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BASIC RESEARCH

APPLIED SCIENCE & ENGINEERING

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RESEARCH NOTES

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COMPARISON OF STRUCTURAL CHANGES IN BOVINE LONGISSIMUS AND SEMITENDINOSUS MUSCLES DURING COOKING

R. C. LEANDER, H. B. HEDRICK, M. F. BROWN, and J. A. WHITE

ABSTRACT

Structural characteristics of aged (10 days) bovine longissimus and semitendinosus muscles as affected by thermal treatment were evaluated with scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Steaks were oven roasted at 177°C to internal temperatures of either 63, 68, or 73°C. At 63°C both muscle samples exhibited coagulation and denaturation of the perimysium and sarcolemma and progressively increased to near complete coagulation and denaturation at 73°C. Well-defined myofibrillar surface features were present in semitendinosus samples at 63°C and increased sarcomere denaturation and shrinkage were observed at the higher temperatures. Whereas, the overall topographical features of longissimus myofibrils were not well-defined and exhibited a granular appearance even at 63°C. TEM of samples heated to 63°C revealed less distinct myofibrils in longissimus than in semitendinosus samples. Disintegration of filaments in the I-band and shrinkage of filaments in the A-band occurred at 63°C. Increased disintegration of actin filaments in the I-band at the junction of the Z-disc and shrinkage and disruption of the A-band material occurred as temperatures were increased. Z-disc material remained intact with some evidence of disruption at the higher temperatures. Although progressive disruption of muscle fiber ultrastructure occurred as steaks were heated to higher internal temperatures, these changes did not result in increased muscle tenderness. Instead the samples became progressively less tender as internal temperatures were increased likely due to the shrinkage and hardening of filamentous material in the A-bands. Increased disintegration of filaments in the I-band as temperature was increased apparently did not contribute to an improvement in tenderness.

INTRODUCTION

TWO STRUCTURES in meat that influence tenderness are muscle fibers and connective tissue (Cover, 1943). These two components have been termed "actin-myosin shortening" and "background toughness," respectively, by Marsh and Leet (1966). The amount and extent to which each of these components contributes to toughness varies considerably.

Numerous studies have been conducted in an effort to elucidate changes that occur in muscle fibers and associated connective tissues during various postmortem treatments, prior to cookery, which are associated with meat tenderness. Comparatively fewer studies have been conducted to determine changes that occur in the structure of muscle fibers and connective tissue during cookery.

Muscle, when heated, undergoes shrinkage due to translocation of water, lipids, and dissolved materials out of the muscle. Accompanying this shrinkage is a concomitant reduction in fiber diameter and sarcomere length (Lowe, 1948).

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Ultrastructural changes in bovine psoas, turkey pectoralis and semitendinosus when heated were studied by Hegarty and Allen (1975). No significant changes in length of sarcomere in bovine psoas were discernible until heated to greater than 70°C. Sarcomere length of turkey semitendinosus dissected in rigor began to decrease in sarcomere length when heated to 40°C but pre-rigor turkey semitendinosus began to shorten at lower temperatures. At 90°C a complete loss in banding pattern was observed for all muscles studied. This finding in not in agreement with the report of Schmidt and Parrish (1971) who observed the principal banding features could still be identified when longissimus muscle was heated to an internal tempterature of 90°C. Wiedemann et al. (1967) cooked bovine semitendinosus to 85°C and observed supercontraction clots along the fibers similar to those observed in rigor contraction. Many details of the fine structure were destroyed with a major portion of the sarcomere breakage occurring at the area of the Z-band.

Scanning electron microscopy (SEM) studies of cooked bovine muscle (Cheng and Parrish, 1976; Jones et al., 1977) revealed that progressive changes occur in myofibril fragmentation at the Z-disc and in collagen denaturation with increased temperatures in the range 50–90°C.

The objective of this paper is to compare ultrastructural changes that occurred when aged steaks from bovine longissimus and semitendinosus muscles were cooked by a dry-heat method of cookery to internal temperatures of 63, 68, and 73°C.

MATERIALS & METHODS

LONGISSIMUS and semitendinosus muscle samples from each of three management groups, comprised of Hereford cattle, were selected for histological examination. All animals were grazed on predominantly fescue grass for 180 days. One group (18 animals) was slaughtered and the two remaining groups (18 animals in each group) were placed on an ad libitum high concentrate diet under drylot conditions for 56 and 112 days, respectively. The first group that was slaughtered graded USDA Standard and the two remaining groups graded USDA Good. After slaughter, the carcasses were chilled and aged at 0–2°C for 10 days. Shortloins (longissimus) and eye of round roasts (semitendinosus) were removed from the right side of each carcass after the 10-day aging period, packaged, frozen in a blast freezer at −23°C, and stored at −15°C.

The short loins and eye of round roasts were removed from frozen storage and cut into steaks while still frozen. Three 3.8-cm steaks were removed from the most anterior end of each cut for cooking. An adjacent 2.5-cm thick steak was removed for uncooked histological examination. Each steak was cut to a uniform diameter of 6.4 cm with a stainless steel coring device and vacuum packaged. Care was taken to insure removal of only the longissimus and semitendinosus muscles.

Prior to cooking the packaged steaks were allowed to equilibrate to 2°C in a holding cooler at 2°C. The steaks were cooked uncovered on a rack in a gas-heated oven at 177°C to internal temperatures of either 63, 68, or 73°C. Internal temperature was monitored throughout the cooking procedure with thermocouples connected to a Micromax temperature recorder. Upon reaching the desired internal temperature, steaks were removed from the oven and allowed to equilibrate to room temperature for approximately 1 hr. After temperature equilibration, three cylindrical samples, 1.3 cm in diameter, were removed from each steak. The most central sample was used for histological evaluation and the other two samples were

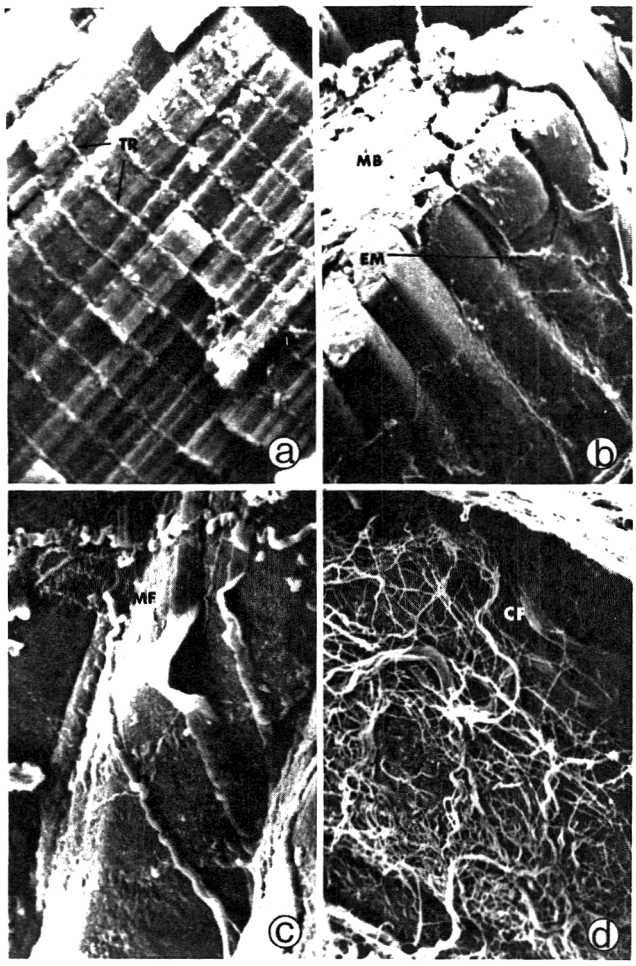

Fig. 1—Scanning electron micrographs of aged unheated bovine semitendinosus muscle: CF, collagen fibrils; EM, perimysium; MB, muscle bundle; MF, muscle fiber; TR, transverse ridges. (a) Muscle myofibrils (magnification x3360). (b) Bundle of muscle fibers (magnification x336). (c) Muscle fiber with endomysial fibers (magnification x1120). (d) Perimysial connective tissue fibers (magnification x1120).

used for Warner-Bratzler shear determinations. A cylindrical sample 1.3 cm in diameter, was similarily removed from each uncooked steak.

Length of sarcomeres was measured on myofibrils obtained from uncooked and cooked steaks for each animal and each cooking treatment. A core 1.3 × 2.5 cm obtained from each steak was placed in a Waring Blendor with reversed blades and blended for 15 sec in 0.25M sucrose. Sample preparations were observed under a microscope and 10 sarcomeres of 10 myofibrils were measured with a filar micrometer. Sarcomere measurements reported represent an average of 100 sarcomeres from each animal for each treatment.

Preparation for transmission electron microscopy (TEM)

Longissimus and semitendinosus muscle samples were obtained from six carcasses selected at random from each of the three management groups. From each carcass, the longissimus and semitendinosus samples were examined uncooked and after being cooked to internal temperatures of 63, 68, and 73°C. Muscle samples (cylindrical sample 1.3 cm in diameter and 2.5 cm in length) were placed on dental wax and immersed with fresh cold (4°C) 2% glutaraldehyde in 0.05M phosphate buffer (pH 6.8). At least 20 samples were cut from each core with a clean, sharp razor blade to approximately 1 mm^3. The cubed samples were fixed in fresh glutaraldehyde for 4 hr at 4°C. After washing in three changes of phosphate buffer (pH 6.8) the samples were postfixed in 1% osmium tetroxide for 4 hr at 4°C. Samples were then dehydrated in a graded series of acetone (20, 40, 60, 80, and 100%). Samples were placed in each dilution for 15 min. The final absolute acetone treatment was repeated three times each for 1 hr. Dehydrated samples were infiltrated with Epon:acetone in a graded series (1:3, 1:1, 3:1, and absolute Epon) under vacuum for a period of 4 hr for each step. The final absolute Epon infiltration was carried out under vacuum overnight. Samples were embedded with Epon and polymerized for 72 hr at 60°C and sectioned with a LKB Ultramicrotome. Silver sections (approximately 60–80 nm thick) were picked up on uncoated 300 mesh copper grids and stained with Uranyl acetate (Sjostrand, 1967) and lead citrate (Reynolds, 1963) and were examined with an RCA 3G electron microscope at 100 KV accelerating voltage.

At least five of the 20 samples prepared from each core were sectioned. From 50–80 sections were removed from each sample. All sections were observed under the microscope and at least 20 pictures were taken of a representative section. Approximately 1000 micrographs were taken during the course of the study.

Preparation for scanning electron microscopy (SEM)

The same number of cooked and uncooked samples from each management group as used for TEM were cut with a clean sharp razor blade to approximately 2 mm^3. Fixation and dehydration procedures were the same as those used in transmission electron microscopy. After acetone dehydration, acetone was replaced by means of a graded series of amyl acetate (20, 40, 60, 80, and 100%). Each step was carried out for 15 min with the absolute amyl acetate treatment repeated twice. The samples were critical point dried by the method of Anderson (1951) using liquid CO_2 as the transitional fluid. The dry samples were then mounted on aluminum discs with copper conductive cement, coated with gold (10 nm thick) and examined with a JEOL JSM-S1 scanning electron microscope operating at an accelerating voltage of 10 KV. Approximately 300 micrographs were taken.

Structural alterations of longissimus and semitendinosus muscles resulting from cooking were evaluated with the scanning and transmission electron microscopes. Surface alterations and overall physical integrity of muscle fibers and associated connective tissue were observed with the scanning electron microscope. Internal structural changes in myofibrillar components were studied with TEM.

RESULTS & DISCUSSION

SEM of unheated and heated muscle

Scanning electron micrographs of aged uncooked semitendinosus muscle are presented in Figure 1. Well defined transverse ridges corresponding to Z-discs (Jones et al., 1977) are orderly arranged along the myofibrils (Fig. 1a). Mean sarcomere length is 1.8μ (measured from this micrograph). An elevated area located centrally within the individual sarcomeres corresponds to the M-line. Figure 1b shows a bundle of muscle fibers surrounded by the perimysium. All observable fibers appear intact, and exhibit minimal structural damage attributable to postmortem aging or sample preparation. A higher magnification micrograph (Fig. 1c) of an individual muscle fiber depicts the endomysial connective tissue traversed by three collapsed tubules believed to be part of the fibers vascular system or large flattened connective tissues of the perimysium. Connective tissue fibers comprising the perimysium are depicted in Figure 1d. These connective tissue fibers were observed to be more abundant and more dense in the semitendinosus samples than in the longissimus samples.

Semitendinosus muscle cooked to an internal temperature of 63°C is shown in Figure 2. Myofibrillar surface features appear slightly affected by the thermal treatment (Fig. 2a). Slight disfigurement of the myofibrils has taken place but the major topographical features as observed in the uncooked sample (Fig. 1a) remain unchanged. There is some evidence of thermally induced swelling in the center of some sarcomeres. Perimysial connective tissue (Fig. 2b) exhibits evidence of thermal induced coagulation. Connective tissue fibers can be seen on the surface of the connective tissue covering. Intact muscle fibers are depicted in Figure 2c. The sarcolemma surrounding individual muscle fibers exhibits some coagulation. The fibrous connective

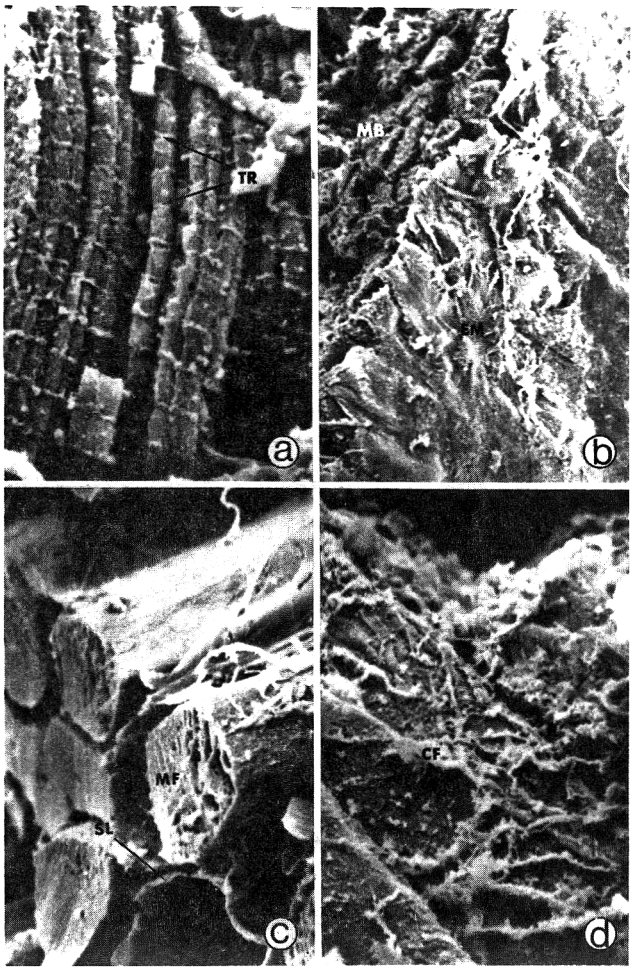

Fig. 2—Scanning electron micrographs of aged bovine semitendinosus muscle heated to 63°C internal temperature: CF, collagen fibers; EM, endomysium; MB, muscle bundle; MF, muscle fiber; SL, sarcolemma; TR, transverse ridges. (a) Myofibrils (magnification x3360). (b) Perimysium (magnification x170). (c) Muscle fibers (magnification x560). (d) Endomysial connective tissue depicting collagen coagulation (magnification x 560).

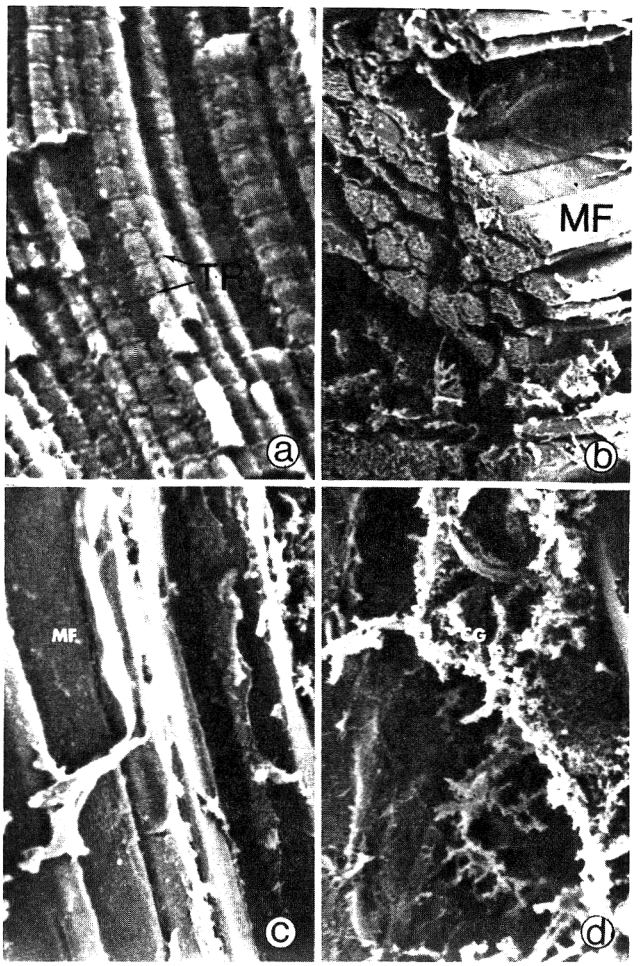

Fig. 3—Scanning electron micrographs of aged bovine semitendinosus muscle heated to 68°C internal temperature: CG, collagen granules; MF, muscle fiber; TR, transverse ridges. (a) Myofibrils (magnification x3360). (b) Bundle of muscle fibers (magnification x225). (c) Muscle fibers depicting sarcolemma degradation (magnification x336). (d) Endomysial connective tissue depicting collagen gelatinization (magnification x336).

tissue observed in Figure 2d shows the initiation of collagen coagulation. Areas of coagulation as well as intact collagen fibers suggest that the smaller collagen fibers are the first affected by heat treatment. The larger fibers appear only slightly affected. Jones et al., (1977) observed endomysial collagenous connective tissue coagulation occurred when muscle was heated to 60°C.

Semitendinosus when heated to 68°C resulted in more swelling in the area of the A-bands due to thermally induced contraction of the sarcomeres (Fig. 3a). This appears to be the only substantial change in the myofibrillar surface structure when compared to semitendinosus heated to 63°C (Fig. 2a). Transverse ridges, corresponding to the Z-line remain unaltered. Muscle fibers remain intact (Fig. 3b), although some separation between muscle fibers has taken place. Connective tissue sheaths surrounding individual muscle fibers (Fig. 3c) have undergone coagulation and are granular in appearance. Closer observation of the connective tissue (Fig. 3d) reveals the process of collagen coagulation to be more extensive than observed for 63°C-heated samples (Fig. 2d).

The greatest effects of heat were observed in the samples heated to 73°C (Fig. 4). Sarcomeres exhibit evidence that thermally induced contraction and breakage of myofibrils at the Z-area are more evident than in samples heated to either 63 or 68°C (Fig. 2a and 3a). Some transverse ridges remain intact. Surface topography of the myofibrils appears granular, evidence of protein coagulation. Perimysial covering of muscle fibers remained intact with the most obvious change being the loss of distinct connective tissue fibers (Fig. 4b). Figure 4c depicts coagulation of the sarcolemma and exposure of myofibrils. Figure 4d depicts coagulation of endomysial connective tissue.

High magnification scanning micrographs (Fig. 5) depict the effects of heating to 68°C on elastin and collagen-type connective tissues. Elastin appears as a thick sheath-like structure surrounded by coagulated collagen (Fig. 5a) and exhibits no evidence of denaturation. Figure 5b shows the beadlike appearance of coagulated collagen. Several unaffected fibers interspersed between the granular material can be seen.

The heat-induced changes in connective tissue described for the semitendinosus were also observed in the longissimus muscle samples. A major difference between these two muscles was the lack of well defined myofibrillar surface features in the longissimus as observed in the semitendino-

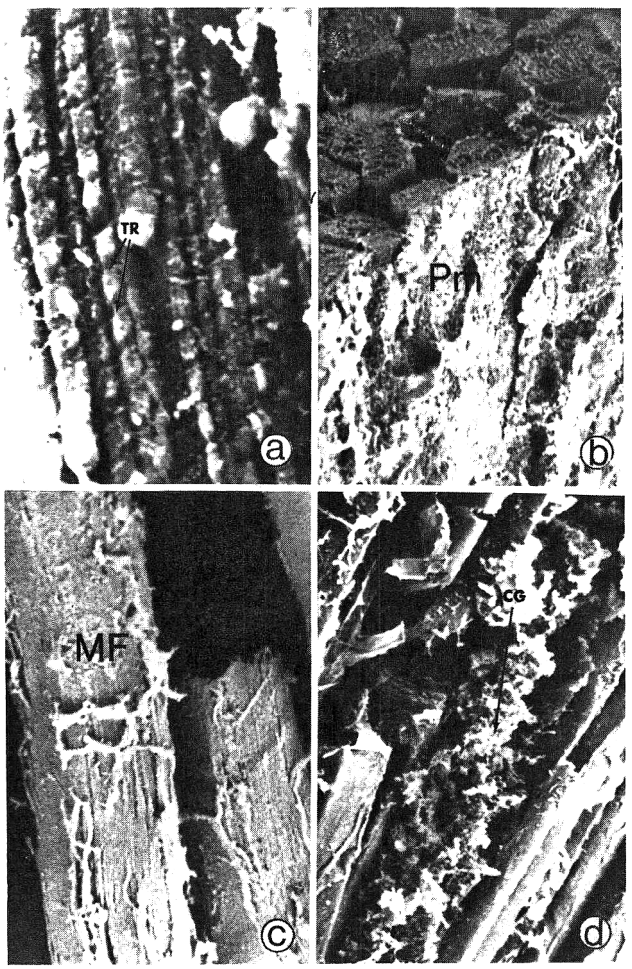

Fig. 4—Scanning electron micrographs of aged bovine semitendinosus muscle heated to 73°C internal temperature: CG, collagen granules; Pm, perimysium; TR, transverse ridges; MF, muscle fiber. (a) Myofibrils (magnification x3360). (b) Perimysium (magnification x225). (c) Muscle fibers depicting degradation of sarcolemma (magnification x560). (d) Endomysial connective tissue depicting collagen gelatinization (magnification x225).

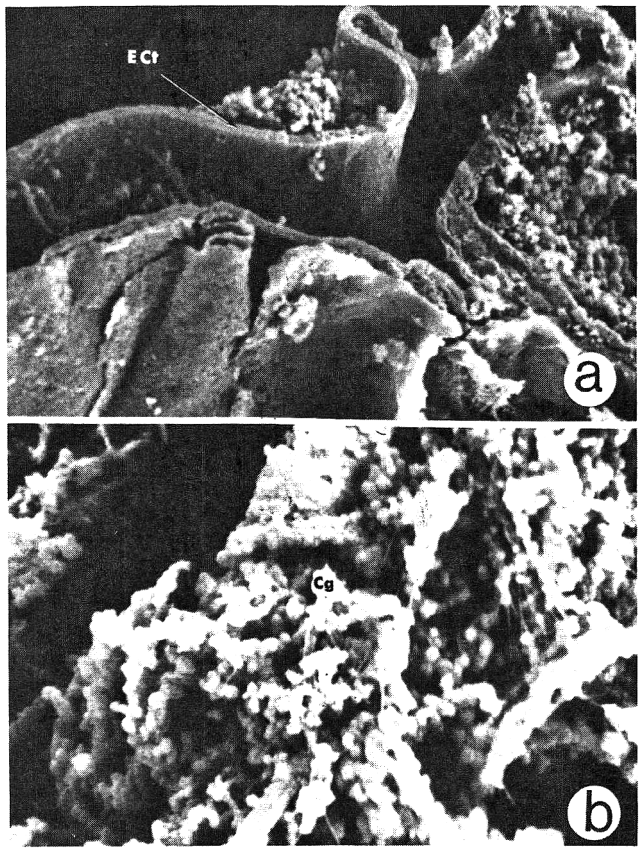

Fig. 5—Scanning electron micrographs of elastin and collagen connective tissues from bovine semitendinosus muscle heated to 63°C internal temperature: Cg, collagen granules; ECt, elastin connective tissue: (a) Elastin connective tissue (magnification x1120). (b) Collagen connective tissue (magnification x3360).

sus. The preparation of samples for scanning electron microscopic evaluation regularly exposed areas where the ssrcolemma had been removed and exposed myofibrils in the semitendinosus. However, exposed myofibrils exhibiting distinctive banding patterns did not occur as frequently in the longissimus samples and consequently these structures could not be as easily studied. The overall surface appearance of the myofibrils was granular and because of the lack of characteristic topographical features, positive identification of myofibrillar structures was difficult.

Micrographs depiciting aged unheated and heated longissimus muscle samples are presented in Figure 6. Unheated muscle fibers with connective tissue fibers are depicted in Figure 6a. When heated to 63°C the sarcolemma remained intact with some evidence of collagen coagulation on the surface of the fibers (Fig. 6b). The fibers appear orderly arranged. Neither the unheated longissimus sample or sample heated to 63°C exhibit as extensive disruption of the sarcolemma as reported by Varriano-Marston et al. (1976). When heated to 68°C the surfaces of the fibers appear granular (Fig. 6c). Coagulation of collagen fibers as well as coagulation of the sarcolemma apparently is responsible for the rough appearance of the fiber surface. Heating to 73°C (Fig. 6d) has resulted in coagulation and removal of the sarcolemma and exposed myofibrils can be observed. Some cross striations can be seen but their identity cannot accurately be determined from this micrograph.

TEM of unheated and heated muscle

Comparison of aged unheated semitendinosus (Fig. 7a) and corresponding muscle heated to 63°C (Fig. 7b) reveals myofibrillar denaturation due to heating. Z-lines, I-bands, A-bands, M-lines and myofilaments are intact in the unheated sample, whereas, the heated sample exhibits coagulation and considerable disappearance of I-band filaments and coagulation of A-band filaments. Although unheated samples exhibit some Z-line breakdown as evidenced by loss of Z-line straightness, the breakdown has proceeded further in the heated samples. Measurements of heated and unheated myofibrils indicate a reduction has occurred in sarcomere length of the heated sample (unheated sample, 2.6μ; sample heated to 63°C, 2.2μ). Most of the shortening occurred in the I-band.

The ultrastructural differences between 63°C heat treatment and 68°C heat treatment are minimal as depicted by the micrographs in Figure 7b and c. Although somewhat decreased in total number, actin filaments in the area of the I-band are still discernible. Heating to 68°C resulted in the shortening of the sarcomere to 2.0μ in length. The additional shortening appears to have occurred in the I-band. Heating to 73°C (Fig. 7d) resulted in a reduction in sarcomere length to 1.85μ. Banding patterns, although somewhat obscured, are still visible. The Z-line appears more dispersed

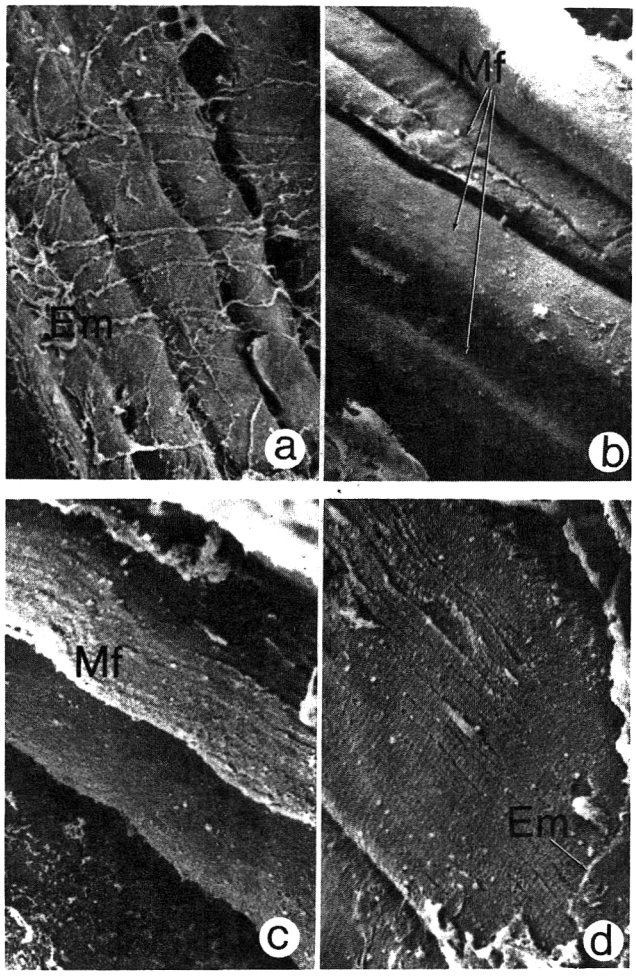

Fig. 6—Scanning electron micrographs of aged longissimus muscle unheated and heated to 63°C internal temperature: Em, endomysium; Mf, muscle fibers. (a) Aged unheated muscle fibers (magnification x375). (b) Aged muscle fibers heated to 63°C (magnification x375). (c) Aged muscle fibers heated to 68°C (magnification x1260). (d) Aged muscle fibers heated to 73°C (magnification x1260).

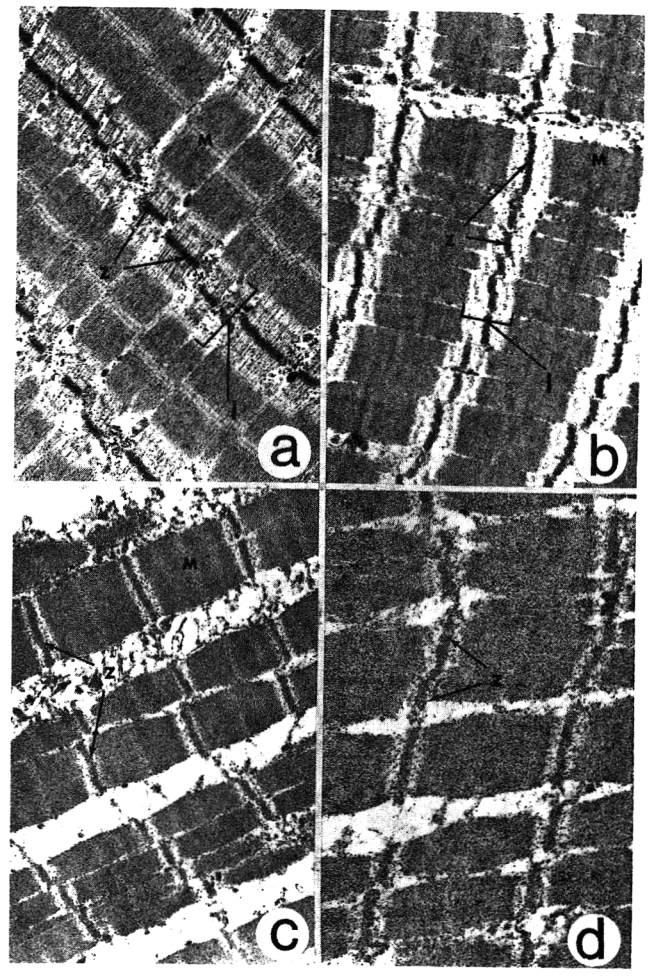

Fig. 7—Transmission electron micrographs of aged bovine semitendinosus muscle unheated and heated to 63, 68, and 73°C internal temperature: I, I-band; M, M-line; Z, Z-line. (a) Aged unheated (magnification x9,260). (b) Aged heated to 63°C (magnification x9,260). (c) Aged heated to 68°C (magnification x9,260). (d) Aged heated to 73°C (magnification x13,900).

and exhibits areas of structural weakness where Z-disc disruption appears to have occurred. In the area of the Z-disc, a corresponding adjacent area of I-band denaturation can be seen. This suggests filamental actin attachments to the Z-disc are broken by thermal treatment, whereas, the filaments in the A-band are less affected by thermal treatment. Crespo and Ockerman (1977) reported that actomyosin in avian breast and leg muscles was relatively heat stable. However, myosin when not in the actomyosin complex was very susceptible to thermally induced denaturation. These researchers suggested that actin in the actomyosin complex may have protected myosin and resulted in the increased heat resistance of the actomyosin complex.

Reaction to thermal treatment was more pronounced in longissimus samples than in semitendinosus samples. More myofibrillar denaturation is observable in the 63°C heated longissimus sample (Fig. 8b) compared to the unheated sample (Fig. 8a). Actin filament denaturation has occurred and there is evidence of thermally induced contraction in the A-band. Heating to 68°C resulted in substantial changes in ultrastructural appearance (Fig. 8c). The remaining discernible features are the rather distinct Z-discs and somewhat indistinct A-bands. Heating to 73°C (Fig. 8d) resulted in the greatest ultrastructural disruption. I-bands were not present, A-bands exhibited denaturation, but Z-discs remained rather distinct. In the center of the sarcomere an interrupted band of material, likely M-line material, is evident in Figure 8d. A 29% reduction in sarcomere length was observed when this sample was heated to 73°C (uncooked sarcomere length, 1.63μ; cooked sarcomere length, 1.17μ). A greater percentage decrease (40% of initial sarcomere length) was reported by Aronson (1966) for sarcomere length of isolated glycerinated rabbit muscle fibers when heated to 70°C. However, Hegarty and Allen (1975) observed that the percentage decrease of thermally induced sarcomere shortening was greater for fibers with longer sarcomeres than for fibers with shorter sarcomeres.

In both longissimus and semitendinosus samples an increase in longitudinal space between the myofibrils was observed when samples were heated to higher internal temperatures. This increase in space between the myofibrils was probably due in part to increased losses of moisture.

Although disruption was evident in the Z-disc, this structure remained remarkably intact in both longissimus and semitendinosus samples after 10 days aging at 0–2°C and after heating to 63, 68, or 73°C. The degree of stability of

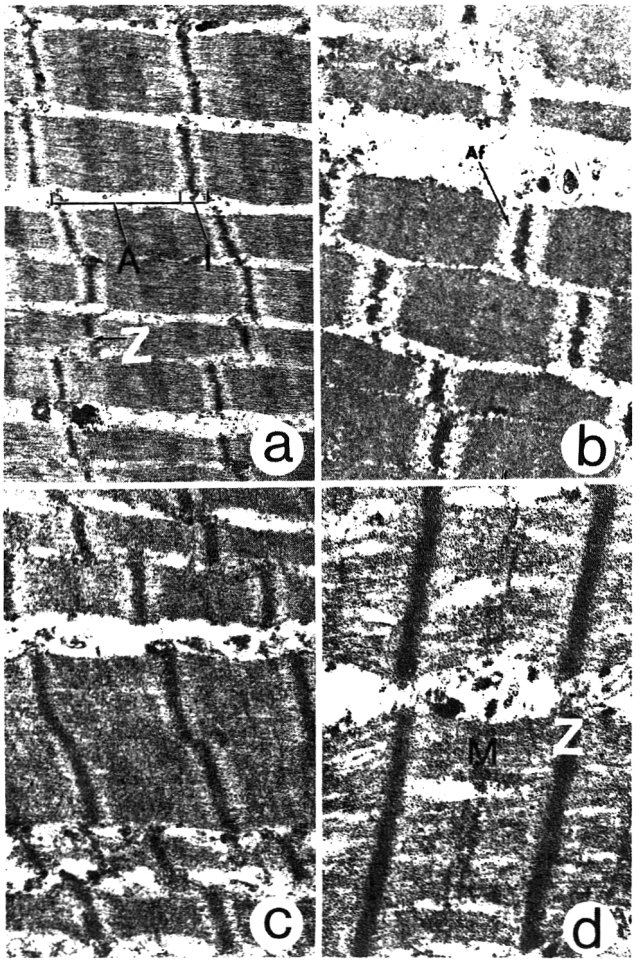

Fig. 8—Transmission electron micrographs of aged bovine longissimus muscle unheated and heated to 63, 68, and 73°C internal temperature: A, A-band; Af, actin filament; I, I-band; Z, Z-line. (a) Aged unheated (magnification x12,750). (b) Aged heated to 63°C (magnification x15,800). (c) Aged heated to 68°C (magnification x12,750). (d) Aged heated to 73°C (magnification x23,300).

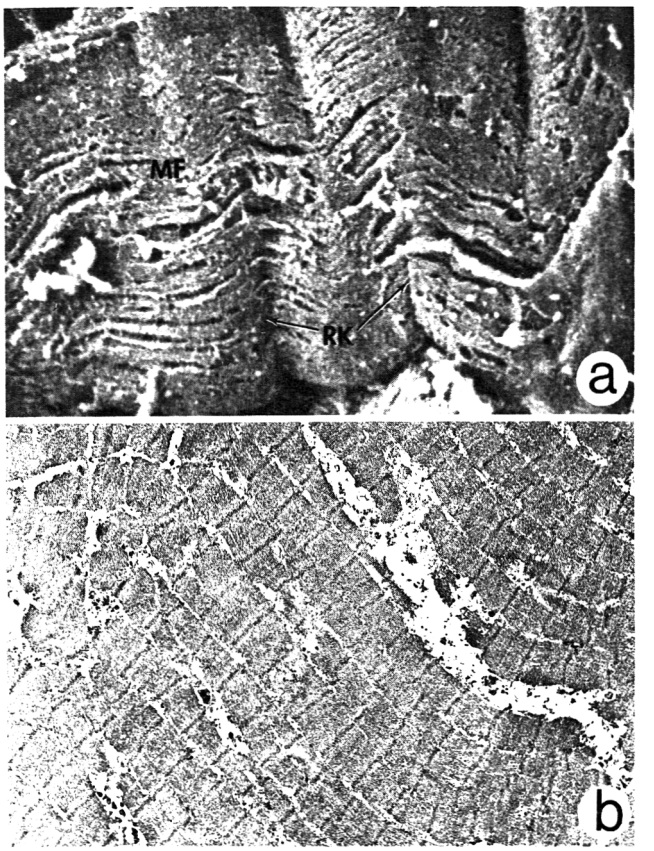

Fig. 9—Electron micrographs of aged bovine longissimus muscle heated to 73°C internal temperature: MF, muscle fiber; RK, rigor kink. (a) Scanning electron micrograph (magnification x1110). (b) Transmission electron micrograph (magnification x4350).

the Z-disc in the present study is not in complete agreement with the reports of Z-disc disintegration during postmortem storage by several other workers (Davey and Gilbert, 1967; Stromer et al., 1967; Takahashi et al., 1967). However, differences existed between the present study and the above cited reports in species of animal, muscle used, and postmortem storage conditions.

Several areas of myofibrillar denaturation due to thermal treatment (73°C) can be seen in the scanning electron micrograph of a longissimus muscle fiber (Fig. 9a). Endomysial connective tissue and the sarcolemma appear coagulated resulting in the exposure of the myofibrils. Distinct separation of myofibrils and rigor kinks can be seen. A corresponding kinked area of this sample is depicted in the transmission electron micrograph (Fig. 9b). Rigor kinks were more numerous in aged unheated longissimus muscle than in aged unheated semitendinosus muscle. Thermally induced breakage of myofibrils was more extensive in areas of rigor kinks than in areas not exhibiting rigor kinks.

The micrographs presented for the longissimus and semitendinosus are representative of all samples studied. No differences in muscle fiber ultrastructure which could be attributed to feeding regimen (0 days, 56 days, and 122 days on an ad libitum high concentrate diet) were observed with either SEM or TEM. The major difference noted was the increased presence of intramuscular adipocytes in the longissimus samples after 56 and 112 days feeding.

Although progressive alteration of muscle fiber ultrastructure occurred as samples were heated to higher internal temperatures, these changes did not result in increased muscle tenderness. Instead the samples became progressively less tender as internal temperatures were increased. The Warner-Bratzler shear values of semitendinosus samples were 2.65, 2.84, and 3.02 kg/cm^2 for 63, 68, and 73°C, respectively. Shear values of longissimus samples were 2.08, 2.30, and 2.48 kg/cm^2 for 63, 68, and 73°C, respectively, These observations are in agreement with the report of Parrish (1974).

From observations of micrographs presented, it appears that the increased toughening associated with increased cooking temperature is due to the contracted and likely hardened filamentous material present in the A-bands of the sarcomeres. Although increased disintegration of filaments occurred in the I-band as temperature was increased, these changes did not result in increased tenderness.

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INFLUENCE OF pH ON Clostridium botulinum CONTROL BY SODIUM NITRITE AND SORBIC ACID IN CHICKEN EMULSIONS

J. N. SOFOS, F. F. BUSTA, and C. E. ALLEN

ABSTRACT

The influence of hydrogen-ion concentration on the effectiveness of sodium nitrite and/or sorbic acid to control *Clostridium botulinum* growth during elevated temperature abuse (27°C) of mechanically deboned chicken meat frankfurter-type emulsions was examined. Toxin production, spore germination (loss of heat resistance) and outgrowth, residual nitrite levels, and total microbial growth (aerobic CFU) were determined at specified times during the incubation period. The effect of sorbic acid (0.2%), alone or in combination with nitrite (40, 156 μg/g), in significantly ($p < 0.05$) inhibiting spore germination, growth, and toxin production was pH dependent. This effect was not observed at pH values above 6.20 and it increased with decreasing pH. Inclusion of nitrite in the formulation increased the effective pH for sorbic acid inhibition of toxin production. The rapid rate of germination and outgrowth in both control and nitrite-containing treatments was not influenced by pH in the range examined (5.93–6.93). When sorbic acid was included in the formulation, there was a slower nitrite depletion during storage. This effect was also pH dependent and it was not observed at higher pH values (7.15). Total microbial growth was affected by decreasing pH, but not by the inclusion of nitrite and/or sorbic acid in the formulation.

INTRODUCTION

THE PRESERVATIVE, sorbic acid, and its salt, potassium sorbate, have received increased attention as possible substitutes for part or all of the nitrite in cured meat products. Several reports have shown that sorbate alone or in combination with low nitrite concentrations (e.g., 40 μg/g) extended the botulinal safety of different meat products such as bacon, comminuted pork, and chicken frankfurters (Ivey and Robach, 1978; Ivey et al., 1978; Robach et al., 1978; Sofos et al., 1979b).

The antimicrobial activity of sorbic acid is pH dependent. The amount of undissociated acid, the effective form, increases at lower pH (approaching 4.80) values (York and Vaughn, 1954; Raevuori, 1976). It was stated by Raevuori (1976) that the free acid enters the bacterial cell and inhibits several enzyme systems. Tanaka et al. (1977) examined *C. botulinum* toxin production in a pork macerate system. Sorbate did not show much botulinal inhibition at pH 6.3 but it was a rather strong inhibitor at pH 5.5. At pH 6.0 some sorbate inhibitory activity could be seen.

Addition of sorbate and especially sorbic acid to food products causes a decrease in the pH of the system. Tompkin et al. (1974) reported that uncured sausage links with 0.1% potassium sorbate had a pH of 6.4, while samples without sorbate had a pH of 7.1. Raevuori (1976) presented data showing that the addition of 0.4% potassium sorbate to the rice filling of Karelian pastry did not change the pH compared to control samples. On the other hand, the addition of 0.3% sorbic acid decreased the pH by one unit.

The effect of nitrite in delaying *C. botulinum* toxin production is increased at lower pH values. A tenfold increase in the inhibitory activity of nitrite on different bacteria was observed as the pH decreased from 7.0 to 6.0 (Castellani and Niven 1955; Eddy and Ingram, 1956; Roberts and Ingram, 1966; Perigo et al., 1967). The effects of nitrite, dextrose, and starter culture on the growth of *C. botulinum* in a summer-style sausage have been investigated by Christiansen et al. (1975). Inclusion of dextrose in the formulation, in combination with the starter culture, decreased the pH of the product during storage to a level low enough for 50 μg of nitrite per g of product to sufficiently control growth and toxin production.

Cured meat products fall within a pH range 5.6–6.6 (Lechowich et al., 1978). In emulsified, cured meats such as frankfurters, the pH is particularly important to emulsifying capacity, emulsion stability, and water retention. Therefore, the objectives of this investigation were to determine the influence of pH on sorbic acid and/or nitrite antibotulinal activity during a 27°C temperature abuse of a mechanically deboned chicken meat (MDCM) emulsified product. Residual nitrite depletion and total microbial flora changes were also determined.

MATERIALS & METHODS

Spore inoculum

The *C. botulinum* inoculum was a composite of equal numbers of five type A (36A, 52A, 62A, 77A, and 12885A) and five type B (ATCC 7949, 41B, 53B, 213B, and Lamanna B) strains sporulated and enumerated as previously described (Sofos et al., 1979b). The mixed spore suspension was heat-shocked at 80°C for 15 min, and equal volumes were used to inoculate all treatments.

Ingredients

The MDCM used was provided by Horace W. Longacre, Inc. (Franconia, Pa.) and it was handled as described by Sofos et al. (1979b). Proximate composition of MDCM, as determined by standard AOAC methods was: protein, 14.1%; fat, 17.1%; and moisture, 67.7%. Microbiological counts of raw MDCM were: aerobic total plate count (APC), in the range 1.1×10^6 to 6.8×10^6 CFU/g; heat-shocked (80°C, 15 min) APC, <10 CFU/g; and, heat-shocked (80°C, 15 min) anaerobic MPN, <2 spores/g.

Three trials (I, II, and III) were conducted, and the common ingredients of all treatments in each trial were the same as in previous studies (Sofos et al., 1979b). Formulation of treatment combinations containing sodium nitrite (Mallinckrodt, Inc., St. Louis, Mo.) and sorbic acid (Monsanto Co., St. Louis, Mo.) was based on the total batch weight (1500g).

Product formation and processing and pH adjustment

The raw ingredients were mixed, inoculated, emulsified, processed, and incubated (27°C) according to procedures described previously (Sofos et al., 1979b).

The pH of the treatments was measured with the aid of an Orion Research pH meter and a probe electrode directly placed in the mixture. It was adjusted to higher or lower levels to provide desired comparisons in order to determine the effect of pH on the nitrite and sorbic acid activity on *C. botulinum* growth and toxin production. Solutions of 0.37M KH_2PO_4 (pH 4.35) and 1M K_2HPO_4 (pH 9.80) were used to decrease or increase the pH of the mixtures, respectively. The same total volume of water, KH_2PO_4, and/or

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K_2HPO_4 was added to all treatments, and in each formulation was 13% of the total weight. Part of the water was used to incorporate the spore suspension, nitrite, and other ingredients into the mixtures. The values given (Fig. 1) represent the pH of each particular treatment after cooking and before temperature abuse of the products.

Two batches of the 40 μg/g nitrite-0.2% sorbic acid treatment of trial III (Fig. 1e) were made. One was extruded into test tubes (Sofos et al., 1979b), as were all other treatments of the study, and the second was extruded into 22 mm cellulose casings. After cooking, the casing samples were peeled, individually vacuum packaged, and incubated along with the test tube samples of the remaining treatments.

Sampling, toxicity testing and residual nitrite analysis

Methods for treatment sampling, residual nitrite analysis, and toxicity testing have been detailed by Sofos et al. (1979b).

Microbiological analyses

Microbiological counts were determined on one sample per treatment and testing time. Eleven grams of sample were blended with 99 ml of 0.1% peptone diluent. A portion of this 1:10 dilution was heat-shocked (80°C, 15 min) before MPN spore count determinations. The remaining unheated portion of the blend was used for spore plus vegetative cell MPN counts and total aerobic plate count (APC) determinations.

The method employed by Christiansen et al. (1978) was used to

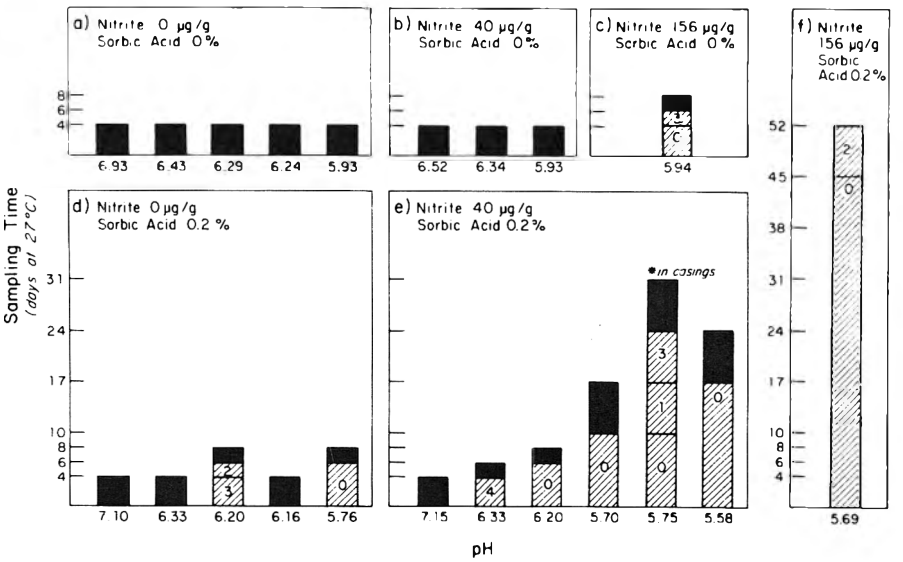

Fig. 1—Influence of pH on the effectiveness of nitrite and/or sorbic acid to control Clostridium botulinum toxin production in mechanically deboned chicken meat (MDCM) frankfurter-type emulsions during 27°C temperature abuse. Numbers represent toxic samples out of five tested, and dark bars indicate that all samples (5) tested were toxic.

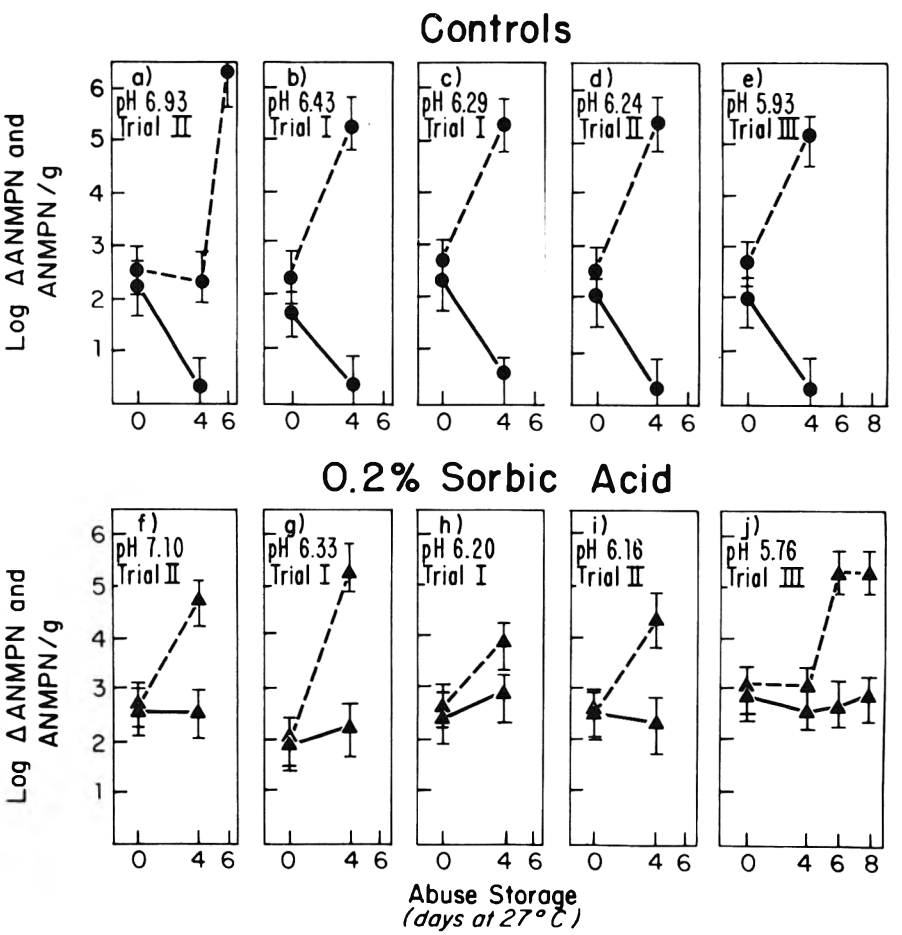

Fig. 2—Influence of pH in control (nitrite/sorbic acid-free) and 0.2% sorbic acid-containing treatments on Clostridium botulinum spore (ΔANMPN-continuous lines) and spore plus vegetative cell (ANMPN-broken lines) counts in mechanically deboned chicken meat (MDCM) frankfurter-type emulsions during 27°C temperature abuse.

determine spore and spore plus vegetative cell counts after heat processing and during temperature abuse of the products. Serial dilutions of the 1:10 heated or unheated dilutions were made, and five tubes of modified peptone colloid medium (Greenberg et al., 1966) were inoculated per dilution. The tubes were capped with vaspar and incubated at 37°C for 7 days. Blackening and putrid aroma of the recovery medium inoculated with cell-free (heat-shocked) or cell-containing (unheated) samples were considered as evidence of botulinal spore or spore plus vegetative cell growth, respectively (Christiansen et al., 1978).

The heat-shocked anaerobic most probable number (ΔANMPN) counts and the unheated anaerobic most probable number (ANMPN) counts were expressed per gram of sample and considered to represent *C. botulinum* spore and *C. botulinum* spore plus vegetative cell counts, respectively. The logarithms of ΔANMPN/g and ANMPN/g (with lower and upper limits) were plotted versus time of temperature (27°C) abuse. For each treatment the last MPN plotted represents the time at which samples of that specific treatment were first found to contain botulinal toxin. An overlapping of the confidence limits of subsequent MPN determinations for a given treatment through the incubation period was considered as evidence of no statistically significant changes in *C. botulinum* spore germination and/or outgrowth. When the confidence limits do not overlap and a decrease in ΔANMPN is shown, significant ($p < 0.05$) spore germination (loss of heat resistance) had occurred.

Total microbial growth (APC), representing mesophilic microorganisms that could grow anaerobically, was determined using standard plate count agar (Difco), and the plates were counted after 48 hr at 37°C.

RESULTS & DISCUSSION

Botulinal growth and toxin production

All five samples tested for each of five control treatments (nitrite/sorbic acid-free) examined at five different pH values (Fig. 1a) ranging from 5.93–6.93 were toxic after 4 days at 27°C. The spore germination and outgrowth results shown in the upper portion of Figure 2 indicate that, in these control treatments, the *C. botulinum* spores germinated (lost heat resistance) quickly and cell outgrowth also occurred rapidly. Similar germination and outgrowth rates were observed at all product pH levels tested, except in part (a) of Figure 2 (pH 6.93) where outgrowth was not observed at 4 days but was high after 6 days of incubation. This discrepancy cannot be readily explained. Based on earlier reports, it was not expected that the pH range studied here (5.93–6.93) would show any inhibitory effect on *C. botulinum* toxin production. The pH of the substrate should be lower than 5.0 and approaching 4.6 in order for botulinal growth to be retarded (Ingram and Robinson, 1951; Townsend et al., 1954). However, these control results (Fig. 1a and upper portion of Fig. 2) were necessary to determine the effects due to nitrite and/or sorbic acid at similar pH levels.

The results shown in Figure 1b indicate that in a product pH range of 5.93–6.52, a nitrite concentration of 40 μg per g of product did not result in any delay in toxin production. The 156 μg/g nitrite level (Fig. 1c) was tested only at one pH value (5.94), and it resulted in a delay in toxin production. Data not presented here indicated that with the nitrite concentrations tested and at the pH values examined, nitrite had no inhibitory effect on *C. botulinum* spore germination. Thus, the delay in toxin production observed in the presence of the higher nitrite concentration (156 μg/g), must have been caused by the inhibitory effect of a higher residual nitrite level, or some related compound, acting on some form of spores or cells after loss of heat resistance and before toxin release (Christiansen et al., 1978; Tompkin et al., 1978a, b).

The results shown in Figure 1d indicate that in chicken frankfurter-type emulsions, ranging in pH from 6.16–7.10, sorbic acid at the 0.2% level was ineffective in delaying *C. botulinum* toxin production with toxic samples detected on the first testing time of 4 days. At the next lower pH value (5.76) tested, toxin was detected after 8 days of temperature abuse. The results shown in the lower portion of Figure 2 demonstrate that botulinal outgrowth, similar to toxin production, was also pH dependent when sorbic acid (0.2%) was included in the formulation. Spore germination, (loss of heat resistance), on the other hand, appeared to be signficantly ($P < 0.05$) inhibited by 0.2% sorbic acid at all product pH values (7.10, 6.33, 6.20, 6.16, and 5.76) tested (Fig. 2, lower portion). Considering the vegetative cell growth (Fig. 2, lower portion) and the toxicity results (Fig. 1d), it would be incorrect to conclude that sorbic acid (0.2%) completely inhibited *C. botulinum* spore germination. Its inhibitory effect, however, was statistically significant ($P < 0.05$) with the MPN procedure used, but inadequate to assure indefinite botulinal safety of the products under abuse conditions. The outgrowth and toxicity results imply that a small number of spores, nondetectable as significant changes by the MPN procedure, germinated even when sorbic acid was included in the formulation and multiplied with eventual production of toxin (Sofos et al., 1979a). These results (Fig. 1d and lower portion of Fig. 2) clearly demonstrate that the effectiveness of sorbic acid in delaying *C. botulinum* growth and toxin production in emulsified MDCM products is pH dependent. It can also be concluded that this effect was not due to lower pH values being restrictive to botulinal growth. However, a lower pH value, besides enhancing sorbic acid activity, could also act synergistically with sorbic acid in delaying toxin production. No treatments were included to study the pH range between 5.76–5.16. Nevertheless, it appears that with the sorbic acid concentration tested (0.2%) and for the product and conditions described, the pH of the substrate should be below 6.0 before any effect from sorbic acid alone can be expected.

An attractive alternative to the use of present nitrite concentrations in meat curing would be the use of lower than presently employed nitrite levels in conjunction with sorbate. Such combinations could retain the desirable characteristics (color and flavor) of cured meat products, and would lower the presently higher nitrite levels required to delay *C. botulinum* toxin production. An important aspect to be considered before such alternatives are adopted is the role of pH in such systems. The data shown in Figures 1e and 3 clearly indicate that the inhibitory activity of 40 μg/g nitrite-0.2% sorbic acid in controlling *C. botulinum* growth and toxin production was pH dependent. According to the results, the inhibitory effect increased with decreasing pH. The system was effective at a product pH value of 6.20 but not at a value of 6.33. A doubling of the time necessary for toxin to be formed was recorded as the pH of the product decreased from 6.33 to 6.20. At the lowest pH value (5.58) examined, a sixfold increase of delay in toxin production was found when compared to the ineffective treatment of ph 6.33.

A comparison of the toxicity results of treatments with similar pH values from Figures 1a–1e indicates that the delay in toxin production observed in 40 μg/g nitrite-0.2% sorbic acid formulations was not a direct effect of lower pH. The inhibitory activity of 40 μg/g nitrite-0.2% sorbic acid, at appropriate pH values, was greater than that of 0.2% sorbic acid alone at similar pH values, and certainly larger than that of the ineffective 40 μg/g nitrite treatments, and the high (156 μg/g) nitrite level. However, an important finding was that the effectiveness of 40 μg/g nitrite-0.2% sorbic acid, although pH dependent, was observed at pH values higher than those where 0.2% sorbic acid used singly was effective. These higher pH values extend the range of effectiveness and increase the importance of nitrite-sorbic acid alternatives as botulinal inhibitors in cured meats. The higher the pH at which the inhibitory

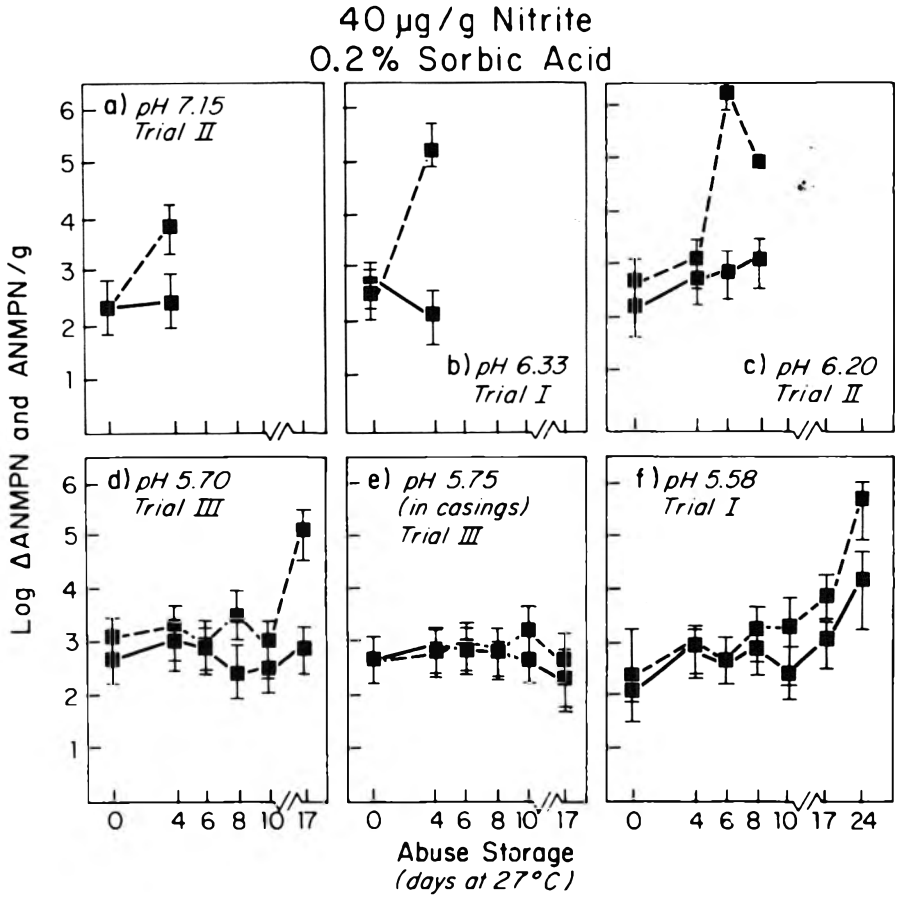

Fig. 3—Influence of pH in treatments formulated with 40 µg/g nitrite-0.2% sorbic acid on Clostridium botulinum spore (ΔANMPN-continuous lines) and spore plus vegetative cell (ANMPN-broken lines) counts in mechanically deboned chicken meat (MDCM) frankfurter-type emulsions during 27°C temperature abuse.

activity starts, the greater the potential for use of antibotulinal agents including the above nitrite (40 µg/g)-sorbic acid (0.2%) combination. Besides the nitrite and/or sorbic acid-free controls and the 156 µg/g nitrite treatment, a 156 µg/g nitrite-0.2% sorbic acid formulation was included for comparison purposes (Fig. 1f and 4). At a pH value of 5.69, this combination greatly increased product safety over that of 156 µg/g nitrite alone or the lower (40 µg/g) nitrite-0.2% sorbic acid treatments. This indicated that the effectiveness of 40 µg/g nitrite-0.2% sorbic acid formulations can be further expanded by increasing the nitrite concentration. However, high nitrite formulations may be impractical in view of current considerations to lower or ban the use of nitrite in meat products.

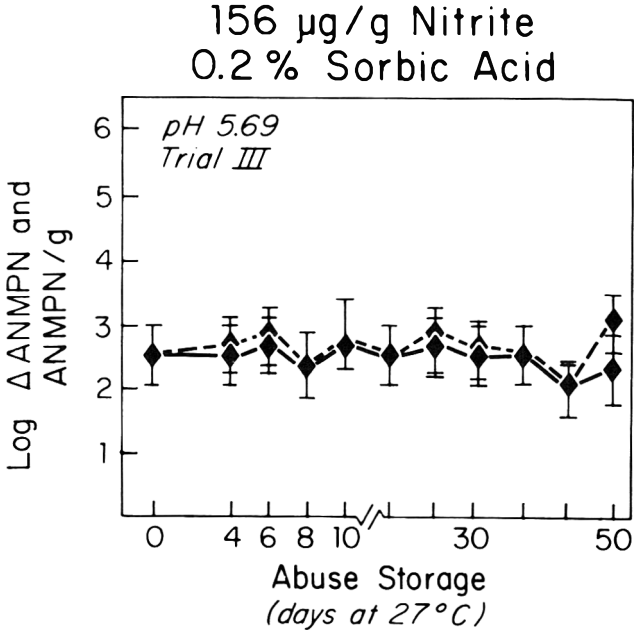

Fig. 4—Effect of 156 µg/g nitrite-0.2% sorbic acid on Clostridium botulinum spore (ΔANMPN-continuous line) and spore plus vegetative cell (ANMPN-broken line) counts in a mechanically deboned chicken meat (MDCM) frankfurter-type emulsion during 27°C temperature abuse.

A comparison of the data of the two 40 µg/g nitrite-0.2% sorbic acid treatments with similar pH values (5.70 and 5.75—Fig. 1e and 3) indicates that results similar to those recorded from test tube experiments can be expected from samples handled in a way customary to industry practice. However, the number of toxic samples from the "casing" treatment increased more slowly than the number of toxic test tube samples. An increased product contamination during peeling and packaging could have resulted in some competition to the growth and toxin production by *C. botulinum*.

The inhibitory effect of 0.2% sorbic acid on germination, as determined by the MPN procedure, appeared to be similar at all pH values tested (Fig. 2, 3, and 4). This was in contrast to the sorbic acid inhibition of cell outgrowth (Fig. 2, 3, and 4) which was pH dependent similar to the toxicity findings (Fig. 1). Generally, at the time that growth occurred during the incubation period, toxic samples were also detected. For sorbic acid-containing treatments, the length of the incubation period before appearance of toxic samples was dependent on the pH of the product, the absence or presence of nitrite, and the level of nitrite present (Fig. 1). The same factors influenced the rate of *C. botulinum* vegetative growth (Fig. 2, 3 and 4). Spore germination might also have been affected by all or some of the

factors that influenced the rate of growth and toxin production. The system studied was too complicated and the methodology was inadequate for identification of these factors and possibly differentiation among *C. botulinum* strains included in the inoculum. The importance and need to obtain this information would justify extensive research on this aspect of germination and outgrowth. Furthermore, the low germinating spore level permits a very important conclusion. A very small number of botulinal spores under the right conditions are capable of growing at a rapid rate and to an extent that detectable toxin is produced. This would support the necessity for the addition of *C. botulinum* growth inhibitors, such as nitrite and sorbic acid, in products of the type examined.

Residual nitrite

Treatments formulated without nitrite were analyzed and found to contain very low (<1 μg/g) nitrite levels. The results presented in Figure 5 indicate that when sorbic acid was included in nitrite-containing treatments, residual nitrite levels during product abuse were higher than those found in treatments formulated with nitrite only. These results confirm previous findings reported by Sofos et al. (1979b). Observations from a single trial indicate that the effect of sorbic acid on nitrite depletion might be pH dependent. Figure 5b shows residual nitrite depletion in one formulation tested at two different pH values (6.20 vs 7.15) in the same trial (II). It appears that at the lower product pH level (6.20) residual nitrite depletion was slower. The reason(s) for such an effect and its relevance to the botulinal safety of nitrite and sorbic acid-treated products is unknown and should be examined more extensively. Such a finding was unexpected since at lower pH values nitrite would be expected to form nitrous acid and disappear more rapidly than at higher pH values.

As shown in Figures 1e, 1f, 3, and 4, inclusion of nitrite in the formulation increased the effectiveness of sorbic acid in retarding growth and toxin production. It also increased the pH value at which these effects were observed. The mechanism(s) for these actions is not known. As postulated earlier (Sofos et al., 1979a, b) there are at least two possible explanations for the observations. First, sorbic acid inhibits spore germination and retards outgrowth, especially at lower pH values (Fig. 2). In addition, there is also evidence that sorbic acid delays nitrite depletion (Fig. 5). These two sorbic acid effects could be additive or more probably synergistic since the nitrite (40, or 156 μg/g)-sorbic acid (0.2%) combination effects were larger than the combined effect of nitrite and sorbic acid used singly (Fig. 1). Another possible mode of action could be the previous speculation by Sofos et al. (1979b) that sorbic acid and nitrite could react and form some compound(s) more inhibitory to *C. botulinum* than either of them individually. These hypothetical compound(s) could be degraded with time until a noneffective level was achieved. Such a degradation could be regenerating nitrite and be responsible for the higher residual nitrite levels observed during product abuse. The results in Figure 5b would suggest that the speculated reaction between nitrite and sorbic acid might be pH dependent. The similarity of the rates of nitrite depletion in the high pH (7.15) 40 μg/g nitrite-0.2% sorbic acid formulation of trial II (Fig. 5b) and the rates in the 40 μg/g nitrite-0% sorbic acid treatments of other trials (Fig. 5a and 5b), would indicate that no nitrite-sorbic acid reaction occurred at higher (7.15) pH values. However, the possibility exists that at lower pH values (6.20) sorbic acid inhibited the development of certain microorganisms potentially responsible for nitrite depletion. The increased effects observed in the presence of 156 μg/g nitrite could be due either to more residual nitrite present for a longer time or to a higher concentration of the possible compound(s) formed from

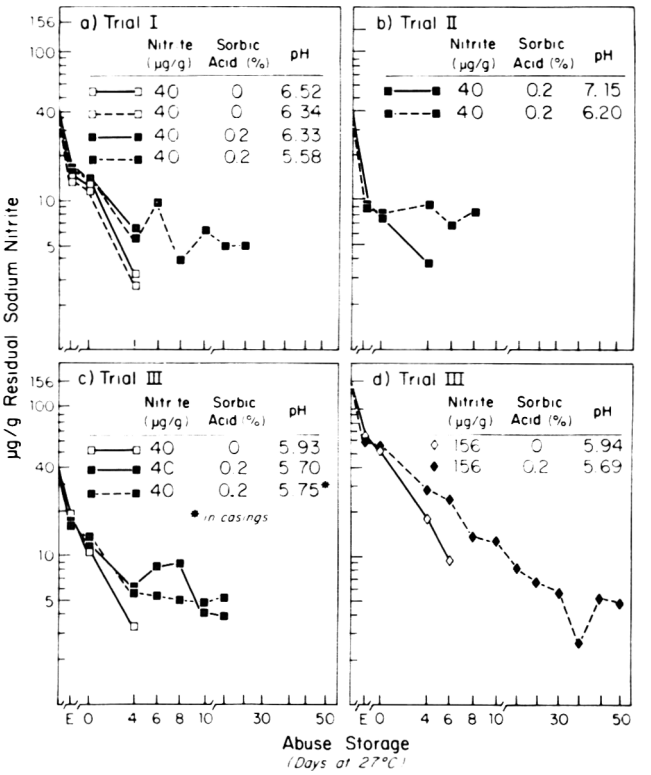

Fig. 5—Effects of pH and sorbic acid on nitrite depletion in mechanically deboned chicken meat (MDCM) frankfurter-type emulsions during processing and 27°C temperature abuse. E, uncooked emulsions.

nitrite and sorbic acid. The above statements constitute logical and justified speculations derived from the results reported. The data presented are insufficient to prove these possibilities and more research is necessary to determine the mechanism(s) of action.

Total microbial growth

Previous work (Sofos et al., 1979a) indicated that sorbic acid depressed total microbial growth during temperature abuse of the same product. It was not evident whether the depressed total growth was the result of sorbic acid, or was due to a lowering of the pH of the product from the addition of sorbic acid. Tompkin et al. (1974), in uncured sausage links found that 0.1% potassium sorbate delayed normal spoilage flora and *Staphylococcus aureus* growth by one day, and *Salmonella* growth was markedly retarded. The pH of the potassium sorbate samples was 6.4 compared to 7.1 for the controls. Inhibition of pathogenic microorganisms such as salmonellae and *S. aureus* by sorbic acid would be favorable, while a depression of normal growth and product spoilage beyond the time of *C. botulinum* toxin production would be unacceptable. A normal appearing product might be used by the consumer, while a spoiled one would normally be discarded and thus a possible intoxication avoided.

Data collected but not presented here demonstrated that the effect of sorbic acid in retarding total microbial growth in the product (Sofos et al., 1979a) was due to a lower pH value. At similar pH values, all treatments including controls, nitrite, and/or sorbic acid-containing samples, showed similar rates and extents of total growth. At pH values below 6.20, the rates were depressed and growth was delayed similarly in all formulations. This would indicate that below pH 6.20 some organism(s) sensitive to these lower pH levels were affected in all treatments. However, different organ-

isms could be affected by sorbic acid and by nitrite. Generally, addition of sorbic acid, as well as nitrite, to emulsified frankfurter-type products for control of *C. botulinum* growth and toxin production would not retard growth of normal spoilage bacteria beyond the time of botulinal safety of these products.

CONCLUSIONS

THE INHIBITION of *C. botulinum* spore germination, outgrowth, and toxin production by sorbic acid (0.2%) in mechanically deboned chicken meat (MDCM) frankfurter-type emulsions was pH dependent. The data indicated that levels below the value of 6.0 were necessary for the effects to appear.

Inclusion of nitrite (40 μg/g) in the formulation increased the pH (6.20) at which the sorbic acid effectiveness developed. Nitrite (40 μg/g) also increased the extent of the effects of sorbic acid. This influence was significant, because not only was a wider pH range included for product safety but also the margin of safety was increased, and the nitrite level could be significantly reduced over that of present formulations. Since sorbic acid-containing samples eventually become toxic, without statistically significant numbers of germinated spores being detected in the low initial spore level (<300/g), it appeared that an extremely low number of *C. botulinum* spores were capable of growing and rendering the product toxic. Such an observation strongly supports the necessity, before any final decisions are reached on the banning or reduction of nitrite concentrations in cured meats, to find and endorse alternative means of preservation to assure botulinal safety of the products.

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CHANGE OF REGULATORY ACTIVITY OF TROPOMYOSIN AND TROPONIN ON ACTO-HEAVY-MEROMYOSIN ATPase DURING POSTMORTEM STORAGE OF MUSCLE

Y. IKEUCHI, T. ITO and T. FUKAZAWA

ABSTRACT

The effect of regulatory proteins on the actin-myosin interaction during postmortem storage of muscle was investigated by using a reconstituted complex of actin, heavy-meromyosin (HMM), tropomyosin and troponin. In the absence of calcium ions, the acto-HMM ATPase activity was maximally inhibited by tropomyosin and troponin, from both at-death and 168 hr postmortem muscles, at a molar ratio of tropomyosin and troponin to actin of more than 0.2. In addition, there was no apparent difference in the molar ratio required for inhibiting the ATPase activity between the regulatory proteins from at-death and 168 hr postmortem muscles. The pCa-dependent ATPase activity of the reconstituted complex prepared from 168 hr postmortem muscle (168 hr acto-HMM and 168 hr tropomyosin and troponin) was higher than that of at-death muscle (0 hr acto-HMM and 0 hr tropomyosin and troponin) and consequently the curve was shifted toward lower calcium ions concentrations. However, little difference was found in Ca^{++}-sensitivity of the regulatory proteins between at-death and 168 hr postmortem muscles. SDS-polyacrylamide gel electrophoretograms showed that there was a slight change of myosin structure and a noticeable degradation of troponin from 168 hr postmortem muscle compared to at-death muscle. These results suggest the possibility that the increase in myofibrillar ATPase activity during postmortem storage of muscle is mainly due to the increase in the actin-myosin interaction.

INTRODUCTION

IN A PREVIOUS PAPER (Ikeuchi et al., 1978), we showed (1) an increase in myofibrillar ATPase activity during postmortem storage of muscle and (2) an increase in Ca^{++}-sensitivity of myofibrils; that is a shift of the pCa-dependent myofibrillar ATPase curve (a regulation curve representing the relation of myofibrillar ATPase activity to the concentrations of free calcium ions) toward lower concentration of calcium ions. The former phenomenon (1 above) has also been observed by other research workers (Goll and Robson, 1967; Greaser et al., 1969; Cheng and Parrish, 1978). Ito et al. (1978) concluded that the cause of this phenomenon was a change of the actin-myosin interaction during postmortem storage. We also suggested in a previous paper (Ikeuchi et al., 1978) that the latter phenomenon (2 above) is due to either (1) the increase in the affinity of actin to myosin which activates myofibrillar ATPase, resulting in the increase in Ca^{++}-sensitivity of myofibrils or (2) the degradation of regulatory proteins, probably due to a Ca^{++}-activation factor (Dayton et al., 1976), which brings about the increase of the actin-myosin interaction and consequently shifts the pCa-dependent curve of myofibrillar ATPase. The former consideration is consistent with the proposal given by Bremel and Weber (1972), Solaro and Briggs (1974) and Moos (1972). However, little has been published about the postmortem change in the function of regulatory proteins on the actin-myosin interaction, although the electrophoretic behavior of regulatory proteins, ATPase activity and superprecipitation of myofibrils and myosin B have been reported by several workers (Fujimaki et al., 1965; Arakawa et al., 1970; Goll and Robson, 1967; Olson et al., 1977).

In the present study, we compared the effect of regulatory proteins on the actin-myosin interaction between at-death and postmortem muscles. Reconstituted complex of actin, heavy-meromyosin (acto-HMM), tropomyosin and troponin prepared from at-death and 168 hr postmortem muscles were used in the present study. This approach provided evidence for elucidating the postmortem change of myofibrillar proteins.

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MATERIALS & METHODS

Materials

Rabbits (about 2.5–3 kg) were anesthesized with sodium pentobarbital and d-tubocurarine chloride. Longissimus and white hind leg muscles were excised from the carcasses within 15 min after exsanguination and served as at-death muscle. Other carcasses sacrificed as above were soaked in 10 mM sodium azide to retard bacterial growth, wrapped in polyethylene bags and stored at 0°C for 1 wk. After storage, the same portions as at-death muscle were excised from the 168 hr postmortem carcasses.

Myofibrillar proteins

Myosins from postmortem muscles were extracted using a modified Guba-Straub solution (0.3M KCl, 0.15M K-phosphate, 2 mM ethyleneglycol-bis-(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 5 mM $MgCl_2$ and 5 mM ATP, pH 6.5). Actin was prepared from acetone dried powder according to the procedure of Spudich and Watt (1971). Polymerization of G-actin was carried out by dialyzing against about 100 vol of dialyzing solution containing 50 mM KCl, 0.5 mM β-mercaptoethanol and 10 mM Tris-maleate (pH 7.0) overnight at 0°C. On measuring pCa-dependent acto-heavy-meromyosin (acto-HMM) ATPase activity the dialyzing solution was exchanged four times with the same solution to eliminate the contaminating free calcium ions in the actin preparation after full polymerization of G-actin. Myosin was prepared according to Tonomura et al. (1961) as described previously (Ito et al., 1978) and stored at −30°C in 50% glycerol solution containing 1 mM ethylenediaminetetraacetic acid (EDTA) until use. After the exclusion of glycerol by dilution, HMM was prepared by the method of Lowey and Cohen (1962). Tropomyosin was obtained using the procedure of Mueller (1966), except that ammonium sulfate fractionation was done between 50–70% saturation. Troponin was prepared by the method of Ebashi et al. (1971). Troponin and tropomyosin preparations were stored at −30°C in the presence of 1 mM β-mercaptoethanol and used within 3 months.

ATPase activity

The steady state acto-HMM ATPase activity was measured in a reaction mixture containing 50 mM KCl, 1 mM $MgCl_2$, 2 mM ATP, 10 mM Tris-maleate (pH 7.0), either 1 mM EGTA or 0.5 mM $CaCl_2$, 0.4 mg/ml HMM, 0.2 mg/ml actin and varying concentrations of troponin and tropomyosin.

The pCa-dependent acto-HMM ATPase was measured under the condition of 50 mM KCl, 1 mM $MgCl_2$, 2 mM ATP, 20 mM Tris-maleate (pH 6.8), 0.6 mg/ml HMM, 0.3 mg/ml actin, 0.145 mg/ml tropomyosin, 0.175 mg/ml troponin and varying concentrations of free calcium ions (pCa). In both cases molar concentration of troponin was equal to that of tropomyosin. The reaction was evaluated at 25°C for 8 min with sampling at 2 min intervals and stopped by adding an equal vol of 10% trichloroacetic acid. The liberated phos-

phorus was determined by the method of Fiske and Subbarow (1925).

For determining the molar concentration, the following molecular weights were conveniently used for this study: tropomyosin, 68,000; troponin, 81,500 and actin, 42,000 (Woods, 1969; Hartshorne and Dreizen, 1972; Elzinga et al., 1973). The calculation of pCa was made as described in a previous paper (Ikeuchi et al., 1978). All of the ATPase measurements were done in triplicate.

SDS polyacrylamide gel electrophoresis

All of the protein preparations were analyzed by SDS polyacrylamide gel (10%) electrophoresis according to the procedure of Weber and Osborn (1969). Prior to the electrophoresis, the protein preparations were dialyzed against 10 mM Na-phosphate buffer (pH 7.0) containing 0.1% SDS and 10 mM β-merceptoethanol overnight at 20°C.

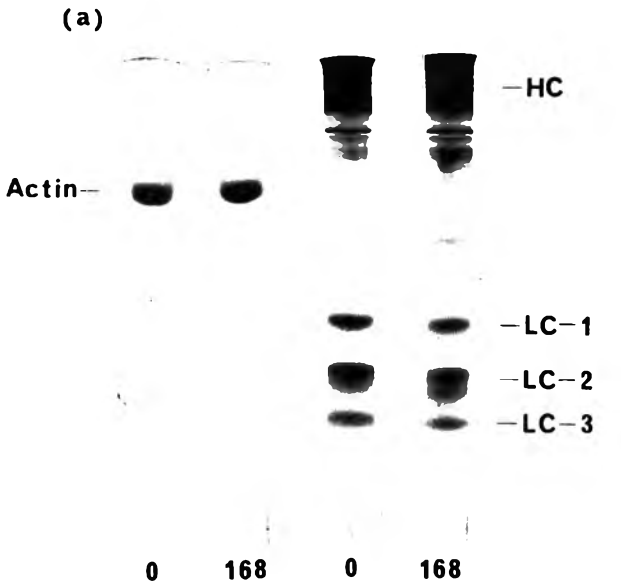

Fig. 1a—SDS gel electrophoretograms of myosin and actin prepared from at-death and 168 hr muscles. 100 μg of myosin and 20 μg of actin were loaded on each gel, respectively. HC, heavy chain; LC, light chain.

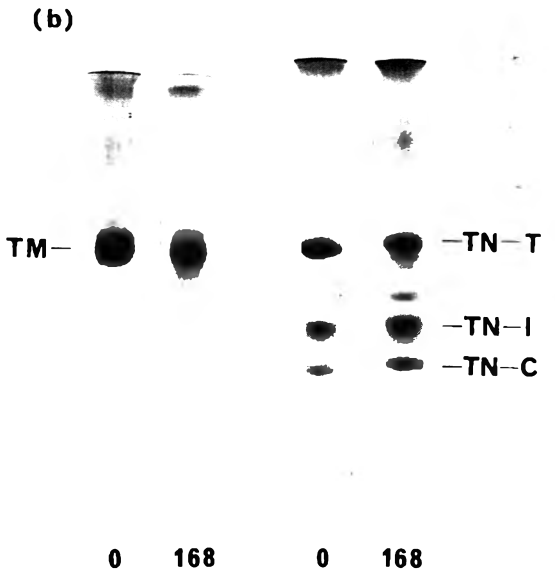

Fig. 1b—SDS gel electrophoretograms of the regulatory proteins prepared from at-death and 168 hr muscles. 15 μg of troponin and 10 μg of tropomyosin were loaded on each gel, respectively. TN-T, troponin-T; TN-I, troponin-I; TN-C, troponin-C; TM, tropomyosin.

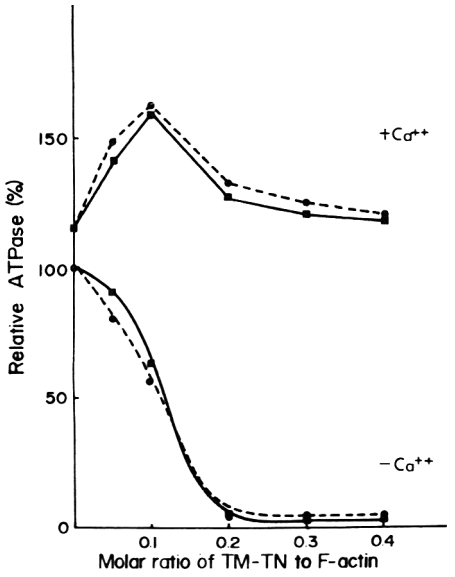

Fig. 2a—Effect of regulatory proteins prepared from at-death and 168 hr postmortem muscles on the ATPase of acto-heavy-meromyosin prepared from at-death muscle. 100% ATPase = 0.097 ± 0.005 μmole Pi per min per mg HMM [means ± S.E.M. (n=3)]. Solid lines (■—■), the regulatory proteins prepared from at-death muscle (0 hr acto-HMM + 0 hr tropomyosin-troponin); dotted lines (●--●), the regulatory proteins prepared from 168 postmortem muscle (0 hr acto-HMM + 168 hr tropomyosin-troponin).

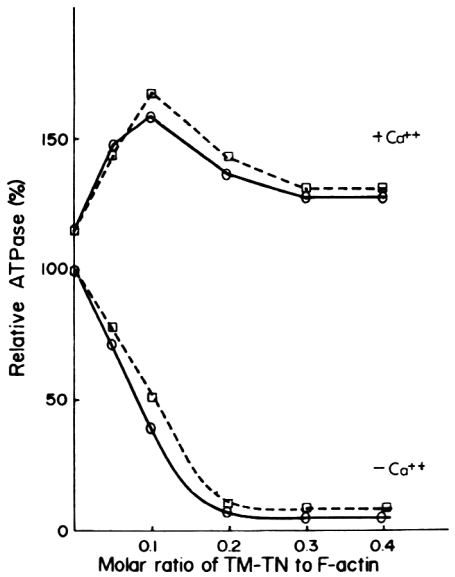

Fig. 2b—Effect of regulatory proteins prepared from at-death and 168 hr postmortem muscles on the ATPase of acto-heavy-meromyosin prepared from 168 hr muscle. 100% ATPase = 0.111 ± 0.002 μmole Pi per min per mg HMM [means ± S.E.M. (n=3)]. Solid lines (○—○), the regulatory proteins prepared from at-death muscle (168 hr acto-HMM + 0 hr tropomyosin-troponin); dotted lines (□--□), the regulatory proteins prepared from 168 hr muscle (168 hr acto-HMM + 168 hr tropomyosin-troponin).

Protein concentration

Protein concentration was determined by the biuret reaction of Gornall et al. (1949) which had been standardized with bovine serum albumin.

RESULTS

FIGURE 1 shows SDS-polyacrylamide gel electrophoretograms of actin, myosin, tropomyosin and troponin prepared from at-death and 168 hr postmortem muscles. Some minor bands between the heavy chain and light chain were found in myosin from 168 hr postmortem muscle as has already been pointed out by Ito et al. (1978) (Fig. 1a). However, it is not clear whether these bands are due to the breakdown products of myosin or some other myofibrillar proteins. While, a noticeable degradation of troponin occurred during 168 hr postmortem storage as has been reported by Olson et al. (1977) (Fig. 1b). On the other hand, no change was observed in the electrophoretograms of actin and tropomyosin during this extended postmortem storage of muscle (Fig. 1a and b).

Figure 2 shows the relative activity of the acto-HMM ATPase as a function of the molar ratio of tropomyosin and troponin to F-actin in the presence or absence of free calcium ions. The acto-HMM ATPase activity in the presence of calcium ions was higher than that in the absence of calcium even when there were no regulatory proteins in the reaction mixture.

In the absence of free calcium ions, the acto-HMM ATPase activity was decreased with increasing molar ratio of the regulatory proteins to F-actin, and was maximally inhibited at the molar ratio of about 0.2 or more for the regulatory proteins both from at-death and 168 hr postmortem muscles in the reconstituted complex containing acto-HMM from both at-death and 168 hr postmortem muscles. Also, there was little difference in the inhibitory effect of regulatory proteins between at-death and 168 hr postmortem muscles.

In the presence of free calcium ions, on the other hand, the ATPase activity was increased at first and then decreased with increasing molar ratio of the regulatory proteins to F-actin, and the highest ATPase activity was obtained at the molar ratio of 0.1. This suggests that the acto-HMM ATPase activity can be maximally activated when the molar ratio of the regulatory proteins to F-actin is close to the physiological one (approximately 0.14 of the in vivo molar ratio; Ebashi and Nonomura, 1973).

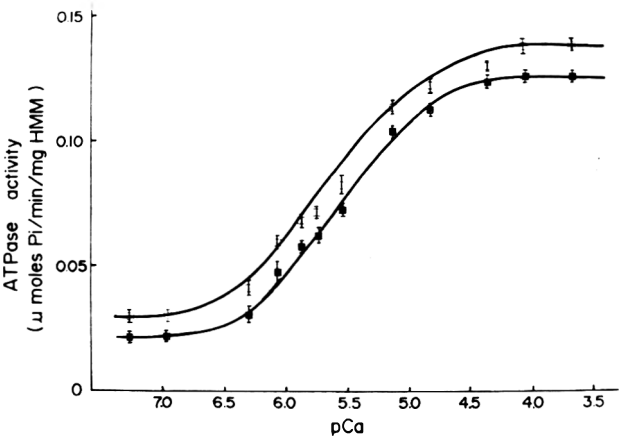

Fig. 3—pCa-dependent ATPase activity of the reconstituted complex of actin, HMM, troponin, tropomyosin prepared from at-death and 168 hr muscles. ■, the reconstituted complex prepared from at-death muscle (0 hr acto-HMM + 0 hr tropomyosin-troponin); □, the reconstituted complex prepared from 168 hr muscle (168 hr acto-HMM + 168 hr tropomyosin-troponin). Vertical lines represent standard errors of the mean (n=3).

As reported previously (Ito et al., 1978), the acto-HMM ATPase activity of 168 hr muscle was also higher than that of at-death muscle in the present study (when the weight ratio of actin to HMM was two). The acto-HMM ATPase activity of at-death muscle and 168 hr postmortem muscle in the absence of the regulatory proteins and free calcium ions was 0.097 ± 0.005 and 0.111 ± 0.002 μmoles Pi/min/mg of HMM [means ± S.E.M. (n=3)], respectively.

The pCa-dependent ATPase activities of the reconstituted acto-HMM-tropomyosin-troponin complex as a function of free calcium ions are illustrated in Figures 3 and 4. The ATPase activity of 168 hr postmortem muscle was consistently higher than that of at-death muscle and the pCa-dependent ATPase curve of 168 hr postmortem muscle was shifted toward the higher pCa value (Fig. 3). This result is quite similar to that for myofibrillar ATPase described in a

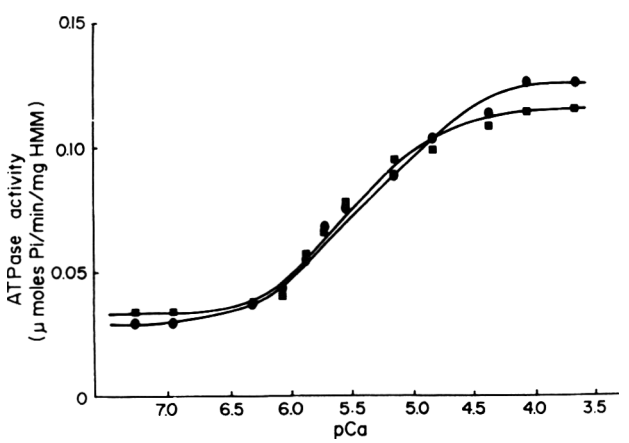

Fig. 4a—Effect of regulatory proteins prepared from at-death and 168 hr muscles on the pCa-dependent ATPase of acto-HMM prepared from at-death muscle. ■, the regulatory proteins prepared from at-death muscle (0 hr acto-HMM + 0 hr tropomyosin-troponin); ●, the regulatory proteins prepared from 168 hr muscle (0 hr acto-HMM + 168 hr tropomyosin-troponin).

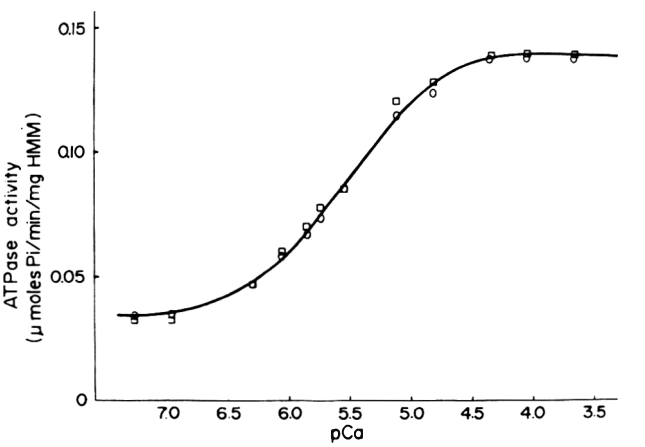

Fig. 4b—Effect of regulatory proteins prepared from at-death and 168 hr muscles on the pCa-dependent ATPase of acto-HMM prepared from 168 hr muscle. ○, the regulatory proteins prepared from at-death muscle (168 hr acto-HMM + 0 hr tropomyosin-troponin); □, the regulatory proteins prepared from 168 hr muscle (168 hr acto-HMM + 168 hr tropomyosin-troponin).

previous paper (Ikeuchi et al., 1978). However, the pCa-dependent myofibrillar ATPase curve was steeper than that of the reconstituted complex and the curve of the reconstituted complex was shifted one pCa value (Compare Fig. 3 in this paper and Fig. 1 in the reference of Ikeuchi et al., 1978). In this experiment the reconstituted actin filaments contained much more regulatory proteins (the molar ratio of the regulatory proteins to actin was 0.3) than myofibrils did (approximately 0.14 molar ratio). This might bring about the shift of the pCa-dependent ATPase curve to the lower pCa value. The gentle slope of the pCa-dependent ATPase curve of the reconstituted complex may be due to the incomplete regulatory function of the reconstituted actin filaments compared to myofibril.

Figures 4a and b show a comparative regulatory effect of troponin and tropomyosin on the acto-HMM ATPase between at-death and 168 hr postmortem muscles. In the case of the acto-HMM prepared from at-death muscle, there was almost no difference in the regulatory function of troponin and tropomyosin between them, except that the ATPase activity was slightly higher for 168 hr regulatory proteins than for at-death muscle regulatory proteins below pCa 4.5. Also, in the case of the acto-HMM prepared from 168 hr postmortem muscle, no difference was found in the pCa-dependent ATPase between the regulatory proteins from at-death muscle and those from 168 hr postmortem muscle.

DISCUSSION

THE CHANGES of myofibrillar proteins during postmortem storage of muscle have well been documented and much information regarding the role of changes in controlling the tenderness of meat have been accumulated (Goll et al., 1970, 1974; Marsh, 1972; Locker, 1960). However, we do not have sufficient knowledge about the change of function of the regulatory proteins during postmortem time. In the present study, we tried to assess the postmortem change of the function of the regulatory proteins by investigating the change of ATPase activity of the reconstituted complex of actin, HMM, tropomyosin and troponin as a function of free calcium ions.

The present results (Fig. 2a and b), which showed a similar tendency to that of Eisenberg and Kielley (1974) and Greaser and Gergely (1971), indicate that the regulatory proteins from 168 hr postmortem muscle maintains almost completely their activity against the actin-myosin interaction, i.e., there was almost no great difference in the inhibitory effect of tropomyosin and troponin between at-death and 168 hr postmortem muscles. In addition, there was no apparent difference in the regulatory function of tropomyosin and troponin on the pCa-dependent acto-HMM ATPase between at-death and 168 hr postmortem muscles (Fig. 4a and b). This indicates that the change of the Ca^{++}-sensitivity of the regulatory proteins during postmortem storage of muscle is very small. However, in the case of the reconstituted complex of actin, HMM, tropomyosin and troponin from the same muscle, the pCa-dependent ATPase of 168 hr postmortem muscle was consistently higher (approximately 10% higher) than that of at-death muscle and consequently the pCa-dependent ATPase curve of 168 hr postmortem muscle was shifted in parallel toward the higher pCa value.

The function of troponin-T is to prevent the function of troponin-C from reversing the inhibitory effect of troponin-I in the absence of calcium ions (Eisenberg and Kielley, 1974). As shown in Figure 1b, the degradative products of troponin from 168 hr postmortem muscle, which may be derived from troponin-T (Dabrowska et al., 1973; Olson et al., 1977), certainly appeared in the SDS-polyacrylamide gel electrophoretogram. However, the present result (Fig. 2) indicates that the regulatory function of troponin was not greatly affected by the degradation of troponin-T during postmortem storage of muscle. The possibility of postmortem change of the function of tropomyosin and troponin which may alter the actin-myosin interaction has been suggested by many workers (Suzuki and Goll, 1974; Cheng and Parrish, 1978), mainly based on the finding of a partial degradation of troponin during postmortem storage of muscle, although it has been found that the tropomyosin-troponin complex still has a Ca^{++}-sensitivity even after 14 days postmortem storage (Arakawa et al., 1970). However, the present results (Fig. 2 and 3) indicate that there was almost no change in the function of the regulatory proteins during postmortem storage of muscle and thereby regulatory proteins from 168 hr postmortem muscle did not take part in the increase of the acto-HMM ATPase during postmortem storage of muscle. On the contrary, the present results (Fig. 2 and 3) suggest that the increase of the acto-HMM ATPase during postmortem storage is mainly due to the increase of the actin-myosin interaction (Ito et al., 1978). The shift of the pCa-dependent ATPase curve toward higher pCa value in 168 hr postmortem muscle may also be due to the increase of the actin-myosin interaction (Solaro and Briggs, 1974). The contribution of the regulatory proteins, if indeed there be any, in increasing the actin-myosin interaction during postmortem storage of muscle is small.

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CHICKEN BLOOD PLASMA PROTEINS: PHYSICOCHEMICAL, NUTRITIONAL AND FUNCTIONAL PROPERTIES

M. T. E. DEL RIO DE REYS, S. M. CONSTANTINIDES, V. C. SGARBIERI and A. A. EL-DASH

ABSTRACT

Chicken blood plasma protein was prepared by collecting blood during the slaughter of animals using 0.5% sodium citrate solution as an anticoagulant. The blood cells were separated by centrifugation, and the plasma recovered by freeze drying either before or after dialysis. Disc polyacrylamide gel electrophoresis gave a pattern with nine protein bands, which were reduced to seven bands when the sample and gels were treated with urea. Sodium dodecyl sulfate (SDS) gel electrophoresis furnished nine protein bands with molecular weights ranging from 24,000–115,000. Gel electrofocusing revealed three protein bands with isoelectric points of 5.7, 5.3 and 4.8, respectively. Digestibility of the proteins was above 90%, and the protein efficiency ratio (PER) was 2.8 in comparison with 2.5 for casein. Addition of plasma to wheat flour for bread making at 2.5 and 5% levels raised the PER of bread from 0.87 to 1.67 and 2.02, respectively.

INTRODUCTION

ALTHOUGH ANIMAL BLOOD, a by-product of the slaughter houses, contains proteins of high biological value, it is generally wasted in most countries throughout the world thus creating also a serious pollution problem. Utilization of this material at present is limited in scope and restricted to animal feeding purposes. The Brazilian poultry industry, for example, has a production capacity of 300 million chickens per year, with a potential yield of 18 million liters of blood or 12.6 million liters of plasma. This plasma contains about 4.5% protein, which amounts to a total of at least 567 tons of protein of high biological value which is wasted annually. When other slaughtering industries are considered, this represents a substantial quantity of protein. In the past 15 years, several authors have studied the utilization of bovine and porcine blood for human consumption (Tybor et al., 1975; Young et al., 1973; Delaney, 1973; Delaney et al., 1975). No study of chicken blood properties and its utilization in food was reported, however. In the present paper some of the physicochemical and nutritional properties of chicken plasma proteins and their utilization in bread making were investigated.

EXPERIMENTAL

Sample preparation

Blood was collected in the bleeding line of a slaughter house. An aqueous sodium citrate solution (5%) was added as an anticoagulating agent at the level of 100 ml/liter of blood. The blood was centrifuged at 4000 × G at 10°C for 15 min. The supernatant (plasma) was either submitted to dialysis against deionized water (48 hr, 5°) with 6–8 changes of water and freeze dried or freeze dried without dialysis.

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Chemical analyses

Moisture, total nitrogen (Kjeldahl), lipid, and ash contents were determined according to the AOAC (1975) methods of analysis. Nonprotein nitrogen was determined according to Cristol and Monnier (1936). Amino acid determinations were performed on acid hydrolysates (6N HCl, 105°C, 22 hr) using a Beckman 120°C amino acid analyzer, following the procedure of Spackman et al. (1958). Tryptophan was determined by the colorimetric method of Spies (1967).

Protein solubility

Protein solubility as a function of pH was determined by suspending freeze-dried plasma in distilled water (1:40 w/v), and the pH was adjusted to 2.3, 4.0, 4.5, 5.0, 7.0, 8.0, 9.0, 10.0 and 11.0 with 2N solutions of HCl or NaOH. The suspensions were then agitated for 2 hr at 25°C and centrifuged (16,000 × G) for 15 min. The soluble nitrogen was determined in the supernatants and the protein content calculated using the factor 6.25.

Protein solubility as a function of NaCl was determined by suspending freeze-dried plasma (1:40 w/v) in solutions of 0.1, 0.25, 0.5, 0.75 and 1.0M NaCl. The pH was adjusted to 4.5 for the undialyzed and to 5.0 for the dialyzed plasma. The suspension was agitated for 2 hr at 25°C and centrifuged; protein content was determined in the supernatants as described above.

Protein isolation

Three procedures were tried for the precipitation of protein

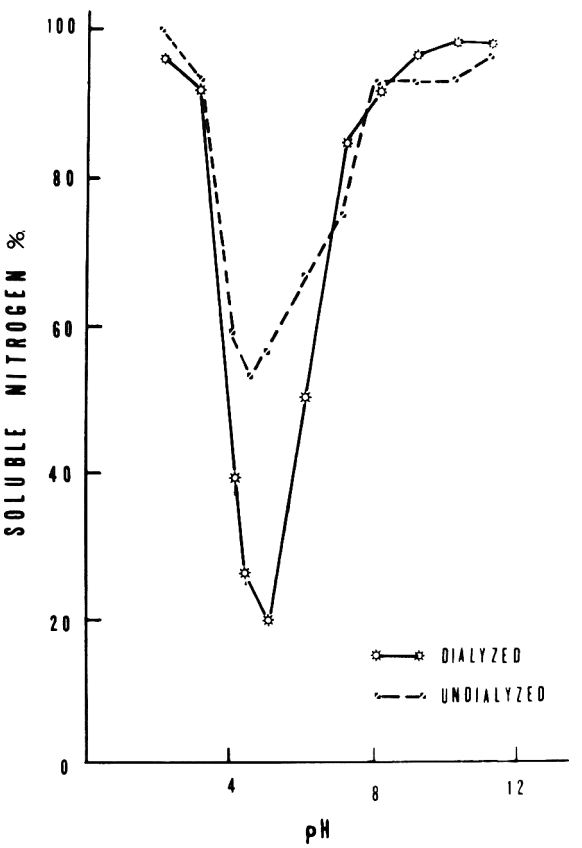

Fig. 1—Solubility curve for dialyzed and undialyzed chicken blood plasma protein as a function of pH.

from the plasma: (1) adjusting pH of the plasma directly to 4.5 with 2N HCl; (2) raising the pH to 9.0 with NaOH solution and then adjusting to pH to 4.5 with 2N HCl; and (3) making the plasma 0.2N with NaOH and then dropping the pH to 4.5 by adding HCl. After adjusting the pH to 4.5, the suspensions were agitated for 3 hr at 25°C and then centrifuged (10,000 × G) for 30 min; the precipitates were freeze dried.

Electrophoretic characterization of the proteins

Electrophoretic patterns of plasma proteins were determined on a simple polyacrylamide gel using vertical tubes of 0.5 × 9.5 cm according to the procedure of Davis (1964), on polyacrylamide gels containing urea according to the Wray and Stubblefield procedure (1970), and by SDS electrophoresis using the method of Weber and Osborn (1969). Electrofocusing on polyacrylamide gels was performed according to the method of Wrigley (1968) using carrier ampholytes in the pH range 3.5–10.

