	3.59
Soybeans, Santa Rosa variety (*Glycine max*)	15.5	43,500	4.41
Alaska dried peas (*Pisum sativum*)	16	42,200	4.21

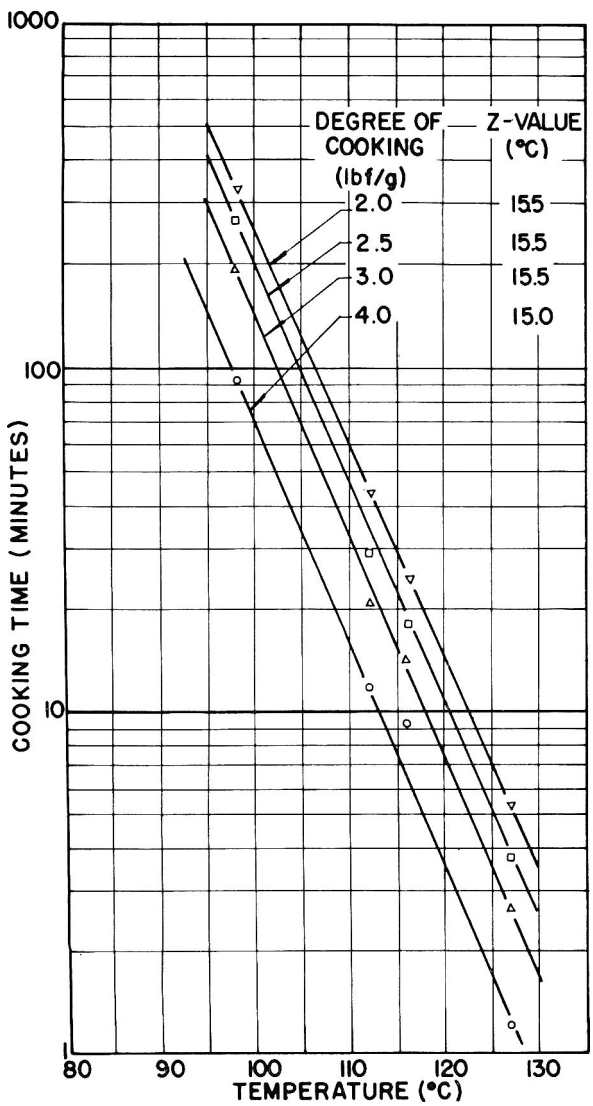

Fig. 6—Effect of temperature on the cooking time of soybeans.

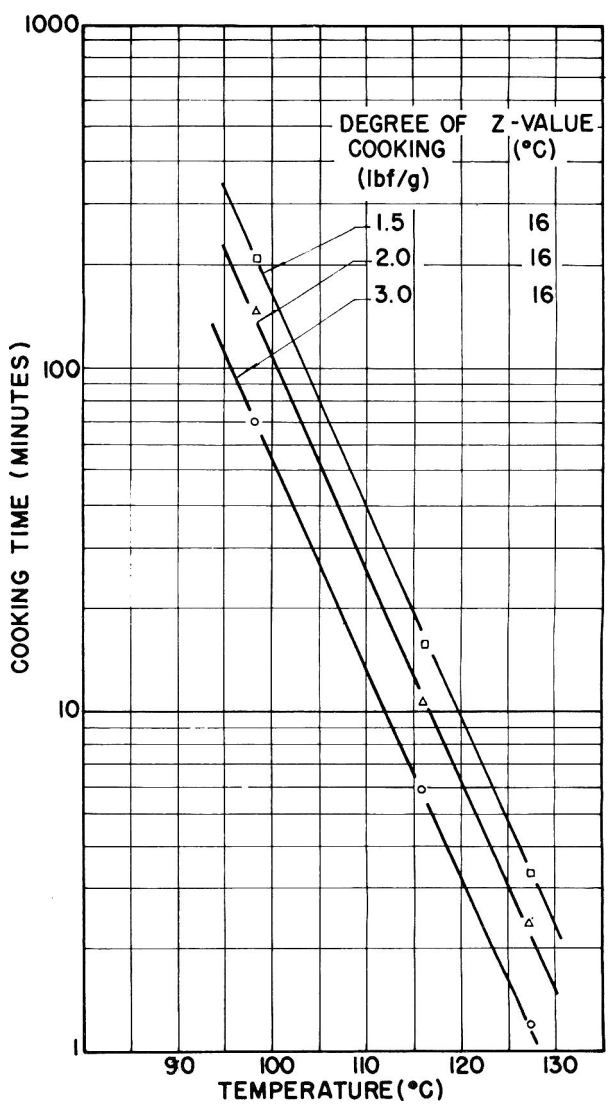

Fig. 7—Effect of temperature on the cooking time of Alaska dried peas.

The cooking time can therefore be reduced by 140 min. Assuming that a typical Brazilian family cooks beans 100 times per years, and since the gas comsumption on a domestic range at the low setting is 75 g/hr of LPG (Brastemp Corp., 1976), the extra comsumption of gas per year, by not using a pressure cooker, amounts to 18 kg. At a cost of 0.40 U.S$/kg, the savings would $7.20 per year. By these savings the pressure cooker could be paid for in 1–2 yr. From a national economic point of view, the subject appears to be of interest. Considering that five million families in Brazil could each save $7.20 per year on gas, a total of $36 million could be saved.

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EFFECT OF BLANCHING, EDTA AND $NaHSO_3$ ON COLOR AND VITAMIN B_6 RETENTION IN CANNED GARBANZO BEANS

ABSTRACT

THE EFFECT of blanching, EDTA and $NaHSO_3$ treatments on color and vitamin B_6 retention in canned garbanzo beans (*Cicer arietinum*) was investigated. *Saccharomyces carlsbergensis* (ATCC 9080) and *Kloeckera apiculata* (UCD FS&T 72-62) were used as comparative organisms for the microbiological assay. Water blanching resulted in approximately 10–15% loss of total vitamin B_6 while steam blanching resulted in only 5–8% loss. The dry garbanzo beans contained 5.5 μg vitamin B_6/g when *S. carlsbergensis* was used as the test organism and 5.8 μg/g when *K. apiculata* was used. Blanching had no significant effect on the color of the canned beans. Vitamin B_6 retention in the canned product was affected by the use of $NaHSO_3$ in the soaking water as a bleaching agent. Samples soaked in water containing 1600 ppm $NaHSO_3$ contained 3.4 ± 0.1 μg vitamin B_6/g on the dry basis while the control samples contained 3.9 ± 0.2 μg/g. The $NaHSO_3$ soaking improved the color of the canned beans significantly. Incorporating 300 ppm of ethylenediaminetetraacetic acid (EDTA) in the canning brine for color improvement did not significantly affect the vitamin B_6 content of the canned product. But, it did improve the color of the canned beans. Use of *K. apiculata* as the test organism resulted in slightly but consistently higher total vitamin B_6 assays in all samples than those using *S. carlsbergensis*. This could be due to the difference in the response of the two organisms toward the different forms of vitamin B_6. *K. apiculata* responds nearly equally to pyridoxine, pyridoxal and pyridoxamine, while *S. carlsbergensis* responds nearly the same to pyridoxine and pyridoxal, but to a lesser degree to pyridoxamine.

INTRODUCTION

THE NUTRITIONAL importance of vitamin B_6 as a coenzyme in body metabolism has been established (Gyorgy, 1971; Sauberlich, 1967). The Food and Nutrition Board of the National Academy of Sciences (1974) included vitamin B_6 in the Recommended Dietary Allowances. The vitamin B_6 content of a variety of foods has been presented in the literature (Dimaunahan et al., 1973; Fiedlerova and Davidek, 1974; Orr, 1969; Polansky, 1969).

The effects of processing methods on nutritive value of foods have been a subject of concern to food processors and consumers and recently there has been greater awareness and concern regarding food additives and their effect on vitamin retention in processed foods. The effects of cooking and processing on vitamin B_6 in some foods have been published (Engler and Bowers, 1975; Everson et al., 1964; Lushbough et al., 1959; Raab et al., 1973; Richardson et al., 1961; Schroeder, 1971; Miller et al., 1973).

Ethylenediaminetetraacetic acid (EDTA) and other sequestrants are used by food processors to minimize undesirable color changes and improve the quality of the final product (Furia, 1972). The effect of such additives on other vitamins has been reported by various researchers but no information on the effect of EDTA on vitamin B_6 was found in the literature.

Sulfur dioxide and sulfites are another group of compounds used in foods to increase storage life, preserve color, and aid in retention of ascorbic acid and carotenoids. These are generally regarded as safe for use but may not be used in foods which are substantial sources of thiamin (Chichester and Tanner, 1972; Schroeter, 1966). While there is extensive information on the application of sulfites to food products, no report on the effect of $NaHSO_3$ on vitamin B_6 was found.

A number of methods have been used to analyze vitamin B_6. Microbiological assays are most commonly used to determine the vitamin B_6 content of biological materials because they are more sensitive and less subject to interference from other compounds than are chemical and physical methods and are less expensive than animal assays (Sauberlich, 1967). *Saccharomyces carlsbergensis* (ATCC 9080) is the test organism used in the AOAC standard method (AOAC, 1970). *K. apiculata* has also been used successfully for vitamin B_6 assay and has been found to have certain advantages over *S. carlsbergensis* (Daoud, 1973; Barton-Wright, 1971).

This work was undertaken to investigate the effect of blanching, EDTA, and $NaHSO_3$ on color and total vitamin B_6 retention in canned garbanzo beans using *S. carlsbergensis* and *K. apiculata* as comparative test organisms for the microbiological assay.

MATERIALS & METHODS

Garbanzo beans

Fourteen pounds of dry garbanzo beans (*Cicer arietinum*) were supplied by a local super market. The beans were thoroughly mixed before use.

Preparation and canning

One part of dried beans was washed and soaked in three parts of distilled water, or in 800 ppm, or in 1600 ppm food grade $NaHSO_3$ solutions for 12 hr at room temperature (22–25°C). The soaked beans were drained, rinsed twice, and blanched either in distilled water, or with steam on a stainless steel screen at 100°C for 10 min. Samples used to study the effect of $NaHSO_3$ soaking were water blanched at 100°C for 10 min. Samples used to study the effect of EDTA in brine were not blanched. Blanched samples were immediately cooled by dipping in cold water (about 10°C) for 2 min and then drained.

Each No. 303 × 407 enamelled can was filled with 250g of beans and 240 ml of 1.5% NaCl solution. For those samples used to investigate the effect of EDTA, 0, 150 and 300 ppm EDTA was added to the brine.

Filled cans were sealed under a vacuum of 22 in. Hg in a Rooney semi-automatic can sealer and heat processed at 118.3°C for 30 min in an upright, stationary retort, allowing 5 min for come-up time. The cans were water cooled in the same retort to 25°C, dried and stored at room temperature until used for analysis (Lin et al., 1975).

Vitamin B_6 content

Preparation of garbanzo bean samples for extraction of vitamin B_6.

Dry garbanzo beans. 500g of dry garbanzo beans were pulverized in an Osterizer blendor and passed through a Tyler standard screen with a 0.0276-in. screen. The pulverized sample was stored in an air-tight bottle at 0°C.

Canned garbanzo beans. The contents of three cans (beans plus

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brine) were blended for 1 min in a Waring Blendor. Portions of the resulting puree were stored in air-tight glass jars at −18°C.

Extraction of vitamin B_6. The extraction procedure described in AOAC (1970) for vitamin B_6 was followed. 1-g samples of dry garbanzo bean powder or 5-g samples of the garbanzo puree were autoclaved with 200 ml of 0.44N HCl for 2 hr at 121°C in 500-ml Erlenmeyer flasks capped with 100-ml beakers. After cooling to room temperature and adjusting the pH to 4.5 with 6N KOH, the solutions were diluted with distilled water to 250 ml and filtered through Whatman No. 40 filter paper under suction. The resulting filtrates were stored in brown bottles at 0°C.

A reagent blank, using 1 ml of distilled water instead of the sample was made.

To test the percent recovery, a known amount (5 μg) of pyridoxine in the form of pyridoxine hydrochloride solution was added to 1g of pulverized dry bean sample just prior to extraction with hydrochloric acid. Also, 2.5 ml of pyridoxine hydrochloride solution containing 2 μg of pyridoxine per ml was subjected to the same extraction procedure as those used for the garbanzo samples and the blank. The data were used to construct an "effective" standard curve from which the total vitamin B_6 content of the garbanzo samples was interpolated.

Assay procedures

The procedures described by Daoud (1973) for the microbiological assay of vitamin B_6 were employed in this investigation.

S. carlsbergensis ATCC 9080 (UCD FS&T 71-35) and *K. apiculata* (UCD FS&T 72-62) were used as the assay organisms.

A synthetic medium containing all the elements necessary for the growth of the two test organisms except vitamin B_6 was used as the basal medium for the assay.

Incubation of the inoculated assay tubes was done on a Rollo-drum at 30°C and the growth response was measured after 15–18 hr of incubation in the case of *S. carlsbergensis* and after 48–60 hr for *K. apiculata*.

The light absorbances at 550 mμ were used as criteria for measuring the growth response of the test organisms to the amount of vitamin B_6 present in the assay tubes. A Spectronic 70 spectrophotometer was used to measure the absorbances.

The amount of vitamin B_6 present in the samples was calculated by interpolating the average OD readings of the triplicate assay tubes from an "effective" standard curve. The results were reported in terms of μg pyridoxine per gram of sample on dry weight basis (allowing for the amount of salt used in making the brine for the canned samples).

Standard curves were constructed for each test organism.

A stock solution containing 2.5 ml of pyridoxine hydrochloride solution (2 μg of pyridoxine per ml) was used. It was treated exactly the same as the garbanzo samples as to extraction, purification, and assay procedure. 0, 1, 2, 3 and 4 ml of the resulting extract solution containing 1 ng pyridoxine/ml was used per assay tube in triplicate. The average OD readings obtained after inoculation and incubation were plotted against concentrations on graph paper.

Total solids. The vacuum oven drying method (AOAC, 1970) as modified by Luh et al. (1975) was used.

Color. The color of the garbanzo samples was evaluated objectively with a Gardner automatic color difference meter, Model AC-1 (Gardner Laboratories, Inc., Bethesda, 14, Md.). A light yellow porcelain plate (LYI) with an Rd = 60.7, a = −2.1, and b = +22.3 was used as a reference (Luh et al., 1975).

Subjective color evaluation. A panel of 12 judges evaluated the color of the canned beans under a standard McBeth Light source on a 10-point scale: excellent, 9–10; good, 7–8; fair, 5–6; poor, 3–4; very poor, 1–2. The results were subjected to analysis of variance, and the least significant difference at 95% confidence level was calculated.

Sulfur dioxide content. The AOAC (1970) pararosanaline hydrochloride colorimetric method was used to determine the SO_2 content in the canned beans as well as in the acid extract containing the vitamin B_6 being assayed for. Results are reported as ppm SO_2.

RESULTS & DISCUSSION

Effect of blanching

The effect of blanching on color and vitamin B_6 retention in canned garbanzo beans is presented in Table 1. Blanching did not affect the color of the canned beans significantly. The water blanched samples were significantly lower in vitamin B_6 than the unblanched samples. Water blanching resulted in approximately a 10–15% loss of total vitamin B_6 while steam blanching resulted in only a 5–8% loss. The greater loss from the water-blanched samples is due to water leaching since vitamin B_6 is water soluble.

The vitamin B_6 content of the dry garbanzo beans used as raw material for canning was 5.5 μg/g when *S. carlsbergensis* was used as the test organism and 5.8 μg/g when *K. apiculata* was used. These values were used as bases for calculating the percent retention in the canned samples. The unblanched, canned samples retained approximately 80–85% of the original vitamin B_6 content. This loss of 15–20% resulted collectively from soaking overnight in water, washing and retorting. No attempt was made to determine the percent loss due to the individual operations. It is known, however, that vitamin B_6 is fairly heat stable and very little loss if any should occur due to retorting; hence most of the loss may be assumed to be due to soaking and washing of the beans in preparation for canning.

Effect of sodium bisulfite

The effect of sodium bisulfite soaking on color, vitamin B_6 retention and the SO_2 residue in the canned beans is presented in Table 2. The $NaHSO_3$ soaking process does improve the

Table 1—Effect of blanching method on color and vitamin B_6 retention in canned garbanzo beans

Treatment	Total solids (%)	Gardner color values			Subjective color score[a] (1–10)	Vitamin B_6			
						S. carlsbergensis		K. apiculata	
		Rd	a	b		Pyridoxine[b] (μg/g)	Retention (%)	Pyridoxine[b] (μg/g)	Retention (%)
Not blanched (control)	22.7 ± 0.1	21.2	+2.4	+20.2	5.7 ± 0.2	4.5 ± 0.3	81.8	4.9 ± 0.3	84.5
Water blanched	21.5 ± 0.2	20.5	+1.5	+19.0	5.8 ± 0.7	3.9 ± 0.2	70.9	4.0 ± 0.2	69.0
Steam blanched	21.6 ± 0.2	19.1	+2.3	+18.5	5.7 ± 0.8	4.2 ± 0.2	76.4	4.4 ± 0.2	75.9
Dry (raw) beans	91.5 ± 0.2	–	–	–	–	5.5 ± 0.1	100	5.8 ± 0.2	100

[a] L.S.D. (P = 0.05) for subjective color score = 0.7

[b] Dry weight basis corrected for solids (salt) added for the brine

Table 2—Effect of $NaHSO_3$ soaking on color and vitamin B_6 retention in canned garbanzo beans

Treatment	Total solids (%)	Gardner color values			Subjective color score[a] (1–10)	Vitamin B_6				
						S. carlsbergensis		K. apiculata		
		Rd	a	b		Pyridoxine[b] (μg/g)	Retention (%)	Pyridoxine[b] (μg/g)	Retention (%)	SO_2 content ppm
Control (soaked in distilled water	21.5 ± 0.1	20.5	+1.5	+19.0	5.8 ± 0.7	3.9 ± 0.2	70.9	4.0 ± 0.2	69.0	0.1 ± 0.02
Soaked in water containing 800 ppm $NaHSO_3$	20.9 ± 0.3	22.0	+2.4	+20.5	7.9 ± 0.5	3.8 ± 0.2	69.1	3.9 ± 0.2	67.2	4.5 ± 0.5
Soaked in water containing 1600 ppm $NaHSO_3$	20.8 ± 0.2	26.8	+3.1	+22.7	9.1 ± 0.5	3.4 ± 0.1	61.8	3.5 ± 0.1	60.3	14.0 ± 1.3
Dry (raw) beans	91.5 ± 0.2	–	–	–	0.5	5.5 ± 0.1	100	5.8 ± 0.2	100	

[a] L.S.D. (P = 0.05) for subjective color score = 0.5
[b] Dry weight basis corrected for solids (salt) added for the brine

color of the canned product significantly, as indicated by the Gardner values and visual color scores. The SO_2 contents in the final products were 4.5 and 14.0 ppm in the samples soaked in water containing 800 and 1600 ppm respectively.

The vitamin B_6 content of the canned product was affected by the sodium bisulfite in the soaking water. Higher levels of sodium bisulfite in the soaking water resulted in lower vitamin B_6 retention in the canned product. Samples soaked in water containing 1600 ppm $NaHSO_3$ were approximately 8–10% lower in vitamin B_6 than the control samples which were soaked in plain water. Since analysis of the garbanzo bean extract solution used to assay for vitamin B_6 was free of any SO_2 residue, the lower vitamin B_6 values in the $NaHSO_3$-soaked samples could not be attributed to any possible inhibitory action of sodium bisulfite on the growth of the test organisms. The SO_2 content of the assay extract from samples soaked in water containing 800 and 1600 ppm was found to be zero.

Effect of EDTA

The effect of incorporating the disodium salt of EDTA into the brine used as the canning medium on color and vitamin B_6 retention in canned garbanzo beans is shown in Table 3. The EDTA treatment does improve the color of the canned product as shown by Gardner Rd value (brightness) and higher visual score in the samples containing EDTA in the brine. However, this color improvement was less than that effected by soaking in $NaHSO_3$ solution. Incorporating 150 and 300

Table 3—Effect of adding EDTA to the canning media (brine) on color and vitamin B_6 retention in canned garbanzo beans

Treatment	Total solids %	Gardner color values			Subjective color score[a] (1–10)	Vitamin B_6			
						S. carlsbergensis		K. apiculata	
		Rd	a	b		Pyridoxine[b] (μg/g)	Retention (%)	Pyridoxine[b] (μg/g)	Retention (%)
Control (no EDTA added)	22.7 ± 0.1	21.2	+2.4	+20.2	5.8 ± 0.8	4.4 ± 0.3	80.0	4.9 ± 0.3	84.5
150 ppm EDTA in brine	22.6 ± 0.2	21.4	+2.7	+19.8	6.3 ± 0.8	4.5 ± 0.2	81.8	4.8 ± 0.3	82.8
300 ppm EDTA in brine	22.5 ± 0.2	22.7	+2.6	+21.4	7.3 ± 0.6	4.5 ± 0.1	81.8	4.8 ± 0.2	82.8
Dry (raw) beans	91.5 ± 0.02	–	–	–	0.6	5.5 ± 0.1	100	5.8 ± 0.2	100

[a] L.S.D. (P = 0.05) for subjective color score = 0.06
[b] Dry weight basis corrected for solids (salt) added for the brine

ppm of EDTA in the canning brine did not significantly affect the final vitamin B_6 content of the canned product. Differences between the various samples were within the range of experimental error.

Test organisms

The use of *K. apiculata* as the test organism resulted in slightly but consistently higher values for total vitamin B_6 content in all samples used throughout this study than those obtained using *S. carlsbergensis*. This could well be due to the difference in the natural responses of the two organisms toward *different* forms of vitamin B_6. *K. apiculata* responds growth-wise nearly equally to pyridoxine, pyridoxal and pyridoxamine while *S. carlsbergensis* responds nearly equally to pyridoxine and pyridoxal but responds to a lesser degree to the pyridoxamine form of vitamin B_6 (Daoud, 1973).

Values for the percent retention of vitamin B_6 obtained with *S. carlsbergensis* were quite comparable to those obtained using *K. apiculata* as the test organism. The mean standard deviations for vitamin B_6 content of the experimental samples were less than 10% of the mean values obtained with the two yeasts.

The responses of the two yeasts toward different levels of a pyridoxine standard solution used for constructing standard curves were greater when the pyridoxine standard solution was exposed to extraction procedures similar to those for food samples than the responses to pyridoxine standards not subjected to extraction procedures. However, the inoculated control blanks (using distilled water instead of samples) that were subjected to extraction procedures produced growth responses similar to that obtained with inoculated blanks not subjected to extraction procedures. Thus one may postulate that the acid treatment with HCl during extraction and the formation of KCl salt after neutralization may have a stabilizing effect on the pyridoxine and that the presence of trace elements as impurities may act as activators in the presence of pyridoxine, thereby stimulating the growth response of the test organisms since no change in the growth response was observed in the inoculated control blanks containing no pyridoxine.

The standard curves (in which the pyridoxine standards were subjected to extraction procedures similar to those used for bean samples) were used for interpolating the vitamin B_6 content of the garbanzo samples. It is believed that the points on this curve are more representative than the points on standard reference curves where the pyridoxine solutions were not subjected to the extraction procedures as were used for the bean samples.

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TITRATABLE ACIDITY OF TOMATO JUICE AS AFFECTED BY BREAK PROCEDURE

ABSTRACT

There is less titratable acidity in tomato juice that has been processed by a "hot-break" procedure to inactivate pectolytic enzymes than in juice of tomatoes from the same lot of fruit that has been extracted cold. These differences in titratable acidity can be attributed to the activity of the pectolytic enzymes in the "cold-break" juice which produce acidic breakdown products from the pectins.

INTRODUCTION

TOMATOES and canned tomato products have been studied extensively with respect to their acid composition, pH, and titratable acidity. In studies on nine lots of canned tomato juice, Rice and Pederson (1954) found that all lots contained citric, malic, and pyrrolidonecarboxylic acids. Some lots also contained lactic and acetic acids. Bradley (1960) reported fumaric and galacturonic acids and three inorganic acids (hydrochloric, sulfuric and phosphoric) as well as the acids reported by Rice and Pederson.

With the exception of studies of the change of glutamine to pyrrolidonecarboxylic acid (Rice and Pederson, 1954) 'Mahdi et al., 1959) only three studies have compared the organic acid composition of tomato juice before and after heat treatments for preservation. Some changes in acid composition do occur, but there is disagreement as to the nature and extent of these changes. Hamdy and Gould (1962) reported the effect of heat processing on dihydroxytartaric, α-ketoglutaric, pyruvic and citric acids in juices from eight varieties of tomatoes. They found that dihydroxytartaric acid disappeared and α-ketoglutaric acid increased while pyruvic and citric acids decreased. They reported substantial decreases for citric acid after processing; yet, with but one exception, the pH of their samples decreased after processing. Eight acids were reported by El Miladi et al. (1969). Their list included succinic acid which had not been found by other investigators. Their observation that α-ketoglutaric acid decreased while citric acid increased with processing is in opposition to the findings of Hamdy and Gould. They noted that their results were in agreement with Scott and Walls (1947) although they did not report values for either pH or titratable acidity (TA) of the juice. Schoenemann and Lopez (1973) studied the changes in acid composition, after 6 months storage at 24°C, for several varieties of tomatoes that had been packed at two different processing temperatures. They concluded "the only acid significantly altered by the length of heat processing was pyrrolidonecarboxylic acid."

In spite of the fact that acidity in heat processed tomatoes has been studied extensively, there has been no study of acidity differences between raw tomato juice and juice from the same lot of tomatoes after hot-break treatments. Only a few studies have been made to determine the effect of the more extensive heat treatments required for preservation on the titratable acidity of tomato products. In an early publication, Scott and Walls (1947) reported that "acidity of processed juice was invariably higher than that of fresh fruit." Adams (1961) measured the pH of tomatoes before and after processing and reported "the average pH value of processed tomatoes (4.23) is actually lower than the average pH value of raw tomatoes (4.41)." Hamdy and Gould (1962) compared eight different varieties of tomatoes with respect to the pH of the juices before and after high temperature-short time processing for 2.5 min at 115.5°C. With but one exception their results show that the pH for each variety was slightly lower after processing.

Since 1967 in the laboratories at the University of California at Davis, TA and pH have been determined for juices from hundreds of tomato samples representing different maturities and many different varieties. For each sample the TA and pH were determined for a laboratory cold-break (CB) juice and for a hot-break (HB) juice prepared by a continuous pilot plant steam injection procedure. The TA of the HB juice was invariably lower than that of the corresponding raw juice, when compared in terms of the soluble solids content of the juices. In almost every case, the lower TA was reflected in a higher pH. These findings are in opposition to published results where tomato juices have been compared before and after heat processing.

This study was undertaken in an effort to explain some of the observed acidity changes and perhaps reconcile some of the differences reported in the literature.

EXPERIMENTAL

Preparation of tomato juices

Tomatoes used for these studies were harvested from field plantings grown for commercial use. Samples for analyses were taken, after the fruit was washed and culls and green fruit removed, by methods shown by statistical studies to yield a representative sample of the harvested lot (800 lb).

Juices were prepared on the day of harvest. From each lot a CB sample for laboratory use was prepared by pulping raw fruit in a Food Processing Equipment Co. laboratory pulper fitted with a 0.027 in. screen. These juices received no heat treatment. The remainder of the lot of fruit was pulped by a procedure designed to inactivate quickly the pectolytic enzyme systems of the tomatoes. Fruit fed through a disintegrator was almost instantaneously heated to 104.4°C by steam injection and held at this temperature for 30–40 sec in a 1 in. diameter holding tube with a back pressure valve at the discharge end. The heated juice was separated from seeds and skin tissue with a Brown extractor equipped with a 0.027 in. screen. The extrusion screw was operated at 800 rpm against 8 lb pressure on the discharge orifice. The juice was then cooled in Cherry Burrell and Creamery Package Co. heat exchangers. Samples of both raw and heated juices not used immediately were sealed in #2-1/2 cans with vacuum, frozen immediately and kept frozen until needed.

Raytheon hot-break juices

A Raytheon microwave oven was used to prepare laboratory hot-break juices for comparison with steam injection HB juices from the pilot plant processing line. Two 4-lb samples of whole unblemished fruit were weighed into separate tared glass baking dishes ca. 12 in. diameter × 3 in. deep. The samples were covered and heated separately for 9 min in the microwave oven and combined afterwards for analytical use.

Alcohol extracted tomato juices

For an "alcohol break" of fresh tomatoes, 200g of firm unblemished fruit were covered with 600 ml of 95% ethyl alcohol in a Waring Blendor and blended for 30 sec. Comparable samples of alcohol-treated HB, CB, and Raytheon heated juices were prepared by thoroughly mixing 200g of each with 600 ml of 95% ethyl alcohol. In each case, the mixture was evaporated to dryness under vacuum in an all glass rotary evaporator equipped with a condenser cooled with acetone and dry ice. Residues were taken up in deionized water and the volumes adjusted to 200 ml. The resulting juices were centrifuged to separate the insoluble solids from the sera.

pH and titratable acidity (TA) were determined on samples of sera separated from insoluble juice solids by centrifugation. pH and titration end points were determined with a pH meter equipped with calomel and glass electrodes. pH was measured on the sera directly, while TA was determined for 10-g samples of serum diluted with 50 ml deionized water and titrated with 0.1N NaOH to a pH 8.00 end point. Values reported in Tables 1 and 2 are the average for triplicate analyses of each serum. Those reported for other experiments are averages of duplicate analyses of each serum.

Serum solids reported are the average of quadruplicate analyses. 10-g samples of serum were weighed into tared aluminum weighing dishes. The solids were taken as the dry weight after 2 hr in a vacuum oven at 70°C.

Paper chromatography was used for qualitative evaluation of pyrrolidonecarboxylic and galacturonic acids in HB and CB sera. For pyrrolidonecarboxylic acid 10 microliter samples were chromatographed for 20 hr using descending elution with ethanol/ammonia/water (8/1/1). Spots were detected with hydroxylamine hydrochloride and ferric chloride reagents according to procedures described by Ramakrishna and Krishnaswamy (1967). For galacturonic acid, 10-ml samples of serum were first passed through a 15 × 200 mm column of Dow-X 50 × 8 resin to remove the cations. Samples eluted with deionized water were collected in 25 ml volumn flasks. 10 microliters of each effluent were chromatographed on paper using butanol/ethanol/water (47%/15.5%/32.5%) as the eluant. Chromatograms were dried and sprayed with an alcoholic solution of m-phenylenediamine dihydrochloride 0.2% and oxalic acid 2% and then heated 20 min at 100°C. Galacturonic acid appeared as a pinkish-brown spot on a white background.

Steam distillation

A 250-g sample of CB tomato juice was placed in a 500 ml RB flask, and steam, generated in a 1000 ml boiling flask, was then bubbled through the juice for 20 min. The vapors were condensed in a water jacketed spiral condenser and collected. The residue was centrifuged to remove the insoluble solids from the sera which was then analyzed for TA and soluble solids.

Pectic substances in tomato juice were determined by inactivating the enzymes with 95% ethanol and further purifying the alcohol-insoluble material with acidified alcohol extraction and washing with acetone. The insoluble residue (marc) was then analyzed for uronic acid carboxyls (free and combined) according to the method described by Gee et al. (1958). The method was modified slightly in that the purified marc was suspended in water and 100-ml aliquots were analyzed rather than dried and pulverized so weighed samples could be used for subsequent analysis.

RESULTS & DISCUSSION

REPORTED IN TABLE 1 are pH and TA values for CB and corresponding HB juices from 20 different lots of tomatoes. In every instance the TA for the HB juice is lower than that for the corresponding CB juice. With but one exception the decrease in TA was reflected in an increase in pH. This relationship between TA for CB and HB juices held for fruit picked at different maturities, (Table 2) and for all varieties of tomatoes analyzed. Since these differences in acidity between raw tomato juices and the corresponding processed juices are at variance with those reported by other investigators, studies were made in an effort to explain the disagreements and cite chemical changes in the juices to account for them. In most instances it was not possible to confirm results already reported. Unfortunately it is not easy to duplicate exactly processing conditions used by other investigators; however, some attempts were made to approximate processing conditions reported in the literature. In order to compare results with published data for juices processed at 104.4°C for 20 min, TA was determined for canned samples of CB and steam injection HB juice that had been removed from freezing storage, allowed to equilibrate at room temperature without being opened, and then processed in a retort at 104.4°C for 20 min. The results from re-heating three samples representing two different varieties of tomatoes are reported in Table 3. It was found that extra

Table 1—Differences in pH and titratable acidities between cold-break and hot-break tomato juices

	pH		TA meq/100g juice		TA diff
Sample	CB	HB	CB	HB[a]	%
R-13-1	4.10	4.30	5.70	5.14	9.8
R-13-2	4.17	4.34	6.24	5.64	9.6
R-14-4	–	4.33	5.96	5.64	5.4
R-16-11	4.12	4.25	6.73	6.19	8.0
R-17-17	4.12	4.20	5.47	5.15	5.8
R-18-19	4.20	4.25	5.60	5.19	7.3
R-19-25	4.20	4.20	6.51	6.28	3.5
R-21-4	4.20	4.30	6.16	5.42	12.0
R-24-23	4.10	4.20	6.97	6.58	5.6
R-26-41	4.11	4.23	6.56	5.51	16.0
11	4.20	4.30	6.01	5.63	6.3
12	4.20	4.34	6.11	5.67	7.2
13	4.26	4.33	6.24	5.74	8.0
14	4.15	4.25	6.60	6.43	2.3
15	4.15	4.20	5.81	5.54	4.6
16	4.18	4.25	5.99	5.48	8.5
17	4.20	4.25	6.96	6.58	5.5
18	4.10	4.30	6.42	5.77	10.1
19	4.15	4.25	6.74	6.09	9.6
20	4.10	4.23	6.44	5.55	13.8

[a] The hot-break values were corrected, on the basis of solids content, for moisture gained during steam injection processing.

Table 2—Titratable acidity of cold-break and hot-break tomato juices with respect to three maturities selected on the basis of color

		Titratable acidity meq/100g juice	
Sample	Maturity	CB	HB[a]
1A		7.72	7.07
2A	Below color	6.57	5.89
3A		5.67	5.16
1B		7.35	6.76
2B	Fairly well	6.45	5.88
3B	colored	5.36	4.85
1C		5.99	5.40
2C	Well colored	5.55	4.69
3C		4.72	4.46
4C		5.48	5.04

[a] The hot-break values were corrected, on the basis of solids content, for moisture gained during the steam injection process.

heating did not significantly change the TA for either the CB or the HB juice for any sample.

One difference between CB and HB tomato juice is the presence of pyrrolidonecarboxylic acid (PCA) after processing (Rice and Pederson,1954; Mahdi et al., 1959, 1961; El Miladi et al., 1969). PCA is readily formed from glutamine when heated in aqueous solution to 100°C. It can also be formed from glutamic acid (Forman, 1914). There are large quantities of both glutamine and glutamic acid in tomatoes (Stadtman, 1972). When PCA is formed from glutamic acid the gamma carboxyl is lost due to the ring closure involving the amino nitrogen group. This would result in a decrease in TA if any of the PCA formed in heated tomato products is derived from glutamic acid. This, however, is probably not the case since it requires heat for a much longer time than used in tomato processing to produce significant amounts of PCA, and other studies have not found significant changes in the glutamic acid concentration of tomatoes after processing or storage, (Stadtman, 1972; Mahdi et al., 1959). The PCA found in processed tomatoes probably all comes from glutamine. When glutamine is converted to PCA there is a release of ammonium ion, but this does not result in a change of ion equilibrium that could affect the TA (Schoenemann and Lopez, 1973); furthermore, there is no significant decrease in glutamine when steam injection is used to produce HB juice (Stadtman, 1972). In this study, when CB and HB sera were chromatographed for PCA, there was none found in the CB sera, and only about 0.3 meq in HB samples—too little to account for TA differences observed.

To check the possibility that some volatile acids might be "flashed off" in the steam injection processing procedures used in the University of California pilot plant, a sample of CB juice was steam distilled. Its TA was then compared with that of unheated CB juice and that of CB juice that was processed in a retort at 104.4°C for 20 min. The results are reported in Table 4. Only 0.0114 meq of acidity was removed by the distillation, and there were no significant differences in TA between any of the three samples. These results indicate that there is in fact, no significant *heat induced* acidity change in tomato juice when heated by steam injection to inactivate pectolytic enzymes.

It became evident that the observed acidity changes actually occurred during the laboratory preparation of CB samples because of pectic enzyme activity. In light of the extensive work done on the activity of pectic enzymes in tomato juice, this should have been obvious. Unfortunately the bulk of confusing data in the literature has obscured the obvious.

To substantiate the conclusion that the pectic enzymes and not heat are, in fact, responsible for the TA differences observed between CB and HB juices, a sample of tomatoes was subjected to an "alcohol break" rather than a hot break. The alcohol served to inactivate the enzymes and precipitate the pectin immediately. For comparison, alcohol-treated samples of CB juice, steam injection processed HB juice, and laboratory HB juice heated in a Raytheon microwave oven were prepared. Results are reported in Table 5. Since there was less TA in the alcohol-break sample than in either of the hot-break samples the alcohol break was apparently more effective than either of the HB procedures for inactivating the enzymes. The higher TA for the CB sample indicates that enzymes had converted the pectin to pectic acid and/or galacturonic acid before the alcohol treatment. This is evidenced by the fact that some galacturonic acid was found in CB juices but not in HB juices when chromatographed on paper.

That differences in TA between CB and HB juices are due to pectic enzyme activity in the CB juice is further substantiated by analyses of the pectic substances in the two juices. Results from one such analysis is presented in Table 6. Significant in these results is the difference in the amount of pectin ester. The ester was not destroyed in HB juice since the

Table 3—Titratable acidity for two varieties of tomato juice after two different heat processing treatments.

Sample	Variety	Method of heating	Titratable acidity meq/100g solids CB	HB[a]
304	145-F5	Steam injection	86.4	80.3
304	145-F5	Retort[b]	86.9	80.1
307	145-7879	Steam injection	119.0	100.9
307	145-7879	Retort[b]	119.0	99.4
310	145-7879	Steam injection	90.6	80.8
310	145-7879	Retort[b]	89.9	78.7

[a] The hot-break values were corrected, on the basis of solids content, for moisture gained during steam injection processing.
[b] 104.4°C for 20 min

Table 4—Comparison of pH and titratable acidity for cold-break tomato juice before and after steam distillation and retort processing

	SS[a]	pH	TA[b]
Raw juice	5.40	4.315	153.3
Steam distillation residue	4.50	4.305	149.9
Retort processed 104.4° C 20 min	5.48	4.275	151.4

[a] Soluble solids %
[b] Titratable acidity in meq/100g solids

Table 5—Titratable acidity in tomato juice after alcohol treatments

Sample	meq/100g solids	meq/100g juice
"Alcohol-break" tomato juice	57.44	2.654
Alcohol-treated HB tomato juice	62.04	2.913
Alcohol-treated CB tomato juice	76.22	3.522
Alcohol-treated "Raytheon Break" tomato juice	63.50	2.934

Table 6—Pectic substances in tomato serum (meq/100g)

Sample	Free carboxyls	Ester	AUA[a]
Q22A (HB)	0.773	1.027	1.800
Q22B (CB)	0.387	0.015	0.402
Difference[b]	0.386	1.012	1.398

[a] Anhydrous uronic acids.
[b] Differences due to enzyme activity in the cold break juice.

pectinesterase was inactivated by heat. The difference, (1.012 meq) of ester between HB and CB juice is a measure of the difference in TA that can be expected between the two juices (Table 1).

The amount of TA difference that occurs between CB and HB juices can vary widely. In these studies the TA differences have varied between 0.23 and 1.58 meq/100g juice. These variations may be due to differences in the pectin content of the different tomatoes studied. Serum viscosity of tomato juice is related to the pectin content of the juice, and there is a positive correlation between TA difference for CB and HB juice and serum viscosity for different varieties of tomatoes.

CONCLUSIONS

1. Differences in TA between CB and HB tomato juices immediately after processing are, for the most part, due to changes in the composition of pectic substances as a result of pectinesterase activity.

2. The TA and/or pH of hot-break tomatoes is a more accurate measure of these factors for the intact, unblemished, raw fruit than TA and/or pH measured in juice samples prepared by a cold-break procedure.

3. When measuring the pH or TA of tomatoes to evaluate the heat required for preservation during canning one should always make these measurements on juice that has been prepared by some hot-break procedure such as an autoclaved sample of whole fruit, or fruit that has been heated in a microwave oven.

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CITRIC ACID FERMENTATION OF BREWERY WASTE

ABSTRACT

Spent grain liquor, a brewery waste, was used as a fermentation medium for the production of citric acid by *Aspergillus foetidus* (formerly called *A. niger* NRRL 337.) The yields of citric acid varied from 3.5–12.3g/liter of the waste fermented, depending on the samples. On the basis of the reducing sugar consumed, the yields ranged from 42–58%. The added nitrogen compounds increased mycelial growth and the consumption of sugar, but markedly reduced the amount of citric acid formed. The addition of phosphate (KH_2PO_4) appeared to have little effect on mycelial growth and citric acid production. Methanol in concentrations of 2–4% markedly increased the formation of citric acid from the waste. There was a reduction in mycelial growth associated with the use of methanol.

INTRODUCTION

SPENT GRAIN LIQUOR, a brewery waste, is characteristically high in biochemical oxygen demand (BOD) and suspended solids (Stein et al., 1973). It presents a serious treatment problem. Hang et al. (1975) recently found *Aspergillus foetidus* (formerly called *A. niger* NRRL 337) capable of rapidly converting about 96% of the BOD into fungal mass. This fungus produced a significant amount of citric acid during the fermentation.

Prescott and Dunn (1959) have reviewed the literature on the production of citric acid by submerged-culture methods. Since the constituents of growth medium are known to have a profound influence on the production of citric acid (Shu and Johnson, 1948), the present investigation was undertaken to determine (1) the variation in the yields of citric acid from the brewery waste samples; and (2) the effect of added nitrogen, phosphate and methanol on the formation of citric acid from the waste.

MATERIALS & METHODS

Source of wastewater

Samples of wastewater used in this work were the liquors resulting from the spent grain recovery process in a nearby brewery. The flow rate of this waste stream ranged from 2,300–3,200 gal per hr. Table 1 shows the characteristics of these waste effluents.

Culture

Aspergillus foetidus NRRL 337 was provided by Dr. J.J. Ellis, Northern Regional Research Laboratory, Peoria, Ill. The culture was maintained on potato dextrose agar slants at 1°C.

Inoculum

The organism was grown on a potato dextrose agar slant at 30°C for 7 days. A spore suspension was prepared by adding 5 ml of sterile distilled water to the slant and shaking vigorously for 1 min.

Fermentation studies

Aliquots of 100 ml of spent grain liquor were dispensed into 500-ml Erlenmeyer flasks and autoclaved at 121°C for 15 min prior to use. Each flask was inoculated with 0.2 ml of the inoculum and incubated for 96 hr at 30°C on a rotary shaker of 200 rpm.

Analytical methods

Mycelial dry weight was determined by filtering, washing with distilled water and drying at 105°C overnight. Reducing sugar was measured by the method of Clark (1964), and citric acid by the method of Taussky (1949). Analyses of 5-day BOD, Kjeldahl nitrogen, total phosphorus, total and suspended solids were conducted according to the standard procedures (APHA, 1971).

All samples were prepared in duplicate and the reported data are the average values.

RESULTS & DISCUSSION

THE YIELDS of citric acid varied from 3.5–12.3g/liter of waste fermented, depending on the samples (Table 2). On the basis of the reducing sugar consumed, the yields ranged from 42–58%. The amount of citric acid formed was apparently dependent on the initial sugar concentration of the waste. The difference in the yields could also be attributed to the variation of other constituents of the samples (Table 1). Shu and Johnson (1948) have observed that a high concentration of sugar was required to produce high yields of citric acid. For example, the highest yield of citric acid (64%) on the basis of the sugar utilized was obtained with a sucrose concentration of 145g/liter. At low concentrations, the yield was poor.

The effect of addition of various nitrogen compounds on citric acid conversion from the brewery waste is shown in Table 3. It is apparent that none of the added nitrogen compounds increased the formation of citric acid. In contrast, these compounds markedly reduced the yields of citric acid although they promoted better mycelial growth. These data clearly indicate that the sugar was mainly used for the synthesis of cellular materials by *A. foetidus* in the medium with

Table 1—Characteristics of brewery waste samples

Sample	BOD (mg/liter)	Reducing sugar (mg/liter)	Kjeldahl nitrogen (mg/liter)	Total phosphorus (mg/liter)	Total solids (mg/liter)	Suspended solids (mg/liter)	pH
A-4	23,500	24,000	376	87	44,000	174	5.0
B-5	16,000	17,750	308	71	32,600	724	3.8
C-6	16,500	20,000	356	74	36,000	931	5.5
D-10	10,800	12,100	280	47	24,800	170	5.7
E-12	19,800	23,900	340	63	35,950	188	5.2
F-15	9,900	12,300	224	41	21,400	507	4.4
G-17	8,000	10,900	236	39	20,500	310	7.1

Table 2—Citric acid conversion from brewery wastes

Sample	Mycelial dry wt (g/liter)	Reducing sugar consumed (g/liter)	Reducing sugar consumed (%)	Yield of citric acid (g/liter)	Yield of citric acid (%)[a]
A-4	11.0	21.2	88	12.3	58
B-5	10.4	12.7	72	6.6	52
C-6	8.3	19.1	96	10.7	56
D-10	7.8	11.1	92	5.5	50
E-12	7.7	20.7	87	9.8	47
F-15	6.6	11.8	96	5.0	42
G-17	6.0	8.0	73	3.5	44

[a] Based on sugar consumed

Table 3—Effect of addition of various nitrogen compounds on citric acid production

Nitrogen source[a]	Mycelial dry wt (g/liter)	Reducing sugar consumed (g/liter)	Reducing sugar consumed (%)	Yield of citric acid (g/liter)	Yield of citric acid (%)[b]
Control	7.0	12.7	78	7.0	55
NH_4Cl	8.1	14.4	89	0.47	3
NH_4NO_3	8.5	15.4	95	1.2	8
$(NH_4)_2SO_4$	8.0	14.0	86	0.37	3
$(NH_4)_2HPO_4$	8.0	14.3	88	1.8	13

[a] Added at a level of 0.1% (wt/vol)
[b] Based on sugar consumed

Table 4—Effect of added phosphate on citric acid production

KH_2PO_4 conc (g/liter)	Mycelial dry wt (g/liter)	Reducing sugar consumed (g/liter)	Reducing sugar consumed (%)	Yield of citric acid (g/liter)	Yield of citric acid (%)[a]
0	7.2	13.5	84	7.7	57
0.5	7.3	14.4	90	8.7	60
1.0	7.5	14.9	93	9.6	64
2.5	7.5	14.5	91	8.7	60
5.0	7.6	13.5	84	7.9	59

[a] Based on sugar consumed

added nitrogen. Our results thus confirm an earlier report (Prescott and Dunn, 1959) that a high nitrogen content increased growth and the consumption of sugar but decreased the amount of citric acid produced.

The addition of increasing amounts of phosphate appeared to have little effect on mycelial growth and the formation of citric acid from the brewery waste (Table 4). Shu and Johnson (1948), however, reported that higher phosphate concentrations (0.5–5.0g/liter) were required by the fungus in a chemically defined medium for maximum production of citric acid. Their highest yield of citric acid (75%) was obtained with a phosphate concentration of 5.0g/liter. They suggested that the phosphate ion might function in some manner other than as a simple nutrient and buffer. The difference in the composition of growth media used may contribute to this descrepency between their data and ours.

Methanol was found to influence the production of citric acid in the brewery waste (Fig. 1). The addition of methanol

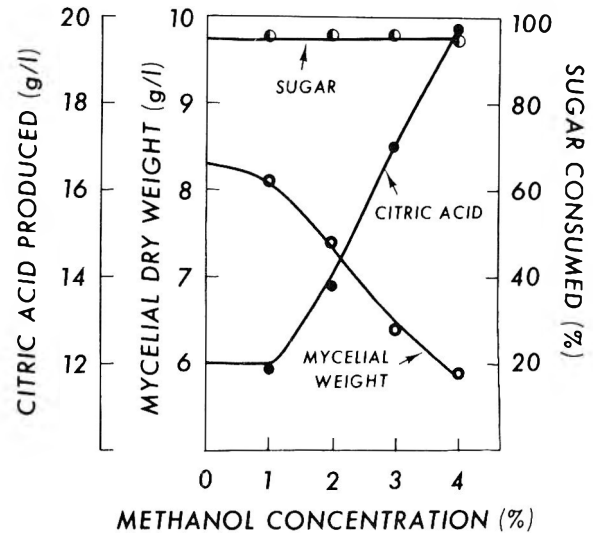

Fig. 1—Effect of methanol concentration on citric acid fermentation in brewery waste.

at concentrations of 2–4% by volume resulted in a marked increase in the amount of citric acid formed. There was, however, a marked reduction in mycelial growth associated with the use of methanol. Moyer (1953) has found methanol capable of enhancing the formation of citric acid from commercial glucose and other crude carbohydrate sources such as corn starch, ground corn, wheat starch, high-test beet and blackstrap molasses. Methanol is not assimilated by *A. foetidus* and its exact role in stimulating the production of citric acid is still not clear.

According to the equation, $C_6H_8O_7 + \frac{9}{2} O_2 \rightarrow 6\ CO_2 + 4\ H_2O$, the theoretical BOD of citric acid may be calculated as 4.5 molecules of O_2 per molecule of citric acid or 144g of oxygen per 192g of citric acid. On the basis of this, if 1% citric acid was removed from the fermented waste, it would reduce the BOD 7,500 mg/l. In our previous studies (Hang et al., 1975), we had observed that the oxidation of 1% citric acid resulted in a reduction of approximately 5,200 mg/l of the waste BOD.

These studies have shown that spent grain liquor could be used as a fermentation medium for the production of citric acid by *A. foetidus*. The commercial potential of this mycological process remains to be assessed.

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COMMERCIAL ORANGE ESSENCE: COMPARISON OF COMPOSITION AND METHODS OF ANALYSIS

ABSTRACT

Quality of three commercial orange essences was related to composition determined by gas chromatography. Two essences were unacceptable in flavor; the third was typical with a high flavor score. The technique of directly analyzing untreated essence was adequate for most components. Of the 21 identified, three were present in reduced concentration in unacceptable essence and six were present in high concentration. The three components found in low concentration – acetaldehyde, ethyl butyrate, and octanal – are known contributors to desirable flavor while two of the components found in high concentration – *trans*-2-hexenal, and α-terpineol – are known contributors to off-flavor. Determination of flavor and off-flavor components by gas chromatography of directly injected essence appears promising as a rapid, objective technique for quality control.

INTRODUCTION

AQUEOUS ORANGE essence, with some of the character of fresh orange juice, is an important citrus product, and is recovered with essence oil by condensation from multiple effect evaporators during production of concentrated orange juice. It contains 10–13% ethanol and more than one-hundred 3- to 15-carbon organic compounds (aldehydes, esters, ketones, alcohols and hydrocarbons) many of which contribute to fresh orange aroma (Shaw and Moshonas, 1974). Certain compounds are essential to orange flavor (Ahmed, 1975), while others cause off-flavor (Tatum et al., 1975). A simple analytical method to determine amounts of components most responsible for flavor quality, would greatly help essence producers to prepare blends of uniform quality.

Quality and flavoring strength may vary from lot to lot because of variations in fruit cultivar, maturity or processing conditions. For controlling quality, commercial essence producers usually base required specifications on ethanol content, total aldehydes and total esters. Some quick tests have been developed to evaluate essence strength and quality (Moshonas and Shaw, 1975). However, these could not replace organoleptic evaluation, which is essential in specifying flavoring quality.

The major components of orange essence have been identified by gas chromatography (GC) and results of previous studies have been summarized by Shaw and Moshonas (1974). In those studies, essence components were usually concentrated before analysis. A commonly accepted concentration technique consists of repeated extraction of an essence sample with methylene chloride and the subsequent vaporization of most of the solvent. A simpler micro-extraction technique was developed by Dinsmore (1973), based on the method of Rhoades and Millar (1965). By this micro-extraction technique, a relatively small quantity of essence is extracted with an even smaller quantity of solvent, such as diisopropyl ether. A sample of the ether solution is then injected directly into a GC column. Another concentration method used involves adsorption on a porous polymer (Moshonas and Lund, 1971; Schultz et al., 1971). By this method, essence components besides water, ethanol, methanol and acetaldehyde are retained on a precolumn of Porapak (Waters Associates, Framingham, Mass), eluted into a GC column, and analyzed without solvent interference. Although many essence components have been determined by GC, some quantitatively, no studies have reported a correlation of essence composition with flavor quality, determined organoleptically.

In our study, commercial essence samples of varying quality were quantitatively analyzed for major components by GC of the untreated samples. Compositional differences were related to flavor score. Three other GC analytical methods of varying complexity were also evalueated. Objectives were to relate chemical composition to essence quality and to demonstrate a simple and rapid GC analytical method which might be applicable to quality control.

EXPERIMENTAL

Essence source

Three essences produced by fractional distillation were obtained from a Florida supplier of commercial essence. Two samples were organoleptically unacceptable (designated A and B) and one was a normal, high-quality essence (designated N). Flavor quality was evaluated by the supplier's expert five-member panel using a synthetic drink base containing 10% sucrose, 0.3% citric acid and 2000 ppm essence. Essence A and B had flavor scores of 5 on a Hedonic scale of 1–10 and essence N was rated 8. Essences A and B had no commercial value due to poor quality ratings.

Quantitative GC analysis

A Hewlett Packard Model 7620A gas chromatograph with a flame ionization detector was used for quantitative analyses. Helium flow was 33 ml/min and temperature of the glass-lined injector and detector was 220°C. Essence samples were injected either directly into a GC column or after concentration as described below.

Analyses for methanol, acetaldehyde and ethanol

For the assay of methanol, acetaldehyde and ethanol, 2-μl samples of essence were directly injected into a 1/8-in. diam by 5-ft stainless steel column packed with 50/80 Porapak Q (Waters Associates, Inc., Framingham, Mass.) maintained at 120°C. The instrument was calibrated with aqueous solutions of known concentrations of methanol, acetaldehyde and ethanol.

Analyses for other components

Less polar compounds of higher molecular weight in 2-μl samples of essence or essence concentrate were analysed with 1/8-in. diam × 15-ft stainless columns. They were packed either with 5% Carbowax 20M on 70/80 Anakrom ABS, or with 5% stabilized diethylene glycol succinate (DEGS) on 70/80 Anakrom ABS, (both packings supplied by Analabs, Inc., North Haven, Conn.). Column temperature was programmed from 80–220°C at 2°C/min. The instrument was calibrated with an external standard of *cis*-hexen-1-ol in methylene chloride.

Calculations

Peak area, determined with a planimeter, was corrected by a factor (Dal Nogare and Juvet, 1962) equal to the molecular weight divided by 12 times the number of carbons not bonded to oxygen (MW/12C).

Reproducibility of peak areas for the compounds determined in this study, except for methanol, acetaldehyde, and ethanol, was estimated to be ± 10–20%, based on repeated injections. Peak area reproducibility for methanol and ethanol was ± 5% and for acetaldehyde, ± 10%.

Concentration by methylene chloride extraction

Anhydrous essence concentrate was prepared according to Wolford et al. (1962). Essence was saturated with sodium sulfate and extracted three times with distilled methylene choloride. Most of the solvent was removed by fractional distillation at atmospheric pressure through a Vigreux column. This method reportedly enables recovery of more than 95% of essence components besides methanol, acetaldehyde and ethanol.

Concentration by microextraction

An essence extract in diisopropyl ether was prepared by the microextraction method of Dinsmore (1973). A 10-ml sample of essence, 0.20 ml of diisopropyl ether and 3.6g of NaCl were added to the test tube of a micro-extraction apparatus (Fig. 1). With the sidearm plugged and the stopcock closed, the mixture was vigorously shaken so that the aqueous phase would by saturated with NaCl and the organic components partitioned by the aqueous and ether phases. After the phases separated, saturated NaCl solution was slowly admitted through the sidearm from a reservoir to displace the upper ether layer through the open stopcock into the 10-cm length of capillary tubing. The ether extract was sampled with a syringe for injection into the gas chromatograph.

A solution of known essence components at about the concentrations in normal essence was prepared for standardization of the microextraction procedures and evaluation of the recovery of specific components in the ether extract.

Concentration by adsorption on Porapak

A modified procedure of Moshonas and Lund (1971) was used to trap essence components other than water, methanol, acetaldehyde and ethanol, on a precolumn (1/8 in. × 5 ft stainless steel packed with 50/80 Porapak Q). Essence samples were injected into the precolumn maintained at 120°C, and the lower molecular weight compounds were allowed to pass through. The precolumn was heated to 160°C and purged by backflushing with helium. As the precolumn was being purged, the eluting essence components were condensed in a liquid nitrogen trap (Dravnieks and O'Donnell, 1971). These were then injected into the Carbowax 20M column by rapid heating of the trap to 240°C in a heated air bath.

Identification of compounds

The anhydrous essence concentrate in methylene chloride was analysed with a Varian Aerograph Series 1400 gas chromatograph coupled to a DuPont 21–490 mass spectrograph. A 4-µl sample was injected onto either the Carbowax 20M or the DEGS column described above. Column temperature was held at 80°C for 10 min, then programmed from 80–220°C at 4°C/min, with helium flow, injector temperature and detector temperature the same as for quantitative GC analysis. Compounds were identified by comparison of their spectra and relative retention times with those of known standards.

RESULTS & DISCUSSION

ESSENCES differing in quality were found to differ in composition, particularly with respect to minor components. As compared to the acceptable essence, both unacceptable essences contained lower concentrations of three components and considerably higher concentrations of six others. The three compounds of lowered concentration have been shown to contribute to orange flavor (Ahmed, 1975). Two of the six compounds that increased are known to contribute off-flavor in orange juice (Ahmed, 1975; Tatum et al., 1975).

Comparison of analytical methods

Figure 2 shows the separation by the Carbowax column of

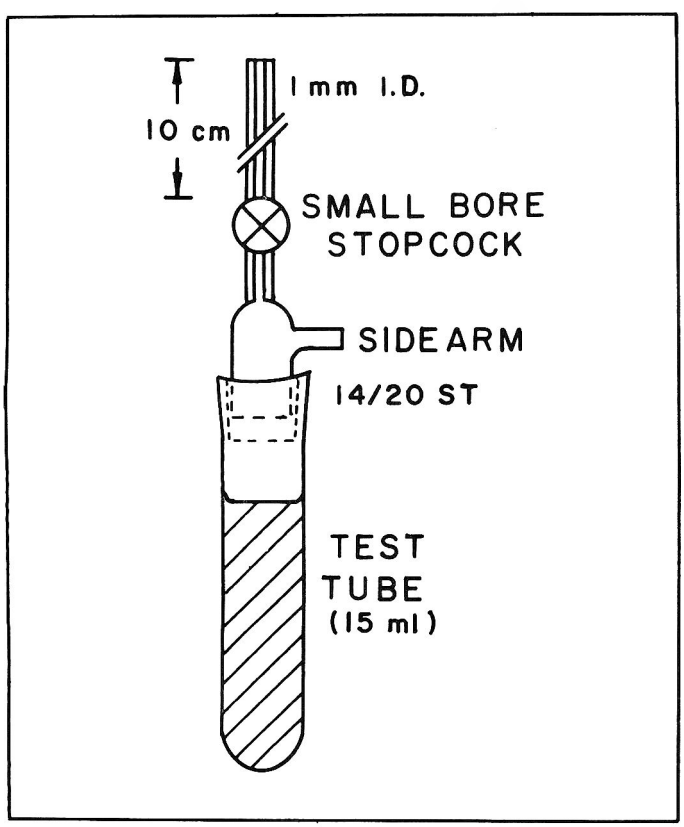

Fig. 1—Microextraction apparatus.

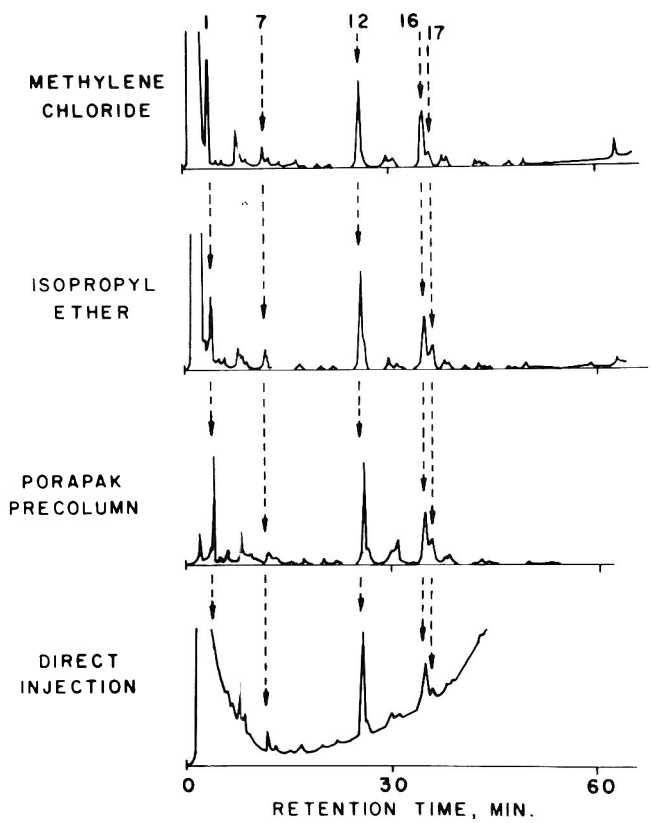

Fig. 2—Gas chromatograms of normal essence on Carbowax 20M.

normal essence injected directly onto the GC column or after concentrations by three methods. The chromatograms have been normalized with respect to the height of the linalool peak (peak 12). Other numbered peaks are: (1)ethyl butyrate, (7) octanal, (16)ethyl-3-hydroxyhexanoate and (17)α-terpineol.

The methylene chloride extraction procedure, which enables high recovery of most essence components (Wolford et al., 1962) was the most accurate. The solvent peak, however, obscured peaks for compounds eluted before ethyl butyrate. This procedure is too complicated and time-consuming to be used for quality control.

The microextraction procedure is relatively simple and rapid, but the extraction of essence components was incomplete. Table 1 shows the recovery by the microextraction procedure of essence components from a standard solution. Recoveries ranged from 8–49% and tended to increase with increasing molecular weight. In spite of low recoveries, the method could be used to determine relative concentrations of individual components among essences. Also, recovery of components in the extract could be improved if the amount of extracting solvent is increased. Peaks detected after solvent elution were as well resolved as for concentrate prepared with methylene chloride.

The Porapak trapping procedure eliminated interfering solvent peaks and permitted quantitative determination of components more volatile than ethyl butyrate. The peak before ethyl butyrate in Figure 2 was caused by residual ethanol retained by the Porapak precolumn. This method must be frequently calibrated with known standards for reliable results.

Chromatograms for directly injected essence showed a broad solvent peak caused by ethanol and water. High instrument sensitivity was required, causing a pronounced baseline shift, as shown in Figure 2. Peaks for the most volatile components, including ethyl butyrate and hexanal were obliterated, and sensitivity for smaller peaks was reduced, particularly for compounds with long retention times. However, most major essence components were quantitatively determined by this simple method, which might be suitable for quality control applications.

Table 1—Recovery of essence components in a standard solution by microextraction[a]

Compound	Conc in std soln mg/100 ml	Recovery %
Ethanol	175	–
Ethyl vinyl ketone	0.327	8.5
Hexanal	0.233	25
1-Penten-3-ol	0.244	14
trans-2-Hexenal	0.262	20
1-Hexanol	0.233	34
cis-3-Hexen-1-ol	0.335	28
Octanal	0.237	25
1-Octanol	0.168	33
Terpinen-4-ol	0.232	42
Citral	0.243	34
α-Terpineol	0.832	45
Carveol	0.208	49

[a] 0.20 ml diisopropyl ether/10 ml essence saturated with NaCl

Table 2—Essence compositions

	Compound[a]	Normal essence (N) mg/100 ml	Concentration ratios[b] A/N	B/N	Determined by direct injection
	Methanol	800	0.9	0.7	x
	Acetaldehyde	120	0.3	0.3	x
	Ethanol1	11,000	0.9	1.0	x
1.	Ethyl butyrate	4	0.3	0.5	
2.	Hexanal	0.14	3.1	2.8	
3.	1-Penten-3-ol	0.3	–	1.3	
4.	3-Methybutan-1-ol + limonene[c]	1.3	0.6	1.3	x
5.	*n*-Amyl alcohol	0.09	2.4	–	
6.	*trans*-2-Hexenal	0.07	12	2.5	x
7.	Octanal	0.5	0.6	0.5	x
8.	1-Hexanol	0.08	9	2.1	x
9.	*cis*-3-Hexen-1-ol	0.3	4	0.7	x
10.	*trans*-Linalool oxide	0.2	1.1	–	x
11.	*cis*-Linalool oxide	0.2	1.0	–	x
12.	Linalool	2.6	0.8	1.0	x
13.	1-Octanol	0.2	1.0	0.8	x
14.	Terpinen-4-ol	0.2	0.8	0.6	x
15.	*trans*-2,8-*p*-Menthadien-1-ol	0.03	3.8	3.9	x
16.	Ethyl-3-hydroxy-hexanoate	6	0.5	0.9	x
17.	α-Terpineol	0.28	2.0	1.4	x
18.	*trans*-Carveol	0.08	2.5	1.8	x
	Unidentified compounds	1.5	1	1	

[a] Numbered in order of retention time on Carbowax 20M
[b] Component concentration ratios, unacceptable: normal essence
[c] An unresolved peak consisting mainly of 3-methylbutan-1-ol
[d] Based on peak area correction factor = 1.0; a total of 29 peaks.

Composition of acceptable and unacceptable essences

Essence compounds which were quantitated after direct injection or preliminary concentration are shown in Table 2. Compounds which were analysed by direct injection methods only are indicated by an "x" in the fourth column. The other compounds were determined by one of the solvent extraction procedures. The underlined ratios indicate substantial difference between normal and unacceptable essences, taking into account the limited number of samples and the accurracy of the data.

Ethanol, methanol and acetaldehyde were the predominant essence components. Of these, only acetaldehyde has been shown to contribute to orange juice flavor (Ahmed, 1975). Concentration of acetaldehyde in either unacceptable essence was about 1/3 that in normal essence.

Remaining components, numbered in Table 2 in order of retention time on Carbowax 20M, were present in far lower concentration than acetaldehyde. Ethyl butyrate and octanal are important contributors to orange flavor (Ahmed, 1975) and their concentrations were significantly lower in the unacceptable essences. Concentration of linalool, another desirable flavor compound, was not significantly reduced.

d-Limonene is the major constituent of essence oil, another flavoring by-product recovered from aqueous essence. It was not quantitated in these analyses, because the limonene peak was obscured by that for 3-methyl-butane-ol. The concentration of limonene would be expected to be about the same in all essence samples and less than 1 mg/100 ml.

Concentrations of several components were substantially higher in one or both unacceptable essences, and some of these are known to contribute off-flavor. Hexanal and *trans*-2-hexenal impart a characteristic immature or "greenish" flavor (Ahmed, 1975). α-Terpineol causes off-flavor in orange juice (Tatum et al., 1975). Other components which were notably more concentrated in the unacceptable essences and which might contribute to off-flavor were: 1-hexanol, *trans*-2-8-p-

menthadiene-l-ol and *trans*-carveol. The 29 unidentified compounds, which totaled 1.5×10^{-3}%, were present in all essences at about the same concentrations.

One possible explanation for the changes in certain of the unacceptable essence components would be the oxidation or hydration of lipid, carotenoid, or terpenoid components of the fruit. The six carbon compounds hexanal, *trans*-2-hexenal and 1-hexanol may be oxidation products of lipids or carotenoids. Similarly, the three terpene alcohols (compounds 15, 17 and 18 of Table 2) are well known oxidation and hydration products of limonene, the main component of orange peel oil. The losses of acetaldehyde, ethyl butyrate, and octanal could have resulted from oxidation or acid catalyzed hydrolysis at some point of essence production.

Another possible cause of the changes is the enzymatic reactions occurring in the normal metabolic process of fruit. The concentrations of volatile aldehydes, ester and alcohols change considerably during the maturation of fruit. Thus, if an over- or underripe batch of fruit were processed, the essence composition would be affected.

A third of possibility could be unusually large amounts of peel components present in the juice used for essence recovery. The volatile aroma from the peel portion of the fruit is lower in ethyl butyrate and ethyl-3-hydroxyhexanoate, and higher in α-terpineol, than the interior of the fruit (Moshonas et al., 1972). Thus, many observed differences could result from the presence of undesired levels of peel components.

CONCLUSIONS

FLAVOR QUALITY of three samples of orange essence appeared to correlate with concentration of certain components as determined by gas chromatography. Two unacceptable essences contained low levels of three compounds, acetaldehyde, ethyl butyrate and octanal, that have been established by previous studies to contribute to typical orange flavor. The two essences also contained high levels of two compounds, *trans*-2-hexenal and α-terpineol, that are known off-flavor components. For the assay of some volatile compounds, such as ethyl butyrate and hexanal, essence first had to be concentrated by solvent extraction or entrapment on a Porapak precolumn. However, most components known to influence typical orange flavor could be determined by GC of directly injected essence. This simple method may be suitable for quality control at citrus plants.

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PRODUCTION OF PROTEIN ISOLATES AND CONCENTRATES FROM OILSEED FLOUR EXTRACTS USING INDUSTRIAL ULTRAFILTRATION AND REVERSE OSMOSIS SYSTEMS

ABSTRACT

The consumption of protein isolates from oilseed flours is expected to continue its upward trend as meat analogs from vegetable proteins are more widely marketed. However, conventional protein isolation procedures are lengthy and somewhat expensive. These processes also result in whey-like liquid by-products which constitute a water pollution threat unless properly processed. Preparation of protein isolates and concentrates from glandless cottonseed and soy flours by extracting the protein and ultrafiltering the solubilized protein directly from the liquid extract was investigated. In the process devised, proteins normally precipitated to produce isolates were recovered together with the conventional whey protein by ultrafiltration (UF). The UF permeate was further processed with a reverse osmosis (RO) membrane. Different industrial UF and RO systems were utilized in 22–60 lb extractions. UF membranes were found which gave desirably high permeation rates and satisfactory constituent separations, especially with cottonseed storage protein (SP) extract. With SP extract, initial UF permeate flux achieved was in excess of 150 gal of permeate per square foot of membrane area per day (gfd). The flux declined to 96 gfd over a period of 8 hr while the volume of original extract in the feed solution was being reduced by a ratio of 12.5 to 1 in the final UF concentrate. As expected, nonstorage protein (NSP) and SP extracts from cottonseed were found to require membranes having different molecular weight cutoff points. Noncellulose-based membranes performed better for this application because of their broader pH and temperature operating ranges.

INTRODUCTION

IT IS THE CONSENSUS of authorities in the area of world food requirements that oilseed proteins for direct consumption in human foods are the most promising means of supplying the protein shortages that exist in the diets of over half the world's population (NSF/MIT Protein Resources Study, 1975). There is little doubt that the production of soy protein isolates and concentrates will continue to increase and that similar types of cottonseed protein products will play an important role in future food systems.

Conventional procedures developed for protein isolation from defatted soy or cottonseed flours, however, are somewhat expensive and in the case of cottonseed, especially, quite lengthy. Cottonseed protein isolation procedures generally require either six or seven centrifuge steps (Berardi et al., 1972, 1969). Also, each of the conventional processes (both soy and cottonseed) result in a whey-like liquid by-product which constitutes a serious water pollution threat unless properly processed (Lin et al., 1974).

Semipermeable ultrafiltration (UF) and reverse osmosis (RO) membrane systems have been used commercially for a number of years to profitably process cheese wheys (Selitzer, 1972; Horton, 1974; Crocco, 1975; Anon., 1975) simultaneously recovering marketable products and reducing pollution loads. Investigations into the use of these techniques for processing both soy wheys (Goldsmith et al., 1972) and cottonseed wheys have also been conducted (Lawhon et al., 1973, 1974, 1975, 1976).

In current research at the Food Protein Research and Development Center (FPRDC), Texas A & M University, an approach to protein isolation different from conventional acid precipitation methods is under investigation for both cottonseed and soy protein. By using semipermeable membranes to process protein extracts from defatted flours the generation of wheys is avoided and process effluents are rendered reuseable. Other investigators reporting results of their work to produce soy protein isolates using RO and UF techniques include Frazeur and Huston (1973) and Pompei and Maletto (1974).

In the work reported here, the industrial membrane systems employed were equipped with the newer noncellulosic, "second generation" membranes and extracts of both fractions of cottonseed protein i.e., nonstorage protein (NSP) and storage protein (SP) were processed in addition to soy protein extracts.

EXPERIMENTAL

Preparation of protein extracts

Protein extracts were prepared in the FPRDC pilot plant. Different preparation procedures were used for soy and cottonseed extracts as shown in Figures 1 and 2, respectively. Multiple runs were made with each procedure.

Soy extracts were prepared by extracting from 22–60 lb per run of a high nitrogen solubility soy flour (Central Soya's Soy Fluff 200W). Extraction was made with tap water (10:1 solvent-to-flour ratio by weight) adjusted to pH 9 with sodium hydroxide. Extraction continued for 40 min at 43°C. After centrifugation to separate the flour-water slurry into soluble and insoluble components, the insoluble residue was resuspended in one-half the original amount of water at the same pH and temperature for an additional 20 min to more completely remove solubilized components. Liquid supernatant from the first and a second centrifugation were then mixed and pasteurized by heating to 63° for 30 min. Pasteurized extract was prefiltered to 10 microns before membrane processing.

Two cottonseed protein extracts were prepared from glandless cottonseed flour which had been produced under mild-heat conditions (simulating commercial processing) using the FPRDC's Crown solvent extractor. The cottonseed protein was divided into NSP and SP fractions by first extracting the flour with tap water (10:1 water to flour ratio) at 28.5°C for 30 min to solubilize the NSP and other water soluble components. The initial extraction was followed by centrifugation and resuspension of the solids in water (8:1 water to original flour by weight) to more completely separate the NSP fraction. The two NSP extracts were mixed, pasteurized and prefiltered as were the soy extracts. SP extract was prepared by reextracting residue from the NSP extraction with tap water (10:1 water to original flour by weight) adjusted to pH 10 with sodium hydroxide. Extraction continued at 28.5°C for 30 min after pH stabilization. Centrifugation was followed by resuspension of the insolubles at the same pH and temperature to more completely remove the SP fraction. The two SP extracts were mixed, pasteurized and prefiltered as the other extracts.

Membrane systems employed

Data obtained using the two UF systems and one RO system that performed quite satisfactorily in producing protein isolates by the new

approach under investigation are reported. The two UF systems were the UCARSEP® system of Union Carbide Corp., Tarrytown, N.Y., and the tubular membrane UF system of Abcor, Inc., Cambridge, Mass. The RO system employed was an RO pak Single-Core Reverse Osmosis Machine manufactured by Rev-O-Pak, Inc., Newbury Park, Calif.

The UCARSEP® system used consists of a stainless steel single tube module equipped with one 0.24 in i.d. UCARSEP® membrane coated porous carbon tube. The tube has an active length of 37 in. between o-ring seals. The membrane coating is placed on its inner surfaces and is of a specially developed inorganic composition that will permit operating temperature of 93°C or higher and a pH range of 1–14 in feed solutions. System pressures up to 600 psi are permissible for use with it.

The Abcor, Inc. tubular membrane unit employed consisted of 2.2 ft^2 of Abcor HFJ organic polymer (noncellulosic) membranes. The membrane is coated on the interior of a 1 in. i.d., 54 in. long support tube. The membrane withstands operation over a wide pH range (pH 2–13) and is limited in operating temperature only by temperature resistance of other components in the system. Operating pressures up to 60 psi may be used.

The RO system yielding the data reported was equipped with Rev-O-Pak, Inc.'s external tubular design cellulose acetate membrane. Two ft^2 of membrane area cast on the exterior of 3-ft length, 5/8 in. diameter ceramic support cores were used in the experimental unit. Plastic-coated wire tubulators helically wound around the cores serve to increase turbulence at the membrane surface during feed flow. Operating temperatures are restricted to below 49°C and pH of feed solution must be maintained between pH 3 and pH 7.5. The membranes used were rated as having a rejection for 5000 ppm NaCl of about 95% at 500 psi.

Membrane processing techniques

After pasteurization and prefiltering, feed solutions were pumped to each membrane system at its manufacturer's recommended pressures and flow rates. Feed temperatures were maintained around 65°C for UF processing and from 46–49°C for RO processing. UF feed solutions were usually processed in batches sized to allow about a 4.5:1 volume reduction in original feed (in the case of soy extract) and a 10:1 volume reduction (for cottonseed SP extracts) prior to employing a dilution technique to further purify the protein retentate. The dilution technique generally consisted of adding an equal amount of filtered tap water to the concentrated feed and reconcentrating it to achieve an "equivalent volume reduction" of around 9:1 for soy extracts and 20:1 for cottonseed SP extract. For NSP extract, batches were concentrated to one-fifth of the starting volume, diluted and reconcentrated to a final concentrate volume of around one-fifth of the initial feed volume for drying. This procedure with NSP extract resulted in an "equivalent volume reduction" of approximately 10:1.

In RO processing, the dilution technique was not employed since the objective was essentially concentration as opposed to concentration and fractionation as achieved with UF membranes. Feed volume reductions of around 20:1, 8:1 and 4:1 were accomplished with the RO membranes for soy UF permeate, SP extract and soy extract, respectively.

Membrane performance calculation methods

A membrane's performance may be assessed by measuring its ability to achieve desired separations of the components in a feed solution and its permeation rates, i.e., the rates at which the separations occur (assuming we do not consider the length of membrane life, etc.).

Separation efficiency or degree of fractionation of feed stream components is conventionally determined by calculating the percentage of each component of interest retained by the membrane as follows:

$$\text{Ret, \%} = \frac{(\%\ \text{Component in feed} - \%\ \text{Component in permeate})}{\%\ \text{Component in feed}} \times 100$$

The above equation will suffice to describe percentage retentions being attained at a given point during feed concentration and fractionation. However, retention of particular components tends to vary (usually increasing) as feeds become more concentrated and membrane pores are restricted by feed solute along the membrane surface. Thus, a calculation method was devised for obtaining a single term that would describe the mean retentive characteristics of a membrane for an individual feed component over the entire processing cycle. Corresponding samples of feed and permeate were taken usually at five somewhat equally spaced points from the beginning to the end of the concentration period prior to dilution. The average % retention of a component between each two consecutive points was calculated and multiplied by the gallons of permeate collected in the interval between the two points. The products obtained were summed and their total divided by the total number of gallons of permeate collected. The quotient obtained represented the mean percentage of an individual component retained. This calculation method expressed in equation form is as follows:

$$\text{Mean Ret, \%} = \frac{\left(\text{Avg ret}_1 \times \text{Vol perm}_1\right) + \left(\text{Avg ret}_2 \times \text{Vol perm}_2\right) + \cdots \left(\text{Avg ret}_n \times \text{Vol perm}_n\right)}{\text{Total vol perm}}$$

Permeation rates through membranes decline continually from beginning to end of a concentration cycle. Therefore, a single value representing the mean flux attainable over the entire processing period was likewise deemed necessary. A method for calculating such a value similar to the one devised for obtaining mean percent retentions was used. In this instance the average permeation rate expressed as gallons of permeate per square foot of membrane area per day (gfd) between each two test points (usually 10–20 rate tests were taken per run) was multiplied by the number of minutes elapsed between the tests. The products were then summed and divided by the total minutes elapsed from the first to the final flux test. The quotient was the mean permeation rate achieved by a membrane while processing a particular feed solution from beginning to end. Expressed in equation form the calculation method is as follows:

$$\text{Mean perm rate, gfd} = \frac{\left(\text{Avg P.R.}_1 \times \text{Time period}_1\right) + \left(\text{Avg P.R.}_2 \times \text{Time period}_2\right) + \cdots \left(\text{Avg P.R.}_n \times \text{Time period}_n\right)}{\text{Total time elapsed (min)}}$$

Analytical procedures

Moisture, total solids, oil and ash were determined according to standard AOCS methods (AOCS, 1971). Nitrogen was determined by the micro-Kjeldahl method. Carbohydrates in terms of glucose were measured colorimetrically by the phenol sulfuric acid method of Dubois et al. (1956). Total phosphorus was determined by the method according to Sumner (1944). Color measurements were made using a Hunter Digital Color and Color Difference Meter, Model 25D. Measurements were first made on freeze-dried products in powdered form and then on the same products as a wet paste prepared by adding water (5:1 water to product ratio by wt). Nonprotein nitrogen (NPN) was determined as nitrogen soluble in 10% TCA solution.

RESULTS & DISCUSSION

THE OBJECTIVE of the investigations reported here was not to evaluate industrial membrane systems per se and rate one system above another for the application under study. The purpose was to demonstrate the feasibility of applying a new approach to protein isolation from oilseed flours. Further, it should be pointed out that the membranes used within a particular manufacturer's system may not be the optimal membranes that manufacturer could supply for the separation task undertaken.

The two industrial UF systems and one RO system from which data are reported performed quite satisfactorily in demonstrating the utility of membrane processing techniques in producing protein isolates and concentrates from the oilseed flours under study.

Protein extracts and UF permeates

Table 1 contains data on protein extracts from soy and glandless cottonseed flours as prepared and processed. The soy extracts were considerably higher in total solids. These solids were composed of a high level of protein (N × 6.25) and a substantial amount of sugars. The cottonseed SP extract (the major fraction of cottonseed protein) contains the high molecular weight proteins. It has the lowest total solids content of the three extracts since prior to its extraction the NSP fraction

has been extracted (see Fig. 2) with tap water along with all the water-soluble carbohydrates and salts. This leaves relatively low amounts of ash and sugar to be removed at pH 10 during the SP extraction. It should be observed that because of this extraction sequence and the low levels of salts and sugars content of the SP extract, it actually requires no fractionation only concentration to reduce the cost of spray drying and conserve water from it for reuse. For this reason RO processing of this extract without first subjecting it to UF was viewed as a viable processing possibility. Actually, both the SP and soy extracts were processed directly with RO membranes and the resulting data will be discussed subsequently.

The NSP extract contains more solids than the SP extract as indicated above because of the salts and sugars that are removed along with these water-soluble, low-molecular weight proteins.

Table 2 contains data on the permeates resulting from UF processing of the three extracts. The solids content of each permeate reflects the composition of the extracts as noted in Table 1. Those extracts highest in sugars and ash, i.e., NSP and soy, had more solids to pass through the UF membranes. The composition of these unretained solids are given on a dry weight basis. These UF permeates as indicated in Figures 1 and 2 would constitute the feed to the RO stage in the proposed process designed.

Table 1—Data on protein extracts from soy and glandless cottonseed flours

Extracts	Total solids (%)	Ash (%)	Nitrogen (%)	Protein (N X 6.25) (%)	Total sugars (%)
Soy	4.75	0.41 (8.72)*	0.51 (10.65)*	3.16 (66.56)*	1.32 (27.79)*
Cottonseed SP	1.63	0.095 (5.82)	0.25 (15.53)	1.58 (97.06)	0.11 (6.53)
Cottonseed NSP	2.54	0.19 (7.59)	0.21 (8.29)	1.32 (51.80)	1.02 (40.16)

* Dry wt basis

Table 2—Data on UF permeates from two UF systems

Permeate source	Membrane system	Total solids (%)	Ash (%)	Nitrogen (%)	Protein (N X 6.25) (%)	Total sugars (%)
Soy Extract	Abcor (HFJ)	1.40	0.26 (18.30)*	0.02 (1.51)*	0.13 (9.45)*	0.70 (50.00)*
SP Extract	Abcor (HFJ)	0.21	0.05 (25.14)	0.01 (4.12)	0.05 (25.75)	0.04 (19.96)
NSP Extract	Abcor (HFJ)	1.63	0.18 (11.22)	0.09 (5.64)	0.57 (35.25)	0.84 (51.53)
Soy Extract	UCARSEP® (ABR)	1.70	0.24 (14.29)	0.04 (2.59)	0.28 (16.19)	1.06 (62.35)
SP Extract	UCARSEP® (ABR)	0.34	0.08 (24.59)	0.02 (6.21)	0.13 (38.79)	0.07 (20.00)
NSP Extract	UCARSEP® (ABR)	1.76	0.18 (10.20)	0.10 (5.58)	0.61 (34.86)	0.79 (44.89)

* Dry wt basis

Retention of components by UF membranes

The percentage of each major component of the three extracts retained by UF membranes are shown in Table 3. Since the percentages retained change from the beginning to the end of a batch concentration, initial and final percentages are given along with a mean value which represents the overall retention for that particular component by a single term.

As would be expected the protein molecules were retained

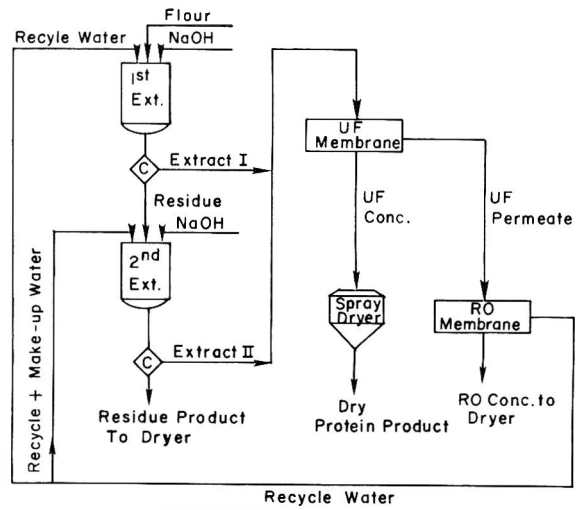

Fig. 1—Simplified flow diagram for soy protein isolation by UF and RO membranes.

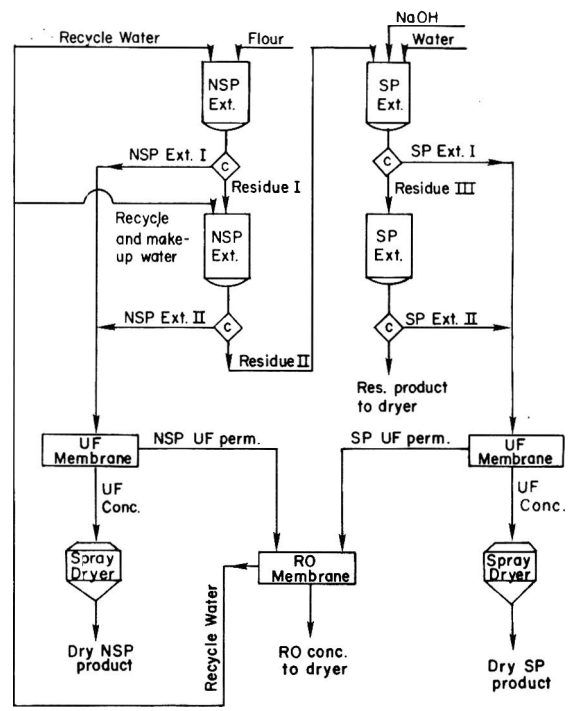

Fig. 2—Simplified flow diagram for cottonseed protein isolation by UF and RO membranes.

Table 3—Percentage of protein extract components retained by two UF systems

Extract processed	Membrane system	Components retained, %											
		Solids			Ash			Nitrogen			Sugars		
		Start	Finish	Mean	Start	Finish	Mean	Start	Finish	Mean	Start	Finish	Mean
Soy	Abcor (HFJ)	66.5	81.0	73.5	45.4	55.3	48.9	97.3	97.2	97.2	23.2	31.7	26.3
SP	Abcor (HFJ)	90.8	96.7	92.6	63.2	71.3	60.2	98.7	98.8	98.1	61.3	88.7	68.1
NSP	Abcor (HFJ)	48.0	66.4	56.0	22.3	28.9	24.0	68.9	84.1	77.3	23.5	45.3	32.1
Soy	UCARSEP® (ABR)	60.2	86.2	72.6	39.4	71.8	51.6	92.1	97.3	94.7	21.3	55.8	34.1
SP	UCARSEP® (ABR)	87.1	96.5	90.2	42.1	61.1	49.8	96.8	98.6	96.5	50.9	83.9	60.9
NSP	UCARSEP® (ABR)	34.3	80.5	49.6	27.0	67.7	36.2	48.3	89.2	63.2	15.0	75.7	36.4

to a much greater degree than smaller salt and sugar molecules. The smaller protein molecules of the NSP extract were retained to a lesser degree than the larger molecules of the SP and soy extracts. While the retention of sugars from the SP extract appears quite high it should be remembered that there were very little sugars in that extract at the beginning. A final observation from these data is the indication that the UCARSEP® ABR membrane was slightly more permeable than the Abcor HFJ in use. This may explain at least in part the differences in permeation rates that will be noted in Figures 3, 4 and 5.

UF permeation rates

Figure 3 depicts permeation rates achieved while processing soy extract by UF. The feed was concentrated to a final solids content of around 13% at overall permeation rates of 26.9 and 84.5 gfd using Abcor and UCARSEP® membranes, respectively. By reducing the original feed volume by a ratio of approximately 4.5 to 1 and then adding back a volume of water equal to the volume of concentrated feed and again reducing the feed volume by a ratio of 2 to 1, one-half of the original feed remaining in the concentrate before dilution is considered to have passed through the membrane and an "equivalent original feed reduction" of around 9 to 1 is generally achieved. Dilution points are indicated in the figure.

Permeation rates achieved during SP extract processing are shown in Figure 4 along with the same information discussed relative to Figure 3. Somewhat different processing tactics were used with the two UF systems during the processing of this important extract. In each instance, however, the final solids content of the concentrated feed was about 14.5% and each UF system accomplished this at the exceptionally high permeation rates of 55.5 and 89.2 gfd for the Abcor HFJ and UCARSEP® ABR membranes, respectively. During processing

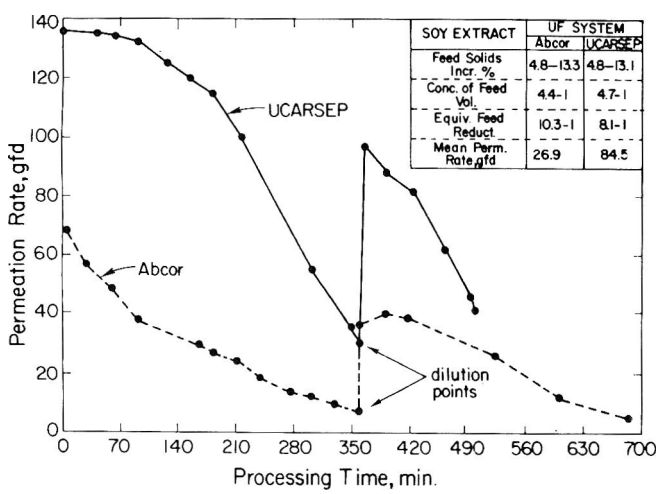

Fig. 3—Relationships between permeation rate and processing time while ultrafiltering soy protein extract using two UF systems with pertinent membrane performance data included.

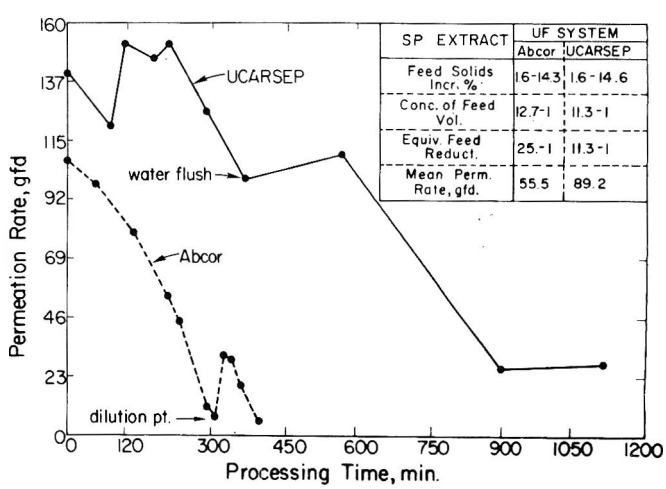

Fig. 4—Relationships between permeation rate and processing time while ultrafiltering glandless cottonseed SP extract using two UF systems with pertinent membrane performance data included.

Table 4—Data on products from UF of soy and glandless cottonseed flour extracts

Product source	UF system	Moisture (%)	Ash	Oil	Nitrogen		Protein (N X 6.25)	Total P	Total sugars	Color	
					Total	NPN*				dry	wet
			% Dry wt basis								
Soy Extract	Abcor (HFJ)	1.72	6.31	0.08	14.05	0.18	87.80	0.83	5.65	82.5	46.0
SP Extract	Abcor (HFJ)	5.23	2.42	0.17	16.86	0.23	105.39	0.33	3.29	75.6	26.0
NSP Extract	Abcor (HFJ)	3.46	4.81	0.77	11.21	1.51	70.05	0.67	17.20	78.4	57.9
Soy Extract	UCARSEP® (ABR)	2.05	6.37	0.00	13.95	0.24	87.13	0.82	7.70	76.3	46.4
SP Extract	UCARSEP® (ABR)	1.33	3.02	0.15	16.72	0.24	104.52	0.29	3.73	69.5	18.5
NSP Extract	UCARSEP® (NJR)	3.54	5.47	2.04	12.24	0.36	76.52	1.11	10.21	69.6	50.1

* Nonprotein nitrogen

with the UCARSEP®, a dilution technique was not used. Instead the feed was removed at the point indicated in Figure 4 and a water flush given to the system before returning the feed and resumption of processing. Thus, a high "equivalent volume reduction" was not attained as for the Abcor system with which the usual technique was employed.

Figure 5 shows permeation rates achieved during NSP extract processing. The same information contained on the previous two figures is shown on it also. Each extract behaves somewhat differently during processing due to their varying composition. The run data plotted were taken using a UCARSEP® NJR membrane rather than the UCARSEP® ABR used with SP extract and in some runs with both SP and NSP extracts. A tighter membrane than those available for use with either system would have been desirable to attain a higher mean retention of NSP. Tighter noncellulosic membranes are now commercially available which would be expected to give higher retention of NSP.

Analytical data on UF products

Table 4 gives analytical data on dry products from the three UF concentrated extract feeds. These products were freeze dried. While the SP product is the only one that could rightly be called an isolate (i.e., 90% protein or greater on a dry weight basis) no difficulty would be anticipated in raising the protein level of the soy isolate by increased purification at the dilution point during processing. NSP protein products from conventional isolation procedures possess protein contents below the recognized isolate level. This will also be true of NSP products from the membrane isolation process (MIP) presented herein.

The higher protein content of the SP product processed with the Abcor membrane is reflective of the dilution (washing) technique. This technique was not applied during the UCARSEP® processing of the SP extract (see Fig. 4). Other proximate analyses are presented in Table 4 and subsequently in Table 5 which permit comparisons of MIP products with

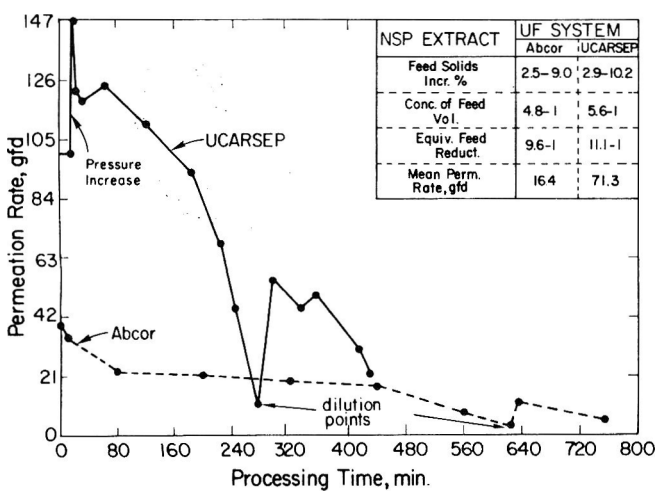

Fig. 5—Relationships between permeation rate and processing time while ultrafiltering glandless cottonseed NSP extract using two UF systems with pertinent membrane performance data included.

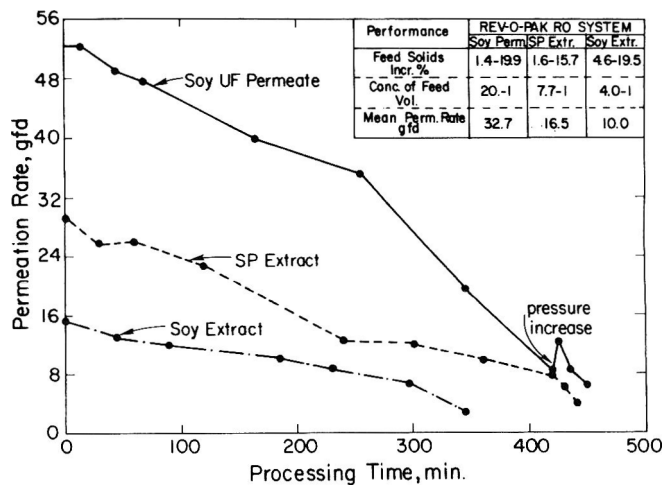

Fig. 6—Relationships between permeation rate and processing time while RO processing soy UF permeate, soy extract and SP extract with pertinent membrane performance data included.

Table 5—Data on products from RO processing of UF permeate and protein extracts[a]

Material processed	Moisture (%)	Ash	Oil	Nitrogen		Protein (N X 6.25)	Total P	Total sugars	Color	
				Total	NPN				dry	wet
		% Dry wt basis								
Soy UF permeate	5.04	16.72	0.00	1.39	0.77	8.66	0.54	46.34	67.5	29.5
Soy extract	1.65	8.33	0.05	11.2	0.35	70.04	0.71	18.71	84.7	43.9
SP extract	2.42	9.61	0.01	15.13	0.38	94.54	0.92	5.10	75.9	27.2

[a] Processed with Rev-O-Pak, Inc. reverse osmosis system

corresponding products from conventional processes where such products are available. For example, the total phosphorus content of the MIP soy product was found to be essentially the same as that of a leading commercial soy isolate. Phosphorus contents of SP and NSP products from the MIP were also comparable to those of similar products from conventional procedures.

It is beyond the scope of the work reported to further compare the quality and quantity of MIP products obtained with that of products from conventional isolation procedures. However, those comparisons along with energy and economic analyses are contemplated in our further investigations.

It is expected that yields of the MIP products will be considerably greater than yields from conventional processes since protein that normally would remain in the wheys after acid precipitation is harvested along with the precipitable protein in the MIP. The highly soluble whey proteins in admixture with the proteins normally going into isolates enhances the nitrogen solubility characteristics of the MIP products and is expected to alter other of their functional properties as well. Nitrogen solubility index (NSI) values on a MIP soy UF product, for example, were more than twice that of a commercial soy protein isolate tested.

RO processing of UF permeates and protein extracts

Figure 6 gives permeation rates and performance data obtained while processing soy UF permeate, SP extract and soy extract with the Rev-O-Pak RO system. These three feed streams, composed of the constituents shown in Tables 1 and 2, were concentrated to solids contents of 19.9, 15.7 and 19.5%, respectively. No dilution step is incorporated in an RO processing cycle since the objective here is essentially concentration alone without component purification. Those feeds which had lower initial solids contents gave better overall permeation rates and the feed with essentially all of its protein previously removed by a UF membrane (soy UF permeate) gave the highest flux of the three.

Although not shown elsewhere the solids content of the RO effluent from processing soy UF permeate was lower than that of the tap water (0.041% as compared with 0.055%) available from the Texas A & M University water system. This fact is indicative of its recyclability as designed into the MIP flow diagrams of Figures 1 and 2.

Table 5 holds analytical data on dry products from processing the three feed streams described. The SP product protein content is still at the isolate level since it needed no fractionation to achieve this as previously mentioned. The soy product protein level changed little from that of the original extract solids as expected. The RO product from soy UF permeate processing is shown in the first row of the table. This product might find utility as a feed ingredient or be marketed for other purposes. A study of its possible utilization was beyond the scope of the work reported.

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DETERMINATION OF VITAMIN A IN FOOD COMPOSITES BY HIGH SPEED LIQUID CHROMATOGRAPHY

ABSTRACT

A method is presented for measurement of Vitamin A in food composites by high speed liquid chromatography. Saponification of the sample is necessary, but no purification or separation is required. Neither phytofluene nor other unsaponifiable material in the sample cause interference. Accurate measurement is possible in samples which contain 0.5–4 μg/ml, and recovery of Vitamin A standard in the presence of high levels of carotenoids is 103.5%.

INTRODUCTION

WHILE TWO OFFICIAL methods (AOAC, 1975) are available for measuring Vitamin A in foods, neither method is adequate in all cases. Limitations and disadvantages of the official method have been described (Erdman et al., 1973). Basically, high sterol content interferes in one method and the presence of high amounts of plant material amplifies problems with the other method.

Recently, Vitamin A has been measured fluorometrically, without many of the disadvantages or problems incurred with the official method (AOAC, 1975). When determinations are made on foodstuffs, substances such as phytofluene may be present and interfere or produce spuriously high readings. Corrections of such data require additional readings and subsequent mathematical manipulation (Thompson et al., 1971; Erdman et al., 1973; Bubb and Murphy, 1973). Garry et al., (1970) used a fluorometric method after chromatographing the sample on a silicic acid column to separate Vitamin A from interfering fluorescent compounds.

Williams et al., (1972), described quantitative analysis of fat-soluble vitamins by liquid chromatography, using a UV detector. They used purified samples which did not require prior saponification, and they did not encounter interfering substances typically present in food material. Van de Weerdhof et al., (1973) used liquid chromatography for routine analyses of foodstuffs. They used a fluorometric detector but did not describe methodology sufficiently to permit use of the method.

The purpose of this work was to develop a method for determination of Vitamin A in food composites, in which interferences would be avoided with minimum clean-up. The method is presented below.

MATERIALS & METHODS

Preparation of standard

A Vitamin A standard reference capsule (obtained from U.S.P. .Reference Standards, U.S. Pharmacopeial Convention Inc., 460 Montgomery Ave., Bethesda, MD 20014) was used. This contained a solution of 34.4 mg of all-trans retinyl acetate per gram in cottonseed oil. The capsule was expressed into a 300 ml glass-stoppered Erlenmeyer flask, a few crystals of EDTA and equal volumes of water and KOH-saturated methanol were added, and the mixture was refluxed in a nitrogen atmosphere for 15 min. The saponification mixture was extracted with acetone-hexane, 1:1, and re-extracted with hexane. The extract was washed free of KOH and acetone, dried with Na_2SO_4, and made to 100 ml in hexane to provide a standard stock solution. A standard working solution was prepared by diluting this solution with hexane to contain 4–5 μg Vitamin A/ml; exact concentration was determined on a scanning double-beam spectrophotometer at 325 nm. A 5-ml aliquot of this standardized working solution was evaporated to dryness and made to 10 ml in $CHCl_3$; this was the reference standard solution used with the high speed liquid chromatograph (LC).

Preparation of food samples

Homogenized mixed food items or composites of complete meals, which had been preserved with KOH and $CHCl_3$, were extracted according to the method of Purcell (1962), except hexane instead of ether was used for extraction of filtrate. Size of sample varied; 50g of the homogenate were used for most samples. When items or composites were estimated to have relatively high Vitamin A content, a known amount ranging from 5–25g was used. Approximately 100 ml aliquots of the hexane extracts were saponified with 50 ml KOH-saturated methanol as above. Samples containing large amounts of Vitamin A were made to known volume in hexane; aliquots were evaporated to dryness in nitrogen atmosphere and dissolved in 10 ml $CHCl_3$ for use with the LC. If items contained a small amount of Vitamin A, the entire sample was used and was fortified with the same amount of Vitamin A as was used for the reference standard.

Most food composites contain carotenoids in varying concentrations. Those containing carrots or tomatoes are especially high in the fluorescing carotenoid, phytofluene. A food sample containing a mixture of items which contained no Vitamin A but a high carotenoid level was prepared by the method described above. This sample was used to demonstrate the influence of carotenoids and to determine the need for clean-up of samples before LC.

The carotenes and xanthophylls were separated by partitioning an aliquot of the above food composite with 95% methyl alcohol. Xanthophylls in the methyl alcohol layer were extracted with diethyl ether and transferred to hexane.

An aliquot of the complete food composite sample was passed through a neutral Al_2O_3 column using the official method (AOAC, 1975). The Vitamin A fraction was fortified and the results with LC were compared to an identical sample that had not been passed through the Al_2O_3 column.

Phytofluene was prepared by treating shredded carrots with methyl alcohol, extracting with acetone:hexane, 1:1, washing free of acetone, and saponifying as described above. The phytofluene was isolated and purified by repeated chromatography through MgO:diatomaceous earth. The final solution was contained in hexane, its purity was checked by its absorption spectrum and the exact concentration determined by its absorbance at 325 nm.

A stock solution of liver extract was prepared by the extraction and saponification method described above. Concentration of the liver extract was determined by LC against the Vitamin A reference standard. Varying amounts of this solution were mixed with constant concentrations of Vitamin A reference standard and recovery of Vitamin A was calculated.

Measurement of Vitamin A

A Varian Model 4200 basic gradient elution liquid chromatograph was utilized. It was fitted with two 5000 psi pumping devices capable of gradient or flow solvent programming and a UV continuously variable wave length detector with flow-through cell. A Fisher Recordall Series 5000 recorder with integrator was connected to the detector.

Dry degassed hexane was used in one pump (A) and degassed

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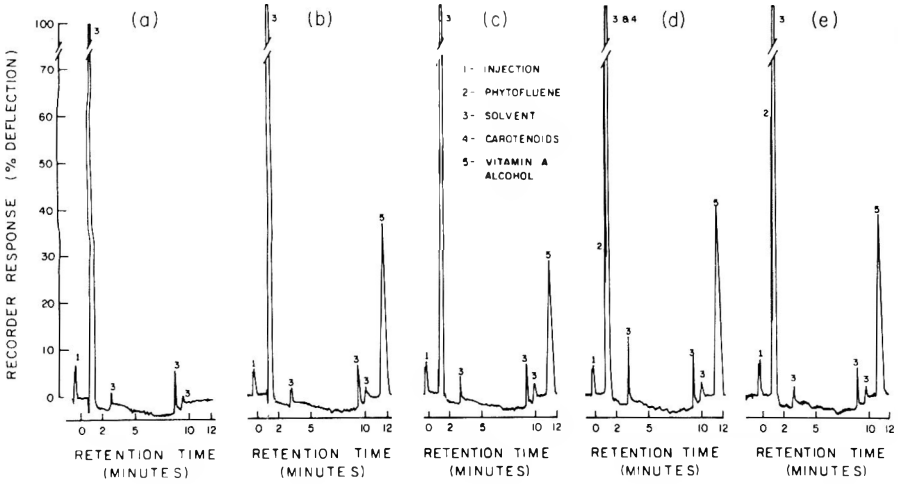

Fig. 1—Chromatograms of Vitamin A reference standard alone and mixed with food materials. (a) solvents; (b) 1.92 μg/ml Vitamin A standard; (c) 0.77 μg/ml Vitamin A standard + 0.5 μg/ml Vitamin A from liver; (d) 1.92 μg/ml Vitamin A standard + 2.0 μg food composite; (e) 1.82 μg/ml Vitamin A standard + 1.71 μg/ml phytofluene.

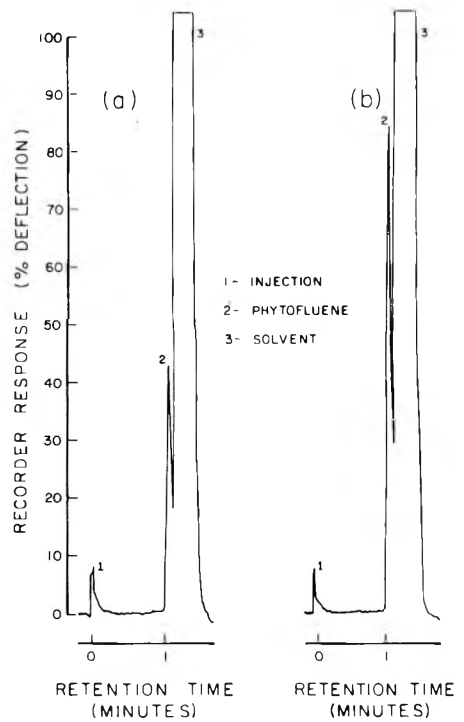

Fig. 2—Chromatograms of separation of phytofluene and solvent peaks. (a) 1.37 μg/ml phytofluene; (b) 2.74 μg/ml phytofluene.

methylene chloride:isopropanol, 9:1, in the other (B). Flow rate was set at 39 ml/hr, pumps A plus B. Detector was set on 325 nm detector scale at 0–0.1 absorbance units, full scale deflection, and slit 2.0 nm. Damping was necessary at this low scale. A 50 cm, 2 mm i.d. Varian MicroPak column packed with LiChrosorb Si60-10 was used. Integration amplitude was set on high, and the volt scale for the recording pen was set at 0.1.

Gradient elution was used and the mobile phase was programmed as follows: Initial solution ratios were 81% from pump A and 19% from pump B; decreased 9%/min from pump B for 2 min; held at 1% for 1 min; decreased 0.2%/min for 1 min; decreased 0.8%/min for 1 min. Pump B was then reset to 19%.

The zero adjustment was made with initial solvent ratios, a 50 μl sample of Vitamin A reference standard was syringe-injected, and the gradient elution program was started. Integrations were counted when Vitamin A was eluted. A 50 μl aliquot of food sample, containing 0.5–4 μg/ml was injected and gradient elution program used as above. This range of accuracy was established from the standard curve. Concentration of vitamin in the sample was determined by ratio with integrations and concentration of the standard.

RESULTS & DISCUSSION

FOOD COMPOSITE samples contain a large amount of pulp. Extracting before saponification separated pulp from the lipid material; therefore, after saponification, there was a decreased problem with washing samples because there was less material entrapped with emulsions. Liquid samples such as milk could be saponified before extraction.

It was necessary to saponify in order to convert all the Vitamin A, whether palmitate or acetate, into the same form as in the standard against which samples were measured. In the work carried out here, no peak was obtained in liquid chromatography with samples that had not been saponified.

Detection of Vitamin A

A chromatogram of the solvent used with all samples is shown in Figure 1a; this include s a typical aliquot of hexane transferred to $CHCl_3$. Chromatograms of Vitamin A reference standard and the standard mixed with liver extract which contained naturally-occurring Vitamin A are shown in Figures 1b and 1c. As can be seen, both the synthetic standard and natural Vitamin A have identical retention times.

There was less than 2% difference in Vitamin A value between liver extracts which had or had not been passed through the Al_2O_3 column. No advantage of this clean-up step was seen. A chromatogram of a food composite which had not been passed through an Al_2O_3 column, and which had been fortified with standard Vitamin A is shown in Figure 1d. Again, no differences in peaks or retention time was noted, and no interference from materials in the food composite was apparent.

When the phytofluene extract was fortified with Vitamin A standard and the mixture passed through the LC, phytofluene was eluted on the leading edge of the first solvent peak, as shown in Figure 1e. Resolution of two concentrations of phytofluene from the first solvent peak, obtained by using a more rapid chart speed, is shown in Figures 2a and 2b. Further proof that carotenes and xanthophylls have much shorter retention times than Vitamin A and do not interfere with its measurement are shown in Figure 3. For these chromatograms, detector scale was changed to 0 to 1 absorbance units, full scale deflection.

Range and recovery

A standard curve plotted for Vitamin A in amounts ranging from 0.24–8.24 μg/ml is shown in Figure 4. At the higher levels, there appeared some distortion toward high readings.

Table 1—Recovery of Vitamin A reference standard in the presence of food materials

Sample		Components included in sample: Vitamin A reference standard (μg/ml)	Components included in sample: Vitamin A from liver (μg/ml)	Components included in sample: Food composite[a] (g/ml)	Total Vitamin A recovered (μg/ml)	Reference standard recovered: μg/ml (Total minus liver)	Reference standard recovered: Percent[b]
I	A	1.71	0.10	0	1.83	1.73	101.2
	B	1.71	0.16	0	1.88	1.72	100.5
	C	1.71	0.20	0	1.98	1.78	104.1
	D	1.88	0.45	0	2.29	1.84	97.9
	E	1.88	1.13	0	2.93	1.80	95.7
	F	0.75	0.45	0	1.17	0.72	96.0
	G	1.32	0.24	0	1.58	1.34	101.5
	H	1.32	0.47	0	1.82	1.34	101.5
	I	1.32	0.71	0	2.06	1.35	102.3
	J	1.32	1.42	0	2.87	1.45	109.8
II	A	1.87	0	0.47	1.94	1.94	103.7
	B	1.87	0	1.87	1.93	1.93	103.2

[a] High carotenoid content but no Vitamin A
[b] Between-duplicate variance = 1.32

The regression formula calculated over the whole range was Y = −2.87 + 48.51 and the residual term was 14.6. However, the regression calculated over the range of 0.24–4.12 μg/ml was Y = 0.24 + 46.10, and the residual term was only 1.64. At levels lower than 0.24 μg/ml, variation between duplicate samples was relatively high.

It was determined that when working with food composites, best data were obtained when Vitamin A levels were 0.5–4 μg/ml.

Food samples containing very low amounts of Vitamin A could be fortified with standard Vitamin A to increase their concentration to a level which could be accurately measured. In analysis of food composites, it was noted that when fewer than 4–5 integration units (based on 100 units in standard chart paper) were contributed by the sample, duplicates often varied beyond an acceptable amount.

Recovery of standard Vitamin A when mixed with the food composite and with natural Vitamin A from liver is shown in Table 1. For samples containing standard Vitamin A and liver extract, low in carotenoid content, mean recovery of standard Vitamin A was 101.1%. Mean recovery of Vitamin A from mixtures with high carotenoid content was 103.5%.

When the stock solution of liver extract was handled as a natural Vitamin A standard and was mixed in varying amounts with food composite containing no Vitamin A, recovery of the natural Vitamin A was determined. Results are shown in Table 2.

