ISSN 0022-1163

Volume 10 Number 5 October 1975

Journal of Food Technology

Published for the Institute of Food Science and Technology (U.K.) by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

JOURNAL OF FOOD TECHNOLOGY

Institute of Food Science and Technology (U.K.)

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The Journal of Food Technology is published bimonthly, each issue consisting of 90–120 pages; six issues form one volume. The annual subscription is £24 ($84.00) post free; the price per issue is £4.50 ($15.00). Back volumes are still available.

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J. Fd Technol. (1975) **10**, 483–496.

Measuring changes that occur during frozen storage of fish: a review

A. MILLS

Introduction

The changes in appearance, in eating quality and in other properties that occur during frozen storage of fish are of great commercial importance. Such changes, especially when caused by poor freezing practice, poor packaging or poor cold storage conditions can be readily detected in the frozen, thawed and cooked state (Banks, 1962). Thus 'freezer-burn' appears on the surface of frozen fish due to loss of moisture, and loss of liquor as 'drip' from fish on thawing can be extensive. The flavour of cooked muscle may possess a characteristic 'cold store' note and the toughness and dryness that can arise often makes frozen fish less acceptable. The development of oxidative rancidity is an additional problem with fatty fish.

Although most changes may be readily detected many have unfortunately proved difficult to quantify and this paper reviews some of the methods that have been applied. Aspects of this topic have been reviewed previously by Soudan (1968) and Gould & Peters (1971).

A universally accepted method for measuring the alterations, in even one attribute, has so far proved elusive. This is due partly to the extremely complex composition of frozen fish, to seasonal variations (MacCallum *et al.*, 1968; Castell & Bishop, 1973) and, as Love (1964) reported, to differences between fish from different fishing grounds. It has been shown that many of the reactions leading to discernible changes have different temperature coefficients: thus a method for predicting changes at one storage temperature may not be applicable to those which have occurred at another. Another difficulty is that changes in flavour (and texture) which occur during frozen storage primarily consist of increases of intensity, whereas in the case of fish stored in ice changes are qualitative and can be recognized as successive but distinct stages in a sequence. Particular problems of assessing and grading have been discussed by Castell (1969) and Rakow (1970).

The purpose and applications of the methods used vary. For commercial application a relatively simple acceptability assessment in terms of quality loss in cold store may be all that is required. In some countries, for example Italy and Holland, there is a legal requirement to distinguish fresh fish from fish that has been frozen and thawed. It may also be necessary from an examination of the frozen and stored product to determine

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the freshness quality at the time of freezing. Methods included in this review have been limited to those that have been applied to measuring changes that have occurred during frozen storage and are classified under three headings, i.e. sensory methods, chemical methods and physical methods.

Sensory methods

Flavour, odour and texture. Tasting remains the pre-eminent method for detecting all the relevant changes during frozen storage and its fundamental importance needs no emphasis. Most frozen fish standards or specifications include assessment by tasting and the applicability of chemical or instrumental methods to assessing changes is usually judged by their performance against sensory assessment. Nevertheless, despite their importance, few papers have been written specifically about tasting methods used for frozen fish. Many authors give scant details of their tasting methods and some merely state that a sensory assessment has been carried out; a review of sensory methods applicable to fish and fishery products is given by Larmond (1969). Sensory methods used to assess frozen fish are of three types:

(1) those in which tasters are asked to assess the fish on a scale of acceptability or palatability;

(2) those in which certain diagnostic features are rated on intensity scales;

(3) those in which comparisons between two samples are made in order to distinguish the sample under test from a control on the basis of the just noticeable difference between them.

The first type of method has produced many useful data. The method for fresh fish of Dyer & Dyer (1949), which incorporates elements of this kind, has been adapted for frozen fish by Dyer *et al.* (1962) and Dyer & Morton (1956), and has been used extensively by other Canadian workers. By assessing palatability, Cutting (1944) derived the frozen storage potential of white fish and smoked fish products. Baines *et al.* (1967) used a nine-point hedonic scale after Peryam & Girardot (1952) although this was considered unnecessarily long by Connell & Howgate (1968, 1969a) when using untrained tasters. The reactions of householders to fresh and frozen halibut have been described by Young (1938, 1939); similar results were obtained from assessment by members of a trained panel.

Various diagnostic sensory factors that change during frozen storage have been examined by several workers. Cowie & Little (1966) used a technique for scoring intensities of toughness and dryness and rated flavour poor to excellent. Kietzmann (1967) devised sensory scoring systems for odour, texture and flavour that would be suitable for use by official fish inspectors. Intensity scales have been used by Banks (1952) and Liljemark (1964) for quantifying the rancid odours and flavours that develop during the cold storage of fatty fish.

Baines *et al.* (1967) identified and scored separately cold storage odour and flavour, firmness and dryness for cod. They detected odours and flavours, differing from those

normally found in fish stored in ice and as these could not be characterized by reference to a single odour or flavour or groups of commonly encountered odours or flavours, the term cold storage odour and flavour was used. Panel members could be trained to recognize this characteristic cold storage odour and flavour and to score for their intensity, separately from what may be described as 'freshness' odours and flavours.

More recently one of the cold storage components has been identified by McGill *et al.* (1974) as hept-*cis*-4-enal, but so far it has not been possible to quantify the development of this compound during frozen storage.

The odour of defrosted flounders has been related, but not necessarily causally, by Castell (1969) to their malonaldehyde content. Measurement of malonaldehyde is reviewed later in the section dealing with changes in the lipid content.

The detection of 'just noticeable differences' is discussed by van Arsdel (1969) who commented on the general usefulness of this approach for determining 'time-temperature-tolerances' of frozen foods, but not specifically frozen fish. The smallest amount of deterioration detectable by untrained tasters was related by Love (1966) to measurements by certain objective methods, while Moorjani, Montgomery & Coote (1960) used paired comparisons for detecting differences between frozen fish samples stored at different temperatures.

Appearance. Although it is well known that freezing and frozen storage affect the appearance of fish on thawing there are no methods available at present that quantify such changes. Measurement of the extent of freezer-burn has been applied in some instances to indicate gross changes during storage, for example in United States standards for grades of frozen fillets (US Dept. of Int., 1971). Increase in drip loss is another visible sign of frozen storage deterioration but this is reviewed more fully in a later section. Freezing whole fish to below $-4{\cdot}8°C$ produces opacity in the lens of the eye (Love, 1956b) but the effect is masked if the fish are stored in ice for more than sixteen days before freezing, or in brine for one day. Further work has been carried out in Italy where a method for distinguishing fresh fish from frozen-thawed fish is required. Results are given by Ciani & Salerni (1964) who state that opacity of the lens 'is not, in itself, sufficient to decide that a fish has been subject to freezing'. In terms of appearance they considered useful the colours of the arterial cone, which turns ochre red on freezing, and of the ventricle which turns soot-black.

Reay (1933) reported that the gloss present in brined and smoked fresh fish was not present, or was reduced, in similar products prepared from frozen-thawed fish. The reduction of the amount of gloss was to some extent dependent on the frozen storage history and this reduction can be assessed by experienced persons to give a crude evaluation of the denaturation undergone by the fish.

Despite the overwhelming importance of sensory methods in general, and of tasting in particular there are situations where alternative methods are required. Such methods can be used to confirm sensory findings, or to examine specific changes occurring during frozen storage.

Chemical methods

Extractable protein fractions. Perhaps the single most useful chemical method that has been used to examine frozen fish for quality changes during cold storage has been measurement of the extractable proteins. Preliminary work was carried out by Reay (1933, 1934) and by Reay & Kuchel (1936), who observed that a decrease in the amount of protein soluble in solutions of neutral salts occurs during frozen storage. It was concluded that this indicated denaturation and modifications and improvements to the method have been carried out by Dyer, French & Snow (1950). The effects of freezing and frozen storage on fish muscle proteins have been reviewed by Dyer (1951), Dyer & Dingle (1961), and by Connell (1962a, 1968), while studies of the mechanism of the changes have been reported by Duerr & Dyer (1952), Love (1958), Connell (1960b, 1962b) and by Sawant & Magar (1961).

Although the method has proved useful there are several drawbacks to its application. It is laborious, results show a high degree of scatter and the amount of extractable protein present in fresh fish varies seasonally (Ironside & Love, 1958; Castell & Bishop, 1973). Below certain storage temperatures (between $-10°$ and $-20°C$) it is alleged that changes in extractable protein fractions do not correlate with certain other detectable changes, for example texture (Love, 1956a; Luijpen, 1957; Castell & Bishop, 1973). This phenomenon may be connected with that observed by Kelly (1967) who showed that pH influences the rate of protein denaturation: from a knowledge of both pH and extractable protein Cowie & Little (1966) demonstrated that acceptability could be predicted better than it could from either factor alone. The rate of freezing has been shown by Love (1956a, 1958) to affect protein extractability after subsequent storage at a steady temperature, as has the stage of rigor mortis at freezing (Nikkila & Linko, 1956; Nikkila, 1957; Nishimoto & Tanaka, 1960; Love, 1962c).

According to Cowie & Mackie (1968) variations in the experimental methodology, especially at the extraction stage, lead to variations in the results obtained. Furthermore many of the discrepancies found in the literature have been attributed by Ravesi & Anderson (1969) to a lack of standardization in the methodology used. Some variation in the amount of soluble protein extracted was eliminated by Ironside & Love (1958) who took only certain myotomes, freed from myocommata, for extraction. It was suggested by Dyer & Morton (1956) and also by Love (1962b) that there may be a relationship between protein denaturation, as measured by decrease in solubility, and the lipid content of fish. This relationship has been reviewed by Ackman (1967) and Connell (1968). Citations of papers presenting conflicting evidence are included later in the section on lipids.

Trimethylamine oxide breakdown. Under conditions of frozen storage bacterial action is prevented so there is no production of trimethylamine from trimethylamine oxide and most authors now agree that measurement of trimethylamine can be used as an index of pre-freezing history (Dyer *et al.*, 1962; Connell & Howgate, 1968, 1969a; Castell, Neal & Dale, 1973). It has been observed that trimethylamine oxide is hydrolysed to

dimethylamine and formaldehyde during frozen storage and measurement of these substances may indicate cold storage history.

Tokunaga (1964a, b) observed increases of dimethylamine and formaldehyde in frozen stored Alaska pollack and measurement of dimethylamine has been proposed by Castell, Neal & Smith (1970) as an indicator of cold storage deterioration. However, when measuring formaldehyde, Mackie (1972) found considerable variation between fish. It has been confirmed that dimethylamine and formaldehyde increase during frozen storage of certain gadoid species, but not in haddock, lemon sole or plaice (Mackie, 1972) nor halibut, redfish, wolf-fish or certain shellfish (Castell, Smith & Neal, 1971). Consequently the method of measuring dimethylamine or formaldehyde would have limited applicability. The situation is reviewed by Amano & Tozawa (1967) and Yamada & Harada (1969).

Amino acids. Studies on changes in the concentrations of amino acids during frozen storage have been carried out by Partmann (1969), Vorob'eva & Sukhanova (1971a) and Danilow, Schewtschenke & Dubskaja (1973). In general it was found that although the types of free amino acids present did not change, their relative concentrations did alter, but measuring such alterations is not considered a useful method of monitoring frozen storage changes.

Sulphydryl groups. Husaini & Alm (1955) reported an initial fall in masked sulphydryl groups (the difference between total and free groups). However, Connell (1960a), suggested these observations were due to examining samples at too infrequent intervals, coupled with large experimental error. Work on actomyosin extracts by Seagran (1956) indicated a slight decrease with storage in reactive sulphydryl groups, but the concentration of total sulphydryl groups remained constant.