Nutritional evaluation of plasma proteins

The nutritional quality of the plasma proteins was evaluated by three different procedures: (1) amino acid profile as determined by chemical analysis (Spackman et al., 1958); (2) determination of digestibility in vitro (Akeson and Stahmann, 1964); and (3) protein efficiency ratio (PER) as described in AOAC (1975) using groups of six weanling rats (35–40g each) of the Wistar strain.

Functional properties in bread making

Either the freeze-dried dialyzed or undialyzed plasma was added to a commercially roller-milled sample of a medium strength flour (ash 0.53%, protein 10.5%, water absorption 56%) at the level of 2.5, 5 and 7.5%. A baking test was performed according to El-Dash (1978) using the following specifications: mixer speed 63 rpm; mixing temperature 30°C; dough consistency of 500 FU at maximum; mixing time until the dough showed a drop of 10 Farinograph Units (FU) after reaching maximum consistency; and a single step fermentation procedure of 105 min at 30°C. The bread was baked at 210°C for 20 min. Specific volume and internal and external quality of the bread were evaluated.

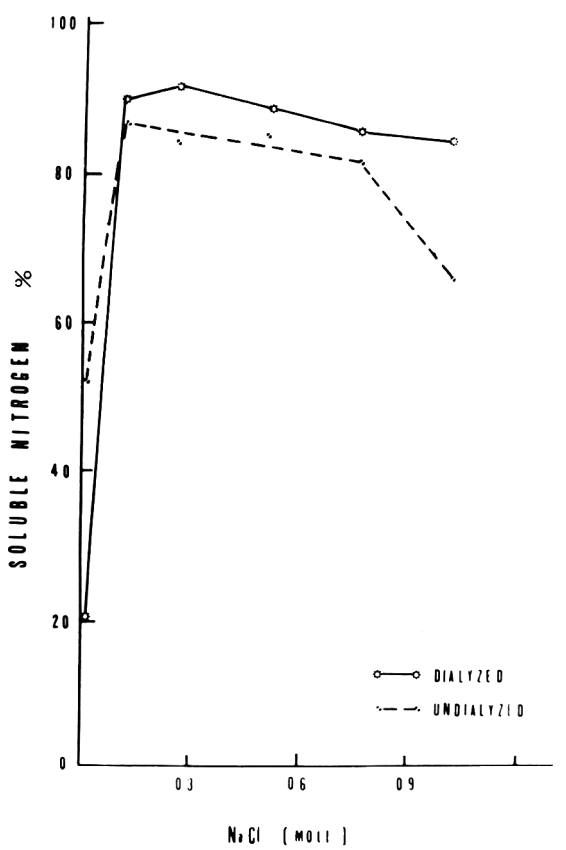

Fig. 2—Influence of sodium chloride concentration on the solubility of the dialyzed and undialyzed chicken blood plasma protein.

RESULTS & DISCUSSION

Chemical analysis of freeze-dried plasma

The composition of the dialyzed and undialyzed freeze-dried plasma is shown in Table 1. The dried plasma is highly rich in protein, varying from a content of 59.4 to 79.6%, depending on the procedure of preparation. The dialyzed plasma is characterized by a higher concentration of protein and a lower content of ash, carbohydrates, and nonprotein nitrogen than the undialyzed plasma.

Influence of pH and NaCl concentration on protein solubility

The plasma protein was found to be 100% soluble in both acid and alkaline solutions. Although the difference in pH at minimum solubility of dialyzed and undialyzed plasma was minimal (4.5 and 5.0, respectively), the differences in solubility were pronounced, as shown in Figure 1. Undialyzed samples showed a low degree of solubility, which may be attributed to the effect of the high concentration of salt present in the undialyzed samples. Sodium chloride greatly increased the solubility of the proteins at the pH of lowest solubility for both the dialyzed and undialyzed plasma. The solubilization effect was more pronounced, however, for the dialyzed plasma, as shown in Figure 2, with the greatest influence in the range 0.1–0.5M of NaCl.

Electrophoretic patterns

Electrophoresis in simple polyacrylamide gels revealed nine protein bands, whereas only seven bands were detected in the gels containing 10M urea. Isoelectric focusing on gels containing a mixture of carrier ampholytes of pH 3.5–10 separated the proteins into three bands of isoelectric pH: 5.7, 5.3 and 4.8 (Fig. 3).

On the other hand, when the plasma was previously treated with urea and mercaptoethanol and run in gels containing SDS, nine polypeptide bands were revealed with molecular weights ranging from 24,000–115,000, as shown in Table 2. A standard semilog plot of molecular weight versus electrophoretic mobility in the SDS gels appears in Figure 4. The fact that an equal number of bands was ob-

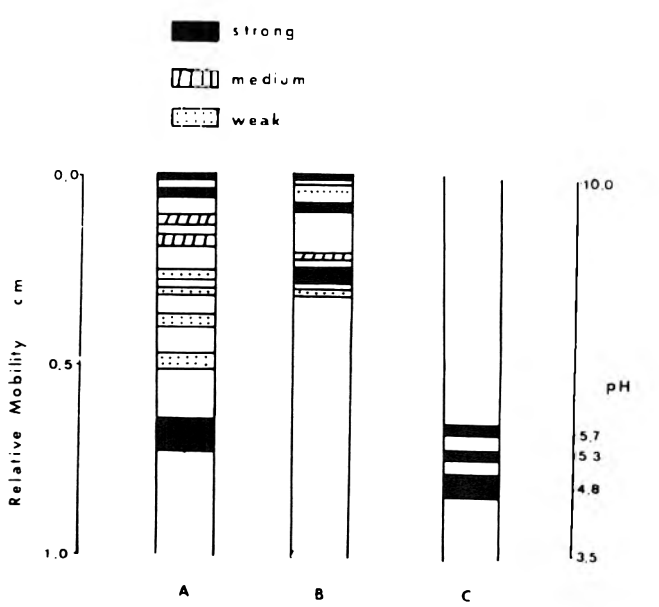

Fig. 3—Electrophoretic pattern of chicken blood plasma protein: A—simple polyacrylamide gel; B—polyacrylamide gel containing 10M urea; and C—electrofocusing (ampholine gradient pH 3.5–10).

served in simple polyacrylamide gels and in the SDS gel electrophoresis appears to indicate a low degree of polymerization of plasma proteins.

Nutritional evaluation of plasma

The in vitro digestibility of the proteins was 92% for the dialyzed plasma and 83% for the undialyzed plasma, as compared with 96% for casein. The protein efficiency ratio (PER) for the dialyzed and undialyzed plasma and for casein appears in Table 3. It is apparent that, if judged only by this parameter, the plasma protein is superior to casein. The growth-promoting capacity of the diet containing casein, however, was greater than that of the diets containing either dialyzed or undialyzed plasma at the same protein concentration (Fig. 5). The superior growth rate of rats on a casein diet could be explained in terms of a greater food intake and protein consumption.

Protein recovery

Upon isolation of plasma protein by directly lowering its pH to 4.5 with 2N HCl, the protein recovery in the precipitate was fairly low (17.4%). When the pH was first raised to 9 and then lowered to 4.5, the protein recovery increased to 19.6%. A substantial increase to 87.2% was obtained when the plasma was first brought to 0.2N with NaOH before dropping the pH to 4.5 with HCl.

Amino acid composition

The amino acid composition of the dialyzed freeze-dried plasma and that of the isolated proteins (NaOH-treated prior to dropping pH to 4.5) is presented in Table 4. The differences in amino acid composition were quite limited, with the exception of cystine, which was reduced drastically in the isolated protein. This is probably due to the alkaline treatment required before isolation of the protein. The blood plasma proteins were found to be well balanced in amino acid composition, with a high concentration of the amino acid lysine. The only limiting amino acid was found to be isoleucine, which provides only 92% of the F.A.O. reference standard (Bender, 1967).

Bread fortification with plasma

From the above-mentioned results, it was evident that the addition of high-lysine protein of plasma to the low-lysine protein of wheat flour should produce a complementary nutritional effect. It appeared essential, however, to test the effect of plasma proteins on the technological quality of bread. The effect of adding undialyzed and dialyzed

Table 1—Proximate composition of freeze-dried plasma

Components[a] (%)	Plasma	
	Dialyzed[b]	Undialyzed[c]
Crude protein	79.60	59.40
Nonprotein nitrogen	0.75	1.16
Lipid	0.20	0.29
Ash	4.60	20.10
Carbohydrate[d]	14.99	19.05

[a] On dry basis
[b] Moisture content 6.7%
[c] Moisture content 5.5%
[d] Calculated by difference

Table 2—Relative mobilities and molecular weights of blood plasma proteins as determined by SDS gel electrophoresis

Protein bands	Relative mobility	Molecular weights
1	0.023	115,000
2	0.046	105,000
3	0.069	90,000
4	0.12	83,000
5	0.16	73,000
6	0.21	64,000
7	0.23	60,000
8	0.25	56,000
9	0.53	24,000

Table 3—Biological evaluation (PER) of casein and of dialyzed and undialyzed blood plasma protein

Protein source in the diet	Body weight gain (g)	Protein consumption (g)	PER[a] (Found)	PER[b]
Casein	106.7	39.6	2.7 ± 0.44	2.5
Dialyzed plasma	89.0	29.5	3.0 ± 0.39	2.8
Undialyzed plasma	80.4	28.7	2.8 ± 0.39	2.6

[a] Groups of six rats were used in each assay; values in this column are PER ± 1 S.D.
[b] Casein = 2.5.

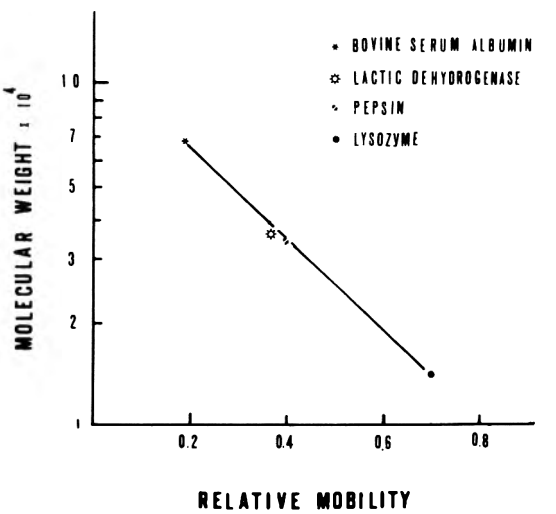

Fig. 4—Semilog standard plot of MW vs relative mobility in the SDS-gel electrophoresis.

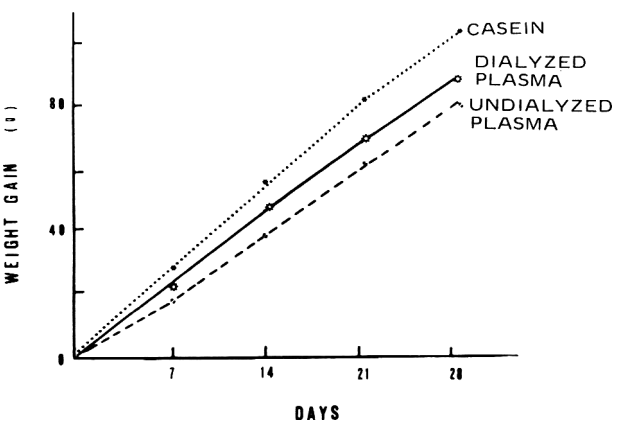

Fig. 5—Growth rate curves for rats on 10% protein diets furnished by the dialyzed and undialyzed chicken blood plasma, compared with a 10% casein diet.

Table 4—Amino acid composition of the proteins in the dialyzed plasma and of the isolated proteins (g/16g N)

Amino acid	Dialyzed plasma	Isolated proteins[a]
Lys	8.2	8.4
His	2.4	2.2
NH_3	2.1	1.6
Arg	6.0	5.6
Asp	14.7	13.1
Thr	7.3	6.0
Ser	9.7	8.0
Glu	25.6	24.9
Pro	6.3	5.6
Gly	5.0	5.2
Ala	6.8	6.8
1/2 Cys	3.9	0.9
Val	7.8	7.1
Met	2.3	2.3
Ile	3.9	3.9
Leu	11.3	9.8
Tyr	4.8	4.3
Phe	5.4	5.8
Trp	1.6	1.4

[a] Isolated after bringing the plasma to 0.2N NaOH before dropping pH to 4.5 with HCl.

Table 5—Effect of undialyzed and dialyzed chicken blood plasma on bread specific volume and total quality score

	Undialyzed		Dialyzed	
Plasma % of flour	Specific volume (cm^3/g)	Total score	Specific volume (cm^3/g)	Total score
0.0	4.89	76.2	4.89	76.2
2.5	4.85	74.1	5.12	73.8
5.0	4.66	62.0	5.35	69.8
7.5	4.06	50.0	5.60	65.1

plasma is presented in Table 5. The use of over 2.5% undialyzed plasma caused a rapid deterioration in the bread specific volume and internal characteristics of the loaf; this was attributed in part to the high ash content of the undialyzed plasma. On the other hand, the addition of dialyzed plasma resulted in a marked improvement in the specific volume, as it increased from an original of 4.89 cm^3/g to a value as high as 5.6 cm^3/g at the 7.5% level. Although an improvement in the bread external characteristics was also noted at the latter level, the internal characteristics evidenced deterioration with 5% plasma. Both dialyzed and undialyzed plasmas affected the crumb color, bread aroma, and taste when used over the 2.5% level. Addition of 5% plasma slightly darkened the crumb, produced a yellowing in the interior of the loaf, in addition to aroma and taste similar to egg bread.

The bread protein, on a dry basis, increased from 10.8% in the control to 14.8% in the bread with 5% of the flour replaced by dialyzed plasma. The PER of the control bread was 0.86 and was raised to 1.64 and 2.02 in the bread fortified with 2.5 and 5.0% dialyzed plasma, respectively. These results indicate a strong nutritional complementary effect between wheat protein and chicken blood plasma protein.

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ULTRASTRUCTURAL POSTMORTEM CHANGES IN ELECTRICALLY STIMULATED BOVINE MUSCLE

P. A. WILL, C. L. OWNBY, and R. L. HENRICKSON

ABSTRACT

Alterations in the morphology of beef longissimus dorsi, psoas major, semitendinosus, and supraspinatus muscles induced by electrical stimulation were studied at the light and electron microscopic levels. Samples of control and electrically stimulated muscles were removed from the carcass at 0.5-, 1-, 6-, and 24-hr postmortem, processed, and embedded in plastic. Changes induced by electrical stimulation were compared with those in normal autolysis in control muscle. Light microscopic examination revealed contraction bands and intracellular edema in the stimulated muscle samples while the control showed no sign of morphological change. At the electron microscopic level, electrical stimulation caused specific structural changes in the muscles. Swollen sarcoplasmic reticulum, mitochondria, and T-tubules were observed in the stimulated muscle samples. In addition, contraction banding, intracellular edema, disintegration of the myofibril, and other morphological deviations were also observed. These data are in agreement with published work that mechanisms other than the prevention of cold shortening bring about tenderization of the resultant meat. This was partially explained by the accelerated autolysis seen in stimulated striated muscles and was an active rather than passive effect as proposed by cold shortening alone.

INTRODUCTION

THE APPLICATION of an electrical current to freshly slaughtered beef carcasses has been shown to increase the rate of glycolysis and reduce the time for onset of rigor mortis (Carse, 1973; Locker et al., 1975; Davey et al., 1976; McCollum and Henrickson, 1977; Shaw and Walker, 1977; Will et al., 1978). These biochemical and physical events have been attributed to the prevention of the development of detrimental effects of cold shortening and enhancement of the tenderness of the resulting meat (Carse, 1973; Locker, 1976; Chrystall and Hagyard, 1975, 1976; Davey et al., 1976; Chrystall, 1976). These conclusions were likewise reached by Gilbert et al. (1976), working with pre-rigor hot boned bovine carcasses.

Reduced cold shortening is one of the major factors given by these researchers as contributing to the tenderization due to electrical stimulation. Work by Pierce (1977) showed that steaks from electrically stimulated sides of beef had significantly lower objective shear force values and preferred ranking by panelists in comparison to control steaks. Sarcomere lengths from the same muscles were not significantly different between control and electrically stimulated sides, indicating no difference in cold shortening (Will, 1978). Based on these experiments, and other agreeing literature (Dutson et al., 1977; Smith et al., 1977; Savell et al., 1977, 1978), it was determined that additional research investigating physical make-up of the striated muscle fiber as it related to the effect of electrical stimulation was warranted. Therefore, the ultrastructural changes resulting from electrical stimulation were compared to those due to normal autolysis in four bovine muscles.

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MATERIALS & METHODS

FOUR STEERS of similar weight and age were slaughtered and the carcasses split in the conventional manner. Both sides were placed in a temperature control chamber cooled to 16°C with circulating air immediately following splitting. At 30 min postmortem samples were removed in both left and right sides of the carcass. The stimulated side then received a DC squarewave pulse of a magnitude (peak) of 300v, 400 cps (frequency) with a duration of 0.5 msec and a current of 1.9 amps for a period of 5 min, while the control side received no electrical stimulation. Cylindrical samples from the longissimus dorsi (LD), psoas major (PM), semitendinosus (ST), and supraspinatus (SS) muscles were taken with a 1.9 cm hand coring device at 0.5-, 1-, 6-, and 24-hr postmortem. Both the control and stimulated sides of beef were fabricated into streamline hindquarters and hot boned 2-hr postmortem. The four muscles studied were placed in Cry-o-vac transparent wrap (without air removal) and held at 1.1°C until the 6-, and 24-hr samples were taken.

Each core sample was diced into small (1 mm^3) pieces and 10 of them fixed immediately in cold (4°C), 2% glutaraldehyde in 0.27M cacodylate buffer, pH 7.4 for 2 hr. After washing in the same buffer, the samples were then fixed for 1 hr in 2% OsO_4 in the cacodylate buffer. Dehydration was followed by embeddment in Epon resin. Thick sections, 1–2μ (microns), were stained with Mallory's Azure II blue (Richardson et al., 1960) and observed with a light microscope. Thin sections (silver) were stained with uranyl acetate and lead citrate (Reynolds, 1963) and then observed with a Philips EM 200 electron microscope.

RESULTS

Longissimus dorsi

Light microscopy. The inspection of light micrographs at 0.5-hr postmortem revealed no morphologic differences in striated muscle between control and experimental samples. At 1 hr, no changes in the morphology of the control were evident (Fig. 1A), but distinct contraction bands were observed in the electrically stimulated longissimus dorsi muscle (Fig. 1B) and by 6 hr, cellular swelling was also present (Fig. 1D). In the 6-hr control samples, these changes were not observed (Fig. 1C). At 24 hr, cellular swelling was noted in the control; however, no contraction banding was present (Fig. 1E). In the 24-hr experimental samples (Fig. 1F), the amount of cellular swelling and contraction bands was similar to that present at the 6-hr treatment.

Electron microscopy. There were no detectable differences between control and experimental treatments at the 0.5-hr period. The nuclei appeared normal, but a slight dilation of the sarcoplasmic reticulum was present in both control and experimental samples.

At 1 hr, no nuclear changes were observed in the control muscle, but the sarcoplasmic reticulum was dilated (Fig. 2A). In the experimental muscle, there was no difference in the nuclear morphology when compared to the control (Fig. 2B). Contraction bands in combination with stretched areas were observed in the myofibrils from the electrically stimulated muscle (Fig. 2C). In addition, areas of disruption of the sarcomere integrity were observed which appeared to

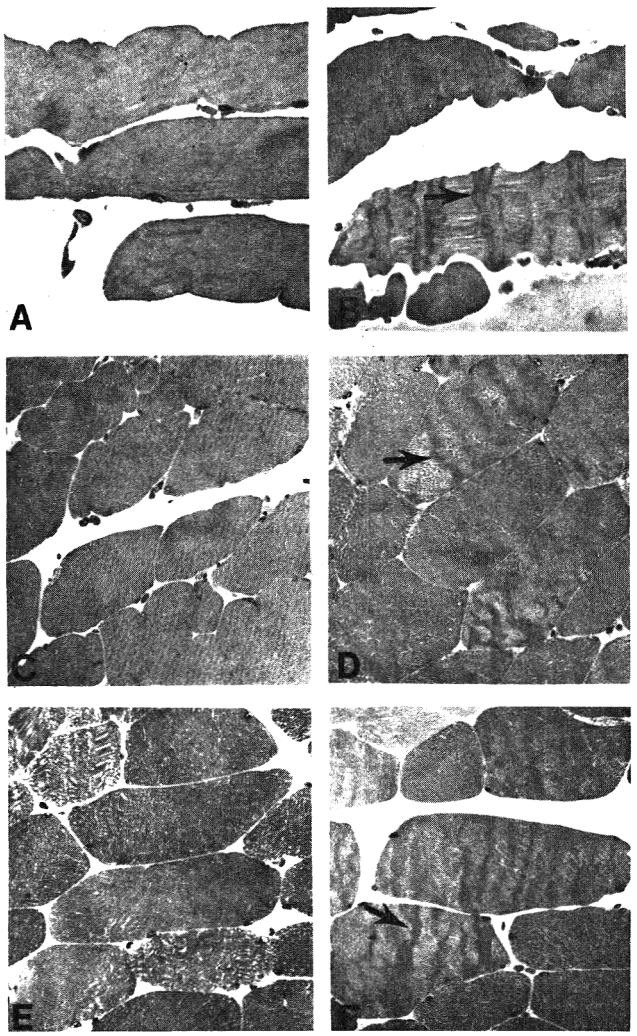

Fig. 1—Light micrographs of longissimus dorsi. (A) 1-hr control. No morphologic changes (225X). (B) 1-hr stimulated. Note contraction bands, arrow (225X). (C) 6-hr control. No morphologic changes (150X). (D) 6-hr stimulated. Note slight cellular swelling and contraction bands arrow (150X). (E) 24-hr control. Note cellular swelling (150X). (F) 24-hr stimulated. Note cellular swelling and contraction bands, arrow (150X).

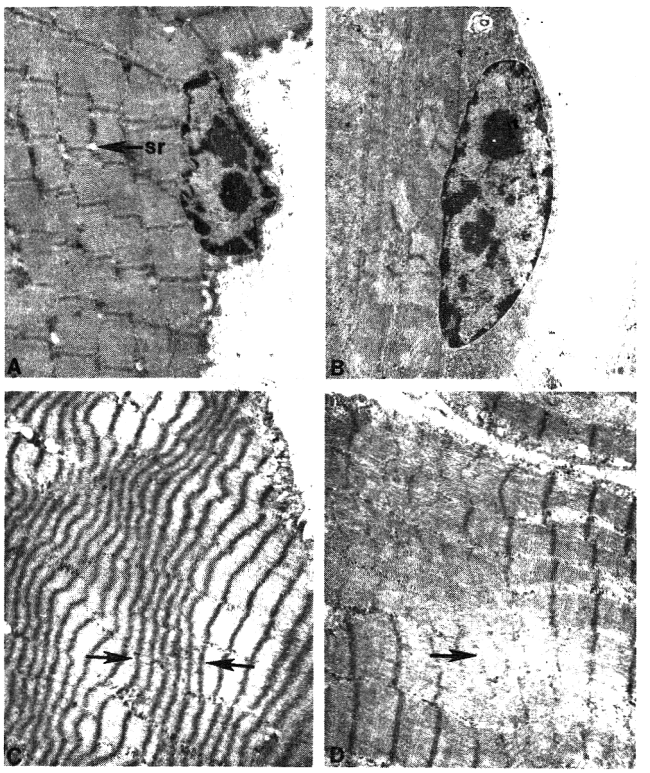

Fig. 2—Electron micrographs of longissimus dorsi. (A) 1-hr control. Nucleus appears normal but sarcoplasmic reticulum is slightly dilated [sr] (4275X). (B) 1-hr stimulated. No change in nuclear morphology (3825X). (C) 1-hr stimulated. Arrows indicate edges of contraction band. Note stretched areas on either side (3600X). (D) 1-hr stimulated. Note area of sarcomere disruption, arrow (4950X).

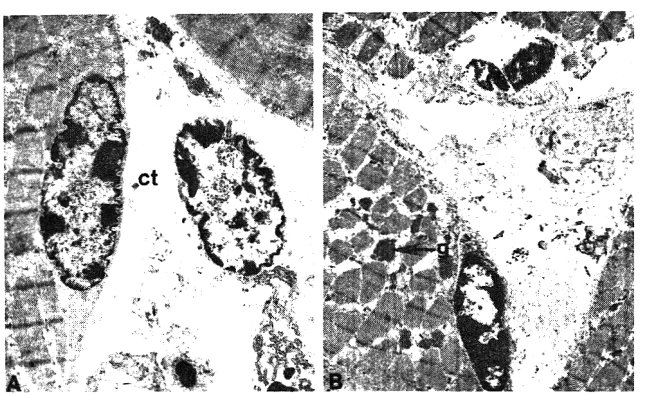

Fig. 3—Electron micrographs of longissimus dorsi. (A) 6-hr control. Nuclear morphology and sarcomere arrangement are normal. Note flocculant material in the corrective tissue, ct (3600X). (B) 6-hr stimulated. Note clumping of chromatin in nuclei and presence of substance similar to glycogen, g (2700X).

be due to Z-line disintegration (Fig. 2D).

In the 6-hr control, a slight intercellular edema and a flocculent material in the connective tissue were noted. However, the integrity of the nucleus and sarcomere was maintained (Fig. 3A). In the stimulated muscle the chromatin material in the nucleus was markedly clumped along the nuclear membrane and around the nucleolus (Fig. 3B). An accumulation or clumping of structures resembling glycogen were observed in slightly swollen areas of the cytoplasm (Fig. 3B). Likewise, cellular swelling was also shown to be present at this time period (Fig. 3B).

At 24 hr the control exhibited cellular swelling and swollen and ruptured mitochondria (Fig. 4A). The experimental treatment revealed a host of abnormalities not present in the control. These included a granular appearance of the connective tissue, swelling of the sarcoplasmic reticulum, a general swelling of the entire muscle cell, and a pyknotic nucleus (Fig. 4B). In addition, the mitochondrial outer membrane and cristae were swollen and ruptured (Fig. 4C). The capillary system servicing the muscle cell was also found to be damaged (Fig. 4C). In addition, a separation of the sarcolemma and basal lamina from the cytoplasm was noted (Fig. 4D). There was also a loss of sarcomere integrity, breakdown of myofibril and myofilaments (Fig. 4E), and an accumulation of a substance similar to glycogen as seen in the 6-hr experimental treatment (Fig. 4F).

Supraspinatus

Light microscopy. Observations of light micrographs indicated that the control samples of this muscle taken at all

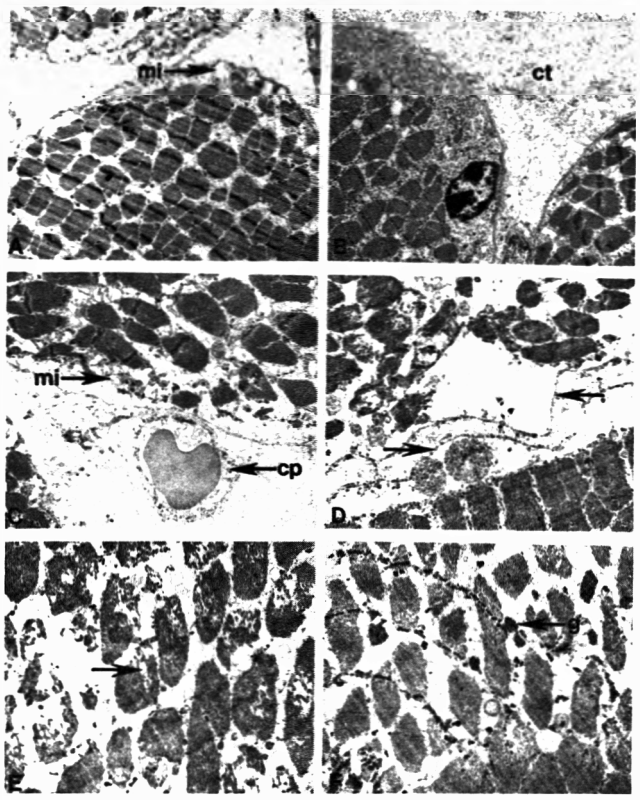

Fig. 4—Electron micrographs of longissimus dorsi. (A) 24-hr control. Note cellular swelling and swollen mitochondria, mi (1800X). (B) 24-hr stimulated. Note granular appearance of connective tissue ct; pyknotic nucleus (3375X). (C) 24-hr stimulated. Swollen and ruptured mitochondria, mi; damaged capillary, cp (2475X). (D) 24-hr stimulated. Note separation of sarcolemma and basal lamina from remainder of cell, arrows (2250X). (E) 24-hr stimulated. Note breakdown of myofibrils, arrow (3825X). (F) 24-hr stimulated. Note presence of substances similar to glycogen, g, accululation, (3600X).

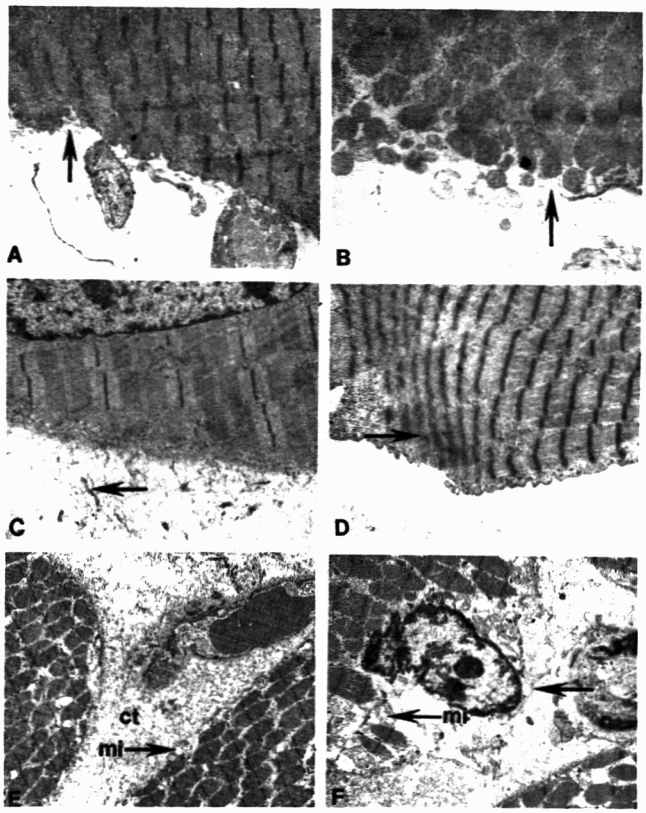

Fig. 5—Electron micrographs of supraspinatus. (A) 0.5 hr control (3375X). (B) 0.5-hr stimulated (3600X). Both A and B show localized rupture of the sarcolemma, arrows. (C) 1-hr control. Note disrupted collagen, arrow, and indistinct sarcolemma (4500X). (D) 1-hr stimulated. Note area of contraction bands arrow (3375X). (E) 24-hr control. Note granular connective tissue, ct; swollen and ruptured mitochondria, mi (1800X). (F) 24-hr stimulated. Note presence of basal lamina and absence of sarcolemma, arrow; note cellular swelling swollen mitochondria, mi, and slightly pyknotic nucleus (2700X).

time intervals (0.5, 1, 6, and 24 hr) were morphologically intact. No morphologic changes were observed in the 0.5- or the 1-hr experimental muscle. Slight cellular swelling was the only morphological change observed in the experimental treatment, and it was present at both 6 and 24 hr.

Electron microscopy. Electron micrographs of the control and experimental treatments showed localized rupture of the sarcolemma at the 0.5 time period (Fig. 5A and 5B). All other intracellular organelles appeared morphologically normal.

At 1 hr it appeared that there was a disruption of collagen and an indistinct sarcolemma present in the control treatment (Fig. 5C). Contraction bands and localized rupture of the sarcolemma were also evident in the electrically stimulated sample at 1 hr (Fig. 5D).

At 6 hr swollen mitochondria with ruptured cristae were evident in both treatments. Similar morphological changes present at 1 hr were present at 6 hr in the electrically stimulated samples.

The control at 24 hr showed swelling of the mitochondria and a granular material in the connective tissue (Fig. 5E). By 24 hr the sarcolemma of experimental muscle was broken, but the basal lamina was intact (Fig. 5F). Cellular swelling was present as well as swollen mitochondria containing ruptured cristae (Fig. 5F). The nucleus appeared slightly pyknotic and glycogen-like structures were clumped in the cytoplasm of the muscle cell (Fig. 5F).

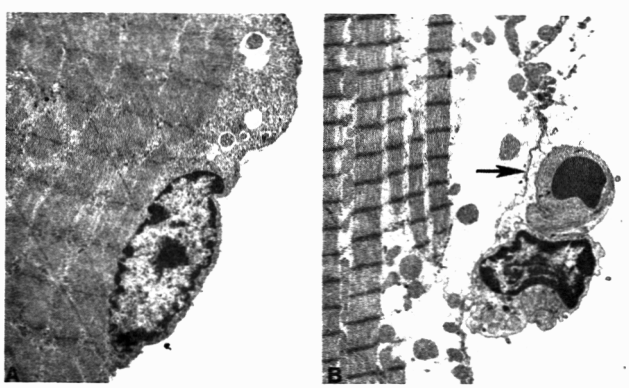

Fig. 6—Electron micrographs of psoas major. (A) 0.5-hr control. Normal morphology with only slight edema (4050X). (B) 0.5-hr stimulated. Note separation of sarcolemma, arrow, and cellular swelling (3150X).

Psoas major

Light microscopy. At all time periods, the control muscle samples appeared normal. Experimental muscle had only slight intracellular edema at 0.5, 1, 6, and 24 hr.

Electron microscopy. At 0.5 hr the control muscle showed only slight cellular swelling (Fig. 6A). In the experi-

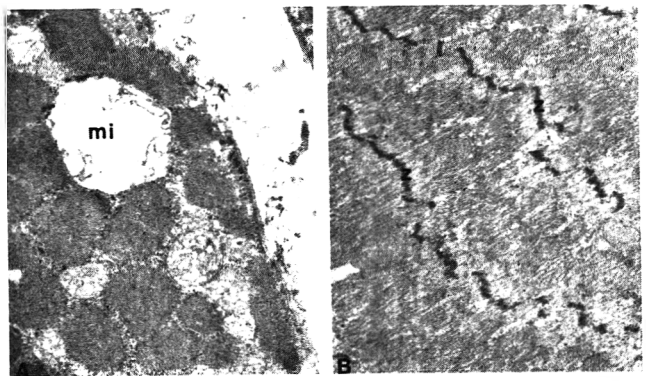

Fig. 7—Electron micrographs of psoas major. (A). 1-hr control. Swollen mitochondria, mi (5850X). (B) 1-hr stimulated. Z lines Z, are disoriented and abnormal sarcomere structure (6075X).

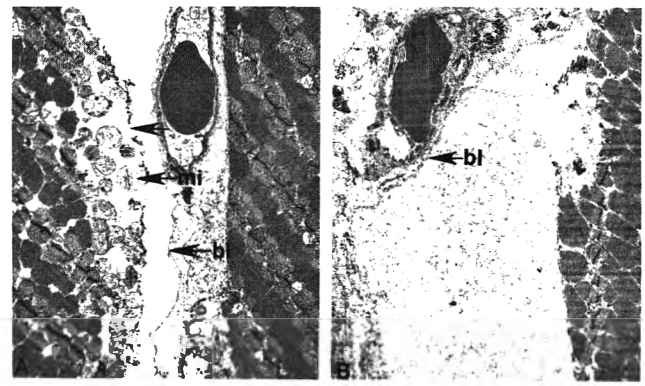

Fig. 8—Electron micrographs of psoas major. (A) 6-hr control. Swollen and ruptured mitochondria, mi; arrow indicates ruptured sarcolemma; basal lamina, bl (2700X). (B) 6-hr stimulated. Note basal lamina, bl, which has separated from the cell (2700X).

mental muscle, the nucleus, myofilament arrangement, and mitochondria appeared normal, but the sarcolemma was pulled away and there was a lot of cellular swelling (Fig. 6B). Also, localized rupture of the sarcolemma was observed in some cells.

At 1 hr the mitochondria were swollen (Fig. 7A) and the nucleus in the control muscle appeared slightly pyknotic. The experimental muscle contained abnormal sarcomere structure along with alteration of the Z-line symmetry (Fig. 7B). The latter two changes (nucleus and sarcomere structure) were not consistently observed.

By 6 hr the control showed swollen and ruptured mitochondria and lifting away of basal lamina with rupture of sarcolemma integrity (Fig. 8A). The experimental muscle showed the above conditions plus breakage of sarcolemma and basal lamina; also, the basal lamina appeared to be lifted away from the main part of the cytoplasm (Fig. 8B).

Twenty-four-hour control muscle samples revealed conditions similar to those present at 6 hr. Experimental samples showed extensive lifting of the basal lamina and highly pyknotic nuclei (Fig. 9A and 9B).

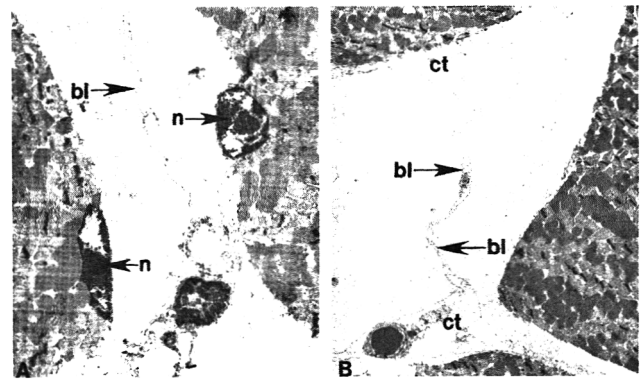

Fig. 9—Electron micrographs of psoas major. 24-hr stimulated. (A) Note separation of basal lamina, bl, and pyknotic nuclei, n (2475X). (B) Note extensive separation of basal lamina from muscle cell, arrows; connective tissue, ct (1575X).

Semitendinosus

Light microscopy. Control samples at the 0.5-, 1-, 6-, and 24-hr time periods had similar morphological appearances. The experimental treatment showed development of slight cellular swelling at the 6- and 24-hr periods. No additional morphological changes were evident at the light level in the electrically stimulated samples.

Electron microscopy. At 0.5 hr, no changes were observed in either treatment at the electron microscope level. At 1 and 6 hr, slight swelling of the sarcoplasmic reticulum and mitochondria were observed in both treatments. At 24 hr swollen mitochondria, sarcolemma breakdown, and debris in the connective tissue were noted in both treatments.

DISCUSSION

THE TWO PRIMARY structural alterations induced by the electrical stimulation of beef muscle were contraction bands and accelerated autolysis. Contraction bands were observed only in the longissimus dorsi and supraspinatus muscles at 1, 6, and 24 hr and at 1 hr, respectively.

Contraction bands have been observed under a host of pathological conditions such as ischemia, cardiomyopathy, catecholamine toxicity, (Adomian et al., 1978), hemorrhagic shock, (Leet et al., 1975. Adomian et al., 1976), catecholamine infusion, open heart surgery (Adomian et al., 1976), malignant hyperthermia (Isaacs et al., 1973; Reske-Nielsen, 1973; Fenoglio and Irey, 1977; Britt and Kalow, 1970), cold shortening (Marsh et al., 1974) and electrical stimulation (Dutson et al., 1977). Work by Adomian et al. (1978) indicates that contraction bands may be considered a morphological index of pathology in myocardial tissue obtained 40-min postmortem. The contraction bands were interspersed between the normal cross striations within the myofibril. Fenoglio and Irey (1977) found these bands in cardiac muscle associated with myofibrillar degeneration or myofiberlysis characterized by dissolution of myofilaments, disruption of sarcomeres and sarcolemmal breaks. Dissolution of myofilaments and disruption of sarcomeres can be observed following prolonged autolysis. However, ever after 12 hr of autolysis, sarcolemmal breaks were not observed (Buja and Roberts, 1974). Therefore contraction bands and sarcolemmal breakage were not directly due to autolysis or ischemia.

The autolysis of control muscle as observed in the investigation agrees with previously published data (Dutson et al., 1974; Cassens et al., 1963; Greaser et al., 1969; Henderson et al., 1970; Abbott et al., 1977; Gann, 1974).

Electrical stimulation accelerated the autolysis in experimental muscle

The experimental longissimus dorsi showed signs of accelerated autolysis as early as 1-hr postmortem. Disruption of sarcomere integrity (I-band) at the 1-hr postmortem period related to 192-hr postmortem muscle degeneration re-

ported by Abbott et al. (1977). A similar autolysis in I-band has been reported by Cassens et al. (1963); Greaser et al. (1969); and Parrish (1977). At 6 hr postmortem, the stimulated treatment showed signs of acute cell injury as, i.e., pyknotic nuclei (Price et al., 1964; Trump and Ericsson, 1965). The cellular swelling present in the stimulated treatment may possibly be explained by reported losses of water-holding capacity of the muscle proteins (Bendall and Wismer-Pedersen, 1962) and the escape of sarcoplasmic protein from the fiber into the intrafiber space (Bendall, 1973).

At 24 hr cellular swelling and swollen and ruptured mitochondria were present in the experimental as well as control. The lifting away of the sarcolemma and basal lamina from the cytoplasm in the experimental muscle agreed with published data of porcine muscle 48-hr postmortem (Abbott et al., 1977). The ultrastructure of electrically stimulated muscle 24-hr postmortem showed accelerated morphological degeneration in comparison to the normal autolysis of control muscle tissue. The breakdown of mitochondria, mitochondrial membrane, sarcoplasmic reticulum, the T-tubule system, myofibril, myofilaments and pyknotic nuclei indicate a physical alteration of the muscle cell. These changes may be partially explained by work of Dayton et al. (1976) and Dutson et al. (1977) which indicated that at lower pH readings, lysosomal catheptic activity is probably responsible for changes in subcellular organelles. In addition, calcium activated factor (CAF) activity which Abbott et al. (1977) indicated as being partially responsible for the normal autolysis of muscle has its greatest activity at a neutral pH and is not thought to be active at an acid pH (Dayton et al., 1976; Dutson et al., 1977). Therefore, it would appear that electrical stimulation through accelerating pH decline increases the free activity of the lysosomal enzymes.

The morphological changes occurring in the nuclei, sarcoplasmic reticulum and myofibrils of the control semitendinosus agreed with results published by Abbott et al. (1977). The breakdown of the sarcolemma 24-hr postmortem in the experimental treatment corresponded to a similar response reported in normal autolysis of muscle 48-hr postmortem (Abbott et al. 1977).

Samples taken from the psoas major control revealed a steady progression of autolysis which was at a faster rate than the longissimus dorsi, supraspinatus, and semitendinosus muscles. This is in agreement with pH data by McCollum (1977). His results showed that psoas major muscle from stimulated and nonstimulated treatments had a significantly faster drop in pH as compared to other muscles studied. A difference in the time of onset of autolysis was observed between the control and experimental treatments. The electrically stimulated muscle showed signs of accelerated autolysis from the standpoint of extensive lifting of the sarcolemma, highly pyknotic nuclei, swollen and ruptured mitochondria, and abnormal sarcomere structure.

The longissimus dorsi muscle responded to electrical stimulation with the greatest amount of morphologic change. This could be due to its location along the axis of the path of the electric current.

Myofibril disintegration was noted in the longissimus dorsi and psoas major muscles. This autolysis of myofibril organization occurs in normal disintegration of striated muscles; however, it occurs at a much faster time sequence in electrically stimulated muscle (Abbott et al., 1977; Henderson et al., 1970; Cassens et al., 1963; Greaser et al., 1969a; Dutson et al., 1974). It is our belief that one of the major effects of electrical stimulation is an acceleartion of the normal autolysis of muscle. However, these changes could be due to mechanical disruption caused by the electrical shock.

The relationship of electrical stimulation of carcasses and resulting tenderness of the meat product has been explained as a simple prevention of cold shortening (Carse, 1973; Locker, 1976; Chrystall and Hagyard, 1975; Davey et al., 1976; Chrystall, 1976; Gilbert et al., 1976).

Research published by Pierce (1977) indicated increased tenderness from the electrically stimulated carcasses. However, the measurement of sarcomere lengths from the same muscles revealed no significant differences (Will, 1978). This indicated that some mechanism, in addition to the prevention of cold shortening, brought about the tenderization of meat (Dutson et al., 1977; Savell et al., 1977; Savell et al., 1978). This tenderness was derived in part from physical or structural changes induced by electrical stimulation in the muscle. This was partially explained by the accelerated autolysis seen in stimulated striated muscles and was an active rather than passive effect as proposed by cold shortening alone.

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PENTANE PRODUCTION AS AN INDEX OF RANCIDITY IN FREEZE-DRIED PORK

C. W. SEO and D. L. JOEL

ABSTRACT

Levels of hydrocarbon in the headspace over freeze-dried pork was measured to evaluate rancidity. At the same time, the pork was given to a sensory panel to describe the degree of rancid odor. When the pentane level in the headspace reached about 0.05 μl, 100% of the panel described the pork as rancid. In the early stage of rancidity, the pentane production levels were not correlated with the rancid odor scores. However, overall pattern of the pentane production was significantly correlated with that of the rancid scores. The correlation coefficient (0.88) was significant at the 99% level. Serum rubber caps used for sealing the sample containers to facilitate headspace gas sampling absorbed hydrocarbons significantly. The extent of the hydrocarbon absorption increased with increasing chain length of the hydrocarbons, i.e., hexane > pentane > butane > propane. Teflon tape-covered silicone rubber septa could alleviate this problem.

INTRODUCTION

SHORT-CHAIN hydrocarbons which are chemically inert can be separated from rancid foods. Among them pentane is known to be exclusively from the oxidation of linoleic acid. This has been confirmed experimentally by different researchers (Horvat et al., 1964; Evans et al., 1967; Johns et al., 1973; Seo, 1976).

Since linoleic acid is the major polyunsaturated fatty acid in meats (Anderson et al., 1975; Anderson, 1976), many attempts have been made to correlate pentane production with the extent of rancidity in foods. Evans et al. (1969) and Jarvi et al. (1971) reported that the amounts of pentane produced from vegetable oils had an inverse linear relationship to flavor score. Fioriti et al. (1974) also reported that there were good correlations between pentane values and the average flavor scores in the various oxidized fats. Warner et al. (1974) studied pentane production from potato chips and vegetable oils in relation to the number of rancid descriptions given by an 18-member taste panel. Vegetable oils with 0.08 ppm pentane were judged to be rancid by all panel members. Seo (1976) reported hydrocarbon production from freeze-dried beef. This report did not give any organoleptic data in relation to the hydrocarbon production. From these reports, it was assumed that pentane production can be correlated with rancid scores and its measurement can be used to follow lipid oxidation in freeze-dried pork.

The purpose of this study was to establish a relationship between pentane values and rancidity scores given by panel members evaluating freeze-dried pork. The established relationship between them can be applied to determine the degree of rancidity in freeze-dried pork. This study was also to show the absorption of hydrocarbons by serum rubber septa which were used to seal the sample containers.

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EXPERIMENTAL

Preparation of freeze-dried pork

Pork-loin was trimmed of excess fat and ground twice with an electric grinder. The ground pork was mixed by hand to obtain a homogenous product and divided into two portions. One portion of meat was treated with sodium tripolyphosphate in a concentration of 0.5%, w/w, of the meat and the other portion was untreated as the control. Meat samples were divided into 420g, filled into short No. 2 cans and heated in boiling water until the internal temperature of the meat reached 70°C. After heating, the cans were cooled in running tap water and opened. The meat was mixed again by hand and dried by the Thermovac FDC-4 laboratory freeze-dryer. The temperature of the heating plate was set to 26.7°C at the gauge and the range of vacuum was 60–100 microns. It took approximately 48 hr to dry the meat. A 100-g portion of the meat gave a final dried weight of 29 ± 1g for control samples and 32 ± 1g for treated samples. The water activity of the control sample was 0.16 and 0.35 for the treated sample. The water activity was measured by the hygroline recorder, Beckman model SMT.

Sample storage

The dried samples were weighed out into 5-g portions, and put into standard serum bottles (100 ml). The bottles were sealed with aluminum seals by a hand crimper to hold the septum firmly in position. The septum was silicone rubber (CHR 300) covered with self adhesive Teflon tape (CHR Tape T). Those were purchased from the Connecticut Hard Rubber Co. Before analysis, the bottles were incubated at 30°C in a water bath for at least 30 min.

Gas chromatograph analysis

A 4-ml sample of headspace gas was taken from each bottle by a gas-tight syringe, and injected onto the gas chromatograph equipped with a flame ionization detector, Hewlett Packard Model 5700A, under the following conditions: Column, 6 ft. long aluminum; Diameter, 1/8 in.; Support, 60/80 mesh activated alumina purchased from the Coast Engineering Laboratory; Flow rate of nitrogen, hydrogen and air were 30 ml/min, 30 ml/min, and 240 ml/min, respectively; Injector port temperature, 200°C; Chart speed, 15 in./hr; Sample size, 4 ml; Range and attenuation varied, usually 1 × 2–16. The alumina column was adapted from the report by List et al. (1965).

For the quantitation of hydrocarbon production, a standard curve was prepared for every analysis by injecting varying concentrations of a standard gas mixture containing six saturated hydrocarbons (C_1–C_6, 100 ppm each) onto the gas chromatograph column under the same experimental conditions. For methane calculation, the blank value was subtracted from the calculated value obtained from the sample. The blank value was obtained from the injection of a 4-ml sample of air onto the chromatograph column. This was done each day of sample analysis. The standard gas mixture was obtained from MG Scientific Company. Hydrocarbon data for all pork samples were based on triplicate sample analysis.

Preparation of freeze-dried model mixtures

One gram fatty acid (linoleic), 24 grams microcrystalline cellulose and 100 ml of 0.1M phosphate buffer (pH 6.4) were combined in a homogenizing flask placed in an ice bath and homogenized with high speed for 7 min by a Virtis Homogenizer. Then 25 ml of 6.78×10^{-5} M $CoCl_2$ (100 ppm of cobalt on a linoleic acid basis) was added to the mixture, and it was again homogenized for 3 min. The homogenized mixture was transferred to a stainless steel pan and dried by a Thermovac-FDC-4 freeze-dryer for 36 hr. The temperature of the heating plate was set at 26.7°C at the gauge and the

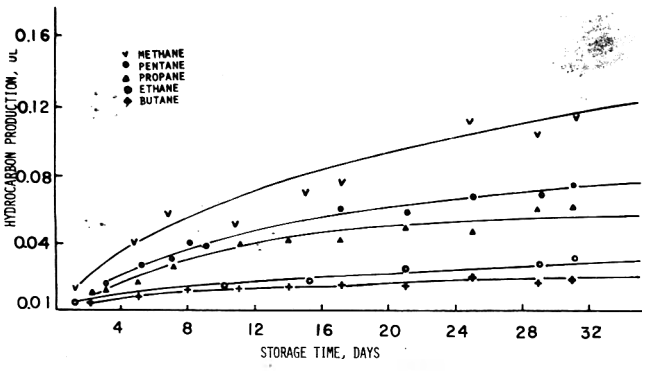

Fig. 1—Hydrocarbon production from the freeze-dried pork during storage (μl/10g dried meat). The sample bottles were sealed with Teflon tape-covered silicone rubber septa.

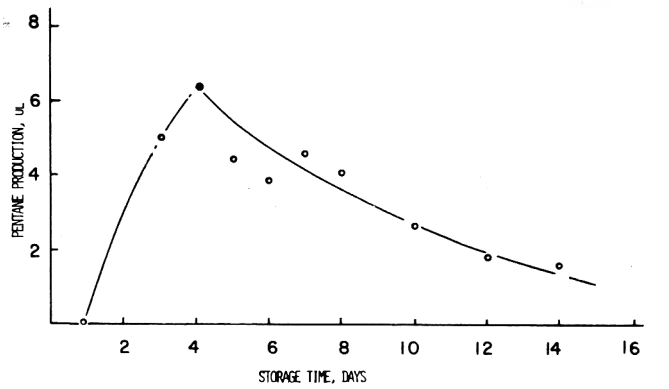

Fig. 2—Pentane production from the cobalt catalyzed oxidation of linoleic acid-cellulose model system during storage at room temperature (μl/g). The sample flasks were sealed with the serum rubber caps.

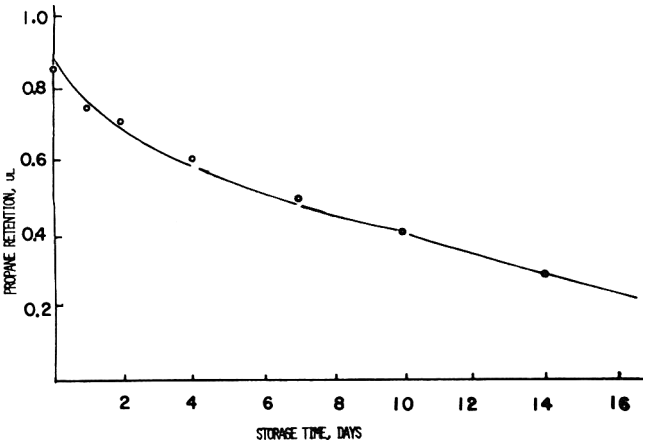

Fig. 3—Retention of propane gas which was introduced to the flasks sealed with serum rubber caps during storage at room temperature. The initial concentration was 1 μl per flask.

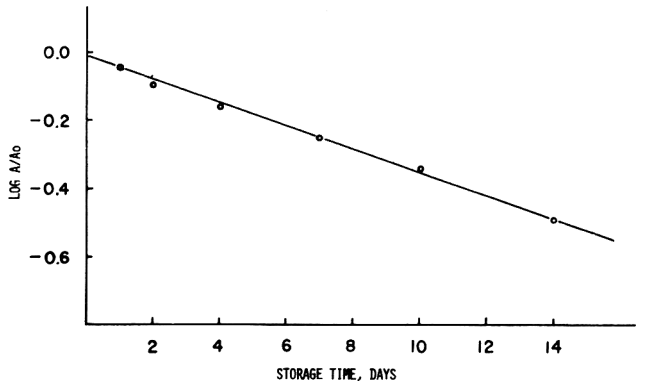

Fig. 4—Rate of loss of propane which was introduced to the flasks sealed with serum rubber caps. Data plotted as first order rates where: A_0 = initial concentration and A = amount present at indicated time.

range of vacuum was 60–100μ. The fatty acids were obtained from Nucheck Prep, Inc. and cellulose (cellex MX) from Bio Rad Chemical Company. The sample preparation method was partially adapted from the report by Heidelbaugh et al. (1971).

The dried samples were crushed and mixed throughly with a spatula. One-half gram portions of the dried samples were put into 125 ml Erlenmeyer flasks sealed with serum rubber caps and kept in a water bath at 30°C for 30 min. The caps were purchased from the Fisher Scientific Company.

Sensory evaluation

Panel members were chosen without regard to special olfactory acuity. The members were given an orientation session to become familiar with the procedures and samples under study. The members were given a strongly rancid sample as the standard and asked to judge the given samples in relation to the rancid sample. The samples were judged by 12–16 panel members depending upon the availability of the panel. This method was adapted from the report by Warner et al. (1974).

Hydrocarbon loss

For propane gas, 1 ml of propane gas, 1000 ppm in helium, was put by a gas-tight syringe into flasks capped with serum rubber cpas, and the flasks containing propane were stored at room temperature. The propane concentrations in the flasks were determined by the gas chromatograph at intervals during storage to monitor its losses. The analytical methods and conditions were the same as those described for the freeze-dried samples. For the hydrocarbon mixture, 1 ml of n-paraffin mixture, C_1–C_6, 100 ppm each in nitrogen was introduced into the flasks.

Samples were analyzed in at least triplicate for each analysis unless otherwise indicated throughout this study.

RESULTS & DISCUSSION

HYDROCARBON determination and rancid odor tests were made simultaneously on the same batch of freeze-dried pork samples. Five saturated hydrocarbons, C_1–C_5 were identified from the headspace over the freeze-dried pork (Fig. 1). Among them methane was the major hydrocarbon and pentane, propane, ethane, and butane were found to be minor constituents. The same fact was found in repeated experiments. In freeze-dried beef, Seo (1976) found that methane was the major hydrocarbon with significant amounts of ethane and propane but traces of butane and pentane. Serum rubber caps were used to seal the containers in the work reported.

Regarding the absence of pentane in the beef samples, it was assumed that pentane was absorbed by the serum rubber caps used to facilitate gas samplings. A model system containing linoleic acid clearly demonstrated the loss of pentane in the same sampling system sealed with serum rubber caps (Fig. 2). The following observations further supported the assumption. Propane gas which had been introduced to the empty flask sealed with the serum caps was steadily lost during storage at room temperature (Fig. 3). The loss was initially rapid and slowed down thereafter. The rate of propane loss followed a first order reaction mechanism (Fig. 4) and the rate constant was 7.9×10^{-2} day^{-1}. The data from Figures 2 and 3 may indicate that the gas production from the samples is initially greater than the

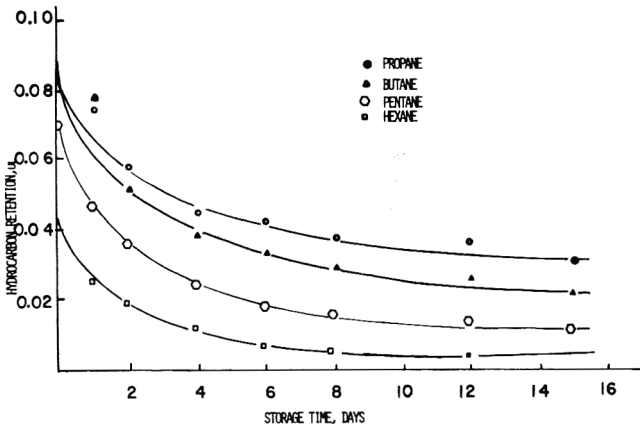

Fig. 5—Retention of the saturated hydrocarbons (C_3—C_6) which were introduced to the flasks sealed with serum rubber caps during storage at room temperature. The initial concentration was 0.1 µl each per flask. The sample flasks were sealed with the serum rubber caps.

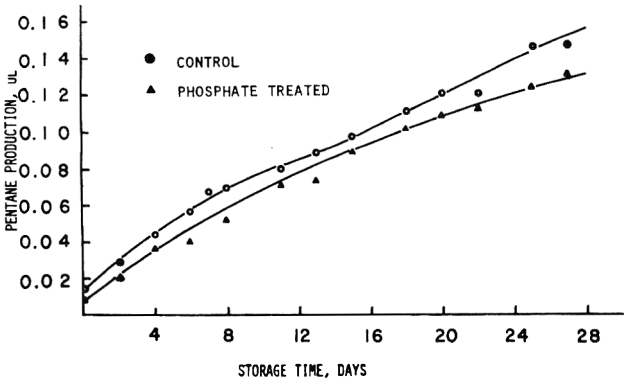

Fig. 6—Pentane production from the freeze-dried control and phosphate treated meat samples (µl/10g).

absorption of the gas by the rubber cap and later it decreases to a rate smaller than the absorption by the cap.

Of two possible causes, absorption and diffusion, for the loss of propane, the latter could be ruled out based upon the data of Figure 5 and the theory of Graham's law of diffusion. Figure 5 shows the loss of the component hydrocarbon mixture which was introduced to the empty flasks sealed with the rubber caps during storage at room temperature. Among four hydrocarbons, the loss of hexane was the greatest. The extent of the hydrocarbon losses increased with increasing chain length, i.e., hexane > pentane > butane > propane. This phenomenon is the opposite of Graham's law of diffusion of gases. The law states that the rate of diffusion of gases varies inversely as the square roots of their molecular weights (Maron and Prulton, 1961). These facts may indicate that the loss by diffusion, if any, may be insignificant and therefore, absorption may be the predominant cause of the loss. Since the serum rubber caps used to facilitate gas samplings from the headspace of flasks absorb hydrocarbons significantly, the sampling system capped with the serum rubber cap for the hydrocarbon analysis is not reliable unless the working concentrations are high enough to neglect the loss.

Regarding the methane production as the major hydrocarbon in the headspace, Seo (1976) suggested that alanine which was one of the major free amino acids in meats might be the precursor of methane; alanine may degrade to methane through a "Strecker Degradation." Since the possibility of pentane production from a nonlipid source such as amino acid is very remote, pentane determination may be a more reasonable approach for the evaluation of rancidity in meats.

Table 1—Pentane production and percentage of sensory panel giving rancid description in stored freeze-dried pork

Days storage at room temp	Pentane[a] (µl/10g)	Rancid description %
1	0.016	0
2	0.017	8
3	0.016	19
4	0.017	33
7	0.031	33
8	0.040	31
9	0.039	42
10	0.042	43
12	0.047	79
14	0.050	94
15	0.052	100

[a] Mean of three samples; these data are from Figure 1.

Table 1 shows pentane production from the pork during storage and the percentage of sensory panel giving a rancid description. According to these data, when pentane concentration in the headspace over the freeze-dried pork reach 0.052 µl, 100% of the panel judged the sample as rancid. In another experiment, similar results were observed. At the 12th day, 79% of the panel indicated that the pork was rancid and 100% of the panel at 15 days of storage (Table 1). In the early stage of rancidity, the pentane production levels were not correlated with the rancid odor scores. However, overall pattern of the pentane production was significantly correlated with that of the rancid scores. The correlation coefficient (0.88) was significant at the 99% level. Table 1 also shows that at the 12th day, the rancid description figure increased drastically while the pentane concentration in the headspace increased slightly. The reason for this is not clear. However, it may be assumed that the threshold for rancid odor in the freeze-dried pork medium may be around 0.05 ml pentane concentration for all panel members assembled for this study; above this level every panel member could detect the odor but some of them could not detect it below this level. As indicated earlier, the panel members participating were given an orientation but were not trained intensively. Pentane is not responsible for rancid odor but the pentane level is indicative of the degree of lipid oxidation. However, the relationship between pentane level and percentage of panel giving rancid descriptions (% rancid description) was a very useful index to establish the degree of rancidity in dehydrated foods. Warner et al. (1974) successfully established the relationship in vegetable oils and potato chips during storage. They reported that samples with as little as 0.08 ppm were judged as rancid by 90% of the panel in vegetable oils and by 100% of the panel in potato chips.

The polyphosphate-treated pork samples produced significantly lower amounts of pentane than the control pork (Fig. 6). The antioxidant activity of the phosphate has been well documented by many workers (Lehmann and Watts, 1951; Tim and Watts, 1958; Tarladgis et al., 1959; Zipser and Watts, 1961; Ramsey et al., 1963; Rao et al., 1975). One of the reasons might be due to the ability of holding moisture in the meat and thus giving the meat a higher water activity. High water activity is known to have a protective effect on lipid oxidation in dehydrated foods (Labuza et al., 1970). The pentane values from this pork were much higher than from the pork used for Figure 1. It might be because of higher fat contents of this pork which was from a different animal.

—Continued on page 92

GROWTH CHANGES IN BOVINE MUSCLE FIBER TYPES AS INFLUENCED BY BREED AND SEX

A. A. SPINDLER, M. M. MATHIAS, and D. A. CRAMER

ABSTRACT

Samples of the biceps femoris were collected at 56-day intervals from 28 days of age to slaughter from 24 Hereford, Angus, Holstein heifer and steer twin calves to define the effects of age, sex, and breed on histochemically estimated muscle fiber type patterns. Red, white, and intermediate fiber types doubled in mean cross-sectional area during growth in all calves, while encounters of white fibers slightly increased and encounters of red fibers slightly decreased. In general, heifers had larger muscle fibers than steers, and Holstein steers had smaller fibers than Hereford or Angus steers.

INTRODUCTION

Meat quality has been shown to be affected by age, sex, breed, diet, and animal husbandry practices (Zinn et al., 1970; Norris et al., 1971; Hunsley et al., 1971; Marchello et al., 1970), as well as by factors which influence growth and differentiation of skeletal muscle fiber types (Cooper et al., 1969; Kowalski et al., 1969; Beatty et al., 1967; Gillis and Hendrickson, 1969).

Luff and Goldspink (1970) reported that the number of muscle fibers within a given muscle was constant within a given strain of mice, but somewhat different among strains of mice. Muscle weight and numbers of fibers were unrelated, indicating that muscle fiber growth occurs by hypertrophy. Swatland and Cassens (1972) found that growth in the size of muscle fibers in the vastus lateralis was significantly greater in male rats which exhibited a high rate of weight gain after weaning. White fiber hypertrophy contributed somewhat more to muscle enlargement than either red or intermediate fiber hypertrophies. Ashmore et al. (1972) evaluated piglet, lamb and calf muscles histochemically to determine growth patterns, and concluded that transformation of fast red to fast white fibers also facilitated muscle enlargement.

Differences within a muscle during growth may be demonstrated through measurements of distribution of particular fiber type and the relative volume occupied by a particular fiber type. In histological sections, distribution may be estimated by the number of encounters and relative volume may be estimated by mean cross-sectional area of particular fiber types. The purpose of this study was to characterize the effects of age, breed, and sex on the encounters (distribution) and cross-sectional area (relative volume) of muscle fibers in beef biceps femoris.

EXPERIMENTAL

MUSCLE BIOPSIES from six Angus, ten Hereford, and eight Holstein heifer and steer twin calves were obtained at 28 days of age and at subsequent 56-day intervals until slaughter (Hecker et al., 1975). Following spinal anaesthesia, the first biopsy was taken from the anterior, distal section of the biceps femoris on the right side. The second biopsy was from the same location on the left side. The third was taken from the distal section of the right side and posterior to the first biopsy. Succeeding biopsies were taken dorsally to previous biopsies in the same manner, carefully avoiding the internal fibroelastic septum which divides the muscle.

Tissue blocks, approximately 3.5 × 3.5 × 5 mm, were immediately frozen in liquid nitrogen and cryostatically sectioned in transverse plane at 10 μm. The sections were stained for NADH-diaphor-

Table 1—Changes in mean cross-sectional area of muscle fiber types with age[a]

Days of Age	Red fibers		White fibers		Intermediate fibers	
	μm^2	SEM[b]	μm^2	SEM	μm^2	SEM
28	750	56	1478	120	1080	73
112	871	30	1784	97	1255	50
168	926	34	1990	73	1403	50
224	1053	46	2237	68	1483	43
280	1230	41	2638	81	1764	53
336	1335	39	2769	78	1774	54
392	1599	53	3067	83	2049	59

[a] Each age period consisted of biopsy samples from 24 calves with four observations per calf.
[b] Standard error of the mean

Table 2—Changes in the percentage encounters of bovine muscle fiber types with age.[a]

Days of Age	Red fibers		White fibers		Intermediate fibers	
	%	SEM[b]	%	SEM	%	SEM
28	38.1	1.4	35.3	1.4	26.7	1.5
112	37.6	1.4	33.4	1.5	29.0	1.4
168	35.1	1.0	36.8	0.8	28.2	0.9
224	36.6	1.1	36.9	0.9	26.4	1.0
280	37.2	1.2	37.9	1.2	25.0	1.0
336	36.2	0.9	37.8	0.9	26.0	0.9
392	33.2	1.0	41.0	1.2	25.8	0.8

[a] Each age period consisted of biopsy samples from 24 calves with four observations per calf.
[b] Standard error of the mean

Table 3—Changes in percentage area in bovine muscle fiber types with age[a]

Days of age	Red fibers		White fibers		Intermediate fibers	
	%	SEM[b]	%	SEM	%	SEM
28	27.8	1.2	45.8	1.6	26.4	1.5
112	26.2	1.5	45.2	1.9	28.5	1.6
168	34.5	1.7	50.2	1.1	27.2	1.0
224	24.1	0.8	51.4	0.9	24.5	0.9
280	24.2	1.0	52.2	1.5	23.3	1.0
336	24.7	1.0	52.2	1.2	23.0	0.7
392	23.2	1.0	53.8	1.3	23.0	0.8

[a] Each age period consisted of biopsy samples from 24 calves with four observations per calf.
[b] Standard error of the mean

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ase activity in order to differentiate cross-sections of red, white, and intermediate fiber types according to their oxidative capabilities (Engel and Brooke, 1966). Photomicrographs taken at 125X were projected onto a point-counting grid system for determination of encounters of each fiber type and their cross-sectional areas at a final magnification of 500X (Weibel et al., 1966). The grid system was calibrated by projection of a photomicrograph of a Bausch and Lomb stage micrometer taken under similar conditions as experimental photomicrographs. The system was adjusted to set the distance between intersections on the grid equal to 10 μm. A photomicrographic field was defined as 10,000 μm^2.

Six slides were made from each sample. Two of them were selected for staining and two randomly selected photomicrographic fields were taken on each slide. Fiber counts were computed as an average of the four fields per biopsy. Only those profiles entirely within the field were counted. Fiber profiles were subjectively classified as red, white, or intermediate by the criteria of Gauthier (1969) and Moody and Cassens (1968).

The cross-sectional area of muscle fibers in μm^2 was determined by counting the number of grid intersections on or within the fiber in the projected image and multiplying by 100. The percentage cross-sectional area of a given fiber type was estimated as the product of the encounters of that fiber type times the mean cross-sectional area.

One-way analyses of variance were computed to test whether variances in percentage encounters of the different fiber types and their mean and total cross-sectional areas could be due to differences in breed, sex, and age of the cattle sampled.

RESULTS

THE MEAN cross-sectional area of each fiber type (Table 1) increased approximately twofold from 28 to 392 days of age. The encounters of white fibers (Table 2) and the percentage occupied by their cross-sectional areas (Table 3) tended to increase from 28 to 392 days of age. The encounters and cross-sectional area of red fibers tended to decrease over the same interval. The effects of breed and sex on the percentage of cross-sectional area for each fiber type are shown by age at biopsy in Table 4. Significant differences and their interpretations are given in Table 5.

Angus and Holstein calves showed a greater difference between sexes in red fiber area than Hereford calves. The patterns for red fiber area in Holstein steers resembled that of all heifers more than that for all steers. In Holstein steers, red fibers were encountered more frequently than in Holstein heifers, although fiber cross-sectional area tended to be somewhat smaller in Holstein steers than in Holstein heifers.

Significant differences in white fiber cross-sectional area existed at all biopsy periods. Greater cross-sectional areas of white fibers were observed in Angus and Hereford steers than in their respective heifers at 6 of 7 biopsy periods. Generally, in Angus steers, white fiber profiles were larger

Table 4—Effects of breed and sex on percentage area of bovine muscle fibers with age

Breed/ Sex[a]	N	%R[b]	%W	%I	%R	%W	%I	%R	%W	%I	%R	%W	%I	%R	%W	%I	%R	%W	%I	%R	%W	%I
		28 Days			112 Days			168 Days			224 Days			280 Days			336 Days			392 Days		
HF	2	26	36	37	26	28	47	23	35	42	21	44	35	20	50	31	17	58	25	22	52	25
HM	8	27	45	28	23	48	29	18	55	27	22	53	25	22	56	22	24	55	21	24	55	21
AF	2	30	35	34	38	40	22	37	39	28	24	50	24	20	53	26	33	37	29	28	41	31
AM	4	21	55	23	19	54	27	19	53	28	22	55	23	24	52	24	25	53	22	19	57	24
Hol F	4	38	39	22	27	47	25	27	53	21	34	45	20	27	50	23	27	51	22	22	55	22
Hol M	4	30	51	19	37	41	21	25	49	25	26	54	20	39	41	20	25	47	28	25	56	19
Pooled SEM		1.0	0.9	1.6	1.4	1.7	1.5	1.2	1.0	1.0	0.9	0.8	0.9	1.0	1.3	0.9	1.0	1.1	0.8	0.9	1.1	0.7

[a] H = Hereford; A = Angus; Hol = Holstein; F = heifers; M = steers.
[b] %R = percent red fibers; %W = percent white fibers; %I = percent intermediate fibers.

Table 5—Differences in percentage area of muscle fiber types during growth by sex and breed

Age[a]	Fiber Type	Significance level	Effect[b]	Interpretation[c]
28	Red	0.001	Combined	Hol F>HF=HM=AM
	Red	0.001	Breed	Hol M>AM
	White	0.001	Sex	AM>HF=AF
	Intermed.	0.02	Breed	HF>Hol F=Hol M
112	Red	0.001	Sex	AF>AM
	White	0.01	Breed	AM>HF=HM
	Intermed.	0.001	Combined	HF>All Others
168	Red	0.001	Combined	AF>HM=HF=Hol F=Hol M
	Red	0.001	Sex	Hol F>HM
	White	0.001	Sex	HM>HF=AF
	White	0.001	Sex	AM>Hol F=HF
	Intermed.	0.001	Combined	HF>All Others
224	Red	0.001	Combined	Hol F>AM=AF=HF=HM
	White	0.003	Sex	AM=Hol M>Hol F=HF
	Intermed.	0.002	Combined	HF>All Others
280	Red	0.001	Combined	Hol M>All Others
	White	0.02	Combined	HM=AF>Hol M
	Intermed.	0.01	Sex	HF>Hol M
336	Red	0.001	Breed	AF>HF
	White	0.001	Sex	AF>HM
392	White	0.01	Combined	AM=Hol F=Hol M=HF>AF

[a] Age in days
[b] Combined refers to the interaction of breed and sex
[c] H = Hereford; A = Angus; Hol = Holstein; F = heifers; M = steers.

and encountered more frequently than in Angus heifers. In Hereford steers, white fiber profiles were encountered less often and cross-sectional areas were smaller than in Hereford heifers until 168 days of age. In Hereford steers over 168 days of age, white fiber profiles were larger than in Hereford heifers. Holstein calves showed an inconsistent pattern of differences between sexes in white fiber cross-sectional area. Holstein heifers tended to have larger white fiber profiles, while Holstein steers had the smallest white fiber profiles of all calves.

Significant differences in intermediate fiber cross-sectional area existed, except at 336 and 392 days of age. Heifers had larger cross-sectional areas of intermediate fibers than steers, generally.

DISCUSSION

THE TWOFOLD INCREASE in mean cross-sectional area in all fiber types from 28 to 392 days of age is indicative of the well-documented hypertrophic pattern of muscle fiber growth (Morita et al., 1970; Rickansrud and Hendrickson, 1967; Nystrom, 1968; Luff and Goldspink, 1970; Joubert, 1956). As judged according to their propensities for oxidative metabolic activities by NADH-diaphorase, the mean cross-sectional area of the various fiber types were found to be the same as those reported by Gauthier (1969), i.e., white > intermediate > red. However, the relative differences might not have been as marked had other staining techniques, especially Ca^{++}-activated ATPase, been employed.

Although the alterations in encounters of each fiber type were not as definitive as alterations in cross-sectional areas over the same interval, there was a slight increase in encounters of white fibers and decrease in encounters of red fibers in all calves. These trends support the hypotheses of Dubowitz (1965), Swatland and Cassens (1972) and Ashmore et al. (1972) that numbers of white fibers increase via differentiation from red fibers without alteration in the total number of fibers.

Results indicated that cross-sectional area of different fiber types was somewhat more related to differences in sex than in breed. Within breed, heifers had red fibers with larger cross-sectional areas. In Holstein calves, steers showed a greater frequency of red fiber profiles than did heifers, but heifers had the larger red fiber profile; therefore, either parameter may have been an influencing factor in sampling within age. No greater overall effect of either parameter may be stated. The lower encounters of red fibers in Hereford heifers in comparison with Hereford steers, as well as, the increase in encounters of intermediate fibers was reflected in the area occupied by these fibers and may have been caused by an inaccurate classification of "true" red fibers.

Frequency of white fiber encounters in Holstein steers was less than that in all other steers until 336 days of age, after which the distribution approached that of other steers. The larger cross-sectional area of white fibers found in Holstein heifers was in disagreement with reported findings from other cattle breeds (Ashmore et al., 1972; Covington et al., 1970; Field et al., 1970). Although Holstein calves consistently showed larger white fiber cross-sectional areas than Angus heifers, and smaller white fiber cross-sectional areas than Angus steers, no consistent pattern of differences in this parameter in Holstein and Hereford calves was apparent.

Inasmuch as the encounters and cross-sectional area contributed by intermediate fibers was relatively unchanged throughout growth, the possibility should be considered that intermediate fibers are transitional forms in the conversion of red fibers to white fibers.

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USE OF CALCIUM SALTS FOR SOYBEAN CURD PREPARATION

J. Y. LU, ELOISE CARTER, and R. A. CHUNG

ABSTRACT

One of the delicate steps in the preparation of soybean curd is the addition of a salt to precipitate soy protein. The commonly employed salt is calcium sulfate. In this study, the use of other calcium salts (calcium chloride, calcium lactate, calcium acetate, calcium carbonate, calcium phosphate, calcium hydroxide, calcium gluconate) and two noncalcium compounds (glucono-δ-lactone and acetic acid) was investigated. The result showed that soy protein was precipitated by calcium chloride, calcium lactate, calcium acetate, calcium gluconate, glucono-δ-lactone, and acetic acid. The amount of chemical added to precipitate soy protein varied depending upon the type of compound used, but in all cases, soy protein was precipitated when the pH of soy milk was at or near 6.0. Calcium acetate and calcium chloride appeared to be good precipitants for soybean curd preparation. The quality and sensory score of the soybean curd prepared from these salts requires less skill than calcium sulfate because of solubility; besides, the amount of the salt needed to precipitate soy protein was less than one-half that of calcium sulfate.

INTRODUCTION

SOYBEAN CURD is one of the important nonfermented soybean products which has been widely used in a variety of dishes by Oriental people for many centuries. This is a highly digestible and nutritive product (Schroder et al., 1973; Liener, 1972), and it also serves as an inexpensive protein source for man.

The traditional way of soybean curd preparation involves first preparation of soy milk, addition of a calcium salt, and then molding the resulting curd into cakes. It has been recognized that the selection and addition of a salt at the proper level is the most important step in the preparation of soybean curd. Insufficient amounts of salt may result in incomplete precipitation of soy protein and make the subsequent filtration difficult, whereas excess amounts of salt make the texture of soybean curd hard and unpalatable. Smith (1949), Smith et al. (1960), Schroeder et al. (1973), and Wu and Salunkhe (1977) used calcium sulfate as the precipitating agent. Wang (1967) reported the use of a mixture consisting of calcium sulfate and magnesium sulfate. It appears that calcium sulfate is the most commonly employed salt in soybean curd preparation. The use of calcium sulfate, however, causes some problems. This salt is practically insoluble; therefore, the addition of this salt needs skill, otherwise, the quality of soybean curd may vary from time to time.

The present investigation was made to study whether other protein precipitants, calcium salts in particular, can be used, and to compare the resulting soybean curds with that of calcium sulfate.

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MATERIALS & METHODS

Preparation of soybean curd

Soybeans (Tracy variety) were purchased from a local seedstore. The beans were soaked in water overnight, then homogenized with 2.5 volumes of water in a Waring Blendor for 2 min at high speed. The homogenized mash was strained through double-layered cheesecloth to obtain soy milk. The soy milk was cooked for 20 min at 95–100°C; after cooling to 70°C, to a 2-liter portion of soy milk, a salt solution was added slowly while stirring until soy protein started to coagulate. The curd was transferred to a plastic box (3 × 4 × 6 in.) lined with cheesecloth to drain off the water. The curd was pressed by a 200-g weight placed on top of the curd for 2–3 hr until draining had almost stopped. The soybean curd thus prepared was examined and compared for its quality.

Precipitating agents

Reagent grade calcium sulfate $CaSO_4 \cdot 2H_2O$, calcium carbonate $CaCO_3$, calcium phosphate $CaHPO_4$, calcium acetate $Ca(CH_3COO)_2$, and calcium hydroxide $Ca(OH)_2$ were obtained from Fisher Scientific Co., Inc. Calcium gluconate $C_{12}H_{22}CaO_{14}$, calcium chloride $CaCl_2 \cdot 2H_2O$ and calcium lactate $C_6H_{10}CaO_6 \cdot 5H_2O$ were U.S.P. grade also obtained from the Fisher Scientific Co. Glucono-δ-lactone $C_6H_{10}O_6$ was a product of Pfizer Chemical Inc., and 5% acetic acid was prepared by diluting glacial acetic acid.

Moisture and protein

Moisture content was determined by drying 10–20g of soybean curd at 100–110°C until constant weight. Protein content was determined by the Kjeldahl method (AOAC, 1970).

Yield

Yield was expressed as grams fresh soybean curd obtained from 1 liter of soy milk One liter of soy milk was made from about 133g of soybeans.

Hardness

Texture qualities were determined by compressing 1 × 1 × ½ in. size soybean curd to 1/8-in. thickness by the compression anvil fixture attached to an Instron Model 1132 texture meter.

Color

The color of soybean curd expressed in L, a and b values were determined by a Gardner XL-10 tristimulus colorimeter.

Taste panel evaluation

A group of panelists familiar with the taste of soybean curd was asked to evaluate the soybean curd samples prepared from various precipitating agents. The samples were ranked according to their preference, and the results were analyzed (ASTM, 1968).

RESULTS & DISCUSSION

TABLE 1 shows that the calcium salts (calcium gluconate, calcium chloride, calcium acetate, calcium lactate, calcium sulfate) and the two noncalcium compounds (acetic acid and glucono-δ-lactone) all precipitated soy protein. The amounts of chemicals added varied depending upon the type of compounds used. In this experiment, the lowest concentrations used were 0.1% for acetic acid and calcium chloride, 0.15% for calcium acetate, 0.2% for calcium lactate and glucono-δ-lactone, 0.3% for calcium sulfate, and 0.5% for calcium gluconate. The pH of soy milk was initially 6.40 but the pH decreased with the increase in the concentration of chemical added. In all the cases, regardless of the type of chemicals used, soy protein started to coagulate when the pH dropped to about 6.0. Calcium carbonate, calcium hydroxide, and calcium phosphate did not precipi-

Table 1—Precipitation of soy protein[a]

	% Added	0	0.05	0.1	0.15	0.2	0.3	0.4	0.5
Calcium gluconate	Coag	–	–	–	–	–	–	–	+
	pH	6.40		6.40		6.40	6.35	6.20	6.05
Calcium chloride	Coag	–	–	+		+	+		
	pH		6.20	6.00		5.85	5.65		
Calcium sulfate	Coag	–		–		–	+	+	
	pH			6.40		6.17	6.00	5.95	
Calcium acetate	Coag	–	–	–	+	+	+		
	pH		6.25	6.11	6.00	5.98	5.85		
Calcium carbonate	Coag	–		–		–	–	–	–
	pH			6.40		6.40	6.40	6.35	6.30
Calcium hydroxide	Coag	–		–		–	–	–	–
	pH			9.45		10.45	11.03		
Calcium lactate	Coag	–		–	–	+	+	+	
	pH			6.20	6.10	6.00	5.95	5.85	
Glucono-δ-lactone	Coag	–		–	–	+	+	+	
	pH			6.20	6.10	6.00	5.84	5.67	
Calcium phosphate	Coag	–		–		–	–	–	
	pH			6.40		6.40	6.40	6.40	
Acetic acid	Coag	–	–	+	+	+			
	pH		6.07	5.98	5.82	5.54			

[a] Mean of three to four determinations. + Coagulation positive; – Coagulation negative.

Table 2—Comparison of soybean curds[a]

	Moisture (%)	Yield[b] (g)	Protein[c] (g)	Chemical added (%)	Hardness (lb)	Color[d]		
						L	a	b
Calcium lactate	84.6 ± 3.1	225 ± 150	7.8 ± 0.7	0.18 ± 0.01	2.66 ± 1.18	66.6	−1.05	9.6
Calcium acetate	84.3 ± 2.1	244 ± 126	7.8 ± 0.6	0.11 ± 0.00	1.66 ± 0.57	66.1	−1.45	9.2
Calcium chloride	86.0 ± 2.6	249 ± 137	7.5 ± 0.6	0.09 ± 0.01	1.52 ± 1.14	66.7	−1.40	9.0
Calcium sulfate	85.2 ± 1.3	225 ± 140	7.6 ± 0.4	0.25 ± 0.08	2.59 ± 1.38	66.1	−1.20	9.3
Glucono-δ-lactone	84.2 ± 0.6	210 ± 110	7.8 ± 0.5	0.24 ± 0.01	3.89 ± 1.10	66.3	−1.21	9.2
Acetic acid	86.1 ± 2.8	229 ± 113	7.6 ± 0.1	0.09 ± 0.01	1.91 ± 0.64	67.5	−1.50	8.4

[a] Mean ± S.D. of three batches
[b] Fresh soybean curd obtained from 1 liter of soy milk
[c] g per 100g wet basis
[d] Average of two determinations

tate soy protein at all concentrations tested. It was noted that with addition of these chemicals, the pH values of the soy milks remained high, and in the case of calcium hydroxide the pH even reached 11.08.

This fact shows that the pH, not the calcium ion, is by far the most important factor in the precipitation of soy protein Soybean curd actually is a soy isolate which is precipitated at pH 6.0.

The quality of soybean curd is indicated by its volume, moisture content, hardness, texture, and color (Smith et al., 1960). Table 2 shows that the moisture content of soybean curds prepared from various precipitating agents ranged from 84–86% with an average value of 85% and the protein content, from 7.5–7.84, with an average value of 7.71. These values are similar to those reported by Watts and Merill (1963).

The exact effect of calcium ion on soy protein precipitation is not clearly understood It forms a smooth gel texture which is high in water content. The data shown in Table 2 also indicate that, in general, soybean curds prepared from calcium salts had a higher yield than the noncalcium compounds. However, the variations were large. The variation may have resulted from slight differences in nitrogen content of soy milk in different batches, from processing errors, and losses of soluble matters during the pressing and rinsing of soybean curd

Softer texture is desirable for soybean curd. The soy. bean curds prepared from calcium chloride, calcium acetate and acetic acid were softer than the other three precipitating agents (Table 2). The cause of this difference may be related to the amount of chemical added. For the softer soybean curds the amounts of chemical added were 0.09% for calcium chloride, 0.09% for acetic acid, and 0.11% for calcium acetate. These levels are only one-half that of the harder soybean curds made from calcium sulfate, calcium lactate, and glucono-δ-lactone. Table 2 also indicates that the variation of adding calcium sulfate was greater than other chemicals employed.

Good soybean curd is white or very pale yellow in color. All the soybean curds prepared in this experiment had a pale yellow color. Although the colorimetric readings showed that the acetic acid soybean curd had slightly higher L and lower a and b values (Table 2), this difference was not detected by the panelists. This result indicates that the precipitating chemicals employed in this experiment had very little effect on the color of soybean curd.

It is desirable for soybean curd to have a very bland taste, and smooth and softer texture. Acceptability of the soybean curds prepared from calcium acetate, calcium sulfate, acetic acid, and glucono-δ-lactone were compared, and the result is shown in Table 3. To avoid confusion in judging, four samples were presented to the taste panelists each time. The samples were ranked according to their preference, and the highest score given was 4, and the lowest score, 1. The result showed that the panelists liked the first two much better than the latter two. The difference was significant ($P < 0.05$). This indicates that calcium ion has a desirable effect on palatability of soybean curd. It was observed that the glucono-δ-lactone soybean curd had a firm and somewhat rubbery texture, and the acetic acid soybean curd had a softer texture but it was easily broken and lacked elasticity.

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Table 3—Taste panel scores of soybean curds made from calcium salts and noncalcium compounds

Subject	Calcium acetate	Calcium sulfate	Acetic acid	Glucono-δ-lactone
1	4	3	2	1
2	3	4	2	1
3	4	3	1	2
4	4	3	2	1
5	3	4	2	1
6	4	3	2	1
Rank Total	22	21	11	7

Table 4—Taste panel scores of soybean curds made from calcium salts

Subject	Calcium sulfate	Calcium acetate	Calcium chloride	Calcium lactate
1	4	3	2	1
2	3	4	2	1
3	3	4	2	1
4	2	3	4	1
5	2	1	3	4
6	4	2	3	1
Rank total	18	17	16	9

In the next experiment the soybean curds prepared from four calcium salts were compared. The result (Table 4) showed that calcium sulfate, calcium acetate, and calcium chloride were rated almost equal, and the difference was not significant ($P > 0.05$). However, calcium sulfate soybean curd was preferred to calcium lactate soybean curd ($P > 0.05$). It was noted that the soybean curd prepared from calcium sulfate had a smooth, firm, and somewhat elastic texture.

The taste panel scores for calcium acetate and calcium sulfate were higher in Table 3 than in Table 4. The reason for this difference was due to the fact that Table 3 and Table 4 were results of separate experiments. The taste panel judges were not exactly the same, therefore the results varied somewhat. Furthermore, in Table 3, acetic acid and glucono-δ-lactone scored low, therefore calcium acetate and calcium sulfate collected higher scores; whereas in Table 4, because of calcium chloride, the scores given for calcium acetate and calcium sulfate were affected, therefore the scores decreased.

It has been recognized that the coagulation of soy protein by addition of a salt is the most difficult step in making soybean curd. The right amount of the salt and the proper rate of its addition are two important factors necessary to attain a satisfactory soybean curd (Wang, 1967). This problem could be eased and controlled if the change of pH in soy milk is carefully monitored during the addition of a salt solution. The present study shows that the addition of a salt should be slowed down or stopped when the pH of soy milk approached 6.0.

Another finding of this study is that it may be advantageous to use either calcium acetate or calcium chloride instead of calcium sulfate. Calcium acetate and calcium chloride are soluble; therefore, it is easier to add and mix these salts uniformly with soy milk and obtain a soybean curd with uniform quality. Taste panel scores have shown that the soybean curds prepared from these salts were rated almost equal to that from calcium sulfate; besides, the amount of the salts used was much less than calcium sulfate. The information obtained by this study may be of value to food scientists, dietitians, and those interested in vegetarian, high protein foods.

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CONVERTING AN F-VALUE DETERMINED ON THE BASIS OF ONE z-VALUE TO AN F-VALUE DETERMINED ON THE BASIS OF A SECOND z-VALUE

IRVING J. PFLUG and RONALD CHRISTENSEN

ABSTRACT

A procedure is presented for converting a heat sterilization process F-value determined on the basis of one z-value (z_1) to an F-value determined on the basis of a second z-value (z_2). The basis of the conversion is explained. Equations and tabular data for determining the several conversion factors are presented. Examples of the calculation of a new F-value when there is a change in z-value are presented.

INTRODUCTION

IN THE DESIGN and monitoring of sterilization processes and in the treatment of process deviations under certain circumstances where the sterilization value F for a z_1-value is known, it is necessary to know the equivalent F-value of the process for a z_2-value that may be either larger or smaller than the original z-value. (For definitions of symbols, refer to Appendix.) In this report we will analyze the problem of converting from an F-value determined on the basis of a z_1-value to an F-value on the basis of a z_2-value and will illustrate the use of the method using two examples.

(1) A sterilization process is monitored using bacterial spores; since a z of 18°F is the basis for both the design and for physical monitoring the sterilization process, a procedure is needed for converting the F-value measured by the bacterial spores with their unique z_1-value to an F-value on the basis of a z_2-value of 18°F.

(2) A product receives a sterilization process (F_0) designed on the basis of a z_2-value of 18°F. What is the F-value at 250°F if the heat resistant microbial spores in the product have a z_1-value of 12°F?

The z-value is the degrees of temperature for the thermal death time (TDT) curve to change by a factor of ten. Its role in calculating the sterilization value (F) of a heat process is well known (Ball 1923, 1928; Ball and Olson 1956; Stumbo 1973). We cannot convert from an F-value determined on the basis of a z_1-value to an F-value on the basis of a z_2-value unless we have heating data either in the form of time-temperature data or in the form of reasonable estimates of the f_h, f_c, and j_c. When the time-temperature data are available, the F-value for z_2 can be directly determined using the general method (Bigelow et al., 1920). Usually, time-temperature data are not available but reasonable estimates of f_h, f_c, and j_c can be made.

The objective of this report is to present an analysis of the problem and a solution in the form of a method for converting an F-value determined on the basis of a z_1-value to an F-value on the basis of a z_2 value assuming that an estimate of the f_h-value is available, that $f_h = f_c$ and that $j_c = 1.40$.

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ANALYSIS OF THE PROBLEM AND DEVELOPMENT OF A SOLUTION

THE ANALYSIS and the development of a general solution will be carried out using the biological indicator problem as an example. In the biological monitoring area the F(BIO)-value of a process is determined using bacterial spores calibrated at temperature T_c. These spores have a z_1-value over the temperature range of use. The product that is being evaluated is heated in a continuous processing machine; the heating medium temperature is T_1. The f_h-value of the product in the container is known. The problem is finding the F-value delivered to the product at a reference temperature, T_{ref}, for a reference z_2-value.

Calculations and data will be based on the mathematical methods of Ball (1923) and tabulated values in Stumbo (1973). Since we are using the mathematical method form, the conversion involving the heating rate of the product must be carried out at the heating medium temperature, T_1. Since the heating medium temperature may be different from the calibration temperature, T_c, and the final reference temperature, T_{ref}, the conversion process requires three steps.

The conversion process is carried out by using the three equations below in consecutive sequence.

$$\text{Step 1: } \underset{(B)}{F(T_1, z_1)} = \underset{(A)}{F(T_c, z_1)} \times Y_1$$

$$\text{Step 2: } \underset{(C)}{F(T_1, z_2)} = \underset{(B)}{F(T_1, z_1)} \times Y_2$$

$$\text{Step 3: } \underset{(D)}{F(T_{ref}, z_2)} = \underset{(C)}{F(T_1, z_2)} \times Y_3$$

The sequence of calculations is: A to B using factor Y_1, B to C using factor Y_2, and C to D using factor Y_3. In simplified form, the three steps can be written as follows:

$$A \times Y_1 = B;\ B \times Y_2 = C;\ C \times Y_3 = D \qquad (1)$$

The conversion process is shown graphically in Figures 1, 2 and 3 for $z_1 < z_2$. The letters above the equations correspond to the letters on the graphs.

The first step, A to B, is to convert the F-value measured at the biological indicator unit (BIU) calibration temperature, T_c, with a z_1-value to an F-value at the heating medium temperature, T_1, on the same z_1-value basis. This conversion requires factor Y_1, min at T_1 per min at T_c, both on the same z_1-value basis; Y_1 is the reciprocal of the lethal rate which can be obtained from a lethal rate table or calculated using the following equation:

$$\text{Factor } Y_1 = 10^{(T_c - T_1)/z_1}. \qquad (2)$$

The second step, B to C, is to convert from the F-value at T_1 with a z_1-value, to an F-value still at T_1 but with a z_2-value. This requires factor Y_2, which can be calculated from the data in Tables 1 through 5. The development of the Y_2 factor is described in a latter section of this manuscript.

The third step, C to D, is to convert from the F-value at T_1 with a z_2-value, to an F-value at the reference tempera-

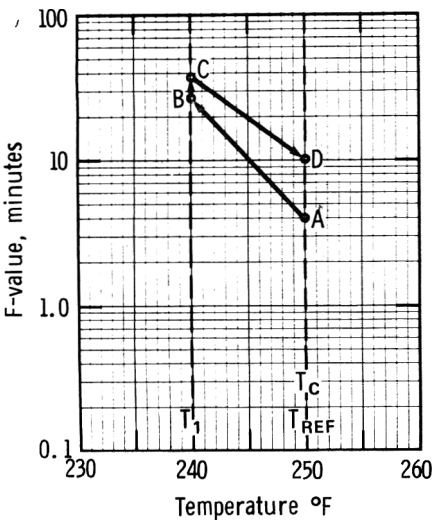

Fig. 1—Graphic example of the F-value correction procedure for a change in z-value where T_c and T_{ref} are both at 250°F, and T_1 is 240°F.

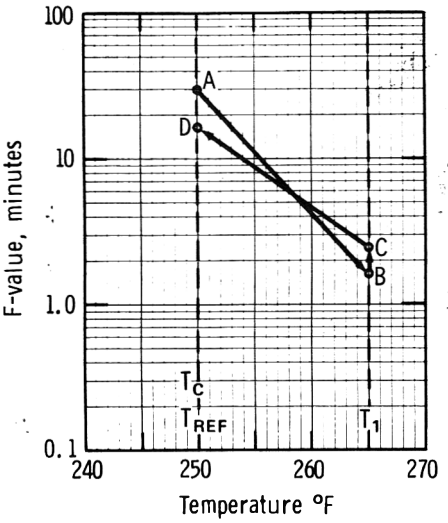

Fig. 2—Graphic example of the F-value correction procedure for a change in z-value where T_c and T_{ref} are both at 250°F, and T_1 is 265°F.

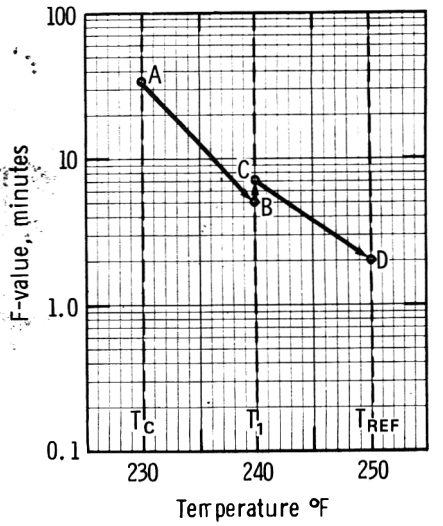

Fig. 3—Graphic example of the F-value correction procedure for a change in z-value where T_c is 230°F, T_1 is 240°F, and T_{ref} is 250°F.

Table 1—Corresponding sterilization values for z-values of 10 and 18°F for several f_h-values computed at the heating medium temperature for a j_c of 1.40

F (T_1, z = 10°F) (min)	F(T_1, z = 18°F), min					
	f_h, min					
	5.00	10.0	20.0	50.0	100.	200.
0.50	1.18	1.52	1.93	2.63	3.35	4.38
1.00	1.84	2.35	3.03	4.17	5.26	6.70
1.50	2.43	3.05	3.92	5.45	6.90	8.71
2.00	2.99	3.68	4.71	6.57	8.35	10.5
2.50	3.53	4.28	5.43	7.58	9.67	12.2
3.00	4.07	4.86	6.10	8.51	10.9	13.8
3.50	4.61	5.42	6.74	9.39	12.0	15.3
4.00	5.14	5.97	7.37	10.2	13.1	16.7
4.50	5.68	6.52	7.97	11.0	14.2	18.1
5.00	6.22	7.06	8.56	11.8	15.2	19.3
6.00	7.29	8.14	9.71	13.2	17.0	21.8
7.00	8.36	9.21	10.8	14.6	18.8	24.1
8.00	9.42	10.3	11.9	15.9	20.4	26.3
9.00	10.5	11.4	13.0	17.2	22.0	28.3
10.00	11.5	12.4	14.1	18.4	23.5	30.3
15.00	16.5	17.8	19.5	24.3	30.5	39.2
20.00	21.5	22.8	24.9	29.9	36.8	47.1
25.00	26.5	27.8	30.2	35.3	42.8	54.3
30.00	31.5	32.8	35.6	40.7	48.6	61.0
35.00	36.5	37.8	40.8	46.1	54.2	67.4
40.00	41.5	42.8	46.0	51.4	59.7	73.7
45.00	46.5	47.8	51.0	56.8	65.2	79.7
50.00	51.5	52.8	56.0	62.2	70.6	85.6
60.00	61.5	62.8	66.0	72.9	81.4	97.1
70.00	71.5	72.8	76.0	83.6	92.1	108.
80.00	81.5	82.8	86.0	94.2	103.	119.
90.00	91.5	92.8	96.0	105.	114.	130.
100.00	102.	103.	106.	115.	124.	141.
110.00	112.	113.	116.	125.	135.	152.
120.00	122.	123.	126.	135.	146.	163.
130.00	132.	133.	136.	145.	157.	174.
140.00	142.	143.	146.	155.	167.	184.
150.00	152.	153.	156.	165.	178.	195.
160.00	162.	163.	166.	175.	188.	206.
170.00	172.	173.	176.	185.	199.	216.
180.00	182.	183.	186.	195.	209.	227.
190.00	192.	193.	196.	205.	220.	238.
200.00	202.	203.	206.	215.	230.	249.

Table 2—Corresponding sterilization values for z-values of 12 and 18°F for several f_h-values computed at the heating medium temperature for a j_c of 1.40

F (T_1, z = 12°F) (min)	F(T_1, z = 18°F), min					
	f_h, min					
	5.00	10.0	20.0	50.0	100.	200.
0.50	.953	1.14	1.33	1.64	2.01	2.59
1.00	1.58	1.91	2.28	2.80	3.28	4.02
1.50	2.14	2.56	3.09	3.84	4.47	5.31
2.00	2.67	3.16	3.81	4.80	5.59	6.56
2.50	3.19	3.73	4.49	5.69	6.66	7.77
3.00	3.71	4.28	5.12	6.53	7.68	8.94
3.50	4.23	4.81	5.73	7.33	8.66	10.1
4.00	4.75	5.34	6.32	8.09	9.60	11.2
4.50	5.27	5.86	6.89	8.83	10.5	12.3
5.00	5.79	6.38	7.46	9.53	11.4	13.3
6.00	6.84	7.42	8.55	10.9	13.1	15.4
7.00	7.90	8.45	9.62	12.2	14.7	17.3
8.00	8.95	9.49	10.7	13.4	16.2	19.2
9.00	9.99	10.5	11.7	14.6	17.7	21.0
10.00	11.0	11.6	12.8	15.8	19.1	22.8
15.00	16.0	16.9	17.9	21.4	25.6	30.9
20.00	21.0	22.0	23.2	26.7	31.6	38.1
25.00	26.0	26.9	28.4	31.9	37.3	44.9
30.00	31.0	31.9	33.7	37.1	42.8	51.2
35.00	36.0	36.9	38.9	42.3	48.1	57.3
40.00	41.0	41.9	44.1	47.5	53.4	63.2
45.00	46.0	46.9	49.1	52.7	58.6	68.9
50.00	51.0	51.9	53.9	57.9	63.8	74.6
60.00	61.0	61.9	63.9	68.4	74.2	85.5
70.00	71.0	71.9	73.9	79.0	84.5	96.2
80.00	81.0	81.9	83.9	89.5	94.9	107.
90.00	91.0	91.9	93.9	99.9	105.	117.
100.00	101.	102.	104.	110.	116.	128.
110.00	111.	112.	114.	120.	126.	138.
120.00	121.	122.	124.	130.	137.	148.
130.00	131.	132.	134.	140.	147.	159.
140.00	141.	142.	144.	150.	158.	169.
150.00	151.	152.	154.	160.	169.	179.
160.00	161.	162.	164.	170.	179.	190.
170.00	171.	172.	174.	180.	189.	200.
180.00	181.	182.	184.	190.	200.	211.
190.00	191.	192.	194.	200.	210.	221.
200.00	201.	202.	204.	210.	220.	232.

ture, T_{ref}, on the same z_2-value basis. This requires factor Y_3 which is the lethal rate, min at T_{ref} per min at T_1. It can be obtained from a lethal rate table or calculated using the following equation:

$$\text{Factor } Y_3 = 10^{(T_1 - T_{ref})/z_2}. \quad (3)$$

Eq (1) is applicable whether the heating medium temperature, T_1, is above or below the BIU calibration temperature and reference temperature, shown graphically in Figures 1 and 2, or between the calibration and reference temperature as shown in Figure 3.

If the BIU's are calibrated at the test (heating medium) temperature that is different from the reference temperature, then the conversion involves only two steps. These are steps two and three above.

The two steps can be written together as:

$$\underset{(B)}{F(T_1, z_1)} \times Y_2 = \underset{(C)}{F(T_1, z_2)}; \underset{(C)}{F(T_1, z_2)} \times Y_3 = \underset{(D)}{F(T_{ref}, z_2)}.$$

This conversion, B to C to D, is illustrated in Figures 1, 2 and 3.

When the BIU's are calibrated at the reference temperature and this is also the heating medium temperature, only the B to C correction is required.

DEVELOPMENT OF THE Y_2 CONVERSION FACTOR

EQUIVALENT sterilization time of a process $F(T_1, z)$ evaluated using the heating medium temperature (T_1) as the reference base is computed by plugging time-temperature data into the following equation:

$$F(T_1, z) = \sum L\Delta t = \sum 10^{(T - T_1)/z}\,\Delta t. \quad (4)$$

When the heating medium temperature (T_1) is used as the sterilization value reference base, the product temperature will be, by definition, equal or less than the heating medium temperature (i.e., $T \leqslant T_1$). Therefore, the equivalent sterilization time $F(T_1, z)$ for specific time-temperature data will unconditionally increase with an increase in the z-value.