Table 2—Recovery of natural Vitamin A in the presence of food composites

Sample	Components included in sample: Vitamin A from liver (μg/ml)	Components included in sample: Food composite[a] (g/ml)	Vitamin A recovered: μg/ml	Vitamin A recovered: Percent
A	2.24	0.29	2.29	102.2
B	1.15	0.29	1.12	97.4
C	2.23	1.17	2.26	100.9
D	1.15	1.17	1.17	101.7
E	1.37	0.46	1.38	100.7
F	1.37	1.87	1.31	95.6

[a] High carotenoid content but no Vitamin A present

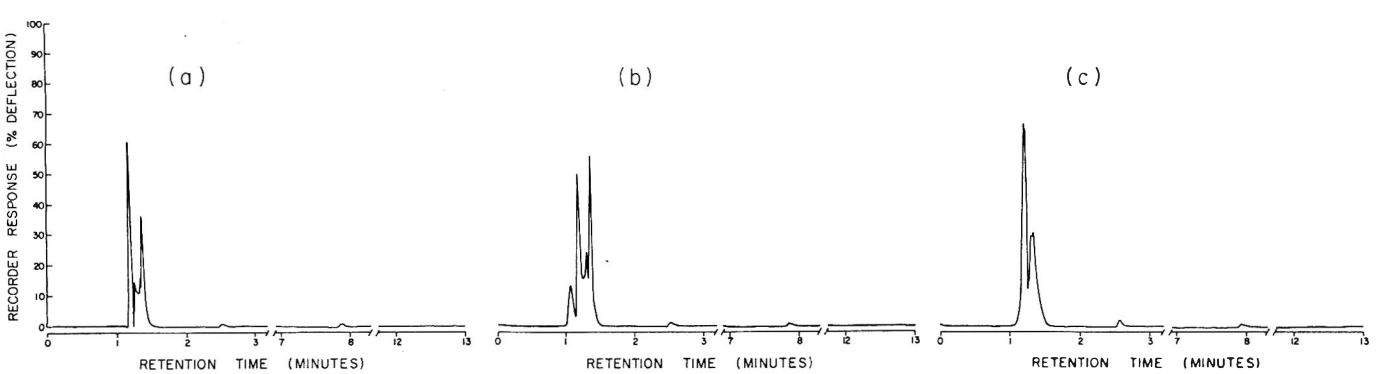

Fig. 3—Chromatograms of carotenoids. (a) solvents; (b) carotenes; (c) xanthophylls.

Hexane vs chloroform

Chromatographing Vitamin A in chloroform necessitated an extra step in the procedure, but chloroform offered several advantages over hexane as the final solvent. Hexane solutions prepared for chromatography were sometimes turbid; recorded peaks from hexane solutions showed more tailing, perhaps because of insoluble material. Recovery of Vitamin A was superior when it was chromatographed in chloroform.

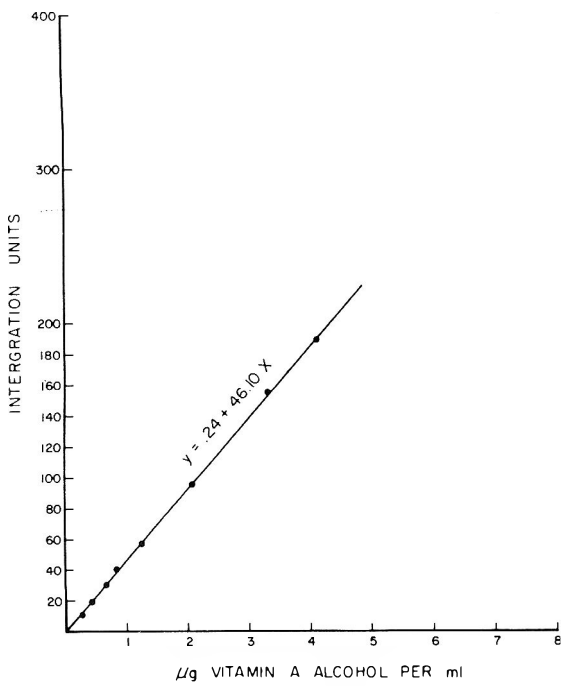

Fig. 4—Standard curve for Vitamin A reference solution obtained by high speed liquid chromatography.

Gradient elution vs constant composition of mobile phase

Results from the liquid chromatograph were compared when programmed gradient elution was used or when mobile phase of constant composition (81% pump A, 19% pump B) was used. Gradient elution gave more reproducible results. With constant composition of mobile phase, recoveries were acceptable but less consistent. Advantages of gradient elution were more pronounced when chromatographing food mixtures than when measuring the Vitamin A standard. The gradient elution method was slower; with the conditions given here, approximately 12 min were required for Vitamin A to pass through the column with gradient elution, while it was eluted in approximately 7 min with mobile phase of constant composition.

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EFFECT OF EMULSIFIERS ON THE TEXTURE OF COOKIES

ABSTRACT

The function of emulsifiers in cookies is well reported as a characteristic of spread ratio. It is understandable that these ingredients would also affect other characteristics. Texture, as related to hardness or softness of the cookie, is one of the most readily discernible features for the consumer. Using the American Association of Cereal Chemists method for determining cookie spread ratio, various emulsifiers were evaluated for their effect on the texture of cookies. Optimum levels for each emulsifier were determined. An Instron, model 1132 with the Ottawa Texture Cell, was used in establishing the procedure for measuring cookie texture. The procedure was designed in which the tenderness of samples could be determined and presented graphicly.

INTRODUCTION

THE EXPLORATION of cookie ingredients and test procedures is well documented. Information has been presented on cookie ingredients (both major and minor ingredients, to include acidulants and alkalinity), mixing conditions and baking variations. The relationship of surfactants to spread ratio, protein fortification and cookie quality has been reported by Tsen et al. (1973, 1975). The textural response to surfactants has not been explored to any significance. It was the purpose of this work to study the textural responses to surfactants used in cookies.

Gross differences in texture (softness vs toughness) can be determined through subjective type evaluations using hedonic ratings or preference selection. Consistency of evaluations during storage and small changes related to richness of mouthfeel can pose some analytical problems. This work used instrumental evaluations to determine textural changes that occur when surfactants are used in cookies.

EXPERIMENTAL

CRITERIA for setting up the evaluation were based on two variations:
(a) Variations between cookie formulae and ingredients; and
(b) Establishing a procedure for textural evaluations.

Of the two available cookie formulae (AACC Method 10-50 (1962) or the Micro Method of Finney et al. (1950)), we used the AACC Method 10-50 (cookie flour spread test) to screen the effects of emulsifier levels.

Ingredients, baking sheets, thickness gauges and cutters were as specified by the test. The soft wheat cookie flour used throughout the investigation had 8.7% protein, 0.41% ash, and 12.5% moisture. The moisture was checked periodically during the project to insure correct water addition. The cookies were mixed in a Hobart N-50 mixer and baked in a Reed reel oven at 204.5°C for 10 min.

Cookies were evaluated, after cooling for 30 min, for spread ratio and top grain. Samples were packaged in heat sealable poly bags and stored at room temperature for 3 months. Initial texture readings were observed after 18 hr storage.

Textural evaluations were measured using an Instron Texturometer, model 1132. The instrument settings were determined through selective observation of chart, print-out data. Critical instrument settings and conditions included load cell, calibration setting, range selector, crosshead speed and chart speed. The load cell used was a 500 kg, combination cell (tension/compression cell) and was calibrated to 50% full scale. This calibration insured that textural measurements would fall within the limits of the chart.

Since the chart speed and crosshead speed affect the appearance of the printed graph, these settings were established to show highest sensitivity and information. Optimum chart speed was 10 mm/sec using the CX/CY gears and the crosshead speed was set for 1 mm/sec using the BX/BY gear combination. Charts produced from these settings gave a moderate peak height at maximum compression and expanded the graph slope to give an indication of internal structure and texture of the cookie.

The range selector for the testing was M-50. The M setting refers to the type of cell used (metric or English) and the 50 is the specific range setting. Variations on the range setting will affect size and shape of the graph.

Cookies were compressed to a depth of one-half the thickness of the cookie or 4 mm. Six cookies were evaluated for each emulsifier level and each storage sample observed.

The emulsifiers selected, have known or expected use in the bakery industry. The samples used were commercial products and were added to the formulation as received with no grinding, blending or preconditioning. The emulsifiers were incorporated into the cookie dough during the first step or creaming phase of the mix. Table 1 summarizes the level of each surfactant. Use levels are based on flour weight.

RESULTS & DISCUSSION

THE DATA collected throughout the evaluations are summarized in Figures 1–5. The higher the Instron units the harder the cookie. The control cookie (no additive) is shown on each graph as a single point, along the vertical axis. Initial results are shown as continous lines, while the 3-month storage points are connected with the dotted lines. All storage was done at room temperature (22–24°C).

The use of sodium stearoyl lactylate (SSL) in cookies, (Fig. 1) shows initially an almost straight line relationship of constant temperature vs level used. After 3 months, SSL gave lower textural scores with the most noticeable change being at the 0.375% level. On initial observation the lactylic esters of fatty acids show constant increase in texture as the use level

Table 1—Surfactants and levels[a]

Surfactant	Levels
Sodium stearoyl actylate (SSL)	0.125, 0.250, 0.375, 0.500, 0.625
Lactylic esters of fatty acids	0.125, 0.250, 0.375, 0.500, 0.750
Succinylated monoglycerides (SMG)	0.125, 0.250, 0.375, 0.500, 0.750
Ethoxylated monoglycerides (EMG)	0.125, 0.250, 0.375, 0.500, 0.625
Plastic mono and diglyceride (40% α-mono)	0.250, 0.500, 0.750, 1.00
Polyglycerol esters (3-1-S)	0.250, 0.500, 0.750
Diacetyl tartaric acid esters	0.250, 0.500, 0.750

[a] Based on % flour weight

[1] Present address: Pillsbury Co., Minneapolis, MN 55414

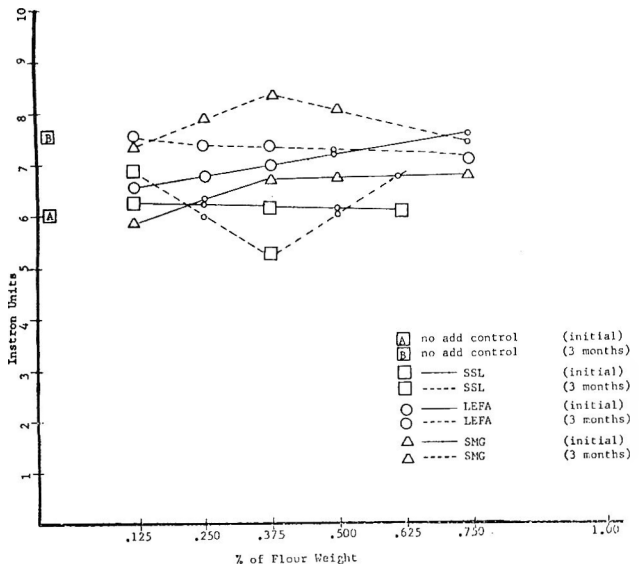

Fig. 1—Sodium stearoyl lactylate (SSL), lactylic esters of fatty acids (LEFA), and succinylated monoglycerides (SMG).

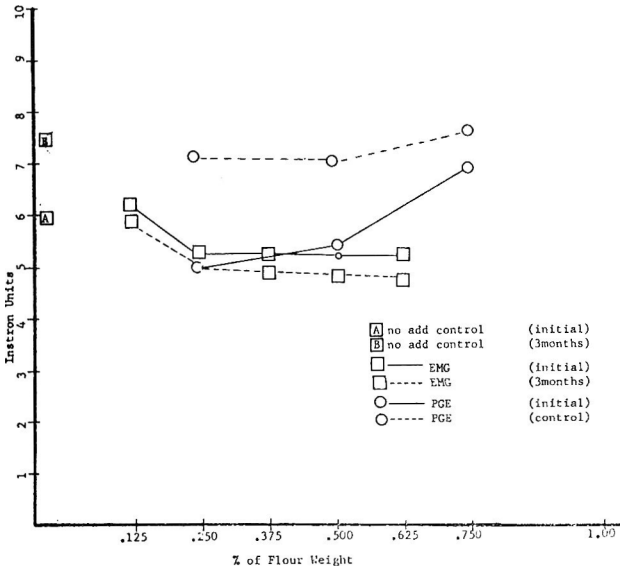

Fig. 2—Ethoxylated monoglycerid (EMG) and polyglycerol esters (PGE).

goes up. At 3 months the higher use levels produced a relatively constant texture and lower levels produced a definite toughening. Succinylated monoglycerides initially increase texture. On storage, the cookies harden at all levels, with the % change being less at the higher levels.

The initial observations with the ethoxylated monoglyceride (EMG) (Fig. 2) show immediate softening and then a leveling out from 0.250% on. After 3 months storage the EMG samples are slightly softer at all levels. The polyglycerol ester (3-1-S type) (PGE) cookies were softer over most levels. The cookies tended to harden as the PGE level increased. Three-month storage samples increased significantly in hardness.

Using a 40% α-monoglyceride product, the texture was slightly softer during initial observations. The slope of the monoglyceride graph paralleled that of the control (Fig. 3). At 3-months storage, the cookies containing monoglycerides approached or exceeded the texture of the control. The diacetyl tartaric acid esters gave a considerably softer cookie at all levels. A slight softening occurs as the level of additive increases. After 3 months some increase is noted; however, most samples were still softer than the control.

Recent interest has been noted in the ability to reduce shortening of fat in bakery and other food systems. The test formula was modified by reducing the shortening by 20% and

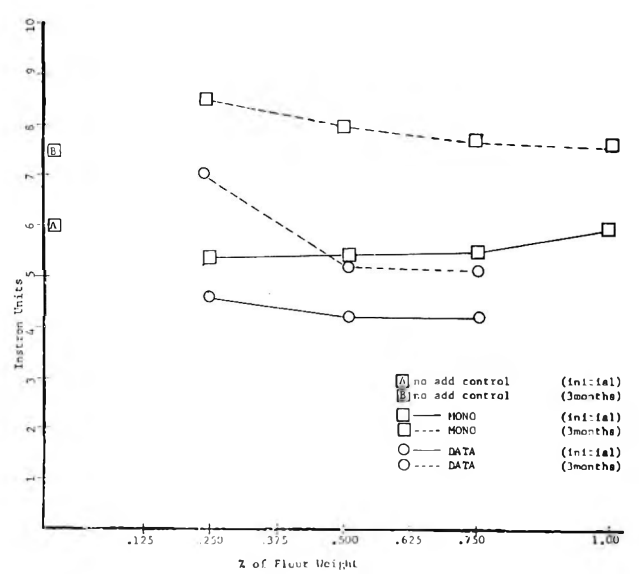

Fig. 3—40% α monoglyceride (MONO) and data esters (DATA).

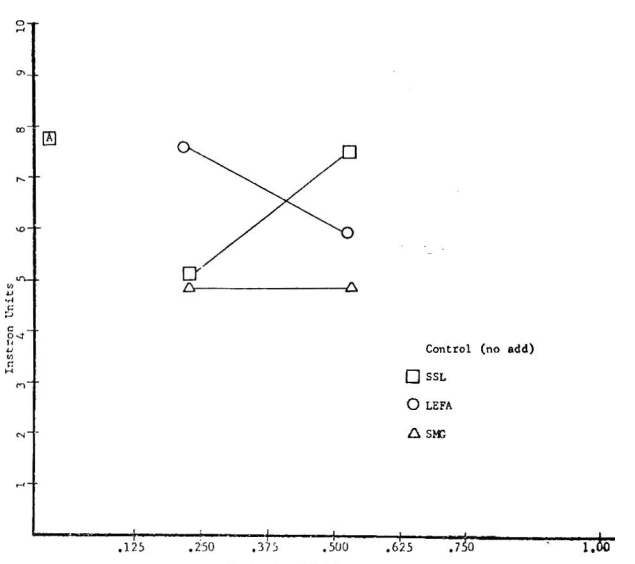

Fig. 4—Reduced shortening with SSL, LEFA and SMG.

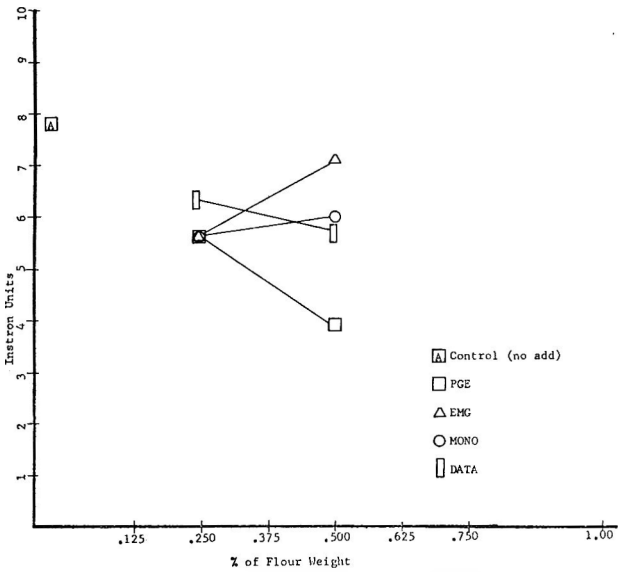

Fig. 5—Reduced shortening with EMG, PGE, MONO's and DATA esters.

at the same time increasing the water by one-half the weight of shortening removed. This is comparable to increasing the water by 32%. This step is necessary to maintain proper formula balance. Initial observations are completed at this time and are presented in Figures 4 and 5. Only two levels of each emulsifier were observed, that being 0.250% and 0.500% of the flour weight.

All of the products observed gave textural values below (softer) that of the control. SMG showed the most stable texture verses levels used and was also the most tender. SSL appears to impart most softening at lower levels while LEFA is just the opposite (Fig. 4).

At the 0.25% level, EMG, monoglycerides and PGE produced equal softness in the cookie (Fig. 5). The polyglycerol esters exhibit the most softening especially at higher use levels. The 40% mono shows moderate increase and the EMG produces a texture approaching that of the control. Data esters show a moderated effect with only slight change as the level increases.

SUMMARY & CONCLUSIONS

SELECTED EMULSIFIERS were tested and their effect on cookie texture has been recorded. The results depend upon the formula used and the specific effect(s) desired. Based on the change in softness vs time, the optimum levels for the discussed surfactants are: sodium stearoyl lactylate (SSL), 0.375%; lactylic esters of fatty acids (LEFA), 0.500%; succinylated monoglycerides (SMG), 0.75%; ethoxylated monoglycerides (EMG), 0.25–0.50%; 40% α-monoglyceride, 1.0%; polyglycerol esters (3-1-S), 0.75%; diacetyl tartaric acid esters, 0.50–0.75%. All percentages are based on flour weight.

When the shortening in the system is reduced, the optimum levels for the surfactants are: SSL–0.250%; LEFA–0.500%; SMG–0.25–0.50%; EMG and 40% α-mono–0.250%; PGE–0.500%; and data esters–0.500%. Again, percentages are based on flour.

The research completed to date can be used as the basis for future work to determine the correlation of surfactant effects on texture, storage and consumer acceptance. Some of this work is currently underway, using commercial formulae and equipment. It is anticipated that the information from these continuing studies will be presented at a later date.

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EFFECTS OF CONVENTIONAL BAKING, MICROWAVE BAKING, AND STEAMING ON THE NUTRITIVE VALUE OF REGULAR AND FORTIFIED BREADS

ABSTRACT

The nutritive value of protein in regular and fortified breads was significantly affected by methods of baking. Rat-feeding tests demonstrated that protein efficiency ratios (PERs) of breads were significantly improved and feed conversion ratios were reduced by substituting steaming or microwave baking for conventional baking. Lysine and other amino acids varied little among conventionally baked, microwave baked, and steamed breads. The significantly low PER of conventionally baked bread indicated that lysine became less available nutritionally with conventional baking than with either microwave baking or steaming. Lysine or soy fortification could effectively raise the PER of conventionally baked bread. Loaf volume and crumb qualities of conventionally baked bread were better than those of microwave-baked or steamed bread. However, conventional baking produced a much darker brown crust and crumb than did microwave baking or steaming. The effect of heating, as shown by browning, on reducing the bread's nutritive value could be greatly minimized by replacing conventional baking with microwave baking or steaming.

INTRODUCTION

BREAD is the most popular food in the world. However, it and other cereal products are nutritionally inferior because they are generally deficient in lysine. Wheat flour is low in lysine, and the baking process can aggravate the lysine deficiency in bread. Several investigators, notably Rosenberg and Rohdenburg (1951, 1952), Rosenberg et al. (1954), Sure (1952), Hutchinson et al. (1959), Ericson et al. (1961), Jansen and Ehle (1965) and Jansen et al. (1964), have shown that lysine is the limiting amino acid in bread and that lysine loses nutritive value during the baking process.

Several simple ways can be employed to remedy bread's lysine deficiency. One is to fortify wheat flour with lysine or such protein-rich additives as soy flour and protein, for breadmaking, as shown by many reports (Scrimshaw and Altschul, 1971; Milner, 1969). The other is to modify the bread processing to reduce the nutritional loss of lysine. However, little information had been available on the effect of various heating processing (conventional baking, microwave baking, and steaming) on the availability of lysine in bread. The study was therefore undertaken to compare the effects of conventional baking, microwave baking and steaming on the nutritive value of protein in regular, lysine-fortified, and soy-fortified breads. Chemical analyses and rat-feeding tests were used to determine results.

MATERIALS & METHODS

Materials

Commercial wheat (hard red winter) flour and defatted soy flour were used. Their moisture, protein, ash and fat contents were:

	Moisture (%)	Protein[a] (%)	Ash (%)	Fat (%)
Wheat flour	12.0	11.0	0.5	–
Soy flour	7.6	51.2	7.8	1.1

[a] %N × 5.7 for wheat flour; %N × 6.25 for soy flour.

We used AACC Methods (1962) for all analyses except for fat by AOCS Method Aa 4-38 (1971) with petroleum ether as the extracting solvent.

L-lysine monohydrochloride (lysine) was purchased from ICN Pharmaceuticals, Inc., Cleveland, Ohio. Sodium stearoyl-2 lactylate (SSL) was obtained from C.J. Patterson Company, Kansas City, Mo.

Processing

We made all doughs using the K-State Process developed by Tsen

Table 1—Composition of diets fed rats

Protein source[a,b] (Protein %)	Casein or ground bread[d] (%)	Fat (oil) (%)	Vit. mix[e] (%)	Min. mix[e] (%)	Sugar (%)	Starch (%)
Casein[c] 82.0	12.20	2.0	2.0	3.0	2.8	77.00
WF(C) 12.4	80.64	2.0	2.0	3.0	2.8	9.56
WF(M) 12.4	80.64	2.0	2.0	3.0	2.8	9.56
WF(S) 12.3	81.30	2.0	2.0	3.0	2.8	8.90
WF + 0.2% L(C) 12.5	80.00	2.0	2.0	3.0	2.8	10.20
WF + 0.2% L(M) 12.5	80.00	2.0	2.0	3.0	2.8	10.20
WF + 0.2% L(S) 12.5	80.00	2.0	2.0	3.0	2.8	10.20
88% WF + 12% SF(C) 17.1	58.47	2.0	2.0	3.0	2.8	31.73
88% WF + 12% SF(M) 17.1	58.47	2.0	2.0	3.0	2.8	31.73
88% WF + 12% SF(S) 17.2	58.13	2.0	2.0	3.0	2.8	32.07

[a] WF, WF + 0.2% L, and 88% WF + 12% SF indicate breads prepared from wheat flour, wheat flour fortified with 0.2% L-lysine monohydrochloride, and 12% soy fortified four (88 parts wheat flour and 12 parts defatted soy flour), respectively.
[b] (C), (M) and (S) denote breads, processed by conventional baking, microwave baking, and steaming, respectively.
[c] Casein diet contains 1% nonnutritive fiber
[d] Amount is added to make diet with 10.0% protein
[e] Vitamin and mineral moistures were adjusted for vitamin and mineral contents of flours, using starch as a filler.

and Tang (1971). The formula, on a wheat flour or soy-fortified flour (88 parts of wheat flour and 12 parts of soy flour) basis, called for 5% sugar, 2% salt, 3% yeast, 70 ppm bromate, 0.5% SSL, 0.2% lysine for lysine-supplemented products only, 60% water for wheat-flour dough, and 70% water for soy-fortified flour dough. Amounts of water used to prepare various solutions and suspensions were included as part of the total water required in each formula.

After each batch of dough fermented, we prepared nine dough-pieces, each weighing 125g. After proofing the dough, we processed three samples each by conventional baking (19 min at 218°C), steaming (25 min), or microwave baking (8 min at 0.3 kW and 0.2 milliamperes). The doughs were not processed in baking pans with the microwave and steaming methods. The microwave oven operated at a frequency of 2450 ± 50 megaHertz.

Methods

Ten min after loaves came from ovens, we weighed them and measured volumes in cc by seed displacement to determine their specific loaf volume (cc/g). Data are averages of triplicate determinations.

Table 2—Analysis of dried and ground breads used in the diets

Bread[a,b]	Protein (%)	Ash (%)	C. Fat (%)	C. Fiber (%)
WF(C)	13.5	2.6	0.9	0.5
WF(M)	13.5	2.6	0.8	0.4
WF(S)	13.4	2.6	1.1	0.5
WF + 0.2% L(C)	13.6	2.7	0.9	0.4
WF + 0.2% L(M)	13.6	2.7	0.9	0.4
WF + 0.2% L(S)	13.6	2.6	1.1	0.4
88% WF + 12% SF(C)	18.5	3.5	0.8	0.8
88% WF + 12% SF(M)	18.5	3.5	0.8	0.6
88% WF + 12% SF(S)	18.6	3.3	0.6	0.9

[a] WF, WF + 0.2% L, and 88% WF + 12% SF indicate breads prepared from wheat flour, wheat flour fortified with 0.2% L-lysine monohydrochloride, 12% soy fortified flour (88 parts wheat flour and 12 parts defatted soy flour), respectively.
[b] (C), (M) and (S) denote breads, processed by conventional baking, microwave baking, and steaming, respectively.

Table 3—Weight gain, feed intake, and feed conversion ratio by rats fed indicated bread diets 28 days

Bread[a,b] in diet	Weight gain (mean[c] ± SEM) (g)	Feed intake (mean[c] ± SEM) (g)	Feed conversion ratio
88% WF + 12% SF(S)	111.5 ± 5.9A	469.0 ± 15.8A	4.2
88% WF + 12% SF(M)	109.0 ± 9.1A	435.3 ± 22.1AB	4.0
WF + 0.2% L(S)	102.0 ± 4.8AB	440.2 ± 13.3AB	4.3
WF + 0.2% L(M)	94.2 ± 5.6B	422.5 ± 14.3B	4.5
Casein (control)	79.2 ± 4.2C	288.7 ± 10.7E	3.6
WF + 0.2% L(C)	61.7 ± 4.6D	363.7 ± 13.8C	5.9
88% WF + 12% SF(C)	49.3 ± 1.2E	328.3 ± 12.3D	6.6
WF(M)	41.0 ± 4.0E	297.3 ± 19.5DE	7.3
WF(S)	40.5 ± 2.3E	308.7 ± 20.6DE	7.6
WF(C)	22.2 ± 1.1F	254.3 ± 12.1	11.5

[a] WF, WF + 0.2% L, and 88% WF + 12% SF indicate breads prepared from wheat flour, wheat flour fortified with 0.2% L-lysine monohydrochloride, wheat flour fortified with 12% soy flour (88% parts wheat flour and 12 parts defatted soy flour), respectively.
[b] (C), (M) and (S): Conventional baking, microwave baking and steaming, respectively.
[c] Duncan's Multiple Range Test (1955): Means without a letter in common differ significantly ($p < 0.05$).

We evaluated the crust and crumb colors of fresh and dried breads with Agtron Multichromatic abridged reflectance spectrophotometer Model M-300A, with monochromatic spectral lines: blue (436 nm), green (546 nm), yellow (585 nm), and red (640 nm). The instrument was standardized with standard discs M-68 and 00 to read 100 and 0, respectively. Crust and crumb-color measurements were on slices (about 1 cm thick), from the crust (top surface) and middle part of a loaf. We used gas-liquid chromatography (Kaiser et al., 1974; Gehrke and Stalling, 1967) to determine amino acid contents of various diets. Since the bread supplied the only protein in the diet, the diets' amino acids also represented the amino acid composition of breads.

Organoleptic evaluation

We used slices without crust from the central portions of loaves, and eight persons judged the crumb for texture, grain, flavor and taste as listed below:

Evaluation of bread-crumbs

	Characteristics			
Factors	Desired	Undesired	Score	Comments
Texture	Smooth, velvety	Rough, crumbly		
Grain	Fine, even	Uneven, close, open-hole		
Flavor	Delicate, neutral	Cheesy, musty, rancid, off-flavor		
Taste	Pleasing, bland	Sour, gummy		
Score key:	Highly desirable	5	Fair	2
	Desirable	4	Unacceptable	1
	Acceptable	3		

Nutritional study

For feeding experiments, we sliced the breads, dried them in a heated oven (air-blowing type) at 37°C for 24 hr, ground them to uniform particle size (20 mesh). We analyzed the ground breads for moisture, protein, ash, fat and fiber (AACC, 1962; AOCS, 1971, Methods) and incorporated them into experimental diets.

We prepared vitamin and mineral premixes according to NRC formulas (1972) to support optimum rat growth, and added 2% vegetable oil to each diet to improve texture and decrease dustiness.

Four-week-old male weanling rats (Charles River CD) were fed experimental diets that contained 10% protein supplied by breads or casein for 28 days. The rats, whose initial weights varied from 75–85g, were randomly divided into groups of six per treatment. Each rat was housed in a screen-bottom cage in an environmentally controlled laboratory and given a test diet and water ad libitum. Fresh water was supplied every second day; feed cups were checked daily and filled as needed. Weights and feed consumed were recorded weekly for each rat and PERs were calculated. Feed wastage (usually small) was subtracted from consumption data. Compositions of diets are listed in Table 1.

RESULTS & DISCUSSION

Analyses of breads

Table 2 shows that processing has little effect on the proximate composition of breads. As expected, fortifying with 0.2% lysine increased bread's protein content slightly; while wheat bread's protein and ash contents were increased 38% and 31%, respectively, by fortifying with 12% soy flour.

Weight gain and feed conversion

Processing significantly affected the nutritive value of breads (Table 3). Replacing the diet containing conventionally baked bread with one containing microwave baked or steamed bread significantly increased weight gained: from 22.2g to 41.0 or 40.5g for wheat bread, from 61.7g to 94.2 or 102.0g for lysine-fortified bread, and from 49.3g to 109.0 or 111.5g for soy-fortified bread, respectively. The increases ranged from 52.7–121.1% for microwave baked breads and from 65.3–126.2% for steamed breads.

Feed intake also varied with different breads (Table 3). The

Table 4—Protein efficiency ratios of bread-diets fed rats 28 days

Bread[a,b] in diet	PER (mean[c] ± SEM)	Adjusted PER
Casein (control)	2.74 ± 0.12B	2.50
88% WF + 12% SF(M)	2.47 ± 0.09C	2.25
88% WF + 12% SF(S)	2.36 ± 0.05C	2.15
WF + 0.2% L(S)	2.30 ± 0.04CD	2.09
WF + 0.2% L(M)	2.23 ± 0.12D	2.03
WF + 0.2% L(C)	1.69 ± 0.11E	1.54
88% WF + 12% SF(C)	1.50 ± 0.04F	1.36
WF(M)	1.37 ± 0.09G	1.25
WF(S)	1.32 ± 0.06G	1.20
WF(C)	0.87 ± 0.05H	0.79

[a] WF, WF + 0.2% L, and 88% WF + 12% SF indicate breads prepared from wheat flour, wheat flour fortified with 0.2% L-lysine monohydrochloride, wheat flour fortified with 12% soy flour (88% parts wheat flour and 12 parts defatted soy flour), respectively.
[b] (C), (M) and (S): Conventional baking, microwave baking and steaming, respectively.
[c] Duncan's Multiple Range Test (1955): Means without a letter in common differ significantly ($p < 0.05$).

feed conversion ratio, calculated from weight gain and feed intake, was reduced by microwave baking or steaming from 11.5 to 7.3 or 7.6 for wheat bread, from 5.9 to 4.5 or 4.3 for lysine-fortified bread, and from 6.6 to 4.0 or 4.2 for soy-fortified bread, respectively. The reductions ranged from 23.7 to 39.4% by microwave baking and from 27.1 to 39.4% by steaming.

PER values

Although there was no significant difference ($P < 0.05$) in PERs between wheat breads, 0.2% lysine-fortified breads or 12% soy-fortified breads when they were processed by microwave baking or steaming, differences in PERs between the conventionally baked bread and the microwave baked or steamed bread were highly significant (Table 4). PERs (adjusted) determined using conventionally and microwave baked breads were 0.79 and 1.25 for wheat bread, 1.54 and 2.03 for lysine-fortified bread, and 1.36 and 2.25 for soy-fortified bread. All favored microwave baking with a respective increase of 58.2, 31.8 and 65.4. For steaming, the respective increases were 51.9, 35.7 and 58.1%. Microwave baking and steaming substantially improved the nutritive value of protein in regular or fortified bread over conventional baking.

Nutritive loss of lysine during heat processing of bread was also reflected by the changes in PERs of fortified breads. Fortifying with 0.2% lysine or 12% soy flour raised the adjusted PER from the nonfortified's 0.79 to 1.54 or 1.36, respectively, for conventionally baked bread, from 1.20 to 2.09 or 2.15, respectively, for steamed bread, and from 1.25 to 2.03 or 2.25, for microwave baked bread, respectively. The marked increases in PERs with lysine or soy fortification, regardless of baking method, suggest that wheat bread is deficient in lysine. The higher response to lysine or soy fortification with conventionally baked bread indicates again that conventional baking can aggravate the lysine deficiency of wheat bread much more than microwave baking or steaming.

Lysine and other amino acids

Lysine and other amino acid contents vary slightly among conventionally baked, microwave baked, and steamed breads (Table 5). Because the different breads were prepared from the same dough, except for the method of baking, the significantly lower PER of conventionally baked bread must have resulted mainly from the browning reaction during oven baking. Lysine, a reactant in the browning reaction, became less available nutritionally with conventional baking than with either microwave baking or steaming despite the small variation in their total lysine contents.

The importance of lysine to bread's nutritive value is reflected by the relation between PERs and lysine contents of the three steamed breads (Table 6). Only the difference in

Table 5—Amino acids (W/W%) of diets containing wheat breads conventionally and microwave baked, and steamed

	Wheat bread		
Amino acid	Conventionally baked (%)	Microwave baked (%)	Steamed (%)
Aspartic acid	0.45	0.49	0.50
Threonine	0.31	0.26	0.28
Serine	0.49	0.41	0.42
Glutamic acid	3.33	3.11	3.20
Proline	1.12	1.06	1.09
Glycine	0.37	0.34	0.36
Alanine	0.31	0.29	0.30
Cystine	0.25	0.22	0.23
Valine	0.42	0.41	0.42
Methionine	0.14	0.15	0.15
Isoleucine	0.35	0.34	0.34
Leucine	0.71	0.67	0.67
Tyrosine	0.22	0.24	0.23
Phenylalanine	0.50	0.47	0.47
Histidine	0.22	0.21	0.21
Lysine	0.20	0.22	0.22
Arginine	0.34	0.36	0.36

Table 6—Amino acids (W/W%) of diets containing wheat, lysine fortified, or soy fortified bread processed by steaming

	Steamed bread		
Amino acid	Wheat (%)	Lysine fortified (%)	Soy fortified (%)
Aspartic acid	0.50	0.40	0.72
Threonine	0.28	0.28	0.30
Serine	0.42	0.46	0.42
Glutamic acid	3.20	3.13	2.59
Proline	1.09	1.04	0.82
Glycine	0.36	0.34	0.35
Alanine	0.30	0.30	0.33
Cystine	0.23	0.22	0.20
Valine	0.42	0.40	0.41
Methionine	0.15	0.14	0.10
Isoleucine	0.34	0.33	0.36
Leucine	0.68	0.67	0.68
Tyrosine	0.26	0.23	0.16
Phenylalanine	0.48	0.47	0.46
Histidine	0.21	0.20	0.21
Lysine	0.22	0.33	0.34
Arginine	0.36	0.34	0.40

Table 7—Specific volumes and colors (Agtron readings) of breads

Bread[a,b]	Specific vol (cc/g)	Agtron reading							
		Crust				Crumb			
		Blue	Green	Yellow	Red	Blue	Green	Yellow	Red
WF(C)	6.43	1	9	12	28	48	79	90	88
WF(M)	5.87	53	77	85	87	58	89	100	100
WF(S)	3.36	22	54	68	70	59	91	100	100
WF + 0.2% L(C)	5.17	0	5	0	19	47	76	73	83
WF + 0.2% L(M)	5.09	49	70	66	71	63	99	100	100
WF + 0.2% L(S)	2.58	28	54	60	68	63	93	100	100
88% WF + 12% SF(C)	5.83	0	0	0	7	41	72	71	79
88% WF + 12% SF(M)	5.16	35	65	70	75	52	77	87	92
88% WF + 12% SF(S)	2.68	30	56	62	68	48	80	92	94

[a] WF, WF + 0.2% L, and 88% WF + 12% SF indicate breads prepared from wheat flour, wheat flour fortified with 0.2% L-lysine monohydrochloride, and 12% soy fortified flour (88 parts wheat flour and 12 parts defatted soy flour), respectively.
[b] (C), (M) and (S) denote breads, processed by conventional baking, microwave baking, and steaming, respectively.
[c] Casein diet contains 1% nonnutritive fiber.
[d] Amount is added to make diet with 10.0% protein.
[e] Vitamin and mineral mixtures were adjusted for vitamin and mineral contents of flours, using starch as a filler.

lysine contents explains the significant difference in PER values among the steamed breads. Little browning reaction takes place during steaming, leaving most lysine available in steamed breads. Plotting steamed bread diets' lysine contents against the PER values shows linear relationship, indicating that lysine content is mainly responsible for the PER differences.

Color and volume

As shown in Table 7, conventional baking resulted in a much darker crust and a slightly darker crumb than microwave baking or steaming. Apparently, the brownings of crust and, to a less extent, of crumb are mainly responsible for the nutritive loss of lysine during conventional baking.

Lysine- and soy-fortified breads were darker than nonfortified breads likely because fortified breads contained more amino acids to serve as reactants for the browning reaction.

The marked differences in crust and crumb colors between conventional baked bread and microwave baked bread or steamed bread were also observed for the crust and crumb of breads after drying or grinding.

Along with the color measurements, data on specific loaf volumes are also given in Table 7, showing that dry heat increases loaf volume more than steam heat, and that conventional baking produces a slightly larger loaf than microwave baking. However, it should be noted that doughs were in pan for proofing and baking during conventional baking but not during microwave baking or steaming.

Evaluation of breads

In addition to the nutritional and compositional data, panel evaluations of the crumb qualities of the breads are presented in Table 8.

For regular bread, mean acceptability scores showed crumb qualities of conventionally baked and steamed breads acceptable. Microwave baked bread was scored low in texture; otherwise it was also acceptable. Statistically, crumb texture, grain, flavor, and taste did not differ significantly between conventionally baked and steamed breads.

Steaming and microwave baking, however, were not suitable for processing soy-fortified bread: soy-fortified bread baked conventionally was rated acceptable except for its crumb flavor, whereas steaming gave soy-fortified bread low rating in all categories and so did microwave baking with the exception of flavor.

DISCUSSION

THE PRESENT STUDY demonstrated that crust and crumb browning during conventional baking reduced the availability of lysine in bread, and consequently decreased PERs, and increased feed conversion ratios measured by growth of experimental rats. Steaming and microwave baking caused less crust and crumb browning and minimized the reduction in lysine availability (Tables 3, 4 and 7). The involvement of lysine in the browning reaction has been well established by many re-

Table 8—Scores (mean ± SEM[c]) of breads' texture, grain, flavor and taste evaluated by panel members

Bread[a,b]	Texture	Grain	Flavor	Taste
WF(C)	3.87 ± 0.47A	4.50 ± 0.18A	3.87 ± 0.44AB	4.12 ± 0.29A
WF(M)	1.75 ± 0.24C	3.00 ± 0.26BC	3.50 ± 0.37AB	3.00 ± 0.26B
WF(S)	3.50 ± 0.42AB	3.75 ± 0.36AB	4.00 ± 0.26A	4.12 ± 0.22A
88% WF + 12% SF(C)	3.50 ± 0.32AB	3.12 ± 0.44BC	2.75 ± 0.52AB	3.37 ± 0.32AB
88% WF + 12% SF(M)	1.87 ± 0.22C	2.00 ± 0.26C	3.00 ± 0.42AB	2.62 ± 0.26B
88% WF + 12% SF(S)	2.12 ± 0.39BC	2.75 ± 0.31BC	2.37 ± 0.32B	2.75 ± 0.31B

[a] WF and 88% WF + 12% SF indicate breads prepared from wheat flour, and wheat flour fortified with 12% soy fortified flour (88 parts wheat flour and 12 parts defatted soy flour), respectively.
[b] (C), (M) and (S) denote breads, processed by oven baking, microwave baking, and steaming, respectively.
[c] Duncan's Multiple Range Test (1955): Means without a letter in common differ significantly ($P < 0.05$).

ports, as reviewed by Reynolds (1965). The nutritive loss of lysine by conventional baking of regular and fortified breads was also observed by such workers as Jansen and Ehle (1965), Jansen et al. (1964), Rosenberg and Rohdenburg (9151), and Jenneskens (1971).

Heating has been reported to reduce the nutritive value of essential amino acids in a protein through racemization (Hayase et al., 1975) and chemical decomposition (Osner and Johnson, 1974). Furthermore, lysinoalanine (Nε(DL-2-amino-2 carboxyethyl)-L-lysine) was recently found to form in proteins with or without alkali treatment during heating (Woodard and Short, 1973; Sternberg et al., 1975) and it could reduce protein digestibility and net protein utilization (De-Groot and Slump, 1969). All indicate that besides crust and crumb browning, other changes during the heating process could also lower the bread's nutritive value. Such changes, like browning, would be more severe with conventional baking at 218°C than with steaming at 100°C. This may also be a factor responsible for the superiority of steaming or microwave baking over conventional baking in preserving the nutritive values of bread.

Since bread is deficient in lysine, its nutritive value primarily depends on the availability of lysine, as shown by the significant raise in PERs with the lysine- or soy-fortification (Tables 4 and 6). However, other amino acids including arginine, histidine, tyrosine, tryptophan, and methionine could also become nutritionally unavailable during conventional baking. The early work of Lea and Hanan (1950) showed that the destruction or combination of such amino acid residues with sugars took place in the reaction between glucose and casein.

In a time of food shortages in many parts of the world, every means should be sought to improve food's utility and reduce food waste. Proper food processing, among various other means, should be employed to preserve and improve the nutritive value of foods. Bread is a staple; its nutritive value affects a great majority of the human population. The marked increase in nutritive value of breads microwave baked or steamed, observed in the present study, indicates that those processes deserve serious attention. Steaming has been the traditional way to process yeast-raised dough in China and other countries in the Far East while microwave has recently been used increasingly to heat foods. Both processes may serve to improve the nutritional value of our daily bread.

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SAFETY EVALUATION OF AN ENZYMATIC FISH PROTEIN HYDROLYSATE: 10-MONTH FEEDING STUDY AND REPRODUCTION PERFORMANCE IN RATS

ABSTRACT

Weanling rats were fed an enzymatic fish protein hydrolysate (EFPH) at 10, 20 and 30% protein levels for 40 wk. Control animals were fed casein at 10% protein level. At 12, 24 and 40 wk, animals of each group were killed to determine hemoglobin conc, hematocrit, total red blood cells count and total and differential white cells count and for histological study. Experimental animals showed higher growth rate when compared to the controls. Hematological and histological studies showed no abnormalities. Fertility was studied in another group of animals and was found comparable to that of the controls. Litter sizes were similar and birth weights were normal in all groups except in the 10% EFPH, in which it was lower. Growth curves in this second generation were significantly higher in animals on 20 and 30% EFPH protein: at 15 days of age they reached the same weight as controls and 10% EFPH groups at 20 days. Results confirm the high quality and safety of this fish protein hydrolysate and open the possibility of using it to supplement the protein intake in developing countries.

INTRODUCTION

PRODUCTION of enzymatic hydrolysates of fish protein has been reported by several groups. According to McBride et al. (1961) pepsin produced better solubilization of herring tissue than did other proteolytic enzymes. Sripathy et al. (1964) developed a method to produce hydrolysate from fish flesh using papain. Hale (1969) investigated the production of soluble fish protein concentrate by enzymatic hydrolysis of whole red hake (*Urophycis chuss*).

Rutman and Heimlich (1974) developed a process to solubilize the proteins from Chilean hake (*Merluccius gayi*) fillets using bromeline. Yáñez et al. (1976) have reported extensively on the chemical composition, nutritive value and supplementary capacity to cereal protein of this hydrolysate.

This paper reports on the safety and influence on reproduction of Rutman's fish protein hydrolysate. For this purpose a 10-month feeding study in rats was designed, using different levels of dietary protein.

MATERIALS & METHODS

THE ENZYMATIC fish protein hydrolysate (EFPH) used in this study was manufactured in the pilot plant of the Fisheries Research Institute of Chile (IFOP) by the method developed by Rutman and Heimlich (1974). Data on chemical composition and biological value of this protein have been reported recently by Yáñez et al. (1976). Adjusted PER values ranged from 2.81–2.96 (casein = 2.50).

Diets

EFPH was incorporated into the experimental diets at the 10, 20 and 30% protein levels. A 10% casein diet, prepared according to Chapman et al. (1959), was used as a control. Fresh batches of each diet were prepared every 2–4 wk and kept at room temperature.

Animal feeding study

Each experimental diet was fed to 15 male and 15 female weanling rats of the Wistar strain from the stock of our Institute. Animals were housed in groups of five, in an environmentally controlled room in wire-mesh screen bottom cages. Temperature was maintained at 24–26°C. Food and drinking water were offered ad libitum. Total body weight of animals housed in each cage was recorded weekly.

At 3, 6 and 10 months, 5 males and 5 females from each group were sacrificed by decapitation. Blood was collected for hematologic studies and organs were fixed for histologic examination.

The following hematologic studies were carried out: hemoglobin concentration and hematocrit, total red blood cell count, reticulocytes and total and differential white cell counts according to Cartwright (1966). In addition the animals were carefully examined for gross pathologic changes. The weight of the liver, heart, kidneys, spleen, adrenals and thyroid was recorded.

Histologic studies were conducted on these organs as well as in samples of lung, stomach and small intestine.

Samples of tissue were fixed in Bouin's solutions, dehydrated in increasing concentrations of ethanol, embedded in paraffin and stained with hematoxylin and eosin for study with the light microscope. Histologic examination was performed without foreknowledge of the diet the animals had been fed.

Reproduction and lactation study

In this study four groups each of 30 weanling rats, 10 males and 20 females, were fed the experimental diets for 3 months. One of these groups was fed rat chow containing 22% protein, served as the control. After 12 wk all the animals in each group were mated and separated into two sub-groups of 5 males and 10 females. The females were caged individually throughout pregnancy and delivery and until weaning. Litters were counted and weighed at birth.

Mothers were allowed to suckle their pups for 3 wk. The litters were weighed every 5 days until 20 days of age.

RESULTS

Physical condition and mortality

During the 10-month period of this study no adverse changes in the appearance, behavior or survival of the rats on the EFPH diets were observed. A male rat on the 30% EFPH diet died after 6 wk of experiment. Autopsy revealed diffuse inflammation of the pulmonary parenchyma.

Growth rates are shown in Figure 1. Weight gains for both sexes were greater in animals fed EFPH at 10% protein level than for those fed the same concentration of casein. As anticipated, animals fed EFPH at 20% and 30% protein grew significantly faster than those fed the casein control diet.

Hematology

Blood values at 12, 24 and 40 wk did not differ markedly between the different groups. At 12 wk hemoglobin and hematocrit values of males on the 20% and 30% EFPH diets were significantly higher than the controls. These differences were not observed later in the trial. Table 1 shows the data at 12 wk of experiment, since no differences were found at 24 and 40 wk.

Organ weights

Table 2 shows liver, kidney and adrenal weights expressed as grams per 100 grams of body weight after 12 wk. No differences were found between the experimental groups and the same was observed at 24 and 40 wk. However, at 12 wk the adrenals of the females were always significantly heavier ($p <$

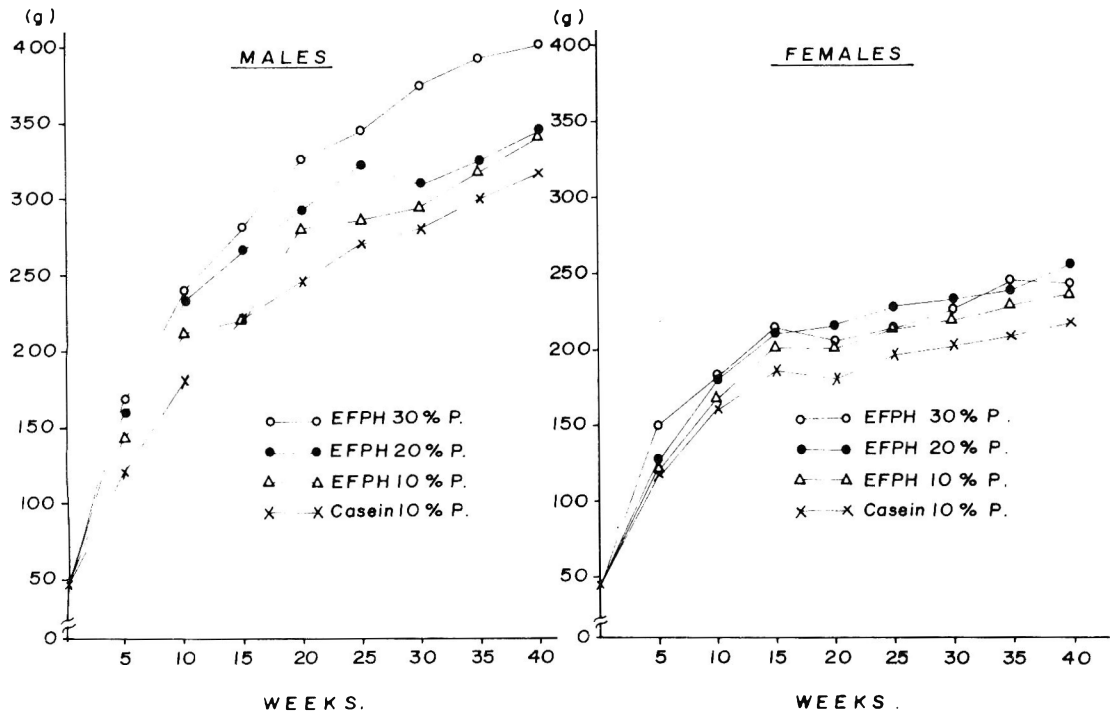

Fig. 1—Growth of rats fed EFPH at different levels of protein.

0.001). These sex differences disappeared at 24 and 40 wk of age.

Histological study

All the organs investigated were histologically normal. No changes could be observed that could be attributed to the test material.

Reproduction and lactation

Reproductive performance is summarized in Table 3. Fertility was similar in all the groups considered and the average number of pups per litter was the same whatever the diet. Pups born to mothers fed 10% EFPH weighed less than those whose mothers received 20%, 30% EFPH or stock diet. Weight gains of pups suckling the mothers receiving 20 and 30% protein level EFPH were significantly greater than the controls. At 15 days of age their weights were similar to those of control pups on 10% EFPH at 20 days.

DISCUSSION & CONCLUSIONS

TESTS for safety and suitability for human consumption are

Table 1—Hematological values for rats fed EFPH at dietary protein levels of 10, 20 and 30% for 12 wk[a]

Dietary protein level %	Hb (g/100 ml)	PCV %	Total $10^3/mm^3$
Males			
Casein (10%)	13.8	39.8	7.88
EFPH 10	14.9	43.0	5.90
EFPH 20	15.6*	44.6	7.52
EFPH 30	16.0**	45.7**	7.32
Females			
Casein (10%)	14.9	43.3	6.14
EFPH 10	14.7	43.0	5.84
EFPH 20	14.9	42.0	7.68
EFPH 30	15.6	42.6	5.60

[a] Each value represents the mean of 4 or 5 observations. Hb = Hemoglobin; PCV = Packed cells volume.
* Significantly different from control ($p < 0.01$)
** ($P < 0.001$)

Table 2—Weight of liver, kidneys and adrenals from males and females after 12 wk of experimental diet[a]

Protein source (%)	Liver[b]		Kidneys[b]		Adrenals[c]	
	Males	Females	Males	Females	Males	Females
Casein 10	3.40 ± 0.28	3.02 ± 0.52	0.56 ± 0.09	0.61 ± 0.10	11.2 ± 2.5	18.0 ± 5.1
EFPH 10	3.24 ± 0.83	2.93 ± 0.54	0.60 ± 0.11	0.57 ± 0.07	11.2 ± 4.4	20.3 ± 3.7
EFPH 20	3.15 ± 0.36	3.04 ± 0.37	0.68 ± 0.02	0.63 ± 0.05	14.6 ± 2.8	17.8 ± 3.9
EFPH 30	2.78 ± 0.32	2.82 ± 0.12	0.65 ± 0.03	0.63 ± 0.03	12.8 ± 2.2	20.0 ± 5.5

[a] All values are calculated as confidence limits of the mean according to the formula: $P(\bar{x} \pm S_{\bar{x}}\, t/2) = 0.95$.
[b] All data expressed as g/100g body weight. Each value is the average for 5 animals.
[c] mg/100g body weight

Table 3—Reproduction of rats fed EFPH at dietary levels of 10–30% protein

Dietary level (%)	Percentage of females with litter	Average no. of rats/litter at birth	Mean body weight (g) of young at day				
			0	5	10	15	20
Stock diet[a]	62.5	9.3 ± 0.3	5.4 ± 0.14	8.8 ± 0.62	15.5 ± 0.66	21.7 ± 1.03	30.0 ± 1.52
EFPH 10	60.0	9.4 ± 0.4	4.8 ± 0.14[b]	9.8 ± 0.45	14.2 ± 0.65	20.7 ± 1.22	32.5 ± 1.31
EFPH 20	66.6	9.6 ± 0.3	5.7 ± 0.15	12.7 ± 1.2[b]	22.5 ± 1.2[b]	32.5 ± 1.33[b]	49.0 ± 2.4[b]
EFPH 30	60.0	10.4 ± 0.6	5.7 ± 0.14	13.1 ± 1.3[b]	22.4 ± 1.3[b]	31.4 ± 1.70[b]	48.3 ± 2.5[b]

[a] Commercial diet containing 22% protein
[b] Significantly different ($p < 0.01$) from stock diet

essential in the development of new foods (NAS-NRC, 1959). Safety is the practical certainty that injury will not result from the substance tested when used in the quantity and in the manner proposed for its use. Commonly, the laboratory rat is used in these experiences to establish the safety of a product because it is easy to handle, economical and, from the enormous experience accumulated, its reactions are well known. Eventually tests have to be carried out in other species, including the one for whom the product is intended. It is common practice to administer at least three dosage levels of the substance under test in the expectation of finding both a non-adverse-effect level and a dose that induces some abnormal response. The present study was performed in weanling rats using EFPH at 10, 20 and 30% protein levels as the sole source of protein for 10 months. Our results indicate that EFPH is a product of good protein quality. It promotes satisfactory growth in rats, it can be ingested at levels as high as 20 or 30% for extended periods of time without apparently causing deleterious effects: hematologic values, relative organ weights and histology were always normal. Fertility and litter size were similar to those of control rats and agreed with the standards of our colony. However, it is noteworthy that 20 and 30% EFPH protein diets induced faster growth rates than in controls or rats fed 10% EFPH since the first days of life. From these data one is tempted to postulate that these animals attained maturity faster than those fed EFPH or casein at 10% protein. However, this is not known with certainty. Widdowson and Cowen (1972) have postulated that earlier sexual maturity of children today as compared to decades ago can be attributed among other factors, to improved nutrition.

In conclusion, EFPH did not induce any obvious toxic effect after 40 wk of feeding up to 30% protein level. Growth rates were normal and clinical illness or adverse biochemical or physiological effects were not detected. Gross and microscopic damage to body tissues or organs were absent and fertility and reproduction seemed unaffected.

Enzymatically hydrolyzed fish muscle has excellent nutritive value and may be fed to rats for long periods of time. It seems advisable to investigate means of introducing this product in human foods to correct the shortage of high quality protein in developing areas.

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BETANINE SEPARATION AND QUANTIFICATION BY CHROMATOGRAPHY ON GELS

ABSTRACT

Published methods for separation and quantification of betalaine pigments from plant extracts are based on electrophoretic and spectrophotometric techniques. These methods are either time consuming, or lack accuracy if interfering substances are present; therefore, a more rapid and accurate method for betalaine separation and betanine quantification is needed. Chromatography on gel filtration supports (Sephadex or Bio-Gel polyacrylamide) is shown to be a rapid and efficient method of separating betalaines from raw beet juice. Raw and fermented beet juice (*Beta vulgaris*) directly applied to a column of polyacrylamide gel (Bio-Gel P-6) resulted in the detection of numerous pigment bands. Observed average distribution coefficients (K_{av}) for betanine on Sephadex G-25 or Bio-Gel P-6 ranged from 0.8–2.0 at a pH value of 4.0–2.0, respectively. These data suggest that the major mechanism of retention of the pigments on gel supports can be adsorption rather than gel-filtration. Resolution of betanine and betanidine was greater on columns packed with Sephadex G-25 compared to columns packed with Bio-Gel P-6. Loading capacity (based on reduced plate height) was greater on columns packed with Bio-Gel P-6 when comparing the two column packings. When Bio-Gel P-6 was chosen as the support for the separation of pigments, excellent separation was obtained using a phosphate buffer at pH 3.0. Elution patterns of betanine were recorded by measuring the absorption at the maximum wavelength. Peak areas obtained were related to standard concentrations of betanine.

INTRODUCTION

RECENT LIMITATIONS on the use of some synthetic food colorants have prompted increased interest in natural pigments. Beet concentrates or powder (*Beta vulgaris*) have been shown to be an applicable colorant in many food systems (Pasch et al., 1975; von Elbe, 1975). Their inherently low betacyanine concentration, organoleptic qualities, and relatively high cost compared to synthetic dyes, have limited their increased food usage. Fermentation of beet juice has been shown to increase the betacyanine content on a dry weight basis and to decrease organoleptic properties (Adams and von Elbe, 1976). Further studies in the application of betacyanines as colorants will require a more efficient large scale method for purification and method of quantification.

Present methods for separation and quantification of betalaine pigments from aqueous plant extracts are based on electrophoretic or chromatographic techniques, and spectrophotometry. Paper electrophoresis is used extensively as a qualitative tool to separate betalaine pigments from raw beet extracts (Powrie and Fennema, 1963; Piattelli and Minale, 1964; Nilsson, 1970; von Elbe et al., 1972). By far the most efficient method for isolation of different betalaine pigments is column chromatography. However, such column methods must be preceded by a crude purification of the plant extract by cationic exchange resin or gel filtration to prevent excessive band spreading on the adsorptive chromatography support (Aronoff and Aronoff, 1948; Peterson and Joslyn, 1958; Piattelli and Minale, 1964; Lempka and Krause, 1970; Nilsson, 1970; Kimler, 1972; von Elbe et al., 1972). Quantification of betalaines in beets involving spectrophotometric measurements at the visible maxima determines only red or yellow pigments and proves slightly inaccurate if sufficient colored impurities are present (Nilsson, 1970). Von Elbe et al. (1972) estimated the betanine content of beets by the use of electrophoresis and subsequent densitometry.

The purpose of this study was to investigate separations of betalaine pigments on gel filtration supports, mode of operation and betanine quantification.

Gel filtration is based on the ability of the solute molecule to diffuse into a cross-linked polymer particle that prevents bulk flow of its imbibed fluid. The molecular size of the solute determines the extent to which it can penetrate the polymeric particle. The chromatographic effect is determined by the ratio of the solute's residence time in the stationary phase relative to the mobile phase. Solute retention on the column is generally determined by a calculation of the average distribution coefficient (K_{av}) using the equation

$$K_{av} = (V_r - V_o)/(V_t - V_o)$$

where V_r is eluate volume of the solute, V_o is the excluded column volume, and V_t is the total column volume. Without solute-gel matrix interactions, solutes totally excluded or totally penetrable in the polymeric particle would have K_{av}'s equal to 0 and 1, respectively.

Gelotte (1960) and Porath (1960) noted that the primary chromatographic effect of Sephadex, the first gel support manufactured, was to separate solutes by molecular sieving. Superimposed on this phenomenon was the possible adsorption of aromatic and heterocyclic organic compounds on the bed material. More recently, Brook and Housley (1969) and Brook and Munday (1970) proposed the mechanism of interaction of phenols, anilines, and benzoic acids with Sephadex. These authors suggested the similarity of titration curves and the elution curves of aromatic acids (K_{av} vs pH), and that the inflection point in either curve was the pK_a of the acid. They further suggested that this adsorption occurs at the epichlorohydrin cross-linkage as hydrogen bonding between the acid's proton and the alcoholic or etheral oxygen. Since this adsorption occurs at the cross-linkage, it would be expected that the capacity of Sephadex as an adsorbent would not be very large; therefore, another gel support (Bio-Gel P-series polyacrylamide) was studied because it has similar gel filtration characteristics to Sephadex, but also contains a matrix which contains possible hydrogen bonding sites.

METHODS

Comparison of Sephadex G-25 and Bio-Gel P-6 as gel support

Sephadex G-25 fine (Pharmacia Fine Chemicals, Piscataway, N.J.) and Bio-Gel P-6 200-400 mesh (Bio-Rad Laboratories, Richmond, Cal.) gel supports were hydrated in 0.025M phosphate buffer of varying pH values. A 2.5 cm diam. column (K25/40, Pharmacia Fine Chemicals, Piscataway, N.J.) was slurry packed to a length of approximately 36 cm. A 1-ml sample (2% solution of a fermented beet juice powder)

(Adams and von Elbe, 1976), adjusted to column pH value with conc HCl) was applied to the column and the eluate collected volumetrically (Golden Retriever Pup, Instruments Specialities Co., Lincoln, Neb.). Fraction collector tube volumes were determined by weighing. Absorbance maxima measurements were made on a Spectronic 20 (Bausch & Lomb Optical Co., Rochester, N.Y.). Excluded column volumes were determined by applying 1 ml of Blue Dextran 2000 solution (Pharmacia Fine Chemicals, Piscataway, N.J.) and determining the leading inflection point of the eluate curve.

Comparison of buffer composition and exclusion limits of polyacrylamide gels

The influence of buffer composition of eluate patterns was investigated on columns containing a Bio-Gel P-6 (-400 mesh) support. Buffers made with 0.025M phosphate, 0.025M citrate, and 0.075M acetate were adjusted to pH 3.0 ± 0.1 by the addition of HCl or NaOH.

The influence of exclusion limits was investigated on columns containing Bio-Gel P-2, P-4, or P-6 (-400 mesh), respectively as supports. The gels were hydrated in 0.025M phosphate buffer at pH 3.00 ± 0.1, deaerated and slurry packed in a 0.9 cm diam. column (K9/30, Pharmacia Fine Chemicals, Piscataway, N.J.) to a length of approximately 28 cm. Absorbent measurements were made on a recording spectrophotometer (ACTA III, Beckman Instruments, Fullerton, Cal.) using on 80 µl flow cell (upward flow). Excluded column volumes were estimated by determining the inflection point of the eluate response (A_{620}) to a step input of Blue Dextran 2000. A 0.1-ml sample (0.4% solution of fermented beet juice powder (Adams and von Elbe, 1976) adjusted to pH 3.00 with conc. HCl) was applied to the column and the elution curve estimated by timed chart response at A_{538} for betanine and A_{542} for betanidine.

Separation of betalaine pigments

Separation of betalaine pigments was accomplished on a 2.6 cm diam. column (K26/40 with A26 applicator, Pharmacia Fine Chemicals, Piscataway, N.J.) containing Bio-Gel P-6 (-400 mesh) as support with 0.025M phosphate buffer (pH 2.95) as eluent. One ml of a 15% solution of fermented beet juice powder (Adams and von Elbe, 1976) or a five-fold concentrate of fresh beet juice (50% solids) were used as samples.

Betanine quantification

Crystalline betanine was obtained in the following manner:

1. 150-ml sample (10% fermented beet juice powder (Adams and von Elbe, 1976) adjusted to pH 2 with conc HCl) was applied to a 5.0 cm diam. column (bed volume 1000 ml, K50/60, Pharmacia Fine Chemicals, Piscataway, N.J.) having a bed material of Bio-Gel P-6, 100-200 mesh with distilled water as eluent. The red fraction was collected (350–400 ml) after essentially all of the salts had eluted. The eluate was concentrated twofold on a rotovaporator (25°C).
2. 20 ml of the above concentrate (adjusted to pH 2.9 with conc acetic acid) was applied to the column and bed material described above with the exception that the eluent was 1% acetic acid in water. The betanine fraction (150–200 ml) was collected and freeze dried.
3. A saturated solution of the freeze-dried powder was prepared with double distilled water at room temperature and filtered. Sufficient conc HCl was added to reduce the pH value below 2. A flocculent precipitate was collected by centrifugation after storage at 4°C for 12 hr. The precipitate was redissolved in double distilled water adjusted to pH 1.0 with HCl, filtered, and placed in a desiccator at 4°C for crystallization.

A series of betanine solutions were prepared and used as samples to relate peak area to mg betanine applied to the column. A 1-ml sample of each solution was placed on a 2.6 cm diam. column with applicator (Pharmacia Fine Chemicals, Piscataway, N.J.) packed with Bio-Gel P-6 200-400 mesh gel. A 0.025M phosphate buffer (pH 3.00) was used as eluent.

Data analyses

A computer program based on Simpson's Rule for integration was written to compute the first, second, and third moments of the chromatograms (absorbance vs volume). A measure of skewness was defined as the third moment divided by the second moment to the 3/2 power and used as a check to substantiate the assumption of symmetrical eluate curves.

The first and second moments of the eluate curve were used as the estimates of its mean (V_r) and the variance (σ^2), respectively. Plate height, reduced plate height, reduced velocity and resolution were calculated as

(a) Plate height (Purnell, 1962)

$$H = N/L = V_e V_r / L\sigma^2$$

where L = column length (cm); N = number of theoretical plates; and $V_e = V_r - \sigma\sqrt{2}$.

(b) Reduced plate height (Giddings and Mallik, 1966)

$$h = H/d_p$$

where d_p = gel particle diameter (= 0.008 cm for Sephadex G-25; = 0.006 cm for Bio-Gel P-6 200-400 mesh; = 0.003 cm for Bio-Gel P-series minus 400 mesh).

(c) Reduced velocity (Giddings and Mallik, 1966)

$$v = d_p v / D_m$$

where D_m (betanine diffusivity in mobile phase) = 5×10^{-6} cm²/sec; v (mobile phase velocity) = FV_t/AV_o; F = flow rate (ml/sec); A = column cross-sectional area (cm²); V_o/V_t = 0.37 for G-25; = 0.32–0.34 for P-6; = 0.36 for P-4; = 0.38 for P-2.

(d) Resolution (Zweig and Sherma, 1972)

$$R_s = \frac{1}{4}\left[\frac{K_{av(betanidine)}}{K_{av(betanine)}} - 1\right]\sqrt{N}\left[\frac{K_{av(betanine)}}{1 + K_{av(betanine)}}\right]$$

RESULTS & DISCUSSION

TO SEPARATE betacyanines from beet juice, gel filtration was considered as a method of desalinating fermented beet juice (Adams and von Elbe, 1976). During these experiments it was found that substantial adsorption of betacyanine pigments ($K_{av} > 1$) occurred when Sephadex G-25 was used as column packing. This property was used to obtain a product of approximately a 50% betacyanine content on a solids basis even with sample loading of 15–20% of the total column volume (von Elbe et al., 1974). This gel filtration-adsorption phenomenon could be useful as an analytical tool particularly when betanine stability and degradation is studied in a variety of solution compositions, and as a commercial method for large scale betacyanine purification.

Dextran and polyacrylamide gel comparison

A performance comparison of Sephadex G-25, fine (dextran matrix) and Bio-Gel P-6 200-400 mesh (polyacrylamide matrix) was made. Figure 1 is a plot of K_{av} for betanine and betanidine vs pH. Table 1 gives data for K_{av}, h, measure of skewness, and resolution of betanine and betanidine. No data were obtained below pH 2.0 because of possible pigment and column support degradation. Above pH 4 reproducible data could not be obtained on P-6 primarily because of excessive band spreading. In Figure 1, and K_{av} values below pH 4.0 increased on both column supports for betanine and betanidine. A polynomial equation (model: $Y_{K_{av}} = A_o + A_1X + A_2X^2 + A_3X^3 + A_4X^4$, where X = pH values) fitted by sequential multi-linear regression yielded only an inflection point of significance (0.05) on P-6 at pH 3.2 and 3.3 for betanine and betanidine, respectively (Fig. 1). The pK_a value of the carboxyl groups at positions 15 and 17 of betanine (Fig. 2) have been reported as 3.4 when determined by alkaline titration (Nilsson, 1970). The data in Figure 1 seem to indicate that the adsorptive mechanism on either G-25 or P-6 is in part due to the protonization of the carboxyl groups at positions 15 and 17 and possible hydrogen bonding with gel cross-linking or matrix.

A sequential multiple linear regression (model: $Y = A_o + A_1X_1 + A_2X_2 + A_3X_3$ was solved for pH values of 2.5–4.0 (independent variables X_1 = pH; X_2 = betanine peak area; X_3 = reduced velocity; dependent variable Y = K_{av}, h, measure of skewness, or resolution of betanine and betanidine). Those

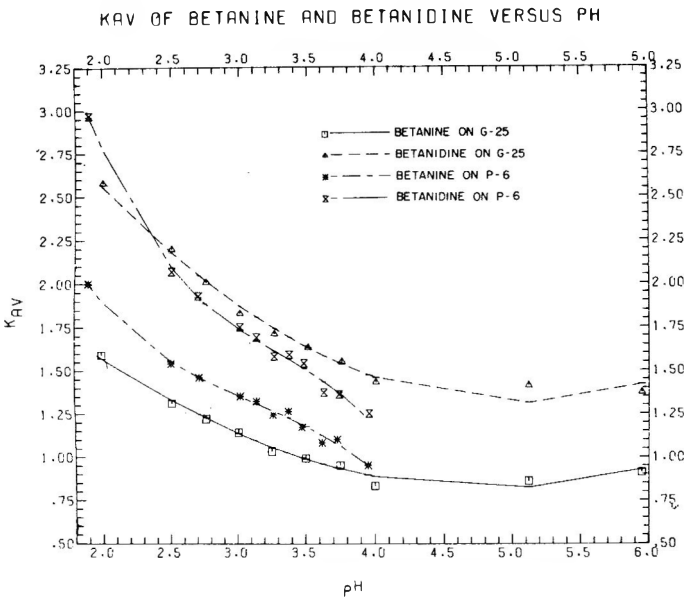

Fig. 1—K_{av} values for betanine and betanidine on Sephadex G-25 and Bio-Gel P-6 vs pH.

relationships having F-ratios significant at the 0.05 level are listed in Table 1. The measure of skewness for the betanine peaks on G-25 and P-6 were independent of pH, peak area and reduced velocity.

The K_{av} and h values for betanine on Sephadex G-25 were dependent on pH and peak area, while on P-6 the K_{av} value was dependent on pH only and the h value was a constant. K_{av} and h value for betanidine on G-25 were dependent on pH only, while on P-6 the results were similar to those of betanine. The resolution of betanine and betanidine was dependent on pH on G-25 but independent of pH on P-6. With dilute betanine sample concentrations on Sephadex G-25 better resolution of betanine and betanidine was achieved compared to that on P-6 (at pH 3.0, 1.79 vs 1.26). However, since h values on P-6 were substantially lower and not dependent on pH and/or peak area, polyacrylamide gel was chosen for further studies.

Buffer composition comparison

Buffer composition was tested as a variable in a more sensitive flow-through spectrophotometric system compared to the previously used fraction collection method. Data in Table 2 compare column variables as a function of the buffer composition of acetate, phosphate and citrate at pH 3.0 ± 0.1. To compare buffer composition, the independent variables of pH, peak areas and reduced velocity were held constant, and the values obtained were not significantly different (Table 2). The measure of skewness indicated symmetrical peaks and, therefore, the assumption made to calculate h and resolution applied. With increasing anion size (i.e., acetate – phosphate – citrate) there was a decrease in K_{av} value for betanine and betanidine and a general increase in h value. The changes in values of both K_{av} and h implied that although adsorption of these betacyanines seemed predominate, there was still a significant molecular exclusion mechanism. Possibly these observations could be understood by considering the buffer anion interaction with the positively charged quaternary nitrogen and the subsequent increase in their associated molecular size. In addition, the resolution of betanine and betanidine decreased as the buffer anion increased in size.

Polyacrylamide gel exclusion limits comparison

Data in Table 3 presents column variables resulting from changing polyacrylamide gel exclusion limits. Independent variables pH, peak area and reduced velocity were, as previously, held constant. For the exclusion limit range studied, the molecular size of betanine and betanidine would suggest significant molecular sieving on P-2 with lessening effects on

Table 1—Dependent column variables for G-25 and P-6 gels as a function of pH (2.5–4.0)[a,b]

Y variable	Column Packing	
	G-25	P-6
Betanine		
Measure of skewness	0.34	–0.14
K_{av}	$2.0 - 0.21X_1 - 0.03X_2$	$2.5 - 0.39X_1$
for $X_1 = 3.0$; $X_2 = 8.1$	$K_{av} = 1.14$	$= 1.33$
h	$-8.3 + 13.5X_1 - 2.6X_2$	7.1
for $X_1 = 3.1$; $X_2 = 8.1$	h = 11.1	–
Betanidine		
K_{av}	$3.4 - 0.49X_1$	$3.5 - 0.57X_1$
for $X_1 = 3.0$	$K_{av} = 1.93$	$= 1.79$
h	$1.44 + 1.93X_1$	4.1
for $X_1 = 3.0$	h = 7.23	–
Resolution of betanine and betanidine		
for $X_1 = 3.0$	$3.62 - 0.61X_1$	1.26
	$R_s = 1.79$	–

[a] Column diam. = 2.5 cm; approx length = 36 cm; reduced velocity on G-25 = 14.5, on P-6 = 10.5

[b] Model: $Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3$ where X_1 = pH, X_2 = betanine peak area, X_3 = reduced velocity

Table 2—Column variables as a function of eluent buffer composition[a] *(pH 3.0 ± 0.1)*

Variables[b]	Buffer composition[c]		
	0.075M Acetate	0.025M Phosphate	0.025M Citrate
Betanine			
Peak area (ml X A_{538})	0.07a	0.08a	0.10a
ν	7.1a	5.8a	6.9a
Measure of skewness	0.0a	0.4a	0.1a
K_{av}	1.45a	1.43a	1.32b
h	4.0a	4.5ab	6.0b
Betanidine			
K_{av}	1.91a	1.87a	1.74b
h	3.7a	2.8b	3.7ab
Resolution of betanine and betanidine	2.3a	2.1ab	1.8b

[a] Column dia. = 0.9 cm; approx. length = 28 cm; P-6 –400 mesh bed material

[b] ν = reduced velocity; K_{av} = average distribution coefficient; h = reduced plate height.

[c] Values followed by the same letter in any one row are not significantly different from each other at the 95% confidence level (t test).

P-4 and P-6, respectively. The K_{av} and h values of both pigments increased with increasing molecular sieving character of the gel. Resolution of betanine and betanidine was a compromise of the desired increase in the ratio of K_{av} values and the undesired increase in h values. On P-2 the differences in K_{av} values of the two pigments was greatest and despite large elution band widths (h values) good resolution was obtained. In this study, because of the narrow elution band and the still adequate resolution of betanine and betanidine, P-6 was chosen for further studies.

The data on column variables presented have indicated that satisfactory separation of betanine and betanidine can be achieved on G-25 and P-series gels. The mechanism involved in column retention of the pigments involve both molecular sieving and adsorption. Betanidine, the aglucone of betanine, was retained on the columns to a greater degree because it is a smaller molecule and may have an additional adsorption site (−OH group at position C-6, Fig. 2) due to the absence of the possible steric hindrance of the glucose moiety.

Betalaine separations

Data in Table 4 show the average distribution coefficients (K_{av}) for compounds from fresh and fremented beet juice detected on columns of polyacrylamide P-6 gel. A compound was considered to be detectable if the elution pattern (ml vs A) reflected a change in sign of the slope. Compounds were considered resolved when the point at which the change in sign of the slope occurred between adjacent peaks was below the half-height of the resolved peak. Areas (ml X A) are given for those peaks which were considered resolved. Fermented as well as fresh beet juice was used as samples because on a dry weight basis fermented beet juice had a higher concentration and greater number of pigments. These data demonstrate that pigment separation was achieved with relatively concentrated but unpurified samples. Measurements at 540 nm easily detected betacyanines while measurements at 475 nm detected both betaxanthines and betacyanines.

Detection of compounds at 220 nm indicated that betanine (K_{av} = 1.23 – 1.32) absorbed considerably less in the UV range than at its visible maxima. The major betaxanthine peak had a column retention (K_{av} = 0.82 – 0.85) approximately equal to other nonexcluded, nonadsorbed compounds, thus greatly increasing the peak area detected (Table 4; col. run 3,

Table 3—Column variables as a function of polyacrylamide gel (Bio-Gel P-series) exclusion limit[a,b]

Variable[c]	Polyacrylamide gel[d] P-2	P-4	P-6
Betanine			
Peak area (ml X A_{538})	0.10a	0.10a	0.08a
ν	7.5a	7.3ab	5.8b
K_{av}	1.54a	1.46ab	1.43b
h	16.7a	10.3b	4.5c
Betanidine			
K_{av}	2.79a	2.06b	1.87c
h	8.1a	5.7b	2.8c
Resolution of betanine and betanidine	3.0a	1.8b	2.1b

[a] Column diam. = 0.9 cm; approx length = 38 cm; 0.025M phosphate buffer; pH 3.0 ± 0.1

[b] Exclusion limits Bio-Gel P-2 = 100–1800, P-4 = 800–4000, P-6 = 1000–6000 daltons

[c] ν = reduced velocity; K_{av} = average distribution coefficient; h = reduced plate height.

[d] Values followed by the same letter in any one row are not significantly different from each other at the 95% confidence level (t test).

Table 4—Average column retention (K_{av}) of peaks detected and peak areas (ml X A) of resolved compounds for P-6 chromatography of fresh and fermented beet juice[a]

	Beet juice									
	Fresh				Fermented					
Column run	1		2		3		4		5	
Detector wavelength (nm)	475		540		475		540		220	
	K_{av}	Area	K_{av}	Area	K_{av}	Area	K_{av}	Area	K_{av}	Area
Tentative Identification										
					0.69				b	
	0.76						0.79			
Vulgaxanthines	0.85	2.66			0.80	2.82	0.83		0.84	11.9
	0.98				1.01					
					1.10		1.06		1.05	
Betanine	1.30	4.42	1.32	13.6	1.30	11.1	1.23	34.6	1.25	17.7
			1.45		1.43				1.37	
					1.52	0.41				
Betanidine			1.77	0.02	1.72	3.03	1.63	11.0	1.66	5.34
									1.74	
					1.99				2.11	0.22
					2.76	0.01	2.78	0.06		
Betalamic acid					3.53	0.77				
									4.16	1.81

[a] Column diam. = 2.6 cm; approx length = 31 cm; bed material P-6 -400 mesh; eluent 0.025M phosphate, pH 2.95

[b] Eight peaks detected with K_{av} values between 0.00 and 0.26.

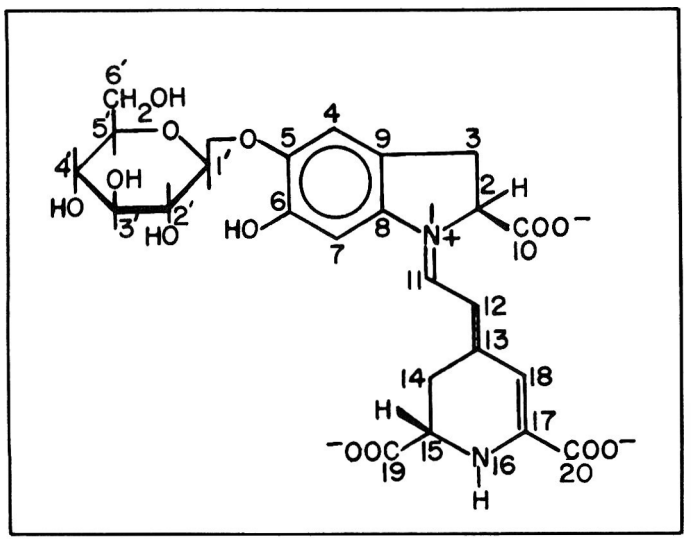

Fig. 2—Structure of betanine.

K_{av} 0.80, area 2.82 vs col. run 5, K_{av} 0.84, area 11.9). UV monitoring could be advantageous for the detection of compounds not absorbing in the visible range which were present (i.e., K_{av} = 1.74 or 4.16, Table 4) in the fermented juice or may be present in future pigment degradation studies.

Betanine quantification

Preparatory columns were used throughout this study since the intention of the research was oriented toward the feasibility of betanine separation and purification. However, even with these limitations a standard betanine curve (peak area vs mg betanine) was established. The equation was solved by regression and found to be linear:

$$Y = 1008x$$

where y = peak area and x = mg betanine. The signicance level of this equation as determined by F-ratio test was 0.0000.

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ENUMERATION OF TEMPERATURE-STRESSED Pseudomonas aeruginosa UTILIZING SELECTIVE PROCEDURES

ABSTRACT

Sublethally stressed cells of *Pseudomonas aeruginosa* were enumerated using the plate count procedure with Plate Count Agar (PCA) and several selective media. Exponential phase (5 hr) cultures were stressed at 1 or 5°C in phosphate buffered distilled water for 0, 5 and 30 min and 24 hr. Counts on all media decreased as time of stress increased. Cold-stressed organisms plated on Acetamide Agar (ACE), King's Medium B with cetrimide (KMB), Trypicase Soy Agar with nitrofurantoin (TSN) and Naladixic Acid Cetrimide Agar (NAC) gave lower counts than those obtained on PCA, but KMB and NAC were the only media that gave statistically different counts at the 95% confidence level. Stationary phase (24 hr) cultures were stressed at 55°C in reconstituted milk-solids-not-fat to obtain approx 99% kill. Counts of heat-stressed organisms on ACE, KMB, TSN, NAC and Pseudosel BBL (PSE) were significantly lower than counts on PCA. Incubation of plates at 41°C reduced the count of heat-stressed cells significantly from counts obtained at 35 or 37°C on all media, including nonselective PCA. Only PSE affected counts of unstressed organisms, the counts being significantly lower than those on PCA. Counts of temperature-stressed *P. aeruginosa* may be substantially decreased when selective procedures are used.

INTRODUCTION

Pseudomonas aeruginosa is currently considered one of the most frequent causative agents of hospital associated infections. *Pseudomonas* infections rarely occur in the normal human host, but some strains can cause disease in debilitated patients. In one report (Bennett, 1974), seven out of 1000 hospital patients developed an infection with *P. aeruginosa*, accounting for about one-tenth of all nosocomial infections.

P. aeruginosa is most commonly isolated from water, soil, dust, food, and animal and human feces. In addition, sampling studies in hospitals have resulted in multiple isolations from a large number of sources in the inanimate environment, including eating utensils, floors, lavatories and kitchen facilities (Young and Armstrong, 1972).

Several studies have concentrated on hospital environments as the source of the organism. Shooter et al. (1969, 1971) reported finding *P. aeruginosa* in cooked and uncooked food in a hospital and suggested that this organism became established as intestinal flora of patients as a result of ingestion in foods. Wright et al. (1976) recovered *P. aeruginosa* from 44% of hospital salad samples. Kominos et al. (1972) isolated *P. aeruginosa* from fresh vegetables, hands of workers, and knives and cutting boards in a hospital kitchen. Pyocine typing of clinical isolates revealed that many of the types were the same as those recovered from vegetables and kitchen sources. This raised the question of whether the source was the plant material or contamination by human handling. Green et al. (1974) detected *P. aeruginosa* in 24% of soil samples, but only 0.13% of vegetable samples from the field contained this bacterium.

Processing and distribution subject foods to conditions which may impose sublethal stress upon the microorganisms. Heat and cold stresses have been reported to affect the recovery of other bacteria (Jackson, 1974; Nelson, 1971; Ordal, 1970; Speck et al., 1975; Traci and Duncan, 1974). Stressed organisms are more demanding in their requirements for initiation of growth than are those which have not been stressed.

Since food is considered a possible source of *P. aeruginosa* in hospital infections, the method of detection employed must be one which can be used with confidence. The purpose of this study was to determine the effects of selective media and incubation temperatures on the enumeration of *P. aeruginosa* which had been subjected to temperature stress.

EXPERIMENTAL

Culture preparation

Two strains of *P. aeruginosa* were employed, ATCC 10145 and a laboratory strain obtained from the Dept. of Microbiology & Medical Technology, University of Arizona. Cultures were held at −20°C in litmus milk. Each strain was grown in nutrient broth (Difco Laboratories, Detroit, MI) at 37°C for 5 hr to obtain exponential phase cells or 24 hr to obtain stationary phase cells. Cultures were mixed by a vortex mixer for 1 min prior to dispensing for stress treatment, in order to diminish cell clumping.

Temperature stress

Cold stress was accomplished by adding 1 ml of exponential phase culture to 99 ml prechilled (1 or 5°C) phosphate buffered distilled water (Hausler, 1972) and holding at that temperature for 5 min, 30 min and 24 hr before plating. All samples were immediately diluted in room temperature (23 or 25°C) phosphate buffered distilled water and plated.

Heat stress was accomplished by adding 1 ml of stationary phase broth culture to 50 ml rehydrated milk-solids-not-fat (110g/liter). A 5-ml aliquot was heated in a screw-capped test tube at 55°C in a thermostatically controlled bath for 5, 6 or 7 min following a predetermined come-up time and then cooled in ice water.

Unstressed cells were diluted in phosphate buffered distilled water and plated out as the control. Platings were made in duplicate and each experiment was repeated three times. Plates were incubated at 37°C, except where otherwise indicated.

Media employed

Standard Methods Plate Count Agar (Difco Laboratories, Detroit, MI) (PCA) (Hausler, 1972) and several media selective for *P. aeruginosa* were used. Acetamide Agar (ACE) was used by Green et al. (1974). The formula is that of Hedberg (1969), utilizing acetamide (Eastman Kodak Co., Rochester, NY) as the sole carbon source. King's Medium B (King et al., 1954) (KMB) with 0.03% cetrimide (hexadecyltrimethyl-ammonium bromide) (Eastman Kodak Co., Rochester, NY) was suggested by Brown and Lowbury (1965). Naladixic acid cetrimide Agar (NAC) of the formula of Goto and Enomoto (1970) utilizes 0.02% cetrimide and 5 μg/liter naladixic acid (Sigma Chemical Co., St. Louis, MO). Trypticase soy agar plus 0.02% nitrofurantoin (Sigma Chemical Co., St. Louis, MO) (TSN) was used. Kominos et al. (1972) used a level of 0.2% nitrofurantoin, but Thom et al. (1971) used a level of less than 0.02%. Pseudosel (BBL, Cockeysville, MD) (PSE) utilized cetrimide as the selective agent.

Statistical analysis used computer program A 3.1 of Sokal and Rohlf (1969). It was modified to conform to CDC 6400 System of the Uni-

Table 1—Influence of plating medium on counts of P. aeruginosa (ATCC 10145) stressed at 1°C for 0 min, 5 min, 30 min, and 24 hr

Medium	Colony forming units/ml			
	0 min	5 min	30 min	24 hr
PCA	820,000	650,000	360,000	3,900
ACE	760,000	510,000	290,000	990
KMB	760,000	1,300	540	90
TSN	880,000	390,000	380,000	2,200
NAC	590,000	24,000	3,400	120

Table 2—Influence of plating medium on counts of P. aeruginosa (lab strain) stressed at 5°C for 0 min, 5 min, 30 min, and 24 hr

Medium	Colony forming units/ml			
	0 min	5 min	30 min	24 hr
PCA	1,500,000	1,100,000	1,100,000	7,500
ACE	1,400,000	560,000	500,000	5,800
KMB	1,400,000	130,000	37,000	1,500
TSN	1,500,000	680,000	550,000	7,600
NAC	1,100,000	380,000	50,000	2,200

versity of Arizona Computer System. Each count was converted to $\log_{10}$ and averaged. Log ratio was expressed as $\log_{10}$ (count on selective medium/count of respective control of that part of the experiment, i.e., PCA at 37°C, with unstressed organisms. Confidence levels of 95% are illustrated by dotted lines in the graphs.

RESULTS & DISCUSSION

TABLE 1 gives the average number of colonies per ml that grew in a representative trial where cold stress at 1°C was used. Selective media had no apparent effect on enumeration of unstressed cells. Cells stressed at 1°C gave lower counts on ACE, KMB, TSN, and NAC than when plated on nonselective PCA.

Statistical analysis of data from trials (Fig. 1) showed that unstressed *P. aeruginosa* gave the same count on all media, so it is not illustrated on the graph. KMB and NAC significantly reduced the count of chilled bacteria. Counts on TSN and ACE were not significantly different from those on PCA.

Data from one representative trial of cells stressed at 5°C are shown in Table 2. This treatment corresponds to possible stress encountered in foods under less rigorous conditions of refrigeration. Unchilled organisms showed little difference in counts between the several media. ACE, KMB, TSN and NAC reduced the counts of stressed organisms. Holding for 24 hr at 5°C markedly reduced counts with all media, including nonselective PCA.

Statistical analysis of data from three trials (Fig. 2) shows that KMB and NAC gave significantly lower counts than PCA when cells are held for 5 min and particularly 30 min at 5°C. Counts on TSN and ACE were not significantly different from those on PCA. After 24 hr stress the total cells enumerated decreased markedly, but no significant difference between counts on PCA and selective media was found.

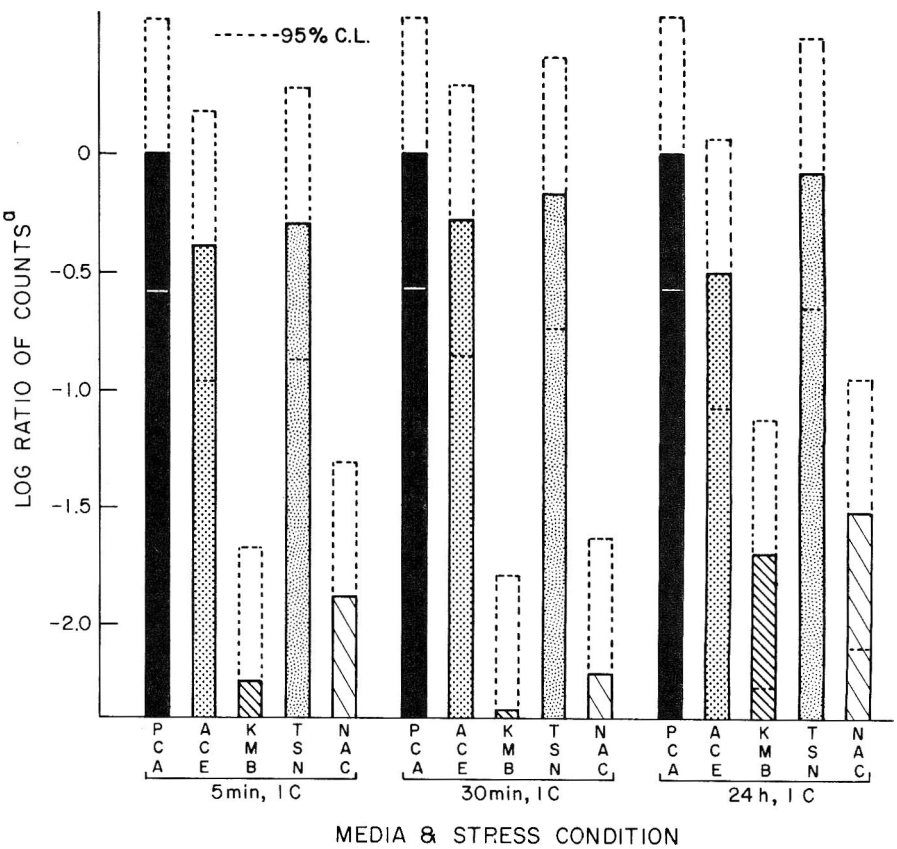

Fig. 1—Comparison of enumeration of cold-stressed (1°C) exponential phase cells of P. aeruginosa (ATCC 10145) on PCA and several selective media. [a]Log of count on selective medium/log of count on PCA for stress times of 5 min, 30 min and 24 hr.

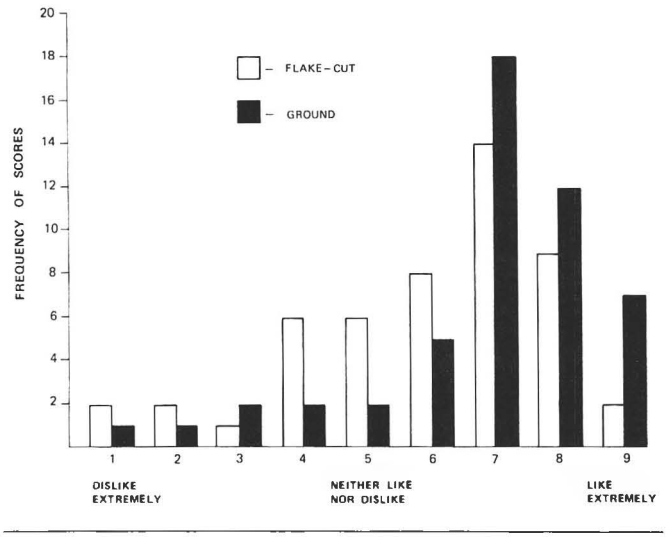

Fig. 2—Frequency of scores assigned by consumer panel.

Sensory evaluation

The trained panel found no significant differences in appearance, flavor or doneness between cooked patties prepared by the two methods (Table 2). The panelists did find, however, that the patties differed significantly in all the other characteristics. The ground meat had a finer grind whereas the flake-cut meat was coarser (2.4 vs 3.7 on the 6.0 scale). The ground meat was more tender (4.3 vs 2.8), less rubbery (2.6 vs 4.0), more juicy (3.9 vs 2.7) and more greasy (3.1 vs 2.3) than the flake-cut meat. The average scores are displayed in Table 2.

The mechanism by which comminution affects texture is not clearly understood. Before being made into patties, the ground meats were in the form of extruded strands whereas the flake-cut meats consisted of finely-cut particles. Visually, it appeared that the flake-cut meats had a finer grind. However, the openings of the grinder plates and cutting heads were of similar size, and particle size should also have been similar. The sensory results on grind would indicate otherwise. It has been claimed (Anon. 1973) that flake-cut meats have improved binding and cohesive properties which could explain why the panelists found the flake-cut patties to have a coarser grind.

Improved binding and cohesive properties could also account for the reduced tenderness and juiciness, increased chewiness and reduced greasiness of the flake-cut patties. It is well documented, in the area of meat emulsions, that the salt-soluble proteins are of major importance in fat and water binding and that these proteins affect textural characteristics. Acton (1972) reported that bind strength of meat loaves (ability of meat particles to bind together) made from various grinds of meats were correlated to the amount of salt-soluble protein extracted. Additional research is required to determine if the sensory characteristics of grind, chewiness, tenderness, juiciness and greasiness of meat patties are related to the type of comminution or, more directly, to the amount of salt-soluble protein extracted by the comminution.

The consumer panel found the patties made by both methods acceptable (Fig. 2). However, a significantly higher score was given to the ground patty (6.8 vs 5.9). The higher acceptability of the ground patty does not necessarily indicate that it is a superior product but may be due to the fact that consumers are accustomed to eating ground patties. The main concern as indicated by the panelists' comments was the difference in texture between the two samples. The flake-cut patty was described as "tough and dry," "pressed too tight," "very firm," "spongy" while the ground patty was "tender and moist," "smoother," "lighter."

The results of this study indicate that some of the desirable characteristics, primarily those related to textural properties, which flake-cutting contributes to a steak-like product are not required in a beef patty or hamburger-type product. In forming restructured meats, blending of the flaked particles increases cohesion; a property which may not be desirable in patties. When patties are produced by flake-cutting only, as in this study, processing parameters that might be controlled in order to reduce the development of cohesion are temperature of the meat and the speed and duration of blending.

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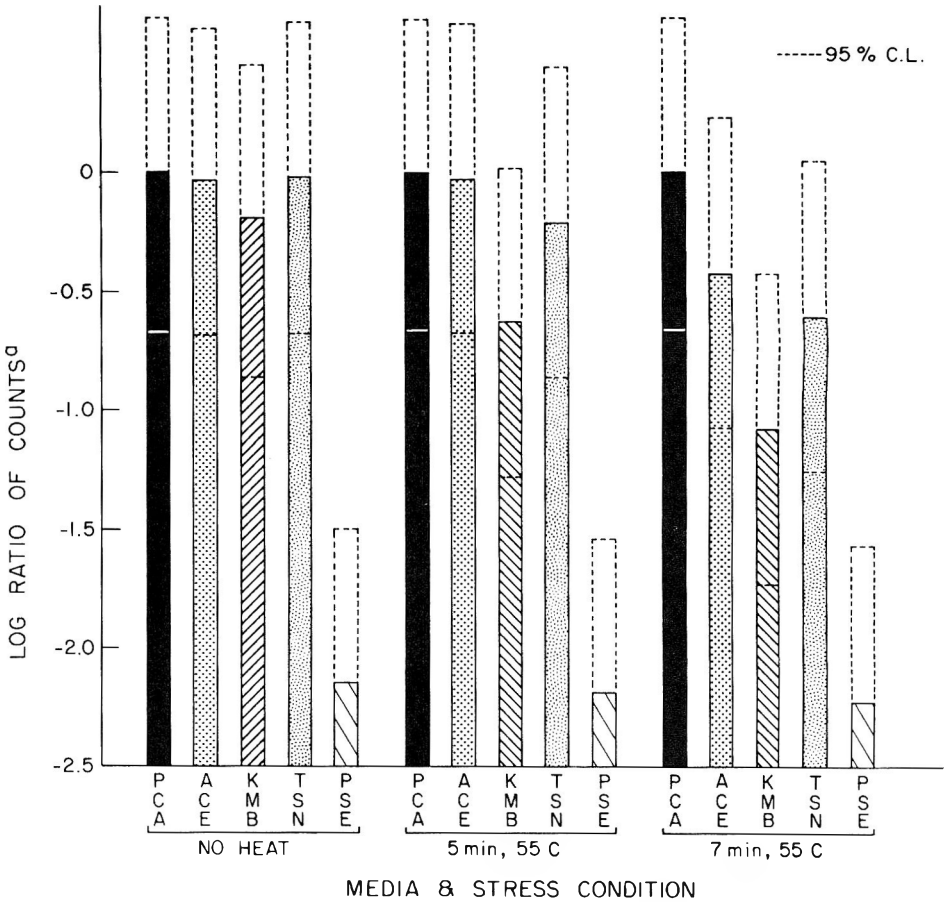

Fig. 3—Comparison of enumeration of heat-stressed (55°C) stationary phase cells of P. aeruginosa (lab strain) on PCA and several selective media. [a]Log of count on selective medium/log of count on PCA for stress times of 0, 5 and 7 min.

5) suggest that incubation at 22 or 41°C lowers the count of heat-stressed cells when compared with 35°C.

Statistical analysis of three trials (Fig. 6) shows that incubation temperatures used had no effect on enumeration of unstressed cells. However, incubation at either 22°C or 41°C significantly decreased the number of heat-stressed cells that formed colonies on all media, including PCA.

Counts by several selective media were essentially the same for unstreseed cells. For both strains of *P. aeruginosa* the selective media used lowered the number of colonies that grew from heat-stressed cultures. This has been demonstrated for other bacteria. Nelson (1943) demonstrated that several heat-stressed bacteria were more difficult to enumerate on selective media than were unstressed bacteria. Maxcy (1970) showed

Table 4—Influence of incubation temperature and plating medium on counts of P. aeruginosa (lab strain) stressed at 55°C for 0 min, 5 min, and 7 min

Medium	Colony forming units/ml		
	0 min	5 min, 55°C[a]	7 min, 55°C[b]
	37°C Incubation		
PCA	10,000,000	1,700,000	23,000
ACE	13,000,000	100,000[c]	2,200[c]
KMB	6,000,000	190,000	2,200
TSN	10,000,000	240,000	2,000
	41°C Incubation		
PCA	9,500,000	270,000	1,200
ACE	14,000,000	15,000[c]	620[c]
KMB	7,000,000	25,000	1,300
TSN	13,000,000	7,000	1,100

[a] 83% kill
[b] 99.8% kill
[c] Counted after 72 hr

Table 5—Influence of incubation temperature and plating medium on counts of P. aeruginosa (ATCC 10145) stressed at 55°C for 0 and 6 min

Medium	Colony forming units/ml	
	0 min	6 min, 55°C
	22°C Incubation	
PCA	53,000,000	2,200,000
NAC	31,000,000	620,000
TSN	52,000,000	1,800,000
	35°C Incubation	
PCA	53,000,000	9,800,000
NAC	30,000,000	2,400,000
TSN	50,000,000	3,200,000
	41°C Incubation	
PCA	55,000,000	3,000,000
NAC	45,000,000	1,000,000
TSN	50,000,000	330,000

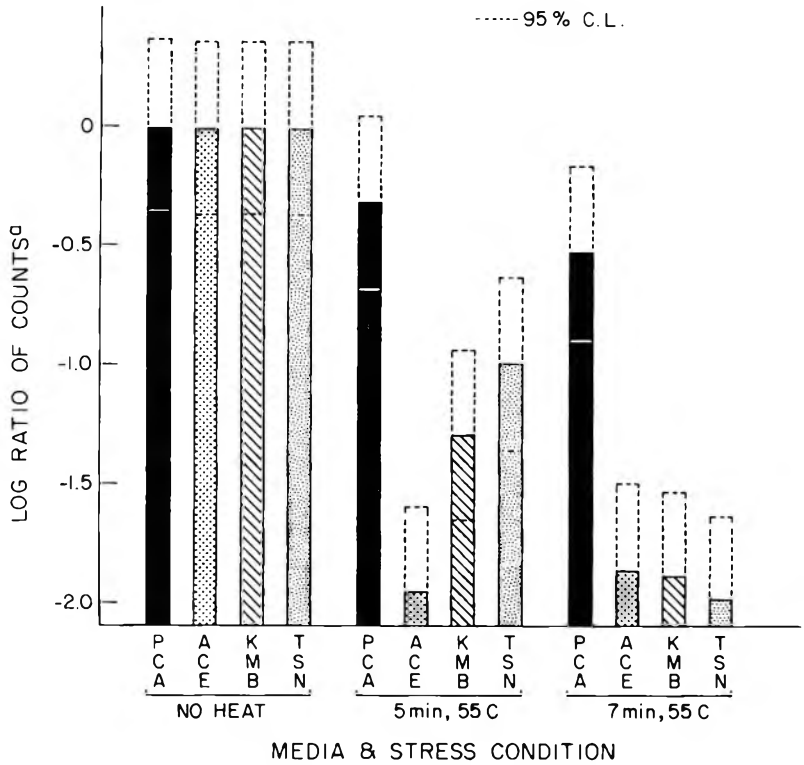

Fig. 4—Comparison of enumeration of heat-stressed (55° C) stationary phase cells of P. aeruginosa (lab strain) on PCA and several selective media. [a]Average log ratio of both incubation temperatures for each medium using ratio of log of count on test medium/log of count on PCA at 37° C for stress times of 0,5 and 7 min.

counts to be comparable for uninjured cells of *Escherichia coli* on PCA and violet red bile agar, but counts of heat-injured cells were much lower on the selective medium. Numerous other examples could be cited.

When cells of *P. aeruginosa* have been stressed by either cold or heat, they definitely are more sensitive to selective conditions employed for enumeration. Media containing cetrimide, naladixic acid, nitrofurantoin and acetamide can reduce the number of stressed cells which will form countable colonies on these selective media, while having no comparable effect on unstressed control cells. Cells stressed by sublethal heat form fewer colonies when plates are incubated at 41°C

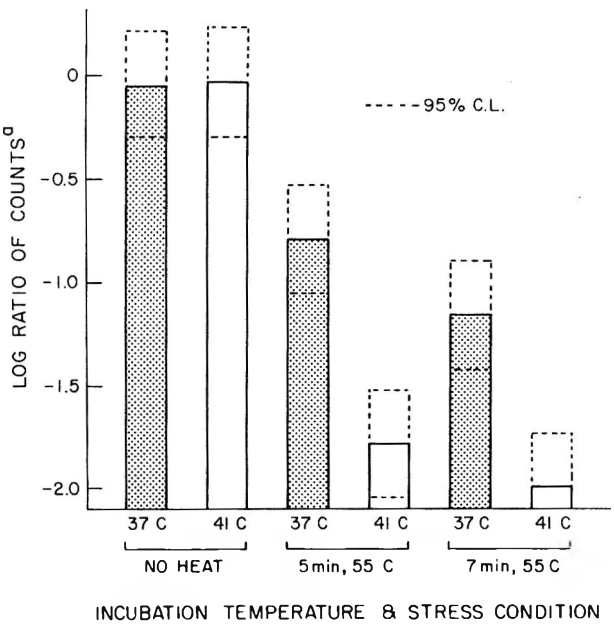

Fig. 5—Comparison of enumeration of heat-stressed (55° C) stationary phase cells of P. aeruginosa (lab strain) incubated at 37 and 41° C. [a]Average log ratio of all media at each incubation temperature using ratio of log of count on test medium/log of count on PCA at 37° C incubation for stress times of 0, 5 and 7 min.

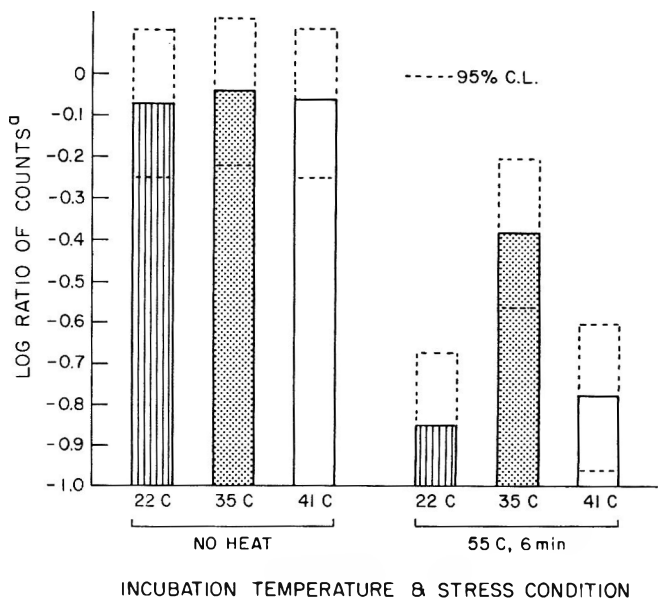

Fig. 6—Comparison of enumeration of heat-stressed (55° C) stationary phase cells of P. aeruginosa (ATCC 10145) at three incubation temperatures. [a]Average log ratio of all three media at each incubation temperature using ratio of log of count on test medium/log of count on PCA at 35° C incubation for stress times of 0 and 6 min.

than at the usual 37°C. Similar data have not yet been obtained for cells stressed by cold, but in view of the similarity of response to other factors by cells stressed by either heat or cold, a reduction in count would not be surprising if an elevated plate incubation temperature were used when enumerating organisms stressed by cold.

Of the selective media studied, TSN gave numbers of countable colonies closest to those obtained on PCA. This might be attributed to the trypticase soy agar base used in TSN which is high in nutritional components that may be required by injured cells for their repair and reproduction. Colonies also are larger and more easily differentiated on the TSN medium than on the other selective media.

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OPTIMAL CONDITIONS FOR ASSAY OF STAPHYLOCOCCAL NUCLEASE

ABSTRACT

Conditions optimal for assay of staphylococcal nuclease were established by spectrophotometric measurement of acid soluble oligonucleotides produced from heat denatured calf thymus deoxyribonucleic acid (DNA) by purified micrococcal nuclease (DNase, E.C. No. 3.1.4.7) as well as crude DNase from *Staphylococcus aureus*. Assay conditions of 50°C, calcium concentration of 0.005M, NaCl concentration of 0.17M and pH of 10.0 were optimal for activity of DNase. There was a sixfold increase in activity of DNase under these conditions over that observed under the most commonly used assay conditions of 37°C and pH 9.0. Use of 50°C and pH 10.0 in a DNA-agar diffusion system also resulted in a reduction in assay time by twofold with crude DNase from growth of weak or strong DNase producing strains of *S. aureus*. Similarly, the assay time of DNase extracted from dried malted milk and cheddar cheese (involved in staphylococcal food poisoning) was also reduced by twofold at 50°C and pH 10.0 over that observed at 37°C and pH 9.0.

INTRODUCTION

THERE IS considerable interest in the use of staphylococcal nuclease (DNase) for detection of staphylococcal growth in heated or fermented foods because DNase withstands both heating and low pH which result in destruction of *Staphylococcus aureus* (Chesbro and Auborn, 1967; Cords and Tatini, 1973; Tatini et al., 1975; Lachica et al., 1972). In order for the DNase assay to be useful as an indicator of staphylococcal growth in finished foods, the assay must be specific for measurement of staphylococcal growth as well as expedient for application as a routine quality control test by the food industry. Consequently, establishment of optimal conditions for activity of staphylococcal DNase would lend the assay for routine quality control of finished foods for possible presence of enterotoxins (Tatini et al., 1976). Varying conditions of temperature, pH, ionic strength, and calcium concentration were reported to be optimal for activity of staphylococcal or micrococcal DNase (Alexander et al., 1961; Cuatrecasas et al., 1967; Heins et al., 1967) and some of these conditions were also shown to be inhibitory to DNase (Cuatrecasas et al., 1967). Of these conditions, temperature and pH are known to exert a significant influence on both the activity and stability of DNase as well as the stability or orientation of the substrate (deoxyribonucleic acid, DNA) with some variable and interactive effects of calcium, magnesium and sodium chloride (VonHippel and Felsenfeld, 1967; Cuatrecasas et al., 1967) on both DNase and substrate DNA. However, the most commonly used assay conditions for measurement of DNase activity were 37°C, pH 8.6 and a calcium concentration of 0.005M.

The purpose of this investigation was to evaluate and establish the optimal conditions necessary for (1) spectrophotometric measurement of crude or purified DNase and (2) for rapid detection of weak nuclease activity from foods with DNA-agar (DA) or DNA-agar-Toluidine Blue-O dye (TDA) diffusion system.

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EXPERIMENTAL

Spectrophotometric assay

The procedure described by Chesbro and Auborn (1967) and Cords and Tatini (1973) was used with minor modification. Purified micrococcal nuclease (E.C. No. 3.1.4.7) was obtained from Sigma Chemical Co. (No. N-3755). A stock solution of enzyme was prepared by dissolving micrococcal nuclease in distilled water to provide 0.146 μg of protein/ml. This solution was stored frozen at −5°C. Before use, the enzyme solution was diluted 12 times in 0.1% Bovine Serum Albumin (also from Sigma Chemical Co.) to provide 0.0122 μg of micrococcal nuclease protein/ml. A solution of 2 mg/ml of heat denatured calf thymus deoxyribonucleic acid (DNA from Calbiochem) was prepared in 0.01M NaCl and boiled for 30 min followed by rapid cooling in ice-water. This DNA solution was also frozen at −5°C until used. A solution of 0.05M glycine buffer and a solution of appropriate molarity of $CaCl_2$ were prepared and stored for <2 wk at 4°C and room temperature, respectively. The reaction mixture contained 0.20 ml of the heat denatured DNA, 0.025 ml of $CaCl_2$ solution, 0.175 ml of the buffer, and 0.10 ml of enzyme solution. The reaction mixture was incubated (in siliconized 13 × 100 glass test tubes) at the desired temperature for 30 min. The reaction was stopped by adding 0.5 ml of 7% cold perchloric acid and placed in an ice-water bath and after 1 min, 3.0 ml of cold distilled water was added to the mixture. After 5 min, the samples were centrifuged at 3000 rpm for 10 min in a refrigerated Sorvall RC-B centrifuge. The supernatant was decanted into a clean cuvette and the optical density (OD) was measured at 260 nm with a Beckman Acta III spectrophotometer. Blanks were prepared in the same way except that the enzyme was added immediately prior to the addition of the perchloric acid. The OD of the blank was subtracted from the OD of the reaction mixture and was expressed in units of activity per ml. The assays (under each set of experimental conditions) were replicated at least twice with duplicate determinations in each trial. One unit of activity is defined as the change in OD of 1.0 at 260 nm resulting from acid soluble oligonucleotides produced from heat denatured calf thymus DNA in 30 min.

Preparation of crude thermostable DNase

Staphylococcus aureus was allowed to grow in Brain Heart Infusion Broth (BHI) under static conditions at 37°C for 18–24 hr. Five ml of BHI was removed and centrifuged at 10,000 rpm for 15 min. The resultant supernatant was boiled for 15 min and cooled for use.

Extraction of thermostable DNase from food products

The procedure described by Tatini et al. (1976) was used to extract DNase from 10g of each food product tested.

Preparation of DNA agar diffusion assay systems

With the exception of omitting Toluidine Blue-O dye both DNA-agar systems (DA and TDA) were prepared by dissolving 0.30 Bacto-DNA (Difco) in 1,000 ml of the appropriate buffer (0.05M Tris or glycine) of the desired pH and containing the desired concentration of calcium and/or sodium chloride and 10g of Bacto-agar as described by Lachica et al. (1971) and by Tatini et al. (1975).

Assay of DNase in an agar diffusion system.

Before using either system, DA or TDA, 2 mm wells were cut with a 13 gauge cannula and the plates were tempered at room temperature for 20 min. Duplicate wells were filled with 5 μl of enzyme solution and incubated at the appropriate temperature. To develop the zone of activity in the DA system, the agar was flooded with 1N HCl. The diameter (mm) of the resultant zone of clearing around the well due to acid soluble oligonucleotides was measured with a calipers. With the TDA system, the pink zone of activity around the well was measured.

All assays were performed at least twice with duplicate wells and two measurements per well per reading.