Nucleotide changes. A review of nucleotide degradation has been given by Hiltz *et al.* (1969) and of the effect of nucleotide catabolites on flavour by Jones (1963). Dephosphorylation of inosine monophosphate to inosine during frozen storage has been observed (Jones, 1963; Dyer, Fraser & Lohnes, 1966; Dyer & Hiltz, 1969; Kemp & Spinelli, 1969). It would, however, be difficult to make practical use of the observation in measuring frozen storage changes since the same reaction proceeds at a faster rate in the unfrozen state. Furthermore freezing and thawing affect the rate of dephosphorylation, as does the presence of red muscle.

Small decreases of nicotinamide adenine diphosphate in several species examined were found by Partmann (1973), but these are too small to be of use as a test. Authors agree that at low frozen storage temperatures the concentration of hypoxanthine does not change and that at higher temperatures (above $-14°C$) the effects are so slight that measurement of this substance may still be used to indicate pre-freezing history (Jones & Murray, 1961; Jones, 1963; Dyer & Hiltz, 1969).

Enzyme activity. Gould (1964) has suggested that the properties of enzymes might be useful for revealing early quality changes in frozen stored fish and she determined the reaction velocity of several enzyme systems in tissue fluid. Two enzymes, α-glycerophos-

phate dehydrogenase and the malic enzyme showed the greatest potential. In all species examined the specific activity of the malic enzyme increases as a result of freezing. At −29°C this enhanced activity is maintained for at least four months, whereas there is a gradual decrease at −7°C. Observations on the α-glycerophosphate dehydrogenase of haddock indicated significant increases in the activity constant (the slope of plotted reciprocals of reaction velocity and substrate concentration) during two to four weeks at −7°C. At −29°C the activity constant dropped slightly over the first two months and then increased. Further work in this field was carried out by Connell (1966a), who suggested that changes in aldolase activity could provide an objective test for frozen storage deterioration. By determining the dehydrogenase activity of muscle Ciani & Salerni (1964) could distinguish fresh fish from frozen-thawed fish.

The changes in adenosine triphosphatase activity during frozen storage have been studied by several workers. Observations by Partmann (1955) led to the development of a method for measuring deterioration based on the loss of muscle fibre contractility (Partmann, 1970). Loss of myofibrillar adenosine triphosphatase activity during frozen storage was observed to be temperature-dependent by Connell (1960a), but Yamanaka & Mackie (1971) considered measurement of sarcoplasmic adenosine triphosphatase activity applicable to measuring storage changes of iced fish but not of frozen fish.

Lipids. Although the amount of total lipid does not change during cold storage (Peters *et al.*, 1968), it has been shown that free fatty acids are formed by hydrolysis (Dyer & Fraser, 1959; Bligh, 1961; Bligh & Scott, 1966). Other changes that have been measured include the production of carbonyls from lipids and the development of rancidity.

The nature and composition of free fatty acids produced during frozen storage have been extensively examined (Awad, Powrie & Fennema, 1969; Wood, Hiltz & Salwin, 1969; Vorob'eva & Sukhanova, 1971b), especially in relation to their effects on the insolubilization of protein (Olley, Pirie & Watson, 1962; Hanson & Olley, 1964; Olley & Duncan, 1965; Ackman, 1967; Anderson & Ravesi, 1969; Castell, 1971). However, estimation of free fatty acids as such is not used as an estimate of frozen storage changes.

Estimation of carbonyls was carried out by Tokunaga (1964a, b) who observed an increase during frozen storage. Similar results using butyric aldehyde as a standard were reported by Altuf'eva, Sokolova & Ushkalova (1970) who thought the method promising.

That fish, especially fatty fish, becomes rancid during frozen storage is well known and various chemical estimations of rancidity have been applied. Banks (1937) measured peroxide values and applied the Kreis test but recommended (Banks, 1945) that a sensory test should also be carried out when determining rancidity. The degree of oxidative rancidity was determined quantitatively by Yu & Sinnhuber (1957) and Sinnhuber & Yu (1958) by measuring malonaldehyde using 2-thiobarbituric acid. Chemical methods for measuring rancidity are reviewed by Banks (1966) who con-

cluded that 'determination of peroxides under anaerobic conditions is probably the most sensitive and reliable procedure for determining early oxidative changes'.

Connell's colour test. Under the action of heated concentrated sulphuric acid it was observed by Connell (1966b) that frozen stored cod muscle gives a blue-purple colouration. By comparing the absorbance at two wavelengths (510 nm and 560 nm) an indication of the intensity of frozen storage changes was obtained. The result is independent of season and storage in ice up to ten days. The method is limited to gadoid species that produce formaldehyde, or other carbonyl compounds yielding similar colours during frozen storage and consequently has the same limitations as the direct estimation of formaldehyde.

Physical methods

Cell fragility. The observations that individual muscle cells of frozen stored fish became progressively more difficult to rupture was made use of in the development of a method that quantifies the degree of muscle denaturation by Love & Mackay (1962). Results obtained from the test have been compared with those obtained for extractable protein (Love, 1962b; Love *et al.*, 1965), for Connell's colour ratio (Connell & Howgate, 1969b) and has been applied to several species (Olley *et al.*, 1967). At present the method is considered unsuitable for fatty fish (Love & Musselmuddin, 1972a, b).

Kelly *et al.* (1966) demonstrated that variation in results obtained by three groups of workers could be attributed to variations in the pH of the fish. Further work was reported (Love & Musselmuddin, 1972a) and a modified technique developed (Love & Musselmuddin, 1972b), which is not influenced by pH. A further improvement by Whittle (1973) in developing a multiple sampling technique enables the operator to increase the rate of sampling.

The method has been tried with varying degrees of success by other workers: Gutschmidt (1963) recommended that samples be cooked before testing and related his results to texture changes; Ciani & Salerni (1964) used the method to distinguish fresh from frozen fish.

Water binding capacity. Measuring the amount of drip produced as a result of freezing and frozen storage has been attempted in several ways, depending on the type of drip measured. In a review of factors affecting drip formation, Miyauchi (1963) defined four types of drip:

(a) 'free drip', or 'weep', is liquid that exudes from frozen fish tissue on thawing without application of force;
(b) 'expressible drip', 'press drip', or 'centrifuge drip', is liquid obtained when external force is applied to the tissue of fish;
(c) 'thaw drip' is the liquid that exudes when frozen fish tissue thaws;
(d) 'cook drip' is the liquid released when the fish tissue is heated or cooked.

Types (b), (c) and (d) have been used in the assessment of frozen stored fish and a

a paper giving an extensive list of references relating to drip formation and drip measurement has been published by Podeszewski (1969). Methods for determining water binding capacity have been given by Connell (1955) who modified the method of Grau & Hamm (1953), and by Podeszewski & Drelichowski (1971) and Podeszewski (1972).

Cook drip has been measured by Miyauchi (1962) and suggested by Kietzmann (1967) as a method suitable for use by official fish inspectors.

Methods of determining thaw drip have been given by Dyer *et al.* (1962), Ota, Hashimo & Murakakami (1973) and used by many others. However, Notevarp & Heen (1940) claim that to obtain a correlation between drip loss and flavour or texture, expressible drip should be measured, and they give a method for its determination. Further work was reported by Banks (1955) who studied the optimum conditions for determining expressible drip. More recently expressible drip has been measured using a centrifuge (Miyauchi, 1962; Kelly & Dunnett, 1969; Vorob'eva, 1969; Podeszewski & Drelichowski, 1971).

Despite several drawbacks, measurement of drip loss does indicate deterioration that has occurred during frozen storage. As measurement is relatively simple it is frequently included in standards for frozen fish.

Texture. There are numerous methods for measuring the mechanical properties of food, but their applications to determining changes in frozen stored fish are few. The requirements of an instrument for measuring texture were described by Dassow, McKee & Nelson (1962) who also described a machine capable of detecting textural differences between fresh and frozen thawed fish. Buttkus & Tarr (1962) considered that there were advantages in using a mechanical measurement of texture, but were not satisfied with results obtained from a Mangold sclerometer. Shearing forces were measured by Gutschmidt (1963), who obtained his best results with cooked fish. A machine capable of measuring the hardness of cooked fish muscle is described by Sutton & Main (1967) who state that the values obtained with the machine were more precise than those obtained with a taste panel.

It appears that because texture includes so many parameters, the majority of which change during frozen storage, it is unlikely that any single instrument will be suitable for measuring frozen storage history.

Refractive index. Elerian (1965) reported that the refractive index of drip rises during frozen storage but that results are too variable to be of use. Todorov (1969) included the refractive index of aqueous humour and of tissue fluid in a list of methods for measuring frozen storage changes. However, Rakow (1970) considered that mechanical damage to the eyes during freezing and thawing precluded this method as a useful measurement of frozen storage changes.

Opacity. It is well known that frozen fish becomes opaque and yellow during storage. Love (1962a) devised a method for measuring opacity but reported that the method could not follow changes during frozen storage. Using the method, however, it was possible to distinguish fresh from frozen-thawed fish.

pH. The literature is conflicting with regard to the changes in pH during frozen storage. Some authors record no change (Connell & Howgate, 1968, 1969a; Carraciolo & Perricone, 1968), or a slight decrease (MacCallum *et al.*, 1968; Lauder, MacCallum & Idler, 1970; Ota *et al.*, 1973), whereas slight increases have also been reported (Vorob'eva, 1969). The above workers thawed the samples before measuring pH, but van den Berg (1966) carried out measurements on frozen muscle and observed decreases in pH during frozen storage. Such contradictory findings are hardly surprising in view of the number of different reactions, producing acids and bases, proceeding in frozen fish.

Although changes in pH may not reflect frozen storage changes, the influence of pH on texture has been clearly demonstrated (Kelly, 1967, 1969; Kelly *et al.*, 1966; MacCallum *et al.*, 1968). It has also been shown that pH changes with season (MacCallum *et al.*, 1968; Connell & Howgate, 1968) and consequently any measurement of texture should take pH into account (see Connell & Howgate, 1968). The fundamental importance of pH to other aspects of fish quality, e.g. gaping, drip loss and flavour, must not be overlooked although this has been considered inappropriate to this review.

Microscopic analysis. Use of the light microscope has not led to a method for following changes in frozen stored fish, but its use has shown differences in size of ice crystals and in tissue structure due to rates of freezing (Birdseye, 1929; Young, 1938), the effect of rigor (Love, 1962d), storage temperature (Kelly & Dunnett, 1969) and fluctuating storage temperatures (Kaminarskaya & Piskarev, 1970). However, the definitive work on effects of rate of freezing and other factors on ice crystal size is that of Love (1968).

Tanaka (1964) observed a decrease in myofilament distances using an electron microscope, and related these decreases to toughness. This was not confirmed by Liljemark (1967) who attributed the toughness to changes within the myofilament.

Conclusions

The literature contains a wide variety of methods that have been applied to measuring quality changes in frozen stored fish. However, it is evident that due to a number of complicating factors (seasonal changes, size, rate of freezing, pH, methodological difficulties, pre-freezing history) finding a method or methods for commercial or even research use has proved difficult. The very fact that so many tests have been proposed and developed is indicative of the complex nature of the problems that exist.