The Y_2 conversion factor is the ratio of the sterilization value for z_2 compared to z_1 using the heating medium temperature (T_1) as a reference for a specific known heat process. It is shown below:

$$Y_2 = \frac{F(T_1, z_2),\ \text{process time-temp data integrated at } T_1 \text{ using } z_2}{F(T_1, z_1),\ \text{process time-temp data integrated at } T_1 \text{ using } z_1}.$$

Therefore, $F(T_1, z_2) = F(T_1, z_1) \times Y_2$.

The sterilization value using the heating medium temper-

Table 3—Corresponding sterillization values for z-values of 14 and 18°F for several f_h-values computed at the heating medium temperature for a j_c of 1.40

F (T_1, z = 14°F) (min)	F(T_1, z = 18°F), min; f_h, min 5.00	10.0	20.0	50.0	100.	200.
0.50	.769	.863	.948	1.08	1.26	1.54
1.00	1.35	1.54	1.73	1.95	2.16	2.51
1.50	1.88	2.14	2.43	2.78	3.04	3.42
2.00	2.40	2.70	3.08	3.57	3.90	4.31
2.50	2.91	3.24	3.69	4.32	4.74	5.20
3.00	3.41	3.76	4.28	5.04	5.56	6.07
3.50	3.92	4.28	4.84	5.73	6.35	6.94
4.00	4.44	4.79	5.40	6.40	7.13	7.80
4.50	4.95	5.30	5.94	7.05	7.89	8.64
5.00	5.47	5.81	6.47	7.69	8.63	9.48
6.00	6.51	6.83	7.53	8.92	10.1	11.1
7.00	7.57	7.85	8.56	10.1	11.5	12.7
8.00	8.62	8.87	9.58	11.3	12.8	14.3
9.00	9.66	9.90	10.6	12.4	14.1	15.8
10.00	10.7	10.9	11.6	13.5	15.4	17.3
15.00	15.7	16.2	16.7	18.8	21.4	24.3
20.00	20.7	21.4	21.9	24.0	27.0	30.8
25.00	25.7	26.3	27.1	29.1	32.4	36.9
30.00	30.7	31.3	32.4	34.1	37.6	42.8
35.00	35.7	36.3	37.6	39.2	42.8	48.4
40.00	40.7	41.3	42.7	44.4	47.9	54.0
45.00	45.7	46.3	47.8	49.5	53.0	59.4
50.00	50.7	51.3	52.6	54.7	58.1	64.7
60.00	60.7	61.3	62.6	65.1	68.3	75.3
70.00	70.7	71.3	72.6	75.7	78.5	85.6
80.00	80.7	81.3	82.6	86.2	88.7	95.8
90.00	90.7	91.3	92.6	96.6	99.0	106.
100.00	101.	101.	103.	107.	109.	116.
110.00	111.	111.	113.	117.	120.	126.
120.00	121.	121.	123.	127.	130.	137.
130.00	131.	131.	133.	137.	141.	147.
140.00	141.	141.	143.	147.	151.	157.
150.00	151.	151.	153.	157.	162.	167.
160.00	161.	161.	163.	167.	172.	177.
170.00	171.	171.	173.	177.	183.	188.
180.00	181.	181.	183.	187.	193.	198.
190.00	191.	191.	193.	197.	203.	208.
200.00	201.	201.	203.	207.	214.	219.

Table 4—Corresponding sterilization values for z-values of 16 and 18°F for several f_h-values computed at the heating medium temperature for a j_c of 1.40

F (T_1, z = 16°F) (min)	F(T_1, z = 18°F), min; f_h, min 5.00	10.0	20.0	50.0	100.	200.
0.50	.626	.665	.688	.716	.773	.883
1.00	1.16	1.25	1.33	1.39	1.43	1.55
1.50	1.66	1.79	1.93	2.05	2.10	2.20
2.00	2.15	2.31	2.50	2.70	2.77	2.86
2.50	2.65	2.82	3.05	3.33	3.44	3.53
3.00	3.15	3.32	3.59	3.94	4.10	4.20
3.50	3.65	3.81	4.11	4.54	4.75	4.88
4.00	4.16	4.31	4.62	5.12	5.39	5.55
4.50	4.67	4.80	5.13	5.69	6.03	6.22
5.00	5.18	5.30	5.64	6.26	6.65	6.88
6.00	6.22	6.30	6.64	7.36	7.88	8.20
7.00	7.27	7.30	7.63	8.44	9.07	9.50
8.00	8.32	8.31	8.62	9.49	10.2	10.8
9.00	9.36	9.33	9.61	10.5	11.4	12.1
10.00	10.4	10.4	10.6	11.6	12.5	13.3
15.00	15.4	15.6	15.6	16.6	17.9	19.3
20.00	20.4	20.8	20.7	21.5	23.1	25.0
25.00	25.4	25.8	25.9	26.5	28.2	30.5
30.00	30.4	30.8	31.2	31.5	33.2	35.9
35.00	35.4	35.8	36.4	36.5	38.1	41.1
40.00	40.4	40.8	41.6	41.6	43.1	46.2
45.00	45.4	45.8	46.6	46.7	48.0	51.3
50.00	50.4	50.8	51.5	51.8	53.0	56.4
60.00	60.4	60.8	61.5	62.2	63.0	66.4
70.00	70.4	70.8	71.5	72.7	73.0	76.3
80.00	80.4	80.8	81.5	83.2	83.1	86.2
90.00	90.4	90.8	91.5	93.6	93.3	96.1
100.00	100.	101.	102.	104.	104.	106.
110.00	110.	111.	112.	114.	114.	116.
120.00	120.	121.	122.	124.	124.	126.
130.00	130.	131.	132.	134.	135.	136.
140.00	140.	141.	142.	144.	145.	146.
150.00	150	151.	152.	154.	156.	156.
160.00	160.	161.	162.	164.	166.	166.
170.00	170.	171.	172.	174.	177.	176.
180.00	180.	181.	182.	184.	187.	187.
190.00	190	191.	192.	194.	198.	197.
200.00	200	201.	202.	204.	208.	207.

ature as a reference F (T_1, z) is a basic parameter of the mathematical method of process calculations and was assigned the specific symbol U by Ball (1923). We will use this symbol in discussing the development of the Y_2 conversion factor.

The U-value is the integrated sterilization value measured at the heating medium temperature (T_1). The data in Tables 1 through 5 for z_1-values of 10, 12, 14, 16 and 20°F for z_2-values of 18°F were developed from the f_h/U vs g data published by Stumbo (1973). In developing the tables, it was assumed that $f_h = f_c$; a j_c value of 1.40 was selected so the resulting data would be in general agreement with processes calculated using the data of Ball (1923, 1928), who used a j_c value of 1.41.

If f_h, j_c, and $U(z_1)$ are known, g can be found from the tables in Stumbo (1973). Similarly, if f_h, j_c, and g are known, $U(z_2)$ can also be found from Stumbo's tables. Starting with $U(z_1)$ and a specific f_h-value, g was calculated; using the same g and f_h-value, we calculated $U(z_2 = 18°F)$. To facilitate this process, equations were developed to approximate the data in Stumbo's tables (less than 5% error). The coefficients for Eq (5) and (6) are displayed in Table 6. In Eq (5), g was established as a function of (f_h/U) for z-values of 10, 12, 14, 16 and 20°F, and in Eq (6), f_h/U as a function of g for a z of 18°F. These equations were fitted using least squares on the data in the tables in Stumbo (1973):

Table 5—Corresponding sterilization values for z-values of 20 and 18°F for several f_h-values computed at the heating medium temperature for a j_c of 1.40

F (T_1, z = 20°F) (min)	F(T_1, z = 18°F), min, f_h, min					
	5.00	10.0	20.0	50.0	100.	200.
0.50	.422	.397	.362	.322	.313	.312
1.00	.869	.844	.795	.701	.644	.626
1.50	1.32	1.29	1.24	1.12	1.01	.947
2.00	1.78	1.74	1.69	1.55	1.40	1.29
2.50	2.25	2.19	2.13	1.99	1.81	1.65
3.00	2.72	2.64	2.58	2.43	2.23	2.02
3.50	3.20	3.10	3.03	2.88	2.66	2.41
4.00	3.69	3.56	3.48	3.32	3.09	2.81
4.50	4.19	4.02	3.93	3.77	3.53	3.21
5.00	4.69	4.49	4.38	4.22	3.97	3.62
6.00	5.71	5.44	5.28	5.11	4.86	4.46
7.00	6.75	6.41	6.20	6.01	5.75	5.32
8.00	7.79	7.39	7.12	6.90	6.65	6.19
9.00	8.84	8.38	8.05	7.80	7.54	7.06
10.00	9.87	9.39	8.98	8.69	8.44	7.95
15.00	14.9	14.5	13.8	13.2	12.9	12.4
20.00	19.9	19.7	18.8	17.8	17.4	16.9
25.00	24.9	24.8	23.9	22.5	21.9	21.3
30.00	24.9	29.8	29.1	27.2	26.4	25.8
35.00	34.9	34.8	34.3	32.0	31.0	30.3
40.00	39.9	39.8	39.5	36.9	35.6	34.8
45.00	44.9	44.8	44.6	41.9	40.2	39.3
50.00	49.9	49.8	49.5	46.9	44.9	43.8
60.00	59.9	59.8	59.5	57.1	54.4	52.8
70.00	69.9	69.8	69.5	67.5	64.1	62.0
80.00	79.9	79.8	79.5	77.9	73.9	71.2
90.00	89.9	89.8	89.5	88.4	83.8	80.5
100.00	100.	100.	99.5	98.7	93.9	89.8
110.00	110.	110.	110.	109.	104.	99.3
120.00	120.	120.	120.	119.	114.	109.
130.00	130.	130.	130.	129.	125.	118.
140.00	140.	140.	140.	139.	135.	128.
150.00	150.	150.	150.	149.	145.	138.
160.00	160.	160.	160.	159.	156.	148.
170.00	170.	170.	170.	169.	166.	158.
180.00	180.	180.	180.	179.	177.	168.
190.00	190.	190.	190.	189.	187.	178.
200.00	200.	200.	200.	199.	197.	188.

$$y = a_o + a_1 x + a_2 x^2 + a_3 x^3 + a_4 (f_h/U) + a_5 (f_h/U)^{-1} \quad (5)$$

and $$x = b_o + b_1 y + b_2 y^2 + b_3 y^3 + b_4 g \quad (6)$$

where $x = \log_e(f_h/U)$ and $y = \log_e g$.

It should be noted that a theoretical result can be applied to improve the tables. With a given f_h and a large enough $U(z_1)$-value, the g-value will be very small; essentially, the product will be at heating medium temperature. Once the product is at heating medium temperature, the z-value no longer plays a role in accumulating equivalent minutes at heating medium temperature. For example, considering the effect of two z-values, if a process has accumulated $U(z_1)$ minutes and $U(z_2)$ minutes, and g is extremely small (less than 0.1°F), additional t minutes of heating will give new values $U(z_1)$ + t minutes and $U(z_2)$ + t minutes or the addition to U is t regardless of z. For extremely small values of g, the approximating equations are less accurate. Therefore, the above relationship was used to establish values in this area.

In Tables 1, 2, 3, 4 and 5 are shown $F(T_1, 18°F)$-values as a function of the temperature response parameter, f_h, and the $F(T_1, z_1)$-value; the values of z_1 are: Table 1, 10°F; Table 2, 12°F; Table 3, 14°F; Table 4, 16°F; and Table 5, 20°F. The factor, Y_2, is the ratio of two corresponding values in a table:

$$Y_2 = \frac{F(T_1, 18°F)}{F(T_1, z_1)}.$$

In the first example problem, a factor, Y_2, is required to convert the $F(T_1, z_1)$-value of 1.69 min at 265°F with a z-value of 12°F to an F(265°F, z = 18°F). Using the equation above and data from Table 2, we can calculate Y_2.

$$Y_2 = \frac{F(T_1, 18°F)}{F(T_1, 12°F)} = \frac{2.33}{1.69} = 1.38.$$

The conversion factor, Y_2, for the change in $F(T_1, z)$ from z_1 to z_2 must be treated carefully because it depends not only on $F(T_1, z_1)$ but also on the f_h- and j_c-value. Since Y_2 is a function of $F(T_1, z_1)$, in a three-step conversion the first calculation must be carried out before the factor, Y_2, can be determined. The second and third calculations can be carried out simultaneously.

The tables were designed to find $F(T_{ref}, 18°F)$-values; therefore, they were indexed for $F(T_1, z_1)$-values. When solving a process deviation problem, the $F(T_1, 18°F)$ is known; this value must be located in the body of the table and then the corresponding value of $F(T_1, z_1)$ found, after which Y_2 can be calculated.

In this development we assumed that the semilogarithmic heating and cooling curves are straight lines with temperature response parameters f_h and f_c, respectively. The conversion factors are for the zone in the container where the f and j parameters are based, and are not integrated for the total contents of the container.

Example problem—Data from biological indicator units

The F(250°F)-value, obtained by inputting the number of survivors per BIU into the calibration curve, was 30.0 min. The z-value of spores in the BIU's was 12°F. The F(250°F)-value was measured in a container where the f_h value was 5.0 min and the j_c was 1.4. The heating medium temperature was 265°F. What was the equivalent F_o (250°F, z = 18°F)?

The problem is solved starting with Eq (1):

$$A \times Y_1 = B, B \times Y_2 = C, C \times Y_3 = D$$

where:

$A = F(T_c, z_1) = F(250°F, 12°F) = 30.0$ min;

$B = F(T_1, z_1) = F(265°F, 12°F)$;

$C = F(T_1, z_2) = F(265°F, 18°F)$;

$D = F(T_{ref}, z_2) = F(250°F, 18°F) = F_o$

Substituting in Eq (2) and (3):

$$\text{Factor } Y_1 = 10^{(250 - 265)/12} = 0.0562,$$

$$\text{Factor } Y_3 = 10^{(265 - 250)/18} = 6.81.$$

We can now solve the problem stepwise:

$B = A \times Y_1$,

$B = 30.0 \text{ min} \times 0.0562 = 1.69$ min at 265°F, z of 12°F.

From Table 2, for: $f_h = 5.0$ min, j = 1.4, factor $Y_2 = \frac{2.33}{1.69} = 1.38$.

$C = B \times Y_2$,

$C = 1.69 \times 1.38 = 2.33$ min at 265°F, z of 18°F.

$D = C \times Y_3$,

$D = 2.33 \times 6.81 = 15.9$ min at 250°F, z of 18°F.

Example problem—Process deviation

The F_o of a process was 4.0 min. The f_h of the product was 10 min, the heating medium temperature was 220°F. It is possible that *C. botulinum* spores in this product will exhibit a z-value of 12°F. What is the F (250°F, z = 12°F)?

The problem is solved using a rearrangement of Eq (1):

$$D \times \frac{1}{Y_3} = C; \quad C \times \frac{1}{Y_2} = B; \quad B \times \frac{1}{Y_1} = A.$$

where:

$D = F(T_{ref}, z_2) = F(250°F, z = 18°F) = F_o = 4.0$ min;

$C = F(T_1, z_2) = F(220°F, z = 18°F)$;

$B = F(T_1, z_1) = F(220°F, z = 12°F)$;

$A = F(T_c, z_1) = F(250°F, z = 12°F)$.

Substituting in Eq (2) and (3):

$$\text{Factor } Y_1 = 10^{(250 - 220)/12} = 316.2,$$

$$\text{Factor } Y_3 = 10^{(220 - 250)/18} = 0.0215.$$

We can now solve the problem stepwise:

$$C = D \times \frac{1}{Y_3},$$

$C = 4.0 \times \frac{1}{0.0215} = 186.0$ min at 220°F, z of 18°F.

$$B = C \times \frac{1}{Y_2}.$$

From Table 2 for: $f_h = 10$, j = 1.4, factor $Y_2 = \frac{186}{184}$

$B = 186 \times \frac{184}{186} = 184$ min at 220°F, z of 12°F.

$$A = B \times \frac{1}{Y_1}.$$

$A = 184 \times \frac{1}{316.2} = 0.58$ min at 250°F, z of 12°F.

DISCUSSION

WHEN THE z-value of the bacterial spores is less than 18°F: (1) if the test temperature (T_1) is below T_{ref}, then the $F(T_{ref}, 18°F)$ will always be greater than the $F(T_{ref}, z°F)$ and (2) if the test temperature (T_1) is above T_{ref}, then the $F(T_{ref}, 18°F)$ will be often, but now always, greater than the $F(T_{ref}, z°F)$. The relative size of the g-value is the determining factor.

The conversion factors, Y_1 and Y_3, are determined, as shown in Eq (2) and (3), by the temperatures T_c, T_1, and T_{ref}, and the z_1- and z_2-values. The magnitude of these factors increases with the temperature difference ($T_c - T_1$) and ($T_1 - T_{ref}$). In both biological monitoring and in process deviation analysis, T_c and T_{ref} are often the same (as shown in Figures 1 and 2). For the same z_1- and z_2-values, the size of the overall correction ($Y_1 \times Y_3$) is a direct function of ($T_{ref} - T_1$). For the same temperature difference, $Y_1 \times Y_3$ will be the same regardless of whether T_1 is above or below T_{ref}.

The Y_2 correction for a specific value of z_1 ($z_1 < 18°F$) for a reference z_2 of 18°F increases as f_h increases and $F(T_1, z_1)$ decreases. For the same minimum public health process for low-acid canned foods, $F_o = 3.0$ min, the $F(T_1, z)$ will be greater than 3.0 min when the heating medium temperature (T_1) is below 250°F and will be less than 3.0 min when the heating medium temperature is above 250°F. Relatively speaking, for the same process F_o-value, the sterilization value at heating medium temperature $F(T_1, z)$ increases as the function (250°F − T) increases. Therefore, the B to C correction using factor Y_2 is of less importance where T_1 is small compared to T_{ref} and greater importance when T_1 is large compared to T_{ref}.

When any of the factors are close to one, they can be ignored. For Y_1 this will happen when T_1 and T_c are close, relative to z_1, and for Y_3 when T_1 and T_{ref} are close,

Table 6—Constants used with Eq (5) and (6) to develop the values in Tables 1 through 5 based on the data of Stumbo

Part A: Constants to be used to find g as a function of f_h/U

z	a_o	a_1	a_2	a_3	a_4	a_5	R^2
10	1.1688	.22962	.048247	−.0063743	.00020611	−2.2669	.99999
12	1.4708	.094972	.099256	−.012514	.00062684	−2.3692	.99997
14	1.5989	.122666	.093303	−.012265	.00066702	−2.3459	.99999
16	1.7060	.16216	.078768	−.010541	.00054179	−2.3151	.99999
20	1.9450	.15784	.082986	−.011563	.00074745	−2.3185	.99999

Part B: Constants to be used to find f_h/U as a function of g

z	b_o	b_1	b_2	b_3	b_4	R^2
18	.12697	.38710	.037988	.0016993	.10930	.99998

relative to z_2. Y_2 nears one as f_h decreases, $U(z_1)$ increases, and z_1 approaches z_2.

In the examples, although each of the factors is relatively large, the numbers are such that the overall effect of the correction is small. This is not always true; it is only after carrying out the computation that the size and direction of the z-value correction will be known.

SUMMARY

A PROCEDURE for converting an F-value determined on the basis of a z_1-value to an F-value based on a z_2-value has been described and demonstrated. This procedure makes possible the correcting of F-values determined using Biological Indicators where the z-value is not 18°F. Correcting these F(BI0)-values should aid in obtaining agreement between biologically and physically determined F-values. This procedure is also useful in evaluating process deviation where the effect of a z-value other than 18°F is to be determined.

APPENDIX

a_i, b_i

Coefficients in the equations fit to Stumbo's data.

$F(T, z)$, F_o, F(BI0)

The equivalent time at temperature T delivered to a container or unit of product for the purpose of sterilization calculated using a specific value of z. F_o indicates that the temperature was 250°F and the z-value was 18°F. F(BI0) indicates that the F-value was measured biologically.

f_h, f_c

The temperature response parameter (f) is the time required for the straight line fitted to the log-linear portion of a heating or cooling curve to traverse one log cycle; it is the time required for the temperature difference between product and heating or cooling medium to decrease by 90%; f_h identifies the heating and f_c the cooling parameter.

g

The heating medium temperature minus product temperature at the slowest heating zone at the end of heating; $g = T_1 - T$ where T is measured at steam off.

j_c

The cooling lag factor $[(j_c = (T_2 - T_{Ba}) / T_2 - T_B)]$.

L

Lethal rate, minutes at T_{ref} per minute at T. $L = 10(T_{ref} - T)/z$.

R^2

Multiple correlation coefficient.

$T, T_o, T_1, T_2, T_{Ba}, T_{ref}$

T is the variable product temperature. T_o is the initial product temperature. T_1 is the heating medium temperature. T_2 is the cooling medium temperature. T_B is the product temperature at steam off. T_{Ba} is the temperature intercept value of the line fitted to the log-linear portion of the cooling curve at steam off. T_{ref} is the reference temperature used in calculating sterilizing values.

t

Real or clock time, generally measured in minutes.

U

The equivalent sterilizing value using the heating medium temperature, T_1, as the reference temperature and with a specified z-value; $U = F(T_1, z)$.

z

Measure of the direction of the thermal death time curve, the number of degrees of temperature change necessary to cause the F-value to change by a factor of ten.

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SUBJECTIVE AND OBJECTIVE EVALUATION OF STRAWBERRY POMACE ESSENCE

J. A. SCHEN, M. W. MONTGOMERY, and L. M. LIBBEY

ABSTRACT

The relationship of incubation parameters (time, temperature, and pH) to the intensity and quality of the aroma of essences distilled from a commercial strawberry pomace was studied. Analysis of variance of sensory panel scores indicated that incubation temperature of the pomace prior to distillation affected the intensity and quality of essence aroma more than holding time or pH. Aroma intensity showed a marked decrease as the pH of incubation of the pomace was increased above pH 4.0. Sensory panels were able to detect statistically significant differences ($p < 0.01$) in the quality of aroma of essences obtained from pomace incubated above pH 4.0 at 20–50°C. Optimum intensity and quality of essence production was obtained in pomace adjusted to pH 4.0 and incubated at 40°C for 4 hr or longer. Gas chromatographic (GC) analysis of several essences collected from strawberry pomace processed under different conditions revealed dissimilar patterns. Essences of highest quality, as determined by the sensory panel, had GC patterns similar to that of essence produced from whole strawberries. All essences obtained from strawberry pomace contained high concentrations of benzaldehyde, 2-heptenal, ethyl hexanoate, limonene, 2-octenal, linalool, benzyl acteate, and ethyl cinnamate. Presence of these compounds may be responsible for the reduced quality of essence produced from strawberry pomace.

INTRODUCTION

DEVELOPMENT of the characteristic flavor of fruits and vegetables has been demonstrated to be enzyme catalyzed (Reed, 1966; Yu et al., 1968; Heatherbell and Wrolstad, 1971; Weurman, 1961). The metabolic pathways and parameters surrounding aroma biosynthesis were reviewed by Nursten (1970) and Salunke and Do (1977). All of these authors commented on the lack of knowledge in this area of flavor research.

Recently, Yamashita et al. (1975; 1976a, b) found that when various aliphatic alcohols were incubated with a whole strawberry, there was a rapid increase in corresponding esters. Further work (Yamashita et al., 1977) showed that aldehydes, when incubated with a whole strawberry, were reduced to the corresponding alcohols, and the alcohols were converted to the corresponding esters. This suggested the presence of an active alcohol dehydrogenase (ADH) system. At least two ADHs were later isolated from strawberry seeds (Yamashita et al., 1976b).

Increasing the strength of natural essences has not been studied extensively. Guadagni et al. (1971a, b) were able to increase the intensity and quality of essence derived from apple peels 2–7 times by holding them for 1–2 days. Karwowska et al. (1969) used pilot plant equipment to produce essences from the pulp juice and pomace of blackberries and found the essence obtained from the pomace had the most intensive and characteristic blackberry aroma. Karwowska and Inchas (1969) later investigated various combinations of the three extracts and found that the richest aroma was obtained by combining the extracts from fruit pulp, pomace, and juice.

Since strawberry pomace from juicing operations is not exposed to temperatures in excess of 50°C, it is possible the flavor precursors are not destroyed and enzymes still active. Presently, disposal of the pomace, which retains a great deal of aroma, is not only costly but also wasteful of a valuable raw material. This disposal cost could be offset by the recovery of an essence with a high market value. Therefore, the purpose of this study was to find the optimum conditions for producing a secondary strawberry essence from pomace.

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EXPERIMENTAL

Preparation of distillates

Juice grade strawberries (Tioga and Hood varieties) and strawberry pomace from the same lot of berries were obtained from a local fruit concentrate processor. The pomace was packed in No. 10 cans as it came from the press chute and placed in insulated carriers containing dry ice. After reaching the laboratory the cans containing the frozen pomace were immediately sealed and stored at –40°C until needed.

After thawing at room temperature overnight, 500g of pomace were placed in a mixing bowl to which 2L of distilled water were added. The slurry was slowly mixed as the pH was adjusted by addition of 6N hydrochloric acid or 6N sodium hydroxide. The pomace was brought up to temperature in a hot water bath (60°C) prior to placing in a water bath of appropriate incubation temperature. After an appropriate holding time, all samples were adjusted to pH 3.5 using 6N hydrochloric acid or 6N sodium hydroxide and placed in a 12-L round bottom distillation flask. The pomace slurry was vacuum distilled at 5 cm Hg for 30 min at 40°C. Two 60 cm Allihn condensors were used: the first was cooled with cold tap water; the second, which refluxed condensate into the collection flask, was cooled with ice water. A 5.5-fold essence was collected and stored at –5°C until analyzed.

To simulate the industrial production of natural strawberry essence on a laboratory scale, whole strawberries, obtained from the same lot as the pomace, were pureed in an Oster Automatic Juice Extractor. To the puree, 0.1% Klearzyme 100 (Wallerstein) was added and incubated for 1 hr in a 46°C water bath. The whole strawberry puree was then distilled using the same equipment and under the same conditions as previously described. A 10-fold essence was collected and stored at –5°C until analyzed. The essence was diluted with distilled water to an equivalent 5.5-fold essence which facilitated the comparison of the essence obtained from the pomace and the whole strawberry essence.

Sensory evaluation

Two separate series of sensory analyses were conducted. The first investigated the effects of incubation temperature (15–55°C), pH (2.5–4.5) and time (1–7 hr) prior to distillation on the intensity and quality of essence produced from strawberry pomace. To investigate these interactions, a 5^3 fractional factorial design was used (Cochran and Cox, 1957). Although a complete design including the three factors at five levels each would require a sensory panel to evaluate 125 samples of recovered essence, Cochran and Cox (1957) described a method to obtain most of the desired information by testing only a fraction of the total number of treatments. Thus a 20 treatment factorial design was used. Distillates were randomly presented to the panel and the samples were evaluated on a seven-point scale. For aroma intensity the scale was from none (1) to very extreme (7) while scores for quality of aroma range from very undesirable (1) to very desirable (7). Multivariate analysis of variance

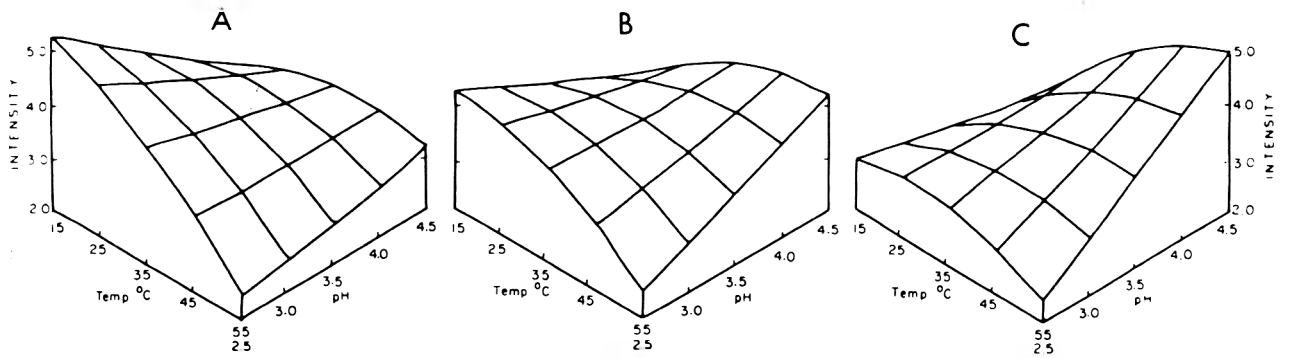

Fig. 1—Effect of incubation time (A-1 hr, B-4 hr, C-7 hr), temperature, and pH on intensity of aroma produced from strawberry pomace. Score range 7, very strong, to 1, very weak.

and a central composite rotatable response surface as described by Cochran and Cox (1957) were used to analyze the data.

The second series of sensory analyses investigated the effects of holding the strawberry pomace at a pH range 4–10 and at temperatures from 20–50°C. All samples were incubated for 4 hr prior to distillation. Presentation of distillates to the sensory panel was determined by an incomplete split plot design (Cochran and Cox 1957), and results were subjected to analysis of variance.

For aroma evaluation, 40 ml of strawberry pomace essence, which had been diluted 300% using distilled water, were measured into a stemmed wine glass covered with a 75 mm watch glass. The judges were instructed to swirl the glass, remove the watch glass, sniff, replace the cover and mark the ballot. After waiting 1 min the judges proceeded to the next sample.

Fifteen judges were selected on their ability to correctly rank the odor intensity of a dilution series of commercial natural strawberry essence. Using distillates prepared from the pomace, judges were trained to develop an internal aroma intensity scale so as to reduce variability. For the first series of sensory evaluations, seven-point scales were used to measure intensity and to measure the quality of the strawberry aroma. Three samples coded with three digit random numbers were presented at each session. Twelve of the original 15 judges were selected for the second series of sensory evaluations. The same ballot was used except that a reference point was included on the scale for intensity, and a reference sample was presented in addition to the three coded samples per session. The reference sample was an equal mix of four essences from pomace samples processed at pH 4, 6, 8, and 10 at 30°C. This represented an average in quality and intensity for pomace essence.

GC analysis

Volatile compounds were trapped using a headspace collection technique as described by Miller et al. (1972). A 250-ml glass bottle, containing a magnetic stirrer, was filled with 25 ml of 5.5-fold strawberry pomace distillate, 25 ml of distilled water, and 20g of anhydrous sodium sulfate. Ethyl esters (C_4–C_{10}) were added to permit calculation of the retention index (I_E) values as described by van Den Dool and Kratz (1963). Ethyl nonanoate was used as the internal standard for calculation of normalized peak areas. Prior to collection, the sample was allowed to stand in a 60°C water bath for 10 min to saturate the headspace. The surface of the agitated sample was swept with prepurified N_2 at 12 cc/min for 30 min and the entrained volatile compounds were collected on 100/200 mesh Porapak Q in a 4 in. × 0.24 in. i.d. stainless steel precolumn maintained at 55°C. At the conclusion of the entrainment procedure, the precolumn was purged with N_2 for an additional 20 min to remove any excess water. The flow of N_2 was then reversed while maintained at 12 cc/min and the temperature of the precolumn increased to 135°C. The volatile compounds eluted were condensed in a 10 in. × 0.03 in. i.d. U-shaped stainless steel trap packed in dry ice. This transfer was completed in 45 min.

The volatile compounds collected from the various strawberry pomace essence samples in the capillary traps were flashed into a 500 ft × 0.03 in. i.d. stainless steel column coated with SF-96 and 5% Igepal CO 880 using the system described by Scanlan et al. (1968). Helium flow through the injection system and the column was 15 cc/min. The column was held at 70°C for 10 min, then programmed at 2°C/min to 160°C. The GC effluent was conducted into the ion source of a Finnigan quadrupole model 1015C electron impact mass spectrometer, and spectra of the eluted compounds were obtained. Data were acquired and processed with a System Industries System 250 data system.

RESULTS & DISCUSSION

First sensory analysis

A very powerful method for the analysis of the results from a factorial experimental design is a second order response surface (Cochran and Cox, 1957). The values assigned by the sensory panel in the present experiment were a function of time, temperature, and pH employed in processing of the pomace prior to distillation, assuming that all other factors surrounding the testing conditions were held

Table 1—Response surface models describing intensity and quality of essence prepared from strawberry pomace when applied to sensory data

Response	Model[a]	R^2 [b]	M.D.[d]
Intensity (Significant at 0.05 level)	$Y = 11.62 - 0.805\ T - 0.117°C - 2.39\ pH - 0.009\ T^2 - 0.0009°C^2 + 0.048\ pH^2 + 0.009\ T \times °C + 0.47\ T \times pH + 0.041°C \times pH$	Total 0.51 Without T 0.20[c] Without °C 0.10[c] Without pH 0.21[c]	0.241
Quality (not significant)	$Y = 7.89 - 0.483\ T + 0.028°C - 2.45\ pH + 0.007\ T^2 - 0.0°C^2 + 0.409\ pH^2 + 0.001\ T \times °C + 0.114\ T \times pH^2 - 0.012°C \times pH$		0.273

[a] Model for pH 2.5–4.5; Temperature 15–55°C; 1–7 hr of incubation (T).
[b] R^2 = regression coefficient.
[c] Regression coefficient if parameter time (T), temperature (°C) or pH was removed from the model.
[d] M.D. = mean difference (see text).

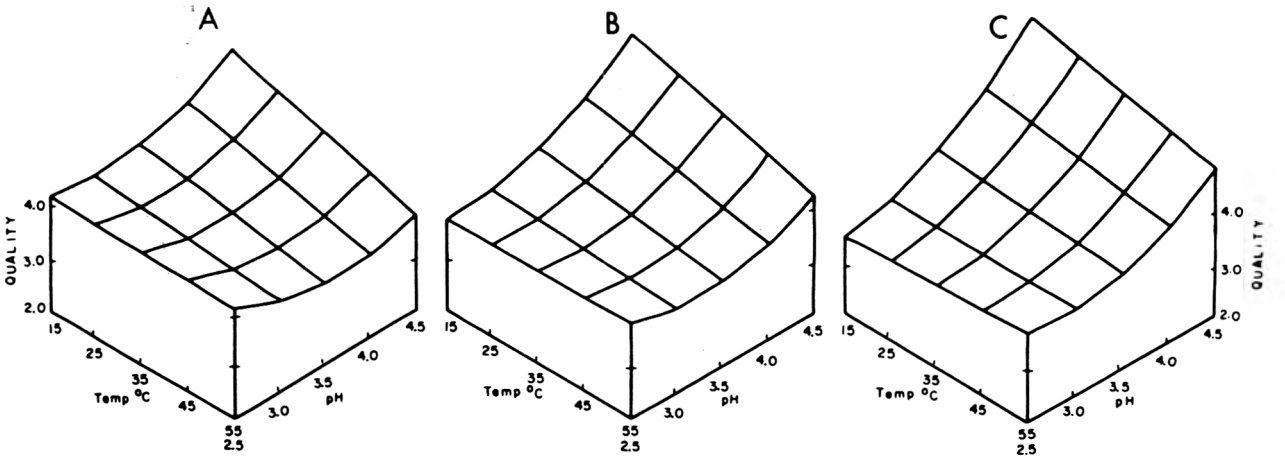

Fig. 2—Effect of incubation time (A-1 hr, B-4 hr, C-7 hr), temperature, and pH on quality of aroma produced from strawberry pomace. Score range 7, very desirable, to 1, very undesirable.

constant. Two models were developed (Table 1) to predict the intensity and quality of strawberry essence produced from pomace. Statistical significance ($p < 0.05$) was found for the intensity model only. The regression coefficient (R^2) indicated that 51% of the total variation could be explained by the intensity model. When one of the three factors (time, temperature or pH) was removed from the model, the R^2 value decreased below the level of significance (Table 1). Therefore, all three of these factors were important elements in the model and in the intensity of the essence prepared from the pomace. It temperature effects were removed from the model, the R^2 value indicated that only 10% of the total variation could be explained by the model. Thus, temperature appears to affect the intensity of aroma more than time of incubation or pH.

The lack of significance with the quality model could have been due to the selection of the panel members on the basis of ability to detect intensity differences and not quality differences in the strawberry essences. Although the panel was screened and trained, a large portion of the uncontrolled variation probably came from the panel itself. This would account for the lack of fit of the model. Kramer (1969) reported that "lack of precision is usually ascribed to the subjective (panel) when correlating to objective measurements." In Table 1, mean difference (M.D.) is the difference between the actual means from the sensory panel and the calculated values computed using the model. Since scoring by the sensory panel was on a seven-point scale for both intensity and quality, a M.D. of approximately 0.2 does not appear appreciably significant.

When values generated by the model were plotted with a three-dimensional projection, the changes in intensity and quality of the essence produced from strawberry pomace can be readily seen (Fig. 1 and 2). Figure 1 shows a shift in the maximum intensity from pH 2.5 and 15°C to pH 4.5 and 55°C occurs when time of incubation was increased from 1 hr to 7 hr. Although time of incubation had little effect on the quality of essence produced, there was a noticeable increase in quality of strawberry aroma as the holding pH was increased to 4.5 and as holding temperature was decreased (Fig. 2). The increase in both quality and intensity of aroma at pH 4.5 was somewhat surprising since the natural pH of strawberries is 3.0–3.5. Yamashita et al. (1976b) found the optimum stability of alcohol dehydrogenase in strawberry seeds to be at pH 7.0. Since the microenvironmental pH of enzymes in whole strawberries may differ greatly from the pH of homogenized strawberries, this increase in quality and intensity with increasing pH might be expected. This suggests the presence of an active enzyme system in strawberry pomace which may account for the observed changes in intensity and quality of essence produced.

Second sensory analysis

Further investigations were conducted to examine the effect of elevated pH on the aroma quality and intensity of essences from the pomace. Time was not investigated in this second series of experiments since the previous experiment had shown that time of incubation had little effect on quality of aroma. All samples were held at the appropriate temperature and pH for 4 hr. To reduce the panels' variability, a reference point was included on the ballot for intensity. The reference sample was prepared by mixing the essences prepared from the pomace under different conditions to represent an average intensity and quality of strawberry aroma. The results presented in Figure 3 show generally that at all temperatures, as pH was increased, intensity decreased. Judges were able to distinguish between the quality of strawberry aroma at the various pH values and temperatures (Fig. 4). An increase, followed by a sudden decrease in quality was obtained for those essences collected from pomace held at 50°C above pH 6. The aroma of these essences contained very little strawberry-like qualities. This could have been due to breakdown products formed due to the high temperature and pH employed. At holding temperatures of 20–40°C, quality remained fairly constant, as holding pH was increased. These observations in Figure 4 indicate that a change in temperature altered quality, suggesting that controlling temperature was important in the utilization of strawberry pomace for the production of essence.

GC analysis

Gas chromatographic (GC) analysis of whole strawberry essence showed a very complex mixture, predominantly made up of esters. A list of major components is shown in Table 2. These compounds have been previously identified by Teranishi et al. (1963), McFadden et al. (1968), and Tressl et al. (1969). To compare essence from strawberry pomace with essence made from whole strawberries, ratios of major peaks from pomace essence to whole strawberry essence identified in Table 2 were computed. A ratio of 1.0 would indicate the normalized peak area from the pomace essence was equal to the normalized peak area from whole strawberry essence. Samples of essences made from strawberry pomace of similar intensities, but which differed in quality, were analyzed (Fig. 5 and 6). Judges found that the

quality of the essence represented in Figure 5 was significantly better than that in Figure 6. As can be seen from the ratios, the distillate judged significantly better (Fig. 5) more closely resembles whole strawberry essence. In all low quality samples examined, there was an increase in n-butyl acetate (26), peak 35, peak 42, methyl hexanoate (45), and α-terpineol (88). These compounds in such large proportions may account for the difference in quality of the two essences, one of which was collected from pomace held at pH 3.5 (Fig. 6) the other at pH 4.5 (Fig. 5). In all high

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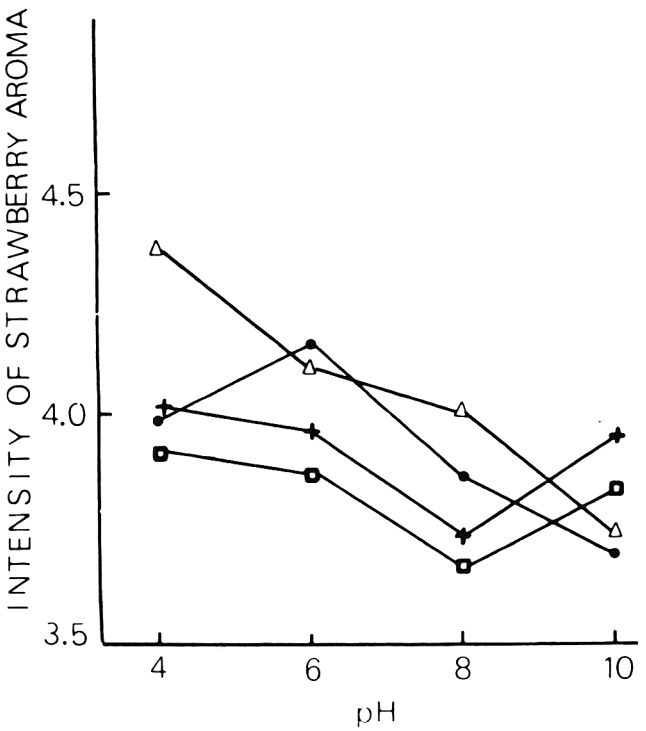

Fig. 3—Effect of temperature and pH on intensity of aroma produced from the strawberry pomace. Score range 1, very much weaker, to 7, very much stronger, with reference sample set at 4. n = 72 judgments/mean. • 20°C, □ 30°C; Δ 40°C; + 50°C

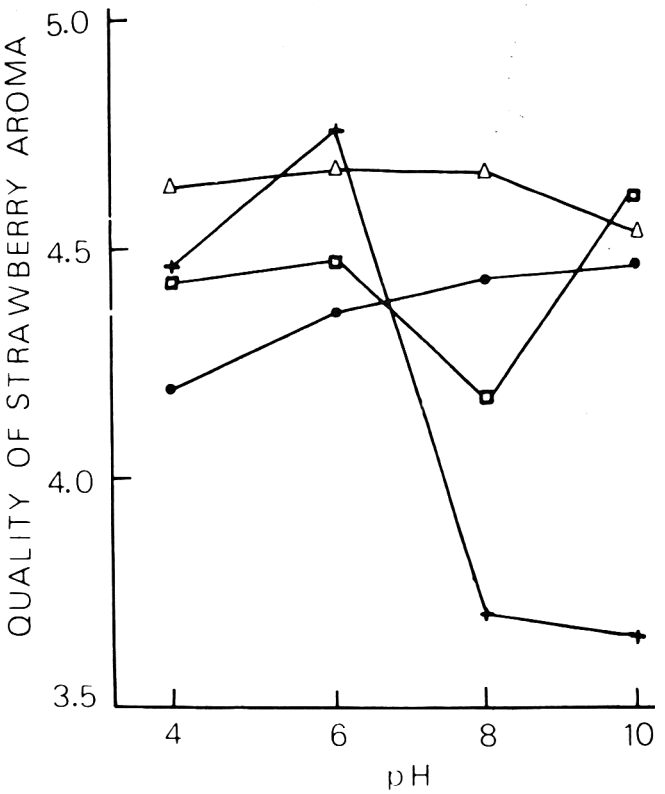

Fig. 4—Effect of temperature and pH on quality of aroma produced from strawberry pomace. Score range 1, very desirable, to 7, very undesirable. n = 72 judgments/mean. • 20°C; □ 30°C; Δ 40°C; + 50°C. p ≤ 0.01 that pH means significantly different. p ≤ 0.01 that pH X temp means significantly different.

Table 2—Identification and retention index of major components in whole strawberry essence

GC peak no.	Compound[a]	Known authentic standard	Unknown	GC peak no.	Compound	Known authentic standard	unknown
3	Diacetyl[R]	1.89	1.69	41	Unknown		5.07
4	Ethyl acetate[R]	2.00	2.00	42	Unknown		5.13
6	Isobutanol[M]		2.05	45	Methylhexanoate[R]	5.23	5.23
7	1-Methoxy-1-ethoxyethane		2.24	50	2-Heptenal[R]	5.57	5.53
9	Unknown		2.55	53	Benzaldehyde[R]	5.80	5.73
11	Unknown		2.71	56	Unknown		5.90
12	Unknown		2.82	57	Ethyl hexanoate[R]	6.00	6.00
13	Methyl butyrate[M]		2.89	59	Trans-2-hexenyl acetate	6.15	6.16
14	1,1-Diethoxyethane		3.05	61	Limonene[R]	6.49	6.40
15	3-Pentene-2-one[M]	3.39	3.13	64	2-Octenal[R]	6.60	6.59
17	3-Methyl-1-butanol[R]	3.48	3.36	69	Unknown		6.90
22	1-Pentenol[R]	3.88	3.86	71	Ethyl n-heptanoate	7.16	7.00
24	Ethyl butyrate[R]	4.06	4.00	74	Linalool[R]	7.27	7.17
26	n-Butyl acetate[R]	4.12	4.11	75	Methyl n-octanoate[R]		7.30
31	2-Hexenal[M]		4.47	83	Benzyl acetate[M]		7.74
32	Ethyl isovalerate[R]	4.52	4.50	87	Ethyl n-octanoate[R]	8.00	8.00
35	Unknown		4.74	88	α-Terpineol[M]		8.07
37	3-Hexenal[T]	4.87	4.82	110	Ethyl n-decanoate[R]	10.00	10.00
38	1-Hexanol[R]	4.87	4.90	119	Ethyl cinnamate[M]		10.35
39	2-Hexanol[T]		4.95	125	Sesquiterpene[T]		
40	Ethyl valerate[R]	5.00	5.00				

[a] R-MS and retention index identification; M-MS identification; T-tentative MS identification

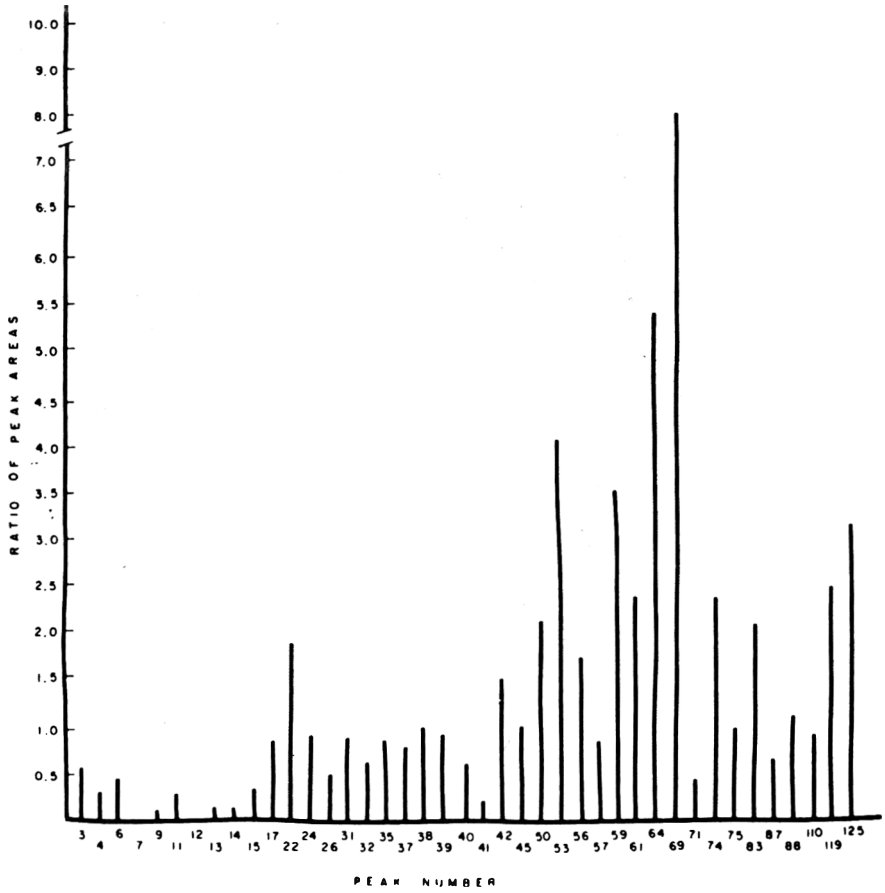

Fig. 5—Ratio of normalized peak areas in strawberry pomace essence to whole strawberry essence. Sensory mean score: Intensity 3.25; Quality 4.69. Experimental conditions: 4 hr, 35°C, pH 4.5.

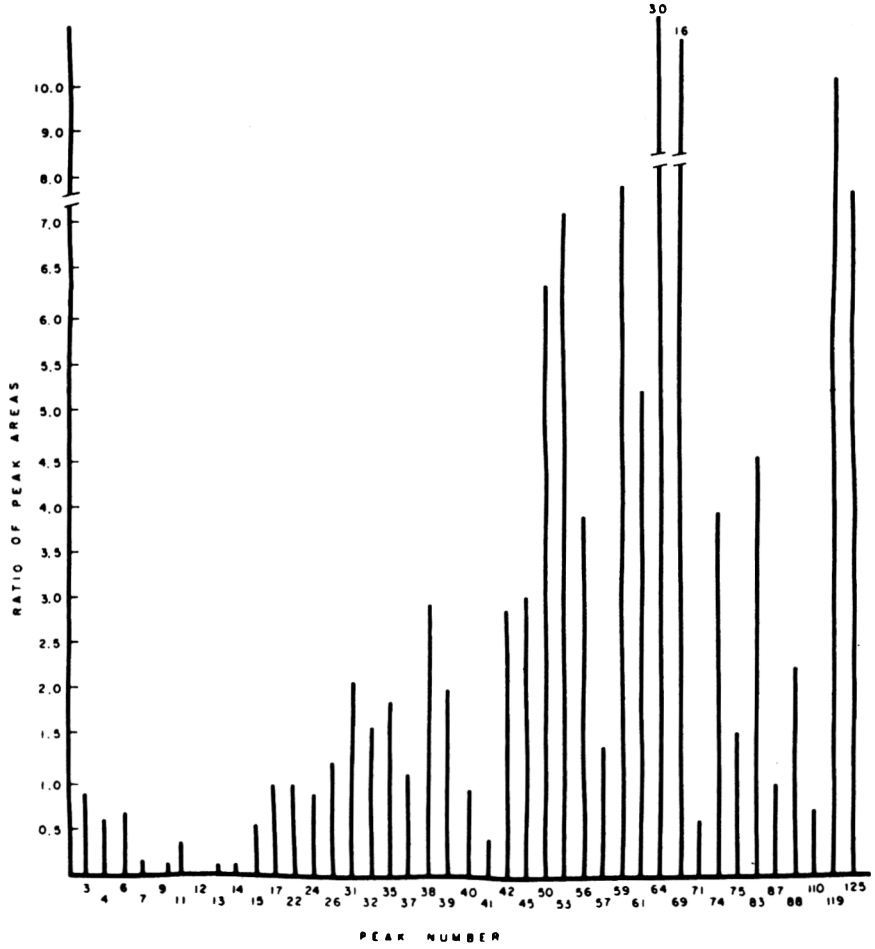

Fig. 6—Ratio of normalized peak areas in strawberry pomace essence to whole strawberry essence. Sensory mean score: Intensity 3.1; Quality 3.2. Experimental conditons: 7 hr, 35°C, pH 3.5.

quality pomace essences examined, 1-pentanol (peak 22) was the only compound whose concentration was consistently higher than in whole strawberry essence.

None of the chromatograms examined duplicated that of whole strawberry essence. All chromatograms of essences made from pomace were characterized by lower concentrations of the more volatile components than those of the whole strawberry essence. Twenty to 30 new peaks, not found in strawberry essence, were present in chromatograms from the pomace essence. None of these compounds was identified in this study. These additional peaks could have been caused, in part, by the filtering aids added to the fruit, such as rice hulls and paper. All pomace essences were abnormally high in benzaldehyde (53), 2-heptenal (50), peak 56, trans-2-hexenyl acetate (59), limonene (61), 2-octenal (64), peak 69, linalool (74), benzyl acetate (83), ethyl cinnamate (119), and peak 125, a sesquiterpene hydrocarbon. This pattern was consistent for all strawberry pomace essences examined, suggesting that orderly enzyme systems acted upon stable aroma precursors in strawberry pomace. The unsaturated aldehydes, found in high concentrations in all pomace essences, might have resulted from action of lipoxidase on unsaturated fatty acids found in strawberry seeds within the pomace. Formation of n-hexanal and trans-2-hexenal from ^{14}C-labeled linoleic and linolenic acids in bananas was reported by Tressl and Drawert (1973). A consistent increase in low molecular weight esters important in strawberry aroma did not occur in any of the pomace essences examined. However, large increases in high molecular weight esters (methyl hexanoate, trans-2-hexenyl acetate, benzylacetate, and ethyl cinnamate) was observed in all pomace essences examined.

CONCLUSION

WHETHER or not enzymic action was responsible for the differences in intensity and quality of the strawberry pomace essence is not certain. Judges found statistically significant differences occurred in quality of the essence when pH was varied from 4.0–10.0 (Fig. 4). A study of the GC patterns of volatiles obtained from these essences again indicated major differences. This suggests that an enzymic process may play a role in production of essence from strawberry pomace.

Although a second order response surface model (Table 1) did not fit the sensory data, multivariate analysis of variance indicated temperature, rather than pH and time, was the most important factor to be controlled in the treatment of strawberry pomace. This is further supported by data in Figure 4 where quality appears to be most influenced by temperature.

From the observations made in this work, duplication of the aroma of whole strawberry essence from strawberry pomace is not possible. However, to obtain the best possible results, the three factors studied (time, temperature, and pH) must be controlled. The optimum conditions found in this study were at pH 4.0 and 40°C for 4 hr or longer. An equivalent strength pomace essence processed under optimum conditions, will be slightly less intense and less desirable than that made from whole strawberries.

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RADIOMETRIC DETERMINATION AND CHICK BIOASSAY OF FOLACIN IN FORTIFIED AND UNFORTIFIED FROZEN FOODS

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ABSTRACT

A radiometric assay for food folacin based on the competitive protein binding procedure was developed. The method is rapid, reliable and comparable to the microbiological assay. Cooking and storage did not significantly affect the folacin content of frozen fortified or unfortified frozen dinners and pies. Food folacin availability was measured by chick bioassay. The procedure involves feeding semipurified diets containing various levels of folic acid and/or cooked or uncooked frozen convenience foods and measuring plasma and RBC folacin. Levels were directly related to folic acid intake from semipurified diets. Availability of folic acid added to TV dinners and pies was demonstrated by elevation of plasma and RBC folacin, but fortification of vegetables did not cause consistent elevation.

INTRODUCTION

FOLACIN deficiency is prevalent in industrialized and in developing countries. In the U.S., dietary deficiency of this vitamin is most frequent among elderly people, particularly those who are housebound, and among alcoholics. Mild folacin deficiency is also common in women of reproductive age during pregnancy and lactation, and when contraceptive steroids are taken. Inadequate intake of this vitamin is due to lack of consumption of folacin-rich foods including green leafy vegetables and liver. Folacin requirements are increased by alcohol abuse and by intake of certain drugs including contraceptive steroids and anticonvulsant agents used in the treatment of seizure disorders. Risks of folacin deficiency are that it can lead to the development of megaloblastic anemia and in the elderly to an organic brain syndrome. Folacin deficiency can also cause melabsorption (Herbert, 1968; Leevy et al., 1965; Johns and Bertino, 1965; Cooper et al., 1970; Kahn et al., 1970; Hurdle and Williams, 1966; Eastham et al., 1975; Tompkins et al., 1976; Reynolds et al., 1973).

The stability of folacin in foods varies with the form of folacin present and with pH. Ascorbic acid stabilizes folacin in foods (O'Broin et al., 1975). Cooking may result in losses of food folacin (Taguchi et al., 1973). Tamura and Stokstad (1973) showed that the availability of food folacin is variable. Factors which alter folacin availability are folacin-binding proteins in foods, mass of food consumed, and probably dietary fiber.

Up to the present time the determination of folacin in foods by microbiological assay has been time-consuming and subject to technical error. In a recent report of the folacin content of foods by Perloff and Butrum (1977), values offered are considered to be provisional because of the limited number of food samples assayed.

Complex methods for determining the bioavailability of food folacin in human subjects have precluded routine measurement of the availability of folacin in different foods and diets processed and prepared in different ways (Brown et al., 1973).

This report describes a simple radiometric method for the determination of food folacin and a chick bioassay for estimation of folacin availability. Frozen convenience foods, including TV dinners and meat (chicken) pies have been used on an experimental basis as vehicles for folic acid fortification. The reason for the selection of this class of foods for use in this study is twofold. First, frozen convenience dinners were shown by Hoppner et al. (1973) to be low in folacin activity, and second, these foods are used in ever increasing quantities by population groups who are at risk of folacin deficiency (Anonymous, 1973; 1974).

EXPERIMENTAL

Radiometric assay for food folacin, serum, and red cell determinations

Test foods. The following frozen convenience foods were used in this study: meat loaf dinner, chicken pot pie, peas, brussel sprouts and collard greens. Samples were tested uncooked or cooked, without or with added folic acid (40 μg/100g of food), and some were stored for 6 months. Following these treatments the samples were homogenized and freeze-dried. Proximate analyses were performed and folacin content of foods was determined by both the competitive protein binding radioassay and microbial assay procedures.

The radio assay methods were modifications of the competitive protein binding procedure developed for determination of folacin in human serum (Waxman et al., 1971). Demineralized cows' milk whey protein ("Hi-Protal-50," Tetroid Co., Hamilton, N.Y.) was used as the binder. It has been shown that when labelled and unlabelled folacin (standard or sample) compete for the binder, the ratio of bound folacin to free folacin diminishes as the concentration of unlabelled folacin increases. Extracts of freeze-dried foods were diluted with freshly prepared pH 9.3 lysine buffer (0.05M lysine buffer, containing 0.1% gelatin and 5 mg/ml sodium ascorbate). Aliquots of the diluted sample (100 μl) were added to 12 × 75 mm polypropylene tubes containing 800 μl of lysine buffer. Then, 100 μl of tritiated folic acid with a concentration of 2.5 ng/ml (^{3}H-PGA, 42 mCi/μM, Amersham-Searle, Arlington Heights, Ill.) was added to the tubes, and the tubes were gently mixed. Binder solution (100 μl), diluted with buffer so that it bound 50-60% of the total radioactivity was then added to the tubes. [The whey protein was initially mixed with 0.9% NaCl solution at a concentration of 100 mg/ml; this stock solution was frozen until the time of assay. Just prior to use, it was defrosted and diluted with lysine buffer; the working concentration was approximately 10 mg/ml.] The tubes were gently mixed and stored at room temperature, in the dark, for 60 min. Ice-cold dextran-coated charcoal (0.4 ml) (2g Darco G-60 activated charcoal: 0.2g dextran, M.W. 40,000 in 100 ml of glass-distilled water) was then added to the tubes. The tubes were then mixed, centrifuged at 1500g for 20 min, and the supernatants were decanted into 10 ml of liquid scintillation fluid (PCS, Amersham-Searle). The samples were counted in a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3375) for 10 min (Tigner and Roe, 1979).

Serum and red cell samples, diluted with buffer, were treated the same as food extracts, except that they were placed in boiling water for 15 min to inactivate endogenous binders, then cooled prior to the addition of tritiated folic acid (Colman et al., 1976).

A set of standards containing 2.5–30.0 ng/ml folic acid was included in the procedure with each set of samples, and a standard curve was constructed using a log-logit transformation (Longo and Herbert, 1976). The protocol for the radiometric assay procedure is summarized in Table 1.

The concentration of folacin in an unknown sample (food ex-

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Table 1—Protocol for radiometric assay of folacin in chick plasma

Tube	Lysine buffer[a] (μl)	Standard (μl)	Plasma sample (μl)	^{3}H Tracer PGA[b] (μl)	Protein binder[c] (μ)	DCC[d] (μl)
1,2 (hot PGA count)	1,400	–	–	100	–	–
3,4 (supernate control)	1,000	–	–	100	–	0.4
5,6 (binder control)	900	–	–	100	100	0.4
7,8	800	100 (1.5 ng/ml)	–	100	100	0.4
9,10	800	100 (3.0 ng/ml)	–	100	100	0.4
11,12	800	100 (5.0 ng/ml)	–	100	100	0.4
13,14	800	100 (10 ng/ml)	–	100	100	0.4
15,16	800	100 (20 ng/ml)	–	100	100	0.4
17,18	800	100 (30 ng/ml)	–	100	100	0.4
Plasma sample[e]	800	–	100	100	100	0.4

[a] Lysine buffer = 0.05M lysine-ascorbate-gelatin
[b] ^{3}H Tracer PGA = tritiated pteroylmonoglutamic (folic) acid
[c] Protein binder = whey protein (Hi-Protal-50, Tetroid Co., Hamilton, N.Y.)
[d] DDC=dextran coated charcoal
[e] Assay of folate in plasma was direct, in red cells using hemolysate and in freeze-dried food using extracts into 1% ascorbate-phosphate buffer.

tract, serum, or red cells) was obtained by comparing the ratio of bound to free folacin observed with that of standard folic acid solutions.

Radiometric assay values for serum, red cell and food folacin were compared with microbiological assays of the same blood and food samples with *L. casei* as the test organism. The *L. casei* method for determining folacin activity was an adaptation of that given in *Methods of Analysis* (Infant Formula Council, 1973). Significant changes were the addition of 1% ascorbate to the extraction medium to protect the folacin, use of the Difco Folic Acid *L. casei* Media, and use of a chick pancreas conjugase in total folacin determinations.

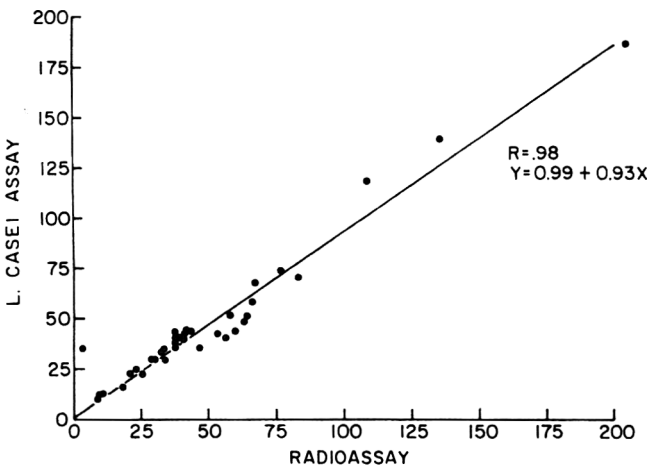

Fig. 1—Relationship between the folacin assay procedures.

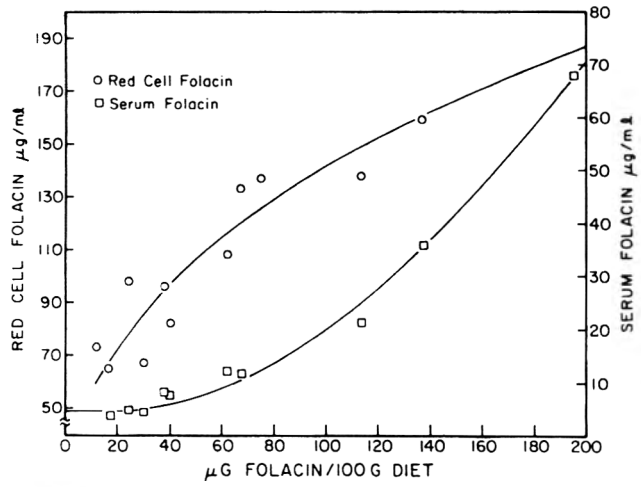

Fig. 2—Relationship between dietary folacin, serum folacin and red cell folacin in chicks on basal diet.

Chick bioassay

Diet preparation. The basal diet was essentially that used by Scott et al. (1969), except that vitamin-free casein and twice the fat level were used. Varying levels of folic acid (0–150 μg/100g of diet) were added to the basal diet. This was the basis for a standard curve. For the experimental diets, the unfortified and fortified frozen convenience foods were substituted on a percent kilocalorie basis for glucose in the basal diet. Chicken pot pie and meat loaf dinner were added at 30% of kilocalories, peas at 5% and brussel sprouts and collard greens at 2%. These levels of substitution were intended to provide comparable amounts of folacin in the experimental diets.

Chick feeding. Day-old male White Leghorn chicks, in groups of 10 each, were fed the various diets ad libitum for 21 days. Feed consumption and weight gain were determined every 2 days.

At the end of the feeding period, the chicks were sacrificed by heart puncture using heparinized syringes and heparinized tubes to

Table 2—Folacin assay of selected foods by microbiological and radiometric methods[a]

Food	Preparation	Micro (*L. casei*) method: Free folacin	Micro (*L. casei*) method: Total folacin (μg/100g)	Radioassay folacin (μg/100g)
Peas	Uncooked (A)	36.2	63.7	86.5
	Cooked (B)	44.1	73.4	79.5
Brussel sprouts	A	3.4	105.0	104.9
	B	3.9		110.2
Collard greens	A	6.1	–	109.1
	B	5.9	93.2	107.1
Chicken pot pie 1	A	3.6	9.7	8.0
	B	1.9	9.8	7.6
Chicken pot pie 2	A	3.7	4.1	3.6
	B	0.6	3.2	3.1
Meatloaf 1	A	2.1	17.0	18.9
	B	2.1	20.3	22.0
Meatloaf 2	A	–	18.4	18.9
		–	16.0	20.5

[a] Microbiologic and radiometric assays are of aliquots of the same food samples. For samples having all three values, H_0:*L. casei* total = radioassay; df = 9; t = −1.586 N.S.; r = 0.99.

Table 3—Average folacin content of frozen convenience foods μg/100g wet weight (Radiometric assay)[a]

	Unfortified				Fortified			
	Raw		Cooked		Raw		Cooked	
Item	I[b]	F[b]	I	F	I	F	I	F
Peas	80.4	92.7	,72.1	86.9	113.8	134.1	112.5	118.2
Brussel sprouts	280.2	104.9	218.5	110.2	308.0	165.7	194.5	160.3
Collard greens	96.0	122.1	116.0	98.1	178.8	148.0	121.6	139.5
Chicken pot pie 1	3.1	4.2	3.2	3.1	32.3	33.1	25.6	40.6
Chicken pot pie 2	7.9	–	8.1	7.1	48.6	36.6	46.3	40.7
Meatloaf dinner 1	23.0	14.7	23.4	17.6	66.2	49.6	72.9	49.2
Meatloaf dinner 2	21.1	16.8	25.1	18.9	71.0	47.3	76.7	52.7

[a] Mean of duplicates
[b] I = Initial value before storage; F = Final value after 6 months storage

Table 4—Serum and red cell folacin levels of chicks on unfortified vs fortified foods[a]

	Serum folacin ng/ml			Red cell folacin ng/ml		
Cooked	Unfortified	Fortified	Level of significance	Unfortified	Fortified	Level of significance
Peas	9.0 ± 2.3	12.3 ± 3.3	$P < 0.01$	103.6 ± 30.8	122.1 ± 22.2	NS
Brussel sprouts	13.2 ± 3.3	10.7 ± 3.8	NS	101.7 ± 21.9	125.9 ± 21.7	NS
Collards	12.1 ± 3.2	13.8 ± 2.6	NS	95.3 ± 15.7	114.0 ± 26.7	$P < 0.05$
Chicken pot pie 1	3.0 ± 1.1	9.4 ± 3.4	$P < 0.001$	83.8 ± 16.6	110.6 ± 25.6	$P < 0.01$
Chicken pot pie 2	3.5 ± 1.7	8.1 ± 3.4	$P < 0.001$	92.9 ± 7.5	107.9 ± 4.0	NS
Meatloaf 1	3.8 ± 1.9	12.0 ± 3.7	$P < 0.001$	115.2 ± 34.6	146.5 ± 22.9	$P < 0.05$
Meatloaf 2	3.3 ± 1.2	10.9 ± 3.1	$P < 0.001$	116.0 ± 24.9	155.9 ± 30.5	NS

[a] Values ± standard deviation

collect and store the blood. Both plasma and red cell folacin were determined. Folacin availability in the foods was estimated by relating the rise in plasma and red cell folacin to the levels of folacin in the foods including those fortified.

Data analysis

Data analysis utilized correlation and linear regression to compare results of radiometric and microbiological assay values for folacin. Analysis of variance and student t-tests were used to compare effects of cooking, storage, and fortification on folacin values in foods and to examine effects of folic acid fortification of foods on blood folacin levels in the chick bioassay procedure.

RESULTS

THE RADIOMETRIC ASSAY for food folacin was rapid, reliable and comparable to the microbiological assay (Fig. 1). The radiometric assay measures total rather than free folacin (Table 2). The folacin content of the TV dinners and chicken pies is low while the frozen vegetables are rich sources of folacin. Except for brussel sprouts, the folacin content of these foods was not significantly reduced either by cooking according to package directions or with 6 months of storage at −20°C with/without subsequent cooking. Fortification of these foods with folic acid (pteroylglutamic acid) supplied stable products (Table 3).

The chick bioassay demonstrated that folic acid in semipurified basal diets is available to the chick as shown by serum and red cell folacin values in the growing birds (Fig. 2). When growing chicks were fed diets containing the frozen foods, blood levels of folacin followed similar values to those obtained when basal diets were fed containing com-

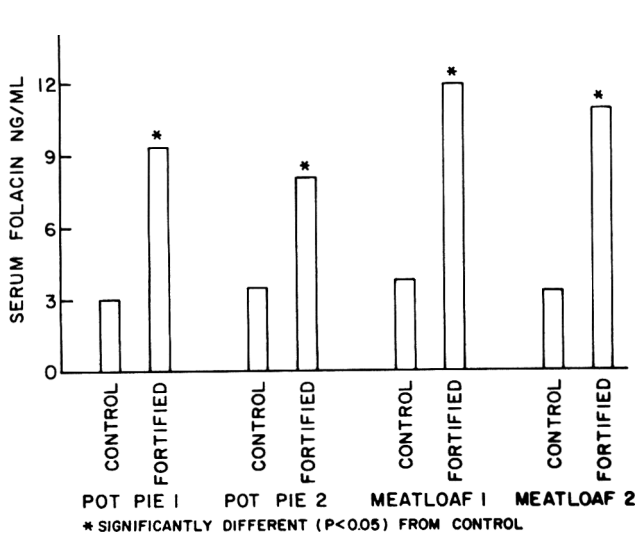

Fig. 3—Changes in serum folacin with folic acid fortification of convenience meals.

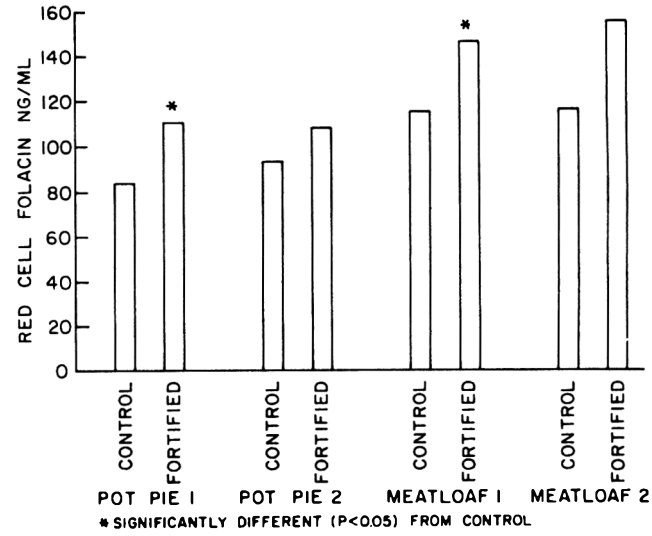

Fig. 4—Changes in red cell folacin with folic acid fortification of convenience meals.

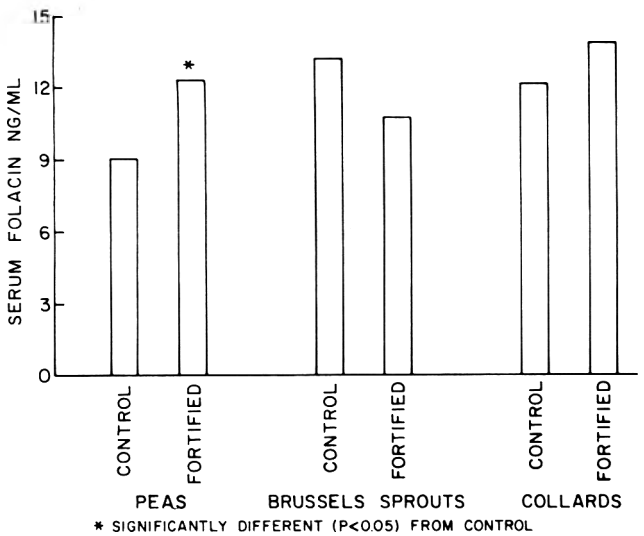

Fig. 5—Changes in serum folacin with folic acid fortification of frozen vegetables.

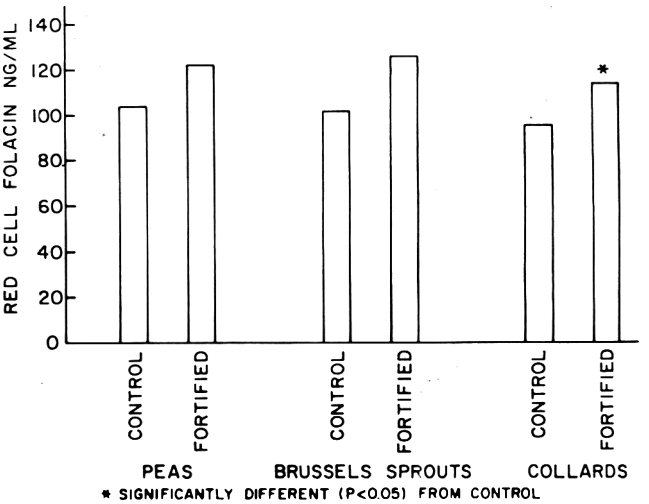

Fig. 6—Changes in red cell folacin with folic acid fortification of frozen vegetables.

parable amounts of folacin as folic acid. Elevation of blood folacin values (serum and red cell) occurred when folic acid fortified TV dinners and chicken pot pies were incorporated into the chicken diets (Table 4, Fig. 3 and 4). Fortification of peas produced a significant increase in serum folacin levels but serum folacin was not significantly altered by fortification of brussel sprouts or collard greens. Red cell folacin was significantly elevated by fortification of collard greens. Fortification of the other vegetables with folic acid was associated with a rise in average red cell folacin values but these changes were not statistically significant (Fig. 5 and 6).

DISCUSSION & CONCLUSIONS

Specificity of the radiometric assay for food folacin

In unfortified foods, only about 5% of the total folacin is in the form of folic acid (pteroylmonoglutamic acid). Food folacin consists mainly of methyl folacin including 5-methyltetrahydrofolate and formyl derivatives including 10-formyltetrahydrofolate and 5-formyltetrahydrofolate (Butterworth et al., 1963). Both monoglutamic and polyglutamic forms of food folacin have been identified (Butterworth, 1968).

Assay of food folacin has until now been by microbiological methods. Different microorganisms have different specificities for food folacins. *Lactobacillus casei* utilizes mono-, di-, and triglutamates. *Streptococcus faecalis* shows a growth response to mono- and diglutamates, other than those with a methyl group at the N-5 position and *Pediococcus cerevisiae* only utilize nonmethylated, reduced forms of food folacin with three or less glutamate residues (Stokstad and Koch, 1967). Since *L. casei* can utilize more folacin derivatives than the other bacteria, it is commonly used as the assay organism.

Hydrolysis of polyglutamic forms of food folacin is achieved by use of a proteolytic "conjugase" (deconjugase) enzyme preparation. The term "free" folacin in food refers to that amount found to be present before use of the conjugase enzyme and total folacin is the whole folacin content as assayed after application of the conjugase.

In the radiometric assay which has been described, total folacin is determined, as shown by comparison of values with microbiological assay. Longo and Herbert (1976) comment that in developing a competitive protein-binding radioassy system, an important step must be to find a binder which measures the substance to be assayed. These investigators showed that at pH 9.3, a milk binder showed similar binding for folic acid (pteroylmonoglutamic acid) and 5-methyltetrahydrofolate. On the other hand, binding of folinic acid to their crude liquid skim milk binder was 12.5% as great as with folic acid. In developing a radiometric assay for serum folacin using crystalline β-lactoglobulin as binder, Dunn and Foster (1973) obtained similar values to those obtained when serum folacin was determined in the same sample using microbiological assay (*L. casei*). Work done in our laboratory (Tigner and Roe, 1979) seems to substantiate the findings of these investigators, namely that the radioassay procedure measures folacins whether they are in the mono- or polyglutamate form. Whey protein, which was used as a binder in the present study, contains approximately 44% by weight of β-lactoglobulin (Smith, 1976).

Variability in the folacin vitamers present in foods is shown by comparison of studies of romaine lettuce and fruit juices. Batra et al. (1977) examined the composition of folacin co-enzymes in lettuce extracts. Folacins were separated into monoglutamic and polyglutamic fractions by chromatography on Sephadex G-15. The fractions were resolved on DEAE cellulose and positive identification of the DEAE peaks was carried out by chromatography with radiolabelled marker folacins and differential microbiological analysis.

It was found that 32% of total folacin is present in romaine lettuce as monoglutamic 5-methyltetrahydrofolate, 40% as polyglutamic forms of 5-methyltetrahydrofolate, and 17% as mono- and polyglutamic formyl derivatives. The remainder of folacins present were not identified.

Using differential microbiological assay of conjugated and unconjugated folacins in citrus and grape juices, Dong and Oace (1973) demonstrated that approximately 50% of the total folacin in these juices was present as polyglutamate. In citrus fruit juices, over 95% of the total folacin activity was as methyl folacins, whereas in grape juice methyl folacins formed only 26% of total folacin activity.

The similar and correlated values which we obtained for total food folacin assayed by the radiometric and microbiological methods, respectively, support methodological validity but further studies of foods containing methyl folacins is indicated. For example, studies are needed to determine the forms of folate, their relative concentrations and binding strengths to whey, in the foods studied here.

Stability of folic acid in different food vehicles

The feasibility of preventing folacin deficiency by fortification of staple foods has been demonstrated by Colman et al. (1975). After feeding maize meal, rice, or bread fortified with folic acid to human subjects, these investigators demonstrated increases in serum folacin levels, using *S. faecalis* and *L. casei* as assay organisms. Elevation of serum folacin produced by fortified maize and rice was similar to that produced with a folic acid solution, but increments produced by the fortified bread were lower. Baking produced a loss of folic acid activity. Fortification of cereal staples with folic acid was proposed as a practical measure of nutrition intervention and preference is given by these investigators to fortification of boiled cereals such as maize and rice. On the other hand, Keagy et al. (1975) found that folic acid is very stable throughout bread processing. Whereas folic acid added to flour showed decreased vitamin activity when stored at high temperatures, no loss of activity occurred when fortified flour was kept for 52 weeks at 28°C or less.

In the present study, folic acid added to frozen convenience foods was found to be stable for at least 6 months and no significant losses were obtained when the foods were prepared by conventional cooking methods described on the packages. Inclusion of cooking fluid in the assay homogenates of vegetables is believed to have minimized folacin losses.

Practical application of the chick bioassay for food folacin

It was shown by Cropper and Scott (1966) that in the chick the predominant folacin in the blood is the N^5-methyl form. More of the blood folacin is in the conjugated form in chicks than in human subjects. In the present study, it was shown that folacin from the natural food sources used and from folic acid added to purified diets or frozen convenience foods (TV dinners and chicken pies) was highly available to the chick.

It is proposed that if it can be demonstrated that availability of food folacins in the chick is similar to that of human subjects, screening of foods for folacin availability could be conducted by chick bioassay rather than by the difficult, expensive method of feeding the foods to human volunteers.

Practical Use of Frozen Convenience Foods as Vehicles for Folic Acid Fortification

Since it was shown that folic acid, added to TV dinners and chicken pies, is stable under conventional methods of storage and food preparation, and available to the test animal (chicks), it is suggested that these foods would be appropriate vehicles for fortification. Use of these convenience foods is high in the elderly and by other people who are unlikely to consume enough of the vitamin from vegetable sources to meet their folacin needs (Anonymous, 1973; 1974).

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IRON AVAILABILITY FROM WHEAT GLUTEN, SOY ISOLATE, AND CASEIN COMPLEXES

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ABSTRACT

Release of iron bound to animal and vegetable protein sources was investigated. Ferrous and ferric iron complexes with wheat gluten, soy protein isolate, and casein were prepared under conditions shown to bind large quantities of iron to the insoluble residue. These mixtures were lyophilized and ground to a fine powder. Treatment of the lyophilized protein-iron-mixtures with 0.1N HCl released 30–68% of the bound iron. Digestion in an HCl-pepsin or an HCl-pepsin-pancreatin system released 64–94% and 85–97% of the bound iron, respectively, indicating that protein-bound iron should be readily freed for absorption within the gastrointestinal tract. Using the hemoglobin repletion technique, protein-bound ferrous iron was statistically as biologically available as the standard, ferrous sulfate. Estimates of the relative biological values for the protein-ferric mixtures were somewhat lower than reported in the literature for ferric pyrophosphate, the free salt used in binding.

INTRODUCTION

NUTRITIONAL availability of iron is extremely complex and the many variables affecting it are not fully understood at this time. The bioavailability of an iron supplement is affected by the body's need for iron, digestibility of the food that supplies the iron, interaction of the iron with other dietary components, and processing effects, as well as the chemical form and particle size of the iron source (Amine and Hegsted, 1974; Fritz et al., 1975, Lee and Clydesdale, 1979; Lee et al., 1979). Several iron sources have been approved for use in foods, yet many of these are of limited biological availability (Fritz et al., 1970; Anon., 1975). Those sources of high biological availability generally are also more chemically reactive in a food system.

Several amino acids have been shown to enhance iron uptake from iron salts when given concomitantly (Kroe et al., 1963; Layrisse et al., 1968; Van Campen and Gross, 1969; Martinez-Torres and Layrisse, 1970). Enhanced absorption is due to the chelation of iron by amino acids. This ability is lost when reactive side groups are modified or removed (Van Campen, 1973). A recent study has shown that iron is bound to both insoluble and soluble protein. The extent of this binding is related to the protein source, the iron source, and conditions imposed, including time, temperature, and pH of incubation (Nelson and Potter, 1979).

Formation of a protein-iron complex may occur as a result of processing, or protein-iron complexes may eventually find use in fortification where free iron salts are too chemically reactive. In either case, the bioavailability of iron from such complexes is of interest. This study reports the in vitro and in vivo availability of protein-bound ferrous and ferric iron.

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MATERIALS & METHODS

Iron sources

Food grade ferrous sulfate and ferric pyrophosphate were obtained from Mallindrodt, Inc. These iron sources meet the criteria of the Food Chemicals Codex. Ferrous sulfate is a grey-white product consisting primarily of $FeSO_4 \cdot H_2O$ with smaller amounts of $FeSO_4 \cdot 4H_2O$. Ferric pyrophosphate, $Fe_4(P_2O_7)_3 \cdot 9H_2O$, is a yellow-green, microcrystalline substance containing sodium citrate as a solubilizing agent.

Protein sources

Wheat gluten was obtained from General Mills Chemicals, Inc., Minneapolis, Minn., as Pro-80 Vital Gluten. Soy protein isolate (Promine D) was obtained from Central Soya, Inc., Chicago, Ill. Land O'Lakes, Inc., Minneapolis, Minn. supplied the casein. Protein contents were determined by the Kjeldahl method (AOAC, 1975). The iron content of the three proteins was determined by atomic absorption spectrophotometry following digestion in concentrated HCl (Anon., 1976).

Atomic absorption spectrophotometry

All atomic absorption analyses were conducted using a Perkin-Elmer Model 360 atomic absorption spectrophotometer. Iron standards were prepared using a certified atomic absorption reference solution from Fisher Scientific Company. All reported values represent averages from triplicate samplings.

Preparation of protein-bound iron

Each protein and iron source was suspended in distilled-deionized water to yield 20 mg Fe/g protein/50 ml H_2O and the pH was adjusted to 6 with 0.5N HCl or 0.5N NaOH. Each mixture was heated with continuous stirring in a steam jacketed vat for 2 hr after reaching a temperature of 87°C. These conditions had previously been shown to result in substantial iron binding (Nelson and Potter, 1979). The slurry was then centrifuged in 250 ml aliquots at 6,000 × G for 10 min. The insoluble residue was resuspended three times in 100 ml distilled-deionized water and centrifuged. The pellets were lyophilized and ground to a fine powder using a mortar and pestle. Following the procedure previously described (Anon., 1976), iron contents of the six protein-bound iron sources were determined.

Acid and enzymatic release of bound iron

The pepsin-pancreatin protein digestion method described by Akeson and Stahmann (1964) and modified by Saunders et al. (1973) was used to study iron release. This method was divided into three parts to quantify iron release after various stages of digestion.

In a 50 ml centrifuge tube, 250 mg of each protein-iron mixture was suspended in 15 ml of 0.1N HCl or 15 ml of 0.1N HCl containing 1.5 mg pepsin (ICN Nutritional Biochemicals, 1 × 10,000) and tubes were incubated with agitation of 100 rpm for 3 hr at 37°C. Immediately following incubation the tubes were neutralized with 0.5N NaOH, centrifuged at 20,000 × G for 5 min, and the supernatant decanted and saved. The insoluble residue was washed five times with 30 ml distilled-deionized water and the washings added to the original supernatant. The solids were filtered through 1.2 μm filter paper (Millipore), air-dried, and weighed.

Another set of samples was incubated in 50 ml centrifuge tubes containing 250 mg of each protein-iron mixture suspended in 15 ml of 0.1N HCl containing 1.5 mg pepsin at 100 rpm for 3 hr at 37°C. After this period the tubes were immediately neutralized with 0.5N NaOH and supplemented with 4 mg pancreatin (ICN Nutritional Biochemicals, 1X) in 7.5 ml of 0.2M borate buffer, pH 8.0, containing 0.005M sodium azide. Borate buffer was used in place of phosphate buffer as outlined in Akeson and Stahmann (1964), to avoid chelation of iron. This mixture was incubated at 100 rpm for 24 hr at 37°C. Immediately following incubation the tubes were centrifuged at 20,000 × G for 5 min, the solids washed five times with 30

Table 1—Iron contents of the six protein-bound iron mixtures prepared at pH 6 and 87°C

Protein-iron mixture	Iron content (mg Fe/g sample)
Wheat gluten-ferrous	9.27
Soy protein isolate-ferrous	17.43
Casein-ferrous	13.58
Wheat gluten-ferric	8.58
Soy protein isolate-ferric	4.68
Casein-ferric	11.42

ml deionized-distilled water, and the supernatant and washings combined. The solids were filtered through 1.2 μm filter paper, air-dried, and weighed.

Iron contents of the supernatants and pellets were determined by atomic absorption spectrophotometry (Anon., 1976) and nitrogen contents by the Kjeldahl method. The experiment was repeated in duplicate and results presented as the percent iron released and percent protein digested. The protected Least Significant Difference Method (Snedecor and Cochran, 1967) was used to assess differences among the means of iron release, among the means of protein digestibility, and for the main effects and two-way interactions over all six protein-iron mixtures. Analysis of variance was used to test the effect of iron source, protein source, and treatment on iron release and protein digestibility.

Biological availability of bound iron

A low-iron diet was obtained from ICN Pharmaceuticals, Inc., Cleveland, Oh. Using the HCl digestion-atomic absorption method previously described, the diet was found to contain 0.04 mg Fe/g.

The low-iron diet was supplemented at levels of 0, 6, 12, and 24 mg Fe/kg diet with standard ferrous sulfate ($FeSO_4 \cdot 7H_2O$) obtained from Mallindrodt, Inc., St. Louis, Mo. The ferrous sulfate was ground to a fine powder using mortar and pestle. These standard ferrous sulfate-supplemented diets, made homogeneous by thorough blending in a Hobart mixer, provided the controls.

Eighteen test diets, representing ferrous and ferric iron bound to wheat gluten, soy protein isolate, and casein, each at three levels, were prepared. The low-iron diet was supplemented with 6, 12, and 24 mg Fe from protein-bound ferrous iron/kg diet and 30, 60, and 120 mg Fe from protein-bound ferric iron/kg diet.

The AOAC (1975) hemoglobin repletion test for iron was used to determine biological availability of the six protein-bound iron sources. Male weanling, Sprague-Dawley rats were housed in individual stainless steel cages and fed the low-iron diet and distilled water ad libitum for 5 wk. At this time, the animals were determined, via blood taken from the tail vein, to be iron depleted (hemoglobin levels less than 6 g/100 ml blood). The animals were then randomly allocated to the test and control diets. Three rats were allocated to each of the 18 test diets and four or five rats to each of the four control diets.

After 2 wk on the various diets, individual blood samples were taken from the tail vein and hemoglobin concentrations determined. Results are presented as the g Hb/100 ml blood increase over the 2-wk feeding period and analyzed using the slope ratio method outlined in Bliss and White (1967). To enable comparison of the six protein-bound iron sources, biological availability is expressed as relative biological value (RBV), which is 100 × the mg iron from the ferrous sulfate standard per kg of diet divided by the mg iron from the sample iron source per kg diet, which give equal curative effect.

RESULTS & DISCUSSION

Lyophilized protein-bound iron mixtures

Bulk preparation and lyophilization of the six protein-bound iron mixtures yielded highly acceptable products. Thorough grinding produced fine powders to enable uniform dispersion in the test diets. Iron contents of the six protein-bound iron mixtures are listed in Table 1.

Ferric iron bound to wheat gluten, soy protein isolate, and casein produced light-tan to cream-colored products. Upon exposure to air and light, darkening occurred. Ferrous iron bound to wheat gluten and soy protein isolate yielded dark-tan products. The casein-ferrous product was a light cream color. There was no color change in the protein-bound ferrous iron products upon exposure to light and air. All protein-iron mixtures were free from off odors even after several months storage in screw-capped glass jars at room temperature.

Table 2—Iron release and protein digestibility of the lyophilized protein-ferrous iron mixtures

Mixture	Iron release (%)	Mean[a] (%)	Protein digestibility (%)	Mean[a] (%)
Wheat gluten-ferrous				
HCl	46.27	36.65ab	19.19	17.81ab
	27.03		16.42	
HCl-pepsin	90.32	87.56hi	63.37	61.61de
	84.80		59.85	
HCl-pepsin-pancreatin	93.49	94.37ij	70.08	72.04ef
	95.25		74.00	
Soy protein isolate-ferrous				
HCl	59.05	57.85c	25.51	25.51abc
	56.65		25.51	
HCl-pepsin	77.61	76.90efg	45.14	52.48d
	76.19		59.81	
HCl-pepsin-pancreatin	93.35	91.96hij	91.41	94.96hi
	90.57		98.50	
Casein-ferrous				
HCl	70.19	68.35de	25.71	28.53bc
	66.50		31.35	
HCl-pepsin	91.96	94.08ij	88.89	89.71gh
	96.20		90.53	
HCl-pepsin-pancreatin	95.89	96.60j	105.53	104.51i
	97.30		103.48	

[a] Means with the same letter do not differ significantly at the 5% level.

Table 3—Iron release and protein digestibility of the lyophilized protein-ferric iron mixtures

Mixture	Iron release (%)	Mean[a] (%)	Protein digestibility (%)	Mean[a] (%)
Wheat gluten-ferric				
HCl	37.75	40.64b	22.57	26.31abc
	43.53		30.04	
HCl-pepsin	66.60	64.26cd	33.52	29.93c
	61.92		26.33	
HCl-pepsin-pancreatin	96.12	96.06ij	74.66	82.54fg
	95.99		90.42	
Soy protein isolate-ferric				
HCl	57.75	57.88c	13.52	16.04a
	58.01		18.56	
HCL-pepsin	80.71	77.29fg	86.10	85.85gh
	73.86		85.59	
HCl-pepsin-pancreatin	84.11	85.00gh	99.08	97.36hi
	85.89		95.64	
Casein-ferric				
HCl	25.93	29.67c	13.84	22.23abc
	33.40		30.62	
HCl-pepsin	71.58	70.12def	66.53	63.51de
	68.65		60.48	
HCl-pepsin-pancreatin	97.46	96.93j	73.66	78.44fg
	96.39		83.21	

[a] Means with the same letter do not differ significantly at the 5% level.

Acid and enzymatic release of bound iron

Results of duplicate trials of acid and enzyme treatment on iron release from, and digestibility of, the six protein-iron mixtures are given in Tables 2 and 3. Iron release and

Table 4—Analysis of variance of the effects of protein source, treatment, and iron source on iron release and protein digestibility

Source of variation	df	Iron release		Protein digestibility	
		Sums of squares	F value	Sums of squares	F value
Protein	2	237.28	6.85[b]	26,350.17	415.20[b]
Treatment	2	12,573.53	362.79[b]	1,809.26	28.51[b]
Protein x Treatment	4	744.39	10.74[b]	1,079.68	8.51[b]
Iron source	1	831.07	47.96[b]	224.55	7.08[a]
Protein x Iron source	2	581.14	16.77[b]	51.25	0.81
Treatment x Iron source	2	310.09	8.95[b]	1,203.41	18.96[b]
Protein x Treatment x Iron source	4	958.51	13.83[b]	2,322.28	18.30[b]
Error	18	311.92			
Total	35	16,547.94			

[a] 5% level of significance
[b] 1% level of significance

protein digestibility were calculated as follows:

$$\text{Iron release (\%)} = \frac{100 \times \text{Supernatant Fe}}{\text{Supernatant Fe + Fe in undigested fragment}}$$

$$\text{Protein digestibility (\%)} = \frac{\text{100 x N in protein-iron mixture} - \text{N in undigested fragment}}{\text{N in protein-iron mixture}}$$

An analysis of variance on the effect of protein source, treatment, and iron source on iron release and protein digestibility is given in Table 4. Release of iron from the six sources was significantly ($P < 0.01$) affected by the interaction of protein, treatment, and iron source. Thus, it is difficult to accurately separate the main effects and two-way interactions.

Ferrous iron was found to be released from both wheat gluten and casein to a significantly greater extent by an HCl-pepsin or an HCl-pepsin-pancreatin treatment, than by HCl alone. The more severe treatments released approximately equal amounts. Ferric iron was released from wheat gluten and casein to a significantly greater extent as the treatment became more severe.

All three treatments significantly differed in the quantity of ferrous iron released from soy protein isolate. Iron release increased with more severe treatment. Release of ferric iron from soy protein isolate was approximately the same with an HCl-pepsin treatment as with HCl-pepsin-pancreatin. Both of these treatments released significantly more iron than HCl alone.

Ferrous and ferric iron were released by the three treatments to much the same extent. Significantly more ferrous than ferric iron was released by mild treatment from casein. Soy protein isolate appears to release ferrous as readily as ferric iron. The overall release of ferrous iron is significantly greater from casein than from wheat gluten or soy protein isolate. Ferric iron is released to a greater extent from wheat gluten and casein than from soy protein isolate by severe treatment.

Protein digestibility is also significantly ($P < 0.01$) affected by the interaction of the protein source, treatment imposed, and the iron source. Significantly more casein was digested as the severity of treatment increased regardless of the iron source bound. Wheat gluten-ferrous and soy protein isolate-ferrous as well as -ferric mixtures exhibited significantly less digestion from HCl treatment alone than when enzymes were added. Digestion of the wheat gluten-ferric mixture was significantly greater following the HCl-pepsin-pancreatin digest than after the two less severe treatments. Over all protein and iron sources, significantly more protein was digested as treatment progressed from HCl alone to HCl-pepsin digestion followed by pancreatin. Significantly more casein and soy protein isolate were digested than wheat gluten over all treatments. When bound to ferrous iron, casein was more easily digested than the other proteins. With ferric iron bound, soy protein isolate was digested to a significantly greater extent than the other proteins.

Release of protein-bound iron parallels protein digestibility with greater iron release and protein digestion occurring as the treatments progress from HCl alone to an HCl-pepsin digestion followed by pancreatin. Significantly less wheat gluten was digested and bound-iron released than occurred with soy protein isolate and casein. The oxidation state of the bound iron did not appear to substantially influence protein digestibility and iron release.

Treatment of the lyophilized protein-iron mixtures with HCl alone released 30–68% of the bound iron. This indicates that disruption of the tertiary and quaternary structures of the protein molecule will release a substantial portion of the bound iron. The remainder appears to be securely associated with the protein molecule and requires almost complete digestion for release. Following digestion the iron may yet be associated with individual amino acids or polypeptides small enough to pass through the filter used. If amino acids do indeed enhance iron uptake, prior binding may produce a protein-bound iron source of higher biological availability than the free salt.

Release of iron was 64–94% complete after digestion in the HCl-pepsin system and 85–97% complete following HCl-pepsin-pancreatin digestion. This suggests that protein-bound iron would be readily available for assimilation by the time it reached the duodenum and upper jejunum. Since the presence of amino acids has been shown to enhance iron uptake, a protein-bound iron system used for fortification would have the added advantage of amino acids present in the system itself.

Biological availability of bound iron

Biological availability of standard ferrous sulfate ($FeSO_4 \cdot 7H_2O$) and the six protein-bound iron sources was measured as the grams hemoglobin/100 ml blood increase over the 2-wk feeding period. A linear regression of the data for standard ferrous sulfate and the protein-ferrous complexes is presented in Figure 1.

Standard ferrous sulfate and the protein-ferrous mixtures showed an increase in hemoglobin concentration as the level of supplementation increased. At supplementation levels greater than 24 mg Fe/kg diet, the increase in hemoglobin concentration is expected to level off. Ferric iron, for which a figure is not presented, yielded near maximum improvement in hemoglobin concentration at 30 mg Fe/kg diet supplementation level with each of the protein-ferric mixtures. This lowest level tested corresponded to 6.0–6.5g

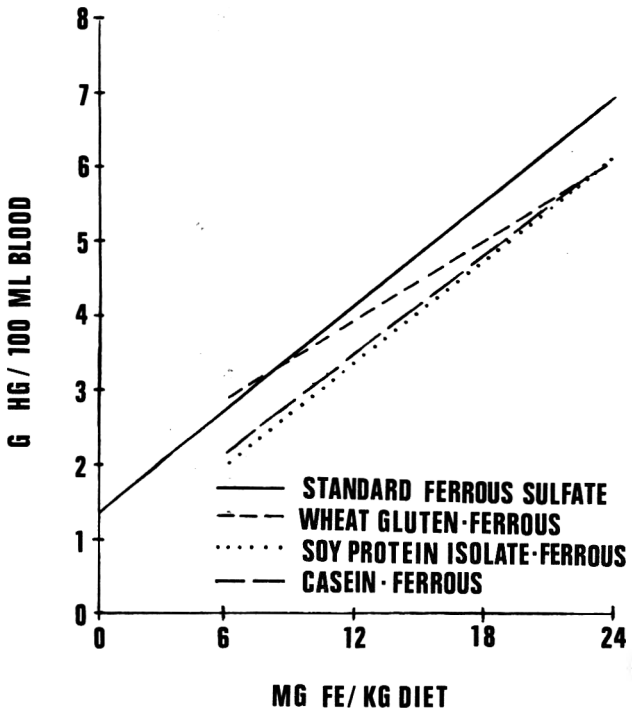

Fig. 1—Linear regression on the biological availability of protein-bound ferrous iron and standard ferrous sulfate.

hemoglobin/100 ml blood. This gradually increased to 6.8–7.1g hemoglobin/100 ml blood as supplementation levels with the protein-ferric mixtures were increased up to 120 mg Fe/kg of diet. Protein-bound ferric iron was more available than expected.

A slope ratio analysis revealed no significant difference in the availability of iron from standard ferrous sulfate and iron from the three protein-ferrous mixtures. Utilization of bound ferric iron was significantly ($P < 0.05$) lower than that of bound ferrous iron and that of the control, standard ferrous sulfate, for all protein sources studied. Among the three proteins studied, utilization of bound ferrous or ferric iron did not differ significantly.

Relative biological values for the six protein-bound iron sources are presented in Table 5. These values are only slightly lower than the ranges reported in the literature for the free iron salts: ferrous sulfate 100, and ferric pyrophosphate 38–52 (Fritz et al., 1970). Biological availability of protein-bound ferrous iron was not statistically different from the standard ferrous sulfate. This substantiates the findings of Motzok et al. (1977) who found the iron in cereals supplemented with ferrous sulfate as available as the free iron salt while other iron salts (ferric orthophosphate and sodium iron pyrophosphate) were less available. In this study, protein-bound ferric iron was significantly less available than the ferrous sources tested.

The enzyme release study indicated that protein-bound ferrous iron was released on the average 94% and protein-bound ferric iron 93% after an HCl-pepsin digestion followed by a pancreatin digestion. The in vivo iron availability measurement substantiates the findings from the in vitro digestion, that protein-bound iron is released during the digestive process and available for assimilation. A similar relationship between in vitro release of nonheme iron from foods and iron bioavailability has been reported by Narasinga Rao and Prabhavathi (1978).

Iron complexes previously have been produced in an effort to find iron supplements of high bioavailability yet low

Table 5—Relative biological values of the protein-bound iron sources

Iron source	Relative biological value
Standard ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	100
Wheat gluten ferrous	90
Soy protein isolate-ferrous	82
Casein-ferrous	82
Wheat gluten-ferric	25
Soy protein isolate-ferric	26
Casein-ferric	21

reactivity in food systems. These have included complexing ferric iron with phenolic compounds (Parliment, 1977; Scarpellino, 1977), a ferripolyphosphate-whey protein complex (Jones et al., 1972; Amantea et al., 1974), and iron-phytate complexes (Morris and Ellis, 1976). The ferripolyphosphate-whey protein powder has been used as an iron supplement in milk, where the iron was 84–107% assimilable (Jones et al., 1975), and in flour and flour-containing products (Nimmo and Fellers, 1975).

Our prior research quantified iron binding to several proteins under various conditions (Nelson and Potter, 1979). The present report shows that in the six systems studied this protein-bound iron is released upon digestion by acid and proteolytic enzymes so as to be readily assimilable. Further, biological availability of the iron was not significantly affected by the protein source involved in the protein-iron complex. This indicates that much of the iron bound to proteins during food processing remains bioavailable. It also indicates that many useful protein-iron complexes for fortification can be produced to meet the specifications of different food systems which cannot now be optimally fortified with free iron salts due to the undesirable reactions associated with such salts.

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STUDIES CONCERNING THE DETERMINATION OF LYSINOALANINE IN FOOD PROTEINS

MARVIN L. RAYMOND

ABSTRACT

A study was made on the use of an amino acid analyzer for determining lysinoalanine (LAL) in food proteins. Protein bound lysinoalanine is liberated by acid hydrolysis, separated on a high pressure cation exchange column with a sodium citrate buffer (0.35N sodium; pH 5.30), and detected colorimetrically after reaction with ninhydrin. The procedure is sensitive to concentrations of 50 μg LAL/g sample and concentrations as low as 20 μg/g sample have been detected. Application to food products, problems encountered, and the use of two different column temperatures (52 and 65°C) for different products to achieve better separation are described.

INTRODUCTION

FORMATION of lysinoalanine (LAL) in alkali and/or heat-treated proteins is of interest to the food industry due to reports concerning its toxic effects. Of particular interest is the reported formation of kidney lesions in a specific strain of rats fed alkaline processed soy protein, as presented in papers by Woodard and Alvarez (1967) and Woodard and Short (1973). They attributed the lesions to LAL found in the soy protein. It is known that LAL can be formed when protein is alkali treated and has been reported in heat-treated proteins as well (Sternberg et al., 1975b). Although it has not been conclusively determined whether LAL has any relevance for man, the concern over processed foods has brought about a need for a rapid and sensitive analytical procedure.