RESULTS & DISCUSSION

DATA IN TABLE 1 show the effects of temperature and pH on the activity of purified nuclease (DNase). As the temperature of assay increased above 37°C there was an increase in the activity of DNase up to 50 or 55°C and decreased at 60 or 65°C. For example, DNase activity was 4.8 units at 37°C and 11.1 units at 55°C and the activity decreased to 9.7 at 60°C and to 4.4 units at 65°C when the pH was 9.5. The DNase activity, which was highest at 55°C and pH 9.5, has more than doubled over that observed at 37°C. Also, as the pH of the assay increased there was an increase in the activity of DNase at each temperature. For example, the activity was 2.3 units at pH 9.0 but was 11 units when the pH was 10.5 at 37°C. The data also indicate that there was an interaction of temperature and pH. The pH optimum was 10.5 at 37 or 45°C whereas it was 10.0 at 50, 55 and 60°C. The activity was highest when the pH was 10.0 and temperature was 50°C (14.8 units). Though the actual values of temperature and pH were different from those reported, these data confirm earlier reports (Cuatrecasas et al., 1967; Alexander et al., 1961; VonHippel and Felsenfeld, 1967) in that there was an interdependence of these factors which affect both the activity and stability of nuclease and substrate DNA. Data in Table 2 show the further dependency of activity on a third factor, calcium. A calcium concentration of 0.0005M was optimum at 45°C but the optimum shifted upward to 0.005M at 50 and 55°C. At each temperature, when the calcium level exceeded the optimum, the activity of DNase decreased. It does not appear that the effects of temperature and pH were on the solubility of calcium (affecting its availability) because the activity of DNase at 0.005M or 0.001M calcium was higher at 55°C or 50°C than that observed with 0.0005M calcium at 45°C. It has been suggested by others that temperature, pH and calcium not only affect the stability of enzyme but also affect the enzyme's preference for sites of attack on DNA (VonHippel and Felsenfeld, 1967) by causing conformational changes. The site of initial attack depends on the conformation of DNA. DNase attacks heat denatured DNA at 60°C randomly, whereas it attacks preferentially regions rich in deoxyadenylic or thymidylic groups in native DNA. Conformational changes in DNase were observed in the range of 55 to 60°C and pH of 10.0–10.5 (Anfinsen et al., 1971; Roberts and Jardetzky, 1970). Since these changes affect both activity and stability of DNase and conformation of DNA, the conditions of 50°C, pH 10.0 and a calcium level of 0.005M which were found optimum, are a reflection of balancing conditions between activity and stability of DNase and the preferred conformation of DNA. In a storage study of the working dilution of purified DNase at 37°C and 50°C and at pH 9.0 and 10.0 at each temperature, it was found that DNase was inactivated substantially (74% decrease) after 4-hr storage at 50°C and pH 10.0. For instance, the activity was 1.6–1.9 units/ml after 1- and 4-hr storage at pH 9.0 and 37°C and the activity was 4.2 units/ml after 1- and 4-hr storage at pH 10.0 and 37°C. When the pH was 9.0, the activity was 2.3–3.0 units/ml after 1- and 4-hr storage at 50°C whereas the activity decreased from 4.2 units/ml after 1 hr to 1.1 units/ml after 4-hr storage at pH 10.0 and 50°C. The assay conditions were 50°C, 0.005M Ca^{++} and 30 min incubation.

The conditions of 50°C, pH 10.0 and calcium level of 0.005M were also found to be optimal for activity of crude nuclease from *Staphylococcus aureus* (Table 3). The activity almost doubled (8.6 vs 4.4 units) at 50°C and pH 10.0 as compared to that observed at 37°C and pH 9.0.

Spectrophotometric assay of DNase activity, though precise, has limitations in application such as (1) expensive instrumentation is needed and (2) cannot be used with weak nuclease activity in the presence of excessive impurities. The DNA-agar diffusion system resulting in an acid soluble zone from DNase activity forms a less expensive assay system. Likewise the DNA-agar system, containing Toluidine Blue-O dye whose blue color changes to pink when DNA is hydrolyzed, is another less expensive system for use with weak nuclease activity in the presence of impurities. The log concentration of DNase/ml is linearly related to the diameter of the zone in both agar diffusion systems. Therefore, conditions found optimal for spectrophotometric assay were evaluated in these two DNA-agar diffusion systems. Data in Table 4 show that DNase activity of crude DNase from each strain of *S. aureus* (Z-88, 196E and 418) was higher when the pH was 10.0 than when it was 9.0 at either assay temperature (11.2 vs 10.3 at 37°C and

Table 1—Activity of purified micrococcal nuclease as influenced by temperature and pH

Temp °C	Units[a] of activity/ml			
	pH 9.0	pH 9.5	pH 10.0	pH 10.5
37	2.3	4.8	8.0	11.0
45	5.3	3.5	10.5	11.9
50	7.9	9.6	14.8	11.4
55	10.7	11.1	14.1	4.3
60	not tested	9.7	12.0	2.6
65	not tested	4.4	2.5	0.3

[a] One unit of activity is equal to a change in optical density of 1.0 at 260 nm resulting from acid soluble nucleotides produced in 30 min by 1 ml of enzyme solution. Assay mixture consisted of 0.2 ml of heat denatured calf thymus DNA (2 mg/ml) in 0.01M NaCl, 0.175 ml of 0.05M glycine buffer, 0.025 ml of 0.1M $CaCl_2$ and 0.1 ml of enzyme solution in 0.1% Bovine serum albumin. Units reported were averages from two separate trials.

Table 2—Activity of purified micrococcal nuclease at pH 10.0 as influenced by temperature and calcium

Temp °C	Final conc of calcium (M)	Units[a] of activity/ml
45	0.0005	11.5
	0.001	10.0
	0.005	10.5
	0.01	9.6
50	0.0005	12.1
	0.001	12.3
	0.005	14.8
	0.01	14.1
55	0.0005	12.2
	0.001	10.9
	0.005	14.1
	0.01	13.8

[a] One unit of activity is equal to a change in optical density of 1.0 at 260 nm resulting from acid soluble nucleotides produced in 30 min by 1 ml of enzyme solution. Assay mixture consisted of 0.2 ml of heat denatured calf thymus DNA (2 mg/ml) in 0.01M NaCl, 0.175 ml of 0.05M glycine buffer, 0.025 ml $CaCl_2$ and 0.1 ml of enzyme solution in 0.1% bovine serum albumin. Units reported were averages from two separate trials.

13.0 vs 12.0 at 50°C with Z88). These observations were analogous with spectrophotometric measurements. Data in Table 5 show the effect of NaCl on the activity of DNase in the DA system. As can be seen, DA with 0.17M NaCl yielded the largest zone of activity.

Data in Figures 1 and 2 show the DNase activity, respectively, of growth from strong and weak nuclease producing strains of *S. aureus* (Z88 and D143). The supernatant from an overnight culture grown in BHI at 37°C was boiled for 15 min and was used as crude DNase for assay. As can be seen from

Table 3—Activity of crude staphylococcal nuclease[a] as influenced by calcium, temperature and pH

Temp °C	Units[b] of activity/ml			
	pH 9.0		pH 10.0	
	Concentration of calcium			
	0.0005M	0.005M	0.0005M	0.005M
37	2.3	4.4	3.9	6.1
50	3.3	6.0	6.0	8.6

[a] Supernatant from an overnight culture of Staphylococcus aureus (Z-88) grown in Brain Heart infusion broth (at 37°C) that was boiled for 15 min and diluted 1:5 in BHI.
[b] One unit of activity is equal to a change in optical density of 1.0 at 260 nm resulting from acid soluble nucleotides produced in 30 min by 1 ml of enzyme solution. Assay mixture consisted of 0.2 ml of heat denatured calf thymus DNA (2 mg/ml) in 0.01M NaCl, 0.175 ml of 0.05M glycine buffer, 0.025 ml $CaCl_2$ and 0.1 ml of enzyme solution in 0.1% bovine serum albumin. Units reported were averages from two separate trials.

Table 4—Activity of crude staphylococcal nuclease[a] in an agar diffusion system as influenced by pH, temperature and calcium

pH	Calcium conc.	Temp °C	Diameter (mm) of acid[b] soluble zone after 4 hr incubation		
			Z-88	196E	418
9.0	0.001M	37	10.3	9.2	9.1
		50	12.0	10.4	11.6
9.0	0.005M	37	not tested	not tested	not tested
		50	13.0	12.0	12.2
10.0	0.005M	37	11.2	10.3	10.2
		50	13.8	12.2	13.4

[a] Supernatant from an overnight culture of S. aureus grown in Brain Heart infustion broth (at 37°C) that was boiled for 15 min.
[b] Zone of clearing caused by 1N HCl soluble nucleotides produced from DNA (0.17M NaCl)

Table 5—Activity of crude staphylococcal nuclease[a] in an agar diffusion system as influenced by NaCl

Temp °C	Conc of NaCl (M)	Diameter (mm) of acid[b] soluble zone after 4 hr incubation		
		Z-88	196E	418
37	0.004	6.7	6.2	6.0
	0.017	7.6	6.0	5.9
	0.17	11.2	10.3	10.2
	0.34	11.5	10.0	10.0
50	0.004	7.2	6.7	6.4
	0.017	7.6	7.1	7.0
	0.17	13.8	12.2	13.4
	0.34	12.0	11.3	12.0

[a] Supernatant from an overnight culture of S. aureus grown in Brain Heart infusion broth (at 37°C) that was boiled for 15 min.
[b] Zone of clearing caused by 1N HCl soluble nucleotides produced from DNA. pH of assay was 10 and Ca^{++} 0.005M.

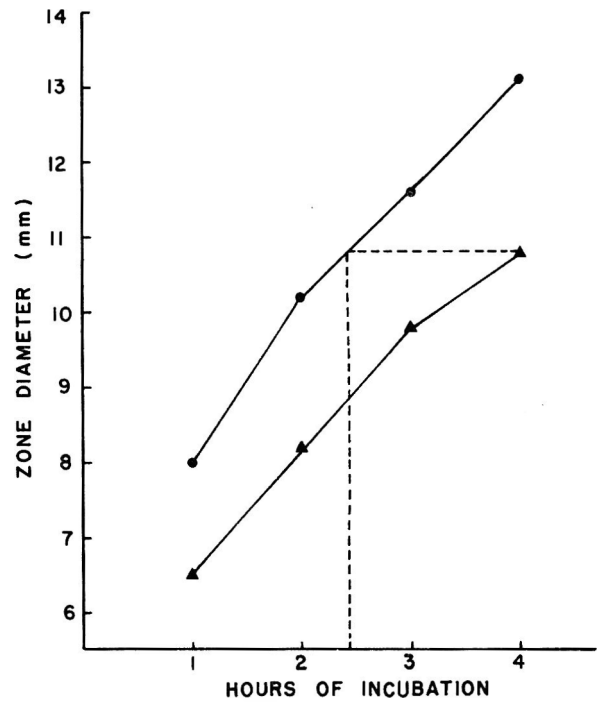

Fig. 1—Nuclease activity of S. aureus (Z-88) culture supernatant in Toluidine Blue-DNA agar. (●—pH 10.0, 50°C, 0.005M Ca^{++}, 0.17M NaCl; ▲—pH 9.0, 37°C, 0.001M Ca^{++}, 0.17M NaCl)

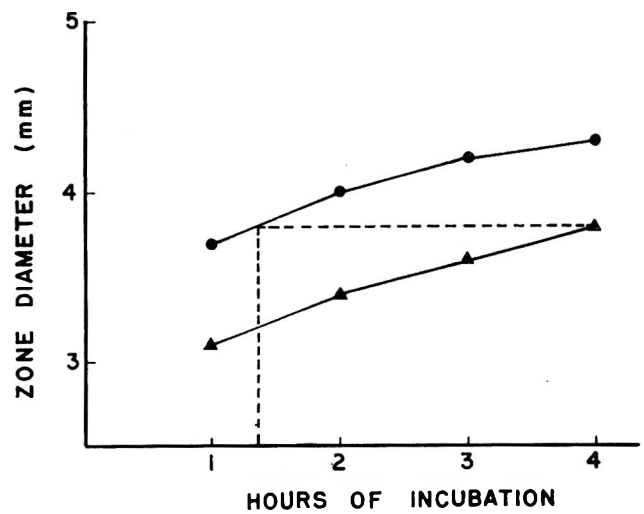

Fig. 2—Nuclease activity of S. aureus (D-143) culture supernatant in Toluidine Blue-DNA agar. (●—pH 10.0, 50°C, 0.005M Ca^{++}, 0.17M NaCl; ▲—pH 9.0, 37°C, 0.001M Ca^{++}, 0.17M NaCl)

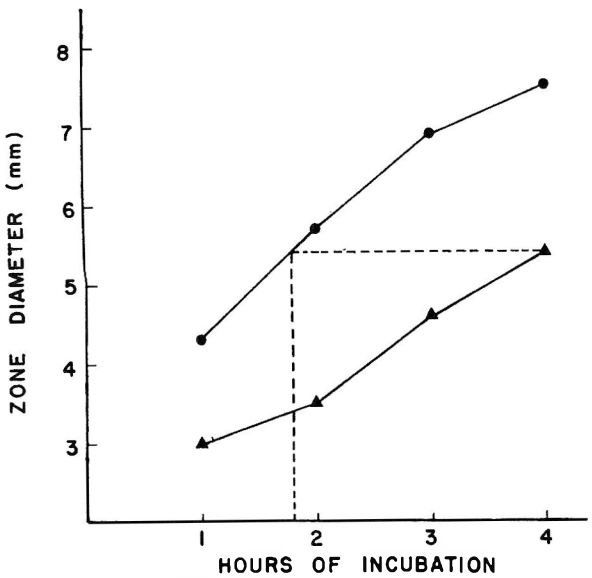

Fig. 3—Nuclease activity of cheddar cheese in Toluidine Blue-DNA agar. (●—pH 10.0, 50°C, 0.005M Ca^{++}, 0.17M NaCl; ▲—pH 9.0, 37°C, 0.001M Ca^{++}, 0.17M NaCl)

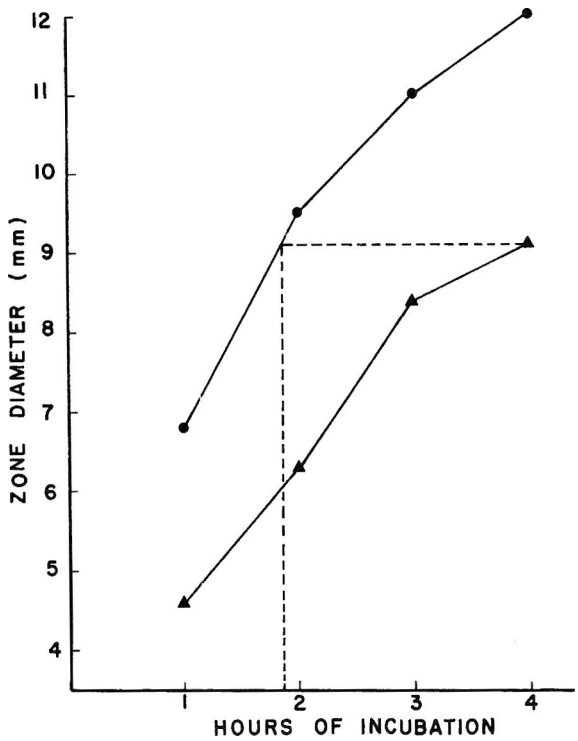

Fig. 4—Nuclease activity of malted milk in Toluidine Blue-DNA agar. (●—pH 10.0, 50°C, 0.005M Ca^{++}, 0.17M NaCl; ▲—pH 9.0, 37°C, 0.001M Ca^{++}, 0.17M NaCl)

the dotted line, a pink zone of activity comparable to a 4-hr assay time at 37°C was obtained within 1.5 and 2.5 hr at 50°C, respectively with weak and strong DNase producing strains. Similar observation was also made with crude DNase obtained from two food products, dried malted milk and cheddar cheese both involved in staphylococcal food poisoning (Fig. 3 and 4). It should be pointed out here that the TDA-system of Lachica et al. (1971) yielded a similar size zone at pH 9.0 or 10.0. However, the zone size according to our findings should have been larger at pH 10.0. It was subsequently found that visualization of pink zone at pH 10.0 was the problem and it was solved by using twice the concentration of dye employed by Lachica et al. (1971). The pink zone was more distinct and was more accurately measured when the dye concentration was higher. The color contrast between background and zone of DNase activity was more distinct with TDA plates of pH 9.0 (blue against pink) than those with pH 10.0 (purple against pink). Increasing the dye concentration in TDA plates by twofold did not change the background purple color though it did make the pink zone more distinct and more accurately measurable. Data shown in Figures 1–4 were obtained through use of TDA system of Lachica et al. (1971) at 37°C and TDA system at 50°C which contained a twofold higher concentration of the dye. The reason for this poor visualization of zone at pH 10.0 with the regular concentration of the dye as in Lachica et al. (1971) may have been due to some limitation of dye molecules to form aggregates.

In any event the TDA system containing a twofold higher concentration of dye than the TDA system of Lachica et al. (1971) and use of 50°C, pH 10.0, 0.005M calcium and 0.17M NaCl would reduce the assay time by twofold over the presently used 4-hr at 37°C and a pH of 9.0. This certainly is advantageous in adapting this assay for routine quality control of foods for detecting *S. aureus* growth in finished foods.

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ENZYME INACTIVATION BY AN IMMOBILIZED PROTEASE IN A PLUG FLOW REACTOR

ABSTRACT

Streptomyces griseus protease was immobilized to porous glass and characterized kinetically. The immobilized protease was then used in a plug flow reactor to inactivate enzymes in solution at low concentrations. The glass-bound protease was effective against fungal glucose oxidase and partially effective against soluble *S. griseus* protease. Some enzymes were more efficiently inactivated by plain or silanized glass without enzyme, presumably due to adsorption. Less tomato pectin methylesterase was inactivated by glass-bound protease than by plain glass. This is most likely due to masking of adsorption sites by the immobilized protease.

INTRODUCTION

IT IS OFTEN desirable to inactivate enzymes in fluid food products like fruit juices. This is usually accomplished by heating. However, where destruction of microorganisms is not necessary for the process, such as in the production of frozen, concentrated orange juice, there may be some advantage in protecting the properties of the material by inactivating the enzymes without heat.

In the work described in this paper we examined the possibility of cold inactivation of enzymes by means of an immobilized protease in a continuous plug flow reactor. The problem basically is one whereby a high molecular weight substrate (the enzyme) at a very low concentration must be acted on by the immobilized protease. With large molecular weight substrates steric hindrance may prevent the approach of the substrate to the immobilized enzyme. In addition, diffusional restrictions are more severe with high molecular weight substrates. With these considerations one would expect that an immobilized protease would be a much poorer catalyst for protein hydrolysis than for esterolytic activity against small molecular weight substrates. Brümmer et al. (1972) found that immobilized trypsin was 20–100 times less active against proteins than it was against small molecular weight amino acid derivatives. However, an alkaline protease from *Tritirachium album* was only six times more active against N-acetyl-L-tyrosine ethyl ester compared to lactalbumin and hemoglobin and less than 10 times more effective against the small molecular weight substrate than against lactate dehydrogenase. This immobilized protease was actually more active against the enzyme ribonuclease than the amino acid ester. Activity of the immobilized protease against the enzymes was followed by measuring the decrease in activities of the substrate enzymes.

In addition to the obvious advantage of adaptability for a continuous processing procedure, immobilized proteases do not digest themselves. Reactions catalyzed by immobilized proteases could be closely controlled by varying the contact time of the enzyme with the substrate (Lill and Hartmann, 1975). Venkatasubramanian et al. (1975) used papain immobilized on collagen to chill-proof beef. Mason et al. (1975) immobilized a neutral protease from *B. subtilis* to porous glass and used it in a fluidized bed reactor for the continuous preparation of a soy protein hydrolysate. The product was low in free amino acids and had good solubility at low pH.

MATERIALS & METHODS

Materials

The enzymes used were as follows: glucose oxidase from *A. niger* (110 I.U. per mg protein), Worthington Biochemical Corporation; pectin methyl esterase from tomato (150 units per mg protein), Sigma; bacterial protease from *Streptomyces griseus* (type VI), Sigma Chemical Co. (hereafter referred to as *S. griseus* protease); and papain, Sigma. The support used was a controlled pore glass of 80–100 mesh with pore sizes ranging from 980–2060 Å obtained from Electro-Nucleonics Inc., Fairfield, N.J. N-benzoyl-L-arginine ethyl ester was a product of Sigma. All other reagents used were the purest available commercially.

The immobilization procedure was similar to that of Lee et al. (1974) who immobilized trypsin to porous glass. The glass was silanized in 10% gamma-aminopropyltriethoxysilane in acetone overnight at 40°C and washed with 1 liter of distilled water. It was then placed in a solution of 25% glutaraldehyde at pH 7.0 and evacuated with an aspirator for 1.5 hr and allowed to stand for 2 additional hr at 1 atmosphere. It was again washed and allowed to react with a solution of *S. griseus* protease at a concentration of 5 mg per ml overnight at 4°C. Five ml of solution were used for every gram of support. After standing overnight the support was again thoroughly washed and stored as a wet cake at 4°C.

Assay of *S. griseus* protease

The *S. griseus* protease was assayed with either α-N-benzoyl-L-arginine ethyl ester (BAEE) or casein as substrate or by its ability to deactivate enzymes. The activity against BAEE was performed in 0.01M tris at pH 7.8 in 0.1M KCl and 0.05M $CaCl_2$ (Walsh and Wilcox, 1970). The pH was kept constant by the automatic addition of standard base in the pH-stat mode of a Radiometer automatic titrator. Activity against casein in 0.1M phosphate buffer, pH 7.6, was determined by measuring the TCA-soluble peptides following digestion using the method of Kunitz (1947) except 20% TCA was used. Activity against the enzymes was determined by measuring the initial activity before treatment and the activity after various times of contact with the *S. griseus* protease. In all cases the reaction medium was kept well mixed with a magnetic stirring bar. Similar assay techniques were used for soluble and immobilized *S. griseus* protease.

Glucose oxidase was assayed in an oxygen polarograph (Gilson Medical Electronics Model KM Oxygraph) by a procedure previously reported (Bouin et al., 1976). Pectin methyl esterase was detemined according to the procedure of Hultin and Levine (1963) using 0.3% pectin in 0.1M NaCl. The amount of base required to maintain a constant pH was followed by a pH-stat technique. Inactivation of papain and *S. griseus* protease were determined using casein as substrate as described abcve.

Reactor

A packed bed reactor was constructed by using a jacketed glass column with a bed diameter of 1 cm. A peristaltic pump was used to maintain fluid flow and temperature was controlled in the column jacket at 38°C. The pump could be regulated to give flow rates up to 160 ml/min. Samples were removed at time intervals from the system for analysis. Results with the column work are expressed in terms of contact time, i.e., the amount of time that the solution actually spent in contact with the bed. With our system it took roughly 30 min to

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obtain 4 min of contact time. Two to 2.3g of support containing 5.2–6.0 mg of immobilized enzyme was used in the column. The exact amount for each experiment is given in the appropriate figure legend.

Determination of bound protein

Determination of the amount of enzyme protein bound to the glass support was performed by a modification of the method of Lee et al. (1974) coupled with the Lowry test for proteins (Lowry et al., 1951). Known weights of the glass-bound protease were shaken with 5 ml of 1N NaOH for 4 hr, filtered and 0.5 ml assayed for protein content. The same procedure was done with silanized glass that had been reacted with glutaraldehyde. This served as a control.

Dry weight determinations

The dry weights of the catalysts were determined on samples comparable to those used in the assay procedures by drying the support at 195°C for 30 min.

RESULTS & DISCUSSION

Characteristics of immobilized protease from *S. griseus*

Determination of the total amount of immobilized protein on the porous glass support using the method of Lee et al. (1974) gave an average value of 3.9 mg/g of glass (dry weight). The amount of immobilized protein was also estimated by determining the activity of the glass-bound protease using BAEE as substrate. The activity of the immobilized enzyme was compared to the activity per unit weight of the original preparation and the amount of protein immobilized calculated therefrom. By this technique an average value of 2.6 mg of protein per gram of dry weight of glass was found. The difference may be due to some inactivation of the enzyme during the immobilization process or to diffusional restrictions during the assay of the immobilized enzyme causing an apparent lower activity in the immobilized preparation.

When stored as a wet cake the immobilized *S. griseus* protease showed no loss in activity over 1 month at 4°C. Thus, the inherent stability was very satisfactory for our experimental work. Experiments were also performed to determine if the protease leached off the column bed during operation. To do this a sample of the casein solution which had been in contact with the bed for 4 min was removed from the system and incubated for 5 hr. The OD at 280 nm of a supernatant from TCA treatment was determined immediately and after the 5 hr incubation. This was used to calculate the activity leached off the column which was then compared with the total activity on the column. It was calculated that approximately 0.06% of the activity of the column was lost by leaching per min of contact time. This was an acceptable level for our experiments.

Maximal velocities (V_{Max}) and apparent Michaelis constants (K_M) were determined for both soluble and insoluble *S. griseus* protease. These kinetic parameters were determined for the immobilized enzyme at two different flow rates through the reactor. The substrate used was casein, varying in concentration from 0.005–0.1% for the insoluble protease and 0.001–1% for the soluble protease (Fig. 1 and 2). At a flow rate of 154 ml per min, the apparent K_M was 0.011% (weight/volume) and the V_{Max} was 10.9 tyrosine units per min. At a flow rate of 13.5 ml per min the apparent K_M was 0.036% and V_{Max} was 30.3 units per min. For the soluble *S. griseus* protease a value of 1.67% was obtained for the apparent K_M and 18.2 tyrosine units per min for V_{Max}. The values of V_{Max} and K_M are summarized in Table 1.

The apparent kinetic constants obtained are somewhat unusual and deserve a comment. The apparent K_M of the bound enzyme at both flow rates was considerably lower than that for the soluble. Assuming that there were no major structural changes in the protease on binding to the support, it is possible that the decrease in K_M on binding is due to an adsorption of casein onto the glass surface thus increasing the effective concentration of substrate in the microenvironment of the enzyme. Strong adsorption of proteins to controlled pore glass has been documented (Messing, 1969; Bock et al., 1976). However, adsorption by itself does not explain the lower apparent K_M at the higher flow rate compared to that at the lower. We suggest that this is due to the smaller unstirred layer at the higher flow rate. Since the diffusion distance would be shorter for the casein at the higher flow rate, the effective concentration of casein at the surface would be higher than that observed at the slower flow rate (larger unstirred layer).

The apparent V_{Max} values are also rather unusual. The

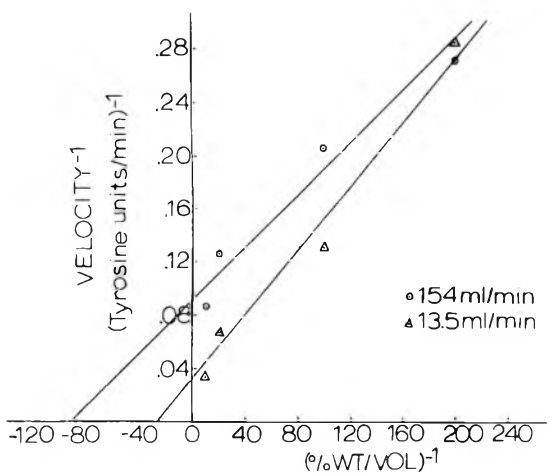

Fig. 1—Double reciprocal plots of initial velocities of immobilized S. griseus protease with casein as substrate in a plug flow reactor at two flow rates. The casein solution (19 ml) was in 0.1M phosphate buffer, pH 7.6, and the temperature was 23° C. Total contact time of the casein solution with the bed was 1.42 min. Approximately 2g of support were used containing 5.2 mg of protein.

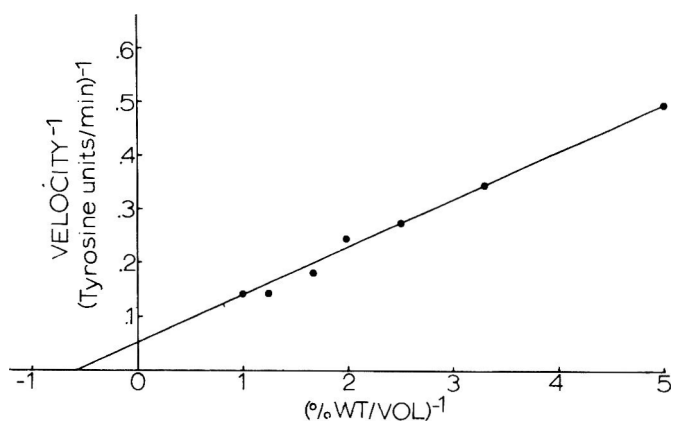

Fig. 2—Double reciprocal plot of initial velocities of soluble S. griseus protease with casein as substrate. The amount of protease used was 200 μg in a total volume of 2 ml. Reaction time was 20 min. All other conditions were as described in the legend to Fig. 1.

total amount of protein used in the flow reactor tests was greater than that in the soluble tests. Therefore, in Table 1 the maximal velocities are also expressed in tyrosine units per min per mg of protein. The apparent V_{Max} of the enzyme was higher at the slower flow rate. This observation is unexpected. We suggest that it is due to the retention of substrate in an unstirred layer. Since this is a macromolecular substrate which can be hydrolyzed several times by the protease, retention of substrate in this unstirred layer gives the enzyme longer to act on it. The unstirred layer is larger at the slower flow rate. This factor would be of particular importance if the enzyme was more active against the polypeptide degradation products than against the native casein. The V_{Max} of the soluble enzyme was considerably greater than that of the immobilized enzyme at either flow rate.

Table 1—Kinetic constants for soluble and immobilized S. griseus protease

Enzyme form	Flow rate (ml/min)	K_M (wt/vol)%	V_{Max} tyrosine units/min[a]	
Immobilized	13.5	0.036	30.3	(5.8)
Immobilized	154.0	0.011	10.9	(2.1)
Soluble	—	1.67	18.2	(91)

[a] Values in parenthesis are expressed on the basis of tyrosine units per min per mg protein.

Inactivation of glucose oxidase by immobilized *S. griseus* protease

Glucose oxidase at a concentration of 200 μg per ml in 0.1M phosphate buffer, pH 6.5, was passed through the reactor containing either glass, silanized glass or the glass-bound protease and the decrease in activity measured as a function of time. Preliminary runs indicated that a significant amount of activity was lost from solution after passing through the plain glass or the silanized glass reactor. Over and above this, additional activity was lost when the glucose oxidase solution was passed over the glass-bound protease. It seemed likely, therefore, that inactivation by the glass-bound protease column was due to both non-specific adsorption as well as inactivation by the immobilized protease.

It was desired to saturate binding sites on the column with glucose oxidase such that any further inactivation would be due solely to proteolytic activity. To this end a silanized glass column reactor was prepared and the glucose oxidase solution passed through the column for a total contact time of 4 min. A contact time of 4 min means that all of the solution was in contact with the bed for a period of 4 min. With our reactor, it took approximately 30 min for this to be accomplished. The glucose oxidase solution was then checked for enzymic activity as well as absorbance at 280 nm (protein) and 450 nm (flavin). After this solution had been through the column, a second was put on and so forth for 10 successive solutions. The results are shown in Figure 3. After three passes of glucose oxidase solution through the column, there was little or no further loss of activity, presumably because the binding sites had been saturated during the first three runs. This experiment was then repeated using protease immobilized to silanized glass (Fig. 4).

The percentage of initial activity remaining after passing through the column is much less than with the plain silanized glass. This difference is presumably due to the proteolytic activity of the immobilized enzyme which hydrolyzed the glucose oxidase and inactivated it. An interesting feature of the results is the absorbance at 280 and 450 nm. These values hover around the 50–60% mark and indicate that the smaller peptides produced by proteolysis are to a large extent adsorbed on to the column. These values should not decrease simply because the protein is hydrolyzed. The ability of the column to inactivate glucose oxidase slowly decreases such that less than 80% of the glycose oxidase is being inactivated after the 10th run. It is possible that this decrease is due to the build-up of adsorbed peptides onto the surface. The smaller fragments of the hydrolyzed glucose oxidase can possibly dif-

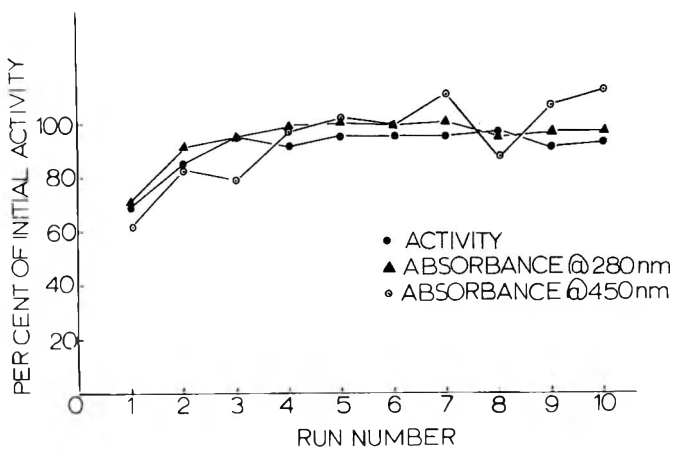

Fig. 3—Inactivation of glucose oxidase by passing through reactor of silanized glass. The graphs indicate the % of activity and material absorbing at 280 and 450 nm which passes through the reactor. The glucose oxidase solution (17 ml) contained 200 μg/ml of enzyme in 0.1M phosphate buffer, pH 6.5; the temperature of the enzyme solution and reactor was maintained at 38°C. Approximately 2.3g of the silanized support was used in the reactor.

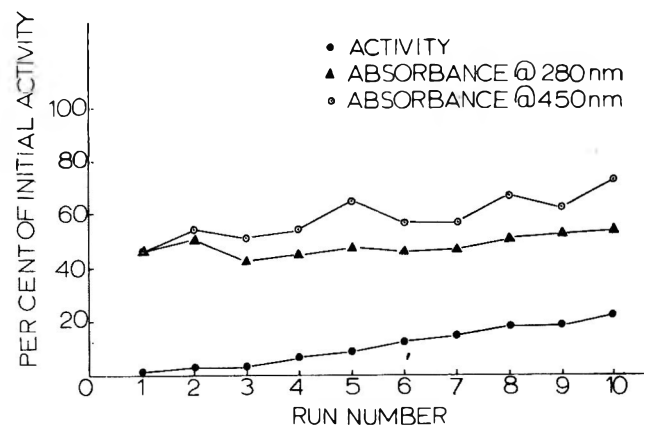

Fig. 4—Inactivation of glucose oxidase by passing through reactor containing S. griseus protease which has been immobilized to silanized glass via glutaraldehyde. All conditions were the same as described in the legend of Fig. 3 except that S. griseus protease was immobilized to the glass support.

fuse more easily into the internal pores of the support than the native molecule.

The silanized glass and the silanized glass-protease columns were washed twice with 1.5M NaCl for 15 min each time to determine if adsorbed protein could be removed. Of the total number of glucose oxidase units put through the silanized glass column, 8.3% was lost. Of this, approximately 0.1% was washed off the column with the 1.5M NaCl. On the other hand, the total loss of activity through the glass-protease column was 89% of the total applied. Twenty-one per cent of the total activity was washed off with the concentrated salt solution. There was indeed considerably more inactivation on the glass-bound protease column than on the silanized glass column, and a good part of the inactivation was due to hydrolysis of the glucose oxidase. However, these data also indicate that in the reactor with the glass-bound protease, more enzyme is reversibly bound than with the silanized glass column. The absorbances of the wash solutions at 280 and 450 nm correlated very closely with the recovered activity. Where the total activity desorbed by the 1.5M NaCl was 21% of the total initial value, the absorbances of the desorbed material at 280 and 450 nm were 26% and 20%, respectively. Thus, most of the protein which was washed off the glass-bound protease column by the strong salt solution was active. The ability to wash rather high levels of protein out of the glass-bound protease support is consistent with the relatively low per cent of materials with absorbance at 280 and 460 nm which came through the column during the runs (Fig. 4). It appears that active enzyme is more easily washed off the glass than are the hydrolyzed fragments.

Adsorption of other enzymes to glass support

Solutions of pectin methyl esterase, papain and the *S. griseus* protease were also passed through plain glass, silanized glass and glass-bound protease columns. Both pectin methyl esterase and papain were completely adsorbed to either plain glass or silanized glass columns. In fact, the loss in activity of pectin methyl esterase was considerably less on the glass-bound protease than on either the silanized glass or the plain glass. In the case of the glass-bound protease, the immobilized enzyme covers some of the binding sites which probably prevents complete adsorption of the pectin methyl esterase.

The protease from *S. griseus* lost about 30% of its activity on passing through the glass or silanized glass column in 4 min of contact time. About 65% of the activity was lost in the same amount of time on passing through the glass-bound protease column. This enzyme is intermediate in its adsorptive properties to pectin methyl esterase and glucose oxidase. It is likely that different proteins would have a very broad range of adsorptive properties.

The efficient binding of enzymes to porous glass may be due in part to the very high surface area of this support. Bock et al. (1976) found that they could adsorb a large number of proteins on controlled pore glass. This procedure followed by elution with chaotropic buffers led to purification of several hundred-fold of proteins from relatively crude mixtures. For the removal of enzymes from fluid food products, a combination of adsorption and deactivation by proteolysis could conceivably be used. The glass support may be regenerated periodically by washing with salts to remove adsorbed proteins. Presumably the covalently linked protease would not be affected by the high salt concentrations and would remain attached to the support.

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COMPARSION OF CHEMICALLY MEASURED AVAILABLE LYSINE WITH RELATIVE NUTRITIVE VALUE MEASURED BY A Tetrahymena BIOASSAY DURING EARLY STAGES OF NONENZYMATIC BROWNING

ABSTRACT

Tetrahymena pyriformis W (TPW) was used as a biological assay to study the loss of overall protein quality due to nonenzymatic browning. The Relative Nutritional Value (RNV) as determined by this organism decreases in a model food system as Maillard compounds are formed during storage at 35°C for 80 days and at three water activities (0.3, 0.5 and 0.7). These results were compared to the fluorodinitrobenzene (FDNB) chemical assay. The latter method shows a greater loss of available lysine than is seen by TPW growth at early stages of browning. Significant accumulation of brown pigment production does not occur until after RNV has diminished by 35–50% and FDNB has decreased by 60–70%. At advanced stages of browning, however, the TPW test showed equivalent or greater losses of nutritional value. Considerable variation in the TPW bioassay occurred due to the problems in the technique but analysis of all stored samples in a single test allowed comparison to a standard casein control for measurement of protein quality loss.

INTRODUCTION

REACTION of sugars with amino acid through Maillard browning results in compounds that cannot be nutritionally utilized. The greatest destruction and loss occurs with lysine, which has a free ε-amino group that can react with reducing sugars (Mauron et al., 1955). The early stages of the reaction are reversible and no color develops; however, some results show that the nutritional availability of the amino acid is reduced (Mauron et al., 1955; Adrian, 1974; Carpenter and Booth, 1973).

Acid hydrolysis used in many chemical assay methods for nutritional losses may release bound amino acids which have reacted but are unavailable for biological enzymatic breakdown. Therefore, chemical assay techniques which rely on reaction with the labile amino acid may underestimate true biological loss. It has been shown that one of the assays most used for lysine measurement, the fluorodinitrobenzene (FDNB) procedure, may underestimate protein losses in nonenzymatic browning (Hurrell and Carpenter, 1974; Mauron et al., 1955). In browning, however, early stage reaction compounds of lysine with carbonyls may not react with FDNB but could be available in the gut so that the FDNB procedure could actually overestimate nutritional losses from a biological standpoint.

The nutritional loss that occurs from nonenzymatic browning affects overall protein quality, usually by reducing the amount of available lysine, an essential amino acid for humans. This could be especially important in cereals that are already low in lysine and in foods formulated to meet the protein requirement for a meal, such as shelf stable intermediate moisture food bars (Labuza et al., 1976). These typically have a water activity (a_w) in the 0.6–0.85 range and contain both protein and reducing sugars.

Lea and Hannan (1949) demonstrated that a_w affected the rate of browning in a casein and glucose system. They found a maximum in browning rate in the a_w range 0.65–0.7 at temperatures from 37–90°C. Warmbier (1975), however, demonstrated that the rate of browning is reduced by the presence of liquid humectants in the high a_w range.

Tetrahymena pyriformis W (TPW) is a protozoan requiring the same essential amino acids as man, including lysine. It has been used over the past 20 yr for research on protein quality evaluation (Fernell and Rosen, 1956; Stott et al., 1963; Helms and Rolle, 1968; Rolle and Eggum, 1971; Srinivas et al., 1975). It has also been used by several workers as a measure of available lysine. In these tests, the sample provides the only supply of lysine in an otherwise nutrient rich media (Stott and Smith, 1966; Boyne et al., 1967; Shorrock and Ford, 1973). Values obtained in such a TPW assay tend to be slightly lower and yet correlate well with FDNB results, particularly if an enzymatic digestion of the sample aids the proteolytic TPW in digesting the intact proteins (Shorrock and Ford, 1973).

The purpose of this project was to evaluate the use of a TPW assay for the study of loss of overall protein quality due to nonenzymatic browning in an intermediate moisture model system. Values obtained by the chemical FDNB assay for available lysine were compared to the TPW assay.

MATERIALS & METHODS

Model food systems

A model food system using ANRC reference casein and glucose in a 3:1 ratio was used. Individual components listed in Table 1 were mixed in descending order. Water was added to obtain the desired water activity. The systems were equilibrated over appropriate saturated salt solutions for 3 days at room temperature. Three systems were prepared to a_w's of 0.3, 0.5 and 0.7.

Ten-gram portions of each system were sealed in 202 × 214 tin cans and dipped in paraffin to prevent moisture changes. Cans representing zero day samples were held at −35°C. The remaining cans were stored at +35°C. Samples were periodically removed for up to an 80 day period and were also placed at −35°C. At the end of storage all samples (12) at each a_w were simultaneously analyzed.

Tetrahymena pyriformis W assay

A microbiological assay using *Tetrahymena pyriformis* W (TPW) was used to measure Relative Nutritional Value (RNV). The method was that used by Stott et al. (1963) and Landers (1975), with an adaption of the nucleic acids which is listed here.

A portion of the model food system containing 100 mg nitrogen (as measured by Kjeldahl) was incubated with 20 ml water and 1% pepsin at pH 1.8 for 3 hr (55°C). The digested sample was cooled, the pH was adjusted to 7.1, and made to a final volume of 50 ml. Triplicate sample suspensions containing 3 mg N (1.5 ml) were added to 1.5 ml water and 2.0 ml of freshly prepared nucleic acid solution. This nucleic acid solution was prepared according to Lander's method (personal communication), except that the final concentrations of phosphate and TRIS buffers (pH 7.1) were at 0.005M and the final concentration of citric acid was 0.001M. These conditions were found to maximize *Tetrahymena* growth.

Sample, water and nucleic acids were autoclaved in 50 ml, gauge stoppered Erlenmeyer flasks for 15 min at 121°C to prevent growth of other organisms. Solutions of vitamin stock and glucose were autoclaved separately and aseptically added to the cooled samples.

Samples were inoculated with 0.1 ml of a 3-day old broth culture of TPW which had been centrifuged for 8 min at 5000 rpm in a Sorvall refrigerated centrifuge. Cells were diluted in 0.07M pH 7.1 phosphate buffer to 5.0×10^5 cells/ml.

After 4 days in a 25°C shaker incubator (100 cpm) 1 ml of the assay

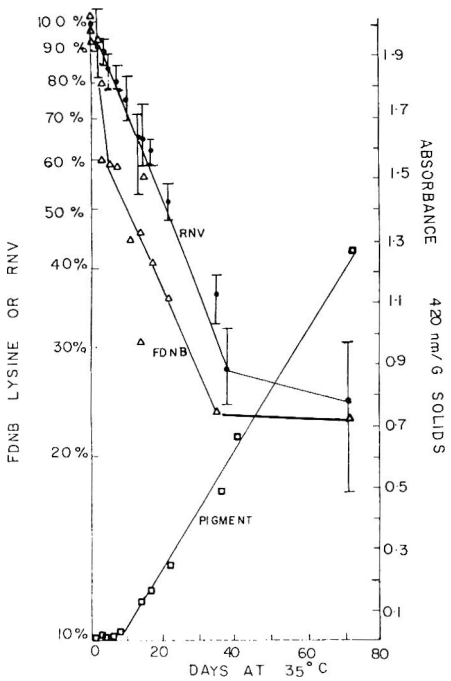

Fig. 1—Lysine loss by FDNB protein quality loss by a TPW bioassay (RNV), and pigment development of 0.3 a_w model food at 35° C.

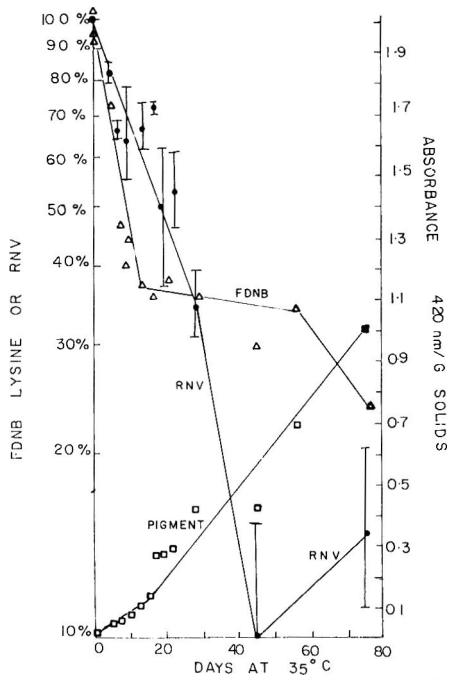

Fig. 2—Lysine loss by FDNB protein quality loss by a TPW bioassay (RNV), and pigment development of 0.5 a_w model food at 35° C.

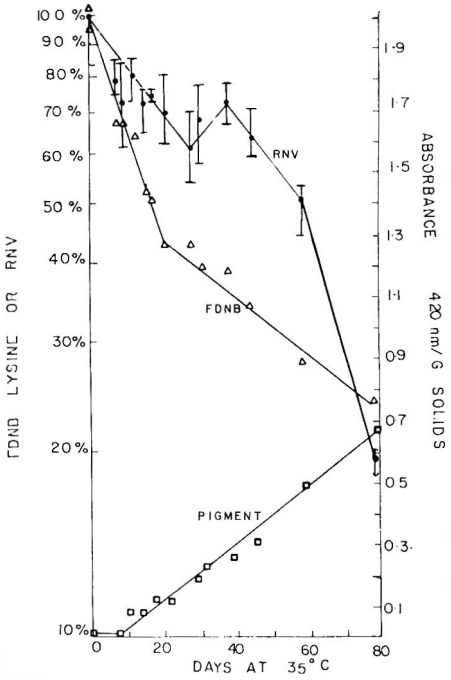

Fig. 3—Lysine loss by FDNB protein quality loss by a TPW bioassay (RNV), and pigment development of 0.7 a_w model food at 35° C.

solution was delivered into 1 ml formaldehyde preserving solution. Cells were then counted in a Fuschs-Rosenthal haemacytometer under a light microscope (300 × 10 power).

RNV was determined as the average total growth of triplicate stored samples per ml assay as a percentage of the total growth of the zero day sample per ml assay material. The range for the TPW is shown in the results. The standard deviation for the control was ± 18.6% RNV based on values for the control done over a two year period. This includes both the variation in the casein as well as the problems in counting. It is felt that most of the error is in the counting procedure however, as experienced by Evancho et al., (1976).

FDNB procedure

Available lysine was also determined using the FDNB method of Carpenter and Booth (Booth, 1971). The FDNB tags to free ε-amino groups at pH 8.5. The sample after treatment with FDNB reagent was refluxed in 8.1N HCl for 16 hr, filtered hot, and diluted with water to a known volume. Available lysine was determined by absorbance at 435 nm.

The available lysine per gram of solid system was compared to the original available lysine determined in the zero day duplicate samples. The standard deviation for FDNB measurement was ± 2.2 mg lysine/gram solids which gives a range of ± 11.8% FDNB lysine.

Nonenzymatic browning pigment production

Brown pigments were measured by the method of Choi et al. (1949). Two grams of the model food system were incubated at 45°C for 2 hr with 20 ml phosphate buffer (pH 7.8) and 2.5 ml 10% (w/v) trypsin. Proteins were denatured with 2.0 ml of 50% (w/w) trichloroacetic acid. Samples were filtered with 0.1g celite filter aid and the filtrate optical density was read at 420 nm against a reagent blank. The OD was reported on a per gram of solids basis against a reagent blank (precision ± 0.001, Warmbier, 1975).

RESULTS & DISCUSSION

THE EFFECT of storage at 37°C on loss of nutritional value and browning in the three model systems is shown in Figures 1, 2 and 3 respectively. Available lysine loss by FDNB assay and protein quality loss (RNV) TPW assay were plotted on a semi-log scale while browning increase was plotted as a zero order reaction (Warmbier et al., 1976). The TPW assay was a comparison of the stored sample to that of the original control at the 3 mg nitrogen/10 ml assay level, so it is a relative nutritional value (RNV). In all cases, loss of lysine occurs as measured by both procedures before significant browning occurs, as was found by Lea and Hannan (1949) and Warmbier et al. (1976).

The early reactions in the Maillard reaction sequence are reversible and the compounds formed are usually colorless. Table 2 compares TPW, RNV and FDNB values when the absorbance reached 0.02/g dry solids. It is obvious that the FDNB procedure overestimates the loss of lysine as compared to the TPW test procedure by about the same amount at each a_w. The organism may be able to enzymatically digest the early reaction products while the FDNB test procedure measures these as being biologically unavailable. It should be noted, however, that the FDNB procedure is specific for available lysine. On the other hand, the TPW procedure measures effect on overall protein quality. Thus, some loss of lysine and other more limiting amino acids may not be detrimental to growth. The early reaction products should be isolated and tested in a limited lysine medium to prove they are available however.

The level of lysine needed to support growth of TPW (as estimated by Rolle, 1975), is sufficient in casein. Thus, total growth capacity should not be affected greatly until lysine becomes the limiting factor. The growth capacity was maximized by choosing the 3 mg nitrogen test level and comparing it to the control to reduce the error in the TPW assay. At lower levels growth is poor and at 4 mg N the assay has high variability (Stott et al., 1963).

The overall results seen in the figures show the browning was more rapid at the lower a_w. This is because of the presence of propylene glycol which is similar to that found by Warmbier et al. (1976). At a_w 0.3 the FDNB procedure showed greater losses over the whole storage time. At the two higher a_w's where the reaction was slower the TPW procedure eventually showed equal or greater protein quality loss. The reasons for this are not clear since one might expect that with

greater browning the protein would become less readily digestible to the organism. Thus the lower a_w sample would be expected to show this crossover. On the other hand, it is possible that reaction pathways change with a_w, so that the change in patterns shown might occur. The data indicate that at stages of advanced browning the TPW test shows equivalent or greater losses in protein quality. The test also indicates the problems that would occur if a similar response is found in rat PER assays. Usually in the latter test, only one system at one storage time and temperature can be evaluated, due to time and labor costs. Thus the value may not be meaningful as to the actual changes that occur during transportation and storage of a food. The TPW test is simple and easy to perform and thus allows comparison of many more test conditions. The problem would be in the evaluation of the reliability of the TPW test. As can be seen, some unexpected fluctuations occur in the TPW assay which cannot be accounted for. This shows that one should not use single point analysis even with reliance on numerous replicates. Rather, one should test more points over a period of time with less replicates, to determine a trend.

Considerable evaluation of the TPW procedure preceded the actual storage study presented here. Variation from trial to trial on total count at a given N level was large even with a standard casein control. Strict control of the inoculant level eliminated part of this problem, although biological variation could not be completely eliminated as might be expected. To eliminate the variation, with respect to the casein standard, stored samples at each a_w, were frozen until they could be analyzed in a single TPW test.

Another consideration examined prior to this experiment was the effect of the individual model food components and possible substitute humectants on TPW growth. Each diluted component was added separately to casein reference samples at levels found in Table 1 and also at a final concentration 25 times that level. As seen in Table 3, only the use of the humectant glycerol completely suppressed growth for this organism. Chloroform:methanol (3:1) extraction of the glycerol from a model food system, resulted in normal growth patterns.

These results indicate the care that must be taken if formulated foods are evaluated with *Tetrahymena pyriformis* W, since they may contain inhibitory substances. It also exemplifies the difficulties that might result from comparison of different food sources to an unrelated reference sample for purposes of protein evaluation.

Table 1—Model food system composition (0.3, 0.5, 0.7 a_w's)

Component	Grams
Potassium sorbate	0.3
Glucose	10.0
Propylene glycol	20.0
Casein (ANRC reference, Sheffield Chem. Co.)	30.0
Apiezon B-oil (J.G. Biddle Co.)	20.0
Microcrystalline cellulose	20.0
Water	variable for a_w used

Table 2—Average loss of available lysine by FDNB and Tetrahymena RNV at the early stage of browning

Model food system	Days stored at 35°C	Pigment development @420 nm OD per g solids	FDNB % original lysine left	RNV at 3 mg N by TPW procedure
0.3 a_w	22	0.2	32	48
0.5 a_w	18	0.2	35	50
0.7 a_w	30	0.2	40	65

Table 3—Effect of model food system components on Tetrahymena RNV

Component	Avg RNV 3 mg N/10 ml[a]
Potassium sorbate	105
Apiezon B oil	102
Microcrystalline cellulose	112
Humectants:	
Propylene glycol	110
Butylene glycol	103
Glycerol	88

[a] Variation was ± 8 RNV for triplicate samples

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EVALUATION OF SEVERAL PULSED NMR TECHNIQUES FOR SOLIDS-IN-FAT DETERMINATION OF COMMERCIAL FATS

ABSTRACT

The critical procedural and instrumental parameters for the determination of the percent solids in commercial fat samples using proton pulsed NMR techniques are reported. The precision and accuracy of two alternate data treatments are identified. The pulsed NMR technique has the advantage of measurement speed on the standard dilatometric and wideline NMR procedures. However, in common with these, for consistent results the samples must be temperature conditioned. Within experimental error, the pulsed NMR method is not sensitive to a tempering step in the conditioning procedure. A pulsed NMR method based on the use of a standar plot has a standard deviation of ±1%. This compares favorably with wideline NMR, but is less precise than dilatometry. The results, however, are linearly related to dilatometry, and are most accurate at low solid levels. For commercial fats, accuracy was found to depend upon their composition.

INTRODUCTION

THE CONSISTENCY of food fats and oils is important to the design of food products. For example, a liquid oil will generally not produce a satisfactory cake in terms of grain and volume. If this oil is hydrogenated to a plastic fat, an acceptable cake is produced. Fat and oil consistency is a function of both composition and temperature, as illustrated by the variation in the spreadability of margarine formulations.

Food fats and oils are principally mixtures of triglycerides. As physical systems, they are liquid crystals. They exhibit polymorphism, and form solid solutions of mixed crystals. Their liquid portions have variable rates of crystallization depending upon composition.

The consistency of fats and oils at a given temperature is determined by the above physical phenomena and measured by the solids-in-fat index (SFI). The temperature dependence of SFI is an important specification for product development and process control purposes.

Methods for determining SFI have been described. These are based on the techniques of differential thermal analysis (DTA), differential scanning calorimetry (DSC), specific volume (dilatometry), continuous wave NMR (wideline NMR), and pulsed NMR. It is also believed that dielectric measurements could be used for this purpose; however, there is no literature describing a method. Regardless of the method, sample temperature history is critical to accuracy and precision.

The DTA and DSC methods (Bentz and Breidenbach, 1169; Miller et al., 1169) have not been widely accepted. They do not measure percent solids directly, since averaged heats of fusion are used in the calculation. The time required for a single determination is long, and the sample size is small. The time-temperature relationships are incompatible with the sample conditioning and tempering procedures used for other methods. This makes comparison of results difficult.

Dilatometry (Braun, 1955, 1957; Bailey, 1950), the oldest and widest used method for SFI, is an official AOCS method (Braun, 1957). This method is time consuming and applicable only below 50% SFI. Dilatometry is not a direct measure of solids.

The other official AOCS technique (Vermaas, 1972) is wideline NMR (Chapman et al., 1959, 1960; Bosin and Marmor, 1968). This technique is a direct measurment of solids since it depends on the dipole-dipole interactions of rotating triglyceride molecules (Pople et al., 1959). It is applicable throughout the entire range of SFI. The method compares the area under a liquid signal at a specified temperature, with that of a reference oil. Haighton (1972) indicates that signal saturation can effect results and recommends the use of specific reference oils depending on sample composition. Comparisons have been made between dilatometry and wideline NMR results (Bosin and Marmor, 1168; Haighton et al., 1971; Wettstrom, 1171), and DSC, dilatometry, and wideline NMR (Walker and Bosin, 1971).

Taylor et al. (1964) reports that dilatometric results at low solids level are more accurate than wideline results. At higher levels, the results are comparable. Pohle et al. (1965) indicates that the precision of the wideline NMR method decreases with increasing solids level.

On first principles, the pulsed NMR approach should produce the same results as wideline NMR (Farrar and Becker, 1971). An automated SFI procedure has been described using the pulsed NMR free induction decay (FID) signals (Van Putte and Vanden Enden, 1974). Two methods were detailed. A method which uses a standard and saturation correction to determine directly the ratio of solid signal to total signal, and an indirect method which tkes only the liquid signal at a time 70 microsec after the pulse and with reference oil data, uses a calculation similar to wideline NMR. Both methods give similar results. Pulsed NMR results have been compared with both wideline NMR and dilatometry (Van Putte et al., 1975). The use of references based on sample composition is suggested.

We report the investigation of critical parameters, and the precision and accuracy of two additional pulsed NMR methods based on the extrapolation of a single FID signal to time zero. The first, the calculation method, is similar to the indirect method of Van Putte and Van den Enden (1974). The second involves the use of a standard plot at each testing temperature.

METHODS & MATERIALS

Fat samples

Samples with known solids content were blends of safflower oil and 5.4 IV tallow supplied by Anderson-Clayton Co., Richardson, Texas. Their composition is given elsewhere (Walker and Bosin, 1971). Unknown samples were commercial shortenings supplied by Glidden-Durkee Co., Cleveland, Ohio. Fatty acid composition and physical properties for these samples are given in Table 1

Sample tempering and conditioning

Three procedures were used: the standard dilatometric method as

described by Braun (1957); the AOCS procedure for wideline NMR (Vermaas, 1172); and an excessive tempering procedure. In this, the samples were tempered in a 60°C waterbath and transferred directly to the testing temperature. After conditioning for 30 min at this temperature, measurements were taken. All samples were retempered at 60°C prior to moving to the next testing temperature. Measurements were made at 10, 20, 30 and 40°C.

NMR measurements

A pulsed NMR spectrometer (Model PR-102, The Praxis Corp., San Antonio, Texas) capable of 10 and 30 MHz operation was used. Sample temperature was controlled to ± 0.1°C in a 10 MHz probe using a Varian Model V-4540 controller (Varian Associates, Palo Alto, Calif.). The 30 MHz probe was maintained at 32°C by magnet heat. A transient recorder (Model 802, Biomation, Cupertino, Calif.) was used to capture the NMR signal. Output was by recorder (Electronik 194, Honeywell, Fort Washington, Pa.), or oscilloscope (Model 5103N, Tektronix, Inc., Beaverton, Or.). An oscilloscope camera was used for permanent records (Tektronix Model C-5).

The logarithm of the free induction decay signal after a 90°C r.f. pulse was extrapolated to time zero. The solids content was calculated from intercept values using a standard curve, or using the following formula.

$$SFI = 100 - \left[\frac{\left(\frac{\text{sample at T°C}}{\text{sample at 60°C}}\right)}{\left(\frac{\text{reference at T°C}}{\text{reference at 60°C}}\right)}\right] \times 100$$

An olive oil reference standard was used.

Dilatometry

The AOCS (Braun, 1957) official procedure was used for all dilatometric measurements.

RESULTS & DISCUSSION

SFI MEASUREMENT by an NMR technique is not expected to agree exactly with a dilatometric measurement. However, there is a linear relationship between them. Figure 1 shows a typical result for a commercial hydrogenated vegetable fat using the standard plot method with wideline sample conditioning. The pulsed NMR results are higher than those of dilatometry. Walker and Bosin (1971) associate this with a lack of sample tempering. The AOCS dilatometric procedure includes a sample tempering step. However, results taken using this sample conditioning sequence are not significantly different than the untempered date (Fig. 2). One concludes that within experimental error, the pulsed NMR method is not sensitive to a tempering step.

The proposed indirect SFI calculation results are consistently lower (Fig. 3) when compared with standard plot results on the same sample. They are linearly related to the later

Table 1—Composition and physical properties of commercial fat samples

Fatty acid composition (wt %)	Fat A	Fat B
C 8:0	0.07	Trace
C 10:0	0.03	0.04
C 12:0	0.26	0.35
C 14:0	0.25	0.42
C 16:0	11.31	13.58
C 16:1	0.10	0.32
C 18:0	9.39	74.60
C 18:1	72.15	10.19
C 18:2	2.71	–
C 18:3	3.40	–
C 20:0	0.33	0.30
Wiley melting point	40.6°C	44.6°C
Iodine value	69.86	69.68

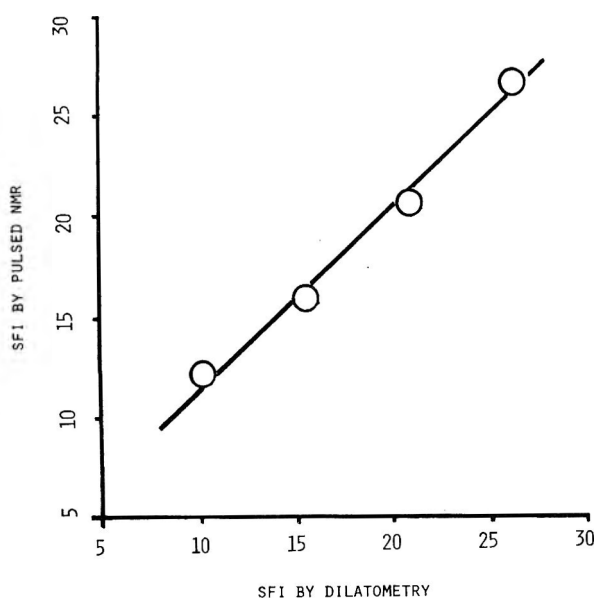

Fig. 1—Comparison of SFI methods on a commercial hydrogenated vegetable fat (Fat B)

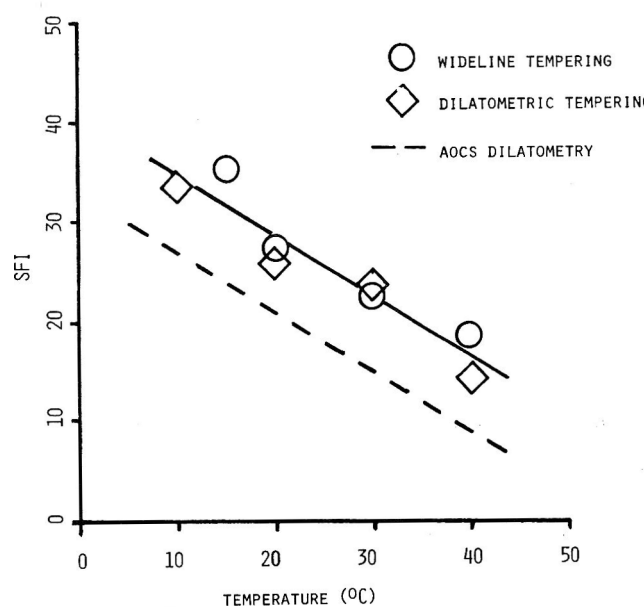

Fig. 2—Comparison of tempering methods

results, and to those of dilatometry. The results in Figure 3 can be explained if molecular exchange were a contributing factor. The standard plot method makes corrections for this if a plot is constructed for each testing temperature.

Other potential sourcs of variance are the type of reference oil used in the calculation technique, sample probe temperature control, and sample composition. Haighton et al. (1971) found that the choice of reference oil did not have much effect on wideline SFI measurements. Safflower oil and olive oil gave the most divergent results. Van Putte and Van den Enden (1974) found that pulsed NMR probe temperature control was necessary only for the most accurate results. Wideline NMR results with compositions having low SFI are more accurate than those with high SFI (Taylor et al., 1964). A two-level full factorial screening experiment (Table 2) was designed to quantify the relative significance of these effects. We also chose to investigage further tempering effects by including an excessive tempering sample conditioning scheme. The fat samples were 30% and 70% SFI safflower oil-tallow mixtures. Data taken for five replications of the eight samples at each solids level at the 30°C testing temperature.

An analysis of variance for the difference between the calculated and known SFI responses is shown in Table 3. The data indicate that solids level (and therefore sample composition) is the most significant factor in the difference between known and measured values. The average difference of the 30 SFI sample was +2.36, while the 70 SFI fat gave +5.23. One concludes that like wideline NMR, the pulsed NMR method is more accurate at lower solids levels. This suggests that saturated fats form mixed crystals (solid solutions) more easily than unsaturated fats. This behavior could also account for the difference seen between the standard plot method and dilatometry (Fig. 1 and 2).

Excessive tempering showed some effect, but it was not significant at the 95% confidence interval. The choice of reference is not important. The AOCS has chosen olive oil for wideline NMR. We see no reason to deviate from this for a pulsed method. Our work confirms the observation that temperature control of the probe is unimportant for routine work.

The limiting precision of these methods is determined by the confidence one can place in the extrapolation of the FID signal. Evaluating all samples as a group, we found this limit to be ± 0.15% solids.

The standard plot method was found to be more precise

Table 2—Variables for full factorial screening experiment

Factor	Variable
Solids level	30%, 70%
Tempering	One step, five steps
Reference oil	Olive oil, safflower oil
Probe temperature	Controlled, uncontrolled

Table 3—Analysis of variance SFI by calculation method

Factor	Percentage of total variance
Solids level	70%
Tempering	21%
Reference oil	8%
Probe temperature	1%

Table 4—SFI of commercial fat samples

Testing temp (°C)	Solids in fat index					
	Standard plot		Calculation		Dilatometry	
	Fat A	Fat B	Fat A	Fat B	Fat A	Fat B
14.5°C	41	34	34	23	34.0	23.5
20°C	34	31	27	18	27.0	20.6
30°C	22	25	8	9	16.0	15.4
40°C	20	23	2	2	5.0	10.2

Table 5—Precision of SFI methods

Method	Standard deviation of method (%)
Dilatometry	0.2
Wideline NMR	1–2
Pulsed NMR calculation	1.8
Pulsed NMR standard plot	1.0

Table 6—Accuracy of proposed SFI methods on commercial fat samples

Method	Slope[a]		Intercept[b]	
	Fat A	Fat B	Fat A	Fat B
Standard Plot	0.70	0.83	+15.4	+13.9
Calculation	1.09	1.54	−3.8	−13.7

[a] Reference is AOCS
[b] % solid content difference

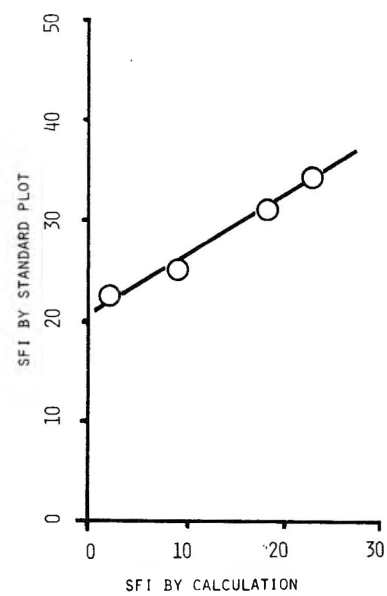

Fig. 3—Comparison of standard plot and calculation methods for SFI determination of a commercial fat by pulsed NMR

Table 7—Effect of temperature on pulse NMR accuracy

Testing temp (°C)[a]	Slope[b]	Intercept[c]
14.5°C	0.960	−6.4
20°C	0.975	−7.0
30°C	0.965	−7.0
40°C	0.995	−13.1

[a] Wideline conditioning
[b] Reference AOCS dilatometry
[c] % Solid fat not measured

than the calculation method when tested on commercial samples (Tables 4 and 5). The precision of the standard plot and calculation methods fall within the range reported for wideline NMR. Both the wideline and pulsed NMR techniques are less precise than dilatometry.

The accuracy of the pulsed NMR calculations compared with dilatometry is determined from a bias plot. The slope of this line would be unity and there would be a zero intercept if both methods were of equal accuracy, and were measuring the same phenomena. The data reported in Table 6 indicates a composition dependence for the slope in both methods. The intercept for the standard plot method is a constant high value within experimental error. The indirect calculation method gives low values, and they appear to vary depending on composition.

In Table 7, bias plots are made at each testng temperature for the entire set of sample data. This information indicates that a temperature dependent phenomenon is responsible for the changes in slope. The low intercept can be explained by molecular exchange between solid- and liquid-like environments. The change in intercept observed between 30 and 40°C has been indicated in other data (Van Putte and Van den Enden, 1974). Molecular exchange would be expected to increase at the melting point. Molecular exchange is composition dependent. The explanation given for Table 6 is therefore consistent with this explanation.

CONCLUSION

AS ANTICIPATED, pulsed and wideline NMR give similar results. The pulsed NMR method has the advantage of speed, ease of automation, and lower instrument cost. Our work has shown that the standard plot technique can be used to measure SFI in food fats with a precision of ±2% at the 95% confidence level. This could be improved to a limit of ±0.15% by replication. The SFI measurement by this technique can be related to dilatometric measurements through regression equations. Our work suggests tha for highest accuracy, standards should be chosen with composition similar to the unknown.

Since tempering does not influence the results within experimental error, we suggest the use of the wideline sample conditioning scheme which emphasizes speed. If samples are rapidly transferred in making measurements, probe temperature control is not necessary. Large 25 mm sample tubes can be used to improve signal to noise ratio. It is important that the sample tube exterior is free of moisture when measurements are taken.

Our work also indicates that molecular exchange has an important influence on NMR measurements in fats and oils.

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DIETARY FIBER: THE EFFECT OF PARTICLE SIZE AND pH ON ITS MEASUREMENT

ABSTRACT

The effect of particle size, acid and alkali on the composition of processed and unprocessed wheat bran, purified corn pericarp, and peanut hulls was studied. Van Soest's method for fiber analyses was used to measure hemicellulose, cellulose, lignin, and cutin. Hemicellulose values for wheat samples ground through a 60-mesh screen of Wiley Mill were 20% lower than samples ground through a 20-mesh screen. Shaking red wheat bran for 24 hr at 25°C solubilized 17% of the hemicellulose at pH 11.5 and 9% at pH 2.2. Refluxing for 60 min reduced the hemicellulose content by 62% at pH 11.5 and by 52% at pH 2.2. Similar losses were observed for purified corn pericarp while little change was found for peanut hulls. Variation in sample particle size and exposing plant fiber to acid and alkali may change fiber composition as measured by the Van Soest procedure.

INTRODUCTION

THE HYPOTHESIS that certain disease states may be related to the lack of plant fiber in the diet has attracted the attention of both nutritionists and food scientists. Many researchers are striving to elucidate the physiological role of plant fiber in man's diet. Others are developing methods of increasing the fiber content of foods without lowering product quality.

Both groups of scientists need quick methods of measuring and classifying various plant fibers. Procedures based on chemical extractions have been suggested by Goering and Van Soest (1970), Morrison (1972) and Edwards (1973). Methods using enzymatic and chemical digestions have been developed by Weinstock and Benham (1951), Fraser et al. (1956), Van Soest and Wine (1967), Southgate (1969), and Hellendoorn et al. (1975). The Van Soest procedure was found to be relevant to the needs of nutritionists and the fastest of four methods of fiber analysis compared by McConnell and Eastwood (1974). Spiller and Amen (1975) reported that they look for Van Soest's measurement of total cell wall, that is the neutral detergent fiber (NDF), to replace the term crude fiber in food composition tables and possibly on food and feed labels.

If nutritionists and food scientists will be using the Van Soest procedure for measuring plant fiber, it is important that we understand factors affecting fiber measurement. The purpose of this research is to show the effect of particle size, acid, and alkali on plant fiber composition as determined by the Van Soest procedure.

MATERIALS & METHODS

Plant fiber sources

Processed and unprocessed red wheat brans were obtained from General Mills. The processed red wheat bran had received a short time, high pressure, high temperature treatment to inactivate enzymes and microorganisms. Purified corn pericarp was obtained from Corn Products Corporation and peanut hulls were received from Gold Kist Inc. The peanut hulls were ground through a hammer mill and then through an attrition mill using a tolerance of 8×10^{-3} inches. All samples were stored at −10°C.

Particle size reduction

Replicate grindings through either a 20- or 60-mesh screen of a Wiley Mill were made using 75-g samples of each of the plant fiber sources. Samples of the stock material were also taken in replicate for comparative analyses. The purified corn pericarp was not ground through the 20-mesh screen since it had been previously commercially gound to pass through a 30-mesh screen.

The treatment and error mean squares were determined by analysis of variance by completely random design for each fiber source. When the computed F value was greater than the tabular F for 95% level of significance, treatment means were compared by the lsd test. Means which differed significantly at the 95% level were given different superscripts as shown in Tables 1–4.

Treatment with acid and alkali

Red wheat bran, purified corn pericarp, and peanut hulls were treated by refluxing for 5 or 60 min, or by shaking at 25°C for 24 hr 6-g samples in 300 ml of each of the following solutions: sodium phosphate-citric acid buffers at pH 2.2, 3.2, 4.8, 8.0 and 0.5M sodium carbonate of pH 11.5. A control of deionized, distilled water was also used. After treatment the solutions were filtered through tall form, coarse porosity sintered glass crucibles of 40μ pore diameter and washed three times with hot water. The filter cake was quantitatively recovered and freezed dried. Recovery factors were computed from the dried weights of the initial and treated samples.

Plant fiber analyses

Plant fiber was analyzed using the Van Soest procedure as described by Goering and Van Soest (1970). This method uses sodium lauryl sulfate in a neutral solution to isolate the cell wall material. The cell wall or neutral detergent fiber comprises the hemicellulose, cellulose, lignin, and cutin components of fiber. When treated with cetyl trimethylammonium bromide in an acid medium the cellulose, lignin, and cutin or acid detergent fiber (ADF) is recovered. Hemicellulose is calculated as the difference between NDF and ADF. The addition of a potassium permanganate solution to the acid detergent residue removes the lignin fraction. Cellulose is removed by applying 72% sulfuric acid to the ADF minus lignin leaving cutin as the remaining substance. When analyzing the samples treated with acid and alkali, the fiber fraction measured was expressed as a percent of the original dry matter using the appropriate recovery factor.

Particle size analyses

Approximately 35g of sample were placed on the largest sieve of the following nested series of 8 in. × 2 in. stainless steel U.S. Standard Sieves: 10, 18, 35, 60, 120 and 230 fitted with a pan and cover. The following procedure was used with a Fisher-Wheeler Sieve Shaker at a setting of 900: place known weight of sample on tared sieves and shake nested sieves and samples for 15 min, disassemble and carefully brush down sieve walls and bottoms, reassemble and shake 10 min, weigh sieves and retained samples, shake for additional 2-min intervals and weigh until $\leq 0.5\%$ of total sample weight passes through any one sieve.

Using probability graph paper, the cumulative percent undersize was plotted on the normal probability axis and the log of the corresponding sieve aperture plotted on the log axis (Lapple, 1968). The aperture at which 50% of the sample passed, the mean log particle size (MLPS), was recorded from the graph.

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RESULTS & DISCUSSION

VALUES for the NDF and hemicellulose content of red wheat bran ground through a 60-mesh screen were significantly lower by 9% and 12% than the samples ground through a 20-mesh screen as shown in Table 1. A significant reduction of 14% and 20% in NDF and hemicellulose values was also found for the finely ground processed wheat samples (Table 2). A 2% decrease in NDF occurred with peanut hulls as reported in Table 3. Particle size had no effect for any of the acid detergent values or for the NDF or hemicellulose values for purified corn pericarp (Table 4).

Since the recommended sample preparation (Goering and Van Soest, 1970) for fiber analyses is grinding to pass a 20–30-mesh screen, samples ground more finely will yield lower values for total cell wall. Differences of up to 20% may occur when measuring the hemicellulose values on finely ground wheat products.

Reduced values for neutral detergent fiber in finely ground wheat were reported by Butcher (1975a). He attributed the decrease to the release of cellular contents from the protein rich aleurone cells which were damaged with severe grinding. Butcher's results are confounded since he used filtering crucibles of maximum pore diameter of 100–120μ (Butcher, 1975b) whereas the crucibles recommended by Van Soest have 40μ pores. Van Soest (1963) has similarly reported an increased nitrogen loss in samples prepared by grinding in a Waring Blendor.

Our results show that the ADF value is not dependent on particle size. Since hemicellulose is calculated as the difference between NDF and ADF, values for hemicellulose will vary by the same amount as NDF.

Lignin values for peanut hulls ground through a 60-mesh screen increased significantly by 20% over samples ground through the 20-mesh screen as shown in Table 3. This increase was attributed to greater oxidation of lignin due to decreased particle diameter. Conversely, the values for the cutin content of the finely ground samples decreased significantly by 25%. This decrease was expected as unoxidized lignin would be included in the cutin fraction.

Particle size had no effect on the values for the cellulose content of peanut hulls, wheat bran, processed red wheat bran or corn pericarp. Similarly no significant differences were found between the 20- and 60-mesh grinds for the lignin and cutin values of red wheat bran or processed red wheat bran. Grinding of the corn pericarp had no effect on either the lignin or cutin values.

The presence of acid or base, and heat resulted in the solu-

Table 1—Fiber composition of red wheat bran[a]

Grind	Neutral detergent fiber	Acid detergent fiber	Hemicellulose	Cellulose	Lignin	Cutin
	% dry weight					
As received 3 Replications MLPS = 1200μ	56.0[a]	15.1[a]	41.0[a]	10.1[a]	5.0[a]	.34[a]
20 Mesh screen 2 Replications MLPS = 320μ	51.7[b]	15.0[a]	36.6[b]	10.6[b]	4.6[b]	.20[a]
60 Mesh screen 3 Replications MLPS = 160μ	47.3[c]	15.1[a]	32.2[c]	10.7[b]	4.7[b]	.20[a]

[a] Values sharing a common superscript letter within a column are not significantly different ($P < 0.05$).

Table 2—Fiber composition of processed red wheat bran[a]

Grind	Neutral detergent fiber	Acid detergent fiber	Hemicellulose	Cellulose	Lignin	Cutin
	% dry weight					
As received 3 Replications MLPS = 600μ	49.9[a]	13.3[a]	36.6[a]	9.2[a]	4.4[a]	.27[a]
20 Mesh screen 2 Replications MLPS = 300μ	47.1[b]	12.9[a]	34.2[b]	9.4[a]	4.2[a]	.19[a]
60 Mesh screen 3 Replications MLPS = 220μ	40.3[c]	13.1[a]	27.2[c]	9.4[a]	4.0[a]	.19[a]

[a] Values sharing a common superscript letter within a column are not significantly different ($P < 0.05$).

Table 3—Fiber composition of peanut hulls[a]

Grind	Neutral detergent fiber	Acid detergent fiber	Hemicellulose	Cellulose	Lignin	Cutin
	% dry weight					
Attrition mill 4 Replications MLPS = 500μ	81.2[a]	71.7[a]	9.5[a]	42.4[a]	12.8[a]	16.7[a]
20 Mesh screen 3 Replications MLPS = 250μ	81.3[a]	70.3[a]	11.0[a]	41.8[a]	14.2[a]	14.6[b]
60 Mesh screen 4 Replications MLPS = 140μ	79.4[b]	69.4[a]	10.0[a]	41.5[a]	17.0[b]	11.0[c]

[a] Values sharing a common superscript letter within a column are not significantly different ($P < 0.05$).

Table 4—Fiber composition of purified corn pericarp[a]

Grind	Neutral detergent fiber	Acid detergent fiber	Hemicellulose	Cellulose	Lignin	Cutin
	% dry weight					
As received 3 Replications MLPS = 250μ	91.5[a]	21.6[a]	69.9[a]	21.3[a]	0.79[a]	.01[a]
60 Mesh screen 3 Replications MLPS = 160μ	89.3[a]	21.4[a]	67.8[a]	21.1[a]	0.85[a]	.03[a]

[a] Values sharing a common superscript letter within a column are not significantly different ($P < 0.05$).

Table 5—Effect of pH on the hemicellulose, cellulose, lignin and cutin content of red wheat bran treated by shaking or refluxing

Fiber fraction	Treatment: Deionized water	pH 2.2	3.2	4.8	6.6	8.0	11.5
	% of dry matter remaining after treatment						
	Shaken 24 hr at 25°C						
Hemicellulose	38.4	35.0	35.4	38.0	38.7	38.6	32.0
Cellulose	10.6	10.2	10.6	10.7	10.7	11.0	11.1
Lignin	4.6	3.9	4.0	4.3	4.4	4.5	4.8
Cutin	0.3	0.5	0.7	0.5	0.4	0.7	0.2
	Refluxed for 5 min						
Hemicellulose	34.7	27.7	34.6	33.1	36.0	35.2	22.3
Cellulose	10.4	10.1	10.6	10.4	10.8	10.9	11.0
Lignin	4.3	4.1	4.2	4.3	4.8	4.6	4.4
Cutin	0.4	0.4	0.4	0.7	0.4	0.1	0
	Refluxed for 60 min						
Hemicellulose	36.4	17.5	22.0	37.1	–	34.0	14.0
Cellulose	10.5	10.1	10.8	10.7	–	10.7	10.3
Lignin	4.9	4.1	4.7	4.5	–	4.8	3.5
Cutin	0.2	0.3	0.2	0.3	–	0.2	0

Table 6—Effect of pH on the hemicellulose, cellulose, lignin and cutin content of purified corn pericarp treated by shaking or refluxing

Fiber fraction	Treatment: Deionized water	pH 2.2	3.2	4.8	6.6	8.0	11.5
	% of dry matter remaining after treatment						
	Shaken for 24 hr at 25°C						
Hemicellulose	69.0	68.5	69.7	69.2	69.0	67.7	60.5
Cellulose	20.5	20.9	21.0	20.9	20.7	20.4	21.0
Lignin	0.7	0.9	0.8	1.3	0.3	0.3	1.1
Cutin	0.3	0	0	0.1	0	0	0
	Refluxed for 5 min						
Hemicellulose	69.6	66.8	69.6	70.4	69.7	69.2	29.7
Cellulose	21.0	21.0	21.0	21.0	21.0	20.9	19.4
Lignin	0.8	1.0	1.0	1.0	1.0	0.5	1.0
Cutin	0	0	0	0	0	0	0
	Refluxed for 60 min						
Hemicellulose	69.8	46.5	65.3	70.2	66.4	65.7	15.4
Cellulose	20.8	20.8	21.0	21.0	20.0	20.6	19.2
Lignin	1.1	0.8	0.4	0.8	0.9	0.8	0.6
Cutin	0.1	0.1	0	0	0	0	0

bilization of the hemicellulose fraction of plant fiber. Since hemicellulose is a part of the cell wall material, the value for NDF reflected the hemicellulose losses. Acid or base had little effect on other fiber components.

After shaking red wheat bran for 24 hr at 25°C the hemicellulose content decreased 17% from its deionized distilled water control at pH 11.5 and 9% at pH 2.2 as shown in Table 5. Refluxing for 5 min reduced the hemicellulose content by 35% under basic conditions and 20% under acidic conditions. A sixty minute reflux resulted in a 62% loss of hemicellulose at pH 11.5 and a 52% loss at pH 2.2.

Purified corn pericarp (Table 6) showed similar trends when treated with acid and alkali. When the fiber was shaken for 24 hr at 25°C a 12% loss in hemicellulose was found at pH 11.5 Refluxing for 5 min increased the loss in alkali (pH 11.5) to 57% while a 4% loss was seen at pH 2.2. The greatest loss occurred after refluxing for 60 min when a 78% decrease in hemicellulose occurred at pH 11.5 and a 33% loss was found under acidic conditions.

The fiber composition of peanut hulls was unaffected by pH when shaken for 24 hr at 25°C (Table 7). Little change in composition was observed when the fiber was refluxed for 5 min. Refluxing for 60 min resulted in a 12% decrease in cellulose at pH 11.5. The NDF and ADF also decreased slightly under alkaline conditions.

These results agree with Van Soest and Wine's (1967) report of the decline of hemicellulose in a basic solution containing a detergent, and a decrease in ADF recovery with an increase in sulfuric acid concentration (Van Soest, 1963). The results do not agree with Van Soest and Wine's (1963) finding of increased lignin solubility under alkaline conditions.

The results show that cell wall material may be solubilized under acidic and basic conditions. This solubility is increased in the presence of heat. Researchers working with plant fibers should be aware that exposing plant fiber to acid and alkali may alter the values determined for hemicellulose and cellulose.

The determination of neutral detergent fiber, hemicellulose and lignin as measured by the Van Soest procedure may be influenced by the particle size of the sample. When analyzing plant fiber, attempts should be made to use samples of similar physical nature.

Table 7—Effect of pH on the hemicellulose, cellulose, lignin and cutin content of peanut hulls treated by shaking or refluxing

Fiber fraction	Treatment: Deionized water	pH 2.2	3.2	4.8	6.6	8.0	11.5
	% of dry matter remaining after treatment						
	Shaken 24 hr at 25°C						
Hemicellulose	9.9	12.2	10.9	15.6	12.2	14.8	14.1
Cellulose	41.0	39.8	40.0	39.8	40.1	40.1	38.7
Lignin	13.6	13.1	13.4	13.0	13.3	13.8	15.5
Cutin	15.7	14.8	14.8	15.1	15.3	15.1	14.4
	Refluxed for 5 min						
Hemicellulose	14.3	11.4	12.0	12.6	14.9	15.7	14.6
Cellulose	39.0	40.7	40.1	40.5	38.9	37.8	38.4
Lignin	13.3	12.4	12.7	12.6	13.0	13.0	13.0
Cutin	13.8	15.5	15.1	14.7	14.4	13.7	14.6
	Refluxed for 60 min						
Hemicellulose	12.0	10.7	11.3	12.5	14.4	14.8	12.9
Cellulose	40.3	40.3	40.7	42.2	39.2	38.7	35.5
Lignin	13.3	13.3	13.2	11.0	13.8	13.9	13.5
Cutin	15.4	15.1	15.3	16.0	14.1	14.2	13.0

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DIETARY HEMICELLULOSE INTERACTIONS INFLUENCING SERUM LIPID PATTERNS AND PROTEIN NUTRITIONAL STATUS OF ADULT MEN

ABSTRACT

The objective of the present project was to determine the effect of graded levels of hemicellulose added to a constant, plant-based diet on serum lipid patterns and protein nutritional status of adult men. The 50-day study was divided into a 2-day depletion period, a 3-day pre-adjustment period, three 14-day experimental periods (randomly arranged) and a 3-day post-adjustment period. During all adjustment and experimental periods, ground peanuts provided 6.0g N/subject/day as the near sole source of dietary protein. In addition to the oil supplied by the ground peanuts, six of the twelve subjects received butter oil while the other six received corn oil on a daily basis. During the three experimental periods, supplements of 4.2, 14.2 and 24.2g of hemicellulose were added daily to the diet. All subjects received all experimental treatments. Graded increases in hemicellulose had no demonstrative effect on nitrogen balances of subjects in strong apparent positive nitrogen balance; however, those individuals in marginal or negative nitrogen balance tended to show poorer nitrogen balances as level of dietary hemicellulose was increased. Mean blood serum cholesterol levels of subjects were slightly lowered as level of dietary hemicellulose was increased regardless of source of dietary fat. While mean serum triglyceride levels of subjects receiving the corn oil and peanut oil combination were quite constant with increases in dietary hemicellulose, the triglyceride levels of all subjects receiving the butter oil and peanut oil combination were increased as levels of dietary hemicellulose were increased.

INTRODUCTION

THE ROLE OF FIBER in nutrition and in maintenance of human health is a topic of current research interest. Authors have suggested possible roles of dietary fiber in the prevention of colon cancer (Burkitt, 1971; Burkitt et al., 1972), diverticular disease (Painter et al., 1972) and coronary heart disease (DeGroot et al., 1963; Mathur et al., 1968; Trowell, 1972). Many of these conclusions are based on correlations and do not constitute proof of cause and effect. Dissemination of information in the lay press has developed an appeal for development of food products with a higher fiber content. Fiber is the term covering several different food components which vary in their chemical/physical characteristics and could well cause different physiological responses when consumed by humans. Furthermore, any recommendations for major change in diets should be carefully studied in order to determine the total as well as isolated effects. Relatively little information is available on purified fibers fed in controlled human metabolism studies.

The purpose of the present study was to investigate the effect of graded additions of hemicellulose to a plant-based diet on serum lipid patterns and protein nutritional status of adult men.

EXPERIMENTAL

THE EXPERIMENTAL PLAN for the study is shown in Table 1. The 50-day study was divided into a 2-day depletion period, a 3-day pre-adjustment period, three experimental periods of 14 days each and a 3-day post-adjustment period. Order of the three experimental periods was randomly arranged for each of the 12 subjects who participated in the study to minimize the effects of time and order of diet presentation.

Initial feeding of a very low level of nitrogen (protein) to research subjects has been found advantageous in our laboratory for speeding adjustment of subjects to the relatively low levels of protein fed in nitrogen balance type studies. During the depletion period, subjects received the basal diet shown in Table 2 from which the peanut butter component was omitted. With this omission, the diet provided 0.8g N per subject per day. Other purposes of this period were to introduce subjects to their duties and responsibilities and to begin to establish that level of caloric intake necessary for each individual to maintain body weight.

During adjustment periods and all experimental periods subjects received a standardized diet shown in Table 2 which provided 6.8g nitrogen (primarily from ground peanuts), 119.8g of fat (59.8g of fat from ground peanuts and 60g from corn oil for subjects 1081, 1082, 1083, 1086, 1088 or from butter oil for subjects 1092, 1094, 1095, 1096, 1097, 1099) and 6.8g of fiber. Hemicellulose was added to the diet to provide 4.2g during depletion, adjustment and one experimental period, 14.2g during a second experimental period and 24.2g during a third period. All subjects received all experimental variables in relationship to hemicellulose intake. Total fiber intake was varied from 10.2 to 20.2 to 30.2g per day during the three randomly arranged experimental periods.

Diets were supplemented with vitamins and minerals so that in addition to those nutrients supplied by the ordinary foods in the diet adequate intake of essential nutrients was assured (Table 2). By varying the amount of starch bread, sucrose, hard candy, jelly and soft drinks among individuals, total caloric intake of subjects was adjusted to that level necessary for weight maintenance. Subjects were weighed daily to ascertain caloric adequacy of the diet. After this level of energy intake was established for each individual, caloric intake was maintained constant for the remainder of the study.

The hemicellulose supplements were added to the ground peanuts and approximately one-third of each day's allotment was served at each of the three meals. Starch bread was prepared using either butter oil or corn oil. The fruits and vegetables used as part of the basal diet were purchased from the same lots at the beginning of the study.

The 12 adult men who were volunteers for this study are described in Table 3. All were inmates of the Nebraska Penal and Correctional Complex for Men–Reformatory Unit who were housed within the facilities of the institution and continued usual institutional assignments of work or study except for consumption of special meals and for collections of excreta. The study was approved for inmate participation by the Human Rights Committee of the University of Nebraska and by the institutions's medical and administrative staff. All subjects were volunteers who were free to withdraw from the project at any time. Clearance for participation on a health basis was obtained on the basis of physical examinations conducted by the institution's medical officer.

Complete collections of urine and feces were made by subjects throughout the 50-day study. Urine collections were divided into 24-hr lots on a time basis for analyses on a daily basis. Creatinine content of urine by the method of Folin (1914) was used to ascertain both the completeness of each 24-hr collection and the accuracy at which divisions were made. Fecal collections were made during the experimental periods on the basis of 7-day composites using orally given carmine dye to mark the division between each lot. Since each period was 14 days long, two fecal composites were made for each subject for each period. Fasting blood samples were drawn from subjects at the beginning of the study and at the end of each experimental period. Food composites were made for analysis twice during the course of the study.

Urine, feces and stools were analyzed for nitrogen basically by the Scales and Harrison (1920) modification of the Kjeldahl method. Blood plasma samples were analyzed for triglyceride (Dade TRI-25, Hantzsch condensation colorometric method) and cholesterol (iron-salt acid, feric chloride method). In addition, blood samples were analyzed for a wide

Table 1—Experimental plan

Period	No. of days	Nitrogen intake (g/day)		Hemicellulose supplement (g/day)	Fat intake (g/day)		
		Peanut	Total[a]		Peanut	Butter oil or corn oil[b]	Total
Depletion	2	0	0.8	4.2	59.8	60	119.8
Pre-adjustment	3	6.0	6.8	4.2	59.8	60	119.8
Expt. 1	14	6.0	6.8	4.2	59.8	60	119.8
Expt. 2	14	6.0	6.8	14.2	59.8	60	119.8
Expt. 3	14	6.0	6.8	24.2	59.8	60	119.8
Post-adjustment	3	6.0	6.8	4.2	59.8	60	119.8

[a] The basal diet shown in Table 2 provided 0.8g nitrogen (excluding ground peanuts) and 6.0g crude fiber per subject per day. Hemicellulose material consisted of a mixture of pentosans, hexosans and galactans from psyllium.
[b] The subjects were divided into two groups of six subjects each. One group received corn oil and the other group received butter oil for the entire feeding period.

spectra of chemical components as a general check on maintenance of physiological condition by autoanalyzer techniques by the Lincoln Pathological Laboratory, Lincoln, Neb.

Statistical analysis on data collected included analysis of variance and Duncan's Multiple Range Test.

RESULTS & DISCUSSION

NITROGEN BALANCES of subjects while receiving the three levels of hemicellulose are shown in Table 4. Mean balances for all subjects while receiving the 4.2, 14.2 and 24.2g of added hemicellulose per day for the first seven days of each period were +0.62, 0 and +0.11 and for the second seven days of each period were +0.56, +0.04 and −0.10g nitrogen per day, respectively. Regardless of whether values for the first seven days or the last seven days of each period were used, subjects retained significantly ($P < 0.05$) less nitrogen while receiving the two higher levels of hemicellulose supplementation than while receiving the 4.2g addition. No significant difference was determined between nitrogen balances of subjects receiving the 14.2g and 24.2g supplement.

Subjects who were in strongest positive nitrogen balance showed the least change in nitrogen balance as a result of dietary hemicellulose addition. Since these individuals were most likely meeting or exceeding all their protein needs at this level of protein intake, it is not surprising that a decrease in protein availability would have little affect on the apparent protein nutriture of the subject as illustrated by nitrogen balance. In a previous study from this laboratory (Kies and Fox, 1974) graded levels of hemicellulose to a low protein wheat diet produced more negative nitrogen balances as levels of hemicellulose were increased. The results of this study are surprising in that it is usually difficult to affect nitrogen balances of subjects when working in the positive range. It is for this reason that most studies of this type are conducted at levels of protein designed to produce a slightly negative nitrogen balance.

Urinary nitrogen and fecal nitrogen excretion values are used in the calculation of nitrogen balance. An examination of these values independently of nitrogen balance is sometimes of value. As shown in Tables 5 and 6, as supplementation levels of hemicellulose were increased, both urinary nitrogen and fecal nitrogen values also increased; however, these differences were not statistically significant at all intake levels. Generally, changes in fecal nitrogen excretion are more difficult to demonstrate than are changes in urinary nitrogen excretion due to inherent errors in fecal collections, division, sampling and analyses. In this situation the increased fecal nitrogen excretion suggests but does not prove that the poorer nitrogen balances achieved with higher hemicellulose intake were due primarily to interference in protein absorption.

Several studies have demonstrated a blood cholesterol-lowering effect of selected dietary fibers or fiber-containing materials in several animal species. For example, inclusion of barley, whole wheat or rolled oats resulted in a lowering of blood cholesterol levels of rats fed cholesterol-containing diets (DeGroot et al., 1963). Pectin has been shown to lower blood cholesterol levels of rats fed cholesterol-containing rations (Leveille and Sauberlich, 1966.). Cellulose additions to rat rations also have been demonstrated to have a cholesterol-lowering effect (Sundaravalli et al., 1971). In several other studies, addition of bran to diets have failed to produce a cholesterol-lowering effect (Eastwood, 1969; Heaton and Pomare, 1974; Jenkins et al., 1975; Elias et al., 1975; Connell et al., 1975; Durrington et al., 1975; Truswell and Kay, 1975; Malinow et al., 1976). Failure to produce a response may be associated with amount of bran, with length of time or with

Table 2—Experimental diet

Item	Amount/day
Ground peanuts	130
Starch bread	Varied[a]
Applesauce	100g
Peaches	100g
Pears	100g
Green beans	100g
Tomato juice	100g
Vitamin supplement[b]	
Mineral supplement[b]	
Soft drinks	Varied[a]
Hard candy	Varied[a]
Jelly	Varied[a]
Nonprotein bouillon	

[a] Fed in amounts to meet caloric needs for weight maintenance of each individual. Although varied between subjects, amounts were constant for each individual. Less than 10% of the calories were from mono or disaccharides, 40–45% of calories from starch and 35–40% of calories from fat.
[b] The mineral supplement (part capsule form, part mixed in starch bread) supplied the following (g per subject per day): Ca, 1.00; P, 1.00; Mg, 0.199; Fe, 0.015; Cu, 0.002; K, 0.323; I, 0.00015; Mn, 0.002; and Zn, 0.0009. Ordinary foods supplied approximately 0.121 mg of additional Mg and approximately 0.850 mg of additional K. NaCl was allowed ad libitum. The vitamin supplement supplied the following (per subject per day, capsule form): 5000 USP units of vitamin A acetate; 600 USP units of vitamin D (ergocalciferol); 2 mg thiamine; 2.5 mg riboflavin; 20 mg niacinamide; 50 mg ascorbic acid, 1 mg pyridoxine; 1 μg cyanocobalamin; 1 mg calcium pantothenate.

Table 3—Description of subjects

Blood Chemistry	Subject Numbers											
	1081	1082	1083	1086	1088	1089	1092	1094	1095	1096	1097	1099
Race	White	White	White	Black	Black	White	White	White	White	White	White	White
Weight, kg	88.4	92.5	85.2	82.5	79.3	83.9	68.0	69.3	68.0	75.7	63.4	83.9
Age, yr	20	19	22	24	23	25	20	18	23	23	23	24
Height, cm	180.3	192.8	177.8	190.5	185.4	185.4	180.3	180.3	179.0	185.4	185.4	177.8
Calcium, mg%	10.2	09.9	09.3	10.0	10.0	9.8	10.1	9.4	10.5	10.3	09.9	09.9
Phosphorus, mg%	03.6	04.7	03.5	04.3	04.3	03.9	04.4	03.5	03.9	03.7	03.2	03.9
Albumin, g%	5.25	5.32	5.49	5.07	4.83	5.49	5.28	4.87	5.45	5.06	5.05	5.52
Bilirubin, mg%	00.3	00.4	00.5	00.3	00.3	00.9	00.6	00.7	01.3	00.7	00.6	00.2
Alk. Phos., m U/ml	118	066	080	108	100	062	060	092	107	125	053	052
LDH, m U/ml	141	124	167	134	182	160	158	161	160	222	121	124
SGOT, m U/ml	022	017	027	022	036	026	054	025	019	196	029	016
Triglycerides, mg%	143	93	106	122	102	84	112	101	62	135	126	84
Glucose, mg%	091	086	092	085	085	092	092	090	080	093	089	081
BUN, mg%	010	011	014	017	013	020	014	013	020	013	009	015
Uric acid, mg%	06.7	05.6	04.7	05.7	05.6	05.6	05.3	06.7	05.5	05.9	05.3	04.3
Cholesterol, mg%	182	153	136	125	271	127	191	153	200	205	169	157
Total protein, g%	07.5	07.6	07.0	07.5	07.6	07.2	07.4	06.9	08.3	08.1	07.3	07.5
Hematocrit	46	50.3	46.5	46.0	47.0	44.5	47.0	45.0	50.7	49.0	49.6	48
Hemoglobin, mg%	15.5	16.4	16.3	15.0	16.0	15.1	15.9	15.7	16.6	16.9	16.4	16.5

Table 4—Nitrogen balances of subjects fed graded levels of hemicellulose at a constant protein intake (6.8g N/day)

Subject no.	N balances (g N/day) of subjects fed hemicellulose at 4.2g/day		14.2g/day		24.2g/day	
	A[a]	B[a]	A	B	A	B
1081	+0.20	+0.01	−0.01	−0.03	−0.32	−0.09
1082	+0.75	+0.73	+0.58	—	+0.66	+0.62
1083	+0.94	+0.97	+0.61	+0.58	+0.85	+0.83
1086	−0.52	−0.36	−1.03	−0.97	−1.21	−1.11
1088	+0.99	+0.92	+0.65	+0.74	+0.77	+0.79
1089	—	−0.20	−1.25	−1.01	−1.32	−1.25
1092	+1.64	+1.65	+0.95	+1.03	+3.82	+1.36
1094	+0.81	+0.84	+0.39	+0.47	+0.22	+0.37
1095	+0.20	+0.27	−0.86	−0.51	−0.70	−0.97
1096	+0.80	+0.88	+0.50	+0.65	+0.81	+0.44
1097	−0.01	+0.08	−1.31	−1.23	−1.34	−1.42
1099	+1.02	+0.93	+0.77	+0.68	−0.94	−1.01
Mean[b]	+0.62a	+0.56a	0b	+0.04b	+0.11b	−0.10b

[a] Part A values are the means of the first 7 days of each period and Part B values are the last 7 days of each 14-day period.
[b] Duncan's multiple range test. Means without a letter in common differ significantly ($P < 0.05$).

Table 5—Urinary nitrogen excretion of subjects fed graded levels of hemicellulose at a constant protein intake (6.8g N/day)

Level of hemicellulose g/day	Mean urinary nitrogen excretion g/day	
	Part A[a]	Part B[a]
4.2	4.86a (2.70–7.78)	4.99a (2.20–8.27)
14.2	5.15b (2.83–8.17)	5.13b (2.75–7.93)
24.2	4.90ab (3.00–6.30)	5.38b (3.05–7.06)

[a] Part A values are the means for 12 subjects for the first 7 days of each period and Part B values are those for the last 7 days. Duncan's multiple range test. Means without a letter in common differ significantly ($P < 0.05$).

other variables. It does not necessarily mean that there is no response under all conditions.

In the present study, mean blood serum cholesterol levels were lowered as dietary hemicellulose was increased (Table 7). The degree of change was approximately the same among subjects receiving corn oil and those receiving butter oil. These results are somewhat suprising since the study involved feeding of the various levels of hemicellulose for short time periods. As previously discussed, peanut oil from the ground peanuts provided approximately half of the fat calories for both groups. Peanut oil is not a good source of polyunsaturated fatty acids and, in previous studies from this laboratory, tends to produce blood serum lipid patterns in human subjects more similar to those typical of animal than plant fats. In studies with rabbits, hydrogenated peanut oil resulted in increased atherosclerosis when added to semi-synthetic diets but not when added to chow diets containing fiber (Kritchevsky et al., 1968). The lowering of blood cholesterol levels even with no fiber additon in comparison to "normal" levels in the butter oil group is surprising since the butter oil plus peanut would be expected to be hypercholesteremic. The observed effect was probably due to the lower total dietary fat and lower intake of cholesterol in comparison to subjects' normal diets, an institutional diet relatively high in fat (50% of calories) and featuring eggs on nearly a daily basis.

Table 6—Fecal (stool) nitrogen excretion of subjects fed graded levels of hemicellulose at a constant protein intake (6.8g N/day)

Level of hemicellulose g/day	Mean fecal nitrogen excretion g/day	
	Part A[a]	Part B[a]
4.2	1.32a (0.66–1.51)	1.25a (0.55–1.41)
14.2	1.55b (1.20–1.83)	1.53b (1.15–1.90)
24.2	1.79b (1.29–2.30)	1.52b (1.22–1.86)

[a] Part A values are the means for 12 subjects for the first 7 days of each period and Part B values are those for the last 7 days. Duncan's multiple range test. Means without a letter in common differ significantly ($P < 0.05$).

Table 7—Blood serum cholesterol levels of subjects fed either butter oil/peanut oil or corn oil/peanut oil at graded intake levels of hemicellulose intake

Subject no.	Cholesterol levels (mg/100cc) of subjects fed hemicellulose			
	Normal	4.2	14.2	24.2g/day
	Corn oil/peanut oil			
1081	182	177	–	158
1082	153	167	162	166
1083	208	169	156	150
1086	125	125	122	118
1088	271	216	194	187
1089	127	124	–	104
Mean	178	163	158	147
	Butter oil/corn oil			
1092	191	182	182	163
1094	153	146	147	110
1095	200	186	177	157
1096	205	210	194	192
1097	169	185	170	160
1099	248	247	221	207
Mean	194	193	182	165

Table 8—Blood serum triglyceride levels of adult men fed either butter oil/peanut oil or corn oil/butter oil at graded levels of hemicellulose intake

Subject no.	Triglyceride levels (mg/100cc) of subjects fed hemicellulose			
	Normal	4.2	14.2	24.2g/day
	Corn oil/peanut oil			
1081	143	76	–	81
1082	93	106	111	105
1083	208	44	63	63
1086	122	91	100	66
1088	102	81	86	78
1089	84	101	–	104
Mean	125	83	90	82
	Butter oil/peanut oil			
1092	112	62	78	85
1094	101	69	90	132
1095	62	102	119	128
1096	135	131	135	230
1097	126	172	122	169
1099	376	448	449	502
Mean	152	154	163	208

Response of subjects' blood serum triglyceride levels to quantitative changes in dietary hemicellulose are shown in Table 8. For the corn oil group, blood serum triglyceride levels were lower than when subjects received their normal diets; however, no consistent changes were demonstrated as a result of hemicellulose supplementation. The group receiving butter oil in addition to the oil provided by ground peanuts responded quite differently to the hemicellulose additions. As dietary hemicellulose increased so too did the blood serum triglyceride levels of these subjects. While it is not unusual for decreases in blood cholesterol levels to be accompanied by increases in blood triglyceride levels, this is not generally considered to be a desirable response. In this situation it is difficult to explain these results.

Blood urea nitrogen levels of subjects were lower while subjects received the experimental diets than while consuming the usual institutional diet. This response would be expected since the protein content of the experimental diet was moderately low. No consistent differences in blood urea nitrogen levels as a result of hemicellulose additions were noted. No consistent changes between blood chemistries of subjects while on normal diets and experimental diets or among experimental diets for the following factors were found: calcium, phosphorus, glucose, uric acid, total protein, albumin, total bilirubin, alkaline phosphatase, LDH, SGOT, hematocrit and hemoglobin.

In conclusion, the results of this study demonstrate that changes in level of dietary hemicellulose result in changes in both apparent protein nutritional status and in blood serum lipid patterns of adult men. These changes may be desirable, undesirable or not important. However, recommendations for radical changes in dietary fiber intake should take into consideration the total possible effects on the physical well-being of humans.

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COMPARISON OF Tetrahymena pyriformis W AND RAT BIOASSAYS FOR THE DETERMINATION OF PROTEIN QUALITY

ABSTRACT

The protein quality of 34 samples of commercially prepared foods was determined using the *Tetrahymena pyriformis* W and rat Protein Efficiency Ratio (PER) bioassays. ANRC Reference Casein was used as the reference protein in both assays. The regression equation relating the *Tetrahymena* Relative Nutritive Value (RNV) and PER was: PER = 0.286 + 0.022 (RNV), r = 0.90 ($P < 0.01$). This relationship could be used to predict the protein quality of food samples. The use of the Coulter particle counter instead of the direct microscopic counting procedure increased the efficiency of the *Tetrahymena* assay by reducing counting error and the length of time required. In addition, quantitative information on cell size in relation to quality and quantity of protein in the test sample was obtained. The results of these studies suggest that the *Tetrahymena pyriformis* W assay may be adapted to provide a rapid, low cost assay for estimation of protein quality of commercially prepared foods.

INTRODUCTION

NUTRITIONAL LABELING requirements, concern for nutrient retention during storage and the effects of processing on the nutritive quality of protein products have created a need for an assay method to measure the quality of protein which is less time consuming and less expensive than the official bioassay for protein efficiency ratio (PER) (AOAC, 1970). The plant breeder, concerned with genetic improvement of protein quality, also has need for a rapid, simple, inexpensive test which requires only a few grams of material (Adams, 1974).

For ethical and practical reasons, human experimentation to determine protein quality is unacceptable, yet any method chosen to test the biological availability of nutrients must correlate with human needs. Numerous in vivo and in vitro methods have been proposed (Porter and Rolls, 1973), but none has met with wide acceptance. Microbial assays employing *Streptococcus faecalis* (Halvey and Grossowicz, 1953), *Streptococcus zymogenes* (Ford, 1962), *Clostridium perfringens* (Boyd et al., 1948) and *Tetrahymena pyriformis* W (Rockland and Dunn, 1949; Anderson and Williams, 1951; Pilcher and Williams, 1954; Fernell and Rosen, 1956; Stott et al., 1963; Stott and Smith, 1966; Sheffner, 1967; Helms and Rolle, 1970; Rolle and Eggum, 1971; Rolle, 1973; Shorrock and Ford, 1973; Srinivas et al., 1975; Frank et al., 1975; Landers, 1975) have been suggested. Of these, *T. pyriformis* appears to be the most suitable since many of the amino acid requirements of the organism are in reasonable agreement with human (Rolle, 1975) and rat (Kidder and Dewey, 1961) requirements.

Limited comparisons have been made between *T. pyriformis* W and rat bioassays (Fernell and Rosen, 1956; Rosen and Fernell, 1956; Srinivas et al., 1975); however, the *Tetrahymena* data were collected using protein samples which had been extracted with organic solvents. Landers (1975), recognizing that this extraction could affect protein utilization by *Tetrahymena*, used unextracted food samples; however, his PER data were obtained from the literature and not determined on the identical samples used in the *Tetrahymena* assay.

The present study was undertaken to determine the rat PER and *Tetrahymena* relative nutritive value (RNV) on identical samples of commercially available food products, to calculate the correlation coefficient between the two methods, and to establish whether the *Tetrahymena* bioassay had application as a rapid screening method in assessing protein quality of commercially available foods.

EXPERIMENTAL

Food samples

Thirty-four commercially prepared foods, representing 15 heat processed canned foods and 19 frozen foods were selected. The composition of the food products examined is given in Table 1.

Sample preparation

Samples of commercially prepared frozen foods were passed through a Hobart commercial grinder with a 1/8 in. stainless steel plate. Heat processed foods were blended in a Waring Blendor. Sufficient sample of each food was prepared for both PER and *Tetrahymena* RNV assay. A portion of the homogenized food sample was removed for the *Tetrahymena* assay and was further homogenized for 5 min using a Tissuemizer (Tekmar Co., Cincinnati, Ohio). The pepsin digests were prepared in Folin-Wu NPN blood digestion tubes. Blended portions of each food, containing 625 mg of protein (100 mg of nitrogen), were weighed into separate digestion tubes. The volume of each sample was made up to approximately 30 ml with distilled water and the pH was adjusted to 1.8 with 1N hydrochloric acid (HCl). Then 1 ml of a 1% pepsin (Sigma Chemical Co., St. Louis, Mo.), the 2X crystallized and lyophilized pepsin, was dissolved in water and the HCl concentration was adjusted to 0.05N with mixing) solution was added to each tube. The stoppered tubes were incubated at 55°C for 3 hr with inversion mixing in a Roto-Torque Heavy-Duty Rotator (No. 0007637, Cole-Parmer Instrument Co., Chicago, Ill.). Pepsin digest pH was adjusted to 7.1 with 1N sodium hydroxide (NaOH); digest volumes were adjusted to the 50-ml mark with distilled water. ANRC (Animal Nutrition Research Council) reference casein (available from Sheffield Chemical Co., Union, N.J.) was digested using the same procedure except homogenization was not required.

Test organism

Tetrahymena pyriformis W (ATCC 10542) was obtained from the American Type Culture Collection, Rockville, Md. The culture was maintained in *Tetrahymena* medium (5g protease peptone, 5g tryptone, 0.2g K_2HPO_4, 1000 ml distilled water). The organism was transferred regularly at 3–4 day intervals into 15 ml of sterile medium in 50 ml micro-Fernback flasks, and grown in the dark at 25°C. Immediately prior to inoculation of sample culture flasks in the assay procedure, a 3-day broth culture of the organism was centrifuged, washed once and resuspended in 15 ml of 0.067M phosphate buffer, pH 7.2.

Tetrahymena assay procedure

The procedure (Fig. 1) of Stott et al. (1963) as modified by Landers (1975) was used with the following modifications. Buffer solution D was prepared by mixing 0.2M solutions of mono- and di-basic potassium phosphate to give a pH of 7.1, and then adding an equal volume of 0.2M Tris-HCl buffer, pH 7.1. This modification was made to help minimize the reduction in pH which occurred in the culture flasks

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during growth. Solution E was prepared by dissolving the following in 14.0 ml of hot water:

	mg/20 ml of sol. E
cytidylic acid	12.5
guanylic acid 3′ (2′) mixed isomers	15
adenosine 2′ (3′) – phosphoric acid	10
uracil	5

After the above was solubilized, 1 ml of the following was added with stirring: 0.02M citric acid, solution B, and solution C. The pH was adjusted to 7.1 with 0.1N NaOH and 2.9 ml of modified solution D added to obtain 20 ml of working solution E. Solution G was prepared in two parts, each autoclaved separately at 121°C for 15 min, cooled and combined aseptically:

Part 1. 3.5g dextrin (bacteriological) (Eastman Kodak Co.) dissolved in 100 ml of hot water.

Part 2. 2.25 ml of stock solution A diluted to 15.0 ml with distilled water.

Vitamin stock solution A was prepared by dissolving the following milligram quantities of the components (available from ICN Nutritional Biochemicals, Cleveland, Ohio) in distilled water and, diluting to 200 ml, followed by heating to 55°C with stirring to dissolve components. Stock solution A was frozen in 10 ml aliquots until needed.

	mg/200 ml
D-calcium pantothenate	12.5
nicotinic acid amide	12.5
pyridoxine hydrochloride	125.0
pyridoxal hydrochloride	12.5
pyridoxamine dihydrochloride	14.8
riboflavin	12.5
folic acid, crystalline	1.25
thiamine hydrochloride	125.0
i-inositol (meso)	12.5
choline chloride	125.0
p-aminobenzoic acid	12.5
D-biotin	1.25
D-L-thioctic acid	0.4

Mineral stock solutions B and C were prepared by dissolving the following quantities of reagent grade chemicals (J.T. Baker Chemical Co., Phillipsburg, N.J.) in distilled water containing 1N HCl to a final volume of 200 ml. The solutions were stored in the refrigerator until needed.