Under commercial conditions a method which evaluates only gross changes is usually adequate and sensory assessment by experienced personnel suffices. Factors in this type of assessment should include measurement of freezer-burn, drip loss on thawing, fillet opacity and assessment of the cooked fish for textural changes and development of cold store odour and flavour. It can also take into account factors influenced by pre-freezing treatment, for example blood discolouration and gaping of the fillet.

In the research environment more time and better facilities are usually available and

so more sophisticated tests, be they sensory, chemical or physical, that reveal other changes, can be applied. Additionally, the results obtained from standardized chemical and physical tests can be used to predict sensory properties as well as overall acceptability. Relationships between various tests have been proved statistically and several correlations published. For examples, see Connell & Howgate (1968, 1969a), Moorjani *et al.* (1960) and Gutschmidt (1963).

Another approach involves keeping a record of storage conditions. This has proved convenient in some instances and it is possible to indicate either the integrated function of time and temperature of storage, or whether a rise above a specific critical temperature has occurred during storage. Various devices available have been reviewed by Schoen & Byrne (1972) and their correlation with quality loss examined by Hayakawa & Wong (1974) and by Olley & Ratkowsky (1973). Such indirect measurement is useful only when the time-temperature-tolerance of a particular product is known (Lane, 1964).

Because of the complexity both of the changes occurring during frozen storage and of the relationship between non-sensory and sensory assessment, it will probably be necessary to carry out more than one test of the former kind. At the present stage of knowledge it is unlikely that any single test or combination of non-sensory tests carried out on frozen fish in general can fully predict all the sensory characteristics of that fish.

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(*Received* 17 *January* 1975)

J. Fd Technol. (1975) **10,** 497–505.

Hydrogen peroxide bleaching of marinated herring

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Summary

Several aspects concerning the application of hydrogen peroxide treatments in the production of marinated herring are discussed. Various treatment groups of marinated herring were prepared using different hydrogen peroxide application methods and different hydrogen peroxide concentrations. The value of these treatments with respect to their actual bleaching effect on the product was assessed. The rates of dissipation of hydrogen peroxide from the dip solutions used in the various treatment groups were determined and the levels of residual hydrogen peroxide in the treated fillets were ascertained. A shelflife study was carried out in order to assess the keeping quality of a product subjected to this type of treatment.

Introduction

Marinated herring are herring fillets which have been cured in a solution of salt and acetic acid. The product is classed as 'semi-perishable' and its shelflife is dependent upon the preservative action of the ingredients, as well as proper handling, packaging and storage. However, only such concentrations of salt and acetic acid as will be palatable can be used because this product is consumed without any further preparation. These ingredients along with sugar, spices and sauces have been used in many combinations in an attempt to obtain a palatable product which at the same time exhibits a satisfactory shelflife.

The production of marinated herring consists of two stages. The first stage consists of curing the raw herring in a solution of acetic acid and salt for 7–10 days. During this period the active ingredients penetrate the fillets. The second stage consists of freshening the herring fillets in tap water or a sugar solution, followed by packing the fillets or sections with a pickle liquor containing any of a variety of sauces or spices. The production can be altered in many ways which will ultimately affect the colour of the final product. This is an important consideration as the whiter product tends to gain a position of preference on the market.

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The whiteness of the product is influenced by the concentrations of salt and acetic acid in the curing mixture, the duration of the cure, the fat content of the raw material, and the temperature during curing and storage. It has been reported that hydrogen peroxide can be used as a bleaching agent in the production of marinated herring (Borgström, 1953). At present, various regulations in many countries, including Canada and the UK, prohibit the use of hydrogen peroxide in this product. The work reported here has a three-fold objective: (1) to assess the bleaching effect of hydrogen peroxide on marinated herring; (2) to measure any residual hydrogen peroxide on the product; and (3) to evaluate the effect of this treatment on the quality and shelflife of the product.

Materials and methods

The application of hydrogen peroxide should be undertaken before the freshening stage of the process. The hydrogen peroxide is usually added by means of a dip. The herring may be treated with hydrogen peroxide before curing (Borgström, 1953) or after curing. Alternatively, the herring can be treated with hydrogen peroxide in the curing mixture

TABLE 1. Initial concentrations of hydrogen peroxide dips, methods of application and duration of application for various treatment groups used in this investigation

Treatment group	Initial concentration of hydrogen peroxide dip (ppm)	Duration of application (hr)
1*	600	24
2	600	4
3	200	24
4	200	4
5	40	24
6	40	4
7†	0	0
8‡	600	4
9	600	1
10	200	1
11	40	1
12§	600	168
13	200	168
14	40	168

* Herring fillets in treatment groups 1–6 placed in hydrogen peroxide dips after curing.
† Control, no hydrogen peroxide treatment.
‡ Herring fillets in treatment groups 8–11 placed in hydrogen peroxide dips prior to curing.
§ Herring fillets in treatment groups 12–14 treated with hydrogen peroxide in the curing mixture.

(Watson & Hess, 1936). In this work, each of these application methods was tried using hydrogen peroxide concentrations of 40, 200 and 600 ppm. The treatment times differed for the various application methods. Reagent grade hydrogen peroxide (30% w/v), which had previously been standardized according to the procedure of Vogel (1961), was used in the preparation of dip solutions. The total process time (curing plus peroxide dip) of each treatment group (Table 1) was 7–8 days. The hydrogen peroxide application was always carried out in earthenware crocks. The curing was carried out in wooden pails or barrels except for the treatment groups in which the hydrogen peroxide application and curing were done simultaneously. In these latter instances, the entire process was carried out in earthenware crocks. The curing pickle used in this work contained sodium chloride (6%) and acetic acid (5%).

On the basis of specificity, the enzymatic method of Bernt & Bergmeyer (1965) was chosen for the chemical analysis of hydrogen peroxide solutions in the part per million range. In this method, hydrogen peroxide is decomposed by a peroxidase and the oxygen so liberated then oxidizes a colourless hydrogen donor to a coloured compound. In this instance the hydrogen donor used was o-dianisidine which is oxidized to give a red-brown dye having a broad absorption maximum in the region of 430–460 nm. This method was applied to standard hydrogen peroxide solutions, as well as to samples of each of the dip solutions. The resultant optical densities of the standard solutions were measured at 440 nm (Unicam SP 800A recording spectrophotometer) and were plotted versus the concentrations of hydrogen peroxide to form a standard curve which obeyed Beer's Law over the concentration range of 2–60 ppm hydrogen peroxide. The resultant optical densities of the sample dip solutions were also measured and the concentrations of hydrogen peroxide in the dip solutions were estimated by reference to this standard curve. Recovery tests from dip solutions indicated that no interferences were present.

The herring samples were blended with an equal weight of distilled water, the blend was filtered and aliquots of the filtrate were subjected to the analytical method previously described. In order to determine the percentage recovery from this procedure, several series of cured herring samples containing no hydrogen peroxide were analysed using different concentrations of hydrogen peroxide as the blending liquid, rather than distilled water. The optical densities were plotted versus the concentrations of hydrogen peroxide in the corresponding blending liquid. The resultant curve (Fig. 1) was linear over a working range of 10–175 ppm and the mean percentage recovery was 26·6 with a 95% confidence interval of 25·7–27·5 (d.f. = 8). This working curve was used for the analysis of hydrogen peroxide in the herring samples. Tests using low concentrations of hydrogen peroxide as the blending liquid indicated that fish containing more than 5 ppm hydrogen peroxide would give a positive response to this test. The low recovery from this method can be attributed either to an interfering reaction such as dye binding by soluble protein, or to the dissipation of hydrogen peroxide during the analyses as additional fish tissue is exposed to reaction.

The dissipation of hydrogen peroxide in dip solutions was monitored for all three

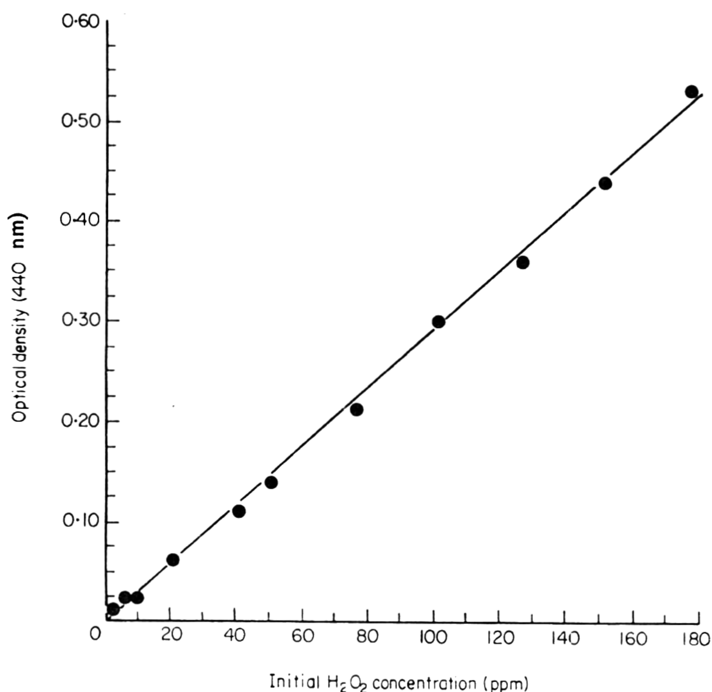

FIG. 1. Beer's Law Plot: Solutions containing different concentrations of hydrogen peroxide (H_2O_2) were blended with equal weights of marinated herring before analysis.

application methods over a 24 hr period or until the hydrogen peroxide was entirely dissipated. At the end of the respective process times, several fillets and a sample of pickle were removed from each treatment group (Table 1) for hydrogen peroxide analysis. The remaining fillets from each treatment group were repacked in curing pickle (about two-thirds the strength of the original pickle) and stored at 40°F in wooden pails. Similar samplings were made from each treatment group after three days storage and after three weeks storage. Although some colour observations were made on the fillets from these samples, the primary aims of this segment of the work were to investigate the dissipation of hydrogen peroxide from dip solutions and to measure any residual hydrogen peroxide in the fillets. Four additional treatment groups, processed solely for colour evaluations, were prepared in a chill room (35–37°F) by treating cured fillets with different concentrations of hydrogen peroxide for 4 hr.

A shelflife study was conducted on three treatment groups. For this study the herring were processed at a local plant. The fish were processed and packed exactly according to the plant's normal procedure (i.e. using their curing solutions, freshening mixture, spices, sauces, etc.) except for the inclusion of hydrogen peroxide treatments in two of these groups. These products were stored at 40°F and samples were withdrawn monthly

in order to evaluate their colour and overall quality. Hydrogen peroxide analyses were performed on fillets from each of these treatment groups within 24 hr of processing. The samples were assessed separately for colour (whiteness) and overall quality by an eight-member panel. Each panelist was asked to assign a score from 0–10 to each sample. A score of 10 denoted the most favourable assessment and a score of 0 denoted the least favourable assessment. The 'mean' scores ('mean overall quality' and 'mean colour') were simply obtained by averaging the appropriate scores of the eight panelists. From previous taste panel work, a sample was considered to be 'reject quality' if the 'mean overall quality' score was 4·5 or less. All panel examinations in this work were carried out on coded samples in order to avoid biased assessments.