Analytical methods for LAL, which have been published, generally involve a chromatographic separation. Ion-exchange column chromatography with a ninhydrin detection system (or amino acid analyzer) is the technique that was first used to analyze for LAL (Bohak, 1964; Patchornik and Sokolovsky, 1964). The short ion-exchange column of an amino acid analyzer (two column system) was used to separate LAL from other basic amino acids in alkali treated ribonuclease. The standard sodium citrate buffer, pH 5.28–5.30, 0.35N sodium ion concentration, was used to elute LAL from the column. The amount of LAL measured in these reports was greater than 1%. Since then, several papers published on the toxicity of LAL or its presence in various alkali-treated proteins have used an amino acid analyzer with the above chromatographic conditions or some modification (Chu et al., 1976; deGroot and Slump, 1969; Nashef et al., 1977; Robson et al., 1967; Sternberg et al., 1975b; Woodard and Short, 1973). A paper devoted to a method of analysis by thin-layer chromatography was published by Sternberg et al. (1975a). This method was also applied to several miscellaneous food products (Sternberg et al., 1975b). An article published by Sakamoto et al. (1977) indicates that LAL and other cross-linked amino acids found in chemically modified wool can be analyzed by gas-liquid chromatography with mass spectrometry detection. Other techniques published have included the determination of LAL in alkali-treated wool by high voltage electrophoresis (Asquith and Carthew, 1972), and the use of paper electrophoresis to identify LAL in fractions isolated from an ion-exchange column (Provansal et al., 1975).

The procedure described as follows involves the use of an amino acid analyzer using high pressure ion-exchange chromatography.

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MATERIALS & METHODS

Reagents

Concentrated hydrochloric acid was diluted to 6N with deionized water and used for protein hydrolysis. Sample dilution buffer was made from sodium citrate to contain 0.20N sodium and adjusted to pH 2.20 with concentrated HCl. The column elution buffer was made from sodium citrate to be 0.35N in sodium and adjusted to pH 5.30 ± 0.01 with concentrated HCl. Ninhydrin solution was prepared according to Moore (1968). A 0.20N sodium hydroxide solution was used for column clean-up. Crystalline lysinoalanine was obtained from Miles Research Products, Elkhart, Ind. Alkaline-treated soy protein isolates were specially prepared (Struthers et al., 1977). Casein was acid precipitated from raw milk. Other food products were used "as is" from grocery shelves.

Sample preparation

Wet samples were freeze-dried prior to grinding. All samples were finely ground and hydrolyzed in 6N HCl for 24 hr at 110°C. Normally, a sample weight of 500 mg was hydrolyzed with 50 ml acid or a sample-to-acid ratio of 10 mg/ml was maintained. Samples were hydrolyzed in Wheaton pressure bottles which were flushed with nitrogen prior to sealing. After heating, the hydrolyzate was diluted to 100 ml with deionized water. An aliquot was evaporated to dryness and reconcentrated in sodium citrate buffer at pH 2.20 to give a sample concentration as high as 20 mg/ml.

Chromatography and detection

A volume of 30 μl of the buffered hydrolyzate was applied to the ion-exchange column of a Dionex (D-500) amino acid analyzer. The column was 0.175 × 48 cm and packed with a sulfonated polystyrene resin, 8 micron bead size. Normal operating pressures were 2200–2400 psig.

The sodium citrate buffer, pH 5.30, 0.35N sodium, was used to separate and elute LAL from the column at a flow rate of 8 ml/hr. A column temperature of 52°C was used for most samples, but 65°C was used for certain problem samples. The column was regenerated with sodium hydroxide solution between runs.

The classical ninhydrin reaction was used for the detection of LAL by a photometer (590/690 nm ratio) which was set at 0.2 absorbance units full scale to determine concentrations below 1000 μg/g sample. Integration of the recorder trace and automation of the procedure were handled by a DEC PDP8 mini-computer. This included measurement of peak areas, determination of elution time, and control of analyzer functions.

RESULTS & DSICUSSION

A CHROMATOGRAM of LAL in relation to some other basic amino acids and some amino sugars is shown in Figure 1. Peaks identified are glucosamine (GluN), galactosamine (GalN), lysinoalanine (LAL), tryptophan (TRP), lysine (LYS), and histidine (HIS). Galactosamine is not always completely resolved and may be an interference in proteins containing relatively high amounts of this amino sugar.

Tryptophan could also be an interference, although most

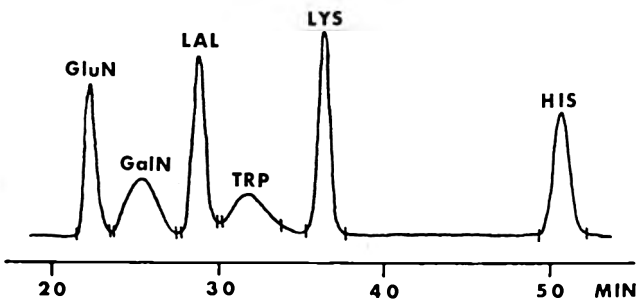

Fig. 1—Analysis of a standard solution containing 0.5 μmol/ml each of glucosamine, galactosamine, lysinoalanine, tryptophan, lysine, and histidine. Analysis made with a column temperature of 52°C.

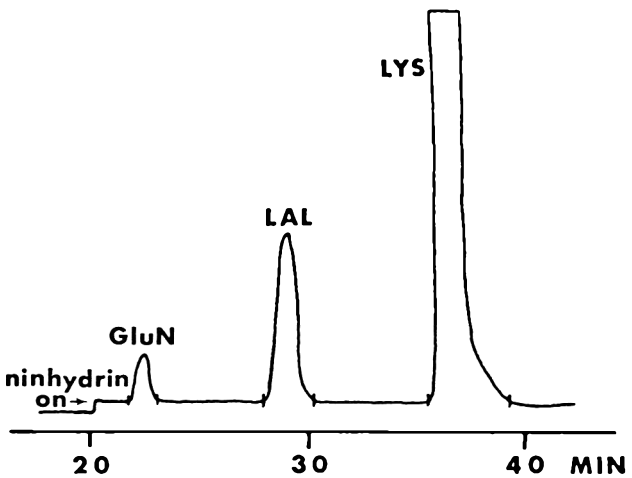

Fig. 2—Analysis of alkali-treated soy protein isolate. Sample was hydrolyzed in 6N HCl, 24 hr, 110°C. A volume of 30 μl of the diluted hydrolyzate (10 mg/ml) was loaded on the column.

of it is destroyed during acid hydrolysis. Our experience indicates that some residual amount may remain and interfere in measurements at low concentrations such as below 100 μg LAL/g sample. Hydroxylysine is not shown in Figure 1 but has been found to be an interference in that it overlaps the LAL peak when the column is operated at 52°C. It is not known to be in soy protein, but would be found in foods containing collagen. Ornithinoalanine was not available for testing as a possible interference.

The use of an automated amino acid analyzer allows for rapid multiple analyses. By using the single buffer as described in this procedure, LAL can be eluted within about 30 min after sample injection. This compares to about 2 hr for the procedure described by Slump (1977) and 75 min for that of Robson et al. (1967). By using the short column of a classical two-column system with the buffer described here, an LAL determination should be completed within an hour.

A chromatogram of an alkali-treated soy protein isolate showing LAL in relation to glucosamine and lysine appears in Figure 2. This sample was used to study the hydrolysis procedure, and contains at least 7000–8000 μg LAL/g sample.

Sternberg's group reported the use of 1 ml acid per 10-mg sample for the TLC procedure (1975a). In an effort to avoid evaporation and use a dilution step instead, the possibility of hydrolyzing a given weight of sample with less acid was examined. It was found, however, that this resulted in a lower hydrolysis yield for LAL. The 10 mg/ml sample/acid ratio was, therefore, used in this study.

The time for hydrolysis was studied as shown by Figure 3. Maximum liberation of LAL is achieved within the range of 20–24 hr with some losses occurring at later times. This is the recommended hydrolysis time for most other amino acids. A mean value of 7100 μg/g sample was obtained at

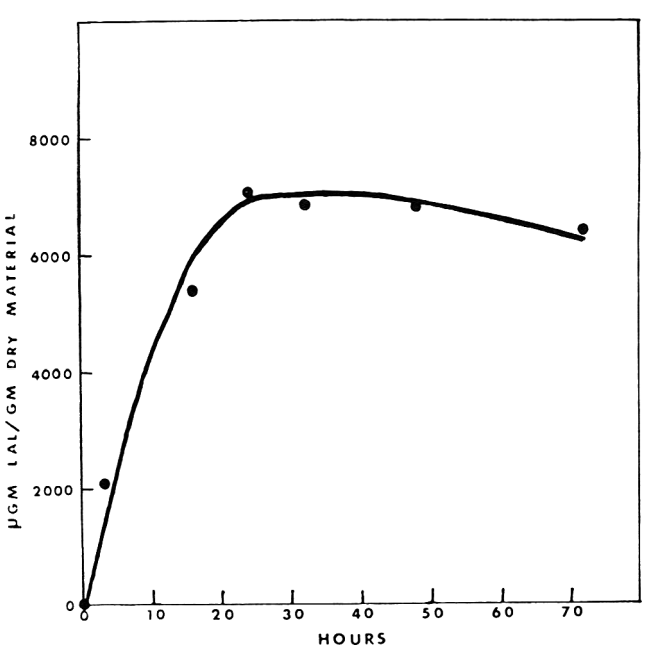

Fig. 3—Hydrolysis of an alkali-treated soy protein isolate in 6N HCl, 10 mg/ml, 110°C to show liberation of LAL as a function of time.

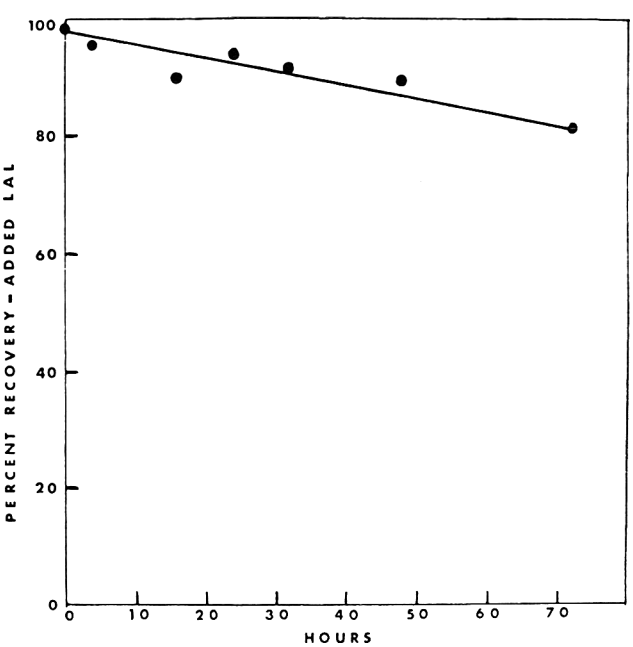

Fig. 4—Hydrolysis of a normal soy protein isolate that was not alkali-treated but contained free synthetic LAL added at a concentration of 2000 μg/g dry sample. LAL was added before hydrolysis and the sample was heated in 6N HCl, 10 mg/ml, at 110°C to show recovery of LAL as a function of time.

24 hr vs 6850 μg/g sample at 48 hr and 6400 μg/g sample at 72 hr. Destruction of free synthetic LAL added to a soy protein isolate that contained no protein bound LAL is demonstrated in Figure 4. Conditions were the same as for Figure 3. A recovery of 90% is obtained at the 24-hr hydrolysis time, but some loss does occur if the time is extended such as up to 72 hr. Recovery at 48 hr was 88%, but only 82% at 72 hr.

Table 1—Determination of sensitivity and recovery

LAL added[a] (μg/g Sample)	Avg LAL found[b] (μg/g Sample)	Std. Dev.	C.V.	Avg Recovery
400	406	29	7.1%	101%
200	195	6.6	4.2%	98%
100	108	9.6	8.9%	108%
80	84	1.7	2.0%	104%
60	45	4.6	10.3%	75%
50	40	5.1	12.5%	81%

[a] An aliquot of an LAL standard solution was added to a portion of a soy protein hydrolyzate prior to evaporation. Each portion was then reconcentrated with buffer to the same sample concentration (10 g/ml), but with varying amounts of LAL.

[b] Average LAL found and average recoveries are each based upon 4 independent analyses per LAL addition level.

Table 2—Analysis of an alkali-treated soy isolate[a]

Day 1 (μg/g Sample)	Day 2 (μg/g Sample)	Avg	Std. Dev.	C.V.
288	347	319	28	8.8%
302	339			

[a] Duplicate analyses were made on two different days and represent different batches of buffer and ninhydrin reagents as well as different hydrolyzates for the same soy isolate.

Table 3—Analysis of food products

Product	μg LAL/g Sample	μg LAL/g Protein
Casein		
From nonpasteurized whole milk	0	0
Two samples (ANRC)	60	70
Sodium caseinate	270	310
Ready-to-eat cereals		
Five samples (corn, wheat, rice, presweetened, instant grits)	0	0
Toasted oat	25	160
Puffed rice	70	1000
Pasta and bread		
Three samples (spaghetti, egg noodles, white bread)	0	0
Cheese		
Four samples (parmesan, cheddar, cheese spread, processed snack)	0	0
Canned vegetables and fruit		
Five samples (tomatoes, tomato paste, catsup, hominy, pears)	0	0
Snack foods and miscellaneous		
Six samples (potato chips, ginger cookies, soy sauce, instant chocolate mix, gelatin, peanut butter)	0	0
Pretzels	20	220
Egg		
Spray dried whole	0	0
Boiled 30 min		
White	0	0
Yolk	0	0
Strained egg yolks (infant)	525	1540
Milk		
Two samples (evaporated)	50	200
	140	550
Two samples (dry powder)	0	0
	0	0
Two samples (infant formula)	50	320
	0	0

The sensitivity of the procedure was examined by adding synthetic LAL in graded levels to a soy protein isolate. No protein bound LAL was found in the soy isolate nor in the soybean meal it was made from. It was found that the optimum sample concentration to achieve the best sensitivity with elimination of interferences and noisy baseline was 10 mg/ml. This means a column load of 0.3 mg of sample when a sample volume of 30 μl is applied to the column. With this sample concentration, the quantitative limit is 50 μg/g sample. Results from this experiment are listed in Table 1. The sensitivity may be increased and LAL concentrations as low as 20 μg/g sample have been measured. Of course, the sensitivity is dependent upon the quality of ninhydrin reagent and column resolution. In addition, the new fluorometric techniques (particularly orthophthalaldehyde) may be applied for increased sensitivities. The thin-layer chromatography procedure (Sternberg et al., 1975a) has a reported sensitivity as low as 6 μg/g protein. With TLC, recoveries of LAL added to soy protein were above 80% for levels greater than 200 μg/g protein, but were as low as 58% for a 100 μg/g protein level. Results of work done in this laboratory indicated that sensitivity was actually reliable to only 100 μg/g protein.

Precision in the measurement of protein bound LAL was examined by analyzing an alkali-treated soy isolate processed under controlled conditions to produce a moderate amount of LAL. The data are summarized in Table 2. Although the overall coefficient of variation of 8.8% is higher than normal for determining most amino acids in protein hydrolyzates, it is acceptable. The usual C.V. found for determining amino acids in this laboratory is 5% or less. However, this is for amino acid concentrations significantly higher than most LAL determinations. As indicated in Table 1, the coefficient of variation can be 10% or greater near the minimum detectable level.

One problem with either thin-layer or ion-exchange chromatography is the possibility of interferences, especially in heterogeneous protein mixtures such as food products. As sensitivities increase, several other ninhydrin reactive compounds may appear that interfere with LAL detection. This may result in a false positive identification for LAL. Some of these interferences include amino sugars, hydroxylysine, and ornithinoalanine, as well as tryptophan that is not completely destroyed during acid hydrolysis of the sample. This problem became evident as the procedure was applied to a variety of commercially available food products. The products were obtained from several different manufacturers. They were analyzed "as is," i.e. no further treatment or processing was made. Data are summarized in Table 3. A zero value indicates no LAL could be measured.

Casein was acid precipitated from raw whole milk. Although it was thought to be a simple sample matrix, it produced some of the most complex chromatograms. No LAL was identified and broad unknown peaks eluting before and after a spiked LAL peak tended to interfere in the measurements. Some LAL was found in ANRC casein. A sample of commercially prepared sodium caseinate indicated a significant amount of LAL (310 μg/g protein), but not nearly as much as previously reported (Sternberg et al., 1975b; Slump and Haagsma, 1978).

Of the several samples analyzed in each food category, only a few samples such as two ready-to-eat cereals, a pretzel sample, and an egg product for infants contained any LAL. Heating a raw egg for as much as 30 min had no effect on LAL formation. An LAL peak was identified in some milk products. However, these products also contain

Table 4—Application to meats

(Column temperature at 65°C to resolve LAL)

Product	μg LAL/g Sample	μg LAL/g Protein
Prepared meats		
Six samples (bologna, thuringer, bratwurst, beef jerky, frankfurter, Vienna sausage)	0	0
Canned fish		
Tuna	0	0
Sardines	120	270
Infant food		
Strained beef	120	160

relatively high concentrations of galactosamine which interfere in the measurements. Although quantitative measurements were carefully made, they may be erroneously high due to overlapping of the broad galactosamine peak with LAL.

Work with meat samples is summarized in Table 4. These were particulary difficult to analyze due to an interference which initially led to a false identification of LAL. It was found that a column temperature of 65°C was adequate for separating LAL from the unknown interference. The interference could be hydroxylysine, but this has not been confirmed. Again, no LAL could be identified in most of the products. Many of the hydrolyzates for these samples were spiked with LAL to make certain that any LAL present could be detected. Two previous papers by Slump (1977) and Slump and Haagsma (1978) have also dealt with interference problems in nonpurified proteins and physiological fluids. An amino acid analyzer technique using a single sodium citrate buffer (0.61N in sodium, pH 4.50) that will separate LAL from hydroxylysine and ornithinoalanine was presented. Also described was a four-buffer separation scheme to give a complete amino acid chromatogram. LAL was well separated from the amino sugars, glucosamine and galactosamine, in these two techniques. For application to food products, a column temperature dependence for removing interferences was also reported (Slump and Haagsma, 1978).

In conclusion, this study indicates that the standard sodium citrate buffer used for determination of basic amino acids on some amino acid analyzers can be used to analyze for LAL in soy protein and some other food products. It is even adequate for measuring low concentrations of LAL. However, the potential for interferences is great in some products such as milk, meats, and even casein. An important conclusion from the food survey data is that anyone involved in the determination of LAL in nonpurified proteins such as food products should watch for interference problems which could lead to a false positive identification of LAL.

It is evident that additional work is necessary to arrive at a method that can be more universally applied. Certainly no one method should be relied upon for conclusive identification of LAL in some food products. Present work involves measurements of residual LAL in the tissues of animals fed an LAL diet. However, there are some interference problems in trying to measure low concentrations of LAL in this type of matrix. Normally these are resolved using the 65°C column temperature as for meat products.

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TOMATO PEROXIDASE: PURIFICATION VIA HYDROPHOBIC CHROMATOGRAPHY

JOSEPH J. JEN, AIKO SEO and WILLIAM H. FLURKEY

ABSTRACT

With a large number of isoenzyme species and substrates, peroxidases have been difficult to purify to homogeneity. By including hydrophobic chromatography in the purification scheme, a homogeneous tomato fruit peroxidase isoenzyme was obtained. The isoenzyme had a clean spectrum in the visible region and a R_z (403 nm/280 nm) value of 2.36. It showed up as a single band in disc gel electrophoresis in basic and acidic buffers, in acetate strip electrophoresis, and in both tube and slab gel SDS electrophoresis. Molecular weight estimation of this tomato peroxidase was 43,000 ± 2,000 daltons. The pH optima were at 5.5 and 7.5 with guaiacol and pyrogallol as substrate, respectively. Kinetic studies showed that pyrogallol and hydrogen peroxide could provide substrate inhibition at 5 mM concentration. Hydrophobic chromatography may be useful for purification of other food enzymes similar to tomato peroxidase.

INTRODUCTION

PEROXIDASE (E.C.1.11.1.7., hydrogen donor oxidoreductase) is an ubiquitous enzyme in living cells. It is related to food quality in processing, particularly in flavors of both raw foods and processed products (Burnette, 1977). Haard (1977) reviewed the physiological roles of peroxidases in postharvest fruits and vegetables and attributed the mystery surrounding peroxidases partly to the presence of unusually large numbers of isoenzyme species and substrates. Hoyle (1978), by isoelectric focusing technique, has demonstrated that up to 42 isoperoxidases could occur in horseradish roots. In tomato plants, Evans and Aldridge (1965) separated out six isoperoxidases by starch gel electrophoresis although only one anionic isoperoxidase was found in tomato fruit. In later reports, Evans (1968, 1970) reported 12 isoperoxidases from dwarf tomato shoots and partially purified four of the isoenzyme species. Huang and Haard (1977) reported the properties of two IAA (indole acetic acid) oxidases from ripening tomato fruit, both of which also exhibited peroxidase activities. Kokkinakis and Brooks (1979a) recently isolated an isoperoxidase from tomato pericarp tissue to 85% purity and studied its kinetic properties in comparison with horseradish peroxidase. It was shown that tomato fruit isoperoxidase did not catalyze the aerobic oxidation of IAA in the presence of 2,4-dichlorophenol and manganese (Kokkinakis and Brooks, 1979b).

Hydrophobic interaction chromatography is based on the availability of a region of exposed hydrophobic surface on the protein molecule under the condition of investigation. The hydrophobic interaction between the protein molecules and the gel matrix can be influenced by a number of factors to facilitate the specific binding of particular enzymes of similar hydrophobicity (Shaltiel, 1974). The technique has been shown to be useful in the studies of polyphenol oxidase in peaches (Flurkey and Jen, 1978) and in separation of lipoxygenase and peroxidases in soybean (Flurkey et al., 1978). The objective of this study was to use hydrophobic chromatography and other means to isolate a major tomato peroxidase to homogeneity and to study some of the kinetic properties of this purified isoperoxidase.

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MATERIALS & METHODS

Tomatoes

Mature green tomatoes (*Lycopersicon esculentum* cv. Walters) were harvested from plants grown in a greenhouse or purchased from a local farmers market. One kilogram of tomatoes were cut into pieces and homogenized in a Waring Blendor for 1 min. The homogenates were squeezed through two layers of cheese cloth to produce approximately 500 ml of juice. Some juices were frozen for future studies without significant loss of peroxidase activities for several months.

Ammonium sulfate precipitation

In a typical purification run, tomato juices from 2 kg of tomatoes were brought to 50% saturation with solid ammonium sulfate. After centrifugation at 10,000 rpm for 15 min at 4°C, the precipitates were discarded. The supernatants were brought to 90% saturation with more ammonium sulfate and left overnight at 4°C. After centrifugation, the precipitates were suspended in 0.1M potassium phosphate buffer, pH 6.0, and dialyzed vs 2M ammonium sulfate in the same buffer at 4°C overnight.

Hydrophobic chromatography

Phenyl Sepharose CL-4B resin (Pharmacia Fine Chemicals, Piscataway, N.J.) was prepared as previously described (Flurkey and Jen, 1978). The column was equilibrated with 2M ammonium sulfate in phosphate buffer, pH 6.0. About 1 ml bed volume of the resin was used for every 3 mg of protein in the sample. After sample application, the hydrophobic chromatographic column was eluted with step gradients of 2M, 1.5M, 1M, and 0.5M ammonium sulfate in phosphate buffer, pH 6.0, followed with plain buffer and 50% ethylene glycol (Flurkey et al., 1978). Seven-milliliter fractions were collected and assayed for peroxidase activities. Fractions with peroxidase activites were pooled and dialyzed vs 4L of 0.05M phosphate buffer, pH 8.0, overnight at 4°C.

DEAE-Sephacel chromatography

DEAE-Sephacel resins (Pharmacia Fine Chemicals) were degassed and equilibrated with 0.05M phosphate buffer at pH 8.0. After sample application, the column was first developed with the same buffer followed with a 0–0.25M sodium chloride linear gradient in phosphate buffer. The fractions with peroxidase activities appeared as a brown band and were pooled, concentrated, and dialyzed vs 4L of 0.05M phosphate buffer at pH 6.0 overnight at 4°C.

Disc gel electrophoresis

The dialyzed peroxidase fractions were introduced onto acrylamide electrophoresis as samples and sucrose solution were layered on top of the samples. Electrophoretic separations were run at pH 8.3 at 5 ma per tube for 30–40 min as described previously (Flurkey and Jen, 1978). Peroxidase appeared as a brown-colored band and could be easily cut out from the rest of the gels. The peroxidase bands were homogenized or soaked in 0.01M phosphate buffer, pH 6.0 and used as samples for re-electrophoresis until only one peroxidase band, which corresponded to one protein band in duplicate gels as stained by Commassie Blue R-250, was obtained. The purified enzyme preparations were used for kinetic studies.

Peroxidase assay

Peroxidase activities were measured using 40 mM guaiacol in

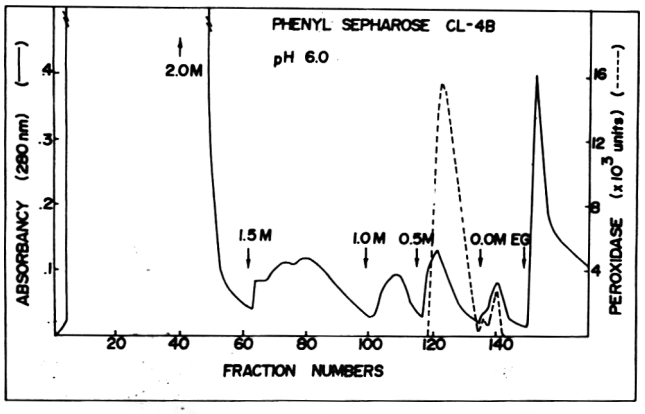

Fig. 1—Hydrophobic chromatography of tomato peroxidase. Proteins (54.9 mg) in 270 ml were applied onto a 20 ml bed volume column. Elution was by stepwise decreasing gradients with 2.0, 1.5, 1.0, 0.5, and 0.0M ammonium sulfate in 0.1M phosphate buffer, pH 6.0, followed with 50% ethylene glycol. 7-ml fractions were collected.

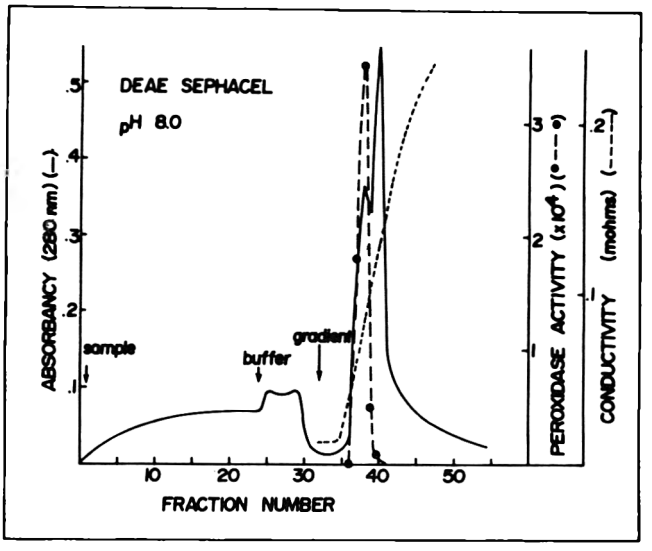

Fig. 2—DEAE-Sephacel chromatography of tomato peroxidase. Proteins (20 mg) in 250 ml were applied onto a 10 ml bed volume column. Linear gradient elution on 0—1.0M NaCl in 5 mM phosphate buffer at pH 8.0 was used to elute the peroxidase. 7-ml fractions were collected. The active peroxidase fraction has a R_z value (403/280 nm) of 0.87.

phosphate buffer, pH 6.0 and 30 mM hydrogen peroxide as substrate as described previously (Flurkey and Jen, 1978). For kinetic studies, various concentrations of guaiacol and pyrogallol were employed at optimum hydrogen peroxide concentration. The assays were carried out similar to that described by Evans (1968) except one unit of peroxidase activity was defined as the amount of enzyme that could cause a change of 0.1 absorbancy units at 470 nm or 430 nm for guaiacol and pyrogallol, respectively, per minute under the experimental conditions employed. Assays were carried out on a recording Beckman model 25 spectrophotometer with temperature of cell compartment set at 30°C.

Protein determination

The amount of protein in each sample was measured either by the standard Lowry method or by the Bio-Rad protein assay kit according to the Bio-Rad Technical Bull. No. 1051 using bovine serum albumin or γ-globulin as standards respectively.

Kinetics studies

Hydrogen peroxide concentrations from 3×10^{-2} mM to 30 mM were used at 40 mM guaiacol or 7.9 mM pyrogallol in 0.05M phosphate buffer at pH 6.0 and pH 6.7, respectively. Various concentrations of guaiacol from 0.8–80 mM and of pyrogallol from 3.2×10^{-2} to 159 mM were employed at 7.35 mM hydrogen peroxide concentration. For pH studies, phosphate buffers of pH 3.0–7.0 and Tris buffers of 7.0–10.0 were employed at optimal substrate concentrations. For temperature studies, enzymes were incubated at a given temperature for 5–45 min and immediately assayed for peroxidase activity.

SDS Electrophoresis

Gel tube SDS (sodium dodecyl sulfate) electrophoresis was performed according to the method of Weber and Osborn (1969) at 8 ma per tube. Slab discontinuous SDS electrophoresis was operated using the method of Laemmli (1970). The gels were fixed in 10% TCA (trichloroacetic acid) and stained with Commassie Blue R-250.

RESULTS & DISCUSSION

TABLE 1 summarizes the results of a typical purification of a tomato peroxidase isoenzyme. A large amount of 280 nm absorbing materials was removed at the hydrophobic chromatography step (Fig. 1) although the amount of protein removed at this step was not substantial. One possible explanation of this result was that there were polyphenol compounds, free sugars, and amino acids existing in green tomato pericarp tissue extract. These compounds absorbed at 280 nm and were not bound to the hydrophobic resin and therefore were eluted off in the void volume of the column. The bulk of the peroxidases were eluted off the Phenyl Sepharose column in the 0.5M ammonium sulfate fractions although some peroxidase activities were more tightly bound to the hydrophobic resin and could only be eluted with buffer with no ammonium sulfate or with 50% ethylene glycol. The step gradient used at this purification step was superior to a linear gradient of decreasing ammonium sulfate in the same buffer. A linear gradient would make the peroxidase spread out in a wide range of fractions and result in considerable dilution of the enzyme. Kokkinakis and Brooks (1979a) have shown that tomato pericarp tissues contain both a soluble and an ionic bound peroxidase. They also reported that 80% of the peroxidase activities in tomato fruit extract was due to a single peroxidase isoenzyme and that green fruit always has higher content of the soluble form of the peroxidase. Since we used green tomato fruit, the peroxidase collected in the 0.5M ammonium sulfate fraction is likely equivalent to the soluble form of peroxidase reported by Kokkinakis and Brooks which they believed was the peroxidase B as named by Evans (1968). Figure 2 shows the elution pattern of

Table 1—Summary of purification of tomato peroxidase isoenzyme

	Volume (ml)	Peroxidase (units/ml)	Total activity (units)	Protein (mg/ml)	Total protein (mg)	Specific activity (units/mg)	Yield (%)	Purification fold
Crude extract	850	364	294,100	0.13	110.5	2661.5	100	1
50% $(NH_4)_2SO_4$ super.	950	290	275,000	0.04	40.7	6769.0	93.9	2.5
Phenyl Sepharose column	184	1232	229,260	0.06	11.2	20841.0	73.2	7.9
DEAE Sephacel column	20.7	8860	183,484	0.25	5.1	35977.0	62.4	13.5
Disc gel electrophoresis	4.6	16703	76.836	0.18	0.82	93702.0	26.2	35.2

DEAE-Sephacel column. Peroxidase activity was eluted off the column at the beginning of the linear salt gradient. When subjected to disc gel electrophoresis, one major peroxidase band accompanied by three minor peroxidase bands were obtained (Fig. 3B). Oftentimes, the major band would spread out as a smear from the top of the separating gel to about one-third the distance down the gels. However, upon cutting off the top one third of the gels and re-electrophoresis, a single sharp peroxidase band corresponding

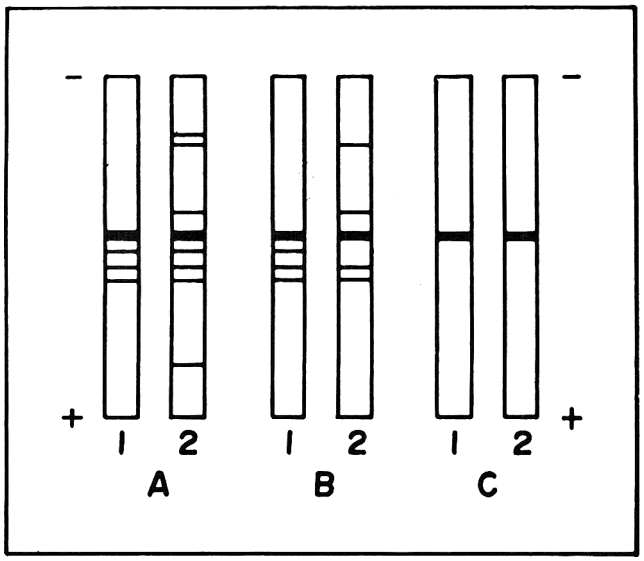

Fig. 3—Disc gel electrophoresis of tomato peroxidase: 1 for peroxidase stain, 2 for protein stain; A is after Phenyl Sepharose column, B is after DEAE-Sephacel column, C is after disc-gel electrophoresis.

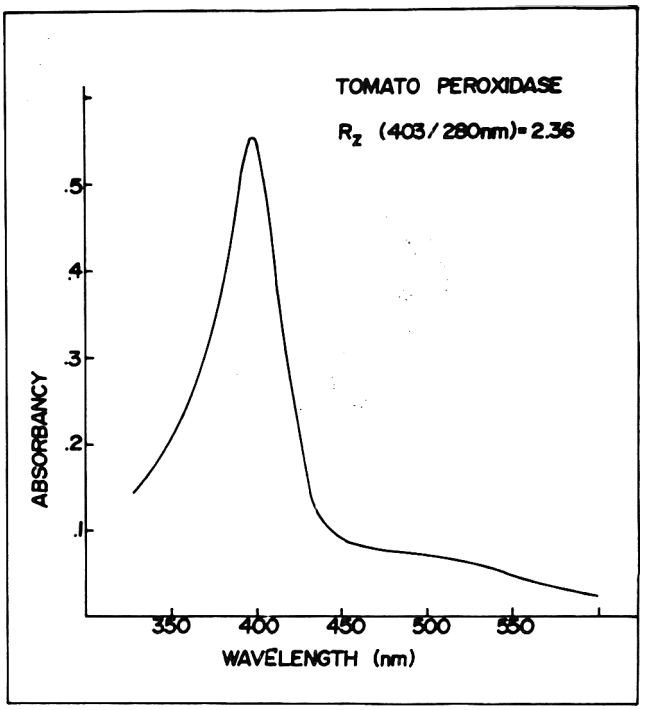

Fig. 4—Absorption spectrum of purified tomato peroxidase isoenzyme.

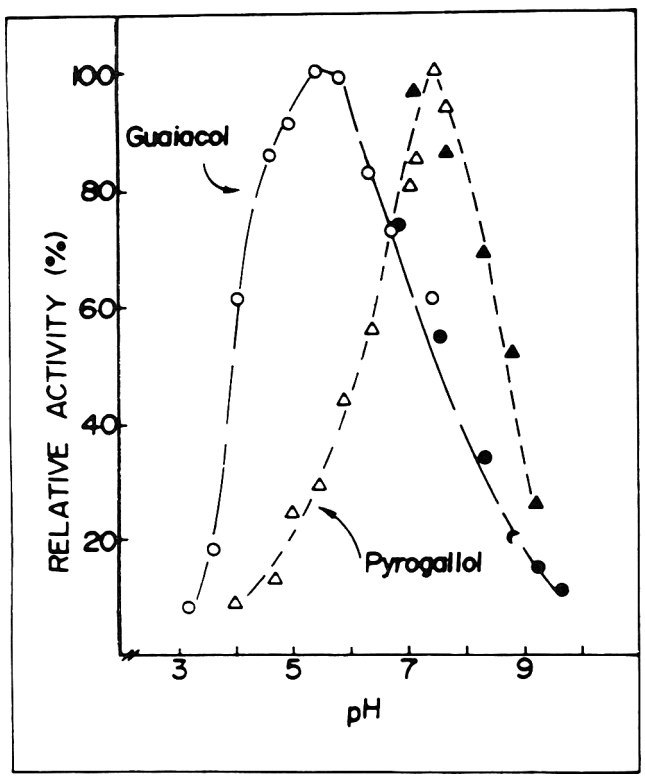

Fig. 5—Optima pH for pure tomato peroxidase isoenzyme. Reaction mixture contained 0.25 ml of 73.5 mM hydrogen peroxide, 0.05 ml enzyme, and 2.2 ml of 50 mM phosphate buffer or 50 mM Tris buffer containing guaiacol or pyrogallol as substrate. ○—○ guaiacol in phosphate buffer; ●—● guaiacol in Tris buffer; Δ—Δ pyrogallol in phosphate buffer; ▲—▲ pyrogallol in Tris buffer.

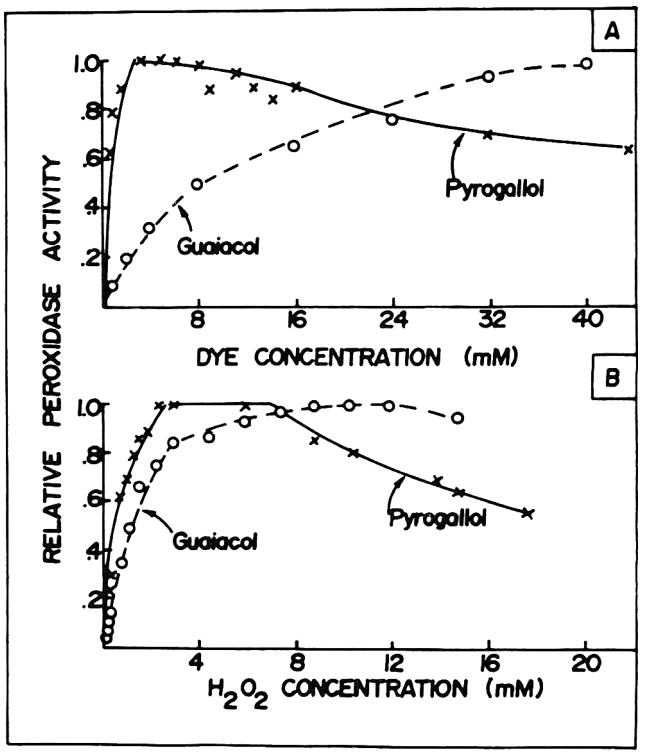

Fig. 6—Effect of substrate concentration on tomato peroxidase activities. (A) Effect of dye concentration: reaction condition same as Fig. 5 except that pyrogallol was in 50 mM phosphate buffer at pH 6.7 and guaiacol was in 50 mM phosphate buffer at pH 6.0. (B) Effect of hydrogen peroxide concentration: reaction mixture contained 2.2 ml of either 40 mM guaiacol or 29 mM of pyrogallol. Other conditions same as in A.

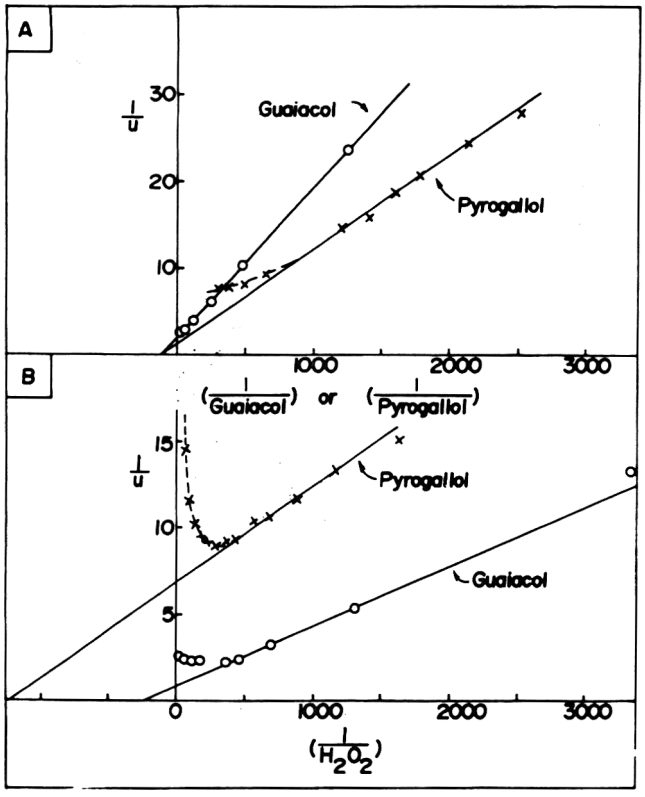

Fig. 7—Lineweaver-Burk plot of tomato peroxidase isoenzyme. Conditions same as in Fig. 6.

to a single protein stain was seen (Fig. 3C). The purified enzyme exhibits a clear spectrum with maximum absorption at 403 nm signifying the Soret band (Fig. 4). The R_z value (A_{403}/A_{280nm}) of the purified peroxidase isoenzyme was 2.36. This value was considerably higher than the purified peroxidase B preparation from tomato shoots with an R_z value of 1.0 reported by Evans (1970). The purity of our tomato peroxidase preparation was checked by running the disc gel electrophoresis at acidic pH, with SDS electrophoresis in both tube and slab gels, and with acetate strip electrophoresis. In all cases, only one protein band was observed.

Molecular weight estimation by SDS electrophoresis and by gel filtration showed our preparation to be 43,000 ± 2,000 daltons. This corresponded well with the heavy fragment of the tomato peroxidase isoenzyme reported by Kokkinakis and Brooks (1979a).

By using guaiacol and pyrogallol as substractes, our peroxidase showed pH optima at pH 5.5 and 7.5, respectively (Fig. 5). This was the same as the tomato shoot peroxidase B reported by Evans (1970). Signoret and Crouzet (1978) in an attempt to purify tomato polyphenol oxidase, reported a tomato peroxidase with a rather braod pH optimum. Substrate concentration studies showed that apparent K_M for hydrogen peroxide was 4 mM at optimum guaiacol concentration and was 0.8 mM at optimum pyrogallol concentration (Fig. 6 and 7). The apparent K_M for guaiacol and pyrogallol at optimum hydrogen peroxide concentration was 10 mM. The Lineweaver-Bulk plots clearly showed that pyrogallol and hydrogen peroxide could provide substrate inhibition at approximately 3 mM and 5 mM, respectively, while guaiacol did not show substrate inhibition until 40 mM concentration. In comparison with kinetic data reported by Evans (1970), our peroxidase preparation was more tolerant to substrate concentration than Evan's preparation.

The complexity evolving peroxidase is partly due to its large number of isoenzyme species. Hoyle (1978) reported 42 isoperoxidases in horseradish by isoelectric focusing. Studies done with partially purified enzyme always leave some doubt as to the contribution of individual isoenzyme species. We feel that we have developed a procedure to provide a homogeneous peroxidase isoenzyme preparation from tomato fruit, and attribute our success partly to the use of hydrophobic chromatography. In addition to tomato peroxidase, we have had good success using this technique for the purification of peach polyphenol oxidase (Jen and Flurkey, 1979) and soybean lipoxygenase (Flurkey et al., 1978). Perhaps similar procedures can be developed to isolate other food enzymes in more homogeneous states.

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EFFECTS OF pH AND SODIUM CHLORIDE ON Yersinia enterocolitica GROWTH AT ROOM AND REFRIGERATION TEMPERATURES

NORMAN J. STERN, M. D. PIERSON, and A. W. KOTULA

ABSTRACT

The effects of pH level and sodium chloride concentration on clinical and environmental strains of *Yersinia enterocolitica* at 3° and 25°C were determined. Bacteriostatic and bactericidal inhibition was observed at 7% w/v sodium chloride at both 3° and 25°C in Brain Heart Infusion Broth. Growth was observed at pH 4.6–9.0. Clinical strains showed significantly greater tolerance to pH level and sodium chloride concentration than did the environmental strains of *Y. enterocolitica* at 25°C. These findings suggest that *Y. enterocolitica* may be resistant to some common methods of food preservation.

INTRODUCTION

Yersinia enterocolitica has been implicated in causing water and foodborne enteritis. This organism has the unusual capability of growing well at refrigeration temperatures. Large outbreaks of *Y. enterocolitica* infections in the United States (Black et al., 1978), Canada (Health and Welfare Canada, 1976), and in Japan (Asakawa et al., 1973; Zen-Yoji et al., 1973) have led to world-wide investigations of foods as a vehicle of *Y. enterocolitica* infection. Substantial evidence now exists showing the presence of the microorganisms in drinking water (Highsmith et al., 1977), raw milk (Schieman and Toma, 1978), meats (Leistner et al., 1975), fish (Kapperud and Jonsson, 1976), oysters (Toma, 1973), and vegetables (Mehlman et al., 1978). *Y. enterocolitica* has also been recovered from tartar sauce in Czechoslovakia (Aldova et al., 1975).

Y. enterocolitica is a heterogenous composite of environmental and clinical strains which have different foci and intensities of virulence (Schiemann and Toma, 1978). Environmental strains may utilize any combination of esculin, salicin or rhamnose, while the clinical strains will not. Rhamnose-positive strains are atypical of the *Y. enterocolitica* clinical reference strains. *Y. enterocolitica* strains may be further subdivided into five biochemical types (Nilehn, 1969; Wauters, 1973). Brenner and co-workers (1976) used biochemical characterization and DNA reassociation to suggest the splitting of the species into four separate groups. Lee (in press) showed a large variation in sensitivity toward recovery media among several clinical strains. Enrichment in modified Rappaport broth (RMC) containing magnesium chloride, malachite green, and carbenicillin (Wauters, 1973) was used to recover several resistant clinical strains from pork and oysters. RMC completely inhibited growth of sensitive clinical strains as well as most of the native environmental strains. Another common enrichment procedure has involved pH 7.6 phosphate buffered saline at refrigeration temperatures. This procedure had inconsistant recovery success among the clinical strains (Lee, in press).

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Because of the multiplicities of characteristic biochemical reactions and chemical resistance, it seems likely that differences in tolerance to chemical and physical stresses may exist within the species. Hanna and co-workers (1977) found that *Y. enterocolitica* ATCC 23715 grew between pH 5–9. The high acidity of sauerkraut and pickles and the survival of *Yersinia* in these products suggests that certain strains are relatively acid tolerant and may be of concern to public health (Lee et al., in press). At present, there is no information on the variation of sodium chloride tolerance among *Y. enterocolitica* strains. Since *Y. enterocolitica* may grow at refrigeration temperatures used in food preservation, additional chemical inhibition may be necessary. The purpose of this study was to determine the levels of pH and sodium chloride concentrations needed to inhibit *Y. enterocolitica* at room and refrigeration temperatures.

MATERIALS & METHODS

Cultures

Yersinia enterocolitica cultures CDC A2635, CDC A2611, IP 867 and IP 955 were obtained from D. Brenner (Center for Disease Control, Atlanta, Ga.). Stock cultures were maintained on Brain Heart Infusion agar (BHIA, Difco). These cultures were transferred at monthly intervals, incubated at 23°C for 24 hr and maintained at 3°C until use. Inocula for growth tolerance studies were prepared by transferring loopfuls of the cultures into screw-cap test tubes containing 5 ml Brain Heart Infusion Broth (BHIB; Difco) and incubating for 15–18 hr at 23°C.

Growth response studies

Studies were done with 250 ml screw cap Erlenmeyer flasks containing 100 ml BHIB. Preliminary studies were used to choose appropriate pH values and sodium chloride concentrations for testing. Sodium chloride was added in the appropriate amounts to achieve final concentrations of 0.5% (the initial sodium chloride concentration of BHIB), 3.0%, 5.0%, 7.0%, and 9.0% (w/v). The media were autoclaved at 121°C for 15 min. Water activity was determined with a Hydrodynamics, Inc. hygrometer, model 15-3001 (Silver Spring, Md.). pH values were adjusted to 4.4, 4.6, 7.2, 8.0, 9.0, and 9.6 by the dropwise addition of either 5N HCl or 5N NaOH. The initial pH of the autoclaved media varied no more than 0.05 pH units after cooling. The growth flasks were aseptically inoculated with predetermined concentrations of the *Y. enterocolitica* strains that were diluted in 0.1% peptone blanks. The target inoculum was ca 10^2 cells per ml of the test medium. The flasks were incubated in a shaker water bath (Fermentation Design, Inc., Allentown, Pa.). at 25°C and 180 oscillations per minute. Studies at 3°C, 190 oscillations per minute, utilized a Lauda constant temperature circulator, model K2RS (Brinkman Instruments, Inc. Westbury, N.Y.). Samples were aseptically withdrawn from the growth flasks at selected time intervals and serial dilutions were made in 0.1% peptone blanks before pour plating with BHIA. The petri dishes were inverted and incubated at 23°C and colonies counted at 36–48 hr. Duplicate experimental trials were performed to obtain the experimental data.

Statistical analysis

Duncan's (1955) multiple range analysis was used to compare the effect of different initial pH values and different sodium chloride concentrations on the log number of the four *Y. enterocolitica* strains tested. Duncan's multiple range analysis also compared strain differences in response to the experimental conditions; the strain differences were measured by log number of cells per ml. The times chosen for the Duncan multiple range analyses coincided with times

Table 1—Effect of initial pH on four strains of Yersinia enterocolitica grown in Brain Heart Infusion Broth at 3°C[a]

Strain	Initial pH 4.4	4.6	7.2	9.0	9.6
A2611	1.49a[b]	2.44a	8.60a	4.51a	1.03ab
IP867	1.09a	—c[c] 1.63a	—c 7.70a	—a 2.35a	—b 1.89a
IP955	1.13a	2.16a	8.74a	4.67a	0.15b
A2635	1.00a	1.63a	8.41a	4.16a	0.00b

[a] Statistical differences in log no. of cells per ml were determined through duplicate experiments at 10 days of incubation. Significantly different values ($P \leq 0.05$) are indicated with different letters (Duncan, 1955). Initial inoculum was 100 cells/ml.

[b] Letters within brackets denote differences between strains for each pH value. Values are log no. cells per ml.

[c] Letters outside brackets denote differences in growth due to differences in initial pH.

of maximum cellular concentration for the variables tested. In the studies at 3°C, the greatest numbers of cells were found after 10 days of incubation. In studies at 25°C, cell numbers were greatest at 48 hr.

RESULTS

TABLE 1 demonstrates the growth response of each *Y. enterocolitica* strain to pH and 3°C incubation. A pH value of 4.4 produced bactericidal or bacteriostatic influences on the different strains over 10-days incubation, and a pH value of 9.6 generally produced lethal effects of *Y. enterocolitica*. The pH value of 7.2 allowed the greatest and most rapid growth of the organism through 10-days incubation. More acidic or more basic BHIB exerted increasingly stringent inhibitory influences and caused longer lag phases and less rapid growth. Significantly lower cellular numbers among all strains tested were observed at the extreme pH values tested (see Table 1). However, some strains continued to survive even at the extreme pH values tested for the duration of the experiment. Generally, with the same initial pH value, individual strain differences at 3°C were not seen in the growth responses as determined by the Duncan multiple range test (Duncan, 1955). Figure 1 depicts the effect of initial pH on the growth response of strain CDC A2635 in BHIB at 3°C.

The growth response of *Y. enterocolitica* CDC A2635 to 0.5, 5.0, and 7.0% sodium chloride is seen in Figure 2. As with the previous set of conditions, the media were inoculated with ca 10^2 cells per ml, incubated at 3°C, and cells enumerated through the 10-day experimental periods. Seven percent sodium chloride produced bactericidal effects on each of the four strains tested (Table 2). Five percent sodium chloride allowed for significantly greater growth among the strains tested in comparison to the 7% sodium chloride concentration. The greatest numbers of *Y. enterocolitica* and the most rapid increase in cell numbers occurred at 0.5% sodium chloride. No differences between strains were detected as determined by the Duncan multiple range test (Table 2).

The effect of initial pH on the growth of *Y. enterocolitica* inoculated at ca 10^2 cells per ml in BHIB at 25°C is shown in Figure 3. Viable cells increased within 24 hr at both an initial pH of 4.6 and an initial pH of 9.6. Substantial increases in cellular numbers at an initial pH of 4.6 ($\geq 10^{7.6}$ cells per ml) were observed for all strains after 48 hr of growth (Table 3). The initial pH value of 4.6 of the inoculated media remained constant throughout the 48-hr growth period, while the inoculated basic media dropped from an initial pH value of 9.6 to a pH of 8.4 within 48 hr. The bactericidal influence of *Y. enterocolitica* at pH 4.4

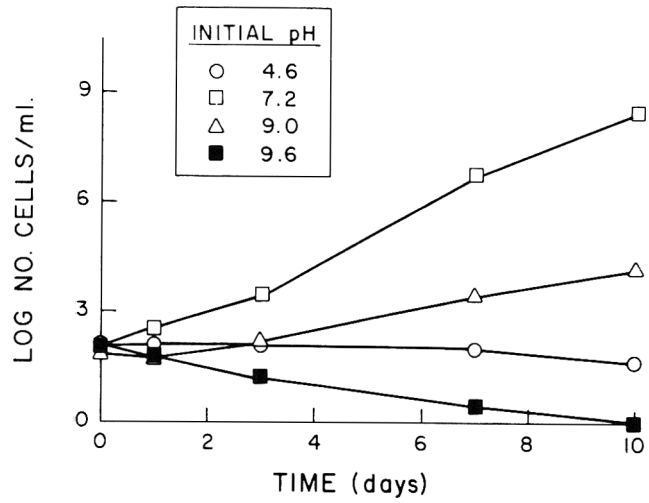

Fig. 1—Effect of initial pH values and incubation time on the growth of Yersinia enterocolitica CDC A2635 in Brain Heart Infusion Broth at 3°C.

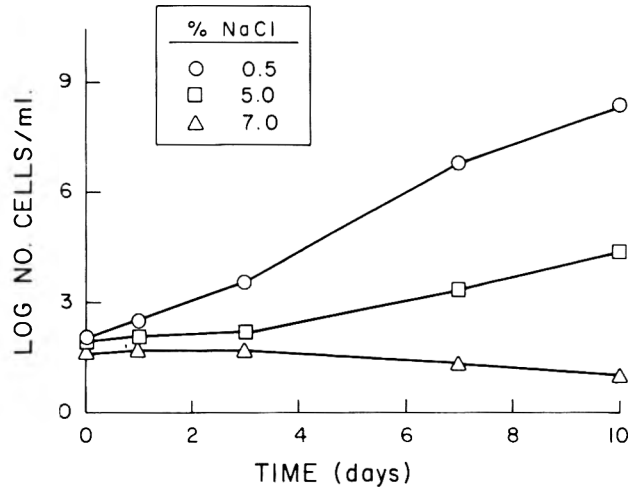

Fig. 2—Effect of sodium chloride concentration and incubation time on the growth of Yersinia enterocolitica CDC A2635 in Brain Heart Infusion Broth at 3°C.

Table 2—Effect of sodium chloride on four strains of Yersinia enterocolitica grown in Brain Heart Infusion Broth at 3°C[a]

Strain	Sodium chloride concentrations 0.5	5.0	7.0
A2611	8.60a[b]	4.05a	0.60a
IP867	7.70a —a[c]	4.78a —b	0.00a —c
IP955	8.74a	4.95a	0.80a
A2635	8.41a	4.36a	1.19a

[a] Statistical differences in log no. of cells per ml determined through duplicate experiments at 10 days incubation. Significantly different values ($P \leq 0.05$) are indicated with different letters (Duncan, 1955). Initial inoculum was 100 cells/ml.

[b] Letters within brackets denote differences between strains for each sodium chloride level. Values are log no. cells per ml.

[c] Letters outside brackets denote differences in growth due to differences in sodium chloride concentration.

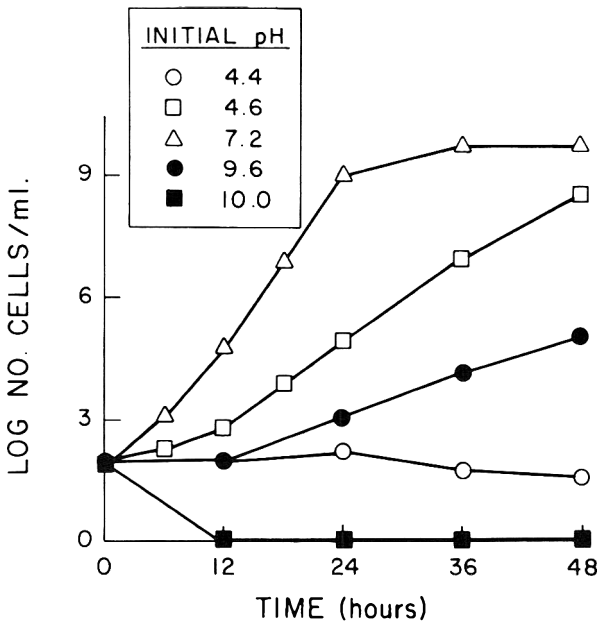

Fig. 3—Effect of initial pH values and incubation time on the growth of Yersinia enterocolitica CDC A2635 in Brain Heart Infusion Broth at 25° C.

was slower in comparison to that at pH 10.0. Viable cells were present in the medium with an intial pH of 4.4 throughout the 48 hr of incubation. Significantly greater ($P \leq 0.05$) inhibition among all the strains tested was found at an initial pH of 9.6 as compared to an intial pH of 4.6 (Table 3). Significant strain variation ($P \leq 0.05$) existed in the growth responses between the environmental and clinical strains at an initial pH value of 9.0. Strains CDC A2611 and CDC A2635 grew to higher numbers than strains IP 867 and IP 955 under this relatively alkaline condition.

The effect of sodium chloride concentration on the growth of *Y. enterocolitica* inoculated at ca 10^2 cells per ml in BHIB at 25°C is demonstrated in Figure 4. At 48 hr the greatest and most rapid growth occurred at 0.5% sodium chloride, while lower numbers and slower growth were observed in increasing sodium chloride concentrations. Seven percent sodium chloride produced either bactericidal or bacteriostatic influences on the strains of *Y. enterocolitica* tested (Table 4). The 7% level of sodium chloride in BHIB which produced these inhibitory influences had a water activity of 0.945. Substantial growth among all strains ($\geq 10^{5.5}$ cells per ml) occurred at 5% sodium chloride within 48 hr while 9% sodium chloride was bactericidal for the strains tested.

Y. enterocolitica proved slightly more tolerant of sodium chloride at 25°C in comparison to 3°C. The four test strains developed nonuniform patterns of growth in response to sodium chloride at 25°C. Duncan multiple range tests for strain variation were made by determining the mean log number of cells per ml for each of the sodium chloride concentrations analyzed. The clinical strains (CDC A2635 and CDC A2611) showed equal or significantly greater growth ($P \leq 0.05$) in comparison to the environmental strains (IP 867 and IP 955) for salt concentrations above 3% at 48 hr of growth at 25°C (Table 4). The clinical strains were relatively tolerant of 7% sodium chloride and their numbers remained essentially unchanged over 48 hr. Numbers of the environmental strains at 7% sodium chloride remained static or diminished.

DISCUSSION

ENTERIC BACTERIA are widely distributed in nature, and generally do not have unusual resistance to most methods of microbial destruction. Typically, enteric organisms may survive in foods with pH values above 4.5 for extended time intervals (Lee and Riemann, 1971). In contrast, *Yersinia pseudotuberculosis* has been recovered from highly acidic foods in the Soviet Union (Kuznetsov et al., 1975). *Salmonella* is completely inhibited in foods with pH values of less than 4.5 (Banwart and Ayres, 1957). Similar results with *Y. enterocolitica* are reported in this study. The organism grew well at a starting pH of 4.6 at 25°C. This finding is in contrast to the work reported by Hanna et al., (1977) who described the growth range of one *Y. enterocolitica* strain as between pH values of 5–9. Although this study was carried out in BHIB, concern about the safety of acid foods would be justified. If an acid food, such as tomato juice, should be contaminated after opening the container, *Y. enterocolitica* may have the capacity to survive or grow at refrigeration and especially at room temperatures.

Seven percent sodium chloride was inhibitory to *Y. enterocolitica* at 3°C and 25°C, producing bacteriostatic or bactericidal effects. This restraining influence may have been caused by specific toxic effects of sodium chloride. Lowered water activity due to increased sodium chloride concentrations might also account for yersinial inhibition. *Y. enterocolitica* and other *Enterobacteriaceae* resist similar levels of sodium chloride. For example, *Salmonella* are also inhibited at water activities below 0.945 (Lee and Riemann, 1971), which corresponds to the sodium chloride levels and water activity found to inhibit *Y. enterocolitica* in this study. The growth of *Y. enterocolitica* in sodium chloride concentrations of 5% at 3°C makes the organism unique among enteropathogens. The pH and sodium chloride levels which commonly inhibit *Enterobacteriaceae* under refrigeration conditions, do not appear adequate for the inhibition of *Y. enterocolitica*.

Significant differences in the growth responses of the clinical and environmental strains were noted in this study. At 25°C (pH 9.0 or 7.0% sodium chloride) the log cell numbers per ml of the clinical strains were significantly

Table 3—Effect of initial pH on four strains of Yersinia enterocolitica grown in brain heart infusion broth at 25° C[a]

	Initial pH					
Strain	4.4	4.6	7.2	9.0	9.6	10.0
A2611	1.52a[b]	7.78a	9.74ab	8.64a	3.68b	0.00a
IP867	1.76a —e[c]	7.95a —b	9.94ab —a	5.37d —c	3.92b —d	0.00a —f
IP955	1.63a	7.66a	10.03a	6.80c	3.91b	0.00a
A2635	1.59a	8.48a	9.60b	7.78b	5.07a	0.00a

[a] Statistical differences in log no. of cells per ml were determined through duplicate experiments at 48 hr of incubation. Significantly different values ($P \leq 0.05$) are indicated with different letters (Duncan, 1955). Initial inoculum was 100 cells/ml.

[b] Letters within brackets denote differences between strains from each pH value. Values are log no. cells per ml.

[c] Letters outside brackets denote differences in growth due to differences in initial pH.

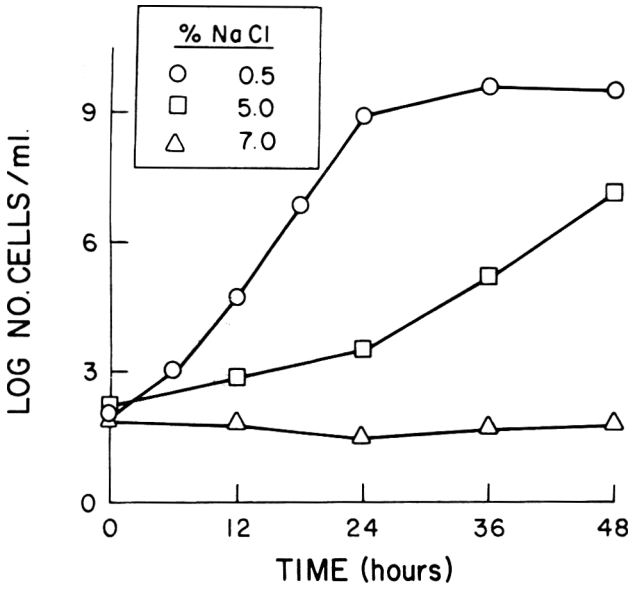

Fig. 4—Effect of sodium chloride concentration and incubation time on the growth of Yersinia enterocolitica CDC A2635 in Brain Heart Infusion Broth at 25° C.

greater than the numbers of the environmental strains. This evidence lends support to Brenner and co-workers' (1976) contention that further subdivision of the present species of *Y. enterocolitica* into different species may be justified.

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Table 4—Effect of sodium chloride on four strains of Yersinia enterocolitica grown in Brain Heart Infusion Broth at 25° C[a]

	Sodium chloride concentration				
Strain	0.5	3.0	5.0	7.0	9.0
A2611	9.89ab[b]	9.51a	6.80a	2.47a	1.70a
IP867	9.94a	9.33a	6.54a	1.56b	0.72ab
IP955	10.03a	9.63a	5.49b	0.00c	0.00b
A2635	9.60b	9.38a	7.11a	1.81b	1.81a
	–a3	–b	–c	–d	–e

[a] Statistical differences in log no. of cells per ml were determined through duplicate experiments at 48 hr of incubation. Significantly different values ($P \leq 0.05$) are indicated with different letters (Duncan, 1955). Initial inoculum was 100 cells/ml.

[b] Letters within brackets denote differences between strains for each sodium chloride level. Values are log no. cells per ml.

[c] Letters outside brackets denote differences in growth due to differences in initial sodium chloride concentration.

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FORMATION OF β-LACTOSE FROM THE STABLE FORMS OF ANHYDROUS α-LACTOSE

F. W. PARRISH, K. D. ROSS, and K. M. VALENTINE

ABSTRACT

Treatment of either of the stable anhydrous crystalline forms of α-lactose with potassium methoxide in methanol at reflux does not result in the formation of β-lactose, whereas the latter is formed from α-lactose monohydrate or hygroscopic anhydrous α-lactose under these conditions. We find that when β-lactose is added to a reaction mixture containing either form of stable anhydrous α-lactose, conversion of α-lactose to β-lactose proceeds readily. Further, we show that this conversion may be a solid-solid transformation rather than dissolution of α-lactose followed by crystallization as β-lactose.

INTRODUCTION

THE USE OF α-lactose monohydrate in a wide variety of baking applications has been studied by Ash (1976) who reported one of the shortcomings of α-lactose monohydrate to be its low solubility in baking formulations. β-Lactose has greater solubility than α-lactose monohydrate (Whittier, 1944), and the formation of β-lactose from deionized whey ultrafiltrate (Kavanagh, 1975) and its application in cake-baking (Goldman and Short, 1977) indicate a renewed interest in this form of lactose. Formation of β-lactose from α-lactose monohydrate with sodium hydroxide (Olano and Rios, 1978) or potassium methoxide (Parrish et al., 1979a) has been reported, but under the same conditions stable anhydrous forms of α-lactose were unaffected (Olano, 1978; Parrish et al., 1979a). This difference in behavior between α-lactose monohydrate and stable anhydrous α-lactose was surprising to us and led us to study further how stable anhydrous α-lactose behaved in basic methanol. We are able now to transform stable anhydrous forms of α-lactose to β-lactose, and we report here on the conditions necessary for this change and its possible mechanism.

EXPERIMENTAL

Materials

α-Lactose monohydrate was obtained from Sigma Chemical Co., St. Louis, Mo. β-Lactose was prepared by the procedure of Buma and van der Veen (1974). Stable anhydrous α-lactose was formed from α-lactose monohydrate by treatment with methanol (α_M) (Tanret, 1896; Lim and Nickerson, 1973) or by heating in air at 130°C (α_S) (Sharp. 1943). An ustable form of anhydrous lactose (α_H), containing variable amounts of β-anomer, was made by heating α-lactose monohydrate in vacuum at 130°C (Herrington, 1948). Amorphous lactose containing the α- and β-anomers was obtained by freeze-drying an aqueous solution of α-lactose monohydrate which had been allowed to attain mutarotational equilibrium (Roetman and van Schaik, 1975). Compounds containing the anomeric forms of anhydrous lactose in the ratio α:β = 4:1 (Olano et al., 1977) or α:β = 5:3 (Hockett and Hudson, 1931) were prepared by treating α-lactose monohydrate with 2% hydrogen chloride in anhydrous methanol and 1% aqueous methanol, respectively.

Potassium methoxide was obtained from Ventron Corp., Danvers, Mass., and potassium hydroxide and methanol were AR grade chemicals from Fisher Chemical Co., King of Prussia, Pa.

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Apparatus

Optical rotations were measured at 589 nm and 20°C with an automatic polarimeter Model 141 (Perkin-Elmer, Norwalk, Conn.). Gas-liquid chromatography of trimethylsilyl ether derivatives of α- and β-lactose, prepared by the procedure of Sweeley et al. (1963), was performed on a 4 ft × 1/8 in. column of 3% SP-2100 on Supelcoport (100–200 mesh) with temperature programming from 150–210°C on a Model 5750 instrument (Hewlett-Packard, Palo Alto, Calif.). Other parameters were helium flow-rate 30 ml/min, injector temperature 215°C, and detector temperature 220°C. The output signal from the flame ionization detector was transferred to an IBM 1130 computing system to calculate peak areas. Moisture determinations were made with an Aquatest II instrument (Photovolt Corporation, New York, N.Y.), and differential scanning calorimetry (DSC) measurements with a Model 990 thermal analyzer (Dupont, Wilmington, Del.). A JEOL FX-60Q NMR spectrometer (JEOL, Inc., Cranford, N.J.) was used to measure methanol and ethanol retained by the lactose samples (Parrish et al., 1979b). Atomic absorption analyses for potassium were made with a Model 306 instrument (Perkin-Elmer, Norwalk, Conn.) after ashing the sample in an electric furnace Type 056-PT (Hevi Duty Electric Co., Milwaukee, Wis.).

General procedure

The lactose sample was stirred at room temperature or at reflux for the required time with eight times its weight of 0.014M potassium hydroxide or potassium methoxide in anhydrous methanol or 1% aqueous methanol. The solid material was removed by filtration and, after being thoroughly washed with methanol, was dried in vacuum at 65°C for 16 hr. Mixtures of different forms of lactose were treated in the same way.

In other experiments, two different forms of lactose (2.5g each) were treated at reflux with basic methanol (100 ml) in the same reaction vessel, but with the two forms physically separated from one another by placing one form inside a cellulose Soxhlet extraction thimble (22 × 80 mm); isolation of products was performed as described above.

Anhydrous α-lactose α_M, or α_S that had been treated with methanol at reflux for 2 hr to incorporate methanol, was mixed with an equal weight of β-lactose. The mixture was stirred at reflux for 3 hr with eight times its weight of 0.014M potassium ethoxide in ethanol. The solid material was removed by filtration and, after being thoroughly washed with ethanol, was dried in vacuum at 65°C for 16 hr. The methanol and ethanol contents of the product were measured by a proton magnetic resonance difference spectra method (Parrish et al., 1979b).

RESULTS & DISCUSSION

WE REPORTED previously (Parrish et al., 1979a) that, while α-lactose monohydrate is converted readily to β-lactose by treatment with methanolic potassium methoxide, the stable anhydrous crystalline forms of α-lactose, α_S (Sharp, 1943), and α_M (Tanret, 1896; Lim and Nickerson, 1973) were unchanged by this treatment (Table 1). This unexpected result led us to consider the possibility that the presence of water (derived from the water of crystallization of the monohydrate) in the methanol was a critical requirement for transformation to β-lactose. However, treatment of either of the anhydrous forms of α-lactose with potassium methoxide in methanol containing up to 1.5% water failed to produce β-lactose, thereby eliminating from consideration a role for water in this transformation. In addition, we examined the reaction of the compounds containing the anomeric forms of anhydrous lactose in the ratio α:β = 4:1 (Olano et al., 1977) and α:β = 5:3 (Hockett and

Table 1—Anomeric composition[a] of lactose samples before and after treatment with potassium methoxide solution at reflux for 3 hr

	Treatment			
	None		Potassium methoxide	
Sample	α-Lactose	β-Lactose	α-Lactose	β-Lactose
α-Lactose monohydrate	100	0	5.0	95.0
Anhydrous α-lactose, α_S	98.4	1.6	97.8	2.2
Anhydrous α-lactose, α_M	98.8	1.2	98.1	1.9
Compound α:β = 4:1	81.6	18.4	3.4	96.6
Compound α:β = 5:3	62.2	37.8	5.0	95.0
Amorphous lactose	55.5	44.5	4.5	95.5
Anhydrous lactose, α_H	91.5	8.5	4.5	95.5

[a] Determined by gas-liquid chromatography of trimethylsilyl ether derivatives

Hudson, 1931) with potassium methoxide in anhydrous methanol (Parrish et al., 1979a) and found that both of these molecular compounds were converted to β-lactose in the absence of water.

Observing that amorphous lactose (containing 2.5% or 5.0% water) (Roetman and van Schaik, 1975) was converted to crystalline β-lactose on treatment with methanolic potassium methoxide (Table 1), we focused our attention on β-lactose addition to effect the transformation of the stable anhydrous forms of α-lactose to β-lactose. At reflux for 3 hr in 0.014M potassium methoxide in methanol, addition of crystalline β-lactose to either of the stable anhydrous forms of α-lactose led to the formation of an additional amount of β-lactose, the increase depending on the initial amounts of the two lactose anomers in the mixture (Table 2).

We then examined the effect of physical separation of either form of stable anhydrous α-lactose from β-lactose under the conditions of 3-hr reflux in 0.014M potassium methoxide in methanol. Separation of equal weights of the two forms of lactose was achieved by use of a cellulose Soxhlet extraction thimble (22 × 80 mm); each component was weighed accurately. Each form of lactose was recovered unchanged in anomeric composition in 86–93% yield. Identical results were obtained irrespective of which form of lactose was placed inside the Soxhlet thimble. Conversion to β-lactose occurred when either form of stable anhydrous α-lactose together with β-lactose was placed inside the Soxhlet thimble and treated with potassium methoxide solution; this showed that the effect of the Soxhlet thimble in preventing transformation of the separated compounds to β-lactose is entirely due to physical separation of the components.

Another form of anhydrous lactose (Herrington, 1948), which we have designated as α_H to indicate its hygroscopic nature, has been shown by optical rotation measurements to contain 5–10% β-lactose (Buma and Wiegers, 1967). We have found that the β-lactose content of α_H prepared on different occasions can vary from 1–25%, although samples prepared in triplicate at any given time were identical. In our initial experiments we were unable to convert α_H to β-lactose with potassium methoxide (Parrish et al., 1979a), and in experiments with alcoholic sodium hydroxide solution Olano (1978) was also unable to transform completely α_H to β-lactose. In our present work we found that when α_H was prepared and treated immediately with alcoholic

Table 2—Effect of amount of added β-lactose on transformation of α-lactose to β-lactose in methanolic potassium methoxide at reflux for 3 hr

% β-Lactose[a]	
Initial	Final
2.0	9.1
5.0	25.2
10.2	39.1
20.2	51.5
27.1	68.2
34.0	95.9
42.2	96.5
48.6	95.9

[a] Determined by gas-liquid chromatography of trimethylsilyl ether derivatives.

potassium methoxide or sodium hydroxide, conversion to β-lactose was complete (Table 1). Possibly when α_H was not processed immediately it was converted during the observed period of rapid weight increase at ambient relative humidity to a form like α_S which is not convertible to β-lactose with potassium methoxide.

The conversion of α_M or α_S to β-lactose through the influence of added crystalline β-lactose can be effected by addition of α-lactose monohydrate or amorphous lactose instead of crystalline β-lactose. This is because both of these forms of lactose with potassium emthoxide produced crystalline β-lactose. Again there was the requirement that the added α-lactose monohydrate or amorphous lactose comprise at least one-third or one-half, respectively, of the mixture with the stable anhydrous forms of α-lactose in order that conversion to crystalline β-lactose be completed under the specified reaction conditions.

These observations that mixtures of α-lactose monohydrate, amorphous lactose, or β-lactose with stable anhydrous forms of α-lactose were converted to β-lactose with potassium methoxide solution precluded the use of this method for analysis of mixtures of different forms of lactose, e.g., determination of α-lactose monohydrate in the presence of other crystalline forms. This analytical approach appeared possible from the previous results of the behavior of the individual forms of lactose (Parrish et al., 1979a) before the interaction effects described above were observed.

Attempted conversions of the stable anhydrous forms of α-lactose, α_M or α_S, to β-lactose by reaction with potassium methoxide solution at 27°C in the presence of β-lactose or α-lactose monohydrate were much slower than reactions at reflux. This is shown for reactions of α_M or α_S with different levels of added β-lactose for 16 hr at 27°C (Table 3). It

Table 3—Effect of addition of crystalline β-lactose to α_M or α_S on extent of β-lactose formation at 27°C in 0.014M potassium methoxide in methanol

	% β-Lactose[a]		
		Final	
Time (hr)	Initial	With α_M	With α_S
140	0.4	0.6	0.5
16	23.9	37.7	37.4
3	48.4	50.0	49.6
6	48.4	59.4	56.7
16	48.4	75.5	70.6
24	48.4	83.8	77.4
48	48.4	95.9	96.0
16	78.3	96.1	96.0

[a] Determined by gas-liquid chromatography of trimethylsilyl ether derivatives

Table 4—Proton magnetic resonance analysis of retention of methanol after treatment of α_M or methanol-treated α_s with β-lactose in 0.014M potassium ethoxide in ethanol

Sample	Methanol content in wt % Before treatment	After treatment	Retention % of methanol
α_M	0.79	0.78	99
α_s (methanol-treated)	0.14	0.14	100
β	0	0	–
α_M + β	0.39	0.34	87
α_s (methanol-treated) + β	0.07	0.06	86

is of interest that α_H was converted to β-lactose at 27°C. Recovery of products from reactions for up to 24 hr at 27°C were 97–100% compared to 86–93% for reactions for 3 hr at reflux.

All products were examined by polarimetry (Buma and van der Veen, 1974) as well as by gas-liquid chromatography (Sweeley et al., 1963). This was done to compare the anomeric composition data from the two methods, the correlation coefficient being 0.99. Purity (as % total anhydrous lactose) of β-lactose samples prepared by potassium methoxide treatment was >99% found by use of accurate equilibrium optical rotation data of Buma and van der Veen (1974). No carbohydrates other than lactose were detected by gas-liquid chromatography in any of the solid products of potassium methoxide treatment of lactose samples, although small amounts of galactose, lactulose, and saccharinic acids in the presence of much larger quantities of lactose were found in the filtrates from the reaction mixtures. The extent of incorporation of potassium into the solid products did not exceed 0.001g atoms/mole lactose as determined by atomic absorption spectroscopy. All products after potassium methoxide treatment were shown to be anhydrous by automated Karl Fischer titration. The lactose samples did retain methanol (0.17–0.80%) as the only detected impurity, the level of impurity being measurable also by DSC (Parrish et al., 1979b).

Olano and Rios (1978) explained the formation of β-lactose from α-lactose monohydrate in methanolic sodium hydroxide solution in terms of a three step process: dissolution of α-lactose monohydrate, rapid mutarotation in solution of α-lactose to an equilibrium anomeric mixture at a rate faster than the rate of crystallization of anhydrous α-lactose, and crystallization of β-lactose after its concentration reaches the saturation value. This interpretation does not explain why α_M and α_s, which we find to have solubilities in methanolic sodium hydroxide or potassium methoxide comparable to that of α-lactose monohydrate, were not converted to β-lactose (Olano, 1978; Parrish et al., 1979a). In addition, the theory involving dissolution of starting materials followed by crystallization of β-lactose cannot explain the lack of formation of β-lactose when either α_M or α_s was separated from β-lactose during the reaction in a Soxhlet thimble. The extent of formation of β-lactose in mixtures of α_M or α_s with β-lactose under our reaction conditions depended on the amount of added β-lactose (Table 2).

These facts lead us to suggest the possibility that the transformation of α_M or α_s to β-lactose in the presence of crystalline β-lactose is a solid-solid reaction. The formation of β-lactose by treatment of amorphous lactose with potassium methoxide may involve the initial formation of a mixture of crystalline α- and β-lactose prior to complete conversion to β-lactose: this crystallization process would be analogous to that observed when amorphous lactose was treated with methanol (Ross, 1978). The same explanation can be advanced for the conversion of the two different anomeric compounds of lactose (Hockett and Hudson, 1931; Olano et al., 1977) to β-lactose (Table 1). In addition, we suggest that the formation of β-lactose from α-lactose monohydrate or α_H by treatment with potassium methoxide is also a solid-solid transformation wherein the expanded crystal form of anhydrous α-lactose (Berlin et al., 1972) reverts to the dense crystalline β-lactose.

In support of the idea that the transformation of α_M and α_s in the presence of β-lactose occurs without dissolution was the following observation. The β-lactose produced from α_M or methanol-treated α_s by treatment with 0.014M potassium ethoxide in ethanol retained the methanol associated with the α-lactose component as judged by alcohol analysis by a proton magentic resonance difference spectra method (Parrish et al., 1979b) (Table 4). If dissolution of the α-lactose component had preceded crystallization in the β-form, no methanol would have been found in the product. No ethanol was incorporated into the β-lactose product (Table 4).

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GELATINIZATION OF STARCH IN BAKED PRODUCTS

D. R. LINEBACK and E. WONGSRIKASEM

ABSTRACT

Scanning electron photomicrographs of starch isolated from white bread, sugar cookies, pie crust, angel food cake, cake doughnuts and cinnamon rolls revealed that the proportion of folded and deformed granules, an indication of the extent of gelatinization and pasting, varied from relatively few granules in sugar cookies to virtually all granules in angel food cake. Starch isolated from angel food cake had no birefringence and was about 97% gelatinized as measured enzymatically, while that from sugar cookies was approximately 91% birefringent and was 4% gelatinized. Starches from other baked products studied had residual birefringence and percent gelatinization intermediate to the values for angel food cake and sugar cookies. Thus starch in the baked products studied ranged from essentially completely gelatinized to that which had undergone very little change.

INTRODUCTION

STARCH, the major component of flour, plays an important role in the physical properties and quality of baked products. The changes that starch undergoes during the baking process are not clearly understood. Of those changes, gelatinization and pasting are the most important. Studies of gelatinization normally have been limited to dilute aqueous starch suspensions, in which gelatinization is a function only of temperature. However, most baked products are made from limited water systems which also contain ingredients that may react with starch or compete with starch for water. Consequently the gelatinization and pasting of starch during baking is controlled not only by temperature, but also by the availability of water. Due to the complex nature of the system it is difficult to evaluate all factors that influence the final condition of starch in baked products. The nature of starch in the final product is a function of baking temperature and time, water content of the dough, and the effect of other ingredients, such as sugar and shortening. The extent of starch gelatinization plays an important role in the textural quality of freshly baked products and may influence the shelf life of the products.

The function or role of starch in baked products, particularly bread, has been the subject of several investigations (Sandstedt, 1961; Schoch, 1965; Medcalf, 1968; Medcalf and Gilles, 1968). However, very little information is available on the extent of starch gelatinization during baking and in baked products. Watson and Johnson (1965) found that susceptibility of starch to β-amylase after 30 min of baking indicated that the starch had undergone maximum gelatinization at the temperature attained and with the moisture available in the bread. Microscopic examination of starch in bread crumb revealed that the granules retained some identity, although they had lost all birefringence (Sandstedt, 1961). Yasunaga et al. (1968) found that the gelatinization of starch in bread was dependent on the location in the loaf; starch in outer layers of crumb was gelatinized to a greater degree than the starch in the center. Using an amylograph, those investigators found that various degrees of starch gelatinization could be obtained during baking of bread. The main factors controlling the extent of starch gelatinization were baking absorption, temperature, and time. Miller and Trimbo (1965) found that changes in the gelatinization temperature of starch altered the consistency of white cake batters and the quality of the resulting cake. Successful layer cakes were obtained when the sugar/water ratio in the batter permitted a starch gelatinization temperature of about 90°C (Bean et al., 1979).

Derby et al. (1975) used light microscopy to compare starch granule structures from limited water reference systems to those extracted from baked products. Swelling of starch was controlled by temperature and the amount of water available to the starch granules. Water availability was determined by the formula or recipe used and by the presence of ingredients such as proteins, pentosans, and sugars. Starch isolated from Russian teacake cookies sustained practically no loss of birefringence while that isolated from pie crust was more than 50% birefringent, indicating little or no change in the starch.

Hoseney et al. (1977) used scanning electron microscopy (SEM) to relate the appearance of starch granules extracted from a variety of commercial baked products to the appearance of granules isolated from three reference series. The appearance of the starch granules extracted from different baked products differed greatly. The extent of gelatinization and pasting, and the subsequent proportion of folded or collapsed granules, varied from relatively little in pie crust and sugar cookies to nearly 100% in angel food cake. Temperature was a major factor responsible for such effects, but sugar and shortening contents and, hence, water availability, also exerted observable influences.

The present study was initiated to obtain more definitive measurements on the extent of gelatinization of starch extracted from commercial baked foods common to U.S. diets.

EXPERIMENTAL

Materials

White bread, sugar cookies, apple pie, angel food cake, cake doughnuts, and cinnamon rolls were purchased from local retail stores. *Rhizopus delemar* glucoamylase (Sumyzyme 3000) was obtained from Shin Nihon Chemical Co., Anjyo Aichi-Prefecture, Japan. Gelatinized starch was prepared in the manner described by Shetty et al. (1974).

Isolation of starch

Representative portions of each baked product were obtained in the manner described by Derby et al. (1975) and Hoseney et al. (1977). For white bread, crumb cut at a distance of at least 0.25 inch from the crust was used; for sugar cookies, cake doughnuts, and cinnamon rolls, the entire product was used; for angel food cake, a center slice with the outer surfaces removed was used; and for pie crust, the crust was separated from the pie filling. Each portion was broken into pieces, blended in a Waring Blendor for a few seconds, and air dried at room temperature.

Starch was isolated in the manner described by Hoseney et al. (1977) except that the aqueous slurry of baked product was mag-

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netically stirred for 1–2 hr before filtering through 120-um Nitex bolting cloth.

Scanning electron microscopy

Dried, isolated starch was sprinkled onto double-sided adhesive tape affixed to specimen stubs. The samples were coated with approximately 500Å of gold in vacuo and were then viewed with an ISI-60 (International Scientific Instruments, Inc., Santa Clara, Calif.) scanning electron microscope operating at 15 kv accelerating voltage. Images were photographed, with the specimen tilted 45°, on Polaroid film, type 52, or on 35-mm film.

Estimation of loss of birefringence

Loss of birefringence was estimated by visually counting the number of starch granules which were not birefringent when viewed at 200X under polarized light as a fraction of the total number of granules in the same sample viewed under ordinary light.

Analytical methods

Moisture was determined according to AACC method 44-40 (1969) using a 2-g sample. Glucose was measured using glucose oxidase in the manner described by Shetty et al. (1974).

Determination of gelatinized starch

Gelatinized starch was determined using the procedure described by Shetty et al. (1974). Determinations were made on 100-mg samples of baked products or of dried starch isolated from those products using 160 I.U. of glucoamylase. A standard curve was prepared with a wheat starch mixture containing 0, 5, 10, 25, 50, 75, and 100% gelatinized starch.

Determination of total starch

Total starch content was determined in the manner described by Thivend et al. (1972) using 500-mg samples of baked products, unextracted and extracted with ethanol. Samples were incubated with 5 ml of 10% by weight of commercial glucoamylase at 55°C for 2 hr with shaking. Native wheat starch was included in the analysis as a known reference.

Extraction of samples with ethanol

To remove low-molecular glucosides and dextrins some samples were extracted with hot 80% ethanol (low-molecular weight glucosides) or hot 40% ethanol (low-molecular weight glucosides and dextrins). Samples (approximately 4.0g) of air-dried baked products were weighed into 250-ml centrifuge tubes to which boiling 80% or 40% ethanol were added at a ratio of about 1:40 (w/v). The contents were magnetically stirred for 10 min and then centrifuged for 15 min. The extract was decanted and the process was repeated two to three additional times. The extracted sample was vacuum dried at room temperature. The ethanol had to be thoroughly evaporated since even small amounts have been reported to inhibit glucoamylase (Thivend et al., 1972).

Table 1—Starch content of baked products and yield of extracted starch

Baked product	Starch content (% dry basis)[a]	Yield of extracted starch (% total starch)[b]
Bread	58	54
Sugar cookies	35	26
Angel food cake	20	17
Pie crust	45	8
Cinnamon roll	42	5
Cake doughnuts	35	2

[a] Results are an average of three determinations on samples extracted with 40% ethanol.
[b] Results are an average of at least three isolations.

RESULTS & DISCUSSION

Starch isolation

Representative portions of each baked product studied were obtained in a manner planned to eliminate potential problems in the isolation and analysis of the starch. For white bread, center slices with the crust removed were used. Pigments formed in the crust interfere with the isolation of a clean, white, prime starch fraction. The extent of gelatinization, as measured by an amylograph technique, also increases from the center of the slice to the crust (Yasunaga et al., 1968). The apple filling was removed from the pie crust prior to isolation of the starch to prevent interference from starch in the filling. The exterior surface of angel food cake was removed before isolation of starch since it made the isolated starch brownish in color.

The yield of starch obtained from each sample is shown in Table 1, based on total starch in the baked product. In several of the baked products the yield of isolated starch was disappointingly low. Previous workers (Derby et al., 1975; Hoseney et al., 1977) did not report yields of starch isolated from baked products by the method used in this study. Several reasons may account for the low yields of isolated starch. The major cause probably involves the entrapment of starch in the protein matrix of the baked products. Derby et al. (1975) found it necessary to treat cake and bread baked for 17 min or longer with ficin in order to effect release of starch through digestion of the protein. Sandstedt (1961) observed that starch granules in bread crumb retain some identity but are stretched or elongated and are embedded in a continuous protein phase. Depending on the moisture present and its distribution between starch and protein, it is likely that very strong protein-starch interactions can occur. Conditions of the isolation procedure, such as solids-to-solvent ratio, extraction time, relative centrifugal force, and duration may not be optimum for each sample, hence affecting the yield (Fellers et al., 1969). Prolonged stirring during the extraction procedure may release more starch embedded in the protein matrix, but the probability of contamination of the starch with finely divided baked product increases. Such contaminants are often not easily separated from the starch. This occured with cinnamon rolls in which the cinnamon was removed by discharging the lowest portion of the starch slurry settled in a separatory funnel, contributing to a decreased yield of isolated starch.

Since complete isolation of starch could not be obtained

Table 2—Extent of gelatinization of starch in baked products

	Microscopic methods		Enzymatic method	
	Scanning electron	Light		
	Degree of deformation and folding	% Loss of birefringence[a,c]	% Gelatinized starch[a,b] in	
Baked product			Isolated starch	Unextracted sample
Angel food cake	very high	100	97 ± 4	119 ± 9
White bread	high	100	96 ± 2	98 ± 3
Cake doughnuts	intermediate	98	93 ± 2	84 ± 24[c]
Cinnamon roll	intermediate	98	75 ± 5	65 ± 9[c]
Pie crust	low	50	9 ± 0.4	49 ± 6
Sugar cookies	low	9	4 ± 0.4	21 ± 6

[a] Values are given as percent of total starch.
[b] Results are an average of three determinations.
[c] Results are an average of two determinations.

from the baked products, it was assumed that the isolated starch was representative of the starch in the product. Such assumptions appear to be inherent in the work of Derby et al. (1975) and Hoseney et al. (1977). Results obtained from studies on the isolated starches were thus extrapolated to the baked product. Such an assumption can be criticized since very low starch yields were obtained from several samples. Until such time as the more difficult task of determining the extent of gelatinization of the starch remaining in the extracted residue is accomplished, the assumption currently used is the only practical alternative.

Morphology and gelatinization of starches

The deformation and folding of starch granules is a relative measure of the extent of gelatinization. Starch granules isolated from most baked products retain a high degree of structural integrity since water is limited and pasting may not have proceeded as far as in a dilute aqueous system (Derby et al., 1975; Hoseney et al., 1977). Gelatinization and pasting appear to proceed through stages of granule morphology similar to those observed in scanning electron photomicrographs of lima bean starch (Rockland et al., 1977) as: (a) swollen; (b) dimpled; (c) doughnut-like; (d) rubber-raft shaped; (e) pancake; and (f) dispersed.

Gelatinization was also estimated by measuring the loss of birefringence in the isolated starches. Loss of birefringence occurs during the initial stages of the gelatinization and pasting sequence; most wheat starches completely lose birefringence before 65°C which is before maximum swelling or viscosity has been achieved. Therefore, this simple rapid measurement is limited to the initial events in gelatinization and pasting and cannot measure further extents of pasting which occur as heating is continued and are dependent on the amount of water available.

The enzymatic measurement of gelatinized starch should give a more realistic measure of the extent of gelatinization and pasting than loss of birefringence. In this case complete gelatinization is defined as complete susceptibility of the starch to the action of fungal glucoamylase (Shetty et al., 1974; Goering et al., 1974). This measurement reflects a later stage in gelatinization and pasting than does loss of birefringence. Goering et al. (1974) compared the loss of birefringence and susceptibility to glucoamylase for several starches. At 65°C, wheat starch was not birefringent and was 70% susceptible to the action of glucoamylase. When wheat starch is gelatinized in excess water, a 2% aqueous suspension in this case, all granules appeared to have lost their inner contents, and were extremely folded (Fig. 1). Aggregation of the granules was clearly evident. Such granules had lost all birefringence and were completely (99.2%) digested by the glucoamylase. This starch sample was the gelatinized starch used as a standard.

The morphologies of the starch granules isolated from baked products in this study were very similar to those reported by Hoseney et al. (1977). The starch isolated from angel food cake was extremely deformed and folded (Fig. 2). Most granules appeared to have lost all their contents, i.e. their fullness, and were aggregated similar to those in scanning electron photomicrographs of starch gelatinized in excess water. The starch had completely lost all birefringence and was 97% gelatinized as measured enzymatically (Table 2).

Starch isolated from white bread had collapsed granules, but folding and deformation were not complete, indicating that pasting was not complete before water became limiting. The starch had lost all birefringence and was 96% susceptible to the action of glucoamylase (Table 2).

Scanning electron photomicrographs of starch isolated from cinnamon rolls and from cake doughnuts showed an intermediate degree of deformation and folding among the baked products studied. Most granules had collapsed and

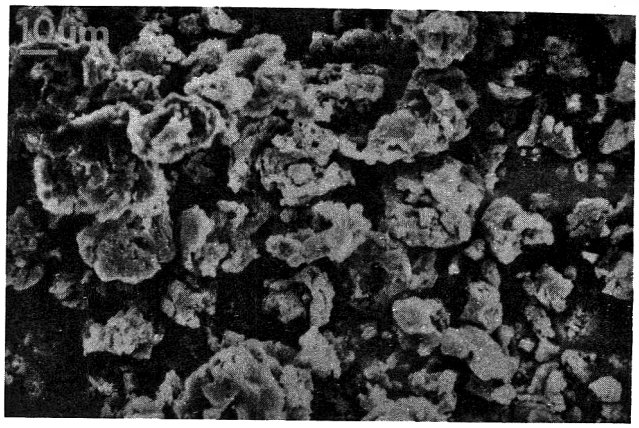

Fig. 1—Scanning electron photomicrograph of wheat starch isolated from a 2% aqueous solution autoclaved for 1 hr.

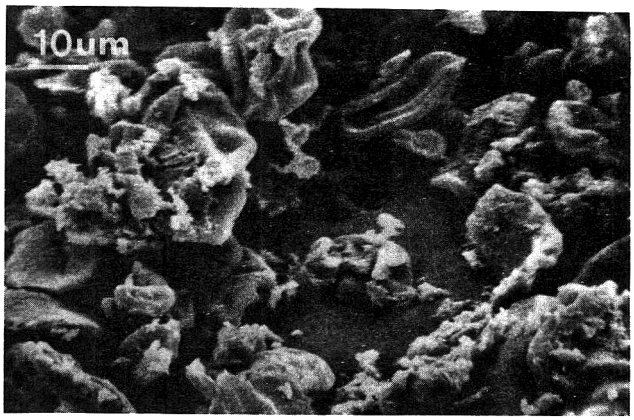

Fig. 2—Scanning electron photomicrograph of starch isolated from angel food cake.

some granules had a pancake-like appearance indicating that starch gelatinization in these products was nearly complete using the descriptive stages proposed by Rockland et al. (1977). Both starches had lost essentially all (98%) birefringence; however, there was a difference in the susceptibility of the starches to glucoamylase. The starch from cake doughnuts was 93% gelatinized while that from cinnamon rolls was 75% gelatinized (Table 2).

Starch isolated from pie crust had a much lower degree of deformation and folding. Most granules appeared to have lost some of their contents with some granules collapsed and doughnut-like while some granules had become flattened (pancake appearance). The starch was 50% birefringent, as also observed by Derby et al. (1975). The starch was only 9% gelatinized as measured enzymatically (Table 2).

Starch isolated from sugar cookies contained a majority of granules which were "full" and showed little deformation (Fig. 3). Some granules appeared to be swollen while others were beginning to have a dimpled appearance (not visible in Fig. 3). Packing marks were still visible on several of the large granules, indicating that the starch had undergone very little change during the baking process. This was further confirmed when the starch was observed to have lost only 9% of the birefringence and to be only 4% gelatinized as measured enzymatically.

Direct measurement of the extent of starch gelatinization in baked products resulted in values which differed greatly from those measured on isolated starch as shown in

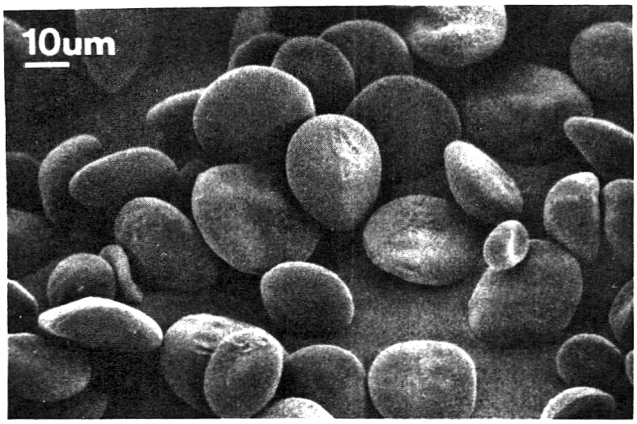

Fig. 3—Scanning electron photomicrograph of starch isolated from sugar cookies.

Table 2. Agreement was obtained only for bread. Differences in the values for the other baked products are attributed to the presence of ingredients which interfere with the determination or yield elevated results (low-molecular weight α-glucosides, dextrins, glucose). Low-molecular weight glucosides can be removed by extraction with hot 80% ethanol while low-molecular weight glucosides and dextrins can be removed by extraction with hot 40% ethanol (Thivend et al., 1972). Unfortunately the amount of gelatinized starch cannot be accurately determined in such extracted samples due to partial gelatinization during the extraction with hot aqueous ethanol.

As previously reported (Hoseney et al., 1978) starch acts as a temperature-triggered water sink in baked products. The extent of starch gelatinization can be related principally to temperature and to the amount of water available to starch granules during the baking process which is also affected by recipe ingredients such as sugar and shortening (Hoseney et al., 1977). In the baked products we studied, the gelatinization of starch varied from relatively little to essentially complete, whether measured as loss of birefringence or by the enzymatic technique. The differences in extent of gelatinization can be attributed primarily to the availability of water to the starch granules as previously reported (Hoseney et al., 1977).

SUMMARY

IN STARCH isolated from baked products, the amount of deformed and folded starch granules as viewed by scanning electron microscopy, the loss of birefringence, and the extent of gelatinization measured as the susceptibility to fungal glucoamylase increased from sugar cookies to angel food cake. Granule deformation and folding reflects the relative extent of gelatinization and pasting of starch, but cannot be easily quantified. While measurement of the loss of birefringence in isolated starch is simple and rapid, it measures only the initial stages of the gelatinization and pasting sequence. The measurement of the susceptibility of starch to fungal glucoamylase gives a more realistic and quantifiable measurement of the gelatinization and pasting of starch, reflecting later events in that sequence.

The extent of starch gelatinization in the baked products studied ranged from 4% in sugar cookies to essentially complete in bread and angel food cake, 96 and 97%, respectively. Loss of birefringence showed the same trend as the enzymatic measurements, although the latter appeared to be a more sensitive measurement of the degree of gelatinization and pasting of starch in baked products. Values measured by the enzymatic method on isolated starch should probably be regarded as minimal values for the extent of gelatinization and pasting.

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NITROGEN ASSIMILATION BY *Salmonella typhimurium* IN A CHEMICALLY DEFINED MINIMAL MEDIUM CONTAINING NITRATE, NITRITE, OR AMMONIA

GREGORY V. PAGE and MYRON SOLBERG

ABSTRACT

A study was conducted to determine whether *Salmonella typhimurium* could assimilate nitrogen from various inorganic nitrogen compounds in a chemically defined minimal medium. The organism was capable of utilizing nitrate, nitrite, and ammonia as sole nitrogen sources, but was unable to utilize hydroxylamine. The generation times calculated for *S. typhimurium* during anaerobic growth with either nitrite or ammonia as the nitrogen source were not statistically different indicating that these two nitrogen compounds were assimilated at comparable rates. Faster generation times were observed when nitrate was provided as the sole nitrogen source while the fastest generation times were observed in anaerobic cultures containing both nitrate and ammonia. *S. typhimurium* utilized $NaNO_2$ as the sole nitrogen source at concentrations as high as 400 μg/ml (5.6 mM) but $NaNO_2$ concentrations of 500 μg/ml (7.0 mM) and above resulted in complete inhibition.

INTRODUCTION

Salmonella typhimurium is capable of reducing nitrate (Le Minor and Rohde, 1974) and recent work has shown that the organism also metabolizes nitrite (Page and Solberg, 1979). *Salmonella* is not a denitrifying organism and has not been reported as capable of assimilating nitrate or nitrite nitrogen. A wide variety of organisms that assimilate nitrogen are capable of growth in a simple salts medium in which ammonia (the reduction product of nitrate and nitrite) provides the sole source of nitrogen. The organisms include *Enterobacter aerogenes, Erwinia carotovora, Pseudomonas fluorescens, Bacillus subtilis, Azotobacter agile,* and *Escherichia coli* (Cole et al., 1974; Meers et al., 1970; Spencer et al., 1957; McNall and Atkinson, 1957). These microorganisms assimilate inorganic nitrogen compounds by forming glutamate from ammonia and α-ketoglutarate (Meers et al., 1970).

It is generally assumed that the biological reduction of nitrate proceeds via an inorganic pathway and that two electron changes are involved for each enzymatic step. The following sequence of intermediates (with the indicated oxidation states of the nitrogen atom) has been postulated for assimilatory nitrate reduction (Doelle, 1975).

$$NO_3 \rightarrow NO_2 \rightarrow [?] \rightarrow NH_2OH \rightarrow NH_4$$

$$+5 \quad +3 \quad +1 \quad -1 \quad -3$$

The +1 oxidation state intermediate has never been identified but some possibilities include the hypothetical nitroxyl (HNO), nitramide (NO_2NH_2) and hyponitrous acid ($H_2N_2O_2$) molecules.

This study was designed to investigate the assimilatory nature of the *Salmonella* nitrate reducing system by determining which of the postulated reduction pathway intermediates could be utilized as a nitrogen source in a minimal medium. The intermediates studied include nitrate (as $NaNO_3$), nitrite (as $NaNO_2$), hydroxylamine (as $NH_2OH \cdot HCl$) and ammonia (as $NaNH_4PO_4 \cdot 4\,H_2O$).

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MATERIALS & METHODS

Culture maintenance and inoculum preparation

Cultures of *S. typhimurium* ATCC 13311 were maintained on nutrient agar slants at 3–5°C. Surface scrapings were inoculated into tryptic soy broth and incubated at 37°C for 18–24 hr. One milliliter of this culture was inoculated into 300 ml of base medium (see below) containing 52 mM $NaNH_4PO_4 \cdot 4\,H_2O$, and incubated for 18 hr at 37°C. Washed inoculum was prepared by harvesting a cell pellet from this minimal medium culture (14,000 × G for 10 min), washing the pellet three times with nitrogen-free base medium and resuspending the pellet in base medium to an absorbance ($A_{560\,nm}$) of approximately 0.5

Base medium preparation

The medium used throughout this study is a modification of the medium developed by Vogel and Bonner (1956). A stock solution was prepared containing 1g $MgSO_4 \cdot 7\,H_2O$, 10g citric acid monohydrate and 50g K_2HPO_4 (anhydrous) dissolved in 67 ml distilled water. Sixteen milliliters of this stock solution were diluted with 704 ml distilled water. A separate solution containing 16g dextrose in 80 ml distilled water was also prepared and the two solutions were autoclaved, cooled and combined yielding a nitrogen free base medium with a final pH of 7.1.