Table 1—Comparison of rat PER, Tetrahymena RNV and calculated Tetrahymena PER of 34 food samples

Sample code	Protein (%)	Fat (%)	Carbohyrate (%)	Solids (%)	Corrected rat PER	Tetrahymena RNV	Calculated Tetrahymena PER[a]
05	9.7	9.6	16.4	37.3	2.9	113	2.8
010	8.1	8.8	12.7	30.9	2.9	115	2.8
012	9.7	10.6	17.4	39.2	2.9	106	2.6
032	6.2	10.9	19.1	37.6	2.9	101	2.5
041	8.5	6.1	11.5	27.4	2.9	110	2.7
042	8.0	5.6	13.7	28.9	2.9	121	2.9
03	8.2	3.6	12.5	25.8	2.8	119	2.9
06	10.7	10.7	13.5	36.7	2.8	111	2.7
07	11.6	11.4	19.1	43.7	2.8	129	3.1
014	8.1	6.4	14.3	29.8	2.8	111	2.7
031	5.7	9.9	19.2	36.3	2.8	107	2.6
033	6.1	11.1	17.7	36.3	2.8	105	2.6
044	6.7	5.7	11.5	25.3	2.8	110	2.7
02	9.7	3.4	10.3	24.6	2.6	120	2.9
08	8.3	13.0	12.4	35.1	2.6	107	2.6
013	6.6	9.4	15.8	33.2	2.6	105	2.6
043	6.4	3.2	13.8	24.6	2.6	102	2.5
020	4.2	3.8	15.9	25.1	2.4	95	2.4
011	9.2	12.9	17.2	41.2	2.3	106	2.6
025	3.0	1.8	7.1	14.2	2.3	76	2.0
037	5.6	2.4	6.9	16.2	2.3	98	2.4
022	3.4	3.2	11.1	19.1	2.2	97	2.4
024	3.3	1.6	6.8	13.8	2.2	77	2.0
016	4.5	4.1	11.2	21.4	2.1	94	2.3
019	3.9	4.6	12.1	22.1	2.1	77	2.0
017	4.1	4.5	11.4	21.5	2.0	84	2.1
036	5.3	1.3	5.7	13.7	2.0	85	2.2
035	4.2	1.8	6.1	13.5	1.9	67	1.8
018	4.6	3.1	14.8	24.0	1.8	73	1.9
038	4.5	2.9	7.6	16.4	1.7	77	2.0
039	3.5	2.6	9.5	17.7	1.7	63	1.7
023	3.0	1.3	8.2	14.1	1.6	84	2.1
029	4.6	1.8	11.0	18.9	1.3	57	1.5
027	1.4	1.6	8.5	12.8	1.2	39	1.1

[a] PER = 0.286 + 0.022 (RNV)

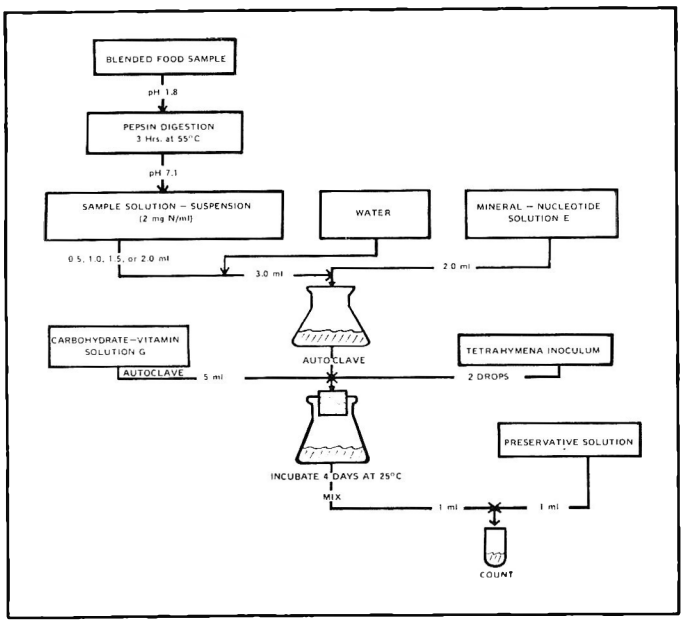

Fig. 1—Tetrahymena bioassay procedure.

Solution B	g/200 ml
$MgSO_4 \cdot 7\ H_2O$	2.8
$Fe(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$	1.25
$MnCl_2 \cdot 4\ H_2O$	0.025
$ZnCl_2$	0.0025
Solution C	**mg/200 ml**
$CaCl_2 \cdot 2\ H_2O$	600
$CuCl_2 \cdot 2\ H_2O$	60
$FeCl_3 \cdot 6\ H_2O$	15

Appropriate aliquots of digested casein or food sample suspensions were pipetted into 50 ml micro-Fernback flasks to provide 0.1–0.4 mg N/ml of final culture medium. Water was added to bring the volume in each flask up to 3 ml. Two ml of freshly prepared solution E were added to each flask. Trays containing 20 flasks each were covered with aluminum trays and autoclaved at 121°C for 10 min. After autoclaving, trays of flasks were cooled rapidly in a cold water bath. Five ml of sterile solution G (dextrin-vitamin solution) were added to each flask aseptically. Each flask was inoculated with 2 drops (approx 10^4 organisms) of the washed inoculum and incubated in the dark at 25°C for 4 days.

Determination of Tetrahymena growth

After 4 days of incubation, each flask was mixed on a Vortex mixer and 1 ml of growth medium was added to 1 ml of preserving solution (86 ml water, 20 ml 36% formaldehyde, and 14 ml of modified buffer solution D).

Organisms were counted by one of two procedures. All samples were counted using a Spiers-Levy eosinophil counting chamber (A.H. Thomas Catalog No. 2936-K10). The organisms in eight adjacent 1-mm squares were counted and the mean number per 1 mm square gave the final population in the test culture in units of 10^4 organisms per ml.

In later experiments, a Coulter counter model Z_B together with a Coulter channelizer (Coulter Electronics Inc., Hialeah, Fla.) were used. Teunnisson (1971) had suggested the use of this instrument to count *Tetrahymena* cells, however, she had used an elaborate extraction procedure to separate the cells from food debris. This extraction procedure greatly restricted the number of samples which could be counted in a day. No extraction procedure was used in this study. A 1:10 dilution of the preserved test culture was made using Isoton balanced electrolyte solution (Coulter Electronics Inc., Hialeah, Fla.). The diluted test culture was then counted using a 100 mm aperature tube. Since the food samples had been homogenized and digested, little interference was encountered from large particles blocking the aperature. In samples other than casein, particles smaller than *Tetrahymena* cells often interfered with the count. However, by using the channelizer to determine the lower threshold setting on the Coulter counter, these small interfering particles could be eliminated from the count. Duplicate or triplicate counts were made on all test cultures.

Calculation of relative nutritive value

The procedure as described by Landers (1975) was used to calculate the RNV of test cultures. Average organism count per ml was plotted against mg nitrogen per ml of incubation medium. The organism count at 0.3 mg nitrogen per ml was determined from the plot or a linear regression calculation.

$$\text{RNV} = \frac{\text{sample organism count}}{\text{ANRC reference casein count}} \times 100$$

Both counts were expressed per ml at 0.3 mg nitrogen per ml of incubation medium.

Animal bioassay

The official AOAC procedures for the biological evaluation of protein quality were followed throughout this study (AOAC, 1970). Weanling male, Sprague-Dawley derived rats, weighing 45–55g, 21–25 days of age were obtained from a commercial supplier (Blue-Spruce Farms, Inc., Altamont, N.Y.). During the 28-day feeding study, the animals were housed in individual galvanized steel (7 in. w × 7 in. h × 15 in. l) cages in a room where humidity (50%), temperature (22–24°C) and lighting (12 hr light/12 hr dark) were controlled.

Assay groups were assembled in lots of 10 rats in a random manner so that the weight differential was less than 5g within each lot and with a mean weight within 2g between lots. Throughout the 28-day test period, feed and water were provided ad libitum. Fresh diet was offered on an every-other-day basis. The composition of the casein control and test material diets were formulated according to AOAC (1970) recommended protocols whenever possible. If the diets could not be prepared according to suggested nutrient levels because of the composition of the test ingredients, the casein control diet was adjusted accordingly to compensate for differences in nutrient content (Hurt et al., 1975).

Individual rat food consumption records and weight gains were determined on a 2-day and 7-day basis respectively. Data collection and processing were done by an automatic electronic balance interphased with time-sharing computer facilities. Upon conclusion of the 28-day feeding studies, individual rat (PER) values were calculated for each rat based upon individual weight gain and consumption of diets for which protein (nitrogen × 6.25) and total solids had previously been determined by official AOAC (1970) procedures.

Statistical analyses

Statistical analyses of the data were patterned after standard regression analysis procedures (Steel and Torrie, 1960). The slope-ratio bioassay technique for the estimation of the potency of an unknown (Finney, 1971) was applied to a portion of the experimental data collected with the Coulter technique as outlined above. Where appropriate, standard analysis of variance and t-test techniques were used to determine the relative significance of observed differences between treatments.

RESULTS & DISCUSSION

A LINEAR GROWTH response was observed with casein and food samples when 0.1–0.4 mg N/ml was contained in the culture medium. The correlation coefficient of the growth versus nitrogen concentration regression lines of over half the samples counted microscopically was greater than 0.95, and all were greater than 0.90. The correlation coefficients of the data obtained by the Coulter counter were greater than those obtained microscopically. This was partly due to less error inherent in the Coulter counting procedure. Coulter counts were done in duplicate or triplicate, whereas, for reasons of time, one count per culuture flask was obtained with the microscopic counting procedure.

For the most part, the magnitude of the slope of the *Tetrahymena* growth versus nitrogen concentration increased as the protein quality of the food sample increased. This was also

Table 2—Linear regression of Tetrahymena count on level of protein from standard casein contained in incubation media.

		95% Confidence level	
	r^2	Y-intercept	Slope
3 point assay Y = 28 + 966 (x)	0.98	± 15	± 71
4 point assay Y = 42 + 880 (x)	0.98	± 13	± 48

Y = *Tetrahymena* Count/0.001 ml media; x Protein level

Table 3—Biopotency of food product protein using 4-point slope-ratio technique

	Potency	PER
Frozen Product FC	1.12 ± 0.03[a]	2.7
Heat Processed TN	0.76 ± 0.06	2.0
Heat Processed HD	0.66 ± 0.04	1.5

[a] Mean and standard deviation of six independent estimates

observed by Srinivas et al. (1975) and Landers (1975); however, there were exceptions, as described by Landers (1975). The growth versus nitrogen curves did not intersect at a common point for proteins of different or even similar quality. Landers (1975) assumed this reflected uncontrolled variables in the method, and emphasized the importance in choosing an optimum and common nitrogen concentration for determining organism counts.

Table 1 shows a comparison of PER and *Tetrahymena* RNV measured on identical samples of the 34 foods studied. The PER values were corrected to the PER of casein set at 2.5. The RNV values were calculated on the basis of the mean count obtained on 15 casein samples.

The RNV and rat PER values from Table 1 were plotted (Fig. 2) to obtain the relationship between the two methods. The regression equation relating *Tetrahymena* RNV and PER was: PER = 0.286 + 0.022 (RNV). The correlation coefficient between RNV and PER was 0.90 ($P < 0.01$).

Using this equation, the *Tetrahymena* PER was calculated from the RNV data (Table 1). 27 of the 34 food samples tested had a calculated *Tetrahymena* PER within 0.2 PER units of that determined by the rat bioassay. Six of the seven samples which disagreed by more than 0.2 PER units, were within 0.4 PER units of that determined by the rat bioassay.

The error inherent in the rat PER bioassay is ± 10%, (Personal experience). This would represent an error of ± 0.25 PER units for casein. In separate experiments designed to determine the error inherent in the *Tetrahymena* microscopic counting procedure, we have observed that a ± 10% error existed in the method. With this degree of inaccuracy, casein could have an RNV of 90–110 which would equate to a calculated PER of 2.3–2.7 (2.5 ± 0.2). Considering the error in both techniques, the PER as measured by the two techniques might be expected to disagree by 0.4 PER units. 33 of the 35 samples tested in this study were within this experimental error.

The error associated with the microscopic counting procedure, as well as the time required to perform the counts, prompted an examination of alternate procedures. Measurement of optical density as an indication of growth was impractical since food digests were often cloudy. Bioluminescence was examined but was found lacking in sensitivity. The Coulter counter appeared promising (Teunnisson, 1971) if time-consuming elution procedures could be eliminated.

Analysis of data obtained by the Coulter counter revealed the counting error had been reduced from 10% to less than 3% (an experimental error of ± 0.1 PER unit). This reduction in error is not surprising when one considers the dilutions involved. Coulter counts were multiplied by 20 to obtain the cell population per ml of growth medium, whereas microscopic

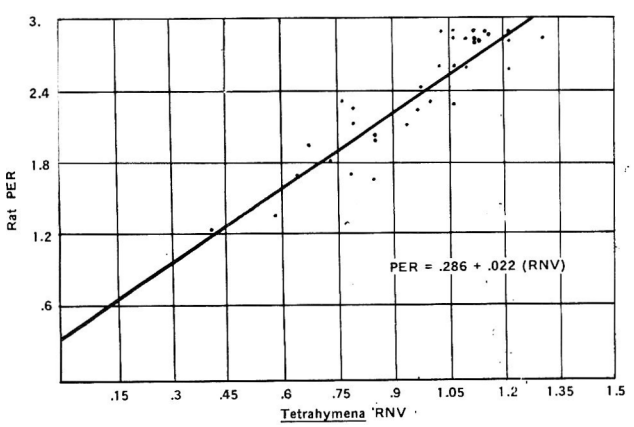

Fig. 2—Correlation of Tetrahymena RNV values with PER of 34 foods. The casein data was not included in the calculation of the regression line.

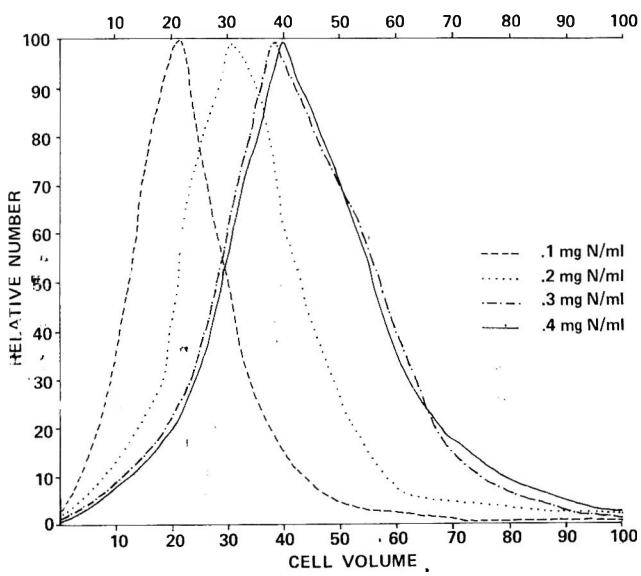

Fig. 3—Increase in cell volume of Tetrahymena pyriformis W cells versus increasing nitrogen concentration in medium.

counts had to be multiplied by 10^4. Miscounting one cell with the Coulter counter would represent 20 cells per ml of growth medium. Miscounting the same cell microscopically would represent 10,000 cells per ml of growth medium. An added advantage of using the Coulter counter was that duplicate or triplicate counts could be made per growth flask. Since the microscopic counting procedure is more time-consuming, and eye strain becomes a problem as the number of samples increases, only one count was performed per growth flask.

While using the Coulter channelizer to determine the lower threshold setting in order to eliminate debris from the count, it was observed that the volume of the cells increased as the amount of protein available to the organism increased (Fig. 3). Therefore, not only did the cell number increase with increasing amounts of protein, but the size of the cells increased as well. With a good protein source, maximum cell size was obtained with 0.3 mg N/ml culture medium, and though cell number increased with the addition of 0.4 mg N/ml, cell size did not. With a poor protein source, maximum cell size was not obtained even at 0.4 mg N/ml culture medium. All investigators who have studied the *Tetrahymena* assay for determining protein quality have used cell number at a particular nitrogen concentration as an index of protein quality. The observation that cell size varies with good and poor protein quality at a particular nitrogen concentration suggests that a good quality protein allows for better cell maintenance than a poor quality protein.

A second approach to utilizing the *Tetrahymena* assay as a predictor of protein quality was attempted by utilizing the concept of the slope-ratio bioassay as described by Finney (1971). *Tetrahymena* growth curves for the four nitrogen levels of the casein and test material were obtained by means of the Coulter procedure previously described. The relative growth curves of the two protein sources were compared according to the slope-ratio bioassay technique. The relative potency of the test material was estimated by the comparison of the slope of the test material to the slope of the standard casein.

The relative linearity of the response of 12 casein protein sources was evaluated using this system. The equations derived from growing *Tetrahymena* on 3 (0.1–0.3 mg N/ml) and 4 (0.1–0.4 mg N/ml) protein levels are presented in Table 2.

All casein protein sources gave the same response curve as determined by multiple comparison test of linear regression. Both the three and four point assays gave highly significant linear response of *Tetrahymena* count with increasing protein concentrations in the incubation media. The four-point assay resulted in a decrease in the variability of both the Y-intercept and slope value. The biopotency (relative to casein) of the protein from three prepared food products as evaluated utilizing this technique is presented in Table 3. These procedures provide a means whereby the biopotency of the protein source can be estimated according to standard statistical techniques.

The *Tetrahymena* bioassay can measure protein quality with accuracy comparable to the rat assay. The method can be used presently as a rapid screening procedure. With further refinement, such as the use of the Coulter counter and additional research on variables in the assay, the technique would be ready for collaborative study. Its greatest application would be in determining changes in protein quality in plant breeding studies, in evaluating the effects of processing on protein quality and in studying changes in protein quality during storage of food products. The technique is relatively simple to perform, requires only a small amount of sample, is less costly and requires relatively little space when compared to the rat bioassay.

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A DIFFERENTIAL MEDIUM FOR THE ISOLATION OF Aspergillus flavus FROM COTTONSEED

ABSTRACT

An amended Aspergillus Differential (BSAD) agar prepared by a modification of Bothast and Fennell's formula [Mycologia (1974) 66: 365] using botran (2,6-dichloro-4-nitroaniline) facilitated the isolation and enumeration of *A. flavus* from cottonseeds based on the characteristic orange yellow under-colony pigmentation after the cultures were incubated for 5 days at 28°C. Pigment production by *A. flavus* cultures on BSAD agar was detected by the third day of incubation. An incubation period of 5 days at 28°C is recommended for routine screening of cottonseed samples for contamination by *A. flavus*. At the concentrations used, botran (10 mg/liter) and streptomycin sulfate (50 mg/liter) did not interfere with the pigment production by *A. flavus* but decreased the numbers and colony size of other fungi and bacteria. Decrease in pH of the medium from 6.5 to 5.5, 4.5 and 3.5 resulted in decrease of the intensity of pigmentation while the sporulation of *A. flavus* colonies increased. When ferric citrate was omitted from ADM containing botran or was replaced with manganous sulfate, zinc sulfate or copper sulfate, orange yellow pigmentation was not produced. Kojic acid produced by strains of *Aspergillus* reacts with ferric citrate in the medium to produce orange-yellow pigmentation. When esculin hydrate (6,7-dihydrocoumarin-6-glucoside) was added to BSAD agar at a 1% level, deep reddish brown pigment was produced by all isolates of *A. flavus* and *A. parasiticus* tested. Similarities between pigment production and nitrification by the *A. flavus* group of fungi was observed. Few isolates of *A. oryzae* produced pigmentation similar to that produced by *A. flavus*.

INTRODUCTION

STRAINS of fungi belonging to the species *A. flavus–A. parasiticus* produce aflatoxins on a wide variety of crops and processed foods. Since aflatoxins are toxic and carcinogenic metabolites, the importance of screening agricultural crops for these mycotoxins cannot be over-emphasized.

Aflatoxin contamination of cottonseed at the time of harvest was correlated with bright greenish-yellow (BGY) fluorescence of the fiber and seed fuzz (Marsh et al., 1969; Whitten, 1966; Ashworth et al., 1968). BGY emission under long wave UV light is diagnostic of *A. flavus* boll rot (Marsh et al., 1973) and a qualitative test for aflatoxin through the detection of BGY fluorescence and removal of fluoresent seed was developed. However, contamination of cottonseed by *A. flavus* may not always be accompanied by BGY fluorescence (Hamsa and Ayres, 1975). Samples of cottonseed free from internal infections at the time of harvest may become contaminated during storage after ginning if the spores of *A. flavus* are present on the surface of the seed at the time of storage. Not all isolates of *A. flavus* produce aflatoxins. However, once a toxigenic strain becomes established within the seed coat, it is able to grow and produce toxins during storage. At the same time the seed coat protects the internal fungi from the chemicals used for their control. To minimize the risk of toxin elaboration during storage, the seed should also be screened for surface and internal contamination with *A. flavus–A. parasiticus*. This prompted us to investigate the possibilities of developing a differential medium that would facilitate isolation and enumeration of *A. flavus* group of fungi by personnel without extensive mycological training.

This paper describes an easily prepared Aspergillus Differential agar which consists of a combination of Bothast and Fennell's Aspergillus Differential Medium (1974) and the Botran Rose Bengal Streptomycin agar developed by Bell and Crawford (1967) for use as a selective isolation medium for strains of *A. flavus*. In addition, information on the factors influencing the formation of orange-yellow undercolony pigmentation by *A. flavus* cultures on BSAD agar is presented.

MATERIALS & METHODS

THE BASAL MEDIUM was Aspergillus Differential (BSAD) agar prepared by modifying Bothast and Fennell's formula to contain 15g tryptone; 10g yeast extract; 0.5g ferric citrate; 10 mg botran; 50 mg streptomycin sulfate; 15g agar; and 1000 ml distilled water. Botran and streptomycin sulfate were added to the medium after it was cooled to 50°C prior to pouring into Petri plates. Initially, an appropriate amount of botran was dissolved in 1 ml of acetone and was then added to the hot medium. Upon contact with the partially cooled medium, acetone volatilizes leaving the botran in suspension. pH of the medium was adjusted to desired values by using sodium hydroxide and hydrochloric acid solutions. The culture media were autoclaved at 15 psi for 15 min before pouring into plates.

Botran Rose Bengal Streptomycin (BRBS) agar medium was prepared according to the procedure described by Bell and Crawford (1967). To determine recovery of *A. flavus* from cottonseed on BRBS agar and BSAD agar, 5-lb samples of cottonseed were obtained from a commercial gin located in the vicinity of the University of Georgia (UGA) Experimental Farm, a commercial oil mill located in Macon, and acid delinted seeds from Farmington, Ga. The seeds were assayed for seed borne *A. flavus* contamination by randomly selecting 400 seeds and the seeds were then plated on BRBS agar and BSAD agar at the rate of 10 seeds per plate. Presence of *A. flavus* colonies on BSAD agar was based on orange yellow under-colony pigment production after incubation for 5 days at 28°C. *A. flavus* cultures were isolated in pure culture from BRBS agar and were identified based on morphological characteristics of cultures grown on Czapek's solution agar and malt extract agar media after incubation for 10 days at 28°C. The moisture content of the seeds collected from UGA Experimental Farm was adjusted to 20% and the seeds were inoculated with dry conidia of *A. flavus* harvested from an 8-day old Czapek's solution agar culture and incubated for 30 days at 28°C. At the end of the incubation period (60 days), seeds were surface disinfected in 5.25% sodium hypochlorite solution for 5 min, followed by several rinsings in sterile tap water and blotted dry. Recovery of *A. flavus* from surface disinfected seeds on BRBS agar and BSAD agar was determined as described earlier.

A modified Czapek's solution agar (Hesseltine et al., 1970) was used in carbon assimilation experiments to which filter sterilized carbon sources were added at levels of 1g/100 ml of the medium. Esculin hydrate (6,7-dihydrocoumarin-6-glucoside) (Sigma Chemical Co., St. Louis, Mo) was added to BSAD agar at a level of 10g/1000 ml and was autoclaved with the medium.

Various trace elements were either added individually to the BSAD agar or were added instead of ferric citrate at the following concentrations: $MnSO_4$, 5 mg/liter; $ZnSO_4$, 5 mg/liter; $CuSO_4$, 5 mg/liter; and $FeCl_3$, 0.5g/liter and their influence on pigment production was noted after incubation for 5 days at 28°C. Two hundred thirty-five cultures of

A. flavus isolated from cottonseed as well as cultures of *A. flavus* obtained from various sources and cultures of *A. oryzae, A. ochraceus, A. tamarii, A. nidulans, A. ustus, A. clavatus, A. fumigatus,* and *A. niger* were plated on BSAD agar to check for the production of characteristic orange-yellow pigmentation under the colony. Fungi outside the Aspergillus group which were known to produce kojic acid, e.g., *Penicillium citrinum, P. purpurogenum, P. rubrum, P. griseofulvum* and 23 genera of fungi isolated from cottonseed, were checked for the production of pigment as described earlier.

RESULTS & DISCUSSIONN

Evaluation of BSAD agar

Table 1 records data on the growth and pigment production by fungi belonging to the genus Aspergillus when the cultures were grown on BSAD agar. Two hundred thirty-five cultures of *A. flavus* series HAF 1-HAF 235 as well as cultures of NRRL Nos. 5917, 5565; H-5642, H-5692; and *A. parasiticus* NRRL No. 2999 and ATCC No. 15517 grew well on BSAD agar and produced under-colony pigment in shades varying from orange yellow to orange red after incubation for 5 days at 28°C. Cultures of *A. oryzae* No. 55–7 produced moderate orange yellow pigment and strain No. 55–3 produced good pigmentation while strain No. H-57 produced no pigment at all. None of the cultures of *A. wentii, A. tamarii, A. ochraceus, A. chevalieri,* or *A. amstelodami* tested produced orange-yellow pigment. Others (Salkin and Gordon, 1975) have reported that cultures of *A. fischeri, A. fumigatus, A. nidulans, A. terrus, A. ustus,* and *A. versicolor* did not produce orange pigments.

Table 2 records data on the under-colony pigment produced by 24 genera of fungi other than *Aspergillus* when such cultures were grown on BSAD agar. None of the cultures tested produced the characteristic under-colony pigmentation.

Recovery of A. flavus on BSAD agar

The performance of any selective medium depends upon the consistent recovery of the desired fungus when wide fluctuations prevail in the frequency of occurrence of the desired fungi. When the seeds are investigated for seedborne fungi, other microorganisms usually influence the results. Interfungal antagonism and fungal-bacterial antagonism can be observed easily in agar tests (DeTempe and Limonard, 1973). Table 3 records data on the recovery of *A. flavus* on BSAD agar and BRBS agar. Pigment production by *A. flavus* cultures was detected by the third day of incubation. The colonies of *A. flavus* were distinct by fifth day of incubation which facilitated their enumeration based on under-colony pigmentation alone. Differences in recovery of *A. flavus* on BRBS agar and BSAD agar from cottonseed with lower levels of *A. flavus* contamination and seeds artificaially contaminated with *A. flavus* were small. Sporulation of fungal colonies was heavy on BRBS agar while on BSAD agar the colonies did not sporulate. Sporulation may be undesirable because of the inaccuracies in count resulting from the development of secondary colonies from conidia disseminated during handling of the culture plates. Addition of botran to Aspergillus Differential agar to inhibit undesired fungi resulted in varying degrees of inhibition. Usually mycelia of *Rhizopus* spp. overgrow in Petri dishes and mask any fungi that grow under the mycelial mat. Growth of this fungus was restricted on BRBS agar when botran was added at a level of 10 mg/liter, while that of *A. flavus* cultures was not. At concentrations above 25 mg/liter botran inhibited the growth of *A. flavus* cultures. Streptomycin-sulfate added to inhibit bacterial contamination (50 mg/liter) did not affect colony growth or pigment production

Table 1—Characteristics of Aspergillus cultures grown on BSAD agar for 8 days at 28°C.

Cultures	Under colony pigmentation	Growth	Sporulation
Aspergillus flavus: HAF: 1 – HAF 235; NRRL 5917, 5565; H-5642, H 5692	Orange-yellow	Good	Nil
A. parasiticus: NRRL 2999, ATCC 15517	Orange-yellow	Good	Nil
A. oryzae: 55–3, 55–7, and NRRL 1988	Orange-yillow	Good	Nil
H-57	No pigmentation	Good	Nil
A. wentii: M-108 ATCC: 10583, 1023, 10584	Colorless	Restricted	Moderate
A. tamarii HAT: 1, 2, 3, 14, 16	Amber	Good	Nil
A. ochraceus HAO: 2, 3, 4, 6, 9	Pale yellow	Restricted	Moderate
A. chevalieri M: 70, 71, 236	Colorless	Restricted	Moderate
A. amstelodami M: 27, 60, 194, 237, 253	Dull gray	Restricted	Nil
A. repens: M-33, 52, 55, 197, 256, 276	Colorless	Restricted	Moderate

Table 2—Undercolony pigment produced by soil fungi after 8 days at 28°C on BSAD agar

Cultures	Strain no.	Undercolony pigment	Colony diam[a] (mm)
Acremoniella	H-157	Pale brown	19
Alternaria	H-17	Black	2
Botryodiplodia	H-124	Black	11
Chaetomium	H-126	Pale yellow	37
Chalara	H-128	Pale yellow	26
Cladosporium	H-6	Black	2
Curvularia	H-147	Pale black	43
Dactylium	H-86,87	Pale brown	22
Diplodia	H-115	Light brown	21
Epicoccum	H-112	Pale black	12
Fusarium	HF-2	Pale brown	19
Gonatobotrys	H-152	Pale yellow	43
Harpographium	H-22	Colorless	3
Humicola		No growth	
Macrophoma	H-151	No growth	
Monodictys	H-120	Pale yellow	24
Nigrospora	H-148	Yellow	39
Penicillium	HP-41	Colorless	2
Phialophora	H-155	Pale black/ brown	41
Pithomyces	H-123	Pale yellow	32
Rhizoctonia	H-117	No growth	
Staphylotrichum	H-121	Yellow	17
Stemphylium	H-122	No growth	
Trichoderma	H-158	Colorless	83

[a] Average of five or more replications

by *A. flavus* isolates. Inhibition of colony growth of undesirable fungi and of *A. flavus* on BSAD agar did not result in reduced recovery of *A. flavus* from naturally or artificially contaminated cottonseed.

Factors affecting pigment production

Carbon source. Utilization of 13 carbon sources by the *A. flavus* group of fungi was investigated according to the procedures described by Hesseltine et al. (1970) to determine if cultures of *A. flavus* and *A. parasiticus* selectively utilize carbon sources and produces pigments indicative of the presence of *A. flavus–A. parasiticus* cultures. The compounds tested were: galactose, glucose, lactose, maltose, melezitose, rhammose, xylose; dulcitol, inositol, mannitol, sorbitol; 5-ketogluconic acid and esculin hydrate. Isolates of *A. flavus* and *A. parasiticus* consistently produced bright red pigments in the media containing esculin hydrate. When esculin hydrate was added to BSAD agar at 1% level pigment production by all isolates of *A. flavus* and *A. parasiticus* was altered. A deep reddish-brown pigment was produced in the BSAD agar medium in contrast to the characteristic orange-yellow pigment produced by *A. flavus*. Growth of isolates of *A. flavus* and *A. parasiticus* was not restricted when esculin hydrate was added. The average colony diameter ranged from 20–35 mm after the cultures had incubated for 10 days at 28°C. The deep reddish-brown pigment had diffused uniformly throughout the agar medium and was stable at room temperature. Since all of the cultures of *A. flavus* isolated in pure culture from BSAD agar produced deep reddish-brown pigments on BSAD agar containing esculin hydrate, addition of esculin hudrate to BSAD agar offered promise as a secondary check on colony counts.

pH. Table 4 shows the effect of the pH of BSAD agar on colony appearance, growth, pigment production, and sporulation by *A. flavus* cultures. The optimum concentrations of hydrogen ions required for fungal growth is low; in higher concentrations the ion can materially affect metabolic processes other than growth. When the pH of the BSAD agar was decreased to 3.5, the under-colony pigment was pale yellow instead of the characterisitc orange-yellow pigment. Sporulation of fungal colonies increased with decrease of the pH of the medium. The pH of unadjusted BSAD agar was 6.5. When the pH of the medium was increased from 6.5 to 9.5, the medium was yellow in color. Growth and pigment production by cultures of *A. flavus* NRRL Nos. 5917, 5565; H-5642; HAF-50; HAF-4; PC 72; and *A. parasiticus* ATCC No. 15517 obtained from various sources was not affected at any pH values between 6.5 and 11.0. Intensity of the pigment produced at pH 9.5 was dark in comparison to the pigment produced at other pH values in the alkaline range. At pH 9.5 there was better contrast between the color of the medium and the pigment produced by *A. flavus* cultures. Culture of *A. parasiticus* NRRL No. 2999 and a culture of *A. oryzae* NRRL No.

Table 3—Recovery of A. flavus from cottonseed

Source	Treatment	% recovery of A. flavus	
		BRBS agar[a]	BSAD agar[b]
UGA farm	Linty seeds, after sterilization, artificially contaminated	100%	100%
UGA farm	Linty seeds, artificially contaminated	98%	93%
Farmington, GA	Acid delented seeds, naturally contaminated	45%	53%
UGA farm	Linty seeds, naturally contaminated	42%	49%
Macon, GA	Linty seeds, naturally contaminated	2%	2%

[a] BRBS agar—The colonies of A. flavus were isolated and identified on Czapek's solution agar and malt-extract agar plates.
[b] BSAD agar—The counts based on undercolony pigmentation

Table 4—Effect of pH on growth and pigmentation production by A. flavus cultures[a] after 8 days at 28° C on BSAD agar

pH of medium	Growth	Avg colony dia (mm)	Undercolony pigmentation	Sporulation
6.5	Regular	38	Orange-yellow	Nil
5.5	Regular	31	Orange-yellow	Nil
4.5	Regular	35	Orange-yellow	Slight
3.5	Aerial felt of cottony mycelium	31	Pale yellow and orange shades	Heavy
2.5	Restricted	7	Pale yellow	Heavy

[a] Observations based on 50 cultures of A. flavus of the HAF series

Table 5—Influence of temperature on pigment production

Cultures	Temperature (°C)				
	25	30	32	37	45
A. flavus: NRRL: 5917, 5565; PC 72; HAF 50	Orange-yellow	Orange-yellow	Orange-yellow	Orange-yellow	No growth
A. parasiticus: NRRL 2999; ATCC 15517	Orange-yellow	Orange-yellow	Orange-yellow	Orange-yellow	No growth
A. oryzae: NRRL 1988	Light orange-yellow	Light orange-yellow	Very light orange-yellow	No pigment	No growth
A. ochraceus: NRRL 3174	No pigment	No pigment	No pigment	Orange yellow	No growth
HAO 30	No pigment	No pigment	No pigment	No pigment	No growth

Table 6—Effect of trace minerals on pigment production

Culture	Undercolony pigmentation				
	Control BSAD Agar	(BSAD Agar-Fe)	(BSAD Agar-Fe) + Zn	(BSAD Agar-Fe) + Cu	(BSAD Agar-Fe) + Mn
A. flavus: NRRL: 5917, 5565; H-5642	Orange-yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
A. parasiticus: ATCC:15517; NRRL: 2999	Orange-yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
A. wentii: M-108	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
A. ochraceus: H-33	Amber yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow
A. amstelodami: M-300	Colorless	Colorless	Colorless	Colorless	Colorless
A. niger HAN-1	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow

1988 also produced the orange-yellow pigment at all pH values. *A. ochraceus* culture HAO-19 produced light orange yellow pigment at pH 9.5 with increased pigmentation at pH 10.0 and 11.0. Culture of *A. ochraceus* NRRL No. 3174 produced brownish-yellow pigment at pH 9.0 and 9.5 while at pH 10.0 and 11.0 the pigment produced was similar to that produced by *A. flavus* cultures. Sporulation of fungal colonies started at pH 9.5 when the cultures were incubated for 5 days at 28°C with increasing sporulation at pH 10.0 and 11.0.

Temperature. Table 5 records data on the influence of temperature on pigment production by *A. flavus* and other selected fungi. Pigment production by the tested strains of *A. flavus* was not affected at 25, 30, 32 and 37°C. With *A. oryzae* NRRL No. 1988 there was light pigmentation at 25°C, the intensity of pigment decreasing as the incubation temperature increased. At 37°C, no pigment was produced at all. *A. ochraceus* NRRL No. 3174 did not produce pigment at 25, 30 and 32°C but produced light orange-yellow pigment at 37°C. *A. ochraceus* culture HAO-30 did not produce any pigment at any incubation temperature. At temperatures above 40°C no growth occurred for any of the fungal cultures tested.

Trace minerals. According to Lillihoj et al. (1974) trace minerals are usually present as impurities in sufficient quantities for the normal growth of fungi. Presence or absence of certain trace elements can materially alter the chemical activities of fungi.

The influence of various trace elements on the pigment produced by *A. flavus* is shown in Table 6. When ferric citrate was omitted from BSAD agar, no pigment was produced. When ferric citrate was replaced with $MnSO_4$, $CuSO_4$, $ZnSO_4$, or $MgSO_4$ at a concentration of 5 mg/liter, orange pigment was not produced but when this iron compound was replaced with ferric chloride at a concentration of 0.5 g/liter, the pigment produced was similar. However, growth of *A. flavus* was more rapid on BSAD agar containing ferric citrate than ferric chloride. At concentrations of ferric citrate of 2.0g/liter and above, pigment production became orange red as contrasted to the orange-yellow shades at a concentration of 0.5g/liter. When either ferric citrate or ferric chloride was added to BSAD agar at concentrations of 0.5g/liter, under-colony pigment permeated into a circular zone around the fungal colony after the cultures were incubated for 10 days at 28°C. At higher concentrations of ferric citrate, the pigment produced was restricted to under the colony.

Table 7—Pigment production after 10 days at 28°C by fungi known to produce kjoic acid

Culture	Strain no.	Undercolony pigment
A. clavatus	H-55	Black
P. rubrum	141-B	Balck-brown
	H-113	Brown
A. ustus	H-611	Pale brown
A. oryzae	55-3	Orange-yellow
	H-57	Pale yellow
	55-7	Orange-yellow
	NRRL-1988	Orange-yellow
A. fumigatus	H-63	Light brown
P. purpurogenum	H-616	Colorless
P. citrinum	H-618	Pale brown
A. nidulans	H-40	Black

Kojic acid. Kojic acid, a metabolite produced by several species of *Aspergillus*, is a reactive multifunctional gamma pyrone. A very sensitive, but nonspecific, test for kojic acid consists of adding ferric chloride to the culture media to produce a cherry-red color. Under-colony pigmentation was produced by strains of *A. flavus* only when ferric citrate or ferric chloride was present in BSAD agar. It was hypothesized that kojic acid produced by strains of *Aspergillus* reacts with ferric chloride in the medium to produce an orange-yellow pigmentation. Two hundred thirty-five cultures of *A. flavus* which produced orange-yellow pigment on BSAD agar also gave a positive test for kojic acid when the cultures were screened for production of kojic acid according to the procedures described

by Hesseltine et al. (1970). However, certain fungal cultures outside *A. flavus* group are known to produce kojic acid. Production of characteristic pigmentation on BSAD agar by such cultures will limit the usefulness of orange-yellow under-colony pigmentation as an indicator of the presence of *A. flavus*. Cultures of *A. clavatus, A. effusus, A. fumigatus, A. flavus, A. oryzae, A. flavus-oryzae, A. nidulans, A. parasiticus, A. tamarii, A. ustus,* and few cultures of *Penicillium citrinum, P. griseofulvum, P. purpurogenum* and *P. rubrum* are reported to produce kojic acid (Parrish et al., 1966). Table 7 records the under-colony pigmentation produced by fungi belonging to the above species. Cultures of *A. clavatus* strain No. H-522, and *A. nidulans* strain No. H-40 produced black pigment while cultures of *A. ustus, A. fumigatus, P. rubrum*, and *P. citrinum*, produced varying shades of brown under-colony pigments. A strain of *P. purpurogenum* produced no pigment under the colony. These cultures produced amounts of kojic acid on BSAD agar inadequate to give orange-yellow pigment under the colony. It was further confirmed when the cultures were grown on BSAD agar minus ferric citrate and the aqueous extract of the medium was tested for the presence of kojic acid. Lack of such a pigment production by the above fungi is desirable since it demonstrates the selective nature of BSAD agar.

The factors affecting pigment production on BSAD agar closely parallel conditions favorable for the formation of nitrate. Nitrate was produced by most *A. flavus* (Marshall and Alexander, 1961), and by occasional *A. oryzae* (Nakamura and Shimota, 1954). Schmidt (1960) observed that during the first 2 days of incubation there was rapid synthesis of mycelium and very little nitrate was produced. At the initial pH level of 6.6 and above, nitrate was formed by the fourth day and increased gradually with further incubation. Under conditions favorable for the formation of nitrate, strains of *A. flavus* cultures produce kojic acid, hydroxyl amines, and N-formyl-N-hydroxyglycine (Doxtader and Alexander, 1966). Orange-yellow pigmentation produced by *A. flavus* cultures is the result of one or more of these colored complexes. Depending upon the amounts of these compounds produced by strains of *A. flavus*, the intensity and shades of pigments produced by the various isolates differs.

SUMMARY & CONCLUSIONS

THESE RESULTS show that BSAD agar can be used as a selective isolation medium for members of the *A. flavus* group. Inhibition of colony growth of undesired fungi by addition of botran (10 mg/liter) did not result in reduced recovery of *A. flavus* from cottonseeds. Differences in the recovery of *A. flavus* on BRBS agar and BSAD agar from cottonseeds with lower levels of contamination and seeds artificially contaminated with *A. flavus*, were small. Pigment production by *A. flavus–A. parasiticus* cultures on BSAD agar was detected by the third day of incubation at 28°C. However, an incubation period of 5 days at 28°C is recommended for routine screening of cottonseed samples to avoid inaccuracies in colony counts. Enumeration of colonies of *A. flavus*, based on under-colony pigmentation alone, could give higher counts since few isolates of *A. oryzea* produced similar pigments. Kojic acid produced by strains of *Aspergillus* reacts with ferric citrate in the medium to produce the characteristic orange-yellow pigmentation. Addition of esculin hydrate to BSAD agar offers promise as a secondary check on colony counts since all the cultures of *A. flavus* isolated in pure culture from BSAD agar produced deep reddish-brown pigments on BSAD agar containing esculin hydrate.

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FUNCTIONAL PROPERTIES OF A SOY CONCENTRATE AND A SOY ISOLATE IN SIMPLE SYSTEMS.

Nitrogen Solubility Index and Water Absorption.

ABSTRACT

Nitrogen solubility index and percent water absorption of a soy protein concentrate and isolate were evaluated as a function of pH and temperature. Although solubility and water absorption tended to increase as pH of the dispersion increased from 5.0 to 7.0 and as temperature increased from ambient to 90°C, pH and temperature effects were interdependent. The concentrate was less soluble and less responsive to changes in pH and temperature than was the isolate. Response surfaces for the concentrate and isolate, particularly those for water absorption, differed in shape.

INTRODUCTION

WITH THE RECENT increase in food uses of edible soy products, the desirability of quantitative information on the functional properties of these products has become apparent (Circle et al., 1964). Soy protein concentrates and isolates with their relatively high protein concentrations seem particularly applicable to assessment of functional characteristics of soy proteins as affected by conditions of use.

Solubility or dispersibility of soy protein is a physicochemical property that is related to the other functional properties and is, therefore, the first property to be studied in systematic investigation of physical properties (Mattil, 1971; Hermansson, 1973; Wu and Inglett, 1974). Ionic strength, pH and temperature, as well as particle size and product processing, have been reported to affect the solubility of soy proteins (Wolf, 1969; Johnson, 1970; Anderson et al., 1973; Hermansson, 1973; Lin et al., 1974; van Megen, 1974; Hermansson and Åksson, 1975).

Water uptake by soy products, attributed primarily to the protein content, also is affected by a number of factors, including pH. Wolf and Cowan (1971) reported the pH-water retention curve of soy proteins to follow the pH-solubility curve. Both solubility and water rentention were minimal at the isoelectric point (4.5) and increased as the pH diverged from this point. Johnson (1970) cited evidence that suggested a less consistent relationship between nitrogen solubility index (NSI) and water absorption of a soy flour.

pH and temperature are variables that are commonly applied to foods and might be expected to be interdependent as to their effects. This study was an investigation of protein solubility and water absorption of a soy concentrate and a soy isolate at several pH-temperature combinations.

EXPERIMENTAL

A SOY CONCENTRATE (Promosoy-100) and a soy isolate (Promine-D) were obtained from Central Soya Company, Chemurgy Division, Chicago. The concentrate and isolate contained approximately 68 and 92% protein, respectively, on the as-is basis. The concentrate was compared with the isolate (P-D) on both an equal weight ($P\text{-}100_1$) and an equal protein ($P\text{-}100_2$) basis. Sample weight for providing the comparable protein content was based on moisture determinations (AOAC, 1970) and nitrogen content on a moisture-free basis. The pH levels used for protein solubility and water absorption measurements were 5.0, 6.0 and as-is (7.0 ± 0.1). The temperature treatments were at 4°C, ambient (22–25°C) and 90°C.

Nitrogen solubility indices (NSI) of the soy products were determined by water extraction (Inklaar and Fortuin, 1969) and micro-Kjeldahl analysis (AOAC, 1970) of the extract. Percent water absorption was determined for the same samples by a modification of the procedure of Sosulski (1962). A 1.25-g sample of Promosoy-100 or Promine-D or 1.684g of Promosoy-100 was transferred to a preweighed 90-ml centrifuge tube. Fifty ml of dispersion medium were added and the sample was dispersed for 30 sec with a magnetic stirrer. The dispersion medium was water to which HCl had been added in the amount previously determined to be needed for achieving the specified dispersion pH. The tubes were placed in a shaker water bath at 4° (cold room), ambient temperature or 90°C and the contents were stirred for 1 hr by polyethylene "policemen" suspended into the tubes from a line stretched between two ringstands. After removal of the tubes from the water bath and centrifugation for 30 min at 1500 × G, the supernatant liquids were decanted into 200-ml volumetric flasks and the residues were re-extracted for 1 hr with 50 ml of distilled water. Supernatant liquids were combined with the corresponding extracts in the 200-ml flasks and made to volume. Extracts were filtered and analyzed for nitrogen by the micro-Kjeldahl method (AOAC, 1970). The unextracted Promosoy-100 and Promine-D samples also were analyzed for nitrogen content and the NSI of the experimental samples was calculated by the following equation:

$$\text{NSI} = \frac{\text{N in water extract}}{\text{N in unextracted sample}} \times 100$$

The centrifuge tubes with residue were placed mouth down at an angle of 15–20° on paper toweling in an air oven at 50°C and allowed to drain and dry for 25 min. The samples were cooled in a desiccator for 30 min and weighed. The water absorption or hydration capacity was calculated as the difference between hydrated weight and original weight, expressed as a percentage of the original dry weight of the sample.

The basis for the statistical analyses was a 3^3 factorial arrangement in which the soy sample, pH and temperature were the factors represented at three levels each. Data were collected in a 9 × 9 quasi-Latin square arrangement (Cochran and Cox, 1962). The Statistical Analysis System (SAS) Package was used to compute the analyses of variance. The effects of soy, pH, temperature, soy × pH, soy × temperature, pH × temperature and soy × pH × temperature, independent of day and order effects, were estimated for NSI and water absorption. Differences in the main effects and interactions were partitioned by the use of polynomials. From the polynomials, the estimated response surfaces of NSI and percent water absorption for the soy products were drawn with a Hewlett-Packard flat-bed plotter.

RESULTS & DISCUSSION

NSI RESPONSE SURFACES are shown for $P\text{-}100_2$ and P-D (Fig. 1 and 2). The surface for $P\text{-}100_1$ was nearly indistinguishable from that for $P\text{-}100_2$.

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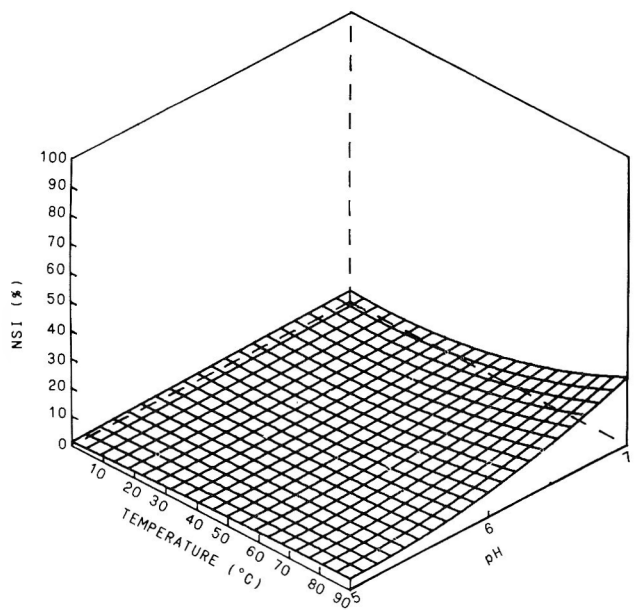

Fig. 1—NSI response surface for Promosoy-100$_2$ with variations in pH and temperature.

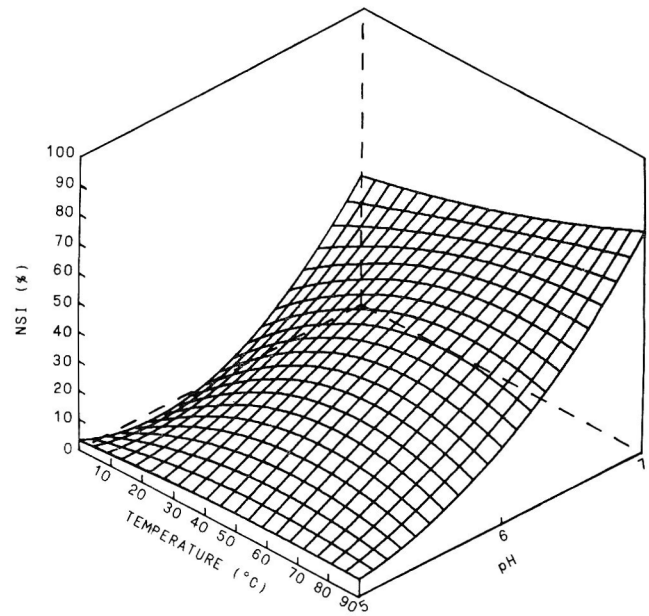

Fig. 2—NSI response surface for Promine-D with variations in pH and temperature.

NSI was much lower for the concentrate than for the isolate, whether the concentrate was equal to the isolate on the dry weight basis (P-100$_1$) or on the protein basis (P-100$_2$). Because NSI is the percentage solubility of the total nitrogen of the sample, the differences in NSI cannot be attributed to the differences in total nitrogen content between concentrates and isolates. Also, the comparison of P-100$_2$ with P-D was on an equal protein (N × 6.25) basis but the NSI values for P-100$_2$ were similar to those of P-100$_1$ samples rather than to those of P-D. The difference in NSI between concentrate and isolate samples undoubtedly reflects variation in processing methods. The concentrate used in this study is denatured to a greater extent during processing and thus is less soluble than the isolate. Another possible contributor to the difference in solubility is the nonprotein portion of the soy products. This component is primarily polysaccharide. Polysaccharides could compete with other system components, protein in this case, for the available water. The carbohydrate content of the soy products used was approximately 22% (as-is basis) for the Promosoy-100 and less than 1% for Promine-D. Because of the

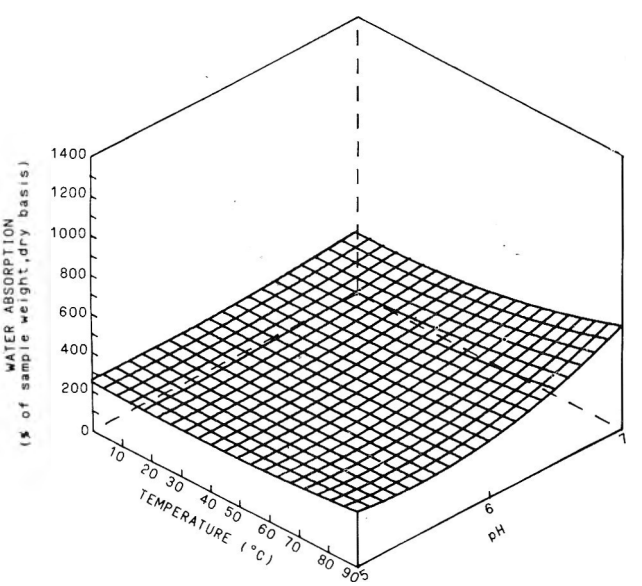

Fig. 3—Water absorption response surface for Promosoy-100$_2$ with variations in pH and temperature.

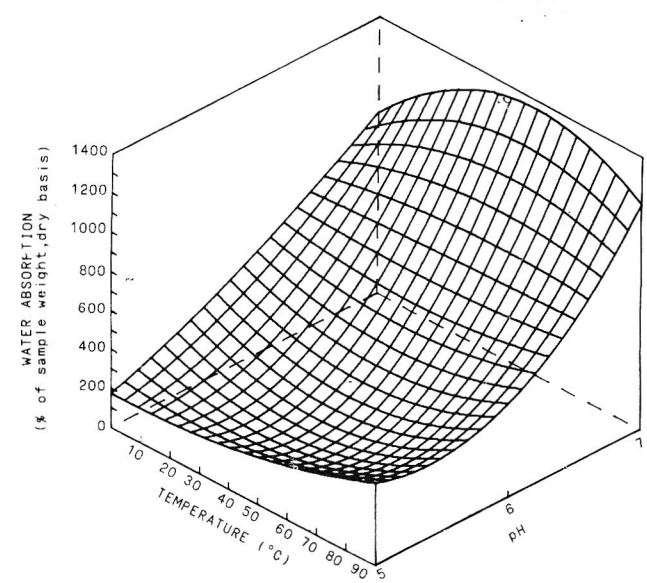

Fig. 4—Water absorption response surface for Promine-D with variations in pH and temperature.

larger amount of Promosoy-100 used for the equal protein comparison, P-100_1 and P-100_2 samples also differed in carbohydrate content but not as much as they both differed from P-D.

NSI in general increased with increased pH. The low solubility at pH 5 reflects the well known response to pH in the vicinity of the isoelectric point. Increased solubility with increased pH was more pronounced for the isolate, P-D, than for the concentrate as a result of the greater extent of denaturation during processing of the concentrate than of the isolate. The effect of pH was temperature-dependent, as seen in the figures, particularly the surface for the more responsive P-D. Whereas the pH slope is roughly linear in the mid temperature range, it is curvilinear at the temperature extremes.

NSI was consistently higher at 90° than at 4°C, but the effect of temperature was dependent both on soy product and on pH. The concentrate was less responsive to temperature than was the isolate, again because of its greater initial degree of denaturation. The pH dependence of the temperature effect is seen particularly in the surface for P-D (Fig. 2).

Water absorption response surfaces are shown for P-100_2 and P-D (Fig. 3 and 4); as with NSI, the water absorption surface for P-100_1 was nearly indistinguishable from that for P-100_2. The water absorption surfaces show the curvilinear nature of the response to both pH and temperature, as well as the pH-temperature interaction and the greater responsiveness of the isolate than the concentrate to changes in pH and temperature.

Effects of pH and temperature on water absorption for the most part paralleled those on solubility, as is to be expected. Comparison of the response surfaces in Figures 2 and 4 does reveal a marked difference in NSI and water absorption results for P-D. At the high end of the pH range used, the temperature effect was increased solubility but increased, then decreased, water absorption. This suggests that solubility and water absorption may be related to a point, perhaps maximum hydration, at which solubility continues to increase and hydration does not. The independent effects of pH, temperature and soy sample on NSI and water absorption were significant at the level $P < 0.0001$, as were the pH-temperature interaction, the other two-factor interactions and the three-factor interaction for each property.

Variations in pH and temperature are commonly imposed on food systems. The highly significant pH-temperature interaction that was observed for effects on protein solubility and water absorption in simple systems containing soy protein might have implications for food systems containing soy protein.

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FUNCTIONAL PROPERTIES OF A SOY CONCENTRATE AND A SOY ISOLATE IN SIMPLE SYSTEMS AND IN A FOOD SYSTEM.

Emulsion properties, thickening function and fat absorption

ABSTRACT

Emulsion stabilizing activity and thickening function of a soy protein concentrate and isolate were evaluated at pH 5.0, 6.0 and 7.0 and at 4°C, ambient temperature (22–25°C) and 90°C; fat absorption was evaluated as a function of temperature. pH and temperature effects on emulsion stability and on apparent viscosity were interdependent. Fat absorption was dependent on protein content of the soy sample. The concentrate and isolate differed as to magnitude of response to treatments. Functional performance of the concentrate and isolate in a food system did not necessarily parallel that in simple systems.

INTRODUCTION

EMULSIFYING PROPERTIES of soy proteins are important in their use as food ingredients. Soy proteins probably play two roles in emulsification. They aid in the formation of oil-in-water emulsions and stabilize the emulsions once formed (Wolf and Cowan, 1971). Proteins lower surface tension and collect at oil-water interfaces. A stabilizing effect of soy protein in emulsions thus possibly results from the protective barrier around fat droplets, preventing their coalescence (Wolf and Cowan, 1971; Lin et al., 1974).

Emulsification capacity of oilseed proteins has been reported to be affected by many factors, including protein solubility, pH and protein concentration. Crenwelge et al. (1974) reported a general positive correlation between emulsification capacity and solubility of a soy protein concentrate. pH influences the emulsifying capacity of protein ingredients indirectly by affecting the solubility of the proteins; as the pH of emulsions diverged from the isoelectric region of the proteins, emulsification capacity of soy isolates increased. Emulsion stability increased as soy protein isolate concentration increased (Inklaar and Fortuin, 1969; Crenwelge et al., 1974).

Rheological properties of aqueous dispersions of soybean globulins also are dependent on many factors, including protein concentration, heat treatment and pH (Circle et al., 1964; Catsimpoolas and Meyer, 1970). According to Hermansson (1972), the viscosity of soy dispersions is related to the degree of protein hydration. The apparent viscosity of soy dispersions increases exponentially with increasing concentration (Circle et al., 1964; Ehninger and Pratt, 1974; Fleming et al., 1974). Heated dispersions exhibited greater viscosity than unheated dispersions at a given concentration (Circle et al., 1964). Ehninger and Pratt (1974) reported that apparent viscosity of soy protein dispersions increased with increased pH. The effect was attributed to increased protein solubility at the higher pH values.

Fat absorption data for soy protein products are meager and the mechanism of fat absorption or binding has not been elucidated. Soy proteins have been added to comminuted meats to promote fat absorption or binding and thus decrease losses and maintain dimensional stability during processing. Wolf and Cowan (1971) reported that in ground meat products fat binding appeared to involve formation and stabilization of an emulsion. A gel formed by a soy protein (Hermansson and Åkesson, 1975) also might interfere with fat globule coalescence and enhance fat absorption.

Interactions between independent variables as to their effects on functional properties of proteins, as well as close relationships among the functional properties themselves, have important effects in food systems. The purpose of this study was to investigate the combined effects of pH and temperature on emulsion properties and thickening function and of temperature on fat absorption of a soy protein concentrate and a soy protein isolate in simple systems. A further purpose was to relate the data for simple systems to functional performance of the concentrate and isolate in a food system.

EXPERIMENTAL

A SOY PROTEIN CONCENTRATE (Promosoy-100) and a soy isolate (Promine-D), obtained from Central Soya Company, were used. For emulsion properties and fat absorption measurements, the concentrate was compared to the isolate (P-D) on both an equal weight (P-100_1) and an equal protein (P-100_2) basis. Viscosity measurements were made on the soy isolate and the equal protein level of the soy concentrate. Sample weight for providing the desired protein content was based on moisture determinations (AOAC, 1970) and nitrogen content on a moisture-free basis. The pH levels of the soy dispersions for all functionality tests except fat absorption were 5.0, 6.0 and as-is (7.0 ± 0.1 pH unit). Fat absorption measurements were made at the as-is pH of the soy and oil dispersion. The temperature treatments were at 4°C, ambient (22–25°C) and 90°C. The food system studied was a base for a dip. The soy concentrate and isolate were compared on an equal protein basis in a base product prepared at pH 5.0 and 6.0 and subjected to holding at 4 and 90°C. The base products were evaluated for emulsion stability and apparent viscosity. Dips formulated from the base products were held and evaluated at 4°C by a consumer panel for viscosity, mouthfeel, oiliness, flavor and general acceptability.

Emulsion properties in simple systems

Emulsifying and emulsion stabilizing activity of the soy products was determined according to a modification of the procedure of Inklaar and Fortuin (1969). Modification involved the use of 30 ml of dispersion medium (adjusted to the predetermined pH) and proportionate reduction of the amounts of soy sample, NaCl and oil used. The emulsion was prepared in a 400-ml beaker. The added oil was a weighed amount and the volume of separated oil was multiplied by its density for conversion to grams. Emulsified oil was expressed as the grams of oil emulsified (total minus free) per gram of sample (dry basis).

Thickening function in simple systems

The thickening function of the soy products was assessed by a modification of the procedure of Circle et al. (1964). The soy sample was added to a blender jar containing dispersion medium at the predetermined pH. Constant low speed was used throughout the 5-min blending

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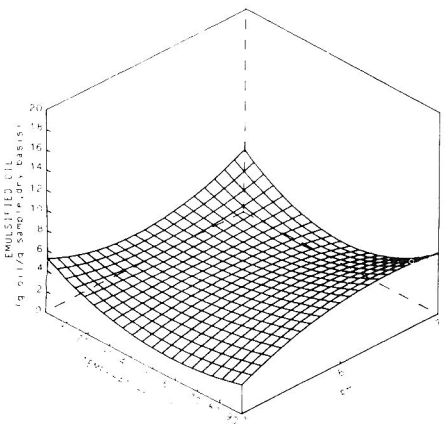

Fig. 1—Emulsified oil response surface for Promosoy-100$_1$ with variations in pH and temperature.

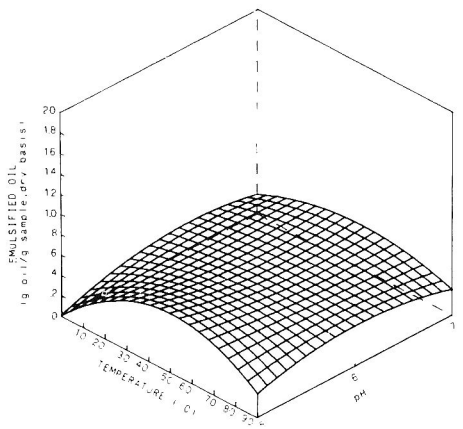

Fig. 2—Emulsified oil response surface for Promosoy-100$_2$ with variations in pH and temperature.

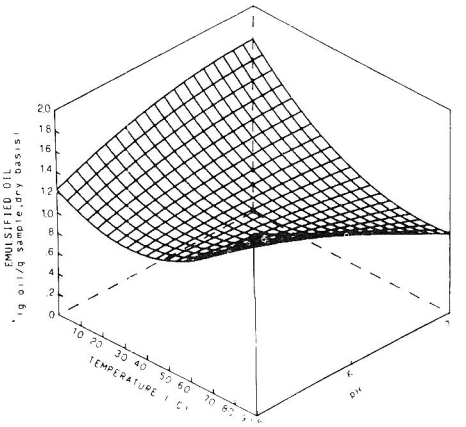

Fig. 3—Emulsified oil response surface for Promine-D with variations in pH and temperature.

period. The dispersions were equilibrated to the predetermined temperatures in water baths for measurement of apparent viscosity, for which the Brookfield LVF viscometer was used on the Helipath stand and with the T-spindles.

Fat absorption in simple systems

Fat absorption of soy products was measured essentially according to the procedure described by Lin et al. (1974). Modification included incubation of the samples at the experimental temperatures for 30 min followed by holding at ambient temperature for 40 min prior to centrifugation. The volume of absorbed oil (total minus free) was converted to weight by multiplying by the density of the oil. The fat absorbed was expressed as percent of soy sample weight on the as-is, dry and protein weight bases.

Preparation of food systems

Preparation of the base product and the dip has been detailed by Hutton (1975). The base contained 250 ml water, 16.9g thin boiling starch (Amaizo Quick-Set 68, American Maize-Products Co.), 8.0g dried whey (Land O'Lakes, Inc.), 26.8g sodium caseinate (Land O'Lakes, Inc.), 90.4g margarine and 9.9g Promine-D or 14.8g Promosoy-100. A portion of the water was acidified as needed to result in base product of the appropriate pH. The freshly prepared base product was held at 4°C in tightly covered 50-ml centrifuge tubes for assessment of emulsion stability, in 100-ml beakers covered tightly with foil for viscosity measurement and in covered jars for use as a dip.

Emulsion stability of base product

After storage overnight at 4°C the subsamples in the centrifuge tubes were held at either 4 or 90°C for 1 hr and then were equilibrated to ambient temperature in a water bath (30 min). The samples were centrifuged at 873 × G for 30 min. Volume of the separated liquid was read directly from the graduated centrifuge tube and was expressed as a percentage of the total volume after centrifugation.

Apparent viscosity of base product

Following overnight storage at 4°C, the subsamples in beakers were held at either 4 or 90°C for 1 hr. Apparent viscosity was measured at the treatment temperatures as described for the simple systems.

Consumer sensory evaluation

Ten percent ham flavored Bontrae (General Mills, Inc.) and 10% minced onion were added to the base product. Each dip was mixed thoroughly and transferred into coded 3/4-oz cups. The cups were covered with plastic wrap and held briefly at 4°C until the time of sensory evaluation. A consumer panel of 115 judges, mostly undergraduate and graduate students enrolled in food science classes, evaluated the dips on descriptive scales for viscosity, mouthfeel, oiliness, flavor and overall acceptability. Four samples were presented simultaneously: dips made from bases containing Promosoy-100 and Promine-D, each at pH 5 and 6.

Treatment of data

A response surface for emulsified oil was plotted for each soy sample as a function of pH and temperature, as previously described (Hutton and Campbell, 1977). The thickening function of the soy products was evaluated in a 2 × 3 × 3 factorial arrangement in which soy product was represented at two levels and pH and temperature at three levels each. Thickening function and fat absorption data were collected in randomized complete block designs for three replications.

Emulsion stability and apparent viscosity data for the base products were collected for two replications in a balanced 2^3 factorial arrangement with soy, pH and temperature represented at two levels each. Sensory panel scores for the dips were collected in a randomized complete block design. Emulsion stability data were not subjected to analysis of variance because only one treatment had a measurable response. All other data were subjected to analysis of variance.

RESULTS & DISCUSSION

Simple systems

Emulsion properties. The emulsified oil response surfaces are shown for P-100$_1$, P-100$_2$ and P-D (Fig. 1, 2, 3). The emulsions containing Promosoy-100 consistently had less emulsified oil after centrifugation than did those containing P-D. Although the difference between P-100$_1$ and P-D can be explained at least partially by a difference in the amount of protein to serve as emulsifier, the difference between P-100$_2$ and P-D cannot. The additional carbohydrate in the P-100$_2$ sample might have competed successfully for water and thus reduced the water available as dispersion medium. Another factor is greater denaturation of the concentrate than of the isolate during processing.

The emulsified oil response surfaces for the three samples (Fig. 1, 2, 3) reflect the interdependence of pH and temperature effects, as well as the differing responses of the soy products to changes in pH and temperature. The effects on emulsification of soy sample, temperature and all two-factor interactions were significant at the level $P < 0.0001$. The three-factor interaction was significant at the level $P < 0.0001$ or $P < 0.05$, depending on the basis of expression. The effect of pH as an individual factor was significant ($P < 0.05$) only for the values based on protein weight. For each soy sample several pH-temperature combinations resulted in relatively effective emulsification. The emulsified oil response surfaces differ considerably in shape from the corresponding surfaces for nitrogen solubility index reported elsewhere (Hutton and Campbell, 1977). While the effects of pH and temperature on protein

Table 1—Apparent viscosity mean values of soy dispersions with variation in pH and temperature

Soy sample	Apparent viscosity (cps)[a]					
	$P\text{-}100_2$			P-D		
Temp (°C)	pH 5.0	pH 6.0	pH 7.0	pH 5.0	pH 6.0	pH 7.0
4	84	166	765	62,693	50,519	2,153
Ambient[b]	86	151	451	55,278	43,326	1,268
90	233	456	19,992	>166,000[c]	>166,000[c]	64,463

[a] Differences attributable to soy sample, pH, temperature and all interactions significant at the level $P < 0.0001$
[b] 22–25°C
[c] Upper limit of measurement

Table 2—Apparent viscosity mean values for Promosoy-100 and Promine-D base products prepared at pH 5.0 and 6.0 and held at 4 and 90°C

Soy product	Apparent viscosity (cps)[a]			
	$P\text{-}100_2$		P-D	
Temp (°C)	pH 5.0	pH 6.0	pH 5.0	pH 6.0
4	>166,000[b]	150,977	>166,000[b]	85,674
90	3,320	3,184	3,537	2,980

[a] Differences attributable to soy product, pH, temperature and all interactions significant at the level $P < 0.0001$
[b] Upper limit of measurement

solubility probably influenced emulsification in specific cases, they apparently were not decisive in their effects on emulsification at all of the pH-temperature combinations.

Thickening function. The apparent viscosity values were consistently lower for $P\text{-}100_2$ than for P-D dispersions (Table 1). $P\text{-}100_2$ dispersions increased in viscosity as pH increased from 5.0 to 7.0 at all temperatures studied. $P\text{-}100_2$ dispersions at 90°C had higher apparent viscosities than $P\text{-}100_2$ dispersions at either ambient temperature or 4°C. The apparent viscosity of $P\text{-}100_2$ dispersions was of greatest magnitude at the combination of pH 7.0 and 90°C. The particularly high viscosity at this pH-temperature combination might be related to the maximum solubility of the concentrate under those conditions (Hutton and Campbell, 1977). The greatest increase in viscosity with increased pH for $P\text{-}100_2$ dispersions was observed as the pH increased from 6.0 to 7.0. This large increase in viscosity from pH 6.0 to 7.0 was evident at all temperatures and is in agreement with the findings of Circle et al. (1964).

As the pH of P-D dispersions increased, the dispersions decreased in apparent viscosity but increased visibly in stability. The effect of pH occurred primarily between pH 6.0 and 7.0 and the decreased viscosity with increased pH was evident at all temperature levels studied. The viscosity response of the isolate dispersions to pH variations in the present study was not in agreement with any data reviewed.

Temperature effects on apparent viscosity were similar for $P\text{-}100_2$ and P-D dispersion (Table 1). Dispersions exhibited minimum viscosity at ambient temperature. Apparent viscosity tended to increase slightly as the temperature was lowered to 4°C and increased dramatically as the temperature was raised to 90°C. The large increase in viscosity with a temperature increase from ambient to 90°C for both $P\text{-}100_2$ and P-D probably can be attributed to irreversible conversion of the sol to a viscous progel (Catsimpoolas and Meyer, 1970).

Fat absorption. Fat absorption, expressed as a percentage of the sample weight on the as-is basis is shown in Figure 4. Fat absorption for the Promosoy was significantly lower ($P < 0.0001$) and the curves are shaped quite differently from that for P-D. The curves for the two levels of Promosoy are similar to each other both as to height and as to shape. The curves for

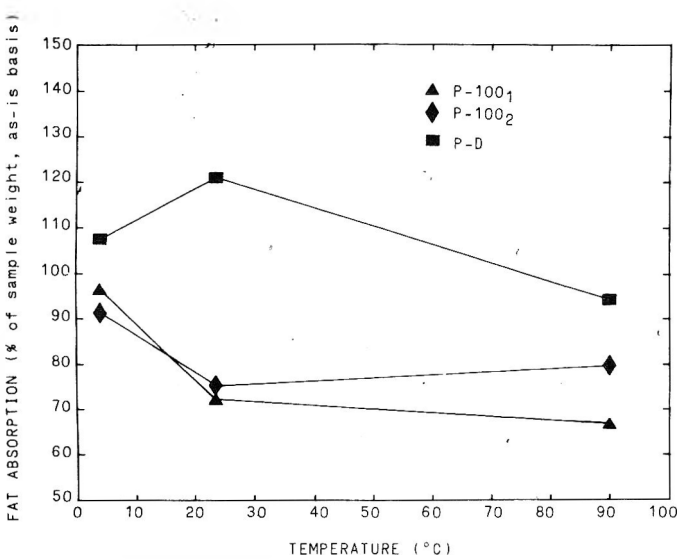

Fig. 4—Fat absorption (% of sample weight, as-is basis) of Promosoy-100$_1$, Promosoy-100$_2$, and Promine-D held at 4°C, ambient temperature (22–25°C) and 90°C.

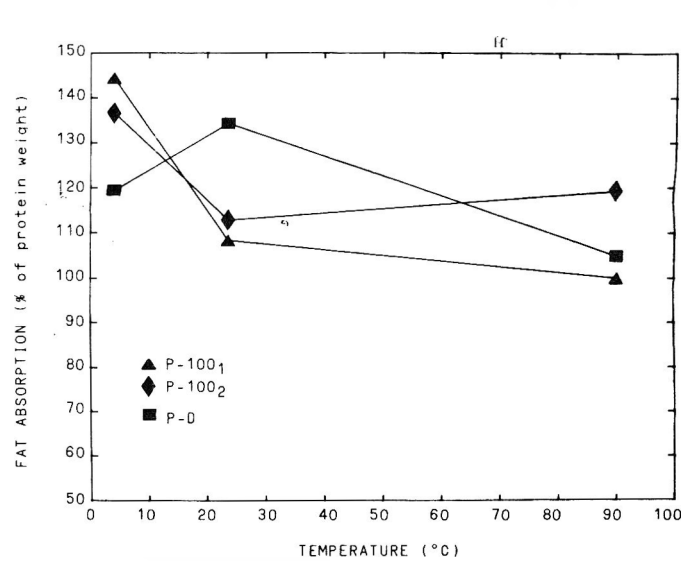

Fig. 5—Fat absorption (% of protein weight) of Promosoy-100$_1$, Promosoy-100$_2$ and Promine-D held at 4°C, ambient temperature (22–25°C) and 90°C.

Table 3—Mean sensory scores for soy dips prepared from Promosoy-100 and Promine-D base products at pH 5.0 and 6.0 and held at 4°C

Soy product	P-100$_2$		P-D	
	pH		pH	
Quality attribute[a]	5.0	6.0	5.0	6.0
Viscosity (6 = very thick)	5.5	3.5	5.7	3.2
Mouthfeel (6 = very smooth)	4.3	4.5	4.0	4.5
Oiliness (4 = very oily)	3.1	2.8	3.0	2.7
Flavor (6 = very desirable)	4.1	3.6	4.1	3.3
Overall acceptability (5 = very good)	3.2	2.9	3.3	2.7

[a] Effect of soy product not significant; effect of pH significant at least at the level $P < 0.001$ for each attribute; only one interaction significant: pH-soy product for viscosity ($P < 0.001$)

fat absorption values expressed on the basis of sample dry weight, not shown, were similar to those in Figure 4. The lower absorption of oil by P-100$_1$ than by P-D when expressed on the basis of sample weight suggests that the protein was largely responsible for fat absorption. Fat absorption values for P-100$_2$ also were lower than those for P-D when expressed on the basis of sample weight, even though the amount of protein was the same; this indicates that the additional carbohydrate present in P-100$_2$ with the use of Promine-D and Promosoy on the equal-protein basis certainly did not absorb as much oil as the protein.

Expression of the fat absorption values as percent of protein weight (Fig. 5) brings the values for all soy samples into a relatively narrow range, further indicating that the protein was responsible for most of the fat absorption by the soy products. The differing shapes of the fat absorption curves for the concentrate and the isolate do not appear to be directly related to the other properties measured.

Differences in fat absorption attributable to temperature and the soy-temperature interaction were significant at the level $P < 0.0001$ for all bases of expression.

Food systems

Emulsion stability. All base products were stable when held at 4°C. At 90°C the concentrate base products were stable and the isolate products were unstable. An average of 4% (volume basis) liquid separated from the isolate base product at pH 5.0 and 90°C. The isolate base product prepared at pH 6.0 and 90°C appeared "crumbled" throughout; however, no separation occurred during centrifugation.

Apparent viscosity. Apparent viscosity was higher at pH 5.0 than at 6.0 and higher at 4°C than at 90°C for both soy products. The products were too viscous at pH 5.0 and a holding temperature of 4.0°C to measure with the available equipment; thus if a difference between the concentrate and the isolate base products existed at this pH-temperature combination, it was not measurable under the conditions of the study. For all other pH-temperature combinations, the concentrate base products were similar in viscosity or more viscous than the corresponding isolate products.

Consumer panel evaluation. With both the concentrate, P-100, and the isolate, P-D, dips prepared at pH 5.0 were rated more viscous and scored higher on flavor and overall acceptability than dips prepared at pH 6.0 (Table 3). On the other hand, dips prepared at pH 6.0 were considered smoother and less oily than those prepared at pH 5.0.

Relation of simple system data to food system data

Of the measurements made on simple systems, emulsion stability and viscosity were applicable to the base products used for dips. In addition, the consumer panel evaluated the dips themselves from the standpoint of oiliness and viscosity, as well as smoothness, which is closely related to solubility.

Panel oiliness scores may be related to emulsion stability. Panel members rated products prepared at pH 5.0 as oilier than those prepared at pH 6.0. Less oil was emulsified at pH 5.0 than at pH 6.0 in the simple systems at 4°C, though the base product emulsion was stable at both pH 5.0 and 6.0 at that temperature.

Concentrate dispersions in simple systems exhibited increased apparent viscosity as pH increased from 5.0 to 6.0, whereas isolate dispersions showed the reverse effect. With both the concentrate and isolate food systems, viscosity of the base product as measured by viscometer and of the dip as evaluated by the consumer panel was higher at pH 5.0 than at 6.0. In spite of the differences in the viscosity responses of the concentrate and isolate in simple systems, the food systems containing the concentrate and the isolate responded similarly to change in pH. In other words, the isolate performed as predicted and the concentrate did not. Combined effect of the low viscosity and low pH-sensitivity of concentrate relative to the isolate and the possiblity of interactions among the product constituents could override the response to pH that would be predicted by behavior of the soy concentrate samples in simple systems. The dips were more complex systems than the dispersions on which viscosity measurements were made in the first part of the study. Possibly other constituents (that contributed to viscosity) in the concentrate base product were affected more by pH than was the concentrate.

Mouthfeel, representing smoothness, was rated higher for the dips at pH 6.0 than for those at pH 5.0. The lower mouthfeel scores for the dips prepared from pH 5.0 base products probably are attributable to the lower solubility of soy proteins at pH 5.0, as observed in simple systems (Hutton and Campbell, 1977).

Many interactions were observed throughout the study. In addition, simple and more complex systems sometimes differed in their response to variations in pH and temperature. Therefore, extreme caution is needed in extrapolation of results from simple systems to food systems.

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CARBOHYDRATE COMPOSITION OF WATER-SOLUBLE PENTOSANS FROM DIFFERENT TYPES OF WHEAT FLOURS

ABSTRACT

Water-soluble pentosans from a hard red winter, a hard red spring and a soft red winter wheat flour contained 50–68% carbohydrate and 8–22% protein, after treatment with α-amylase. Carbohydrate compositions of those pentosans were: arabinose, 39–40%; xylose, 35–42%; and galactose, 18–24%. Fractionation of the pentosans on a column of DEAE-cellulose yielded five fractions, two of which were fractionated into two subfractions, indicated to be heterogeneous by polyacrylamide disc electrophoresis and by gel filtration. The carbohydrate composition of fractionated pentosans from the three types of wheat differed, particularly in the distribution of carbohydrate components in fractions II, III, and V. Gel filtration patterns were similar for pentosans from all three wheat flours, and indicated that the fractions probably contain glycoprotein components, but part of the protein is present as non-dialyzable protein that is not glycoprotein.

INTRODUCTION

WHEAT FLOURS contain 2–3% pentosans of which about 20–25% are soluble in water forming viscous solutions (Neukom et al., 1962). Kuendig et al. (1961a) fractionated water-soluble pentosans from wheat flour into five fractions on a column of DEAE-cellulose. The first fraction was an arabinoxylan, free of galactose and protein. The other four fractions were more complex and contained various amounts of protein and galactose. Fractions 2, 4 and 5 contained xylose, arabinose, and galactose as components of their carbohydrate polymers, while fraction 3 contained only galactose and arabinose. Kuendig et al. (1961b) suggested that about half of the pentosan preparations investigated were glycoproteins. Fractions II, III, and V have been fractionated into subfractions with various carbohydrate or protein contents during chromatography of the water-soluble pentosans on DEAE-cellulose (D'Appolonia et al., 1970; Patil et al., 1975a).

Treating wheat flour with 80% ethanol prior to extraction with water resulted in isolating water-soluble pentosans containing only 2% protein (Fincher and Stone, 1974). The 2% protein was firmly bound to a polysaccharide. A high molecular weight arabinoxylan and a low molecular weight arabinogalactan, the latter being associated with a hydroxyproline-rich peptide, were separated by fractional precipitation of the water-soluble pentosans with ammonium sulfate.

An arabinoxylan containing protein was isolated from water-soluble pentosans of wheat flour by precipitation with ammonium sulfate (Geissman and Neukom, 1973). Chromatographic purification of that fraction yielded an arabinoxylan with only a trace of protein. Ferulic acid was bound to the polysaccharide.

Carbohydrate components in the five fractions resulting from chromatography of water-soluble pentosans on DEAE-cellulose have been investigated by several groups (Kuendig et al., 1961a; Cole, 1967; Wrench, 1965; Lin and Pomeranz, 1968). Medcalf et al. (1968) isolated water-soluble pentosans from the endosperm of durum and hard red spring wheats using two isolation procedures. In all cases, durum pentosans contained a higher proportion of arabinose than hard red spring wheat did, indicating a more highly branched structure. D'Appolonia and MacArthur (1975) isolated water-soluble pentosans from three conventional-height and five semidwarf varieties of hard red spring (HRS) wheats. High amounts of glycoproteins were reported in those water-soluble pentosans. Based on the carbohydrate properties investigated, it was not possible to separate conventional-height wheat varieties from semidwarf samples by any one particular character measured. However, certain differences were noted between varieties.

To obtain a better understanding of differences and similarities between water-soluble pentosans from different types of wheat flours, we isolated water-soluble pentosans from a hard red winter (HRW, Scout R-70), a hard red spring (HRS, Chris), and a soft red winter (SRW, Logan) wheat flour. The carbohydrate components of the five fractions, obtained from chromatography of the pentosans on DEAE-cellulose, were determined after acid hydrolysis.

EXPERIMENTAL

Materials

Flours were milled (Miag Multomat mill) from different types of wheat: hard red winter (Scout R-70, 9.5% protein, 14% moisture basis), hard red spring (Chris, 13.1% protein, 14% m.b.), and soft red winter (Logan, 10.5% protein, 14% m.b.).

Isolation of crude pentosans

Crude, water-soluble pentosans were isolated as described previously (Patil et al., 1975a) using 250-g samples of flour. Extracts from five 250-g samples were combined for further purification and isolation of the pentosans. Solutions of crude pentosans were freeze dried.

Preparation of purified pentosans

Soluble starch in crude water-soluble pentosans was removed by treatment with bacterial α-amylase according to the procedure of Kuendig et al. (1961a) except the enzyme was precipitated with 5% trichloracetic acid (TCA) using 25 mg TCA per 1 mg α-amylase (Patil et al., 1975a). Solutions of purified pentosans were freeze dried.

Fractionation of purified pentosans on a column of DEAE-cellulose

DEAE-cellulose (Brown Company, Berlin, N.H., exchange capacity 0.86 meq/g) was prepared in the borate form according to the method of Neukom et al. (1960) with minor modifications.

α-Amylase-treated pentosans (2g in 100 ml water) were applied to the top of a column (5 × 70 cm) of DEAE-cellulose and fractionated as described previously (Patil et al., 1975a) using 4 liters of each solvent. Fractions were combined on the basis of protein and carbohydrate content, dialyzed against distilled water for 3 days at room temperature, and freeze dried.

Analytical methods

Carbohydrate contents of column effluents and of freeze-dried fractions were determined using the phenol-sulfuric acid procedure (Dubois et al., 1956). A standard curve was constructed with D-xylose (10–60 μg).

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Protein content of each fraction was determined using the Lowry (Lowry et al., 1951) procedure. A standard curve was constructed with bovine serum albumin (10–100 μg). Protein components were located in effluent fractions by automatically scanning the effluent at 280 nm.

Gas-liquid chromatography of alditol acetate derivatives

Freeze-dried samples (40 mg) of purified pentosans and DEAE-cellulose fractions I-V from Scout R-70, Chris, and Logan flours were hydrolyzed with 3 ml of 1M hydrochloric acid in a boiling-water bath for 6 hr. The hydrolyzates were diluted with 10 ml distilled water and were neutralized to pH 6 with AG 3-X4 resin (OH^- form, Bio-Rad Laboratories, Richmond, Calif.). The resin was removed by filtration, washed thoroughly with water (4 × 20 ml), and the filtrates were freeze dried. myo-Inositol (10 mg) was added to each freeze-dried sample as an internal standard. The aldoses in the hydrolyzates were reduced to the corresponding alditols with 10 ml sodium borohydride (10 mg/ml). After 2 hr at room temperature, the samples were placed in an ice-water bath and excess borohydride was decomposed by dropwise addition of glacial acetic acid until gas evolution ceased. The solutions were concentrated to 10 ml each. Methanol (3–4 ml) was then added and the samples were evaporated to dryness to remove excess borate as the volatile methyl borate.

Methanol was added and evaporated to dryness eight times. The samples were acetylated in screw-capped vials by adding 0.5 ml of acetic anhydride and 0.5 ml of pyridine and heating for 10 hr in an oven at 100°C. Aliquots (1–2 μl) of the acetylation mixtures were injected into the gas-liquid chromatograph for analyses of individual carbohydrate components.

Chromatography was accomplished on a Hewlett-Packard Model 5751B Research Gas Chromatograph equipped with dual flame ionization detectors using a stainless-steel column (1/8 in. × 10 ft) packed with 3% ECNSS-M on Gas Chrom Q (100/200 mesh) (Sawardeker et al., 1965). The column was operated isothermally at 180°C with nitrogen as carrier gas (35 ml/min).

Carbohydrate components were identified by comparing their retention times with those of known alditol acetates chromatographed under identical conditions. Areas under component peaks were determined using a disc integrator. A response factor was determined for each alditol by chromatography of standard alditol acetates.

Gel filtration of purified pentosans and DEAE-cellulose fractions

Purified pentosans (15 mg/15 ml 0.1M acetic acid) and DEAE-cellulose fractions (<0.16% in 0.1M acetic acid) were subjected to gel filtration on a column (2.5 × 40 cm) of Bio-Gel P-150 which had been calibrated for molecular size. Calibration of the column and gel filtration were accomplished as described previously (Patil et al., 1975a) except 0.1M acetic acid was used to prepare and to elute the column at a flow rate of 15 ml/hr. Fractions (5 ml) were collected automatically.

Disc electrophoresis

DEAE-Cellulose fractions II_a, II_b, III_b, IV, and V were characterized by disc electrophoresis (pH 4.3) on polyacrylamide gels (5%) using a modification of the procedure described by Davis (1964). Samples (0.1 ml), prepared 1 day before use to insure complete dissolution, of fractions II_a (2%), II_b (2%), III_a (4%), III_b (2% plus 20% sucrose), IV (2% plus 20% sucrose), and V (4%) were subjected to electrophoresis at 1.6 ma per tube for the first 10 min and then 4 ma per tube for an additional 60 min. Immediately after being removed, gels were fixed in 20% sulfosalicylic acid for 40 min and stained with 0.25% Coomassie Brilliant Blue R-250 (Colab Laboratories, Chicago Heights, Ill.) for 1 hr (Chramback et al., 1967). The stained gels were stored in 7% acetic acid.

RESULTS & DISCUSSION

Yield of water-soluble pentosans

Yields of crude water-soluble pentosans ranged from 0.55–0.81% (Table 1) while those of α-amylase-treated (purified) pentosans ranged from 0.44–0.55% of the flour. Both results are similar to those reported by other investigators (Neukom et al., 1967; Lin and Pomeranz, 1968; Patil et al., 1975a). Chris flour yielded more crude pentosans than either of the other two flours and the yield of purified pentosans from Chris flour exceeded that of either of the other two flours about 20%. Our yields are somewhat lower than those reported by D'Appolonia and MacArthur (1975) from hard red spring wheat flours; 1.1–1.5% for crude pentosans and 0.6–0.7% for amylase-treated pentosans. Those authors obtained 1.4% crude pentosans and 0.7% amylase-treated pentosans from Chris flour.

Purified pentosans from Scout R-70 flour were highest in carbohydrate content but lowest in protein content (Table 1). Purified pentosans from Chris and Logan flours contained similar amounts of carbohydrate and protein. The protein contents of the purified pentosans from Chris and Logan flours agree well with values (16.9–22.6%) reported for Marquis and Omar flours (Lin and Pomeranz, 1968). D'Appolonia and MacArthur (1975) reported 28.0% protein in the amylase-treated pentosan from Chris flour. Purified pentosans from Scout R-69 flour were previously reported (Patil et al., 1975a) to contain 16.4% protein.

In our study, carbohydrate content was expressed as xylose, the predominant carbohydrate, although other carbohydrates are present. The carbohydrate values must be considered as minimal because D-xylose gives the highest absorbance values of common monosaccharides (Dubois et al., 1956).

Table 1—Yield and distribution of carbohydrate and protein in water-soluble pentosans

Source	Crude pentosans	α-Amylase-treated pentosans		
	Yield (%)	Yield (%)	Carbohydrate[a] (%)	Protein[b] (%)
Scout R-70 (HRW)	0.55	0.44	68.5	8.0
Chris (HRS)	0.81	0.55	51.2	22.5
Logan (SRW)	0.70	0.47	50.5	20.6

[a] Determined by the phenol-sulfuric acid procedure (Dubois et al., 1956) using D-xylose as a standard
[b] Determined by the Lowry procedure (Lowry et al., 1951) using bovine serum albumin as a standard.

Table 2—Yield of freeze-dried fractions obtained from chromatography of purified water-soluble pentosans on DEAE-cellulose

Pentosan source	Fraction number						
	I	II_a	II_b	III_a	III_b	IV	V
Scout R-70 (HRW)	39.9[a]	10.8	5.6	15.9	1.1	17.7	8.9
Scout R-69[b] (HRW)	16	3	22	21		20	17
Chris (HRS)	30.1	8.6	9.2	18.0	9.2	15.6	9.1
Chris[c] (HRS)	31.1	15.9		17.4		24.4	11.1
Logan (SRW)	27.9	9.7	7.6	17.8	12.5	10.7	13.7

[a] Values cited are distribution percentages
[b] Values from Patil et al. (1975b)
[c] Values from D'Appolonia and MacArthur (1975)

Table 3—Distribution of carbohydrate in fractions obtained from chromatography of purified pentosans on DEAE-cellulose

Pentosan source	Fraction number						
	I	II_a	II_b	III_a	III_b	IV	V
Scout R-70 (HRW)	29.7[a]	23.3	14.5	14.2	0.9	7.5	9.9
Scout R-69[b] (HRW)	20	4	32	17		16	10
Chris (HRS)	27.3	21.5	18.2	12.7	1.4	9.5	9.4
Logan (SRW)	23.9	23.2	16.1	13.4	3.6	11.2	8.6

[a] The value represents the distribution (%) of carbohydrate relative to total carbohydrate obtained from each flour.
[b] Values from Patil et al. (1975b)

Table 4—Distribution of protein in fractions obtained from chromatography on DEAE-cellulose

Pentosan source	Fraction number						
	I	II_a	II_b	III_a	III_b	IV	V
Scout R-70 (HRW)	0	3.5[a]	15.5	16.9	7.8	18.3	38.0
Scout R-69[b] (HRW)	2	4		17	19	33	26
Chris (HRS)	0	3.5	8.9	13.6	25.7	20.2	28.0
Logan (SRW)	0	1.4	4.8	11.0	46.7	23.4	12.7

[a] The value represents the distribution (%) of protein relative to total protein obtained from each flour.
[b] Values from Patil et al. (1975b)

This may account for the carbohydrate and protein values not summing to 100%. Neukom et al. (1962) found 16% protein and 84% carbohydrate in purified pentosans from Manitoba II flour.

Fractionation of purified pentosans on DEAE-cellulose

After fractionation on columns of DEAE-cellulose according to the method of Kuendig et al. (1961a), the purified pentosans from Scout R-70, Chris and Logan flours each yielded the five major pentosan fractions expected. Peak II was separated into two subpeaks, designated II_a and II_b, on the basis of carbohydrate and protein contents. Such behavior has been reported by D'Appolonia et al. (1970) and Patil et al. (1975b). Peak III was separated into III_a and III_b according to protein contents, in agreement with results reported by Patil et al. (1975a). The yield of each fraction is shown in Table 2. Fraction I was obtained in highest yield. The largest amount of fraction I in Scout R-70 flour supports the observation that pentosans from Scout R-70 flour contained the most carbohydrate, since fraction I has been identified as an arabinoxylan (Kuendig et al. 1961a; Wrench, 1965). Other fractions were obtained in much lower yields than fraction I and were composed of carbohydrate and protein. They have been postulated to by glycoproteins (Kuendig et al., 1961a). Lin and Pomeranz (1968) did not find fraction I to be the largest component from two hard red winter wheat flours, a hard red spring wheat flour, one soft red winter, durum, and soft white club flour. Neukom et al. (1962) and Wrench (1965) obtained fraction I as the largest component of their pentosan preparations. Considerably larger proportions of fraction II_b and V and smaller proportions of fractions I and II_a were reported (Patil et al. 1975b) for the pentosans from Scout R-69 flour (Table 2) than we obtained for those from Scout R-70 flour. D'Allolonia and MacArthur (1975) reported less fraction III and more fraction IV in the pentosans from Chris flour (Table 2) than we obtained. The reasons for those differences are not known.

Distribution of carbohydrates and proteins in the fractions are shown in Tables 3 and 4, respectively. As expected, fraction I had the highest carbohydrate content and contained no protein. Fraction III_b from Logan, a soft wheat flour, was remarkedly high in protein content compared with the other two types of wheat. No general protein content trends could be ascertained in the fractions from the three flours, although differences among the pentosans were obvious. The highest protein content in fractions from Scout R-70 and Chris flours was in fraction V, while the highest from Logan flour was in fraction III_b. Additional varieties from each type of wheat must be investigated before valid generalizations can be made. Carbohydrate and protein distribution reported by Lin and Pomeranz (1968) differ considerably from ours. Patil et al. (1975b) reported less carbohydrate in fractions I and II_a and more carbohydrate in fractions II_b and IV from Scout flour (Table 2) than we observed. The total carbohydrate in fraction II (fractions II_a and II_b) was very similar from both Scout flours. Larger percentages of protein were reported in fractions I, III_b, and IV from Scout flour (Patil et al., 1975b) than we obtained, while smaller percentages were reported for fractions II (II_a and II_b in our data) and V (Table 2). Reasons for those differences are not known.

Table 5—Carbohydrate composition (% of total[a]) of acid-hydrolyzed α-amylase-treated pentosans and DEAE-cellulose fractions[b] II_a, II_b, III_a, and III_b

Fraction		Source of pentosans		
		Scout R-70 (HRW)	Chris (HRS)	Logan (SRW)
α-Amylase-treated pentosans	Arabinose	39.3	40.6	40.2
	Xylose	42.6	37.4	35.3
	Galactose	18.1	22.0	24.5
II_a	Arabinose	41.8	43.5	38.6
	Xylose	26.2	32.5	60.7
	Galactose	32.0	24.0	0.7
II_b	Arabinose	43.5	44.7	49.2
	Xylose	29.0	47.4	37.3
	Galactose	27.4	7.9	13.5
III_a	Arabinose	59.8	47.5	54.5
	Xylose	4.2	3.7	2.0
	Galactose	36.0	48.8	43.5
III_b	Arabinose	40.8	19.4	21.0
	Xylose	11.8	20.9	8.8
	Galactose	47.4	59.7	70.2

[a] Summing individual aldoses obtained by GLC analysis
[b] See Table 6 for fractions I, IV and V.

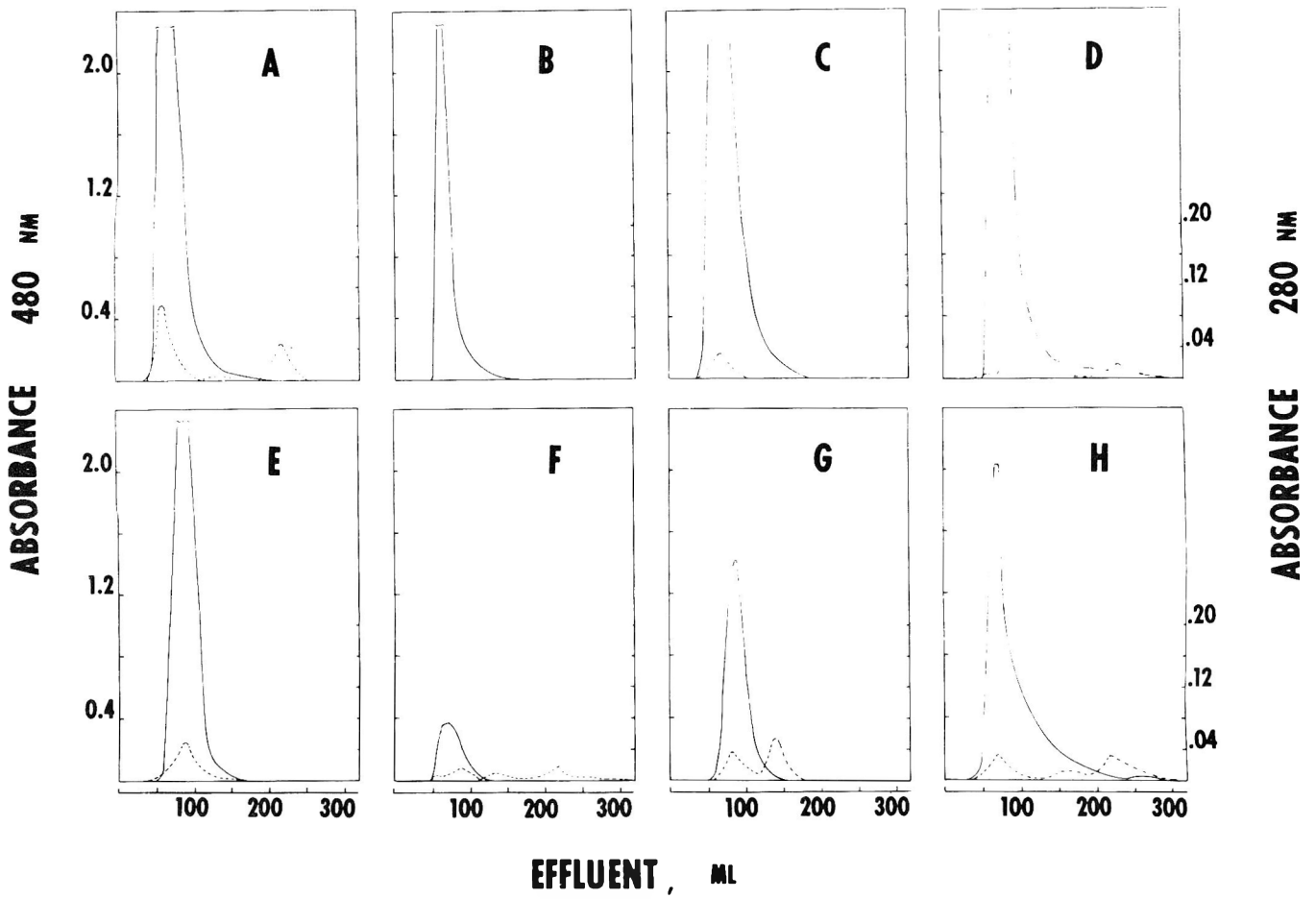

Table 6—Carbohydrate composition (% of total) of acid-hydrolyzed fractions of pentosans eluted from chromatography on DEAE-cellulose

		Investigator[a]											
	Fraction	1	2	3	4	5	6	7	8	9	10	11	12
I	Arabinose	37	37	40	–	43	45	27	40	36	41	40	45
	Xylose	62	63	60	–	49	55	66	60	64	59	60	55
	Galactose	1	0	0	–	0	0	–	–	–	–	–	–
	Glucose	0	0	0	–	0	0	1	–	–	–	–	–
II	Arabinose	43[b]	44[b]	44[b]	43	41	36	32	50	45	47	47	36
	Xylose	27	40	49	49	42	38	66	50	55	53	53	38
	Galactose	30	16	7	8	17	26	–	–	–	–	–	26
	Glucose	0	0	0	0	0	0	5	–	–	–	–	–
III	Arabinose	50[c]	33[c]	38[c]	40	35	46	53	50	50	32	37	46
	Xylose	8	12	5	21	0	0	53	35	45	17	7	–
	Galactose	42	54	57	39	65	54	–	–	5	51	56	54
	Glucose	–	–	–	–	–	–	trace	15	–	–	–	–
IV	Arabinose	42	41	36	38	43	43	45	53	50	32	30	43
	Xylose	6	6	2	6	0	8	50	11	10	8	9	8
	Galactose	52	54	61	56	57	49	1	–	40	60	61	49
	Glucose	–	–	–	–	–	–	3	37	–	–	–	–
V	Arabinose	26	21	8	22	–	17	20	43	42	42	44	17
	Xylose	38	23	25	31	–	81	35	39	46	58	56	81
	Galactose	7	10	7	0	–	2	trace	–	12	–	–	2
	Glucose	27	42	48	47	–	0	13	17	–	–	–	–
	Mannose	2	3	12	0	–	0	–	–	–	–	–	–

[a] 1, 2, 3 Present investigation, source of pentosans from Scout R-70, Chris, and Logan flours, respectively; 4 From Lin and Pomeranz (1968) from HRS (Marquis) wheat flour; 5 from Wrench (1965) from "typical Australian baker's flour;" 6 from Kuendig (1961a) from HRS (Manitoba II) wheat flour; 7 From Cole (1967) from HRW (Montana) wheat flour (sodium hydroxide-soluble flour hemicelluloses); 8, 9 from Medcalf et al. (1968) from durum (Leeds) and HRS (Thatcher) wheat flour, respectively; 10, 11 From D'Appolonia and McArthur (1975) from HRS (Justin and World Seeds 1809, respectively) wheat flours; 12 From Neukom et al. (1962) from HRS (Manitoba) wheat flour.

[b] Values for fractions II_a and II_b averaged

[c] Values for fractions III_a and III_b averaged

Fig. 1—Gel filtration of α-amylase-treated pentosans and DEAE-cellulose fractions from Scout R-70 (HRW) flour. The samples were eluted from a column (2.5 × 40 cm) of Bio-Gel P-150 with 0.1M acetic acid, flow rate 15 ml/hr. Top curve (solid line) represents carbohydrate (phenol-sulfuric acid method). Lower curve (dotted line) represents protein (automatically scanned at 280 nm.). [(A) α-Amylase-treated Pentosans; (B) DEAE-cellulose Fraction I; (C) DEAE-cellulose Fraction II_a; (D) DEAE-cellulose Fraction II_b; (E) DEAE-cellulose Fraction III_a; (F) DEAE-cellulose Fraction III_b; (G) DEAE-cellulose Fraction IV; and (H) DEAE-cellulose Fraction V].

Carbohydrate composition of α-amylase-treated pentosans and DEAE-cellulose fractions

The carbohydrate compositions of the unfractionated, acid-hydrolyzed, α-amylase-treated pentosans from Scout R-70, Chris, and Logan flours are shown in Table 5. Pentosans from Scout R-70 flour differed from those from the other two flours by containing slightly more xylose than arabinose and by having the least galactose. No glucose was found in any purified pentosan before fractionation on DEAE-cellulose.

The carbohydrate compositions of acid-hydrolyzed DEAE-cellulose fractions from Scout R-70, Chris and Logan flours are shown in Tables 5 and 6. Fraction I from Chris and Logan flours was an arabinoxylan as expected, but fraction I from Scout R-70 flour contained a trace of galactose. Fraction II_a from Chris and Scout R-70 flours had similar carbohydrate compositions but that from Logan flour differed considerably by having twice as much xylose and only a very small amount of galactose. Fraction III_a from all three flours had extremely low xylose content. Kuendig et al. (1961a) and Wrench (1965) reported no xylose in fraction III. Fraction III_b from Logan flour had the highest galactose content as well as the highest protein content. In general, in fractions II to IV, a decrease in xylose content was accompanied by an increase in galactose content. The inverse relation between changes in galactose and xylose contents in the fractions and the direct relation between galactose and protein contents might be related to a structure

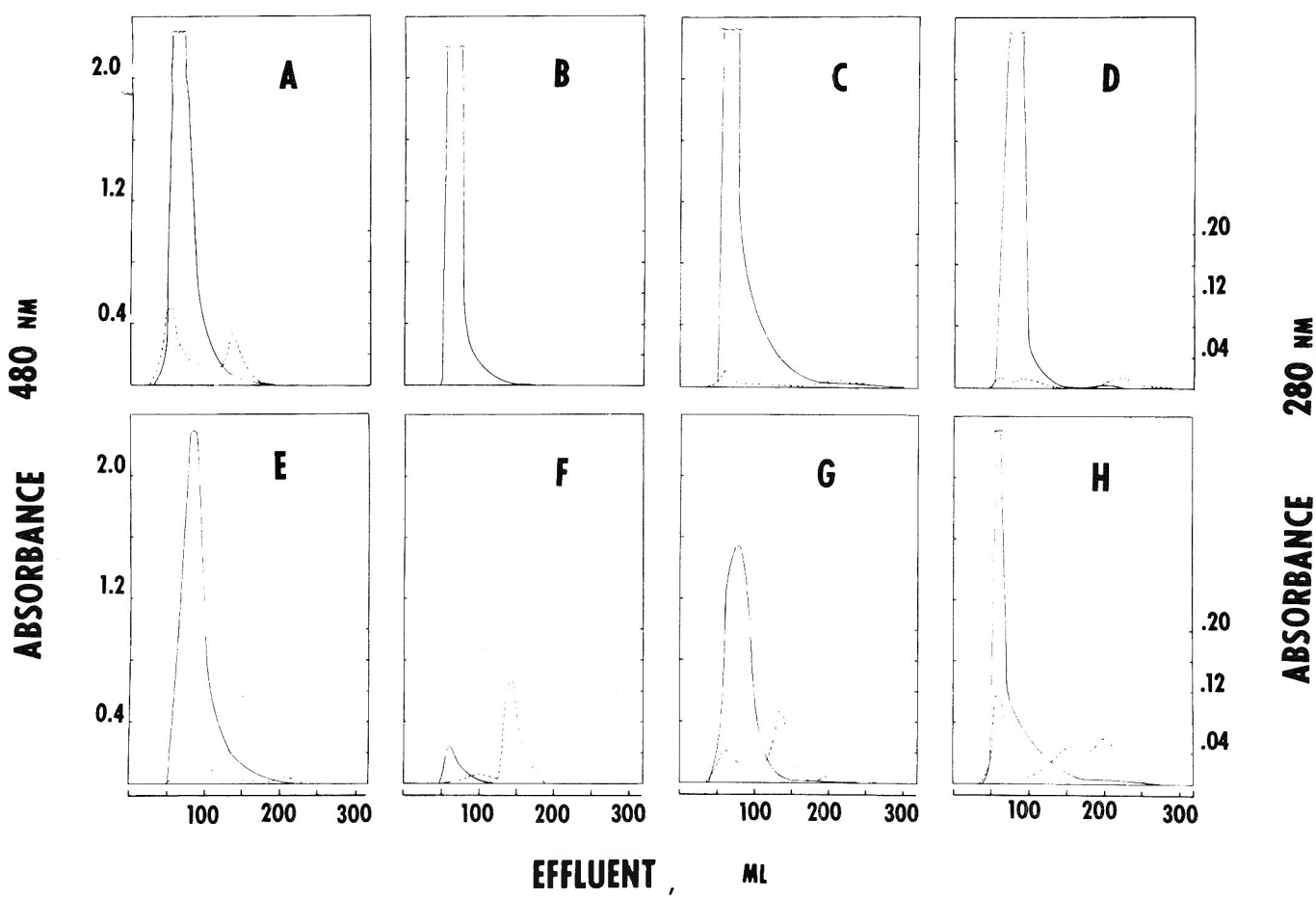

Fig. 2—Gel filtration of α-amylase-treated pentosans and DEAE-cellulose fractions from Chris (HRS) flour. The samples were eluted from a column (2.5 × 40 cm) of Bio-Gel P-150 with 0.1M acetic acid, flow rate 15 ml/hr. Top curve (solid line) represents carbohydrate (phenol-sulfuric acid method). Lower curve (dotted line) represents protein (automatically scanned at 280 nm.). [(A) α-Amylase-treated Pentosans; (B) DEAE-cellulose Fraction I; (C) DEAE-cellulose Fraction II_a; (D) DEAE-cellulose Fraction II_b; (E) DEAE-cellulose Fraction III_a; (F) DEAE-cellulose Fraction III_b; (G) DEAE-cellulose Fraction IV; and (H) DEAE-cellulose Fraction V].

proposed for pentosans (Neukom et al., 1967) of an arabinogalactan linked to an arabinoxylan via a peptide bridge. Fractions with high galactose and protein contents may contain more of such a structure and perhaps less arabinoxylan. An arabinogalactan-peptide has been isolated from the water-soluble pentosans from wheat flour (Fincher and Stone, 1974); its chemical and physical properties have been studied (Fincher et al., 1974). Interestingly, fraction V contained glucose and mannose. Neither of these aldoses was detected in the starting material. They may have arisen from eluting DEAE-cellulose with a fairly strong alkaline solution.

Carbohydrate compositions of fractionated pentosans we obtained from Chris, Logan and Scout R-70 flours are compared with results by other investigators in Table 6. They agree reasonably well. Differences are noted among the three types of wheat, particularly in the distribution of carbohydrate components in fractions II, III, and V. The composition of fraction I, an arabinoxylan, was similar in all pentosans. The degree of branching may differ slightly as indicated by the small variation in arabinose:xylose ratios; increasing arabinose:xylose ratios indicate increased branching since the arabinose units are present as single unit side-chains (Perlin, 1951). Considerable differences are noted in the composition of fraction II from different pentosans, particularly in galactose content. The xylose and galactose contents of fraction III differ among the pentosans. In virtually all the pentosans, fraction IV contains the smallest amount of xylose and the greatest amount of galactose. This fraction may contain the arabinogalactan isolated as a peptide by Fincher (Fincher and Stone, 1974; Fincher et al., 1974) and also reported by Kuendig and Neukom (1963). The composition of fraction V shows large differences among the pentosans. No consistent differences in carbohydrate composition are readily apparent to allow pentosans from different sources to be separated.

Gel filtration of α-amylase-treated pentosans and DEAE-cellulose fractions

Gel filtration of α-amylase-treated pentosans and DEAE-cellulose fractions on a column of Bio-Gel P-150 resulted in the elution patterns shown in Figures 1–3. The elution patterns are similar for the three different wheats, although differences do exist in some of the fractions. It is evident that part of the protein present in most of the fractions is not glycoprotein, since the protein and carbohydrate components do not coincide. Glycoproteins probably are present in all fractions, except 1, as indicated by a symmetrical overlapping of carbohydrate and protein components. It definitely appears that only a limited portion of the pentosans are glycoproteins.

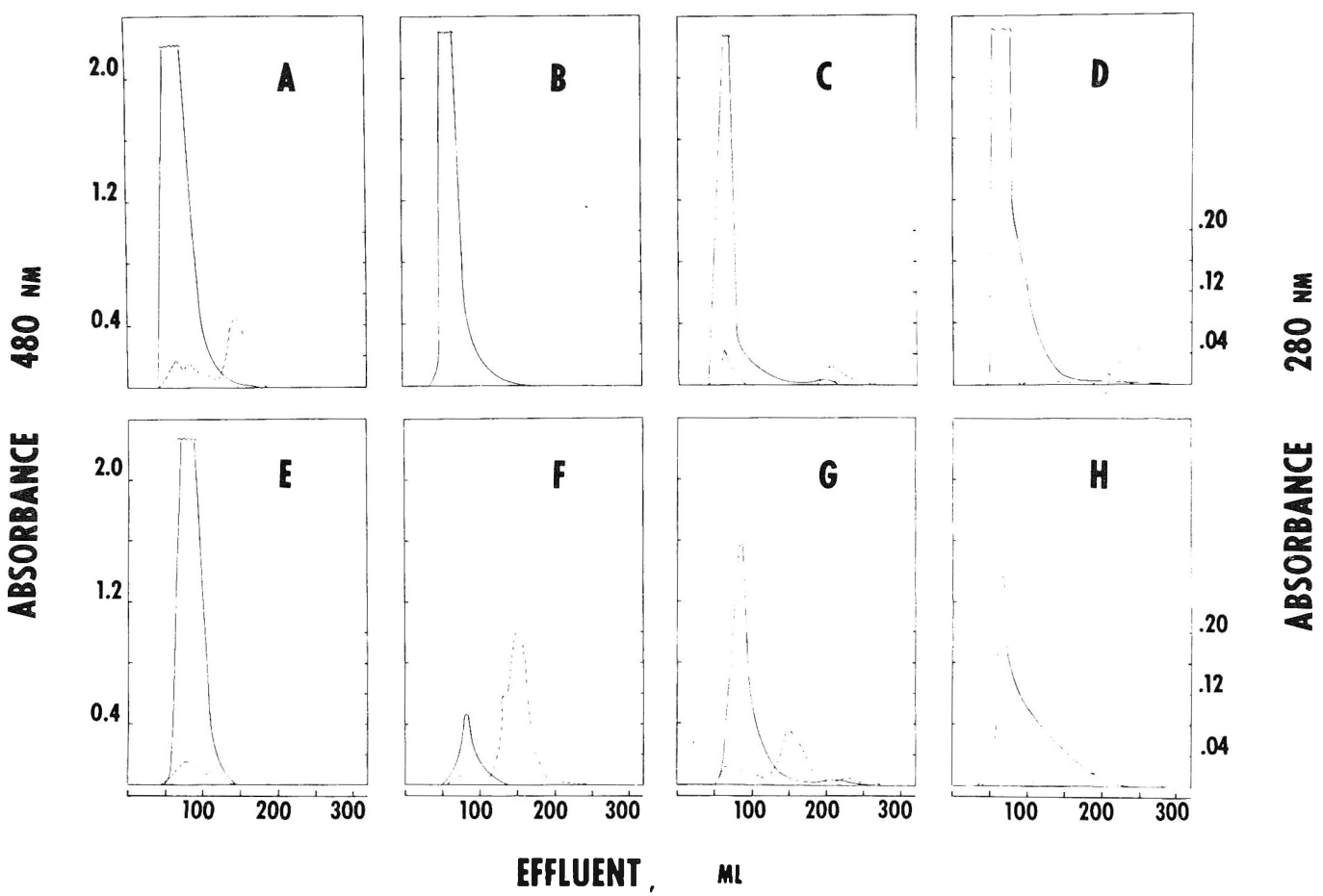

Fig. 3—Gel filtration of α-amylase-treated pentosans and DEAE-cellulose fractions from Logan (SW) flour. The samples were eluted from a column (2.5 × 40 cm) of Bio-Gel P-150 with 0.1M acetic acid, flow rate 15 ml/hr. Top curve (solid line) represents carbohydrate (phenol-sulfuric acid method). Lower curve (dotted line) represents protein (automatically scanned at 280 nm). [(A) α-Amylase-treated pentosans; (B) DEAE-cellulose Fraction I; (C) DEAE-cellulose Fraction II_a; (D) DEAE-cellulose Fraction II_b; (E) DEAE-cellulose Fraction III_a; (F) DEAE-cellulose III_b; (G) DEAE-cellulose Fraction IV; and (H) DEAE-cellulose Fraction V].