Results

The dissipation of hydrogen peroxide in the dip solutions was measured from 'time zero', which was defined as the time when the fillets were immersed in their respective treatment dips. The results indicated that hydrogen peroxide was entirely dissipated

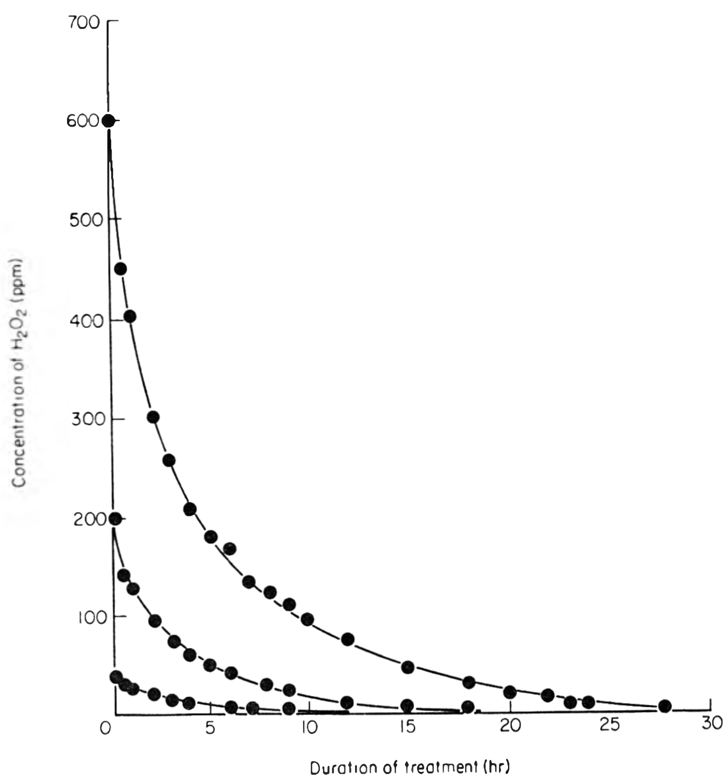

Fig. 2. Dissipation of hydrogen peroxide (H_2O_2) in dip solutions; different initial concentrations applied to fillets in the curing mixture.

within 0·5 hr when raw uncured fillets were immersed in dip solutions containing up to 600 ppm (the maximum concentration tested) hydrogen peroxide. Figure 2 shows the curves representing the dissipation of hydrogen peroxide from treatment dip solutions in which the fillets were treated with hydrogen peroxide in the curing mixture. These curves were obtained by plotting the residual hydrogen peroxide in samples of dip solutions taken at different times in the treatment periods versus the times (measured from 'time zero') at which these samples were removed for analysis. Similarly, Fig. 3 shows the corresponding relationship for the treatment groups in which the fillets were

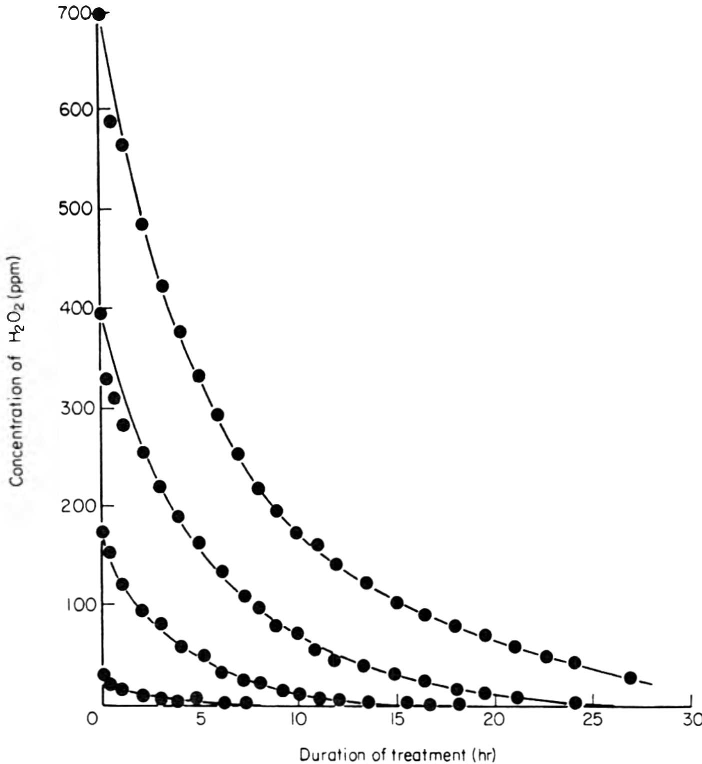

Fig. 3. Dissipation of hydrogen peroxide (H_2O_2) in dip solutions; different initial concentrations applied to fillets after curing.

treated with hydrogen peroxide after curing. In the latter two methods of application, hydrogen peroxide was dissipated rapidly at first. These rates of dissipation decreased considerably as treatment time elapsed. Dip solutions containing initial concentrations of 600 ppm hydrogen peroxide contained traces of hydrogen peroxide even after 27 hr of treatment time while dip solutions containing initial concentrations of 35 ppm hydrogen peroxide retained lingering traces for 7–8 hr.

It will be recalled that fillets which had been processed by various methods (Table 1)

were also analysed for residual hydrogen peroxide. The results from the analysis of fillets taken at the first sampling indicated that only the fish from Treatment Group no. 2 showed any detectable residue (17 ppm hydrogen peroxide). The dip solution from which these fillets were taken contained 308 ppm hydrogen peroxide at the time of sampling. The fillets in this group had been treated with 600 ppm hydrogen peroxide for a 4-hr period after curing. Hydrogen peroxide residues were not detected in any fillets from the later samplings (after three days and three weeks of storage).

The colour observations carried out on samples from the treatment groups shown in Table 1 indicated that the fillets treated with hydrogen peroxide were considerably whiter than those of the control group. There was no appreciable difference in colour

TABLE 2. Mean overall quality and mean colour scores of samples withdrawn monthly from the shelflife study treatment groups

Storage time (months)	Mean scores*					
	Treatment A		Treatment B		Treatment C	
	Quality	Colour	Quality	Colour	Quality	Colour
0	7·9	7·3	7·7	3·6	6·6	5·9
1	8·3	7·6	7·8	4·5	7·8	6·4
2	7·3	7·3	5·7	3·3	7·0	6·8
3	6·4	7·6	4·9	3·6	6·4	7·5
4	6·4	7·3	4·4	3·6	5·6	7·3
5	5·3	7·1	4·1	2·9	4·7	7·3
6	3·9	6·3	2·1	2·3	3·4	4·6

* Each mean score was derived by averaging the appropriate scores of eight panelists.

among fillets subjected to the three application methods, at any of the hydrogen peroxide concentration levels tested. The samples prepared solely for colour evaluations consisted of four groups of cured fillets which had been treated for 4 hr in dip solutions containing 0 (control), 200, 400 and 800 ppm hydrogen peroxide respectively. At the end of the treatment period, the coded fillets were ranked according to whiteness. The panel agreed unanimously that the 800 ppm hydrogen peroxide treated fillets were whitest in colour, followed by fillets from the 600 ppm, 200 ppm and control groups, in that order.

The shelflife study conducted at a local plant was comprised of the following three treatment groups: (A) a 600 ppm hydrogen peroxide dip applied for 2 hr after curing; (B) a control, no hydrogen peroxide treatment; and (c) 50 ppm hydrogen peroxide applied in the curing pickle. Samples from each of these treatment groups were analysed

within 24 hr of processing and were found to contain no detectable hydrogen peroxide residue. The overall quality and colour scores of these treatment groups are shown in Table 2. The samples from Treatment A were off-white in colour with a yellowish cast occasionally dominant. The untreated samples, Treatment B, were pink in colour while the samples from Treatment C were light grey. Samples from Treatment A consistently received higher scores than samples from Treatment C, for both colour and overall quality. The colour scores for samples from Treatments A and C were considerably higher than those for samples from Treatment B (control). Samples from Treatments A and C were considered to be of 'reject quality' (mean overall quality score $\leq 4 \cdot 5$) after five to six months and five months of storage respectively; samples from Treatment B (control) were considered to be reject quality after four months of storage.

Discussion and conclusions

The rates of dissipation of hydrogen peroxide in dip solutions were determined in order to assess their potential periods of effectiveness. If hydrogen peroxide was applied to the fillets during the cure or after the cure, treatments could be continued for several hours (Figs 2 and 3) without complete dissipation of the hydrogen peroxide. However, if the treatment was applied to the raw fish, treatment could only be continued for up to 0·5 hr, after which no detectable hydrogen peroxide remained. Dip solutions containing an initial concentration of more than 600 ppm hydrogen peroxide were not investigated for this application method.

The hydrogen peroxide residue (17 ppm) detected in the fillets of Treatment Group no. 2 (Table 1) was probably superficial and could be attributed to the presence of dip solution adhering to the sample. It should be noted that, at the time when this sample was taken, the treatment dip solution still contained 308 ppm hydrogen peroxide. Furthermore, this sampling was conducted at a point before the fillets reached the freshening and packing steps. Therefore, since this was the only residue detected in any of the fillet samples analysed, it can be concluded that it is very unlikely that residual hydrogen peroxide would remain on treated (up to 600 ppm hydrogen peroxide) fillets by the time they reached the consumer. This was further confirmed by the negative results from samples from the shelflife study which were analysed within 24 hr of processing.

The colour evaluations carried out on the various treatment groups clearly indicated that hydrogen peroxide has some value in bleaching herring so as to obtain a whiter marinated herring product.

A major point of concern had been that hydrogen peroxide treatments would lead to excessive oxidative rancidity, thereby causing a marked decrease in the overall quality of the product. However, the results from the shelflife study indicated that hydrogen peroxide acted as a preservative as well as a bleaching agent, thus yielding a higher quality product with an extended shelflife.

Acknowledgments

The authors are indebted to B. H. Lauer for his assistance in the preparation of this manuscript.

The authors also thank S. Varga and G. E. Mack for their suggestions regarding various aspects of this work.

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(*Received* 27 *October* 1974)

J. Fd Technol. (1975) **10**, 507–513.

Bacterial infection/invasion in fish flesh

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Summary

Experiments with mechanical treatment of trout have been carried out to show whether there exists any correlation between the stress of the animal before death and the level of bacterial infection in the flesh and/or the quality of the fish determined by organoleptic assessment.

The quality and keeping time of the trout is reduced when the fish is exposed to physical stress. The reduction increases with increasing mechanical treatment and is bigger in feeding than in starving fish.

The infection level in the fish muscle increases with increasing physical stress and is higher for feeding than for starving trout.

When the count of bacteria is higher than 100/g there is a very good correlation between the log count and the organoleptic score.

Introduction

It is generally accepted that the tissue of healthy living animals is sterile. In consequence it has been presumed that the bacteria invade the muscle post mortem through the skin and belly cavity. If this was true it could be anticipated that the level of infection of the flesh would be related to the initial load on skin and belly cavity and thus affect the quality and keeping time of the fish.

However, Huss *et al.* (1974), reported experiments on storage of cod and plaice in ice with varying levels of initial contamination which showed that the initial bacterial contamination had only little influence on the quality and keeping time of the fish.

In 1974 van Spreekens reported the presence of micro-organisms in the flesh of whiting, cod and plaice after the fifth, sixth, eighth and twelfth days of storage which were of the same type as those normally found in the intestines. Evisceration of the fish immediately after catching had little effect on the numbers of these organisms in the flesh. The flora of the skin on the other hand was shown to be mixed and it was suggested that penetration of the flesh by micro-organisms occurred primarily from the intestines, the skin flora only being found in the flesh during the later stages of spoilage (eight to seventeen days depending on species).