Minimal medium preparation (nitrogen source addition)

$NaNO_3$, $NaNO_2$, $NH_2OH \cdot HCl$, and $NaNH_4PO_4 \cdot 4\,H_2O$ were individually added to 300 ml aliquots of base medium at equivalent nitrogen concentrations of 1.24 mM. In addition, each compound was tested with 1.24 mM $NaNH_4PO_4 \cdot 4\,H_2O$ added as a supplementary nitrogen source so that the final nitrogen concentration in the supplement containing cultures was 2.48 mM. All media were at pH 7.1 after addition of the various nitrogen sources. Twenty-milliliter aliquots of this chemically defined minimal medium were dispensed into sterile cuvettes and inoculated with 0.5 ml of washed inoculum. The cuvettes were incubated in a 37°C water bath without agitation. This system was defined as a mixed aerobic/anaerobic system. Separate studies were conducted in which the system was made anaerobic by bubbling nitrogen gas into the minimal medium and overlaying the medium with mineral oil.

Three replicates of each of the eight nitrogen sources and the nonnitrogen containing control were observed in each experimental trial and at least two trials were conducted for both the mixed and anaerobic systems.

Growth measurement

Culture growth was measured at 30 min intervals by observing the change in absorbance at 560 nm using a Coleman Jr. Spectrophotometer (Model 6A). Coleman cuvettes, measuring 25 × 105 nm, were used. Aerobic plate counts (APC) were determined for each culture once stationary phase had been reached. Serial dilutions were made in 0.1% peptone water. Plates were poured with Plate Count Agar (Difco) and colony forming units were counted after incubation for 24 hr at 37°C.

$NaNO_2$ inhibition study

A study was conducted to determine the level at which nitrite becomes inhibitory, under anaerobic conditions, in a minimal medium in which nitrite is the sole nitrogen source. A solution was prepared containing 20 mg $NaNO_2$/ml base medium. Dilutions of

Table 1—Generation time (GT), slope of the exponential growth curve and stationary phase aerobic plate count (APC) for Salmonella typhimurium cultured in a chemically defined minimal medium containing various inorganic nitrogen sources

Nitrogen source	Mixed aerobic anaerobic			Anaerobic		
	Slope ± sd[a]	GT ± sd (min)	APC (X 10^8 CFU/ml)	Slope ± sd	GT ± sd (min)	APC (X 10^8 CFU/ml)
$NaNO_3$	NG[b]	NG	NG	0.193 ± 0.006	93.6 ± 2.9d	2.0
$NaNO_2$	0.167 ± 0.012	108.1 ± 8.0a[d]	1.8	0.166 ± 0.003	108.8 ± 2.0e	1.9
$NH_2OH \cdot HCl$	NG	NG	NG	NG	NG	NG
$NaNH_4PO_4 \cdot 4H_2O$	0.214 ± 0.005	84.4 ± 2.0b	2.2	0.174 ± 0.006	103.8 ± 3.5e	1.9
$NaNH_4PO_4 \cdot 4H_2O + NaNO_3$	0.286 ± 0.005	63.2 ± 2.1c	5.2	0.224 ± 0.007	80.6 ± 2.4f	5.3
$NaNH_4PO_4 \cdot 4H_2O + NaNO_2$	0.207 ± 0.003	87.3 ± 1.3b	5.2	0.177 ± 0.007	102.0 ± 3.8de	5.3
$NaNH_4PO_4 \cdot 4H_2O + NH_2OH$	NM[c]	NM	NM	NM	NM	NM
$NaNH_4PO_4 \cdot 4H_2O + NaNH_4PO_4 \cdot 4H_2O$	0.217 ± 0.004	83.2 ± 2.0b	5.3	0.173 ± 0.003	104.4 ± 1.8e	5.3

[a] sd = standard deviation
[b] NG = no growth
[c] NM = not measured
[d] Values followed by the same letter are not significantly different at $\alpha = 0.01$ level.

this solution were added to 10 ml of base medium to yield $NaNO_2$ concentrations ranging from 50–2000 μg/ml. The medium was reduced with nitrogen gas and the tubes were overlayed with 2 ml of mineral oil. Each tube was inoculated with 0.5 ml of washed inoculum. Cultures were checked regularly for signs of visible growth during a 30-day incubation period at 37°C.

RESULTS

THE SLOPE of the growth response curve representing exponential growth and the generation time (GT) was determined by linear regression analysis. An overall average slope and the standard deviation (sd) was determined by analysis of variance for each nitrogen source. The results are presented in Table 1. A comparison test was conducted on the exponential growth data to determine which nitrogen sources gave rise to statistically different generation times. This would be indicative of the rates at which the various nitrogen sources were assimilated. $NaNO_3$ was not assimilated in the mixed aerobic/anaerobic system but was assimilated in the anaerobic system. NH_2OH was not assimilated in either system. The generation time was shortest when the combination of nitrate and ammonia served as the nitrogen source in both systems. Cell density was approximately doubled when twice as much nitrogen was made available in the form of an ammonium supplementation. Of those nitrogen sources which supported growth, only $NaNO_2$ yielded identical generation times in both systems. Generation times were usually longer in the anaerobic system. Generation time was longest when $NaNO_2$ was the nitrogen source in both systems but in the anaerobic system this generation time was statistically equivalent to nitrogen supplied as $NaNH_4PO_4 \cdot 4H_2O$, $NaNH_4PO_4 \cdot 4H_2O + NaNO_2$, and 2 × $NaNH_4PO_4 \cdot 4H_2O$.

The cultures containing nitrite exhibited a 5-hr adjustment phase in the mixed system and a 2.5-hr adjustment phase in the anaerobic system after normalizing all responses relative to the ammonia containing culture which was assigned a 0 time adjustment phase duration. The cultures containing nitrate were unable to grow under mixed aerobic/anaerobic conditions and exhibited a 3–5 hr adjustment phase in the anaerobic system. The *Salmonella* cultures were unable to utilize hydroxylamine alone but cultures containing hydroxylamine with ammonia supplementation were able to grow after an adjustment phase which exceeded 12 hr but was less than 24 hr. Although no slope or APC data were obtained for this nitrogen source combination, absorbance measurements made after 24 hr of incubation indicated that the culture had grown to a level comparable to that reached in cultures containing 1.24 mM ammonia as the sole nitrogen source.

Anaerobic cultures containing 50 and 100 μg $NaNO_2$/ml (0.7 and 1.4 mM, respectively) showed heavy growth within 24 hr and similar growth was observed in cultures containing 200 μg/ml (2.8 mM) within 32 hr. Cultures containing 300 μg/ml (4.2 mM) showed limited growth after 72 hr and very limited growth was evident in the cultures containing 400 μg/ml (5.6 mM) on the 8th day of incubation. The cultures containing 500–2000 μg/ml (7.0–28 mM) showed no signs of growth up to 30 days.

DISCUSSION

THE ASSIMILATION of nitrate, nitrite and ammonia nitrogen by *S. typhimurium* under the conditions of this study is similar to the results reported by Cole et al. (1974) in which anaerobic cultures of another member of the *Enterobacteriaceae* family, *E. coli*, maintained steady state growth with either nitrite or ammonia as the sole nitrogen source. These workers also report that the total bacterial yields from equivalent concentrations of nitrite and ammonia were identical, which is in agreement with the data presented in Table 1 for *Salmonella*.

Attempts to demonstrate the growth of *E. coli* with nitrate as the sole nitrogen source have yielded conflicting results possibly due to variations in media components, culture conditions and strains used but there is little doubt that some strains assimilate nitrate in media supplemented with small quantities of organic nitrogen compounds (McNall and Atkinson, 1956; Zarowny and Sanwal, 1963; Fujita and Sato, 1966). This study shows that *S. typhimurium* is capable of utilizing nitrate as a sole nitrogen source only under anaerobic conditions, but if the medium is supplemented with ammonia the organism will utilize both the ammonia and the nitrate when inoculated into a mixed aerobic/anaerobic system.

The statistical comparisons of the slope data show that under mixed aerobic/anaerobic conditions virtually all of the nitrogen sources are utilized at different rates. This variability may be a reflection of the fact that the mixed system is not a well defined system in terms of oxygen concentration and availability. Under the much more well defined anaerobic conditions the comparison tests show that NH_4, NO_2, $NH_4 + NH_4$ and $NH_4 + NO_2$ are all utilized at similar rates which indicates that nitrite is utilized as efficiently as ammonia by the cells.

Increasing concentrations of $NaNO_2$ resulted in greater growth inhibition and ultimately total inhibition of *S. typhimurium* under anaerobic conditions. The minimal medium is well buffered and remained at pH 7.1 as $NaNO_2$ concentration increased. Therefore, the observed inhibitory effect could not be accounted for by pH dependent dissoci-

—*Continued on page 83*

EXAMINATION OF THE CRYSTALLINITY OF FOOD GELS BY X-RAY DIFFRACTION

JOYCE LYNN JOHNSON, G. CURTIS BUSK and THEODORE P. LABUZA

ABSTRACT

Various concentrations of agar, carrageenan, and gelatin gels made by adsorption and desorption processes were examined for evidence of crystallinity by x-ray diffraction methods in an attempt to correlate this with a measure of water binding. As an approach to quantify the degree of crystallinity, the diffraction photographs were also measured for the amount of adsorbed light using a general purpose densitometer, and for the amount of reflected light using a computerized digitizer and false coloring program. Only an amorphous phase was found in the unstressed desorption gels, indicating that the hypothesis of a high degree of oriented crystalline regions forming a capillary-like matrix is not representative of the true nature of a dilute gel and thus water binding can be based on water-water and water-solute interactions alone. Adsorption gels of carrageenan did show a higher degree of crystallinity in the intermediate water activity range. It is suggested that the thermal and mechanical stress during preparation forced the helices to orient into a capillary network. Densitometer scans were unsuccessful in measuring the degree of order in the gel systems as compared to visual observation of the film.

INTRODUCTION

WATER, a major component of all food, comprising 75–95% of many food products, contributes to the physical, mechanical, chemical and organoleptic microbiological properties of food.

Gel-forming macromolecules are well known for their ability to bind or imbibe large quantities of water. The application of food gels for binding water includes use in dessert gels, jellies, fish gels, pet foods and whipped toppings. Functionally, they add to the structural feature of the food system via their properties of gelation, viscosity, suspension and emulsion stabilization. Water-gel interactions allow a considerable variation in final moisture content and thus can strongly influence the final texture of the food itself.

A question remains as to the mechanism by which water is held in a gel formed by a macromolecule. One hypothesis is that water is bound in gels by entrapment in a three-dimensional network of channels and pores set up by the crystalline network of the gel (Dransfeld et al., 1962; Labuza, 1975). This could be enhanced by the hydrogen bond interactions between the polar sites of the macromolecule and water as well as water-water hydrogen bonds. It has been suggested in fact that water molecules can exist in these pores as polarized bound layers covering the polymer surface to a thickness greater than 0.1μ (Ling, 1965, 1972; Derjaguin, 1970). On the other hand, the water entrapped by the macromolecular three-dimensional arrangement has been proposed to have properties similar to that of pure water (Clifford and Child, 1971; Child and Pryce, 1972; Child et al., 1970). Thus the water could exist as a free solvent entrapped in a mixed amorphous crystalline network of the macromolecule.

Several approaches have been used to investigate the perplexing questions concerning the relationship between solute and solvent in gel systems. The properties of the water – both free and bound – have been measured by the following methods: amount of unfreezable water (Kuprianoff, 1958), differential thermal analysis (Duckworth, 1971; Parducci and Duckworth, 1972), water activity (van den Berg et al., 1975; Labuza, 1974; 1975), nuclear magnetic resonance (Clifford and Child, 1971; Child and Pryce, 1972; Belton et al., 1972, 1973), vapor pressure lowering effect (Labuza, 1974), suction potential (Lewicki et al., 1978a, b) and predictions based on theories developed by Flory (1942), Huggins (1942a, b) and Busk (1978). Thus there still remains several questions concerning the mechanism of the measurement of water binding in gels. A technique which was originally used for structural determination of biological macromolecules but more recently for water-solute relationships in crystals is x-ray diffraction. Since the early work of investigators such as Sponsler and Dore (1926), Ferry (1948), Cochran and coworkers (1952), Rich and Crick (1955; 1961), and Fuller and coworkers (1967), the application of x-ray diffraction techniques to determine the structure of macromolecules has become a standard research technique. A considerable amount of x-ray research has been conducted on gels, polymers, and helical nucleic acids (Anderson et al., 1965; 1968; Arnott et al., 1974; Harrington and von Hippel, 1961; Ramachandran, 1967; Ramachandran and Kartha, 1955; Rees, 1969a, b; Rees et al., 1969). To identify the structural and functional component of the complex macromolecules, it was necessary in past research to treat the macromolecule in such a way that the straight chains or helical components would be ordered and therefore could produce in-phase diffraction of the x-ray beam, giving adsorption features peculiar to complex helical structures of large molecular weight.

The purpose of the present investigation was twofold. First, an x-ray diffraction technique was applied to examine (as a function of water activity) the extent of crystallinity in gels of some common high water binding food gelling agents which have not been altered, such as by stretching, so as to be able to determine how much ordering occurs. In past research by Lewicki et al. (1978a) in measuring water binding by suction pressure it was found that the gels exhibited a region of low solids concentration in which water binding increased slowly, presumably due to lack of a three-dimensional structure and thus the macromolecule was in true solution. Above this concentration, water binding increased exponentially with concentration, presumably due to formation of a three-dimensional capillary structure with increased crystallization points in the gel with little effect on water properties. Finally, at higher concentrations the gels showed only a small increase in water binding with increased solids content, due probably to no increase in net crystalline junction points. Labuza and Busk (1979) however, interpreted the same data using the Flory-Huggins

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theory to show that there was no need to assume a high degree of junction zones to propose water binding. They felt that the water binding could be based solely on ionic, hydrophillic and hydrophobic interactions between the water and the macromolecule. Thus their theory would be supported if x-ray analysis shows a very low degree of crystallinity. The second purpose of the research was an attempt to devise a crystallinity index for the gels employing the use of both a manually operated and computerized densitometer for scanning the x-ray picture results.

MATERIALS & METHODS

Desorption direct mix process

The desired concentrations of the gel samples were determined from their respective suction potential curves as reported by Lewicki et al. (1978a) to represent the low, intermediate and high crystallinity regions.

Gelatin (Velvoter – 250 Bloom – lots #09552 and 09774; General Gelatin Company) was made to concentrations of approximately 0.5% (weight percent), 10% and 30%. Kappa carrageenan (gelcarin HWG – lot #RE-7691; Marine Colloids, Rockland, ME) was prepared to concentrations of 0.5%, 2.5% and 9.0%. Agar (gum agar agar – lot #61476; Tragacanth Importing Company) was made to concentrations of approximately 0.25%, 2.5% and 9.0% (all weight percents).

Each of the weighed gel samples was slurried in the appropriate amount of cold distilled water while stirring, then heated in a water bath until the temperature of the solution reached 70°C (agar and carrageenan) and 80°C (gelatin) and the solution appeared translucent. Each solution was allowed to cool in a 6°C refrigerator for 24 hr before processing for x-ray diffraction.

Adsorption process

Four percent by weight gels of kappa carrageenan and agar were prepared as previously described for the desorption gels. After the cooling period, the gels were cut into approximately 1/2 cm cubes, then frozen in liquid nitrogen. The frozen cubes were freeze-dried in a Virtis Freeze-Dryer for 24 hr at 21°C and 10^{-2} mm Hg. Samples of each gel were weighed into small glass petri dishes before being placed in desiccators over saturated salt solutions in the 0.52–0.93 a_w range. The desiccators were then evacuated and stored in a 4°C refrigeration unit to equilibrate. Isotherm points were measured by the vapor pressure manometric technique of Karel and Nickerson (1964) as modified by Lewicki et al. (1978a, b). Moisture content was determined by weighing the samples initially, after 1 month, then at 2-wk intervals. A weight difference of less than 0.0003g between successive weighings indicated the systems had reached equilibrium. Equilibration time varied from 7–10 months.

Refrigeration was required during the long equilibration period because the samples at water activities greater than 0.75 were potentially subject to microbial spoilage, especially from mold. At the very high a_w's (greater than 0.91) small vials of toluene were stored in the desiccators to ensure growth inhibition.

X-Ray technique

A 0.5 mm diameter glass capillary (Charles Supper Co., Natick, MA) was cut to a length of 3 cm, then inserted into the gel until at least a 0.3 mm length of the capillary was filled. The capillary was shortened to 1.5 cm, then sealed closed on both ends with an epoxy cement which had thickened and almost set.

Diffraction scattering from water and air was measured by preparing two blank capillaries: one with air sealed inside and the other with distilled water.

The adsorption gels were cut with a razor in a mortar to as fine a powder-like consistency as possible, then packed into a 1.0 mm diameter glass capillary to a depth of approximately 5 mm. Care was taken to pack the fibers so air pockets were avoided. The capillary was shortened to 1.5 cm followed by heat sealing by passage through a flame.

Each capillary was mounted on a GE-XRD5 x-ray unit using a Weissenberg camera (Charles Supper Co., Natick, MA) and exposed to CuKα radiation while rotating 360° for 1 hr. Duplicate samples of each were photographed.

Densitometer

Chemical. The developed pictures of both the adsorption and desorption gels were scanned with a Beckman Model R-112 Densitometer with integrator. 600 nm wavelength of light through the narrow slit was found to give maximum light absorption and peak sensitivity. The pictures were scanned through the center of the concentric halos at the narrowest diameter. Peak baseline was zeroed against an exposed portion of the background film outside of the gel picture. The resulting graphs were examined for variation in peak heights and contours as a function of gel concentration and crystallinity.

Computer. Representative films of an adsorption gel and desorption gel were digitized by a Dicomed Digitizer and Film Recorder (Special Interactive Computations Laboratory, Univ. of Minnesota, Minneapolis, MN) which in turn is run by a PDP 11-40 mini-computer (DEC). The digitized pictures were processed into color by a "false-coloring" program which projects the image on a television screen with colored filters and later projects the image back onto standard Polaroid fast processing film. The object of this technique is to numerically and pictorially differentiate the more dense por-

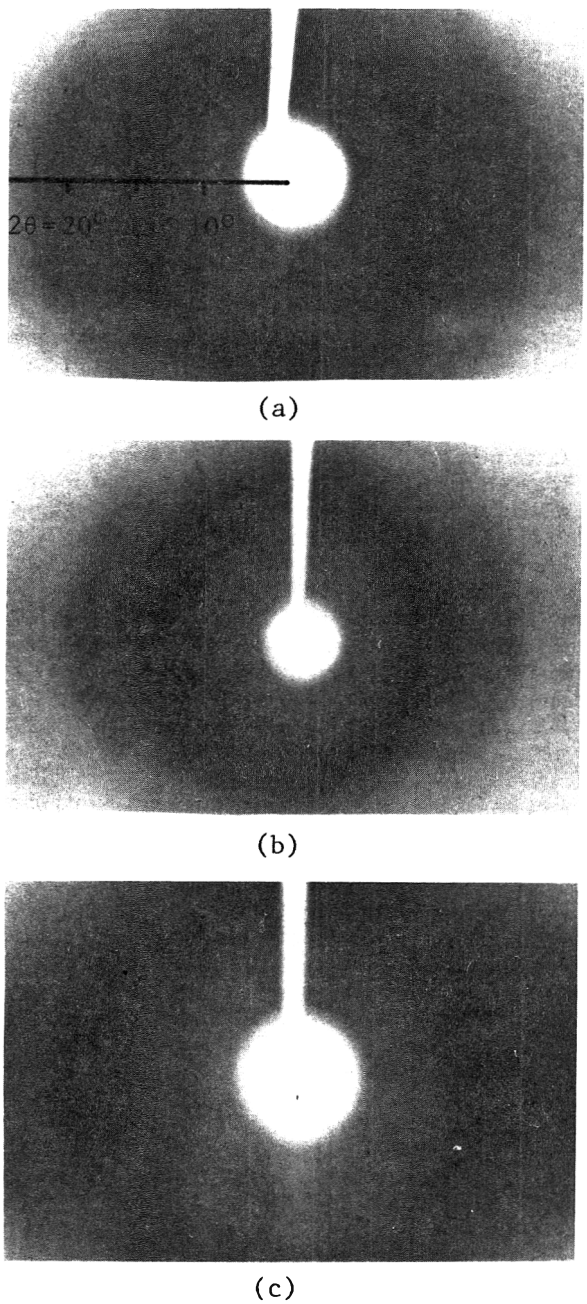

Fig. 1—Diffraction pictures of carrageenan desorption gels: (a) 0.5% solids; (b) 2.5% solids; (c) 9% solids.

tions of the gel's halos and rings (if present) as a function of gel concentration and crystallinity. This technique has the advantages of increased sensitivity to color variation since it "reads" reflected light as opposed to absorbed light used in the chemical densitometer and better reproducibility of results since it scans the entire film as opposed to passing light through a small section of the picture.

RESULTS & DISCUSSION

FIGURE 1 shows the x-ray diffraction pattern of kappa carrageenan desorption gels at different solids concentrations. The large white area in the middle of all the pictures results from the beam stop on the x-ray unit. Without this the center of each film would appear over-exposed as a result of those x-rays passing straight through the sample. It is the changes in the intensity of the darker regions that would be indicative of changes in gel-water interactions. Figure 2 shows the diffraction picture for water. As seen, the halo is at 14° which is the value associated for water (Danford and Levy, 1962). The water halo appears at the same location of 14° in all the gel pictures of Figure 1, but the intensity is much greater and the band is broader. This could be due to enhanced water structuring, but since there are no sharp concentric rings or distinct point in Figure 1, it can be concluded that there is little if any crystallinity or high concentrations of helix junctions at any of the three gel concentrations. This correlates well with the fact that the Flory-Huggins interaction parameter χ determined by Lewicki et al. (1978a) decreased over this concentration range, indicating that the gel behaved as a solution of increasing solute-solvent interaction with enhanced water binding through hydrophillic hydrogen bonds. One would expect sharp concentric ring formation if there were a highly organized crystalline structure in the dilute gel.

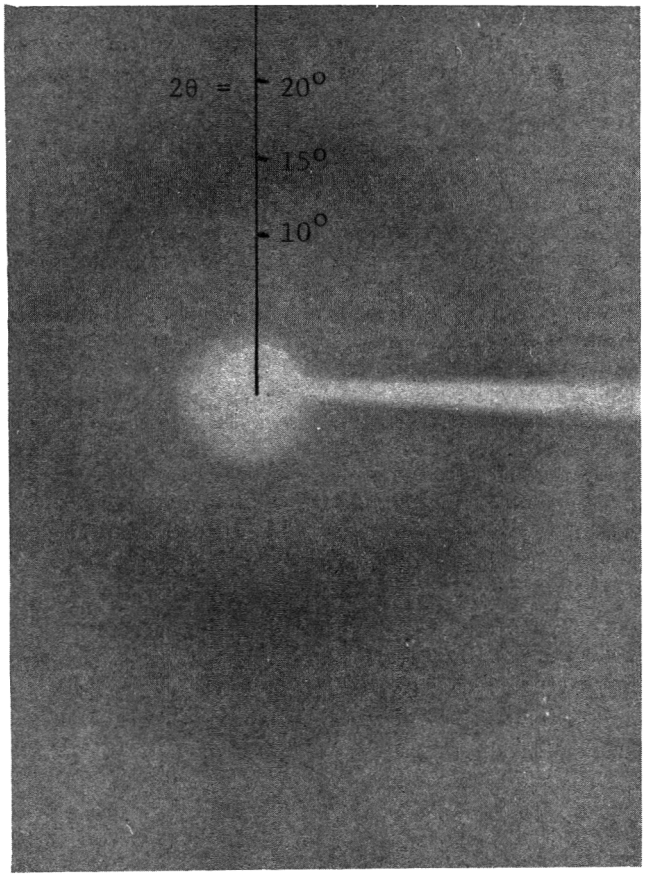

Fig. 2—Diffraction picture of distilled water in a capillary.

With respect to gelatin in Figure 3, as the concentration of gelatin increased, the intensity of the amorphous water halo at 14° appears to increase slightly. Below a concentration of 25% solids for gelatin, the value of the Flory-Huggins interaction parameter χ was constant. Above this concentration χ increased such that water-polymer interactions are decreasing as more energetic polymer-polymer interactions increase (Booth et al., 1957); i.e., less polymer-water interaction. At the same time, however, gelatin induces greater water-water interaction through hydrophobic regions (i.e., more water clusters). This would induce a greater intensity in the water halo. The binding site energy for desorption gels, 823 cal/mole (Lewicki et al., 1978a) indicates that gelatin exhibits a small degree of polymer/water interaction. The spacing of the amorphous halo at all concentrations of gelatin measured 14° which as noted is

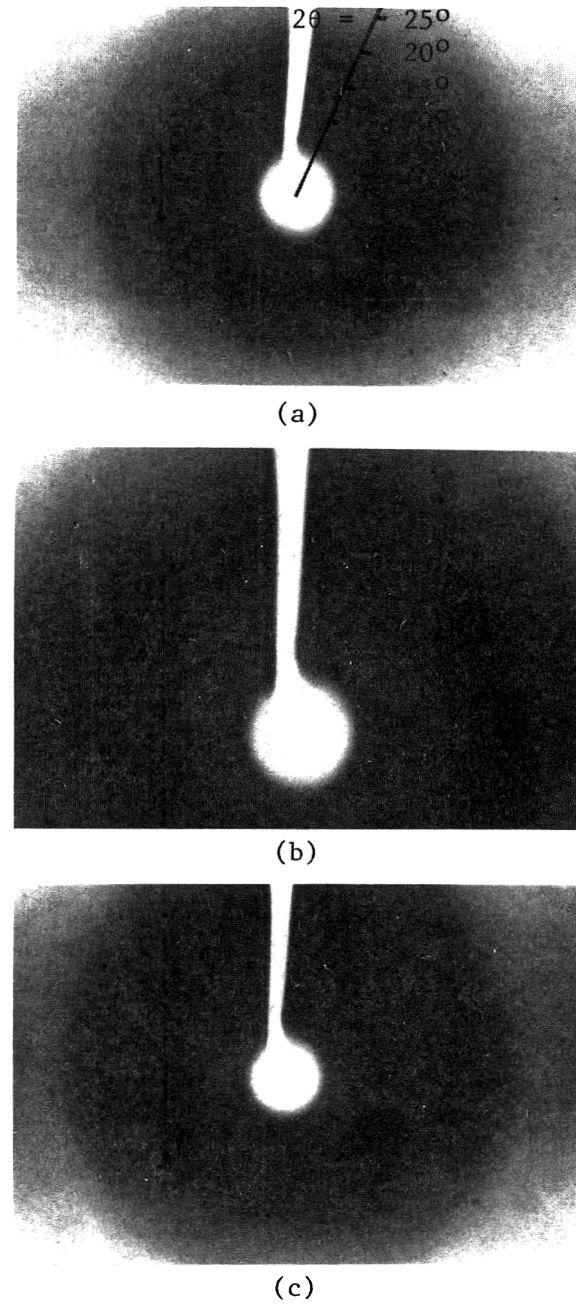

Fig. 3—Diffraction pictures of gelatin desorption gels: (a) 0.5% solids; (b) 10% solids, (c) 30% solids.

associated with the diffraction value of water and not with the values of layer lines (i.e., distinct sharp halos) for a dehydrated gelatin gel as reported by von Gerngross et al. (1932).

The absence of crystallinity evidenced in the diffraction films for both gelatin and carrageenan confirms the belief that helical zone orientation and interaction in the unstressed gel is not favored although the macromolecule may be in a helix form. Thus, the random orientation of the helices and few junction zones would result in a small ratio of crystalline to amorphous material and, consequently, as found, would be undetectable by the x-ray diffraction technique used in this study.

The diffraction films of agarose in Figure 4 show only slight visual evidence that the intensity or darkness of the amorphous halo at 14° is increasing with concentration.

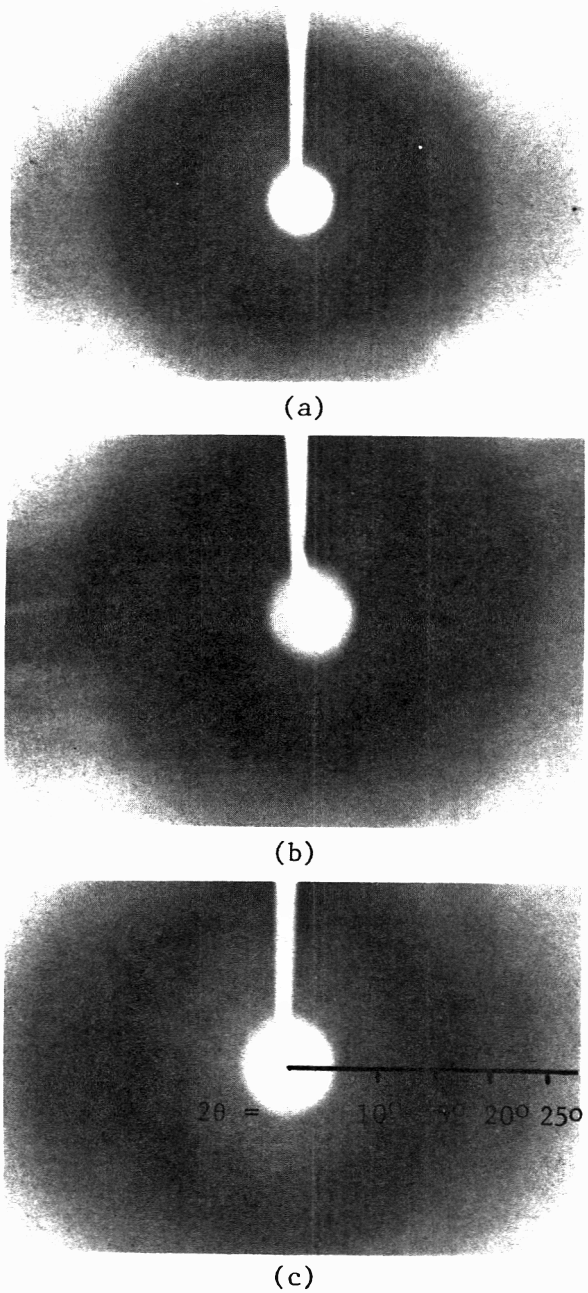

Fig. 4—Diffraction pictures of agar desorption gels: (a) 0.25% solids; (b) 2.5% solids; (c) 9% solids.

This indicates more water structuring since water can bind to three of the four hydroxyl groups pointing outward from the agar internal helix as well as within the helix cavity. As the concentration of the gel increases, more hydroxyl binding sites become available while at the same time some are lost at the point of junction zone formation (Labuza and Busk, 1979). Thus, with increased concentration, the x-ray patterns show only a water effect suggesting, as with the other macromolecules, a lack of high density regions of crystallinity.

The x-ray diffraction patterns of the kappa carrageenan gels prepared by adsorption from the freeze-dried state are shown in Figure 5. At water activities of 0.15–0.56 there is no visual evidence of concentric ring formation and, therefore, no evidence of significant crystallinity as found for the regular gels. The adsorption gels were stressed thermally during the freezing and freeze drying process and mechanically from shrinkage during humidification. The macromolecular network in the gels of a_w 0.15–0.56 may be ordered in clusters as a result of the process conditions, but are not oriented. At low water activities there may not be sufficient water molecules present to promote interaction between macromolecules and neighboring polymer chains. Consequently, the crystalline regions in the amorphous matrix are not large enough to produce sharp, diffracted beams. The amorphous halos in Figure 5 for a_w 0.15–0.56 show little variation in halo width or in apparent darkness or intensity. The more highly oriented the polymers are in the matrix, the more densely packed they would be than the unoriented polymers (Alexander, 1969). Visually, they would be represented by a decrease in the integral breadth of the radial section of the halo. Therefore, there is minimal short or long range order within the gels of a truly crystalline nature, but there would exist a minimal amount of short range organization consisting of most probable distances between neighboring atoms.

At a water activity of 0.66 (Fig. 5e), relatively sharp concentric circles superimposed on a background of diffuse x-ray scatter are present. The interactions between sulfate groups on the polysaccharide and the gel-inducing cations (naturally present in carrageenan) required for the gelation process must be correctly balanced by the increased water content. The numerous circles extend outward with Bragg equation angle values of 20–22°, values greater than the distance to the amorphous water halo. The evidence of crystallinity at 0.66 a_w does suggest that carrageenan forms crystalline junction points. Observation of all six diffraction diagrams of kappa carrageenan indicate that even under the stress of the freezing and freeze-drying processes, regions of ordered crystalline clusters are concentration-dependent and concentration sensitive. These regions were only visible in the intermediate moisture range, disappearing above and below a_w 0.66.

X-ray diffraction of the agar samples in Figure 6 showed no concentric ring formation at any water activity. The amorphous halo at all a_w's occurred at the 2θ of 10° which is in agreement with the reported axial periodicity of 9.5 Å

Table 1—Comparison of water content of adsorption agar and carrageenan gels in the intermediate moisture range

	g H_2O/g dry matter	
a_w	Agar	Carrageenan
0.56	0.30	0.20
0.66	0.34	0.23
0.71	0.37	0.25
0.80	0.43	0.33

(2θ = 10.34) (Arnott et al., 1974) for agar. At the higher water activities of 0.71–0.94 there was slight visual evidence of a second halo area forming near the periphery of the film. This is associated with first and second nearest neighbor effects without specific orientation and may represent primary periodicies of the helicies.

Table 1 presents the moisture contents at equilibrium of selected agar and carrageenan adsorption gels. At an a_w of 0.56 agar already has as much water per gram of dry material as a carrageenan gel at a_w 0.80. Crystallinity was detected in the carrageenan sample with a moisture content of 0.23 grams water per gram dry matter. Agar may have passed its optimal water content for crystallite formation and cluster orientation once its moisture content was 0.30g per gram dry matter (a_w = 0.56) and at a_w of 0.56 was thus influenced by a dilution effect as suggested by the well defined intense halos.

In trying to quantify the ring intensity by spectrometric methods, it was found that results from the light scan of the densitometer could be changed by simply rotating the film under the slit. Consequently, this procedure would be difficult to standardize because of variations in placement of the film between the light and detector, variation in the intensity of the concentric halo, variation in the initial

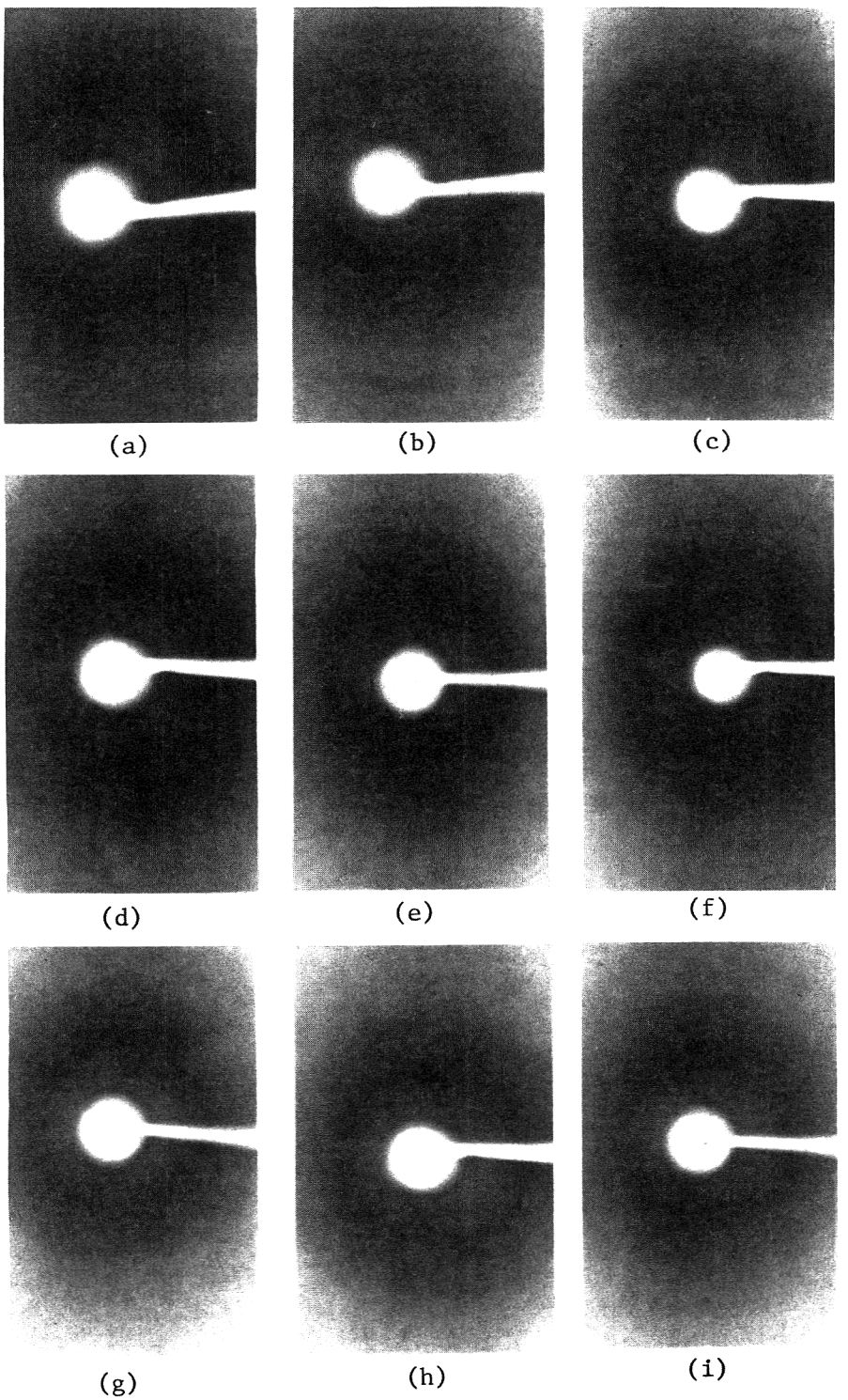

Fig. 5–Diffraction of adsorption gels of kappa carrageenan at different water activities: (a) 0.15; (b) 0.32; (c) 0.42; (d) 0.56; (e) 0.66; (f) 0.71; (g) 0.80; (h) 0.91; (i) 0.93.

packing density of the sample within the capillary and lastly, because of the extreme faintness of crystallite concentric circles. Thus the desitometer method is not adequate. The computerized scan of the kappa carrageenan adsorption gel (a_w = 0.66) film which visually showed specific concentric rings were obvious. The computer false coloring program does have the best potential as a means of quantifying gel crystallinity; however, the program at present cannot adjust for varying degrees of background color and thus this adversely affected the final picture obtained. The major benefit it offers as an index tool is based on the method of scan; i.e., it scans and digitizes the entire image, thus eliminating variation in film placement and results, but it was not quantitative enough in these studies to indicate the ring pattern.

In summary, the application of x-ray diffraction to investigate solvent-solute interactions of an unmodified dilute gel supports the previous work in suggesting that a high degree of crystallinity is not present and therefore is not necessary to form a capillary matrix to hold water (Labuza and Busk, 1979). Crystallite formation is present but is in such a small amount that it is obscured by the patterns from the random orientation of the macromolecules, and the diffuse halo of water itself. In terms of trying to quantify the degree of crystallinity, the use of two scanning techniques did not indicate any benefit over visual observation.

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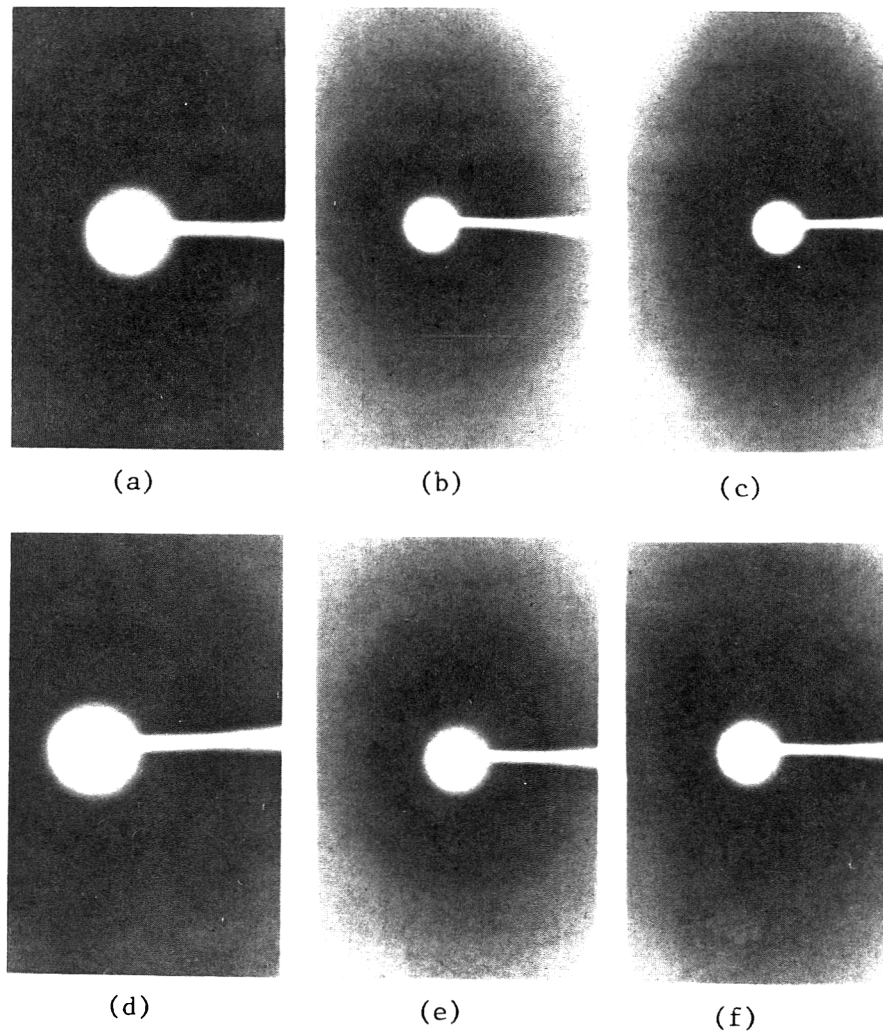

Fig. 6—Diffraction of adsorption gels of agar at different water activities: (a) 0.56; (b) 0.66; (c) 0.71; (d) 0.80; (e) 0.91; (f) 0.93.

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ation related changes. It is possible that a $NaNO_2$ concentration build-up within the cell, resulting from an increased diffusion rate, causes cell death. The shortest generation times were found in cultures containing $NH_4 + NO_3$ under either mixed or anaerobic conditions. The cultures containing NO_3 alone showed shorter generation times than nonnitrate containing cultures, under anaerobic conditions. These shorter generation times may be the result of two separate, possibly independent, enzyme functions, each metabolizing one substrate (either NO_3 or NH_4) such that when functioning together the result is more rapid nitrogen assimilation and more rapid growth. The fact that nitrite and ammonia are utilized at similar rates may indicate involvement of a single enzyme function which gives rise to slower nitrogen assimilation from these two sources and therefore a slower growth. The adjustment phase durations exhibited by the cultures containing NO_2 or NO_3 may indicate the presence of inducible enzymes.

Work is currently under way to isolate, purify and characterize these nitrogen assimilating enzymes.

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BIOAVAILABILITY OF VITAMIN B-6 IN NONFAT DRY MILK AND A FORTIFIED RICE BREAKFAST CEREAL PRODUCT

J. F. GREGORY III

ABSTRACT

The results of microbiological and high performance liquid chromatographic (HPLC) methods for total vitamin B-6 were compared with rat bioassay results for biologically available vitamin B-6 in nonfat dry milk and a rice base breakfast cereal product. Excellent agreement was observed between assay values for total and available vitamin B-6 in the nonfat dry milk, indicating full bioavailability. In contrast, the rat bioassay results indicated that the bioavailability of the vitamin B-6 in the cereal product was low. The factors responsible for the apparently poor absorption or utilization of the vitamin B-6 from the cereal product are not known. The results of this study indicate that the bioavailability of vitamin B-6 may be strongly influenced by food composition.

INTRODUCTION

EVALUATION of the nutritional quality of foods requires accurate data concerning both the total content and bioavailability of the nutrients which are present. Because a direct determination of the biological availability of nutrients is not yet feasible, animal bioassays must be employed to quantitatively determine the biologically available nutrients in a food. Animal bioassay results are compared with the total nutrient content, as determined chemically or microbiologically, to provide an estimation of bioavailability.

Relatively little research has dealt with the bioavailability of the various forms of vitamin B-6 in foods. The bioavailability of vitamin B-6 was first examined by Sarma et al. (1947). Generally good agreement between rat bioassay and microbiological assay results was shown; however, an apparent bioavailability of approximately 70% was reported for liver fractions, whole wheat, and yellow corn. Yen et al. (1976) reported bioassay results which suggested that the biological availability of vitamin B-6 in yellow corn and soybean meal was low, although the bioavailability could not be quantitatively calculated from their data. The rat bioassay results of Toepfer et al. (1963) indicated that the bioavailability of vitamin B-6 in nonfat dry milk and whole wheat flour may be incomplete (70–80% available), while the availability of the vitamin in lean beef and lima beans was found to be high. Lecklem (1977) recently reported the results of a human bioavailability study in which the naturally occurring vitamin B-6 in whole wheat bread was utilized less rapidly than the B-6 in white bread with or without pyridoxine (PN) hydrochloride fortification.

The effects of food processing and storage have been studied to a limited extent. The retorting of milk and infant formula has been reported to induce large losses in the bioavailability of the naturally occurring vitamin B-6 in these products (Tomarelli et al., 1955), although conflicting data have been presented (Davies et al., 1959). Recent research by Gregory and Kirk (1978a, b) indicated that the roasting and storage of low-moisture model food systems does not affect the bioavailability of the remaining vitamin B-6.

Another possible influence on the apparent bioavailability of vitamins is the activity of their degradation products. Analyses of the biological activity of ϵ-pyridoxyllysine, a vitamin B-6 degradation product (Gregory and Kirk, 1977, 1978b), indicate that this compound may metabolically retard the utilization of normally active B-6 vitamers (Gregory and Kirk, 1978c).

The present research was begun as an investigation of the accuracy of a recently developed high performance liquid chromatographic (HPLC) method for the determination of vitamin B-6. The food samples examined, commercial nonfat dry milk and a rice base fortified breakfast cereal, were chosen arbitrarily to provide specimens of widely differing composition and B-6 vitamer content. This paper deals with the results of vitamin B-6 analyses of these products using HPLC, conventional microbiological, and rat bioassay methods.

Table 1—Compsition of diets for rat bioassay determination of biologically available vitamin B-6 in nonfat dry milk and rice base cereal

Component	Basal and standard diets (Percentage by Weight)	Test diets
Casein (vitamin-free test)[a]	19.8	18.8
Sucrose	60.5	56.5
Cellulose (Alphacel)[b]	9.4	9.4
Corn oil	5.0	5.0
Mineral mixture[a,c]	4.0	4.0
Vitamin mixture[b,d]	1.1	1.1
DL-Methionine	0.2	0.2
Nonfat dry milk or powdered cereal	—	5.0

[a] Teklad Test Diets

[b] ICN Pharmaceuticals, Inc.

[c] Wesson modified Osborne-Mendel; $ZnCO_3$ added to provide 15 ppm Zn in all diets

[d] Vitamin mix provided (per kg diet): vitamin A, 9900 units; vitamin D, 1100 units; α-tocopherol, 55 mg; choline chloride, 825 mg; menadione, 25 mg; niacin, 50 mg; riboflavin, 11 mg; calcium pantothenate, 33 mg; thiamin, 0.22 mg; folic acid, 0.99 mg; vitamin B_{12}, 0.015 mg; biotin, 0.22 mg.

EXPERIMENTAL

Materials

Pyridoxal hydrochloride, pyridoxamine dihydrochloride, pyridoxine hydrochloride, and pyridoxal 5'-phosphate were purchased from Sigma Chemical Co. Aspartate aminotransferase assays were performed using commercially available reagents (Calbiochem; UV GOT reagent Stat-Pack). Microbiological vitamin B-6 assays were performed using Pyridoxine Y Medium obtained from Difco Laboratories. All other chemicals used were reagent grade.

Determination of total vitamin B-6

The method of Haskell and Snell (1970) was employed for the microbiological determination of total vitamin B-6 using *Saccharomyces uvarum* ATCC 1080 as the test organism. Each sample was extracted by the method of Toepfer and Polansky (1970). Parallel samples containing 7.5 μg added PN/250 ml were run to permit the correction of the assay results for the recovery of the internal stand-

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ard. Recovery values ranged from 85–100%. Standard curves were prepared using pyridoxine hydrochloride over the range 0–8.0 ng PN free base per assay tube. Each sample extract was assayed using 0.05, 0.10, and 0.15 ml volumes of addition to the assay tubes, each level in triplicate.

The individual B-6 vitamers in the cereal product were quantitatively determined using a recently developed HPLC method (Gregory, 1979a) which was based on the procedure described by Gregory and Kirk (1978b). This technique involves a reverse phase separation of the extracted B-6 vitamers using an octadecylsilica column (μBondapak C_{18}; Waters Associates) and 0.033M potassium phosphate, pH 2.2, for the mobile phase. The HPLC system employed was an Altex Model 312 chromatograph and an Aminco FluoroMonitor detector.

All assay procedures were performed under gold fluorescent lights or in subdued light to minimize photochemical degradation of the B-6 vitamers.

Rat bioassay methods

The concentration of biologically available vitamin B-6 in the nonfat dry milk and cereal samples was determined using the rat bioassay method previously described (Gregory and Kirk, 1978a). In this bioassay, a 14-day depletion period preceded an 18-day assay period. Standard diets (Table 1) were prepared using pyridoxine hydrochloride fortification over the range 0–2.5 μg PN free base per gram. In contrast to previous bioassays in which a dry PN premix was added, the PN fortification was performed by the addition of aqueous pyridoxine hydrochloride solutions of 0–152 mg PN per liter at a level of 40 ml per 2415g of diet. This method of fortification provided easier dispersion of the added PN. Two test diets were separately prepared containing nonfat dry milk or finely ground rice base breakfast cereal at 5% (w/w; Table 1). The replacement of the basal diet ingredients for test diet formulation was performed on the basis of the mean proximate composition of the nonfat dry milk and cereal as indicated on the product label (Table 2). A 40-ml volume of distilled water was added to each test diet (2415 g) to maintain a water content equivalent to that of the standard diets. As in previous bioassays, the diets were fed ad libitum. Coprophagy was not prevented.

The bioassay was evaluated using growth, growth per gram feed consumed (feed efficiency), liver pyridoxal 5′-phosphate (PLP) concentration, erythrocyte aspartate aminotransferase (AspAT) activity, and AspAT stimulation (%) by PLP added in vitro as the response criteria. Blood was collected and hemolysates were prepared as previously described (Gregory and Kirk, 1978a). Immediately after sacrificing the animal and collecting blood, the liver was excised, rapidly cooled on ice, and stored at −20°C until analyzed for PLP. PLP was quantitatively determined as the semicarbazone derivative by an HPLC method (Gregory, 1979b). Erythrocyte AspAT activity was determined by the Calbiochem kinetic method designed for serum AspAT (Calbiochem, 1975). The in vitro stimulation of AspAT by added PLP was determined by assaying the enzyme activity in the presence and absence of 0.8 mM PLP added as an aqueous solution (Bayoumi and Rosalki, 1976). AspAT activity was expressed as mU/mg hemoglobin, where one unit catalyzes the reaction of one micromole of substrate per min at 30°C. The hemoglobin concentration was determined by the cyanomethemoglobin procedure (Crosby et al., 1954).

Standard log dose-response curves were plotted for each response criterion. The content of biologically available vitamin B-6 in the nonfat dry milk and cereal test diets was determined using the various standard log dose-response curves. The apparent available dietary vitamin B-6 value was calculated for each individual rat using the growth, feed efficiency, and liver PLP criteria. Because of the wide within-group variability observed in the AspAT activity and percent stimulation data, group mean values were used to estimate the content of available vitamin B-6 in the test diets. The estimated standard errors of the vitamin B-6 results based on AspAT data were calculated to provide relative standard error values equal to those of the enzyme activity and stimulation data.

Statistical analysis

The bioassay estimates of available vitamin B-6 in the test diets were compared with the total vitamin B-6 results using a completely randomized distribution analysis of variance (ANOVA). As previously discussed (Gregory and Kirk, 1978a), the lack of independence between the growth, liver PLP, and feed efficiency results necessitated the use of a separate ANOVA for testing each of these bioassay response criteria. To achieve a family error rate of $\alpha = 0.05$, the critical probability employed in each of the ANOVAs for each sample was $\alpha/3 = 0.017$. The presence of significant differences between the rat bioassay estimates of available vitamin B-6 using the various response criteria (growth, liver PLP content, or feed efficiency) was determined for each test material by one-way ANOVA. All statistical methods were described by Neter and Wasserman (1974).

RESULTS & DISCUSSION

THE MAIN OBJECTIVE of this research was to provide initial data concerning the correlation between vitamin B-6 values obtained by microbiological, HPLC, and rat bioassay methods of food analysis. The composition of the products which were examined is shown in Table 2. Nonfat dry milk has been reported to contain approximately 3.8 μg total vitamin B-6 per gram, with 73% of the total as pyridoxal (PL), 24% as pyridoxamine (PM), and 3% as PN (Polansky and Toepfer, 1969). The cereal sample was expected to contain vitamin B-6 predominantly in the form of PN be-

Table 2—Composition of nonfat dry milk and rice base breakfast cereal samples (data from package labels)

Component	Percentage by weight	
	NFDM	Cereal
Protein	35.2	7.1
Carbohydrates		
Total	52.9	88.3
Starches and related carbohydrates	–	77.7
Sucrose and other sugars	–	10.6
Fat	0	<4.4

Table 3—Rat bioassay data used for determination of biologically available vitamin B-6 in nonfat dry milk and breakfast cereal samples.[a]

Diet	Growth (g)	Feed efficiency (g growth/g feed)	Liver PLP[b] (μg/g)	Erythrocyte AspAT[c]	
				Activity (mU/mg Hb)	PLP Stimulation (%)
Standard					
0.00 μg PN/g	10 ± 3	0.08 ± 0.01	2.9 ± 0.2	1.11 ± 0.12	102 ± 11
0.25 μg PN/g	34 ± 4	0.27 ± 0.04	3.4 ± 0.3	1.29 ± 0.05	85 ± 13
0.50 μg PN/g	80 ± 5	0.39 ± 0.02	5.1 ± 0.3	1.41 ± 0.07	66 ± 10
1.00 μg PN/g	112 ± 3	0.44 ± 0.01	5.9 ± 0.2	1.42 ± 0.17	68 ± 6
2.50 μg PN/g	125 ± 5	0.45 ± 0.02	10.1 ± 0.4	1.81 ± 0.17	28 ± 6
5 % Test materials					
Nonfat dry milk	70 ± 3	0.33 ± 0.01	3.9 ± 0.4	1.33 ± 0.22	85 ± 8
Cereal	108 ± 11	0.34 ± 0.04	4.0 ± 0.4	1.43 ± 0.18	40 ± 10

[a] Mean and standard error, 7 rats per group. Growth and feed efficiency values are for the 18-day assay period. AspAT parameters and PLP concentrations are those determined at the end of the assay period.

[b] Liver pyridoxal 5′-phosphate concentration

[c] Erythrocyte aspartate aminotransferase

Table 4—Biologically available and total vitamin B-6 in nonfat dry milk and breakfast cereal samples. Data represent μg vitamin B-6 per gram of test material[a,b]

	Test material	
	Nonfat dry milk (μg/g)	Cereal (μg/g)
Biologically available vitamin B-6		
Indicator		
Growth	8.1 ± 0.4A	15.3 ± 1.9A
Feed Efficiency	7.1 ± 0.4A	9.3 ± 1.6B
Liver PLP[c]	5.9 ± 1.7A	6.2 ± 1.9B
AspAT activity[d]	9.0 ± 1.4	15.0 ± 1.8
AspAT stimulation[d]	4.8 ± 0.5	37.2 ± 9.4
Total vitamin B-6		
Assay		
Microbiological	6.7 ± 0.3A	33.9 ± 1.1C
HPLC[e]	—	35.5 ± 0.8C

[a] Mean and standard error

[b] For each column, values with a different capital letter were significantly different at the 0.017 critical probability level.

[c] Liver pyridoxal 5'-phosphate

[d] Estimates based on erythrocyte aspartate aminotransferase activity and percentage stimulation by added PLP were calculated using group means and, thus, were not included in the statistical analysis.

[e] High performance liquid chromatography

cause of the fortification to 25% US-RDA/oz with pyridoxine hydrochloride.

The data from the rat bioassay are presented in Table 3. The dose-response relationships observed for growth, feed efficiency, AspAT activity, and AspAT stimulation were very similar to those of previous studies (Gregory and Kirk, 1978a, b) and were in agreement with other published results (Sarma et al., 1946; Beaton and Cheney, 1965; Brin and Thiele, 1967). The liver PLP concentration values were in fair agreement with the recent results of Lumeng et al. (1978).

The results of the assays for total and biologically available vitamin B-6 in the test materials are shown in Table 4. For the nonfat dry milk, generally good agreement was found between the rat bioassay estimates of available vitamin B-6 and the microbiologically determined value for total vitamin B-6. These results indicate that the vitamin B-6 in the nonfat dry milk sample was fully available for absorption and utilization by the rats. The HPLC assay procedure could not be successfully applied to the nonfat dry milk. Further refinement of extraction and extract purification procedures is required. The observed agreement between the rat bioassay and microbiological assay procedures is in contrast with the data reported by Toepfer et al. (1963) in which microbiological results were significantly lower than rat bioassay growth data.

The lack of agreement between the estimates of total and biologically available vitamin B-6 in the cereal sample (Table 4) was unexpected. The rat bioassay results based on growth, feed efficiency, and liver PLP concentration for available B-6 were significantly lower than either HPLC or microbiological assay data for total vitamin B-6. The bioassay estimates of available vitamin B-6 based on growth, feed efficiency, AspAT activity, and liver PLP concentration ranged from 18–44% of the mean value for total vitamin B-6 in the cereal product. The close agreement between the results of the microbiological and HPLC assay procedures supports the accuracy of the total vitamin B-6 values. These results indicate that, under the conditions of this bioassay, the apparent bioavailability of the vitamin B-6 in the breakfast cereal sample is relatively low. In contrast, the estimate of available vitamin B-6 in the cereal determined on the basis of in vitro AspAT stimulation by added PLP was in close agreement with the microbiological and HPLC results. Thus by the enzyme stimulation criterion alone, the PN in the cereal product was fully available.

The divergent results of the rat bioassay for the cereal sample cannot be explained. The concentration of PLP in tissues (e.g. blood, liver, muscle) has been shown to be the most definitive index of vitamin B-6 status because of the key coenzymatic function of this vitamer (Lumeng et al., 1978). The liver PLP results therefore strongly support the validity of the estimates of available vitamin B-6 based on growth, feed efficiency, and AspAT activity.

As a check of the accuracy of the rat bioassay, microbiological assays were performed on the nonfortified basal diet and the two diets. The nonfortified basal diet was found to contain 0.62 ± 0.15 μg total vitamin B-6/g ($\bar{x}$ ± S.D.), while the test diets containing either nonfat dry milk or cereal contained 0.83 ± 0.01 and 2.57 ± 0.17 μg total vitamin B-6/g, respectively. These data indicate that the actual level of vitamin B-6 in the test diets which was provided by the added nonfat dry milk and cereal was 0.21 ± 0.15 and 1.95 ± 0.23 μg/g, respectively. On the basis of the values determined for total vitamin B-6 in the nonfat dry milk and cereal samples (Table 3), the predicted levels of added vitamin B-6 in the test diets would be 0.32 and 1.74 μg/g. These data, within the precision of the microbiological assay, confirm the accuracy of the diet formulation and thus support the accuracy of the bioassay results. Greater bioassay precision could be obtained by using several levels of dietary incorporation for each test substance (Bliss and White, 1967). Such a design would also permit a determination of the dose-dependence of vitamin B-6 bioavailability in these products.

Relatively little is known concerning the food composition and processing factors which affect the bioavailability of vitamin B-6 in foods. Recent research has indicated that the roasting and storage of low moisture food systems similar to cereal products has little effect on the bioavailability of the remaining vitamin B-6 (Gregory and Kirk, 1978a, b). PN was the sole detectable form of vitamin B-6 found in the HPLC assay of the cereal sample. The other vitamers, if present, were below the detection limit (signal/noise = 3) of less than 0.5 μg/g under the assay conditions employed. In view of the previously reported resistance of PN to losses in bioavailability (Tomarelli et al., 1955; Gregory and Kirk, 1978a, b), the results of this study cannot be readily explained. Research has shown that covalently complexed forms of vitamin B-6 such as the pyridoxylamino compounds may retard the utilization of vitamin B-6 under certain dietary conditions (Gregory and Kirk, 1978c). Because of the relatively nonreactive nature of PN, the formation of such complexes in unlikely.

In summary, nonfat dry milk and rice base breakfast cereal samples have been analyzed for vitamin B-6 by microbiological, HPLC, and rat bioassay procedures. The results indicate that the vitamin B-6 of the nonfat dry milk is fully available, while the apparent bioavailability of the vitamin B-6 in the cereal product is low. Further research is needed to identify the factors which influence vitamin B-6 utilization and to determine the adequacy of animal models as indicators of vitamin bioavailability in humans.

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EFFECT OF GERMINATION ON NUTRITIVE VALUE AND BAKING PROPERTIES OF DRY PEAS, LENTILS, AND FABA BEANS

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ABSTRACT

Dry yellow peas, lentils, and faba beans were germinated under laboratory conditions. Periodic rinsing with water at 2-hr intervals was effective in controlling microbial growth. Marked increase in ascorbic acid of the legumes was observed during germination. Amino acid contents did not change appreciably after 4-day germination. Wheat flour blends containing ungerminated and germinated legume flours at 5, 10, and 15% levels were used in baking studies. Addition of 15% legume flours to bread resulted in only small deleterious effects on loaf volume, crumb grain, and flavor. Germination adversely affected the baking properties of peas and lentils, but not faba beans. Blanching of the germinated peas further impaired its baking properties.

INTRODUCTION

IT HAS LONG BEEN recognized that germination generally enhances the nutritive value of seeds (Rudra, 1938; Everson et al., 1944; Banerjee et al., 1955; Kakada and Evans, 1966). Increases in several vitamins in certain pulses during germination were observed by Banjeree and coworkers (Chattopadhyay and Banerjee, 1954; Banjeree et al., 1955). Recently, optimizing nutrient availability of seeds through germination has received renewed interest (Chen et al., 1975; Kylen and McCready, 1975; Wang and Field, 1978; Hofsten, 1979). Among all the nutritive changes during sprouting of seeds, the most remarkable is the dramatic increase in ascorbic acid (Fordham et al., 1975; Venugopal and Rao, 1978).

Recently Wang and Field (1978) reported that germination increased the relative nutritive value (RNV), and lysine, methionine, and tryptophan contents of corn and sorghum proteins. Significant increase in RNV was also observed in wheat, barley, rice (Hamad and Fields, 1979), and corn (Hasim and Fields, 1979) after sprouting.

Germination is also beneficial in reducing some of the antinutritional factors in cereal and legume seeds. Trypsin inhibitor activity of soybeans was found to decrease by 13% after 3 days of germination (Collins and Saunders, 1976). Increased phytase activity and decreased phytate content were reported in germinated seeds (Mandal et al., 1972; Chen and Pan, 1977; Reddy et al., 1978). The decrease in phytate content may increase the availability of many essential minerals. Subbulakshmi et al. (1976) also showed that hemagglutinin activities of horsegram decreased markedly after 72 hr germination.

Fortifying wheat and corn products with legume flours to improve the protein quality has received considerable interest recently. Jeffers et al. (1978) reported that yellow pea flour was superior to soy flour in mixing and breadmaking properties at supplemental levels of 5–20%. Pomeranz et al. (1977) found that replacement of 10% wheat flour by germinated soy flour resulted in highly satisfactory bread quality. Using a different approach, Finney (1977) showed that replacement of 7% wheat flour by wet, mashed germinated soybeans produced breads with no objectionable taste or odor. Although germination and processing procedures may influence the functional properties and acceptability of the final product, little information regarding these aspects is available.

In spite of the extensive studies on the effect of germination on nutritive values of dry seeds, information regarding sprout production for food uses is lacking. Most of the germination studies were limited to laboratory or home conditions. The germination methods used varied among researchers with little emphasis on optimizing sprouting conditions. Microbial growth during germination was recognized by most workers as a potential problem. Yet, no specific information on microbial counts was given. The effect of germicide, such as hypochlorite, on germination and sprout development has not been thoroughly studied.

This study was designed to determine the effects of germination conditions on the quality of sprouts and to ascertain changes in ascorbic acid, riboflavin and amino acid composition of dry yellow peas (*Pisum sativum*), lentils (*Lens esculenta*), and faba beans (*Vicia faba*) during germination. The bread baking properties of wheat flour blends fortified with germinated and ungerminated peas, lentils, and faba beans were also compared.

MATERIALS & METHODS

Germination of seeds

Dry yellow peas (Plain Latah) and lentils (Common Chilian) were obtained from Dumas Seed Company, Moscow, Id. Faba beans were purchased from a food store in Seattle, Wash. Only dry yellow peas were used to study the effect of chlorine and soaking on germination.

Percent of weight increase of peas soaked over a period of 12 hr at room temperature was determined. Based on the hydration curve, four different points (3, 6, 9, and 12 hr) were selected to evaluate the effect of soaking time on germination. To study the effect of chlorination on sprout development and microbial growth during germination, peas were soaked in freshly prepared solutions containing 0, 50, 100, or 200 ppm chlorine for 6 hr. Rinsing medium was the same as the soaking medium. Purex containing 5.25% sodium hypochlorite was used as the source of chlorine.

For each treatment, 20g of whole yellow peas were thoroughly rinsed and soaked in 100 ml of tap or chlorinated water as described above. The soaked peas were spread on a perforated tray lined with cheesecloth. The tray was placed on a cake pan and covered with aluminum foil. The peas were germinated in the dark at 25°C for 4 days. They were rinsed twice daily with fresh soaking medium. This method will be referred to as manual rinsing later.

Germinated seed quality

Percent germination was determined by direct counting of sprouted and unsprouted seeds in each sample. Twenty randomly selected germinated seeds from each treatment were used for hypocotyl and epicotyl length measurement. General appearance and odor of the sprouts were also noted.

Microbial study

Surface counts were determined by placing 11g of 4-day sprouted seeds in dilution blank bottles containing 99 ml phosphate buffer followed by vigorous shaking prior to plating and dilution.

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For bacteria counts, the Standard Plate Count (SPC) Method was used (Speck, 1976). The SPC agar plates were incubated at 22°C for 48 hr. Mold and yeast counts were determined using acidified Potato Dextrose Agar. The plates were incubated at 22°C for 96 hr.

Table 1—Effect of rinsing and chlorine on percent germination and sprout development of yellow peas

Treatment		% Germination	Hypocotyl length[b] (cm)	Epicotyl length[b] (cm)
Manual rinsing[a]	Tap water	83.7	5.84 ± 1.05ac	3.30 ± 0.83a
	50 ppm chlorine	88.1	5.72 ± 1.59abc	2.70 ± 0.39bc
	100 ppm chlorine	86.0	5.03 ± 1.44b	2.54 ± 0.43b
	200 ppm chlorine	83.3	4.43 ± 1.50	2.48 ± 0.51b
Automated rinsing	200 ppm chlorine soak and tap water rinse	94.0	6.33 ± 1.63c	2.96 ± 0.49ac
	Tap water soak and rinse	92.0	6.39 ± 1.69c	2.99 ± 0.49ac

[a] Same soaking and rinsing medium

[b] Expressed as mean of 20 samples ± standard deviation. Values in a column followed by a common letter are not significantly different at the 5% level.

Table 2—Effect of rinsing and chlorine on microbial counts of yellow peas after four days of germination

Treatment		Standard plate count (counts/g)	Mold yeast count (counts/g)
Manual rinsing[a]	Tap water	318 X 10^6	2316
	50 ppm chlorine	0	0
Automated rinsing	200 ppm chlorine soak and tap water rinse	0.96 X 10^6	0
	Tap water soak and rinse	1.72 X 10^6	187

[a] same soaking and rinsing medium

Table 3—Nutrient analyses of freeze-dried germinated and ungerminated legumes

Nutrient[a]	Sample	Germination time, days: 0 (dry seed)	1	2	3	4
Protein (%) (N X 6.25)	yellow peas	25.3	25.8	25.8	26.7	26.4
	lentils	26.7	27.1	27.7	28.0	28.9
	faba beans	29.7	30.8	30.5	30.4	31.1
Ascorbic acid (mg/100g)	yellow peas	2.2	13.3	39.3	44.7	64.1
	lentils	0.9	6.0	22.5	44.6	77.5
	faba beans	1.4	5.1	32.7	63.2	75.8
Riboflavin (mg/100g)	yellow peas	0.24	0.35	0.36	0.40	0.50
	lentils	0.32	–	–	–	0.39
	faba beans	0.95	–	–	–	1.33

[a] dry weight basis

Automated laboratory germinator

In order to provide better environmental control for the germination, a specially designed laboratory germinator was converted from a household refrigerator (Fig. 1). The germination compartment was fully enclosed to provide control for temperature, relative humidity (RH), air circulation, lighting and automatic periodic rinsing. Germination capacity was 300–400g of dry seeds. After thorough washing, dry yellow peas and lentils were soaked for 8 hr while faba beans were hydrated for 18 hr. Soaked seeds were spread on a perforated tray lined with cheesecloth and rinsed at 2-hr intervals. The automated spraying device provided a total water volume of 4.0–4.5 gallons per rinse. The peas were germinated for 4 days in the dark at 24°C and 95% RH. The sprout development and microbial counts of the yellow pea sprouts germinated with automated rinsing were evaluated and compared with those germinated using manual rinsing as described previously.

Sample preparation

Yellow peas, lentils, and faba beans were germinated using the automated germinator. Faba beans were dehulled by hand prior to sample analyses, while peas and lentils were ground whole. Legumes and freeze-dried germinated seeds were ground into flour using a Hobart or Udy mill. Flours were stored in air tight jars at −20°C until analyses or baking.

Part of the 2-day and 4-day pea sprouts were blanched using live steam. Adequacy of blanching was ascertained using the peroxidase test (Hart and Fisher, 1971). The blanched pea sprouts were freeze-dried and milled into flour.

Chemical analyses

Flour moisture and protein contents were determined using the AACC method (AACC, 1962). Ascorbic acid and riboflavin contents of legume flours were analyzed using the AOAC fluorometric methods (AOAC, 1975). Amino acids of the acid hydrolysates were determined with a Beckman 121 automatic amino acid analyzer as described by Robbins et al. (1971) and Hubbard and Finney (1976). Methionine and cystine were determined after performic acid oxidation (Moore, 1963).

Baking studies

An unmalted, commercial straight grade baker's flour with a medium mixing time of 4 min and good loaf volume potential was used throughout the study. Bread baking formula included 100g legume-wheat flour blends (14% M.C. basis), 7.6% fresh baker's yeast (Fleischmann's Standard Brands), 1.5g NaCl, and 3.0g vegetable shortening (Crisco). Baking absorption, mixing time, and oxidation (ascorbic acid and potassium bromate) were optimized for each formulation. The legume flours were formulated on a replacement basis at levels of 5, 10, 15, or 20%. Doughs were fermented at 30°C for 70 min prior to panning and 45 min after panning. Doughs were degassed after 40 and 60 min, and once immediately prior to panning. Breads were baked at 217°C for 24 min and weighed. The loaf volume was determined by rape-seed displacement immediately after removing from the oven. Crumb grain and texture, crust color, oxidation response, and overall appearance of breads were subjectively evaluated by members of the SEA-USDA Western Wheat Quality Laboratory.

The 10-g mixograph was used to estimate physical dough properties including mixing time, mixing tolerance, and water absorption of the legume wheat-flour blends according to the method of Finney and Shogren (1972).

RESULTS & DISCUSSION

Germination studies

Effect of hydration. Hydration rate studies showed that dry yellow peas soaked for 6 hr at room temperature increased in weight by 84%. The most rapid water uptake occurred during the first 3 hr of soaking, while water imbibition rate began leveling off after 9 hr of hydration. Pea seeds soaked for 6 and 9 hr were similar in percent germination; both treatments produced better sprout quality than those soaked for 3 or 12 hr. In subsequent germination trials, it was found that 6–9 hr soaking was also satisfactory for lentils. However, faba beans required 18 hr of soaking time.

Effect of chlorination and periodic rinsing. Microbial growth during germination must be controlled in order to

Table 4—Amino acid composition of ungerminated and 4-day germinated legume flours (g/100g total amino acids)

	Yellow pea		Lentil		Faba bean	
	Ungerm	Germ	Ungerm	Germ	Ungerm	Germ
Lysine	7.26	7.06	7.85	7.93	7.00	6.88
Histidine	2.66	3.07	2.79	2.87	2.80	2.92
Ammonia	2.44	3.10	2.66	4.07	3.14	3.49
Arginine	9.35	8.90	7.23	6.81	8.82	8.20
Aspartic acid	11.15	12.04	11.61	15.98	11.28	13.31
Threonine	3.70	3.40	3.75	3.67	3.68	3.57
Serine	5.01	5.48	5.38	5.52	5.45	5.84
Glutamic acid	17.02	18.68	16.93	14.03	17.16	17.08
Proline	6.08	5.08	5.35	3.96	5.19	5.26
Cystine (half)	3.84	2.84	3.85	3.72	3.24	3.01
Glycine	4.25	3.82	3.97	3.55	4.29	3.98
Alanine	4.31	4.49	4.24	3.96	4.46	4.46
Valine	4.21	4.05	4.43	4.44	4.05	3.85
Methionine	1.21	1.27	1.20	1.16	1.09	1.08
Isoleucine	3.46	3.13	3.87	3.63	3.39	3.00
Leucine	7.00	6.70	7.29	7.01	7.56	7.08
Tyrosine	2.56	2.34	2.64	2.81	3.05	2.77
Phenylalamine	4.54	4.60	4.99	4.92	4.39	4.28

obtain wholesome sprouts and high yield. Often in laboratory studies, sterile germination conditions or chlorination were used for microbial control (Chattopadhyay and Bannerjee, 1954; Fordham et al., 1975; Cunningham et al., 1978). As shown in Table 1, seeds soaked in tap water with rinsing twice daily had high bacterial and mold counts. Fifty ppm chlorine in the soaking and rinsing media was effective in inhibiting microbial growth. However, chlorine in soaking or rinsing medium had an adverse effect on sprout development (Table 1). Shriveled hypocotyls and epicotyls, yellow discoloration, and pronounced chlorine odor were noted among samples treated with 200 ppm chlorine. Although the control samples had the most vigorous growth, microbial spoilage was clearly visible. Thus, percent germination was affected. It appears that a chlorine concentration of 50 ppm in the soaking and rinsing medium would be sufficient to reduce microbial counts to an acceptable level.

Frequent rinsing during sprout production has been the practice in China. The germinator with automated spraying device developed in this study provided automatic rinsing at desired intervals. As shown in Table 2, 200 ppm chlorine soak and periodic rinsing with tap water at 2-hr intervals produced sprouts of greatly reduced SPC and mold/yeast counts. Soaking pea seeds in tap water, followed by periodic rinsing in the germinator produced sprouts with slightly higher but acceptable SPC and mold/yeast counts (Table 2) as judged by their appearance. Percent germination and sprout development of yellow peas germinated for 4 days in the automated germinator were better than those by manual rinsing (Table 1). The percent germination for lentils and faba beans was also above 90% when sprouted in the germinator. The sprouts of consistently high yield and vigorous sprout development produced by the germinator may be attributed to desirable, controlled germination conditions and reduced microbial build-up by effective rinsing.

Chemical analyses

The results of chemical analyses are shown in Table 3. The ascorbic acid content of the three legumes increased markedly during germination. The 29–86 fold increases in ascorbic acid observed in these legumes after 4 days of germination were comparable to the results reported for other germinated seeds (Kylen and McCready, 1975). Riboflavin content of peas increased by twofolds after 4 days of germination. This finding is in agreement with the values

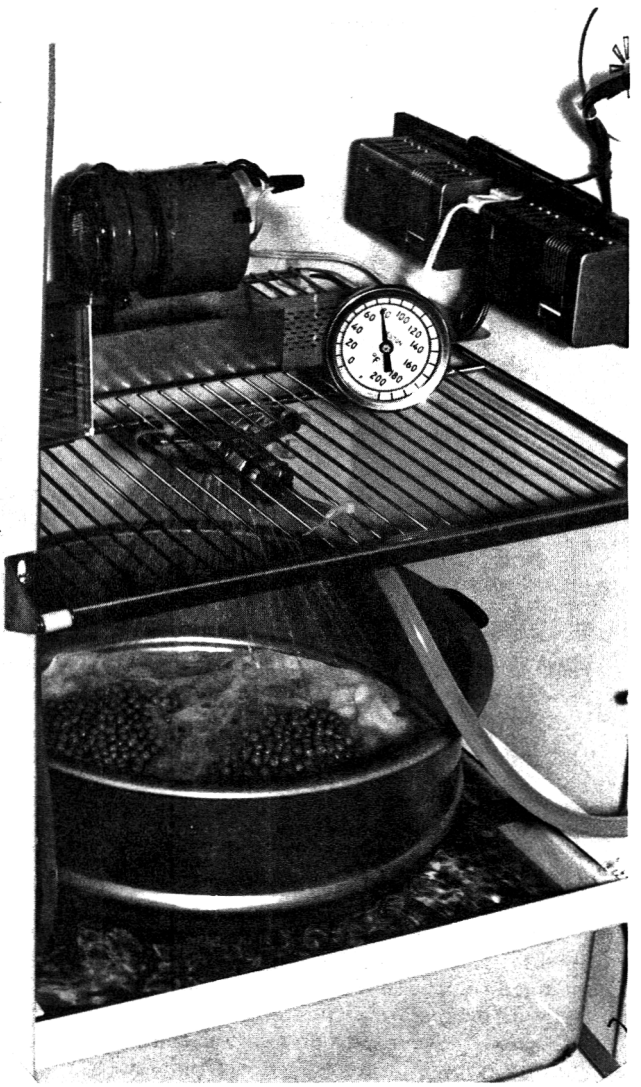

Fig. 1—Automated laboratory germinator.

for Alaska peas (Chen et al., 1975; Fordham et al., 1975). Riboflavin in lentils remained unchanged after germination. These data agree with the findings of Kylen and McCready (1975). A slight increase in riboflavin in faba beans was observed after germination. No data on germinated faba beans were found in the literature.

Little change in protein content of the three legumes was observed after 4-days germination (Table 3). Amino acid content of the germinated and ungerminated yellow pea, lentil, and faba beans flours are presented in Table 4. Little or no change in the essential amino acid content occurred after germination.

Although some studies have shown that the nutritive value of legume protein improved after germination (Everson et al., 1944; Desikachar and De 1950), Chen and Thacker (1978) reported little change in essential amino acid content of pea seeds after 5 days of germination. Recent studies of cottonseed and soybean proteins also showed little change in essential amino acid content after germination (Cunningham et al., 1978; Bau and Debry, 1979).

Physical dough tests

The mixograms which reflect the dough properties of

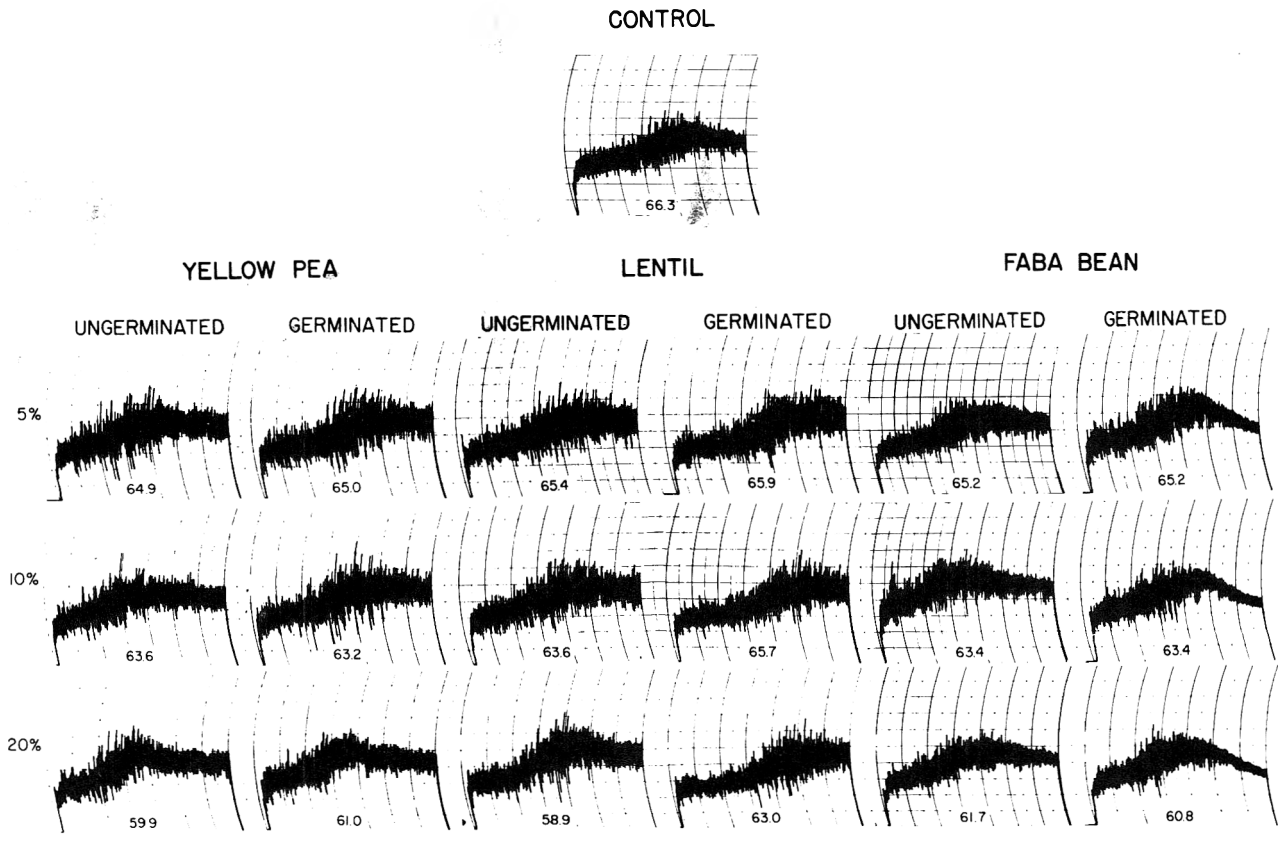

Fig. 2—Mixograms (10g) for ungerminated and germinated legume-wheat flour blends at 5, 10, and 20% replacement levels.

the commercial straight grade control flour and the legume-wheat flour blends are shown in Figure 2. In either the germinated or ungerminated legume-wheat flour blends, the overall mixograph properties of yellow peas, lentils, and faba beans were similar to those of the control, except that germinated lentils prolonged mixing time and germinated faba beans reduced mixing tolerance. In all cases, water absorption decreased with increased level of supplementation. This observation was in agreement with the results reported by Jeffers et al. (1978) when they fortified wheat flour with raw pea flour. No significant differences in water absorption were noted between germinated and ungerminated yellow peas or faba beans, but germinated lentil flour blends absorbed slightly more water than the ungerminated samples.

Blanching affected the mixing properties of germinated pea flour (data not shown). Water absorption increased when blanched germinated pea flour was added. At 15% substitution level, water absorption exceeded the control by approximately 7%. Jeffers et al. (1978) also observed increases in water absorption when cooked pea flour was added to wheat flour. Mixing time of the blanched, germinated pea blends was shorter than those of control and the unblanched germinated pea flour blends. At 5 and 10% levels, differences in the mixing tolerance between blanched and unblanched pea flour blends were not noticeable; however, at the 15% level, the mixing tolerance was better in the unblanched germinated pea flour than in the blanched sample.

Baking studies

The data obtained from comparative baking studies on wheat flour supplemented with germinated or ungerminated legumes are shown in Table 5. All the samples were optimized for oxidation, baking absorption, and mixing time. With increasing levels of legume supplements, baking absorption decreased. The decrease was approximately the same for both germinated and ungerminated legumes. Addition of germinated lentil flour increased mixing time greatly; while all 3 levels of germinated faba bean fortified flour blends had approximately the same mixing requirement as control (Table 5).

Only small deleterious effects on loaf volume, crust color and flavor were noted in breads made from flour blends supplemented with 15% regular legume flours (Table 5). The results were similar to those reported by D'Appolonia (1977) and Jeffers et al. (1978).

Germination adversely affected the functional properties of pea and lentil flours, resulting in decreased loaf volume, and inferior crumb grains (Table 5). The mixograph properties of germinated yellow pea and lentil flour blends which did not differ significantly from the ungerminated counter parts (Fig. 2) cannot account for the reduced bread baking ability of these two germinated legume flour blends. However, germination had little adverse effect on the baking properties of faba beans. In certain respects, 4-day germination improved the fucntionality of faba bean flour blends. When wheat flour was supplemented with 5–10% germinated faba bean flour, the breads produced were similar if not superior in quality to that of the control (Fig. 3 and Table 5).

Off-flavor characterized by beany odor and bitter taste was especially pronounced in breads fortified with germinated pea flour and to a lesser degree in germinated lentils. Only a slight difference in flavor was detected when 15% of wheat flour was replaced by germinated faba bean. At this level, the flavor was mild and acceptable.

The dark crust of breads made from germinated legume flour blends may be due to increased Maillard browning.

Reducing sugars in the legume seeds may increase due to hydrolysis of starch during germination. The adverse effects of germination on peas and lentils were probably due to increases of certain enzyme activity. It is not clear why germination did not adversely affect the baking properties of faba beans.

Table 5—Baking data of legume wheat flour blends

Flour blends	Baking absorption[a] (%)	Mixing time (min:sec)	Loaf vol. (cc)	Crumb grain[b]	Crust color[c]
Control (comml std)	67.7	3:40	1050	S	5
Ungerminated yellow pea (%)					
5	66.0	2:53	1111	S	5
10	63.9	2:50	1050	S	6
15	61.8	2:51	1002	Q	6
faba bean (%)					
5	65.4	3:05	1061	S	5
10	64.5	3:05	1033	S	5
15	62.8	3:07	970	Q-S	5
lentil (%)					
5	66.6	3:25	1066	S	5
10	64.3	3:40	1092	S	6
15	62.0	3:23	988	Q-S	6
Germinated, 4 days yellow pea (%)					
5	66.1	3:15	1005	Q-S	6
10	65.7	3:10	868	Q	6
15	63.4	3:11	792	Q-U	7-8
faba bean (%)					
5	65.4	3:30	1134	S	5
10	64.1	3:30	1035	S	6
15	61.8	3:33	906	Q	6
lentil (%)					
5	64.7	4:25	998	S	5
10	68.4	4:23	889	Q-S	6
15	64.0	4:50	821	Q-U	6

[a] 14% M.C. basis
[b] S = satisfactory, Q = questionable, U = unsatisfactory
[c] crust color rated subjectively on a 1 to 10 scale with increasing darkness

Table 6—Effect of blanching on the baking quality of germinated pea-wheat flour blends.

Treatment	Yellow pea %	Baking absorption[a] (%)	Mixing time (min:sec)	Loaf vol. (cc)	Crumb grain[b]	Crust color[c]
Control	0	67.7	3:40	1050	S	5
Unblanched Germinated 2 days	5	68.7	3:25	1081	S	5
	10	65.4	3:15	1050	Q-S	6
	15	64.3	2:47	905	Q-S	6
Germinated 4 days	5	66.1	3:15	1005	Q-S	6
	10	65.7	3:10	868	Q-S	6
	15	63.4	3:11	792	Q-U	7-8
Blanched Germinated 2 days	5	68.7	2:40	1019	Q-S	6
	10	71.4	2:20	889	Q-U	7
	15	74.2	2:10	780	U	7
Germinated 4 days	5	70.5	2:46	918	Q-S	7
	10	74.1	2:44	863	Q-U	8
	15	70.8	2:50	793	U	8

[a] 14% M.C. basis
[b] S = satisfactory, Q = questionable, U = unsatisfactory
[c] Crust color rated subjectively on a 1 to 10 scale with increasing darkness

Effect of blanching

Breads containing germinated pea flour had bitter, beany odors and tastes which were highly unacceptable. An attempt was made to eliminate the off-flavors by inactivating the enzymes using steam blanching. It was noted that the time required to reach a negative peroxidase test was related to germination time. Peas soaked for 8 hr required only 1/2 min of blanching time. Two-day and 4-day germinated peas required 2 and 4 min, respectively, for peroxidase inactivation.

Table 6 shows the effect of blanching on the functional properties of germination. Water absorption increased in blanched germinated pea flour blends, while mixing time decreased when compared to that of the control wheat flour or the unblanched pea sprout flour blends. Although blanching of germinated peas eliminated the off-flavor problem, it further impaired the baking properties of the germinated pea flour. The adverse effects of blanching on baking properties are probably due to heat denaturation of proteins and/or gelatinization of starch.

CONCLUSIONS

A SOAKING and rinsing medium containing 50 ppm chloride was effective in controlling microbial growth during germination of peas. A laboratory germinator with automated rinsing and temperature control was developed to produce high quality sprouts for this study. Ascorbic acid content of dry yellow peas, lentils, and faba beans increased by 29–86 fold and riboflavin content of peas doubled after 4-days germination. Germination had little effect on the protein content of amino acid composition of the legumes. Replacement of wheat flour with 15% legume flours had little deleterious effect on loaf volume. Four-day germination adversely affected the baking qualities of peas and lentils, resulting in decreased loaf volume, off-flavor, and dark crust. However, the baking properties of faba beans were not adversely affected by germination. Blanching of germinated peas eliminated off-flavor but further impaired baking properties.

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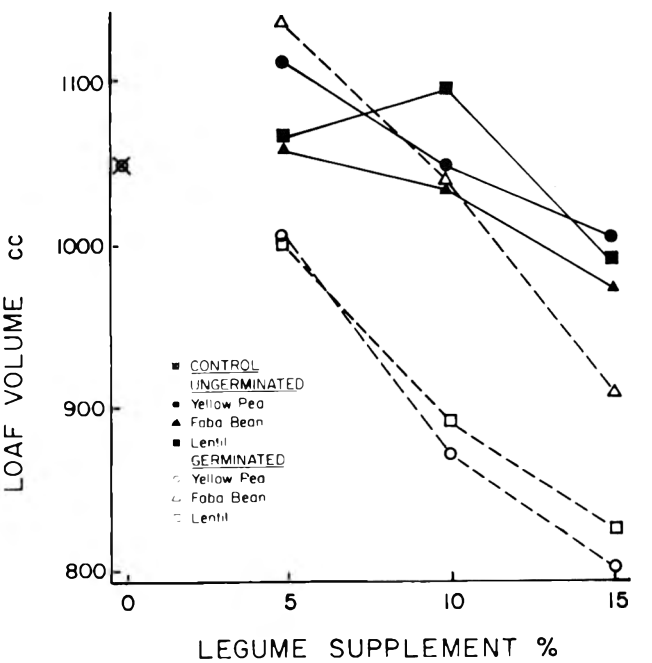

Fig. 3—Effect of legume supplement on loaf volume.

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PENTANE PRODUCTION IN FREEZE-DRIED PORK. . .From page 28

In conclusion, pentane measurement can be used to determine the shelf life of freeze-dried pork.

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MODIFIED ATMOSPHERE STORAGE OF ROCKFISH (Sebastes miniatus) AND SILVER SALMON (Oncorhynchus kisutch)

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ABSTRACT

Rockfish (*Sebastes miniatus*) fillets and salmon (*Oncorhynchus kisutch*) steaks were held in atmospheres containing 20% or 40% carbon dioxide, with or without 1% carbon monoxide. Controls were stored similarly in air. At intervals of refrigerated storage up to 14 days, samples were removed for sensory, chemical, and microbiological analyses. At 7 days, all treatment groups were significantly different visually, with appearance of slime on the air controls, but not on samples in the gas treatments. Samples held in air were judged by panelists to have stronger aromas than others held under carbon dioxide at either level. The higher level of carbon dioxide was more effective. Values for thiobarbituric acid were low in all groups; hypoxanthine values varied widely, with no particular effect due to modified atmospheres. Storage under carbon dioxide was effective in reducing the formation of trimethylamine and ammonia, and markedly inhibited microbial growth.

INTRODUCTION

THE PIONEERING WORK of Coyne (1932, 1933) demonstrated that high levels of carbon dioxide (CO_2) would inhibit microbial growth and make possible prolonged storage times of fish held in such atmospheres. He used concentrations of CO_2 ranging from 20–100%. His findings corroborated an earlier study (Killeffer, 1930) which involved a variety of food commodities, including cod, held under CO_2, but in less well controlled circumstances. Stansby and Griffiths (1935) in a careful study demonstrated that whole haddock stored in CO_2 atmospheres from the time of capture keep approximately twice as long as controls stored in air. Later, Shewan (1950) summarized studies following up the earlier work of Coyne and suggested that the use of carbon dioxide showed sufficient promise to warrant further investigation. He suggested that the best percentage of CO_2, while not known with certainty, was probably 30–40%.

In spite of these early promising findings, the application of high levels of CO_2 for storage of fishery products has not received a great deal of attention. Yokoseki et al. (1956) found CO_2 atmospheres were useful for the storage of fish cakes. Windsor and Thoma (1974) investigated the use of CO_2 atmospheres in conjunction with a variety of chemical preservatives for use with industrial fishery products. They found such atmospheres beneficial, but suggested that the exclusion of air was probably as important as the replacement gas used, the results with nitrogen or a vacuum being about the same as with CO_2. Other studies have demonstrated that CO_2 dissolved in refrigerated brines improved quality of rockfish and chum salmon (Barnett et al., 1971); silver hake (Hiltz et al., 1976); and pink shrimp (Bullard and Collins, 1978; Barnett et al., 1978) held in such brines.

The present study was designed to evaluate sensory, chemical, and microbial changes occurring in rockfish and salmon stored in 20 or 40% CO_2 or in air. Preliminary studies with a variety of fish indicated that high levels of CO_2 might promote surface darkening in more highly pigmented species due to oxidation of oxymyoglobin. Consequently, low levels of carbon monoxide, which cause the formation of carboxymyoglobin, which is more resistant to oxidation, were included in some of the gas mixtures employed in this investigation.

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MATERIALS & METHODS

Fish

Fresh rockfish (frequently sold in California as "red snapper") and salmon were purchased from a wholesale outlet in Sacramento (Ocean Beauty). Iced fish were brought in by truck from the San Francisco or Monterey Bay areas during the night. Some rockfish were landed in Oregon and flown to the wholesaler. Samples were picked up at the wholesaler early the following morning and brought to Davis for analysis.

Rockfish were received in iced plastic bags containing 10 lb of fillets. Samples were taken to yield approximately 4.5-in lengths from each fillet; a second length was taken from larger fillets. Such samples were distributed among the treatment groups.

Salmon were received in ice, gutted, and headed. The fish were scaled in running water after removal of dorsal and ventral fins. Approximately 10–12 cross-sectional steaks 1-in. thick were cut between the pectoral and anal fins. These were divided into groups to equal the total number of treatments × the number of storage periods. Each group was loaded sequentially onto shelves (see below) to minimize effects of fish-to-fish variation.

Fish samples were placed onto racks of 20 superimposed (1.75-in. intervals) circular shelves (8.5-in. diam, with four parallel, equally spaced cross pieces) made of 1/8-in. stainless steel welding rod. Each rack was placed into a cylinder fashioned from polyvinychloride pipe (10-in. diam by 36-in. long). Plexiglass closures (1/2-in. thick) were sealed with 1/4-in. rubber gaskets and secured with bolts and wing nuts. Gas tightness was verified in preliminary work by a cylinder's ability to hold a 20-in. vacuum for several minutes.

Gas mixtures employed for modified atmosphere storage were 20% CO_2, balance air; 20% CO_2 plus 1% CO, balance air; 40% CO_2, balance air; 40% CO_2 plus 1% CO, balance air; and room air controls. Gas mixtures were obtained as analyzed grade mixtures from Matheson Co. Actual values for gas composition in different lots ranged from 19.22–20.16% CO_2 (called 20%); 38.06–39.02% CO_2 (called 40%); and 0.95–1.07% CO (called 1%).

Closed cylinders were evacuated to 22-in. vacuum three times. Each time the vacuum was released with the treatment gas mixture (calculated 98% conversion). Controls were evacuated similarly, but released with room air. Cylinders were stored at 4.5°C, in the dark.

For sampling, weighed portions of fish were removed from various locations on the rack; remaining samples were quickly regassed, and returned to storage. The storage schedule was planned for a random presentation of treatment groups for sensory analyses. Samples used for chemical analyses were taken in parallel with those to be used for organoleptic evaluation (except salmon 3-day samples, held in air). Six fillets or steaks were used per assay. All analyses were performed on each of the six samples. The same day's pack was followed throughout the duration of each treatment. All handling of samples throughout the study was done with disposable vinyl gloves.

Sensory analysis

Eighteen judges were given training panels with fish of about the largest and mid-range amounts of expected difference using the

same tests to be used in the experiment. Visual differences between treatments were judged under ordinary flourescent lighting by triangle tests. The judges were asked to look for simple differences and were not limited to one parameter of visual difference. All judges saw the same samples. Each sample included three 1.5-in. out-surface sections of random fillets or three half-steaks. Aroma differences were judged in paired comparison for the stronger aroma intensity. Aroma samples for each judge were randomly chosen from at least six diced fillets or steaks; about eight ½-in. dices for each sample were presented in covered ceramic cups. Samples from the three treatment atmospheres were compared to each other and to fresh samples. Each pair was replicated twice through the complete treatment procedure. Significance was estimated using the statistical tables provided by Roessler et al. (1978).

Chemical analysis

Thiobarbituric acid (TBA) assay for lipid oxidation was done by the procedure of Yu and Sinnhuber (1957) slightly modified for low levels. Values are reported as absorbance at 535 nm of the final reaction mixtures. Five grams of tissue were used. In salmon, the belly flap material, containing a larger amount of lipid material, was sampled for the TBA assay.

Neutralized perchloric acid extracts were used for hypoxanthine, trimethylamine (TMA) and ammonia (NH_3) analyses. For such extracts 25g of fish were blended with 50 ml of 0.6N perchloric acid, filtered, and neutralized with 5N KOH, chilled in ice, and centrifuged free of potassium perchlorate. A lipid layer observed in salmon extracts was by-passed when aliquots were taken.

Hypoxanthine levels were determined by the procedure of Beuchat (1973) as modified to utilize the buffer system of Jones et al. (1964).

Ammonia was assayed using a specific ion electrode and following the instructions of the manufacturer (Orion). Samples were 5 ml neutralized perchloric acid extracts diluted to 50 ml after addition of 0.5 ml of 10M NaOH.

Trimethylamine values were obtained using the electrode described by Chang et al. (1976) except that the internal filling solution for the electrode was 0.01M TMA·HCl:0.05M KCl and the formaldehyde concentration was increased to 0.37% to increase the selectivity for TMA over NH_3 (Chang, personal communication).

Microbiological analysis

Portions of approximately 15g were taken from each sample at indicated time intervals and placed individually into sterile plastic bags (Whirl Pac, Nasco) and quickly frozen below −40°C in a blast freezer. After freezing, samples were stored at −15°C prior to shipment by air in dry ice containers to a commercial laboratory (Silliker Labs, Carson, CA 90746) for microbiological analysis. Each day's pack was sampled for initial loading. Extracts of the muscle samples were made with sterile distilled water containing 0.1% peptone (Difco). Using conventional dilution procedures, counts were done on Plate Count Agar (Difco) for total counts; Azide Blood Agar (Difco) for gram positive organisms; and MacConkey Agar (Difco) for gram negative bacteria. Plates were incubated at 20°C for 3 days, then counted.

RESULTS & DISCUSSION

Sensory analysis

The fresh fish, particularly rockfish, varied somewhat in aroma intensity and color when received. Fish obtained on any given day were more similar within that batch than when compared with a batch purchased on another day. This may influence comparisons between stored and fresh samples, and show in the sensory data as differences between replicates of the same comparison groups. It was impossible, however, to anticipate this variance which probably has more influence on the comparison of 2-day treatment groups versus fresh samples than on comparisons involving samples stored for longer treatment times.

At 7 days, all treatment groups were significantly different visually (Table 1). The most obvious differences were the appearance of slime on the air controls at 7 days, but not on the samples in the gas treatment groups, and the redder color of the samples held in atmospheres containing carbon monoxide due to the formation of carboxymyoglobin.

Panelists readily detected aroma differences between fresh samples of rockfish and those held in air or 20% CO_2. In paired comparisons, both fresh and air samples were considered to have stronger aromas than samples held in CO_2 (Table 2). Interestingly, the addition of only 1% CO to the CO_2 atmosphere eliminates this difference. After 7 days in storage in 20% CO_2, with or without low levels of CO, the gas mixtures were ineffective, in that stored samples consistently were found to have a stronger aroma than fresh samples, and there were no differences between samples held in air or in the gas mixtures (Table 3). We now believe, based on experiments in progress, that 20% levels of CO_2 are too low to be maximally beneficial.

Table 1—Triangle tests for visual differences among storage treatment groups and fresh samples of rockfish and salmon[a]

	CO_2	CO_2 CO	Air
	Rockfish, 20% CO_2, 2 days		
Fresh	19/30***	20/27***	28/33***
Air	10/29NS	26/29***	
CO_2 CO	29/32***		
	Rockfish, 20% CO_2, 7 days		
Fresh	30/30***	34/34***	30/30***
Air	20/28***	25/28***	
CO_2 CO	32/32***		
	Salmon, 40% CO_2, 3 days		
Fresh	14/34NS	29/34***	18/32**
Air	12/32NS	18/30**	
CO_2 CO	20/32***		
	Salmon, 40% CO_2, 7 days		
Fresh	22/31***	24/30***	
Air	27/29***	27/30***	
CO_2 CO	15/30*		

[a] Numbers are correct answers per number of judgements. NS=Not significant; *5% level; **1% level; ***0.1% level.

Table 2—Paired comparison tests for stronger aroma between rockfish samples of fresh and 2-day storage treatment groups, 20% CO_2[a]

Pair		Fresh	CO_2	CO_2 CO	Air
Fresh–CO_2	***	26	4		
Fresh–CO_2 CO	NS	13		14	
Fresh–Air	NS	17			16
Air–CO_2	***		5		24
Air–CO_2 CO	NS			17	12
CO_2 CO–CO_2	NS		20	12	

[a] Values in Table indicate number of agreeing judgements for treatment groups. NS=Not significant; ***significantly different at the 0.001 level of probability.

Table 3—Paired comparison tests for stronger aroma between rockfish samples of fresh and 7-day storage treatment groups, 20% CO_2[a]

Pair		Fresh	CO_2	CO_2 CO	Air
Fresh–CO_2	***	1	29		
Fresh–CO_2 CO	***	1		33	
Fresh–Air	***	0			30
Air–CO_2	NS		13		15
Air–CO_2 CO	NS			14	16
CO_2–CO_2	NS		17	16	

[a] Values in Table indicate number of agreeing judgements for treatment groups. NS=Not significant; ***significantly different at the 0.001 level of probability.

With salmon held for 3 days storage, the atmospheres containing 40% CO_2 were effective. Samples stored in either 40% CO_2 or CO_2 plus CO were found to have a less strong aroma than those held in air. Also, panelists could not distinguish between atmosphere-treated samples and fresh ones (Table 4).

Salmon held for 7 days in the 40% CO_2 atmosphere systems were found to have a stronger aroma than fresh samples; however, comparison of samples held in atmospheres with those held in air demonstrate the effectiveness of the modified atmospheres in reducing strong aroma (Table 5). In this series, the presence of low levels of CO was without effect.