Disc electrophoresis

Aqueous solutions of the α-amylase-treated pentosans and DEAE-cellulose fractions were subjected to disc electrophoresis on 5% polyacrylamide gel at pH 4.3 with Sephadex G-25 as the upper spacer. The α-amylase-treated pentosans showed three bands in Scout R-70, six in Chris and five in Logan flours. All bands were stained blue with Coomassie Brilliant Blue dye, a protein stain. Fraction II_a from all three flours showed no bands, perhaps because of its high viscosity when dissolved in the upper buffer, pH 4.5, and its low protein content.

Fraction III_a from all three flours contained four bands when stained with Coomassie Brilliant Blue dye. Fraction III_b from Scout R-70 and Logan flours contained three bands; that from Chris flour, four bands. Fraction IV from Logan and Chris flours showed four bands; that from Scout R-70 flour, three bands. Thus disc electrophoresis also indicated heterogeneity of fractions III through V. In some cases (all fraction III_a, fraction III_b from Chris flour, fraction IV from Scout R-70 flour, and the α-amylase-treated pentosans from Chris and Logan flours) disc electrophoresis revealed greater heterogeneity (more components) than did gel filtration on Bio-Gel P-150. However, fraction II_b from all three flours showed less fractionation with disc electrophoresis than during gel filtration – perhaps because the high viscosity of the solution containing fraction II_b prevented clear separation on the polyacrylamide gel. The remaining fractions (fraction III_b from Logan and Scout R-70 flours, fraction IV from Chris and Logan flours, fraction V from all flours, and the α-amylase-treated pentosans from Scout R-70 flour) showed the same number of protein bands by the two techniques.

SUMMARY

PENTOSANS isolated from Scout R-70 flour contained the most carbohydrate and the least protein. Pentosans from Chris and Logan flours were similar in carbohydrate and protein content. Pentosans from Scout R-70 flour also gave the highest amount of fraction I, an arabinoxylan.

Pentosans are a complex class of materials defined on the basis of solubility. As such they tend to be heterogeneous, lacking well-defined structures. The carbohydrate compositions of fractions of pentosans from different sources are not sufficiently different to separate the pentosans by source. DEAE-cellulose fraction I is an arabinoxylan and fraction IV apparently contains an arabinogalactan. The structures of the other fractions are not known. Glycoprotein components appear to be present in the pentosans but part of the protein is present as nondialyzable protein that is not glycoprotein.

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FUNCTIONAL CHARACTERIZATION OF PROTEIN STABILIZED EMULSIONS: EFFECT OF PROCESSING

ABSTRACT

In order to evaluate the emulsifying characteristics of a protein the effect of processing of a protein stabilized soybean oil-in-water emulsion on the creaming stability of the emulsion was investigated. Three protein systems were used, namely soybean protein isolate, sodium caseinate and whey protein concentrate (WPC). The effects of four different types of emulsifying equipment, an ultra-turrax, a Sorvall omnimixer, a valve homogenizer and an ultrasonic apparatus were studied. It was found that the type of emulsifying apparatus, the emulsifying time and intensity had a marked influence on the properties of the protein stabilized emulsions. It was demonstrated for all the types of apparatus and proteins used, that by increasing emulsifying intensity and time better emulsions, when characterized by the creaming method, are obtained up to a certain limit whereupon there is nothing to be gained by an additional increase of these factors. The necessary emulsifying intensity and time to obtain a certain stability range differed considerably according to the various proteins investigated as well as environmental conditions. This intensity-time dependence may be considered as an emulsifying characteristic of the protein.

INTRODUCTION

PROTEIN STABILIZED oil-in-water emulsions are to be found in various branches of the food industry. These include milk, cream, ice-cream, salad dressings, mayonnaise, gravies and meat emulsions. Ice-cream and meat emulsions differ from "true" emulsions as they contain one or two additional structures such as foam, suspension and gel. Moreover "true" emulsions can differ considerably in oil phase volume, in fat solid/liquid ratio and content and in viscosity of the continuous phase. This leads to different stability problems. It is, therefore, difficult to develop one single model system applicable in all cases for measuring the emulsification properties of a protein.

The determination of the emulsifying characteristics of proteins has evolved via two main approaches: emulsifying capacity and emulsion stability measurements.

The method of Swift et al. (1961) to investigate the emulsifying capacity is to determine the maximum amount of fat emulsified by a protein dispersion. Oil is added at a given rate to a protein dispersion being constantly stirred, until the emulsion inverts into a water-in-oil emulsion, as indicated by a sudden drop in emulsion viscosity. This method has been widely used, though modified in certain respects. The registration of the inversion point varies from visual appearance of the viscosity decrease (Swift et al., 1961; Pearson et al., 1965; Carpenter and Saffle, 1964) to change in amperage required to drive the blender (Crenwelge et al., 1974) and to change in electrical resistance of the emulsion (Webb et al., 1970; Satterlee and Free, 1973; Smith et al., 1973). Alternating blender speed from 1000 up to 20000 rpm has a large effect on both the amount of fat emulsified and the character of the emulsion produced. The amount of fat emulsified is also related to the rate of oil addition and to the maximum temperature attained during emulsification (Swift et al., 1961). All these influencing factors raise doubts about the possibility of evaluating the field of application of a protein in food emulsions by this type of method.

There is a great difference between the emulsifying capacity and the emulsion stability produced with proteins. Emulsion stability is not a measure of maximum oil addition, but rather of the ability of the product to remain durable and unchanged.

Instability of an emulsion can visually appear as creaming and fat separation, which is usually caused by flocculation and coalescence, as shown in Figure 1.

Emulsion stability can be estimated from oil separation (Smith et al., 1973; Inklaar and Fortuin, 1969; Neelakantan, 1971). However, to be able to measure any fat separation from protein stabilized emulsions, "weak" emulsions have to be made – for instance by choosing an oil concentration near the inversion point – or stable emulsions have to be ultra-centrifuged. These criteria limit the research field, and it is questionable whether ultra-centrifugation provides a valid stability test for highly stable emulsions (Kitchener and Musselwhite, 1969). Time-consuming, but more informative, is to register the size distribution of the fat particles as a function of time (Mita et al., 1973; Kako and Sherman, 1974).

Another approach is to measure the extent of creaming as a rapid test to characterize the emulsion produced. Various parameters can be chosen to evaluate creaming, such as depth of cream layer, percentage of the total fat collected in the cream layer (Mol, 1963) or the percentage of the total fat left in the aqueous lower phase (Vakaleris and Sabharwal, 1972; Kurzhals, 1973). The percentage of water (Acton and Saffle, 1970, 1971) or of total solid (Smith and Dairiki, 1975) in the aqueous lower phase can also be recorded. Mild centrifugation is often used to accelerate the creaming rate of the fat particles. The tests vary depending on storage temperature and storage time of the emulsions.

Although the same stability test has been used by several authors it is difficult to compare the results, due to the fact that the formation of the emulsion has been made differently. It has been shown for milk that processing parameters strongly affect the character of the emulsion (Goulden and Phipps, 1969; Kurzhals, 1973; Mulder and Walstra, 1974; Walstra, 1975).

The aim of this work is to investigate the effect of processing of a protein stabilized soybean oil-in-water emulsion on the stability of the emulsion in order to obtain information on the evaluation of the emulsifying characteristics of a protein. Four different types of emulsifying equipment have been studied, as processing conditions may differ considerably for various types of food emulsions. They are an ultra-turrax, a Sorvall omni-mixer, a valve homogenizer and an ultrasonic apparatus. Three protein systems were used, a soy protein isolate (Promine-D), a sodium caseinate and a whey protein concentrate (WPC). In this paper low-viscous emulsions with a fat content of 40% are considered as model systems.

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MATERIALS & METHODS

Soy protein isolate

Promine-D (Central Soya), a commercially available sodium soybean proteinate. Analysis: protein (N × 6.25) 89.6% (dry wt), solubility in distilled water at pH 9, denoted as (0–9), 65%.

Caseinate

Spray blend caseinate (DMV, Holland), a commercially available sodium caseinate. Analysis: protein (N × 6.37) 89.3% (dry wt), fat 1.2% (dry wt), solubility in distilled water and in 0.2M sodium chloride solution at pH 7, denoted as (0–7) and (0.2–7), is 97.4% and 95.4%, respectively.

Whey protein concentrate (WPC)

WPC concentrated by gel filtration on an industrial scale was used. Analysis: protein (N × 6.25) 65.6% (dry wt), fat 1.9% (dry wt). Solubility in distilled water at pH 9, denoted as (0–9), 45%.

Soybean oil

A commercially available soybean oil (AB Karlshamns Oljefabriker, Karlshamn, Sweden) was used. Analysis: fatty acid composition C 18:2 53.3%, C 18:1 23.0%, C 16 10.8%.

Preparation of samples

Protein dispersions composed of 2.5% (w/w) based on the protein content in distilled water or sodium chloride solution were made with the Sorvall omni-mixer. The pH was adjusted with 0.2M NaOH or 0.2M HCl. Soybean oil was added directly to the protein disperison to attain 40% oil by weight.

Emulsion formation

Omni-mixer. A sample of 40g was emulsified in a stainless steel container with a capacity of 50 ml. A Sorvall omni-mixer, consisting of a rotating six-bladed knife suspended into the mixture, was used as a disperser. The rotor speed could be adjusted up to 15000 rpm.

Ultra-turrax. A quantity of 40g was emulsified in a 100 ml centrifuging tube of stainless steel with an Ultra-turrax type TP 18/2N (Janke & Kunkel) driven by a universal motor giving 20000 rpm. The motor speed was adjustable with a variable voltage transformer.

Ultrasonic Emulsions of 30g were formed in a 40 ml tube with a Branson Sonifier Cell Disruptor model B-12 (Branson Sonic Power Co.) consisting of a power supply, a sonic converter and a disruptor horn operating at a nominal frequency of 20 kHz. The power supply had a timing control, an activity output control with a dial marked in arbitrary units from 1 to 10. Before emulsification the mixture was shaken sufficiently to mix the two liquids, and thereafter the disruptor horn was suspended into the emulsion.

Valve homogenizer. Homogenization was accomplished on an aliquot of 50g with a single piston valve homogenizer equipped with a high pressure pump driven by a hydraulic system and having a pressure gauge and an adjustable valve opening (To be published). The mixture was shaken before it was poured into the feeder. Repeated homogenization was carried out.

Cooling was performed during all the emulsification procedures to keep the temperature of the emulsion near 25°C during processing.

Emulsion characterization. In this study the stabality rating (SR) was determined on the basis of the percentage change of fat in the aqueous lower phase after creaming. The following equation was used:

$$SR = \frac{F_{test}}{F_{original}} \times 100\ (\%)$$

F_{test} is the fat percentage of the bottom 5 ml of the sample, and $F_{original}$ is the initial fat percentage of the whole sample. 30g of the emulsion were stored for 24 hr at 20°C. After storage the samples were centrifuged at room temperature at low speed (180 × G) for 15 min. The centrifugation was supposed not to lead to any desorption or removal of proteinacious material from the fat globule interface. Preliminary experiments with different storage times and temperatures and various centrifuging speeds were done to establish conditions giving reproducible results.

After centrifugation of the emulsion 5 ml of the lower phase was carefully removed with a syringe for fat determination by the Gerber method. The Gerber method was calibrated for the soybean oil over the range 0 – 50% (w/w) in the emulsions. No significant deviation of the fat content determination due to the various techniques of emulsion formation was evident.

RESULTS

Effect of different emulsifying equipment

The two mixers, the ultra-turrax and the omni-mixer, running at a maximum speed of 20000 rpm and 15000 rpm, respectively, were used to produce Promine-D (0–9) stabilized emulsions. The resulting stability rating as a function of the emulsifying time can be seen in Figure 2, which obviously shows that emulsions produced by the two mixers can differ considerably in creaming stability. This behavior was most pronounced at shorter emulsifying times, when the difference in creaming stability could be as high as 45%.

Comparisons between the ultra-turrax and the ultrasonic equipment can be made in Figures 3 and 5. The stability rating is plotted as a function of the intensity factor of the emulsifying apparatus; which for the ultra-turrax is the motor speed (emulsifying time of 2 min) and for the ultrasonic equipment is the power supply (emulsifying time of 1 min). The ultrasonic device seemed to be more efficient than the ultra-turrax.

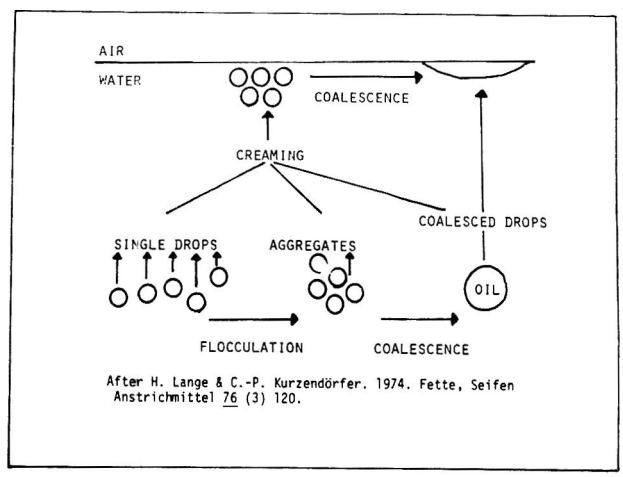

Fig. 1—A schematic representation of emulsion instability.

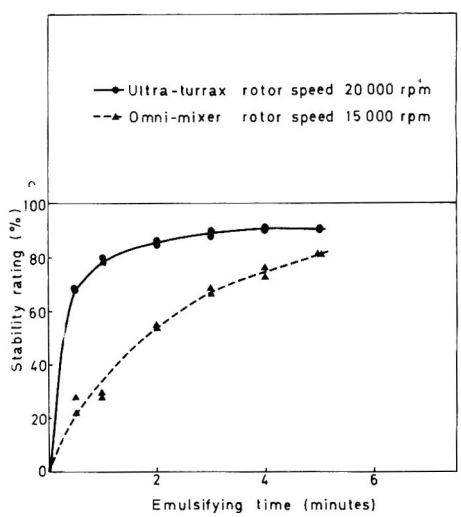

Fig. 2—Stability rating of a Promine-D (0–9) emulsion, emulsified with an ultra-turrax and an omni-mixer running at 20000 rpm and 15000 rpm, respectively, as a function of the emulsifying time.

The differences in creaming stability are pronounced for the WPC (0–9) and the caseinate (0.2–7) emulsions. It is also interesting to note that the WPC (0–9) emulsion had a higher stability score than the caseinate (0.2–7) emulsion when produced in the ultrasonic device, whereas the reverse was found when the emulsions were produced in the ultra-turrax.

The intensity factor of valve homogenization is the pressure drop and this has been varied for a caseinate (0.2–7) emulsion. The derived creaming stabilities when emulsifying for three minutes are plotted in Figure 6. This figure clearly shows that valve homogenization is more effective compared to the ultra-turrax in giving emulsions of high creaming stability.

Effect of emulsifying time and intensity

So far it has been shown that the type of emulsifying apparatus being used strongly influences the creaming stability of the protein emulsions. In determining the characteristics of the formed emulsion, the emulsifying time and intensity also play an important role, which is illustrated in Figures 2 to 7.

The dependence of stability rating on processing time can best be seen in Figures 2 and 7. In Figure 7 caseinate dispersions of various ionic strength were used for emulsification in the valve homogenizer at two pressures, 7.5 and 15 MPa. In Figure 4 the resulting creaming stabilities of Promine-D (0–9) emulsions are plotted against various ultrasonic intensities, where the different curves represent various emulsifying times. The figures show that prolonged emulsification gave more creaming stable emulsions up to a certain limit whereupon nothing further could be gained in stability with time. The upper limit of stability rating is dependent on the apparatus used, the emulsifying intensity, the type of protein and environmental factors such as pH and ionic strength.

Increasing emulsifying intensity also gives rise to more creaming stable emulsions, which is clearly demonstrated in Figures 3, 4, 5 and 6 for all the types of apparatus and protiens used. In the case of emulsifying intensity a limit of creaming stability is also attained where the curve flattens out. This feature, however, varies according to the apparatus used, as can be seen in the case of caseinate (0.2–7): a stability rating of about 90% is achieved at the higher intensities with

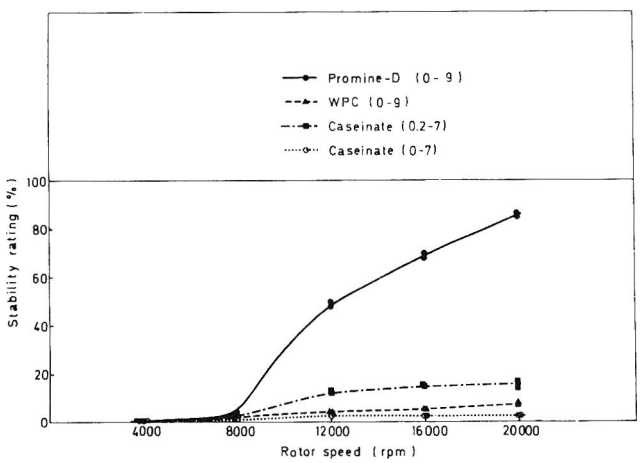

Fig. 3–Stability rating of different protein stabilized emulsions, emulsified for 2 min with an ultra-turrax, as a function of the rotor speed.

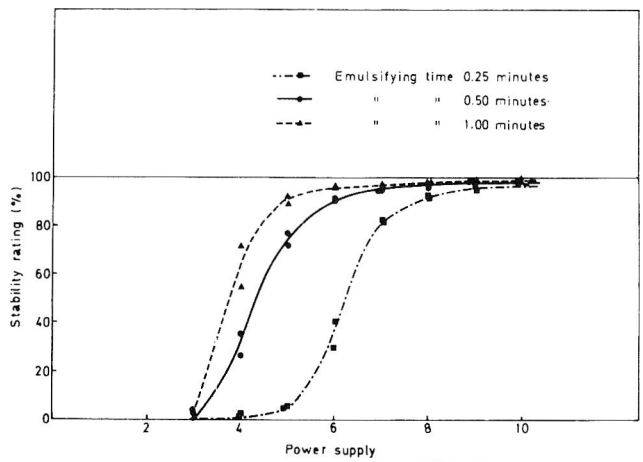

Fig. 4–Stability rating of a Promine-D (0–9) emulsion, emulsified for various times, as a function of the ultrasonic power supply.

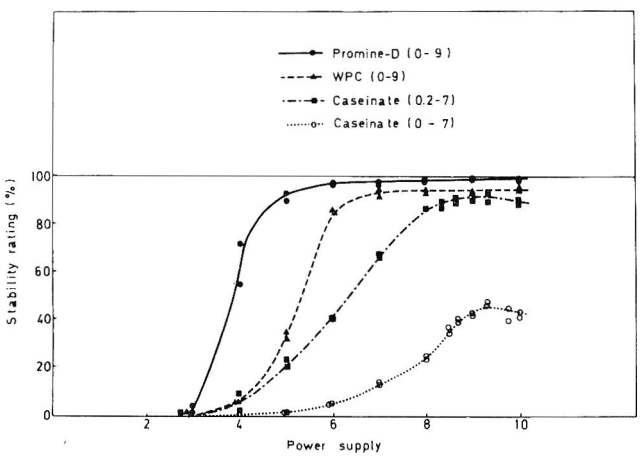

Fig. 5–Stability rating of different protein stabilized emulsions, emulsified for 1 min, as a function of the ultrasonic power supply.

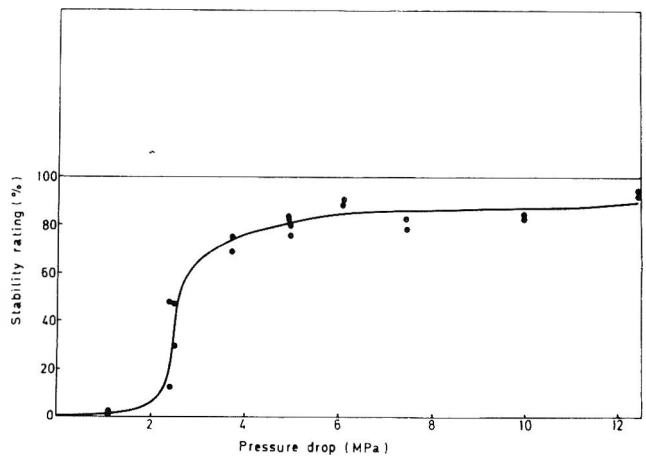

Fig. 6–Stability rating of a caseinate (0.2–7) emulsion, valve homogenized for 3 min, as a function of the pressure drop.

the ultrasonic and the valve homogenizer, whereas the greatest stability rating obtained with the ultra-turrax is about 15%.

Effect of protein and ionic strength

Although increasing emulsifying time and intensity could give better emulsions, the intensity-time dependence differed considerably according to the protein used and the environmental conditions. This is clearly demonstrated in Figures 3, 5 and 7.

Comparisons between the proteins used as stabilizers can be made from Figures 3 and 5. It can be seen that the Promine-D (0–9) emulsions needed less intensity and shorter times to reach a stability rating of 90% than all the other protein stabilized emulsions when emulsified with the ultra-turrax or the ultrasonic device. On the other hand, caseinate (0–7) emulsions required a lot of energy input to achieve stabilities as low as 20%, whereas WPC (0–9) and caseinate (0.2–7) emulsions had an intensity-time dependence somewhere between the two first mentioned proteins.

The creaming stability of the emulsion in the case of caseinate as a stabilizer showed a strong dependence on changes in ionic strength. Increasing the ionic strength to 0.2, drastically improves the intensity-time dependence for all the types of apparatus used, as is clearly visible in Figures 3, 5 and 7.

DISCUSSION

THE RESULTS obtained in this investigation have demonstrated that it can be hazardous to draw any general conclusions about the stabilizing power of a protein when emulsifying with only one apparatus at a fixed intensity and time level. The emulsifying apparatus used in investigations on protein stabilized emulsions varies from mixers of different outfit (Mita et al., 1973; Neelakantan, 1971; Inklaar and Fortuin, 1969; Smith et al., 1973) to valve homogenizers (Acton and Saffle, 1970, 1971; Vakaleris and Sabharwal, 1972) and to ultrasonic equipment (Smith and Dairiki, 1975). Comparisons between the results obtained from these different investigations are therefore difficult to make.

Mulder and Walstra (1974) have already pointed out that mixers usually employed in emulsification give globules of the order of 10 μm, whereas the great "power per unit volume" generated in valve homogenizers and in ultrasonic machines generally produces very small particles. Our results also showed a better emulsifying efficiency in using the valve homogenizer or the ultrasonic device than the ultra-turrax.

Our findings that WPC, caseinate and Promine-D under certain conditions were good emulsifiers had already been reported by several authors (Vakaleris and Sabharwal, 1972; Smith and Dairiki, 1975; Pearson et al., 1965; Acton and Saffle, 1970, 1971; Inklaar and Fortuin, 1969; Guy et al., 1972). Comparisons can hardly be made, as no or little attention has been paid to the emulsification procedure in these investigations.

The stability-time and the stability-intensity dependence of the proteins used in this investigation showed similar behavior. At a certain emulsification intensity or time the creaming stability started increasing but this tendency levelled out with additional intensity or time. Hence there is nothing to gain in creaming stability by increasing emulsifying intensity or time past a certain limit. Another feature, not observed to any extent for our protein stabilized emulsions in the range studied, is that an emulsion can be overprocessed, i.e., continual processing or excessive pressure will increase the particle size.

A protein being a biopolymer, has the possibility to adsorb on a surface at many points as illustrated by Figure 8. The number of attachments can vary to a greater or lesser extent depending on such factors as time, protein concentration, oil phase volume etc. If a protein is attached to an interface at many points and the adsorption at each individual site is reversible, the activation energy for desorption of the whole molecule is still very high, because the probability of all sites to desorb at the same moment is extremely small. Therefore, the adsorption of the protein as a whole often can be considered as irreversible. The irreversible adsorption of the protein to the fat surface can explain why the character of a protein stabilized emulsion is so strongly influenced by the emulsification procedure, as all irreversible processes are dependent on the pre-history. Lankveld and Lyklema (1972) have clearly demonstrated with a polymer, polyvinylalcohol, as stabilizer for a paraffin-in-water emulsion, that the properties of the emulsion are primarily determined by the kinetics of the emulsification process, as also suggested by the results of this study.

Evidently, a wider approach in investigation proteins as stabilizers for emulsions is needed, in order to get any comparative results. A factor describing the need of emulsifying time and intensity to reach 90% stability for a given protein could perhaps be a better description of the emulsifying characteristics of the protein. To be able to compare between different

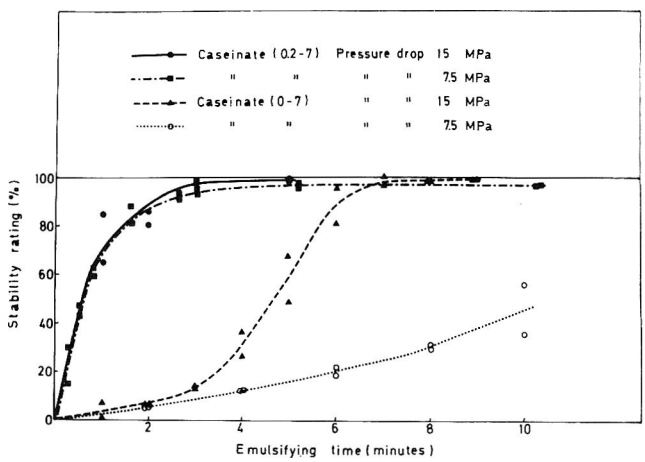

Fig. 7—Stability rating achieved, when valve homogenizing caseinate (0–7) and caseinate (0.2–7) emulsions at different pressures, as a function of the emulsifying time.

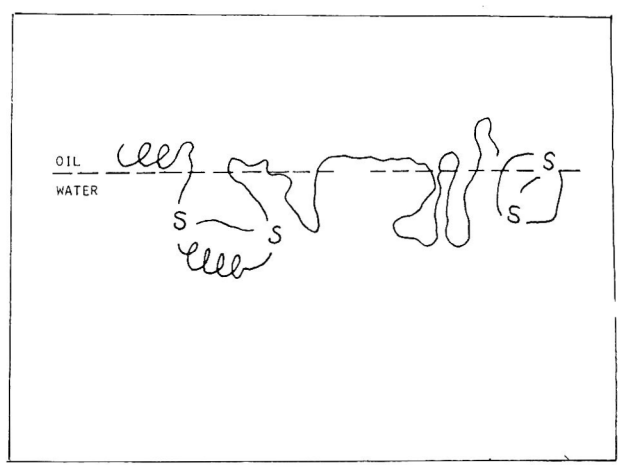

Fig. 8—A schematic representation of the structure of a protein adsorbed at an interface.

types of emulsifying apparatus with respect to their emulsifying efficiency, flow conditions, effect and energy input in the emulsifying unit must be controlled. These problems are under investigation.

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CALCIUM BINDING AND ITS EFFECT ON THE PROPERTIES OF SEVERAL FOOD PROTEIN SOURCES

ABSTRACT

A study was conducted to evaluate the ability of four protein sources to bind calcium during an alkaline, calcium saturated incubation. Leaf protein concentrate and gluten bound the most calcium. Soy protein isolate and bovine blood protein isolate (plasma fraction) bound less calcium but both had large (80%) reductions in sodium content. Phytic acid was prevalent in both soy protein isolate and gluten, and was found to be entirely in their residue protein fractions after Ca binding. After incubation, essential amino acid losses were noted for all protein fractions, yet the losses were not significant. Following Ca binding, changes in molecular weights of the soluble proteins were noted as determined by gel filtration chromatography. Isoelectric points were noted to decrease in the soluble protein fractions, following Ca binding.

INTRODUCTION

INGESTION of excessive amounts of sodium over long periods of time has been shown to cause irreversible hypertension in rats (Dahl and Schockrow, 1964). Even though there is controversy concerning the exact role of sodium in human hypertension (Lancet, 1975), there is a general concern over prolonged high daily sodium intake by humans.

As the human consumption of plant protein concentrates and isolates increases, these food sources will contribute large amounts of sodium to the human diet. Sodium is introduced into the proteinate during alkaline extraction. At present, calcium proteinates are produced only for therapeutic diets and are quite expensive. A possible alternative is to exchange calcium for sodium in an existing and less expensive sodium proteinate. Secondly, with the decrease in consumption of milk products, the added calcium could contribute to the development of a new dietary calcium source.

Appu Rao and Narasinga Rao (1975) have described the ability of the 11S fraction of soybean protein to bind calcium. These authors found insignificant binding at pH 5.5, but substantial binding at pH 7.8. They demonstrated that the imidazole groups of the histidine residues were the binding site for the calcium ions. It was noted that phytate impurities, inherent in plants, also bind calcium ions. Saio et al. (1968) described the effect of phytic acid on the combination of calcium and soybean meal protein. Their results indicated that the extent of calcium binding increased as the content of meal phosphorus compounds, such as phytic acid, increased.

This study reports the ability of four protein sources: soy protein isolate, wheat gluten, leaf protein concentrate and bovine blood protein isolate (plasma fraction); to bind calcium with concurrent removal of sodium and the effect of pH and temperature on this binding. It was of interest to evaluate the biochemical changes that occurred during the binding of calcium, since De Groot and Slump (1969) reported a detrimental effect on proteins exposed to excessive alkali and temperature treatments, with corresponding formation of lysinoalanine and reduction in nutritive value.

MATERIALS & METHODS

Protein sources

Soy protein isolate (Promine D) was supplied by Central Soya Co., Chicago, Ill. Wheat gluten was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Leaf protein concentrate processed from alfalfa was prepared by the method of Free and Satterlee (1975). Bovine blood protein isolate (plasma fraction), prepared by the method of Tybor et al. (1975), was supplied by Dr. C.W. Dill, Texas A & M University. Protein contents were determined by the Kjeldahl method (AOAC, 1975).

Effect of pH on Ca binding

The equivalent of 1g of protein from each source was incubated separately in a calcium ion rich system containing 100 ml distilled-deionized water and 0.02% sodium azide, a bactericide. The protein was allowed to solubilize for 15 min before titration to the appropriate pH with a saturated $Ca(OH)_2$ solution. The pH conditions ranged from 7–11 for each source studied. Differences in calcium ion concentration among the various pH conditions were adjusted with a $CaCl_2$ solution to achieve a final calcium ion concentration of 49 mg%. All calcium solutions were monitored for exact calcium ion content by atomic absorption spectrophotometry. The calcium rich protein solutions or suspensions were incubated for 36 hr at the specified temperature. Protein solubility was measured by the method of Lawhon and Cater (1971) with the following modifications: (1) after incubation, a final pH adjustment was made (if needed) with 2M KOH, and the solution was centrifuged for 20 min at 27,000 × G; (2) a 1-ml aliquot of the supernatant was diluted to 25 or 50 ml with distilled-deionized water and water soluble protein was measured by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

The supernatant fraction obtained after centrifuging the incubation mixture was freed of unbound Ca and Na ions by ultrafiltration using 500 ml of a 10 mM tris buffer of the appropriate pH, in an Amicon Model 52 ultrafiltration chamber with a PM 10 ultrafiltration membrane. The above procedure was based on preliminary studies, which demonstrated that 99+% of the total removable calcium ions were removed during the ultrafiltration process with this specific volume of buffer. The washed protein solution was then lyophilized and stored for later analysis.

The insoluble residue fraction was resuspended twice in 20 ml of 10 mM tris buffer of the same pH, and recentrifuged for 20 min at 27,000 × G. The residue was collected, lyophilized and stored for later analysis.

After preparation of both residue and supernatant fractions for each pH value, a portion of each sample was ashed in a muffle furnace at 500°C for 12 hr. The ashed samples were dissolved in 50 ml 6N HCl in preparation for atomic absorption spectrophotometry.

Atomic absorption spectrophotometry

All atomic absorption analyses were conducted using a Perkin-Elmer Model 303 atomic absorption spectrophotometer. Calcium standards ranging from 0.1–10 μg/ml were prepared, as well as sodium standards ranging from 0.1–4 μg/ml. Final sample dilutions were made using a 1% lanthanum and a 1.5 mM lithum (I) solution for the calcium and sodium samples, respectively.

Temperature effect on Ca binding

Following analysis of the pH effect on Ca binding to the protein samples, an optimal pH was chosen and used for temperature studies at

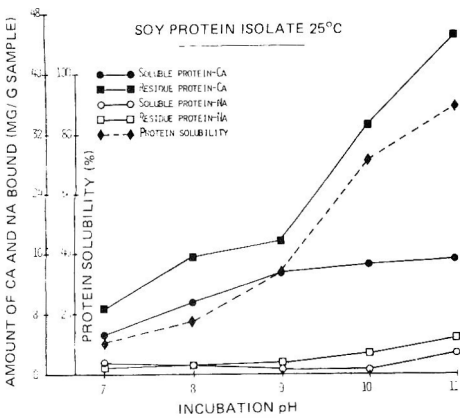

Fig. 1—Effect of incubation pH w/excess Ca^{++} on amount of Ca-Na binding and solubility of soy protein isolate.

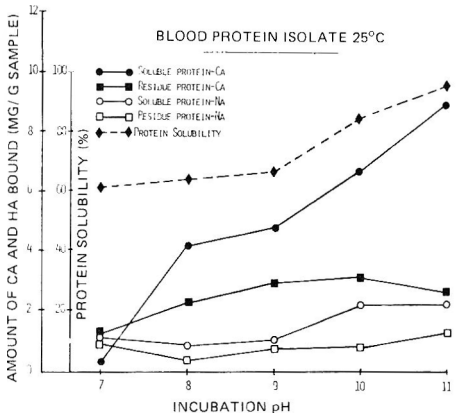

Fig. 2—Effect of incubation pH w/excess Ca^{++} on amount of Ca-Na binding and solubility of blood protein isolate.

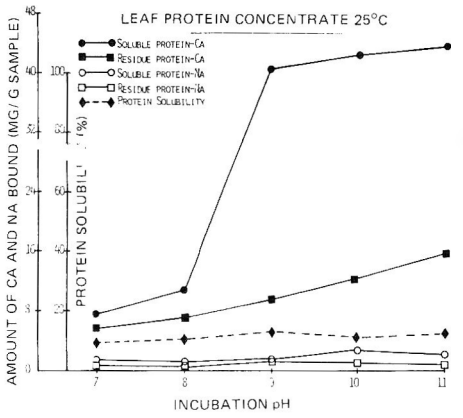

Fig. 3—Effect of incubation pH w/excess Ca^{++} on amount of Ca-Na binding and solubility of leaf protein concentrate.

25°, 35° and 45°C. All Ca incubation parameters and procedures were identical to those described for the pH studies, except that the incubation temperature was held constant throughout all steps of the removal of unbound Na and Ca ions.

Phytate determination

Phytate was isolated from the soy protein isolate, leaf protein concentrate and gluten, and their fractions from Ca binding by the methods of Common (1940) and Singsen et al. (1950), as modified by Hansmeyer (1976). Phosphorus was determined spectrophotometrically as described by Fiske and Subbarow (1925).

Amino acid composition

Amino acid compositions of all protein sources and their fractions after Ca binding were determined using a Beckman 120C amino acid analyzer. All amino acids except methionine, cysteine and tryptophan were obtained by a 6N HCl hydrolysis for 24 hr at 110°C. The sulfur amino acids were determined using a performate-HCl hydrolysis as specified by Moore (1963). Tryptophan was determined using a $Ba(OH)_2$ hydrolysis as described by Pataki (1968).

Molecular weight determination

Protein molecular weights were obtained by gel filtration chromatography for the soluble fraction of the original protein sources using a 10 mM tris buffer, pH 7.0. Solubilization was accomplished by stirring the protein source in the buffer for 30 min, followed by centrifugation to remove suspended particles. Ten mg of the Ca-incubated, lyophilized, supernatant fraction samples were dissolved in 1 ml distilled-deionized water. One ml of sample was applied to 0.9 × 60 cm columns of Ultrogel AcA 34 and AcA 54 (LKB Instruments, Inc.), which were used to separate the large and small molecular weight proteins, respectively. Bovine serum albumin (BSA), bovine metmyoglobin, eggwhite lysozyme, and bovine insulin were used to calibrate the AcA 54 column. Urease, catalase, and BSA were used to calibrate the AcA 34 column. A flow rate of 0.20 ml/min was used on both columns and was controlled by an LKB Instruments, Inc., peristaltic pump.

Isoelectric focusing

Isoelectric focusing of the soluble proteins from the original protein source and the Ca-binding supernatant proteins was conducted in 7% polyacrylamide gels using pH 3.5–10 ampholyte (LKB Instruments, Inc.), as described by Wrigley (1968). Focusing was performed in 125 × 5 mm glass tubes for approximately 3 hr at 300V. Protein staining of the gels took 24 hr using a 0.02% Coomassie Blue stain containing 18% methanol, 5% trichloroacetic acid, and 5% sulfosalicyclic acid. Individual pH gradients were obtained by transversely slicing a duplicate gel into equal portions. Each gel slice was placed in 1 ml distilled-deionized water for 3 hr, then the pH of each slice was measured using a Corning Model 109 digital pH meter.

RESULTS & DISCUSSION

Effect of pH on protein solubility

Soy protein isolate (SPI) demonstrated a very pH dependent solubility curve, increasing from 10.6% at pH 7 to 89.9% at pH 11 (Fig. 1). The solubility of blood protein isolate (BPI) increased from 61.2% at pH 7 to 95.6% at pH 11, with the major increase in solubility occurring just above pH 9 (Fig. 2). Leaf protein concentrate (LPC) was only slightly soluble throughout the pH range studied (Fig. 3), with 9.4% solubility at pH 7 and 13.0% at pH 11. Gluten solubility decreased from 14.3% at pH 7 to 10.6% at pH (Fig. 4), with a large increase in solubility to 39% between pH 9 and pH 11. SPI and BPI were both highly soluble proteins, gluten was moderately soluble, and LPC was only slightly soluble.

Effect of pH on Ca binding

Soy protein isolate had original Na and Ca levels of 10.0 and 2.5 mg/g, respectively. After incubation in the presence of excess Ca, both the insoluble proteins (residue) and soluble proteins demonstrated higher calcium levels. The residue protein fraction bound considerably more Ca than did the soluble protein fraction (Fig. 1). The calcium content of the soluble fraction increased to 5.2 mg Ca/g at pH 7 and to 15.3 mg Ca/g at pH 11. The residue fraction increased to 8.5 mg Ca/g and to 45.5 mg Ca/g at pH 7 and pH 11, respectively. The sodium level for the soluble fraction was below 2.6 mg Na/g for all pH levels. The same was found for the residue fraction through pH 9, with a slight increase to 4.5 mg Na/g at pH 10 and pH 11. When considering optimal protein solubility of SPI, pH 9 was chosen for temperature studies. At this pH the calcium content of the Ca-incubated soluble and residue protein fractions were 548% and 727% above the level in the original protein. Sodium levels in the fractions were reduced to near or below 10% of the original Na level.

Blood protein isolate had original Na and Ca levels of 10.0 and 0.9 mg/g, respectively. The soluble protein fraction had a greater ability to bind Ca ions, when compared to the residue proteins (Fig. 2). At pH 7, the soluble proteins contained only 0.3 mg Ca/g, but increased to 8.95 mg Ca/g at pH 11. The residue proteins at pH 7 contained 1.2 mg Ca/g, and increased to only 2.6 mg Ca/g at pH 9 and pH 10. Sodium levels for the soluble proteins were near 1.0 mg Na/g through pH 9, but

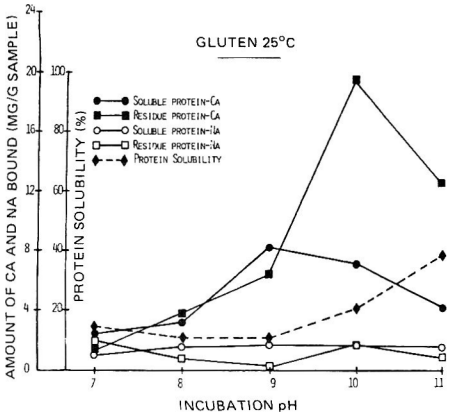

Fig. 4—Effect of incubation pH w/excess Ca^{++} on amount of Ca-Na binding and solubility of gluten.

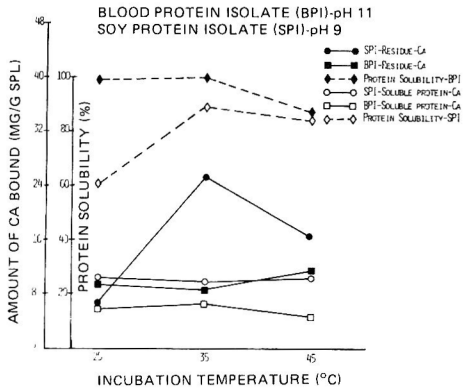

Fig. 5—Effect of incubation temperature w/ excess Ca^{++} on amount of Ca binding and solubility of blood protein isolate (pH 11) and soy protein isolate (pH 9).

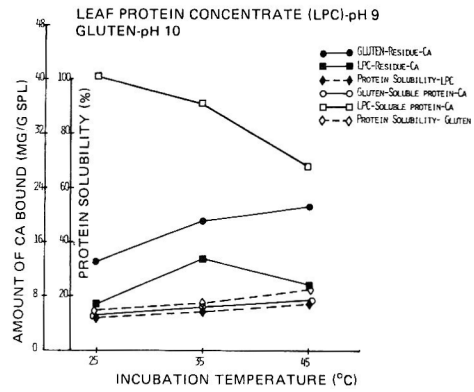

Fig. 6—Effect of incubation temperature w/ excess Ca^{++} on amount of Ca binding and solubility of leaf protein concentrate (pH 11) and gluten (pH 10).

increased to 2.1 mg Na/g at pH 10 and pH 11. In the residue proteins Na levels were slightly lower, ranging from 0.3 mg Na/g at pH 8 to 1.3 mg Na/g at pH 11. Based on these data, pH 11 was chosen for the temperature study. At this pH the calcium level of the soluble and residue proteins increased above the original levels, 1017% and 290%, respectively. Sodium levels in both fractions were reduced to less than 20% of the original value in the BPI.

The original Na and Ca levels of LPC were 1.2 and 1.6 mg/g, respectively. The soluble proteins bound considerably more Ca than did the residue proteins (Fig. 3), but this soluble fraction was rather insignificant when protein solubility was considered. The soluble proteins bound 7.7 mg Ca/g and 10.7 mg Ca/g at pH 7 and pH 8, respectively. A significant increase in binding was seen (40 mg Ca/g) at pH 9, pH 10 and pH 11. The residue proteins responded in a linear fashion, ranging from 5.9 mg Ca/g at pH 7 to 15.8 mg Ca/g at pH 11. Sodium levels for the soluble proteins were consistently near 2.0 mg Na/g, with the residue proteins having less than 1.0 mg Na/g. After considering solubility and Ca binding, pH 11 was chosen for temperature study. At this pH the Ca level of the soluble and residue fractions excelled the original level, 2655% and 966%, respectively. Sodium content was slightly higher for the soluble proteins when compared to the original level. The residue's Na level was reduced by 56%.

Gluten had original Na and Ca levels of 0.9 and 3.1 mg/g, respectively. The residue proteins demonstrated a greater ability to bind Ca. This binding was linear through pH 9, reaching 6.7 mg Ca/g at that pH (Fig. 4). A sharp increase in Ca binding occurred at pH 10, with maximum binding (19.4 mg Ca/g) then decreased at pH 11 to 12.2 mg Ca/g. The soluble protein Ca-binding curve was similar to the residue protein Ca-binding curve, but reached a maximum at pH 9 (8.5 mg Ca/g). Sodium levels for both protein fractions ranged between 1.9 mg Na/g and 0.3 mg Na/g. Due to the high Ca binding at pH 10 by the residue proteins, this pH was chosen for the temperature study. At this pH, the Ca levels of the soluble and residue proteins were 235% and 637% higher than the level in the original gluten. Both protein fractions increased their Na contents about 100%; however, the final levels of Na were not considered high. The 100% increase in Na content is due to the lower limit of the ultrafiltration system to remove this ion; the Na ions were contributed by the sodium azide used in each incubation.

Effect of temperature on Ca binding

The SPI at pH 9 generally exhibited the same types of curves for both solubility and residue protein Ca binding (Fig. 5). Protein solubility increased from 60.1% at 25°C to 89.7% at 35°C, then decreased to 84.5% solubility at 45°C. The amount of Ca bound to the residue proteins increased from 12.7 mg Ca/g at 25°C to 26.1 mg Ca/g at 35°C, and dropped to 17.7 mg Ca/g at 45°C. The soluble proteins were not significantly affected by temperature, with all Ca levels near 10.0 mg Ca/g.

The BPI at pH 11 (Fig. 5) was very soluble (near 100%) at both 25°C and 35°C, but decreased to 87.2% at 45°C. The residue proteins responded linearly to temperature and extent of Ca binding (8.5 mg Ca/g at 25°C), increasing to 11.7 mg Ca/g at 45°C. The soluble proteins remained stable, having 6.0 mg Ca/g at both 25° and 35°C, then decreasing to 4.7 mg Ca/g at 45°C.

The LPC solubility (Fig. 6) was only slightly affected by temperature, increasing from 11.3% at 25°C to 16.5% at 45°C. The soluble proteins showed decreased Ca-binding ability as temperature increased, from 40 mg Ca/g at 25°C to 27 mg Ca/g at 45°C. The more prevalent residue fraction exhibited an increased Ca-binding ability at 35°C, from 6.7 mg Ca/g at 25°C to 13.6 mg Ca/g, decreasing at 45°C (10.0 mg Ca/g).

The gluten protein solubility (Fig. 6) at pH 10 was slightly elevated with increased temperature, from 14.8% at 25°C to 22.9% at 45°C. The Ca binding of the residue proteins increased from 13.0 mg Ca/g at 25°C to 21.0 mg Ca/g at 45°C. The soluble proteins Ca-binding ability increased from 4.6 mg Ca/g at 25°C to 6.9 mg Ca/g at 35°C, decreasing to 6.6 mg Ca/g at 45°C.

It is clear that all four protein sources have an ability to bind calcium and that the extent of binding is pH dependent. Temperature effects upon Ca binding are not as clear nor as consistent. But the amounts of calcium bound to these proteins could easily allow for their use as new calcium sources in the human diet.

The Ca binding process resulted in an exchange between calcium and sodium with SPI and BPI, causing a reduction in sodium and an increase in calcium contents. However, with the LPC and gluten, when sodium was low initially, there was no exchange. This appears to be caused by the ability of these systems, with their low sodium content, to rapidly bind Ca.

Phytate content

Both Saio et al. (1968) and Appu Rao and Narasinga Rao (1975) indicate that phytic acid contained in proteins of plant origin can effectively bind calcium ions. Therefore, it was of interest to evaluate the phytate content of the three plant proteins and determine the final distribution of the phytate in the protein fractions from Ca binding. The phytic acid content of SPI and gluten were found to be 1.15 and 0.60 mg phytic acid phosphorus/g of sample, respectively (Table 1). The LPC contained no phytic acid. Analysis of both residue and soluble fractions of incubated SPI and gluten showed that all phytic acid was in the residue fractions.

Final distribution of the phytate contained in SPI and gluten in the residue proteins substantiates the increased Ca binding seen in these fractions, when compared to their soluble protein counterparts.

Table 1—The phytic acid content of the three plant proteins and their fractions

Protein source	mg Phytic acid phosphorus gram sample
Soy isolate	1.15
Soy isolate soluble fraction[a]	0.0
Soy isolate residue fraction[a]	1.78
Gluten	0.60
Gluten soluble fraction[b]	0.0
Gluten residue fraction[b]	0.53
Leaf protein concentrate and its fractions	0.0

[a] Extracted with 10 mM tris, pH 9.0 at 25°C in the presence of excess Ca.
[b] Extracted with 10 mM tris, pH 10.0 at 35°C in the presence of excess Ca.

Amino acid compositions

Based on the research reported by De Groot and Slump (1969) and the prolonged alkali treatment used during the Ca incubation, changes in the amino acid compositions of the four proteins were expected to occur. To detect any such changes, the amino acid compositions of the original proteins and their Ca-incubated fractions were determined.

All comparisons were made back to the amino acid content of the original protein source. Samples analyzed were chosen based upon the sample's greatest Ca binding and the least severe treatment; therefore, the SPI sample incubated at 25°C was analyzed. The only amino acid losses by the proteins of SPI (at pH 9, 25°C) were a 10.8% lysine loss from the residue proteins and a 14.3% loss of tryptophan from the soluble proteins (Table 2). A very small quantity of lysinoalanine (LAL) was qualitatively detected in the original SPI; however, slightly increased quantities of this amino acid were found in the residue proteins, possibly explaining the lysine loss. The SPI soluble proteins did not form this amino acid.

The residue proteins of BPI (pH 11, 35°C) incurred losses of lysine and methionine + cysteine, of 15.0% and 16.8%, respectively. No formation of LAL was noted. The soluble proteins lost small but near equal amounts of lysine and cysteine + methionine, and a small amount of LAL was detected, possibly the product of lysine and cysteine reaction. Trace amounts of LAL were found in the original BPI protein.

The LPC (pH 11 at 35°C) proteins had the most substantial overall loss of amino acids, especially the residue proteins which had losses of 24.8, 14.5, 14.2, and 16.4% for lysine, methionine + cysteine, isoleucine, and valine, respectively. The soluble proteins lost 17.1% isoleucine, 16.7% leucine, and 13.0% tyrosine + phenylalanine. No significant quantities of LAL were detected in either of the protein fractions or original protein source.

The residue proteins of gluten (pH 10, 35°C) had a substantial loss of lysine (21.5%), but no formation of LAL. The soluble proteins had small losses of all amino acids except the sulfur containing and tryptophan. However, only the lysine and threonine losses were significant, at 26.5 and 11.6%, respectively. The gluten residue did produce a small quantity of LAL.

Table 2—The essential amino acid content of the original protein sources and their soluble and residue fractions after calcium binding

Essential amino acid	Essential amino acids (g/100g protein)											
Lysine	5.92	5.82	5.28	2.00	1.47	1.57	8.20	7.91	6.97	7.30	7.37	5.49
Methionine + cysteine	2.86	2.98	2.85	4.64	4.98	4.64	5.52	5.27	4.59	4.06	5.34	3.47
Threonine	3.31	3.24	3.32	2.76	2.44	2.53	5.80	5.93	5.82	3.95	4.38	4.96
Isoleucine	4.80	4.73	4.76	3.74	3.61	3.93	2.92	2.98	2.92	5.51	4.57	4.73
Leucine	7.47	7.48	7.78	6.87	6.41	7.13	8.50	8.81	8.41	8.67	7.22	8.79
Valine	4.76	4.62	5.05	3.99	3.73	4.18	6.02	6.2	6.45	7.20	6.99	6.02
Tyrosine + phenylalanine	8.55	8.64	8.52	8.44	8.03	8.13	9.44	9.74	8.98	9.84	8.56	10.92
Tryptophane	0.98	0.84	0.97	0.30	0.32	0.44	1.24	1.24	1.65	1.45	1.68	1.71
	Soy isolate	Soy isolate soluble protein	Soy isolate residue	Gluten	Gluten soluble protein	Gluten residue	Blood protein isolate	Blood protein isolate soluble protein	Blood protein isolate residue	Leaf protein concentrate	Leaf protein concentrate soluble protein	Leaf protein concentrate residue

Table 3—The molecular weights of the soluble proteins from soy isolate, blood protein isolate, leaf protein concentrate and gluten

Soluble protein fraction	Molecular weight (daltons)	Relative concentration (as %of soluble protein)
Soy isolate – pH 7.0,	260,000	39.1
10 mM tris, 25°	125,000	10.7
	62,000	50.2
Soy isolate – pH 9.0,	260,000	50.5
10 mM tris w/excess Ca, 25°	125,000	25.8
	62,000	23.7
Gluten – pH 7.0,	150,000	4.3
10 mM tris, 25°	90,000	15.5
	66,000	79.1
Gluten – pH 10.0,	66,000	100.0
10 mM tris w/excess Ca, 35°		
Blood protein isolate – pH 7.0,	69,000	92.0
10 mM tris, 25°	11,000	8.0
Blood protein isolate – pH 11.0,	69,000	97.5
10 mM tris w/excess Ca, 35°	11,000	2.5
Leaf protein concentrate, pH 7.0,	90,000	4.3
10 mM tris, 25°	6,900	95.7
Leaf protein concentrate, pH 9.0,	90,000	0.2
10 mM tris w/excess Ca, 35°	6,900	99.5
	<1,000	0.3

The slight, but insignificant, production of LAL in these proteins was expected (De Groot and Slump, 1969). No toxicity caused by LAL would be expected because the amino acid is protein bound. Also, this amino acid is now being shown to be prevalent in many of our foods (Sternberg et al., 1975).

Lysinoalanine is only one possible explanation for the loss of lysine which was consistent throughout the protein fractions from Ca binding, except SPI soluble and LPC soluble proteins. Other condensations and racemization are also possible (Sternberg et al., 1975). At present, there is no explanation for the loss of some of the stable essential amino acids; however, in most cases the losses are of no nutritional significance.

Molecular weights

The alkaline treatment used during the Ca incubation of the proteins and the resulting Ca binding could have an effect upon the structure of these proteins. To evaluate subunit or molecular species changes, gel filtration chromatography was conducted for the soluble proteins from both the original protein source and from the Ca incubation.

Three large molecular weight fractions at 260,000, 120,000, and 62,000 daltons (Table 3) were obtained from the original soluble SPI. The largest and smallest fractions were the most prominent, representing 39.1 and 50.7%, respectively, of the total soluble protein. Following Ca binding at pH 9.0, 25°C, there was a shift in relative concentrations toward the larger molecular weight fractions. The 260,000 fraction made up 50.5% of the total soluble protein, with the remainder evenly distributed between the other two fractions.

One large and one small fraction, at 69,000 and 11,000 daltons, was eluted from the original soluble BPI. The larger molecular weight fraction made up 92.0% of the total soluble protein. Ca binding at pH 11, 35°C, enlarged the relative concentration of the 69,000 dalton fraction to 97.5% of the total soluble protein.

Three large molecular weight fractions (150,000, 90,000 and 66,000 daltons) comprised the original soluble gluten proteins. The 66,000 dalton fraction comprised nearly 80% of the total soluble protein. Following Ca binding at pH 10, 35°C, the only fraction present was the smaller 66,000 dalton protein.

The original soluble protein from LPC contained two fractions (90,000 and 6,900 daltons). The smaller protein was most abundant, representing 95.7% of total soluble protein. This fraction became 99.5% of the soluble protein after Ca binding at pH 11, 35°C. A very small (<1,000 dalton) fraction also appeared but comprised only 0.3% of the total soluble protein.

The shift in the major protein fraction of the SPI soluble proteins from the smallest to the largest fraction is due to the solubilization of larger proteins at the alkaline pH. Gluten soluble proteins appear to dissociate to their smallest component at 66,000 daltons. The 6,900 dalton protein fraction from LPC appears to be the same chlorogenic acid binding fraction reported by Lahiry et al. (1977).

Table 4—The isoelectric points of the soluble protein fractions both before and after calcium binding

Soluble protein fraction	Isoelectric point							
	Major components			Minor components				
Soy isolate – pH 7, 10 mM tris, 25°C	5.25	5.20	3.65					
Soy isolate – pH 9, 10 mM tris w/Ca, 25°C	4.97	4.70		6.95	6.75	6.30		
Gluten – Ph 7, 10 mM tris, 25°C	5.59			7.43	7.35	7.25	7.02	6.90
Gluten – pH 10, 10 mM tris w/Ca, 35°C	3.65			6.97				
Blood protein isolate – pH 7, 10 mM tris, 25°C	6.35	5.30		6.50	6.08	5.95	5.42	
Blood protein isolate – pH 11, 10 mM tris w/Ca, 35°C	4.34	4.15		4.95	4.60			
Leaf protein concentrate – pH 7, 10 mM tris, 25°C	5.75	5.45	5.25					
Leaf protein concentrate – pH 9, 10 mM tris w/Ca, 35°C	4.75	4.45	4.15					

Isoelectric focusing

If calcium ions were bound to protein amino acid residues (histidine), then a masking of the negative charges would take place and the isoelectric point of that protein would decrease. To verify this hypothesis, soluble proteins from both the original proteins and the Ca-incubated proteins were isofocused in polyacrylamide gels.

The original soluble SPI protein contained three major components with isoelectric points (IP) of 5.25, 5.20, and 3.65. Following incubation with excess Ca at pH 9, 25°C, two major components (IP = 4.97 and 4.70) and three minor components (IP = 6.95, 6.75, and 6.30) were present (Table 4).

The original soluble BPI protein contained two major and four minor components, having respective IP's of 6.55 and 6.30, and 6.50, 6.08, 5.95, and 5.42. After incubation at pH 11, 35°C, with excess Ca, two major fractions and two minor fractions were found at IP's of 4.34 and 4.15, and 4.95 and 4.60, respectively.

The original soluble LPC protein included three major components with IP's of 5.75, 5.45, and 5.25. The sample incubated at pH 11, 35°C, with excess Ca also had three major protein peaks but they possessed lower IP's of 4.75, 4.45, and 4.16.

The soluble gluten protein contained one major fraction with an IP of 5.59 and five minor components with IP's of 7.43, 7.35, 7.25, 7.02, and 6.90. The Ca-incubated, pH 10, 35°C, protein had one major component (IP = 3.65) and a wide minor band (IP = 6.97).

The binding of calcium ions decreases the IP of the soluble protein. This is clearly seen with the LPC soluble proteins whose IP's decreased 1 full pH unit.

CONCLUSIONS

ALL FOUR PROTEIN sources studied possessed an ability to bind calcium which generally increased with increasing pH and temperature up to 35°C. The level of calcium is sufficiently high to possibly establish a new calcium source in the human diet when consumption of plant proteinates reaches a higher level.

Leaf protein concentrate bound the most calcium, approximately twice the quantity bound by gluten. Blood protein isolate and soy protein isolate bound the least amount of Ca. The two sources, BPI and SPI, which had high initial sodium levels both lost substantial amounts of this ion during the Ca-binding procedure.

High phytate content of both gluten and SPI residue proteins explain their greater ability to bind calcium when compared to their respective soluble proteins.

There was a consistent loss of lysine for all proteins from all the protein sources, excluding the soluble proteins of LPC and SPI. The lysine loss of SPI residue, BPI soluble, and gluten soluble proteins is partially explained by very slight formation of lysinoalanine. The lysine loss which occurred with wheat gluten was considered critical because of its low initial level in the gluten. The loss of essential amino acids occurred for the residue proteins of LPC, but even these losses were not considered extremely significant from a nutritional standpoint.

Soluble protein molecular weight changes were noted for gluten, which dissociated to its smallest molecular weight (66,000 daltons) component following Ca binding. The relative concentrations of protein in SPI changed from smaller to larger molecular weight as more of the larger molecular weight proteins solubilized at the alkaline pH. The only appearance of a previously unseen molecular weight species, after Ca binding, was a $< 1{,}000$ dalton component in the LPC.

Isoelectric points of the soluble proteins generally moved to more acidic ranges following Ca binding, the only exception being three minor components of SPI.

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SOME VISCOELASTIC AND MACRONUTRIENT CHARACTERISTICS OF PEANUTS

ABSTRACT

Some of the viscoelastic properties of four peanut varieties were investigated by a series of uniaxial compression tests. The uniaxial tests were performed with a universal testing machine at temperatures of 5, 20 and 30°C, all at 55% relative humidity. Individual amino acid contents of the peanuts were determined by ion exchange chromatography. A proximate analysis was run to determine Kjeldahl nitrogen and oil. The results of the mechanical tests were analyzed to obtain uniaxial modulus of elasticity at various times and a decay constant. A correlation analysis of viscoelastic properties with chemical composition yielded significant correlations between modulus of elasticity and some individual amino acids, protein and oil. The amino aicds and protein were positively correlated while oil exhibited a negative correlation to the viscoelastic properties.

INTRODUCTION

PEANUTS are a very important cash crop in the Southeast United States, amounting to $317 million in cash receipts for 1972 (Sullivan, 1974). The production of peanuts has been steadily rising since the late 1940's; however, the domestic demand for conventional peanut products has remained fairly constant for the last 15 yr. This has led to much research into the potential use of peanut protein in applications similar to those of soy. These would include meat extenders, meat analogs, and formulation into existing products in order to increase their nutritive value and/or appeal. It is estimated that the market potentials for peanut flour and grits is 265 million pounds per year (Chiang, 1973).

It is very likely that peanuts will find their way into more and more foods in one form or another. In each of these applications the texture of the product must be accurately predicted so that a desirable product is produced. However the definition of the physical behavior of any biological material is always a complex problem. It is felt that some light may be shed on this problem by measuring the viscoelastic properties of the peanuts and attempting to correlate this with the chemical makeup of the unprocessed peanut. It is hoped that by knowing more about the unprocessed peanut it will be possible to better predict the behavior of the peanut as an ingredient in a food product. This research is intended only to be a start in this direction, but it does serve to point out some interesting correlations that merit a more detailed examination.

EXPERIMENTAL METHODS

THE PEANUTS used for this study were planted May 10, 1974 at the Southwest Branch Station of the University of Georgia, located near Plains, Ga. The four varieties used were: Early Runner (#45), F 416-12-4-8-2 (#81), F 393-8-2-1-4-1-4 (#94), and Bradford Big Boy (#102). These peanuts and their protein levels have been previously described by Young and Hammons (1973). Recommended cultural practices were used (McGill, 1974) in the growing of the samples. The peanuts were dug on Oct. 2, 1974, placed in onion mesh bags and dried at 35°C to about 8–10% moisture. These peanuts were stored at 5°C and 50% RH until ready for analysis.

Before analysis the peanuts were hand shelled to prevent damage and to leave the testa (skin) intact. While shelling the peanuts were sorted according to maturity. Mature peanuts were classified as those with a dark colored interior pericarp surface or with some white on the interior pericarp and a thin pink or very thin faded pink colored testa (Young et al., 1974). Only those samples meeting this requirement were used in the analysis. No attempt was made to select for basal and apical seeds.

For the amino acid analysis full-fat peanuts were ground in a Laboratory Wiley mill equipped with a stainless steel rotor and blades. The duplicate samples (200 mg) were first hydrolyzed in 20 ml of 6N HCl with high purity nitrogen flushing for 2 hr at 145°C. following this, the pH of the samples was adjusted to 2.1–2.2 with 12N NaOH. The samples were then diluted to 50 ml with pH 2.2 citrate buffer. Total amino acid analyses were performed by the ion-exchange chromotography technique of Spackman et al. (1958). A Durrum Model D-500 amino

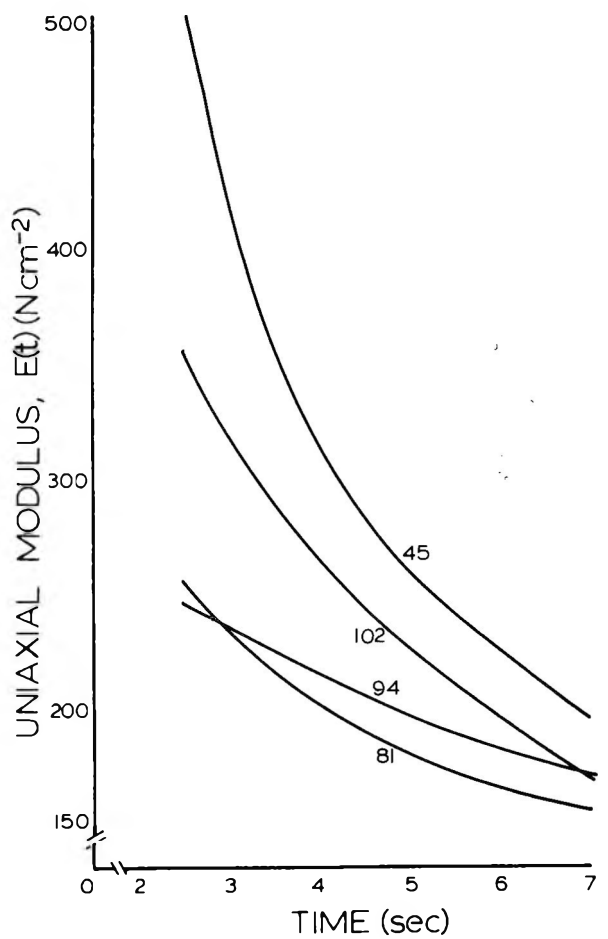

Fig. 1—Uniaxial modulus master curves for the four peanut varieties.

acid analyzer was used with a 1.75 mm (i.d.) × 48 cm column packed with Durrum high resolution cation exchanger (bead diameter 8 ± 1μ). Running time was 70 min including column regeneration.

Kjeldahl nitrogen was determined by the AOCS Method (AOCS, 1951) and protein calculated by using the 5.46 conversion factor (Jones, 1941). Total oil was determined by drying 1-g samples for 5 hr at 110°C, suspending the dried meal in 20 ml of diethyl ether, letting stand overnight or until clear, removing 10 ml of clarified ether containing dissolved fat, evaporating the ether in a tared vessel, weighing and calculating the percentage oil originally contained in the meal. Previous unpublished data from our laboratory indicates that this method of oil quantitation compares favorably to the Soxhlet extraction procedure. Carbohydrate content was calculated by differences.

The uniaxial compression tests were performed with a table model Instron universal testing machine (Model 1130). A strain gage load cell with 100 lb (455 N) capacity was used as the force sensing element. The universal testing machine was fitted with a compression cage consisting of two parallel plates made up of hardened stainless steel. The peanuts were compressed (with testa in place) in a direction perpendicular to the longitudinal axis. The compression tests were run at 0.2 in/min (0.508 cm/min) with a decade crosshead speed reducer. This slow speed allowed the peanuts to be compressed for the longest period of time without failure. A chart speed of 50 in/min (127 cm/min) was used to provide a mixmum magnification of the time scale. The desired temperature and relative humidity were maintained by a Hotpack Model 882 environmental chamber.

Prior to mechanical testing, the four varieties of peanuts were maintained at an equilibrium relative humidity of 55% and 5°C for 2 wk for preconditioning. Then, the peanuts were allowed to equilibrate for 3 days at each of the test temperatures of 20 and 30°C before testing. At each temperature, 10 replications of the compression tests were performed. The longitudinal and cross-sectional dimensions of each peanut were measured with a micrometer and the peanuts were compressed until the failure point, which was indicated in the force-displacement graph, by a sudden drop in force.

The force-displacement curve for each temperature and replication was analyzed using the general method of Hammerle and Mohsenin (1970). The actual displacement of the peanut was obtained as the ratio of crosshead to chart speed. For each displacement value the corresponding force was determined from the graph. Since, the tests were run under quasi-static conditions and other assumptions of Hertz's theory (Kozma and Cunningham, 1962) are valid, the modulus of elasticity was calculated at each "time" by the following equation (Mohsenin, 1970).

$$E(t) = 0.531 \frac{(1-u^2)}{D^{1.5}} F(t) \left(\frac{1}{R_1} + \frac{1}{R_2}\right)^{0.5}$$

where u = Poisson's ratio; F = force at time t; D = displacement at time t; and R_1, R_2 = radii of curvature.

As the tests were conducted at constant loading rate, the displacement was linearly proportional to time and the "time" was obtained as the ratio of displacement to displacement rate. At each "time" the modulus of the 10 replications were averaged and the standard deviation determined. The curves of uniaxial modulus function against time were drawn through a range representing the mean, plus and minus one standard deviation. The three curves for the three temperatures were shifted horizontally using an average horizontal shift factor (Rao and Hammerle, 1973) of 0.056 sec/°C. This shift factor was experimentally determined as the one which would produce the smoothest curve. The master curves, thus obtained for the four varieties are shown in Figure 1.

RESULTS & DISCUSSION

From the shape of the master curves shown in Figure 1, it can be seen that the peanuts obeyed a generalized Maxwell model which corresponds to two or more Maxwell models arranged in parallel. Since it was desired to correlate the viscoelastic properties of the peanut with the chemical properties, values of the uniaxial modulus were taken 3, 5 and 7 sec from the master curve for each variety. These points, along with selected data from the chemical analysis are presented in Table 1. In order to correlate the points, linear regression was performed using x and y data points from each variety of peanut.

Table 1—Results of chemical and physical analysis for the four peanut varieties

	Early Runner (#45)	F 416-12-4-8-2 (#81)	F 393-8-2-1-4-1-4 (#94)	Bradford Big Boy (#102)
$E_{(3.0)}(Ncm^{-2})$[a]	417	249	245	345
$E_{(5.0)}(Ncm^{-2})$	258	182	198	225
$E_{(7.0)}(Ncm^{-2})$	206	153	171	168
$E_{(3.0)}/E_{(7.0)}$	2.024	1.627	1.433	2.054
Oil (%)	52.06	53.92	55.45	53.25
Protein (%)	29.53	26.01	26.94	28.96
Carbohydrate (%)	8.93	11.14	9.06	8.46
Lys (mg/g)	12.49	11.59	11.88	11.97
Arg (mg/g)	41.89	35.71	38.47	40.29
Ala (mg/g)	14.26	12.38	13.22	13.44
Gly (mg/g)	21.70	20.24	19.65	20.68
Asp (mg/g)	43.34	38.27	40.88	41.13
Glu (mg/g)	59.50	51.70	55.10	55.62
Ile (mg/g)	11.45	10.60	11.05	11.38
Tyr (mg/g)	11.08	9.507	10.14	10.85
Phe (mg/g)	19.09	17.05	17.98	18.25

[a] E(t) refers to uniaxial modulus of elasticity at time t.

In all cases the uniaxial modulus and the $E_{3.0}/E_{7.0}$ value (which indicates how rapidly the modulus decayed with time) represented the x data point. To determine how well the regression line fits the original data points the (r) value was calculated. Using the technique of Snedecor and Cochran (1968) it was determined that an (r) value of greater than 0.900 was needed for a 95% confidence limit. This was the limit chosen in this experiment to determine significance. The (r) values of all parameters desplaying a significant relationship are presented in Table 2. Any parameters not showing a significant correlation are not shown.

The % oil values gave a negative correlation with the uniaxial modulus at low times and also correlated inversely with the decay of the modulus ($E_{3.0}/E_{7.0}$). This would seem to indicate that the oil in the peanut tends to make the material dissipate stress more rapidly. Oil could have this effect by providing some sort of lubrication, which would cause the peanut to undergo a strain more readily.

Nine of the amino acids displayed a significant correlation to the uniaxial modulus at one or more of the times. All of these correlations were positive and are listed in Table 2. The % total protein also showed a positive correlation with the uniaxial modulus at two of the times. These facts indicate that the protein in the peanut provides some of its strength. The fact that some of the amono acids correlate with the uniaxial modulus at time intervals where the total protein does not, may come from at least two factors. First, it is known that the peanut contains several different proteins (St. Angelo and Mann, 1973). The two classical protein fractions found in peanuts are arachin and conarachin. It has been confirmed (Neucere and Ory, 1970) that the arachin exists in aleurone grains or protein bodies in the cell. The conarachin fraction seems to exist as two separate proteins, one of which exists "free" in the cytoplasm of the cell. It is likely that protein found in a spherical body in the cell and protein found "free" in the cytoplasm will have different effects on the viscoelastic properties of the cell. It has been shown (Dieckert et al., 1962) that these two protein fractions have different nitrogen contents and it is likely that they have different amino acid profiles (Jacks et al., 1972). In light of this information it can be

Table 2—Correlation coefficient (r) values from linear regression of uniaxial modulus on oil, carbohydrate, protein and some amino acid contents for peanuts

	Oil (% w.b.)	Carbohydrate (% w.b.)	Protein (% w.b.)	Lys (mg/g)	Arg (mg/g)	Ala (mg/g)	Gly (mg/g)	Asp (mg/g)	Glu (mg/g)	Ile (mg/g)	Tyr (mg/g)	Phe (mg/g)
$E_{(3.0)}$[a]	−0.905*	−0.556	0.945	0.907*	0.893	0.878	0.953*	0.836	0.870	0.837	0.902*	0.877
$E_{(5.0)}$	−0.792	−0.683	0.962*	0.971*	0.961*	0.963*	0.882	0.938*	0.957	0.907*	0.944	0.967*
$E_{(7.0)}$	−0.696	−0.561	0.791	0.985*	0.870	0.950*	0.777	0.945*	0.970*	0.771	0.803	0.971*
$E_{(3.0)}/E_{(7.0)}$	−0.900*	0.479	0.864	0.630	0.719	0.640	0.855	0.561	0.592	0.721	0.791	0.566

[a] $E_{(t)}$ refers to uniaxial modulus of elasticity at time t.
* Denotes significance at 5% level.

understood that the % total protein and the individual amino acid contents are really measuring different things; therefore, they may display different correlations with the uniaxial modulus at different times. A second explanation for the discrepancy between the individual amino acid correlations and the % total protein correlations could lie with the method of measuring protein. For this research the Kjeldahl method was used with the accepted conversion factor of 5.46 used for peanuts. However, this factor could change quite drastically from one peanut variety to another. This would produce an inaccurate % total protein measurement and erroneous correlations. There is some indication at this time that the conversion factor is not constant for all varieties of peanuts. More work needs to be done to determine if this factor does indeed change significantly from one variety to another.

It can be seen from Table 2 that the % carbohydrate did not correlate with the uniaxial modulus of the peanuts. This is somewhat surprising since the cell walls of the peanut contain cellulose and the cell walls provide strength to the cell. The lack of a correlation could be due to the fact that true carbohydrate was not measured, instead the nitrogen free extract percentage obtained from the proximate analysis was used to obtain the % carbohydrate data. An improvement over this would be to measure the cell wall polysaccharides and correlate this with the uniaxial modulus.

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TOXICITY OF *Aspergillus amstelodami*

ABSTRACT

ISOLATES of *Aspergillus amstelodami* from country cured hams were tested for toxicity to chick embryos and to mice. Seven of eight culture filtrates from isolates grown 30 days in yeast extract (2%)-sucrose (20%) media were lethal to chicken embryos. Six of eight culture filtrates were lethal when injected intraperitoneally into mice. Eight of eleven strains grown on sterile corn were toxic when fed to mice. Deaths occurred after only 3 days. A single isolate, toxic to both mice and chick embryos was selected for further study. Culture filtrates of this strain were lethal to male, white, weanling rats. Symptoms and gross lesions were indicative of an effect on the circulatory system. Incorporation of the mycelial mat into the diet of rats resulted in increased liver and kidney weights although deaths were not observed within 30 days. Rats fed corn inoculated with this mold had significantly lower average body weight gains than controls. Because of limited solubility of the toxic substance in most organic solvents, gel permeation chromatography was used to isolate a single toxic fraction. Purification of the compound was accomplished by ion exchange and paper chromatography. The toxic material (R_f = 0.94) fluoresces blue when viewed under short wave UV light. The melting point of the material was 159–162°C. The UV absorption maximum of the material in 95% ethanol was 211 nm.

INTRODUCTION

THE TOXICITY of *Aspergillus amstelodami* to animals was first reported by Rabie et al. (1964). They found that yellow corn inoculated with *A. amstelodami* (Mangin) Thom and Church (*Eurotium amstelodami* Mangin) was lethal to rabbits and, although not causing death of poultry, reduced the growth rates of Pekin ducklings and White Leghorn chickens appreciably. In screening tests, Semeniuk et al. (1971) evaluated the toxicity of 10 strains of *A. amstelodami* together with other fungi. Of 10 *A. amstelodami* strains tested for toxicity, one strain when grown on wheat was found to be lethal to mice and another strain grown on soybeans caused significantly lower average weight gains in chicks. These findings led these investigators to classify *A. amstelodami* with fungi that were "moderately to mildly toxigenic."

Results of screening tests done by Saito et al. (1971, 1974) showed culture filtrates of two strains of *A. amstelodami* isolated from Japanese foodstuffs to be toxic to Hela cells and to mice. Mycelial extracts were not found to be toxic to mice. Histological examination of selected organs revealed hepatic lesions (Saito et al., 1971) and damage to proliferating cells of the intestinal mucose (Saito et al., 1974). Recently in screening tests, Wu et al. (1974b) found chloroform extracts of two of seven *A. amstelodami* strains isolated from cured meats to be toxic to chick embryos. Culture filtrates were not found by these investigators to be toxic.

Not all screening tests have implicated *A. amstelodami* to be toxic. Diener et al. (1963) fed ducklings sterilized defatted residues of peanuts inoculated with a single strain of each of nine species of fungi for a period of 3 days. *A. amstelodami* was not toxic to the ducklings. Kurata et al. (1968) found culture filtrates and methanol extracts of rice cultures of two strains of *A. amstelodami* to have no toxic effect when injected subcutaneously into mice in one single dose.

There is a need for a more thorough investigation of the toxic effects resulting from ingestion and other routes of administration of metabolites elaborated by this mold. The toxic metabolites still must be isolated and characterized.

The present investigation was undertaken to study the toxicity of *A. amstelodami* isolated from country cured hams to chicken embryos, mice and rats and to isolate the toxic components produced by this mold.

EXPERIMENTAL

Microorganisms

Twelve cultures of *Aspergillus amstelodami* isolated previously from country cured hams by Leistner and Ayres (1968) and Sutic et al. (1972) were used in the experiments. All cultures were maintained on Czapek Dox Agar (Difco Laboratories, Detroit, Mich) slants.

YES medium

The yeast extract-sucrose (YES) medium of Davis et al. (1966) containing 2% yeast extract and 20% sucrose was used for the cultivation of the molds. 100-ml aliquots of the medium were dispensed into 250 ml Erlenmeyer flasks. The flasks were stoppered with cotton plugs, autoclaved for 15 min at 121°C, cooled and inoculated using 1.0 ml of a suspension of 10^6 spores per ml harvested from CDA slants. Unless otherwise specified, each flask was incubated without agitation at 28°C for 30 days.

Chicken embryo inoculations

The method of Verrett et al. (1964) was used for the chicken embryo air cell inoculations. Groups of 10 or 20 fertile White Leghorn eggs were inoculated with 0.04 ml of the test solution, sealed, then incubated and candled daily from the fifth day of incubation. Infertile eggs were discarded. Nonviable embryos were removed and grossly examined. Relative toxicity of a test solution was based on the number of embryos that did not survive through the hatching period compared to control eggs.

Intraperitoneal injections

Animals were swabbed around the area of injection with 70% ethyl alcohol. One ml of the test solution was injected intraperitoneally into the test animal with a 1 ml tuberculin syringe equipped with a 7/8 in 25 guage needle.

Screening tests

Toxicity of culture filtrates. Eight flasks containing YES medium were inoculated with 10^5 spores of one of 8 strains of *A. amstelodami* and incubated as described above. After 30 days, the cultures were filtered through a fluted Whatman No. 1 filter paper and the filtrate sterilized through a 0.45 μm Millipore (Millipore Filter Corp., Bedford, Mass.) membrane into sterile vials.

The sterile culture filtrates were tested for toxicity by chicken embryo air cell inoculations and by intraperitoneal injections into mice. In the chicken embryo tests, 10 eggs were used in each group. Two groups of controls, one inoculated with YES and the other composed of drilled, uninoculated eggs were used for comparison. 20–25g male, white mice were used in tests for toxicity by intraperitoneal injection. Four mice were used in each group. Two groups of mice, one group injected with sterile physiological saline solution and the other group injected with sterile YES, were used as controls. Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) and water were supplied ad libitum. The mice were dosed once a day for 21 days. One mouse from each group was sacrificed for histopathological examination after the

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7th and 14th day of the experiment. All animals that died were examined for gross lesions.

Toxicity of moldy corn. Corn cultures were prepared by combining 1000g corn and 1400 ml distilled water in a 2800 ml Fernbach flask. The flasks were stoppered with cotton plugs and autoclaved for 1 hr at 121°C. After the media were sufficiently cooled, 10^6 spores of one of 11 strains of *A. amstelodami* were added to each flask. The inoculated flasks were incubated at 28°C for 2 months. The moldy corn was dried at room temperature then ground in a Wiley mill. The ground moldy corn was used as the test diet. The control diet was uninoculated gound corn.

Groups of 4 mice, each weighing 20–25g, were used in the feeding experiments. Water and feed were supplied ad libitum. The mice were maintained on the diets for 20 days.

Determination of toxicity of *A. amstelodami* VIII/24

45–50g Sprague-Dawley male rats and 20g CF_1 mice were used as the test animal. They were caged individually in wire cages 25.4 × 20.3 × 17.8 cm, in a room maintained at 24–26°C.

Toxicity of culture filtrate. The culture filtrate from a 30-day culture was tested for toxicity to rats and mice by injecting 1 ml filter-sterilized culture filtrate intraperitoneally into the animals. Symptoms were observed. The rats that died were examined for gross lesions.

Toxicity of mycelia The mycelial mat from 20 day YES cultures were shaken with chloroform, dried at room termperature and ground. The ground mycelia were then incorporated into diets in the following proportions of mycelia to laboratory chow: 1:10, 1:20, and 0:100 (control). The rats were maintained on the diets for 30 days. The animals were weighed at regular intervals. After 30 days the animals were sacrificed. Liver, heart, kidney, spleen and lung weight were recorded.

Toxicity of moldy corn. Moldy corn was prepared according to the method of Wu et al. (1974a). After 2 months, the moldy corn was autoclaved for 15 min at 121°C, ground and fed to rats. A second group of rats were fed uninoculated, autoclaved corn. A third group of rats was fed laboratory chow. Ten rats were used in each group of rats fed moldy corn and control corn. Four rats were fed laboratory chow. Water was provided ad libitum. The rats were maintained on the diets for 42 and 71 days. The animals were weighed at regular intervals for 35 days. After the 42nd day and 71st days the rats were sacrificed. Their body weight and liver, heart, kidney, spleen and lung weights were recorded.

Effect of time and temperature of mold growth and toxicity to chicken embryos

YES cultures incubated at 10, 25, 28 and 37°C were held at these temperature for 10, 20, 30, 40, 50 and 60 days. Growth was measured by mycelial dry weight per 100 ml of medium. The filter sterilized culture filtrates were tested for toxicity to chicken embryos. Ten eggs were used in each test group.

Effect of heat on culture filtrates of 30-day cultures of *A. amstelomali* VIII/24

10 ml of filtrate from a 30-day culture of *A. amstelodami* VIII/24 were placed in each of four test tubes. One test tube was heated at 50°C for 1 hr, another was heated at 75°C for 1 hr while still another was sterilized at 121°C for 15 min. The fourth test tube was not subjected to any heat treatment. The material in each of the test tubes was inoculated into chicken embryos.

Isolation of the toxic metabolites

Selection of suitable solvent. Ethyl acetate, chloroform, chloroform-methanol (8:2), ether and petroleum ether extracts were prepared by adding 100 ml of the solvents to a 30-day culture filtrate which was then agitated on a rotary shaker for 10 min. The liquid was decanted and filtered through a fluted Whatman No. 1 filter paper into 250 ml separatory funnel and shaken vigorously. The aqueous layer was transferred back to the original culture flask while the solvent layer was transferred into a 500 ml or 1000 ml round bottom flask. The extraction was repeated twice or five times as indicated with all extracts being combined in the round bottom flask. The extracts were concentrated to a volume of 10 ml using a rotary vacuum evaporator. The aqueous layer of the culture filtrate after extraction was filtered through Whatman No. 1 filter paper and filter sterilized into sterile vials. These solutions were tested for toxicity to chicken embryos without further treatment or evaporated to dryness and dissolved in propylene glycol when indicated and used in chicken embryo toxicity tests.

Methanol, ethanol, acetone, methylene chloride and propylene glycol extracts were prepared by freeze drying 10 ml of the culture filtrate and suspending this in 4 ml of each of the specified solvents. These suspensions were filtered and filtrates were used in chicken embryo toxicity tests.

Control groups inoculated with each of the solvents were used for comparison.

Gel permeation chromatography. Because of the limited solubility of the toxic substance(s) in any of the solvents tested, gel permeation chromatography was used to separate the filtrate into fractions.

A 30-day culture of *A. amstelodami* VIII/24 was filtered through Whatman No. 1 filter paper and the filtrate layered onto a 2.5 × 58 cm Biogel P-2 (Biorad Labories, Richmond, Calif.) column. Distilled water was used to elute the fractions. 5-ml fractions were collected. The fractions were visualized under both visible and UV light and combined into 10 major fractions. These fractions were freeze dried, dissolved in 5 ml distilled water (unless otherwise specified) and filtered through a 0.45 micron Millipore membrane and tested for toxicity to chicken embryos.

Comparison with fungal metabolites. Culture filtrate and Band 2 from the Bio-gel P-2 column were spotted together with aflatoxin B_1, B_2, G_1 and G_2, kojic acid, citritnin, patulin, penicillic acid, gliotoxin, ochratoxin, sterigmatocystin, zearalenone and emodin standards onto Adsorbosil-5 thin-layer plates (5g Adsorbosil-5:70g water) which had been previously activated at 110°C for 2 hr and cooled in a desiccator. All the standards, with the exception of kojic acid, were dissolved in ethyl acetate.

The thin-layer plates were developed using toluene:ethyl acetate:90% formic acid (6:3:1 TEF) according to the method of Scott et al. (1970).

Paper chromatography. Fraction 2 from the Bio-gel P-2 column was run through Dowex 1 ion exchange resin (Fig. 1). The material from the Dowex 1 resin was freeze dried, spotted on Whatman No. 1 filter paper sheets, developed in a descending fashion for 24 hr using n-butanol, acetic acid, water (4:1:5 BAW) as the solvent system. The paper was run in a descending fashion.

After 24 hr, the paper was dried, bands visualized and cut-off. The bands were eluted with distilled water. The material was freeze dried, then 70% ethanol was added to the dried material to serve as a carrier for the material to be used in the chicken embryo inoculations.

A measured amount of material was placed in tared aluminum dishes. The material in the dishes was dried and weighed and the amount injected into each egg was calculated.

Test for purity and partial characterization

The material from band 12 from the paper chromatogram that was most toxic to chicken embryos was tested for purity.

Thin-layer chromatography was carried out using silica gel plates developed in chloroform:acetone (95:5), toluene-ethyl acetate-formic acid (6:3:1 TEF) and methanol:water (1:1). Compounds were visualized under visible and UV light then sprayed with sulfuric acid solutions and charred. A melting point determination was carried out using a Mettler melting point apparatus.

The UV spectrum of the material in absolute ethanol was determined in the 190–370 nm wavelength range using a Coleman Model 124 Hitachi double beam grating spectrophotometer and cell with a 1 cm light path.

RESULTS & DISCUSSION

Screening tests

Table 1 shows results of the tests for toxicity of culture filtrates of 8 strains of *A. amstelodami* to chicken embryos. Inoculation of culture filtrates of *A. amstelodami* VIII/24 and VIII/31 resulted in lethal toxicity to 100% of the eggs. No toxicity was observed in eggs inoculated with filtrates of *A. amstelodami* VIII/21. Culture filtrates of *A. amstelodami* VIII/2, VIII/29, VIII/32, M27 and M60 resulted in varying degrees of toxicity ranging from 67% to 90% mortality to chick embryos. No deaths were observed in the control groups.

Intraperitoneal injections of the culture filtrate into mice resulted in 0–100% mortality (Table 2). Injection of culture filtrates of *A. amstelodami* VIII/21 and M27 did not cause any deaths. Injection of culture filtrates of *A. amstelodami* VIII/2, VIII/24, VIII/29, VIII/31, VIII/32 and M60 resulted in lethal toxicity to all the animals injected. All four mice injected with the filtrate of *A. amstelodami* VIII/24 died within 3–6 days.

No deaths occurred in the group of mice injected with physiological saline solution. One mouse injected with sterile YES died after the second day of the experiment as a result of peritonitis. No histopathological lesions were found in the organs examined.

Before death, the mice were first observed to be able to use only their fore legs and had to drag their bodies and hind legs when forced to move. They exhibited continuous tremors of the entire body. A few hours before their death they lay quietly on their ventral side with both fore and hind legs spread apart away from their bodies. Even when forced to move they seemed unable to use both pairs of legs properly. The control mice appeared to healthy and active until the experiment was terminated.

The results of the feeding experiments are presented in Table 3. Moldy corn inoculated with *A. amstelodami* VIII/1, VIII/3, VII/24, VIII/33 and M60 caused lethal toxicity to all the mice in the groups. Deaths occurred only 3 days after the animals were placed on these diets. Two groups, those fed corn inoculated with *A. amstelodami* VIII/2 and M27, did not manifest any toxic effect and appeared to be as healthy and active as those in the control group. The groups of mice fed corn inoculated with *A. amstelodami* VIII/27, VIII/29, VIII/31 and VIII/32 exhibited 25–75% mortality.

Table 1—Toxicity of 30-day culture filtrates of eight strains of Aspergillus amstelodami to chicken embryos

Treatment	Infertile	Dead	%Mortality
A. amstelodami VIII/2	0	8	80
A. amstelodami VIII/21	0	0	0
A. amstelodami VIII/24	0	10	100
A. amstelodami VIII/29	0	8	80
A. amstelodami VIII/31	0	10	100
A. amstelodami VIII/32	1	6	67
A. amstelodami M 27	0	9	90
A. amstelodami M60	0	9	90
YES Control	0	0	0
Drilled uninoculated control	0	0	0

Table 2—Toxicity of culture filtrates of eight strains of Aspergillus amstelodami injected intraperitoneally into mice

Treatment	Mortality	% Mortality	No. of days to death
A. amstelodami VIII/2	3/3	100	2–11
A. amstelodami VIII/21	0/4	0	–
A. amstelodami VIII/24	4/4	100	3–6
A. amstelodami VIII/29	2/2	100	11–20
A. amstelodami VIII/31	3/3	100	7–9
A. amstelodami VIII/32	3/3	100	11–13
A. amstelodami M 27	0/4	0	–
A. amstelodami M 60	3/3	100	11–14
YES control	1/4	25	2
Physiological saline control	0/4	0	–

Table 3—Toxicity of corn inoculated with Aspergillus amstelodami when fed to mice

Treatment	Mortality	% Mortality	No. of days to death
A. amstelodami VIII/1	4/4	100	6–16
A. amstelodami VIII/2	0/4	0	–
A. amstelodami VIII/3	4/4	100	6–7
A. amstelodami VIII/24	4/4	100	4–10
A. amstelodami VIII/27	3/4	75	6
A. amstelodami VIII/29	2/4	50	10–13
A. amstelodami VIII/31	1/4	25	6
A. amstelodami VIII/32	2/4	50	8–20
A. amstelodami VIII/33	4/4	100	3–6
A. amstelodami M 27	0/4	0	–
A. amstelodami M 60	4/4	100	3–5
Uninoculated corn control	1/4	25	11

Toxicity of *A. amstelodami* VIII/24

Based on its toxicity in all three tests, one strain, *Aspergillus amstelodami* VIII/24 was selected for further study.

Intraperitoneal injections of culture filtrate of *A. amstelodami* VIII/24 into rats resulted in lethal toxicity. One ml of the fivefold concentrated culture filtrate produced immediate response in the form of rapid breathing, palpitations, pronounced thirst, and inability to stand within 7–10 min after injection. All these animals died within 15 hr. Gross lesions consisted of congestion of the heart muscle, congestion of the lungs with pale areas, accumulation of fluid which was either colorless or tinged with blood in the thoracic cavity. The plantar surface of the paws and the nail bed were so congested they appeared dark. The symptoms and gross lesions indicate an effect on the circulatory system. Two of 5 rats and 5 of 5 mice injected intraperitoneally for 1 or 2 days with 1 ml of a 30-day culture filtrate died.

Rats fed diets containing defferent proportions of mycelia of *A. amstelodami* VIII/24 to laboratory chow did not show any significant differences in body weight gain after the 30-day feeding period. However, organ weights as shown in Table 4 had some statistically significant differences. Rats fed one

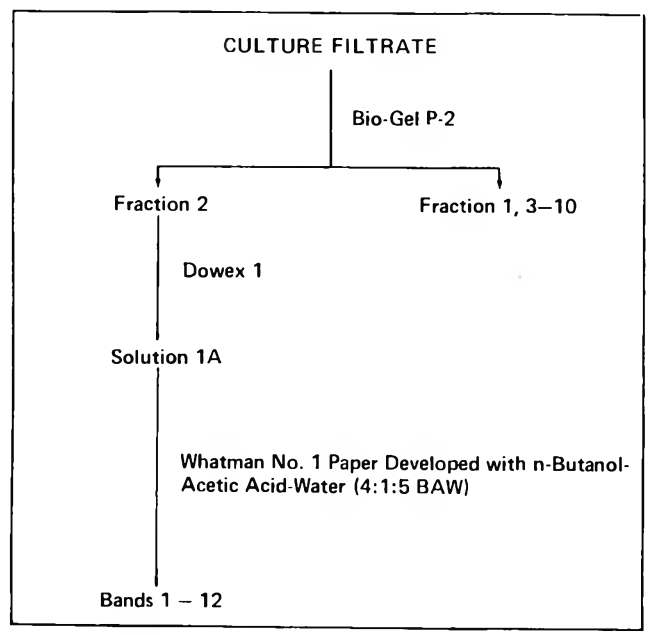

Fig. 1—Scheme for the isolation of the toxic compounds.

Table 4—Mean weight gain and organ weights of rats fed diets containing mycelia of Aspergillus amstelodami VIII/24 in the diet

Ratio of mycelia to chow in the diet	Mean wt gain	Mean organ weights expressed as % body weight				
		Liver	Heart	Lungs	Kidney	Spleen
1:10	138.6	4.39	0.40	0.64	0.90	0.38
1:20	131.0	3.66	0.41	0.58	0.80	0.26
0:100	119.2	3.31	0.32	0.58	0.77	0.26
F value	0.11783	2.54597	1.82116	0.94334	4.09924	2.00111
Probability > F	0.8895	0.1224	0.2066	0.5789	0.0460	0.1806
LSD, 1% Level	88.3	1.37	0.11	0.18	0.15	0.22
LSD, 5% Level	62.6	0.97	0.08	0.13	0.11	0.15

part mycelia to ten parts chow had significantly ($P < 0.5$) kidney weight from those in the control group.

Average body weights of rats fed corn inoculated with *A. amstelodami* VIII/24, uninoculated corn and laboratory chow for 35, 42 and 71 days are shown in Table 5. Average body weight gains of rats fed the moldy corn for 35, 42 and 71 days were significantly different ($P < 0.01$) from those maintained on the uninoculated corn and the laboratory chow. There were no significant differences in the relative organ weights.

Effect of time and temperature of incubation on mold growth and toxicity to chicken embryo

Growth of the mold as indicated by dry mycelial weight/100 ml medium (Fig. 2) is best at 37°C. There was very little growth at 10°C.

Results of toxicity tests to chicken embryo are depicted in Figure 3. After 10 days, only cultures incubated at 37°C showed any toxicity. Cultures held for 20 days at 15, 28 and 37°C were lethal to all embryos inoculated. After 30 days, only the cultures held at 25°C remained toxic to 100% of the embryos inoculated. Cultures held at 10°C were not appreciably toxic to chicken embryos.

Gross examination of nonviable embryos showed them to be very congested. Several of the chicks that survived through the hatching period showed bilateral leg paralysis. Affected birds rested on their hocks which were enlarged and deformed and the toes curled inward. Some of these chicks attempted to walk on their hocks. Two birds examined had slipped tendon of Achilles. The affected chicks died within one to three days probably due to their inability to secure food and water even when it was made easily available to them.

Effect of heat treatment on culture filtrates

Results of the toxicity tests of culture filtrates subjected to different heat treatments are shown in Table 6. Culture fil-

Table 5—Means of weight gain of rats fed moldy corn for 35, 42 and 71 days

Diet	Weight gain (g)		
	35 Days	42 Days	71 Days
Moldy corn	– 4.9	– 4.7	1.2
Control corn	29.0	16.2	25.8
Laboratory chow	132.8	177.0	287.0
F value	115.3873	620.6943	251.3183
Probability > F	0.0001	0.0001	0.0001
LSD, 1% Level	21.5	14.2	44.3
LSD, 5% Level	15.8	10.0	29.9

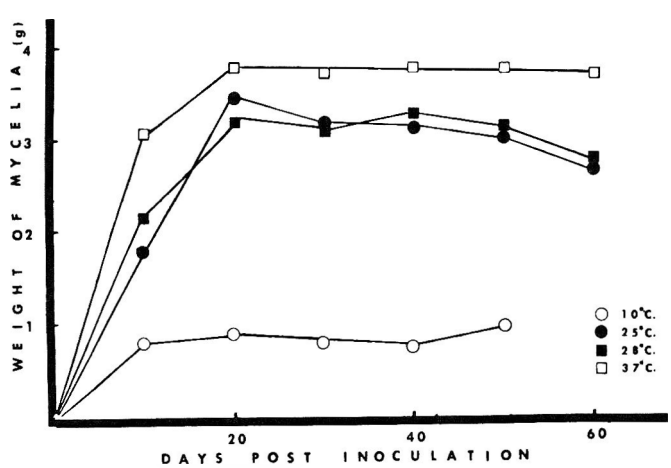

Fig. 2—Growth of A. amstelodami VIII/24 (dry mycelial wt/100 ml medium).

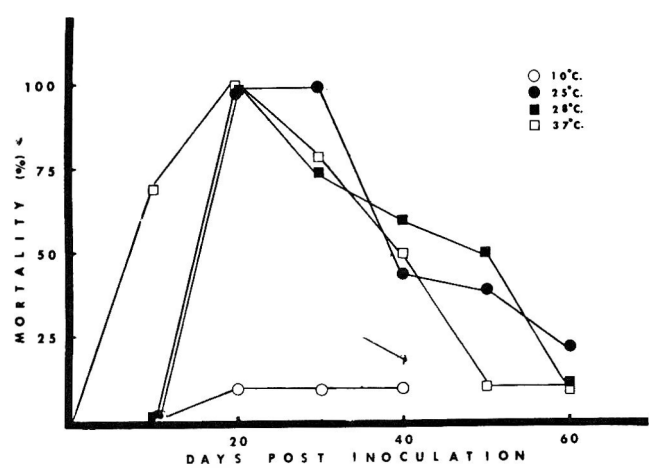

Fig. 3—Toxicity of A. amstelodami VIII/24 cultures grown at various temperatures to chicken embryos.

trates of *A. amstelodami* resulted in 89% mortality when inoculated into chicken embryos. After heating at 50°C for 1 hr, 80% mortality was observed. Heating culture filtrates for 1 hr at 75°C resulted in 60% mortality to chicken embryos inoculated. Sterilization of the filtrates at 121°C for 15 min did not appreciably lower the toxicity of the culture filtrate.

Table 6—Toxicity of Aspergillus amstelodami culture filtrate subjected to different heat treatments

Treatment	Infertile	Dead	% Mortality
Culture filtrate			
No heat treatment	1	8	89
50° C, 1 hr	0	8	80
75° C, 1 hr	0	6	60
121° C, 15 min	0	7	70
Drilled, uninoculated control	0	0	0

Table 7—Toxicity of extracts of A. amstelodami to chicken embryos

Treatment	% Mortality
Control, uninjected	5
YES control	0
Culture filtrate	55
Ethyl acetate extract	70
Ethyl acetate control	10
Methanol extract[a]	0
Methanol control	10
Acetone extract[a]	0
Acetone control	10
Methylene chloride extract[a]	0
Methylene chloride control	0
Ethanol extract[a]	10
Ethanol control	0
Propylene glycol extract[a]	10
Propylene glycol control	0
Chloroform extract	20
Chloroform control	30

[a] Extracts were made by freeze-drying the culture filtrate and dissolving in the different solvents.

Table 8—Toxicity of extracts and culture filtrate of A. amstelodami after extraction with various solvents

Treatment	% Mortality
Control, uninjected	5
YES control	5
Propylene glycol control	11
Ether extract[a]	45
Culture filtrate after ether extraction	63
Ethyl acetate extract[a]	5
Culture filtrate after ethyl acetate extraction	100
Chloroform-methanol (8:2) extract[a]	20
Culture filtrate after chloroform-methanol (8:2) extraction	75
Petroleum ether extract[a]	11
Culture filtrate after petroleum ether extraction	79

[a] Extracts were evaporated to dryness then dissolved in propylene glycol.

Isolation of the toxic components

In order to isolate the toxic principle in the culture filtrate of *A. amstelodami* VIII/24, the efficiency of various solvents in the extraction of the toxic components was determined.

Results of the test for toxicity of extracts to chicken embryos is shown in Table 7. Of the seven solvents selected, ethyl acetate seemed to be the preferred solvent for extracting the toxic component. However, comparing the toxicity of the concentrated extract to the toxicity of the filtrate, its efficiency as a solvent system is questionable.

Another test for the extraction efficiency of ethyl acetate and three other solvent systems was conducted. Extractions were repeated five times. The six extracts were combined and evaporated to dryness, then dissolved in propylene glycol prior to injection. The culture filtrates after extraction were also inoculated into eggs. Results of the toxicity tests are shown in Table 8. In all instances the culture filtrate after extraction with the different solvents remained more toxic than the extracts.

Gel permeation chromatography. Because of the limited solubility of the toxic substance(s) in any of the organic solvents tested, gel permeation chromatography of the culture filtrate was used to fractionate the toxic filtrate.

The 5-ml fractions obtained from the Bio-gel P-2 column were combined into ten major fractions according to color of each of the fractions under visible and ultraviolet light. The characteristic color of the fractions is shown in Table 9.

Results of the toxicity tests are also shown in the table. Fraction 2 was found to be the most toxic to chicken embryos. The toxicity of the fivefold diluted Fraction 2 was greater than any of the other fractions. Fraction 3, 5 and 8 also showed some toxicity to chick embryos.

Since Fraction 2 was more toxic than any of the other fractions, all further effort was concentrated on the purification of the toxic components in this fraction.

Comparison with fungal metabolites. Comparison of migration on thin-layer chromatography aflatoxin B_1, B_2, G_1 and G_2, kojic acid, citrinin, patulin, penicillic acid, gliotoxin, ochratoxin, zearalenone, sterigmatocystin and emodin standards with the culture filtrate and Fraction 2 from the Bio-gel P-2 column confirmed the absence of these compounds.

Table 10—Bands obtained after spotting the toxic fraction on Whatman No. 1 and developing 24 hr with N-butanol, acetic acid water (4:1:5)

Band	Average R_f	Appearance		
		Visible	Short Wave UV	Long Wave UV
1	0.03	Lt brown	Green	Green
2	0.10	–	Purple	Purple
3	0.14	–	–	Lt blue
4	0.16	–	Blue	Purple
5	0.21	–	Lt blue	Blue-Green
6	0.26	–	–	Lt purple
7	0.33	–	Lt blue	–
8	0.42	–	–	Purple
9	0.49	Yellow	–	–
10	0.54	–	–	Purple
11	0.69	–	–	–
12	0.94	–	Bright Blue	–

Table 9—Toxicity of Biogel P-2 fractions obtained from culture filtrate of A. amstelodami

Treatment	No. of ml in fraction	Appearance		% Mortality
		Visible	UV	
Control, uninjected				17
Fraction 1	80	–	–	16
2	155	Yellow	Yellow	39[a]
3	25	Lt. yellow	–	25
4	60	–	–	21
5	40	–	Blue	37
6	20	–	Lt. yellow	11
7	20	–	Yellow	22
8	140	–	–	26
9	65	Lt. yellow	–	15
10	135	–	–	20

[a] Diluted fivefold.

Table 11—Toxicity of bands eluted after paper chromatography of the toxic fraction

Band	mg Dry wt/egg	Infertile	Dead	% Mortality
1	7.18	1	4	44
2	23.64	0	1	10
3	15.50	0	1	10
4	18.41	1	2	22
5	15.21	1	2	22
6	4.64	2	4	50
7	1.92	0	3	30
8	1.54	0	–	0
9	1.33	0	2	20
10	1.30	0	1	10
11	1.02	0	1	10
12	1.43	0	7	70

Paper chromatography. To further isolate and purify the toxic material(s), paper chromatography was carried out using the top layer of n-butanol, acetic acid and water (4:1:5 BAW) as the solvent system. Twelve distinct bands were obtained. R_f values and the appearance of these bands under visible and ultraviolet light is shown in Table 10.

The results of toxicity tests of the material eluted from the different bands is shown in Table 11. Three of the bands gave a high mortality to chicken embryos. These were bands 1, 6 and 12 which gave % mortalities of 44, 50 and 70, respectively. However, if the amount of material injected is taken into consideration, the material from band 12 appears to be most toxic to chicken embryos. 70% mortality was observed when each egg was inoculated with 1.43 mg of the material.

Test for purity and partial characterization. Band 12 which appeared to fluoresce bright blue when viewed under short wave ultraviolet light was isolated and partially characterized.

Thin-layer chromatography of the material from band 12 and visualization under visible and short and long wave ultraviolet light then charring with sulfuric acid showed that no more than two components were present.

The melting point of the material was found to be 159–162°C.

The toxic material was found to have a UV absorption maximum at 211 nm.

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QUANTITATIVE EVALUATION OF Rubus FRUIT ANTHOCYANIN PIGMENTS

ABSTRACT

The distribution of individual anthocyanin pigments in the fruits of 43 *Rubus* clones was determined by TLC and densitometry. Quantitative estimates of anthocyanin pigments were based on absorbance at 533 nm. The fruit of all red and black raspberries and blackberries contained cyanidin 3-glucoside. Some blackberries also contained cyanidin 3-rutinoside. Cyanidin 3-rutinoside and cyanidin 3-glucosylrutinoside were also present in most red raspberries. Cyanidin 3-sophoroside was present only in red raspberry and red raspberry hybrids while cyandin 3-sambubioside and cyanidin 3-xylosylrutinoside were found only in black raspberries. *R. crataegifolius*, *R. morifolius*, *R. illecebrosus* and *R. parviflorus* each contained pelargonidin glycosides as major pigments. Black raspberry had the highest anthocyanin content, over 200 mg/100g of fruit, and red raspberry had the lowest, less than 60 mg/100g. Blackberry was generally intermediate in anthocyanin content between red and black raspberries.

INTRODUCTION

RED AND BLACK RASPBERRIES, blackberries, Boysenberries and Loganberries are commercially important *Rubus* species. Their fruits are usually frozen for (a) direct consumer use, (b) production of jams and preserves, (c) concentration for flavor and coloring agents, and (d) wine production. With each of these uses the quality and quantity of the natural anthocyanin pigments affect processing characteristics and consumer acceptance.

In *Rubus* the major fruit pigments are anthocyanins, glycosides of cyanidin and in a few species glycosides of pelargonidin (Harborne and Hall, 1964; Nybom, 1968; Barritt and Torre, 1973). Quantitative estimations of individual anthocyanin pigments in *Rubus* are lacking although Blundstone and Crean (1966) determined total anthocyanin content for Loganberry and three red raspberry cultivars. The objectives of this study were to determine the fruit anthocyanin pigments in a wide range of *Rubus* species and to provide quantitative estimates of individual anthocyanin pigments.

MATERIALS & METHODS

FRUITS from 43 *Rubus* species, advanced selections and cultivars were harvested from Western Washington Research and Extension Center field plots, frozen and stored at −22°C until needed.

Quantitative determination of total anthocyanin

With minor modifications the procedure of Lees and Francis (1972) was used to determine total anthocyanin content. 20g of frozen fruit were blended for 2 min with 10 ml of extracting solvent (95% ethanol:1.5N HCl, 85:15) and quantitatively transferred to a Buchner funnel under vacuum fitted with a GF/A (glass fiber) filter. Approximately 60 ml of extracting solvent was used to facilitate washing, after which an additional 70 ml of extracting solvent was added to the residue and allowed to stand for 2 hr before vacuum was reapplied. Another 10 ml of extracting solvent was used to wash any remaining anthocyanin from the funnel and residue. The filtrate was then quantitatively transferred to a 200 ml volumetric flask and made up to volume. The pH was 1 ± 0.1. Absorbance readings were taken using a Hitachi Perkin-Elmer Model 124 double beam spectrophotometer. Readings of the undiluted extract using a 1 mm cuvet at absorbance maximum of 533 nm were converted to total mg cyanidin 3-galactoside/100g of fruit using the following formula: (absorbance × 10 mm cuvet × 200 ml × 5)/98.2. For four species with pelargonidin glycosides, *R. crataegifolius*, *R. illecebrosus*, *R. morifolius* and *R. parviflorus*, absorbance maximum was 515 nm. The complete procedure was repeated with three 20-g samples and the means are presented in Tables 1 to 4.

Qualitative determinations

A 5-g sample of frozen fruit was thawed, macerated and centrifuged. To the supernatant was added 2-propanol to precipitate the pectinatious material. After filtering through glass wool, the filtrate was transferred to a 0.8 × 10 cm column containing 32–60 mesh polyvinylpyrrolidone (PVP) Polyclar AT. After washing the column with H_2O and methanol the anthocyanin was released from the PVP with the addition of 0.1% HCl in meOH. The filtrate was concentrated to a small volume with a rotary flash evaporator and spotted onto 10 × 10 cm microcrystalline cellulose TLC plates (Brinkmann Instruments, Inc.). The plates were developed in the first direction with water-concentrated HCl-propionic acid (10:2:3) and in the second direction with n-butanol-concentrated HCl-water (5:2:1). For each *Rubus* species, cultivar and selection, an extract was spotted on three TLC plates.

After the plates were dried each spot was scanned using a Photovolt densitometer at response setting 5, slit aperture 1 mm × 6 mm and with a green 525 nm filter attached to the search head. Pigment densities were determined twice for each plate. The integrated pen response was converted to mg/100g fruit based on the proportion of the total anthocyanin content. Method for identification of individual anthocyanin pigments was described previously (Barritt and Torre, 1973, 1975).

RESULTS

SEVEN CYANIDIN and three pelargonidin pigments (Tables 1–4) in addition to five unidentified minor pigments (Table 5)

Table 1—Anthocyanin content in mg/100g of fruit for black raspberry species

	Cy 3-Gl	Cy 3-Ru	Cy 3-Sam	Cy 3-XylRu	Total
R. leucodermis — Washington native	30.0	100.4	67.8	127.7	325.9
R. occidentalis — New Hampshire native	35.9	49.8	43.6	84.5	213.8
R. occidentalis — 'Munger'	76.3	85.7	33.5	59.1	254.6
R. occidentalis — 'Cumberland'	89.0	167.7	55.2	115.8	427.7

Table 2—Anthocyanin content in mg/100g of fruit for red raspberries and red raspberry hybrids

	Cy 3-Gl	Cy 3-Ru	Cy 3-Sop	Cy 3-GlRu	Cy 3,5-diGl	Pel 3-Sop	Pel 3-GlRu	Total
R. idaeus var. *strigosus* —								
New Hampshire native	2.9	3.4	8.9	9.5		T[a]	0.8	25.5
R. idaeus — 'Augustred'	3.7	7.1	4.7	6.7		0.4	0.8	23.4
R. idaeus — 'NH TXP15'	9.2	3.6	30.4	6.2		2.5		51.9
R. idaeus — 'Willamette'	16.8		35.6		2.4	4.3		59.1
R. idaeus — 'Lloyd George'	7.7	2.2	30.4	10.6				50.9
R. idaeus — 'Meeker'	10.1	2.7	18.6	8.3				39.7
R. idaeus — 'Glen Clova'	7.6	2.5	16.6	6.9				33.6
R. idaeus — 'Puyallup'	3.8	4.1	11.3	9.3				28.5
Raspberry-Blackberry Hybrids —								
'Boysenberry'	42.5	5.3	71.6	41.5				160.9
'Loganberry'	16.7	5.2	37.2	18.3				77.4

[a] T equals trace amounts

Table 3—Anthocyanin content in mg/100g of fruit for blackberries

	Cy 3-Gl	Cy 3-Ru	Pel 3-Gl	Other unidentified pigments[a]	Total
R. allegheniensis	129.2	51.0			180.2
R. amabilis	137.5			(A) T[b]	137.5
R. caesius	75.6	6.9			82.5
R. canadensis	95.7				95.7
R. caucasicus	92.9		4.9		97.8
R. dregeri	221.6	75.6		(A) T; (C) T; (E) 28.7	325.9
R. laciniatus	148.7				148.7
R. picetorum	171.1				171.1
R. plicatus	171.4	41.5		(E) 21.3	234.2
R. procerus	143.6			(A) T	143.6
R. radula	145.6			(B) T	145.6
R. scanicus	138.1	9.6			147.7
R. scheutzii	161.9			(A) T; (B) T	161.9
R. shankii	159.9			(A) T; (E) T	159.9
R. wahlbergii	186.7	25.3		(A) 11.7; (D) 2.9; (E) 17.8	244.4
R. ursinus	64.0	45.0			109.0
'OR-US 1122'	121.4	17.6		(B) 3.0; (E) 8.7	150.7
'Black Satin'	142.1			(C) 3.9; (E) 9.0	154.8
'Dirksen Thornless'	131.4			(E) T	131.4

[a] Upper case letters refer to pigment designations in Table 5.
[b] T equals trace amounts.

Table 4—Anthocyanin content in mg/100g of fruit for a miscellaneous group of Rubus clones

	Cy 3-Gl	Cy 3-Ru	Cy 3,5-diGl	Pel 3-Gl	Pel 3-Ru	Other unidentified pigments[a]	Total
R. crataegifolius	6.5			30.2			36.7
R. illecebrosus	4.4			30.2			34.6
R. morifolius	1.2			18.1			19.3
R. neo-mexicanus	11.0	35.9	3.2			(C) 2.8; (D) 2.7; (E) 11.6	67.2
R. parviflorus	5.4			33.4	18.2		57.0
R. parvifolius — 'NC 701-4'	13	30.6					43.8
R. parvifolius — 'NC 1164-4'	20.8	24.0					44.8
R. parvifolius — 'ARK 57-4-5'	16.8	25.0					41.8
R. phoenicolasius	24.0	28.6					55.0[b]
'Dormanred'	3.3	19.1					22.4

[a] Upper case letters refer to pigment designations in Table 5.
[b] Includes 2.4 mg/100g of Cy 3-XylRu.