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It is known (Pusztai, 1970) from work on slaughter animals under stress that a non-specific invasion of muscle tissue by micro-organisms can take place. The purpose of this investigation was to determine if a similar situation exists with fish, and whether there is any correlation between the degree of stress before death, the level of micro-organisms in the flesh and post mortem storage properties of the fish.

Materials and methods

Although cod and plaice might be the most obvious test material, they are not easily obtainable in a suitable condition. It was decided to utilize rainbow trout, which are obtainable in a reasonably relaxed condition and whose feeding can be controlled.

The trout were obtained from the Danish Experimental Trout Farm.

To our knowledge nothing is known about the stress to which fish are exposed in the gear during catching. It was therefore arbitrarily decided to treat the trout in a tumbler, where they were given a gliding fall of about 0·5 m every 3 sec. In a preliminary experiment the trout were tumbled for 20 and 60 min, respectively. Those tumbled for 20 min had no signs of damage, but 60 min resulted in damage to the fins and gill covers, although no scratches were observed on the skin. It was decided to tumble the trout for 20 and 40 min in the further experiments. Tumbling for 40 min gave some injuries to fins and gill covers, but not more than what could be expected during catching.

After the treatment the trout were bled and gutted and transported to the laboratory packed in ice. A sample of untreated trout was bled and gutted and used as the control.

Each treatment was carried out on thirty-six fish, and six fish from each treatment were sampled on each of six occasions during the storage experiment. Each sample was tested for total bacterial counts and organoleptic quality.

Since the objective of organoleptic quality assessment was to evaluate bacterial spoilage, the trout needed for the main experiment were packed in sixes under vacuum in Mylar bags (polyester/polyamide laminate) to minimize fat oxidation.

It is realized that contrary to normal storage in ice the packaging will change the oxygen concentration in the fish. However, the oxygen concentration in the fish muscle will be low anyway so that the packaging might affect the surface flora only.

Microbial counts were carried out on samples cut aseptically from the fish. The trout were nailed to a board with two spikes, one through the head and one through the tail. An area between the lateral line and the dorsal fin was rubbed with a cotton pad wetted with alcohol until the scales were removed. With a sterile knife a window of about 4×4 cm^2 was cut and the skin removed with a pair of tweezers. From the naked surface of the muscle a print was taken by the agar sausage technique (ten Cate, 1963). With a knife a piece of flesh was then cut from the fillet within the cutting line of the window, and the piece was weighed in a mixer glass and mixed with 45 ml of sterile isotonic saline solution. One millilitre of this suspension was spread on the surface

of plate count agar enriched with 0·5% yeast extract and 0·5% salt, and the plates incubated for five days at 20°C. Each result is the average from six fish.

From the other side of the fish a fillet was cut for organoleptic assessment. The organoleptic score ranges from 10 to 0 with 4 as the acceptability level.

Results

Preliminary experiment

The bacterial counts found in the preliminary experiment are presented in Table 1. The trout were stored unpacked in ice and examined after three and ten days; no organoleptic assessments were made.

TABLE 1. The log count/g

	3 days		10 days	
	G	U	G	U
G 0	0		1·52	
U 0		0		1·08
G 20	1·57		3·30	
U 20		1·08		2·90
G 60	1·34		3·79	
U 60		1·11		3·65
Guts	>6		>6	

G, gutted; U, ungutted.
0, no treatment in tumbler; 20, 20 min in tumbler; 60, 60 min in tumbler.

These results show an increase in the bacterial count in both gutted and ungutted fish.

The main experiment was carried out on trout starved for three days and on feeding trout. The changes in organoleptic quality is shown in Figs 1 and 2. With starving trout there is no difference in keeping time between the control fish and those tumbled for 20 min. Those tumbled for 40 min, however, became unacceptable five days earlier. With feeding fish bigger differences were found, the controls were rejected after twenty-two days, those treated for 20 min after sixteen days and those treated for 40 min after eleven days. A combination of feeding fish and mechanical damage will therefore result in a considerable reduction in keeping quality.

The changes in total viable bacterial counts are shown in Figs 3 and 4. In feeding fish the count is generally 1 log cycle higher than in starved fish. In both starved and feeding fish the controls showed a slow rise in counts towards the end of the storage period and the counts increased with increasing mechanical stress.

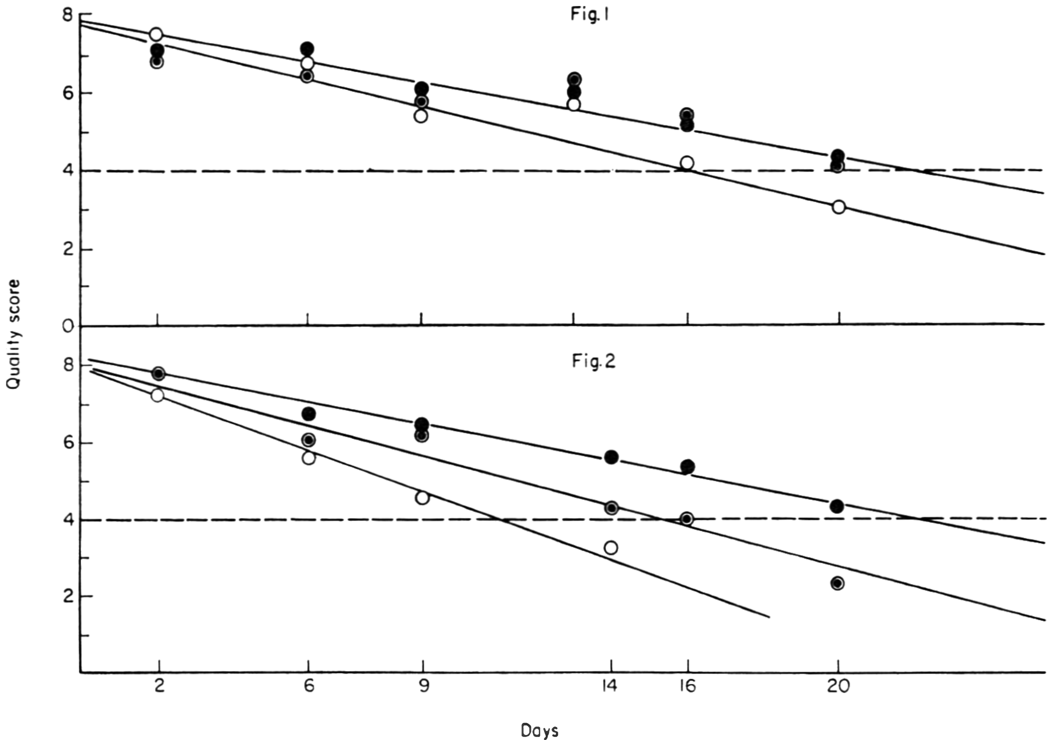

FIG. 1. Starved trout. Quality scores for mechanically treated trout vacuum-packed and stored in ice. One untreated code (●) and codes treated for 20 (⊙) and 40 (○) min before slaughtering. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 0·81 units.
FIG. 2. Feeding trout. Quality scores for mechanically treated trout vacuum-packed and stored in ice. Same symbols as in Fig. 1. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 0·73 units.

Figures 5 and 6 show a good correlation between organoleptic score and the log bacterial count where the count is greater than 100/g.

Examination of the muscle surface by the agar sausage technique (Fig. 7) shows that no colonies appeared on the 'prints' from the controls until the fourteenth day of storage. Colonies were found from the tumbled fish after six days, increasing during further storage.

Discussion

It has been demonstrated that quality and keeping time are reduced by mechanical treatment (physical stress). The keeping time decreased with increasing stress and is shorter for feeding than for starving fish.

When gutting the fish it was observed that the intestines appeared pale and bloodless in the treated samples. This could be the result of a stress condition, e.g. due to an

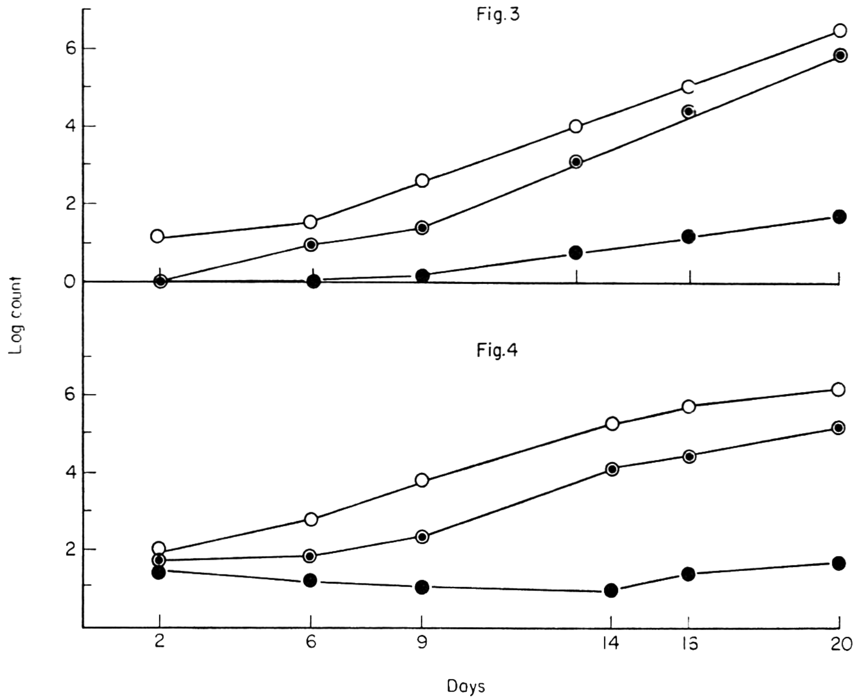

FIG. 3. Starved trout. Bacterial growth in mechanically treated trout vacuum-packed and stored in ice. Same symbols as in Fig. 1. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 0·50 log units.

FIG. 4. Feeding trout. Bacterial growth in mechanically treated trout vacuum-packed and stored in ice. Same symbols as in Fig. 1. Half confidence limit ($t \times s/\sqrt{n}$) on the calculations is 0·49 log units.

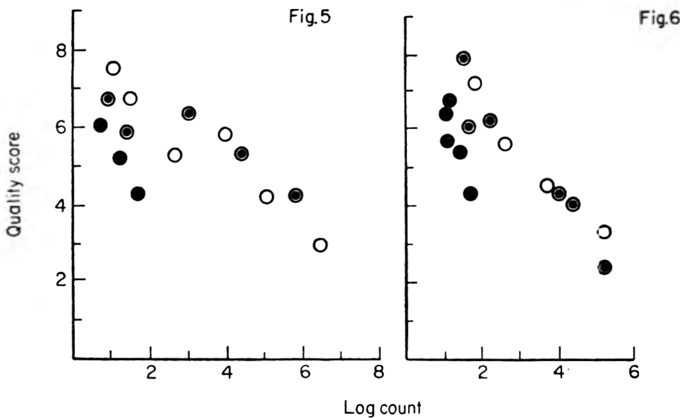

FIG. 5. Starved trout. The quality scores plotted against the log bacterial count. Same symbols as in Fig. 1.

FIG. 6. Feeding trout. The quality scores plotted against the log bacterial count. Same symbols as in Fig. 1.