Chemical analysis

Results of the chemical analyses are summarized in Tables 6 and 7. There was a general trend of increasing TBA

Table 4—Paired comparison tests for stronger aroma between salmon samples of fresh and 3-day storage treatment groups, 40% CO_2 [a]

Pair		Fresh	CO_2	CO_2 CO	Air
Fresh—CO_2	NS	17	17		
Fresh—CO_2 CO	NS	18		16	
Fresh—Air	***	1			31
Air—CO_2	***		5		27
Air—CO_2 CO	***			2	28
CO_2 CO—CO_2	NS		17	15	

[a] Values in Table indicate number of agreeing judgements for treatment groups. NS=Not significant; ***significantly different at the 0.001 level of probability.

Table 5—Paired comparison tests for stronger aroma between salmon samples of fresh and 7-day storage treatment groups, 40% CO_2 [a]

Pair		Fresh	CO_2	CO_2 CO	Air
Fresh—CO_2	***	3	28		
Fresh—CO_2 CO	***	1		29	
Fresh—Air	***	1			31
Air—CO_2	***		0		24
Air—CO_2 CO	***			1	29
CO_2 CO—CO_2	NS		17	13	

[a] Values in Table indicate number of agreeing judgements for treatment groups. NS=Not significant; ***significantly different at the 0.001 level of probability.

Table 6—Mean chemical and microbiological data for rockfish, 20% CO_2 [a]

Treatment	TBA values Net A^{535}	Hypoxanthine μmoles/g	TMA μmoles/g	NH_3 μmoles/g	Microbial counts X 10^{-6} Total	Azide	MacConkey
0 days	0.12a	5.8ab	0.4a	8.2a	0.7a	0.6a	0.3a
			Air				
2 days	0.34bc	5.4a	1.8a	9.0a	2.4ab	0.1a	0.9a
7 days	0.52cd	6.4bc	30.6cd	131.7c	334.0e	6.8a	117.8b
14 days	0.37bc	6.5c	42.2e	191.0e	114.3d	3.4b	1.2a
			CO_2				
2 days	0.18ab	8.8c	0.4a	9.1a	0.1a	0.0a	0.0a
7 days	0.27ab	8.5de	15.1b	49.5b	0.3a	0.2a	0.1a
14 days	0.54cd	5.5a	34.1d	167.5d	57.9c	3.1b	2.9a
			CO_2 CO				
2 days	0.28ab	6.7c	0.3a	8.5a	0.9a	0.0a	0.0a
7 days	0.71d	9.0e	28.3cd	123.6c	7.3abc	0.6a	1.8a
14 days	0.62d	7.8d	30.7cd	152.2d	54.0bc	2.6b	1.1a

[a] Means not followed by the same letter differ significantly at the 5% level.

Table 7—Mean chemical and microbiological data for salmon, 40% CO_2 [a]

Treatment	TBA values Net A^{535}	Hypoxanthine μmoles/g	TMA μmoles/g	Microbial counts X 10^{-6} Total	Azide	MacConkey
0 days	0.23ab	2.5a	0.6a	0.1a	0.1a	0.0a
		Air				
3 days	0.44b	4.3cd	4.0c	13.1a	0.2a	0.5a
7 days	0.72c	4.9d	7.6e	484.4b	232.7a	352.3b
		CO_2				
3 days	0.16a	3.0ab	2.0b	8.4a	0.1a	0.1a
7 days	0.97cd	3.9bc	6.6de	45.2a	0.2a	2.0a
		CO_2 CO				
3 days	0.30ab	2.7a	0.6a	0.1a	0.0a	0.0a
7 days	1.10d	4.5cd	6.0d	18.9a	2.7a	0.2a

[a] Means not followed by the same letter differ significantly at the 5% level.

Table 8—Mean microbiological data for rockfish, 40% CO_2 [a]

Treatment	Microbial counts X 10^{-6}		
	Total	Azide	MacConkey
0 days	0.1a	0.0a	0.0a
	Air		
3 days	0.7a	0.0a	0.0a
7 days	551.1b	10.9a	7.5b
	CO_2		
3 days	0.2a	0.0a	0.0a
7 days	0.9a	0.2a	0.0a
	CO_2 CO		
3 days	0.1a	0.0a	0.0a
7 days	2.1a	0.1a	0.2a

[a] Means not followed by the same letter differ significantly at the 5% level.

values with no particular effect shown in the modified atmosphere treatment groups. However, all values were quite low, indicating that lipid oxidation was not particularly significant in this study. Determinations of peroxide values using rockfish samples stored in 20% CO_2, and with a limited number of salmon samples, resulted in similar findings. Since the values paralleled the TBA results and the analyses required much more material and yielded more erratic values at the low levels of oxidation encountered, determinations of peroxide values were not continued throughout the study and the values are not reported here.

Hypoxanthine values varied widely and there was no particular effect of the modified atmospheres. The values found in rockfish are quite high overall, including those of fresh samples. We included hypoxanthine analyses as a measure of tissue autolysis; in our own experience, hypoxanthine levels are not too meaningful.

As would be expected, the TMA and NH_3 values were reasonably consistent with the microbiological data. The modified atmosphere treatment was effective throughout for rockfish held in 20% CO_2, i.e. lowering levels of TMA, and was also effective in salmon, particularly for the 3-day treatment groups held in 40% CO_2. Ammonia levels were lower in the 7-day rockfish groups held in modified atmospheres (20% CO_2) than in controls. Due to the redundant pattern of build-up of TMA and NH_3, NH_3 analysis was not done on salmon samples. The TMA assay was selected due to its larger percentage increase in the course of the treatment.

Microbiological analysis

Microbiological data are presented in Tables 6, 7 and 8. Microbial growth was markedly inhibited by the presence of CO_2 in all treatment groups used throughout the study. As a typical example, rockfish held in 20% CO_2 for 7 days had total microbial counts of 300,000 compared to values of approximately 33,000,000 for controls stored in air (Table 6). Microbial growth was somewhat greater in salmon, but again, comparative values for total counts demonstrate the effectiveness of CO_2 atmospheres (Table 7). In work in progress, we are finding that atmospheres containing higher amounts of CO_2 (60–80%) produce even greater inhibition of microbial growth.

CONCLUSIONS

THE POTENTIAL for use of carbon dioxide containing atmospheres for the preservation of fresh fish samples is great. Such modified atmospheres dramatically inhibit microbial growth and are effective in preventing undesirable sensory changes at the levels (20 and 40%) employed. Levels of TMA and NH_3 were markedly lower in samples held in the atmosphere treatment groups. These may well be the most useful chemical indices for similar studies in the future.

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PROCESSING OF CURED MULLET ROE

W. H. HSU and J. C. DENG

ABSTRACT

In processing of cured mullet roe, the desalting step was required to prepare an acceptable product. When mullet were salted with various levels of solid salt, the data of salt diffusion followed first order kinetics. With a 10% curing salt, the time constant (hr) increased from 42.25 and 45.27 for whole roe and central region, respectively, to 44.39 and 50.07 for those cured with a 20% salt. Cured roe (15% salt for 28 hr and desalted for 6 hr) open sun-dried (T ≃ 30°C) for 7 days contained 5.1% salt, 26.5% moisture and 0.832 a_w.

INTRODUCTION

BLACK MULLET, *Mugil cephalus* Linnaeus, is one of the most widely distributed food fish in the world. It is found in coastal waters and estuaries throughout the tropics and subtropics (Futch, 1976). In the United States, production of mullet is concentrated in seven southeastern states from North Carolina to Louisiana with an annual production from 1967–1971 of 32.4 million pounds valued at 2.6 million dollars. Florida normally produces slightly over 80% of U.S. landings (Cato et al., 1976). The catch of mullet is the largest among the finfish landed in Florida. In 1973, approximately 30 million pounds of mullet or 27.8% of the total finfish landed were from the Gulf of Mexico.

In Florida, mullet roe is available primarily from November through January. The roe, as well as the mullet, have traditionally been sold fresh or frozen at retail seafood markets. Despite its low market demand in Florida, it is a popular seafood in many other countries. Mullet roe, a by-product, is especially popular in certain Asian countries where it is processed into a dried and cured product. Its delicious flavor but limited catch make mullet roe one of the more expensive food items in these countries.

Recently, foreign countries have purchased large quantities of mullet roe during the spawning season in November. It was estimated that 2 million pounds of mullet roe were frozen and sold in 1974 (Cato et al., 1976). The market for mullet roe in the future seems very promising.

The method of processing mullet roe in some Asian countries traditionally involves salting, desalting and sun-drying. The final product is a yellow-brown color with about 4% salt content and 20–30% moisture content. It is generally reported that dried mullet roe have a refrigerated shelf life of 1–3 months; however, no actual data have been reported in this area. The object of this research was to study some factors that may affect processing of cured mullet roe.

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EXPERIMENTAL

Materials

Freshly caught mullet (*Mugil cephalus*) were purchased from Placida, Fla., (Gasparilla Store) at the end of November (spawning season), in 1975 and 1976. The fish were transported on ice to Gainesville, Fla., where the fish roe, encased in an ovarian membrane, were carefully separated from the fish without rupturing the membrane. Dirt and other extraneous materials, as well as the blood in the surface vessels, were either washed or squeezed out. The cleaned roe were tied at the end. Three or five roe were placed in a

Table 1–Definition and levels of independent variables for salt penetration experiment

	Code	Levels				
		$-\sqrt{2}$	−1	0	1	$\sqrt{2}$
Percent of salt (%)[a]	X_1	5.0	7.9	15.0	22.1	25.0
Salting time (hr)	X_2	1.0	9.6	30.5	51.4	60.0

[a] Based on wet weight of thawed roe

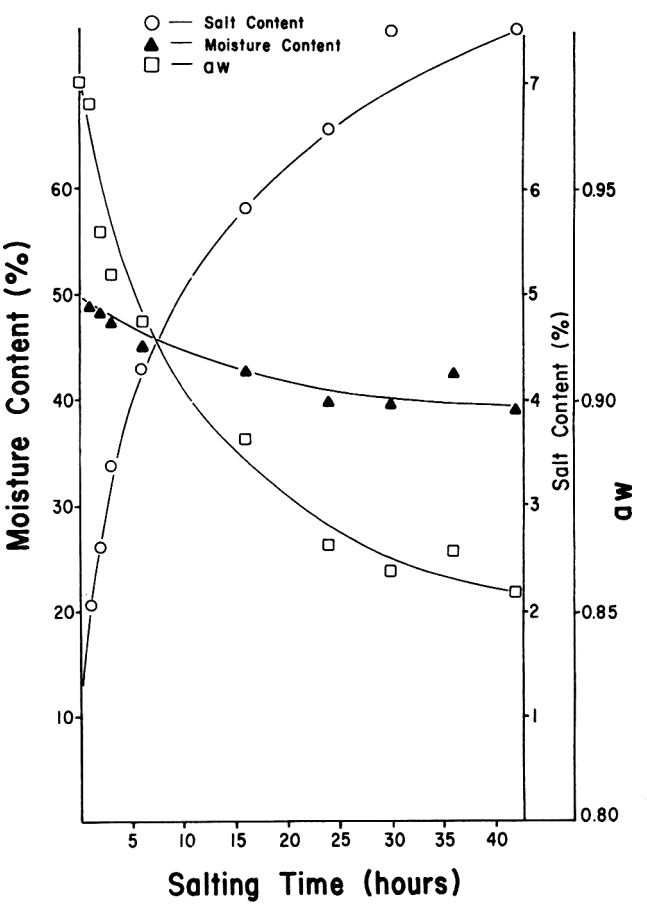

Fig. 1–Effect of salting time (15% salt) on water activity, moisture, and salt content of mullet roe.

Table 2—Number of raw mullet in various sizes

Weight range[b] (g)	1975[a] No. of samples	1975[a] % of Total count	1976[a] No. of samples	1976[a] % of Total count	Total No. of samples	Total % of Total count
106–149	14	11.76	19	12.18	33	12.00
150–199	46	38.66	60	38.46	106	38.55
200–249	41	34.45	53	33.97	94	34.18
250–299	16	13.45	19	12.18	35	12.73
300–360	2	1.68	5	3.21	7	2.54
Total count	119	100.00	156	100.00	275	100.00
Average wt	196.51g		202.91g		200.14g	

[a] Obtained at the end of November

Table 3—Proximate composition[a] of frozen mullet roe

Composition	%
Moisture	52.00
Crude protein	25.96
Crude fat	15.05
Carbohydrate[b]	5.68
Ash	1.31
Total	100.00

[a] Mean of two samples in duplicate
[b] Calculated by subtraction from total

plastic bag and frozen at −34°C until used. In each experiment, the desired number of frozen roe were thawed for 1 day at 2°C before further processing.

Salt penetration

Thawed roe were salted at 27°C on trays with various amounts of salt for certain intervals according to a two factor (percent of curing salt and salting time) central composite experimental design (Cochran and Cox, 1957), each at five different levels (Table 1). Eleven combinations were chosen in random order according to the design for two factors. The range of salting time was decided according to preliminary experiments (Fig. 1) and the range of salt content was chosen based on literature (Lee, 1977). Salt and moisture contents for overall and central regions were determined in duplicate for each sample. The data were analyzed using the Statistical Analysis System (SAS) program package for the analysis of variance calculations. Surface contours were also calculated by computer.

The thawed roe were also salted at 27°C with 15% salt on the roe weight basis for 42 hr. At various time intervals, two samples were taken for the determination of water activity, moisture content, and salt content.

Dehydration of salted roe (without desalting)

The production of cured and dried mullet roe generally includes salting, desalting and sun-drying for 1–2 wk. A short salting time (1 hr) with 15% salt was tried in an attempt to eliminate the desalting process. Both open sun-drying, and mechanical air dryer with controlled temperature, air flow rate, and humidity were used to dry the mullet roe.

Thawed roe were salted with 15% salt for 1 hr and pressed. The roe were either hung and dehydrated in a room with a temperature of 27°C, a relative humidity of 74% and an air flow speed of 150 ft/min for 74 hr, or they were sun-dried on a wooden board. Two roe were taken for the determination of water activity, moisture content, and salt content at various dehydration intervals.

Open sun-drying of cured roe

Thawed roe were placed on trays (15 in. wide × 20 in. long) and salted at 27°C with 15% salt for 28 hr; 10 and 20% salt could also be used, but the salting time would be different. The trays were overlapped during salting to press the roe. The salted roe were washed to free solid salt from the surface and desalted in a plastic tub containing twice the amount of water on the roe weight basis for 6 hr. The roe were placed on the trays and sun-dried for 7 days. Twice each day the roe were sun-dried for a total of 6 hr (3 hr after sunrise and 3 hr prior to sunset). The study was conducted in August with the temperature around 30°C. Two roe were taken from each step of processing for the determination of water activity, moisture content, and salt content.

Proximate analysis

All analyses were performed in duplicate according to AOAC (1970). In addition to ether extraction, the chloroform-methanol method developed by Bligh and Dyer (1959) was also used to determine the total fat for comparison.

Determination of water activity

Water activity was determined by an Electric Hygrometer (Hygrodynamics, Inc., Silver Spring, Md) at 27°C. Samples were sliced, weighed (20g), and sealed in air-tight jars in which a temperature-humidity sensor of the appropriate range was fitted through the lid. The sensors were checked against standard salt solutions for accuracy. After approximately 24 hr, an equilibrium between the product and the surrounding atmosphere inside the jar was reached. The dial reading was recorded and converted to water activity or relative humidity using a humidity calibration chart (Karmas and Chen, 1975).

Salt content

Salt content was determined in duplicate according to AOAC (1970).

RESULTS & DISCUSSION

Proximate composition of raw mullet roe

Mullet roe is largest from mullet caught right before spawning. Table 2 shows the size of mullet roe used in this experiment. The weight of mullet roe ranged from 106–360g with an average weight of 200g. A major portion of the roe (slightly over 72% of the total samples used) was found in the range 150–249 g. Iyengar and Schlenk (1966) reported that mullet caught in the fall of 1964 and 1965 on the coast of the Gulf of Mexico (near Pascagoula, Miss.), had roe ranging from 15–55g. This indicates that the size of roe varies with the location. The roe harvested along the southwest coast of Florida are larger than those landed along the northwest coast of Florida, which correlates with the size of mullet (Deng et al., 1977).

The eggs were encased in a very thin ovarian membrane and located in the cavity of the mullet. Frequently the membrane was broken during separation of the roe from the fish, and the damaged roe was not suitable for further processing. In order to get perfect roe out of the mullet without breaking the membrane, extreme care has to be taken during separation and handling of the roe. It was found that by using a pair of scissors with dull heads instead of using a knife, the possibility of breaking the membrane was reduced. Most of the roe were light in color; however, some were dark. It was observed that dark roe had a higher blood content than light roe. The color of raw mullet roe affects the appearance of the final product. Blood was difficult to separate after the roe were frozen; therefore, the blood was removed from the fresh roe. Both mullet and roe have a high content of polyunsaturated fatty

acid (Deng et al., 1976; Iyengar and Schlenk, 1966) which is susceptible to oxidation. Fischer and Deng (1977) reported that heme iron was the major catalyst responsible for oxidation in mullet dark flesh; therefore, the reduction of blood in the roe may reduce the development of oxidative rancidity during storage.

Table 3 indicates the proximate composition of frozen mullet roe which held in a freezer (−34°C) for 14 months before they were thawed and analyzed. A high lipid content (19.68%) was found by using the chloroform-methanol method (Bligh and Dyer, 1959). This result was similar to that reported by Iyengar and Schlenk (1966). Using $CHCl_3$-CH_3OH (2:1) solvents, they extracted 20% lipids from the roe (ranging in size from 15–55g). Generally, very fine particles resulted from blending mullet roe with organic solvents, and filtration of the homogenate was difficult and slow. Consequently, smaller amounts of sample (about 5–10g) were used for extraction. Moisture (53.50%) detected in fresh mullet roe was greater than that of frozen roe (52%). This is due to dehydration of roe during frozen storage.

Salt penetration using surface response methodology

Regression analysis. The raw experimental data obtained for four dependent variables (overall moisture and salt uptake as well as moisture and salt uptake of central region) are shown in Table 4. Analysis of variance (ANOVA) summaries are given in Table 5, for the four dependent variables. Multiple correlation coefficients (R^2) reveal the correlation between predicted responses and observed responses. The higher the multiple correlation coefficient (approaching 1), the better the empirical model fits the actual data.

The results show that R^2 values for all dependent variables are large except for moisture content in the central region. Regression coefficients of mathematical models for the responses are presented in Table 6.

A possible explanation of the low R^2 in the response moisture content of the central region may be the experimental variation due either to the difficulty in collecting from the same central region of roe or to the nonuniform sample size of roe. Predictions concerning both responses, salt uptake in whole mullet roe, and central region of roe may be made with 99% confidence. Regression coefficients and their statistical significance for these dependent variables are shown in Table 6. According to the tests, salting time is probably the primary factor influencing these responses since it is highly significant ($\alpha<0.01$) for all responses. There is an exception with Y_2 (moisture content of the central region) being significant at a 5% level. Percent of curing salt had a significant effect on the salt uptake in the central region.

Salt uptake. Predicted responses of salt uptake for three levels of curing salt and various intervals of salting time are shown in Figure 2. The amount of salt uptake of mullet roe

Table 4—Effect of independent variable combinations upon dependent variable

Trial no.	Independent variables[a]		Dependent variables[b]			
	X_1	X_2	Y_1	Y_2	Y_3	Y_4
1	−1	−1	45.94	47.05	5.00	2.43
2	1	−1	42.83	45.25	4.78	3.47
3	−1	1	42.97	46.22	7.05	7.37
4	1	1	38.06	43.27	9.98	10.91
5	$-\sqrt{2}$	0	44.28	45.15	4.57	3.93
6	$-\sqrt{2}$	0	40.10	40.20	7.80	6.89
7	0	$-\sqrt{2}$	49.47	50.36	1.78	1.55
8	0	$\sqrt{2}$	35.46	39.40	9.75	9.34
9	0	0	40.70	43.27	8.70	7.88
10	0	0	40.77	41.53	6.80	7.01
11	0	0	41.60	44.04	6.99	6.93

[a] X_1, X_2 (independent variables) refer to Table 1.
[b] Y_1, Y_2, Y_3, Y_4 (dependent variables) refer to Table 5.

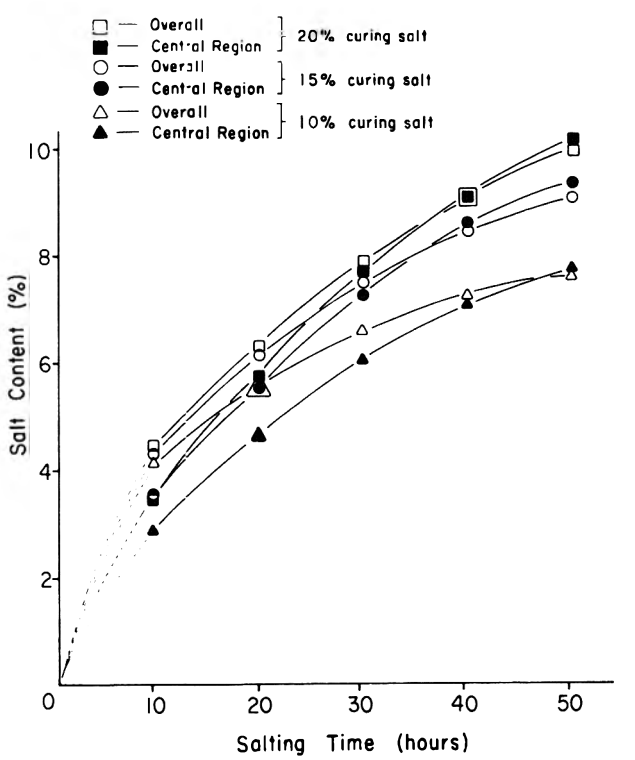

Fig. 2—Predicted salt uptake in mullet roe cured in 10, 15, and 20% salt.

Table 5—Definitions and analysis of variance (ANOVA) summaries for dependent variables

Dependent Variables	Code	R^2	Mean square regression	Mean square error	F	Probability
Moisture content (overall)	Y_1	0.865	24.81	3.87	6.40	0.03*
Moisture content (central region)	Y_2	0.704	14.08	5.93	2.37	0.18
Salt uptake (overall)	Y_3	0.906	11.01	1.15	9.58	0.01**
Salt uptake (central region)	Y_4	0.981	16.95	0.32	53.52	0.0002**

* Significant at α <0.05
** Significant at α <0.01

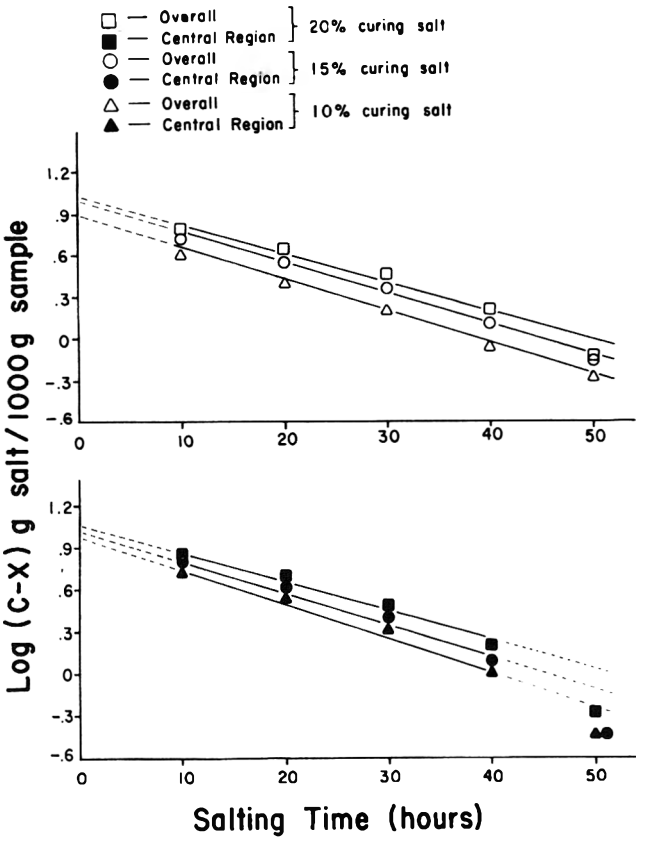

Fig. 3—Predicted salt penetration into mullet roe cured in 10, 15, and 20% salt (semi-logarithmic plot). log (C − X) = (t/k) + log C where C = maximum salt concentration, X = salt concentration at time t, and K = time constant.

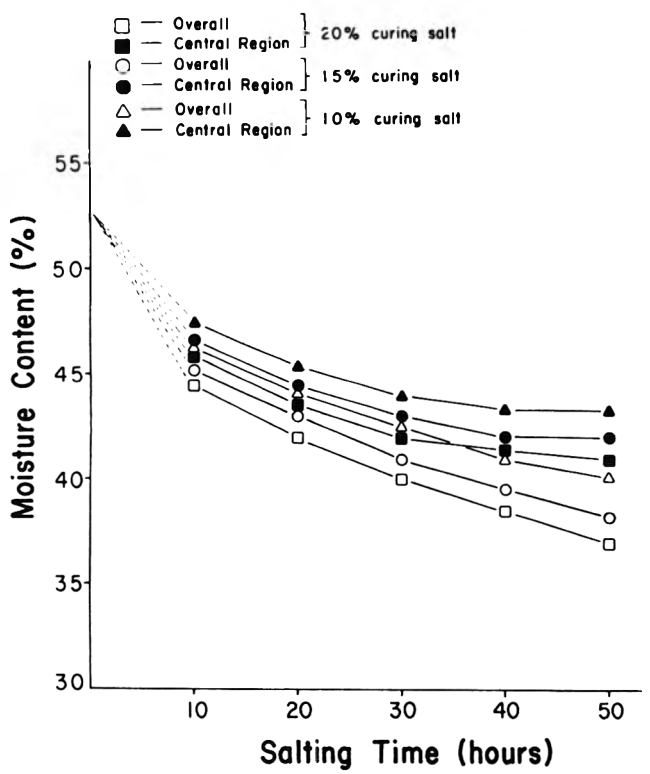

Fig. 4—Predicted moisture content in mullet roe cured in 10, 15, and 20% salt.

cured with various percents of solid salt increased as salting time increased. The difference between the maximum salt concentrations and the concentration at any time in the salting were plotted on semi-logarithmic paper as a function of the salting time (Fig. 3). The data points follow first order kinetics, $X = C(1 - e^{-(t/k)})$, where X is the concentration at time t, C is the maximum concentration, and k is the time constant. With a 10% curing salt, the time constant (hr) increased from 42.25 and 45.27 for whole roe and central region, respectively, to 44.39 and 50.07 for those cured with a 20% salt.

Moisture change. Predicted values of moisture change with three levels of curing salt and various intervals of salting time are shown in Figure 4. The whole roe lost more moisture than the central region of the roe which indicates less water was remaining in the exterior than interior. First order kinetics appeared to occur only during 10-hr salting. The water change of mullet roe between 10- and 50-hr salting appeared to follow zero order kinetics, especially the change in whole roe.

Dehydration of salted roe (without desalting)

Although the processing time for both open sun-drying and mechanized air-drying was different, the quality of final product was all undesirable. Thus, only the data with the mechanical dryer are discussed. Generally, a high flow rate will cause a wrinkled roe surface, which results from a too quick drying rate on the surface without permitting the moisture equilibrium between interior and exterior of the roe. A low flow rate was thus used in the drying process.

The changes in water activity, moisture, and salt content in salted mullet roe during the drying process are shown in Figure 5. Moisture content and water activity decreased

Table 6—Regression coefficient for four dependent variables

Model term	Regression coefficient[a]			
	Y_1	Y_2	Y_3	Y_4
Constant	52.71	52.75	1.05	2.49
X_1[b]	−0.52	−0.32	0.25	0.49**
X_2[c]	−0.22**	−0.28*	0.12**	0.18**
X_1^2	0.01	0.006	−0.009	−0.02
X_2^2	0.002	0.003	−0.002	−0.002*
X_1X_2	−0.003	−0.002	0.005	0.004

[a] Y_1, Y_2, Y_3, Y_4 (dependent variables) refer to Table 5.
[b] X_1 = percent of curing salt.
[c] X_2 = salting time.
* Significant at $\alpha < 0.05$
** Significant at $\alpha < 0.01$

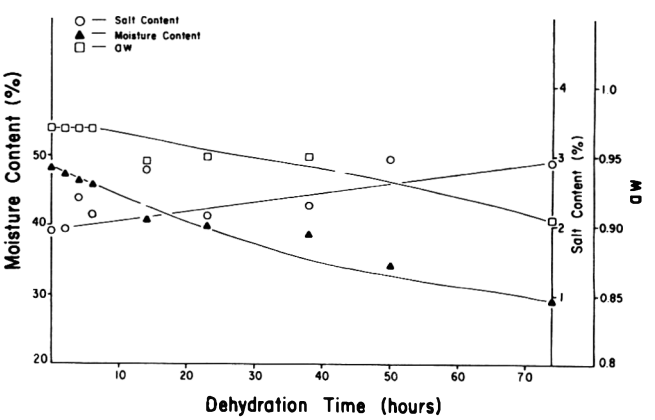

Fig. 5—Effect of dehydration (mechanical method) on the water activity, moisture, and salt content of salted mullet roe.

from 48.6% and 0.970 to 29.6% and 0.905, respectively, after 74-hr dehydration. The salt content increased from 1.9% to 2.9%. Approximately 48-hr additional drying would be needed to lower the water activity level to 0.850; however, the experiment was not continued due to hard exterior and soft interior after 74-hr drying. Greater salt uptake in the external layer in comparison with the interior caused more denaturation of surface protein and hardened the exterior of the roe during the initial drying process. Moisture diffusion rate from interior to exterior was thus slower than the drying rate on the roe surface.

Since a short salting (1 hr) process without desalting would result in an undesirable product, a long salting procedure with a desalting step was used in the later experiments. It was also determined that desalted roe could be shaped easier than roe without desalting during the pressing step. Final roe shape is one of the important factors contributing to product quality.

Open sun-drying (with desalting)

Figure 6 shows the changes in moisture content, salt content, and water activity of mullet roe at each processing step of open sun-drying. Salting (15% solid salt) resulted in water loss and salt uptake which depressed the water activity from 0.975 to 0.858. On the other hand, water activity increased after desalting due to water absorption and decreased salt content. During sun-drying, water activity decreased and salt content (on a wet basis) increased as dehydration proceeded. The water activity of the product reached 0.832 after 7 days sun-drying. This level is generally considered bacterially stable (Brockmann, 1973; Desrosier, 1970). The product had 26.5% moisture and 5.1% salt. A composition for Karasumi (cured mullet roe) was reported by a Japanese researcher (Anonymous) as 23% moisture and 4.2% salt. With these levels of moisture and water activity, the cured roe may be considered an intermediate moisture food (Potter, 1970). Growth of fungi on the roe during the drying stage was found. This was expected since no fungi inhibitor was used. It is difficult to control the quality of traditional products by using sun-drying due to the varation of processing parameters such as temperature, humidity, etc. In Florida, the frequent rain and varied humid climate limit application of the conventional sun-drying method. An improved method, using a mechanical drier equipped with humidity, temperature, and air flow control would be desirable to process and prepare a uniform intermediate moisture mullet roe.

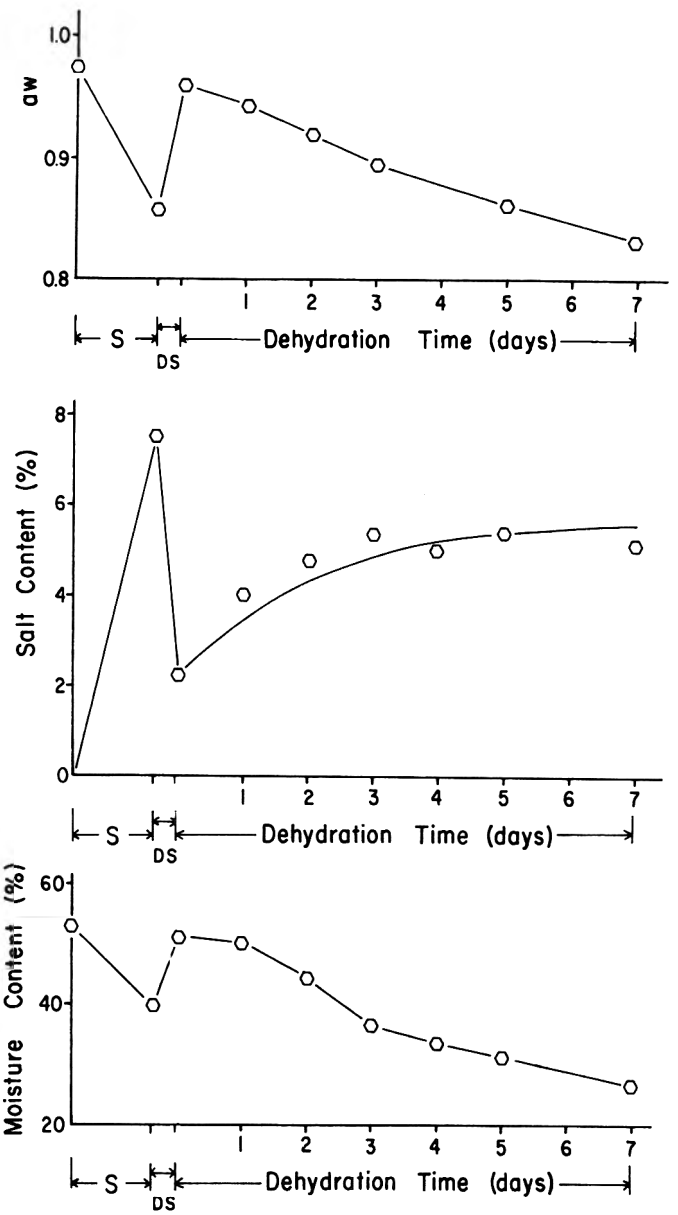

Fig. 6—Water activity, moisture, and salt content of mullet roe during processing with traditional method (S = 28-hr dry salting; DS = 6-hr desalting; dehydration = sun-drying).

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EFFECT OF SALTING TIME, DEHYDRATION TEMPERATURES AND DEHYDRATION TIME ON QUALITY OF INTERMEDIATE MOISTURE MULLET ROE

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ABSTRACT

A mechanical drying method for processing an intermediate moisture mullet roe was developed to replace the traditional sun-drying method. Using a central composite experimental design, 20 combinations of three independent variables (salting time, dehydration temperature, and dehydration time) with constant desalting time and relative humidity were performed and analyzed for salt content and water activity value. It was discovered that various processing combinations exist that might produce products with the same level of salt and water activity. Product color was used in the selection of processing combinations in addition to salt content and water activity.

INTRODUCTION

BLACK MULLET is the most abundant of the fin fish in Florida coastal waters. The average landing of black mullet from 1969 to 1973 in Florida was 27.4 million pounds; the total value was approximately 2.5 million dollars (Pierce, 1975). Mullet are landed along the coastal area during all months of the year; however, the spawning period of mature mullet is generally from October to February. Mullet roe has better flavor quality during the spawning period because of the higher lipid content.

Recently several foreign countries have begun importing mullet roe from Florida (Cato et al., 1976). The market for mullet roe in the future looks very promising. The cost of mature raw mullet roe in certain Asian countries is about $10.00/lb as opposed to about $1.00/lb in America. A delicious taste but limited catch makes mullet roe one of the food delicacies in certain Asian countries.

Generally, mullet roe are salted, pressed, and sun-dried for a certain period of time to produce a yellow-brown product (Hsu and Deng, 1980). This open sun-drying process, however, has a high labor cost and is limited by climate. An improved method could be developed which would make it beneficial for the Florida fish industry to process the mullet roe and sell the higher priced products instead of the cheaper raw mullet roe to foreign countries as well as to supply the potential domestic customers. Thus, in this study the method of drying cured mullet roe by a mechanical drier was investigated.

A response surface analysis was performed to determine the possible optimum combination levels of three factors (salting time, dehydration temperature, and dehydration time) with other factors constant to produce a desirable intermediate moisture roe.

MATERIALS

MULLET (*Mugil cephalus*) were purchased from Placida, Fla., (Gasparilla Store) at the end of November (spawning season) in 1976. The fish were transported on ice to Gainesville, Fla., where the fish roe, encased in an ovarian membrane, were separated from the fish and cleaned. The cleaned roe were tied at the end, placed in a plastic bag, and frozen at −30°F until used. The desired number of frozen roe were thawed for 1 day at 36°F before further processing.

Table 1—Definition and levels of three independent variables

Independent variables	Code	Levels				
		−1.682	−1	0	1	1.682
Salting time (hr)	X_1	1.0	5.9	13.0	20.1	25.0
Dehydration temp (°F)	X_2	35.0	48.2	67.5	86.8	100.0
Dehydration time (hr)	X_3	24.0	43.5	72.0	100.5	120.0

Preparation of intermediate moisture mullet roe

A three factor (salting time, drying temperature, and drying time) central composite design was used to obtain empirical models so that prediction of the most desirable processing conditions for preparation of intermediate moisture mullet roe could be made. Five levels of each of the three factors were chosen for study. Twenty combinations, including six replicates of the center point, were tested in random order according to a central composite rotatable design configuration for three factors (Cochran and Cox, 1957). Actual values of the three independent variables are shown in Table 1. The data were analyzed using the SAS program package for the analysis of variance calculations and the APL system for generating the surface contours.

The thawed roe were placed on trays and salted with 15% salt on the roe weight basis for the interval according to the design. The salted roe were desalted in a tub containing twice the amount of water on the roe weight basis for 6 hr. The desalted roe were hung in a room (7'3" × 6'4" × 7'3") at various combinations of temperatures and time intervals according to the design. The room was controlled at 74% relative humidity and air velocity at about 150 ft/min (Alnor series 6000-P velometer with Lo-Flow probe, Alnor Instrument Company, Niles, Ill.).

Chemical analysis

Proximate composition and salt content were measured according to the procedure of AOAC (1970). Water activity was determined with an electronic hygrometer. Sample preparation was similar to Hsu and Deng (1980).

RESULTS & DISCUSSION

Regression analysis

In the processing of intermediate moisture mullet roe with a mechanical drier, the product quality is affected by such factors as amount of curing salt, salting time, volume of water for desalting, desalting time, air flow rate, relative humidity, dehydration temperature, and dehydration time. Of these factors, salting time, dehydration temperature, and dehydration time were considered the most important. The effect of these three factors at various levels was investigated; the other factors were kept at a constant level.

Raw experimental data for three dependent variables, water activity, total moisture, and salt content, were obtained from 20 combinations of three independent vari-

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ables, salting time, dehydration temperature, and dehydration time, as shown in Table 2. Analysis of variance (ANOVA) summaries are given in Table 3 for the three dependent variables.

The results indicate the R^2 values for all dependent variables were high; therefore, predictions concerning these responses may be made with confidence ($\alpha = 0.01$). Regression coefficients and their statistical significance (probability level) for the dependent variables are given in Table 4.

Table 2–Effect of combinations of three independent variables on three dependent variables

Independent variables			Dependent variables		
X_1 Salting time (hr)	X_2 Dehydration temp (°F)	X_3 Dehydration time (hr)	Y_1 Moisture (%)	Y_2 Salt (%)	Y_3 A_w
13.0	35.0	72.0	44.01	3.79	0.937
5.9	48.2	43.5	50.39	2.36	0.970
5.9	48.2	100.5	38.74	2.65	0.944
20.1	48.2	43.5	49.14	3.92	0.940
20.1	48.2	100.5	36.63	5.26	0.893
13.0	67.5	24.0	48.71	3.25	0.950
13.0	67.5	120.0	37.06	4.17	0.915
1.0	67.5	72.0	40.17	1.20	0.978
25.0	67.5	72.0	36.86	4.41	0.910
13.0	67.5	72.0	44.15	3.34	0.959
13.0	67.5	72.0	42.18	3.12	0.954
13.0	67.5	72.0	40.95	3.16	0.959
13.0	67.5	72.0	38.49	3.81	0.937
13.0	67.5	72.0	43.89	3.95	0.952
13.0	67.5	72.0	37.64	3.74	0.941
5.9	86.8	43.5	43.47	2.22	0.974
5.9	86.8	100.5	31.88	2.43	0.935
20.1	86.8	43.5	41.22	3.97	0.945
20.1	86.8	100.5	31.08	5.63	0.857
13.0	100.0	72.0	31.15	4.10	0.889

Table 3–Analysis of variance summaries for three dependent variables

Dependent variables	Code	R^2	F	Probability
Moisture content (%)	Y_1	0.904	10.43	0.0005**
Salt content (%)	Y_2	0.956	24.01	0.0001**
Water activity	Y_3	0.908	10.99	0.0004**

** Significant at $\alpha<0.01$

Table 4–Regression analysis of three dependent variables

Model term	Regression coefficient[a]		
	Y_1	Y_2	Y_3
Constant	59.0000	4.5660	0.7554
X_1[b]	0.2398	0.1119**	0.0024**
X_2[c]	0.1794**	–0.0705**	0.052**
X_3[d]	–0.3420**	–0.0252**	0.0017**
X_1^2	–0.0157	–0.0047**	0
X_2^2	–0.0030	0.0004	0
X_3^2	0.0009	0.0001	0
X_1X_2	0.0003	0.0007	0
X_1X_3	0.0004	0.0015**	0
X_2X_3	0.0006	0	0

a Y_1, Y_2, Y_3 (dependent variables) refer to Table 2.
b X_1 = salting time.
c X_2 = dehydration temperature.
d X_3 = dehydration time.
* Significant at $\alpha<0.05$
** Significant at $\alpha<0.01$

According to significance tests, the moisture content (Y_1), salt content (Y_2), and water activity (Y_3) were all highly influenced by salting time, dehydration temperature, and dehydration time.

Quality factors

Contours of constant response values were computed for the three responses: salt content, water activity, and moisture content.

Some representative contours are shown in Figures 1, 2, and 3 for four fixed levels of dehydration temperature. The contour lines represent estimated responses. For example, any point on the 0.9 contour (Fig. 3) should represent a particular combination of salting time and dehydration time which gave a water activity value of 0.9 at a fixed level of dehydration temperature. As dehydration temperature increased, the processing time required for salting and drying to reach the same levels of water activity and salt content decreased (Fig. 2 and 3). For example, with 13 hr of salting, 72 hr of dehydration at 67.5°F was necessary for mullet roe to reach water activity of 0.95; however, at 77.2°F, only 61 hr of dehydration were necessary for roe to obtain the same level of water activity (Fig. 3).

Generally, at a fixed level of two independent variables, an increase in the level of the third variable decreased the water activity (Fig. 3) but increased the salt content (Fig. 2) of mullet roe after processing.

The main concern in the development of intermediate moisture mullet roe of H_2O activity since it is the basic control factor in the preservation of this type of roe. Brockmann (1970) indicated that spores cannot germinate and relatively few species of bacteria, including only one food pathogen, can multiply at a 0.9 water-activity level. Under favorable conditions, *Staphylococcus aureus* has been observed to grow at water activity as low as 0.86. Halophilic bacteria may grow at water activity as low as 0.75, which corresponds to a saturated solution of sodium chloride. The

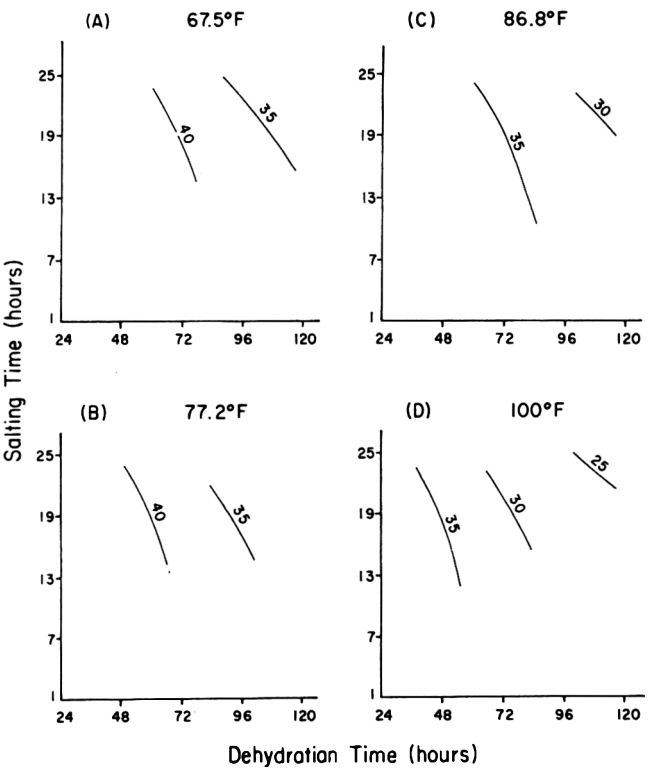

Fig. 1–Response surface contours for moisture content as functions of salting and dehydration time at four dehydration temperatures.

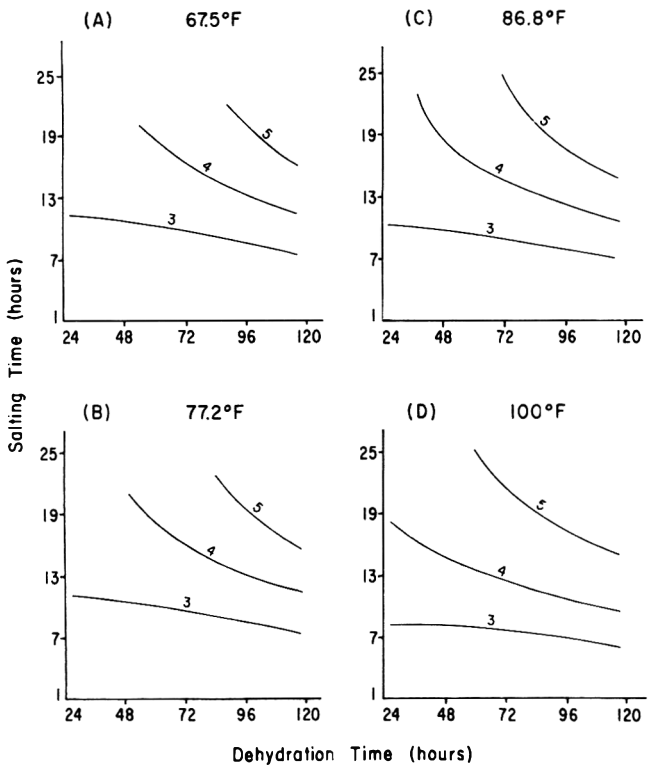

Fig. 2—Response surface contours for salt uptake (%) as functions of salting and dehydration time at four dehydration temperatures.

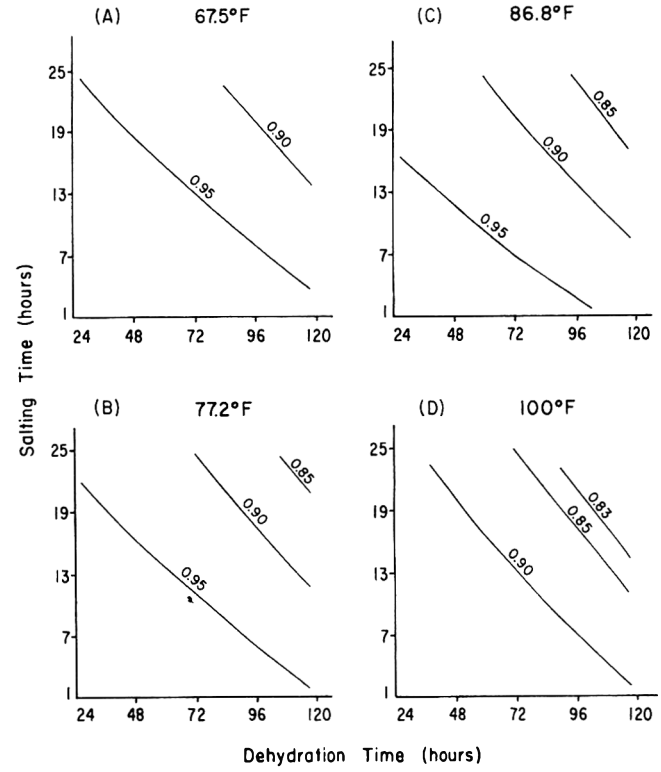

Fig. 3—Response surface contours for water activity as functions of salting and dehydration time at four dehydration temperatures.

common species of yeast and mold are suppressed at water activity of 0.88 and 0.80, respectively, while the limits of xerophilic molds and osmophilic yeast are stated to be 0.65 and 0.62, respectively. Based solely upon the potential limits of microbial growth, an intermediate moisture food would be required to have a water activity level of 0.60 or less; however, at this water activity it would be difficult to distinguish most common foods from their dry counterparts. A study by Collins and Yu (1975) indicated that catfish flesh, essentially free of bacteria, yeasts, and molds, could be prepared as an intermediate moisture deep-fried product with water activities of 0.76 and 0.80. Processed and packaged meat products normally require a water activity of 0.85 (Kramlich et al., 1973). Thus, the water activity level for an acceptable intermediate moisture mullet roe product would be 0.85 or less.

Salt is used for depressing water activity and imparting desirable flavor to foods. Salt levels are varied, and they are a matter of personal preference. Because of the availability of home refrigerators to prolong the shelf-life of lightly salted foods, these foods are in greater demand than heavily salted foods. An acceptable level of salt in bacon is 2%, whereas in ham it is 3%. The average salt content of a lightly salted fish may be as low as 4% (Cream, 1961). It was reported that traditionally dried and cured mullet roe had 4.2% salt and 23% moisture (Anonymous, 1973). Therefore, both a water activity of 0.85 and a salt content of 4% are considered desirable levels in intermediate moisture mullet roe. A 25% moisture level in cured mullet roe is within the moisture range of intermediate moisture food (Potter, 1970).

Prediction of expected values of dependent variables (water activity, salt, and moisture contents) may be calculated by substituting appropriate values of independent variables (salting time, dehydration temperature, and dehydration time) into the appropriate regression models. According to Figure 3, mullet roe would reach water-activity levels of 0.85 or less at dehydration temperatures of 86.8 and 100°F, a salting time up to 25 hr, and a dehydration time up to 120 hr. By using the dehydration temperatures of 86.8 and 100°F, the predicted desirable qualities (water activity of 0.83–0.85 and 4% salt content) for the product were calculated from the combination of certain levels of the other two factors (salting time and dehydration time). The predicted responses were compared with the observed responses (actual experimental data) as shown in Table 5. Generally, the prediction outside the region of experimentation is discouraged because no estimate of error exists (prediction variance) for outside of the region. However, the estimates are quite close to the actual values and the authors feel reasonably confident about extrapolating outside the experimental region. Response surface plots were

Table 5—Predicted and observed values of water activity and salt content for intermediate moisture mullet prepared by various combinations of independent variables

Combination of independent variables[a]	Dependent variables	Predicted values	Observed values	Difference
X_1 = 8.0 X_2 = 100.0	a_w	0.830	0.843[b]	±0.013
X_3 = 140.0	salt cont (%)	4	3.96	±0.04
X_1 = 7.4 X_2 = 86.8	a_w	0.830	0.835[c]	±0.005
X_3 = 164.0	salt cont (%)	4	4.07	±0.07
X_1 = 8.4 X_2 = 86.8	a_w	0.850	0.838[d]	±0.012
X_3 = 149.0	salt cont (%)	4	3.7	±0.3

[a] X_1 = salting time (hr); X_2 = dehydration temperature (°F); X_3 = dehydration time (hr).
[b] Mean of three samples
[c] Mean of two samples
[d] Mean of four samples

Table 6—Effect of processing on quality of intermediate moisture mullet roe

Processing conditions	A 8-hr salting 140-hr dehydration at 100°F	B 7.4-hr salting 164-hr dehydration at 86.8°F	C 8.5-hr salting 149-hr dehydration at 86.8°F
	Product quality		
a_w	0.83	0.83	0.85
Salt content	4%	4%	4%
Color	dark	dark brown	golden brown

Table 7—Composition[a] of freshly prepared intermediate moisture mullet roe (Type C)

Composition	%
Moisture	24.19
Crude protein	41.16
Crude lipid	18.17
Carbohydrate[b]	12.19
Ash[c]	4.29
Total	100.00

[a] Means of two samples in duplicate
[b] Calculated by subtraction from total
[c] Including salt (NaCl) 4.18%

generated by computer to aid in visualizing the effect of these independent variables (Fig. 4 and 5). Dotted points indicate the estimated response outside the region of design. Any intersection of contours from two dependent variables represents predictions of combinations of three independent variables. For example, point A (Fig. 5) indicates the estimated response of 0.83 water activity and 4% salt content in final intermediate moisture mullet roe that results from the combination of 8-hr salting time and 140-hr dehydration time at 100°F (with other factors constant as discussed in the Experimental section).

Although many combinations of these three independent factors may be used to produce the same response of the two dependent variables, water activity and salt content, it is possible that different colors may be detected in the final products (Table 6). Roe A was processed with 8-hr salting and 140-hr dehydration at 100°F. Roe B was processed with 7.4-hr salting and 164-hr drying at 86.8°F. Roe C was processed with 8.4-hr salting and 149-hr drying at 86.8°F. A golden brown color is generally considered acceptable for cured and dried mullet roe (Anonymous, 1973). In this study, color acceptability of the roe increased in the following order: A, B, and C. Although Roe A and B had the same levels of water activity (0.83) and salt content (4%), Roe A was darker than Roe B. The higher drying temperature of 100°F for Roe A in comparison with 86.8°F for Roe B probably caused a greater degree of nonenzymatic browning reaction and oxidation that resulted in the darker color. On the other hand, Roe B and C had the same salt content but different levels of water activity. Labuza et al. (1970) reported that at water-activity levels above 0.80, oxidation and browning reaction increased as the water-activity value decreased; therefore, the darker color of Roe B might be the result of the lower water activity of Roe B (a_w=0.83) as compared with Roe C (a_w=0.85). After considering these three quality factors—color, water activity, and salt content—the processing combinations of Roe C were found to be the most acceptable and were used for further study. The three selected processing factors were 8.4-hr salting and 149-hr dehydration at 86.8°F. Acceptable products could be produced with a dehydration temperature lower than 86.8°F; however, a longer processing time would be needed which is not economical. Microbiological quality is one of the important factors contributed to food acceptability. Additional studies are underway concerning the product safety and storage stability.

Proximate analysis

Proximate composition of intermediate moisture mullet roe (type C) are shown in Table 7. Protein content was the highest (41.6%), followed by moisture (24.19%), lipid

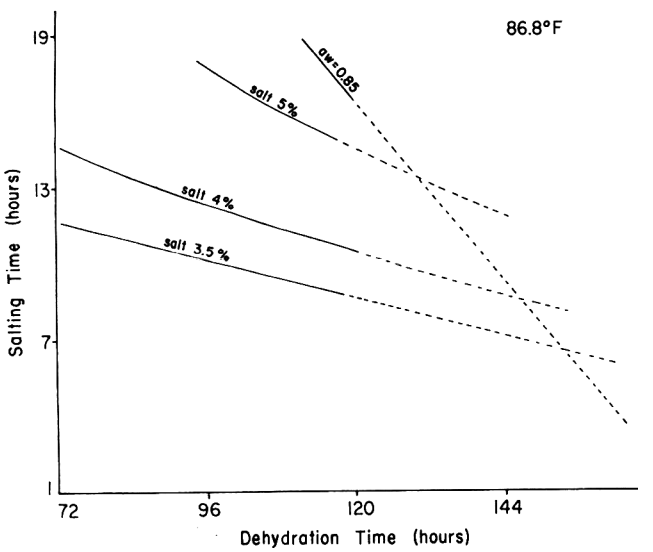

Fig. 4—Response surface contours for water activity and salt content as functions of salting and drying times (dehydrated at 86.8°F).

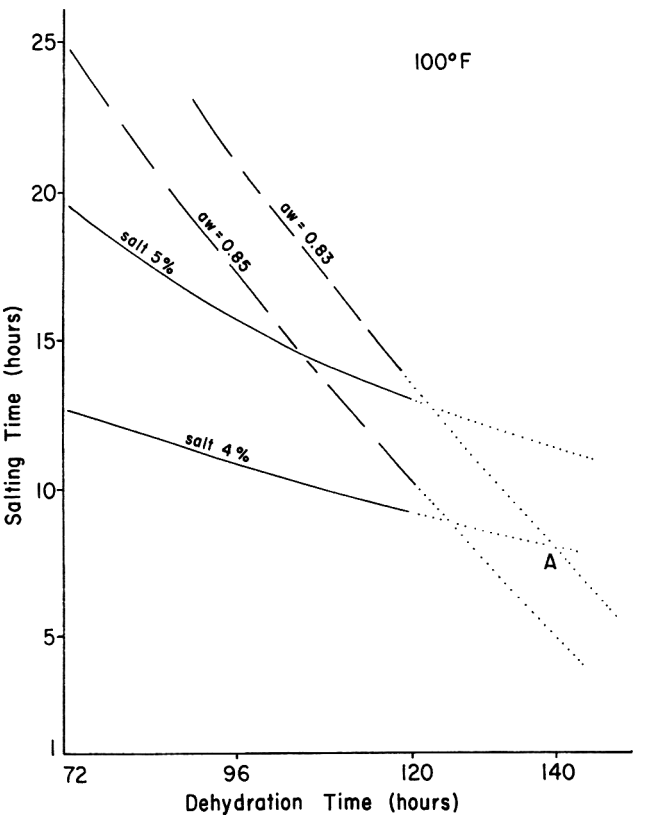

Fig. 5—Response surface contours for water activity and salt content as functions of salting and drying times (dehydrated at 100°F).

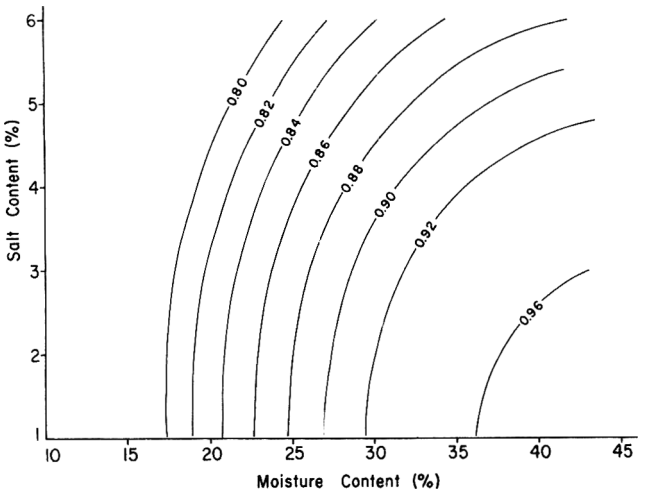

Fig. 6—Response surface contours for water activity (Y) as functions of moisture (X_2) and salt content (X_1).

(18.17%), carbohydrate (12.19%), and ash (4.29%). The yield (wet basis) of intermediate moisture mullet roe was 64.23% (calculated from 57 samples) of fresh wet roe. Processing procedure for the roe were as follows: The thawed roe were salted with 15% salt for 8.4 hr and pressed with overlapped trays. The roe were desalted for 6 hr with twice the amount of water and dehydrated for 149 hr at 86.8°F, 74% RH, and 150 ft/min air velocity.

Water activity as functions of moisture and salt contents in mullet roe

Although different foods may have the same moisture content, the water-activity levels are not necessarily the same due to varied compositions with different binding properties (Brockmann, 1973; Kaplow, 1970). The interrelationship of moisture content and water activity is described by a moisture sorption isotherm. Salt can be used as an additive for controlling the water activity (Brockmann, 1973). Salt depresses water activity uniformly in the solution as concentration increases (Karmas and Chen, 1975). It was assumed that relationship existed between moisture content, salt content, and water activity in the mullet roe. An investigation was conducted using the SAS program package to analyze data from 51 observations of moisture content, salt content, and water activity in mullet roe processed with various combinations of the three independent factors. Since the multiple correlation coefficient is high (0.967), the mathematical model $Y = 0.516 + 0.0121\ X_1 + 0.0198\ X_2 - 0.0033\ X_1^2 - 0.002\ Y_2^2 - 0.0002\ X_1X_2$ can be used for prediction with confidence ($\alpha<0.01$). According to significance tests on the estimates of the regression model, both moisture content (X_2) and salt content (X_1) influence water activity (Y) since the coefficient values of the X_1 and X_2 term are highly significant for the response. Thus, the predicted value (water activity) can be calculated by substituting appropriate values of moisture content and salt content. Contours of constant response for water activity were computed and shown in Figure 6. With two factors known, the third factor can be calculated in the cured mullet roe.

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CUTTING YIELDS AND PALATABILITY TRAITS OF HAND-CUT OR PRESSED-CLEAVED, AND FRESH, CRUST-FROZEN OR FROZEN-TEMPERED BEEF SUBPRIMALS

J. W. SAVELL, G. C. SMITH, and D. L. HUFFMAN

ABSTRACT

Beef carcasses of similar grade characteristics provided 15 pairs each of strip loins, top sirloin butts and tenderloins. Subprimals were prepared according to Institutional Meat Purchase Specifications (IMPS) for boneless cuts, vacuum packaged, stored for 13 days at −1°C and then trimmed to steak-ready form. Trimmed subprimal cuts were assigned to one of three treatment groups: (I) fresh (not frozen), hand-cut; (II) crust-frozen (2.33 hr at −34°C, 0.75 hr at −1°C), pressed-cleaved; and (III) frozen (8 hr at −34°C), tempered (24 hr at −1°C), pressed-cleaved. Percentage yield of salable cuts from pressed-cleaved subprimals (Treatments II and III) was significantly higher than that for hand-cut subprimals (Treatment I). Few differences in thawing-cooking characteristics were observed among steaks from the three treatments. Treatment had no ($P > 0.05$) effect on sensory panel ratings for longissimus, biceps femoris or psoas major muscles but gluteus medius muscles from Treatment III were less tender ($P < 0.05$) than those from Treatment II and more tender ($P < 0.05$) than those from Treatment I. There were no differences ($P > 0.05$) in shear force values in 20 of 21 comparisons. Beef loin subprimals can be hand-cut or pressed-cleaved in fresh, crust-frozen, or frozen-tempered form with little or no differences in cooking or palatability traits among steaks.

INTRODUCTION

THE HOTEL, restaurant, and institutional trade demands products of consistent and uniform quality. Standard portion weight is not the only criterion to be met by the H.R.I. trade; standard shape and consistent thickness are important from the standpoint of uniformity in cooking properties and appearance. Mechanical portioning systems may make it possible for purveyors to sell a highly uniform product with the possibility of increasing yields of portions from each primal cut without having to employ highly-trained workmen.

Roberts et al. (1974), Davis et al. (1975), Neer et al. (1978) and Goldner and Mandigo (1974) have mechanically portioned beef or pork. Davis et al. (1975) reported that pressed-cleaved steaks had more uniform size and shape, improved eye appeal and more precisely controlled weights when compared to steaks processed by conventional methods.

Historically, the U.S. Department of Defense purchase specifications for meat products included steaks produced by mechanical portioning for use by the military. However, the Department of Defense now uses the Institutional Meat Purchase Specifications (IMPS) as an interim guideline until new Federal specifications can be developed. Under IMPS, there are no provisions for incorporation of pressing-cleaving systems because "pressing" before steak cutting is not allowed.

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Table 1—Experimental design

	Treatment		
Group	(I) Fresh, hand-cut	(II) Crust-frozen, pressed-cleaved	(III) Frozen-tempered, pressed-cleaved
A	5	5	
B	5		5
C		5	5
Total sides	10	10	10

The present study evaluated mechanical portioning of strip loins, top sirloin butts, and tenderloins into steaks under commercial production conditions. Further, because of the possible savings in time and energy which might accrue from such systems comparisons of (a) crust-frozen, pressed, and cleaved steaks with (b) frozen, tempered, pressed and cleaved steaks were made. Finally, evaluations were made of the cooking and palatability characteristics of steaks produced by (I) fresh, hand-cut; (II) crust-frozen, pressed-cleaved; and (III) frozen-tempered; pressed-cleaved systems.

EXPERIMENTAL

Selection of subprimals

Fifteen beef carcasses were selected (on the first day postmortem) at a commercial beef slaughter plant to have the following: USDA quality grade, U.S. Choice; marbling score, Modest^{00} to Modest^{100}, warm carcass weight, 295–340 kg; and USDA yield grade 2.8–3.3. On the second day postmortem, both hindquarters from each carcass were fabricated to obtain 30 each of: IMPS 180 (Strip Loins, Short Cut, Boneless); IMPS 184 (Top Sirloin Butts); and IMPS 189 (Full Tenderloins). Each subprimal was identified, vacuum packaged, boxed, and shipped to a commercial meat purveying company.

Product preparation

The 90 subprimals were held (−1°C) at the purveyor's plant for 13 days; after aging, paired subprimals were assigned to treatment groups (Table 1). All subprimals were removed from the vacuum bags, weighed, and boneless strip loins and top sirloin butts were blade tenderized once before further processing. Subprimals that were assigned to the crust-frozen, pressed-cleaved (treatment II) and the frozen-tempered, pressed-cleaved (treatment III) groups were trimmed to steak-ready form before freezing. Subprimals that were assigned to the fresh, hand-cut (treatment I) group were trimmed to steak-ready form immediately before they were cut into steaks.

Freezing and tempering

Steak-ready subprimals in the crust-frozen, pressed-cleaved (treatment II) and frozen-tempered, pressed-cleaved (treatment III) groups were tightly-wrapped with polyvinyl chloride film to avoid open seams between muscles on the prepared meat log and to give the meat log preliminary shape before pressing. Subprimals in the frozen-tempered, pressed-cleaved (treatment III) group were frozen (−34°C) for 8 hr and tempered (−1°C) for 24 hr before pressing. Subprimals in the crust-frozen, pressed-cleaved (treatment II) group were frozen (−34°C) for 2.33 hr and tempered (−1°C) for 0.75 hr before pressing.

Pressing

After tempering, the polyvinyl chloride film was removed from each subprimal and the steak-ready subprimals in treatments II and III were pressed using a Bettcher Dyna-Form Model 75 Press using the appropriate die shapes for each subprimal. Subprimals in treat-

ment II were pressed with 28 kg/cm² of pressure while subprimals in the frozen-tempered, pressed-cleaved treatment were pressed with 49 kg/cm² of pressure.

Cleaving and hand-cutting

After pressing, steak-ready subprimals were cleaved with a Bettcher Power Cleaver Model 81 to yield 283.5g (10 oz) strip loin steaks, 226.8g (8 oz) top sirloin butt steaks and 170.1g (6 oz) tenderloin steaks. Subprimals in the fresh, hand-cut group (treatment I) were cut into steaks by a highly trained steak cutter. Steaks were sized to the appropriate weight range, when necessary, by additional trimming.

Steak handling

Each steak was weighed, identified, vacuum packaged, frozen (−34°C) and held (−24°C) until shipment by truck to the Texas A&M University Meat Laboratory. After arrival, two steaks were used for palatability tests at Texas A&M University and two steaks were assigned for use in Instron testing at Auburn University. The steaks assigned to Auburn University were placed in plastic foam-lined containers and shipped by air freight to Auburn.

Palatability tests

Two steaks from each subprimal were thawed (2°C) for 24 hr and broiled on Farberware Open-Hearth broilers to an internal temperature of 70°C (monitored by use of copper constantan thermocouples and a recording thermometer). One steak was evaluated by a 10-member trained sensory panel for the following traits: juiciness, muscle fiber tenderness, connective tissue amount, overall tenderness, flavor, and overall palatability. Cores (1.3 cm diam) were removed from the second steak and shear force was measured by the use of the Warner-Bratzler shear machine.

Instron analysis

Two steaks from each subprimal were thawed (2°C) for 24 hr and oven-roasted in a Blodgett gas convection oven at 176°C to an internal temperature of 70°C (monitored by use of copper constantan thermocouples and digital thermometer). Steaks were equilibrated 30 min at 22°C to an internal temperature of 24°C. Four cores from each top sirloin butt and strip loin steak and three cores from each tenderloin steak were removed and degree of doneness was evaluated using a photographic scale. Each core (2.54 cm diam) was sheared twice using the single blade shear cell (Warner-Bratzler shear device) attachment on the Instron Model 1122. A crosshead speed of 200 mm/min and a full scale load of 20 kg was used. Eight measurements were obtained for each strip steak and butt steak and six measurements for each tenderloin steak.

Data analysis

The data were analyzed by the use of paired-t distribution to determine the significance of differences between treatments within groups (Steel and Torrie, 1960).

Table 2—Mean percentage yields of steaks from steak-ready subprimals from fresh, crust-frozen, and frozen treatments[a]

	Group A[b]		Group B		Group C	
Item	Fresh, hand-cut	Crust-frozen, pressed-cleaved	Fresh, hand-cut	Frozen-tempered, pressed-cleaved	Crust-frozen, pressed-cleaved	Frozen-tempered, pressed-cleaved
Strip loins	88.3	95.8	88.4	94.0	95.9	94.1
Top sirloin butts	80.9	95.3	82.4	90.5	94.8	93.7
Tenderloins	86.3	96.4	82.0	91.9	94.9	90.4

[a] Calculated by dividing weight of salable steaks by weight of steak-ready subprimal.
[b] Means within a group underscored by a common line are not significantly different ($P > 0.05$).

Table 3—Mean values for certain cooking characteristics of strip loins, top sirloin butts, and tenderloin steaks from fresh, crust-frozen, and frozen treatments

	Group A[a]		Group B		Group C	
Item	Fresh, hand-cut	Crust-frozen, pressed-cleaved	Fresh, hand-cut	Frozen-tempered, pressed-cleaved	Crust-frozen, pressed-cleaved	Frozen-tempered, pressed-cleaved
Strip loins						
Broiling						
Thawing loss (%)	1.12	1.10	0.96	1.33	0.58	0.80
Cooking loss (%)	30.5	32.1	30.4	33.6	34.5	33.8
Cooking time (min)	25.5	25.9	25.1	24.2	27.0	25.1
Degree of doneness[b]	3.8	3.6	3.0	3.2	2.8	3.4
Oven-roasting						
Cooking loss (%)	27.1	28.6	23.4	29.3	29.1	30.1
Degree of doneness[b]	3.2	3.0	3.4	3.1	3.0	2.8
Top sirloin butts						
Broiling						
Thawing loss (%)	2.74	2.74	2.30	2.24	2.42	1.76
Cooking loss (%)	28.9	30.7	30.9	34.2	33.8	32.8
Cooking time (min)	23.8	26.3	25.7	27.9	24.6	26.0
Degree of doneness[b]	3.8	4.3	4.0	4.3	4.0	3.3
Oven-roasting						
Cooking loss (%)	30.0	30.3	28.5	32.1	29.6	29.3
Degree of doneness[b]	3.1	3.1	3.3	2.8	3.0	2.7
Tenderloins						
Broiling						
Thawing loss (%)	1.56	0.68	0.94	0.82	0.76	0.72
Cooking loss (%)	34.2	35.1	33.7	33.3	34.2	33.7
Cooking time (min)	34.2	34.4	33.5	31.8	37.2	36.0
Degree of doneness[b]	4.8	4.0	4.6	4.4	3.6	4.0
Oven-roasting						
Cooking loss (%)	28.9	28.6	27.1	28.3	23.9	25.6
Degree of doneness[b]	3.0	3.0	3.0	3.1	3.1	3.1

[a] Means within a group underscored by a common line are not significantly different ($P > 0.05$).
[b] Degree of doneness was determined by pictorial standards: 8 = extremely rare, 1 = extremely done.

RESULTS

MEAN PERCENTAGE YIELDS of steaks from steak-ready subprimals in the fresh, hand-cut; crust-frozen, pressed-cleaved; and frozen-tempered, pressed-cleaved groups (treatments I, II, and III, respectively) are presented in Table 2. In 5 of 6 comparisons, subprimals from the pressed-cleaved groups (crust-frozen or frozen-tempered) had superior yields ($P < 0.05$) of salable steaks when compared to the fresh, hand-cut treatment. When comparisons were made between pressed-cleaved steaks from crust-frozen and frozen-tempered groups (Group C) there were no significant differences ($P > 0.05$) in salable steak yields for strip loins or top sirloin butts but tenderloins from subprimals in the crust-frozen treatment had higher yields than did frozen-tempered subprimals. Increased yields of salable steaks from steak-ready subprimals suggest economic advantages for use of mechanical portioning (either from crust-frozen or frozen-tempered states). Mechanical portioning of steaks makes possible use of semi-skilled labor to operate pressing and cleaving machinery rather than employing highly trained and proficient labor to hand-cut, weigh, and size steaks, when necessary, to achieve exact ranges needed for portion controlled steaks in the H.R.I. trade.

Mean values for certain cooking characteristics of steaks from each subprimal are presented in Table 3. In 8 of 9 comparisons no significant differences ($P > 0.05$) were observed between treatments for thaw loss; however, thaw loss was significantly lower in the crust-frozen treatment, Group A, tenderloins as compared to that for fresh, hand-cut tenderloins. Cooking loss was negatively affected in

Table 4—Mean values for palatability characteristics of longissimus muscles from fresh, crust-frozen, and frozen treatments

Item	Group A[a]		Group B		Group C	
	Fresh, hand-cut	Crust-frozen, pressed-cleaved	Fresh, hand-cut	Frozen-tempered, pressed-cleaved	Crust-frozen, pressed-cleaved	Frozen-tempered, pressed-cleaved
Juiciness[b]	5.2	4.9	5.6	5.0	4.7	5.2
Muscle fiber tenderness[c]	6.3	6.6	6.7	6.4	6.4	6.8
Connective tissue amount[d]	7.2	7.2	7.3	7.4	7.3	7.2
Overall tenderness[c]	6.2	6.5	6.7	6.5	6.4	6.7
Flavor[e]	6.1	5.9	6.3	5.8	6.2	6.1
Overall palatability[e]	5.8	5.7	6.3	5.7	5.8	5.9
Warner-Bratzler shear force (kg)	3.0	2.7	2.6	2.3	2.6	2.7
Instron Warner-Bratzler shear force (kg)	6.1	5.5	5.2	5.2	5.4	5.5

[a] Means within a group underscored by a common line are not significantly different ($P > 0.05$).
[b] 8 = extremely juicy; 1 = extremely dry.
[c] 8 = extremely tender; 1 = extremely tough.
[d] 8 = none; 1 = abundant; determined by sensory panel.
[e] 8 = extremely desirable; 1 = extremely undesirable.

Table 5—Mean values for palatability characteristics of gluteus medius muscles from fresh, crust-frozen, and frozen treatments

Item	Group A[a]		Group B		Group C	
	Fresh, hand-cut	Crust-frozen, pressed-cleaved	Fresh, hand-cut	Frozen-tempered, pressed-cleaved	Crust-frozen, pressed-cleaved	Frozen-tempered, pressed-cleaved
Juiciness[b]	5.4	5.5	5.2	5.2	5.3	4.7
Muscle fiber tenderness[c]	6.3	6.7	6.2	6.8	6.6	6.0
Connective tissue amount[d]	7.3	7.5	7.2	7.7	7.6	7.3
Overall tenderness[c]	6.2	6.6	6.3	6.7	6.6	5.9
Flavor[e]	6.4	6.6	6.3	6.7	6.7	6.1
Overall palatability[e]	6.2	6.4	6.2	6.5	6.2	5.7
Warner-Bratzler shear force (kg)	3.7	4.0	3.5	3.5	3.3	4.1
Instron Warner-Bratzler shear force (kg)	7.3	7.2	7.4	7.3	6.3	6.8

[a] Means within a group underscored by a common line are not significantly different ($F > 0.05$).
[b] 8 = extremely juicy; 1 = extremely dry.
[c] 8 = extremely tender; 1 = extremely tough.
[d] 8 = none; 1 = abundant; determined by sensory panel.
[e] 8 = extremely desirable; 1 = extremely undesirable.

only 3 of 18 comparisons by use of pressing and cleaving (frozen-tempered subprimals). Cooking time was not affected ($P > 0.05$) by any subprimal treatment. Degree of doneness was not significantly affected as a result of either freezing or tempering treatment or by pressing and cleaving rather than hand-cutting of subprimals into steaks. These data suggest that cooking parameters of steaks from mechanically portioned subprimals, regardless of method of freezing and/or tempering, are not seriously affected.

Mean values for palatability traits of steaks from each subprimal are reported in Tables 4, 5, 6, and 7. There were no significant differences between treatments for juiciness, connective tissue amount (determined by sensory panel), overall palatability, or Warner-Bratzler shear force for any of the muscles evaluated. Gluteus medius muscles from the frozen-tempered, pressed-cleaved subprimals were more tender than were those from fresh, hand-cut subprimals but less tender than those from crust-frozen, pressed-cleaved subprimals. Tenderloin steaks produced by the crust-freezing process were more tender ($P < 0.05$) than their counterpart fresh, hand-cut steaks. The only other significant differences were those for overall tenderness (Group C, gluteus medius, Table 5) and flavor (Group B, gluteus medius, Table 5). Although a few statistically significant differences were observed between treatments, 88 of 93 comparisons (group × muscle × palatability trait) resulted in nonsignificant differences in palatability attributes between treatment means within groups. These data suggest that palatability traits of steaks produced by pressing and cleaving after either freezing-tempering or crust-freezing, are not deleteriously affected when compared to steaks produced in the conventional manner—hand-cutting of fresh subprimals.

DISCUSSION

MECHANICAL PORTIONING of meat could not be a vi-

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Table 6—Mean values for palatability characteristics of biceps femoris muscles from fresh, crust-frozen, and frozen treatments

Item	Group A[a]		Group B		Group C	
	Fresh, hand-cut	Crust-frozen, pressed-cleaved	Fresh, hand-cut	Frozen-tempered, pressed-cleaved	Crust-frozen, pressed-cleaved	Frozen-tempered, pressed-cleaved
Juiciness[b]	5.1	5.5	5.2	5.0	5.3	4.8
Muscle fiber tenderness[c]	6.5	6.9	6.9	6.4	6.9	7.0
Connective tissue amount[d]	6.5	6.4	6.9	6.5	7.0	6.9
Overall tenderness[c]	6.1	6.3	6.6	6.1	6.6	6.8
Flavor[e]	5.9	6.3	5.9	5.8	6.4	6.3
Overall palatability[e]	5.6	5.8	5.9	5.7	6.2	6.0
Warner-Bratzler shear force (kg)	3.2	3.1	2.8	3.0	3.2	3.0

[a] Means within a group underscored by a common line are not significantly different ($P > 0.05$).
[b] 8 = extremely juicy; 1 = extremely dry.
[c] 8 = extremely tender; 1 = extremely tough.
[d] 8 = none; 1 = abundant; determined by sensory panel.
[e] 8 = extremely desirable; 1 = extremely undesirable.

Table 7—Mean values for palatability characteristics of psoas major muscles from fresh, crust-frozen, and frozen treatments

Item	Group A[a]		Group B		Group C	
	Fresh, hand-cut	Crust-frozen, pressed-cleaved	Fresh, hand-cut	Frozen-tempered, pressed-cleaved	Crust-frozen, pressed-cleaved	Frozen-tempered, pressed-cleaved
Juiciness[b]	5.6	5.6	5.7	5.9	5.2	5.4
Muscle fiber tenderness[c]	6.7	6.7	7.0	6.9	7.1	6.7
Connective tissue amount[d]	7.5	7.3	7.4	7.5	7.4	7.3
Overall tenderness[c]	6.7	6.7	7.0	6.9	7.0	6.7
Flavor[e]	6.1	6.2	6.2	6.4	6.5	6.0
Overall palatability[e]	6.2	6.1	6.3	6.4	6.3	6.0
Warner-Bratzler shear force (kg)	3.5	2.9	3.2	3.0	2.8	3.0
Instron Warner-Bratzler shear force (kg)	6.9	6.1	6.7	6.4	5.8	5.8

[a] Means within a group underscored by a common line are not significantly different ($P > 0.05$).
[b] 8 = extremely juicy; 1 = extremely dry.
[c] 8 = extremely tender; 1 = extremely tough.
[d] 8 = none; 1 = abundant; determined by sensory panel.
[e] 8 = extremely desirable; 1 = extremely undesirable.

FRAGMENTATION INDEX OF RAW MUSCLE AS A TENDERNESS PREDICTOR OF STEAKS FROM USDA COMMERCIAL AND UTILITY CARCASSES

C. R. CALKINS, G. W. DAVIS, and W. L. SANDERS

ABSTRACT

Loin steaks were removed from USDA Commercial (n=38) and Utility (n=42) beef carcasses to facilitate study of Fragmentation Index (FI). Tenderness was assessed by the Warner-Bratzler shear (WBS) and a trained, 8-member sensory panel. To determine the minimum time required to obtain the FI for fresh and frozen raw longissimus muscle, three drying times were studied (10 min, 40 min, and 22 hr). Simple correlation coefficients relating FI to WBS force value and sensory tenderness rating were: fresh muscle FI (10 min), 0.60 and −0.60; frozen muscle FI (40 min), 0.73 and −0.69, respectively. USDA grade factors and simultaneous consideration of all all measures of fragmentation accounted for 14.1 and 61.1%, respectively, of the observed variation in WBS force values. FI determined from frozen longissimus muscle accounted for 18.6–23.8% more of the observed variation in cooked meat tenderness than FI of fresh muscle. The best two-variable regression model accounted for 56.6% of the observed variation in WBS force value.

INTRODUCTION

MEASUREMENT of tenderness in the cooked product by use of instruments (Warner, 1928; Bratzler, 1932, 1949; Kramer et al., 1951a, b) or by highly trained sensory panels is essential to meat palatability research. However, assessments of the cooked product are slow and expensive. As an alternative, Kapsalis and Szczesniak (1976) suggested that a unified practical test of raw meat for prediction of cooked meat tenderness could be extremely useful for the practical aspects of quality control, purchase specifications and consumer acceptance. The application of a mechanical test to raw meat for prediction of cooked product tenderness is very difficult, since it is complicated by biochemical changes and altering of the material during cookery (Kapsalis and Szczesniak, 1976). Extensive progress has been made, but additional work is needed before a satisfactory predictive test can be established for raw meat. Several raw meat tenderness prediction instruments or procedures have been recently reported: The Poisson's Ratio Device (Segars et al., 1974); Myofibril Fragmentation Index (Davey and Gilbert, 1969; Olson et al., 1976); and Fragmentation Index (Reagan et al., 1975; Davis et al., 1980). Continued study of these procedures is necessary for reduced costs and for improvements in prediction, accuracy, and speed which may lead to commercial acceptance for one or more of the raw meat tenderness prediction methods. The Davis et al. (1980) fragmentation procedure necessitated the use of frozen bovine longissimus muscle and required approximately 50 min of laboratory time to perform. The objectives of the present study were to compare the use of fresh to frozen longissimus muscle and to determine the minimum drying time required to obtain the Fragmentation Index.

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EXPERIMENTAL

A TOTAL of 80 beef carcasses (USDA Commercial n=38, USDA Utility n=42) were selected at two commercial firms, evaluated for USDA grade factors, and shipped to the University of Tennessee meat laboratory. After aging 10–14 days in a 2°C cooler, six steaks (A and B, 1.3 cm thickness; C and D, 0.65 cm thickness; E and F, 3.2 cm thickness) were removed from the anterior end of the short loin for subsequent palatability (steaks E, F), chemical (steak A), physical (steak B, C, D) and histological (steak B) analysis.

Proximate analysis samples (steak A) were trimmed of all subcu-

Table 1—Means, standard deviations, and coefficients of variation for independent variables (carcass traits, fragmentation index, and laboratory assays) and dependent variables (shear force value and sensory tenderness rating) determined from cooked steaks.

Variable	Mean	S.D.	C.V.
	Independent variables		
Carcass traits			
Marbling degree[a]	45.10	16.87	37.40
Overall maturity[b]	37.50	5.38	14.36
Carcass weight (kg)	226.08	35.04	15.50
Adjusted fat thickness (mm)	9.89	7.12	75.80
Lean texture[c]	4.25	1.63	38.27
Lean color[c]	4.24	1.08	25.53
Fragmentation of fresh or frozen longissimus muscle			
Fresh (10 min)[d]	201.66	94.59	46.90
Fresh (40 min)[d]	175.06	82.96	47.39
Fresh (22 hr)[d]	47.95	21.79	45.44
Fresh (filtrate volume, ml)	53.97	1.90	3.52
Frozen (10 min)[d]	325.89	133.23	40.88
Frozen (40 min)[d]	289.90	124.60	42.98
Frozen (22 hr)[d]	83.31	35.84	43.02
Frozen (filtrate volume, ml)	51.62	2.55	4.94
Laboratory assays			
Sarcomere length (mμ)	1.82	0.13	7.03
Percentage fat (WTB)[e]	3.91	2.08	53.25
Percentage fat (MFB)[f]	14.22	6.67	46.92
Percentage moisture	73.33	2.00	2.72
Percentage expressible juice	52.24	5.16	9.87
Percentage cook loss	29.98	2.82	9.41
	Dependent variables		
Response			
Shear force value (kg)	4.45	1.45	32.49
Sensory tenderness rating	5.15	1.31	25.48

[a] Mean based on 100-unit scale (70=Slightly abundant°°, 30=Slight°°).
[b] Mean based or 50-unit scale (40=D°°, 30=C°°).
[c] Mean based on 8-point rating scales (8=extremely fine or very light cherry red; 4=extremely coarse or black).
[d] FI = 100 X weight (g) after air drying at 22°C (10 min and 40 min) and over drying at 35°C (22 hr).
[e] WTB=whole t ssue basis.
[f] MFB=moisture free basis.

Table 2—Simple correlation coefficients relating measures of fragmentation to certain beef carcass traits and laboratory assays

Trait	Fragmentation of fresh muscle				Fragmentation of frozen muscle			
	10 min	40 min	22 hr	filtrate volume (ml)	10 min	40 min	22 hr	filtrate volume (ml)
Carcass traits								
Marbling degree	0.00	0.01	0.19	−0.09	−0.07	−0.07	0.09	0.05
Overall maturity	0.24*	0.26*	0.21	−0.29**	0.13	0.13	0.06	−0.16
Carcass weight	−0.18	−0.17	−0.04	0.11	−0.27*	−0.27*	−0.15	0.27*
Adjusted fat thickness	0.03	0.05	0.22*	−0.13	0.04	0.03	0.15	−0.05
Lean texture	−0.27*	−0.26*	−0.15	0.31**	−0.17	−0.16	−0.07	0.20
Lean color	−0.07	−0.07	0.03	0.18	0.02	0.02	0.13	0.04
Laboratory assays								
Sarcomere length	−0.16	−0.17	−0.13	0.27*	−0.26*	−0.26*	−0.21	0.34**
Percentage fat (WTB)	0.09	0.12	0.32**	−0.14	−0.02	−0.02	0.17	0.03
Percentage fat (MFB)	0.09	0.12	0.32**	−0.14	−0.04	−0.04	0.15	0.05
Percentage moisture	−0.04	−0.06	−0.28*	0.09	0.05	−0.05	−0.15	−0.07
Percentage espressible juice	0.06	0.06	0.12	0.14	0.13	0.14	0.18	0.01
Percentage cook loss	0.08	0.08	0.20	−0.03	0.18	0.20	0.30**	−0.09
Shear force value	0.60**	0.60**	0.53**	−0.48**	0.71**	0.73**	0.68**	−0.72**
Tenderness rating	−0.60**	−0.60**	−0.52**	0.43**	−0.68**	−0.69**	−0.63**	0.68**

* $P < 0.05$
** $P < 0.01$

taneous fat and epimysial tissue, frozen in liquid nitrogen and powdered in a Waring Blendor. Percentage fat and moisture were determined by AOAC (1975) procedures.

Fragmentation Index (FI) was derived by adding 10g of 7 mm cubed longissimus muscle [fresh (steak C) or frozen (steak D)] to 50 ml of cold sucrose (0.25M) and potassium chloride (0.02M) solution in a 150 ml stainless steel homogenization cup. After 5 min, each sample was blended for 40 sec at full speed in a Virtis Macro-Model "45" homogenizer. The blades were in reverse position and parallel with the dorsal blade positioned at the surface of the solution (Calkins and Davis, 1978). The resulting homogenate was filtered through a "Nitex" screen (250 μm pore size) by use of a 115 ml "Nalgene Filter Unit" and a plastic stir rod. Screens were blotted on Whatman No. 3 filter paper and the residue was allowed to air dry at 22°C. Weights were taken at 10 min, 40 min, and after a 22-hr oven drying (35°C) period (FI = wt in g × 100). Volume of filtrate remaining after filtration also was recorded.

Duplicate, 500-mg samples of longissimus muscle (steak B) were subjected to the press-filter paper absorption technique of Grau and Hamm (1953) as modified by Briskey et al. (1959). Percentage expressible juice was calculated using the Carpenter (1962) formula.

Sarcomere length was assessed on samples blended for 90 sec in approximately 25 ml of a 4% formalin solution. A light microscope (1500X) and filar micrometer were used to measure 10 sarcomeres on each of 12 myofibrils.

Sensory evaluation (steak E) and shear force value (steak F) were determined on steaks cooked to a final internal temperature of 70°C on individual, preheated broiling units. Thermocouples were used to monitor endpoint temperature. Steaks E and F (wrapped in polyethylene coated freezer paper, frozen at −31°C and stored at −18°C) were thawed for 24 hr in a 5–7°C cooler and weighed prior to and after cooking for the purpose of calculating percentage cook loss. Eleven potential sensory panel members were interviewed prior to panel training. Nine persons were selected and subsequently trained by an experienced panel leader during 16 sessions with use of reference samples varying in tenderness, juiciness, and connective tissue amount. Following cooking, each steak was boned, divided into several 1.3 cm × 1.3 cm × 1.9 cm pieces with use of a plexiglass grid (Cross et al., 1978) and immediately served to panel members in individual sensory booths. Five samples (two pieces per sample) were evaluated during each panel session. The trained, 8-member sensory panel evaluated tenderness, juiciness, flavor desirability, connective tissue amount, and overall satisfaction for each sample using 8-point rating scales (8=extremely tender, extremely juicy, extremely desirable flavor, no connective tissue, or extremely desirable overall; 1=extremely tough, extremely dry, extremely undesirable flavor, abundant amount of connective tissue, or extremely undesirable overall). Four 1.3-cm cores were removed from steak F (cooked in the same manner and cooled to 25°C) and sheared in duplicate on a Warner-Bratzler shear machine.

Statistical analysis included estimate of coefficients of determination, partial coefficients of determination, simple correlation coefficients, and multiple and stepwise regression using the Statistical Analysis System (SAS) of Barr and Goodnight (1972). Dependent variables were shear force value and sensory tenderness rating while independent variables included carcass traits, fragmentation measure, and laboratory assays.

RESULTS & DISCUSSION

MEANS, standard deviations, and coefficients of variation for various independent and dependent variables are presented in Table 1. FI and various estimates of fatness (marbling degree, adjusted fat thickness, and percentage fat) contained the greatest variation among the independent variables. Reagan et al. (1975) obtained coefficients of variation for fragmentation ranging from 12.28–14.66%. In the current study, relatively high coefficients of variation for shear force and tenderness ratings are also shown (Table 1).

Simple correlations between measures of fragmentation and tenderness (shear force value and sensory rating) were highly significant (Table 2). FI of fresh muscle was related to overall carcass maturity and lean texture. Sarcomere length and carcass weight were significantly associated with FI of frozen muscle. Marbling degree, adjusted fat thickness, and lean color were not related to fragmentation in 23 out of 24 comparisons (Table 2). These data suggest that the FI of fresh muscle is related to carcass physiological maturity while FI of frozen muscle may be a measure of the myofibrillar component of tenderness. Nevertheless, these relationships are low and care should be taken in interpretation of the results.

Correlation coefficients (Table 2) between FI of frozen muscle and sensory tenderness (−0.68) and between sensory tenderness and sarcomere length (−0.26) substantiate observations of Parrish et al. (1973) and McBride and Parrish (1977), who indicated that MFI was a more important beneficial contributor to tenderness than sarcomere length for carcass aged bovine longissimus. Simple correlation coefficients between WBS force value or tenderness rating and FI (fresh or frozen muscle, 10 or 40 min drying time) presented in Table 2 range from −0.69 to 0.73 ($P < 0.01$). These values are in general agreement with significant correlations for cooked meat tenderness rating or WBS force value to FI (r = 0.71, Davis et al., 1980; r = 0.91, Calkins and Davis, 1978; r = −0.74, Calkins and Davis, 1980; r =

−0.79, Reagan et al., 1975); and to MFI (r = −0.73, Olson and Parrish, 1977; r = 0.78, Davey and Gilbert, 1969; r = −0.78, Moller et al., 1973; r = −0.90, Culler et al., 1978). This substantial evidence indicates that fragmentation of raw bovine longissimus muscle may be the most important currently known physical, chemical, or histological predictor of cooked loin steak tenderness.

The FI is primarily a measure of the myofibrillar component of tenderness. This is in agreement with the relationship between Z-line degradation and MFI reported by Olson et al. (1976) and reduced sized muscle fragments associated with proteolysis of high temperature aged muscle (Moeller et al., 1977). The FI may also measure the bulk density effect of tenderness, even though results presented in Table 2 do not support this hypothesis. An increased knowledge of factors measured by objective assessments could lead to a better understanding or appreciation of organoleptic tenderness (Bouton and Harris, 1972). Thus additional studies are needed to further elucidate the scientific basis of the Fragmentation Index. Six carcass traits (marbling degree, overall maturity, carcass weight, adjusted fat thickness, lean texture, and lean color), six fragmentation measures (FI of fresh and frozen muscle at 10 min and 40 min and filtrate volume) and six laboratory assays (sarcomere length and percentage fat, moisture, expressible juice, and cook loss) were selected as candidate variables to develop prediction models for beef tenderness. The decisions regarding variable selection for this model were based on the influence of each variable on final USDA grade (all quality and yield factors were considered), the simplicity and time involved in the various fragmentation measurements, and the increased precision and objectivity of certain laboratory assays which have been shown to be related to tenderness. Since the six beef carcass traits can be evaluated rapidly, they were chosen to be included first in the regression model. Simultaneous consideration of these indicators accounted for approximately 14 and 21% of the variation in tenderness for shear force and tenderness rating, respectively (Table 3).

Because measures of fragmentation necessitate less time and skill than is required for the laboratory assays, the six fragmentation variables were given next priority in the model. Partial coefficients of determination accounting for variation in shear force values and sensory tenderness (not previously explained by the carcass variables) were approximately 51 and 41%, respectively. This is substanitated by Culler et al. (1978) who reported that from use of A, B, C and E maturity carcasses, MFI accounted for more than 50% of the variation in loin steak tenderness. The third major addition to the regression model was laboratory assays which increased the C.D. ($R^2 \times 100$) by approximately 3–4% (Table 3). The magnitude of these increases were not significantly different from zero. Also, none of the partial regression coefficients for either the carcass traits or laboratory assays was significantly different from zero. Clearly, the fragmentation variables were more highly related to beef tenderness than either the selected carcass or laboratory variables. To further simplify and define a usable prediction equation for beef tenderness, subsequent analyses were conducted comparing the use of fresh to frozen muscle for determination of FI. It is realized that these measures are multicolinear. Due to ease of application, fresh muscle fragmentation measures were included first in the predictive model and resulted in coefficients of determination (Table 1) of approximately 37% (shear value) and 40% (sensory tenderness). Frozen muscle fragmentation variables increased the explained variation in tenderness to 61.06% (shear value) and to 58.14% (sensory tenderness) (Table 4). These data suggest that fragmentation of frozen longissimus muscle may contain sufficient information to be utilized independently as a predictor of muscle tenderness. Thus, a model containing raw frozen muscle fragmentation measures accounted for approximately 49–58% of the variation in shear force value and sensory tenderness, respectively (Table 4). It appears the increased time required to freeze raw longissimus muscle prior to performance of the fragmentation procedure can be justified.

In data not shown in tabular form, the best fresh muscle fragmentation model contained one variable (FI at 10 min) and accounted for 36.50% of the observed variation in WBS force value. Addition of a second variable contributed only 0.71% to the model. Conversely, the best frozen muscle fragmentation model contained two variables (FI at 10 min and 40 min) and accounted for 56.6% of the variation in WBS force value.

The conclusions of the present study are as follows: (a)

Table 3—Coefficients of determination and partial coefficients of determination between dependent variables (shear force value and sensory tenderness rating) determined from cooked beef loin steaks and selected independent variables (carcass traits, fragmentation, and laboratory assays) determined from beef longissimus muscle

	Dependent variable	
Model	Shear force value $R^2 \times 100$	Sensory tenderness rating $R^2 \times 100$
Carcass,[a] fragmentation,[b] laboratory[c]	68.05	65.76
Carcass	14.10	21.38
Fragmentation/carcass[d]	50.56	40.72
Laboratory/carcass, fragmentation[e]	3.40	3.66

[a] Carcass traits include marbling degree, overall maturity, carcass weight, adjusted fat thickness, lean texture, and lean color.
[b] Fragmentation includes fresh and frozen muscle measures of filtrate volume (ml) and indexes at 10 min and 40 min drying times.
[c] Laboratory assays include sarcomere length, percentage fat (whole tissue basis), percentage fat (moisture free basis), percentage moisture, percentage expressible juice, and percentage cook loss.
[d] Partial coefficients of determination accounting for variation in WBS force values and sensory tenderness which was not previously explained by the carcass trait variables.
[e] Partial coefficients of determination accounting for variation in WBS force values and sensory tenderness rating which was not previously explained by the carcass trait and fragmentation variables.

Table 4—Coefficients of determination and partial coefficients of determination between dependent variables (shear force value and sensory tenderness rating) determined from cooked beef loin steaks and selected models containing fresh and frozen muscle fragmentation measures.

	Dependent variable	
Model	Shear force value $R^2 \times 100$	Sensory tenderness rating $R^2 \times 100$
Fresh muscle fragmentation,[a] frozen muscle fragmentation[a]	61.06	58.14
Fresh muscle fragmentation	37.25	39.51
Frozen muscle fragmentation/ fresh muscle fragmentation[b]	23.81	18.63
Frozen muscle fragmentation	58.09	49.36

[a] Fresh muscle fragmentation and frozen muscle fragmentation includes measures of filtrate volume (ml) and indexes at 10 min and 40 min drying times.
[b] Partial coefficients of determination accounting for variation in WBS force values and sensory tenderness ratings which were not previously explained by the fresh muscle fragmentation variables.

since the FI can be accurately determined with either a 10 min or 40 min residue drying time, the 10 min period is recommended; (b) FI of fresh or frozen muscle is superior to USDA carcass grading factors and/or laboratory determinations for prediction of cooked meat tenderness of USDA Commercial and Utility carcasses; (c) FI of frozen muscle accounted for approximately 20% more of the observed variation in tenderness rating than FI of fresh muscle; (d) the best two-variable regression model for FI (frozen longissimus muscle and 40 min drying period) accounted for 56.6% of the observed variation in WBS force values. The magnitude of the relationship between FI of frozen muscle and shear force value suggest the possibility of a commercial application for this objective measure of tenderness.

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EFFECT OF SODIUM ERYTHORBATE AND PACKAGING CONDITIONS ON COLOR STABILITY OF SLICED BOLOGNA

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ABSTRACT

Retention of cured color and shelf life of sliced bologna manufactured with various erythorbate levels and packaged with different films and vacuum levels were studied. Good barrier films (7.0 ml/m²/24 hr or less oxygen permeability), film rigidity, and high vacuum levels (686–737 mm) are most critical for less faded color, higher cured pigment conversion, and lower TBA numbers. Erythorbate aided curing developments but could not compensate for low barrier films or low vacuum levels during storage.

INTRODUCTION

SODIUM ERYTHORBATE together with its isomer, sodium ascorbate, has been used effectively in cured meat systems to accelerate curing (Fox et al., 1967; Counsell, 1971) and counteract light-induced discoloration (Cassens et al., 1974; Walters et al., 1975). Erythorbate and ascorbate accomplish these effects by functioning as reductants and thus also very effectively reduce residual nitrite content of cured meat (Brown et al., 1974).

Residual nitrite in cured meats has been a source of concern for carcinogenic nitrosamines and for nitrite itself. Both concerns are at least somewhat alleviated by reduction of nitrite to the lowest levels that will retain cured product characteristics. Reduction of added nitrite while maintaining product stability can be accomplished through use of high barrier packaging films and high vacuum levels, with the vacuum levels seeming the most important (Lin and Sebranek, 1979).

Commercially, vacuum levels vary considerably among packages, and the effectiveness of ascorbate or erythorbate for retaining cured characteristics under various vacuum levels is not clear. This study was initiated to examine the effect of sodium erythorbate and packaging conditions (vacuum levels and film barrier properties) on storage stability of sliced bologna, and to determine how effective the reductants might be in overcoming packaging conditions that are less than ideal.

EXPERIMENTAL

A LARGE DIAMETER bologna was made in the Iowa State University Meat Laboratory and was composed of a mixture of 3% salt, 0.5% Heller's number 531 seasoning, 10% ice, 156 ppm sodium nitrite and three different sodium erythorbate levels of 0 ppm, 500 ppm and 940 ppm. Fresh beef and pork were trimmed to approximately 10 and 50% fat content. All trim was ground through the 9.5-mm plate of a grinder, after which samples weighing 5.9 kg were taken from each batch of ground meat and placed in an Anyl-Ray (Anyl-Ray Corp.) machine for rapid fat analysis. The weights of analyzed meat were prepared in the proper proportions to give a 25 ± 1% fat mixture. The beef trim was first placed in a Kramer Grebe Silent Cutter, followed immediately by ice and salt. After 3 min of chopping, the pork trim and other ingredients were added. The final temperature was about 10–11°C. The emulsion was stuffed (Vemag Robot 1000S Type 116 model) into large-diameter fibrous casing and smokehouse processed to 65°C internal temperature. After processing, samples were sliced and randomly packaged in five different types of film characterized in Table 1.

Three different initial vacuum levels of maximum attainable, 90% of maximum and 70% of maximum were used in a Multivac Pouch machine MC 2 equipped with heat sealing bar. Measuring the vacuum levels inside packages immediately showed the three vacuum levels to be 686–737 mm Hg, 584–635 mm Hg, and 457–533 mm Hg, respectively. All samples were displayed under 200 ft-c of cool white fluorescent light at 2–5°C in a display case, for 1, 4, 10, 21, 35, and 56 days. Prior to packaging, the sliced bologna was very fresh and very bright pink in color, free of surface discoloration and very desirable in general appearance.

Residual nitrite was determined according to the method of AOAC (1970).

Rancidity measurements were made by the TBA (2-thiobarbituric acid) number according to the method of Tarladgis et al. (1960). The vacuum level inside packages was determined by placing each package in a plastic bell jar. The chamber was evacuated by an electric vacuum pump, and the equilibrium vacuum pressure between package and chamber was determined. As the film separated from the meat surface, a measurement in millimeters was recorded as the equilibrium vacuum level.

Quantitation of nitroso pigment was performed by acetone-water extraction according to techniques described by Hornsey (1956). Pigment conversion was the percentage of total heme pigments converted to nitric oxide haemochrome.

Reflectance spectroscopy also was used to determine the degree of pigment nitrosation. A ratio of K/S values for percent reflectance readings at 570 and 650 nm was used with a Beckman DK-2A spectroreflectometer (Wodicka, 1956; Judd and Wyszecki, 1963; Giddy, 1966).

A photovolt with a green filter also was used to measure color change as total reflectance values.

Subjective evaluation of surface discoloration was included by use of an 8-point scale scored from 8 = dark reddish-pink to 1 = brown-green abnormal. Evaluations were conducted under cool white fluorescent lighting.

Data were analyzed by using analysis of variance (Snedecor and Cochran, 1967). The significance of the difference between means was determined by the least significant difference method.

RESULTS & DISCUSSION

EFFECT OF packaging films on color and rancidity is given in Table 2 as means for all time periods. Superior cured meat color development was indicated by color score, K/S

Table 1—Characteristics of packaging films[a]

Film code no.	Oxygen permeability[b] ml/m²/24 hr
1	7.0
2	7.0
3	60.0
4	0.3
5	0.2

[a] All films and permeability data were provided by American Can Company.
[b] Permeability was measured at 23°C and 0% RH.