Table 5—R_f values for unidentified minor anthocyanin pigments

Pigment designation	Water:HCl:propionic acid (10:2:3)	n-butanol:HCl:water (5:2:1)
A	40	57
B	27	53
C	59	59
D	57	72
E	67	71

were found in the *Rubus* clones examined. Based on the distribution of individual anthocyanin pigments and total anthocyanin content, the *Rubus* clones examined were separated into four groups.

Black raspberry group

Fruits were characterized by two xylose-containing pigments, cyanidin 3-sambubioside (Cy 3-Sam) and cyanidin 3-xylosylrutinoside (Cy 3-XylRu), and by having a total anthocyanin content of more than 200 mg/100g of fruit (Table 1). *R. leucodermis* and *R. occidentalis* are closely related species native to Western and Eastern North America, respectively. Black raspberry cultivars such as 'Cumberland' appear to be an excellent highly concentrated source of natural cyanidin pigments.

Red raspberry and raspberry hybrid group

Fruits were characterized by the presence of cyanidin 3-sophoroside (Cy 3-Sop) as a major pigment, and by total anthocyanin concentrations in the low range between 20 and 60 mg/100g of fruit (Table 2). Two red raspberry/blackberry hybrids, 'Boysenberry' and 'Loganberry', had pigments typical of red raspberry and a total anthocyanin content more typical of blackberry (see below). They are morphologically blackberries as the drupelets do not separate from the torus (core) when picked as do raspberry fruits.

Blackberry group

Blackberry fruits were characterized by one major pigment, cyanidin 3-glucoside (Cy 3-Gl), and, in some cases a second pigment, cyanidin 3-rutinoside (Cy 3-Ru) in lower concentrations (Table 3). Total anthocyanin content was higher than red raspberries and generally lower than black raspberries, although 3 of 19 blackberry species studied, *R. dregeri, R. plicatus* and *R. wahlbergii,* had high total anthocyanin contents similar to black raspberries.

Miscellaneous Rubus group

Neither the Cy 3-Sop and cyanidin 3-glucosylrutinoside (Cy 3-GlRu) typical of red raspberries or the Cy 3-Sam and Cy 3-XylRu typical of black raspberries were major pigments in this group, and the total anthocyanin content was less than 70 mg/100g of fruit (Table 4). Pelargonidin 3-glucoside (Pel 3-Gl) and pelargonidin 3-rutinoside (Pel 3-Ru) were major pigments in *R. crataegifolius, R. morifolius, R. illecebrosus,* and *R. parviflorus,* while Cy 3-Gl was found in very low concentrations. Fruits of 3 clones of the Asiatic trailing raspberry, *R. parvifolius,* and 'Dormanred', a *R. parvifolius* hybrid, contained only Cy 3-Gl and Cy 3-Ru, and had total anthocyanin contents of less than 50 mg/100g of fruit. None of this group could be morphologically classed as blackberries as the torus is not imbedded in the fruit, but the fruit, when pulled from the plant is hollow.

DISCUSSION

THE DISTRIBUTION of major cyanidin fruit pigments in a wide range of red and black raspberries and blackberries found in this study confirm previous reports (Barritt and Torre, 1973, 1975; Francis, 1972; Harborne and Hall, 1965; Nybom, 1968). Only *R. parviflorus,* the Thimbleberry, and *R. odoratus,* the flowering raspberry, have been reported to have major amounts of pelargonidin glycosides (Barritt and Torre, 1973; Harborne and Hall, 1964). We report three additional species, *R. crataegifolius, R. illecebrosus,* the strawberry raspberry, and *R. morifolius,* the Korean tree berry, which have Pel 3-Gl as their major fruit pigments.

Total anthocyanin content for black raspberry fruits was high with over 200 mg/100g of fruit while red raspberry fruits contained less than 60 mg/100g. Blackberry fruits usually contained intermediate amounts. The quantitative determinations of total anthocyanin content for red raspberry and Loganberry are in agreement with those of Blundstone and Crean (1966).

Based on the distribution of individual pigments it would be possible to determine if processed products such as puree and jelly were prepared from either blackberry, red or black raspberry fruits. If xylose containing pigments, Cy 3-Sam and Cy 3-XylRu, were found black raspberry fruit would be present, if Cy 3-Sop were found red raspberry fruit would be present, and if these pigments were absent and Cy 3-Gl and Cy 3-Ru were found to be the only major pigments blackberry fruits would be present.

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INVOLVEMENT OF ETHYLENE IN THE HARDCORE SYNDROME OF SWEET POTATO ROOTS

ABSTRACT

Evidence presented supports our thesis that an ethylene mediated reaction(s) functions in the series of events which lead to a market disorder of sweet potatoes called "hardcore." Storage of roots in an environment conducive to ethylene accumulation or containing exogenous ethylene resulted in a higher incidence of hardcore. Alternatively, storage conditions designed to minimize endogenous ethylene accumulation decreased the extent of hardcore. Induction of hardcore was markedly reduced by carbon dioxide, a competitive inhibitor of ethylene mediated reactions. Sweet potato roots weighing less than 200g had a lower incidence of hardcore compared to larger roots. Also, low levels of ethylene gas were detected in the volatiles of sweet potato roots after long-term chilling.

INTRODUCTION

HARDCORE is a market disorder of sweet potatoes where areas of the cooked root retain the firm texture of raw tissue. Recent evidence has demonstrated hardcore is induced by storing roots below 15°C (Daines et al., 1974; 1976) and that it is caused by modification of the intercellular cement (Daines et al., 1976; Haard et al., 1976). Buescher (1976) has reported that treatment of sweet potato tissue with tannic acid results in tissue firming analagous to hardcore. These data suggest that a tannin-like substance is responsible for hardcore. It is known that treatment of sweet potato root with exogenous ethylene evokes the accumulation of phenolic substances (Imaseki et al., 1968). The purpose of this study was to examine the involvement of ethylene gas in the induction of hardcore by chilling temperatures. The data indicate that an ethylene mediated reaction functions in the chain of events leading to the hardcore syndrome of sweet potato root tissue.

MATERIALS & METHODS

SWEET POTATO ROOTS (*Ipomea batatas,* Jewel) were purchased from a local farmer at harvest time, dry cured at 20°C, 50% relative humidity for 2 wk and subsequently stored at 15°C, 85% relative humidity. A different lot of Jewel sweet potatoes, originating from North Carolina, were purchased from a local market for an experiment designed to measure endogenous ethylene concentration and the influence of carbon dioxide on hardcore development.

Hardcore was detected by boiling roots in tap water for 45 min as described by Daines et al. (1974). Hardcore tissue was separated from normal tissue by rapidly running water. Tissue which remained intact under the water stream was indicated as hardcore. The firmness of this tissue varied but in all cases the tissue indicated as hardcore was judged inedible because of its texture. The "percent severity" of hardcore was determined by dividing the weight of roots exhibiting hardcore by the total weight of the cooked tissue. The "percent incidence" of hardcore was determined by dividing the number of roots exhibiting any degree of hardcore by the total number of roots examined.

Roots were incubated under the various environmental regimes of temperature, atmospheric pressure and atmospheric content as described in the results section. In most experiments storage time under chilling conditions was 6 days as described by Daines et al. (1976). In all experiments roots were boiled and analyzed for hardcore immediately after removal from cold storage. In certain experiments roots were stored at 22°C for up to nine days after chilling prior to analysis for hardcore.

The headspace of 30 liter jars containing 20 roots each was analyzed for ethylene. Jars were incubated at various temperatures as described in the text and flushed at 24-hr intervals. Ethylene was analyzed by gas-liquid chromatography on a chromosorb 104 (6 ft × ¼ in.) column. The oxygen concentration of jars was monitored with a Servomex oxygen analyzer.

RESULTS & DISCUSSION

Hypobaric storage

The incidence and severity of hardcore in roots stored at 2°C for 6 days was considerably lower at 0.1 atmosphere containing 100% oxygen than at 1 atmosphere of air or 1 atmosphere of 10% oxygen and 90% nitrogen (Fig. 1). Hardcore was most severe immediately after cold treatment and declined markedly on storage at 22°C. These data are contrary to previous findings that hardcore increases after removal from a chilling environment and subsequent storage at ambient temperatures (Daines et al., 1974, 1976). The size of the root influenced the development of hardcore and its reversal by subatmospheric pressure (Fig. 2 and 3). Roots weighing greater than 200g exhibited a higher incidence of hardcore under all storage conditions (Fig. 2). Larger roots also showed a slower reversal of hardcore as a result of storage at 22°C after chilling. Also, hypobaric conditions were more effective in reducing hardcore in the smaller roots.

The reduction in chill-induced hardcore by hypobaric conditions can be attributed to an increased diffusivity of endogenous ethylene gas from internal tissue spaces. Hypobaric storage of fruits and vegetables retards physiological events known to be mediated by ethylene such as fruit ripening and chilling disorders (Burg, 1975; Salunkhe and Wu, 1975). While it is possible that volatile substances other than ethylene may be involved these data rule out the possibliity that reduced oxygen tension was solely influential since 1 atmosphere at the same partial pressure of oxygen as hypobaric samples (10%) resulted in a comparable induction of hardcore as air. The severity of hardcore in larger roots was significantly greater after chilling in 10% O_2 than in air (Fig. 3). The suggested involvement of volatiles like ethylene is also supported by the observation that small roots, having a greater surface to volume ratio, exhibited a lower incidence of hardcore. The greater surface area of small roots may facilitate diffusion of ethylene from internal tissue spaces.

Hyperbaric storage

The incidence and severity of hardcore was greater for roots stored at 1.7 atmosphere of air containing 100 ppm ethylene than in roots stored at 1 atmosphere of air (Table 1). Again, the induction of hardcore was most pronounced in larger roots; however, hyperbaric conditions did not increase, but rather decreased, hardcore in small roots. This indicates that some factor other than, or in addition to, the diffusivity of volatiles is related to the low susceptibility of small roots. The data with large roots shows that a hyperbaric environment containing ethylene increases the chilling induction of hardcore and are accordingly consistent with our thesis.

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Exogenous ethylene

Roots stored in a continuous air stream containing 92 ppm exogenous ethylene exhibited a greater incidence and severity of hardcore under chilling conditions than roots stored in air (Table 2). In this study, significant levels of hardcore were detected in roots stored at 22°C and the severity of hardcore was increased somewhat with ethylene treatment. The tissue exhibiting hardcore in roots stored under ethylene was also observed to be firmer by subjective analysis than was hardcore tissue from air-stored roots. The hardcore in roots stored at 22°C was not observed in previous trials and may reflect changes occurring during storage at 15°C after harvest.

Endogenous ethylene

Roots stored at various temperatures in sealed jars did not evolve detectable levels of ethylene until 6 days storage (Table 3). There was no relationship between ethylene concentration in the headspace (0.7–11 ppm) and hardcore at the different temperatures (Table 3). The finding that considerable hardcore occurred at nonchilling temperatures (Table 4) is again contrary to previous findings (Daines et al. 1974, 1976) and is apparantly related to the postharvest age of the roots. As storage of the sweet potatoes used in this study in a continuous air stream or in a ventilated incubator resulted in negligible hardcore the use of a static environment may also by influencial. These data do not fully support our contention that ethylene

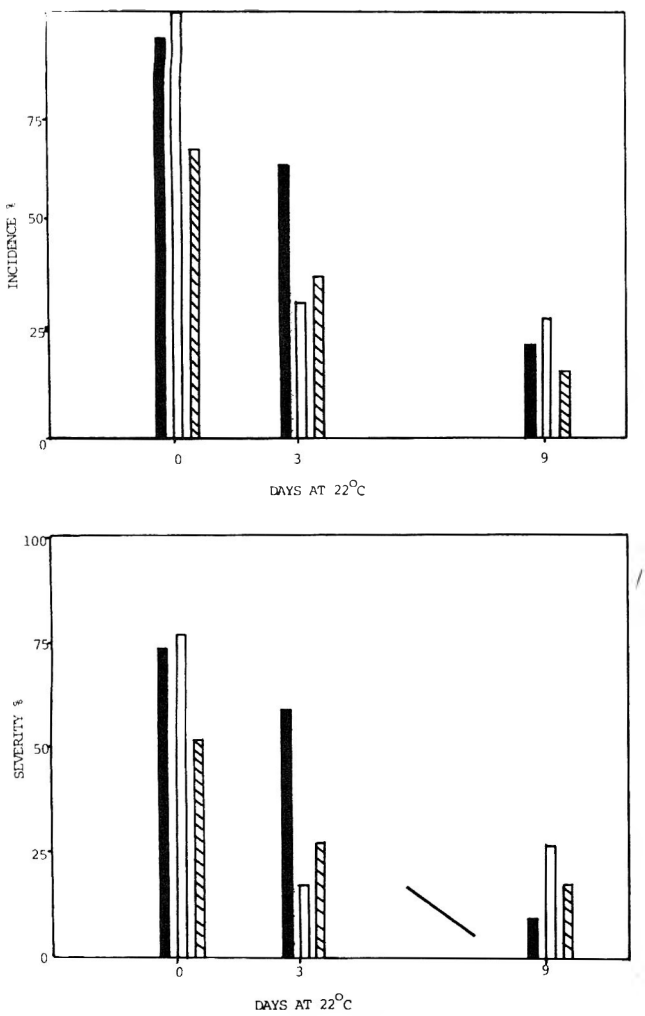

Fig. 1—Influence of hypobaric conditions on chilling induced hardcore. Roots (50 for each treatment) were incubated in the indicated atmospheres for 6 days at 2°C. One lot of 50 roots was boiled immediately after removal from chilling environment and additional lots were analyzed for hardcore after 3 and 9 days storage at 22°C in air. Incubation environments were 1 atmosphere air ■, 1 atmosphere 10% O_2 in N_2 □, 0.1 atmosphere 100% O_2 ▨.

Table 1—Influence of hyperbaric conditions on hardcore induction

Sweet potatoes (wt range, g)	Hardcore severity (%)[a]	
	Air (1 atmos.)	Air (1.7 atmos.)
125–480	41.4	59.6
125–199	12.2	0.0
200–480	62.6	86.0

[a] Roots (20 in each treatment) were stored at 2°C for 6 days under continuous flow at the indicated pressure. Air employed for hyperbaric conditions contained 100 ppm ethylene. Roots were analyzed for hardcore immediately after removal from cold storage.

Table 2—Influence exogenous ethylene on hardcore induction

Storage environment[a]	Hardcore severity (%)	Hardcore incidence (%)
Air, 2°C	64.0	73.3
Air + C_2H_4 (92 ppm), 2°C	82.0	100
Air, 22°C	3.9	26.6
Air + C_2H_4 (92 ppm), 22°C	14.0	20.0

[a] Roots (15 in each treatment) were stored in a continuous stream of air or air containing ethylene for 3 days at the indicated temperatures and maintained at that temperature for 3 more days and analyzed for hardcore immediately after removal from storage.

Table 3—Influence of storage temperature on hardcore and ethylene evolution

Storage[a] temp (°C)	Hardcore severity (%); C_2H_4 (ppm)	
	6 Days	12 Days
2	39.1 (n.d.)	89.5 (4.1)
5	26.1 (.74)	79.6 (n.d.)
7	30.3 (n.d.)	87.4 (n.d.)
9	10.6 (n.d.)	38.5 (n.d.)
18	10.6 (n.d.)	21.3 (11.1)
22	12.0 (n.d.)	52.8 (n.d.)

[a] Roots (20 at each temperature) were stored in a static environment for 24-hr intervals and flushed. The limit of ethylene detection was 0.5 ppm. No ethylene was detected during the initial 5 days storage at 2°C.

Table 4—Influence of exogenous CO_2 on hardcore

Storage conditions[a]	Hardcore severity (%)	
	22°C	7°C
Air, static	14.7	80.5
Air, ventilated	6.6	78.3
Air + CO_2 (5%), static	35.2	24.9

[a] Roots (15 in each treatment) were stored as indicated for 6 days and analyzed for hardcore immediately.

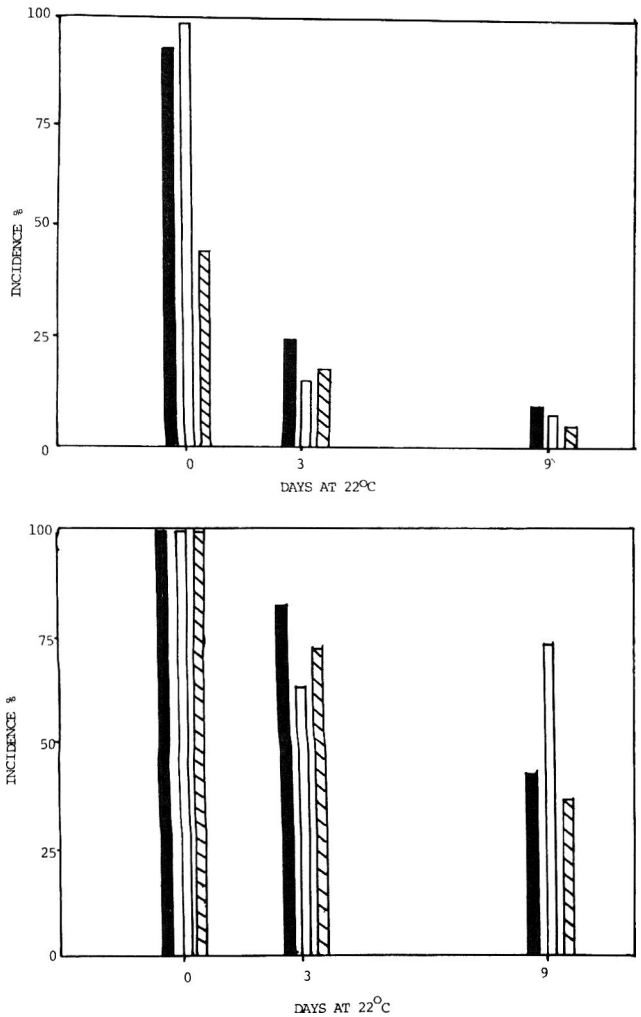

Fig. 2—Influence of root size on the incidence of hardcore after chilling in different atmospheres. Sampling was as described in the legend of Fig. 1.

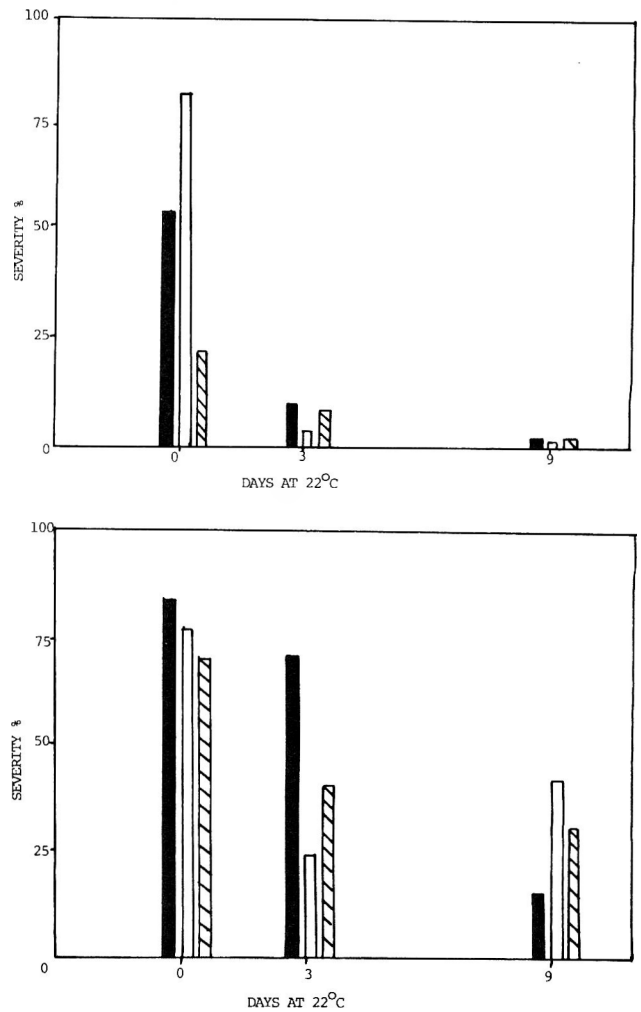

Fig. 3—Influence of root size on the severity of hardcore after chilling in different atmospheres. Sampling was as described in the legend of Fig. 1.

is causally related to hardcore induction. It is, however, possible that ethylene concentration in the surrounding atmosphere is not representative of ethylene concentration in internal spaces of the tissue.

Exogenous carbon dioxide

Storage of roots in a static environment (flushed after 24-hr intervals) containing 5% CO_2 significantly reduced the severity of hardcore induced by chilling (Table 4). It is known that CO_2 is a competitive inhibitor of ethylene mediated events (Abeles, 1973). Roots maintained in an atmosphere containing 5% CO_2 at 22°C also showed appreciable hardcore. These data indicate accumulation of CO_2 in the storage environment affects induction of hardcore differently at chilling and nonchilling temperatures. The inhibitory effects of CO_2 at low temperatures does, however, support our thesis that the hardcore syndrome is, in part, dependent on ethylene. Again, hardcore tissue in roots stored at 22°C was considerably softer than that found in chilled roots.

The data presented implicate ethylene in the induction of hardcore by chilling temperatures. Exogenous ethylene and hyperbaric conditions increase the severity of hardcore. Alternatively, hypobaric conditions and CO_2 decreased hardcore. Evolution of ethylene by sweet potatoes was low and did not correlate consistently with the incidence of hardcore. Measurement of internal ethylene concentration may provide a more reliable measure of ethylene. This study also showed that small roots are less susceptible to chilling induced hardcore and that carbon dioxide can induce hardcore at nonchilling temperatures.

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THE USE OF A COMMERCIAL Pediococcus cerevisiae STARTER CULTURE IN THE PRODUCTION OF COUNTRY-STYLE HAMS

ABSTRACT

Hams were inoculated with *Pediococcus cerevisiae* cells to determine effects of a lactic acid starter culture on country-style ham quality. Treatments consisted of two different cure mix levels, three aging times and five inoculum levels. Hams were subjected to taste panel, physical, chemical, and microbial analyses. Acidity and white film formation on cut surfaces were higher for inoculated hams although taste panel analyses indicated little difference from control hams. Increased aged flavor and nuttiness flavor were noted at the higher NaCl level. Inoculated hams decreased in pH during the initial days of aging but increased later in the aging period. Increases in pH and the concomitant development of aged flavor confirmed previous work done on flavor at this station that at least 80 days processing time must elapse when using our processing conditions before aged flavor becomes acceptable. The total time required to produce aged hams may be shortened by inoculating hams with *P. cerevisiae*.

INTRODUCTION

COUNTRY-STYLE ham is a specialty item which is very popular in the southeastern United States. A natural aging process is responsible for flavor development that is both temperature and time dependent (Cecil and Woodroof, 1954).

Using current processing methods, assurance of top quality hams is not as controllable as processors would like.

Commercial concentrated starter cultures containing different species of lactic acid bacteria, as well as nonlactic species, have been used extensively in recent years in the production of dry and semi-dry sausages. A more consistent and higher quality is attainable in these sausages by using starter cultures rather than having to depend on chance contamination (Niven et al., 1958). However, other workers have reported good results by natural contamination under controlled processing conditions (Palumbo et al., 1973; 1976). Sharpe (1962) reported that lactobacilli were responsible for flavor production in some fermented sausages.

This study was undertaken to explore the effects of *Pediococcus cerevisiae* on quality characteristics of country-style hams.

EXPERIMENTAL

42 SHORT-CUT, skinned hams (7.3 ± 1.6 kg) were obtained from a local meat processor, wrapped in freezer paper, placed by pairs in cardboard boxes, and held in frozen storage at −29°C until used (2–12 months). Melo et al. (1974) found practically no difference in hams stored up to 3 yr under these conditions. Hams were alloted to three treatments or factors in a 2 × 3 × 5 factorial randomized block design (Cochran and Cox, 1957). The first factor, the curing mixture (78.5% sodium chloride; 19.7% sucrose; 1.5% sodium or potassium nitrate; 0.3% sodium nitrite), was applied at the rate of 50 or 70 g/kg of ham. The second factor was sampling time at 80, 95, and 110 days after initiation of curing. The third factor was the inoculum size of lactic acid bacteria at five different levels: 1.2×10^4, 4.0×10^6, 7.5×10^6, and 2.3×10^7 cells/g of ham, and controls with no inoculum. The choices of inoculum sizes were based on the pH response and the number recovered from the meat. The hams were randomly assigned to the three factor treatments with inoculum levels 7.5×10^6 and 2.3×10^7 cells/g of ham being duplicated because of time and ham number restrictions.

Concentrated lactic acid starter culture

A frozen concentrate of *Pediococcus cerevisiae* was obtained from a commercial source (Chr. Hansen's Lab., Inc., Milwaukee, Wis.) 2 days prior to use. The starter culture is marketed under the brand name of "Redi-Set Meat Cultures #PC-1 (American Meat Institute, U.S. Patent No. 2,907,661) and is recommended for use in fermented sausages. All cultures were shipped and stored in dry ice prior to use. Each shipment of starter cultures was tested for viability in APT (BBL) agar from which serial dilutions were made and NaCl tolerance was tested in increasing concentrations in APT broth.

Inoculation and processing procedures

Frozen concentrated starter cultures were thawed in lukewarm water for 20–25 min and mildly agitated prior to use; whereupon, thawed weighed hams were inoculated immediately with viable *P. cerevisiae* cells at 10 different locations via a 10 ml syringe equipped with a 20 gauge, 3.8 cm length needle. The inoculum volume was minimal (1.00 to 11.67 mls/ham) to not appreciably alter ham moisture content. This volume is insignificant compared to the total water (ca 4.5 kg) present in the ham. The important consideration is the number of cells present and not the volume of the injection. The butt was inoculated in muscle tissue on each side of the ilium. Inoculum was injected into the cushion area with three injections in the quadriceps and adductor muscle area and two injections in the semimembranosus, biceps femoris, and semitendinosus muscle area. The shank was inoculated in muscle tissue on each side of the tibia and fibula.

Curing, salt equalization, and aging were done under the same conditions as described by Butz et al. (1974). One-third of the curing mixture required for each ham was applied on the first, third and tenth days of cure. The hams remained in cure for 4 days/kg of ham at 4°C and about 80% relative humidity (RH). Ultraviolet light was used in the curing room to control the surface growth of microorganisms.

After curing, the hams were weighed, placed in stockinettes, reweighed, and held at 11 ± 2°C, RH 61 ± 5% for 30 days to permit equalization of curing ingredients. They were then aged with the butt end hung downward for 19, 34, or 49 days at 29 ± 4°C and RH 49 ± 5% and the weight of each ham recorded. In both aging and equalization, an airflow of 10.5 M/min was maintained at the ham surfaces.

pH determination

The pH was determined on each ham before and after curing, after equalization, and during aging at 70, 80, 95 and 110 days. A Leeds and Northrup pH meter model no. 7401 fitted with a Corning combination electrode was used for pH measurements. The pH measurements were made in the gluteus medius muscle of the butt and the semimembranosus muscle of the cushion; incisions were made approximately 1.9 cm deep for electrode insertion. One milliliter of distilled water was placed in the incision to facilitate ease of pH measurement in the aged ham.

Sampling procedure

After aging, hams were trimmed free of subcutaneous fat and skin, the outside area treated with 95% ethanol, then flamed and a core 2.2 cm in diameter removed aseptically in cross-sectional area of muscles for microbial analyses. The coring instrument was a metal tube sharpened on one end, 37 cm long and of the above diameter. The cored plug was removed from corer with a sterile metal rod. To obtain sufficient samples for analyses, three cores were removed from the butt, divided into thirds and labeled butt inside (BI) from adductor, semimembranosus and/or quadriceps; butt middle (BM) from semitendinosus and/or quadriceps; and butt outside (BO) from bicips femoris and/or quadriceps; in like manner the sections from the cushion were labeled cushion inside (CI) from adductor, semimembranosus, semitendinosus and/or quadriceps and cushion outside (CO) from biceps femoris and/or quadriceps.

Table 1—Percent moisture, pH, fat and NaCl content by processing time

	Moisture	pH	Fat	NaCl
T_1[a]	64.08	5.77	4.29	4.52
T_2	62.77	5.90	5.02	4.48
T_3	60.59	5.91	4.57	5.33

[a] T_1 = 80 days processing; T_2 = 95 days processing; T_3 = 110 days processing. Total of 14 hams used for each sampling time.

Subjective evaluations of ham quality

Three trained taste panel members evaluated ham quality on a broiled, 9.5 mm thick slice taken from the cushion of the ham (biceps femoris muscle). The following factors were evaluated by the taste panel: saltiness, tenderness-elasticity, crumbliness, softness, juiciness, aged flavor, acidity, nuttiness, color, fruitiness, bitterness, moldiness, feed sack, and rancidity. Samples were rated using a scale of 1–7 with a mean of 4.00 considered as optimum except for the factors fruitiness through rancidity which were considered as undesirable when scored greater than 4. The meat was broiled for 12 min at ca 180°C and 15 cm from the heat source. The white film which may form on the cut surface of the ham during storage was rated subjectively by one panel member on a 0–5 scale: 0, none; 1, very slight; 2, slight; 3, moderate; 4, heavy; and 5, very heavy.

Identification procedures

Plate counts were made with APT and LBS agars (BBL) incubated at 30°C for 48–72 hr. A modified LBS agar (Costilow et al., 1964) of pH 5.6 was used. Organisms growing on LBS agar were considered to be lactic acid bacteria. Details of identification procedures, including sample size and sample preparation, are given in Bartholomew and Blumer (1977).

Physical and chemical measurements

The percent moisture was determined on duplicate homogenized samples according to AOAC (1965). Duplicate samples agreed within 0.50%. Aliquots from the same homogenized sample were used for each of the measurements listed in this section.

The pH of homogenized samples was determined with a pH meter on homogenized aged ham samples taken after 80, 95, and 110 days processing independent of the pH measurements made in the intact ham muscles during processing. A titration was also made on 10g of homogenized sample with 0.5N HCl to determine milliequivalents of acid needed to change the pH by 1.0 pH unit.

Percent fat was determined according to AOAC (1965) on duplicate homogenized samples. Duplicate samples had no more than 10% error for samples containing less than 5% fat and a difference no greater than 0.50% between duplicates containing more than 5% fat.

Sodium chloride was determined to ± 0.2% accuracy on duplicate homogenized samples by the method of Glasstone (1946) as adapted by Graham and Blumer (1972).

Data were analyzed using a regression procedure of Snedecor and Cochran (1967).

RESULTS & DISCUSSION

Physical and chemical measurements

Moisture, fat and NaCl content were similar to those encountered in other studies (Graham and Blumer, 1972; Melo et al., 1974) (Table 1). Percent moisture decreased, while pH, percent fat, and salt increased. The increase in pH is due in part to free amino acid production by catheptic enzymes (Melo et al., 1974). A linear reduction in pH was observed between pH 6.0 to 5.0 in titration of the homogenized tissue with HCl. Approximately 0.38 meq of HCl were required to lower pH of 10g of aged country-style ham 1.0 pH unit; about 0.41 meq of lactic acid would be required by titration to reduce the pH from 6.0 to 5.0. Lactic acid of microbial origin causes some of the pH reduction in dry-cured ham with microorganisms acting on glucose or other carbohydrates present. This acid is a weak acid and does not completely dissociate in aqueous solution as does HCl. The percent dissociation of lactic acid ranges from 93.2% at pH 5.0 to 99.3% at pH 6.0. The usual pH range of dry-cured ham is from 5–6.

Quality evaluation

Subjective evaluations indicated that control and inoculated hams were similar in flavor and other quality characteristics, although aged flavor increased as acidity increased, but not significantly. Nuttiness was highest at the I_1 inoculum level, but further work is needed to confirm the value of this finding. *Pediococcus cerevisiae* is known to produce possible flavor related compounds (Bothast et al., 1973). Sharpe (1962) found that experimental inoculation of hams with high populations of *Lactobacillus casei* and *Lactobacillus plantarum* varied little organoleptically from the controls. In Table 2, both NaCl and acidity appeared to enhance aged flavor significantly ($P < 0.05$). Hams receiving the 7% cure mix treatment were rated best for aged flavor and nuttiness ($P < 0.05$). The increases in acidity were not significant ($P > 0.05$).

White film formation increased generally in inoculated hams and with increased aging time. A significant increase ($P < 0.05$) in ham film was observed between 80 and 95 days (Table 3). Film formation was positively correlated ($P < 0.05$) with increased crumbliness, softness, and tenderness. It is well

Table 2—Taste test factors[a] by cure mixture and time periods of storage

	Saltiness	Tenderness-elasticity	Crumbliness	Softness	Juiciness	Aged flavor	Acidity	Nuttiness	Color	Fruitiness	Bitterness	Moldiness	Feed sack	Rancidity
S_1[b]	3.83	4.16	4.06	4.10	4.65	3.42	4.56	4.01	4.00	4.17	4.11	4.01	4.03	4.00
S_2[b]	3.94	4.08	4.03	3.96	4.67	3.63*	4.72	4.09*	3.94	4.06	4.12	4.01	4.00	4.00
T_1[c]	3.93	4.56	3.69*	3.44	4.46	3.34	4.54	4.02	3.93	4.02	4.10	4.04	4.02	4.00
T_2[c]	3.91	3.84*	4.21	4.34	4.67	3.56	4.74	4.06	3.90	4.11	4.15	4.00	4.00	4.00
T_3[c]	3.81*	3.96	4.25	4.29	4.84	3.67	4.64	4.07	4.08	4.21	4.09	4.00	4.02	4.00

[a] Mean of 4.00 was considered optimum.
[b] Average of 21 hams; S_1 = 5% cure mix; S_2 = 7% cure mix.
[c] Average of 14 hams; T_1 = 80 days processing; T_2 = 95 days processing; T_3 = 110 days processing.
* Values are significantly different ($P < 0.05$) than other values within a column.

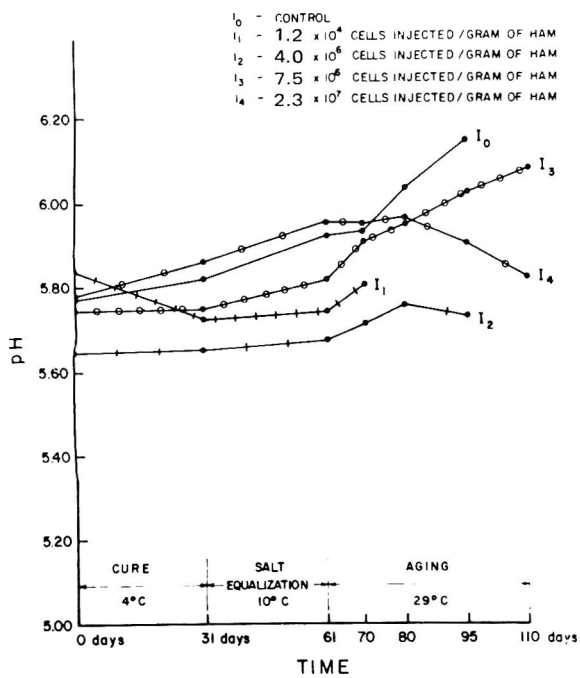

Fig. 1—Ham butt pH by inoculum levels.

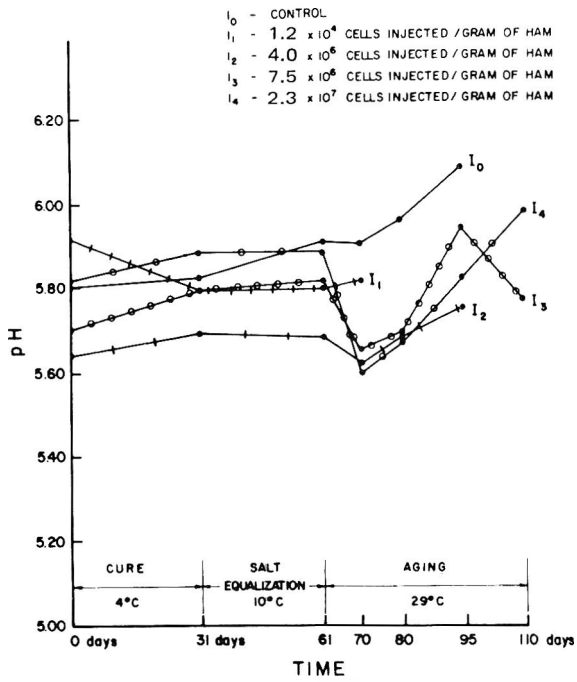

Fig. 2—Ham cushion pH by inoculum levels.

known that as the pH of meat decreases, so does tenderness. Based upon past studies, we might expect that a lowering of pH by increased acid production would decrease tenderness. Ham film was also significantly greater ($P < 0.05$) with higher lactobacilli numbers and LBS counts, but tended to decrease appreciably when a greater number of staphylococci were present ($P < 0.05$). Ham film was also greater with higher lactobacilli numbers and LBS counts, but tended to decrease when more staphylococci were present. LBS counts included both lactobacilli and pediococci numbers. Increased film production was probably due to lactic acid production and/or staphylococcal inhibition affecting free amino acid concentration. Ham film formation results from free amino acid precipitation (mainly tyrosine) on sliced ham surfaces (Butz et al., 1974).

Table 3—Film scores by time and by inoculum levels[a]

Time[b]	Film score	Inoculum	Log_{10} LBS counts	Film score[e]
80 days	0.3d	I_0[c]	1.48	0.8d
95 days	3.1e	I_1[c]	7.11	1.8d,f
110 days	2.8e	I_2[c]	7.73	3.3e
		I_3[d]	7.90	2.3f
		I_4[d]	7.79	1.9f

[a] Film scoring: 0 = none; 1 = very slight; 2 = slight; 3 = moderate; 4 = heavy; 5 = very heavy. I_0 = no inoculum; I_1 = 1.2 X 10^4 cells/gm; I_2 = 4.0 X 10^6 cells/gm; I_3 = 7.5 X 10^6 cells/gm; I_4 = 2.3 X 10^7 cells/gm

[b] Total fo 14 hams used for each sampling time

[c] 6 hams used for each inoculum level

[d] 12 hams used for each inoculum level

[e] Film scores between time or inoculum levels followed by different letters are significantly different ($P < 0.05$).

Lactobacilli and staphylococci from these hams were identified in another study (Bartholomew, 1975).

Inoculum size and pH of ham muscle tissue

Inoculated hams were significantly lower in pH in both the butt and cushion compared with control hams after about 70 to 80 days processing (Fig. 1 and 2). However, the number of cells injected into the ham did not result in a uniform pattern of pH response for either the ham butt or cushion areas. An inoculum level of about one million *P. cerevisiae* per gram was sufficient to cause pH reduction which occurred primarily during the aging period. The other processing periods at 4°C and 10°C had temperatures too low for sufficient acid to be produced and appreciably affect the pH. Graham and Blumer (1972) found that ham pH prior to cure was lower than that encountered after cure; but, for the inoculated hams of this study, the pH was usually the lowest during the early part of aging. However, it may be observed in Figure 1 that, as time in processing progressed beyond 70 days, the pH increased in the ham butts for all inoculum levels except I_2 and I_4. In the cushion area shown in Figure 2, the pH increased rather abruptly after 70 days for all inoculum levels except. I_1. The variation in pH may be due to the nonhomogeneous nature of the hams, variability of growth factors, and to variable numbers of bacterial cells within the cores sampled. These data support work previously done in this laboratory that at least 80 days of processing time must elapse under the above conditions before aged flavor becomes acceptable. The increase in pH is due to the hydrolysis of amino acids and the exposure of basic groups to a level where the acid produced by the lactic acid bacteria is neutralized and the pH is elevated. The level of free amino acids appears to be associated with aged flavor in country-style ham (McCain et al., 1968).

Processing time

The I_2 inoculum level hams, after being stored at equalization temperature (11°C) for 2 wk, were subjected inadvertently to an internal temperature of 38°C for perhaps 36 hr

without any apparent effect on odor or flavor acceptability. The presence of lactic acid bacteria and reduced pH of the tissue probably inhibited spoilage organisms. Ordinarily, 2 wk equalization time is insufficient for the curing ingredients to equalize within the ham tissue at this temperature. Under usual curing conditions, spoilage, or at least the production of off-flavors, would be expected, since the abnormal internal temperature obtained during storage would favor the growth of spoilage bacteria in ham areas where NaCl concentration was low with an accompanying high moisture level in the muscle tissues. The feasibility of reducing processing time when using lactic acid bacteria needs further study.

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MICROBIAL INTERACTIONS IN COUNTRY-STYLE HAMS

ABSTRACT

Hams were inoculated with *Pediococcus cerevisiae* cells to determine effects of a lactic acid starter culture on resident microflora of hams. Butt microflora of control hams was predominated by lactobacilli, whereas coagulase negative staphylococci were most prevalent in the cushion. Inoculated hams were predominated by *P. cerevisiae* in both butt and cushion areas. Other bacteria isolated included fecal streptococci, *Enterobacteriaceae*, micrococci, bacilli, and yeasts. The natural flora of country-style ham appeared to be antagonized by *P. cerevisiae*, while a staphylococcal isolate and fecal streptococci were most antagonistic to the natural flora on agar media.

INTRODUCTION

SALT tolerant microorganisms predominate in the resident microflora of country-style hams and apparently have little effect on ham quality (Graham and Blumer, 1971). However, earlier unpublished work done in this laboratory indicates that hams with few total bacteria or none present after aging were rated higher in flavor than those with large numbers present.

Coagulase negative *Staphylococcus aureus* is the predominant microorganism found in the cured and aged ham. Lance-

Table 1—Methods of identification and isolation

Microorganism	Isolation procedure	Incubation °C	Incubation Hours	Identification procedure
All microorganisms	APT agar (BBL)	30	48–72	Streaked on APT agar. Purified cu tures carried on BHI[a] agar slants. Gram stain, pH reductions in APT broth; nitrate reduction and catalase production were determined for each organism.
	Colonies selected by morphology			
Bacilli and Clostridia	Heated at 80°C Nutrient broth (BBL)	30	72	Heated at 80°C for 10 min. Aerobic or anaerobic growth, spore confirmation, and biochemical reactions.[d] (Heat treatment from Barbe and Henrickson, 1967.)
Coryneform bacteria	Growth up to 9% NaCl in APT broth	30	168	Irregular Gram stain, cell morphology and biochemical reactions.[d]
Enterobacteriaceae	MacConkey agar (Difco)	35	16–18	IMViC tests, morphology, sugar fermentations (Bergey's, 8th ed., 1974) and other biochemical reactions.[d] Lactose preenrichment technique used to identify lactose negative organisms (North, 1961).
Lancefield Group D Streptococci	M-Enterococcus agar (BBL)	35	48	No growth on LBS agar and biochemical reactions.[d]
Lactobacilli	LBS agar (BBL)	30	48	YTG pseudo-catalase test (Felton et al., 1953), CO_2 production (Hayward, 1957), sugar fermentations (Cavett, 1963) and biochemical reactions.[d]
P. cerevisiae	LBS agar	30	48	Cell morphology, YTG pseudo-catalase test, sugar fermentations and biochemical reactions.[d]
Staphylococci and Micrococci	TPEY agar (BBL)	37	24	Glucose fermentation in Brewers Thioglycolate[b] shake cultures and Phenol Red Broth Base (1% glucose). Coagulase positive cultures confirmed using Rabbit Blood Plasma (Direct Tube Method, BBL, 1973). Cell morphology and biochemical reactions.[d]
Yeasts and molds	Acidified[c] Potato Dextrose agar (BBL)	20	120–168	Cell morphology and biochemical reactions.[d]

[a] Brain Heart Infusion agar (BBL).
[b] Methods of Evans and Kloos (1972).
[c] Acidified to pH of 3.5 with sterile tartaric acid.
[d] Biochemical reactions used are given in the identification procedure for all microorganisms.

field Group D streptococci, *Staphylococcus epidermidis,* micrococci, *Proteus* and bacilli have also been isolated from country-style ham (Graham and Blumer, 1971). Yeasts and molds are commonly associated with surface growth (Langlois and Kemp, 1974).

Bacteria of public health concern are normally not associated with dry-cured ham as evidenced by a lack of food poisoning or food illness outbreaks involving country-style ham in the last 14 years (Bryan, 1974). Only a small number of coagulase positive *S. aureus* have been isolated from ham samples within this laboratory. It is unlikely, even by chance contamination of *S. aureus,* that food poisoning outbreaks would occur in processing; however, it would be possible for this to occur in the home preparation and storage of certain ham-containing food recipes.

Concentrated starter cultures have been used extensively in recent years in the production of dry and semi-dry sausages in order to control microbial lactic acid fermentation. Certain species of lactic acid bacteria have been found to be antagonistic to bacteria of public health concern (Daly et al., 1972; Hurst, 1973) and provide a more acceptable microflora for human consumption.

This study was undertaken to investigate the effects of *Pediococcus cerevisiae* and ham environment (e.g., moisture, pH, NaCl) on resident microflora of hams.

EXPERIMENTAL

HAM INFORMATION, concentrated *P. cerevisiae* starter culture information, inoculation and processing procedures, pH determination, sampling procedures, subjective evaluations of ham quality, physical and chemical measurements, and statistical analyses are referred to in Bartholomew and Blumer (1977).

Sample preparation

An 11-g aliquot was removed aseptically from each core section from the ham butt and cushion areas (Bartholomew and Blumer, 1977) and blended with 99 ml of distilled water for 3 min at high speed, using a commercial Waring Blendor and sterilized stainless steel blender cups. From this dilution (1:10), serial dilutions were prepared to 10^7 for use in making plate counts.

Isolation and identification procedures

All organisms were isolated from APT (BBL) enumeration plates and were carried on Brain Heart Infusion agar (BBL) slants and tested for catalase production (Seeley and Vandermark, 1965), nitrate reduction (Difco Manual, 9th ed.) and growth in APT broth. Four or more organisms selected by morphology were isolated from each sampled ham location unless fewer organisms were present in the sample. A total of 1256 organisms were isolated and identified in this study. The identification scheme used is shown in Table 1. A modified LBS agar (Costilow et al., 1964) was used to enumerate lactobacilli and *P. cerevisiae.*

Interactions of microbial groups

Bacteria were tested for inhibitory action by an agar overlay procedure. Multiple isolates of each genus were grown in Trypticase Soy broth (BBL) for 24–48 hr at 30°C and one drop of each culture was

Table 2—Percent composition of microflora of dry-cured ham after aging

Organisms	Langlois[a] study	Graham[b] study	Control hams no inoculum[c]	P. cerevisiae inoculated hams[d]
Pediococci	—[e]	—[e]	12.4	88.0
Lactobacilli	Present	—[e]	20.9	5.0
Lancefield group D Streptococci	Present	0.0[f]	8.9	0.4
Enterobacteriaceae	Present	0.0[f]	6.7	1.3
Staphylococci	Present	88.9	31.9	1.7
Micrococci	—[e]	0.0[f]	7.7	1.3
Bacilli	—[e]	11.1	0.0	0.3
Yeasts & molds	Predominate	0.0[f]	11.6	1.8

[a] Ten hams (controls) were cured 2 days postmortem and held at 1.7°C prior to curing. Microbial analyses of samples obtained from surface of hams through 150 days of processing.
[b] Represents a total of 30 prefrozen hams. Microbial analyses from hams cured and aged 62 days (no equalization period of curing ingredients was used).
[c] Represents a total of 6 prefrozen hams used for controls. Microbial analysis performed on surface and internal samples used from core.
[d] Represents a total of 36 prefrozen hams. Microbial analysis performed on surface and core samples.
[e] (—) indicate organisms were not determined in the Langlois study were not isolated in the Graham study.
[f] Organisms isolated only before aging.

Table 3—Percent microflora comparison of inoculated and control hams

	CI[a,f]		CM[a,f]		CO[a,f]	
	Inoculated[b]	Control[c]	Inoculated[b]	Control[c]	Inoculated[b]	Control[c]
P	92.1	0.0	87.4	0.0	87.3	0.0
L	5.3	0.0	9.0	0.0	4.2	0.0
D	0.0	13.4	0.0	20.0	1.6	20.0
E	0.0	10.0	0.6	10.0	5.8	20.0
S	0.7	60.0	2.3	30.0	0.8	60.0
M	1.4	0.0	0.0	0.0	0.0	0.0
B	0.0	0.0	0.7	0.0	0.3	0.0
Y	0.5	16.6	0.0	40.0	0.0	0.0
	BI[d,f]		BM[e,f]		BO[e,f]	
	Inoculated[b]	Control[c]	Inoculated[b]	Control[c]	Inoculated[b]	Control[c]
P	82.6	10.0	88.0	33.3	90.8	31.1
L	3.7	43.4	7.3	50.0	1.6	32.0
D	0.7	0.0	0.0	0.0	0.0	0.0
E	0.0	0.0	0.0	0.0	1.2	0.0
S	6.1	20.0	0.0	16.7	0.5	4.6
M	3.2	23.4	1.0	0.0	2.0	22.6
B	0.0	0.0	0.0	0.0	0.8	0.0
Y	3.7	3.2	3.7	0.0	3.1	9.8

[a] 1 ham in each sample (CI, CM, CO) of controls contained no microorganisms.
[b] 36 inoculated hams sampled by core sections shown
[c] 6 control hams sampled by core sections shown
[d] 2 inoculated and 1 control ham contained no detectable microorganisms.
[e] 4 inoculated and 2 control hams contained no detectable microorganisms.
[f] Samples containing no microorganisms were not used in calculation of percentages.
P = P. cerevisiae; L = Lactobacilli; D = Lancefield Group D streptococci; E = Enterobacteriaceae; S = Staphylococci; M = Micrococci; B = Bacilli; Y = Yeasts.
CI = Cushion inside core section from adductor, semimembranosus and/or quadriceps.
CM = Cushion middle core section from adductor, semimembranosus, semitendinosus and/or quadriceps.
CO = Cushion outside core section from biceps femoris and/or quadriceps.
BI = Butt inside core section from adductor, semimembranosus and/or quadriceps.
BM = Butt middle core section from semitendinosus and/or quadriceps.
BO = Butt outside core section from biceps femoris and/or quadriceps.

placed on prepoured Trypticase Soy agar plates containing 15 ml of agar and incubated for 24 hr at 30°C. The broth cultures were each mixed with melted Trypticase agar and poured in an agar overlay on each drop-inoculated plate. The overlay plates were incubated for 72 hr at 30°C and inhibition zones (zones of decreased and/or no growth) and stimulation zones (zones of increased colony growth) were measured around drop-inoculated cultures. The inhibition area measured was the diameter of inhibited growth zone. This is a common procedure for determining antagonism among microorganisms (Fleming et al., 1975).

Interactions were postulated within the ham by correlation coefficients of variations of percentages of the microflora within the inoculated and the control hams.

Statistical analyses

Data obtained from this 2 × 3 × 5 factorial randomized block design were analyzed using a regression procedure of Snedecor and Cochran (1967).

RESULTS & DISCUSSION

Control and inoculated ham microflora

Microflora from control hams (I_0) was comparable to that found in other country-style hams (Langlois and Kemp, 1974; Kemp et al., 1975; Graham and Blumer, 1971) except that a few pediococci were isolated in some of the butt areas, with lactobacilli predominating the butt microflora (Table 2). Coagulase negative staphylococci were the most prevalent organisms isolated from the cushion area. Graham and Blumer (1971) reported coagulase negative *S. aureus* to predominate the microflora within the ham. Other microorganisms isolated included Lancefield Group D streptococci, *Enterobacteriaceae*, micrococci, bacilli, and yeasts. Control and inoculated hams contained no anaerobic organisms or organisms of public health concern. *Pediococcus cerevisiae* predominated in both the butt and cushion areas of all inoculated hams (Table 3).

Butt and cushion sections provided differing microenvironments that were selective for microbial groups probably due to water activity (a_w), redox potential, NaCl concentration, or other chemical and physical aspects. *Enterobacteriaceae* isolates were most numerous in ham areas with the highest water content, their growth being limited by low a_w (Mossel and Ingram, 1955) (Table 3). Yeast and micrococci were most prevalent in the butt and CI samples. Microbial associations varied within the three aging periods, the T_2 time period yielded the highest percentage of *P. cerevisiae* (Table 4). Lactobacilli increased directly with aging time in the cushion while decreasing significantly ($P < 0.05$) with inoculum size in the butt; staphylococci and micrococci showed the opposite trend by decreasing in the cushion and increasing in the butt with aging time. Yeast numbers increased significantly ($P < 0.05$) with aging time, showing their tolerance at this low moisture content. Numbers of *P. cerevisiae* increased significantly ($P < 0.05$) with inoculum size for the butt samples; however, the cushion samples remained fairly constant (Table 5). Staphylococci and micrococci decreased with higher inoculum levels and the same trend ($P < 0.05$) was observed in the cushion area for yeasts which might be indicative of antagonism by the *P. cerevisiae* inoculum.

Table 4—Percent microorganisms isolated/ham core section by time levels

	CI			CM			CO		
	T_1[a]	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
P	85.71[b]	81.38[b,c]	75.57[c]	78.00	85.00	67.86	70.86	80.77	78.57
L	0.00	7.08	7.14	3.57	5.77	14.29	2.36[b]	1.31[b]	7.14[c]
D	0.00	0.00	4.79	0.00	0.00	7.14	4.07	0.00	7.14
E	3.57	0.00	0.00	3.57	1.54	0.00	12.50	10.23	0.00
S	10.71	7.69	5.36	13.07[b]	0.00[c]	3.57[c]	9.21	7.69	7.14
M	0.00	3.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.00	0.00	0.00	1.79	0.00	0.00	1.00	0.00	0.00
Y	0.00[b]	0.00[b]	7.14[c]	0.00	7.69	7.14	0.00	0.00	0.00
	BI			BM			BO		
	T_1[a]	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
P	76.21	78.50	65.38	82.08	97.00	68.00	83.93	88.58	80.00
L	14.29[b]	1.42[c]	9.62[b,c]	17.92	0.00	16.67	12.50[b]	0.00[c]	0.00[c]
D	1.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.70
S	4.79	9.67	9.62	0.00	0.00	5.58	0.00	2.83	0.00
M	1.14[b]	9.08[c]	7.69[c]	0.00	3.00	0.00	0.00	6.92	5.30
B	0.00	0.00	0.00	0.00	0.00	0.00	1.79	0.00	0.00
Y	1.79[b]	1.33[b]	7.69[c]	0.00[b]	0.00[b]	9.75[c]	1.79	1.67	10.00

[a] Total of 14 hams used for each sampling time

[b,c] Mean percentages of a microbial group between time levels for a ham sample (CI, CM, CO, BI, BM, BO) followed by different superscripts are significantly different ($P < 0.05$).

T_1 = 80 days processing; T_2 = 95 days processing; T_3 = 110 days processing.

P = P. cerevisiae; L = Lactobacilli; D = Lancefield Group D streptococci; E = Enterobacteriaceae; S = Staphylococci; M = Micrococci; B = Bacilli; Y = Yeasts.

CI = Cushion inside core section from adductor, semimembranosus and/or quadriceps

CM = Cushion middle core section from adductor, semimebranosus, semitendinosus and/or quadriceps.

CO = Cushion outside core section from biceps femoris and/or quadriceps.

BI = Butt inside core section from adductor, semimembranosus and/or quadriceps.

BM = Butt middle core section from semitendinosus and/or quadriceps.

BO = Butt outside core section from biceps femoris and/or quadriceps.

Interactions of microorganisms from dry-cured hams

Interactions of different microbial groups were studied during growth on agar media and in the ham. Streptococci and one staphylococcal isolate (*Staphylococcus #3*) were found to have broad spectrums of inhibition on agar media (Table 6) including antagonism to staphylococci, micrococci, streptococci, *P. cerevisiae* and lactobacilli. The staphylococcal isolate also inhibited bacilli and the streptococci inhibited *Enterobacteriaceae* isolates. Staphylococcal inhibition may have been due to epidermidins (Hsu and Wiseman, 1967). Enterococci have been found to be inhibitory to Gram negative bacteria (Hurst, 1973), bacilli, clostridia, and lactobacilli (Kafel and Ayres, 1969; Pohunek, 1961).

Neutralism was noted among several bacterial groups and with yeasts. Two populations of microorganisms almost always react with each other. Neutralism, when present, is probably exhibited more often by extremely diverse organisms than by closely related organisms. For example, two strains of a species would be so similar in nutritional and environmental requirements that they would compete for a common resource, whereas, diverse organisms might not compete (Brock, 1966).

Enterobacteriaceae and bacilli stimulated some of the bacterial groups including staphylococci (in agreement with McCoy and Faber, 1966 for bacilli) and micrococci. The *Bacillus* isolate was auto-stimulatory and the *Enterobacteriaceae* were stimulatory to some of the other *Enterobacteriaceae* isolates. Although only slight inhibition by *P. cerevisiae* was observed in the agar overlay study, statistical analyses indicated this lactic acid producer to be inhibitory to most of the microorganisms in dry-cured ham tissue including staphylococci, micrococci, lactobacilli, *Enterobacteriaceae* and yeasts (Table 7), indicated by negative correlation coefficients ($P < 0.05$) which agrees with results found by other workers except for yeast

Table 5—Percent microorganisms isolated/ham core section by inoculum levels

		P	L	D	E	S	M	B	Y
CI	I_0 [a]	0.00[c]	0.00[c]	13.40	10.00	60.00	0.00	0.00	16.60[c]
	I_1 [a]	91.67[d]	0.00[c]	0.00	0.00	0.00	8.33	0.00	0.00[d]
	I_2 [a]	83.33[d,e]	12.50[d]	0.00	0.00	4.17	0.00	0.00	0.00[d]
	I_3 [b]	98.58[d,f]	0.00[c]	0.00	0.00	0.00	0.00	0.00	1.42[d]
	I_4 [b]	90.25[d,e]	9.75[d]	0.00	0.00	0.00	0.00	0.00	0.00[d]
CM	I_0 [a]	0.00[c]	0.00	20.00	10.00	30.00[c]	0.00	0.00	40.00
	I_1 [a]	95.83[d]	0.00	0.00	0.00	0.00[d]	0.00	4.17	0.00
	I_2 [a]	67.50[d,e]	20.83	0.00	3.33	8.33[d,e]	0.00	0.00	0.00
	I_3 [b]	91.67[d]	8.33	0.00	0.00	0.00[d,f,g]	0.00	0.00	0.00
	I_4 [b]	88.92[d]	8.33	0.00	0.00	2.75[d,e,g]	0.00	0.00	0.00
CO	I_0 [a]	0.00[c]	0.00[c]	20.00	20.00	60.00	0.00	0.00	0.00
	I_1 [a]	83.33[d]	0.00[c]	9.50	0.00	4.83	0.00	2.30	0.00
	I_2 [a]	72.33[d,e]	22.17[d]	0.00	5.50	0.00	0.00	0.00	0.00
	I_3 [b]	85.42[d]	0.00[c]	0.00	14.58	0.00	0.00	0.00	0.00
	I_4 [b]	98.58[d]	1.42[c]	0.00	0.00	0.00	0.00	0.00	0.00
BI	I_0 [a]	10.00[c]	43.40[c]	6.25	0.00	20.00[c]	23.40[c]	0.00	3.20[c]
	I_1 [a]	62.50[d]	0.00[d]	0.00	0.00	25.00[c]	0.00[d]	0.00	6.25[c]
	I_2 [a]	62.50[d]	12.50[d,e]	0.00	0.00	0.00[d]	8.33[d]	0.00	16.67[d]
	I_3 [b]	93.08[d,e]	0.00[d,f]	0.00	0.00	4.83[d]	2.08[d]	0.00	0.00[c]
	I_4 [b]	88.92[d,e]	4.17[d,e,f]	0.00	0.00	4.17[d]	2.75[d]	0.00	0.00[c]
BM	I_0 [a]	33.25[c]	50.00	0.00	0.00	16.75	0.00	0.00	0.00[c]
	I_1 [a]	100.00[d]	0.00	0.00	0.00	0.00	0.00	0.00	0.00[c]
	I_2 [a]	75.00[d]	5.00	0.00	0.00	0.00	0.00	0.00	20.00[d]
	I_3 [b]	93.75[d]	6.25	0.00	0.00	0.00	0.00	0.00	0.00[c]
	I_4 [b]	84.75[d]	11.08	0.00	0.00	0.00	2.75	0.00	1.42[c]
BO	I_0 [a]	32.50[c]	31.25[c]	0.00	0.00	4.25	20.75	0.00	11.25
	I_1 [a]	87.50[d]	0.00[d]	0.00	0.00	0.00	0.00	12.50	0.00
	I_2 [a]	83.33[d]	0.00[d]	0.00	0.00	0.00	0.00	0.00	16.67
	I_3 [b]	91.67[d]	0.00[d]	0.00	3.08	0.00	5.25	0.00	0.00
	I_4 [b]	94.42[d]	4.17[d]	0.00	0.00	1.42	0.00	0.00	0.00

[a] 6 hams used for each inoculum level

[b] 12 hams used for each inoculum level

[c,d,e,f,g] Mean percentages of a microbial group between inoculum levels for a ham sample (CI, CM, CO) or (BI, BM, BO) followed by different superscripts are significantly different ($P < 0.05$).

I_0 = No inoculum; $I_1 = 1.2 \times 10^4$ cells/gm; $I_2 = 4.0 \times 10^6$ cells/gm; $I_3 = 7.5 \times 10^6$ cells/gm; $I_4 = 2.3 \times 10^7$ cells/gm.

P = P. cerevisiae; L = Lactobacilli; D = Lancefield Group D streptococci; E = Enterobacteriaceae; S = Staphylococci; M = Micrococci; B = Bacilli; Y = Yeasts.

BI = Butt inside core section from adductor, semimembranosus and/or quadriceps.

BM = Butt middle core section from semitendinosus and/or quadriceps.

BO = Butt outside core section from biceps femoris and/or quadriceps.

CI = Cushion inside core section from adductor, semimembranosus and/or quadriceps.

CM = Cushion middle core section from adductor, semimembranosus, semitendinosus and/or quadriceps.

CO = Cushion outside core section from biceps femoris and/or quadriceps.

inhibition (Daly et al., 1973; Al-Mashat, 1973; Haines and Harmon, 1973). Fleming et al. (1975) reported only two isolates of pediococci from cucumber brine were inhibitory to other Gram positive organisms while 13 other isolates of pediococci from cucumber brine and various other sources were not. The combined effects of lactic acid and the ham environment, including pH, NaCl concentration, and moisture content appear to contribute to the antagonistic effects of our starter culture. Inhibitory and stimulatory mechanisms were not analyzed in this study; therefore, it was not determined if lactic acid was the primary inhibitor produced by *P. cerevisiae*. More work would appear to be justified in an effort to identify the factor(s) that are responsible for growth inhibition among microorganisms present in the hams. It may be possible to eliminate undesirable microorganisms (e.g., staphylococci, bacilli and *Enterobacteriaceae*) by inoculating with approved concentrated starter cultures to produce hams free from these bacteria of public health hazard and with superior flavor and quality characteristics.

Table 6—Microbial interactions by agar[a] overlay method

	Staphyloccocci	Micrococci	Streptococci	P. cerevisiae	Lactobacilli	Enterobacteriaceae	Bacilli	Yeasts
Staphylococci (3 isolates)	–	(+)	0	0	0	0	0	0
Micrococci (4 isolates)	(–)	(–)	0	0	0	0	0	0
Enterococci (2 isolates)	–	–	(–)	–	(–)	–	0	0
P. cerevisiae (6 isolates)	–	–	0	(–)	(–)	0	0	0
Lactobacilli (4 isolates)	–	–	0	0	0	–	0	0
Enterobacteriaceae (6 isolates)	(±)	+	0	0	0	(+)	0	0
Bacilli (1 isolate)	(+)	(±)	0	0	0	0	+	0
Yeasts (2 isolates)	0	0	0	0	0	0	0	0
Staphylococcus #3	–	–	–	–	–	0	–	0

[a] Trypticase Soy agar (BBL): + = Stimulation; – = Antagonism; 0 = Neutralism (organisms that can exist together and grow with undiminished vigor); () = Interactions involving less than half the isolates of a microbial group.

Table 7—Microbial population interactions[a]

	Staphylococci	Micrococci	Enterococci	P. cerevisiae	Lactobacilli	Enterobacteriaceae	Bacilli	Yeasts
Staphylococci	X	+	0	0	0	0	0	0
Micrococci	0	X	0	0	0	(+)	0	0
Enterococci	0	0	X	0	0	0	(+)	0
P. cerevisiae	–	–	0	X	–	–	0	–
Lactobacilli	0	0	0	0	X	0	0	0
Enterobacteriaceae	(+)	0	0	0	0	X	0	0
Bacilli	0	0	0	0	0	0	X	0
Yeasts	0	0	(+)	0	0	0	0	X

[a] Significant correlation coefficients ($P < 0.05$) of percent of microflora within hams showing stimulation or antagonism: X = Interactions within group not determined; + = Stimulation (significantly positive r); 0 = Neutralism (nonsignificant r) (organisms that can exist together and grow with undiminished vigor); – = Antagonism (significantly negative r); () = Interaction was observed only at one location in butt or cushion.

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THE ALKYLBENZENES IN ROAST BEEF

ABSTRACT

The volatile components of roast beef were isolated by a specially designed gas entrainment apparatus. The isolated compounds were separated into acidic, basic and neutral fractions. The neutral fraction was further separated by repeated gas chromatography on two different stationary phases and collected as subfractions. The individual subfractions thus obtained were analyzed by gas chromatography-mass spectrometry. Mass spectral identifications were confirmed by comparing the mass spectra and relative retention times with authentic compounds. A total of 20 n-alkylbenzenes and substituted alkylbenzenes were positively identified, many for the first time in cooked beef. These alkylbenzenes are probably derived from the fat present in the beef by thermal oxidation.

INTRODUCTION

DUE TO BOTH economic importance and academic interest, considerable research has been carried out on the volatile flavor compounds of cooked beef during the last 20 yr (for reviews see Herz and Chang, 1970; Dwivedi, 1975). As many as 231 compounds have been identified in the volatile fractions of cooked beef. However, the compounds thus far identified represent neither a complete spectrum of volatile compounds, nor the unique compound or compounds having the characteristic cooked beef aroma.

Among the compounds identified are alkyl substituted benzenes (for a review see Johnson et al., 1969; in cooked beef or beef fat, Watanabe and Sato, 1971; Liebich et al., 1972; Hirai et al., 1973; Persson and von Sydow, 1973; Peterson et al., 1975).

The present communication reports the identification of a homologous series of n-alkylbenzenes and other alkyl substituted benzenes in the neutral volatile fraction from roast beef.

EXPERIMENTAL

THE VOLATILE FLAVOR constituents of 150 lb of roasted top-round beef were isolated by bubbling nitrogen through its water slurry. The nitrogen current was then passed through traps cooled with dry ice and acetone. The condensate thus collected was saturated with sodium chloride and extracted with ethyl ether. The ether extract was dried with anhydrous sodium sulfate and then concentrated with the use of an Oldershaw column.

The concentrated ether extract was separated into acidic, basic, and neutral fractions by extraction first with 10% aqueous Na_2CO_3 and then with 5% aqueous HCl.

The neutral fraction was separated into 13 broad fractions on a Beckman GC-5 gas chromatograph with a thermal conductivity detector, according to the method of Chang (1973). A 12 ft × 1/8 in. o.d. stainless steel column packed with 10% SE-30 on 60/70 mesh Anakrom ABS (Supelco, Inc., Bellefonte, PA) was used. The flow rate was 20 ml/min with a column temperature program of 60°C held for 10 min then 4.5°C/min to a holding temperature of 220°C. The 13 fractions thus collected were each chromatographed for a second time, using a 10 ft × 1/8 in. o.d. stainless steel column packed with 10% OV-101 on 60/80 mesh Chromosorb W (AW, DMCS) (Johns-Manville, Denver, CO). Each of the subfractions was again collected and identified with the use of a DuPont Model 21-490 mass spectrometer with a jet separator interfaced with a Varian Moduline 2700 gas chromatograph with flame ionization detector. A 10 ft × 1/8 in. o.d. stainless steel column packed with 10% Carbowax 20M on 60/80 mesh Chromosorb W (AW, DMCS) was used. The flow rate was 30 ml/min and the column temperature was programmed to provide maximum resolution of each fraction analyzed. Conditions for the mass spectral scans were: ionization voltage 70 eV, ion source temperature 270°C, ion source pressure 10^{-6} Torr, separator temperature 250°C.

RESULTS & DISCUSSION

AMONG the compounds identified in the neutral fracion of the roast beef volatiles, were a homologous series of n-alkylbenzenes and other variously substituted alkylbenzenes. The alkylbenzenes were characterized by their mass spectra. Identification of the alkylbenzenes was confirmed by comparing the mass spectra and gas chromatographic retention indices relative to ethyl esters with those of the authentic compounds (Aldrich Chemical Co., Milwaukee, Wis.). Table 1 lists the alkylbenzenes which were identified.

The alkylbenzenes were characterized by their prominent molecular ion and characteristic fragmentation pattern. In long chain n-alkylbenzenes, β-cleavage with hydrogen rearrange-

Table 1—Alkylbenzenes identified in roast beef

Compounds	Mass fragmentation, m/e					Retention index (I_E) SF-96
o-Xylene	91,	106,	105,	39,	51	4.83
m-Xylene	91,	106,	105,	77,	51	4.73
p-Xylene	91,	106,	105,	77,	51	4.78
1,2,4-Trimethylbenzene	105,	120,	119,	91,	77	6.10
1,3,5-Trimethylbenzene	105,	120,	119,	106,	77	5.83
1-Ethyl-2-methylbenzene	105,	120,	106,	91	77	5.93
1,2,3,5-Tetramethylbenzene	119,	134,	91	133,	39	7.34
1,2,4,5-Tetramethylbenzene	119,	134,	133,	91,	39	7.37
Styrene	104,	103,	78,	51,	77	4.94
Benzene	78,	52,	51,	77,	50	–
Toluene	91,	92,	39,	65,	51	3.62
n-Ethylbenzene	91,	106,	51,	39,	65	4.65
n-Propylbenzene	91,	120,	65,	39,	92	5.63
n-Butylbenzene	91,	92,	134,	39,	65	6.70
n-Pentylbenzene	91,	92,	148,	65,	105	7.69
n-Hexylbenzene	91,	92,	162,	43,	65	8.80
n-Heptylbenzene	91,	92,	176,	43,	65	9.74
n-Octylbenzene	92,	91,	190,	41,	65	10.78
n-Nonylbenzene	92,	91,	204,	41	43	11.82
n-Decylbenzene	92,	91,	218,	41,	43	12.84

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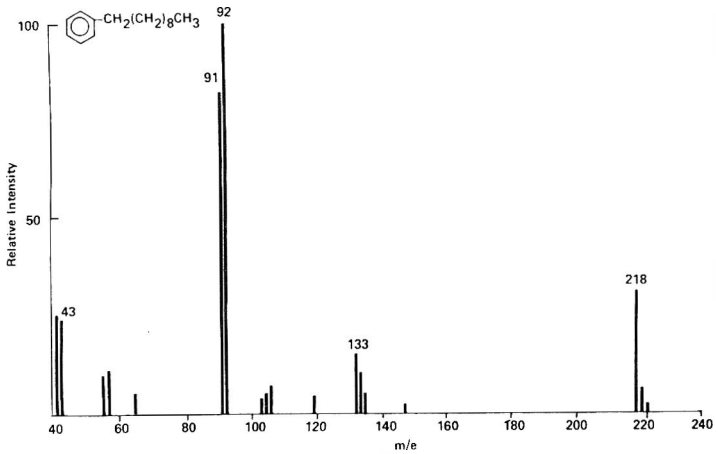

Fig. 1—Mass spectrum of n-decylbenzene.

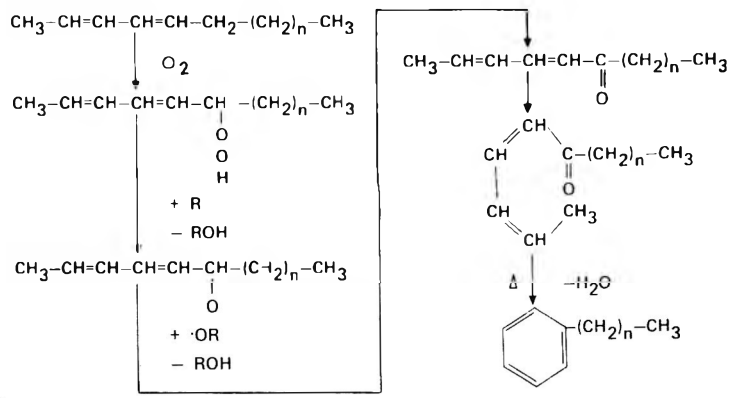

Fig. 2—One possible mechanism for the formation of n-alkylbenzenes from hydrocarbons.

ment gives rise to the strong m/e 92 peaks with elimination of a neutral olefin molecule (Budzikiewicz et al., 1967). Figure 1 shows a typical mass spectrum of a long chain n-alkylbenzene.

The odor descriptions of some of the higher n-alkylbenzenes and of the more highly substituted alkylbenzenes identified are given in Table 2. The alkylbenzenes probably play little if any role in the odor of the lean portion of roast beef; however, they could play a somewhat more important role in the odor of cooked beef fat.

Most of the alkylbenzenes identified in this study have not been previously reported in meat flavor isolates. Liebich et al. (1972) identified toluene, 1,2,- and 1,4-dimethylbenzenes, n-butylbenzene and a number of C_3 and C_4 uncharacterized alkylbenzenes in roast beef meat and/or roast beef drippings. Hirai et al. (1973) reported benzene, toluene and n-propylbenzene in boiled beef. Persson and von Sydow (1973) identified benzene, toluene, xylenes, n-propylbenzene, ethylmethylbenzenes, trimethylbenzenes, and methylpropylbenzenes in canned beef. Peterson et al. (1975) identified benzene, toluene, ethylbenzene, and eight other more highly substituted or uncharacterized alkylbenzenes in canned beef stew. Watanbe and Sato (1971) identified benzene, xylenes, isopropylbenzene and a number of uncharacterized C_4, C_5 and C_6 alkylbenzenes in fried beef fat. Alkylbenzenes have also been identified in various other food systems such as corn oil used for deep-fat frying (Krishnamurthy and Chang, 1967), boiled chicken (Nonaka et al., 1967), and roasted peanuts (Walradt et al., 1971).

Table 2—Odor descriptions of some alkylbenzenes identified in roast beef

Compound	Odor description
n-Propylbenzene	Odor similar to that of toluene; somewhat mothball-like
n-Butylbenzene	Fruity; licorice-like; somewhat mothball-like
n-Amylbenzene	Slight licorice note; somewhat ethereal
n-Hexylbenzene	Weak; slightly fruity
n-Heptylbenzene	Somewhat pungent, goaty cheese-like odor
n-Octylbenzene	Vinyl plastic-like odor with notes similar to those of the higher fatty acids
n-Nonylbenzene	Waxy, fatty odor
n-Decylbenzene	Weak, waxy odor
n-Dodecylbenzene	Very weak odor
1,2,4-Trimethylbenzene	Mothball-like; slightly green
1,3,5-Trimethylbenzene	Slight floral odor with green, moss notes
1,2,3,5-Tetramethylbenzene	Slight fecal, sewer-like odor
1,2,4,5-Tetramethylbenzene	Weak mothball-like odor with slight floral notes

There are a number of precursor molecules which could give rise to alkylbenzenes when heated under the proper conditions. Heynes et al. (1966) identified benzene, toluene, xylenes and trimethylbenzenes when pure D-glucose was pyrolyzed at 300°C. However, Johnson et al. (1969) have questioned the relevance of such data to most foodstuffs. Merrit and Robertson (1967) reported that benzene and toluene were obtained on pyrolyzing free phenylalanine and tyrosine, respectively. Nonaka et al. (1967) found p-xylene, n-propylbenzene and n-butylbenzene among the oxidation products of trans-2-trans-4-decadienal, a thermal oxidative decomposition product of fat. Long chain 2,4-dienals may form n-alkylbenzenes by a six-membered ring cyclization reaction involving loss of H_2O.

Alkylbenzenes may also be formed from long chain unsaturated hydrocarbons, which have been reported to be auto and thermal oxidative decomposition products of fatty acids. Krishnamurthy and Chang (1967) and Reddy et al. (1968) identified C_{13}–C_{18} straight chain, saturated and unsaturated hydrocarbons in corn oil and hydrogenated cottonseed oil used in deep-fat frying respectively. Liebich et al. (1972) and Watanabe and Sato (1971) have found long chain saturated hydrocarbons in roast beef and fried beef fat, respectively. These compounds are formed from long chain fatty acids by a peroxide initiated free radical decomposition. The possible formation of n-alkylbenzenes from long chain fatty acid derived hydrocarbons is shown in Figure 2.

Thus it is possible that the 20 alkylbenzenes identified in roast beef are primarily derived from the fat present in the beef by a thermal oxidative cyclization.

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RELATIONSHIP OF MYOFIBRIL FRAGMENTATION INDEX TO MEASURES OF BEEFSTEAK TENDERNESS

ABSTRACT

Samples were removed from 6 veal, 35 A-maturity and 12 C-maturity bovine longissimus (L) muscles at 1 and 7 days postmortem. Myofibril fragmentation index (MFI), Warner-Bratzler (W-B) shear-force, sensory panel evaluation and sodium dodecyl sulfate (SDS) polyacrylamide gels of myofibrils were determined on muscle samples and steaks. Correlation coefficients between MFI and W-B shear-force were −0.95, −0.73 and −0.65 and, between MFI and sensory tenderness, were 0.97, 0.75 and 0.72 for steaks postmortem aged for 7 days at 2°C from veal, A-maturity and C-maturity carcasses, respectively. SDS-polyacrylamide gels showed that the intensity of the 30,000-dalton component corresponded to the tenderness level of the steaks. These results demonstrate that MFI accounts for about 50% of the variation in tenderness and that myofibril fragmentation and the intensity of the 30,000-dalton component offer potential usefulness as indices of tenderness.

INTRODUCTION

OUR PREVIOUS WORK has indicated that myofibril fragmentation (Parrish et al., 1973; Olson et al., 1976) and the appearance of a 30,000-dalton component seemed to be related to postmortem bovine muscle tenderization. Both Moller et al. (1973) and Olson et al. (1976) have been able to successfully employ a light-absorbance method to measure the amount of fragmentation of a myofibril suspension from bovine muscle. Moller et al. (1973) found a correlation coefficient of 0.78 between the light absorbance of a myofibril suspension and beef tenderness at 7 days postmortem storage. Olson et al. (1976) showed that amount of myofibril fragmentation was muscle and storage temperature dependent, and coincided with W-B shear values and microscopic observations. A potential practical application of the information on myofibril fragmentation would be to use it to predict level of steak tenderness by segregating carcasses into different tenderness groupings.

The purpose of this paper is to report that MFI is highly related to beef loin steak tenderness at both 1 and 7 days postmortem storage at 2°C and at three maturities, veal and maturities A and C. Moreover, the increased intensity of the 30,000-dalton component seems to coincide with greater tenderness.

MATERIALS & METHODS

Source of muscle tissue

Wholesale short loins from 35 A-maturity carcasses grading USDA Choice and 12 C-maturity carcasses were randomly selected 24–30 hr after death (1 day postmortem) from a commercial packing company (Wilson and Co., Cedar Rapids, Iowa). The wholesale short loins were taken to the Iowa State University Meat Laboratory where five 3.1-cm steaks were removed from the anterior end of the loin and packaged in freezer paper. Two steaks (one for sensory panel and one for W-B shear) were randomly selected, frozen and stored at −20°C. Two other steaks were stored at 2–4°C for 7 days postmortem and then frozen and stored at −20°C. The frozen steaks were stored for 14–40 days for subsequent sensory panel evaluation and W-B shear-force determination. A 4-g sample was removed from the fifth steak at 1 and 7 days postmortem and used immediately for myofibril fragmentation determination.

Bovine longissimus muscles from the wholesale short loin of six veal were obtained from animals originating from the Iowa State University Dairy Farm and slaughtered at the Iowa State University Meat Laboratory. Muscle samples and steaks were removed at 1 and 7 days postmortem and used in the same manner as previously described for A- and C-maturity bovine carcasses.

Myofibril fragmentation preparation and index determination

Myofibrils were prepared, and MFI was determined as described previously (Olson et al., 1976).

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis

Myofibrils were analyzed by SDS-10% polyacrylamide gel electrophoresis as previously described (Olson et al., 1977).

Warner-Bratzler (W-B) shear-force and sensory panel evaluation

W-B shear-force was made on steaks as previously described (Olson et al., 1976), and sensory panel evaluation was conducted with a 10-member panel as described by Parrish et al. (1973). Steaks were oven-broiled to an internal temperature of 65°C. Panelists evaluated each meat sample for tenderness, flavor and juiciness on the basis of a hedonic scale of 8 to 1, with 8 being extremely tender, desirable flavor, or juicy, and 1 being extremely tough, undesirable flavor or dry. The average of the 10 panelists' values was used as the tenderness, flavor and juiciness score for each sample.

Data analysis

The data from the various experiments were analyzed for means, standard errors, simple correlations and significant mean differences by the analysis of variance according to methods described by Snedecor and Cochran (1967) and Steel and Torrie (1960). The Statistical Analysis System of the Iowa State Computation Center was used for data computations.

RESULTS

MFI of longissimus muscle increased significantly ($P < 0.05$) from 1–7 days postmortem storage at 2°C for all three maturities (Table 1); however, there were no significant differences in W-B shear values and sensory scores (with the exception of flavor score for maturity A). W-B shear values and sensory tenderness scores both indicated that tenderness improved with aging, but variation within each postmortem period was too large for these to be significantly different between 1 and 7 days postmortem.

The correlation coefficients (Table 2) between MFI and W-B shear-force values were significant ($P < 0.05$, $P < 0.01$) for both 1 and 7 days postmortem for all three maturity groups, with the lowest coefficient being −0.65 for the A-maturity group at 1 day postmortem. Likewise, the correlation coefficients between MFI and sensory panel tenderness score were significant ($P < 0.05$, $P < 0.01$) for both 1 and 7 days postmortem for all three maturity groups, with the lowest coefficient being 0.65 for the C-maturity group at 7 days post-

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Table 1—Effect of postmortem storage (2°C) on myofibril fragmentation index (MFI), Warner-Bratzler (W-B) shear-force and sensory panel tenderness (TEND), flavor (FLA) and juiciness (JU) of longissimus muscle from 35 A- and 12 C-maturity carcasses and 6 veal[a]

	A-maturity, days		C-maturity, days		Veal, days	
	1	7	1	7	1	7
MFI[b]	64.8 ± 0.7	71.4 ± 0.6	59.4 ± 0.9	74.2 ± 0.8	44.4 ± 1.1	58.6 ± 1.2
W-B[c]	2.85 ± 0.08	2.72 ± 0.07	3.36 ± 0.16	2.94 ± 0.13	4.53 ± 0.55	3.62 ± 0.49
TEND[d]	5.29 ± 0.20	5.52 ± 0.15	4.72 ± 0.34	5.42 ± 0.27	3.91 ± 0.54	5.17 ± 0.55
FLA[d]	5.86 ± 0.08	5.62 ± 0.08	5.57 ± 0.14	5.42 ± 0.17	3.36 ± 0.18	3.22 ± 0.17
JU[d]	5.94 ± 0.09	5.78 ± 0.09	5.73 ± 0.20	5.56 ± 0.18	5.32 ± 0.27	5.34 ± 0.23

[a] Means ± standard errors. Means not underscored by the same line are significantly different ($P < 0.05$).
[b] Absorbance per 0.5 mg myofibril protein X 200
[c] Kg of shear-force per cm^2
[d] Hedonic scale of 1 to 8 with 8 being extremely tender, flavorful or juicy.

mortem. In addition, the correlation coefficient between W-B shear-force values and sensory panel tenderness scores for veal steak was significant ($P < 0.01$) at 7 days postmortem (−0.94), but not at 1 day postmortem (0.76). Also the correlation coefficient between sensory panel tenderness and juiciness scores for veal was significant ($P < 0.05$) at 7 days postmortem (0.82), but not at 1 day postmortem (0.75). All other correlation coefficients for veal carcasses were not significant ($P < 0.05$). The correlation coefficients between sensory tenderness and sensory flavor and juiciness scores were moderately high (0.62–0.69) for the L muscle of A-maturity carcasses. Sensory flavor and juiciness scores also were moderately correlated with W-B shear-force and fragmentation index; these correlations, however, probably were not direct correlations, but resulted from their mutual relationship to sensory tenderness scores.

MFI is related to the degradation of the myofibrillar protein during postmortem storage (Olson et al., 1976, 1977). Therefore, myofibrils were selected at 1 and 7 days postmortem at 2°C from two longissimus muscles of veal and of A-maturity and C-maturity carcasses that had differences in myofibril fragmentation index, W-B shear-force and sensory tenderness scores. These selected myofibrils were analyzed by SDS-10% polyacrylamide gel electrophoresis and are shown in Figures 1–3. The gels of myofibrils (Fig. 1) show that troponin T is not extensively degraded in L muscle from A-maturity carcass 1; therefore, the 30,000-dalton component appears only faintly even at 7 days postmortem storage.

Troponin T seems to be more degraded in L muscle from A-maturity carcass 2, however, and therefore, the 30,000-dalton component appears more intense. Amount of degradation of troponin T and the intensity of the 30,000-dalton component coincide with lower W-B shear and higher sensory tenderness scores and MFI. Similarly, gels of myofibrils from longissimus of maturity C (Fig. 2) and veal (Fig. 3) show that the absence of troponin T and the presence of the 30,000-dalton component parallel high MFI and sensory tenderness scores and low W-B shear values.

DISCUSSION

THESE RESULTS add further evidence to the importance of

Table 2—Effect of postmortem storage (2°C) on correlation coefficients among myofibril fragmentation index (MFI), Warner-Bratzler (W-B) shear-force and sensory panel tenderness (TEND), flavor (FLA) and juiciness (JU) of longissimus muscles from veal and from A-maturity and C-maturity carcasses

	Veal[a]		A-maturity[b]		C-maturity[c]	
	Days of postmortem storage					
	1	7	1	7	1	7
MFI vs W-B	−0.95**	−0.97**	−0.65**	−0.75**	−0.68*	−0.72*
MFI vs TEND	0.88*	0.95**	0.67**	0.73**	0.68*	0.65*
MFI vs FLA	0.52	0.06	0.43**	0.54**	0.57*	0.06
MFI vs JU	0.58	0.78	0.50**	0.57**	0.36	0.52
W-B vs TEND	−0.76	−0.94**	−0.86**	−0.62**	−0.59*	−0.48
W-B vs FLA	−0.54	−0.01	−0.50**	−0.49**	−0.47	0.01
W-B vs JU	−0.59	−0.79	−0.47**	−0.31	0.04	−0.32
TEND vs FLA	0.55	0.06	0.63**	0.69**	0.75**	0.55
TEND vs JU	0.75	0.82*	0.64**	0.62**	0.66*	0.83**
FLA vs JU	0.22	−0.27	0.49**	0.49	0.46	0.66*

[a] Correlation coefficients of six veal carcasses
[b] Correlation coefficients of 35 A-maturity carcasses
[c] Correlation coefficients of 12 C-maturity carcasses
* Significant at the 5% level
** Significant at the 1% level

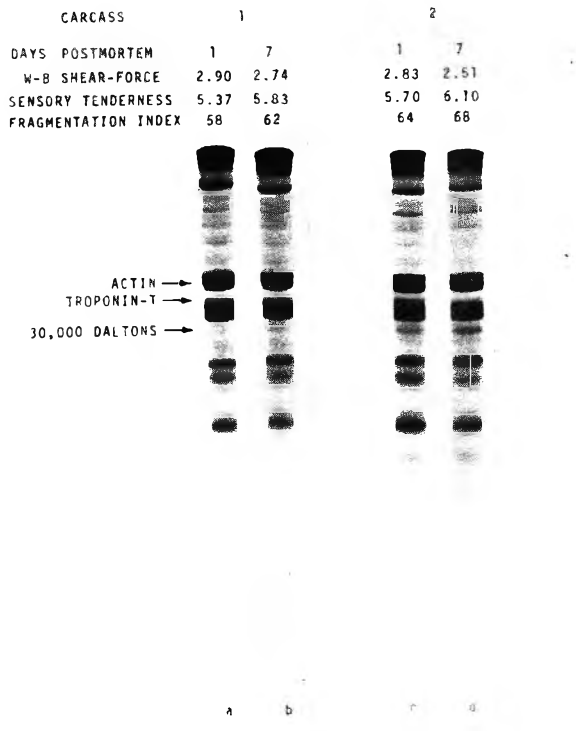


Fig. 1—SDS-10% polyacrylamide gels of myofibrils prepared from longissimus muscles of two A-maturity bovine carcasses at 1 and 7 days postmortem storage at 2°C having different Warner-Bratzler (W-B) shear force, sensory tenderness and myofibril fragmentation index values. [(a) Gel of myofibrils at 1 day postmortem; (b) Gel of myofibrils at 7 days postmortem; (c) Gel of myofibrils at 1 day postmortem; (d) Gel of myofibrils at 7 days postmortem]

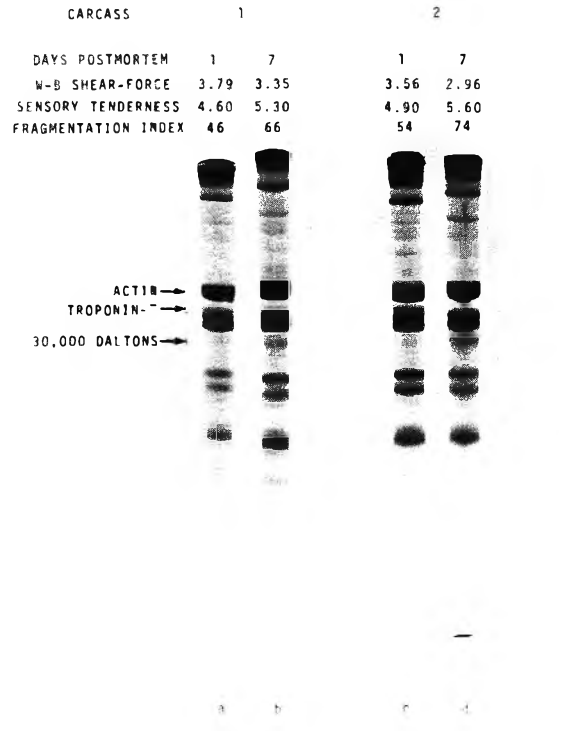
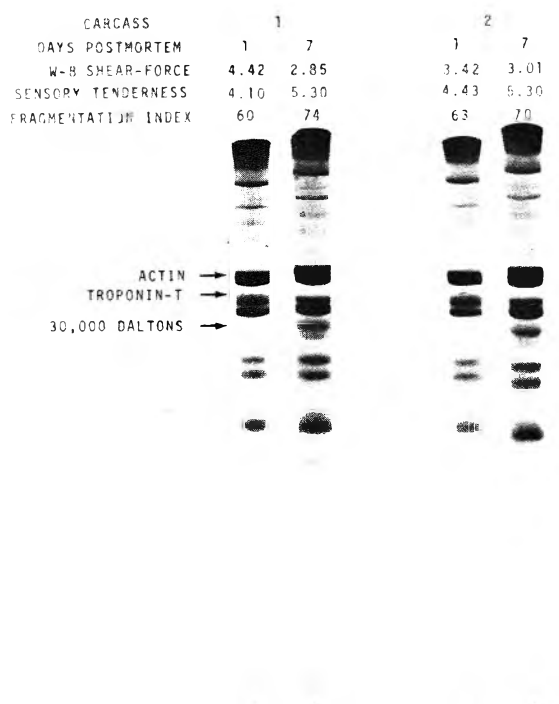


Fig. 2—SDS-10% polyacrylamide gels of myofibrils prepared from longissimus muscles of two C-maturity bovine carcasses at 1 and 7 days postmortem storage at 2°C having different Warner-Bratzler (W-B) shear force, sensory tenderness and myofibril fragmentation index values. [(a) Gel of myofibrils at 1 day postmortem; (b) Gel of myofibrils at 7 days postmortem; (c) Gel of myofibrils at 1 day postmortem; (d) Gel of myofibrils at 7 days postmortem]



myofibrillar proteins and their relationship to improved beef tenderness during postmortem storage. Particularly, the relationship between MFI, determined by measuring the light absorption of a myofibril suspension, and sensory evaluation and W-B shear is of significance. Our results closely agree with Moller et al., (1973), who found by using a method of measuring myofibril fragmentation similar to that used in this investigation, a correlation coefficient of −0.78 between myofibril fragmentation and W-B shear force of longissimus muscles from A-maturity bovine carcasses at 7 days postmortem at 2°C. Hence, our results clearly show that changes in myofibrils isolated from postmortem muscle and determined as a fragmentation index is a good indicator of cooked muscle tenderness, accounting for about 50% of the variation in tenderness of longissimus from A-maturity carcasses. Evidently, the changes in the myofibril during postmortem muscle storage are not greatly altered during heating to an internal cookery tem-

Fig. 3—SDS-10% polyacrylamide gels of myofibrils prepared from longissimus muscles of two veal carcasses at 1 and 7 days postmortem storage at 2°C having different Warner-Bratzler (W-B) shear force, sensory tenderness and myofibril fragmentation index values. [(a) Gel of myofibrils at 1 day postmortem; (b) Gel of myofibrils at 7 days postmortem; (c) Gel of myofibrils at 1 day postmortem; (d) Gel of myofibrils at 7 days postmortem]

perature of 65°C, but the effect of cookery temperature on myofibril fragmentation requires further exploration. That fragmentation of myofibrils occurs early during postmortem storage of muscle at 2°C and that it increases significantly during postmortem storage is indicative that calcium activated factor (CAF) activity on myofibrillar proteins occurs during the early stages of postmortem storage. CAF is an endogenous neutral protease active in postmortem beef muscle and is responsible for myofibrillar protein degradation (disappearance of troponin T and appearance of a 30,000-dalton component) during postmortem aging (Olson et al., 1977).

A practical application of MFI would be to specify tenderness groups. That MFI could be used to predict beef tenderness by segregating carcasses into different tenderness groupings is supported by the significant correlation coefficients between MFI and W-B shear values (ranging from −0.65 to 0.97) and between MFI and sensory tenderness scores (ranging from 0.65−0.95) in all three maturity groups at 1 and 7 days postmortem. Although no correlation coefficients were reported, our earlier results (Olson et al., 1976) demonstrated that myofibril fragmentation and W-B shear values were closely related for beef aged on the carcass. MFI was a sensitive method of detecting differences in myofibril fragmentation during postmortem storage at 2°C, but the W-B shear and sensory panel did not detect significant tenderness differences during the postmortem aging period, although tenderness improved during aging. This further demonstrates that significant changes in tenderness occur early during postmortem aging. It could be argued that if aging was carried out for a longer time, significant changes could have been detected in tenderness; however, our earlier work demonstrated that W-B shear values change little between 7 and 28 days of postmortem time (Parrish et al., 1969).

Myofibrils from longissimus muscles of veal and of A-maturity and C-maturity carcasses at 1 and 7 days postmortem at 2°C that were: (a) selected on the basis of differences in myofibril fragmentation index, W-B shear-force and sensory tenderness of the muscles; and (b) analyzed by SDS-polyacrylmide gel electrophoresis showed a relationship between beef steak tenderness (high myofibril fragmentation index and sensory tenderness and low W-B shear-force) and the degradation of myofibrillar proteins (absence of troponin T and presence of 30,000-dalton component). Conversely, less tender muscles showed less myofibril degradation (more intense troponin T band and less intense 30,000-dalton band on SDS-polyacrylamide gels). These results, obtained by using SDS-polyacrylamide gel electrophoresis, add further evidence to the important relationship of myofibrillar proteins to bovine muscle tenderization. Further investigations must be carried out to determine what ante- and postmortem variables regulate myofibril fragmentation and how MFI can be implemented for segregating carcasses into different tenderness groups.

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HIGH TEMPERATURE EFFECTS ON LYSOSOMAL ENZYME DISTRIBUTION AND FRAGMENTATION OF BOVINE MUSCLE

ABSTRACT

Bovine muscle samples were fractionated and assayed to assess the effects of high postmortem temperatures on lysosomal enzymes and muscle fragmentation values. Samples of the longissimus dorsi muscle were excised from both sides of six animals. One muscle was held at 37°C (HT) and the other was maintained at 2°C as control (C). The pH of the muscles was determined at 1, 4, and 12 hr postmortem. After 12 hr the muscles were homogenized and centrifuged to separate sedimentable and unsedimentable fractions which were assayed for β-glucuronidase and cathepsin C activities. A fragmentation value was also determined for each sample. The pH of the HT samples dropped more rapidly and was significantly lower at both 4 and 12 hr. No detectable difference in total β-glucuronidase activity was observed between HT and C samples but the distribution was markedly altered as shown by significant differences in the percent of total activity that was unsedimentable ($HT > C$, $P < 0.025$) and specific activities of the sedimentable ($HT > C$, $P < 0.025$) and unsedimentable ($HT > C$, $P < 0.025$) fractions. For cathepsin C there was a significant drop in total enzyme activity ($HT > C$, $P < 0.005$) resulting from an apparent degradation of the unsedimentable enzyme which had been released by the HT treatment. The fragmentation values were significantly different showing that the HT samples had probably undergone limited proteolysis resulting in a reduction of muscle fragment size after homogenization. These results add support for the role of lysosomal enzymes in postmortem tenderization.

INTRODUCTION

TENDERNESS is the predominant meat quality determinant and therefore must be of major concern. Low temperatures can cause a decrease in tenderness through cold shortening (Marsh et al., 1968) and elevated temperatures during the early postmortem period produce marked tenderness improvements (Parrish et al., 1969; Fields et al., 1976). That these higher temperatures have effects other than preventing cold shortening was demonstrated by Dutson et al. (1975) who showed tenderness differences between high and low temperature sides which had been restrained to maintain equal sarcomere lengths. Initial studies (Moeller et al., 1976) have shown significant changes in lysosomal enzyme levels and distribution in tissue fractions at early postmortem stages between high temperature conditioned and normally chilled carcasses. The present investigation magnifies temperature differences in an attempt to amplify the enzyme changes and study the effects on muscle fragmentation.

EXPERIMENTAL

SIX USDA Good grade cattle, each weighing approximately 383 kg (live weight), were utilized in this study. Sections of the longissimus dorsi were removed from both the right and left sides of the animal in the region of the first to fifth lumbar vertebrae at approximately 45 min after exsanguination. One section of longissimus muscle from each animal was placed in an incubator at 37°C for 12 hr (HT samples) while the companion muscle from that animal was placed in a cold room at 2°C (C samples). Muscles were placed in covered Pyrex dishes during the incubation period. After 12 hr, muscle samples were trimmed of all fat and epimysial tissue and the outer surface of each muscle sample was removed to eliminate any bacterial growth that might have occurred during the incubation period. Muscle samples were ground through a 3 mm plate and 10g of each ground muscle sample were homogenized and centrifuged according to the procedures of Moeller et al. (1976) except that the adjusted filtrate was centrifuged at 105,000 × G for 2 hr to obtain the sedimentable (pellet) and unsedimentable (supernatant) fractions. The protein content of these fractions was determined by the Conway (1958) micro-Kjeldahl procedure.

Muscle fiber fragmentation was determined by a modification of the method of Reagan et al. (1975). Duplicate 10-g samples of ground muscle were placed in 50 ml of 0.25M sucrose containing 0.02M KCl and homogenized in a Virtis "23" homogenizer at full speed for 40 sec. The homogenate was filtered through one layer of cheese cloth. A 20-ml aliquot of filtrate was placed in a pre-weighed 50 ml polyethylene centrifuge tube and centrifuged at 48,000 × G for 10 min. The supernatant was decanted, leaving the protein residue in the centrifuge tube. The remaining fat particles and supernatant were removed from the inside of the centrifuge tube using a metal spatula covered with cheese cloth. The tube containing the protein residue was again weighed and the total weight of the residue determined. Residue weight (muscle fragment weight) was used as an index of muscle fiber fragmentation.

The muscle pH at 1, 4 and 12 hr postmortem was measured following homogenization of muscle samples in 0.005M sodium iodoacetate to prevent glycolytic changes after homogenization.

Cathepsin C and β-glucuronidase activities were assayed fluorometrically as previously described (Moeller et al., 1976).

Statistical analysis was by the Student's paired t method (Li, 1964).

RESULTS & DISCUSSION

THE RATES of pH decline for both temperatures are shown in Figure 1. The high temperature samples had significantly lower pH values at both 4 and 12 hr postmortem ($P < 0.05$ and $P < 0.01$, respectively). This is in essential agreement with the results of Cassens and Newbold (1967). The combination of lower pH and elevated temperature approaches optimum conditions for lysosomal hydrolase activity (Barrett, 1972). The release of these enzymes into such an environment capable of maintaining their activity can lead to subtle changes in muscle tissue.

The localization of enzyme activity was examined by isolating a sedimentable and an unsedimentable fraction. The sedimentable fraction represents the amount of enzyme remaining in fragmented tissue and membranous material. The unsedimentable fraction represents the amount of enzyme released from the lysosome. Table 1 presents the total enzyme activities recovered for each fraction of each animal. To remove the variability due to differences in protein extraction, the enzyme specific activities of the fractions are presented in Table 2. While the trends are obvious, the large animal variation necessitates the use of a paired analysis dealing with differences between high and low temperature samples from each animal. Table 3 summarizes the differences in enzyme distribution patterns.

No significant difference in total β-glucuronidase activity was observed between the two treatments (Table 3). However, marked changes occurred in the distribution of β-glucuronidase

Table 1—Total enzyme activities[a] in each fraction for β-glucuronidase and cathepsin C in the sedimentable and unsedimentable fractions at both high (HT = 37° C) and low (C = 2° C) temperatures

		β-glucuronidase		Cathepsin C	
Animal	Temp	Sedimentable	Unsedimentable	Sedimentable	Unsedimentable
1	HT	1.20	2.35	33.40	91.14
	C	2.25	1.88	89.85	83.56
2	HT	1.80	1.56	52.00	51.54
	C	2.25	1.12	89.10	92.72
3	HT	3.35	2.81	79.75	51.18
	C	2.55	2.21	105.10	82.92
4	HT	1.90	2.56	32.45	70.49
	C	2.75	2.11	62.00	83.95
5	HT	3.20	2.18	102.80	68.94
	C	3.15	1.61	133.80	72.73
6	HT	2.55	2.14	100.90	110.05
	C	2.48	1.76	190.95	135.68
Mean	HT	2.33	2.27	66.88	73.89
	C	2.57	1.78	111.80	91.93

[a] Total activity of β-glucuronidase expressed as nanomoles of product released per minute. Total activity of cathepsin C expressed as nanomoles product released per min.

Table 2—Specific activities[a] for β-glucuronidase and cathepsin C in the sedimentable and unsedimentable fractions at both high (HT = 37° C) and low (C = 2° C) temperatures

		β-glucuronidase		Cathepsin C	
Animal	Temp	Sedimentable	Unsedimentable	Sedimentable	Unsedimentable
1	HT	2.1	4.5	0.0798	0.1784
	C	4.0	3.7	0.1586	0.4328
2	HT	3.9	3.4	0.1130	0.1152
	C	4.9	2.4	0.1956	0.2008
3	HT	4.4	7.3	0.1070	0.1341
	C	8.4	4.3	0.3593	0.2247
4	HT	4.4	5.6	0.0753	0.1562
	C	6.2	5.0	0.1417	0.2014
5	HT	4.1	5.3	0.1349	0.1679
	C	9.2	4.1	0.3941	0.1868
6	HT	3.9	5.7	0.1540	0.2917
	C	4.3	4.3	0.2309	0.3287
Mean	HT	3.8	5.3	0.1107	0.1739
	C	6.2	4.0	0.2467	0.2625

[a] Specific activity of β-glucuronidase expressed as picomoles of product released per minute per milligram protein. Specific activity of cathepsin C expressed as nanomoles product released per min. per mg. protein.

between the sedimentable and unsedimentable fractions. The high temperature samples contained a larger amount of unsedimentable activity ($P < 0.025$). When considering the altered distribution of this enzyme relative to the changes in distribution of other proteins, i.e., the specific activity of the enzyme, the same pattern emerges. The specific activity of the unsedimentable fraction is higher ($P < 0.025$) for the high temperature samples, while for the sedimentable fraction, this is reversed ($P < 0.025$).

For cathepsin C a more complicated picture is observed (Table 3). First, the high temperature samples contain much less total activity ($P < 0.005$) than the control samples. This result corroborates our earlier findings (Moeller et al., 1976) on the lability of this enzyme. Additionally, there is no significant difference between treatments in the proportion of the total enzyme activity which is unsedimentable. The explanation for these two results is found in an examination of the specific activity patterns for cathepsin C. The sedimentable fraction of the high temperature sample has a lower specific activity than the low temperature sample ($P < 0.025$), agreeing with the data on β-glucuronidase. However, the specific activity of the unsedimentable fraction was lower in the high temperature samples as compared to those held at the low temperature ($P < 0.05$). This complete reversal, compared to β-glucuronidase, indicates that at the higher temperature, the cathepsin C activity not associated with the lysosomal membrane is more labile. The sedimentable enzyme is apparently able to maintain its stability while associated with the membrane fraction.

The fragmentation data in Table 4 show a greater residue weight for the high temperature samples (mean difference significant at $P < 0.05$). This is probably due to a limited proteolysis which results in a reduction of muscle fragment size due to homogenization, allowing more fragments to pass through

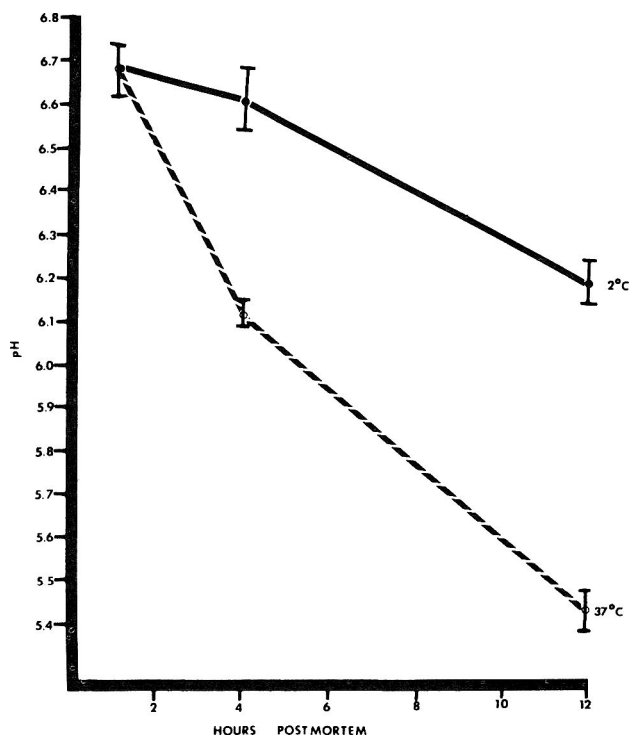

Fig. 1—A plot of pH vs hours postmortem for 2° C and 37° C incubated muscle samples showing the increased rate of pH decline for the 37° C incubated muscle (average of six animals). The pH at 4 and 12 hr is significantly less ($P < 0.05$ and $P < 0.01$, respectively) for the 37° C incubated samples. Vertical bars indicate standard deviations.

Table 3—Mean differences in enzyme activity distribution patterns for high (HT) and low (C) temperature treated samples[a]

	β-glucuronidase	Cathepsin C
Total activity[b]	0.067 ± 0.298	−62.95 ± 12.16[d]
% in unsedimentable fraction	11.00 ± 2.80[e]	7.30 ± 4.30
Specific activity of sedimentable fraction[c]	−2.42 ± 0.74[e]	−0.135 ± 0.038[e]
Specific activity of unsedimentable fraction[c]	1.33 ± 0.35[e]	−0.089 ± 0.035[f]

[a] A positive mean difference indicates more activity for the high temperature samples, while a negative mean difference indicates less activity for these samples. Values are mean difference ± standard error of mean for six samples.
[b] Differences in total enzyme units per 10g of muscle (total nanomoles product formed/minute)
[c] Specific activities defined as in Table 1
[d] $P < 0.005$
[e] $P < 0.025$
[f] $P < 0.05$

Table 4—Residue weights of sample aliquots used in fragmentation analysis

	Residue wt (g)		
Animal	HT(37°C)	C(2°C)	H-L
1	0.885	0.405	0.480
2	0.980	0.590	0.390
3	1.145	0.380	0.765
4	0.730	0.515	0.215
5	1.375	0.370	1.005
6	1.295	1.440	−0.145
Mean	1.068	0.617	0.451[a]

[a] Mean difference significant at $P < 0.05$

the cheese cloth. Such degradation is not characterized by the release of free amino acids, but rather the cleavage of a few sites resulting in a deterioration of the muscle superstructure, allowing it to be broken more easily by homogenization. This probably contributes to overall tenderness differences between high temperature treated and normally chilled carcasses (Parrish et al., 1969; Fields et al., 1976; Dutson et al., 1975). With the current trend in this country against food additives and, since economic considerations have dictated shorter carcass aging periods, the maximum use must be made of endogenous factors controlling tenderness. These studies underscore the possible role of lysosomal hydrolases in the aging of meat and the need to maximize their effects.

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PROFILE OF FIBER TYPES AND RELATED PROPERTIES OF FIVE BOVINE MUSCLES

ABSTRACT

Histochemical, histological and physico-chemical parameters of the longissimus, psoas major, gluteus medius, semitendinosus and semimembranosus beef muscles were investigated. Significant differences ($P < 0.05$) were observed among muscles for fiber type, cross-sectional fiber area, sarcomere length, visual color, percent reflectance, myoglobin content, hemoglobin content, water-holding capacity, transmission value, pH, moisture content, ether extractable constituents and protein content. Inner and outer areas of the semitendinosus and semimembranosus muscles differ histochemically in fiber type and color.

INTRODUCTION

RECENT REVIEWS by Cassens and Cooper (1971), Anderson and Parrish (1972) and Ashmore (1974) indicated a relationship between ultimate meat quality and the muscles' fiber type composition. Most of the histochemical literature pertaining to meat quality has been obtained, however, from porcine muscle. The reported histochemical data on bovine muscles have not established a "normal" fiber type composition for various economically important muscles within the same animal or carcass, but have related fiber characteristics to postnatal fiber development (Ashmore et al., 1972), double muscling (Holmes and Ashmore, 1972; Hendricks et al., 1973; West, 1974), carcass maturity and marbling (Rao et al., 1968), animal age, nutrition and sex (Reddy, 1971), biopsy prediction of carcass composition and palatability (Melton et al., 1974, 1975) and color stability of frozen meat (Schafer, 1972). The objective of this study was to characterize and interrelate various histochemical, histological and physico-chemical parameters of five major bovine muscles removed from Choice, A maturity carcasses.

MATERIALS & METHODS

Sample selection and location

Six USDA Choice (USDA, 1965) steer beef carcasses were selected at a commercial packing plant 24 hr postmortem. Carcass selection criteria were: yield grade near 3.0; typical A maturity; uniform distribution of a small or modest amount of marbling; normal ribeye color, firmness and exudation; and no physical evidence of double muscling. All muscle samples were removed from the carcasses on the fourth or fifth day postmortem and were either frozen or analyzed immediately.

A steak 3 cm in thickness was removed from the longissimus muscle adjacent to the 12th thoracic vertebra and used for ultimate pH, transmission value, reflectance and pigment determinations. A steak 5 cm in thickness was removed from the 13th thoracic-first lumbar area for studies on longissimus (LD) muscle histochemistry and sarcomere length determination. Psoas major (PM) and gluteus medius (GM) muscle samples were removed between the fourth and sixth lumbar vertebrae and the second and third sacral vertebrae, respectively. Semitendinosus (ST) and semimembranosus (SM) muscle samples were taken near the midpoint of the shaft of the femur. All histological and histochemical samples were removed from the center of each muscle except for the ST and SM which were divided into inner and outer portions. The large central portions of the ST and SM muscles were not used. The inner ST (IST) and the outer ST (OST) did not differ in visual color; therefore, the ST muscle was divided into dark and light portions (Beecher et al., 1968). The inner SM (ISM) and outer (OSM) varied considerably in visual appearance (pale and dark red, respectively). The excess tissue from the histochemical steaks and from ST and SM muscles was pulverized in a Waring Blendor with liquid nitrogen (Borchert and Briskey, 1965) and used for studies on water-holding capacity (WHC), moisture and protein and for ether extraction.

Histochemical and histological methods

Muscle samples were frozen immediately after excision in isopentane that had been cooled in liquid nitrogen and stored on dry ice until further processing. Transverse frozen sections (10μ) were mounted on cover slips and incubated at pH 9.4 (2-amino-2-methyl-1-propanol buffer) for ATPase assay by the method of Padykula and Hermann (1955a, b) as modified by Guth and Samaha (1969) and for reduced nicotinamide-adenine dinucleotide tetrazolium reductase (NADH-TR) assay using the procedure of Engle and Brooke (1966). Preincubation at pH 10.4 of fixed muscle sections for ATPase assay prior to the usual incubation at pH 9.4 did not enhance fiber differentiation of the post-rigor tissue; therefore no alkaline preincubation was done. ATPase sections preincubated in an acetate buffer (pH 4.3) were used only as a check to establish fiber classification. Fibers possessing ATPase activity were classified according to the nomenclature of Ashmore and Doerr (1971); dark staining fibers as αWhite (αW), intermediate staining fibers as αRed (αR) and the light staining fibers as βRed (βR). Fibers were also classified as red (includes intermediate staining fibers) or white on the basis of their NADH-TR reaction.

Photomicrographs were taken with a Leica M-2 camera mounted on a Leitz Ortholux microscope. Sufficient photomicrographs of ATPase fibers were taken so that approximately 1000 fibers per LD per carcass and approximately 400 fibers per muscle for all other muscle areas per carcass could be measured with a Zeiss TGZ-3 particle size analyzer. Parameters calculated were: percentage of βR, αR, αW fibers; mean area of βR, αR, αW and all fibers; percent area for each fiber type to the total fiber area measured; anaerobic fiber ratio (percent-area α-fibers to percent-area β-fibers); and an aerobic fiber ratio (percent-area R-fibers to percent-area W-fibers). Percentage of NADH-TR red fibers in each muscle area was determined on enlarged photomicrographs of approximately 350 fibers.

Sarcomere lengths were determined from myofibrils isolated by blending 5g of tissue with 40 ml of cold 0.25M sucrose for 45 sec (Stromer and Goll, 1967). The suspensions were examined at 1000× under a phase contrast microscope. Sarcomere lengths were measured using an eyepiece filar micrometer and reported as the average of 250 sarcomeres from each muscle.