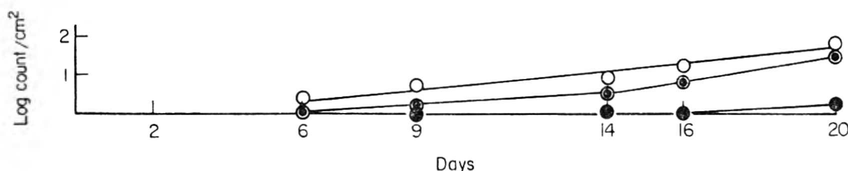

FIG. 7. Feeding trout. The log bacterial count/cm² on the naked surface of the muscle. The determination was made by the agar sausage technique. Same symbols as in Fig. 1.

increased production of adrenalin, thus forcing the blood into the muscles and gills. If the blood and/or lymph were infected during mechanical stress by bacteria from the intestines, these bacteria could be rapidly spread to the muscle tissue via the blood and lymph vessels.

The bacterial counts all show the same pattern, i.e. the counts in the flesh increase with increasing physical stress and are ten times higher in feeding fish than starved fish.

Correlation between organoleptic score and bacterial counts is good where the counts are higher than 100/g. The lack of correlation at lower bacterial counts should perhaps be expected since autolytic spoilage processes will be more active in the earlier storage period and will be the predominant factor influencing organoleptic assessment. There is, however, still a more rapid spoilage of the treated fish in the earlier storage period and this could possibly be explained by activation of tissue enzymes and acceleration of the autolytic processes.

The observed contamination under the skin could originate from either the skin or from deeper layers of the muscle through the vascular tissue. The low surface count compared to the relatively high volume counts seem to indicate contamination from deeper more heavily contaminated layers. This could be verified by a taxonomic study of the flora though interpretation will be difficult since intestinal contents exude from the trout during tumbling and contaminate the skin.

Penetration of bacteria from the skin cannot be excluded, especially in the later stages of storage; however, the data presented suggests that the main cause of spoilage of physically stressed fish is contamination of the flesh by micro-organisms from the intestines. This agrees with the findings of van Spreekens (1974), and would explain to some extent the findings of Huss *et al.* (1974) who showed that initial bacterial load did not have a large effect of subsequent spoilage.

Conclusion

The quality of the trout is reduced when the fish is exposed to physical stress. The quality decreases with increasing mechanical impact and is poorer for feeding than for starving fish.

The infection level in the fish muscle is increasing with physical stress and is higher for feeding than for starving trout.

At a higher infection level than a log count value of 2 there is a good correlation between the log count and the quality score.

There might be a penetration of bacteria from the surface flora through the skin during storage in ice. However, it is not considered a major cause of the reduction of the quality and keeping time.

Acknowledgments

The authors wish to express their thanks to Dr Knud Ølgaard, Danish Meat Research Institute, Roskilde for helpful discussions.

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J. Fd Technol. (1975) **10**, 515–522.

The effect of smoking and drying on the lipids of West African herring (*Sardinella* spp.)

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Summary

The fate of the lipids of smoke dried *Sardinella aurita* has been investigated. Peroxide values showed that smoke drying initiated lipid oxidation. Crude fractionation of the lipids showed that the site of initiation was bound lipids in contact with the proteins. Comparison of the oxygen uptake of smoke dried fish under different time–temperature conditions indicated that final moisture content was the predominant factor affecting rate of oxidation. The use of smoke did not appear to affect the rate of oxidation.

Introduction

Smoke drying is an ancient, well accepted method of preserving fish and is widely used in the tropics (Rawson, 1966). In West Africa, fish smoked on the coast is transported to the interior where it is often the major source of animal protein. Although the exact proportion varies from year to year, *Sardinella* spp. are usually the most abundant of the fish landed in Ghana.

Traditional smoking in mud ovens does not easily lend itself to the control of the quality of the finished product, its final moisture content, infestation and microbial contamination. In addition, the main catch of *Sardinella* spp. occurs in two to three months of the year. This throws a heavy burden on a basically inefficient processing system, which can result in poor quality smoke dried fish. Recently, attempts have been made to improve oven design and temperature control, and to package the final smoke dried product (Youngs, 1974). Long term storage of packaged smoke dried *Sardinella* spp. can prevent insect and microbial spoilage. Little is known about the influence of chemical changes such as lipid oxidation on storage life.

The oxidative deterioration of fish oils and fish products has been reviewed (Banks, 1967). Problems of rancidity in dried fish products were also mentioned. As fish lipids are highly unsaturated, they are extremely susceptible to oxidation. The use of wood smoke in preventing lipid oxidation in meat and fish products has been investigated (Lea, 1933; Banks, 1952). Polyphenols derived from the smoke acted as antioxidants.

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In this investigation, the oxidation of fish lipids was followed with the aim of minimizing chemical changes which might occur during the storage of smoke dried *Sardinella* spp.

Materials and methods

A catch of fresh *Sardinella aurita* was purchased from local fishermen and kept in ice until required. After the large and small fish had been rejected, the remainder consisted of fish of approximately equal size (about 200 mm in length). The fish to be used were divided into seven batches of about thirty-five fish each. Three of the batches were dried further, three smoke dried and the seventh was kept frozen at −20°C. The fish were not gutted before drying since this is the normal procedure in locally produced dried fish. The temperatures used for drying were 60, 80 and 100°C.

Those fish which were smoke dried were processed in an electrically heated smoke house (Griffith Laboratories Ltd) using wood shavings as a source of smoke. Those dried without smoke were processed in a laboratory oven, containing a fan, set at the same temperature as the smoke house.

Immediately after drying three fish were taken from each batch. The heads, tails and skins of the three fish were removed and the muscle tissue carefully peeled away from the backbones. The tissue was then ground up using a pestle and mortar and sieved. The fraction between B.S. 10–20 mesh was retained. This sample was used for oxygen uptake and peroxide measurements as well as moisture determinations. The remaining fish from each batch were stored in polythene bags at ambient temperature (30–35°C) and assayed as required.

Moisture content

This was determined in triplicate on approximately 2 sg samples heated in a vacuum oven at 70°C for 24 hr. The results were calculated on a dry weight basis.

Peroxide values

Determinations were carried out in duplicate on samples of muscle tissue. The method used was essentially that described by Rockwood, Ramsbottom & Mehlenbacher (1947). The results were expressed as milliequivalents of oxygen/100 g of oil.

Oxygen uptake

About 1 g of the ground muscle tissue was weighed into a previously calibrated manometer flask (total volume 35 ml) which was fitted to the manometer. The temperature of the flask was maintained at 40 ± 0·05°C by a stirred water bath. The oxygen uptake was calculated as μl O_2/g of dry matter at 40°C.

Fish lipid fractionation

Three *Sardinella aurita* (each approximately 300 mm in length) were smoke-dried at 80°C for 33 hr; and the muscle tissue prepared as described previously. The free

lipids were extracted from the ground smoke-dried tissue by blending with petroleum ether (40–60°C) for 2 min. Three extractions were made. The petroleum ether was filtered, pipetted into the manometer flask and evaporated under vacuum. A sample of the petroleum ether extracted fish (bound lipids plus protein) was also weighed into a manometer flask and dried in a vacuum oven.

The bound lipids were extracted by blending petroleum ether extracted fish with chloroform : methanol (3 : 1 v/v) three times. Samples of the extract (bound lipids) and solvent extracted fish (lipid free protein) were transferred to manometer flasks and the solvent evaporated under vacuum as before.

Oxygen uptakes of the free lipids, bound lipids plus protein, bound lipids and lipid free protein and of the whole fish, were followed. The results were expressed as $\mu l\ O_2/$ equivalent gram of whole fish, which allowed the uptake of the individual components to be compared and related to oxidation of the whole fish.

Results

The processing conditions and final moisture content of the six dried samples are summarized in Table 1.

Examination of the change of peroxide values with storage time (Fig. 1) indicates that oxidation of the lipids has been initiated by the drying process. Comparison of these peroxide values with the results of the frozen control sample would also indicate that the hydroperoxides in dried fish are more unstable than in the control. The information obtained from Fig. 1 is a measure of hydroperoxide stability rather than of the overall oxidation process, and is therefore of limited value. Fish lipid hydroperoxides are known to be unstable (Banks, 1967). Other workers have found that oxygen uptake is a good index of lipid oxidation in dried meat products (Chipault & Hawkins, 1971).

Oxygen uptake of the six samples of dried fish are shown in Fig. 2. The shape of the curves shows a high initial rate of oxygen absorption, which decreases with time. Comparison of processing parameters with oxygen uptake does not reveal any relationship.

TABLE 1. Moisture content of dried *Sardinella* spp.

Sample no.	Temperature of drying (°C)	Time of drying (hr)	Treatment	Final moisture content g water/ 100 g solids
1	60	35	Smoke	15·0
2	60	41	No smoke	19·9
3	80	24	Smoke	12·3
4	80	20	No smoke	11·3
5	100	13·5	Smoke	18·2
6	100	13·5	No smoke	7·4

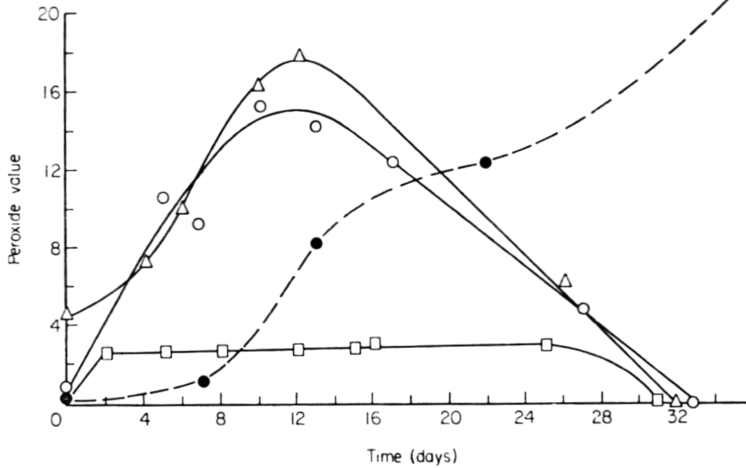

FIG. 1. Peroxide values of stored smoke dried *Sardinella* spp. □, Smoked at 60°C; ○, smoked at 80°C; △, smoked at 100°C; ● - - - ●, unsmoked, frozen control sample.

However, Fig. 3 shows that there appears to be a definite relationship between oxygen uptake and moisture content. The curve obtained shows that moisture content is the major factor influencing lipid oxidation in smoke dried fish.

The oxygen uptakes of the fish lipid fractions and whole fish are shown in Fig. 4. The bound lipids plus protein fraction is oxidizing at a similar rate to whole fish. The bound lipids also have a high initial rate of oxidation, whereas the free lipids have an induction period before oxidizing more rapidly.

Discussion and conclusions

The smoke drying process, which involves a heat process appears to cause lipid oxidation, as shown by peroxide values (Fig. 1). The site of initiation would seem to be the bound lipids in contact with protein (Fig. 4). The bound lipids are oxidized first as the bound lipids plus protein fraction contributes most to the oxygen uptake in the early stages. It is only later that the main contribution to oxygen uptake is made by the free lipids. Lipid free protein appears to be stable and does not oxidize at all.

The role of haeme pigments in muscle tissue in initiating lipid oxidation has been well established (Banks, 1937; Tappel, 1962; Uri, 1973) and this is confirmed by the results shown in Fig. 4. The dark muscle of pelagic fish such as *Sardinella aurita* has a high myoglobin content, and so is very effective in catalysing the oxidation of lipids. The bound lipid has a considerable phospho-lipid content. In other fish, phospholipids have been shown to contain highly unsaturated fatty acids (Notevarp, 1961; Hardy & Keay, 1972) which would be extremely susceptible to oxidation.