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Table 2—Effect of packaging films on color and rancidity development over all time periods[a]

Items	Films 1	2	3	4	5
Color score	4.13 ± 1.52a	4.06 ± 1.63a	2.37 ± 1.72a	4.06 ± 1.72a	3.39 ± 2.05b
Reflectance	34.12 ± 3.22bc	34.55 ± 2.97b	35.59 ± 3.38a	33.87 ± 3.64c	34.60 ± 3.83b
K/S 570:K/S 650	4.67 ± 1.56a	4.39 ± 1.44a	2.92 ± 1.39c	4.61 ± 1.82a	3.91 ± 1.83b
Nitric oxide heme pigment (ppm)	81.97 ± 15.77a	79.72 ± 19.35a	51.09 ± 25.78c	78.82 ± 22.29a	68.01 ± 30.29b
Total pigment (ppm)	129.26 ± 19.67a	133.67 ± 25.08a	122.87 ± 23.52b	133.47 ± 22.74a	125.39 ± 29.52b
Pigment conversion (%)	63.71 ± 9.69a	60.14 ± 11.69a	40.87 ± 18.70c	59.00 ± 14.15a	52.45 ± 18.98b
Terminal vacuum (mm)	634.5 ± 86.1a	613.4 ± 115.8a	609.1 ± 127.8a	616.7 ± 137.7a	453.6 ± 229.6b
TBA no.	0.72 ± 0.23b	0.71 ± 0.18b	1.05 ± 0.62a	0.79 ± 0.25b	1.12 ± 0.69a

[a] All means in a row with the same letter are not significantly different ($P < 0.05$); X ± SD = mean ± standard deviation.

570:650 ratio, nitric oxide heme pigment, and pigment conversion in the following order: films 1, 2, 4, 5, and 3. Overall means for film 3 indicated that this film was not acceptable as measured by a color score of 2.37, spectral ratio of 2.92 and pigment conversion of 40.87%. There were no significant differences for terminal vacuum among films 1, 2, 3, and 4. Film 5 had the poorest vacuum retention, particularly at the 70% initial vacuum level. This seemed to result from the rigid type of material and may mean that more residual air existed inside the package. This caused more rancidity, as indicated by higher TBA numbers for the samples of films 3 and 5 compared with the others.

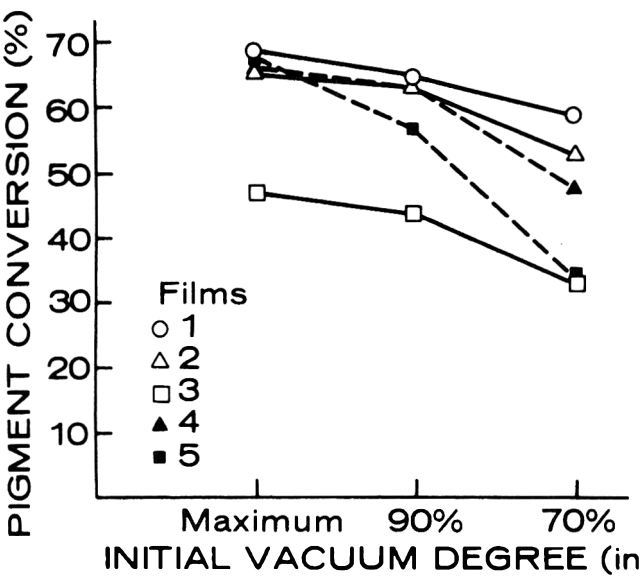

Fig. 1—Effect of films and initial vacuum on pigment conversion for all time periods.

The significant effect of films and initial vacuum level on pigment conversion is shown in Figure 1. Film 1 in all three vacuum groups and films 2, 4, and 5 in maximum and 90% initial vacuum levels exhibited more than 55% pigment conversion, which was acceptable. Film 3 showed low values (less than 50% pigment conversion) with all three vacuum groups because of oxidation of pigment, which resulted from higher oxygen permeability (60 ml/m^2 /24 hr). Film 5 was found to have serious discoloration at the 70% vacuum level because of the low terminal vacuum previously discussed.

Initial vacuum level had a significant influence on color and rancidity development (Table 3). The package with the maximum initial vacuum level seemed to have high color retention as indicated by higher color score, 5.28; lower total reflectance, 33.4; higher spectral ratio, 5.56; higher nitric oxide heme pigment, 85 ppm; and higher pigment conversion, 62.80%. The maximum vacuum level, when compared with the other two initial vacuum levels, also showed higher terminal vacuum, 710 mm of Hg; higher residual nitrite, 15.07 ppm; and lower TBA number, 0.75. The 70% initial vacuum level did not show acceptable color; the color score was less than 3.5, the spectral ratio was less than 3.5, pigment conversion was less than 50%, and the TBA number was more than 1.

Influence of films, initial vacuum and storage time on pigment conversion is given in Table 4. Good barrier films interacted with maximum initial vacuum to retain good color development. The maximum and 90% initial vacuum for films 1, 2, 4, and 5 still retained acceptable pigment conversion up to 56 days of storage. The 70% initial vacuum level remained acceptable for 56 days with film 1, 21 days with film 2, and was not acceptable after only 1 day

Table 3—Effect of initial vacuum on color and rancidity development for all films and time periods[a]

	Degree of vacuum		
	Maximum	90%	70%
Color score	5.28 ± 1.51a	3.54 ± 1.33b	1.98 ± 0.92c
Reflectance	33.40 ± 3.17c	34.35 ± 3.35b	35.88 ± 3.38a
K/S 570:K/S 650	5.56 ± 1.52a	4.11 ± 1.29b	2.63 ± 0.87c
Nitric oxide heme pigment (ppm)	85.00 ± 21.64a	76.33 ± 20.04b	54.43 ± 25.23c
Total pigment (ppm)	135.46 ± 22.33a	132.63 ± 22.78a	118.34 ± 25.02b
Pigment conversion (%)	62.80 ± 13.51a	57.89 ± 14.07b	45.01 ± 18.02c
Terminal vacuum (mm)	709.9 ± 28.9a	599.2 ± 103.4b	447.3 ± 177.5c
Residual nitrite (ppm)	15.07 ± 9.37a	14.12 ± 9.14b	13.47 ± 10.41b
TBA no.	0.75 ± 0.31b	0.81 ± 0.32b	1.08 ± 0.66a

[a] All means in a row with the same letters are not significantly different ($P < 0.05$); X ± SD = mean ± standard deviation.

Table 4—Effect of films, initial vacuum degree, and display time on pigment conversion for all erythorbate levels[a]

Storage time (days)	Initial vacuum degree	Films				
		1	2	3	4	5
1	Maximum	62.82 ± 9.89	61.19 ± 9.35	61.44 ± 6.14	67.76 ± 5.56	63.11 ± 9.06
	90%	54.94 ± 10.43	57.11 ± 15.13	54.11 ± 13.00	54.55 ± 11.91	50.15 ± 10.67
	70%	52.70 ± 14.26	51.45 ± 14.70	43.24 ± 11.17	40.29 ± 21.29	26.09 ± 1.69
4	Maximum	68.36 ± 3.61	65.74 ± 1.63	58.54 ± 2.47	64.61 ± 5.52	66.38 ± 6.98
	90%	63.03 ± 6.03	58.84 ± 6.77	52.07 ± 18.13	63.50 ± 10.23	55.92 ± 8.96
	70%	62.72 ± 13.68	45.38 ± 13.69	47.57 ± 11.31	43.92 ± 26.40	28.55 ± 15.98
10	Maximum	75.58 ± 8.85	73.51 ± 7.96	52.65 ± 26.13	67.16 ± 2.99	68.70 ± 2.80
	90%	70.52 ± 6.32	68.47 ± 10.46	50.28 ± 28.69	62.34 ± 3.04	57.58 ± 10.72
	70%	58.52 ± 6.98	64.30 ± 10.28	33.62 ± 11.91	43.60 ± 16.86	24.23 ± 21.41
21	Maximum	72.58 ± 7.80	69.62 ± 7.42	43.35 ± 22.27	69.09 ± 13.59	71.80 ± 14.49
	90%	74.65 ± 6.07	66.59 ± 10.13	41.80 ± 12.09	69.95 ± 14.42	63.47 ± 13.50
	70%	64.27 ± 12.08	59.01 ± 13.55	35.72 ± 15.88	60.32 ± 16.24	25.61 ± 17.36
35	Maximum	66.49 ± 9.33	62.39 ± 2.64	35.69 ± 18.36	63.51 ± 2.21	63.78 ± 8.77
	90%	62.16 ± 4.30	61.81 ± 4.31	31.59 ± 21.91	59.16 ± 4.55	62.48 ± 3.90
	70%	54.84 ± 8.07	42.49 ± 12.98	20.83 ± 15.66	43.72 ± 13.20	25.59 ± 12.32
56	Maximum	64.03 ± 5.25	62.53 ± 0.90	27.62 ± 22.06	67.64 ± 1.70	65.76 ± 2.75
	90%	60.85 ± 8.97	61.38 ± 6.41	29.29 ± 8.91	66.69 ± 4.83	61.35 ± 3.19
	70%	57.74 ± 0.81	50.12 ± 13.88	16.09 ± 6.51	44.19 ± 13.34	43.63 ± 22.53

[a] X ± SD = mean ± standard deviation.

Table 5—Effect of sodium erythorbate levels on color related factors and rancidity development for all films and vacuum levels[a]

Items	Levels of sodium erythorbate		
	0 ppm	500 ppm	940 ppm
Nitric oxide heme pigment (ppm)	80.20 ± 3.03a	71.13 ± 24.38b	64.44 ± 19.23c
Total pigment (ppm)	149.20 ± 15.59a	128.30 ± 24.34b	108.94 ± 12.62c
Pigment conversion (%)	52.97 ± 19.01c	54.27 ± 16.19b	58.46 ± 15.35a
Residual nitrite (ppm)	22.27 ± 11.11a	9.25 ± 3.75b	11.13 ± 6.58b

[a] All means in a row with the same letters are not significantly different ($P < 0.05$); X ± SD = mean ± standard deviation.

of storage for films 4 and 5. In addition, the poor barrier film 3 remained acceptable after 4 days with maximum initial vacuum and for 1 day with 90% initial vacuum. Greater pigment conversion occurred, particularly in the maximum and 90% initial vacuum levels, for films 1, 2, 4, and 5. This was also true in the 70% initial vacuum level but only for film 1. As previously stated, anaerobic conditions are extremely important with regard to pigment conversion and how long it will remain acceptable.

Sodium ascorbate and erythorbate act by reducing nitrite to nitric oxide, which reacts with the meat pigment myoglobin and forms the stable pink nitric oxide hemochrome upon heating. The higher nitric oxide heme pigment found in groups without erythorbate or with 500 ppm erythorbate compared with the 940 ppm erythorbate samples was due to higher total pigment content in these groups resulting from different raw meat sources. The 940-ppm erythorbate group had a higher pigment conversion ($P < 0.05$) compared with the 500-ppm and zero erythorbate groups. This suggested that erythorbate was accelerating the development of cured color (Table 5). Similar findings have been reported by other authors (Fox et al., 1967; Counsell, 1971).

Residual nitrite levels were higher in bologna cured without erythorbate than in bologna cured with erythorbate (Table 5).

Erythorbate accelerated the nitrite depletion rate as shown in Figure 2. Bologna with erythorbate had lower

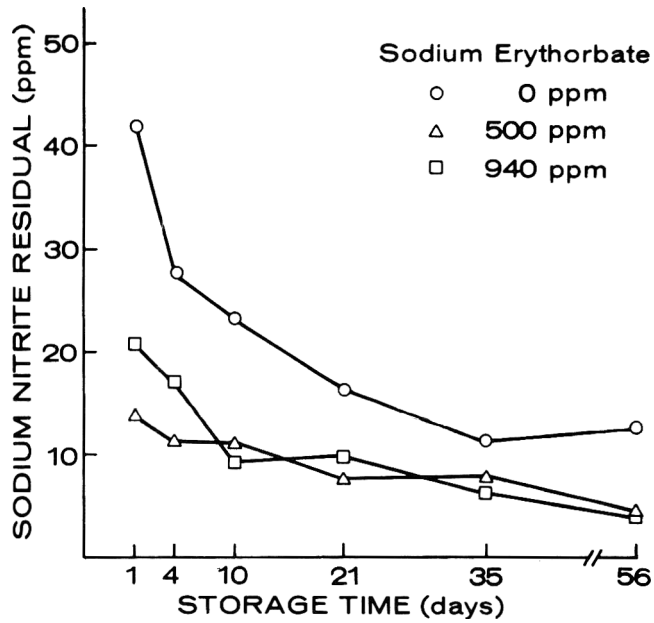

Fig. 2—Effect of sodium erythorbate and storage time on residual nitrite.

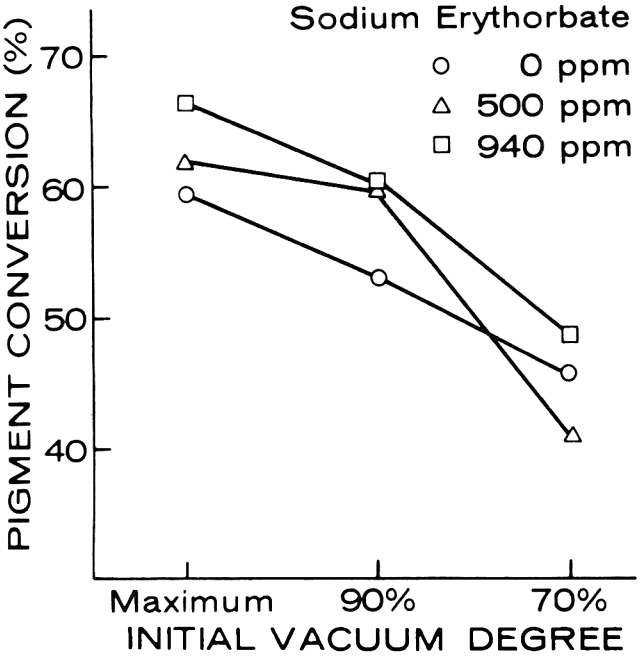

Fig. 3—Effect of sodium erythorbate and initial vacuum on pigment conversion for all time periods.

residual nitrite from the first day of display as compared with the zero erythorbate level. Residual nitrite was also found to decrease with storage time, and the depletion rate was much faster during the first few days of storage.

Cured meat with 500 ppm and 940 ppm sodium erythorbate and packaged with maximum initial vacuum was found to have the best pigment conversion (Fig. 3). The pigment conversion of the 940-ppm erythorbate samples was better than the 500-ppm or zero erythorbate groups at all time periods when compared at both maximum and 90% initial vacuum levels, respectively. The samples of three erythorbate levels at maximum or 90% initial vacuum level were acceptable with a pigment conversion value of above 55%. All the 70% initial vacuum samples, however, had conversion values of less than 50%, even the 940-ppm erythorbate group. Thus, even erythorbate could not help those packages that had higher quantities of residual air inside as indicated by spectral ratio in Table 6. In addition, the initial vacuum level was more important for zero erythorbate samples than for groups with erythorbate. Because the difference in spectral ratio between the maximum and 90% vacuum levels in the zero erythorbate group was more than in 500-ppm or 940-ppm erythorbate groups, the improvement of cured color by maximum vacuum was more important in the zero erythorbate group.

Residual nitrite was significantly lower in samples with erythorbate with only a slight difference for residual nitrite between the 500-ppm and 940-ppm erythorbate groups.

The 940-ppm erythorbate level was somewhat better for color retention in general as described by pigment conversion at all time periods (Table 7). Acceptable color development was indicated for film 1 at all three initial vacuum and erythorbate levels, for films 2, 4, and 5 at the three erythorbate levels, but only at maximum and 90% initial vacuum levels, and for film 3 at only 500-ppm erythorbate with maximum and 90% initial vacuum. Therefore, it seems the use of a reductant such as erythorbate, although aiding in cured color development, is not nearly as important as maintaining good packaging conditions. The reductant does offer a significant advantage in faster depletion of residual nitrite.

High barrier films as well as film rigidity and an adequately maintained vacuum level within packages seem the most critical factors for storage stability and will be particularly so as nitrite levels are decreased.

Finally, when erythorbate is used, there seems to be

—Continued on page 121

Table 6—Effect of sodium erythorbate and initial vacuum degree on spectral ratio and residual nitrite for all films[a]

Items	Initial vacuum degree	Levels of sodium erythorbate		
		0 ppm	500 ppm	940 ppm
K/S 570:K/S 650	Maximum	4.17 ± 1.59	4.73 ± 1.05	5.79 ± 1.51
Residual nitrite (ppm)	Maximum	23.24 ± 10.82	10.36 ± 3.48	11.60 ± 6.01
K/S 570:K/S 650	90%	3.65 ± 1.14	4.23 ± 0.95	4.46 ± 1.59
Residual nitrite (ppm)	90%	21.40 ± 10.79	9.65 ± 3.44	11.29 ± 6.66
K/S 570:K/S 650	70%	2.82 ± 0.63	2.46 ± 1.01	2.60 ± 0.92
Residual nitrite (ppm)	70%	22.17 ± 12.00	7.74 ± 3.93	10.51 ± 7.18

[a] X ± SD = mean ± standard deviation.

Table 7—Effect of films, sodium erythorbate, and initial vacuum degree on pigment conversion for all time periods[a]

Level of sodium erythorbate	Initial vacuum degree	Films				
		1	2	3	4	5
0 ppm	Maximum	67.11 ± 6.09	65.54 ± 5.98	30.13 ± 20.68	67.01 ± 5.05	69.39 ± 7.17
	90%	62.22 ± 10.89	60.91 ± 12.69	25.97 ± 9.88	61.48 ± 11.61	56.07 ± 16.07
	70%	56.33 ± 14.02	52.97 ± 17.17	24.45 ± 8.60	30.96 ± 16.33	24.09 ± 19.96
500 ppm	Maximum	65.82 ± 11.52	64.18 ± 9.51	58.52 ± 10.17	62.52 ± 6.09	59.00 ± 5.19
	90%	63.02 ± 10.59	64.33 ± 7.73	56.92 ± 14.07	58.94 ± 6.34	57.39 ± 6.74
	70%	59.16 ± 9.39	55.69 ± 11.33	30.02 ± 18.37	30.92 ± 9.96	27.63 ± 16.08
940 ppm	Maximum	72.01 ± 4.91	68.03 ± 4.44	51.04 ± 17.34	70.36 ± 4.35	71.38 ± 4.48
	90%	67.84 ± 5.13	61.87 ± 6.93	46.69 ± 16.29	67.68 ± 8.27	57.01 ± 11.29
	70%	59.90 ± 5.15	47.71 ± 13.61	44.07 ± 13.11	41.13 ± 7.69	30.14 ± 17.79

[a] X ± SD = mean ± standard deviation.

QUALITY, APPEARANCE, AND TENDERNESS OF ELECTRICALLY STIMULATED LAMB

R. R. RILEY, J. W. SAVELL, G. C. SMITH, and MAURICE SHELTON

ABSTRACT

Fourteen wether lambs were slaughtered, split longitudinally and the left sides were electrically stimulated (ES) with 17 impulses (1.8 sec duration) of 550 volts for 1 min prior to chilling. At 24 hr postmortem, USDA maturity scores were assigned to certain lean surfaces of the carcass. Following storage at 0–2°C for 5 days, four boneless loin chops were removed from each side; two chops were displayed under retail conditions (1–3°C) for 4 days and two chops were used for shear force measurements. Longissimus muscles from ES sides were more youthful ($P < 0.001$) in color, and body cavity muscles (primary flank, secondary flank, intercostal) were brighter ($P < 0.05$) in color at 24 hr postmortem. There were no significant differences between chops from ES and control sides for muscle color, surface discoloration and overall appearance after 3 days of retail display; however, loin chops from the ES sides were more desirable ($P < 0.10$) in appearance on the fourth day of retail display than were the chops from the control (unstimulated) sides. Although there was no difference in cooking loss percentage, there was a significant difference ($P < 0.10$) in thawing loss percentage (0.83 for ES chops; 1.23 for control chops). Shear force measurements revealed that samples from ES sides were significantly ($P < 0.01$) more tender than were samples from the control sides. These data suggest that lamb lean color can be made brighter and more youthful in appearance by use of electrical stimulation. Other advantages associated with use of electrical stimulation are extended retail caselife and markedly improved tenderness of loin chops.

INTRODUCTION

ELECTRICAL STIMULATION of lamb has gained wide acceptance in New Zealand. Researchers in that country (Carse, 1973; Chrystall and Hagyard, 1976) have documented the positive benefits of utilizing electrical stimulation to prevent "cold shortening" and/or "thaw rigor" in lamb that is frozen immediately after slaughter and dressing. Much of this research was conducted because of complaints received from the United Kingdom about the tenderness of New Zealand frozen lamb (Chrystall and Hagyard, 1975). Although the chilling procedures are different in the United States (refrigeration instead of freezing), electrical stimulation has been shown to result in substantial improvements in the tenderness of lamb and goat (Savell et al., 1977; McKeith et al., 1979).

Interest in the incorporation of electrical stimulation in the slaughter-dressing sequence of U.S. beef packing plants has been due to the multiple benefits realized by the packer–increased tenderness and improved quality-indicating characteristics (Savell and Smith, 1979). Research has not been conducted to ascertain whether some of the improvements in the quality-indicating characteristics observed in beef (such as lean color) would also be found in lamb. Brighter lean color and more attractive lamb chops in the retail case, in addition to the benefits of increased tenderness, could make the use of electrical stimulation for lamb in the United States a reality.

This study was conducted to investigate the effects of electrical stimulation on some of the quality-indicating characteristics of lamb. Also, evaluations were made of loin chops displayed under retail conditions in order to determine the possible effects of electrical stimulation on muscle color, surface discoloration and overall appearance. Finally, shear force determinations were obtained for cooked loin chops from electrically stimulated and control sides to further document tenderness improvement due to electrical stimulation.

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Table 1–Means and standard deviations for certain carcass traits[a]

Trait	Mean	S.D.
Carcass weight, kg	24.3	2.8
Adjusted fat thickness-12th rib, mm	5.33	1.7
USDA leg conformation	Ch^{55}	12.9
Kidney and pelvic fat, (%)	3.0	0.7
Longissimus muscle area, cm^2	14.84	1.4
USDA maturity score	A^{67}	15.9
Feathering	Sl^{86}	76.6
Flank streaking[b]	Sm^{08}	67.3
Flank firmness	TMF^{34}	72.1
USDA carcass conformation	Ch^{66}	11.5
USDA quality grade	Ch^{55}	30.8
USDA yield grade	3.2	0.6

[a] All lambs were graded using USDA (1960) grade standards.
[b] Average of scores for primary and secondary flank streaking.

EXPERIMENTAL

FOURTEEN wether lambs were exsanguinated, dressed, split longitudinally and the left side of each carcass was electrically stimulated within 30 min postmortem and before chilling. The source of electrical stimulation was an experimental "Lectro-Tender™" unit manufactured by the LeFiell Company, San Francisco, Calif. The single probe of the unit was inserted in the muscles between the first rib and scapula of each side with the rail serving as the ground for the completion of the circuit. The stimulator setting was 550 volts (AC), 5 amps and 17 impulses were given during a period of 1 min (1.8 sec duration, 1.8 sec interval between impulses).

Quality and yield grade measurements

Approximately 24 hr following slaughter, quality and yield grade factors were assigned by T.A.E.S. personnel. The factors evaluated were adjusted fat thickness, 12th rib; USDA leg conformation; kidney and pelvic fat percentage; USDA skeletal, lean, and overall maturity; intercostal feathering; flank streaking; and longissimus muscle area.

Fabrication

The longissimus muscle was removed from the wholesale loin and cut into four chops (3.8 cm in thickness) on the fifth day postmortem. Loin chops were cut using strict sanitation procedures which consisted of continuous cleansing of hands and equipment as well as knife sterilization between fabrication of loins.

Retail display

Two boneless loin chops from each side were placed on foam trays, overwrapped with polyvinyl chloride film and displayed in a

Table 2—Comparison of muscle color, cooking characteristics, and shear force values for samples from electrically stimulated and untreated sides

Trait	Electrically stimulated		Untreated (control)		Level of probability[a]
	Mean	S.D.	Mean	S.D.	
Longissimus muscle color[b]	A^{51}	18.8	A^{67}	23.1	$P < 0.001$
Primary and secondary flank and intercostal muscle color[b]	A^{59}	11.7	A^{65}	13.5	$P < 0.05$
Thawing loss, (%)	0.83	0.3	1.23	0.8	$P < 0.10$
Cooking loss, (%)	24.90	5.3	25.19	3.9	N.S.
Warner-Bratzler shear force, kg	3.24	1.1	4.63	1.9	$P < 0.01$

[a] Probability that the difference between treatments is statistically significant based on the paired-t analysis (Steel and Torrie, 1960). $P > 0.10$ was reported as nonsignificant (N.S.).

[b] Lambs slaughtered at chronological ages of 3–8 months generally produce carcasses with physiological maturity indicators described as A^{00} to A^{100}, respectively, in USDA (1960) grade standards for lamb carcasses.

Table 3—Comparison of certain traits for boneless loin chops subjected to retail display for four days

Trait	Day of retail display	Electrically stimulated		Untreated (control)		Level of probability[a]
		Mean	S.D.	Mean	S.D.	
Muscle color[b]	0	5.1	1.03	5.2	0.93	N.S.
	1	5.0	0.64	5.0	0.86	N.S.
	2	5.0	0.61	5.0	0.77	N.S.
	3	4.7	0.61	4.6	0.80	N.S.
	4	4.5	0.64	4.3	0.77	N.S.
Surface discoloration[c]	0	6.9	0.17	7.0	0.07	N.S.
	1	6.3	0.42	6.4	0.34	N.S.
	2	5.9	0.48	5.8	0.43	N.S.
	3	5.6	0.46	5.6	0.43	N.S.
	4	5.0	0.45	4.9	0.53	N.S.
Overall appearance[d]	0	6.9	0.65	6.7	0.58	N.S.
	1	5.9	0.72	5.7	0.66	N.S.
	2	5.2	0.79	4.9	0.68	N.S.
	3	4.9	0.73	4.6	0.79	N.S.
	4	4.4	0.72	4.0	0.69	$P < 0.10$
Bacterial count, $\log_{10}$	0	1.11	–	1.23	–	–
	4	1.83	–	3.06	–	–
Shrink loss, (%)	4	3.53	0.67	3.56	0.68	N.S.

[a] Probability that the difference between treatments is statistically significant based on the paired-t analysis (Steel and Torrie, 1960). $P > 0.10$ was reported as nonsignificant (N.S.).

[b] 9 = very light cherry red; 1 = very dark purple.

[c] 7 = no surface discoloration; 1 = total surface discoloration.

[d] 8 = extremely desirable; 1 = extremely undesirable.

standard retail display case at 1–3°C under 883 lux of incandescent light for 4 days. At 24-hr intervals, a nine-member trained panel evaluated the chops for muscle color (9=very light cherry red, 1=very dark purple), surface discoloration (7=no surface discoloration, 1=total surface discoloration), and overall appearance (8=extremely desirable, 1=extremely undesirable). Chops were weighed at the beginning and end of retail display in order to determine shrink loss.

Bacterial counts

Samples for bacterial counts were taken from the lean surface of the retail cuts at the beginning and end of retail display by the use of a moist sterile dacron swab, a 9 cm^2 sterile template, and buffered rinse solution technique. After swabbing, samples were diluted in phosphate buffer, plated (within 30 min of swabbing) and incubated at 22°C for 5 days to obtain psychrotrophic counts.

Shear force determinations

On the fifth day postmortem two chops from each side were wrapped with polyethylene-coated paper, frozen, and stored (−23°C) for 2 wk. Each chop was removed from the freezer, thawed, and cooked to 70°C internally in a 190°C gas oven. Chops were weighed before and after thawing and after cooking in order to determine thawing and cooking loss percentages. After chops were cooled to room temperature, two cores (1.3 cm diam) were removed from each chop (four cores per side) and shear force measurements (two shear force values per core) were obtained by the use of the Warner-Bratzler shear machine.

Statistical analysis

The data were analyzed by the use of paired-t distribution analysis (Steel and Torrie, 1960) to determine significance of difference between treatments.

RESULTS

IN ORDER TO adequately describe the lambs utilized for this research study, certain carcass traits were obtained for each lamb and the mean and standard deviation for each trait are presented in Table 1. The mean USDA quality grade was Ch^{55} and the mean USDA yield grade was 3.2. Grade data and other values obtained suggest that these lambs were quite typical of the type of lambs slaughtered commercially in the United States.

Comparisons of untreated (control) and electrically stimulated sides for muscle color, cooking characteristics, and shear force values are reported in Table 2. Electrically stimulated sides had significantly more youthful longissimus muscle color ($P < 0.001$) and internal (primary and secondary flank and intercostal) lean color ($P < 0.05$) when evaluated at 24 hr postmortem, than did control sides. Longissimus muscle samples from electrically stimulated sides had less thaw loss ($P < 0.10$) and lower Warner-Bratzler shear force values ($P < 0.01$) than did samples from the untreated

sides. Cooking loss did not differ ($P > 0.10$) between samples from electrically stimulated and untreated sides.

Presented in Table 3 are comparisons of retail caselife for boneless loin chops from electrically stimulated and untreated sides. No significant differences ($P > 0.10$) were observed between retail cuts from electrically stimulated and untreated sides for muscle color, surface discoloration, and overall appearance after 3 days of retail display; however, loin chops from the electrically stimulated sides were more desirable ($P < 0.10$) in appearance on day 4 than were the chops from the untreated sides. Final bacteria counts from samples of electrically stimulated lamb were substantially lower numerically than were those from samples of untreated sides after 4 days of retail case display

DISCUSSION

SINCE COLOR is undoubtedly related to decisions on purchasing, production of young slaughter animals might help alleviate a color problem in lamb since physiological maturity is closely related to color (Carpenter, 1966). Because electrical stimulation has been shown to improve lean color of beef (Savell et al., 1978a, b; 1979), improved lean color in retail cuts as a result of electrically stimulating lamb may greatly enhance their attractiveness to retail customers without necessarily having to market very youthful animals. In the present study, lean color (longissimus muscle and body cavity muscle surface) was significantly improved by the use of electrical stimulation but muscle color of chops from electrically stimulated sides displayed under retail conditions was not different from that of chops from sides in the control group. The color advantage for chops from electrically stimulated sides at 24 hr may be negated by chilling and storage for 5 days before fabrication. It is possible that use of differing voltages, impulses, etc. for electrical stimulation could prevent deterioration in lean color associated with additional postmortem chilling time.

Extending the retail caselife of lamb is important because lamb is a slow-moving item at retail in many parts of the U.S. In this study, overall appearance of chops from electrically stimulated lambs was significantly improved in comparisons to that for chops from control lambs after 4 days of display. Bacterial counts (especially final) were substantially lower for chops from electrically stimulated sides. It is possible that electrical stimulation may have a deleterious effect on either bacteria or on their growth medium. Further studies on retail caselife and bacterial growth on cuts from electrically stimulated carcasses are needed to determine whether or not these factors are significantly influenced by the ES process.

In conclusion, it is possible that electrical stimulation can be utilized to improve the lean maturity of certain lean surfaces of the lamb carcass, to increase tenderness, to improve appearance of chops during retail display and to reduce bacterial growth on retail cuts. Such advantages for electrically stimulated lamb may lead to utilization of the ES process by lamb slaughterers in the United States.

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little advantage to concentrations higher than the currently maximum approved levels of 550 ppm.

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MODELING AND SIMULATING A DISTRIBUTED PARAMETER TUNNEL-DRIER

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ABSTRACT

In this paper a distributed parameter model is proposed for the discontinuous drying operation of a tunnel-drier. This model is more complete than the previous ones in which continuous loading is currently assumed. The model is used for spherical fruits placed in a California tunnel of the parallel flow type. Numerical results are in good agreement with experimental information. The model can be used for optimum designing and automatic control.

INTRODUCTION

SOME PREVIOUS PROJECTS have been devoted to modeling tunnel-driers with the assumption of continuous loading and have been the topic of extended papers: Kilpatrick et al. (1955), Gentry et al. (1965), Keey (1972). In some actual cases, such as prune driers, loading is discontinuous (Fig. 1). In this paper we present an extension to the case of discontinuous loading of a previously published model (Bertin et al., 1976).

The mathematical simulation is based on the analysis of the heat and mass transfer, at two different scales, inside the product and for the overall tunnel. From this analysis, and with complementary assumptions, a set of nonlinear partial differential equations is derived, the main dependent variables of which are average values of temperature and water content for both fruit and air.

The functions which appear as the coefficients of the equations are determined by fitting theoretical curves from these equations to experimental data (identification methods). By this way, the coefficients can be deduced from the simple model with continuous loading (Bertin et al., 1976), from a numerical simulation of the drying of fruit in a laboratory oven (Bertin et al., 1978), or from the distributed parameter model (Bertin and Srour, 1979).

Heat and mass balances for the product

Inside the product, the local temperature and water content obey a system of coupled heat and mass transfers described by partial differential equations (Luikov, 1966). Assuming (1) that all the coefficients appearing in these equations are constant, and (2) that the temperature and water content profiles are parabolic functions of the radius inside the product (Babukha and Shraiber, 1974), an averaging procedure over volume is applied (Bertin et al., 1978) by taking into account the boundary conditions at the surface of the product. Two equations are then derived for the average values of both temperature and water content:

$$\frac{\partial T_p}{\partial t} = \frac{3a}{R^*}(1 + Bi^*/5)^{-1}\left[\frac{Bi^*}{R^*}(T_a - T_p) + \frac{\Delta hv}{\lambda}\frac{R^*}{3}\rho_d^*\frac{\partial W}{\partial t}\right] \quad (1)$$

$$\frac{\partial W}{\partial t} = -\frac{3\alpha_m}{\rho_d^* R^*}(p_{surf} - p_v) \quad (2)$$

The symbol * is used to point out a parameter on which shrinkage effect is possible.

One can remark that these two equations are now often considered as classical and appear in a lot of engineering papers devoted to drying. For instance, Eq (1) is mentioned for textile materials (Nordon and Bainbridge, 1971) and for steady state food drying (Kilpatrick et al., 1955), and Eq (2) for prunes (Guillou, 1942), various foods (Krisher, 1963), and different materials (Keey, 1972, 1978).

Heat and mass transfer for the tunnel

To describe the overall drying process we must add to the previous equations the equations for heat and mass balances for the overall tunnel itself. The entire drying process consists in loading and unloading trucks at given times. During this operation fans and heater are stopped. Therefore, boundary conditions at the inlet end of the tunnel are changed. Then the following assumptions are introduced which are justified by the usual operating conditions of a tunnel:

- Hydrodynamic transient state times are short compared to thermal regimes.
- When the fans are on, the dry air flow G is constant.
- Inside the trucks the velocity of the gas phase v can be considered as decreasing slowly (experimental results are in good agreement with this assumption which can be justified theoretically).

Then enthalpy and mass balances applied to both gas flow G and solid flow L phases yield (See Fig. 2 for details):

$$\left(\frac{\partial}{\partial t} + v\frac{\partial}{\partial x}\right)\left[(C_g T_a + \Delta h_v w)\frac{G}{v}\right] + \left(\frac{\partial}{\partial t} + u\frac{\partial}{\partial x}\right)\left[(C_s T_p)\frac{L}{\bar{u}}\right] = 0 \quad (3)$$

$$\left(\frac{\partial}{\partial t} + v\frac{\partial}{\partial x}\right)\left(w\frac{G}{v}\right) + \left(\frac{\partial}{\partial t} + u\frac{\partial}{\partial x}\right)\left(W\frac{L}{\bar{u}}\right) = 0 \quad (4)$$

In these equations G/v and L/$\bar{u}$, respectively, represent the dry mass of gas and solid phases per unit of length and the instantaneous truck speed u has been assumed negligible compared to the gas velocity; the dry mass flow of product is divided in Eq (3) and (4) by $\bar{u}$ which is the ratio of the overall drying time to the tunnel length.

These balance equations have the usual form encountered in any drying procedure (Keey, 1972; 1978) such as mentioned for grain by Sutherland et al. (1971) and for textile materials by Nordon and Bainbridge (1971).

Plant model

We transformed this first order system of partial differential equations to the normal form (Ames, 1972):

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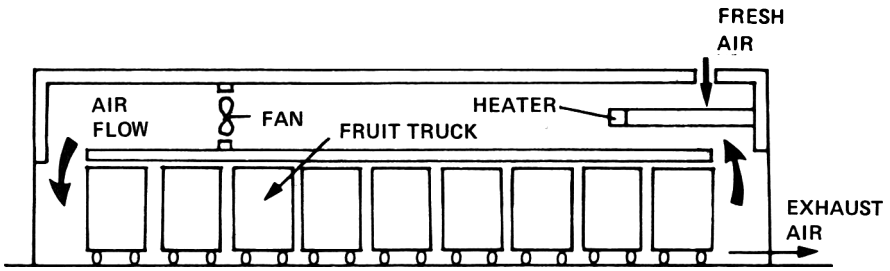

Fig. 1—A typical prune tunnel-drier. Fruits are placed on trays. They progress along the tunnel, step by step, when a new truck is introduced.

$$A\,U_x + B\,U_t + D = 0 \qquad (5)$$

in which U is the unicolumn vector $(T_a, w, T_p, W)^T$; A, B being matrices; and D a vector. Initial and boundary conditions impose the values of some components of the vector U.

$$\text{For } t = 0:\ U(x,t) = U_i(x) \qquad (6)$$

At the upstream side of the truck (x = 0)

$$T_a(0,t) = h(t) \text{ and } w(0,t) = g(t) \qquad (7)$$

where h(t) and g(t) are known functions which are measured directly in the tunnel.

RESULTS

Simulation: method of characteristics

This hyperbolic system is transformed by the method of characteristics to a system of ordinary differential equations along with characteristic directions given by the roots of

$$\det(A - \nu B) = 0, \text{ i.e. } \nu_1 = \nu_2 = v \text{ and } \nu_3 = \nu_4 = 0 \qquad (8)$$

With the operators:

$$\frac{d}{ds_1} = \frac{\partial}{\partial t} + v\frac{\partial}{\partial x} \quad \text{following } x = vt + \text{constant} \qquad (9)$$

$$\frac{d}{ds_2} = \frac{\partial}{\partial t} + 0\frac{\partial}{\partial x} \quad \text{following } x = \text{constant} \qquad (10)$$

Hence the model is written in the characteristic normal form (Appendix):

$$dT_a/ds_1 + E_1 = 0 \qquad (11)$$

$$dw/ds_1 + E_2 = 0 \qquad (12)$$

$$dT_p/ds_2 + E_3 = 0 \qquad (13)$$

$$dW/ds_2 + E_4 = 0 \qquad (14)$$

High air velocity makes the hydrodynamic transient states through trucks sufficiently short to be neglected. Therefore, characteristic directions dx/dt = v could be replaced by the direction t equal to a constant.

The equation set (11) to (14) is integrated by the fourth-order Runge-Kutta-Gill method following t = constant and x = constant but other methods given by library computer programs are possible.

Further complementary approximations

The model is applied with some simplifications which are now presented and discussed.

The temperature of the fruit surface obeys:

$$T_{surf} = T_p \qquad t = 0 \text{ and } t \geq t_1$$

$$T_{surf} = (T_a + T_p)/2 \qquad t \in]0, t_1[$$

The duration t_1 is derived from transient regime during which the surface temperature is different from the average product temperature.

The overall mass transfer coefficient α_m which could have been put under the form of a mass Biot number, is described as a function of W as well as the density ρ_d^* and the radius R* of the sphere equivalent to the product; this is a way of taking into account the piece-size effect on drying rate which is then an implicit function of T_p, T_a, x, t:

$$\frac{\partial W}{\partial t} = f(T_{ps}, W, p_v, R^*, \rho_d^*) \qquad (15)$$

In the formula given for α_m in the Appendix, α_m is described as proportional to W (x,t) rather than to the difference between W and the equilibrium moisture content (Guillou, 1942); this approximation is justified by a low equilibrium water content of the product compared to the final water content.

We must notice that the system of equations (11) to (14) has been stated assuming negligible product shrinkage, i.e., with constant values of R* and ρ_d^*. However, this equation set is used with R* and ρ_d^* varying with time. Because of the low values of the drying rate, the procedure which is proposed here appears quite correct and convenient.

DISCUSSION & CONCLUSIONS

Continuous loading

Some previously published results (Bertin et al., 1976) are devoted to the application of the model to the sole case of continuous loading. In Figure 3 average profiles along the tunnel are presented which have been obtained for ex-

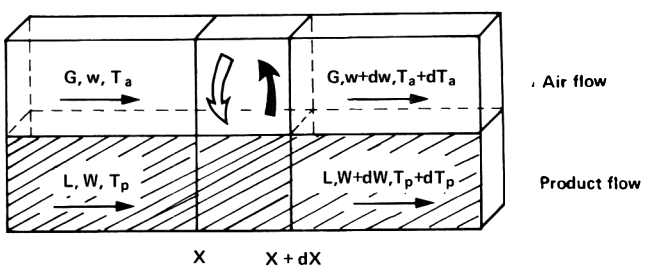

Fig. 2—Sketch shows the enthalpy and mass balances between the air and product flows along the tunnel-drier. The following symbols refer to heat flow rate ⇝ and to mass flow rate ➞.

perimental conditions described in Table 1 and assuming a continuous loading with constant air flow rate at the injection abscissa. The influence of various parameters on these profiles has been studied; for example, heat and mass transfer coefficients (Bertin et al., 1976). The results are consistent with what can be inferred from a qualitative analysis of the phenomena. For instance, as far as with air flow rate is concerned, when G is increased, variations with x of both temperature and water content of the air are decreased; complementarily the overall drying time is reduced.

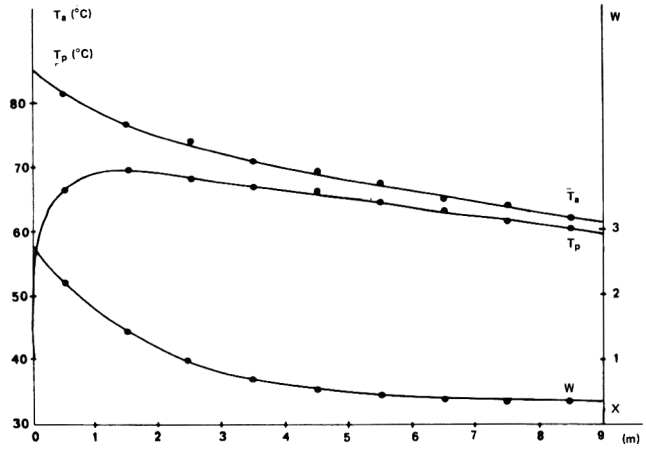

Fig. 3—Assumption of continuous loading: Distribution vs drier length of air temperature T_a, product temperature T_p, and moisture content of product W for the nine positions of the truck in the tunnel-drier.

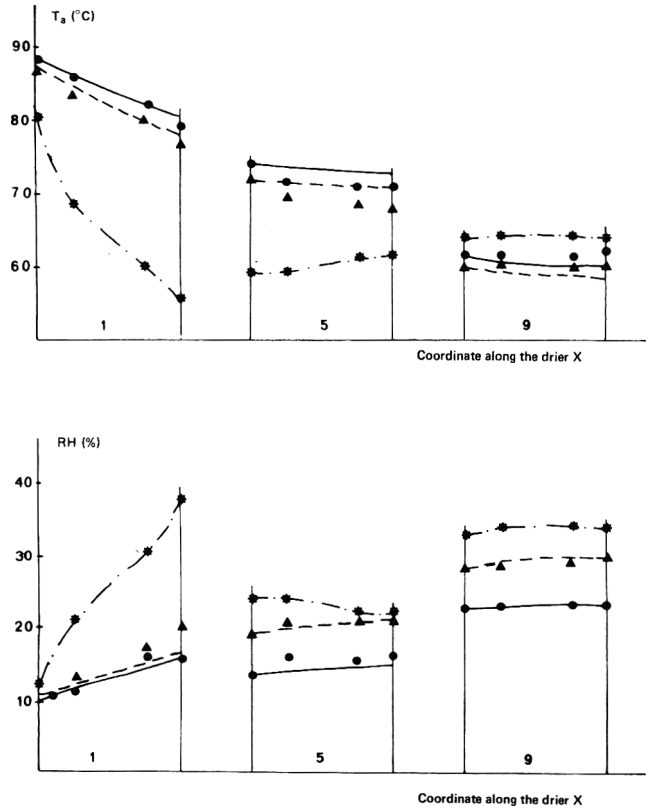

*Fig. 4—Discontinuous loading: Distribution of temperature T_a and relative humidity RH in the air vs drier length when time shifts. These diagrams show three particular positions of the truck in the tunnel. For each position (n° 1, 5 or 9) the curves are displayed at time 0 min, 45 min and 90 min. Time starts from the moment the truck arrives at the given position. The symbols for experimental points at 0, 45, and 90 min are respectively *, ▲, ●, and theoretical curves •—•—, – –, ————.*

Discontinuous loading

The results which are presented in Figures 4 and 5 concern the modeling of a real test during which a loaded test truck equipped with different sensors has been run through the drier, step by step, numbered from 1 to 9. The overall duration of the experimental test was about 16 hr (each time step about 105 min). The measured variables are (1) temperature and humidity of air; and (2) temperature and moisture content of product. The sensors have recorded their distributions along the tunnel, and these variables are time dependent.

For computation we used as boundary conditions the experimental results given by the downstream side of the test truck. The numerical results presented in Figures 4 and 5 have been obtained with the values of the parameters given in Table 1.

The complementary operating conditions are:

- steady state temperature and relative humidity of the air at the inlet of the tunnel: $T_{ai} = 87°C$ and $RH_i = 11\%$
- tray loading: 15 kg/m^2
- average dry air flow rate: G = 6.3 kg/s
- average dry product flow rate: $L = 24.10^{-3}$ kg/s

Numerical profiles obtained by the theoretical model are in a relatively good agreement with experimental data.

We notice that the average profiles along the tunnel are consistent with the results given by the simple continuous loading theory applied to the same experiment (Fig. 3; Bertin et al., 1976)

The model presented here is based on a more complete description of the phenomena than the models considered in previous projects (Kilpatrick et al., 1955; Bertin et al., 1976).

It appears to be useful for analyzing the real drying conditions of prunes in one particular truck. Simulation with the mathematical model can be used to estimate the real values of the heat and mass transfer coefficients. On the other hand this model can be used for both the designing and automatic control of discontinuous tunnel driers.

Table 1—Experimental conditions

$P = 1.013 \times 10^5$	N/m^2	$\Delta h_v = 2.5 \times 10^6$	J/kg
$C_{ad} = 1.00 \times 10^3$	J/kg·°C	$C_{pd} = 1.34 \times 10^3$	J/kg·°C
$C_v = 1.84 \times 10^3$	J/kg·°C	$C_\ell = 4.18 \times 10^3$	J/kg·°C
$\rho_\ell = 1.00 \times 10^3$	kg/m^3	$\rho_d = 1.40 \times 10^3$	kg/m^3
$a = 1.6 \times 10^{-7}$	m^2/s	$\lambda = 0.546$	W/m·°C
$\alpha = 63.9$	W/m^2·°C	$\beta = 4.78 \times 10^{-8}$	kg/N·s
$\ell = 1$	m	$R_f = 0.979 \times 10^{-2}$	m
$t_1 = 540$	s	$\mu = 77$	

NOMENCLATURE

a	Thermal diffusivity of the product	(m^2/s)
Bi	Biot number	($\alpha R_f/\lambda$)
C	Specific heat	(J/kg·°C)
d_w	Product desorption coefficient	
G	Dry gas flow rate	(kg/s)
ℓ	Length of the truck	(m)
L	Dry solid flow rate	(kg/s)
P	Total air pressure	(N/m^2)

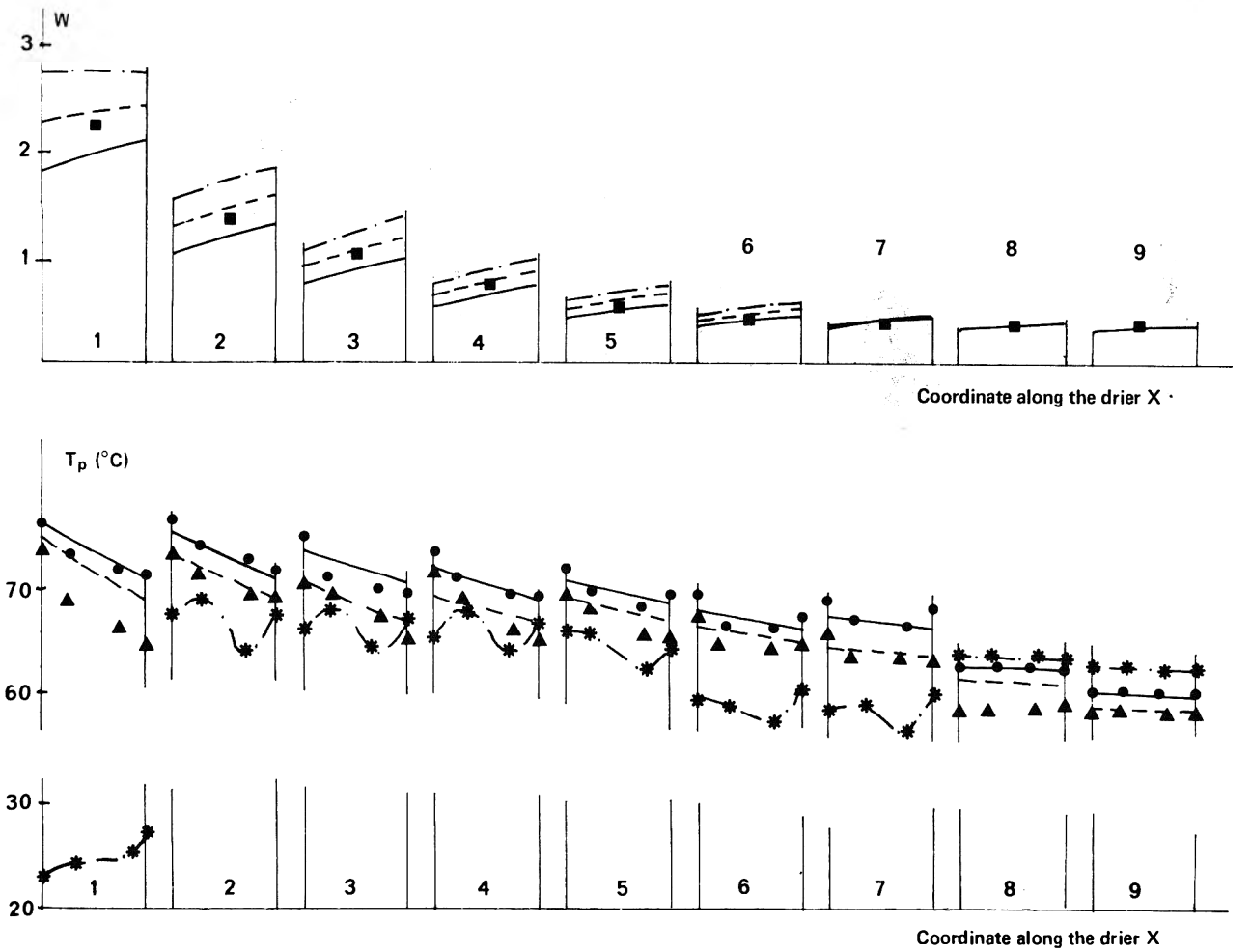

Fig. 5—Discontinuous loading: Distribution of moisture content and temperature T_p of the product versus drier length when time shifts. These diagrams show the nine positions of the truck in the tunnel. For each position, the curves are displayed at time 0 min, 45 min, and 90 min, starting from the arrival of the truck at the given position. The symbols for theoretical curves are the same as in Fig. 4. The symbol ■ refers to the experimental data of moisture content W.

p_v	Water vapor partial pressure in air	(N/m^2)
R	Product external radius	(m)
RH	Relative humidity of air	
T	Temperature (averaged over volume)	(°C)
t	Time	(s)
u	Truck speed ($\bar{u}$ averaged over time)	(m/s)
v	Air velocity	(m/s)
W	Water content in the product dry basis (averaged over volume)	(kg/kg)
w	Water content in the air dry basis (averaged over volume)	(kg/kg)
x	Longitudinal coordinate	(m)

Greek Letters

α	Heat transfer coefficient between air and the product surface	($W/m^2 \cdot °C$)
α_m	Mass transfer coefficient between air and the product surface	(kg/N·s)
Δh_v	Latent heat of vaporization	(J/Kg)
λ	Thermal conductivity of the product	($W/m \cdot °C$)
ρ	Density	(kg/m^3)

Subscripts and superscripts

a	air
d	dry
f	final
g	gas phase
i	initial
ℓ	liquid
p	product
s	solid phase
sat	satured water vapor
surf	surface
t	time derivative
v	vapor
x	space derivative
*	used for pointing out shrinkage effect

A, B, D, U, E, s_1, β, Δh, μ, ν, σ, ψ, are used in equations or in appendix.

APPENDIX

Formulas

$C_g = C_{ad} + C_v w$ $\qquad$ $C_s = C_{pd} + C_\ell W$

$p_v = P\, w(0.622 + w)^{-1}$ $\qquad$ $p_{surf} = p_{sat}(T_{surf})\, d_w$

$d_w = 0.4\, W + 0.48$ $\qquad$ for $W \leqslant 1.3$

$d_w = 1$ $\qquad$ for $W \geqslant 1.3$

$Bi^* = Bi(1 + W\rho_d/\rho_\ell)^{1/3}$ $\qquad$ $\alpha_m = \beta(\rho_d^*/\rho_d)\cdot(W/W_i)$

—Continued on page 137

EFFECTS OF MODIFIED PROCESSING PROCEDURES ON QUALITY OF SOUTHERN CROWDER PEAS

L. F. FLORA

ABSTRACT

Two lots of freshly harvested Crowder-type southern peas were subdivided and processed to study the effects of soaking, exhausting, EDTA, and acidification on percent drained weights, color, texture, and ascorbic acid of canned and frozen peas. Canned peas acidified with citric acid were lighter in color and firmer in texture than nonacidified canned peas. Though peas picked up moisture during soaking, the percent drained weight of canned soaked peas was not significantly different from unsoaked peas. Neither was there any difference in drained weights between blanched and unblanched, exhausted canned peas. Peas exhausted rather than blanched prior to closing were slightly lighter in color and had higher levels of ascorbic acid. Canned peas with EDTA in the brine were lighter in color than peas canned in plain brine. Processing variables affected quality of canned peas more than that of frozen peas.

INTRODUCTION

CANNED BEANS AND PEAS are often prepared from mature seeds or dry beans which must be soaked before processing. Soaking is usually considered necessary to reduce cooking times and increase drained weights. Quast and da Silva (1977) reported that soaking of several dry legumes prior to cooking did not significantly decrease the cooking times. They observed higher drained weights for peas soaked before cooking, but found no effect of soaking on drained weights of black beans. Luh et al. (1975) found that citric acid, calcium chloride and Na_2EDTA improved color and other sensory properties of canned dry lima beans. Sistrunk and Bailey (1965), studying factors affecting discoloration of canned blackeye peas, found that more immature peas showed more discoloration of liquor after canning and that lower blanch temperatures produced more discoloration in the peas. They also found that the effects of EDTA and other chelaters in preventing discoloration were influenced by processing factors such as fill-in weight and headspace. Rizley and Sistrunk (1979) reported that pyrophosphate soak solutions and boiling, rather than steam cooking, yielded blackeyed peas with lighter color. They also suggested that by controlling the pH and time in bicarbonate soaking solutions the cooking time for blackeyed peas could be reduced without affecting quality. Losses of ascorbic acid from peas during processing and storage are well documented (Cain, 1967; Fennema, 1977; Kramer, 1979; Lund, 1979; Selman, 1978).

The purpose of this study was to evaluate effects of EDTA and citric acid acidification on quality of freshly harvested canned Crowder peas. Other objectives were to investigate the possibility of eliminating soaking of freshly harvested peas for canning and to compare blanching and hot closing to exhausting prior to closing and cooking.

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MATERIALS & METHODS

CROWDER-TYPE southern peas, cv "Knuckle Purplehull," were harvested on two dates in August, about 8 days apart, from an experimental plot near Griffin, Ga. Approximately 21 kg of peas from the first harvest were shelled in a pilot size sheller (Taylor Mfg. Co., Moultrie, Ga.). Half the peas were soaked in tap water for 4 hr at ambient temperature (20°C) and the other half were processed immediately. Half of the soaked peas and half of the unsoaked peas were blanched in water at 76.5°C for 3 min and water cooled. Blanched and unblanched peas were filled separately into No. 300 plain cans with "C" enamel ends (200g peas/can) and topped with hot 1.5% (w/w) salt brine solution containing 150 ppm Na_2EDTA. The cans of blanched peas were immediately closed while the unblanched peas were exhausted 6 min prior to sealing. Several cans of the soaked, blanched peas were acidified by direct addition of citric acid to the can prior to closing. The amount of citric acid added was determined by titrating 200g blended blanched peas to pH 4.5 with a standard citric acid solution. Canned peas were thermally processed for 19 min at 121°C.

Approximately 20 kg of peas from the second harvest were shelled and processed. Part of the peas were soaked for 2 hr at ambient temperature, blanched 2.5 min at 100°C, and equally divided for canning and freezing. Another fraction of shelled peas was processed as just described except that the soak water contained 150 ppm Na_2EDTA. A third fraction was processed without soaking. Part of these peas were blanched and equally divided for canning and freezing. Another part consisted of several cans that were topped with brine, exhausted, and canned. Yet another fraction of nonsoaked peas were blanched in a 0.5% (w/w) citric acid bath and equally divided for canning and freezing. No EDTA was used in the brine to top the cans of peas. Canned peas were thermally processed as described above. Frozen peas were stored at −18°C in heat-sealed freezer bags.

Processed peas from both harvests were analyzed within 2 wk of processing and again after 5 months for drained weight, pH, can vacuum, Gardner color, shear values, and ascorbic acid. Drained weight was determined according to standard methods (AOAC, 1975). Canned peas were drained and 100g blended with 20 ml distilled water for pH measurements on a Beckman Model 3500 pH meter. Can vacuum was read using a standard can vacuum gauge. Color L, a, and b values were determined on 30g peas or liquor in a clear sample cup using a Gardner C-4(L) Color Difference Meter (CDM) previously standardized to an ivory tile (L = 76.6, a = −1.1, b = +24.2). Shear values were determined on a Food Technology Corporation TP-1 Texture Test System; a 300-lb ring was used to shear canned peas and a 3,000-lb ring was used to shear thawed frozen peas. Total ascorbic acid was determined using the AOAC microfluorometric procedure (1975). Alcohol insoluble solids (AIS) were determined on the processed peas by standard methods (AOAC, 1975). Though sufficient numbers of samples were prepared for adequate replication of analyses, leakers caused by poor seals in some of the cans of peas from the second harvest resulted in insufficient samples of some treatments for 5-month analyses. The data were submitted to analyses of variance and Duncan multiple range tests to determine significant differences using the SAS program developed by Barr et al. (1976). A composite sample of frozen peas left after analysis at 5 months was divided into four lots of 200-g each and two of the lots were cooked in an equal amount of water in a saucepan for 25 min. CDM color and ascorbic acid were determined for the lots of cooked and uncooked peas and for the cooking liquor and shear was determined on the peas. Acidified canned peas were informally tasted to determine if flavor were objectionable.

Although the two harvests were designed as separate experiments, some comparisons could be made. Freshly harvested peas were soaked in parts of the study because this is commercial prac-

Table 1—Effects of processing on Gardner CDM values of Crowder peas[a]

	Harvest	Peas L	Peas a	Peas b	Liquor L	Liquor a	Liquor b
Acidification							
Acidified	1	46	+0.2	+17.7	54	0	+12.1
Not acidified	1	35	+4.1	+13.4	30	+2.1	+ 9.2
EDTA							
EDTA Brine[b]	1	35	+4.1	+13.4	30	+2.0	+ 9.2
Plain Brine	2	29	+2.0	+ 9.2	23	−0.3	+ 5.2
Process							
Canned	2	29	+2.0	+ 9.2	23	−0.3	+ 5.2
Frozen	2	41	−1.9	+13.8	—	—	—
		(35)[c]	(−2.2)	(+ 4.2)	(38)	(−2.5)	(+ 3.5)
Blanching Method							
Acid blanch	2	39	+0.4	+14.4	26	−8.0	+ 6.3
Water blanch	2	36	−1.0	+11.7	22	−0.6	3.3
Closing Method							
Exhausted	1&2	34	+3.7	+14.2	30	+2.8	+ 9.6
Blanched, hot brine[b]	1&2	32	+4.3	+12.1	25	+0.7	+ 6.7

[a] Pairs of values not connected by a line are significantly different at $p \leq 0.05$.
[b] Adjusted for influence of acidified samples.
[c] Values in parentheses are for cooked frozen peas.

tice in some operations. Blanching conditions used in this study were based on the blanch time-temperatures used by two commercial processors of southern peas.

RESULTS & DISCUSSION

PEAS from the second harvest were more mature than those from the earlier harvest. They contained an estimated 50–60% purple pods as opposed to 10–20% purple pods from the first harvest. The canned peas from the second harvest were higher in AIS than peas from the first harvest (24% vs 20%). The more mature late peas were easier to shell and gave a higher shelling yield—52.2% for the late peas vs 40.5% for the early peas.

The vacuum in cans of peas exhausted prior to closing was double that in cans of peas topped with hot brine before closing (20 in. vs 9 in.), as expected.

There was no significant effect of process treatment on percent drained weight of the canned peas. Luh et al. (1975) had reported that acidification of dry lima beans in the can prior to cooking resulted in lower drained weight. They attributed this to decreased hydration of proteins and starch resulting in some shrinkage in volume of the canned beans. Although mean drained weight of acidified Crowder peas (64%) in this study was lower than mean drained weights of nonacidified peas (66%), the difference was not statistically significant. Drained weights of canned peas from the first harvest were significantly lower after storage for 5 months. There was no significant difference in percent drained weights between peas canned from the first harvest and peas canned from the second harvest. Peas from the second harvest picked up 8–10% (by weight) water after soaking for 2 hr at room temperature. It might therefore be assumed that soaked and nonsoaked canned peas might yield different drained weights but this was not the case.

The pH of the peas acidified in the can was reduced from about 6.1 to about 4.8. This is higher than the pH 4.5 projected from titration trials on blanched peas. However, Flora et al. (1978) found that pH was also higher in acidified pimiento peppers after thermal processing than values projected from titration trials on blanched peppers. Blanching peas in a 0.5% citric acid bath reduced pH to 5.8. Luh et al. (1975) reported about the same result for dry lima beans rehydrated in a 0.25% citric acid bath at room temperature for 12 hr. There were some statistically significant differences in pH due to treatments other than acidification, but these were relatively minor in magnitude and biologically insignificant, all being in the range pH 6.0–6.2. A slight rise in pH of the canned peas was observed with storage.

Acidification had a dramatic effect on color of canned peas. Peas acidified prior to canning were much lighter in color and resembled frozen peas more than peas canned without acid. The higher Gardner L and b values (Table 1) for acidified canned peas and frozen peas than for nonacidified canned peas are evidence of the lighter color of the former. Luh et al. (1975) found that acidification improved color of canned dry lima beans. They postulated that citrate ion can form complex ions with trace elements such as copper or iron, making them unavailable for reactions with phenolic compounds and sulfides which tend to cause discoloration in canned beans. EDTA had a similar but less drastic effect on color. Peas from the first harvest canned with EDTA brine had higher L and b values than peas from the second harvest canned in brine without EDTA (Table 1). This difference was most noticeable after 5 months' storage when peas canned with EDTA were a medium tan color while peas from the second harvest canned in plain brine were dark gray. This effect seems clearly due to EDTA since Sistrunk and Bailey (1965) found that more mature blackeye peas exhibited less discoloration after canning than immature peas. Blanching in dilute acid yielded peas slightly lighter than the control (Table 1). Although acidified and frozen peas were lighter in color than peas canned without acid, variations in color due to maturity differences were much more striking in the former. Canned peas, though darker in color, looked more uniform.

Peas from the first harvest that were exhausted before cooking were slightly lighter in color than blanched peas; this difference was most noticeable in can liquors (Table 1). The higher vacuum in peas exhausted prior to closing probably results in less oxygen being available for reaction. Canned peas from the first harvest that were soaked and blanched, but not acidified, usually had relatively low L and b values. Peas from the second harvest that were soaked before canning appeared darker than nonsoaked peas, an observation that did not appear to be born out by CDM measurements. Rizley and Sistrunk (1979) observed an increase in visible discoloration of blackeyed peas with longer soaking time.

There was no significant effect of processing treatment

Table 2—Effects of processing on shear values of Crowder peas[a]

	Harvest	Shear (kg/g) 2 wk	Shear (kg/g) 5 mo
Acidification			
Acidified	1	0.75	1.01
Not acidified	1	0.54	0.90
Process			
Canned	2	0.53	0.99
Frozen	2	3.94	4.33 (1.04)[b]

[a] All pairs significantly different at $p \leq 0.05$.
[b] Value in parentheses is for cooked frozen peas.

Table 3—Effects of processing on ascorbic acid (mg/100g) values of Crowder peas[a]

	Harvest	Peas	Liquor
Process			
Canned	2	6.5	8.0
Frozen	2	19.8 (5.6)[c]	— (9.3)
Storage[b]			
2 weeks	1&2	8.0	10.8
5 months	1&2	5.2	5.9
Soaking			
Soaked	2	6.7	9.7
Not soaked	2	8.9	11.5
Closing Method			
Exhausted	1&2	10.5	13.1
Blanched, hot brine	1&2	5.5	7.4

[a] All pairs significantly different at $p \leq 0.05$.
[b] All canned.
[c] Values in parentheses are for cooked frozen peas.

on the color of uncooked frozen peas, nor were there any significant changes in Gardner CDM values of canned or frozen peas with storage, except for b values of canned peas from the second harvest which registered a decline. Cooking had a dramatic effect on color of frozen peas; Gardner L and b values were lowered substantially (Table 1). The peas took on a darker, grayer cast than canned peas. This may have been due to reactions of components with oxygen during cooking and iron in the cooking water. These values were not compared statistically because they were composite analyses.

Acidified canned peas were firmer than nonacidified canned peas (Table 2). Luh et al. (1975) reported the same results for canned lima beans and attributed the phenomenon to denaturation of some proteins and suppression of hydration of proteins and starch in the beans by the acid. Firmness increased with storage and the difference between acidified and nonacidified canned peas was reduced after 5 months. There were no significant effects of soaking or exhausting on the texture of canned peas, though canned peas exhausted prior to closing had a tendency to be slightly less firm than canned peas that had been blanched. Frozen peas not soaked prior to blanching were less firm than those that were soaked or those blanched in dilute acid before freezing. Cooking reduced the texture of the frozen peas to the same level as the canned peas (Table 2).

More than half of the ascorbic acid in the peas was leached into the liquor during canning (Table 3), and about half of the vitamin in canned peas was destroyed by storage at ambient temperature for 5 months. Uncooked frozen peas still contained several times as much ascorbic acid as canned peas after 5 months' storage. Ascorbic acid in cooked frozen peas resembled values recorded for canned peas.

No effect of soaking on ascorbic acid was evident in peas canned from the first harvest, but peas from the second harvest that were soaked prior to further processing were significantly lower in ascorbic acid than peas that were not soaked (Table 3). The difference might be attributable to some leaching of the water-soluble vitamin during soaking. Soaking times longer than those used in this study might allow leaching of more ascorbic acid into the soak water. Canned peas that were exhausted rather than blanched prior to cooking had higher levels of ascorbic acid (Table 3). This difference might be attributable, as were differences in color, to higher vacuum and less oxygen for reaction in the exhausted peas.

There were no treatment effects on percent AIS of peas. Frozen peas had higher AIS levels than canned peas, as expected.

In summary, canned Crowder-type southern peas, acidified with citric acid, were lighter in color and firmer in texture than conventionally canned peas. Flavor of acidified canned peas was not particularly objectionable, but further study should be given to their acceptability and the optimum acidification level. Investigation into the possibility of reduced cooking times for acidified canned peas is warranted. EDTA incorporated into packing brine reduced discoloration of canned peas and was more effective than equal amounts of EDTA in soak water. It appeared that soaking and/or blanching could be eliminated in favor of exhausting Crowder peas prior to canning without sacrificing drained weight or quality. Unblanched peas exhausted prior to canning were slightly lighter in color and higher in ascorbic acid than blanched, canned peas. Soaking allowed peas to absorb moisture and thus increased in weight but, because canned soaked peas showed no differences in drained weight and quality, soaking may be of more benefit for frozen peas. The effects of soaking, blanching, and exhausting should be examined using dry peas, and benefits of substituting one process in place of another should be evaluated in terms of energy usage as well as quality.

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PARTIAL REPLACEMENT OF NONFAT MILK SOLIDS AND CANE SUGAR IN ICE CREAM WITH LACTOSE HYDROLYZED SWEET WHEY SOLIDS

E. J. GUY

ABSTRACT

Evaluation of vanilla-flavored ice creams that contained either 67 or 79% lactose hydrolyzed sweet whey solids (LHW) as a replacement for both milk solids nonfat (MSNF) and cane sugar showed that replacement of 2.75% of combined MSNF and cane sugar solids (13.6% of the total MSNF and 8.3% of the total cane sugar solids) with LHW can be made with no loss of ice cream quality. Increasing levels of LHW above 2.75% progressively lowered the quality of ice creams stored at −20°C. Replacement with 5.5% of 67% LHW produced significant losses of flavor quality but did not change hedonic texture. Replacement with 5.5% of 79% LHW did not change either hedonic flavor or texture. Replacement with 8.25 and 11% LHW significantly decreased mix viscosities and freezing points, ice cream flavor scores (increased saltiness and caramel-like flavors) and consistencies although hedonic texture ratings were not always significantly lowered. Except for the 11% substitution of LHW for MSNF, ice creams had satisfactory heat shock stabilities.

INTRODUCTION

CURRENT ICE CREAM standards permit replacement of up to 25% milk solids nonfat (MSNF) with unmodified whey solids. A change in the standards of identity for frozen desserts was proposed in 1978, permitting ice creams to be formulated from any milk derived ingredient with a minimum of 2.7% protein. This proposal stimulated research into the replacement of skim milk solids with caseins, wheys, and whey protein concentrates. Although this proposal was dropped, it is believed the results of these studies should be reported.

Frazeur (1967) showed that replacement of 25% MSNF with excellent-flavored dry whey in ice creams of 10.5–14.5% milk fat produced taste and texture scores equivalent to those of control ice creams. Steinholt (1974) reported that substitution of more than 40% of MSNF with whey solids produced lower flavor and melt down scores; 80% replacement produced a significantly lower score for consistency. Lowenstein et al. (1975) reported that up to 100% replacement with 50% lactase hydrolyzed neutralized cottage cheese whey for nonfat dry milk (NDM) produced ice creams of good body and texture, but 50 and 100% substitution gave poorer heat shock flavor and texture scores.

Bhursi et al. (1976) reported that neutralized acid whey can replace 25%, and this same whey with 80% of its lactose hydrolyzed can replace 40% of the MSNF in ice creams. Both unmodified and hydrolyzed sweet wheys can replace 60% of the MSNF in ice creams.

The objective of the present research was to develop acceptable ice creams exceeding a minimum of 2.7% protein when whey solids are used. It was thought this objective would best be achieved by replacing both MSNF and cane sugar with hydrolyzed lactose sweet whey solids (LHW) because (1) the literature reports that wheys containing hydrolyzed lactose produce better ice creams than unmodified wheys, and (2) hydrolyzed lactose may be substituted for a portion of the cane sugar in ice creams (Guy et al., 1974). The lactose in sweet whey solids was hydrolyzed by lactase enzyme to either 67 or 79%; the notation used is LHW-A for the 67% hydrolyzed and LHW-B for the 79% hydrolyzed solids.

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MATERIALS & METHODS

Ingredients

Extra grade low heat NDM (Queen's Farm Dairy), Domino brand extra-fine grade granulated sugar, commercial pasteurized 40% fat-containing cream, 49% total solids (TS) hydrolyzed sweet whey concentrates, Kontrol brand (Germantown Manufacturing Co.) stabilizer-emulsifier, and McCormick onefold pure vanilla extract were used in ice cream mixes. Lehigh Valley sweet whey solids were used in mixes prepared for flavor evaluation by an expert panel of five judges.

Preparative methods

HL sweet whey. Maxilact brand (40,000 ONPG μ/g) of *Saccharomyces lactis* lactase (either 0.8 g/gal of milk for HWS-A or 1.2 g/gal of milk for HWS-B) was added to raw whole milk held 16–18 hr at 3°C. The milks were pasteurized at 71°C for 15 sec, cheddar cheese was made, and the wheys were drained and pasteurized at 63°C for 30 min. The wheys were then clarified, and, after the fat was separated, cooled to 4°C overnight. The next day the wheys were concentrated to 49% TS in an APV evaporator and stored in plastic jugs at −20°C.

Ice creams. Fifty pounds (22.7 kg) ice cream mixes were batch pasteurized for 30 min at 74°C, homogenized at 2000–500 psi (140–35 kg/cm²) in a two-stage homogenizer, cooled on a plate exchanger to 15°C, and aged overnight at 4°C. Ice creams of 88–95% overruns were frozen in a 10-qt Emery Thompson batch freezer, then portioned into waxed 2-qt cartons, immediately placed in a hardening box at −30°C, held overnight, and then transferred to a −20°C storage cabinet.

Analytical methods

Total protein (N × 6.38) was determined by the micro-Kjeldahl procedure (AOAC, 1970), ash by the standard method for milk (AOAC, 1970), and total solids and fat by the Mojonnier procedures (Milk Industry Foundation, 1959). Degree of lactose hydrolysis was determined by the glucose-oxidase procedure (Jasewicz and Wasserman, 1961).

Ice cream mix evaluation

Viscosity. Duplicate apparent viscosities of ice creams at 4.4°C were determined by use of a Brookfield Sychrolectric Viscosimeter (#2 spindle, 60 rpm).

Freezing time and temperature. The freezing time and temperature are those required to obtain the proper stiffness of the mix as visually judged by an experienced operator. After freezing the ice cream mix, the refrigerant was turned off, the mix was whipped 1–1½ min to the desired overrun, and the ice cream was drawn from the freezer.

Ice cream evaluation methods

Melting resistance. Twelve centimeter diameter tea strainers containing 150–160g cut blocks of ice cream were suspended over large funnels, and the melted material was collected. Melt down was determined at 37°C as the weight percent of ice cream melted in 90 min.

Overrun. Overrun was computed from average net weights of 2-qt cartons of ice cream using a Pelauze Y-70 ice cream scale for reading overrun.

Firmness. Samples of ice cream mix were filled to the top of 4-oz plastic cups, placed in the hardening box overnight at −30°C, and then transferred to the freezer boxes. Five-second penetration

Table 1—Analyses of milk-derived ingredients used in ice creams

Ingredient	Percentage			
	Moisture	Dry weight basis		
		Fat	Protein	Ash
Nonfat dry milk	4.4	1.28	37.0	8.3
67% lactose hydrolyzed sweet whey	48.9	0.78	13.1	6.9
79% lactose hydrolyzed sweet whey	49.3	0.66	12.5	8.0

Table 2—Formulation and analyses of ice creams containing, 12% fat, 0.28% stabilizer, and 0.5% vanilla

Percent				
Lactose hydrolyzed sweet whey solids	Milk solids nonfat	Cane sugar	Protein[a]	Ash[a]
0.00	11.0	15.0	4.1	0.9
2.75	9.5	13.75	3.9	1.0
5.50	8.0	12.50	3.6	1.1
8.25	6.5	11.25	3.4	1.2
11.00	5.0	10.00	3.2	1.3

[a] Calculated in mix containing 79% hydrolyzed whey

Table 3—Freezing characteristics of ice cream mixes containing lactose hydrolyzed sweet whey

Percent whey solids	67% Lactose hydrolyzed sweet whey			79% Lactose hydrolyzed sweet whey		
	Min freezing time	Freezing temp °C	Avg % overrun	Min freezing time	Freezing temp °C	Avg % overrun
0.00	8 3/4	−4.5	91	8	−4.5	94
2.75	8 3/4	−4.7	90	8 1/2	−5.0	87
5.50	9	−4.6	88	8 3/4	−5.3	95
8.25	9	−5.0	94	9	−5.6	91
11.00	9 1/2	−5.0	95	9 1/2	−6.0	94

values were obtained in triplicate by use of a Precision Scientific Penetrometer set up outside the freezer room. To minimize melting of the sample after being withdrawn from the freezer, one reading per sample was taken within an elapsed time of 15–20 sec.

Heat shock. Heat shock tests were made by removing 2-qt cartons of ice cream from the freezer (−18°C) twice daily and holding at 23–25°C for ½-hr periods over 5 consecutive days. The ice cream was returned to the freezer after each ½-hr period. Both heat shocked and nonheat shocked samples of each test formula were evaluated in triplicate against control NDM containing ice cream treated similarly.

Panel evaluation. Ice creams for panel evaluation were removed from the cartons, placed in 4-oz plastic cups, refrigerated at −18°C, and withdrawn for evaluation just prior to being tested. Taste and texture were evaluated on a 9-point hedonic preference scale (Peryam and Pilgrim, 1957) by a panel of 20–25 judges. The judges consisted of employees of ERRC who were trained in evaluating a variety of food products. Four samples were evaluated at each session, at least one being the control. Standard deviations measured on different days (between days) for nonheat shocked control NDM ice cream up to 1 month averaged ±0.23 for flavor scores and ±0.19 for texture scores, and for heat shocked ice creams, ±0.32 for flavor and ±0.40 for texture scores. Saltiness and sweetness were judged on a 7-point descriptive scale with the mid point of 0 designated as being just right, positive number 1 slightly, 2 moderately, and 3 excessively sweet or salty. Negative numbers were designated as 1 slightly lacking, 2 moderately lacking, and 3 excessively lacking in saltiness or sweetness. Iciness/coarse ness scores were judged on a 4-point scale of 0 = none, 1 = slight, 2 = moderate, and 3 = pronounced.

Table 4—Analysis of ice cream mixes[a]

Percent whey solids	Percent lactose hydrolysis of added sweet whey					
	67			79		
	Percent total solids	Centipoise viscosity 4.4°C	pH	Percent total solids	Centipoise viscosity 4.4°C	pH
0.00	37.9	182a	6.59	37.7	183a	6.60
2.75	37.9	177ab	6.47	37.9	173ab	6.51
5.50	38.0	170ab	6.40	37.9	164bc	6.43
8.25	37.9	159c	6.39	38.2	162c	6.34
11.00	37.3	132d	6.31	38.4	141d	6.26

[a] Data followed by different letters are significantly different $P < 0.05$).

Table 5—Percent ice creams containing lactose hydrolyzed sweet whey solids melted in 90 min at 37°C[a]

Percent whey solids	Percent melted	
	67% Lactose hydrolyzed sweet whey	79% Lactose hydrolyzed sweet whey
0.00	38.8	42.3
2.75	44.0	44.7
5.50	45.2	44.1
8.25	45.2	42.8
11.00	46.3	44.2

[a] Differences nonsignificant ($P > 0.05$)

Statistical methods

All results on panel testing of ice creams were processed by the ERRC computer and analyzed by analysis of variance with Duncan's multiple range test used to determine significance of results. Significant differences of viscosities of mixes, melt down, and firmness of ice creams were analyzed statistically by use of the formula.

$$SD = \frac{2s\sqrt{a}}{\sqrt{n}}$$

where SD = significant difference; s = standard deviation; z = numerical rank apart in array of data; and n = number of replications.

The standard deviations for the above were evaluated from ranges, i.e., the difference between the largest and smallest measurements (Snedecor, 1959).

RESULTS & DISCUSSION

THE MOISTURE, fat, protein, and ash analyses of the milk-derived ingredients are shown in Table 1. The formulations of ice cream and the calculated protein and ash contents of ice cream mixes containing LHW-B are shown in Table 2. The MSNF, sugar, and LHW in each ice cream totaled 26%. The protein and ash values in ice creams containing LHW-A varied slightly from those containing LHW-B because the wheys were from different milks. MSNF were calculated on a moisture-free basis from both the added NDM and serum solids of the cream (calculated as 5.4% of the weight of 40% fat cream). Small levels of fat contained in wheys and NDM were disregarded in calculations of the fat composition of the mix.

The use of 8.25% and 11% LHW slightly increased the time required to freeze the mix and slightly decreased the freezing temperatures (Table 3). This may be expected because of increased levels of salts and monosaccharides from

Table 6—Firmness of ice creams containing 79% lactose hydrolyzed sweet whey solids[a]

Percent whey solids	Average mm penetration in 5 sec		
	Lot 1	Lot 2	
	−21°C	−19°C	−14°C
0.00	8.9a	10.0a	12.2a
2.75	10.0b	11.5b	15.3b
5.50	11.4c	13.1c	16.4b
8.25	12.6d	15.1d	18.4c
11.00	13.7e	13.2c	16.3b

[a] Data followed by different letters in one column are significantly different ($P < 0.01$).

LHW. Increasing LHW content progressively decreased mix viscosity and pH (Table 4). Replacement of MSNF and sugar with greater than 5.5% LHW significantly decreased mix viscosities.

Melt down times of all ice creams containing LHW were not significantly different from that of the control (Table 5). Firmness of two lots of ice creams decreased significantly with increased content of LHW-B (Table 6). An exception was the ice cream (Lot 2) containing 11% LHW, which might be explained by its somewhat lower overrun (86% vs 90–95%). The temperatures of −21° to −14°C approximate the temperature range of home freezers. The increasing softness of ice creams containing increasing levels of LHW and stored at −14°C was especially evident when portions of the ice creams were spooned and tasted.

Hedonic flavor scores of ice creams stored at −20°C containing LHW-A or -B, progressively decreased with increasing levels of LHW above 2.75% and to some extent with storage time (Fig. 1 and 2). For each storage period, average of duplicate values for the control NDM ice creams and single values of LHW samples were obtained for flavor and texture ratings. Ice creams with 5.5% LHW-A and 8.25% LHW-A or -B had significantly ($P < 0.05$) lower flavor scores, and those containing 11% LHW-A or -B had very significantly ($P < 0.01$) lower flavor scores than the control NDM ice cream. Flavor defects in ice creams with high levels of LHW were salty, caramel-like, or sirup flavor, and lacking vanilla. When evaluated by a panel consisting of five expert judges, mixes prepared in 400-g lots containing 11% either hydrolyzed or unmodified whey solids had caramel-like flavors. When the fat was omitted from a mix batch pasteurized at 74°C for 30 min or the complete mix was HTST pasteurized at 80°C for 25 sec, no caramel-like flavor was detected. Thus these flavors were judged to be formed by the result of heating milk fat and whey at batch pasteurization temperatures.

Increasing levels of LHW-A or -B progressively increased saltiness of ice creams (Fig. 3 and 4), which correlated with the increased ash of these mixes. Ice creams containing 2.75% LHW were not significantly different ($P > 0.05$) from their controls. Those containing 5.5% LHW, on the average, were saltier ($P < 0.05$) and those with higher levels of LHW were very significantly saltier ($P < 0.01$) than the controls. Average saltiness scores of all ice creams containing LHW-A were lower than those containing similar levels of LHW-B, consistent with the lower ash (6.9%) of LHW-A. Even though the ice creams containing 11% LHW were rated saltiest, their scores averaged only close to slightly salty on the 4-point scale used.

Hedonic texture scores decreased slightly to moderately with increasing LHW (Fig. 5 and 6). Only the ice creams at the initial and 2-month storage period containing 11% LHW-B had lower texture scores ($P < 0.05$). Although the panelists noted the softer body of the ice creams containing

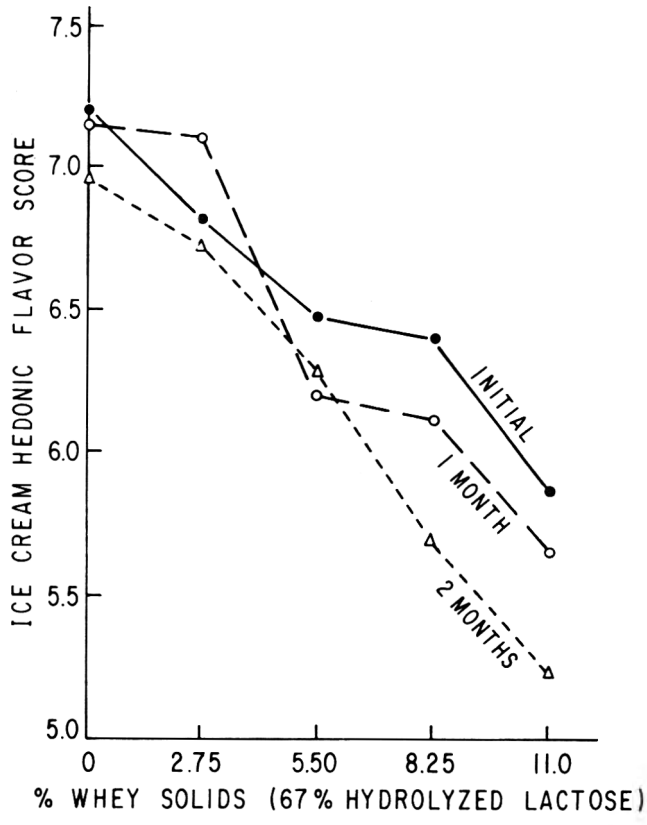

Fig. 1—Hedonic flavor scores of ice creams (LHW, 67%) stored at −20°C. All 5.5 and 8.25% LHW, $P < 0.05$; all 11% LHW, $P < 0.01$ from the control.

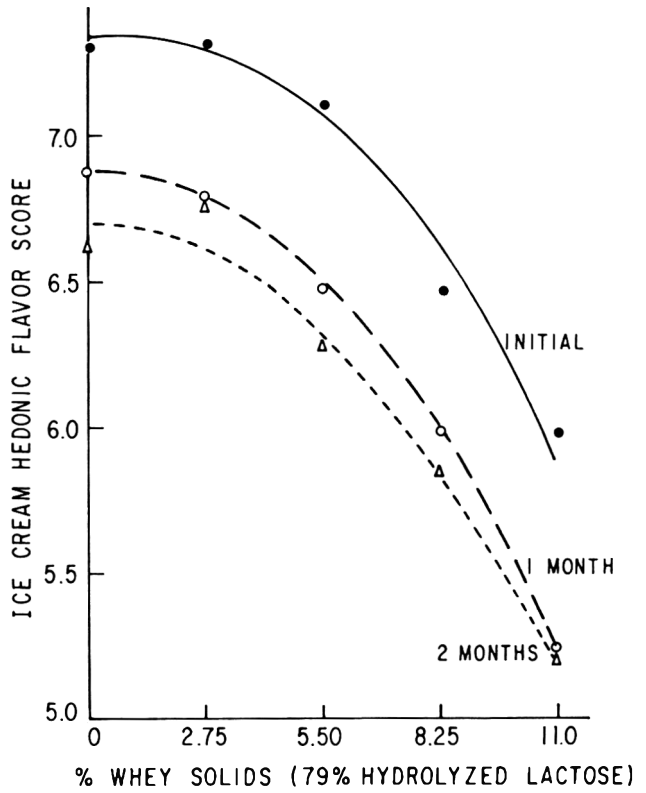

Fig. 2—Hedonic flavor scores of ice creams LHW, 79%) stored at −20°C. All 8.25 and 11% LHW, $P < 0.05$ and $P < 0.01$, respectively, from the control.

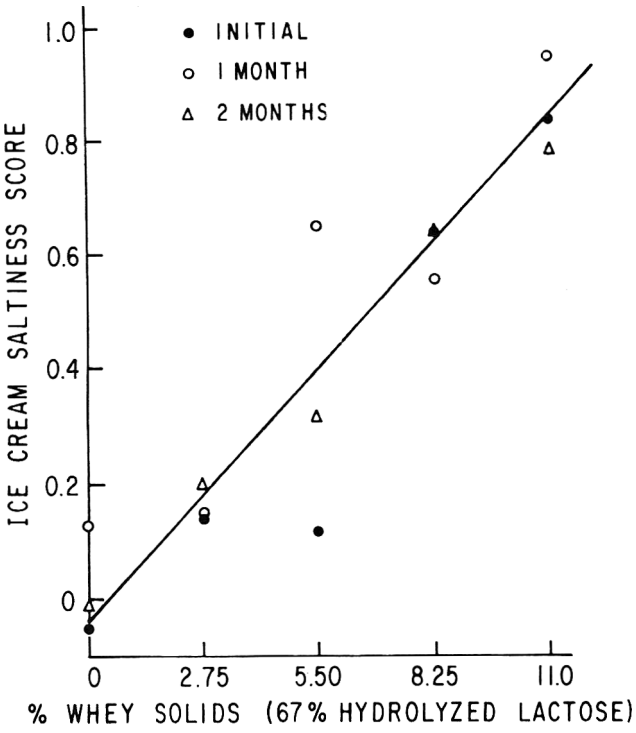

Fig. 3—Saltiness scores of ice creams (LHW, 67%) stored at −20° C. 1 month, 5.5% LHW, $P < 0.01$; 2 months, 5.5% LHW, $P < 0.05$; all 8.25% and 11% LHW, $P < 0.01$ from the control.

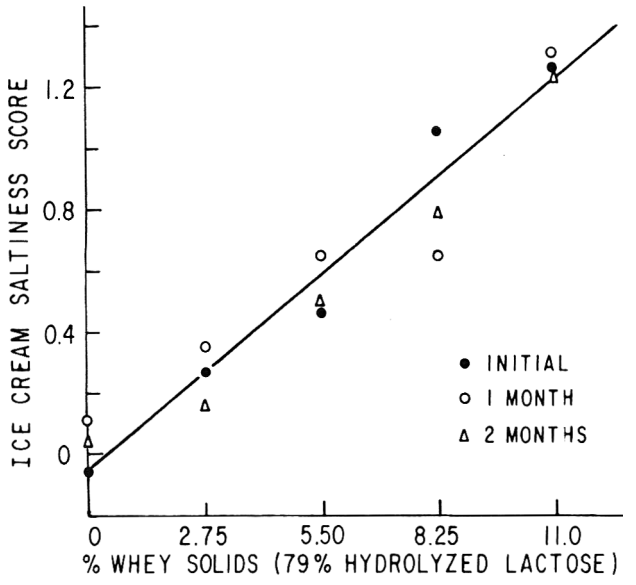

Fig. 4—Saltiness scores of ice creams (LHW, 79%) stored at −20° C. All 5.5% LHW, $P < 0.05$; all 8.25% and 11% LHW, $P < 0.01$ from the control.

higher levels of LHW, they did not penalize this severly as a texture defect.

Sweetness scores of all ice creams averaged from 0.16–0.46 and showed no significant consistent pattern with respect to whey solids level, type used in the formulation, or time of storage. Neither did iciness/coarseness scores which averaged from 0.30–0.51. No sandiness was detected in any ice cream. Triplicate tests run on ice creams containing LHW-B showed that average texture scores de-

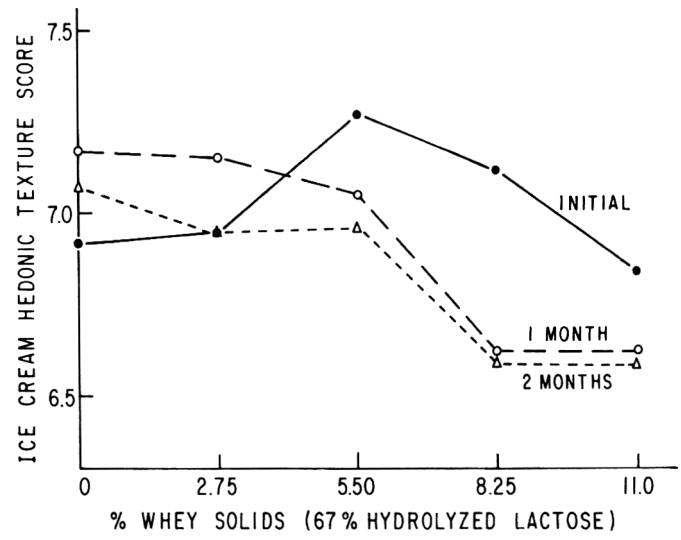

Fig. 5—Texture scores of ice creams (LHW, 67%) stored at −20° C. All LHW, $P > 0.05$ from control.

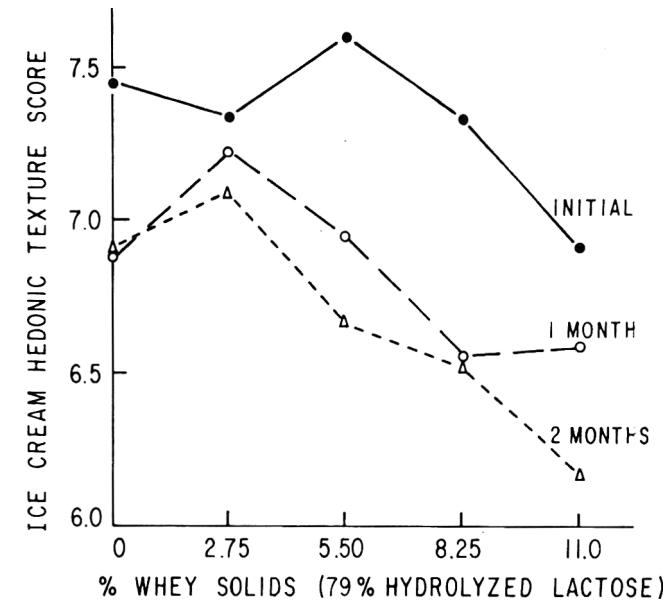

Fig. 6—Texture scores of ice creams (LHW, 79%) stored at −20° C. Initial and 2 months, 11% LHW, $P < 0.05$ from control.

creased (exception 2.75%) and coarseness scores of heat shock samples increased, although only the coarseness scores of 11% LHW were significantly higher in all three trials (Fig. 7). Heat shocking of the 12 controls on the average decreased texture and increased coarseness scores significantly in about a third of the samples and very little or not at all in the rest of the samples, indicating the possible variations that may be encountered in running these tests. Heat shocking was not any more deleterious to the LHW samples, with exception of the ice cream containing 11% LHW, than control samples. Heat shocking had no significant effect on the flavor score of any one sample.

CONCLUSION

UP TO 13.6% replacement of milk solids nonfat and 8.3% sugar with lactose hydrolyzed sweet whey solids can be

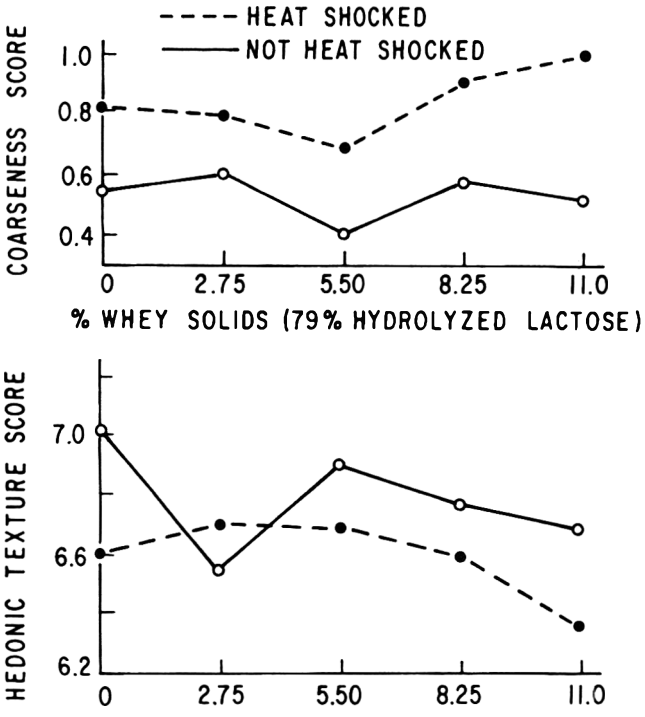

Fig. 7—Average texture and coarseness scores of heat shocked ice creams (LHW, 79%) compared to their respective controls. Texture: 9 NDM controls ($P > 0.05$), 1 ($P < 0.05$), 2 ($P < 0.01$); 11% LHW, 2 ($P > 0.05$), 1 (< 0.05). Coarseness: 7 NDM controls ($P > 0.05$), 3 ($P < 0.05$), 2 ($P < 0.01$); 5.5% LHW, 2 ($P > 0.05$), 1 ($P < 0.05$); 8.25% LHW, 2 ($P > 0.05$), 1 ($P < 0.01$); 11% LHW, 1 ($P < 0.05$), 2 ($P < 0.01$).

made with no loss of ice cream quality. Higher levels of replacement contribute to progressive loss of flavor quality and consistency. Caramel-like flavor defect can be corrected by high temperature short time pasteurization of the mix, and saltiness most probably by partial demineralization of the whey.

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Reference to brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

MECHANICAL PROPORTIONING OF BEEF. . .From page 110

able method of producing portion controlled steaks if palatability was sacrificed for increased production and yields. Results of the present study and those of Davis et al. (1975), Neer et al. (1978), and Roberts et al. (1974) reveal very few differences between steaks from pressed and non-pressed subprimals in juiciness, flavor, tenderness, overall palatability, and shear force values.

Use of a system involving partially frozen product (such as that involved in crust-freezing systems) would minimize tempering time allowing purveyors to incorporate pressing-cleaving without prolonged tempering. An in-line continuous production process whereby subprimals are trimmed to the steak-ready form, wrapped with polyvinyl chloride film, crust-frozen quickly by means of air or cryogenically with liquid CO_2 or N_2, tempered for a short period, and pressed and cleaved would allow packaging, boxing, and freezing of steak portions in a matter of hours. From the findings of this study, crust-frozen, pressed and cleaved product was comparable or superior in all parameters to that produced via the frozen-tempered or fresh subprimal treatments. Because of drastically shorter freezing and tempering times, strict adherence to recommended timetables for product freezing and tempering would have to be followed since the margin of error would be increased by using a crust-freezing instead of a freezing-tempering system. Further studies into the economic benefits of a crust-freezing system must be conducted before a recommendation could be made to purveyors who may wish to use this procedure.

Results of this study suggest that mechanical portioning of subprimals will increase yields of salable steaks, will not substantially affect steak thawing and cooking parameters, and does not result in lower quality or less palatable product when compared to fresh, hand-cut subprimals. When systems of product preparation prior to mechanical portioning were compared (crust-freezing, or freezing-tempering) there was little evidence (except that of time required for crust-freezing vs freezing-tempering) favoring one over the other of the two systems in terms of the traits evaluated in this study.

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BOD AND COD DETERMINATIONS ON CITRUS WASTE STREAMS AND COMPONENT PARTS

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ABSTRACT

Triplicate grapefruit and orange samples of about 500 kg each were processed under controlled pilot plant conditions. Composite samples of the wash water and the products of each unit operation were analyzed for oxygen demand by the long chemical oxygen demand (L-COD), short-chemical oxygen demand (S-COD) and biochemical oxygen demand (BOD). Additionally, samples of grapefruit and orange were washed by hand with 0.4L of H_2O/kg fruit before dividing the fruit into six component parts: flavedo, albedo, membrane, juice sacs, seeds, and juice. Samples of fruit were also prepared by grinding the whole fruit in a comminuting machine. The L-COD values for the pilot plant runs ranged from 68 ppm on the water used to rinse grapefruit before it was washed, to more than 1,180,000 ppm for the dried orange peel. The weight of rinse water was 40% of the fruit weight and dry peel weights were 8% of the fruit weight. For the component parts, the L-COD ranged from 416 ppm for the rinse water to more than 1.5 million ppm for the grapefruit seeds. Covariance analysis comparing differences in oxygen demand measurements within a cultivar in addition to the differences in percentage of total solids and °Brix is discussed.

INTRODUCTION

IN THE UNITED STATES, citrus fruit processing utilizes more raw material than the processing for all other types of fruit combined. In 1975, of the estimated 11.1 million metric tons of raw fruit processed, citrus constituted almost 64%. Of the 146.5 million cubic meters of water discharged during fruit processing, citrus accounted for almost 60% (Woodroof, 1975). Eidsness et al. (1971) estimated that the combined effluent from the 52 citrus processing plants in Florida was approximately 492,000 cubic meters per day, with a combined 5-day biochemical oxygen demand (BOD) loading equivalent to a population of 2 million people.

In spite of these large volumes of water discharged, there is a surprising lack of basic information in the literature. One study detailed the various waste streams discharged by two citrus processing plants (Black, Crow and Eidsness, Inc., 1970). But by and large, the available information characterized only the final effluent (Hong, 1977; Koo, 1976) or was conducted some time ago (McNary et al., 1956) when larger volumes of water diluted the waste to give relatively low BOD values. For example, in the 1950's, one plant was using almost 38 million liters of water with a resulting BOD of only 25 (Lee, 1955). Today this situation has changed. Strict government effluent discharge standards and recycling efforts to conserve water are making the wastes more concentrated. So future waste treatment efforts must improve to keep pace with changing government and social needs.

For more than 25 years, citrus processors have been making salable products from the entire citrus fruit by fractionating the fruit into specialty products. Estimates have been made on the amount of waste discharged based on the weight of fruit purchased minus the weight of products produced. Scott and Morgan (1959) calculated that only 2.9% of the total fruit solids purchased did not end up in the total solids of the extracted juice. On a Florida statewide basis, only 2.8% of the fruit purchased by processing plants could not be accounted for by the weights of juice and specialty products produced (Gerwe, personal communication).

The purpose of the current study was to gather basic information on various waste streams produced when the fruit is divided into its component parts. This study was done under carefully controlled pilot plant conditions using replicate orange and grapefruit samples to determine: (1) oxygen demand loads generated when washing and rinsing a known amount of fruit with a known amount of water; (2) the oxygen demand potential of the various waste streams generated in citrus processing; and (3) a materials balance for the amount of fruit processed into the various fractions and the cost in terms of oxygen demand.

In the second part of this study, replicate samples of orange and grapefruit were fractionated into their component parts, some of which are not made commercially but were included for completeness of the study. Estimations were made on: (1) the oxygen demand potential of these components in a waste stream, and (2) the variability associated with the materials balance and oxygen demand measurements.

MATERIALS & METHODS

Preparation of samples

Unit processing operations. Samples of 'Marsh' grapefruit were processed on Jan. 13 and Feb. 10, 1978; 'Valencia' oranges on March 24 and April 25, 1978. Triplicate samples of fruit each weighing approximately 500 kg were processed for the replicate runs. The fruit was processed as detailed by Kesterson and Braddock (1978).

For each of the three replicates, composite samples were prepared from those collected over a period of time or at different sampling points for the following. Composite waste water samples were taken from the water used to rinse off the loose dirt from the fruit (prewash rinse water, PWR). The fruit was washed using a Food Machinery Corporation (FMC) 220 Fruit Cleaner. Samples were taken of the water used to rinse off the fruit cleaner and dirt after washing (after wash rinse water, AWR). Next the fruit was extracted with a FMC In-Line-Juice-Extractor and samples were taken of the finished juice, oil emulsion, and juice sacs. The extractor, finisher, tanks, and floor were cleaned using a measured amount of water and a composite waste water sample was taken for all three samples. The peel, membrane, and seeds were ground together and a sample was taken prior to the addition of lime [$Ca(OH)_2$]. After liming and pressing, the peel was dried and samples were taken of the dry peel and press liquor for each triplicate.