Physico-chemical parameters

Muscle color, firmness, and exudation were subjectively evaluated using seven-point scales (1 = very pale, soft or exudative; 4 = typical characteristics; 7 = very dark, firm or dry). Subjective scoring was done by the two authors. The scoring system was based on a preliminary study of these beef muscle characteristics. Percent reflectance at 685 nm (Ockerman and Cahill, 1969) was measured on fresh cut and bloomed muscle surfaces using a Beckman DU-2 spectrophotometer with a reflectance attachment which was standardized with a MgO block. Muscle pH was determined by placing a combination electrode directly on a freshly cut, cross-sectional surface of the muscle. Water-

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holding capacity (WHC) was determined by a modification of the procedure of Grau and Hamm (1953). Tissue frozen in liquid nitrogen and pulverized, (500–700 mg) was placed on filter paper stored in a desiccator above a saturated KCl solution for at least 24 hr and subjected to a pressure of 455 kg/6.45 cm^2 with a Carver press for 5 min. WHC was calculated from duplicate samples as a ratio of the total area to the meat film area; a larger value suggests a lower WHC. Transmission value of each muscle was determined according to the procedure of Hart (1962) as described by Ockerman and Cahill (1968). Total muscle pigment, myoglobin (Mb), and hemoglobin (Hb) concentrations were determined in duplicate by a slight modification of Poel's (1949) method. Tissue which had been blended fresh into a homogenous paste and frozen was extracted twice with a 0.01N acetate buffer, pH 4.5 (deDuve, 1948) instead of water. The individual pigments were quantitated using a Beckman DU-2 spectrophotometer in their carboxy forms at wave lengths of 538 and 568 nm and the total muscle pigment was measured at 540 nm by conversion to the cyanomet compounds.

Statistical analyses

The data were subjected to analysis of variance (Snedecor and Cochran, 1967) to detect significant effects among muscles and Duncan's new multiple range test (Duncan, 1955) was used to separate means. Simple correlation coefficients were computed between many of the variables.

RESULTS & DISCUSSION

SIGNIFICANT EFFECTS among muscle were observed for all variables studied except for the area of βR fibers and Duncan's multiple range test separated mean differences ($P < 0.05$) for this variable (Table 1).

Percent fiber types

Histochemically the PM was the reddest (Table 2) and the OST the whitest amongst the muscles examined. The LD and GM muscles were similar in fiber type composition. The IST had a greater proportion of ATPase βR-fibers and NADH red fibers than the OST muscle area. Similar results have been reported for pig semitendinosus muscle (Beecher et al., 1965b). However, the ISM muscle area had significantly fewer NADH red fibers and more ATPase αW fibers than the OSM muscle area. Thus, the distribution of different fiber types within the semimembranosus differs from that of the semitendinosus. The combined percentage of ATPase βR and αR fibers was positively correlated ($r = 0.91$) with the percent NADH red fibers in each muscle. The reciprocal relationship observed between the ATPase R-fibers and the NADH positive fibers supports the work of Ashmore and Doerr (1971) suggesting that bovine ATPase R-fibers are oxidative in their metabolism. These data enhance the role of ATPase αR fibers in the overall classification of bovine muscle redness and whiteness, i.e., the LD would be only 30% red compared to 53% red if ATPase αR and intermediate staining NADH fibers were excluded in the redness classification.

Fiber type areas

βR fibers (Table 2) were most uniform in size or area but these were not necessarily the smallest type fiber in each muscle. The αW fiber was definitely the largest of the ATPase fiber types in all muscles except the PM muscle. Fiber types are dynamic in their physiology (Guth and Yellin, 1971) and interconversions of one type fiber to another have been postulated (Ashmore and Doerr, 1971; Ashmore et al., 1972), but data from the present study indicate that dramatic changes in muscle size would result if αR fibers were converted to αW fibers.

All fiber types in the muscle samples contained a few fibers which were similar in appearance to the intra-fascicular terminating fibers of bovine muscle reported by Swatland and Cassens (1972). A limited number of very large round shaped fibers, usually ATPase αW and NADH negative, were observed in all of the muscles. Similar observations for porcine muscle were reported by Cassens et al. (1969), Cooper et al. (1969), de Bruin (1971), Hendricks et al. (1971) and Linke (1972).

Percent fiber areas and fiber ratios

A major reason for histochemical classification of muscle fibers is to evaluate the metabolic potential of the total muscle. Data expressed as percent fiber areas and/or fiber ratios of the dual nomenclature ATPase classification system provide such estimates. ATPase βR and αW fibers have been shown to be primarily oxidative and glycolytic, respectively in their metabolism, while the αR fibers contain enzymes of aerobic and anaerobic pathways (Ashmore and Doerr, 1971).

Comparison of the percentage area of different fibers to the percent of the same fibers in all the muscles revealed an increase in the percent area for αW fibers and a decrease for αR and βR fibers (Table 2). Hence, all muscles, except the PM, were potentially more anaerobic in their metabolism than indicated by fiber type percentages per se.

Ratio values (Table 2) calculated from percent fiber areas clearly indicate major metabolic differences between and within muscles. The degree of utilization of certain pathways of metabolism as revealed by the ratios were expected, i.e., the LD and GM muscles were moderately anaerobic, the PM muscle was very aerobic and the IST and OST muscles were aero-

Table 1—Mean squares for histochemical, histological, chemical and physical characteristics of bovine muscles[a]

Variable	Source of variation and degrees of freedom: Muscle (6)	Error (35)
	Histochemical and histological	
% βR fiber	1312.03**	29.34
% αR fiber	188.72**	17.22
% αW fiber	862.62**	20.88
Area βR fiber	433685.00	236733.69
Area αR fiber	985090.50**	191604.38
Area αW fiber	1927891.00**	410075.13
Area, average	976013.69**	244178.31
% area βR fiber	0.15**	0.0026
% area αR fiber	0.018**	0.0012
% area αW fiber	0.11**	0.0030
Anaerobic ratio	62.02**	2.12
Aerobic ratio	2.06**	0.12
% NADH-TR red fiber	776.82**	22.78
Sarcomere length	1.38**	0.050
	Chemical and physical	
Reflectance	204.18**	21.07
Visual color	3.73**	0.31
Myoglobin	3.17**	0.16
Hemoglobin	0.17**	0.022
Total pigment	3.29**	0.13
Firmness	0.99**	0.19
Exudate	1.00**	0.062
WHC	1.51**	0.063
Transmission value	0.69**	0.058
pH	0.03**	0.005
Moisture	6.17*	2.10
Ether extract	12.54**	3.25
Protein	1.82*	0.59

[a] Muscles: longissimus, psoas major, gluteus medius, inner semimembranosus, outer semimembranosus, inner semitendinosus, outer semitendinosus.
* Significant ($P < 0.05$).
** Significant ($P < 0.01$).

Table 2—Means for histochemical and histological characteristics of bovine muscles

Variable	Muscle[a]						
	LD	PM	GM	ISM	OSM	IST	OST
% βR fiber	29.3[d]	52.4[b]	25.8[d]	11.7[e]	15.5[e]	35.8[c]	12.2[e]
% αR fiber	24.7[cb]	14.9[e]	21.7[d]	27.8[c]	32.9[b]	24.7[cd]	20.6[d]
% αW fiber	46.0[e]	32.7[g]	52.6[d]	61.3[c]	51.1[de]	39.2[f]	67.2[b]
Area βR[j]	2589[bc]	2211[c]	2309[c]	2646[bc]	2621[bc]	2613[bc]	3046[b]
Area αR fiber[j]	2580[bc]	1569[e]	2028[cde]	2660[b]	1956[be]	2562[bc]	2355[bcd]
Area αW fiber[j]	3899[b]	2228[e]	2931[be]	3048[cd]	3133[bcd]	2870[be]	3770[bc]
Area, average[j]	3135[bc]	2127[b]	2561[cd]	2887[bc]	2612[cd]	2645[cd]	3346[b]
% area βR	24.0[d]	54.2[b]	23.2[d]	10.5[e]	15.0[e]	34.7[c]	10.8[e]
% area αR	20.0[cd]	10.8[f]	17.2[be]	25.0[b]	24.5[b]	23.6[bc]	14.3[ef]
% area αW	56.0[d]	35.0[f]	59.6[cd]	64.6[c]	60.5[cd]	41.7[e]	75.0[b]
Ratio							
Anaerobic[h]	3.33[d]	0.91[e]	3.38[d]	8.84[b]	5.82[c]	1.90[d]	8.93[b]
Aerobic[i]	0.80[d]	2.01[b]	0.69[de]	0.55[de]	0.66[de]	1.43[c]	0.34[e]
% NADH-TR red	53.3[cd]	69.6[b]	49.1[de]	42.1[f]	48.1[de]	56.2[c]	33.3[g]
Sarcomere length, μ	1.83[d]	3.06[b]	1.73[d]	1.80[d]	1.85[d]	2.39[c]	2.22[c]

[a] LD = longissimus, PM = psoas major, GM = gluteus medius, ISM and OSM = inner and outer semimembranosus, IST and OST = inner and outer semitendinosus.
[b,c,d,e,f,g] Means bearing different superscripts on the same line are different ($P < 0.05$).
[h] ATPase percent area α-fibers to percent area β-fibers
[i] ATPase percent area R-fibers to percent area W-fibers
[j] Areas expressed as μ^2

bic and anaerobic, respectively. While ISM showed higher anaerobiosis than the OSM, the OST showed higher anaerobiosis than the IST muscle.

Sarcomere length

Differences in sarcomere length presented in Table 2 were typical of muscles from vertically suspended carcasses and were similar to those reported by Herring et al. (1965) and Hostetler et al. (1972). The longest sarcomeres were from the reddest muscle (PM) and the sarcomeres from the red portion of the SM and ST muscles were slightly longer than those from the white area of the corresponding muscle. These trends support the conclusions in the review by Cassens and Cooper (1971) that red myofibrils have longer sarcomeres than white myofibrils.

Relationship between fiber characteristics and other muscle properties

Subjective and objective measurements of color (Table 3) were similar for the LD, PM and GM muscles and were not significantly correlated with myoglobin, hemoglobin or total pigment content. Inner and outer portions of the ST muscle

Table 3—Means for chemical and physical characteristics of bovine muscles

Variable	Muscle[a]						
	LD	PM	GM	ISM	OSM	IST	OST
% reflectance[g]	32.0[d]	38.0[c]	36.3[cd]	48.7[b]	31.7[d]	34.4[cd]	33.9[cd]
Visual color[h]	4.1[b]	4.2[b]	4.3[b]	2.3[d]	4.7[b]	3.3[c]	3.3[c]
Myoglobin[i], mg/g	3.48[c]	3.71[bc]	4.11[b]	3.91[bc]	3.56[c]	2.97[d]	1.95[e]
Hemoglobin[i], mg/g	0.19[c]	0.61[b]	0.53[b]	0.44[b]	0.23[c]	0.23[c]	0.26[c]
Total pigment[i], mg/g	3.54[d]	4.07[bc]	4.18[b]	3.95[bcd]	3.71[ad]	3.10[e]	2.05[f]
Firmness[h]	4.0[bc]	4.4[b]	3.6[c]	3.2[d]	4.0[bc]	4.1[bc]	4.0[bc]
Exudate[h]	4.0[c]	4.4[b]	3.8[c]	3.1[d]	3.8[c]	4.0[c]	4.1[c]
WHC	2.61[de]	2.87[cd]	2.90[cd]	3.82[b]	2.52[e]	3.09[c]	2.72[de]
Transmission value	11.1[d]	48.7[c]	48.8[c]	89.6[b]	17.8[d]	40.6[c]	13.1[d]
pH	5.46[c]	5.60[b]	5.47[c]	5.48[c]	5.36[d]	5.54[bc]	5.49[c]
Moisture[j]	71.9[cd]	71.7[cd]	73.2[bc]	71.3[b]	72.5[bcd]	71.8[cd]	74.1[b]
Ether extract[j]	4.9[bcd]	6.7[b]	3.5[d]	5.8[bc]	3.7[cd]	5.9[bc]	2.9[d]
Protein[j]	22.4[b]	20.7[c]	21.7[b]	21.8[b]	22.3[b]	21.5[bc]	22.0[b]

[a] LD = longissimus, PM = psoas major, GM = gluteus medius, ISM and OSM = inner and outer semimembranosus, IST and OST = inner and outer semitendinosus.
[b,c,d,e,f] Means bearing different superscripts on the same line are different ($P < 0.05$).
[g] Reflectance at 685 nm
[h] Subjective codes: 1 = very pale, soft or exudative; 7 = very dark firm or dry.
[i] Determined as COMb for myoglobin, COHb for hemoglobin and cyanomet for total pigment in wet tissue.
[j] Expressed as percentage of wet tissue weight

were similar in color even though the IST muscle had more ($P < 0.05$) red fibers and more total pigment than the OST muscle. The ISM muscle, however, was significantly more pale in color and had more myoglobin and hemoglobin than the OSM muscle; yet, histochemical data indicated that the ISM muscle was considerably more anaerobic in its metabolism. These data are contradictory to results obtained from pig muscle (Beecher, 1965a, b; 1968) which indicate the inner, red portions of certain muscles were darker in color and had more myoglobin content than the outer portions. Resons for these discrepancies, particularly with the SM muscle, are not clear but may be related to time–temperature cooling relationships of the more massive round muscles in beef carcasses compared to pork muscles or subtle differences in cytochrome content between muscle area differing in proportions of fiber types.

Hemoglobin concentrations of the LD, PM, GM, ISM, OSM, IST, and OST muscles were approximately 5%, 14%, 11%, 10%, 6%, 7%, and 12% respectively, of the total pigment. These values are within the range reported for hemoglobin concentration in beef muscle (Fleming et al., 1960; Rickansrud and Henrickson, 1967; Hamm and Bunning, 1972). The greater blood flow and/or greater capillary density per unit muscle area in red muscles (Smith and Giovacchini, 1956; Romanul, 1965) cannot fully explain the above differences in hemoglobin concentrations of the different muscles; further the whitest of the muscles such as the ISM and OST had nearly as much hemoglobin as the PM muscle. Variation in degree of blood removal at the time of slaughter may have contributed to variation in hemoglobin levels among the muscles studied.

The ISM muscle was softer, more exudative, and had higher transmission and WHC values (lowest actual WHC) than the other six muscle areas studied. Transmission value was related positively to the percentage content of ATPase αW and αR fibers and negatively to βR fibers; however, the correlation coefficients were low and nonsignificant. Water-holding capacity was strongly correlated (0.83) with transmission value. Ultimate pH was not significantly correlated with either WHC or transmission value; however the lack of correlation was not surprising since the rate of postmortem pH decline has been observed by numerous workers to affect these parameters to a greater extent than ultimate pH when only small differences in ultimate pH occurred. Conditions similar to those of the SM muscle also occurred in the IST vs OST muscles although to a lesser extent. These data indicate that the physico-chemical differences between the ISM and OSM muscles may result from the same circumstances which cause pale, soft and exudative muscle in pigs; these are rapid postmortem glycolysis and decline in pH while muscle temperature is high and a subsequent denaturation of sarcoplasmic proteins (Briskey, 1964). Recent studies by Khan and Bullantyne (1973) and Khan and Lentz (1973) have revealed wide variation in the pH of the semimembranosus muscle of beef carcasses after one hour postslaughter.

Percent moisture in the various muscles was inversely related to percentage of ether extractable material (Table 3). Red muscle is generally considered to contain more lipid than white muscle. Excepting the ISM muscle, a similar trend was observed for all muscles in this study; the PM had the highest percentage of ether extractable material and the OST the least. Apparently the lipid content of the ISM muscle was not related to its fiber type composition. Percent protein ranged from 20.7% for the PM muscle to 22.4% for the LD muscle.

CONCLUSIONS

BASED ON FIBER characteristics, the muscles examined can be ranked in the following descending order for anaerobic potential: OST > ISM > OSM > GM > LD > IST > PM and in the following descending order for aerobic potential: PM > IST > LD > GM > OSM > ISM > OST. Inner and outer areas of the bovine semitendinosus muscle are histochemically red and white, respectively. Visual colors of the two areas are similar. Fiber type composition of the inner and outer areas of the bovine semimembranosus muscle tends to be white and red, respectively. Visual color of the inner and outer areas are light and dark, respectively. These patterns do not follow the classical histochemical pattern of inner and outer muscle areas being red (dark) and while (light), respectively. Histochemical, histological and physico-chemical characteristics of bovine tissues vary among muscles and within cross-sectional areas of a muscle.

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SURVIVAL OF Clostridium perfringens IN REFRIGERATED AND FROZEN MEAT AND POULTRY ITEMS

ABSTRACT

Refrigerated and frozen meats and poultry products from a retail source were examined for incidence of *Clostridium perfringens* vegetative cells and spores. Foods included raw ground beef, beef and pork liver, and precooked frozen beef and poultry items. Inoculated samples were also frozen and stored for various periods up to 42 days at −29°C. Survival of *C. perfringens* spores in frozen food samples and in a bacteriological medium was high, but vegetative cells were virtually eliminated. Thawing at low temperature for 2 days caused further reduction of vegetative cells, but not of spores. Approximately 50% of 32 retail frozen precooked food samples were positive for vegetative cells, and about 15% demonstrated spores of *C. perfringens*.

INTRODUCTION

Clostridium perfringens is a normal inhabitant of the gut of man and animals (Turner and Wong, 1961; Hall and Hauser, 1966; Sutton, 1966a, b; Oka, 1966; and Shapiro and Sarles, 1949). It has been implicated in food poisoning outbreaks associated with poultry meat as well as with other meat products (McClung, 1945; McKillop, 1959; Hobbs, 1960; and others). Food-borne illness caused by *C. perfringens* has recently been reviewed by Walker (1975). Many instances have been reported of the organism as a contaminant of meat and meat products. Canada and Strong (1964) reported an incidence of 26% in bovine livers from animals at the time of slaughter. In a survey by Ladiges et al. (1974), *C. perfringens* was recovered from 47% of ground beef samples in a retail store, with counts commonly in the range of 5–100 per g. Raw, boneless beef yielded *C. perfringens* in 30% of the samples obtained from a fast food service restaurant (Bryan and Kilpatrick, 1971).

C. perfringens has been isolated from poultry during processing operations and from further processed products (Lillard, 1971; 1973).

The ubiquitousness of this organism makes it a probable contaminant of various foods including meat and poultry items via the raw material or during processing and handling. Freezing kills many vegetative cells but is not as harmful to spores of microorganisms. They appear to be highly resistant to freezing and those of *C. perfringens* are no exception (Barnes et al., 1963; Strong and Canada, 1964; and Canada et al., 1964). In considering the epidemiology of pathogenic organisms, *C. perfringens* food poisoning is usually connected with large scale institutions and with reheated meats and meat dishes. The heat resistant *C. perfringens* spores can survive heat and cooking processes (Woodburn and Kim, 1966; Strong and Ripps; 1967; Sutton, 1966a). They can also survive in frozen foods. When frozen foods are reheated, the heat may cause activation of spore germination. With favorable conditions, the spores germinate and multiply rapidly. Precooked frozen foods sometimes are given a mere oven warming which cannot kill all organisms, but instead may activate spore-forming bacteria with subsequent multiplication. A potential hazard exists when foods are held on warming trays. It has been suggested that improper handling of such foods may cause food poisoning outbreaks from *C. perfringens*.

Traci and Duncan (1974) reported that a progressive loss of viability occurred in cold-shocked *C. perfringens* with increasing time of low temperature exposure. As many as 75% of viable cold shocked cells suffered injury when exposure occurred late in the exponential growth phase.

The present study was undertaken to determine the effect of freezing and subsequent frozen storage on the survival of *C. perfringens* populations in frozen meat and poultry foods. This study also considered the effect of thawing and holding at refrigeration temperature on germination and survival of spores of *C. perfringens* in the frozen-defrosted foods. Refrigerated or precooked frozen foods from retail stores were examined to aid in selection of specific foods for further study.

EXPERIMENTAL

FOR STUDIES in which food products were inoculated, a heat resistant *C. perfringens* strain (S-45) was used. The culture was maintained in cooked meat medium (Difco) stored at room temperature. Inocula were from cultures grown in Ellner's medium (Ellner, 1956).

The medium used for recovery of *C. perfringens* from food samples was similar to that of Angelotti et al. (1962), but with no polymyxin B sulfate and sodium sulfadiazine. D-cycloserine was substituted, as in the medium of Harmon et al. (1971). D-cycloserine inhibits growth of fecal streptococci and most of the common contaminants in food without excessive inhibition of *C. perfringens* (Füzi and Csukás, 1969; Harmon et al., 1971; Walker and Rey, 1972). The medium differed from the TSC agar of Harmon et al. (1971) in that no soytone was added, the concentration of yeast extract was 1.0%, and the level of ferric citrate was 0.05%.

Recovery of *C. perfringens* cells was determined by use of the pouch method described by Bladel and Greenberg (1965). The incubation time was 37°C for 18–24 hr.

For spore counts, the heat activation for spore germination was 80°C for 15 min. Confirmed tests for *C. perfringens* were made by using motility nitrate medium described by Angelotti et al. (1962).

Preliminary examinations were made on raw hamburger, beef liver, pork liver, and several precooked frozen food items purchased from a local retail store. Fresh hamburger and three types of precooked frozen foods, i.e., meat loaf dinner, beef pot pie and chicken pot pie were selected for further studies on freezing and thawing and survival of *C. perfringens*.

For quantitative determinations, each item was weighed aseptically into 30-g portions and added to 270 ml of sterile peptone water to make a 10^{-1} dilution in the blender jar. The appropriate dilutions were then made using 99 ml sterile 0.1% peptone solution blanks. Previous work in this laboratory demonstrated recoveries of vegetative cells in inoculated meat (Baran et al., 1970) and uninoculated meat (Rey et al., 1970).

On the day of examination, the food items were purchased from the local store. Frozen items were thawed at room temperature (25–27°C) for a time long enough only to enable aseptic removal of a portion from each. Then a representative portion of 30g of each sample was plated for detection of *C. perfringens* (both vegetative cells and spores) immediately to determine initial counts. The rest of the sample was weighed aseptically into eleven 30-g packages and inoculated with *C. perfringens* S-45 grown in Ellner's medium. The culture was shaken by hand for 10 min for uniform distribution of cells, then successive inoculations of

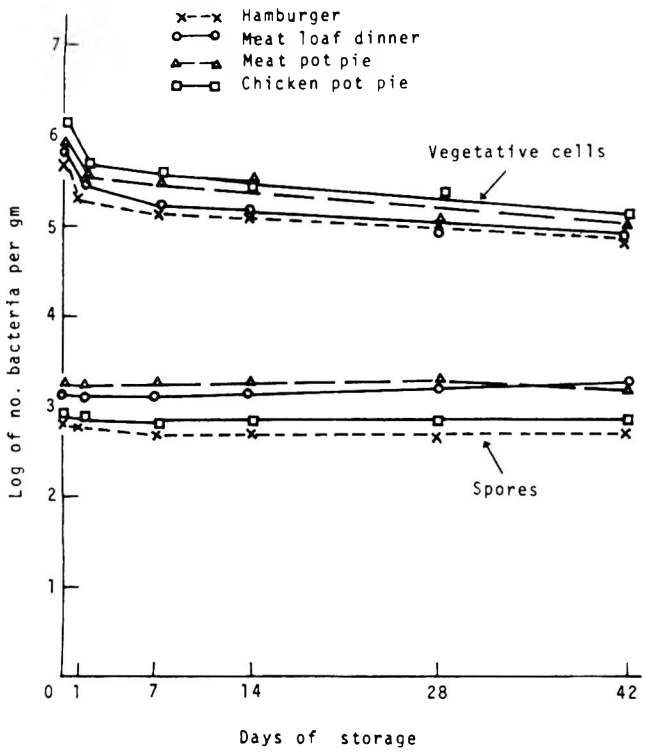

Fig. 1—Survival of C. perfringens in different food products at −29° C.

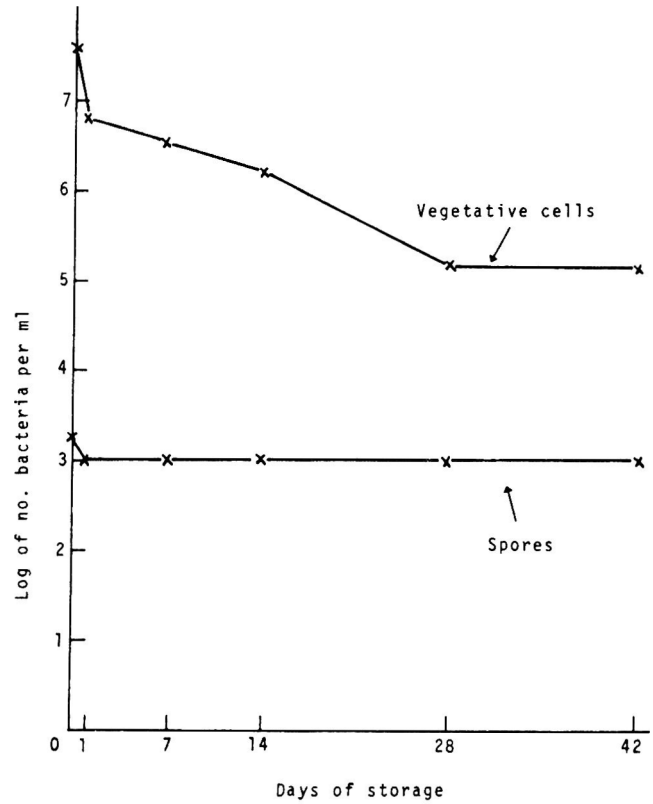

Fig. 2—Survival of C. perfringens in Ellner's medium at −29° C.

one ml were made into each of the eleven sample packages. Each package represented a portion of the food sample. One inoculated package was immediately examined for the initial numbers of *C. perfringens* vegetative cells and spores. The remainder of the culture was transferred to 11 sterile test tubes (16 by 150 mm), one-fourth full; one tube from eleven tubes was sampled for making a count before freezing. Ten packages of inoculated food samples and ten culture tubes were stored at −29° C (±2° C).

At periods of 1, 7, 14, 28, and 42 days of storage, two packages and two culture tubes were taken from the freezer. One package was opened and added to 270 ml of 0.1% peptone water to make the first dilution in a blender jar. Subsequent dilution and plating was performed immediately for *C. perfringens* determinations. Another package was transferred to a 5° C display case for 2 days, and then examined for *C. perfringens*. The culture tube was also examined in the same manner. Behavior of the culture in Ellner's medium and in food samples was determined and compared.

RESULTS & DISCUSSION

IN FRESH MEAT surveyed, *C. perfringens* vegetative cells occurred more frequently than spores. This is in agreement with Hobbs (1962) who indicated that although vegetative cells of *C. perfringens* were frequently found in meat products, spores had seldom been observed in raw meats. Goepfert and Kim (1975) reported that *C. perfringens* was unable to grow in ground beef at low temperature. The frozen precooked foods generally had a lower incidence of *C. perfringens* vegetative cells than the fresh meats. This may be due to the fact that the processing operations inhibited or destroyed the organisms. Spore contamination was more frequent in the frozen precooked foods examined. Spores may also have been contributed by ingredients other than meat. Survival of spores of *C. perfringens* in cooked meat has been observed by several investigators (Sylvester and Green, 1961; Barnes et al., 1963; Woodburn and Kim, 1966). Resistance of spores to heat and freezing temperature makes the spores themselves extremely difficult to destroy in food.

Food samples were inoculated with *C. perfringens* and frozen at −29° C to study survival of the vegetative cells and spores during a period of 6 wk. Figure 1 shows number of *C. perfringens* vegetative cells and spores in frozen ground hamburger, meat loaf dinner, meat pot pie and chicken pot pie, recovered after 1, 7, 14, 28 and 42 days of storage. Generally, in all types of foods tested there was a rapid decrease in *C. perfringens* vegetative cell counts. At day 1, 38–75% of the cells were killed. Subsequent declines were slow for the remainder of the storage period. In contrast, spores of *C. perfringens* remained fairly constant in all types of frozen food samples throughout storage. Figure 2 illustrates the number of vegetative cells and spores of *C. perfringens* maintained in Ellner's medium for 42 days in frozen storage. A rather sharp reduction occurred in vegetative cells at the end of 28 days of storage with no decrease throughout the remainder of frozen storage. About 99.5% of the vegetative cells were killed after 28 days; changes after that were not perceptible. However, the spore count in Ellner's medium was quite stable throughout the storage period. The loss of viable vegetative cells in Ellner's medium was quite pronounced (99.5% compared to 89.5% decrease in food samples). In the medium, suspensions tested were in small tubes which quickly became equilibrated with environmental temperature (Canada et al., 1964). In addition, since no food was present, protection to organisms offered by components of foods was lacking. More destruction probably occurred than would be true under usual conditions of food

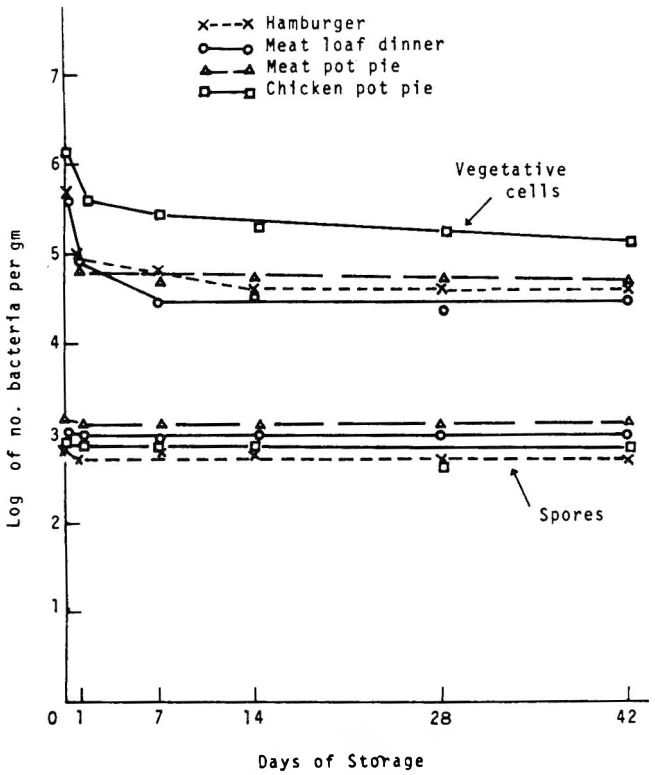

Fig. 3—Survival of C. perfringens in different frozen food products after storage at −29° C and 2 days of thawing at 5° C.

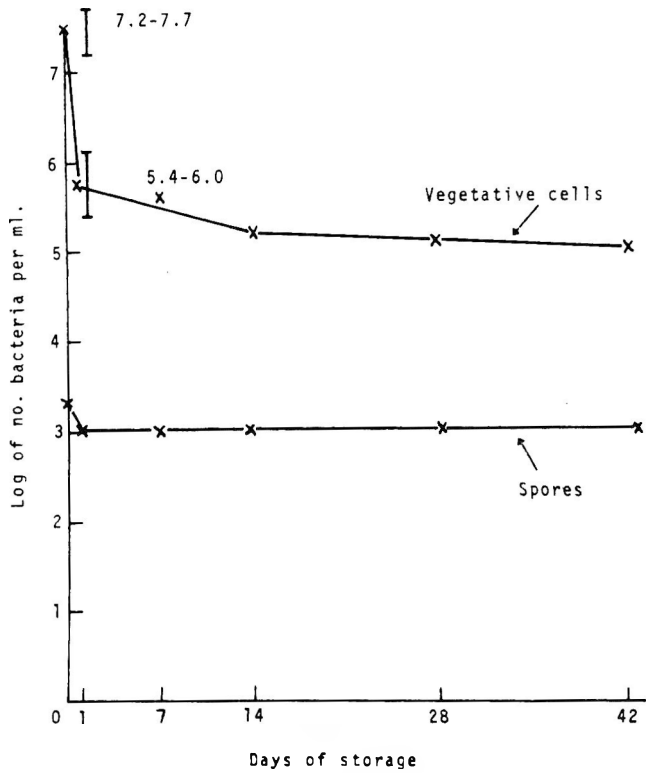

Fig. 4—Survival of C. perfringens in frozen Ellner's medium after storage at −29° C and 2 days of thawing at 5° C.

storage. Blanco and Dawson (1974) found that freezing and thawing inoculated chicken caused greater reduction of vegetative cells and spores when the chicken was subsequently cooked by microwave energy than when no freezing or thawing was done. Freezing and thawing heat resistant spores had no effect on recoveries, but later heat activation by microwave cooking did cause a reduction in *C. perfringens*.

Samples held frozen at −29°C for different periods of time and then thawed for 2 days at 5°C showed only slight changes in numbers of spores. In all cases the reduction was quite pronounced initially and rather gradually in considering the total storage period (Fig. 3 and 4). In comparing Figures 1 and 3, it may be observed that freezing without the thawing treatment (Fig. 1) did not produce as much destruction during the storage period than did freezing and thawing for 2 days (Fig. 3). Percent survival of spores remained fairly constant when observed along with the vegetative cells. Apparently, thawing for 2 days had little effect on spore counts, whether recoveries were made from frozen foods or from Ellner's medium.

From a practical standpoint, one important consideration is that caution must still be observed with respect to *C. perfringens* in fresh and precooked frozen meat and poultry. All such foods may be expected to contain vegetative cells and spores of *C. perfringens*, and the contamination by this organism may become great if growth conditions are favorable. Contamination by spores presents a more serious problem because of their resistance to destruction by freezing and thawing.

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PHYSICAL AND CHEMICAL INFLUENCES ON MEAT EMULSION STABILITY IN A MODEL EMULSITATOR

ABSTRACT

A new method of preparing laboratory meat emulsions utilizing the concept of continuous-flow emulsification was developed to simulate industrial conditions. High speed centrifugation was used to separate phases of preblends and emulsions for the investigation of protein solubility Studies of the effect of water:meat ratio on the protein solubility or preblended and emulsified meat indicated that increasing water:meat ratios resulted in preblends and emulsions with larger soluble phases and lower soluble protein concentrations in the soluble phase. The effects of preblending method, temperature of meat prior to preblending, level of added water and temperature of the added water on the temperature of preblends and emulsions, protein solubility, NaCl concentration, cooked emulsion stability and firmness of the cooked emulsions were established. Temperature of the meat (−30, −10 or 0°C) accounted for the largest proportion of the variation in temperature of preblends and emulsions, protein solubility and cooked emulsion stability. Lower temperatures in preblends and emulsions were associated with the colder meat temperatures. The data indicated that emulsions prepared with −10°C meat had the poorest cooked emulsion stability as compared to those prepared with −30 and 0°C meat. Level of added water (10, 20, 30 or 40%) significantly affected the temperature of preblends, protein solubility, cooked emulsion stability and firmness of the cooked emulsions. The 10% level of added water resulted in firmer emulsions with greater cooked stability. Temperature of the added water (0, 30, 60, or 90°C) had a significant effect on the temperature of preblends and emulsions, cooked emulsion stability and firmness of the cooked emulsions. Adding 90°C water resulted in emulsions with the poorest cooked stability while adding 0°C water resulted in firmer cooked emulsions.

INTRODUCTION

INDUSTRIAL EXPERIENCE in meat manufacturing indicates that many factors lead to the instability of meat emulsions during thermal processing and account for an estimated 2–3% loss of production. Swift et al. (1961) developed a model system to study meat emulsions and this development led to numerous investigations by many different researchers into the areas of emulsifying capacity, emulsion stability, protein solubility, nonmeat additives and water-binding capacity in meat emulsions. Some 160 investigations employing model systems, industrial production systems, or biochemical and biophysical methods have been reported during the past 15 yr to give food scientists a better understanding of the mechanisms of meat emulsion formation. However, the development of our knowledge of meat emulsion stability during heat processing is still in its infancy.

The recent work by Webb (1974) with model systems has given meat emulsion technologists some insight into the roles of water and lipids in processing. While this investigator and other (Saffle et al., 1964; Haq et at., 1972) have attempted to simulate production systems, the use of efficient scale model emulsion processing equipment resembling that presently used in the industry for high-volume, high-speed production has not been developed or utilized.

Model systems have contributed a vast amount of understanding of the basis for emulsion stability. However, many of the results obtained with model systems cannot be duplicated under practical conditions.

Since frozen meats are of major economic importance in sausage making (Saffle, 1968) and their role in maintaining or destroying stability in heat processed emulsions is poorly understood (Webb, 1974; Gorbatov and Gorbatov, 1974), studies were designed with the following objectives:

1. Develop a prototype sausage emulsitator to measure parameters heretofor unreported.
2. Determine the effect of the level of water added to meat on the extraction of salt-soluble proteins.
3. Measure the responses of protein solubility and cooked emuslion stability to various preblending methods.
4. Compare various meat temperatures, levels of added water and added water temperatures during preblending and emulsification with respect to their effects on cooked emulsion stability.

EXPERIMENTAL

Prototype emulsitator

A model emulsitator was developed and a series of experiments were designed to study the effects of low temperature on protein solubility, emulsion formation and emulsion stability.

The concept of continuous-flow emulsification to simulate current industrial practices was used in the development of a laboratory model emulsitator rather than that of step-wise addition of formulae ingredients previously utilized by other researchers (Ockerman and Cahill, 1970; Morrison et al., 1971; Haq et al., 1972). A formulation (Table 1) in the approximate proportions of a controlled commercial meat emulsion (30% fat, meat protein % × 4 + 10% added moisture) was used to evaluate model emulsitators. The muscle tissue samples were ground through a 3 mm plate and then thoroughly mixed. The formulations were prepared and mixed in the manner outlined by Waldman (1973) and passed through the scale model, continuous-flow emulsitator illustrated in Figure 1. The plunger apparatus was designed in order to accommodate control of pressure and dwell time of the formulae during emulsification. A 3-hp motor (3 phase, 220V), operating at 1750 rpm, was used as the power supply. Through preliminary experimentation, the degree of heat rise and particle size reduction during comminution was found to be similar to conditions found in industry.

Protein solubility

Previous researchers (Swift et al., 1961; Saffle and Galbreath, 1964; Anderson and Gillet, 1974) have used large quantities of saline to extract and quantitate salt-soluble protein concentration. These methods involve the use of high speed blenders for extraction with varying salt concentration and time. A method to extract the salt-soluble proteins from ground meats and uncooked emulsified formulae using levels of water and salt concentrations found in commercial production was developed.

The method of Anderson and Gillett (1974) employed a centrifugation speed of 22,000 × G for 15 min and that of Saffle and Galbreath (1964) required centrifugation at 3020 × G for 10 min. However, soluble phases could not be obtained by either method when ratios less than 5:1 saline to meat were used. Differences in the total salt-soluble proteins extractable were also noted to be significantly ($P < 0.01$) greater due to increased extraction time before centrifugation or when

Table 1—Ingredients of preblends used in the preparation of meat emulsions for model emulsitator studies.

Ingredient	Amount (g)	Proportion of total (%)
70% fat pork trim	454	25.94
25% fat pork trim	908	51.89
Water	340	19.43
NaCl	48	2.74
Total	1750	100.00

a 10:1 ratio of saline to meat (Swift et al., 1961) was used for extraction (Fig. 2). However, the values obtained for salt-soluble proteins were similar to those previously reported (Saffle and Galbreath, 1964; Anderson and Gillett, 1974).

Higher centrifugation speeds (40,000 rpm; approx 130,000 × G) than those cited above were obtained using a Beckman Spinco preparative centrifuge (Model L) and a type 50 Ti rotor. The rotor and centrifuge tubes were chilled overnight to 4°C and samples were centrifuged at 4°C. Ground or emulsified samples previously held at 4° or −8°C could be separately into three distinct phases using this centrifugation method (Fig. 3).

During the development of a similar centrifugation method, Macfarlane (1974) used a speed of 35,000 rpm (100,000 × G) to study the effects of pressurization on protein solubility. Saline to meat concentrations of less than unity were used; however, these ratios were adjusted to 10:1 (by the addition of 0.5M saline solution), homogenized and then centrifuged. Macfarlane (1974) reported no differences in the amount of salt-soluble proteins which could be solubilized from various homogenates with ratios ranging from 0.25:1 to 10:1 after adjustment to 10:1.

Macfarlane (1974) reported that rotor speeds did not affect protein concentration in the soluble phase. In the present study, time of centrifugation (Table 2) did affect the amount of salt-soluble proteins as a precentage of total protein which could be extracted. Duplicate samples of the formulation shown in Table 1 were subsampled and no statistical differences were attributed to sampling error. Therefore, a centrifugation time of 60 min at 130,000 × G was used for extraction.

Soluble phase

Accurately weighed centrifuge tubes were filled with the emulsified or ground samples, reweighed and centrifuged by the method previously described (40,000 rpm for 60 min at 4°C). After centrifugation, the soluble portion and the loss was claculated as a percent of the original sample weight and referred to as the soluble phase. The soluble phase was used to quantitate the salt-soluble protein concentration in each extract by the biuret method using bovine serum albumin as a standard (Gornall et al., 1949). Soluble protein was calculated as a percent of total protein in the original sample.

Cooked emulsion stability

Samples were stuffed into preweighed 2 × 7 cm glass tubes to simulate micro-bolognas. After weighing, the emulsions were cooked in a RAPIDAIRE oven (Eurelra Company, Arpington, England) started at 36°C and increased 6°C at each 20-min interval until an internal temperature of 68°C in the emulsion was attained on a final oven setting of 72°C. The weight loss after cooking and draining (by inverting the tubes) was determined and cooked emulsion stability was calculated as a percent of the original sample weight.

Penetrometer readings

Firmness of the cooked emulsions was measured using a Universal Precision Penetrometer with timer and equipped with a 5-prong attachment. Depth of penetration in 5 sec was measured to the nearest 0.1 mm.

Protein solubility of preblended and emulsified meat

Five levels of added water (0°C) were preblended with boneless pork shoulder meat (ca 33% fat, 15% protein, 4°C) to study the effects of blending and comminution on the size of the soluble phase, protein concentration in the soluble phase and percent of total protein which

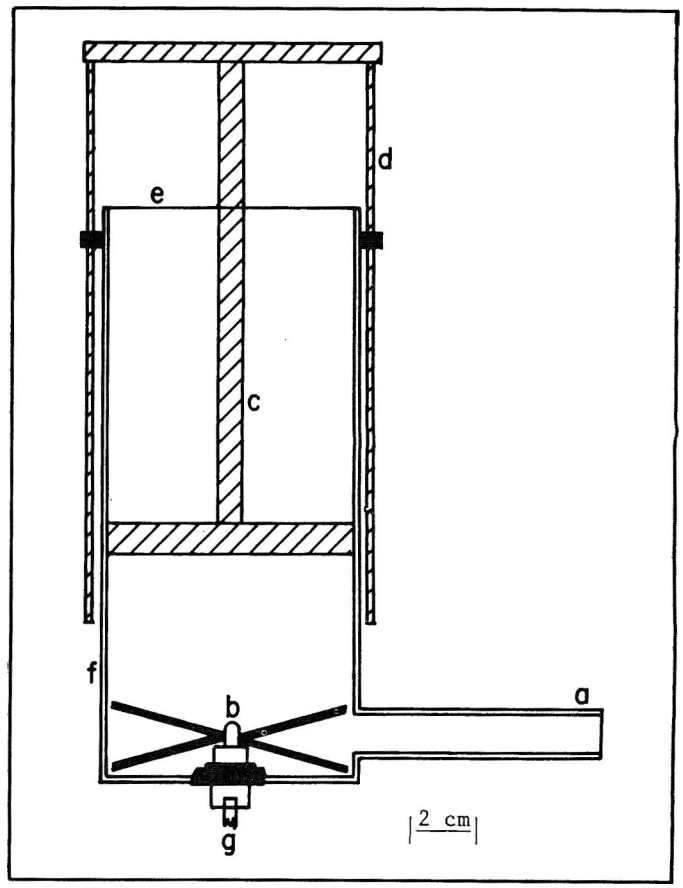

Fig. 1—Schematic diagram of the prototype emulsitator. (a) Exit tube; (b) blade assembly; (c) plunger; (d) plunger guide; (e) infeed opening; (f) side wall; (g) to drive motor.

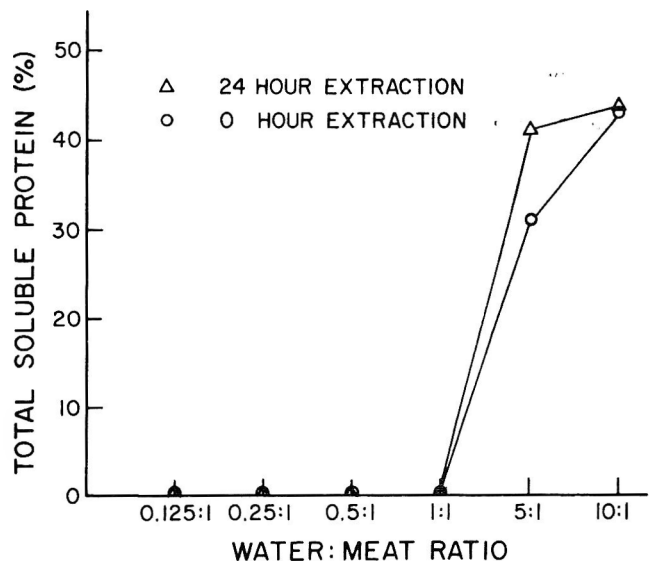

Fig. 2—Effect of water:meat ratio on the protein solubility of meat centrifuged at 22,000 X G.

Table 2—Effect of time of centrifugation on the soluble protein in emulsified meat

	Time of centrifugation[a] (min)					
	15	30	45	60	75	F value
Soluble protein[b]	1.15c	2.40d	4.45e	8.75f	8.69f	49.598**

[a] At 40,000 rpm (approx 135,000 X G) at 4°C
[b] % Soluble protein of total protein. Means with a common letter are not significantly different ($P < 0.01$).
**Significant at 1% level

Table 3—Ingredients of preblends and emulsions

Water:meat ratio	Bnls pork shoulder[a] (g)	NaCl (g)	Water[b] (g)
0:1	750	22.50	0
0.125:1	750	25.32	94
0.25:1	750	28.14	188
0.5:1	750	36.75	375
1:1	750	45.00	750

[a] 33% fat by analysis, 15% protein assumed
[b] 0°C

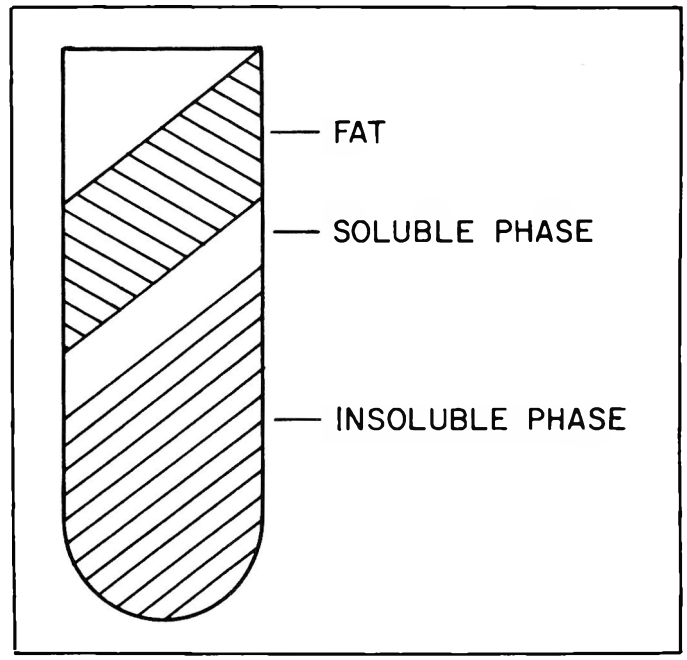

Fig. 3—Phase separations in meat centrifuged at 130,000 X G in ground meat or emulsions centrifuged.

Table 4—Ingredients of preblends containing unfrozen[a] or frozen[b] meat

Water:meat ratio	Bnls pork shoulder[c] (g)	NaCl[d] (g)	Water[e] (g)
0.125:1	25	0.825	2.5
0.25:1	25	0.9	5.0
0.5:1	25	1.125	12.5
1:1	25	1.5	25.0
5:1	25	3.75	100.0
10:1	25	6.75	225.0

[a] 4°C
[b] −8°C
[c] 33% fat by analysis, 15% protein assumed
[d] If used
[e] 0°C

was salt-soluble. Formula compositions are outlined in Table 3. NaCl was maintained at a constant 3% of the total weight of meat and water, and the preblends and emulsions were maintained at 4°C during preparation. Approximately 40 min elapsed from the preparation of preblends or emulsions until centrifugation. Since a precise estimate of the total protein content of the meat was not required in order to compare the percentages of total soluble protein and since only one source of meat was used, total protein was not measured but was assumed to be 15% of the weight of the meat. Samples were prepared in duplicate utilizing a completely randomized design (CRD) for each experiment. Duplicate analyses were performed for soluble protein.

Protein solubility of unfrozed and frozen preblended meat

The six water:meat ratios used in each of the three independent experiments are listed in Table 4. CRD designs with replication were used for each study. The frozen samples were ground (3 mm plate) while in the frozen state, packaged in freezer paper and allowed to equilibrate at −8°C for 2 days before preblending. Preblends were prepared and protein solubility was characterized in the manner described in the first experiments. Duplicate analyses were performed for soluble protein.

Effects of preblending, temperature of meat, level of added water and temperature of added water

A CRD 2 × 3 × 4 × 4 factorial design with replication that was utilized to investigate the treatment effects in shown in Table 5. Preblended and emulsion temperature, protein solubility, percent NaCl, cooked stability and firmness of the cooked emulsions were the variables studied. Preblend temperature refers to that temperature of the preblends immediately prior to emulsification whereas emulsion temperature corresponds to the temperature of the formed emulsion as it exited the prototype emulsitator. Approximately 40 min elapsed from preblend preparation until emulsification. Boneless pork shoulder meat was ground through a 2.5 cm plate, mixed and adjusted to 35% fat content and allocated to the three meat temperatures. Meat samples were frozen at their respective temperature for 4 days, reground (3 mm plate) before formulation and maintained at their frozen temperatures. The samples to be processed at 0°C were frozen at −10°C, thawed and ground as above and maintained at 0°C until formulation was accomplished. Preblends and emulsions were prepared in a 10°C room. Pork shoulders were used because they approximate the chemical analysis of raw materials utilized in commercial meat emulsions. Their proximate composition was determined (moisture, AOAC; fat, Babcock; protein, by difference) and is shown in Table 6. NaCl was kept constant at 3% of total formualtion weight. Added water levels approached the limitations used in industry.

RESULTS & DISCUSSION

Protein solubility of preblended and emulsified meat

Table 7 shows data on the solubility of proteins, after centrifugation of preblended and emulsified meat at the various water:meat ratios. The effects of dilution were highly significant ($P < 0.01$) in both preblended and emulsified meat.

As more water (0°C) was added, the soluble phase increased in both preblended and emulsified meat. Even without statistical comparison, it is obvious that a portion of the soluble phase was rendered insoluble during the emulsification process. Gorbatov and Gorbatov (1969) reported that, as water is increased, the muscle fibers separate faster during comminution forming larger surface areas for protein-water bonding.

In both preblended and emulsified samples, the decrease in

Table 5—Treatment levels and combinations studied in 2 X 3 X 4 X 4 CRD factorial

Temp of added water (°C)	Preblending method: NaCl in added water			NaCl after added water		
	Temperature of meat (°C)					
	−30	−10	0	−30	−10	0
	Level of added water (%)					
0	10 20 30 40	10 20 30 40	10 20 30 40	10 20 30 40	10 20 30 40	10 20 30 40
30						
60						
90						

concentration of protein in the soluble phase from approximately 27 to 17 mg/ml appears to be a straight-line relationship with increasing water:meat ratio. Swift et al. (1961), using saline phases larger than those reported here, increased dilution ratios and found similar reduction of salt-soluble protein concentration. These researchers (Swift et al., 1961) found that increasing the saline:meat ratio resulted in more total protein being solubilized which increased the ability of the meat to emulsify large quantities of fat. Saffle (1968) reported data on the effect of protein concentration in meat slurries on fat emulsifying capacity. As the concentration of protein exceeded 28 mg/ml a curvilinear relationship resulted between the concentration of salt-soluble protein and the amount of fat emulsified. Doubling the number of blades on the model emulsifying equipment raised the maximum concentration to a value of 39 mg/ml before which a curvilinear relationship occurred. High concentrations of salt-soluble proteins in the continuous phase of a meat blend could therefore adversely affect emulsion stability. Data from the present study indicate that protein concentration in the soluble phase of unfrozen meat does not exceed 27 mg/ml when water is not added to aid extraction.

Table 6—Proximate composition of boneless pork shoulder meat

Component	Composition (%)
Fat[a]	35
Protein[b]	13
Water[c]	49
Ash[d]	3
Total	100

[a] Babock
[b] 100 − (% fat + % water + % ash)
[c] AOAC (1970)
[d] Constant assumed

The effects of increasing the water:meat ratio on protein in the soluble phase as a percent of total protein in preblended and emulsified samples are shown in Table 7. At all levels of water added to either preblended or emulsified meat, the soluble protein as a percent of total protein increased significantly ($P < 0.01$). The increase in total protein solubility from the 0:1 to 1:1 ratio was nearly threefold. Even though no statistical comparisons can be made between the independent studies of preblended and emulsified meat, it appears that 20–42% (difference in total soluble protein between blended and emulsified meat as a percent of preblended) of the soluble protein available in preblended meat is tied up in some way during emulsification. Other researchers have reported that

Table 7—Effect of water:meat ratio on the protein solubility of preblended and emulsified meat

Measurement	Water:meat ratio[a] 0:1	0.125:1	0.25:1	0.5:1	1:1	S.E.	F value
Soluble phase[b] (%)							
Preblended	22.88a	30.87b	34.60c	46.06d	61.08e	0.67	441.4**
Emulsified	17.75a	16.24a	24.52b	31.51c	40.84d	0.58	208.0**
Soluble protein conc[b] (mg/ml)							
Preblended	26.83a	24.06b	23.13c	19.93d	17.24e	0.17	55.5**
Emulsified	23.40a	26.70b	23.86a	20.83c	18.99d	0.15	64.6**
Total soluble protein[d] (%)							
Preblended	4.22a	5.74b	6.87c	9.48d	14.47e	0.15	39.9**
Emulsified	2.86a	3.30a	5.07a,b	6.76b,c	10.65d	0.51	35.0**

[a] Means in the same line bearing a common letter are not significantly different ($P < 0.05$).
[b] % of total sample wt
[c] Mg soluble protein/ml soluble phase
[d] % Soluble protein of total protein
** Significant at 1% level

Table 8—Effect of water:meat ratio on the protein solubility of unfrozen (4°C) and frozen (−8°C) preblended meat

	Water:meat ratio[a]							F
	0.125:1	0.25:1	0.5:1	1:1	5:1	10:1	S.E.	value
Soluble phase[b] (%)								
Unfrozen—no NaCl	33.14a	39.04	50.59c	63.66d	83.39e	89.31f	0.35	1066.0**
Unfrozen + NaCl	13.81a	17.23b	34.17c	50.95d	61.14e	77.54f	0.39	1253.7**
Frozen + NaCl	–	14.30a	34.87b	52.59c	71.90d	85.79e	1.11	2198.5**
Soluble protein conc[c] (mg/ml)								
Unfrozen—no NaCl	21.42a	19.75b	15.84c	14.16d	5.03e	2.14f	0.22	245.9**
Unfrozen + NaCl	25.01a	22.90b	18.94c	17.38d	9.81e	4.61f	0.42	244.4**
Frozen + NaCl	–	21.46a	18.88b	17.71b	14.66c	6.85	0.54	270.1**
Total soluble protein[d] (%)								
Unfrozen—no NaCl	5.20a	6.17b	8.01c	12.05d	13.97e	12.72f	0.32	54.7**
Unfrozen + NaCl	2.63a	3.25a	6.71b	12.16c	20.62d	24.47e	0.23	337.1**
Frozen + NaCl	–	2.57a	6.58b	12.55c	34.72d	38.54e	0.73	1105.7**

[a] Means in the same line bearing a common letter are not significantly different ($P < 0.05$).
[b] % of total sample wt.
[c] Mg soluble protein/ml soluble phase
[d] % Soluble protein of total protein
** Significant at 1% level

only 30% of the soluble proteins available are used in the emulsification process (Saffle and Galbreath, 1964). Using saline phases of 10:1, Swift et al. (1961) reported that up to 84% of the proteins became insoluble during emulsification of very large amounts of fat. However, in the ratios used in commercial meat emulsions (0.125:1, 0.25:1 and possible 0.5:1) these data indicate that the amount of soluble protein rendered insoluble during emulsification varies considerably and lies somewhere between the values previously reported (Saffle and Galbreath, 1964; Swift et al., 1961).

Protein solubility of unfrozen and frozen and preblended meat

The effects of water:meat ratio on the protein solubility of unfrozen (4°C) and frozen (−8°C) preblended meat in the three independent experiments utilizing unfrozen meat with and without 3% NaCl and frozen meat with added NaCl (3%) are reported in Table 8. Highly significant ($P < 0.01$) differences were observed for the effects of water:meat ratio on the variables measured in all three studies.

Soluble phase

Adding water (0°C) to preblends of meat resulted in significantly larger ($P < 0.01$) soluble phases. Even without statistical comparison, it is obvious that the omission of NaCl during blending resulted in large soluble phases when compared to preblending frozen and unfrozen meats with NaCl, particularly at the lower ratios of added water. This is expected since NaCl increased the hydration of muscle proteins (Hamm, 1960). Without NaCl availability, much of the added water is not bound in any form and is expressed in the soluble phase during centrifugation. These data suggest that the soluble phase could be increased in preblends using unfrozen meat at the water:meat ratios used in commercial conditions (0.125:1, 0.25:1, and 0.5:1) by blending without NaCl. It might be possible to subsequently mix these preblends with NaCl to extract more of the soluble proteins, thus increasing the potential for greater fat and water binding during emulsification.

When the water:meat ratios reached or exceeded unity, the soluble phases in all three studies surpassed 50% of the total volume. Water:meat ratios exceeding unity have been used by other researchers for extraction of soluble proteins in the study of basic meat emulsion properties (Swift et al., 1961; Saffle and Galbreath, 1964; Johnson et al., 1974). However, it is unlikely that such high water:meat ratios would ever be used in actual practice. When the preblending conditions (unfrozen meat with or without NaCl and frozen meat with NaCl) are compared with respect to volume of the soluble phase at the level of added water most frequently used in industrial preblending conditions (0.25:1), the addition of NaCl to unfrozen meat greatly reduced the soluble phase. Frozen meat with NaCl added had the smallest soluble phase. Since added water increases the surface area for protein-water bonding during comminution (Gorbatov and Gorbatov, 1969), the addition of water to increase the soluble phase could play an important role in emulsion formation when unfrozen and frozen meats are used.

Total soluble protein

Figure 4 shows the percentage of the total protein in the preblends that was in the soluble phase. When all three studies are compared, the most interesting result is the similarity in the percentage total protein in the soluble phases at the 1:1 ratio of water to meat. These data suggest when frozen (−8°C) and unfrozen (4°C) meat is preblended with equal amounts of 0°C water, neither the addition of NaCl nor the temperature of the preblend at the time of extraction greatly alter the amount of total protein that is solubilized. Isolated proteins (myosin, actin, tropomyosintroponin and sarcoplasmic protein) have been shown to be equal to each other in emulsifying capacity at a protein concentration greater than 12 mg/ml (Tsai et al., 1972). The 1:1 ratio resulted in similar protein concentrations in these studies. Therefore, when frozen and unfrozen meats are preblended with water at a 1:1 ratio, differences in emulsifying capacity are probably small. Dearth and Lindle (1972) used this concept in converting meat to semi-liquids during blending to achieve full utilization of meat proteins in commercial emulsification systems.

It is obvious from Figure 4 that a greater proportion of the total protein is in the soluble phase in frozen preblends as compared to unfrozen preblends at the 5:1 and 10:1 water to meat ratios. These results and those of Bard (1965) suggest that, in the presence of large volumes of water, the temperature of the meat influences total protein extractability. The proteins soluble in the unfrozen preblend without NaCl are

Table 9—Analysis of variance of the data obtained for the influence of preblending method, temperature of meat, level of added water and temperature of added water

Source of variation	df	Mean squares							
		Preblend temp (°C)	Emulsion temp (°C)	Soluble phase[a] (%)	Soluble protein conc[b] (mg/ml)	Total soluble protein[c] (%)	NaCl conc[d] (%)	Cooked emulsion stability[e] (%)	Penetrometer reading[f] (mm)
Preblending method (M)	1	1.860	8.114	0.051	1626.515	8.953	0.018	1.978	0.141
Temp of meat (T)	2	2578.203**	2106.093**	1062.527**	21311.199**	1131.978**	0.184	427.916**	0.021
Level of added water (L)	3	122.510**	16.098	572.582**	2653.242**	604.173**	0.130	85.444**	7.599**
Temp of added water (W)	3	164.902**	140.920**	4.292	116.146	6.886	0.295	105.404**	0.945**
M X T	2	1.580	7.620	4.818	999.233	27.987	0.634*	3.515	0.255
M X L	3	2.902	18.583	7.302	1564.821**	47.362**	0.228	8.924	0.100
M X W	3	1.999	5.384	1.822	195.411	0.488	0.295	3.238	0.390
T X L	6	16.138*	19.354*	112.395**	1371.528*	128.363**	0.140	74.860**	0.905**
T X W	6	11.474	19.255*	16.836**	447.909	29.782**	0.147	11.917	0.659**
L X W	9	11.603	10.291	6.386	6.33.976	12.664	0.072	23.439**	0.529**
M X T X L	6	8.468	9.297	7.866	294.137	16.248	0.107	8.162	0.29
M X T X W	6	5.300	7.953	1.096	183.156	3.779	0.072	4.684	0.315
M X L X W	9	4.959	5.372	6.004	628.130	15.161	0.115	4.293	0.341
T X L X W	18	7.507	9.518	11.202**	740.163	16.016	0.094	8.983	0.357*
M X T X L X W	18	5.451	9.120	5.420	494.569	10.033	0.089	6.400	0.288
Error	96	7.136	7.305	4.634	628.596	9.629	0.145	6.422	0.198

[a] % Of total sample wt
[b] Mg soluble protein/ml soluble phase
[c] % Soluble protein of total protein
[d] % NaCl in insoluble phase
[e] % Of original sample wt
[f] Depth of penetration in mm
* $P < 0.05$
** $P < 0.01$

most likely the sarcoplasmic proteins that are available for extraction. Sonce sarcoplasmic proteins lack gelling ability (Trautman, 1966), they probably contribute little to emulsification. Although the total water-soluble proteins in this preblend are lower than those reported by Macfarlane (1974), they follow the same general trend (i.e. decreasing with decreasing water:meat ratios).

Influence of preblending method, temperature of meat, level of added water and temperature of added water

Table 9 shows the combined effects of preblending method, temperature of meat, level of added water and temperature of the added water for the variables measured. The experimental treatment levels, means and standard errors are shown in Table 10.

Temperature of preblends

No significant differences were found in the preblending method (NaCl with added water during blending or NaCl after blending with added water) for temperature of the preblends. Salt and added water have been reported to increase the hydration of muscle proteins (Hamm, 1960) and the increase is thought to be time dependent (Draudt, 1974). Since approximately 40 min elapsed from the time of preblending until emulsification, the NaCl and added water probably equilibrated with the meat in both preblending methods and is the likely explanation for the similarity in temperature of the preblends.

Temperature of the meat before preblending with added water and NaCl accounted for a large proportion of the variation in temperatures of the preblends (Table 9). It is obvious that increasing the temperatures of the meat prior to preblending resulted in warmer temperatures of the preblended meat mixes. The equilibration of preblends containing the −30°C meat to an average temperature of −4°C after preblending is facilitated by the effects of quantity and temperature of added water.

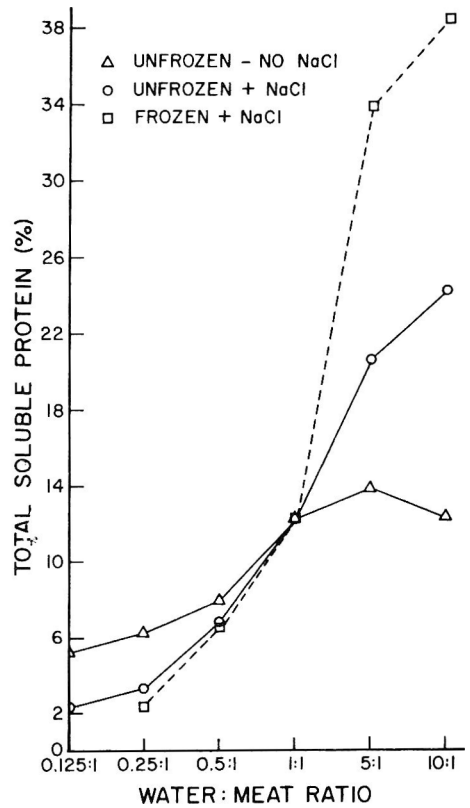

Fig. 4—Effect of water:meat ratio on the total soluble protein of blended meat.

Table 10—Mean values[a] and standard errors of the data obtained for the influence of preblending method, temperature of meat, level of added water and temperature of added water

Treatment	Preblend temp (°C)	Emulsion temp (°C)	Soluble phase[b] (%)	Soluble protein conc[c] (mg/ml)	Total soluble protein[d] (%)	NaCl conc[e] (%)	Cooked emulsion stability[f] (%)	Penetrometer reading[g] (mm)
Method of preblending:								
NaCl in added water	0.9a	6.7a	9.92a	61.93a	7.19a	3.16a	71.78a	58.5a
NaCl after added water	1.1a	7.2a	9.89a	56.11a	6.76a	3.15a	71.58a	58.6a
S.E.	0.64	0.59	0.55	3.02	0.62	0.03	0.42	0.65
Temp of meat:								
−30°C	−4.0a	2.0a	12.18a	64.74a	8.52a	3.14a	73.62a	58.6a
−10°C	−1.1b	5.7b	12.33a	73.72b	10.29b	3.12a	68.75b	58.6a
0°C	8.1c	13.2c	5.20b	38.60c	2.12c	3.20a	72.68c	58.6a
S.E.	0.67	0.77	0.68	3.70	0.76	0.04	0.51	0.80
Level of added water:								
10%	−1.1a	6.3a	5.46a	50.19a	2.77a	3.22a	73.64a	58.1a
20%	0.7b	6.9a	9.15b	57.58a,b	5.61b	3.12a	71.08b	58.5b
30%	1.9c	7.0a	11.50c	60.10a,b	8.59c	3.09a	71.35b	58.7c
40%	2.2c	7.7a	13.50d	68.2b	10.93d	3.18a	70.66b	59.0d
S.E.	0.36	1.39	0.78	4.28	0.88	0.04	0.59	0.93
Temp of added water:								
0°C	−1.2a	4.7a	9.82	60.40a	7.21a	3.15a	73.29a	58.4a
30°C	0.5b	6.6b	9.56a	60.00a	6.43a	3.23a	72.54a	58.6b
60°C	1.4b	7.9c	9.95a	56.95a	7.01a	3.18a	70.78b	58.7b
90°C	3.3c	8.6c	10.28a	58.72a	7.25	3.05a	70.12c	58.6b
S.E.	0.36	1.39	0.78	4.28	0.88	0.04	0.59	0.93

[a] Means within each variable bearing a common letter are not significantly different ($P < 0.05$).
[b] %Of total sample wt
[c] Mg soluble protein/ml soluble phase
[d] % Soluble protein of total protein
[e] % NaCl in insoluble phase
[f] % of original sample wt
[g] Depth of penetration in mm

A linear relationship of increasing preblend temperature with increasing added water (10, 20, 30 or 40%) was observed under these experimental conditions. Temperatures of the preblends were significantly ($P < 0.01$) increased by the 10, 20, and 30% levels of added water. The 40% level of added water had little effect on the temperature of preblends above that obtained with 30% added water. The significant ($P < 0.05$) interaction of meat temperature with added water level resulted from the similar temperatures of preblends at the 10% level of added water when −30 and −10°C meats were used. It is possible that the eutectic point was reached in the −30°C meat and not in the −10°C meat. Buttkus (1974) reported that the eutectic point of myosin is −10°C and that maximum protein aggregation occurs during freezing at this temperature when compared to −30, −20 and 0°C freezing temperatures. If proteins are more closely aggregated at −10°C than at −30°C, it could be postulated that the −30°C meat would have a greater interstitial area with smaller ice crystals which could allow for rapid thawing. Consequently, the rapid thawing of −30°C meat in the presence of 10% added water probably accounted for a faster rise in temperature than did −10°C meat with 10% added water. As the level of added water increased above 10%, the increases in the temperature of the preblends were similar when −30 and −10°C meats were used in formulation.

As expected, temperature of the added water (0, 30, 60 or 90°C) significantly ($P < 0.01$) affected the temperature of the preblends. However, the differences caused by 60 and 90°C added water were not significant (Tables 9 and 10). The temperature of the meat and the large surface area of the ground meat were most likely responsible for the rapid cooling of the added water and accounted for the similarity between 60° and 90°C added water with respect to temperatures of the preblends.

Temperature of emulsions

Only the main effects of meat temperature and added water temperature were significant ($P < 0.01$) for temperature of the emulsified preblends (Tables 9 and 10). Meat temperature accounted for the largest proportion of variation in emulsion temperature. It is obvious that the use of meats with higher temperatures resulted in emulsions with significantly higher ($P < 0.01$) temperatures.

Highly significant ($P < 0.01$) effects of the added water temperatures were observed for emulsion temperatures, except between the 60 and 90°C added water temperatures. Thus, adding 90°C water had very little effect on emulsion temperature beyond that of 60°C water. The cold meat temperatures may allow for rapid cooling of the added water at the warmer temperatures. The very slight over-all increase (1°C) in emulsion temperature due to warmer water temperatures for 0°C meat and the significant ($P < 0.01$) increase in emulsion temperature for −30 and −10°C meat with 0, 30 and 60°C added water resulted in the significant ($P < 0.05$) meat temperature vs added water temperature interaction.

Temperature of the meat and added water temperature had more influence than preblending method or added water level on emulsion temperature, except for added water at 90°C.

Table 11—R^2 for prediction variables of cooked emulsion stability[a]

Variables in equation	R^2
Total soluble protein[b]	0.185
Emulsion temp[c], soluble phase[d]	0.385
Preblend temp[c], emulsion temp, soluble phase	0.405
Preblend temp, emulsion temp, soluble phase, total soluble protein	0.417
Preblend temp, emulsion temp, soluble phase, total soluble protein, conc[e]	0.427
Preblend temp, emulsion temp, soluble phase, total soluble protein, soluble protein conc, NaCl conc[f]	0.428

[a] % Of original sample wt
[b] % Soluble protein of total protein
[c] °C
[d] % Of total sample wt
[e] Mg soluble protein/ml soluble phase
[f] % NaCl in insoluble phase

More importantly, using 0°C meat resulted in emulsion temperatures nearly identical to the ideal industrial conditions described Saffle (1968), but the temperature and level of added water had little effect on temperature of emulsions at this meat temperature. Therefore, the addition of large amounts of warm water appears to be neither beneficial nor detrimental to temperatures of emulsions when using 0°C meat in formulations. Adding water at increasing levels and at warmer temperatures did increase the emulsion temperatures when frozen meats were used. This is often the only means a processor has to control temperatures of preblends and emulsions.

Soluble phase

The soluble phase after emulsification and centrifugation was significantly reduced ($P < 0.01$) when 0°C meat was used in the formulation as compared to −30 and −10°C meat. Emulsions prepared with frozen (−30 and −10°C) meat did not differ significantly in the amount of soluble phase. These results indicate that emulsions formulated with frozen meat, regardless of the meat temperature did have a larger proportion of total volume expressed as soluble phase. The results of the first study on preblended and emulsified meat demonstrated that the emulsion forming process renders a portion of the soluble proteins insoluble. It is likely that a greater proportion of the available salt-soluble proteins were used in the formation of the continuous phase of emulsions prepared with 0°C meat as compared to those prepared with frozen (−30, −10°C) meats. In frozen meats a large part of the water is most likely unavailable before emulsification due to the subfreezing temperatures of the preblends. These data suggest that during emulsification the temperature of preblends made from frozen meats rises to a point where ice crystals are thawed and subsequently a proportion of the available water and solutes are expressed during centrifugation. Lumry (1973) reported that there is a sizeable fraction (30% of the dry weight of protein) of water about proteins and polypeptides which does not freeze at temperatures below −50°C. It is probable that this unfrozen water along with the added water is involved in solubilization of protein. Therefore, the expressed water seen in the soluble phase is most likely thawed ice crystals released during and/or after emulsification.

The soluble phase volume was significantly ($P < 0.01$) different at all four added water levels. It is obvious that increased added water resulted in increased soluble phase volume. The main reason for adding water during the emulsion forming process, other than improving the yield, is to aid in the solubilization and extraction of proteins to form the continuous phase (Saffle, 1968). Thus, one would expect larger soluble phases to contain more total protein *before* emulsification.

Cooked emulsion stability

Numerous investigators (Meyer et al., 1964; Rodney, 1965; Swift, 1965; Saffle et al., 1967; Ivey et al., 1970) have reported that the proportions of fat, water, protein and frozen meat can influence cooked emulsion stability. The emulsions prepared with −30°C meat had the highest cooked emulsion stability while −10°C meat resulted in emulsions with the lowest stability. All three meat temperatures were significantly ($P < 0.01$) different with respect to cooked emulsion stability. It is likely that the process of emulsification greatly reduced the particle size of the preblends containing −30°C meat thus allowing for larger surface areas for protein-water-lip interactions. Buttkus (1974) indicated that −30°C meat is very close to 0°C meat in rate of protein aggregation, and that proteins are more tightly compressed in meat at −10°C. These results

Table 12—Correlation matrix[g] for preblend temperature,[a] emulsion temperature,[a] soluble phase,[b] soluble protein concentration,[c] total soluble protein,[d] NaCl concentration,[e] and cooked emulsion stability[f]

	Preblend temp	Emulsion temp	Soluble phase	Soluble protein conc	Total soluble protein	NaCl conc	Cooked emulsion stability
Preblend temp	1.00	0.93	−0.36	−0.40	−0.32	0.03	−0.18
Emulsion temp		1.00	−0.41	−0.43	−0.38	0.04	−0.23
Soluble phase			1.00	0.62	0.90	−0.10	−0.42
Soluble protein conc				1.00	0.78	−0.00	0.25
Total soluble protein					1.00	−0.06	−0.43
NaCl conc						1.00	0.15
Cooked emulsion stability							1.00

[a] °C
[b] %of total sample wt
[c] Mg soluble protein/ml soluble phase
[d] % Soluble protein of total
[e] % NaCl in insoluble phase
[f] % of original sample wt
[g] For significance at the 1% level a coefficient must exceed 0.18.

indicate that limited quantities of water and fat are bound and stabilized by meat emulsions made from −10°C meat that hard frozen (−30°C) meat results in emulsions that are more heat-stable.

Adding water at the 10% level resulted in significantly greater ($P < 0.01$) cooked emulsion stability than adding water at higher levels. None of the cooked emulsions approached acceptable cooked yields (i.e., 90%; Morrison et al., 1971) since extreme conditions of meat temperature were employed in the formation of emulsions. This experiment confirms the findings of Morrison et al. (1971) that frozen and thawed meat near 0°C produces emulsions with poor stability.

The quantity of fat emulsified to give maximum cooked emulsion stability is dependent on the volume of water in an emulsion and the relationship of interfacial film thickness to fat droplet diameter (Ivey et al., 1970). The higher cooked emulsion stability at 10% added water was probably due to smaller fat droplet formation associated with the mechanical reduction of frozen fat during emulsification. It was noted, but not measured, that preblends containing −30°C meat took slightly more time to exit the emulsitator. Thus, further particle size reduction could have occurred due to extended time around the emulsitator blades. Consequently, the significant interaction ($P < 0.01$) between meat temperature and level of added water probably resulted from the reduced fat particle size and protein aggregation in −30°C meat at 10% added water vs 0°C meat with 30 and 40% added water.

Added water temperatures >60°C resulted in emulsions with significantly ($P < 0.01$) less cooked stability as compared to emulsions prepared with 0 and 30°C added water. Since the temperature of the added water did not significantly affect (Table 9) the protein solubility or NaCl concentration in this experiment, decreases in cooked emulsion stability with warmer water temperatures are probably not related to protein denaturation but most likely due to changes in protein hydration as suggested by Webb (1974). Temperature of the fat prior to emulsification could also have been raised to the point of minimum binding when 60 and 90°C water was added. This theory is supported by the findings of Webb (1974) and Townsend et al. (1968) which showed that pork fat melts at temperatures greater than 8°C. The emulsion temperature studies indicated that temperatures after emulsification were 7.9 and 8.6°C when added water temperatures of 60 and 90°C respectively, were used (Table 10).

When the combined treatment effects were examined, the most striking finding was that preblending with NaCl and formulating −30°C meat with 0°C water at the 10% added water level resulted in the greatest cooked emulsion stability. This probably resulted from decreased protein hydration and reduction in fat particle size to assure maximum fat and water binding.

Table 13—Analysis of variance for regression of preblend temperature,[a] emulsion temperature,[a] soluble phase,[b] soluble protein concentration,[c] total soluble protein[d] and NaCl concentration[e] on cooked emulsion stability[f]

Source of variation	df	Mean square	R^2	F value
Regression	6	229.902	0.428	23.055**
Residual	185	9.972		

a °C
b % Of total sample wt
c Mg soluble protein/ml soluble phase
d % Soluble protein of total
e % NaCl in insoluble phase
f % Of original sample wt
** Significant at 1% level

Prediction of cooked emulsion stability was accomplished using the variables previously discussed. Table 11 shows the squares of regression coefficients for the "best" regression equations of all possible regression equations using the parameters in this study to predict cooked emulsion stability. The single most important predictor was the percentage of total protein appearing in the soluble phase and it accounted for 18.5% of the variation in cooked emulsion stability. Since soluble protein was negatively correlated (Table 12) with cooked emulsion stability, these data indicate that the reciprocal of soluble protein (i.e., protein in the insoluble phase of the emulsion) was the single most important variable measured in this experiment for explaining the variation in cooked emulsion stability. The analyses shown in Table 11 indicate that the best combination of two variables for predicting cooked emulsion stability was emulsion temperature and the size of the soluble phase; the best combination of three variables was temperature of the preblends, emulsion temperature and the size of the soluble phase. In fact, prediction equations using four, five, or six variables accounted for only an additional 2.3% of the variation in cooked emulsion stability over that equation including the three best variables. Since soluble protein, soluble protein concentration and NaCl concentration were directly or indirectly related to the volume of the soluble phase, this is understandable.

Table 13 indicates the parameters of the regression equation using all six variables to predict cooked emulsion stability. Although the regression is significant ($P < 0.01$), a large proportion of the variation is still unexplained. Since this experiment used only frozen or near freezing meat temperatures, it should be emphasized that the precition Eq (1) below applies only to the boundries of this experiment.

$$\hat{y} = 81.97 + 0.135PT + 1.489\,NaCl - 0.149\,Pro - 0.005C - 0.318SP - 0.339ET \quad (1)$$

where $\hat{y}$ = predicted value of cooked emulsion stability (%); PT = preblend temperature (°C); NaCl = NaCl in insoluble phase (%); Pro = total soluble protein (%); C = soluble protein concentration (mg/ml); SP = soluble phase (%); and ET = emulsion temperature (°C).

These observations suggest that cooked emulsion stability could be predicted, under the treatment combinations of this experiment, by measuring the six variables above. It might also be possible to improve cooked emulsion stability when using frozen meats at the added water levels investigated by increasing preblend temperature, reducing the emulsion temperature, and by reducing the size of the soluble phase.

Penetrometer reading

Only quantity and temperature of added water significantly ($P < 0.01$) affected cooked emulsion firmness (Table 9). Although the differences in penetrometer readings were small for the effects of level of added water, all means were significantly ($P < 0.05$) different. It is obvious that increased levels of added water resulted in cooked emulsions that were less firm These results compare favorably with those of Morrison et al. (1971) who used a trained panel for measuring physical properties of cooked emulsions and reported that increased water levels resulted in samples which were less firm. These data suggest that the penetrometer reading is a good indicator of the physical firmness of cooked meat emulsions, even if differences are small.

SUMMARY & CONCLUSIONS

THE ADDITION of increasing quantities of water to unfrozen (4°C) preblended meat resulted in emulsions with significantly larger soluble phase volumes. As the size of the soluble phase became larger in preblended and emulsified samples, the soluble protein concentration within the soluble phase decreased. The data indicate that the emulsification process renders a

portion of the total soluble protein insoluble and that increased volumes of water added to meat results in increased solubilization of the total protein. The increase in total protein solubility from the 0:1 to 1:1 ratio of water to meat was nearly threefold. These data imply that the salt-soluble proteins in unfrozen meat are more readily available for the formation of the continuous phase of emulsions by the increased addition of water and 3% NaCl.

When large (>5:1) volumes of water and meat were preblended, the use of frozen meat as compared to unfrozen meat resulted in more total protein being solubilized. At the 1:1 ratio of water to meat, the data indicate that neither the addition of NaCl nor the temperature of the preblend as influenced by the use of frozen meat greatly altered the solubilization of meat proteins, especially when formulating with frozen meats, by first preblending with large quantities of water. It is probable that emulsion products such as imitation frankfurters and bologna (>30% fat; water >10% plus four times the total meat protein) utilize the larger amounts of water to increase protein solubilization thereby increasing the binding of large amounts of fat and water which are stabilized during heat processing.

The temperature of the meat before preblending and emulsification accounted for the largest proportion of the variation in temperature of the preblends, emulsion temperature, protein solubility and cooked emulsion stability. Preblends and emulsions prepared with −30°C meat had the coldest temperatures while those formulated with 0°C had the warmest temperatures. The most interesting observation for the effect of meat temperature was that emulsions formulated with −10°C meat had large amounts of soluble protein and poor cooked stability. Cooked emulsion stability was highest in emulsions prepared with −30°C meat. The results of this study indicate that when meat processors formulate with frozen meats in emulsion preparation, the use of hard frozen rather than tempered (−10°C) meat would result in greater yeilds of cooked product.

Level of added water significantly affected the temperature of preblends, protein solubility, cooked emulsion stability and firmness of the cooked emulsions. As the quantity of added water was increased, the temperature of preblends and protein solubility also increased. However, the addition of 20, 30 and 40% added water resulted in significantly poorer cooked emulsion stability as compared to adding 10% water. Increased levels of added water resulted in emulsions that were less firm.

The temperature of the added water significantly affected the temperature of preblends and emulsions, cooked emulsion stability and firmness of the cooked emulsions. As the temperature of the added water was increased from 0 to 90°C, the temperature of the preblends and emulsions increased. However, adding 90 vs 60°C water did not significantly increase the emulsion temperatures. Emulsions prepared with 90°C added water and then cooked had the lowest emulsion stabilities. The use of 0 and 30°C added water in the preparation of preblends resulted in emulsions with the greatest cooked stability. Therefore, the practice on the part of meat processors of using large amounts of hot water in conjunction with frozen meats should be curtailed.

Of particular interest was the finding that the frozen meat temperature most frequently utilized (−10°C) in industry resulted in the poorest cooked emulsions. More soluble protein was removed from the continuous phase of the emulsions at this meat temperature as compared to colder or warmer raw materials. The poorest cooked emulsions were associated with high concentrations of protein in the soluble phase and large soluble phase volumes. The available literature and the results of the present study indicate that the soluble protein concentration in the soluble phase of emulsions formulated with −10°C meat adversely affect the binding of fat and water.

These data indicate that the continuous phase of meat emulsions was separated into two components called soluble and insoluble in this study. The soluble component was present in very small amounts in emulsions prepared with fresh (<0°C) meats. However, this soluble component was found in amounts of as high as 20% of the total weight in emulsions prepared with frozen meats and was associated with poor cooked emulsion stability. Most previous researchers have used unfrozen meats in their study of basic emulsion properties. The techniques developed in this investigation of frozen meats allowed the observation of the separation of the continuous phase of meat emulsions into two parts. The data indicate that the insoluble component of the continuous phase is particularly important in stabilizing meat emulsions.

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A Research Note

PRODUCTION OF BEER FROM CASSAVA

ABSTRACT

At present malt for beer production in Nigeria is imported. Substitution of malt with cassava, an indigenous crop, would conserve foreign exchange and also create employment opportunities for many workers in cassava plantations. Preliminary results show a satisfactory product can be made with cassava, comparing favorably with beer manufactured from conventional malt. Among the cultivars used, Nwugo gave the best results.

INTRODUCTION

SOURCES of carbohydrate other than malt for the production of beer has been reported by many workers. Rothschild (1972) used plain sorghum, sprouted sorghum and maize grits for the production of Bantu beer in South Africa. The process was automated and during the first month of operation over 10 million liters of beer were produced. Pfeniger et al. (1971) have been able to substitute malt with up to 15% maize for the production of beer. Noort (1971) has reported the use of sorghum malt in place of brewers' malt. Cassava is one of the richest fermentable substances for the production of alcohol. The fresh roots contain about 30% starch and 5% sugars and the dried roots contain about 80% of fermentable substances. Nigeria is the third largest producer of cassava in the world with a production of 7.3 million tons annually (Grace, 1971). This cassava is readily available, easy to cultivate, particularly in the Southern parts of the country. At present, malt which is used as a source of carbohydrate for the production of beer in Nigeria, is imported. Substitution of indigenously available cassava in place of malt for the production of beer would help in having a source close at hand, conserve foreign exchange and also create employment opportunities for many workers in cassava plantations. This work was therefore undertaken to study how far cassava could be used in the production of beer.

MATERIALS & METHODS

Materials

The following varieties of cassava (*Manihot utillisima*) were collected from a farm at Umuahia: (a) cultivar *"Nwugo;"* (b) cultivar *"Nwanyi Oji;"* (c) cultivar "60566;" and (d) cultivar "60447," and used as a source of carbohydrate. The tubers of *Discorea dumetorium* (called *'onu'* locally) were collected from and around Umuahia. *Discorea dumetorium* is a variety of edible yam grown around Umuahia. It was used as a source of α and β amylases because of its easy availability. Further, preliminary experiments had shown the tuber to be rich in α and β amylases. Maize grit was obtained from the agricultural farm of the University of Nigeria. Caramelized maize was prepared by roasting the maize until it had a rich brown color. Natural hops and *Saccharomyces carlbergenesis* were obtained from the Golden Guinea Breweries, Umuahia. *Saccharomyces elliposideus*, a yeast isolated from palm wine, was obtained from the culture collection of the University of Nigeria, Nsukka.

Extraction of enzymes from Discorea dumetorium

Three-month old tubers of *Discorea dumetorium* were grated after removing the outer peels. The grated mass was then blended in a dilute solution of papain at a temperature of 57°C and a pH of 3.3. This treatment gave a mixture of α and β amylases and was used as a source of enzymes for the saccharification of cassava.

Preparation of wort

The following procedure for the production of beer was employed for each of the cultivars. Each fermentation was carried out both separately by both *S. carlsbergenesis* and *S. ellipsoideus.*

The corky outer and inner cortical layers of the cassava were peeled off. The body of the root was pulverized using local graters; the quantity of grated cassava used was 4 kgs. The grated cassava was steeped in water in a stainless steel vessel containing 40 liters of water. About 400 ml of a mixture of the amylase extract from *Discorea dumetorium* was added to the mash. 0.2 mg of iron filings and 5.0g of potash were added at this stage. After 4 days of steeping at room temperature it was found that most of the HCN had been removed. The mash was then mixed with a wooden stirrer and subjected to the following treatment: about one-third of the mash was withdrawn, boiled separately for about 2 min and returned to the main mash. This raised the temperature of the mash to 50°C. At this temperature the pH was adjusted to 5.0 and the mash held for 15 min. One-third of the mash was again removed, boiled separately for 2 min and returned to the main mash. The temperature of the total mash was about 65°C. The pH was again adjusted to 5.0 and the mash allowed to stand for 15 min. Finally another one-third of the mash was withdrawn, boiled for 2 min and added to the main mash raising the temperature to about 70–75°C. The pH was found to still be 5.0 and the mash was allowed to stand at this temperature for 15 min. The mash was then filtered first using a wire gauze netting and then a Buchner funnel under suction. The filtrate (wort) was boiled for 1½ hr. After it had boiled 30 min, 45g of hops, 700g of granulated sugar, 80g of a mixture of roasted *Discorea dumetorium* and maize grits and 3 liters of a mixture of salts of the following concentration (NaCl

Table 1—Analysis of beer from four varieties of cassava using S. carlsbergensis as fermenting organism

Cultivar	Alcohol content (% by wt)	CO_2 content (%)	pH	Color	Direct microscopic count	Flavor
"Nwugo"	3.55	0.4	4.5	Very bright amber yellow	Nil	Palatable beer like
"Nwanyi Oji"	3.25	0.4	5.0	Dull yellow	Nil	Palatable beer like
"60506"	2.80	0.4	5.5	Dull yellow	Nil	Flat; No beer flavor
"60447"	2.55	0.4	6.0	Dull yellow	Nil	Flat; No beer flavor

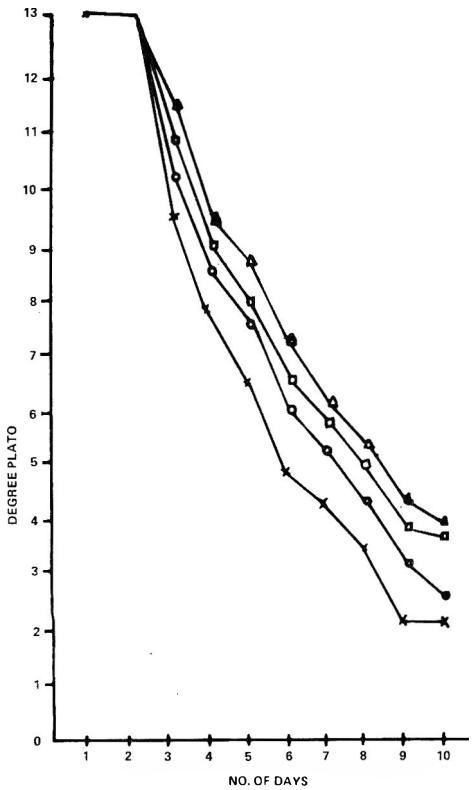

Fig. 1—Daily saccharometric readings of fermenting wort using Saccharomyces carlsbergensis [x—x, cultivar Nwugo; ○—○, cultivar Nwanyi Oji; □—□, cultivar 60506; △—△, cultivar 60447. Degree Plato = standardized hydrometric reading at 15.56° C].

4.5 mg/liter; $CaCl_2$ 4.5 mg/liter; KCl 4.5 mg/liter; $(NH_4)_2SO_4$ 1.17 mg/liter; K lactate 1.5 mg/liter; $FeSO_4$ 1.0 mg/liter; $ZnSO_4 \cdot 7H_2O$ 1.0 mg/liter; and $CuSO_4$ 1.0 mg/liter) were added. The pH was now adjusted to 5.4 and the precipitated tannins removed as sludge. The boiled wort was then filtered and cooled. The resulting product had a sugary taste and gave a negative iodine test for starch indicating complete saccharification. Tests for sugars at this stage showed presence of dextrins and small quantities of maltose and glucose. HCN was found to be completely removed. The original sp. gravity was found to be 11° Plato. The hot sweet wort was refrigerated for 2 hr to reduce the temperature to 8° C.

Fermentation

30g of a lyophilized culture of *S. carlsbergenesis* was grown in 400 ml of glucose yeast extract broth and a 24-hr old vigorously growing culture used for pitching into the cold wort. The temperature of the wort was gradually raised to and maintained at 10°C for the fermentation period which lasted for 10 days. The specific gravity of the wort was measured at daily intervals. A hydrometer marked in degrees Plato (standardized hydrometric reading at 15.56°C) was used for measuring specific gravity.

The pH of the wort was also measured daily. On the 10th day the wort was fined using isinglass which was prepared by dispensing 10g of isinglass in 200 ml of a mixture of 1N tartaric acid and 1N sulfurous acid at a ratio of 1:1. This solution was added to the product which was chilled for 1 hr. The chilled product was centrifuged at 1000 rpm and the clear liquid separated out. The final product was transferred into bottles and carbonated by a direct introduction of carbon dioxide. The carbonation was stopped when about 2 volumes of the gas per volume of beer had been introduced. Clipper corks were then put on to maintain the pressure and the bottles were pasteurized.

RESULTS & DISCUSSION

PRELIMINARY EXPERIMENTS had shown that addition of potash and iron filings (a common practice in Southern Nigeria for hastening the saccharification of "stubborn" cassava) helped in reducing the time needed for the saccharification process. Further detailed study would be needed to find how the saccharification process is aided by the addition of these adjuncts.

S. ellipsoideus did not show any increase in the alcoholic content as needed for beer production with any of the varieties of cassava. This is in agreement with the findings of Steinkraus and Robinson (1967) who showed that the enzymes of *S. ellipsoideus* strain 223 did not attack higher polysaccharides present in corn syrups and were thus unable to give theoretical yields of alcohol.

Figure 1 gives daily saccharometric readings during the fermentation by *Saccharomyces carlsbergenesis* of the four varieties of cassava. Among the cultivars used, the cultivar *"Nwugo"* gave the best results. Further tests confirmed that the beer obtained from this variety of cassava compared favorably with beer manufactured from conventional sources such as malt. The chemical and physical properties of the beer obtained from the four varieties of cassava are shown in Table 1.

Over 200 tasters, among them staff and students of the University of Nigeria, Nsukka and other institutions nearby have reacted favorably to the product and expressed satisfaction. However, due to the small quantity of the final product and paucity of technical help it has not yet been possible to carry out a statistically designed taste trail.

It is evident from these results that it would be possible to use cassava as a source of carbohydrate for the production of beer. Further large scale experiments would have to be carried out to (1) see how far the process could be automated, (2) estimate the cost of each operation, and (3) to carry out consumer taste-trials.

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RESEARCH NOTES

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A Research Note

ANALYSIS OF GERMAN WINES BY GAS CHROMATOGRAPHY AND ATOMIC ABSORPTION

ABSTRACT

An analytical profile of a total of 49 samples of 1970 and 1971 vintage Rhine and Moselle German white wine is presented. The data include atomic absorption analysis for the elements K, Na, Mg, Ca, Fe, Cu, and Zn; gas-liquid chromatographic analysis for methanol, ethyl acetate, n-propyl alcohol, iso-butyl alcohol, and iso-amyl alcohol; the traditional specific gravity determination for ethanol; and the determinations for total acids, solids, and ash. For each of the parameters determined the range, mean and standard deviation is given. A comparison of the data for the 1970 and 1971 vintage years and the Rhine and Moselle wine regions is included. The conclusion is drawn that the data are insufficient to determine distinguishing and identifying characteristics of either vintage year or wine region.

Table 1—Analyses of Moselle and Rhine wines, 1970

Sample no.	g/100 ml			% v/v		mg/100 ml				ppm						
	Total acids	Solids	Ash	Ethanol	Methanol	Ethyl acetate	n-Propyl alcohol	i-Butyl alcohol	i-Amyl alcohol	Na	K	Mg	Ca	Fe	Cu	Zn
Moselle wines																
22	0.73	3.05	0.249	9.40	0.007	2.68	5.49	4.20	15.84	18.4	907	89	62	0.27	—	0.45
23	0.73	2.97	0.229	9.27	0.006	4.91	7.06	4.54	17.60	20.7	868	84	50	0.24	0.34	1.08
24	0.78	2.73	0.238	7.74	0.006	3.08	12.54	3.80	14.78	23.0	880	88	54	0.26	0.08	0.35
34	0.56	3.93	0.205	6.27	0.003	1.72	1.62	1.86	11.88	29.9	813	58	61	0.27	0.13	0.10
35	0.28	3.63	0.211	6.23	0.004	1.37	1.81	2.35	11.88	46.0	649	70	95	0.32	0.13	0.09
37	0.75	3.09	0.211	9.31	0.005	1.72	1.81	3.35	14.52	11.5	684	73	60	0.34	0.08	0.08
38	0.59	3.26	0.151	9.89	0.006	5.49	3.02	4.69	13.20	9.2	547	84	53	0.14	0.23	0.15
39	0.37	3.89	0.222	7.84	0.004	2.40	2.41	3.35	14.52	20.7	739	64	46	0.26	0.00	0.81
42	0.64	3.32	0.226	8.57	0.005	2.40	1.81	3.35	13.20	20.7	790	85	67	0.47	0.33	0.50
43	0.70	3.02	0.237	8.56	0.006	3.43	1.81	4.69	14.52	16.1	813	88	60	0.49	0.35	0.40
Avg	0.61	3.29	0.218	8.31	0.005	2.92	3.94	3.62	14.19	21.6	769	78	61	0.31	0.19	0.40
High	0.78	3.93	0.249	9.89	0.007	5.49	12.54	4.69	17.60	46.0	907	89	95	0.49	0.35	1.08
Low	0.28	2.73	0.151	6.23	0.003	1.37	1.62	1.86	11.88	9.2	547	58	46	0.14	0.00	0.08
S.D.	0.17	0.40	0.027	1.28	0.001	1.36	3.54	0.97	1.75	10.4	114	11	14	0.11	0.13	0.33
Rhine wines																
1	0.80	3.90	0.266	9.72	0.008	2.97	2.18	4.64	11.71	11.5	989	82	55	0.20	0.43	0.45
4	0.74	3.49	0.231	9.72	0.007	2.79	3.48	5.26	13.78	9.2	880	73	49	0.30	0.20	0.05
7	0.66	2.79	0.204	9.70	0.005	4.09	3.05	5.26	13.77	0.5	892	59	50	0.16	0.38	0.11
11	0.73	2.93	0.211	9.53	0.011	8.16	6.14	10.84	29.42	9.2	895	81	46	0.46	0.40	0.11
12	0.65	2.68	0.241	Insufficient amount of sample for distillation						11.5	981	76	67	—	0.78	0.12
15	0.46	3.16	0.224	9.40	0.005	3.23	3.07	5.23	14.33	27.6	876	142	59	0.32	0.12	0.78
16	0.58	3.06	0.306	9.30	0.007	5.65	4.06	5.90	15.73	23.0	915	98	49	0.30	0.35	0.38
17	0.61	2.99	0.190	9.18	0.005	8.92	2.85	3.07	15.84	29.9	895	90	52	0.33	0.08	0.23
18	0.40	3.83	0.154	6.59	0.006	5.10	2.85	6.75	16.53	0.0	669	60	60	0.33	0.40	0.89
19	0.64	3.25	0.205	9.56	0.004	2.55	2.14	3.22	11.71	39.1	907	79	43	0.30	0.08	0.15
20	0.61	3.36	0.263	9.17	0.006	3.79	7.06	6.03	15.84	13.8	923	85	48	0.42	0.13	0.20
56	0.73	2.77	0.252	10.30	0.005	3.44	2.16	2.47	13.86	16.1	985	78	55	0.35	0.45	0.37
58	0.59	3.44	0.279	9.22	0.006	4.39	3.09	7.31	15.27	16.1	1056	93	81	0.20	0.39	1.31
61	0.38	3.80	0.207	7.80	0.004	1.98	1.90	3.17	14.71	23.0	751	64	45	0.37	0.18	0.04
Avg	0.61	3.25	0.231	9.17	0.006	4.39	3.39	5.32	15.58	16.5	901	83	56	0.31	0.31	0.37
High	0.80	3.90	0.306	10.30	0.011	8.92	7.06	10.84	29.42	39.1	1056	142	90	0.42	0.78	1.31
Low	0.38	2.68	0.154	6.59	0.004	1.98	1.90	2.47	11.71	0.0	669	59	43	0.16	0.08	0.04
S.D.	0.13	0.41	0.040	0.96	0.002	2.11	1.56	2.24	4.42	11.1	98	21	14	0.08	0.19	0.38

INTRODUCTION

GERMAN white wines are ranked among the finest quality wines in the world, and this is especially surprising considering the geographical handicaps of Germany's vineyards. Geographically, Germany's vineyards are situated the farthest north of any major wine growing nation, and Germany has the smallest land area under cultivation for vineyards. However, the soil and climatic conditions are favorable for the wine production and the application of German wine laws regulate wine production. The German wine regions are divided into the four areas of the German rivers: The Rhine, the Moselle, the Main, and the Neckar (Simon and Hallgarten, 1963). German white wine is generally acknowledged to be a dry, light, delicate and mature wine with distinctive flavor.

The purpose of this work was to develop an analytical profile of German white wine from the 1970 and 1971 vintages of the Rhine and Moselle regions to determine if vintage year and wine regions could be distinguished by a chemical and spectrographic analysis.

EXPERIMENTAL

THE TOTAL ACIDITY, solids, ash, ethanol, ethyl acetate, methanol, n-propyl alcohol, iso-butyl alcohol, and iso-amyl alcohol were determined by the methods in the AOAC, 1975.

The metallic ion components were determined by atomic absorption spectrophotometry with a Jarrell-Ash 82-500 MVAA instrument. Metallic ion standards were prepared by diluting commercially manufactured (Fisher Scientific Co., Pittsburg, PA) reference solutions with 10% ethanol-water diluent. There was no sample treatment except for dilutions where necessary. The single optical pass system through a laminar flow burner flame with a 10 cm slot was utilized. Air was used as the support gas and acetylene as the fuel for all determinations. The entrance slit was 100 μm and the exit slit was 150 μm. Parameters such as burner height, gas flow rates, and hollow cathode lamp current were optimized for each element. For all spectrographic determinations the

Table 2—Analyses of Moselle and Rhine wines, 1971

Sample no.	g/100 ml			% v/v		mg/100 ml				ppm						
	Total acids	Solids	Ash	Ethanol	Methanol	Ethyl acetate	n-Propyl alcohol	i-Butyl alcohol	i-Amyl alcohol	Na	K	Mg	Ca	Fe	Cu	Zn
Moselle wines																
5	0.75	3.50	0.181	9.48	0.007	3.16	3.05	4.64	13.09	2.3	622	82	48	0.15	0.28	0.18
6	0.70	3.46	0.160	9.46	0.007	3.16	3.92	5.26	15.84	2.3	622	86	47	0.15	0.28	0.20
8	0.63	3.66	0.188	7.06	0.009	2.60	3.05	5.57	15.15	0.0	740	78	53	0.10	0.20	0.12
9	0.78	3.77	0.208	8.28	0.010	4.80	3.48	5.26	13.40	2.3	849	81	63	0.14	0.28	0.16
21	0.83	5.02	0.266	7.83	0.006	3.35	4.70	4.54	19.36	11.5	915	84	64	0.29	0.13	0.2
25	0.60	3.68	0.213	8.70	0.005	2.85	3.87	3.45	14.76	32.2	841	82	58	0.36	0.08	0.27
26	0.64	2.42	0.230	9.72	0.005	3.35	5.49	3.85	15.84	18.4	817	96	57	0.54	0.40	0.21
27	0.75	4.43	0.364	8.05	0.006	2.68	4.70	4.20	14.96	20.7	1013	97	56	0.29	0.00	0.45
28	0.64	3.22	0.251	8.72	0.006	3.50	3.84	3.76	15.51	13.8	1017	90	52	0.56	0.20	0.71
29	0.65	3.95	0.310	8.39	0.007	1.78	3.92	3.50	14.08	0.0	1021	95	50	0.19	0.08	0.00
30	0.64	2.59	0.251	9.49	0.005	3.12	4.70	4.20	17.60	25.3	760	144	52	0.10	0.68	0.50
31	0.64	4.78	0.199	8.09	0.004	2.39	1.87	4.23	7.65	18.4	759	86	59	0.51	0.18	0.51
32	0.88	5.26	0.356	7.94	0.009	1.53	1.87	4.65	8.19	16.1	1181	99	61	0.20	0.13	0.20
33	0.62	2.49	0.228	9.87	0.005	2.35	1.62	1.86	11.88	16.1	1193	90	55	0.42	0.33	0.19
40	0.74	3.56	0.246	8.97	0.006	2.74	2.41	4.02	13.20	18.4	860	74	65	0.19	0.23	0.07
41	0.66	3.97	0.210	9.01	0.005	1.89	1.81	2.68	11.88	13.8	751	61	55	0.07	0.13	0.00
Avg	0.70	3.74	0.241	8.69	0.006	2.83	3.39	4.10	13.90	13.2	873	89	56	0.27	0.23	0.25
High	0.88	5.26	0.364	9.87	0.010	4.80	5.49	5.57	19.36	32.2	1193	144	65	0.56	0.68	0.71
Low	0.60	2.24	0.160	7.06	0.004	1.53	1.62	1.86	7.65	0.0	622	61	47	0.07	0.00	0.00
S.D.	0.08	0.84	0.059	0.79	0.002	0.79	1.21	0.95	3.04	9.6	173	18	5	0.16	0.16	0.20
Rhine wines																
2	0.80	4.29	0.219	8.79	0.013	4.92	2.56	5.15	18.90	6.9	751	85	47	0.15	0.28	0.44
13	0.51	2.89	0.262	10.87	0.001	11.58	5.11	10.84	36.21	18.4	1013	87	45	0.33	0.18	0.24
14	0.52	3.35	0.203	9.67	0.006	4.93	3.41	6.29	16.59	25.3	923	90	57	0.32	0.08	0.22
44	0.61	5.90	0.215	8.47	0.007	3.43	1.81	5.35	14.52	4.6	673	73	60	0.33	0.28	0.04
45	0.82	2.98	0.247	10.99	0.008	3.60	1.35	1.55	9.90	6.9	997	89	69	0.12	0.23	0.14
47	0.71	4.82	0.345	9.76	0.009	1.88	2.16	3.71	13.86	9.2	1372	86	65	0.08	0.60	0.09
49	0.65	2.58	0.210	8.94	0.007	1.25	2.70	5.57	12.54	0.2	692	80	61	0.21	0.63	0.61
50	0.57	2.88	0.249	9.97	0.007	4.07	3.24	6.49	16.50	11.5	657	75	60	0.46	0.23	0.70
62	0.64	4.59	0.182	8.31	0.008	1.27	2.61	5.60	12.45	9.2	716	76	59	0.29	0.33	0.47
Avg	0.65	3.81	0.237	9.53	0.007	4.10	2.77	5.62	16.83	10.2	866	82	58	0.25	0.32	0.33
High	0.82	5.90	0.345	10.99	0.013	11.58	5.11	10.84	36.21	25.3	1372	90	69	0.46	0.63	0.70
Low	0.51	2.58	0.182	8.31	0.001	1.25	1.35	1.55	9.90	0.2	673	73	45	0.08	0.08	0.04
S.D.	0.11	1.14	0.048	0.98	0.003	3.14	1.09	2.47	7.74	7.5	236	6	8	›0.12	0.18	0.24

elements were analyzed at the following analytical lines: Na-5890; K-7665; Ca-4227; Mg-2852; Cu-3247; Zn-2139; Fe-2483 (Angstrom units). The metallic ion components are expressed in parts per million (ppm).

RESULTS & DISCUSSION

THESE SAMPLES were received directly from the U.S. Customs Service in the original sealed bottle with cork closure and protective wrap. As traditionally bottled the Moselle wines were packaged in green glass bottles and the Rhine wines were packaged in brown glass bottles.

Tables 1 and 2 give the results of the analyses of samples of Moselle wine and Rhine wine for vintage years 1970 and 1971. In the first column, each sample is identified by a number.

The wines were analyzed for these particular 16 parameters since they represent some of the major components in wine. In general, the concentration levels of these components agree with the data given by Amerine, 1974.

Due to the lack of a sufficiently large sample population, it is unlikely that an extensive statistical evaluation will uncover any relationship which would classify a particular wine sample according to vintage year or wine region; consequently, no elaborate statistical calculation was carried out.

As can be seen from the tables, there is little difference for the averages of the parameters determined for either vintage year or wine region, and there is considerable overlap of the values. There appears to be no definite pattern of distribution for any of the parameters. These samples were evaluated organoleptically and considerable differences were observed; however, the authors do not consider themselves "experts" to the degree to enable them to identify the wines according to vintage year or wine region.

CONCLUSION

IT IS READILY APPARENT from the data presented that there is no component quantitated which would characterize or distinguish the vintage year 1970 or 1971 of the Moselle or Rhine wine region from which the wine originated. After a thorough examination of the collected data, the conclusion is drawn that the data are insufficient to determine vintage year (1970 or 1971) or wine region (Moselle or Rhine).

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A Research Note

AN IMPROVED METHOD FOR THE DETERMINATION OF RESIDUAL HYDROGEN PEROXIDE IN MILK

ABSTRACT

The method of hydrogen peroxide determination in milk described by Ferrier et al. (J. Dairy Sci. 53: 598, 1970) was modified. This modification increased the sensitivity threefold and permitted the detection of less than 1 ppm hydrogen peroxide in milk.

INTRODUCTION

GILLIAND (1969) developed an enzymatic method for determining low levels of hydrogen peroxide in milk. In the following year, Ferrier et al. (1970) described a relatively simple colorimetric method, using titanium tetrachloride for detecting residual hydrogen peroxide in milk. The method was based on the formation of a colored complex between titanium and hydrogen peroxide and could detect levels of 2–3 μg/ml in milk. This paper reports a modification of this method, involving precipitation of the titanium-hydrogen peroxide complex.

EXPERIMENTAL

Materials

All reagents were of analytical grade. Titanium tetrachloride was obtained from British Drug houses. Hydrogen peroxide was standardized against sodium thiosulfate. Homogenized, pasteurized whole milk was obtained from the Modern Dairies, Winnipeg. In order to destroy possible residual enzyme activity, the milk prior to use was heated to 80°C for 5 min and subsequently cooled to room temperature (23°C).

Method

Five milliliters of aqueous solution of hydrogen peroxide (0–240 ppm) were added to 45 ml of the heated milk. The resulting concentration of hydrogen peroxide in milk ranged between 0–24 ppm. An equal volume of 1% trichloroacetic acid was immediately added to the 50 ml milk sample containing hydrogen peroxide. The mixture was then shaken and filtered through a Whatman No. 50 filter paper. Aliquots of filtrate were then used for hydrogen peroxide determination using the original and modified procedures.

Procedure of Ferrier et al. (1970). Six milliliters of filtrate were transferred to 12 ml centrifuge tubes. To each tube was added 1 ml of titanium reagent (20% $TiCl_4$ in conc HCl) and the solutions allowed to stand for 10 minutes to permit full color development. The tubes were centrifuged at 2000 × G for 10 min to remove possible influence from residual turbidity in the colored solutions. The clear solutions were then measured for absorbance at 415 nm against an equivalent blank.

Modified Method. Twenty-seven ml of filtrate were transferred to a 50 ml centrifuge tube. To each tube was added 1.0 ml of titanium reagent (20% $TiCl_4$ in conc HCl) and the contents mixed thoroughly. The tubes were allowed to stand for 10 min for color development. The titanium-hydrogen peroxide complex was then precipitated by the addition of 3.0 ml conc NH_4OH to each tube. The tubes were then mixed and centrifuged at 3000 × G for 10 min. The supernatant was discarded and the precipitate dissolved in 8N H_2SO_4 to a final volume of 9 ml. The colored solutions were then centrifuged and the absorbance read at 415 nm against an equivalent blank.

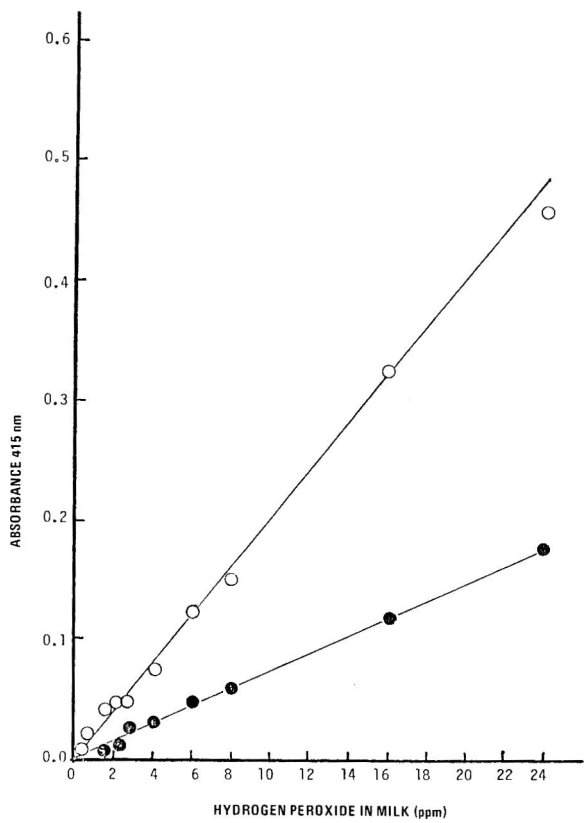

Fig. 1—Standard curves for hydrogen peroxide in milk as determined by the method of Ferrier et al. (1970) •; modified procedure ○.

RESULTS & DISCUSSION

THE STANDARD CURVES for hydrogen peroxide levels in milk using the "original" and "modified" titanium methods are shown in Figure 1. A linear relationship was evident for both methods over an H_2O_2 concentration range 2–24 ppm. The sensitivity of the modified titanium method was approximately three times that of the original method of Ferrier et al. (1970). This permitted levels as low as 0.4 ppm to be detected in milk compared to 1.6 ppm using the original method. The increased sensitivity and low detection limit using the modified method described should provide a more effective procedure for determining residual hydrogen peroxide in milk.

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A Research Note
ABSORPTION OF A TAINT AROMA BY EGGS

ABSTRACT

The extent of absorption of ethyl propyl sulphide into whole shell eggs was examined both for eggs of different ages and for eggs stored in the tainting atmosphere for different lengths of time. The latter proved the more important factor but an absorption plateau is approached with time. Significant amounts of the taint aroma were absorbed in all instances.

INTRODUCTION

SINCE the normal flavor of the hen's egg is so bland, any slight off-flavor or taint is immediately noticeable. Such a problem therefore can be a severe one in that only a minute amount of a tainting agent might be involved. In recent years there has been a number of reports of work on tainted eggs, the causative factors having a variety of origins, mainly the hen itself, its environment or its feed.

Off-flavors in eggs due to factors other than these have been investigated less frequently, even though it is well known by experience that eggs can become contaminated by absorption of taint aromas through the shell. The main study in this field to date has been by Kato et al. (1971) who used gas chromatography to study the absorption of various single compounds by eggs. It was found that in some homologous series, rates of absorption and dispersion were inversely proportional to the number of carbon atoms and were independent of the molecular weights or boiling points of the compounds. Aldehydes were absorbed into shell eggs more easily than other compounds studied, namely ketones, esters and alcohols, with the latter showing only slight absorption. In this project reported here, only one compound was studied, but this is a highly odorous sulphide, and in addition its absorption into eggs of different ages was considered. This work formed a logical progression from our previously reported detailed systematic analysis of the natural flavor components of the egg (Mac Leod and Cave, 1975), and comparative survey of the effects on these volatile components of variations in the nature and history of the egg (including consideration of eggs of different ages) (Mac Leod and Cave, 1976).