The rate of oxidation of fish lipids, at moisture contents between 7 and 20 g water/

100 g dry matter (Fig. 3), decreases with decreasing moisture content. The effect of water on lipid oxidation has been reviewed (Labuza, 1971), and has been found to be complex. In the low moisture content range, that is below the BET (Brunaeur, Emmett, Teller) monolayer value, water acts as antioxidant and hence lipid oxidation decreases with increasing moisture content (Labuza, Tannenbaum & Karel, 1970). In the intermediate moisture range, water acts as a prooxidant and hence lipid oxidation increases

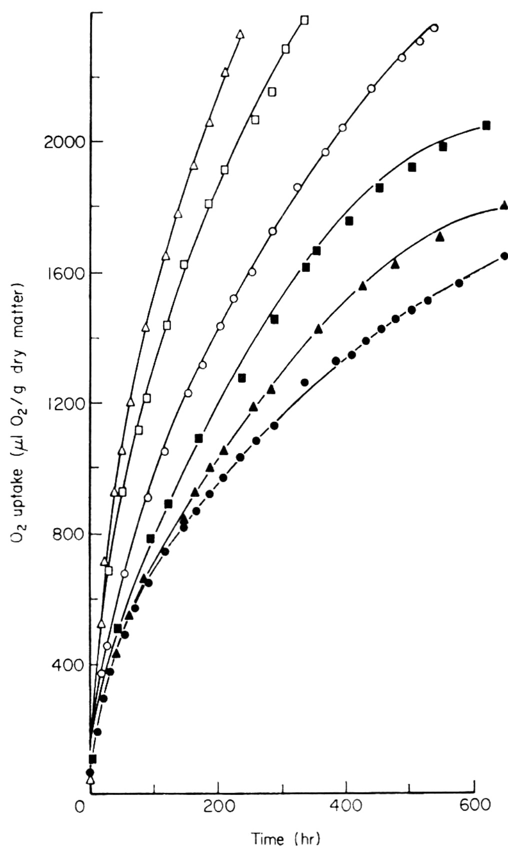

Fig. 2. Oxygen uptakes of smoke dried and dried *Sardinella* spp. immediately after drying. ○, Smoked at 60°C; △, dried at 60°C; ▲, smoked at 80°C; ■, dried at 80°C; □, smoked at 100°C; ●, dried at 100°C.

with increasing moisture content (Labuza & Chou, 1974). Recent work by Youngs (1974) in determining the sorption isotherm of smoke dried *Sardinella* spp. has indicated that the BET monolayer value is about 5 g water/100 g dry matter, but the exact value varies with lipid content. Therefore the fish used in the experiment, as well as the smoke dried fish produced traditionally are in the intermediate moisture range. Fish dried to moisture contents of 5–7 g water/100 g dry matter should have minimum rates of oxidation. Lipids in whole, dried fish will oxidize more slowly than in the samples

specially prepared for the oxygen uptake measurement, as oxygen has to diffuse through the muscle. The experiment does give relative values of oxidation and shows that if smoke dried *Sardinella* spp. is to be stored for some time it must be dried to a low moisture content, i.e. 5–7 g water/100 g solids. Unfortunately, high ambient temperatures and humidities make drying to these low moisture contents difficult and slow by traditional methods in Ghana.

Smoke had no effect on lipid oxidation, but this is not surprising since the fish is smoked intact, the skin acts as a barrier to the penetration of the flesh by smoke components.

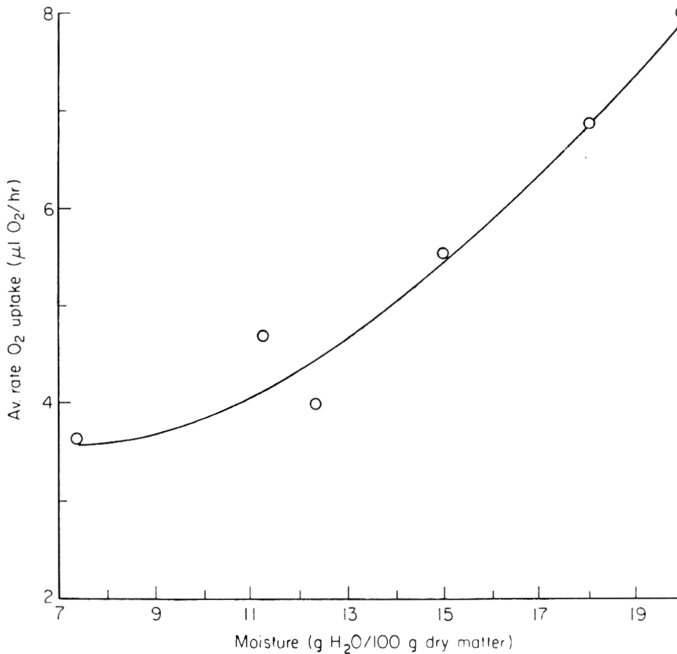

FIG. 3. Relationship between average rate of oxygen uptake and final moisture content of dried and smoke dried *Sardinella* spp.

Within the range 60–100°C (the normal range for the traditional process) temperature has little effect on lipid oxidation. That this appears dependent on moisture content rather than processing parameters is important to the traditional process of smoke drying in Ghana, which involves uncontrolled heating. Fish in the lower parts of the oven are frequently overheated. However, provided that moisture is equalized by changing the position of fish in the oven, this should have little effect on the oxidation of the lipids.

The smoke drying process initiates lipid oxidation which can be kept to a minimum by control of moisture levels. The storage life of the processed fish will eventually be limited by the production of off flavours, and possible loss of nutritional value via

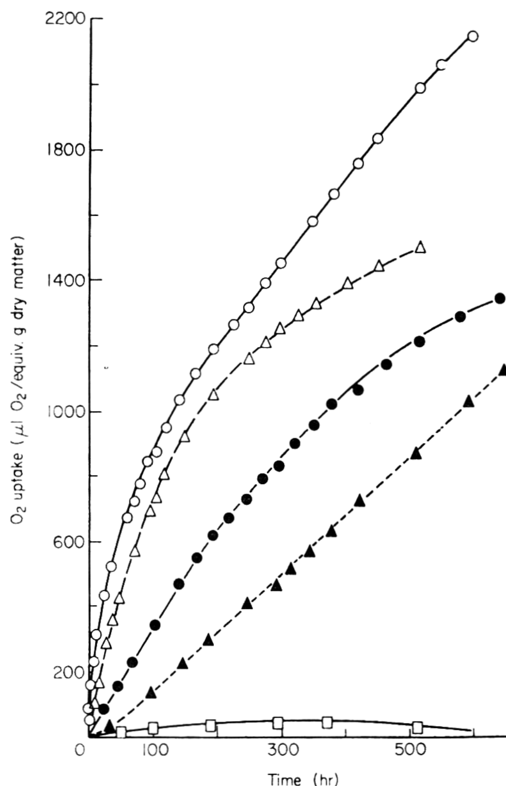

Fig. 4. Oxygen uptakes of the lipid fractions of smoke dried *Sardinella* spp. ○ - - - ○, Whole fish; △——△, bound lipids plus protein; ●——●, bound lipids; ▲ - - - ▲, free lipids; □——□, lipid-free protein.

oxidation of protein (Karel, 1973). Sensory evaluation of smoke dried *Sardinella* spp. is now in progress, but samples stored for twelve months have already been found acceptable when prepared in local dishes (Youngs, 1974).

Acknowledgments

The work was carried out as part of the Ghana Government–I.D.R.C. (International Development and Research Centre, Ottawa, Canada)–Rural Fisheries Project. The author would like to thank Mr K. Wilson for the determination of peroxide values and the Department of Biochemistry, Legon for the use of the manometers.

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J. Fd Technol. (1975) **10**, 523–532.

Utilization of edible protein from meat industry by-products and waste

V. The characterization of extracted proteins, using polyacrylamide gel electrophoresis incorporating sodium dodecyl sulphate

R. H. YOUNG* AND R. A. LAWRIE

Summary

Characteristic patterns for soluble proteins extracted from meat waste tissues at alkaline pH have been established using polyacrylamide gel electrophoresis incorporating sodium dodecyl sulphate (SDS). Whilst there were distinct differences between the character of proteins recovered from lung, stomach and plasma, little interspecies variation was detected. The compositions of the protein isolates from lung and stomach differed from those of the soluble protein extracts since a major component of molecular weight 70 000 remained soluble at the isoelectric pH. Electrophoretograms of spun proteins were characterized by the presence of a dense component of high molecular weight (about 130 000) which was not apparent in the proteins prior to spinning.

Introduction

Preliminary work in this study using the technique of SDS-polyacrylamide gel electrophoresis has indicated that compositional differences exist between proteins extracted from ovine lung and stomach (Young & Lawrie, 1974a). In view of the lack of information on the character of offal proteins, it was thought desirable to extend the electrophoretical analyses and to elucidate any interspecies variation between the recovered protein extracts. In addition, changes in the nature of the protein extracts prior to and during the texturization procedure were investigated and the significance of the findings is discussed.

Materials and methods

Preparation of protein samples for electrophoresis

Soluble proteins were recovered at pH 10 from bovine lung, rumen, reticulum, omasum and abomasum, ovine lung and rumen and porcine lung and stomach. About

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200 g of each tissue was taken and the protein extracts were purified, dialysed and freeze dried. Seven milligrams of dried protein was treated with electrophoresis buffer containing 1% (w/v) SDS and 1% β-mercaptoethanol.

Protein isolates were prepared from bovine lung and porcine stomach by adjusting the pH of alkaline protein extracts to 4·0, 4·5 and 5·0 respectively. The isolated proteins were recovered by centrifugation at 12 000 **g** for 20 min. Protein contents were determined on 2-ml aliquots of the supernatants and 1-g samples of the isolates by the microkjeldahl method. For application to the gel, the concentration of protein in the supernatants was adjusted to 5 mg/ml with distilled water and 0·01 g SDS and 0·01 ml β-mercaptoethanol added to 1 ml of the sample. Similarly, an amount of isolate containing 5 mg protein was dissolved in SDS/β-mercaptoethanol in electrophoresis buffer.

For electrophoretical analysis of fibrous proteins, prepared by coagulation of an alkaline dope in an acetic acid/salt solution (Young & Lawrie, 1974b), the protein contents of spun samples from plasma, lung and stomach proteins were measured by the microkjeldahl method. A quantity of protein fibre containing 5 mg of protein was taken and treated with SDS/mercaptoethanol solution.

Plasma samples were prepared by diluting fresh plasma to a protein concentration of 5 mg/ml with distilled water. A 1-ml aliquot was taken for electrophoresis to which was added 0·01 g SDS and 0·01 ml β-mercaptoethanol.

Each sample was incubated overnight at 25–35°C and sucrose (10% w/v) added to the solution prior to electrophoresis.

Electrophoretical procedure

The technique employed for electrophoresis was similar to that previously described (Young & Lawrie, 1974a) but was modified slightly for the present study. A single 10% (w/v) acrylamide gel was cast between the vertical plates and the voltage applied during electrophoresis was 20 v for 15 min followed by 140 v for 1 hr. Thus, in comparison with the former system the time required for electrophoresis was considerably reduced. Concomitantly, these modifications resulted in improved resolution of protein bands. Staining was effected using 0·5% (w/v) coomassie blue in 40% (w/v) methanol/1 M acetic acid solution.

Laser densitometry

The thin layer polyacrylamide electrophoretograms were scanned using the technique of laser beam densitometry described by Parsons *et al.* (1969).