Component parts. On each individual processing date, component part samples were also prepared. Samples of ten grapefruit or ten oranges were taken prior to washing and another ten fruit were taken after washing and before extraction. The replicate samples of the washed fruit were ground as whole fruit in a Model D Fitzpatrick Comminuting Machine (W.J. Fitzpatrick Co., Chicago, Ill.). The samples of fruit taken prior to washing were washed by hand in 0.4L of water per kg of fruit. The fruit was then separated by hand

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Table 1—Characteristics of waste water generated during four unit processing operations on 'Valencia' orange and 'Marsh' grapefruit[a]

Sample description		Settleable solids (ml/L)	Turbidity (FTU)	L-COD (mg/L)	S-COD (mg/L)	BOD (mg/L)
Prewash rinse H_2O	GF[b]	0.42 ± 0.09	7.5 ± 1.5	68.2 ± 18.6	59.3 ± 8.2	14.8 ± 4.0
	O	0.58 ± 0.39	6.6 ± 1.8	83.3 ± 24.2	71.3 ± 24.6	18.3 ± 2.9
After wash rinse H_2O	GF	0.97 ± 0.77	41.7 ± 6.5	263. ± 54.	205. ± 57.	43.5 ± 17.7
	O	0.92 ± 0.56	41.8 ± 5.3	335. ± 50.	279. ± 34.	73.2 ± 29.5
Clean-up H_2O	GF	22.00 ± 7.07	54.5 ± 0.7	1,640. ± 267.	1,360. ± 201.	929. ± 228.
	O	25.50 ± 4.95	50.5 ± 0.7	1,470. ± 286.	1,410. ± 330.	807. ± 220.
		Recoverable oil % by volume				
Oil emulsion	GF	0.14 ± 0.03		28,700. ± 5,470.	26,200. ± 5,320.	16,400. ± 1,890.
	O	1.22 ± 0.07		27,900. ± 7,010.	28,800. ± 13,400.	10,800. ± 2,550.

[a] Each value is the mean of 12 measurements.
[b] GF = grapefruit; O = orange

into flavedo, albedo, membrane, juice sacs, seeds, and juice similar to the method of Rouse et al. (1962, 1964).

Materials balance

During each of the triplicate unit processing runs, separate data were kept on weight of fruit, water used, and total amount of products.

In the study of the fractional components, the weight of pollutants washed from the fruit by a given amount of water was measured. The weights of the six component parts for each processing date were also tabulated.

Methods of analysis

Waste water analysis. For the triplicate grapefruit and orange processing runs, AWR, PWR, and clean-up water, as well as the samples from washing the component parts fruit were all analyzed for settleable solids, turbidity, long-chemical oxygen demand (L-COD), and biochemical oxygen demand (BOD) according to Rand et al. (1976) and short-chemical oxygen demand (S-COD) as described by Crandall et al. (1977). The juice samples were analyzed for percent acid, °Brix and recoverable oil percent by volume according to the procedures outlined in Praschan (1976). The press liquor was analyzed for °Brix, the oil emulsion was analyzed for percent oil by volume. All of the other samples were analyzed for percent total solids by drying in an oven at 80°C for 18 hr.

Statistical analysis. The data were subjected to a covariance analysis (Steel and Torrie, 1960) because of differences between the two cultivars and processing dates within a cultivar. These differences occurred in the percent total solids and, in some constituents, in °Brix which influenced the oxygen demand measures making direct comparisons unfeasible.

The covariance procedure compared the two cultivars as if they had the same mean percentage total solids or °Brix on the assumption of a linear regression between the covariate (solids) and response (oxygen demand parameter measured).

RESULTS & DISCUSSION

Unit processing operations

The relative amounts of the pollution measurements made on the PWR, AWR, clean-up water, and oil emulsion water are shown in Table 1. For all parameters measured, PWR consistently was the lowest. This rinse water was so clean that it probably should be recirculated to concentrate the pollution load and reduce the amount of fresh water used. The increase in load found in AWR reflects the increased amount of dirt removed with the fruit cleaner. The amounts of fruit cleaner ranged from 2–10 ppm when measured as methylene blue active substance (according to Rand et al., 1976) and by itself was a negligible contribution to the oxygen demand.

Clean-up water had a surprisingly large load indicating the pollution could be lessened by an initial clean-up with sweeping instead of washing the floor and equipment with water. Part of the water used in clean-up could be reused water from the first two rinsing operations.

As expected, the oil emulsion had high oxygen demand due to its oil content from the flavedo. Table 1 presents the mean oil values for the emulsion on a percent by volume basis. The oxygen demand values were difficult to determine on these samples because the oil is approximately 90% d-limonene, which is volatile, toxic to bacteria (Murdock and Allen, 1960), and has a theoretical COD of more than 3 million (Rebeck, 1971). We found the pure oil had a L-COD of approximately 1.2 million and an approximate BOD of 1 million.

For the rest of the unit processing operations oxygen demand values, there seemed to be a relationship between the percentage total solids (% TS) or °Brix and oxygen demand (Table 2). For grapefruit, the fresh juice had the lowest values for all three oxygen demand measures and averaged 8.0 °Brix. Finisher pulp had the next lowest long and short COD with press liquor having the next lowest BOD. The % TS for the finisher pulp averaged 9.7 and press liquor averaged 8.9 °Brix. Ground peel had the second highest oxygen demand measurements of all three tests and the average total solids content was 15.9% while the dry peel had the highest measurements with an average 90.1% TS.

For the orange samples, the ranking of the three oxygen demand measurements was the same. These were, from lowest to highest: press liquor, fresh juice, finisher pulp, ground peel, and dry peel. Their respective values were 8.8 °Brix, 9.8 °Brix, 14.9, 17.9, and 90.1% TS.

The relationship between oxygen demand measurements and solids is discussed in the results of covariance analysis section.

The oxygen demand figures (from Tables 1 and 2) can be used in conjunction with the relative amounts of pro-

Table 2—Oxygen demand characteristics of waste water generated during five unit processing operations on 'Valencia' orange and 'Marsh' grapefruit[a]

		L-COD	S-COD	BOD
Fresh juice	GF[b]	77 ± 5	76 ± 5	51 ± 4
	O	106 ± 13	99 ± 12	69 ± 2
Finisher pulp	GF	99 ± 10	90 ± 15	64 ± 14
	O	171 ± 26	138 ± 3	89 ± 6
Ground peel	GF	158 ± 25	146 ± 24	103 ± 22
	O	204 ± 27	175 ± 18	101 ± 8
Press liquor	GF	152 ± 7	93 ± 10	59 ± 13
	O	96 ± 9	92 ± 8	63 ± 4
Dry peel	GF	861 ± 137	1,160 ± 142	554 ± 103
	O	1,180 ± 99	1,020 ± 90	440 ± 137

[a] Each value multiplied by 1,000 = mg/L COD or BOD.
[b] GF = grapefruit; O = orange

Table 3—Weight of products from the unit processing operations (% total fruit weight[a])[b]

	Grapefruit (%)	Orange (%)
Prewash rinse H_2O	40.2 ± 3.9	46.6 ± 3.8
After wash rinse H_2O	39.1 ± 3.5	37.2 ± 2.8
Clean up H_2O	43.3 ± 1.8	79.0 ± 3.7
Oil emulsion	31.1 ± 10.0	51.1 ± 11.2
Fresh juice	49.0 ± 1.1	50.2 ± 7.7
Finisher pulp	4.3 ± 0.3	1.8 ± 0.2
Ground peel	47.6 ± 1.4	50.0 ± 6.5
Press liquor	13.1 ± 1.7	10.9 ± 2.2
Dry peel	8.3 ± 0.2	9.4 ± 0.3

[a] (Component wt/total fruit wt) X 100
[b] Each value is the mean of 6 readings.

Table 4—Characteristics of waste water generated during hand washing (0.4L water/kg fruit) per kg fruit

	Grapefruit	Orange
Settleable solids (ml/L)	3.0 ± 0.7	3.3 ± 1.0
Turbidity (FTU)	27 ± 3	48 ± 5
L-COD (mg/L)	416 ± 41	469 ± 74
S-COD (mg/L)	350 ± 37	380 ± 60
BOD (mg/L)	98 ± 10	119 ± 14

ducts obtained (Table 3) to get an indication of the overall strength of each unit operation. The values are expressed as percentages to facilitate their use with any unit of weight or amount of fruit. These values are similar to those reported in other studies concerning dividing fruit into its component parts and not pollution parameters (Kesterson et al., 1978).

Component parts

The pollution parameters from the single hand washing of the fruit in 0.4L of water per kg of fruit (Table 4) approximated the value of the combination of the two wash solutions, PWR and AWR seen in Table 1. This was because only one hand washing was used and the values in Table 4 reflect the more thorough hand washing.

The potential oxygen demand from the various component parts is shown in Table 5. These values show the oxygen demand in mg/L that the various component parts exert when they are washed into the waste treatment stream. For grapefruit, all three oxygen demand measures of the component parts were ranked the same way, from the lowest to the highest: juice, juice sacs, whole fruit, membranes, flavedo, albedo, and seeds. Their respective solids were 8.1 °Brix, 10.8 °Brix, 12.4, 14.1, 21.6, 21.7, and 41.5% TS. The rank was the same for the orange samples except the flavedo and albedo were switched in the ranking of the COD's. The respective solids were 10.9 °Brix, 15.2 °Brix, 16.8, 20.5, 30.9, 28.4, and 47.5% TS. A good correlation exists between the rank of the oxygen demand values and the solids content, the only exceptions being the orange flavedo and albedo.

Table 5—Oxygen demand characteristics of the fractionated component parts of 'Valencia' orange and 'Marsh' grapefruit[a]

		L-COD	S-COD	BOD
Whole fruit	GF[b]	130 ± 16	125 ± 16	89 ± 10
	O	187 ± 30	171 ± 26	104 ± 14
Flavedo	GF	205 ± 34	208 ± 50	105 ± 28
	O	420 ± 31	319 ± 42	135 ± 24
Albedo	GF	244 ± 24	214 ± 14	150 ± 15
	O	354 ± 37	290 ± 43	182 ± 20
Membrane	GF	138 ± 16	142 ± 8	94 ± 18
	O	234 ± 28	220 ± 35	114 ± 22
Juice sacs	GF	123 ± 7	107 ± 3	70 ± 10
	O	171 ± 16	154 ± 16	96 ± 19
Juice	GF	93 ± 16	79 ± 5	59 ± 4
	O	119 ± 13	107 ± 16	85 ± 14
Seed	GF	1,589 ± 153	1,251 ± 114	838 ± 106
	O	1,625 ± 266	1,232 ± 234	678 ± 161

[a] Each value multiplied by 1,000 = mg/L COD or BOD.
[b] GF = grapefruit; O = orange

The oxygen demand values from Table 5 can be used with the relative weights of the component parts from Table 6 to obtain the components with the highest oxygen demand. Table 6 also shows the weight of the clean, whole grapefruit and orange. The values in the rest of the Table are expressed in terms of percent. These values are similar to those reported by Rouse et al. (1964) where grapefruit were divided into their component parts once each month for 10 months during two processing seasons. In that study, the amounts of peel (flavedo and albedo combined) were the same, the amount of membrane was more, and juice sacs less than in the current study. There was considerably more juice and less seeds in this variety of fruit. In the orange, there was slightly more peel, more membrane, less juice sacs, slightly more juice, and the same amount of seeds in the current study compared with Rouse's where 'Valencia' fruit was separated into its fractional components throughout two processing seasons. Rouse et al. (1962) found little variation in the percentage of individual components in grapefruit or oranges during the season.

Results of covariance analysis

Up to this point, we have been discussing the results of the orange and grapefruit runs separately. This is because an analysis of variance on each sample prior to the covariance analysis showed significant differences between the two cultivars in all but the unit processing runs of dry peel and press liquor.

No comparison has been made between significant differences in oxygen demand measurements within a cultivar because of the differences in these values caused by differences in % TS or °Brix. The covariance analysis first adjusted the oxygen demand measurements to a common % TS or °Brix value then tested for differences beyond the effect of solids. As for the unit processing runs, there were significant differences between cultivars in the dry peel and press liquor L-COD, dry peel S-COD and fresh juice BOD. In the component parts, there were significant differences between cultivars except in whole fruit L-COD, S-COD, al-

Table 6—Weight of the fruit and distribution of the component parts in 'Valencia' orange and 'Marsh' grapefruit[a]

	Grapefruit	Orange
Whole fruit	451g ± 26	238g ± 36
Flavedo	14.0%[b] ± 1.9	15.2%[b] ± 4.0
Albedo	14.9 ± 2.1	7.6 ± 3.1
Membrane	14.9 ± 2.4	17.5 ± 3.1
Juice sac	6.6 ± 1.9	5.4 ± 3.2
Juice	49.1 ± 2.4	53.4 ± 4.5
Seeds	0.5 ± 0.1	0.9 ± 0.5

[a] Each value is the mean of at least 6 readings.
[b] (Component wt/total fruit wt) X 100

bedo, and membrane BOD and all three pollution measurements on the juice.

However, only a few of the oxygen demand measurements were found to have a significant linear regression with their respective % TS or °Brix. For the component parts, these were: membrane L-COD, juice sac S-COD and the three juice responses. With the samples from the unit processing runs, there were a few pollution measurements that had a significant linear regression with their respective % TS or °Brix, exceptions being finisher pulp, fresh juice, press liquor and ground peel L-COD, and fresh juice and press liquor S-COD.

This implies that the covariance, °Brix or total solids, adjustment was not required since the linear coefficient was not significantly different from zero. However, there may have been a relationship between the measured pollution parameters and % TS and °Brix but the test failed to show this because it lacked sensitivity due to the large amount of variation in the data compared to the sample size.

An additional implication is that the sample may be very heterogeneous and small particles of high oxygen demand material (d-limonene with an oxygen demand of 3.29 g/g, mixed with low oxygen demand, glucose; with an oxygen demand of 1.07 g/g) cause the results of the pollution measure to depend more on what was in that particular sample i.e. mixture of high and low oxygen demand material rather than the % TS or °Brix.

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TUNNEL-DRIER MODELING. . .From page 125

$$R^*\rho_d^* = R_f\,\rho_d\,(1 + W\rho_d/\rho\ell)^{-2/3}$$

$$R^* = R_f\,(1 + W\rho_d/\rho\ell)^{1/3}$$

In these last formulas, we replaced overall prune density by flesh density because they are close (Perry, 1944).

With $\Delta h = C_v T_a + \Delta h_v - C\ell\, T_p$; $\mu=(L/G)\cdot(v/\bar{u})$; and $\sigma = C_s/C_g$ and $\psi = \Delta h/C_g$, we obtain:

$$E_1 = \mu\psi\, E_4 - \mu\sigma E_3$$

$$E_2 = -\mu E_4$$

$$E_3 = -\frac{3a}{R^*}(1 + Bi^*/5)^{-1}\left[\frac{Bi^*}{R^*}(T_a - T_p) - \frac{\Delta h_v}{\lambda}\frac{R^*\,\rho_d^*}{3}E_4\right]$$

$$E_4 = \frac{3\alpha_m}{\rho_d^*\,R^*}(p_{surf} - p_v)$$

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GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF SUGARS IN READY-TO-EAT BREAKFAST CEREALS

B. W. LI and P. J. SCHUHMANN

ABSTRACT

A wide selection of ready-to-eat breakfast cereals was analyzed for sugars with a gas-liquid chromatographic technique. Cereal products from General Foods, General Mills, Kellogg, Nabisco, Quaker Oats, and Ralston-Purina were purchased locally. Sugars found in the cereals varied from sucrose alone to any combinations of fructose, glucose, lactose, maltose, and sucrose. The amount of a given sugar ranged from <0.5% to >50% of dry weight. Amounts of but not types of sugar varied from lot to lot; usually only slightly more than the analytical variations between replicates. The analytical data were verified on several occasions by comparisons with data from a liquid chromatographic technique in our own laboratory, and in other laboratories by similar methods.

INTRODUCTION

SOME of the presweetened ready-to-eat breakfast cereals have been on the market since the early 1950's; however, recently there has been much discussion about their nutritive value, possible role in dental caries (Glass and Fleisch, 1974), and even the ethics of their recommendation to children in television advertising (Snyder, 1978). All these and other controversies are centered not on the cereal portion of the food but rather on the added sugars.

Cereal manufacturers are not required by law to provide labeling information on sugars, but some of them do. A value for "sucrose and other sugars" appears on the label of certain products of three manufacturers. According to information provided by them, these values were based on formulation and were checked with TLC or GLC analysis by two manufacturers. The only other data for sugar in cereals are those of Shannon (1974, 1977). He reported on the amounts of glucose and sucrose in 79 dry breakfast cereals. His data were from enzymatic analysis of two samples of each product.

In view of the interest in cereals, we undertook analyses of individual sugars in the same samples. To date, 62 different cereals, three lots of each have been analyzed in duplicate. A "lot" is a separate box obtained from a separate retail outlet and/or having a different expiration date. The method routinely gives quantitative data for any of the following sugars: fructose, glucose, lactose, maltose, and sucrose.

EXPERIMENTAL

Apparatus

Gas-liquid chromatograph: Hewlett-Packard 5840A equipped with flame ionization detector, automatic sampler, and 6 ft × 1/8 in. stainless steel column packed with 3% SP2250 (Supelco, Inc.) on 80/100 mesh Supelcoport. Operating conditions: injection port 200°C, detector 300°C, column 150–300°C programmed at 10°/min; helium carrier flow 30 ml/min, hydrogen flow 40 ml/min, air flow 300 ml/min. Injection volume: 1 μl.

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Stock solutions

The pyridine reagent can be conveniently prepared in 500-ml quantities by adding the calculated amounts of hydroxylamine hydrochloride (25 mg/ml) and β-phenyl-D-glucopyranoside (2 mg/ml) as an internal standard to a freshly opened bottle of pyridine. Pyridine serves as an excellent solvent and reaction medium. Care must be taken to dry the reagents before they are weighed and to avoid exposure of the pyridine to air. After the solution is completely clear, we transferred the reagent under N_2 to 50-ml capped serum bottles.

We prepared standards from the dried (over P_2O_5 in a vacuum desiccator) sugars (Supelco, Inc.) as follows: 20 mg of each of the five sugars was weighed into a 10-ml volumetric flask, dissolved and made up to the mark with deionized water. Aliquots (0.5 ml) of this stock solution were pipetted into reaction tubes and stored in the freezer. One or two of these were dried and derivatized along with a batch of samples.

Procedures

Sampling. Cereals from freshly opened boxes were ground in a Wiley Mill to pass through a 30-mesh screen. Composition of most products was fairly uniform (flakes, donuts, stars, etc.) and about 1/4 of the boxes' contents was processed. The ground samples were thoroughly mixed and portions were removed and dried in a vacuum

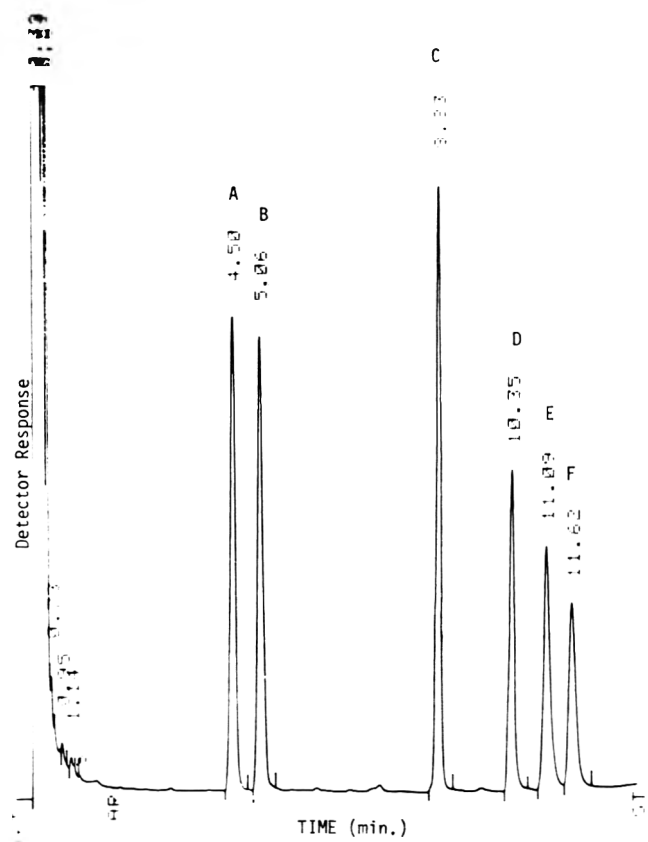

Fig. 1—Gas chromatogram of TMS oximes of reducing sugar standards and TMS ethers of nonreducing sugar standards: (A) fructose; (B) glucose; (C) β-phenyl-D-glucopyranoside; (D) sucrose; (E) lactose; (F) maltose.

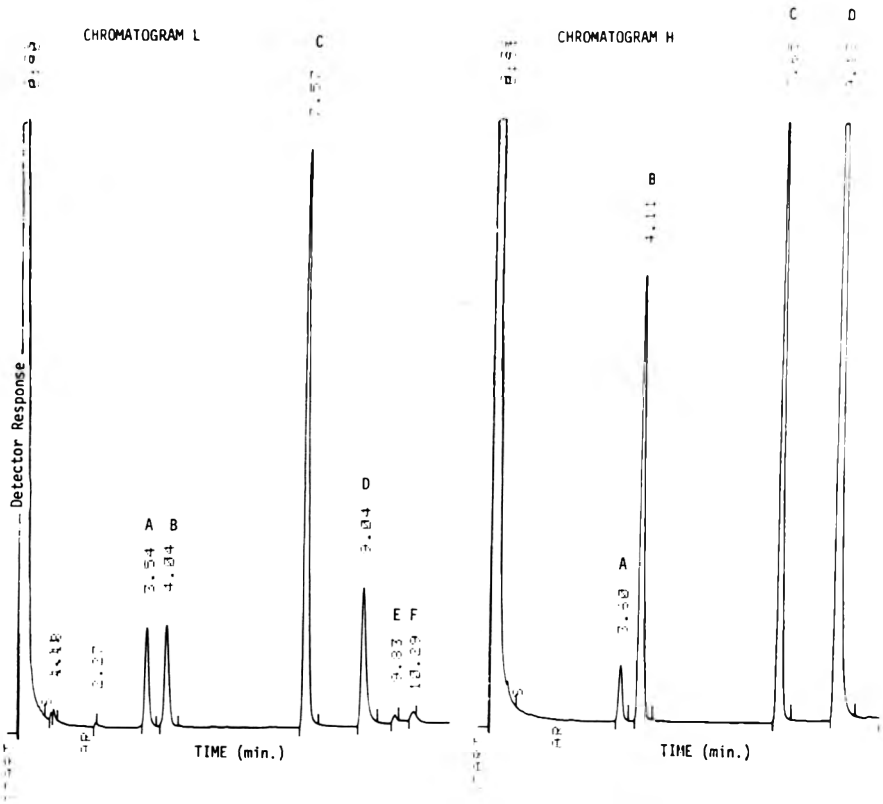

Fig. 2—Gas chromatograms of TMS oximes of reducing sugars and TMS ethers of nonreducing sugars for a low sugar cereal (chromatograph L) and a high sugar cereal (chromatograph H): (A) fructose; (B) glucose; (C) β-phenyl-D-glucopyranoside; (D) sucrose; (E) lactose; (F) maltose.

desiccator prior to weighing. Test-tubes with Teflon-lined screw-caps were convenient for both the extraction and derivatization of the samples. Accordingly, duplicate 100-mg samples of each lot of each cereal were weighed into such tubes.

Extraction. An initial extraction with 3 ml of n-hexane removed fat and other nonpolar material. When free of hexane, one sample was extracted with either 5 or 10 ml of water and the other with the same volume of 80% methanol. The volume of extracting solvent used depended on the estimated total amount of sugar in the sample. Extractions were carried out at room temperature with vigorous mixing at regular intervals for about 2 hr. When extraction was complete, the tubes were centrifuged at 2000 rpm for 5–6 min.

Derivatization. Three 1-ml aliquots of the supernatant were transferred into another set of tubes; the extracts were concentrated under a stream of N_2 in a 50°C water bath; then taken to complete dryness in a vacuum desiccator. The dried sugar extracts were heated at 75°C with 1-ml of pyridine reagent. After 30 min the tubes were cooled to slightly above room temperature, 0.5 ml of hexamethyldisilazane was added, followed by 4 drops of trifluoroacetic acid. The contents were mixed vigorously for 30 sec and after standing for at least 30 min were centrifuged and ready to be injected into a GLC column.

RESULTS & DISCUSSION

GAS-LIQUID CHROMATOGRAPHIC method (Sweeley et al., 1963; Brobst and Lott, 1966) is one of several techniques applicable to carbohydrate analysis. Other procedures include HPLC, LC, TLC, and enzymatic determinations to name a few. Among gas-liquid chromatographic methods various derivatives of sugar can be used for quantitative analysis. We analyzed the trimethylsilylated sugar oximes of reducing sugars (Mason and Slover, 1971; Toba and Adachi, 1977; Li and Stewart, 1978) and the trimethylsilyl ethers of sugars such as sucrose and β-phenyl-D-glucopyranoside. Derivatization is straight forward and was highly reproducible; the method is specific and gives good separation of the sugar derivatives (see Fig. 1–2). These derivatives may be kept below 0°C for several months without deterioration.

The coefficient of variation for standard sugars, fructose, glucose, and sucrose, was not greater than 2%. Variation was sometimes greater for lactose and maltose but not more than 5%; measured variation averaged around 5%. In most cases, the variation for samples was not greater than that for the standards but individual cereals produced problems related to sampling and/or measuring the aliquots. The overall error of the method was estimated at ± 7% for levels of sugar between 0.5% and 55% of the dry sample. The range of recovery for the five sugars is between 94–106%. This was checked by repeated extractions and spiking before derivatization.

The sampling and extraction procedures described above were satisfactory for most of the cereals we analyzed except for the few that contained raisins, nuts, or frosted sugar. These were found difficult to grind to 30 mesh, so were ground with no screening in a Krups Mill (Robert Krups, North American) and 5–10g samples were used for extraction. In all cases where both water and 80% methanol were used, the results obtained from the water extract and methanol extract were comparable, with the exception of four which contain more than 3% maltose. In these cases, 80% methanol extraction gave significantly lower maltose values than the water extract. On the other hand, cereals which have added raisins could not be extracted with water,

Table 1—% of dry weight

	Fructose	Glucose	Sucrose	Total sugar
Cereal A				
Our Lab	0.93	1.2	28.2	30.3
General Mills' LC Lab	1.2	1.5	26.2	28.7
General Mills' GLC Lab	0.7	1.0	26.2	27.9
Cereal B				
Our Lab	0.77	0.66	9.6	11.0
General Mills' LC Lab	1.2	1.2	10.8	13.2
General Mills' GLC Lab	0.9	0.9	10.9	12.7

since in water, the presence of invertase in raisin will lead to extensive hydrolysis of sucrose during extraction. Of the data we report here, approxmately one-third were obtained from the average of both water and methanol extraction, another third from only water extraction, and the remainder from only 80% methanol extraction. Except for special cases mentioned above, we found the 80% methanol extract contains less extraneous material and requires less time for drying before derivatization.

The sums of our values for individual sugars generally were comparable to the total sugar given on the labels. Data from several of our analyses were verified with those from other laboratories using similar methods (Table 1).

Among the 62 varieties of cereals we analyzed (Table

Table 2—% of dry weight—General Foods (Post)

Product	Fructose	Glucose	Lactose	Maltose	Sucrose	Total sugar
Alphabits	<0.5	<0.5	ND[a]	ND	35.4 – 39.9	35.4 – 39.9
Bran Flakes, 40%	0.85 – 1.32	0.76 – 1.17	ND	0.75 – 1.22	9.22 – 10.7	11.6 – 14.4
C. W. Post (plain)	0.61 – 0.76	1.64 – 1.83	1.69 – 1.86	2.61 – 4.72	19.7 – 20.4	27.1 – 28.5
C. W. Post (raisin)	3.84 – 5.48	4.28 – 5.90	1.22 – 1.26	ND	18.1 – 18.5	27.4 – 31.1
Cocoa Pebbles	0.25 – 0.27	0.34 – 0.35	ND	ND	40.5 – 43.6	40.5 – 43.6
Country Crisp	1.70 – 1.79	1.97 – 2.08	ND	< .5	17.5 – 18.2	21.3 – 22.0
Fortified Oat Flakes	0.20 – 0.24	0.20 – 0.32	ND	< .5	17.5 – 17.7	18.2 – 18.4
Frosted Rice Krinkles	0.25 – 0.28	0.41 – 0.46	ND	ND	40.0 – 43.5	40.0 – 43.5
Fruity Pebbles	0.21 – 0.23	0.33 – 0.36	ND	ND	41.3 – 41.8	41.3 – 41.8
Grape Nuts	0.74 – 0.77	1.38 – 1.41	ND	4.82 – 4.98	ND	7.01 – 7.11
Grape Nuts Flakes	0.28 – 0.35	0.70 – 0.85	ND	4.09 – 4.93	6.83 – 7.72	11.9 – 13.7
Honey Combs	0.12 – 0.14	0.10 – 0.12	ND	ND	35.7 – 39.0	35.7 – 39.0
Post Toasties	1.17 – 1.19	1.40 – 1.45	ND	ND	2.59 – 2.92	5.23 – 5.49
Raisin Bran	6.40 – 7.62	6.22 – 7.41	ND	0.96 – 1.66	14.4 – 15.4	28.0 – 32.1
Super Sugar Crisp	2.20 – 2.40	4.49 – 4.93	ND	2.37 – 3.02	35.0 – 36.6	44.9 – 45.8

[a] ND—Not detectable. The detection limit was 0.01% for fructose, glucose, and 0.1% for lactose, maltose, and sucrose.

Table 3—% of dry weight—General Mills

Product	Frustose	Glucose	Lactose	Maltose	Sucrose	Total sugar
Buckwheats	0.77 – 0.86	0.66 – 0.90	ND[a]	0.1 – 0.4	9.56 – 11.4	11.0 – 13.0
Cheerios	0.06 – 0.14	ND	ND	ND	2.58 – 3.48	2.58 – 3.48
Cocoa Puffs	0.10 – 0.16	1.65 – 1.89	ND	1.01 – 1.22	31.0 – 32.1	34.0 – 35.0
Count Chocula	0.13 – 0.41	3.01 – 4.88	0.15 – 0.32	ND	33.2 – 37.3	36.5 – 42.2
Crazy Cow, Chocolate	0.11 – 0.34	2.05 – 2.46	0.81 – 0.84	0.28 – 0.62	41.0 – 43.7	43.9 – 46.6
Crazy Cow, Strawberry	0.10 – 0.15	1.79 – 2.19	ND	< .5	36.1 – 40.6	37.9 – 42.6
Frankenberry	0.36 – 0.42	3.94 – 4.42	0.26 – 0.29	1.16 – 1.42	37.7 – 38.9	43.7 – 45.2
Golden Grahams	0.91 – 0.93	1.14 – 1.20	ND	ND	26.6 – 28.2	28.7 – 30.4
Kix	0.25 – 0.69	0.14 – 0.53	ND	ND	3.06 – 4.83	3.06 – 6.05
Lucky Charms	0.46 – 0.82	3.98 – 5.89	0.21 – 0.47	0.22 – 0.32	34.1 – 37.4	38.5 – 44.1
Total	0.69 – 0.90	0.44 – 0.62	ND	ND	5.54 – 6.88	6.67 – 8.16
	0.80 – 0.89	0.54 – 0.66			6.99 – 7.58	8.48 – 9.13
Trix	0.12 – 0.17	1.63 – 2.11	ND	0.57 – 1.06	28.5 – 37.2	30.8 – 40.5
Wheaties	0.60 – 0.86	0.36 – 0.58	ND	ND	6.70 – 7.52	7.98 – 8.48

[a] ND—Not detectable. The detection limit was 0.01% for fructose, glucose, and 0.1% for lactose, maltose, and sucrose.

Table 4—% of dry weight—Kellogg

Product	Fructose	Glucose	Lactose	Maltose	Sucrose	Total sugar
All Bran	1.09 – 1.64	0.90 – 0.98	ND[a]	1.32 – 1.54	15.6 – 16.0	19.3 – 19.4
Apple Jacks	0.21 – 0.32	0.27 – 0.42	ND	ND	52.4 – 55.6	52.4 – 55.6
Cocoa Krispies	0.32 – 0.39	0.77 – 0.85	0.72 – 0.82	ND	39.0 – 43.8	40.8 – 45.8
Concentrate	0.20 – 0.30	0.03 – 0.11	ND	ND	8.45 – 10.2	8.65 – 10.6
Corn Flakes	1.02 – 1.20	1.15 – 1.34	ND	< .1 – 0.50	2.17 – 2.65	4.80 – 5.21
	1.08 – 1.18	1.16 – 1.58		0.35 – 0.40	2.23 – 3.33	5.39 – 6.05
Corny Snaps	0.19 – 0.30	0.20 – 0.35	ND	ND	35.1 – 45.4	35.7 – 45.8
Cracklin' Bran	0.64 – 0.70	1.23 – 1.52	ND	0.82 – 0.96	25.3 – 28.7	28.4 – 31.8
Froot Loops	ND	ND	ND	ND	47.7 – 49.1	47.7 – 49.1
Frosted Mini Wheats	ND	ND	ND	ND	25.0 – 26.1	25.0 – 26.1
Frosted Rice	0.59 – 0.83	0.76 – 1.02	ND	ND	32.4 – 37.4	33.8 – 39.2
Product 19	0.84 – 0.94	0.82 – 0.91	ND	ND	7.84 – 8.36	9.53 – 10.0
Raisin Bran	9.36 – 9.51	7.87 – 8.23	ND	ND	11.4 – 11.6	28.7 – 29.4
Rice Krispies	0.38 – 0.53	0.30 – 0.44	ND	ND	6.9 – 7.3	6.9 – 7.3
Special K	0.24 – 0.30	0.22 – 0.24	ND	ND	4.85 – 4.99	4.85 – 4.99
	0.23 – 0.25	0.19 – 0.24			4.58 – 5.20	5.03 – 5.68
Sugar Corn Pops	1.02 – 1.21	5.60 – 6.32	ND	ND	37.6 – 40.2	44.4 – 47.6
Sugar Frosted Flakes	0.72 – 0.93	0.89 – 1.03	ND	ND	35.8 – 42.0	37.5 – 43.6
Sugar Smacks	1.23 – 1.34	11.3 – 11.8	ND	ND	41.4 – 44.0	53.9 – 57.1

[a] ND—Not detectable. The detection limit was 0.01% for fructose, glucose, and 0.1% for lactose, maltose, and sucrose.

Table 5—% OF DRY WEIGHT—Nabisco, Quaker Oats, and Ralston-Purina

Product	Fructose	Glucose	Maltose	Sucrose	Total sugar[a]
Nabisco					
100% Bran	1.04 – 1.08	0.88 – 0.92	ND[b]	18.7 – 19.2	20.7 – 21.1
	0.82 – 1.26	0.64 – 1.03		17.5 – 18.3	18.9 – 20.4
Shredded Wheat-Spoon Size	ND	ND	ND	0.64 – 0.78	0.64 – 0.78
				0.45 – 0.49	0.45 – 0.49
Team	0.87 – 1.07	0.67 – 0.91	0.25 – 0.39	11.7 – 12.1	13.2 – 14.0
Quaker Oats					
Cap'n Crunch	<0.5	<0.5	ND	39.2 – 40.5	39.2 – 40.5
Cap'n Crunch, Crunchberries	0.53 – 0.80	0.46 –0.70	ND	41.4 – 43.4	42.8 – 44.6
Cap'n Crunch, Peanut Butter	0.34 – 0.45	0.71 – 0.87	ND	30.1 – 31.7	30.9 – 32.5
Life	ND	ND	ND	15.3 – 17.7	15.3 – 17.7
Life, Cinnamon	ND	ND	ND	20.9 – 21.5	20.9 – 21.5
Puffed Rice	ND	ND	ND	< 0.1	< 0.1
Puffed Wheat	0.1 – 0.3	ND	ND	0.1 – 0.4	< 0.5
Quisp	0.37 – 0.43	0.31 – 0.35	ND	38.3 – 42.0	38.3 – 42.0
Ralston-Purina					
Cookie Crisp, Chocolate Chip	0.40 – 0.50	0.58 – 0.73	ND	40.3 – 40.6	41.2 – 41.7
Cookie Crisp, Oatmeal	0.28 – 0.71	0.36 – 0.85	ND	38.1 – 40.7	39.6 – 40.7
Cookie Crisp, Vanilla	0.20 – 0.30	0.29 – 0.35	ND	39.8 – 45.6	39.8 – 45.6
Corn Chex	ND	ND	ND	3.45 – 3.79	3.45 – 3.79
Rice Chex	0.15	0.16 – 0.20	ND	3.95 – 4.23	4.28 – 4.58
Wheat Chex	0.68 – 0.84	0.51 – 0.56	0.26 – 0.31	2.06 – 2.11	3.51 – 3.79

[a] All cereals in this table have no detectable lactose.

[b] ND—Not detectable. The detection limit was 0.01% for fructose, glucose, and 0.1% for lactose, maltose, and sucrose.

2–5) 9 contained only sucrose; 19 had maltose and other sugars; 6 had a combination including detectable lactose. Of the 29 cereals that had a combination of only three sugars, sucrose, fructose, and glucose, about half had equal but low levels of the invert sugars that indicated sucrose was hydrolyzed during processing. The total sugar contents of all cereals ranged from less than 0.5% to more the 50%. Of the cereals, 15 contained less than 10% sugar and 3 had no added sugar. Another 17 had no more than 30%, and 21 had between 32 and 44% sugar. Finally, 8 cereals had as much as 45–56% total sugar.

Sugar contents seldom differed markedly among the three lots of the same cereal. For a few cereals we purchased lots more than 9 months apart, and found that their sugar contents were similar.

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The research conducted by the USDA on the commercial samples as reported in this manuscript was limited to analyses of their sugar content. The data are reported solely as factual information and are limited to the samples analyzed. No warranty or guarantee is made or implied that other samples of these products would have the same or similar composition. It is the policy of the USDA not to endorse those commercial products tested in research over those that were not tested. Use of company or product names by the USDA does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

A Research Note

VARIATION OF CHERIMOYA (Anona cherimolia) TEXTURE DURING STORAGE, AS DETERMINED WITH AN INSTRON FOOD TESTING INSTRUMENT

C. FÚSTER and G. PRÉSTAMO

ABSTRACT

Two varieties of cherimoya (known in U.S as custard apple) were harvested at the green-ripe stage and subsequently ripened under the following conditons: 5°C and 40% relative humidity (RH); 10°C and 85% RH; 20°C and 40% RH. Samples of the fruits were taken every 2 or 3 days and the following determinations were made: weight losses, specific weight, total soluble solids content (relating it with a taste panel) and texture measurements (compression, puncture, and shearing tests) by means of an Instron Food Testing Instrument. The deformation increased with the storage time and the temperature. In the puncture test the force required to break the skin decreased with the storage time down to limit value, which was maintained. The force-distance curves obtained in the shearing test showed a decrease of the force during storage until ripeness was reached.

INTRODUCTION

ONE OF THE BASIC characteristics of food quality is texture (Kramer and Szczesniak, 1973; Clark and Rao, 1977). The degree of softness contributes to the organoleptic characteristics of the fresh fruit, but it might also play a major role in the processing operations.

The subjective estimate of softness is based on measurements of the degree of deformation of the food (that depends on the food, Szczesniak and Bourne, 1969) under the influence of a compression force applied by the fingers. An objective method by a Universal Testing Machine was used by Bourne et al. (1966). Food deformation tests were made by different authors (Bourne, 1967; Shipman et al., 1972). Bourne (1965) and Watada et al. (1976) made puncture tests. Peleg (1974) and Peleg and Gomez Brito (1975) investigated some tropical fruits by penetration tests. The Kramer Shear Press was used by Binder and Rockland (1964), Bolin (1976), and Neumann et al. (1961). The total solids content of the release juice was determined in the punction tests (Abbott et al., 1968).

On the basis of the above reports, we have chosen to study the texture changes in the cherimoya, by compression, punction, and shearing tests using an Instron Instrument.

EXPERIMENTAL

CHERIMOYAS of the "Campas" and "Fino de Jete" varieties were harvested at the green-ripe stage of maturity and subsequently ripened under the following conditions: 20°C and 40% RH, ambient conditions; 5°C and 40% RH; 10°C and 85% RH. Samples of the fruit were taken every 2 or 3 days.

Weight losses were measured and analyses of regression between the storage days and the weight losses were evaluated. Standard errors (e) and correlation coefficients (c) were obtained. The total soluble solids content (TSS) was determined for each fruit in the liquid obtained from the punction test. The results are expressed in °Brix. Sensory panel tests were made with a score range from 1–5:

Flavor	Firmness
1. Very bad	1. Very hard
2. Bad	2. Hard
3. Acceptable	3. Slightly soft
4. Good	4. Soft
5. Very good	5. Very soft

The specific weight was determined.

Compression, puncture, and shearing tests were made by using an Instron Food Testing Instrument, Model 1140. Whole intact fruits were compressed by using a cylindrical, flat-surfaced anvil with a 57 mm diam running at a speed of 50 mm/min. The compression force used was that necessary to compress the fruit 3 mm. The compression force was recorded, with the chart running at a constant speed of 500 mm/min. The compression was measured at the equator position of the fruit as deformation caused by a given force (mm/kg). Force-distance curves were plotted directly by the recorder of the Instron Machine. Puncture tests were made on the whole intact fruits with a plunger of 6.4 mm diam. A maximum of three punctures were made in each fruit. All punctures were made at the equator, with the crosshead and the chart running at the same speed of 50 mm/min. Puncture force-distance curves are obtained. Shear tests were made by using a Kramer Shear Press. Determinations were made of the force required to push a series of metal plates through 50g of cherimoya flesh without skin. The shear press and the chart was operated at a speed of 50 mm/min. The standard shear-compression box was used as the test cell. Characteristic curves are plotted.

RESULTS & DISCUSSION

THE REGRESSION ANALYSIS was applied to the % of weight losses (y) during the days of storage (t), showing the following lineal regression curves, with their standard errors (e) and correlation coefficients (c), these coefficients being higher than 0.98.

Regression equations at 20°C and 40% RH:

$y = -1.30 + 1.88t$; $c = 0.98$; $e = 2.05$ (Fino de Jete variety)

$y = 0.90 + 1.82t$; $c = 1.00$; $e = 0.56$ (Campas variety)

Regression equations at 5°C and 40% RH:

$y = 2.03 + 0.80t$; $c = 0.99$; $e = 0.80$ (Fino de Jete variety)

$y = 1.53 + 0.66t$; $c = 0.99$; $e = 0.74$ (Campas variety)

Regression equations at 10°C and 85% RH:

$y = 0.88 + 0.45t$; $c = 0.99$; $e = 0.35$ (Fino de Jete variety)

$y = 0.78 + 0.51t$; $c = 0.99$; $e = 0.38$ (Campas variety)

From the slopes of the regression lines we can see easily the effect of temperature and relative humidity, according to the varieties investigated. The RH showed a stronger influence on weight losses than the temperature.

There was a correlation between the total soluble solids content and the flavor of the fruit. When the cherimoya was unripe, the value of °Brix was lower than 22 (taste

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panel score 2–3), but in the ripe fruit with a good flavor (score 4–5), the °Brix had values ranging from 22–28.

The specific weight increased with storage time until the fruit was ripe. If the fruit is unripe, the specific weight is lower than 1 g/cm^3.

Texture measurements

Deformation (mm/kg) increased with the temperature.

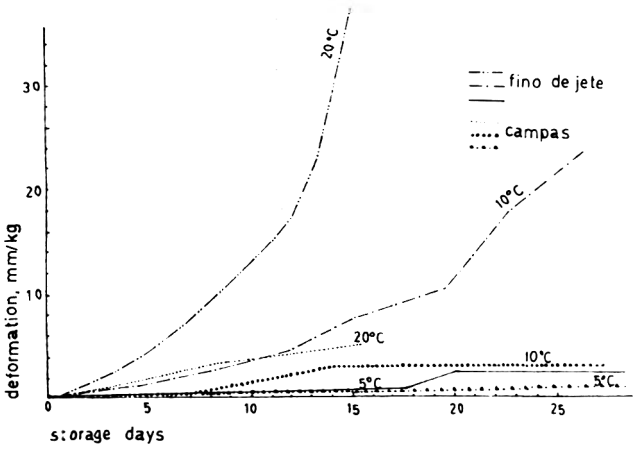

Fig. 1–Deformation-storage time in the two varieties of cherimoya.

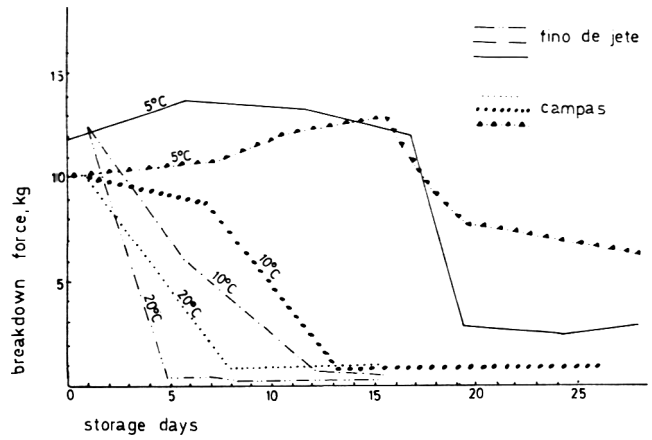

Fig. 2–Force required to breakdown the first skin (kg) on both varieties of cherimoya.

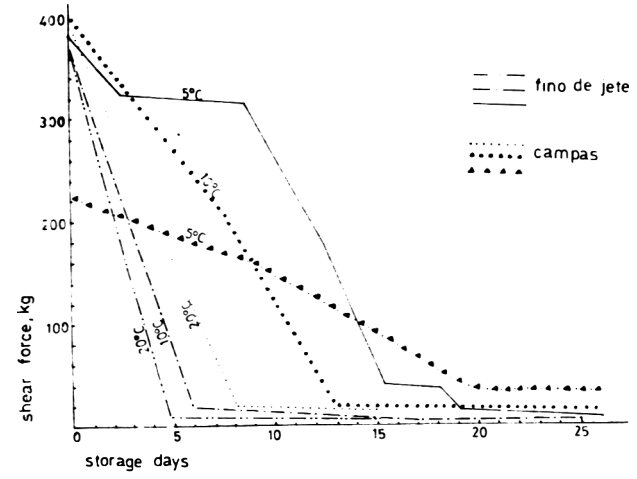

Fig. 3–Shear force-storage time in the two varieties of cherimoya.

At 5°C and 40% RH, deformation increased very little during storage, because the skin became hard. The cherimoya was kept under these conditions for more than 30 days. After this time the fruit (without skin) was ripe, with white flesh and good flavor, but the appearance was poor, i.e. dark and with a hard skin. At 5°C after 5 days, dark spots appeared on the skin and with the storage time the whole fruit became dark, probably due to the phenomenon of chilling injury (Pentzer, 1951), as the most subtropical fruits are susceptible to chilling injury at temperatures below 10°C. In Figure 1, the deformation-storage time in both varieties of cherimoya is plotted. The Campas variety had a very little deformation as compared with the Fino de Jete variety.

The puncture force required to break the skin (yield point) decreased at 10° and 20°C with the storage time down to a limit value and afterwards this value remained approximately constant. At 5°C the break force increased slightly (chilling injury) with the storage time and after 15 days began to decrease. The penetration force in the Fino de Jete variety is lower than in the Campas variety and the limit value of the force was reached faster at 20°C than at 10°C and 5°C (Fig. 2).

The shearing force decreased with the storage time until ripeness was reached. When the cherimoya was ripe, the Fino de Jete variety was softer than the Campas variety. The shearing force is a quality index of the fruit ripeness. Fino de Jete variety was ripe in 5 or 6 storage days at 10° and 20°C, but when stored at 5°C the ripeness was reached in 15 days. Campas variety ripened in 10 days when stored at 10° and 20°C and in 20 days when stored at 5°C (Fig. 3). There is a correlation between the shearing force and the firmness determined by a taste panel. When the fruit was ripe the score for firmness ranged from 3–4.

Influence of temperature at the same RH

At 5°C, Fino de Jete ripened in 15 days and Campas in 20 days, the limit value of shearing force was then reached, the breakdown force began to decrease, there was practically no change in deformation, the °Brix value was larger than 22, and the values from the sensory panel were: flavor, score 4–5, and firmness, score 3–4. At 20°C, Fino de Jete ripened in 5 days and Campas in 8 days, the limit value of shearing force was then reached. The force needed to break the skin decreased with the storage time, with a parallel increase in the cherimoya deformation until the fruit was ripe.

Influence of temperature at different RH

At 10°C and 85% RH, Fino de Jete ripened in 6 days and Campas in 13 days, the limit value of shearing force was then reached. The deformation was smaller than at 20°C and 40% RH (Fig. 1, 2, and 3).

CONCLUSIONS

CHERIMOYAS cannot be kept at 5°C, because they are susceptible in chilling injury and the skin became hard and dark.

The maturity and quality of cherimoya can be measured by the texture of the sample by using an Instron. The deformation increased with storage time and temperature. Campas variety was less deformed than Fino de Jete. In the puncture test the force required to break the skin decreased with storage time down to a limit value, which was maintained. The force-distance curves obtained in the shearing test showed a decrease of the force during storage until ripeness was reached.

The best storage conditons were 10°C and 85% RH. In this case the optimal time of storage was 15–21 days in Campas and 9–21 days in Fino de Jete. Fino de Jete vari-

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A Research Note

LOW VOLTAGE ELECTRICAL STIMULATION OF BEEF CARCASSES

D. G. TAYLOR and A. R. MARSHALL

ABSTRACT

Studies were carried out on the effect of low voltage stimulation of beef carcasses on the onset of rigor mortis and meat tenderness. One side of each of 14 beef carcasses was electrically stimulated using a step-wise increase in voltage to a peak of 32V direct current; the other side being used as the unstimulated control. Application of this low voltage treatment produced marked muscle contraction and carcass distortion. The rate of fall of muscle pH (indicating the onset of rigor mortis) was appreciably more rapid in the stimulated than the control sides. Meat samples from stimulated sides assessed by the use of objective and subjective methods were found to be significantly ($P < 0.01$) more tender than samples from unstimulated sides. The data suggest that low voltage stimulation (32V) of beef carcasses was associated with hastening of rigor mortis and improvement in tenderness comparable to that reported for carcasses subjected to voltages between 110V and 3600V.

INTRODUCTION

ELECTRICAL STIMULATION of the carcasses of sheep (Carse, 1973; Bendall, 1976; Chrystall and Hagyard, 1976) and cattle (Bendall et al., 1976; Davey et al., 1976; Gilbert and Davey, 1976; Shaw and Walker, 1977; Savell et al., 1977; Bouton et al., 1978; Chrystall and Devine, 1978) has been shown to increase the rate of glycolysis in the musculature and to hasten the onset of rigor mortis. The anticipated benefit of this procedure to the meat industry would be the reduction in the toughening effects of cold shortening and thaw rigor (Carse, 1973; Chrystall and Hagyard, 1976; Davey et al., 1976), and the consequent reduction in the post-slaughter chilling and processing time (Gilbert and Davey, 1976).

Most of the published work on this subject involved the application of high voltages (3600V: Chrystall and Hagyard, 1976; Gilbert and Davey, 1976, 1600V: Davey et al., 1976, 700V: Bendall et al., 1976 and 250V: Bendall, 1976); this being undesirable as pointed out by Shaw and Walker (1977) and Bouton et al. (1978) due to the possible dangers in commercial abattoir situations.

The present studies were carried out to investigate the effect of 32V direct current on rigor mortis development and the subsequent meat quality of beef carcasses.

EXPERIMENTAL

Animals and stimulation treatment

Fourteen beef carcasses from animals representing a range of breeds, ages, weights and body conditions were used. Immediately after slaughter, the carcasses were dressed and split using conventional abattoir procedures. One side of each carcass was electrically stimulated and the other side was left as the unstimulated control. The period from stunning of the animal to the time of stimulation of the carcass side was approximately 30 minutes.

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Stimulation was applied by using two multi-point electrodes as described by Shaw and Walker (1977). One electrode was inserted at the distal end of the junction of the biceps femoris muscle and semitendinosus muscle and the other into the brachiocephalicus muscle. The direct current supplied to these electrodes was from a transformer suitably constructed to deliver voltages from 0–32 volts, with peak amperage of 2 amps. Total stimulation time was 90 sec, with gradually increasing voltage applied in steps: 10 sec, 2.5V; 20 sec, 8V; 30 sec, 16V and 30 sec, 32V.

Both sides of each carcass were placed in a chiller at 1°C approximately 2 hr after stunning.

pH measurements

The pH at two specific muscle locations was taken at 1, 4 and 24 hr postmortem; the locations being mid-way from the proximal to the distal ends of the semitendinosus muscle, and the longissimus dorsi (l. dorsi) muscle over the thirteenth rib. The probe of the pH meter (Townson meat pH meter) was inserted 2 cm into the muscle at these locations through a slit made in the epimysium with a scalpel blade.

Tenderness determinations

Sample preparation for Warner-Bratzler shear force determinations involved excision of a 5 cm long section of l. dorsi muscle from the area above the first lumbar vertebra. This muscle section was trimmed of excess fascia and fat and cooked in sealed plastic bags in a water bath at 80°C for 90 min. Using a double bladed scalpel, cores with dimensions of 1.5 × 1.5 × 4 cm were cut with the core parallel to the long axis of the muscle fibers. Eight such cores were taken from each muscle sample and subjected to shear force determination using the Warner-Bratzler shear. The mean of these eight values was taken as being the shear value for the sample.

Taste panel evaluation was carried out using a strip of l. dorsi muscle similar to that used for shear force determination. The samples were deep fried to an internal temperature of 75°C before being trimmed and cut into 5 × 2 × 2 cm pieces for serving to the taste panel. The taste panel consisted of 9 males and 3 females aged 20–24 yr. The taste panel members were requested to subjectively rate the tenderness of the meat sample on a 0–10 point scale, with 10 being the extremely tender classification.

RESULTS & DISCUSSION

PRONOUNCED CHANGES in carcass posture were observed during electrical stimulation. Contraction of most of the visible carcass muscles occurred with the initial low voltage current (2.5V and 8V) and progressed to bending of the back and rigid extension of the fore and hind limbs at higher voltages (16V and 32V). With the step-wise increase in voltage, it was observed that muscle contraction occurred when the low voltage (2.5V) was applied, but after 5–8 sec, some loss of contractability was noted. When the voltage was raised to 8V, marked contraction again occurred but it began to subside within 10–15 sec. Following the last stage of stimulation, the carcass musculature showed relaxation as soon as the current was turned off and the carcass returned to a normal posture.

Similar observations on muscle contraction during stimulation using much higher voltages have been reported by Bendall (1976), Bendall et al. (1976) and Chrystall and Hagyard (1976). It has been observed that if high voltages are applied to a beef carcass side the massive muscle contraction produced may result in breaking the vertebrae and inter-vertebral joints in the mid-back region and causing tearing of muscles in adjacent areas. Therefore it would

Table 1—Mean pH values for longissimus dorsi and semitendinosus muscles taken at 1, 4 and 24 hr post-slaughter for electrically stimulated and unstimulated control beef sides

Muscle	Time (hr)	Stimulated Mean	Stimulated S.D.	Control Mean	Control S.D.	Difference
L. dorsi	1	6.34	0.17	6.80	0.21	0.46**
	4	5.89	0.19	6.37	0.23	0.48**
	24	5.55	0.12	5.63	0.15	0.08
Semitendinosus	1	6.45	0.20	6.76	0.26	0.31**
	4	5.95	0.17	6.40	0.22	0.45**
	24	5.59	0.09	5.63	0.12	0.04

** $P < 0.01$

Table 2—Mean Warner-Bratzler shear values and taste panel results for longissimus dorsi muscle samples from electrically stimulated and unstimulated control beef sides

	Stimulated Mean	Stimulated S.D.	Control Mean	Control S.D.	Difference
Shear value[a] (kg)	7.62	1.30	12.29	3.03	4.67**
Taste panel score[b]	5.84	0.91	3.70	1.46	2.14**

[a] For a 1.5 × 1.5 cm square end section core

[b] On a 10-point scale: 0 being very tough, 10 being very tender.

** $P < 0.01$

appear that a step-wise increase in voltage up to the peak voltage selected would be most desirable.

The means of the pH readings taken at 1, 4 and 24 hr postmortem on the l. dorsi muscle and the semitendinosus muscle are presented in Table 1. The rate of pH fall reported here using a peak voltage of 32V is comparable to that reported by other workers such as Davey et al. (1976) and Gilbert and Davey (1978) using 1600V, and Shaw and Walker (1977) and Bouton et al. (1978) using 110V. It is to be noted that although the rate of fall of pH was significantly more rapid in the stimulated sides than in the control sides, a similar ultimate, or near ultimate pH was achieved at 24 hr post-slaughter.

The results of objective and subjective tenderness assessment are presented in Table 2, and demonstrate the significant improvement in tenderness resulting from electrical stimulation. The mechanism by which this tenderness improvement is brought about is not clearly known but some reports have suggested that it may be due to a reduction in cold shortening (Chrystall and Hagyard, 1976; Davey et al., 1976; Gilbert and Davey, 1976; Bouton et al., 1978), increased activity of proteolytic enzymes (Savell et al., 1977) or physical disruption of the muscle fiber ultrastructure (Savell et al., 1978).

This study showed that application of very low direct current voltages (2.5–32V) to beef carcasses can produce a significantly more rapid onset of rigor mortis and a marked improvement in the tenderness of meat.

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CHERIMOYA TEXTURE VARIATION DURING STORAGE...From page 143

ety has better flavor than Campas variety as judged by a taste panel.

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A Research Note

SOME FLAVOR AND COLOR CHANGES DURING LOW TEMPERATURE DEHYDRATION OF GRAPES

M. GEE

ABSTRACT

Seedless grapes were dried at 45–50°C at air flow rates of 750 fpm to yield a raisin product that differs from sun-dried grapes in having a lighter color, sweeter flavor, and better sanitation. Development of color in the raisin product is strongly affected by the water activity of the grape tissue during drying. Raisin color can be controlled by adjusting drying time and water activity in and near the skin to affect the enzymatic browning rate. Carbohydrates are converted to free sugars during the drying process and storage. As raisin production is being shifted from solar to mechanical drying, these mechanisms become important.

INTRODUCTION

THE AVERAGE yearly production of raisins in California has been between 200–250 thousand tons. The majority of the grapes are dried on the ground using sunlight for energy. In 1976, the yield was 141,924 tons (Anon. 1978), and in 1978, the production will be less than average by 50% or more (Anon. 1979). These years 1976 and 1978, represent years of natural disaster. Early seasonal rainfall occurred while grapes were drying on the ground and initiated mold growth. Although consumer demand for raisins is high, if production is small and prices high, the consumer will substitute or do without raisins.

There has been a recent trend to dry grapes mechanically, but this conversion has been slow to develop. When raisins were reconditioned after mold damage in 1976, mechanical hot-air drying was necessary for a second drying to salvage the crop. The near loss of the 1978 crop will make mechanical drying even more attractive for raisin production.

This study is part of a continuing examination (Gee et al., 1977) of low temperature (45–50°C) drying of untreated fruit and vegetable pieces. The dehydration of seedless grapes to raisins by low temperature mechanical heat was carried out to better understand the role of water activity and drying time on enzymes influencing appearance, flavor, and quality of the mechanically dried raisins as compared with the sun-dried product.

MATERIALS & METHODS

Materials

Thompson seedless grapes were purchased in 1976 and 1977 and Flame seedless grapes in 1978 from local markets for this study. The grapes were dehydrated as whole and as cut grapes at 45–50°C to a_w 0.55 or less using air flow rates of 750 fpm as previously described (Gee et al., 1977). The grapes were cut so that an exposed cut surface was available for the ease of water loss. The cut grapes dried in 16 hr or less, while the whole grapes took 3–4 days under the conditions described. Samples were stored at room temperature (20°C) for up to a year in air in the absence of light.

Alcohol extractable color

A 7.5-g portion of a well-mixed, chopped sample was blended for 4 min with 100 ml of 50% ethanol. The slurry was filtered through filter paper and the absorbance of the filtrate read on a Bausch and Lomb Spectrophotometer (Spectronic 20) at 440 mμ after the method of Nury et al. (1960).

Table 1–50% Ethanol extractable color[a]

Sample	Absorbance at 440 mμ: Freshly prepared	Absorbance at 440 mμ: After 1 yr[b]
Fresh Thompson seedless grapes	0.10	
Whole, dried seedless raisins	0.20	0.24
Cut, dried seedless raisins	0.09	0.12
Fresh Flame seedless grapes	0.20	
Whole, dried seedless raisins	0.27	
Cut, dried seedless raisins	0.25	

[a] Samples corrected for water content to give readings at same dried weight.
[b] Samples stored in air, in the dark.

Moisture determination

Moisture was determined by 16 hr, 60°C, vacuum oven drying of a 10-g or larger sample chopped in a food processor.

Sugar determinations

Extracts for sugar determinations were prepared following AOAC (1975) methods. Total and reducing sugars were obtained by the method of Potter et al. (1968) and fructose by the method of Williams et al. (1960).

RESULTS & DISCUSSION

MECHANICALLY DRIED Thompson seedless grapes do not develop as dark a color as sun-dried grapes. This probably can be explained, in part, by the extent of enzymatic browning taking place during dehydration. Sun-dried grapes dry much slower than mechanically dried grapes. Therefore, the time of enzymatic browning is longer so more extensive brown color is developed. Whole grapes dried in the sun take 2 wk or more to reach a storage stable water activity (a_w = 0.55 or less). Cabinet-dried grapes take 3–6 days or less to reach the same a_w at 50°C. Both Thompson seedless and Flame seedless grapes were dried as described and the optical density of the alcohol extracts was recorded in Table 1. Whole dried Thompson seedless raisins were brown in color, but cut and dried grapes gave yellow or greenish-yellow raisins. The absorbance values observed were lower than those found by Nury et al. (1960) for sun-dried grapes and the cut and dried grapes compared with golden sulfured raisins. The cut grapes dried in less than 24 hr while the whole grapes took several days. Ivanov and Ivanova (1968) have reported that the enzymes responsible for browning in grapes are highly concentrated in the skin area. The cut grape pieces dried rapidly losing moisture through the cut surface, allowing the skin area to maintain a lower a_w during drying. Whole grapes could lose water only through the skin surface which maintained a high a_w in the skin area during drying. The rate of enzymatic browning is reduced as the level of a_w is lowered (Labuza et al., 1972). At the final a_w of 0.55 or less, 14–15% water, these light-colored raisin pieces remained color stable for 1 year's storage at 20°C. Mechanically dried cut raisins could compare with golden raisins in color and had a rich flavor reminiscent of a concentrated fresh Thompson seedless grape. The flavor of

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Table 2—Changes in sugar content during conversion of fresh grapes to raisins[a]

Sample	% Sucrose	% Glucose	% Fructose	% Total sugars
Thompson seedless, 1976				
Fresh	0.0	10.0	59.0	69.0
Whole, dried	0.0	5.9	76.3	82.2
Cut, dried	0.9	8.2	71.3	80.5
Thompson seedless, 1977				
Fresh	1.8	37.4	41.8	81.0
Whole, dried	0.0	34.2	38.3	72.5
Cut, dried	2.6	38.1	37.4	78.1
Above samples stored for 1 year				
Whole, dried	0.0	27.0	49.7	76.7
Cut, dried	0.0	28.9	52.9	81.8
Flame seedless, 1978				
Fresh	7.0	27.0	40.0	74.0
Whole, dried	1.2	30.2	47.3	78.7
Cut, dried	3.9	26.5	47.8	78.2

whole brown raisins was more similar to typical raisin flavor though the color was not as dark. Both dried products had a pleasant plump texture even at the low a_w. The Flame seedless grapes did not develop the dark raisin color on dehydration. The original tokay-pink skin color intensified to a mahogany color on dehydration, but the interior was very light in color. From the absorbance readings in Table 1, the fresh, whole-dried and cut-dried fruits were found not to be greatly different. This variety of grapes probably does not have either the necessary substrates or enzymes to develop extensive enzymatic browning. The flavor of the whole or cut Flame seedless raisin was rich in fruity overtones as compared to ordinary raisins.

The current practice of setting the harvest date of grapes for raisin production is based on the sugar content of the grapes (USDA, 1944). A sugar content of 20–25% in the fresh grapes is desired. In this study, the sugar content was followed from fresh fruit to dried product and after storage as reported in Table 2. The grapes used in this study were table grapes and some samples were not initially at the desired high sugar content for raisin production. However, changes in sugar content did occur during dehydration and storage of the 1976 and 1978 samples. Sugar levels increased. The final sugar content compares with the sugar levels from high sugar grapes. Sweetness in the raisins was enhanced by increased free sugar content as well as isomerization of glucose to fructose. This sugar conversion during mechanical dehydration could make it possible to permit harvest of grapes over a longer period of maturity at the farmer's convenience. Though these raisins are reasonably stable at 20°C storage, lower storage temperatures would give increased shelf-life (Barger et al., 1948).

As the California raisin production season continues to be plagued with crop destructive rainfall (Anon. 1979), understanding mechanisms of product conversion in mechanical dehydrators will make the phasing out of direct sun-ground drying more desirable. Low temperatures (45–50°C) are desired for quality dehydration and can be obtained from indirect solar or waste heat sources.

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Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

A Research Note

ELECTRICAL STIMULATION OF CALF CARCASSES: RESPONSE OF VARIOUS MUSCLES TO DIFFERENT WAVEFORMS

P. E. BOUTON, R. R. WESTE and F. D. SHAW

ABSTRACT

The effectiveness of electrical stimulation of calf carcasses by two different waveforms, one with a pulse frequency of 14.3 pulse sec^{-1}, the other with a pulse frequency of 40 pulse sec^{-1}, was compared by measuring muscle pH and Warner Bratzler (WB) shear values. Different muscles, in particular the semimembranosus (SM) and the longissimus (LD), responded differently to the two waveforms. For the LD, the 14.3 pulse sec^{-1} waveform was superior in that muscles from calf sides stimulated with this waveform had lower pH values and lower WB shear values than muscles from sides stimulated with the 40 pulse sec^{-1} waveform while for the SM the latter waveform was superior. These differences were confirmed by taste panel tests. The findings indicate that, when assessing the effectiveness of a particular stimulation system, it would be unwise to study only one muscle.

INTRODUCTION

WHEN BEEF SIDES or carcasses are electrically stimulated the magnitude of the applied voltage is the most important electrical parameter whether the success of stimulation is judged by the fall in pH or by the tenderness of muscles (Bendall et al., 1976; Bouton et al., 1979). Practical systems of stimulating carcasses have been developed using voltages as high as 1100 (Gilbert, 1978). However it has recently been shown that stimulation of beef sides with a maximum voltage of 45v, while not as effective as stimulation with higher voltages, can be of value in the prevention of cold shortening and thus of value in the production of tender meat (Bouton et al., 1979). The use of low voltages has many practical advantages with respect to electrical safety requirements; in Australia guarding of carcasses and devices for the protection of employees are not required if voltages do not exceed 45v. During the course of experiments to determine an optimal stimulation waveform using a maximum applied voltage of 45v, variations in muscle response to different waveforms were observed and the purpose of this research note is to record these variations.

MATERIALS & METHODS

Animals and stimulation treatments

Six 6-month old Hereford calves of carcass weight 28–38 kg were used. Stimulation commenced after the dressed carcass was split into sides (25–30 min after stunning) and was applied for 1½ min with the voltage being varied as follows: 0–10 sec 7v; 10–30 sec 14v; 30–60 sec 28v, and 60–90 sec 45v. The current was applied via two multipoint electrodes (Bouton et al., 1978), one inserted at the distal end of the junction of the biceps femoris muscle with the semitendinosus muscle and the other into the brachiocephalicus muscle. Alternate left and right sides were stimulated with either pulsed D.C. at 40 × 2 ms pulses sec^{-1} (Bouton et al., 1978) or pulsed A.C. at 14.3 × 10 ms half-sinusoid pulses sec^{-1} (Gilbert, 1978). The D.C. pulses were monodirectional; the A.C. pulses were of alternating polarity.

After stimulation the sides were transferred to a holding room (air temperature 10°C, air velocity 0.1m sec^{-1}) until 2 hr after stunning when they were then transferred to a chiller (air temperature 1°C, air velocity 0.5m sec^{-1}). During stimulation and chilling the sides were suspended by the Achilles tendon.

Measurement of pH

The pH of each of five muscles [m. semimembranosus (SM), m. biceps femoris (BF), m. vastus lateralis (VL), m. longissimus (LD) and m. triceps brachii (TB)] was measured at 1 hr after stunning. Measurements were made with a portable digital pH meter with a probe-type combined electrode. The probe electrode was inserted into a shallow cut made in the muscle. The pH values for individual muscles were taken as the mean of readings from three different sites.

Removal and treatment of muscles

After the sides had been in the chiller for 22 hr, seven muscles [m. gluteus medius (GM), m. psoas major (PM), SM, BF, VL, LD and TB] were removed. Samples of approximate weight 200g were taken from these muscles and were cooked for 90 min in polyethylene bags totally immersed in water maintained at 80°C (± 0.5°C). After cooking, the samples were cooled in cold running water for 30 min. Excess surface moisture was removed with an absorbent paper towel and the samples were rewrapped in polyethylene and stored at 0–1°C overnight. Warner Bratzler shear determinations were carried out on samples from all seven muscles using the technique described by Bouton et al., (1973).

Taste panel assessments

400-g samples were taken from the SM and LD muscles and cooked as described above. A duo-trio difference test (Amerine et al., 1965) was used to determine if panelists could distinguish between muscles from the two treatments. Panelists were presented with three cold samples of 10 mm cubes of cooked muscle, one being marked as control (14.3 pulse sec^{-1} and 40 pulse sec^{-1} samples were both used as controls, being partitioned equally, where possible, among the panelists at each session). One of the other two samples was identical to the control, the other being from the alternative pulse system. Panelists were asked to identify the odd sample of the pair and to specify which sample was tougher. At any one session only samples of the one muscle (SM or LD) were used.

Reference ranges

pH measurements and WB shear values were determined on several calf sides used in other experiments at this laboratory. These sides were not stimulated but in other respects (carcass weight and storage conditions) were similar to the stimulated sides used in the present experiment. Values from these unstimulated sides were used to provide a reference range (Table 1).

Statistical analysis

An analysis of variance was used to determine the significance of differences in pH and WB shear values due to the different pulse systems. The analysis included a partitioning of the interaction of treatment × muscles degrees of freedom to allow a specific comparison between the SM and LD muscles. Taste panel data were analysed using the one-tailed test described by Roessler et al. (1978).

RESULTS & DISCUSSION

WB SHEAR VALUES and 1-hr pH values for SM, LD and the seven muscles combined are listed in Table 1. A comparison with the reference range for unstimulated sides indicated that stimulation with either pulse system using a maximum stimulation voltage of 45v caused a marked decrease

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Table 1—1-hr pH values and Warner Bratzler shear values (WB) for muscles from stimulated and unstimulated calf sides (n = 6)

Muscle		Waveform (Pulse sec^{-1}) 14.3	40	Reference range
SM	pH	6.50	6.37	6.71– 6.77
	WB	11.95	7.55	14.81–17.56
LD	pH	6.18	6.41	6.64– 6.91
	WB	6.70	8.95	12.44–22.94
All muscles tested	pH	6.32	6.33	6.66– 6.75
	WB	9.28	7.62	10.63–13.83

Table 2—Results of duo-trio taste panel tests

Muscle	Taste panel[a] assessments Total	Correct	Tougher sample[b] 14.3 pulse sec^{-1} Waveform	40 pulse sec^{-1} Waveform
LD	91	54	14	40
SM	87	58	44	14

[a] Results for both muscles are statistically significant ($P < 0.05$).
[b] Results for both muscles are statistically significant ($P < 0.01$).

both in 1-hr pH values and in muscle toughness assessed by the Warner Bratzler shear device.

When the interaction of muscles with treatment was partitioned, there was a statistically significant effect ($P < 0.05$) for the comparison between SM and LD muscles. For the SM the 40 pulse sec^{-1} system was superior (lower 1-hr pH values and lower shear values) while for the LD muscles the 14.3 pulse sec^{-1} system was superior. The other five muscles contributed little to the muscle × treatment interaction.

Taste panel results for the SM and LD muscles are presented in Table 2. These results agree with the 1-hr pH values and the WB shear values i.e. for the LD muscle the 14.3 pulse sec^{-1} waveform was superior while for the SM muscle the 40 pulse sec^{-1} waveform was superior.

A comparison with the reference sides indicated that both stimulation treatments had a marked effect on the SM and LD muscles and this indicates that these muscles have a different degree of response to the different waveforms rather than a lack of response to one of the waveforms. The waveforms differed in pulse frequency, duration, shape and polarity, and it is not possible to state which of these variables was responsible for the different responses in the various muscles. In experimental studies using one muscle (bovine sternomandibularis), Chrystall and Devine (1978) showed that pulse frequency has a considerable effect on the magnitude of the pH fall that occurs during stimulation but that variations in pulse shape and polarity caused minor changes only. The greatest falls in pH were achieved with pulse frequencies between 5.0 and 16.6 pulses sec^{-1}. If the differences in the present experiment are due to differences in pulse frequency alone then this would indicate that, for some muscles, a far wider range of pulse frequencies than that suggested by Chrystall and Devine could be used with equal effectiveness.

There are at least two possible explanations for the variation in muscle response to different stimulation waveforms. Muscles may respond differently per se to different waveforms or different waveforms may be transmitted with varying degrees of effectiveness along nerve-muscle pathways so that the effective stimulus received by a particular muscle may vary.

Our results emphasize the difficulties in endeavoring to find an optimal stimulation system. If muscles differ in their response to a particular waveform then misleading conclusions may be drawn when the response to stimulation is assessed on the basis of studies involving one muscle only. The LD muscle is commonly used in stimulation studies. In the present experiment there was clear evidence that for this muscle the 14.3 pulse sec^{-1} system was superior to the 40 pulse sec^{-1} system. However, for the SM muscle the latter pulse system was superior to the former.

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A Research Note

CORRELATION OF NONDESTRUCTIVE MEASUREMENTS AND SOLUBLE SOLIDS FOR ROME BEAUTY AND YORK IMPERIAL APPLES

G. M. SAPERS, J. G. PHILLIPS, and O. PANASIUK

ABSTRACT

Relationships between nondestructive measurements and other indices of fruit composition or condition in Rome Beauty, York Imperial, Red Spur Delicious, and Golden Delicious apples were studied. Fruits were subjected to different storage and ripening treatments before being analyzed individually by various nondestructive and destructive procedures. Of the procedures tested, potentially useful correlations were obtained between nondestructive spectrophotometric measurements and juice soluble solids with Rome Beauty and York Imperial apples.

INTRODUCTION

A NUMBER of objective indices have been proposed for the measurement of apple maturity or ripeness (Anon, 1965). Such measurements, especially those performed nondestructively, might be used to grade or sort apples for fresh market or processing.

Relationships between nondestructive measurements and other indices of apple condition or composition must be established if nondestructive measurements are to be used as predictors of the state of the fruit. Previously, we reported correlations between values of the stiffness coefficient (or firmness index), determined by nondestructive measurement of sonic resonance frequency, and levels of volatile components, acidity, and firmness in McIntosh apples (Sapers et al., 1977). Finney et al. (1978) obtained correlations between the firmness index, Magness-Taylor firmness, and sensory ratings of textural attributes for five apple cultivars. At this time, we report correlations between juice soluble solids and various nondestructive measurements obtained with Rome Beauty and York Imperial apples.

MATERIALS & METHODS

Materials and experimental design

Samples of Rome Beauty, York Imperial, Red Spur Delicious, and Golden Delicious apples were provided by the USDA's Beltsville (Md.) Agricultural Research Center from fruits obtained from a commercial Pennsylvania orchard. The apples were harvested at weekly intervals over a 3–4 wk period in 1975, bracketing the time of normal harvest, and stored in Beltsville at 0°C and 80–90% RH. Random samples of 20 apples per harvest were removed from storage after 1–2 months and again after 5–6 months; half of each sample taken from storage was held at 18–20°C for 1 wk to permit ripening. Apples were coded so that analytical data for individual fruits could be collated.

Analytical measurements

Nondestructive measurements were made at the Beltsville laboratory on ripened and unripened apple samples. Visible absorption spectra (absolute optical density values taken at 10 nm intervals between 640 and 880 nm) were obtained with a computer-assisted high intensity spectrophotometer which was capable of measuring light transmitted through intact fruits (Massie and Norris, 1975). Differences in absorbance between 700 and 740 nm (ΔA 700/740), a potential index of maturity based on the internal chlorophyl content of apples (Aulenbach et al., 1972), were used to select six apples from each sample to provide a range of maturities. Differences in absorbance for a second wavelength pair representing a maximum (680 nm) and a minimum (720 nm) were also calculated from the spectra and expressed as ΔA 680/720. In addition, the selected apples were analyzed by the procedure of Finney (1972) for sonic resonance frequency, and values of the firmness index f^2m (dynes/cm) were calculated.

After the completion of the nondestructive measurements at Beltsville, the coded apple samples were shipped to our Center where they were analyzed individually by destructive procedures described previously (Sapers et al., 1977), thereby permitting the direct comparison of nondestructive and destructive data obtained for each apple. Apple firmness (in pounds) was measured with a Magness-Taylor pressure tester. The juice of individual apples was extracted with an Acme Supreme Juicerator Model 6001 (Acme Juicer Manufacturing Co., Lemoyne, Pa.) and analyzed for soluble solids with a refractometer and for titratable acidity (as % malic acid).

Statistical analyses

Relationships between objective measurements were examined by correlation analysis, performed on the entire data set for each cultivar as well as on subsets representing different ripeness and storage combinations since these treatments were expected to influence fruit condition. Tests of significance were based on at least 16 degrees of freedom.

RESULTS & DISCUSSION

DURING RIPENING and storage, the apple samples changed in firmness and juice composition, producing a wide range of analytical values for the individual fruits (Table 1). Correlations between physical measurements performed on individual apples and the composition of the juice were examined for relationships which might be of potential value in predicting fruit maturity, ripeness, or quality.

Significant and potentially useful correlations were found between nondestructive spectrophotometric measurements, performed on intact fruit, and the soluble solids content of juice from Rome Beauty and York Imperial apples (Table 2). Correlation coefficients were similar for data acquired at the two wavelength pairs (680/720 and 700/740 nm). Firmness index values for these apples also correlated with juice soluble solids, although correlation coefficients were lower. Correlations were usually higher with ripened than with unripened fruits. Red Spur Delicious and Golden Delicious apples generally did not yield significant correlations (at the 5% level) between these measurements. Correlations between other nondestructive and destructive measurements were too low or limited in applicability to be of any practical value.

The relationship between juice soluble solids and nondestructive spectrophotometric measurements can be described by regression equations such as Eq (1) and (2) for ripened Rome Beauty and York Imperial apples, respectively, both analyzed at 680 and 720 nm.

$$\text{Soluble solids} = 14.6 - 0.91\ \Delta\ A\ 680/720 \quad (1)$$

$$\text{Soluble solids} = 12.9 - 0.44\ \Delta\ A\ 680/720 \quad (2)$$

Each of the Rome Beauty subsets in this study yielded

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Table 1—Analytical measurements for Rome Beauty and York Imperial apple samples[a]

Measurement	Rome Beauty			York Imperial		
	Mean	Std. dev.	Range	Mean	Std. dev.	Range
Destructive						
Magness-Taylor (kg)	4.6	0.4	3.1 – 6.0	6.9	1.0	4.6 – 9.3
Acidity (% malic)	0.38	0.09	0.18 – 0.64	0.38	0.09	0.20 – 0.58
Soluble solids (%)	12.1	1.1	8.9 – 14.4	12.2	0.7	10.6 – 14.4
Nondestructive						
ΔA 700/740[b]	0.18	0.35	(–0.38) – 0.87	0.02	0.25	(– 0.30) – 0.72
ΔA 680/720[b]	2.79	1.02	0.89 – 4.16	1.98	1.00	0.62 – 4.03
Firmness index f^2m (dynes/cm X 10^{-6})	148	12	130 –185	176	15	142 –219
No fruits		94			72	

[a] For all storage and ripening treatments
[b] Absorbance difference between 700 and 740 or 680 and 720 nm

Table 2—Correlation between nondestructive measurements and juice soluble solids

Cultivar	Nondestructive measurement	Correlation coefficient: Stored 1–2 months, Ripened[a]	Stored 1–2 months, Not ripened	Stored 5–6 months, Ripened[a]	Stored 5–6 months, Not ripened
Rome Beauty	ΔA 700/740	–0.91[b]	–0.78[b]	–0.84[b]	–0.82[b]
	ΔA 680/720	–0.91[b]	–0.73[b]	–0.86[b]	–0.83[b]
	f^2m	0.64[b]	0.44[c]	0.45[c]	0.42[c]
York Imperial	ΔA 700/740	–0.79[b]	–0.40	–0.77[b]	–0.61[b]
	ΔA 680/720	–0.77[b]	–0.38	–0.78[b]	–0.65[b]
	f^2m	0.66[b]	0.57[c]	0.72[b]	0.62[b]

[a] 1 wk at 18–20°C
[b] Significant at 1% level
[c] Significant at 5% level

similar regression coefficients; slopes differed, however, for York Imperial subsets. Relationships such as Eq (1) might be made the basis of automated procedures for grading, sorting or blending Rome Beauty apples for juice production. Practical applications of this kind, however, must be preceded by further research to establish the validity of the method for widely differing raw material sources, growing conditions, and storage conditions.

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Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

A Research Note

RATES OF ASCORBIC ACID DEGRADATION DURING THERMAL PROCESSING OF CANNED PEAS

P. J. LATHROP and H. K. LEUNG

ABSTRACT

Thermal degradation rates of ascorbic acid in peas during a simulated retort operation were investigated at various temperatures. Fresh peas were blanched and vacuum sealed in thermal death time cans with brine. The canned peas were processed at 110–132°C for different time intervals. The ascorbic acid degradation reaction was found to be first order with an activation energy of 41 kcal/mole.

INTRODUCTION

THE NUTRITIONAL QUALITY of processed foods has recently been the subject of a great deal of controversy among consumers and processors alike. A substantial amount of work has been done on the effects of thermal processing on nutrients (Bender, 1966; Lund, 1975). However, there exists a lack of kinetic data necessary to describe the time/temperature effects on nutrients in food systems so that processes can be designed with optimizing nutrient retention as a consideration (Lund, 1977).

The presence of ascorbic acid in processed food is considered to be associated with quality due to its relative instability to heat, oxygen, and light (Birch et al., 1974). Several kinetic studies have been conducted on the degradation of ascorbic acid in buffer solutions (Huelin, 1953; Finholt et al., 1963; Blaug and Hajratwala, 1972). However, virtually no kinetic data are available for the thermal destruction of ascorbic acid in food systems.

Recently, Kirk and co-workers conducted some extensive studies of ascorbic acid degradation during storage in different food systems (Singh et al., 1976; Lee et al., 1977; Kirk et al., 1977; Dennison and Kirk, 1978). However, the kinetic data obtained from these storage studies at 7.2–37°C may not be applicable to canning due to the large difference in temperature ranges. Therefore, it is worthwhile to investigate the kinetics of ascorbic acid loss in canned food systems over a temperature range used in commercial processing. Canned peas were chosen for this study because they contain a substantial amount of ascorbic acid (40% U.S. R.D.A. per 8-oz serving). The objective of this study was to determine the reaction rate constants and the activation energy for ascorbic acid degradation in canned peas during thermal processing.

MATERIALS AND METHODS

FRESHLY HARVESTED PEAS were collected from the receiving area of a local pea processing plant and cooled in dry ice until blanching could be accomplished. The peas were blanched for 35 sec in saturated steam with periodic agitation. They were vacuum sealed in thermal death time (TDT) cans (208 × 006) with 4 ml of hot (88°C) brine containing 3.4% sugar and 1.7% salt. The canned peas were kept refrigerated until processing was accomplished.

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The peas were processed at temperatures of 110.0, 115.5, 121.1, 126.7, and 132.2°C in an oil bath equipped with a HAAKE model Type F 4391 thermoregulator. The internal temperatures of the control can samples (blanks) were monitored using copper-constantan thermocouples (Bee and Park, 1978) together with an Esterline Angus Model D-2020 Digital Aquisition System. The time for each process was started when the processing temperature was reached within the blanks. The blanks were used to correct for any ascorbic acid loss incurred during the preheating period. Duplicate can samples were taken at various periods throughout each processing run. After heating, the cans were immediately cooled in ice water and stored at −40°C until ascorbic acid analysis.

The total ascorbic acid concentration of the peas and brine was assayed with the microfluorometric method (AOAC, 1975) using a Turner Model 111 Fluorometer. The dissolved oxygen content of the brine was determined using a Transidyne General Chemical Microsensor. The pH of the system was at its natural value of 5.9.

RESULTS AND DISCUSSION

THE THERMAL INACTIVATION curves of ascorbic acid at temperatures between 110–132°C are shown in Figure 1. Some of the curves did not intercept at A/Ao of 1.0 but do fit the linear regression. The correlation coefficients of these plots ranged from −0.96 to −1.00. Linearity of these curves indicated that the reaction was first order. Previous studies on aerobic or anaerobic degradation of ascorbic acid in buffer solutions (Huelin, 1953; Finholt et al., 1963; Blaug and Hajratwala, 1972), infant formula (Singh et al., 1976), tomato juice (Lee et al., 1977) and dehydrated model food system (Dennison and Kirk, 1978) also confirmed that the reactions followed first order kinetics.

An attempt was made to determine the concentration of dissolved oxygen in the brine in an effort to correlate ascor-

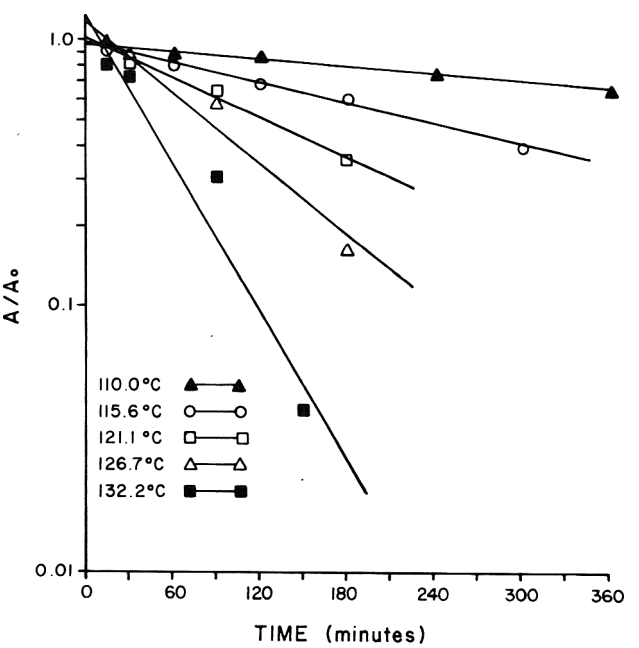

Fig. 1—Rates of ascorbic acid degradation in canned peas.

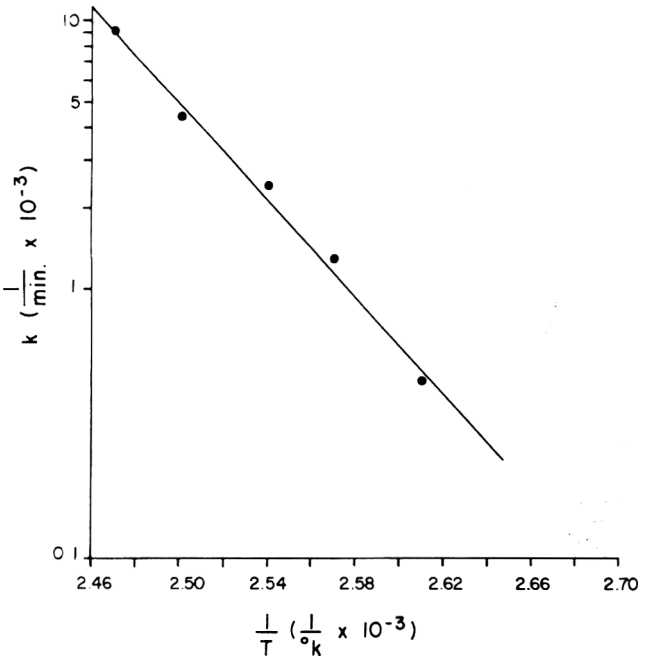

Fig. 2—Arrhenius plot for ascorbic acid degradation in canned peas.

bic acid degradation with oxygen levels inside the can. The dissolved oxygen concentration in the samples processed at temperatures between 110–121°C varied between 0–0.014 mmoles/L. However, for those samples processed at 126.7 and 132.2°C, the apparent oxygen concentration was much higher (0.023–0.115 mmoles/L) and increased with heating time. This was probably caused by interfering substances leached from the peas. Since the canned peas contained a limited amount of oxygen, both aerobic and anaerobic degradation of ascorbic acid may occur during heating.

The logarithm of the first order reaction rate was plotted as a function of the reciprocal of absolute temperature in Figure 2. The reaction rate at 110°C was 0.5×10^{-3} min^{-1}. This value is in close agreement with the reaction rate of 0.2×10^{-3} min^{-1} for the anaerobic decomposition of ascorbic acid at 100°C in citrate-phosphate buffer of pH 6.0 (Huelin, 1953). The reaction rate of aerobic oxidation of ascorbic acid in acetate buffer (pH 5.5) at 85°C was 0.9×10^{-3} min^{-1} (Blaug and Hajratwala, 1973).

The Arrhenius activation energy (Ea) for ascorbic acid degradation in canned peas at 110–132°C was calculated to be 41 kcal/mole. This value is comparable to the Ea for other vitamins in similar temperature range. The reported Ea values for thiamin and panthothenic acid degradation in pea puree were 27.5 kcal/mole (Mulley et al., 1975) and 36 kcal/mole (Hamm and Lund, 1978), respectively. The higher Ea obtained in this study indicates that the ascorbic acid degradation is more temperature dependent than the degradation of thiamin and panthothenic acid in peas.

The Ea value of 41 kcal/mole obtained in this study is considerably higher than those for ascorbic acid in buffer solutions. For aerobic oxidation, the Ea was 18 kcal/mole at pH 5.6 in the temperature range 60–85°C (Blaug and Hajratwala, 1972). For anaerobic decomposition, the Ea was 22.4 kcal/mole at pH 6.0 in the temperature range 30–100°C. This Ea was calculated by the authors based on the results of Huelin (1953). The comparison of Ea values for canned peas and buffer solutions at similar pH demonstrates that the kinetic data obtained from model buffer systems may not be applicable to food systems.

The Ea value for ascorbic acid in canned peas during thermal processing is quite different from those in other food systems during storage. The Ea was found to be 18.3 kcal/mole for ascorbic acid degradation in a dehydrated model food system at water activity of 0.65 (Dennison and Kirk, 1978). Lee et al. (1977) investigated the anaerobic ascorbic acid degradation in canned tomato juice during storage and found the Ea to be 3.3 kcal/mole. One reason for the differences in Ea values is the temperature range over which these values were determined. The storage studies of the dehydrated food system and tomato juice were conducted at 10–37°C, much lower than the temperature used in this study. Therefore, different reaction mechanisms for ascorbic acid degradation may be involved. Another factor to be considered is the different environmental conditions such as oxygen, moisture, pH and composition of the food products. The marked differences of Ea as discussed above indicate that kinetic studies should be conducted for different food systems.

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A Research Note

CONTROL OF pH IN GENERATING CHLORINE DIOXIDE FOR BACTERICIDAL USE IN POULTRY PROCESSING WATER

H. S. LILLARD

ABSTRACT

This study showed that there is no significant difference in total aerobic counts, fecal coliform levels, and salmonellae incidence of chiller water or broiler carcasses when pH of the chlorine dioxide generating system is controlled with either hydrochloric acid or chlorine gas. Also, there is no difference in mean number of days of shelf-life of carcasses processed in water treated by either method.

INTRODUCTION

The bactericidal use of chlorine (Cl_2) to reduce microbial contamination and increase shelf-life of poultry carcasses has been extensively investigated (Mead and Thomas, 1973; Blood and Jarvis, 1974; Nillson and Regner, 1963; Ranken et al., 1965; Patterson, 1968; May, 1974). However, the use of chlorine dioxide (ClO_2) as a bactericide in poultry processing has received limited attention. Baran et al. (1973) used a mixture of ClO_2 and Cl_2 in a plant with an in-plant chlorination system. Microbial levels were reduced but simultaneous use of two bactericides made the results hard to interpret. Dougherty (1974) reported that stabilized ClO_2 was ineffective as a bactericide in poultry processing. Lillard (1979a, b), however, reported that ClO_2, generated on site with Olin Water Services' system, was an effective bactericide in poultry processing. When ClO_2 was added to bird chiller water at about 1/7 the concentration of Cl_2 gas, the shelf-life of broiler carcasses was extended significantly. White (1972) listed the probable reasons for the effectiveness of ClO_2 at low levels: ClO_2 is five times as soluble as Cl_2 in water, has over two times the oxidizing capacity of hypochlorous acid, does not combine with ammonia or nitrogenous compounds, including the simple amino acids, and its action is not impaired at a high pH. However, the efficient generation of ClO_2 is pH dependent.

ClO_2 is generated on site by reacting a precursor, sodium chlorite, with Cl_2. The generation of ClO_2 is optimum between pH 3 and 3.5. The pH of the reaction mixture can be adjusted with Food Grade hydrochloric acid or chlorine gas. Because chlorine gas is easier to handle than hydrochloric acid, I wanted to determine whether the bactericidal effect of the ClO_2 generated differed between reaction mixtures in which pH was adjusted with either hydrochloric acid or chlorine gas.

MATERIALS & METHODS

PRELIMINARY WORK showed that 5 ppm ClO_2 in chiller water (based on total volume for an 8-hr shift) resulted in a free residual of 0.5–1 ppm with a concomitant reduction of fecal coliforms and salmonellae to nondetectable levels (Lillard, 1978). Therefore this level (5 ppm ClO_2) was selected to test the bactericidal effect of ClO_2 generated by reaction mixtures in which pH was controlled with acid or chlorine gas. The effectiveness of each treatment was judged by the microbiological quality of the chiller water treated with the ClO_2, microbiological quality of carcasses processed in this water, and shelf-life of the broiler carcasses.

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Bactericidal treatment

Chlorine dioxide was generated on site by reacting a solution of sodium chlorite precursor (Olin 4107) with chlorine, in an Olin Water Services system. Optimum pH (3–3.5) was maintained with Food Grade hydrochloric acid (J.T. Baker Chemical Co.), in one experiment and by the addition of chlorine gas to the reaction mixture in another experiment. For safety, ClO_2 was introduced into the bottom of the chill tanks, close to the circulating pumps, after the chillers were filled with potable water as described by Lillard (1979a). ClO_2 was measured by published methods (Lillard, 1979a).

Sampling

Water. From the midway point of the chiller, two water samples (500 ml) were collected in the morning, 2 hr after start-up, and in the afternoon, 7 hr after start-up. An appropriate amount of sodium thiosulfate (105 mg) was added to each container in which treated water was collected.

Carcasses. Two carcasses were taken from the exit end of the chiller at each sampling time (2 and 7 hr after start-up) for microbiological analyses, and four carcasses were taken 7 hr after start-up for studies of shelf-life.

Microbiological analyses and shelf-life

Methods for microbiological analyses and shelf-life determinations were described by Lillard (1977; 1978).

Statistical analyses

Data for total aerobes, fecal coliform levels, and shelf-life were treated by standard analysis of variance. The Chi Square test was applied to data on salmonellae incidence. Significance is reported at the 5% level.

RESULTS & DISCUSSION

TOTAL AEROBIC COUNTS, levels of fecal coliforms, and incidence of salmonellae did not differ significantly between samples of chiller water treated with ClO_2 generated by the two reaction mixtures (Table 1). Carcasses from chiller water treated with ClO_2 acidified by either method also were of equal microbiological quality: there is no significant difference in total aerobic counts, levels of fecal coliforms, incidence of salmonellae, or mean days of shelf-life. These data showed that the pH of the reaction mixture for ClO_2 generation can be controlled with either chlorine or hydrochloric acid without significantly affecting the bactericidal action of ClO_2 on quality of either the processing water or the broiler carcasses.

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A Research Note

INTESTINAL RESPONSE TO FEEDING HEAT DAMAGED CASEIN IN RATS

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ABSTRACT

A previous study demonstrated that feeding rats a heat damaged casein diet caused a decrease in body weight and in the pancreatic content of chymotrypsin and amylase. However, the present study shows that leucine aminopeptidase (LAP) activity in the small intestinal mucosa is not affected by this dietary stress, indicating that the mucosa still has amino acids available for enzyme synthesis. The LAP activity of the mucosa did increase in response to feeding a meal probably due to an increase in the rate of synthesis.

INTRODUCTION

A PROTEIN that has been severely damaged by heat is less digestible and feeding it lowers the pancreatic level of chymotrypsin and amylase (Percival and Schneeman, 1979). The major factor causing this adaptation appears to be decreased availability of amino acids from the heated protein. The small intestine is also known to adapt to diet composition (Nasset, 1964) and reports show that mucosal peptide hydrolases are affected by the protein content of the diet (Nicholsen et al., 1974; McCarthy et al., 1977; Saito and Suda, 1975). Although adaptation of the small intestine to various levels of dietary protein has been investigated, the effect of heat damaged protein on mucosal peptide hydrolases has not been determined. The lower availability of amino acids or the elevated levels of peptides in the lumen from a heat damaged protein are two factors which could affect peptidase activity in the gut. This study was undertaken to determine the effect of a heat damaged protein on the mucosal enzyme leucine aminopeptidase (LAP).

METHODS & MATERIALS

HEAT DAMAGED CASEIN was prepared by autoclaving at 121°C, 2 atm for 24 hr. Previous studies from this laboratory confirmed the effect of this heat treatment on reducing protein digestibility (Percival and Schneeman, 1979).

Twenty Wistar rats weighing 200g each were housed individually in stainless steel wire-bottomed cages with distilled water provided ad libitum. Animals were fed the heated casein (HC) or casein (C) diets for 10 days. The diet contains 24% protein, 60% dextrin, 8% corn oil, 6% salt mix, 2% vitamin mix, and 0.2% BHT, details of the diet mixture and experimental protocol are published elsewhere (Percival and Schneeman, 1979). Five animals from each group were killed after a 12-hr fast. The remaining animals were offered 3g of their respective diets, allowed to eat for 3 hr at which time the food cup was removed and the animals killed 30 min later. Both groups consumed about 2.1g of this meal.

The whole intestine was removed and flushed with 5.0 ml of cold 0.9% NaCl. The intestine was slit lengthwise, and the mucosa collected by gently scraping with a glass slide. Samples were frozen on dry ice and stored at −60°C. After thawing, the mucosa was weighed and prepared for peptidase assay by a modification of the method of Saito and Suda (1975). The total mucosa was homogenized in 0.01M Tris at 0°C with a Teflon mortar and pestle. Aliquots were taken for determination of dry weight and protein content (Dorsey et al., 1977). The homogenate was incubated with a papain solution (0.2% in 0.1M $NaHPO_4$, pH 7.0, containing 0.1% L-cysteine) and detergent (Triton X-100) to liberate the peptidases from the brush border. After 30 min of incubation at 37°C, the reaction was stopped by rapid cooling. The homogenate was centrifuged at 100,000 × G, the supernatant decanted, and a 1:10 dilution made for leucine aminopeptidase determination.

Leucine aminopeptidase activity was determined according to Sigma Technical Bull. No. 251, using L-leucyl-β-naphthylamide as the substrate. Results are expressed as micrograms β-naphthylamine formed per hour.

Statistical evaluation of the data was by a two way analysis of variance comparing the effects of C vs HC and feeding vs fasting (Nie et al., 1975).

RESULTS & DISCUSSION

THE RESULTS of this study are shown in Table 1. Although amino acids are less available from the heated casein diet, the small intestinal mucosa was able to maintain normal weight, protein, and LAP activity (Table 1, unfed animals). The pancreas of rats fed this heated casein diet for the same length of time had lower levels of chymotrypsin and amylase per 100g body weight. Additionally, rats fed the heated casein diet were not able to maintain normal body weight (Percival and Schneeman, 1979). The lack of alteration in the intestinal mucosa when both body weight and pancreatic enzyme activity had been affected by the poor amino acid availability of this diet illustrates the advantageous position of the mucosa during a dietary stress. Since it is the first organ to receive amino acids from the diet, it can retain what is needed for synthesis and consequently functions normally for a longer period of time (Fauconneau and Michel, 1970). With a prolonged fast, the pancreas has been shown to lose protein at a more rapid rate than the small intestine, and upon realimentation, the protein is restored at a slower rate than the intestine. Additionally, during protein depletion the mucosal cells may be able to maintain their normal composition by using amino acids from endogenous protein (Fauconneau and Michel, 1970). The present study demonstrates that feeding a poorly digested protein will have a more pronounced effect on pancreatic function than on intestinal function.

Feeding per se increased the dry weight of the mucosa and decreased the protein per milligram mucosa; both effects are probably due to the diet components present in the intestine, not an actual change in composition.

LAP activity was higher in the mucosa of fed vs fasted animals (Table 1). Feeding has been shown to increase the rate of cell synthesis (Dawson et al., 1964; McManus and Isselbacher, 1970) and presumably enzyme synthesis would also occur at this time. At the time rats were killed in our experiment (3 hr after feeding), cell synthesis and probably enzyme synthesis would be proceeding rapidly (Dawson et al., 1964). During the 12-hr fast, the rate of cell proliferation would have slowed down (McManus and Isselbacher, 1970), consequently, sloughed cells would not have been readily replaced. This is another factor which could have led to lower (LAP) activity in the mucosa of fasted animals.

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Table 1—Mucosal weight, protein content, and leucine aminopeptidase activity (LAP) in fed and fasted rats fed casein (C) or heated casein (HC) diets

		Unfed	Fed	Significant main effects[b]
Mucosal wet wt (g)	HC	2.73 ± 0.08[a]	2.88 ± 0.17	
	C	3.05 ± 0.10	2.89 ± 0.18	
Mucosal dry wt (g)	HC	0.41 ± 0.01	0.50 ± 0.03	
	C	0.46 ± 0.02	0.51 ± 0.04	Feeding
g protein/g dry wt	HC	0.69 ± 0.06	0.29 ± 0.02	
	C	0.62 ± 0.04	0.42 ± 0.04	Feeding, FxD[c]
LAP units[d] 4/mg dry wt	HC	7.24 ± 0.74	12.24 ± 1.28	
	C	6.99 ± 0.47	13.51 ± 0.31	Feeding
Total mucosal LAP units[c]	HC	2979 ± 278	5983 ± 476	
	C	3251 ± 340	7096 ± 1149	Feeding

[a] Mean ± SEM
[b] Significant F value ($P < 0.05$)
[c] FxD = Feeding by diet interaction based on ANOVA
[d] LAP units = mg β-naphthylamine/hour

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pH FOR ClO_2 GENERATION. . .From page 154

Table 1—Microbiological evaluation of water and carcass treated with 5 ppm ClO_2 generated from reaction mixtures acidified with hydrochloric acid or chlorine gas[a]

Sample type and mode of pH control of ClO_2 reaction mixture	Mean $\log_{10}$/ml: Total aerobic count a.m.[b]	Total aerobic count p.m.[b]	Fecal coliforms a.m.[b]	Fecal coliforms p.m.[b]	Salmonellae incidence a.m.[b]	Salmonellae incidence p.m.[b]	Mean No. Days Shelf-life
Chiller water - 5 ppm ClO_2 + acid	2.49k	2.89k	0.64m	0.77m	2/24h	0/24h	N.A.
Chiller water - 5 ppm ClO_2 + Cl_2	2.86k	3.00k	0.66m	0.53m	0/24h	0/24h	N.A.
Carcass - 5 ppm ClO_2 + acid	3.49e	3.85e	1.88f	1.69f	0/24g	0/24g	27.15j[c]
Carcass - 5 ppm ClO_2 + Cl_2	3.66e	3.91e	1.97f	1.70f	0/24g	1/24g	28.58j[d]

[a] The same lower case letter in rows and columns follows values which are not significantly different at the 5% level.
[b] Based on 24 samples taken 2 on each of 12 sampling days.
[c] Based on 52 carcasses taken 4 on each of 13 sampling days.
[d] Based on 48 carcasses taken 4 on each of 12 sampling days.

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