EXPERIMENTAL

THE EGGS employed in this project were brown and were from hens of the Warren breed kept under battery conditions. All eggs were from birds of the same age, although for the experiments studying the effect of the age of the egg on the absorption of a taint aroma, eggs were used 1 day after laying, 2 wk after laying and 4 wk after laying. The taint aroma selected for this work was ethyl propyl sulphide. This had a number of advantages including the fact that it is a highly odorous compound and its presence in a sample egg could be detected (but not measured) by the nose as well as by the gas chromatograph. In addition, under the conditions of analysis normally employed with egg aroma samples, (Mac Leod and Cave, 1975; 1976) this compound was eluted from the gas chromatography column separated from all natural egg volatiles and thus could be recognized easily and could be measured quantitatively very accurately. Due to the complexity of the gas chromatogram of egg aroma volatiles it was not in fact easy to locate a reasonable gap for resolved elution of a suitable tainting compound.

For exposure to the taint six whole eggs, accurately weighed, were placed in an air-tight tank (capacity 13.8 liters) in the presence of 0.1 ml of ethyl propyl sulphide at a temperature of 4°C (i.e., general refrigerator temperature) for a period of 18 hr. To study the effect of time of exposure on absorption of taint, eggs were also exposed to the sulphide for 48 hr and 168 hr. All experiments were done at least in duplicate and results were consistent within experimental error.

The concentration of contaminant in the tank atmosphere was estimated by headspace analysis and gas chromatography using the same chromatographic conditions as for the analysis of the egg itself. After exposure to the taint, eggs were analyzed for their volatile flavor composition exactly as already described for the analysis of untainted eggs (Mac Leod and Cave, 1975; 1976). Blank determinations were carried out in the absence of the tainting compound. It was also necessary to allow for the efficiency of the analytical procedure and this was assessed by adding 5 μl of ethyl propyl sulphide to a sample of uncontaminated eggs in the Likens and Nickerson extractor apparatus immediately before sample preparation.

RESULTS & DISCUSSION

TABLE 1 summarizes the results obtained for the absorption of taint aroma both by eggs of different ages and by eggs exposed to the taint for different lengths of time. It can be seen from the fourth column that the absorbed taint can contribute a significant proportion of the natural aroma volatiles. In the blank determination when 5 μl of the sulphide were added directly to the eggs immediately prior to extraction it provided only 2.2% of the resultant total volatiles. So the figure quoted of 29.2% sulphide in one tainted sample represents an amount of about 76 μl (or 63 mg) absorbed into the eggs. This calculation allows for determined recovery of the analytical procedure for ethyl propyl sulphide, namely 87%. The figure of 76 μl absorbed in this particular instance is nearing the total amount of sulphide available for absorption in the contaminant atmosphere (100 μl) and it is possible that the result for this one experiment might be slightly low due to the excessive depletion of taint molecules from the atmosphere.

The results given in columns five and six of Table 1 are all corrected for the 87% recovery of the analysis. The figures in column five quote the determined volume of ethyl propyl sulphide per ml of tainted egg divided by the original volume of ethyl propyl sulphide per ml of the tainting atmosphere. In other words this is a concentration factor expressing the relative intensity of taint absorption by the egg under the particular conditions.

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Table 1—Contamination of eggs by ethyl propyl sulphide

Time after eggs laid (days)	Time of storage in presence of sulphide (hours)	Sulphide content of tainting atmosphere (μl/l)	Sulphide in total analyzed volatiles (%)	Concentration factor[a]	Weight of sulphide absorbed by egg (μg/g)
1	18	7.25	11.3	11	56
14	18	8.39	12.3	10	59
28	18	7.59	10.0	9	48
28	48	7.34	18.8	19	98
28	168	7.25	29.2	42	214

[a] See text for definition

The time elapsed after the egg is laid has little influence, if any, upon the amount of contaminant absorbed, all other factors remaining constant. However, perhaps predictably the variation in the amount absorbed with time of storage in the presence of the contaminant is appreciable. A plot of concentration factor against storage time indicates that an absorption plateau is being approached with time (the log/log plot is perfectly linear). Whether this is indicative of the attainment of a saturation level for the egg or whether it is simply due to relative lack of tainting medium after a certain time, as mentioned earlier, is debatable, although the former is the more likely explanation. Kato et al. (1971) observed a linear variation between the amount of contaminant absorbed per egg and the number of hours of storage both for the yolk and for the white, but generally the exposure times employed were far less than those used in this work (usually only a few hours). In such a short time an absorption limitation need not be attained. Interestingly, the previous workers also report far greater absorption than observed in this investigation; butanone was absorbed by egg white to the extent of 400 μg/g after only 7 hr. However, the concentration of butanone in the contaminant atmosphere was very high at more than fifteen times that of the ethyl propyl sulphide. In addition, Kato et al. (1971) generally conducted their experiments at the relatively high temperature of 27°C although they showed that less contamination was effected at lower temperatures, and this could also partly explain the generally lower absorption figures reported here (experiments conducted at 4°C).

In summary, it would appear that in the absorption of taint aromas by whole eggs the length of time of exposure is the critical factor and the age of the egg seems to matter little. It is significant how readily quite a large amount of a foreign aroma is absorbed through the egg shell, and all the eggs tainted in this project had very noticeable off-aromas. It is clear therefore that eggs are very susceptible to this type of tainting at refrigerator temperature.

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A Research Note

SENSORY COMPARISON OF FOUR POTATO VARIETIES BAKED CONVENTIONALLY AND BY MICROWAVES

ABSTRACT

A trained 20-member panel was asked to rank the eight potatoes (one of each variety baked conventionally and one of each baked by microwave) from best to worst on the basis of external appearance, internal appearance, aroma and flavor. Different potatoes were randomly presented for each sensory property evaluated with each evaluation being repeated three times. Statistical evaluation of the pooled data revealed that an experimental variety (WC-230-14) was significantly superior in external appearance, odor and flavor for both baking procedures. Among the other varieties, conventionally baked potatoes ranked ahead of the corresponding microwaved potato.

INTRODUCTION

THE SENSORY PROPERTIES of a baked potato are quite unique and as demonstrated in a recent study by Twomey and Maga (1974) a wide range in overall sensory acceptability among potato varieties can exist. In the case of potatoes, few reports exist discussing the influence of potato variety on quality (Heinze, 1955).

Thus this study was designed with a twofold purpose. The first dealt with attempting to ascertain if a trained sensory panel could demonstrate a preference based on various specific sensory properties such as external appearance, internal appearance, aroma, and flavor of baked potatoes as influenced by potato variety. The second phase was an effort to determine if this same panel could distinguish between conventionally baked and microwave baked potatoes again as influenced by potato variety.

EXPERIMENTAL

Potatoes

Three experimental potato varieties (WC 284-146, WC 285-18, WC 230-14) that had previously demonstrated good potato qualities (Twomey and Maga, 1974) along with Russet Burbank were grown at the San Luis Valley-Colorado State University Experiment Station using the same horticultural practices. After harvest, potatoes within each variety were grouped for similar weights (200–210g) and specific gravity. Specific gravity was found to range from 1.080–1.090 among varieties and thus tubers from each variety with a specific gravity of 1.085 were choosen. All evaluations were performed after 1 month of storage in the dark at 22°C and 30% relative humidity to more closely simulate supermarket-consumer storage conditions.

Baking conditions

Whole potatoes of each variety were baked plain in either a conventional oven operating at 205°C for 60 min or in a consumer model microwave oven for 5 min. Preliminary baking trials had demonstrated that the four varieties did not vary significantly in degree of doneness under the baking conditions employed. At the end of each respective time period, one potato from each variety and baking method was randomly arranged for presentation to the sensory panel.

A 20-member sensory panel composed of 14 women and 6 men ages 20–35 who had previously taken a 16-wk college-level course in the sensory evaluation of food was used. For each sensory property evaluated, 8 potatoes, coded with 3-number random digets, which represented each of the 4 potato varieties baked by the 2 methods were randomly presented and the panel asked to rank the 8 potatoes from the most acceptable to the least acceptable. All evaluations were done 5 min after their respective baking times.which were arranged to be completed at the same time. Each sensory property was judged separately, used a new set of potatoes, and was repeated 3 times.

External appearance was on whole potatoes while internal appearance was judged by making a longitudinal slit through each potato. Aroma was judged by sniffing but not consuming samples of slit potatoes. Flavor was evaluated by permitting the panel to freely ingest samples of each as required.

Textural properties were not evaluated due to the difficulty of employing a sensory panel to evaluate texture, especially in ranking 8 samples.

Statistical evaluation

Ranking data obtained for each of the 3 replicates were pooled for each sensory property evaluated and subjected to analysis of variance.

RESULTS & DISCUSSION

A STATISTICAL SUMMARY of the sensory data obtained is shown in Table 1. As seen, significant differences were apparent as influenced by variety for all sensory properties evaluated except internal appearance. Also, relative to baking method, all sensory properties were found to be significantly different. All other variables and their interactions were found not to be significantly different.

When the data for specific potato varieties were compared

Table 1—Factorial analysis of variance of pooled data

Variable	Critical F-value ($\alpha = 0.05$)	Appearance		Aroma	Flavor
		Ext	Int		
Variety	2.64	48.63	0.06	194.21	267.56
Baking method	3.88	9.22	9.05	20.28	17.17
Var. X Bkg. Meth.	2.64	0.43	0.29	1.77	1.31
Judge	2.80	0.00	0.00	0.00	0.00
Var. X Judge	2.10	1.28	1.08	0.61	1.68
Bkg. Meth. X Judge	2.80	1.45	0.79	1.60	0.72
Var X Bkg. Meth. X Judge	2.10	0.81	1.03	0.76	1.74

(Table 2), it was found that in all cases microwave baking resulted in significantly inferior products for all sensory properties evaluated as compared to the corresponding conventional baking treatment.

Table 2—Statistical evaluation of variety and baking method on sensory properties

		Method of baking	
Sensory property	Pototo variety	Conventional	Microwave
Ext appearance	WC 285-146	6.87a	7.17a
	Russett Burbank	4.50b	5.00b
	WC 230-14	1.87c	2.23c
	WC 285-18	3.73b	4.67b
Int appearance	WC 285-146	4.22a	4.92a
	Russett Burbank	4.28a	4.60a
	WC 230-14	4.15a	4.85a
	WC 285-18	4.06a	4.92a
Aroma	WC 285-146	6.60a	6.98a
	Russett Burbank	4.53b	5.50b
	WC 230-14	1.88c	2.13c
	WC 285-18	3.70b	4.67b
Flavor	WC 285-146	5.10a	5.87a
	Russett Burbank	5.37a	5.68a
	WC 230-14	2.63c	3.02c
	WC 285-18	3.78b	4.55b

[a] Microwave significantly inferior ($\alpha = 0.05$) to comparable conventional. Column data with the same letter within each sensory property grouping are not significantly different ($\alpha = 0.05$).

From an external appearance standpoint, both baking methods resulted in variety WC 230-14 being found significantly superior to the other 3 varieties and variety WC 285-146 being significantly inferior. Variety WC 230-14 was said to be more symmetrical, have fewer eyes, and undergo little if any skin shrinkage after baking as compared to the other 3 varieties. The same statistical differences were noted for aroma properties.

Interestingly no significant differences were found among varieties with regard to internal appearance. However, several significant differences were noted for flavor properties. Variety WC 230-14 was found to be significantly superior to the other varieties by both baking methods whereas the Russett Burbank and variety WC 285-146 were found to be significantly inferior. An earlier study by Eheart and Gott (1964) had concluded that no significant difference existed between the flavor properties of conventional and microwave cooked potatoes. However, their study utilized boiled potatoes while the current one employed baked.

Thus it would appear that a sensory panel can distinguish sensory differences among baked potatoes as influenced by variety and method of baking.

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A Research Note

CHANGES IN NONVAOLATILE ACIDS OF CONCORD GRAPES DURING MATURATION

ABSTRACT

Separation by GLC indicated the major nonvolatile acids in Concord grapes to be tartaric and malic with lesser quantities of succinic, citric, quinic and ascorbic acids present. In the less mature fruit, malic acid was present in greater quantities than tartaric acid; however, as the grape berries ripened malic acid was apparently metabolized more rapidly than was tartaric acid so that tartaric became the dominant acid present in the fully ripe fruit.

INTRODUCTION

THE RIPENING PROCESS in Concord grapes, as in all fruits, is accompanied by a number of changes in form and structure as well as significant changes in chemical composition. The changes in organic acids and the resulting effect on the pH of the grape berries may influence several factors such as flavor, anthocyanin (ACY) extraction during processing, and color stability of grape products.

Numerous studies have shown that tartaric and malic acids are the principal organic acids in several different grape cultivars (Carroll et al., 1971; Sistrunk et al., 1974; Amerine and Winkler, 1942; Johnson and Carroll, 1973; Amon and Markakis, 1968). Amerine (1951) and Kliewer (1964, 1965) have shown that concentrations of both tartaric and malic acids in *Vitis vinifera* cultivars decrease rapidly during ripening. Sistrunk et al. (1974) found this to be the case in Concord grapes and showed that malic acid exhibited the greatest change during maturation. In the mature green fruit, malic was the predominant acid, with tartaric representing a much smaller percentage of the total acids present. As the grapes ripened, malic acid decreased appreciably, so that, in the fully ripe fruit tartaric represented the major percentage of the total acids. Johnson and Carroll (1973) observed the opposite situation in Scuppernong grapes (*Vitis rotundifolia*). In this cultivar, tartaric acid decreased continually from a maximum observed on the first sampling date, while malic acid content rose to a peak just prior to verasion and then decreased.

The present study was initiated to determine the composition of nonvolatile acids in different maturities of Arkansas grown Concord grapes.

MATERIALS & METHODS

CONCORD GRAPES (*Vitis labrusca*) were hand harvested from mature plants in a vineyard on the University of Arkansas Main Experiment Station at Fayetteville, Ark. Harvest began when grape berries were at the hard green stage and continued at 5-day intervals until berries were obviously over-mature. Clusters were stripped and the grape berries were frozen in enamel-lined cans at −20°C until all harvesting was completed. Triplicate samples from each harvest date were drawn for analysis and data were reported as averages of the three replications.

Organic acids were extracted from the grape berries according to the method of Sistrunk and Cash (1973) with the following modifications. Elution of the sample from the anion exchange column was accomplished by passing 6N formic acid through the column until 30 ml of eluate were collected. This eluate was then evaporated to dryness at room temperature under a fume hood and the residue was taken up in 10 ml of 95% ethanol (EtOH). One ml of EtOH sample solution and 1 ml of 95% EtOH containing 100 μm fumaric acid as an internal standard were pipetted into a glass vial and evaporated to dryness in preparation for silylation. The dried acids were silylated by adding 1 ml of "Tri-Sil" (hexamethyldisilasane, trimethylchlorosilane and pyridine: Pierce Chemical Company, Rockford, Ill.) to each vial, which was then shaken until the sample residue was completely dissolved. Ethanol solutions containing 100 μm per ml of known organic acids were prepared and silylated in a similar manner. After silylation was complete (1 hr at room temperature), 5 μl of each sample were injected into a Varian Aerograph Series 1800 gas chromatograph which had the following operating conditions:

Detector	Thermal conductivity
Columns	Dual stainless steel 10 ft × ¼ in.
Stationary phase	Chromosorb W, 10-100 mesh DMCS treated
Liquid phase	SE-52 (3%)
Carrier gas (He)	35 ml/min
Injector temperature	220°C
Detector temperature	250°C
Column temperature	80–250°C
Program rate	8°C/min
Recorder speed	0.5 in/min

Acid concentration was calculated using the equation of Philip and Nelson (1973).

RESULTS & DISCUSSION

GLC SEPARATION of organic acids of Concord grapes revealed that malic and tartaric acids were the principal acid components of this cultivar (Fig. 1), which agrees with results obtained using other methods of separation (Amon and Markakis, 1968; Sistrunk et al., 1974). Other important acids identified by GLC were succinic, citric, quinic and ascorbic. Malic acid was found to be the dominant acid in less mature fruit but as ripening proceeded, the concentration of malic decreased more rapidly than the concentration of tartaric, so in full-ripe and over-ripe fruit, tartaric was the most abundant acid (Fig 1 and 2). In the full sized green grape, malic acid represented 55% of the total acids, while tartaric represented 40% of the total. In the fully-ripe fruit, the concentration of malic acid had changed to 28% of the total acids, while tartaric acid had changed to 62% of the total acids present (Fig. 1).

The changes in malic and tartaric acid may be due to the fact that malic acid is localized within the immature fruit in

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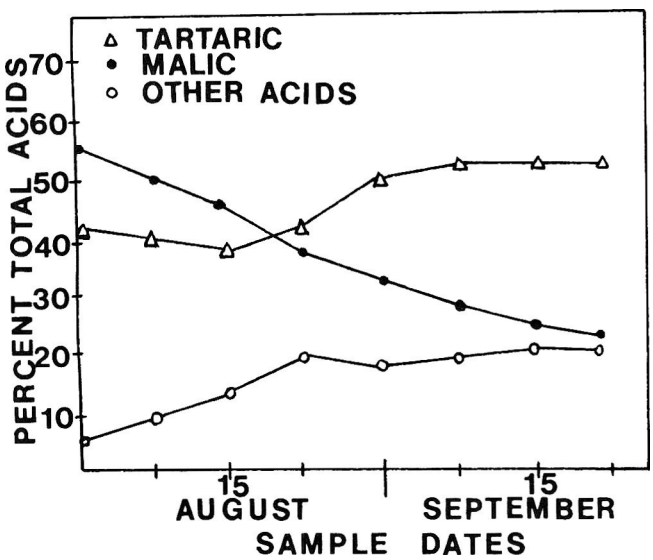

Fig. 1—Changes in organic acids of Concord grapes during ripening.

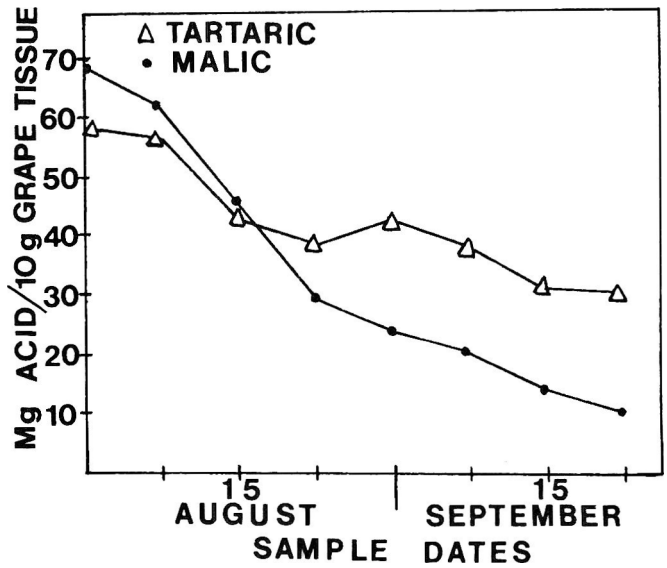

Fig. 2—Changes in malic and Tartaric acid of Concord grapes during ripening.

such a way that it is inaccessible to malic enzymes. As the ripening process causes cell membrane permeability to increase, then the malic acid may be made available for decarboxylation by the malic enzymes. Hawker (1969) has documented the changes in activity of several enzymes during grape ripening, and he noted that at the time of veraison, malic acid concentrations decrease, while malic dehydrogenase and malic enzyme increase to their highest levels in the grape berries.

Tartaric acid synthesis is very active in the immature grape berry, but as ripening continues, it decreases rapidly and has practically ceased by veraison. At this point, however, tartaric acid metabolism is much slower than that of malic acid (Peynaud and Riberau-Gayon, 1971). Therefore, once it is formed, tartaric acid does not undergo rapid losses or changes. The synthesis and metabolism of these acids in Concord grapes has been documented by Mattick and Moyer (1973).

Amon and Markakis (1968) have found malic to be the dominant acid in Concord juice and concentrate, with tartaric acid present in slightly lower concentrations but this may have been due to the maturity of the grapes from which the products were manufactured or to climatic conditions during the growing season (Kliewer, 1964). In a 4-yr study of New York State Concord juice, Rice (1974) showed that tartaric acid is the dominant acid in juice from mature, New York Concord grapes. It would appear that the nonvolatile acid constituents of Concord grapes and juice are similar in fruit from different growing regions of the country.

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A Research Note

LETHAL EFFECT OF FOOD TEMPERATURE ON LINEAR PORTION OF A HEATING OR COOLING CURVE

ABSTRACT

The purpose of this note is to clarify the ambiguity in the presentation in a heat processing text re using an exponential integral function to estimate analytically a sterilizing value of a linear portion of a heating or cooling curve.

THE MATHEMATICAL estimation of thermal processes is based on the use of sterilizing value, which may be estimated through the integration of lethal rate values, L, with respect to processing time. A sterilizing value of a linear portion on a heating curve (Ball and Olson, 1957; Klostergaard, 1965; Dickerson, 1969, 1970, 1971) may be estimated analytically by using an exponential integral function of one type, and that on a cooling curve by using an exponential integral function of a different type. However, the use of these exponential integrals is not clearly presented in one of the most standard textbooks on heat processing (Ball and Olson, 1957). The present note was prepared to clarify this ambiguity.

Any linear line segment of a heating or cooling curve may be represented with a formula, which contains two experimental parameters as shown below:

Heating curve

$$t_h = f_h \log_{10}\left(j_h \frac{T_1 - T_0}{T_1 - T}\right) \tag{1}$$

Cooling curve

$$t_c = f_c \log_{10}\left(j_c \frac{T_a - T_c}{T - T_c}\right) \tag{2}$$

The lethality of these thermal processes is given by:

$$F = \int \exp\left(\frac{T - T_r}{z'}\right) dt \tag{3}$$

Where dt is determinated from either (1) or (2),

$$dt_h = \frac{f_h}{2.303}\frac{dT}{T_1 - T} \text{ and } dt_c = -\frac{f_c}{2.303}\frac{dT}{T - T_c} \tag{4}$$

It is important to note that dt_h is positive while dt_c is negative. From (3) and (4) we have as follows:

For a linear line segment of a heating curve

$$F_h = \frac{f_h}{2.303} \exp\left(\frac{T_1 - T_r}{z'}\right) \int_{X_o}^{X_g} \frac{\exp(-X)}{X} dX \tag{5}$$

where $X = (T_1 - T)/z'$. The integral in (5) can easily be expressed by means of an exponential integral defined by (Gautschi and Cahill, 1964):

$$E_1(x) = \int_x^\infty \frac{\exp(-\mu)}{\mu} du \tag{6}$$

where $0 < x < \infty$. The lethality of a logarithmic heating phase considered between the temperatures T_o and T_g will thus be written:

$$F_h = \frac{f_h}{2.303} \exp\left(\frac{T_1 - T_r}{z'}\right) \left\{E_1 \frac{T_1 - T_g}{z'} - E_1\left(\frac{T_1 - T_o}{z'}\right)\right\} \tag{7}$$

This expression is identical to Ball and Olson's formula (p. 315, Eq 12.18).

For a linear line segment of a cooling curve

$$F_c = -\frac{f_c}{2.303} \exp\left(\frac{T_c - T_r}{z'}\right) \int_{Y_a}^{Y_v} \frac{\exp(Y)}{Y} dY \tag{8}$$

where $Y = (T - T_c)/z'$. This integral can also be expressed by means of an exponential integral defined by (Gautschi and Cahill, 1964):

$$Ei(x) = \int_{-\infty}^{x} \frac{\exp(\mu)}{\mu} du \tag{9}$$

where $0 < x < \infty$. After interchanging the limits of integration in (8) to eliminate the minus sign, the lethality of a logarith-

mic cooling phase considered between the temperature T_a and T_v can thus be written:

$$F_c = \frac{f_c}{2.303} \exp\left(\frac{T_c - T_r}{z'}\right)\left\{Ei\left(\frac{T_a - T_c}{z'}\right) - Ei\left(\frac{T_r - T_c}{z'}\right)\right\} \quad (10)$$

It is quite unfortunate that Ball and Olson (1957) used $E_1(x)$ in (10) instead of the correct integral $Ei(x)$. The error ϵ, owing to the incorrect use of the exponential integral, is:

$$\epsilon = \int_{Y_v}^{Y_a} \frac{\exp(\mu) - \exp(-\mu)}{\mu}\, du = 2[Shi(Y_a) - Shi(Y_v)]$$

where Shi(Y) is the integral hyperbolic sinus (Gautschi and Cahill, 1964; page 231, formula 5.2.3). When the arguments of Ei in (10) are greater than unity, which is generally the case, the relative value of ϵ is 100%, since $E_1(x) \simeq 0$ and since $E_1(x) \ll Ei(x)$.

Ball's value of F_c is thus underestimated. Consequently, a safety factor is incorporated into estimated lethal effect through the incorrect use of the exponential integral. For average processes, the lethality of the cooling phase is about 25% of the total process (Hayakawa, 1974). The relative importance of this safety factor will depend on the value of T_a and T_v. For product heating by pure conduction this factor is low, a few per cent (Flambert, 1973). When convection occurs the safety factor may increase up to about 25% or more.

For a broken heating curve

Frequently, there is more than one linear line segment on a heating curve. The sterilizing value for each line segment may be easily estimated by using Eq (7).

For a curvilinear cooling

There is also more than one linear line segment on some cooling curves. However, the estimation of a sterilizing value from an initial curvilinear portion and only one subsequent linear portion produces a sufficiently accurate result since the lethal rate values of food temperatures on the latter portion of the curve are negligibly small, especially for microbiolcgical z values.

NOMENCLATURE

$E_1(x)$, $Ei(x)$	Exponential integrals defined in Eq (6) and (9)
f_h, f_c	Slope of a logarithmic temperature curve for heating or cooling; letters h and c refer to heating and cooling phase
F, F_h, F_c	Lethality of a thermal process
j, j_h, j_c	Lag factor
L	Lethal rate value $L = 10^{(T - Tr)/z}$
t, t_h, t_c	Elapsed time of thermal process: in general, in a heating phase, in a cooling phase.
t_o, t_g	Initial and final time of the logarithmic heating phase
t_a, t_v	Initial and final time of the logarithmic cooling phase
T, T_o, T_g, T_a, T_v	Temperature at time t, t_o, t_g, t_a and t_v
T_1	Retort temperature
T_c	Temperature of the cooling medium
T_r	Reference temperature
X	$(T_1 - T)/z'$
Y	$(T - T_c)/z'$
z	Slope of thermal death time curve
z'	z/2.303

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A Research Note

STORAGE STABILITY OF RAISINS DRIED BY DIFFERENT PROCEDURES

ABSTRACT

The storage stability of on-the-vine dried and tunnel dehydrated raisins treated with a spray or dip of a water emulsion of fatty acid esters prior to drying were compared to the traditional sun-dried raisin at 70 and 90°F. The flavor and color stability of samples held at 90°F was relatively short, varying from 8.8–14 wk. At 70°F, storage stability varied from 31–43 wk. This study showed that the color and flavor stability of on-the-vine dried and tunnel dehydrated raisins should be equal or superior to that of the traditional sun-dried raisins. It was also shown that 90°F storage causes rapid flavor and color loss regardless of drying method.

INTRODUCTION

CALIFORNIA produces over 250,000 tons of raisins annually. Currently, 90% of these raisins are sun dried in the vineyards on paper trays, and the remainder is tunnel dehydrated. The traditional sun-drying procedure is time consuming and labor intensive, and the raisins are susceptible, during drying, to rain and other damage. The disadvantages of the presently used tunnel dehydration procedures are that they often produce raisins with sticky surfaces, and consume considerable energy. The use of fatty acid ester mixtures to accelerate the sun and tunnel dehydration of Thompson Seedless grapes has been studied at this laboratory by Ponting and McBean (1970) and at California State University, Fresno (Petrucci et al., 1974).

Even though dried fruits store well for extended periods, all dried fruits have definite storage lives in which they retain acceptable flavor, color, nutrients and overall appearance. The traditional sun-dried raisins maintain these qualities for a year or more at storage temperatures of 50°F or less (Nury et al., 1960). Studies by Barger et al. (1948) showed that 32°F was the preferred storage temperature, yet most dried fruit is stored at much higher temperatures in distribution channels, and at the consumer's storage location. This study was undertaken to determine if the storage stabilities of on-the-vine dried and tunnel dehydrated raisins treated respectively with a spray or dip of fatty acid esters prior to drying are comparable to the traditional sun-dried raisins.

MATERIALS & METHODS

THE RAISINS used in this study were from the 1973 Thompson Seedless grape crop. The on-the-vine dried raisins were obtained from a grower near Fresno, Calif. Grapes from vineyard plots at California State University, Fresno, were brought to this laboratory for tunnel dehydration. High oleic safflower oil was used as the starting material for the production of ethyl esters. One lot of sun-dried raisins (A) was obtained from the same vineyard as the on-the-vine dried raisins. Another lot (C) was obtained from a processor near Fresno. The moisture level of the raisin lots, as determined by the AOAC (1970) procedure, ranged from 16.1–16.5%. The color changes were checked visually and measured by the method described by Nury et al. (1960). The concentrations of esters in the processed vine dried and tunnel dehydrated raisins were 31 and 74 ppm, respectively, as determined by the method of Stafford et al. (1974).

Processed raisins were stored at 32°, 70° and 90°F, with the 32°F stored raisins serving as controls. The 90° and 70°F samples were withdrawn from storage at 3- and 6-wk intervals, respectively, for flavor and color analysis. For the flavor evaluation the raisins stored at 70 and 90°F were presented to 20 trained judges as a duo-trio in which the sample stored at 32°F was labeled control and one of the two coded samples was held at a higher storage temperature. One duo-trio was presented at each session with control and stored samples first in each pair an equal number of times. The duo-trio data were handled exactly as described by Guadagni et al. (1975), to give storage stability and 95% confidence intervals for the samples held at 70° and 90°F.

RESULTS & DISCUSSION

CORRECTING the duo-trio data for chance by the relation Pc = (Po − 50) × 2 and plotting Pc values against storage time on log-probability paper gave reasonably good straight lines for the stimulus-response relationship (Fig. 1). At Pc = 50, one-half of the responses actually detected flavor differences between control samples and those stored at 70° and 90°F. This value corresponds to the familiar LD_{50} in dose-response experiments, or to 75% correct responses in the uncorrected duo-trio data. Flavor stability values in weeks were determined graphically at the point Pc = 50. The slopes of the stimulus response lines were fairly similar for 70° and 90°F storage, indicating that the flavor changes occurring at these temperatures were not grossly different in nature. Therefore, Pc 50 values for 70° and 90°F represent storage times required to produce essentially the same degree of flavor change. Table 1 shows the Pc 50 values for the different raisin samples held at 70° and 90°F, along with their 95% confidence intervals. The stability of samples held at 90°F was relatively short, varying from 8.8–14.0 wk. The on-the-vine dried and tunnel dehydrated samples were the most and least stable respectively. Statistical analysis of the data indicated that the vine-dried samples were significantly more stable than the tunnel-dried sample. At 70°F, storage stability varied from 31–43 wk with the on-the-vine dried sample again among the more stable samples. At this temperature, both on-the-vine dried and sun-dried (A) were significantly more stable than tunnel dehydrated and sun dried (C). Therefore, it would appear that the flavor stabil-

Table 1—Effect of drying procedure and storage temperature on flavor stability

	Stability (Pc 50) (wk)		95% Confidence interval	
Sample	90° F	70° F	90° F	70° F
Tunnel dehydrated	8.8	34	7.6–9.7	32–37
On-the-vine dried	14.0	41	11.5–17.1	38–44
Sun-dried A	11.5	43	10.1–13.1	38–49
Sun-dried C	11.0	31	9.2–13.2	29–34

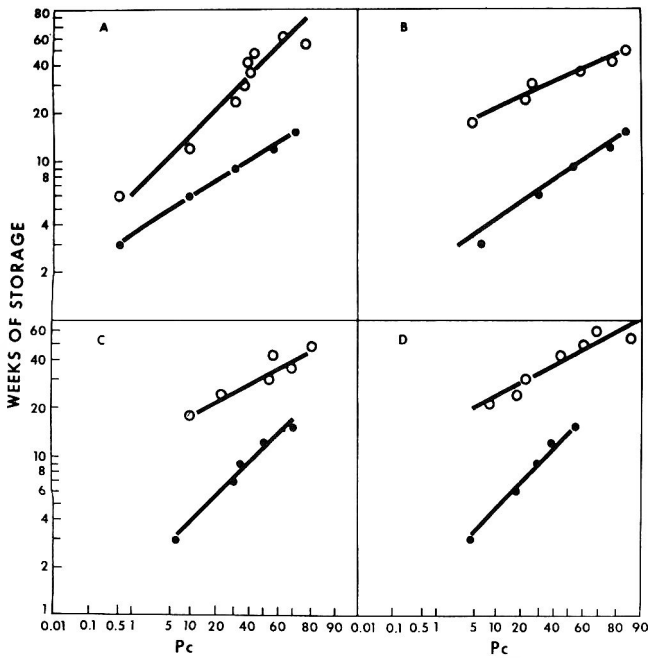

Fig. 1—Effect of storage on detection of off-flavor in raisins at (○) 70 and (●) 90° F. Pc = (Po − 50) X 2 where Po = percentage correct responses in duo-trio test, and 50 is the percentage correct responses by chance and Pc can be regarded as the percentage of responses actually detecting the difference in stimulus between control and experimental samples, (A) sun dried A; (B) tunnel dehydrated; (C) sun dried C; (D) on-the-vine dried.

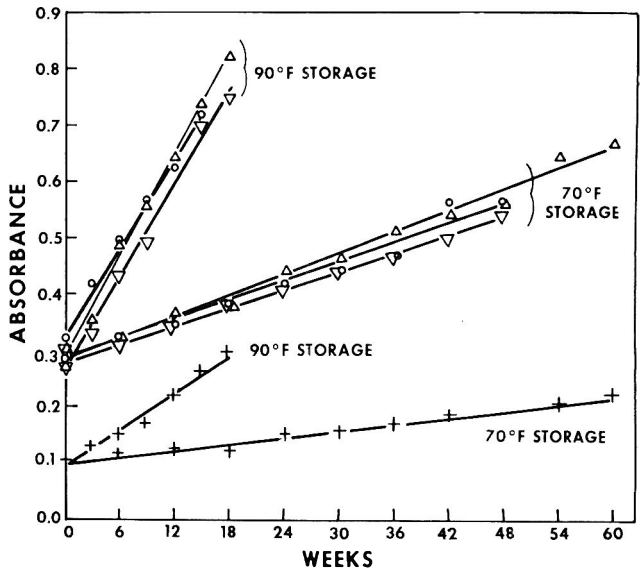

Fig. 2—Alcohol extractable color changes of raisin samples during storage, (+) on-the-vine dried, (▽) tunnel dehydrated, (○) sun dried A, (△) sun dried C.

ity of on-the-vine dried raisins would be equal or superior in flavor retention to tunnel dehydrated or sun-dried raisins.

Color stability of the stored raisins, when visually compared to their respective 32°F controls, followed a pattern similar to that found in the flavor stability part of this study. Samples stored 6–9 wk at 90°F showed minor color darkening when compared to their respective controls. These color changes were noticeably different at 12 wk; at 15 wk all samples appeared significantly darker and of poorer color quality, than their respective controls. The rates of color change of the sun-dried and tunnel dehydrated raisins during storage were similar as may be seen in Figure 2. The on-the-vine dried samples had the lowest initial and final color values, as well as a lesser rate of color change.

All raisin samples stored at 70°F showed much lower rates of color change (Fig. 2), and color differences were not visually detected between the 70°F stored samples and their respective controls until 36 weeks. All samples were darker than their respective controls at 48 wk and of fair to poor color quality. The rates of change of extractable color for the samples stored at 70°F were greatly reduced over those samples stored at 90°F for comparable time periods.

The results of this storage stability study indicate that the color and flavor stability of on-the-vine dried and tunnel dehydrated raisins should be equal or superior to that of the traditional sun-dried raisins. However, this storage study does indicate that 90°F storage causes rapid flavor and color loss regardless of drying method and should be avoided.

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A Research Note

NITRITES IN INOCULATED CARROT JUICE AS A FUNCTION OF NITRATE CONTENT AND TEMPERATURE

ABSTRACT

High levels of nitrite accumulated in fresh carrot juice inoculated with a nitrite-producing bacterium isolated from carrots when the nitrate levels were over 500 ppm. The rate of accumulation increased with temperature (20–35°C) over a 6-hr period. Nitrite continued to increase in carrot juice at 4.5°C following incubation for 1–4 hr at 30°C.

INTRODUCTION

FRESH CARROT JUICE was considered by Keating et al. (1973) to be the cause of a case of infantile methemoglobinemia because the juice was found to contain 775 ppm of nitrite. The carrots, from which the juice was made in the home, were produced on organic soil in Florida.

Research was initiated to determine the probable cause of the high nitrite content. Burdine and Hall (1973) found less than 1 ppm of nitrite in carrots from five harvests during the 1972–73 growing season although the nitrate levels ranged from 650–1350 ppm. Only slight changes in nitrite were found when samples from the five harvests were stored at 5°C for up to 38 days. Hicks et al. (1975) found an association between bacteria and nitrite content in fresh carrot juice. The bacterial population of freshly made juice was in the range 10^5–10^6 cells per ml. Nitrite did not accumulate in the juice if the bacterial population was held in the above range by refrigeration or by addition of dehydroacetic acid. The nitrite content of juice held at 20°C increased rapidly when the bacterial population exceeded 10^6 cells per ml.

Results are presented in this papaer on the effect of nitrate level, temperature and storage period on nitrite accumulation in fresh carrot juice.

MATERIALS & METHODS

CARROTS produced in California and purchased from a local wholesaler were made into juice with an automatic juice extractor (Oster). The carrots contained 106 ppm of nitrates (fresh wt). Samples consisted of 25 ml of unfiltered juice in a stoppered 50-ml flask. Each treatment was replicated three times in all experiments with separate lots of juice for each replication. Time sequence samples were individual samples and not aliquots from a single flask.

All juice samples were inoculated with a nitrite-producing bacterium isolated from carrot juice to give a population of 10^7 cells per ml of juice. The type of bacteria, conditions of bacterial growth and nitrite determinations were as reported by Hicks et al. (1975).

Nitrate level

Potassium nitrate was added to fresh juice to give levels of 0, 443, 886 and 1329 ppm of nitrate (in addition to the 106 ppm in the carrots). Bacteria were added and the nitrite determined after incubation at 30°C for 2, 4 and 6 hr. The fresh juice contained 0.56 ppm of nitrite.

Temperature

Inoculated juice samples were incubated at 20, 25, 30 or 35°C for 2, 4 or 6 hr without added nitrate and with 886 ppm nitrate added.

Incubation at 30°C followed by storage at 4.5°C

Inoculated juice samples (886 ppm nitrate added) were incubated at 30°C for 0, 1, 2. 3 and 4 hr followed by 1 and 2 days at 4.5°C. This experiment was designed to determine the stability of the various levels of nitrite produced at 30°C during subsequent storage at 4.5°C.

RESULTS

Nitrate level

The nitrate level by hours of incubation interaction was highly significant due to no difference between levels at 2 hr (mean of 6 ppm) and no increase with hr at the zero level (mean of 5 ppm). The nitrite content at 4 hr was 111, 143 and 135 and at 6 hr was 518, 574 and 525 ppm, respectively, for the added nitrate levels of 443, 886 and 1329 ppm. Thus the rate of nitrite accumulation was not substrate dependent at nitrate levels of 549 ppm (443 plus 106 of endogenous) or above during a 6-hr incubation period.

Temperature without added nitrate

Nitrite increased through the 6-hr period at 20°C, whereas at 25 and 30° the highest nitrite level was at 4 hr with a subsequent decrease at 6 hr (Table 1). At 35°C, nitrite was highest at 2 hr with very low values at 4 and 6 hr. None of the nitrite levels approached the level of nitrate (106 ppm) in the carrots. Loss of nitrites after an initial increase was reported by Hicks et al. (1975). The temperature by hr interaction was highly significant.

Temperature with added nitrate (886 ppm)

Nitrite production increased with time and temperature (Table 1) but not to the same degree. There was only a small difference in nitrite content of samples at different temperatures after 2 hr. During the 2- to 4-hr period, nitrite increased greatly at 30 and 35°C with only small increases at 20 and 25°. After 6-hr incubation, high nitrite levels were found at 25, 30 and 35° but not at 20°C. The percent conversion of nitrate to nitrite was 2, 35, 49 and 60, respectively, for 20, 25, 30 and 35°C samples. The temperature–hours interaction was highly significant.

Nitrite production was similar with both nitrate levels for each temperature after 2 hr and over the 6-hr period at 20°C.

Incubation at 30°C followed by storage at 4.5°C

As in the previous experiment, nitrite increased by the end

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Table 1—The interaction of temperature X hours on the nitrite content (ppm fresh wt) of inoculated carrot juice[a]

Hours of incubation	Incubation temperature °C				Mean
	20	25	30	35	
	(ppm nitrite)				
	Without added nitrate				
2	4.3	5.3	7.6	14.4	7.9
4	8.6	29.2	26.9	2.1	16.7
6	24.0	7.2	1.2	1.0	8.4
Temp. mean	12.3	13.9	11.9	5.8	
	With added *nitrate* (886 ppm)				
2	2.8	4.4	5.8	11.1	6.0
4	7.4	23.4	124.1	401.1	139.0
6	20.9	350.3	481.7	598.6	362.9
Temp. mean	10.4	126.0	203.9	336.9	

[a] The juice had an endogenous nitrate level of 106 ppm.

Table 2—The interaction of days at 4.5°C X hours at 30°C on nitrite content (ppm fresh wt) in inoculated carrot juice[a]

Days at 4.5°C	Hours at 30°C					Mean
	0	1	2	3	4	
	(ppm nitrite)					
0	0.6	–	3.9	–	120.4	41.6
1	3.4	6.2	18.1	137.9	379.6	109.1
2	6.9	11.6	52.5	199.9	437.2	141.6
Mean	3.6	8.9	24.9	168.9	312.4	

[a] Inoculated with 886 ppm nitrate (K) and bacteria

of 4 hr (Table 2). Nitrites continued to increase during each day at 4.5°C. The nitrite levels attained during storage at 4.5° were much greater following incubation for 3 or 4 hr than following incubation for 1 or 2 hr at 30°C.

DISCUSSION

FRESH CARROT JUICE held at temperatures above 20°C for more than 2 hr can accumulate high nitrite levels if nitrite-producing bacteria and as much as 500 ppm nitrate are present. Carrots containing a low level of nitrate (106 ppm) produced only a small amount of nitrite at any temperature. The range of temperatures used, especially 25 and 30°C, could be found in homes in the summer. The unexpected continued increase in nitrite during storage at 4.5°C is especially significant and points out the need of prompt refrigeration. A large volume of juice placed in a home refrigerator would cool at a much slower rate than the juice under the conditions used in this research. Storage of fresh carrot juice does not seem advisable unless rapidly cooled or made from cooled carrots and promptly refrigerated.

According to computations made by Phillips (1968), 1-oz of juice (30 ml) containing 400 ppm nitrite could result in cyanosis in a 1-yr-old child. Nitrite levels in carrot juice reached or exceeded 400 ppm in several of the treatments reported here.

None of the juice in these experiments developed off-odors. The juice held at 35°C for 6 hr separated and had some foam. An occasional sample at 30°C for 6 hr showed slight separation, but most appeared normal. Thus, there was no indication in most cases of any change in the juice.

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A Research Note

FERULIC ACID AND OTHER PHENOLICS IN OAT SEEDS (Avena sativa L. Var Hinoat)

ABSTRACT

This note presents results of a survey of the free and bound phenolic acids and some other polyphenols in the seed meat from the oat (*Avena sativa* L. Var Hinoat) which is of some interest as a potential high protein cereal food.

INTRODUCTION

THE RECENT WORK of Maga and Lorenz (1973) has shown that phenolic acids, widely distributed in plants, can contribute significantly to objectionable flavor in foods. The taste sensation described, is one of astringency. These authors also found that free phenolic acids occur in various cereals and oilseeds in quantities well within their taste threshold values (Maga and Lorenz, 1974a). Combinations of different acids lower the threshold value considerably.

Caffeic and ferulic acids were reported in oats as ether-soluble fatty acid esters (Daniels et al., 1963). However, no other phenolic acids were reported.

This communication presents the result of a survey of the free and bound phenolic acids and some other polyphenols in the seed meal from the oat (*Avena sativa* L. Var Hinoat) which is of some interest as a potential high protein cereal food.

MATERIALS & METHODS

Materials

The oat seed meal was provided by Dr. D. Paton of the Food Research Institute and contained most of the bran. The phenolic markers for chromatography were obtained from commercial sources and used without further purification. β-glucosidase was obtained from the Sigma Chemical Company.

Methods

Phenolic acids. The oat seed meal was extracted with 70% ethanol at room temperature. The phenolic acids derived from the acidified aqueous residue, alkaline hydrolysates of this residue and the alcohol-insoluble material, were extracted with ethyl acetate and separated on paper chromatograms and identified by methods previously described (Durkee and Thivierge, 1975).

Estimation of ferulic acid. The total alkali-labile ferulic acid (free and soluble–insoluble ester ferulic acid) was obtained as the diazonium dye derivative, by spraying the paper chromatogram with diazotized sulfanilic acid, followed by sodium carbonate (Block et al., 1958). The purple spot obtained was cut from the paper and placed in a syringe filter holder (Canlab). Methanol (70%) was flushed through the paper several times until the color was removed, and the final eluate made up to a specified volume. The optical densities of the standards and the unknowns were read in a spectrophotometer at 490 nm, using a reagent blank removed from the paper in the same manner. Ferulic acid gave a linear response at low concentrations (0–50 ppm).

Proanthocyanidins. The presence or absence of proanthocyanidins was determined by spraying with vanillin-HCl (Ribereau-Gayon, 1972), the chromatograms were developed in butanol-acetic acid-water (4:1:5) (BAW). The proanthocyanidins were also detected by digesting the seed meal with butanol-HCl (5:1) at 100° in order to transform to the corresponding anthocyanidins, which were identified, as necessary, by chromatography and spectrophotometric data.

RESULTS & DISCUSSION

THE PHENOLIC ACIDS found in oatseed meal are given in Table 1. Their identity was confirmed by specific color reaction, R_f values and comparison with authentic compounds (Durkee and Thivierge, 1975). Ferulic acid was the major acid, although traces were found in the free form, the bulk of the ferulic acid was derived from soluble or insoluble bound forms, after alkaline hydrolysis. Incubation of the soluble fraction with β-glucosidase yielded ferulic acid also, which suggests the presence of soluble glucose ester. The largest amounts of ferulic acid were liberated by alkaline hydrolysis of the insoluble residue. This was essentially true of the other acids found in a bound form: vanillic, *p*-coumaric, sinapic and *p*-hydroxybenzoic acids. Traces of free *p*-coumaric acid were found in the 70% ethanol extract.

The results are indicative of the presence of simple soluble esters and to a greater extent of more complex insoluble substances where these acids are found in ester linkage, probably bound to polysaccharides, protein or certain cell wall material.

Daniels et al. (1963) found both ferulic and caffeic acids in defatted oat seeds after hydrolysis of a direct ether extract. Our ether extraction, following a prior defatting with hexane, only revealed a small amount of ferulic acid after alkaline hydrolysis. This supports their evidence that ferulic acid may be bound as a fatty acid ester (e.g., glyceride).

The values for the total alkali-labile ferulic acid are shown in Table 2. These values were obtained from two different

Table 1—Major phenolic acids in oats (Hinoat)

Substance	Free	Esters (alkali and β-glucosidase labile)		
		Sol. bound	Insol. bound	Ether ext.
Ferulic acid	trace	++[a]	+++	+
Vanillic acid	trace	+		
Sinapic acid		+	++	
p-Coumaric acid	trace	+	+	
p-Hydroxybenzoic acid		+	+	

[a] Plus marks (+) indicate the relative intensity of the spots.

Table 2—Total alkali-labile ferulic acid (Hinoat)

Extract	Optical density	ppm
10 grams (final vol 5 ml)	0.45	300 ppm
5 grams (final vol 2.5 ml)	0.40	290 ppm

samples of seed meal extracted with 2N NaOH for 4 hr at room temperature and are in good agreement. As can be noted from the Table the concentration of 300 ppm ferulic acid is a high value. The total soluble bound ferulic acid in wheat and triticale flour is in the range, 20–30 ppm (Maga and Lorenz, 1974b). This suggests that most of the ferulic acid of oats is in an insoluble bound form.

Proanthocyanidins were not detected in the oat grain extract by either vanillin-HCl test on paper chromatograms or by the digestion method. Only brownish-yellow colors were obtained at 100°C (water-bath). This result is not entirely unexpected since only a few cereals contain condensed tannins. *Sorghum Vulgare* (Strumeyer and Malin, 1975) and *Hordeum Vulgare* (Bate-Smith and Rasper, 1969) and the nature of these tannins have not as yet been fully clarified.

There were no indications of flavone glycosides on two-dimensional chromatograms developed in one direction with BAW and in the other with 5% acetic acid. However, since some C-glycosylfavones were reported in leaves of *Avena sativa* (Harborne, 1967), further work is necessary to prove the presence or absence of these unhydrolyzable flavones in the oat seed.

The results obtained here so far show that free phenolic acids can occur in oat flours. These could be liberated during processing procedures or cooking and baking under either acid or alkaline conditions. Ferulic acid, however, is readily destroyed at higher temperatures under acid conditions and caffeic acid under even mild alkaline conditions (Van Sumere et al., 1172). The other acids are much more stable (e.g., vanillic and *p*-hydroxybenzoic acids). These stable acids, if produced by hydrolysis during extraction or by processing and cooking might contribute to taste. The taste threshold for a 1:1 mixture of vanillic and *p*-hydroxybenzoic acids is 10 ppm (Maga and Lorenz, 1973).

From the results obtained it appears that objectionable taste due to phenolic acids in protein meal is possible. Discoloration, due to phenols may be minimal because of the absence of tannins and other easily oxidizable phenols in the grain. Further work is in progress to understand the complex nature of the bound insoluble polyphenols and to explain the binding or association between phenols and proteins.

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A Research Note

PROTEIN QUALITY EVALUATION OF AN INSTANT BEAN POWDER PRODUCED BY DRY HEAT PROCESSING

ABSTRACT

Instant bean powders were prepared by roasting navy beans in a bed of salt at 190°C for 20 sec or 220°C for 10 sec followed by grinding in a Wiley Mill. The powders were evaluated for antitrypsin activity and for relative protein value (RPV). The RPV's of methionine-supplemented and unsupplemented samples were superior to casein and equal to 110% and 60% of the RPV of casein, respectively. The heat treatment destroyed 70–80% of the antitrypsin activity originally present. Potential applications of the dry heat process are discussed.

INTRODUCTION

A MACHINE for puffing pieces of food product with a heated granular material was developed by Bateson and Harper (1970). It is a modification of Benson's equipment used to puff cook cereal pieces very rapidly (Benson, 1966). It has been further developed in the Department of Agricultural Engineering at Colorado State University for use in dry roasting beans and other crops (Raghavan and Harper, 1974). The process consists of roasting the beans in a heated bed of salt (NaCl) and then cooling and grinding. It presents obvious economic and ecological advantages. The unique feature of the dry heating method is that it eliminates contamination and spoilage as well as waste water associated with other bean processing methods.

Feeding trials carried out with large turkeys showed no difference in weight gain or gain/feed between birds fed a commercial diet containing soybean meal or a modification of such diet in which the meal was replaced by either the CSU salt-bed roasted whole soybean or by a Roastatron roasted whole soybean (Turner et al., 1973). The salt-bed roasted full-fat soy flour was evaluated as a potential protein supplement for white bread and no differences in bread performance were noted when compared to a commercial full-fat soy flour (Harper and Lorenz, 1974). In this communication protein quality evaluation of navy bean powders made using this process is described and possible product uses suggested.

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EXPERIMENTAL

NAVY BEANS, purchased from Lakeland Bean Company, Olivia, Minn., were roasted in a bed of heated salt under conditions of time and temperature to be specified by the method previously described (Harper and Lorenz, 1974).

Samples used in this study were raw, autoclaved (120°C for 8 min) and roasted (at 190°C and 220°C for 30 sec and 10 sec, respectively) navy beans. The roasting in either case resulted in a maximum average bean temperature of 110°C when the beans were removed from the hot salt. The samples were cooled in air and were at 65°C after 2 min of cooling. Raw and processed bean samples were ground in a laboratory Wiley Mill to pass through a 0.5 mm sieve. Trypsin inhibitor activity was measured by a modification (Kakade et al., 1974) of the original procedure of Kakade et al. (1969).

The method utilized for estimating the nutritive value of the proteins was the Relative Protein Value (RPV) method as described in PAG Guideline 16 (PAG, 1975). The diets were prepared by incorporating the protein sources at the expense of corn starch into a protein-free basal diet to furnish either 2%, 5% or 8% protein (N × 6.25). All bean samples were evaluated unfortified or when fortified with 0.6% DL-methionine. The nitrogen content of the dietary protein sources was determined by the macro-Kjeldahl method (AACC, 1962). The basal diet contained (grams/100 grams diet) corn starch 85, corn oil 10, salt mixture USP XVII 3 and vitamin mix (Nutr-Biochem) 2. The reference protein was ANRC reference casein (Sheffield Chemical Co.).

The diets were fed to young male rats (Carworth CFE) weighing 70–83g (mean 76g) for 14 days. A total of 15 rats were fed each

Table 1—Relative protein value of heat-processed bean powders

	Casein	Aut + Met.	190°C + Met	220°C + Met	Aut.	190°	220°C
Slope	29.4	26.9	31.6	33.9	16.8	17.6	17.6
Intercept	−12.1	−12.3	−17.0	−16.1	−15.4	−16.7	−14.6
Correlation coef. (%)	99.2	97.1	97.5	97.3	95.9	91.2	86.5
95% Conf. limit (slope)							
Lower	27.2	22.9	27.3	29.1	13.8	12.9	11.5
Upper	31.7	30.9	35.9	38.7	19.8	22.4	23.6
95% Conf. limit (intercept)							
Lower	−14.8	−16.9	−21.2	−21.5	−18.1	−20.3	−19.0
Upper	− 9.5	− 7.7	−12.7	−10.7	−12.8	−13.0	−10.3
Relat. Prot. Value	100.0	91.4	107.3	115.1	57.2	59.9	59.6

protein source. The slopes of the regression lines relating body weight gain to nitrogen intake were calculated for the test samples and expressed as a percentage of the slope for casein. 95% confidence intervals based on the t-distribution were set on each slope and intercept.

RESULTS & DISCUSSION

THE RESULTS of the rat feeding study are shown in Table 1. The relative protein values of the bean samples roasted at 190°C and 220°C were 59.9 and 59.6 respectively compared to a value of 57.2 for autoclaved beans. In the presence of added methionine, relative protein values were 107.3 and 115.1 for beans roasted at 190°C and 220°C respectively compared to 91.4 for methionine fortified autoclaved beans. Trypsin inhibitor activities (TIU) expressed in terms of TIU/mg of dry sample were as follows: raw beans 17.3, autoclaved beans 2.4, 190°C roasted beans 4.9, and 220°C roasted beans 3.1. The percentages of inhibitor destroyed were 86%, 72% and 82% respectively for autoclaved, 190°C roasted and 220°C roasted beans. The results of the biological evaluation and determination of trypsin inhibition activity strongly suggest that the dry heat processing was equivalent to autoclaving in the inactivation of anti-growth substances. From the standpoint of protein quality and digestability, the instant bean powders prepared from the roasted beans are likely to be fully satisfactory for human use. Another possible application of the dry heat process could be in reducing the moisture content of whole beans for storage. It has been reported that low moisture content is a very important factor in maintaining good quality in stored beans (Morris and Wood, 1956; Burr and Kon, 1967). The optimum conditions of dry heat processing for this process were not determined in the present study. It seems likely that a much lower temperature, perhaps around 90–120°C would be desirable to reduce splitting and cracking.

The present report suggests several potential applications of a dry heat process for making an "instant" powder from navy beans. Potential applications could include meatloaf extender, refried beans and bean dips and soups. It is also suggested that the process may have value in pre-treating beans prior to storage, particularly in less developed tropical countries where storage facilities are inadequate. Experiments in this laboratory are currently in progress to define further the practical applications of the process.

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A Research Note
Bdellovibrio IN FOODS

ABSTRACT

Bdellovibrio bacteriovorus is a unique parasitic microorganism that is capable of inhabiting and growing in the intraperiplasmic space of other bacteria. The ecological role of this organism has been reported in soil, sewage and polluted rivers. The analysis of various foods revealed parasitic organisms, but no *Bdellovibrio*. The inoculation of *Bdellovibrio* into foods resulted in a slight increase or decrease of the parasite and no apparent effect on the host cell population.

INTRODUCTION

AN UNUSUAL PARASITIC, gram-negative bacterium, *Bdellovibrio bacteriovorus* was discovered in 1962. This organism is capable of attaching to, penetrating, and growing in the intraperiplasmic space of other bacteria (Stolp and Starr, 1963; Starr and Siedler, 1971). Although mutant strains of *Bdellovibrio* have been isolated that grow on artificial media, in general, the parasite needs a host population in which to grow. The life cycle of *Bdellovibrio* is similar to phage development and consists of attachment of the organism to a host bacterium, followed by penetration of the parasite through the host cell wall. The next step involves growth of the *Bdellovibrio* at the expense of host cell cytoplasm, until fragmentation into daughter cells occurs.

Bdellovibrio occurs widely in nature. The interaction of this parasite with normal flora in a particular ecosystem was postulated as being a significant factor in controlling the bacterial populations that were present. Research has centered around the relationships of *Bdellovibrio* and potential host populations in soil, sewage, and polluted rivers (Dias and Bhat, 1965; Klein and Casida, 1967; Fry and Staples, 1974; Hendricks, 1974). However, no research has been reported on the role of *Bdellovibrio* in foods. The purpose of this study was to determine if *Bdellovibrio* are present in food products and what effect the organism might have on bacterial populations that are present.

MATERIALS & METHODS

Bdellovibrio bacteriovorus 109J and *Escherichia coli* ML35, the host organism, were kindly furnished by Dr. Sydney C. Rittenberg, University of California, Los Angeles, Calif. Dilute nutrient broth (DNB) medium, described by Rittenberg and Shilo (1970), was used for growth of *Bdellovibrio*. Host and parasite populations were inoculated simultaneously into 30 ml of DNB medium in a 125 ml Erlenmeyer flask and incubated on a shaker at room temperature. After 24 hr incubation, a microscopic examination revealed few *E. coli* host cells and by means of the double-layer agar technique (Stolp and Starr, 1963) the yield of *Bdellovibrio* was determined to be 10^9 plaque-forming units (PFU) per ml. Stock cultures of *Bdellovibrio* were maintained in sterile, screw-cap test tubes at 4°C.

The foods analyzed in this study were obtained from stores or a home garden in the Columbus, Ohio, area. The foods were prepared for analysis by the rinse technique, using a plastic bag, or by blending in a Waring Blendor jar. The liquid for rinsing, blending, or making further dilutions was sterile 0.1% peptone water. A double-layer agar technique was used to determine if *Bdellovibrio* were present. The blended sample or rinse water was also filtered through a 1.2 μM Millipore filter and then the filtrate was analyzed for the possible presence of *Bdellovibrio*. Filtering removed part of the normal flora from the sample, but allowed the smaller bdellovibrios to pass through. This procedure facilitated the identification of plaques which were characteristic of *Bdellovibrio*, when using the double-layer agar technique, by preventing interference from the normal flora. Plates were incubated at 32°C and checked daily for the presence of plaques. Plaque morphology was observed with a stereo microscope. In addition, material was removed from within the plaques for examination under a phase-contrast microscope.

Ground beef and milk samples were inoculated with *Bdellovibrio* to determine the effect of the organism on the normal flora of the product. *Bdellovibrio* cultures were filtered through a 1.2 μM Millipore filter, prior to inoculation of a food sample, to remove any *E. coli* host cells that remained. The double-layer agar technique was employed for enumeration of *Bdellovibrio*. Plates were incubated at 32°C and plaques were counted after 4 days incubation. Plate count agar (Difco) and violet red bile agar (Difco) were used for determining total and coliform counts, respectively, using duplicate plates with the pour plate method. The plates were incubated at 32°C for 24 hr and colonies were counted with the aid of a Quebec colony counter. Control samples were left uninoculated and examined as described above.

RESULTS & DISCUSSION

THE GROWTH REQUIREMENTS of *Bdellovibrio* limited the types of foods that were analyzed. The organism has an optimum pH of 7.5, a host population of 10^7 cells/ml, and requires aerobic conditions in order to sustain its growth. Many food products do not satisfy the conditions necessary to support growth of the parasite. However, *Bdellovibrio* is a common soil inhabitant and is associated with sewage and polluted rivers. Therefore, fresh fruits and vegetables, which might have the parasite present on their surfaces if the product had not been washed properly, were analyzed for the presence of *Bdellovibrio*.

Sixty-eight food samples were examined, and plaques were observed on the plates of 19 of these foods. Observation of these plaques with a phase microscope revealed that 12 samples contained myxobacteria, six contained amoebic protozooans and one sample contained both types of microorganisms. Plaques characteristic of *Bdellovibrio* are much smaller than plaques formed by myxobacteria and amoeba, and also take more time to develop. Because of their small size, *Bdellovibrio* plaques could be overgrown by the nonhost bacteria in the sample. The rinse water was filtered through 1.2 μM Millipore filters to aid in the removal of the normal flora and alleviate this problem; however, no plaques characteristic of *Bdellovibrio* were found.

Bdellovibrio was added to ground beef and pasteurized milk to determine if this parasite would influence the host population in food products. After storage of the ground beef sam-

ples for 1 day at room temperature, the meat was spoiled; however, the samples were analyzed to determine if the high bacterial population would stimulate *Bdellovibrio* growth. The coliform count reached 1.0×10^8 cells/g after 1 day and continued to increase during the 4-day incubation period, providing a host population capable of supporting the growth of *Bdellovibrio*. However, the *Bdellovibrio* count decreased from 2.0×10^6 PFU/g to 3.1×10^4 PFU/g during the storage period, a survival of only 1.5%. At 4°C, the low coliform population and lower temperature were not suitable for growth of the parasite. However, the lower temperature did enhance the survival of the organism, since 65.4% of the original *Bdellovibrio* inoculum remained after 4 days incubation.

Pasteurized milk was inoculated with *Bdellovibrio* to determine if the organism could grow in a liquid food product. Because pasteurized milk has a low coliform population, *E. coli* was added to provide a host concentration suitable for *Bdellovibrio* growth. In the milk seeded with *E. coli*, *Bdellovibrio* was capable of reproduction, although at a very slow rate. Within 48 hr, the parasite population reached its highest concentration; however, the increase was slight, and after 48 hr the parasite population began to decrease.

It also was apparent that the presence of *Bdellovibrio* in ground beef or milk had no effect in reducing the bacterial populations in these products. The coliform counts of *Bdellovibrio*-seeded and unseeded samples showed similar results.

In both ground beef and pasteurized milk, the results showed two important points: (1) that *Bdellivibrio* could not grow in these food products; and, (2) that the presence of *Bdellovibrio* had no effect on reducing the bacterial populations inherent in the food products.

The growth of *Bdellovibrio* is best when a growth-limiting medium for the host organism is used. In this type of medium, the slower growing *Bdellovibrio* can compete with the host organism and eventually result in its elimination. Ground beef and pasteurized milk are not growth-limiting media for *E. coli*. Even if the bdellovibrios had been able to grow, their presence would not play an important role in reducing the bacterial populations that were present.

The results from the addition of *Bdellovibrio* into food products agree with other studies on the ecological significance of bdellovibrios in soil, sewage and polluted rivers, by having no effect on reducing the indigenous bacterial populations (Dias and Bhat, 1965; Hendricks, 1974; Keya and Alexander, 1975).

Bdellovibrio could not be detected on any of the food samples analyzed, and when the parasite was added to a food product, it could not multiply. Thus, it is doubtful that *Bdellovibrio* has any significant role in food products.

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A Research Note
EFFECT OF HANDLING AND PROCESSING ON DISCOLORATION OF ALBACORE TUNA

ABSTRACT

Occasionally, in the canning of tuna, normal appearing tuna flesh will turn from a desirable white color to an undesirable green color after precooking and prior to retorting. Results demonstrated that the different systems used by the fishermen to hold local albacore tuna prior to delivery to the processor had little influence on the incidence of green discoloration of precooked tuna. Furthermore, frozen storage of local tuna, up to 3 months, did not increase the incidence of green discoloration of precooked tuna.

INTRODUCTION

THE OCCURRENCE in canned tuna of an off-color known as "greening" is a matter of concern and of financial loss to the processing industry. The discoloration of the meat generally takes place during the precooking of the tuna prior to canning.

Nagaoka and Suzuki (1964) have proposed that the heme pigments and TMAO (trimethylamine oxide) content of tuna flesh were involved and that they may be used in predicting the discoloration after the flesh is cooked. Further work showed that reducing agents were involved and cysteine in the presence of metmyoglobin and TMAO produced a green pigment after heating (Koizumi and Matsuura, 1967; Ok-Koo et al., 1969). Since then, a number of investigators have reported the distribution of TMAO in tuna flesh and the use of TMAO as a tool for predicting the color to be expected when the flesh is cooked (Yamagata et al., 1971; Chung et al., 1973).

Recently, there was an outbreak of greening in our local albacore tuna industry. Local processors, sharing the concern of distant skipjack tuna processors (Nonaka and Koizumi, 1973), felt that the outbreak may have been the result of the rapid growth of brine freezing systems being used by the fishing industry. This study was undertaken to determine the effect of current handling and processing procedures on discoloration of albacore tuna. The relationship of TMAO to the handling and processing procedures was also investigated.

EXPERIMENTAL

Materials

Local albacore tuna (*Thunnus alalunga*) were randomly selected from seven vessels fishing off the coast of North America, North of the Columbia River to Vancouver Island. The fishing vessels were making 14- to 20-day trips. Upon delivery, the temperature of the loin muscle of eight tuna from each vessel was immediately measured using an Esterline Angus multipoint recorder. The tuna were tagged and, after thawing in cold water, were processed and evaluated by a local processor. A sample of muscle, free of connective tissue and skin, was removed from between the tail and anal opening for chemical analyses. After visually inspecting the subsequently precooked and cleaned tuna by a local processor, samples were taken for evaluating the color using a Hunter Color and Color Difference Meter.

For the 3-month storage trial, 20 tuna from each of the fishing vessels were randomly selected during unloading. A sample of muscle, free of connective tissue and skin, was removed from the area between the tail and anal opening of each of the 20 tuna. The exposed flesh of the tuna was sealed by wrapping the tuna several times with plastic PVC wrapping film to prevent desiccation during subsequent frozen storage. Five tuna were immediately processed and evaluated by a local processor. The remaining 15 tuna were held at −18°C until processed. At monthly intervals, five tuna from each lot were removed from frozen storage and sampled. The sample of flesh for the chemical analyses was removed directly opposite the backbone of the initial sampling. The tuna were then thawed in cold running water (14 hr), processed and evaluated by a local processor.

Chemical analyses

The method of Yamagata et al. (1969) for the reduction of TMAO (trimethylamine oxide) to TMA (trimethylamine), was used to determine TMAO. TMA was determined using the picric acid procedure of Dyer (1959) as modified by Murray and Gibson (1972), where 45% potassium hydroxide (KOH) was substituted for saturated potassium carbonate (K_2CO_3). The absorbance was read at 410 nm and the difference between total TMA (TMAO + TMA) and TMA values represents the amount of TMAO.

The pH of a blend of tuna flesh (10g flesh to 40g distilled water) was determined using an Orion digital pH meter.

The color of the precooked tuna flesh was determined visually and by the use of Hunter Color and Color Difference Meter. The meter was standardized against a Standard Panel 31, Ivory, having an "L" value of 75.1, "a" value of −1.3, "b" value of 23.1.

The method of Vander Werf and Free (1971) was used to determine the salt content.

RESULTS & DISCUSSION

Effect of handling

Coil systems were introduced several years ago and instead of holding the tuna in ice, the tuna are frozen on coils. Recently, brine tanks have been installed on some of the vessels. During good fishing periods, the tuna are partially frozen in the brine tank (held up to 4 hr) and then placed on the coils. At the same time, spray brine systems were being introduced. In this system the tuna are frozen and held with a cold brine being sprayed over them.

The tuna examined off the various vessels using these different systems were in very good condition. The internal temperatures of the tuna loin muscle from the iced, coil and spray-brine vessels were found to average 4.1°C, −4.9°C, and −3.1°C, respectively. The salt content of tuna held with ice or coil systems was less than 0.1%, but varied from 0.2−1.2% (0.7 ± 0.3) in tuna held with the spray-brine system. The pH of the tuna flesh was 5.9 ± .08 and did not vary between the vessels.

The results in Table 1 indicate that the presence of greening can be observed in tuna from each of the various systems being used on the vessels. All the tuna examined were in very good condition and none of them would have been rejected because

Table 1—Relationship of systems used in vessels to greening of albacore tuna[a]

Boat system	Raw meat TMAO (μg/g)	Precooked meat: Hunter "a" value	Precooked meat: No. of tuna with visual traces of greening
Ice			
Vessel A	644.8 ± 210.2	1.96 ± 1.12	1
Vessel B	784.5 ± 315.0	2.60 ± 0.60	3
Coil			
Vessel C	696.4 ± 129.0	0.97 ± 0.87	1
Brine-coil			
Vessel D	554.4 ± 277.9	2.62 ± 0.70	4
Vessel E	625.7 ± 72.2	1.50 ± 0.50	1
Spray brine			
Vessel F	448.4 ± 154.5	3.00 ± 0.90	0
Vessel G	613.1 ± 84.1	2.00 ± 0.50	3

[a] Mean values ± std dev for eight tuna randomly selected from each vessel during unloading.

Table 2—Relationship of handling and storage at −20° C to greening of albacore tuna[a]

Boat system	Raw meat[b]: pH	Raw meat[b]: TMAO (μg/g) Initial	Raw meat[b]: TMAO (μg/g) After storage	Cooked meat: No. of tuna with visual traces of greening
Ice				
0	5.83 ± .05	618.4 ± 143.4	—	0
1 mo	5.87 ± .05	527.3 ± 103.4	476.3 ± 77.5	0
2 mo	5.77 ± .06	684.9 ± 250.7	540.4 ± 221.8	1
3 mo	5.76 ± .06	546.1 ± 101.7	430.5 ± 77.9	0
Coil				
0	5.81 ± .08	741.4 ± 286.5	—	1
1 mo	5.92 ± .08	766.8 ± 100.8	573.0 ± 104.4	0
2 mo	5.83 ± .04	610.0 ± 185.8	468.6 ± 90.6	0
3 mo	5.76 ± .13	842.0 ± 288.9	673.4 ± 184.6	2
Spray brine				
0 mo	5.83 ± .06	829.9 ± 272.3	—	0
1 mo	5.95 ± .03	777.8 ± 319.4	538.0 ± 122.6	1
2 mo	5.74 ± .03	435.4 ± 147.5	290.4 ± 152.8	0
3 mo	5.74 ± .12	583.5 ± 223.9	510.8 ± 157.1	2

[a] Group of 20 tuna selected randomly from each vessel during unloading
[b] Mean values ± std dev for five tuna examined each month

of the discoloration. The plant personnel examining the tuna were especially critical for any sign of greening which included odor ("grassy" aroma) and texture ("stringy"), as well as color. The TMAO content between the individual tuna varied greatly. The various systems of cooling and freezing used on the vessels did not affect the level of TMAO in the tuna.

Handling and storage of the tuna at −20°C did not affect the incidence of greening (Table 2). Even the tuna with traces of greening were still in very good condition and would not have been rejected because of the discoloration.

However, a more pronounced greening was predicted to have occurred. Yamagata et al. (1971) reported that yellowfin tuna containing 9 mg % TMAO-N would most probably turn green after precooking. This level corresponds to 481 μg TMAO/g tissue. The low incidence of greening may be related to the myoglobin content of the tuna (Yamagata et al., 1970) or differences between the two species.

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A Research Note

UTILIZATION OF A FISH PROTEIN ISOLATE IN WHIPPED GELATIN DESSERTS

ABSTRACT

A fish protein isolate developed for its whippability was evaluated in a whipped gelatin dessert. The isolate was substituted for 30, 60 or 100% of egg albumin. No significant differences in the desserts were found for appearance, texture, tenderness, mouthfeel, and flavor. Increased specific gravity and rigidity, decreased index of volume and pH accompanied increased levels of the isolate. However, these differences are of relatively little importance in terms of an acceptable product. Panel members indicated a preference for the product with higher levels of the isolate which had a more intense fruit flavor. The isolate can be used as a substitute for egg albumin in production of foams.

INTRODUCTION

IN RECENT YEARS, much attention has been given to the development of various protein products from fish, including fish protein isolates (FPI). Many forms of the isolate have been developed. Each form was an attempt to improve the isolation procedure, functional characteristics or other aspects of the isolates (Spinelli et al., 1972b). Enzymatically modified succinylated myofibrillar protein (EMSMP) is a fish protein isolate that was developed to have excellent whipping properties (Groninger and Miller, 1975). Although some laboratory experiments have been conducted, little research has been published concerning the whipping properties of EMSMP in an actual food product. It is widely recognized that the performance of a protein product in a laboratory situation is not necessarily an indication of its potential in food system (Wolf, 1970). This study evaluated substituting EMSMP for egg albumin as a whipping agent in a whipped gelatin dessert. The EMSMP was prepared as outlined by Spinelli et al. (1972a).

EXPERIMENTAL

Preliminary tests

Preliminary tests were conducted to study the whippability of EMSMP and water. Since the EMSMP foam was to replace a foam of dried egg albumin and water, the EMSMP was substituted directly for the egg albumin on an equal protein basis. However, the consistency of the EMSMP and water mixture using these proportions was extremely viscous and would not whip. A lower ratio of EMSMP to water was necessary to allow for hydration and extension during whipping. Several levels of EMSMP to water were tested. Percentage drip loss and specific gravity were used to determine the desired ratio of EMSMP to water for use in a foam. The foam with the lowest ratio of EMSMP to water that remained stable for 1 hr was used. Therefore, total weight of the foams was the basis for substitutions. Eight grams of egg albumin were needed to make 60g of foam; whereas, only 1.8g of EMSMP were needed to make the same weight of foam. The EMSMP foam thus contained more water by weight and less solids by weight than the egg albumin foam.

Preparation of foams and desserts

Egg albumin foams were substituted with EMSMP foams at 0, 30, 60 and 100% levels in a whipped gelatin dessert. Treatment group A incorporated 100% dried egg albumin as a protein source and treatment group D incorporated 100% EMSMP as a protein source. Treatment group E evaluated the contribution of EMSMP (0%) to a foam when a low level (40%) of egg albumin was used.

To prepare the dessert, 250g of a gelatin dessert mix (Strawberry Jello) were dissolved in 700 ml of boiling water. Cold water (530 ml) was added, and the mixture chilled at −20°C for 70 min. The chilled mixture was whipped for 4 min at high speed then divided into five 240-g portions. Egg albumin and/or EMSMP and water foam was prepared by whipping the protein substances and water for 4 min at high speed. The foam (60g) was added to a 240-g portion of whipped gelatin mixture and whipped at high speed for 30 sec. The product was placed in a 1000-ml beaker and chilled for 18 hr at 4°C. Aliquots were then taken for sensory and objective tests.

Subjective evaluation

A sensory panel of seven food and nutrition majors was trained to evaluate the quality factors of whipped gelatin desserts. The factors were defined and discussed until uniform scoring of a standard was obtained. Six replications were judged. Fifteen minutes before evaluation, 15-g portions were taken with a melon ball scoop and put on coded dishes. All samples were presented randomly to each judge in individual booths illuminated with incandescent light.

Sensory panel members judged the desserts for appearance, texture, tenderness, mouthfeel, and flavor using a descriptive scoreing scale of seven points (See Table 1). Subjective test scores were evaluated statistically using ANOVA and Duncan's multiple range test (Duncan, 1955).

Objective evaluation

Specific gravity and stability measurements of the desserts were conducted using the methods of Funk et al. (1971). The index to volume was estimated using the method of Finklin and Vail (1946). Rigidity of the dessert was determined with a precision penetrometer equipped with a cone weighing 10.2g and released for 3 sec. The pH of the dessert was measured with a Beckman pH meter (Miller et al., 1959). Objective tests were evaluated statistically by using ANOVA and Duncan's multiple range test (Duncan, 1955).

RESULTS & DISCUSSION

PROPERTIES that a protein product must display in order to be used as a whipping agent in an unbaked food product include dispersability, foam formation, and foam stability (Hammonds and Call, 1970). Subjective and objective tests were conducted to test the EMSMP for these characteristics.

The results of the subjective tests are given in Table 1. No significant differences were found. However, the general trend of the scores reflected an improved product as the amount of EMSMP level increased. This is illustrated consistently for tenderness, mouthfeel anf flavor values. The appearance or air cell size increased as the level of EMSMP increased. A comparison

Table 1—Means, standard deviations and significant differences of subjective quality factors for whipped gelatin desserts

	Treatment group				
	% Total egg albumin foam/% total EMSMP foam				
	A 100/0	B 70/30	C 40/60	D 0/100	E 40/0
Appearance[a]	0.50 ± 0.74	0.45 ± 0.77	0.91 ± 0.76	1.14 ± 1.07	0.98 ± 1.02
Texture[b]	0.17 ± 0.70	0.26 ± 0.67	−0.05 ± 0.62	0.12 ± 0.59	0.21 ± 0.68
Tenderness[c]	−0.81 ± 0.74	−0.67 ± 0.65	−0.24 ± 0.66	0.17 ± 0.70	−0.31 ± 0.56
Mouthfeel[d]	−0.93 ± 1.05	−0.71 ± 0.86	−0.26 ± 0.73	0.05 ± 0.73	−0.36 ± 0.73
Flavor[e]	−0.67 ± 1.00	−0.67 ± 0.72	−0.17 ± 0.58	0.07 ± 0.46	−0.12 ± 0.67

[a] Increasing score indicates an increasing air hole size
[b] Increasing positive score indicates increasing gumminess and a negative score indicates increasing graininess
[c] Increasing positive score indicates increasing rubberiness and a negative score indicates increasing tendency to fall apart
[d] Increasing positive score indicates increasing dryness and a negative score indicates increasing slimness or wateryness
[e] Increasing positive score indicates increasing off-flavor and a negative score indicates decreasing fruity flavor

Table 2—Means, standard deviations and significant differences of objective quality factors for whipped gelatin desserts

	Treatment group					Significant differences[a]	
	% Total egg albumin foam/% Total EMSMP foam						
Objective tests	A 100/0	B 70/30	C 40/60	D 0/100	E 40/0	at 1.0%	Additional at 5.0%
Specific gravity	0.395 ± 0.019	0.427 ± 0.024	0.481 ± 0.025	0.605 ± 0.023	0.417 ± 0.020	AEBCD	C < D
Index to vol, cm	7.87 ± 0.42	7.31 ± 0.30	6.62 ± 0.34	5.16 ± 0.23	7.68 ± 0.35	AEBCD	B < A
Rigidity, cm	1.41 ± 0.08	1.41 ± 0.08	1.30 ± 0.07	1.29 ± 0.03	1.40 ± 0.06	BAECD	None
pH	4.48 ± 0.13	4.41 ± 0.10	4.28 ± 0.07	4.17 ± 0.05	4.18 ± 0.07	AB CED	None

[a] All items underscored by one consecutive line are not significantly different (Duncan, 1955).

of treatment groups C and E resulted in a definite indication of the functional role of the EMSMP in the quality of the foam.

The objective test results of the whipped gelatin desserts are summarized in Table 2. Specific gravity and rigidity of the products increased, while volume and pH decreased as the level of EMSMP increased. The specific gravity of treatment D was significantly ($P < 0.05$) greater than that of all other treatment groups. Volume of the products as determined by the index of volume decreased significantly as the percentage of EMSMP was increased above 60%. The significant difference in volume of treatments E and C indicates that the EMSMP has a suppression effect on the volume of these products.

The specific gravity and subjective data of air cell size of the foams indicate no difference in treatment C and E. However, the increase in specific gravity and decrease in volume in a foam-type product yields a more compact texture. This was also paralleled by an increase in the intensity of the fruit flavor, which the judges liked. Differences among groups in rigidity were not significant. The pH of groups A and B was significantly ($P < 0.01$) different from the pH of groups C, D and E. All whipped gelatin desserts remained stable for the test period, as indicated by a lack of drip loss.

EMSMP can be used as a whipping agent to replace egg albumin. The foam formed is stable with a smooth texture. It can be manipulated as an egg albumin foam. The flavor and mouthfeel are acceptable in a delicately flavored product. EMSMP is of high nutritional quality making it possible to substitute it for egg albumin or soy protein isolates.

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A Research Note

MIGRATION OF PLASTICIZERS FROM POLYVINYLCHLORIDE PACKAGING FILMS TO MEAT

ABSTRACT

The migration of plasticizers used in polyvinylchloride films was investigated. Meat and meat/fat mixtures were covered with film placed between glass plates and stored at 4 ± 0.5°C. At predetermined storage times, samples were extracted, extracts saponified, and alcoholic constituent of plasticizer determined by gas chromatography. In the case of di (2-ethylhexyl) adipate, up to 23.5 mg/dm² migrated to the high fat meat. The migration to lower fat contents meat was proportionally lower.

INTRODUCTION

POLYMERIC MATERIALS (plastics) are used in a wide array of products directly in contact with food and biological materials. The commercial versions of these plastics contain various additives used for heat and light stabilization, process facilitation, surface properties and many other special purposes.

Among these additives often the largest component is the plasticizer used to modify mechanical properties. At various times, questions have been raised concerning the safety of such additives in view of their tendency to migrate. Such questions of safety involve both the immediate hazards posed, for example, by polymeric devices used in human therapy and the chronic case as from continued ingestion of foods packaged in various types of polymeric containers. Phthalates and adipates often used as plasticizers in films for fresh meat packaging seem to have a low order of toxicity; however, their effect on health is still not fully known.

In addition to the toxicological considerations, there is great need for accurate and reproducible methods for the determination of such migrants in biological materials to enable proper evaluation of risk factors involved.

There are a number of methods for determination of kind and amount of plasticizers in polymers, but there are no accepted methods for determination of such substances in biological materials. The degree of difficulty between these two cases is quite extreme since smaller amounts must be determined as migrants in a much more complex medium.

There are excellent publications dealing with the migration of additives from plastics into foods applying radiotracer techniques. A review of this work may be found in a paper by Figge (1972). However, phthalates and adipates were not investigated in these studies. Phthalates were detected in different foods including butter (Morita et al., 1973), cheese and lard (Pfab, 1967) and milk (Wildbrett, 1973).

Less information is found in the available literature concerning adipates. They were detected in blood as a result of its contact with plastic biomedical devices (Rubin, 1973). A method for the determination of dioctylphthalate and dioctyladipate in meat has been published by Van Battum and Wouters (1969). These authors did not give, however, any numerical values of plasticizers detected in meat. No data were found in the available literature on the amount of di (2-ethylhexyl) adipate (DEHA) migrating from film into meat.

Our study was undertaken to prepare a relatively simple analytical method for the determination of DEHA present in meat as a result of a contact with plasticized film

Another objective was to investigate the influence of fat content and time on the amount of plasticizers migrating from packaging films into meat.

EXPERIMENTAL

Sample preparation and storage

Meat and meat/fat mixtures (100g) were ground, thoroughly mixed and placed between sheets of commercially available highly plasticized polyvinylchloride film (film thickness approx 0.5 mil, plasticizer content approx 30%). The samples were pressed between two glass plates using 500g weight. An approximately 2.5 mm thick layer of the material was formed with two sides 3.87 dm² each exposed to the film. Samples were stored at 4°C and at various intervals analyzed for plasticizer content.

Extraction and saponification

Samples were carefully removed from the plates, mixed with equal amounts of anhydrous sodium sulfate and extracted for 6 hr with hexane in a Soxhlet apparatus. The extract was quantitatively transferred to a distillation flask and the solvent removed by evaporation. Potassium hydroxide (2N solution in methanol) was added at the rate of 0.8g per 1g of fat and saponification was conducted for 4 hr under reflux.

Separation of alcoholic constituent of plasticizer

After saponification, methanol was removed by evaporation. Then residue was subjected to steam distillation until about 200 ml of distillate was collected. The distillate was shaken with 50 ml portions of ethyl ether for 10 min. The extraction was repeated four times. All portions of ethyl ether were combined and left overnight with 25g of anhydrous sodium slufate. Ethyl ether was separated from Na_2SO_4 and evaporated to an approximate volume of 50 ml. This solution was then used for gas chromatographic examination.

Gas chromatographic (GC) analysis

GC determination of the alcoholic constituent of the plasticizer was conducted under the following conditions:

Gas chromatograph: Hewlett Packard Model 5750 with flame ionization detector
Column: Aluminum 10-ft long, 1/4–in. i. d.
Stationary phase: SE 30 10% on Anachrom ABS
Temperatures:

Column	150°C
Injection port	180°C
Dectector	220°C

Quantitation was made on the basis of standard curve prepared with known amounts of 2 ethyl hexanol.

To obtain recovery factor, meat samples were prepared with known amounts of DEHA. Extraction of plasticizer, saponification, separation of alcoholic constituent of plasticizer, and GC analysis were conducted using the same procedure as for experimental samples.

Table 1—Di (2-ethylhexyl) adipate migration into ground meat and fat (stored at 4°C for 72 hr)

Sample	Approx fat content (w/w)	Di (2-ethylhexyl) adipate mg/dm² [a]
Beef	20%	15.5
Beef	20%	14.5
Beef 50% Pork 50%	25%	17.0
Beef 50% Pork 50%	25%	19.0
Beef Fat	90%	21.0
Beef Fat	90%	23.5

[a] Values include correction for recovery

Table 2—Dynamics of di (2-ethylhexyl) adipate migration into meat containing various amounts of fat (mg/dm²)[a]

	Time (hr)			
	24	48	72	96
Beef (14.4% fat)	2.0	5.5	6.0	–
Beef (21.1% fat)	4.5	13.5	–	14.0

[a] Values include correction for recovery

RESULTS & DISCUSSION

THE VALUES for DEHA adipate migration into ground meat and fat, as well as the dynamics data on migration, are shown in Tables 1 and 2. Recovery factor of 69.4% was obtained in the experiments with known amounts of DEHA added to the meat.

DEHA migrated to meat from plasticized PVC film in the range of 20 mg/dm² under conditions of high ratio of film area to meat weight.

The rate of migration is increased in the samples with higher fat content. After 48 hr, the migration is almost complete.

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A Research Note

EFFECT OF LIGHT ON THE COLOR STABILITY OF STERILE AQUEOUS BEEF EXTRACT

ABSTRACT

Warm white fluorescent light (120 ft-c) had a significant ($P < 0.01$) effect on the color stability of sterile aqueous beef extract free of fat. At the end of 26 days there was a complete loss of oxymyoglobin in samples stored in a 1°C walk-in cooler and exposed to light for 12 hr each day. There was a 60% loss of oxymyoglobin in the control samples stored in dark for the same period. A possible mechanism for the photochemistry of the conversion of oxymyoglobin into metmyoglobin is suggested.

INTRODUCTION

IN THE SELF-SERVICE merchandising of prepackaged meats, lighting is recognized as one of the most powerful of merchandising tools. The adverse effect of light on fresh meat color has been established by Marriott et al. (1967), Wiles (1971), Solberg and Franke (1971) and Satterlee and Hansmeyer (1974). The photochemistry of this reaction is not fully understood. In intact meat samples it is also possible that photo-oxidation products of lipids could trigger the oxidation of myoglobin.

An effort was made to determine the color stability of myoglobin present in sterile aqueous beef extract, by exposing it to warm white fluorescent light; the influence, if any, of the photo-oxidation products of lipids was avoided thereby.

MATERIALS & METHODS

Preparation of sterile aqueous beef extract

The sterile aqueous extract was prepared using the semitendinosus muscle of USDA Good beef. After the removal of external fat, the muscle was cut into 2.5 cm squares and ground thoroughly. One hundred grams of ground meat was mixed with 250 ml of cold sterile deionized water and 12g of Hyflo Super-Cell. These were blended for 6 sec at low speed in a chilled stainless steel blender jar. The meat slurry was filtered under vacuum using Whatman No. 1 filter paper. The clear filtrate was cold sterilized by membrane filtration. The sterile aqueous beef extract was aseptically transferred into sterile test tubes (10 ml each) in a transfer room under UV lights. During transfer, the aqueous beef extract was covered with aluminum foil to prevent the influence of UV light on color.

Storage

The test tubes with sterile aqueous beef extract were stored in a 1°C walk-in cooler. The solution temperature was measured by thermocouples placed in the center of the test tubes containing the sterile aqueous beef extract. The solutions were oxygenated by frequent shaking. Aqueous beef extract samples stored under light were illuminated with 120 ft-c of warm white fluroescent light for 12 hr each day. Tests for bacterial contamination were made by the standard plate count method.

Spectrophotometric analysis of aqueous beef extract

The absorbancy of the extract at 473, 507, 573, and 597 nm was determined at 48-hr intervals using a Model 24 Beckman Spectrophotometer. The relative concentration of myoglobin (MB), oxymyoglobin (O_2Mb) and metmyoglobin (MMb) was determined by the absorbancy ratio method devised by Broumand et al. (1958). Data were subjected to analysis of covariance (Snedecor and Cochran, 1971).

RESULTS & DISCUSSION

AS ILLUSTRATED in Figure 1, light had a significant ($P < 0.01$) effect on color of the sterile aqueous beef extract. In our studies, 50% of the oxymyoglobin was lost in 13 days from light exposed samples and in 21 days from dark storage samples. There was complete loss of oxymyoglobin in light exposed samples in 26 days; 60% of oxymyoglobin was lost during the same period from dark storage samples. Myoglobin and oxymyoglobin absorb light, with attendant radiation and energy, and undergo a photo-oxidation reaction in which oxymyoglobin and myoglobin are converted into metmyoglobin. Fe^{2+} in the heme part of myoglobin loses electrons and is oxidized to Fe^{3+}. Histidine in the globin part of myoglobin is susceptible to photo-oxidation. Histidyl residues in proteins such as β-lactoglobin can undergo photo-chemical oxidation without causing any proteolytic change (Weil and Buchart, 1951). Studies on photo-oxidation of horse myoglobin showed that photo-oxidation stopped after the consumption of 2 moles of oxygen per mole of metmyoglobin and concomitant disappearance of two moles of histidine but there was no difference in the oxygen binding capacities of native and photo-

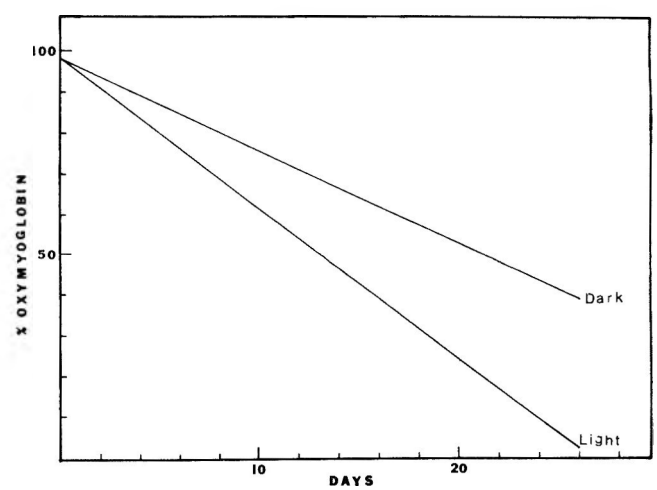

Fig. 1—Effect of light on color stability of sterile aqueous beef extract.

oxidized myoglobins (Sajgo, 1961, 1963). Histidine, in the oxidized state, cannot accept more electrons from Fe^{2+}; but the transfer of electrons is more likely from Fe^{2+} to oxygen than to nitrogen, since oxygen is more electronegative than nitrogen. It is postulated that light catalyzes the transfer of electrons from Fe^{2+} to oxygen of oxymyoglobin after the initial oxidation of histidine; Fe^{2+} is oxidized to Fe^{3+} and oxygen to O_2^-. Light breaks the bond between Fe^{3+} and negatively charged oxygen to produce the free radical O_2^-. This free radical O_2^- catalyzes further the chain oxidation of myoglobin and oxymyoglobin to metmyoglobin.

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A Research Note

FATE OF Staphylococcus aureus IN BEEF-SOY LOAVES SUBJECTED TO PROCEDURES USED IN HOSPITAL CHILL FOODSERVICE SYSTEMS

ABSTRACT

Staphylococcus aureus was inoculated into uncooked mixtures of ground beef and soy protein and survival of the organism was determined during the stages of food handling that would occur in a hospital with a chill foodservice system. Although the initial inoculum was only approximately 5000/g, heating the mixture as loaves in a convection oven at 121°C to an internal temperature of 60°C was not lethal to *S. aureus*. Numbers of *S. aureus* in loaves decreased during holding at 5°C for 24, 48 and 72 hr. Numbers of *S. aureus* in the center of the final product were less than 3/g after loaves were portioned, held chilled at 5°C for 2 hr, and portions heated to 80°C in a microwave oven.

INTRODUCTION

EXTENDING the storage of partially cooked food beyond 24 hr by refrigeration is becoming common in hospital and school foodservice systems. Such storage periods of 24, 48, or 72 hr, in combination with the quantity of food and the temperature at which the food is held, could afford an opportunity for bacterial growth (Bunch et al., 1976).

Meats are frequently reported as the vehicle of transmission in outbreaks of staphylococcal food poisoning (CDC, 1976). After reviewing the literature on outbreaks of staphylococcal food poisoning traced to meat and bakery products, Minor and Marth (1972) suggested that critical products were nonsterile foods, frequently of animal origin, which are subjected to substantial handling by people. Problems with staphylococcal food poisoning are frequently associated with mishandling of food in foodservice establishments and in the home. A major factor responsible for all outbreaks of staphylococcal intoxication in 1974 was improper storage or holding temperatures of food (CDC, 1976).

Since outbreaks of staphylococcal food poisoning are often associated with the foodservice industry and since the microbiological aspects of chill foodservice systems have received only limited attention, we believed it necessary to determine the fate of *Staphylococcus aureus* in a food handled according to procedures employed in hospital chill foodservice systems. Observations on the fate of *S. aureus* in beef-soy loaves handled according to these procedures are described in this paper.

EXPERIMENTAL

BEEF-SOY LOAVES were selected for these experiments because they are currently used in chill foodservice systems and because ground beef has a relatively high initial bacterial count (Duitschaever et al., 1973) and when it is combined with such ingredients as milk and eggs, the resulting mixture offers a suitable substrate for formation of enterotoxin if toxigenic bacteria are present and can grow. Ingredients, equipment and detailed procedures used to prepare, partially cook, store chilled, portion onto plates, hold chilled, and reheat beef-soy loaves were reported previously (Bunch et al., 1976).

Procedure

On the first day of the experiment three lots of beef-soy loaves were prepared in sequence. Twenty pounds of fresh gound beef were purchased from a local retail outlet. Sufficient of a 24-hr-old broth culture of *S. aureus* (strain 100, produces enterotoxin A, from the Food Research Institute, University of Wisconsin, Madison) was added to the beef-soy mixture to provide approx 5,000 staphylococci per gram of finished product. To facilitate distribution of staphylococci in the beef-soy mixture, the broth culture was diluted with 0.1% phosphate buffer solution and the diluted culture was mixed with milk that was an ingredient of the beef-soy mixture. This resulted in 4,500–5,500 staphylococci per gram of the unheated mixture. Material for the three lots of loaves was inoculated, mixed, and packed into 30.5 × 50.8 × 6.4 cm (12 × 20 × 2-1/2 inch) pans. Product in each pan was divided into four equal loaves and loaves were coded 1 to 4. Loaves were baked to 60°C in a convection oven at 121 ± 8°C and then were stored at 5 ± 3°C for 72 hr. At the end of 24, 48, or 72 hr, portions approx 1.6 cm (5/8 inch) thick were cut from the middle sections of a loaf toward the center of each of the three pans. One sample from this loaf in each of the three pans was placed on a paper plate and the plate was covered with plastic film. Each plate of three samples was held in a pass-through refrigerator at 5 ± 1°C for 2 hr and then it was covered with a fiberglass dome cover and heated for 55 sec in a microwave oven to an internal temperature of approximately 80°C.

The internal temperature of loaves in pans was measured continuously as described previously (Bunch et al., 1976). Temperatures of refrigerators were indicated by recording thermometers. A pyrometer was used to measure the temperature in the loaves before heating in the convection oven and after heating in the microwave oven.

Sampling and bacteriological analyses

Samples from each pan of loaves were collected for analysis as follows: (1) immediately after inoculation, center of the mixture; (2) after cooking, corner of loaf 1; (3) after cooking, center section of loaf 3; (4) after 24, 48, or 72 hr of chilled storage, center section of loaf 3; and (5) after heating in a microwave oven, center section of loaf 3.

The knife used to take samples was sanitized by dipping it in a solution containing 200 ppm of chlorine. Samples were placed in sterilized jars with screw caps and then held in ice until taken to the Food Microbiology Laboratory for the analysis. Samples were never held longer than 30 min before they were tested. From each of the five samples 11g of beef-soy mixture were homogenized with 99 ml of sterile 0.1% peptone water, and blended in a Waring Blendor for 2 min. Appropriate decimal dilutions of the samples were made in tubes of nutrient broth. Diluted samples in broth were incubated at 37°C for 16 hr. A loopful of broth was taken from each tube and was streaked on Vogel-Johnson agar. These plates were incubated at 37°C for 36 hr. Colonies typical of coagulase-positive staphylococci were picked and tested for the coagulase reaction with coagulase plasma (Difco) using the tube method (Thatcher and Clark, 1968).

RESULTS & DISCUSSION

TIME AND TEMPERATURE histories recorded during cooking and chilling of beef-soy loaves are shown in Table 1. An average cooking time of 43 min was required to raise the internal temperature of 5.2 kg of loaves from a mean of 11 to 60°C. Internal temperatures in the loaves, recorded just before chilled storage, averaged 61°C for the three lots. During the first 7 hr of chilled storage and for some of the cooking time in the oven, the internal temperatures in the loaves were in the range (6.5–45.5°C) that permits growth of *S. aureus* (Angelotti et al., 1961a). Loaves were at or near the optimum temperature (37°C) for growth of *S. aureus* within the first 20 min

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Table 1—Time in convection oven at 121°C required to raise the temperature of 5.2 kg of beef-soy loaves from a mean of 11—60°C and time at 5°C required to lower the internal temperature of the loaves from a mean of 61°C to a mean of 6°C

Lot (no.)	Initial temp in loaves before cooking (°C)	Temperature in loaves in oven °C					Initial temp in loaves before chilled storage (°C)	Hours							
		32	43	49	54	60		1	2	3	4	5	6	7	8
		(min)						(°C)							
1	11	11	21	29	33	42	61	45	32	26	19	16	10	7	6
2	12	11	19	28	35	44	60	43	31	26	19	16	9	7	6
3	11	11	20	26	34	43	61	46	32	26	20	17	9	7	6
Mean	11	11	20	28	34	43	61	45	32	26	19	16	9	7	6

of cooking in the oven and during the first 2 hr of chilled storage.

Samples from the uncooked beef-soy mixture contained between 4,500 and 5,500 *S. aureus*/g (Table 2). Although 2 ml of the diluted culture of *S. aureus* were added to each lot, the slight variance in the numbers of the organism may have resulted, in part, from an initial difference in population of *S. aureus* in the raw ground beef. The inoculum used was thought to be reasonable since Duitschaever et al. (1973) found up to 440,000 *S. aureus* per gram of hamburger.

Data in Table 2 show that the numbers of *S. aureus* in samples from the corner and center of loaves after cooking ranged from 3—4/g and 23—430/g, respectively. Loaves in the second trial were at the higher temperatures (54 and 60°C) during cooking slightly longer than were loaves in the other trials (Table 1). This may account for the small number of surviving staphylococci that were observed in this trial. Since *S. aureus* did survive cooking to 60°C in each of the three trials, the possibility of foodborne disease exists if partially cooked beef-soy loaves are handled improperly. Angelotti et al. (1961b) concluded that foods heated to 65.5°C, and every particle of food held at this temperature for at least 12 min, were essentially free of *S. aureus* even though the initial count was 1×10^7/g. These authors concluded that food must be held 78—83 min at 60°C before the same degree of destruction is achieved in similarly contaminated foods. In our study heating to 60°C was not lethal to all cells of *S. aureus* (Table 2).

Although beef-soy loaves remained at temperatures in the range for growth of *S. aureus* (6.5—4.5°C) for over 7 hr (Table 1), no increase in numbers occurrred during that time (Table 2). In the first and third trials there was a further reduction in numbers of *S. aureus* after chilled storage for 24 hr (Table 2). However in the second trial the numbers remained the same. In each trial the numbers of *S. aureus* in the loaves decreased between 24 and 72 hr of chilled storage at 5°C. This may have happened because heat-injured cells were damaged further by the extended refrigerated storage and thus were unable to initiate growth.

Angelotti et al. (1961a) found no growth of *S. aureus* in chicken a la king incubated at 5.5°C for 5 days. Growth of *S. aureus* is related to the number of cells present, the substrate, temperature, and the degree of injury suffered as a consequence of heat treatment.

After storing partially cooked beef-soy loaves chilled at 5 ± 3°C for 24, 48, or 72 hr followed by heating in a microwave oven, *S. aureus* was undetectable in any samples. Although there were few, if any, *S. aureus* present after heating in a microwave oven, preformed toxin, if present, would not be inactivated by the short exposure to 80°C. Hence products in a chill foodservice operation must be handled with care during all phases of the operation to prevent excessive microbial contamination and to minimize growth of contaminants that might be in the food.

Table 2—Numbers of Staphylococcus aureus in inoculated beef-soy loaves

Sampling stage and location	Trial 1	Trial 2	Trial 3	Mean
	(no./g)			
After inoculation, center of mixture	4900	4500	5500	5000
After cooking[a]				
corner of loaf 1[b]	4	3	3	3
center of loaf 3[c]	430	23	230	230
After chilled storage[d]				
center of loaf 3				
24 hr	150	23	93	90
48 hr	93	21	23	46
72 hr	19	9	3	10
After reheating[e]				
center of loaf 3				
24 hr	<3	<3	<3	<3
48 hr	<3	<3	<3	<3
72 hr	<3	<3	<3	<3

[a] Convection oven at 121 ± 8°C to 60°C
[b] Loaf 1 (side of pan)
[c] Loaf 3 (center of pan)
[d] 5 ± 3°C
[e] Microwave oven for 55 sec to 80°C

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A Research Note

NUTRITIONAL EVALUATION OF ALL-MEAT AND MEAT-SOY WIENERS

ABSTRACT

Nutritive values of all-meat and soy-containing wieners were evaluated by protein efficiency ratio (PER), amino acid composition and sodium analyses. The effect of beef muscle reported to have high (shank) and low (skeletal muscles) levels of connective tissue was also evaluated. Replacement of 25% beef skeletal muscle by hydrated soy protein (BSMSP) gave a lower ($P < 0.05$) PER (2.67) than the PER (3.11) for all-beef skeletal muscle weiners (BSM). However, the PER for the BSMSP wieners was not significantly different from the PER (2.54) for the casein control. Beef shank, all-meat wieners (BS) and wieners where 25% of the beef shank (BSSP) was replaced by hydrated soy protein had similar PER values of 2.18 and 2.02, respectively. Both BS and BSSP wieners had lower ($P < 0.05$) PER values than the BSM and BSMSP wieners. BSM wieners contained 1.95–4.17 mg/100g protein more total essential amino acids (minus tryptophan) than the beef shank and soy-containing formulations. Soy incorporation caused a 10–22% decrease in the methionine content compared to the all-meat formulations. Sodium levels ranged from approximately 990–1070 mg/100g in the four wiener formulations. The high sodium levels in these formulations were derived primarily from the 2% salt which was necessary to form the emulsions. Incorporation of the soy protein resulted in less than an 8% increase in the sodium content of the wieners.

INTRODUCTION

EMULSION TYPE SAUSAGES, such as wieners and bologna are two of the most popular processed meat products in the United States. Incorporation of plant proteins into comminuted meat products may be advantageous relative to economics and product composition. Presently, according to the USDA regulations (Mussman, 1974), 3.5% soy protein concentrate, flour or grits, and 2% isolated soy protein can be incorporated in regular-labeled cooked sausages. In addition, nonspecified sausage-type products containing much higher levels of nonmeat proteins, such as soy, are commercially available and the market for such products is expected to increase (Ziemba, 1974). The texture, composition, sensory evaluation and other characteristics of wiener-type products containing 20% or more soy protein have been studied (Sofos et al., 1977; Sofos and Allen, 1977).

Nutritive evaluation of meat products blended with plant proteins is an essential consideration, since these proteins are limiting in one or more amino acids (Kakade, 1974). Several workers showed that soy-blended meat products containing up to 30% soy did not have significantly lower protein efficiency ratios (PER) than all-meat products (Kies and Fox, 1971; Wilding, 1974). In wiener-type products, Terrell and Staniec (1974) showed that incorporation of hydrated soy protein (25%) resulted in a PER not less than 15% lower than that of all-meat products.

High levels of connective tissue in meat products may also lower protein quality. Elastin and collagen are lower in essential amino acids (Bodwell and McClain, 1971) and, consequently, are lower in biological protein quality. Since all meat wieners do not specify the type of meat, the protein quality of such wieners may vary depending upon the relative proportion of connective tissue and contractile proteins. It should also be noted that protein quality varies among different soy protein products (Horan, 1974).

In this study, nutritive values from four different wiener formulations were obtained, and the effect of soy protein replacement as well as the use of low quality lean meat was evaluated. The objective of this study was to determine the effect of two types of meat and the replacement of these with 25% soy protein on PER, amino acid composition and sodium content in an emulsion-type product.

MATERIALS & METHODS

WIENERS used for this study were produced at the Meat Science Laboratory of the University of Minnesota (Sofos et al., 1977). Four different formulations were prepared as shown in Table 1. The textured soy protein (Textratein No. 18 plain, manufactured by Cargill, Inc., Minneapolis, MN), was hydrated with two parts by weight of cold tap water. The isolated soy protein (Pro-Fam 90/HS, Grain Processing Corp., Muscatine, Iowa) was hydrated with four parts by weight of cold tap water. Fat level was adjusted to 19.3% by addition of pork fat in each treatment. All wieners were cooked to an internal temperature of 68°C, cooled and then peeled. Proximate compositions were determined by AOAC methods (1970). Protein content was obtained from Kjeldahl nitrogen x 6.25.

Protein quality was determined by PER (AOAC, 1970) and amino acid analyses. After manufacture, all wieners were frozen, ground, freeze dried and powdered for preparation of the diets. All diets were standardized to 10% protein and 12% fat with cottonseed oil when necessary. Sprague-Dawley (Holtzman, Madison, Wisc.) rats of 51–75g body weight and 24 days of age were used in the PER trial. The 12 rats in each group were fed the respective diets for a 4-wk period. A control casein diet, (10% protein, 12% cottonseed oil) was included.

Amino acid composition was obtained by the chromatographic method of Spackman et al.(1958). Sodium levels were determined by the AOAC (1970) method using a Perkin Elmer 303 Atomic Absorption Spectrophotometer. Duncan's multiple range test was used for statistical analyses.

RESULTS & DISCUSSION

THERE WAS little variation in moisture and protein content of the four formulations (Table 1). Approximately one-fourth of the protein originated from soy protein in beef skeletal muscle plus soy protein (BSMSP) and beef shank plus soy protein (BSSP) wieners. The lower content of fat in the beef shank (BS) and BSSP formulations was the result of losses during cooking caused by a less stable emulsion in these two formulations. Beef shank is known to be high in connective tissue due to anatomical characteristics of these muscles (Briskey and Kauffman, 1971). Connective tissue proteins have a low emulsifying capacity (Kramlich et al., 1973) and become self-limiting in the formulation.

Results obtained from PER analyses are also shown in Table 1. Beef skeletal muscle wieners (BSM) gave a higher ($P < 0.05$) PER than the PER for BSMSP (2.67), BS (2.18) and BSSP (2.02) wieners. The PER for BSM wieners was 122% of the PER (2.54) for the casein control. Replacement of 25% of

Table 1—Formulation, proximate composition, sodium content and PER, for all-meat and meat-soy wieners

	Beef skeletal muscle (BSM)	BSM and soy protein (BSMSP)	Beef shanks (BS)	BS and soy protein (BSSP)	Casein control
Ingredients					
Lean beef (%)	77.3	53.2	77.3	53.2	
Fat[a] (%)	19.3	19.3	19.3	19.3	
Hydrated, textured soy protein[b] (%)	0	19.3	0	19.3	
Hydrated, isolated soy protein[c] (%)	0	4.8	0	4.8	
Salt (%)	2.0	2.0	2.0	2.0	
Ascorbate-spice (%)	1.4	1.4	1.4	1.4	
Sodium nitrite (mg/kg)	156	156	156	156	
Composition and PER of wieners					
Moisture (%)	55.0	54.5	58.7	56.6	
Protein (%)	17.4	17.2	18.7	17.8	
Fat (%)	21.3	20.7	16.5	17.3	
Sodium content (mg/100g)[d]	990.4 ± 5.44a	1021.5 ± 3.34a	1009.5 ± 7.74a	1069.7 ± 8.83b	
Protein efficiency ratio (PER)[d]	3.11 ± 0.11a	2.67 ± 0.09b	2.18 ± 0.13cd	2.02 ± 0.13c	2.54 ± 0.19bd
PER relative to casein (%)	122.4	105.1	85.8	79.5	100.0

[a] Pork fat was added to achieve 19.3%.
[b] Textured soy protein was hydrated with two parts water. Textratein No. 18 plain, Cargill, Inc., (Minneapolis, Minn.).
[c] Isolated soy protein was hydrated with four parts water. Pro-Fam 90/HS, Grain Processing Corp., (Muscatine, Iowa).
[d] Means on a line with different letters differ significantly ($P < 0.05$). Means without letters were not statistically analyzed.

the beef skeletal muscle in wieners with hydrated soy protein reduced ($P < 0.05$) the PER from 3.11 (BSM) to 2.67 (BSMSP). However, 25% replacement of beef shank in wieners with hydrated soy protein did not change ($P > 0.05$) the PER. PER for BS wieners (2.18) was similar ($P > 0.05$) to the casein control (2.54), but the PER for the BSSP wieners (2.02) was lower ($P < 0.05$) or about 80% of the PER for the casein control. Horan (1974) reported that textured soy protein products had a PER which was 80% of that for casein. Kies and Fox (1971) determined that beef-flavored, textured soy protein had 85% of the casein PER. Fortification with 1% DL-methionine in these products raised the PER to 113% of casein.

The PER values for BSM (3.11) and BSMSP (2.67) were higher ($P < 0.05$) than the values of BS (2.18) and BSSP (2.02). This can be explained by the high content of connective tissue present in shank meat which is low in essential amino acids. Other work (Anon., 1974) demonstrated that 10% collagen replacement in lean beef lowered the PER by 0.3 unit. From these PER results, it can be concluded that 25% substitution of shank meat by hydrated soy protein products used in these studies does not significantly lower the PER. In addition, the use of beef shank caused a greater reduction in the PER of wieners than the replacement of beef skeletal muscle with 25% hydrated soy protein (Table 1). The report of Kies and Fox (1971) suggests that the PER of wieners containing 25% hydrated soy protein might be increased by addition of 1% DL-methionine.

Amino acid analyses (Table 2) showed a relatively lower level of lysine and methionine in soy-blended wieners (BSMSP and BSSP). Although not demonstrated in this study, Terrell and Staniec (1974) reported that addition of other protein sources such as egg albumin may improve the nutritive value, when beef skeletal muscle is only partially replaced with soy protein. Happich et al. (1975) found a high correlation between essential amino acid content and PER of protein from different sources. Total essential amino acid (minus tryptophan) content in shank products, BS and BSSP was 5.17 g/100g protein and 2.14 g/100g protein lower than that in skeletal meat products, BSM and BSMSP, respectively (Table 2). Even larger differences in total essential amino acid content would be expected if values for tryptophan had been determined.

Sodium level is often a major concern to consumers, since the relationship between hypertension and salt intake has been demonstrated (Dahl and Love, 1956). Hegarty and Ahn (1976) found high sodium levels in some meat analogs manufactured

Table 2—Amino acid composition of all-meat and soy-containing wieners[a]

Essential amino acids[b]	Beef skeletal muscle (BSM)	BSM and soy protein (BSMSP)	Beef shank (BS)	BS and soy protein (BSSP)
Arginine[c]	5.92	6.53	5.97	6.12
Histidine[d]	3.28	3.01	2.64	2.62
Isoleucine	4.05	3.79	3.33	3.59
Leucine	7.15	6.83	6.15	6.54
Lysine	7.79	6.80	6.73	6.51
Methionine	1.89	1.49	1.63	1.46
Phenylalanine	3.73	3.97	3.15	3.56
Threonine	3.84	3.60	3.49	3.59
Valine	4.37	4.05	3.76	3.94
Total	42.02	40.07	36.85	37.93

[a] Grams of amino acid per 16 grams total nitrogen. Based on a single analysis of each formulation and therefore no statistical comparisons were possible.
[b] Tryptophan not determined
[c] Essential for growing mammals
[d] Essential for the human infant

from soy protein products. However, most of the sodium was derived from flavor compounds, such as monosodium glutamate (MSG). The results of sodium analyses on these wiener formulations showed some variation among treatments (Table 1). However, these differences are probably not biologically significant, since wieners are a very high sodium-containing food. Two percent salt was added to each treatment for emulsification purposes; this was the major sodium source in all products. Sodium derived from other ingredients was negligible. As reported by the manufacturer, the textured soy protein used was low in sodium (25.4 mg/100g) and no MSG was added to the spice mixture. The average sodium level for beef is 65 mg/100g (Watt and Merrill, 1963). The increased sodium levels of the soy-containing treatments could be due to the high sodium content of this isolated soy protein (1200 mg/100g, as reported by the manufacturer). However, the level of incorporation of the isolated soy protein was not high enough (5% hydrated) to cause a marked effect on sodium levels of the soy-containing formulations. Therefore, it can be concluded that unflavored textured soy protein does not increase the sodium level in wieners to a degree that is of major biological importance.

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