Molecular weight determination

Further modifications have been applied to the method of molecular weight determination. A calibration graph of mobility v. log-molecular weight was constructed using the following proteins: trypsin (23 300), aldolase (40 000), 3 phosphoglycerate

kinase (48 500), albumin (69 000), fructose-6-phosphokinase (74 000), phosphorylase B (92 500) and lipoxidase (97 440).

A sample of plasma protein was included alongside the market proteins. This allowed direct determination of the molecular weights of the plasma protein components using the calibration graph obtained from the standard proteins. Plasma was then used as a standard protein mixture and incorporated in the same gel as the unknown components. Consequently, molecular weights could be derived from a calibration graph constructed under identical electrophoretical conditions.

An additional modification was that the mobilities of protein bands were measured from the densitometer traces. Mobility was then defined as the distance of a peak from the origin divided by the total chart width. This was found to facilitate and improve the accuracy of the measurement.

Results

Effect of species and tissue type on protein character

Facsimiles of densitometer traces showing patterns for the full range of soluble protein extracts studied are given in Fig. 1. Comparison of the traces obtained for lung protein extracted from the three different animal species indicated that the patterns are essentially similar. The stomach proteins extracted from the three species also exhibit a high degree of similarity.

Molecular weights of all the detectable components were estimated from the calibration graph (Fig. 2) derived from plasma protein separated on the same gel and are presented in Table 1. It was apparent that a subunit of molecular weight 114 000–119 000 was common to both lung and stomach proteins extracted at pH 10. Subunits of molecular weight 66 000–71 000 and 16 500–17 500 were also present in all extracts. The characteristic feature of the lung protein extracts, however, was that the subunit of molecular weight 17 000 appeared as a particularly dense band. This was not the case for stomach protein extracts which possessed a group of three components at high concentration with molecular weights in the range 36 500–43 500. Although a number of similar components were detectable in the lung protein electrophoretograms, their proportion was substantially reduced.

Notwithstanding the fact that proteins extracted from corresponding tissues were essentially similar irrespective of species, a number of minor differences were elucidated. In particular, two additional components of molecular weight 18 500 and 13 500 occurred in bovine lung protein so that the densitometer trace (Fig. 1e) showed some variation over those obtained for ovine and porcine lung protein. Also, porcine stomach protein (Fig. 1d) contained diminished concentrations of two low molecular weight subunits (19 000 and 20 000) in comparison with ovine and bovine stomach protein.

Another feature which became apparent was that compositional differences occurred

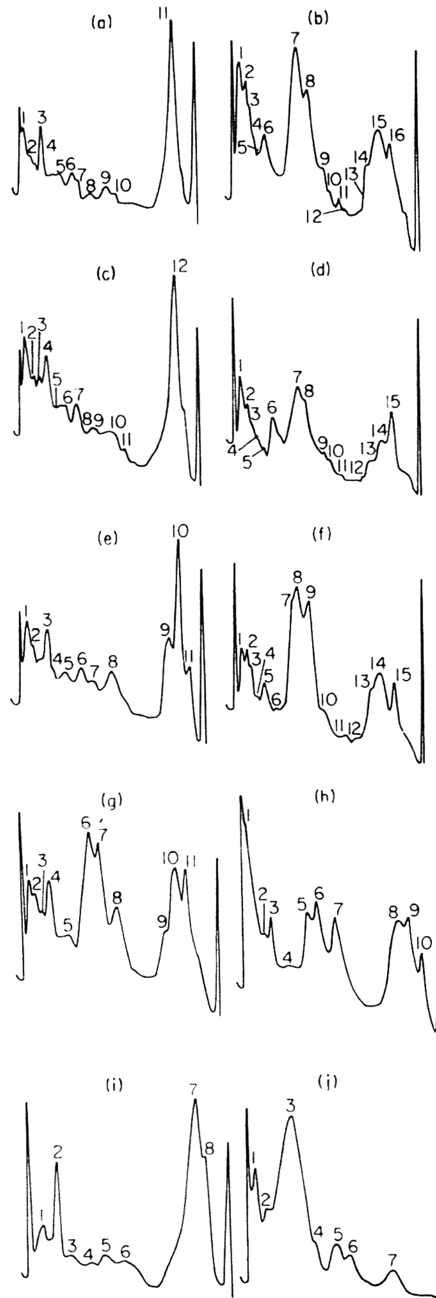

FIG. 1. Densitometric tracings (by laser beam) of polyacrylamide electrophoretograms in SDS of proteins extractable at pH 10 from (a) ovine lung, (b) ovine rumen, (c) porcine lung, (d) porcine stomach, (e) bovine lung, (f) bovine rumen, (g) bovine reticulum, (h) bovine omasum and (i) bovine abomasum. The pattern for bovine plasma is also shown (j). The estimated molecular weights represented by the numbered peaks are listed in Table 1.

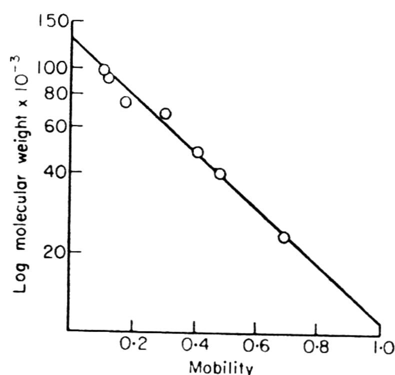

FIG. 2. Plot of the logarithms of the molecular weights of plasma protein components treated with SDS versus their mobilities on a 10% polyacrylamide gel.

between protein extracts from the four compartments of the bovine stomach. It appeared that the protein extracts from reticulum, omasum and abomasum comprised a lower number of components than the rumen extract and differences in the electrophoretical patterns were observed. The molecular weights of individual components are again compared in Table 1. Reticulum and omasum were characterized by the appearance of

TABLE 1. Molecular weights of protein subunits extracted at pH 10 from meat waste tissues as estimated by SDS-polyacrylamide gel electrophoresis (major components in bold)

Component no. (see Fig. 1)	Ovine lung	Porcine lung	Bovine lung	Ovine rumen	Porcine stomach	Bovine rumen	Bovine reticulum	Bovine omasum	Borine abomasum	Bovine plasma
1	**114 000**	**119 000**	**115 000**	**119 000**	**119 000**	**118 000**	**115 000**	**125 000**	**115 000**	**115 000**
2	78 000	100 000	102 000	109 000	110 000	111 000	96 000	76 000	**68 000**	89 000
3	**71 000**	78 000	**70 000**	100 000	100 000	109 000	75 000	**66 000**	47 000	**65 000**
4	55 000	**66 000**	62 000	93 000	93 000	98 000	**67 000**	52 000	**41 500**	40 500
5	52 000	57 000	49 000	81 000	80 000	**67 000**	55 000	**42 000**	**36 000**	**29 000**
6	**43 000**	46 000	**41 500**	**70 000**	**67 500**	57 000	**41 500**	**36 500**	29 000	**25 000**
7	37 500	**40 500**	38 500	**43 500**	**43 500**	**43 500**	**36 500**	**28 500**	**17 000**	**18 800**
8	32 000	37 500	**26 500**	**41 500**	**41 500**	**39 000**	**28 000**	**18 000**	13 500	
9	**26 500**	34 000	**18 500**	**36 500**	**38 000**	**36 500**	**20 000**	**17 000**		
10	24 500	**26 000**	**17 000**	34 000	36 500	31 500	**18 500**	13 500		
11	**17 000**	21 500	**13 500**	32 000	29 000	17 000	17 000			
12		**16 500**		27 000	22 500	23 000				
13				22 000	20 000	20 000				
14				21 000	18 500	**19 000**				
15				**19 000**	**17 500**	**17 000**				
16				**17 500**						

a component of molecular weight 28 000–28 500 giving rise to a distinct densitometric peak (Fig. 1g and h). This component was either absent or its concentration much reduced in the extracts from rumen and abomasum. Moreover, a large proportion of high molecular weight material (125 000) was apparently present in the extract from omasum. Abomusum protein exhibited an electrophoretical pattern quite dissimilar from the other stomach proteins. Figure 1i indicates that the densitometric trace obtained for abomasum protein extract shows a greater similarity to the trace for lung protein, being typified mainly by a high density of low molecular weight components.

The plasma protein pattern (Fig. 1j) comprising mainly albumin (69 000) differed appreciably from all the other patterns.

Effect of isoelectric precipitation on protein character

The densitometer traces obtained from the bovine lung protein electrophoretograms show the distribution of the original protein components between the isolates and the supernatants at pH 4·0, 4·5 and 5·0 respectively (Fig. 3). By referring to Fig. 3a and b,

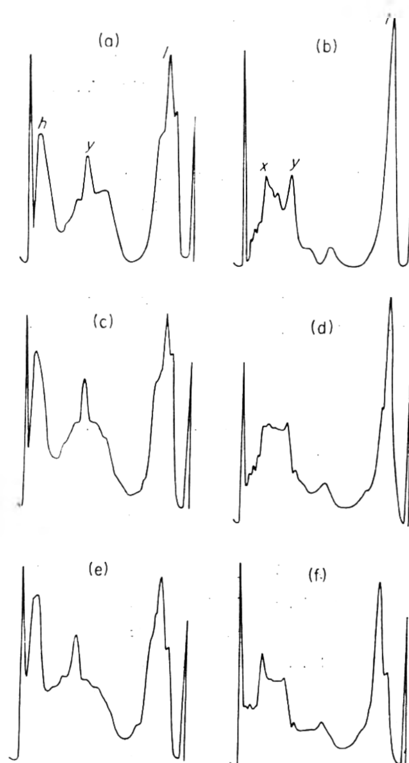

Fig. 3. Densitometric tracings (by laser beam) of polyacrylamide electrophoretograms in SDS characteristic of bovine lung protein present in (a) isolate at pH 4·0, (b) supernatant at pH 4·0, (c) isolate at pH 4·5, (d) supernatant at pH 4·5, (e) isolate at pH 5·0 and (f) supernatant at pH 5·0.

it can be seen that the component of molecular weight 115 000 (h) is present only in the isolated protein whereas the component of molecular weight 70 000 (x) retains its solubility at this pH and is common only to the protein of the supernatant. The other major subunits of lung protein, y and l (of molecular weights 42 000 and 17 000 respectively) appear to be fairly evenly distributed between the isolate and the supernatant. Variation of the pH between 4·0 and 5·0 had little effect on these observations.

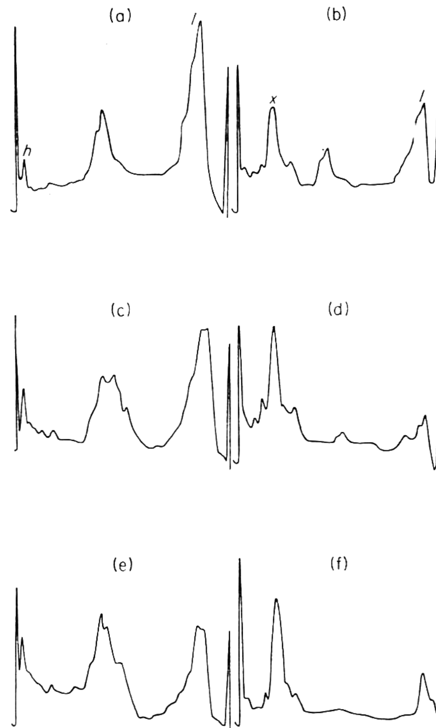

FIG. 4. Densitometric tracings (by laser beam) of polyacrylamide electrophoretograms in in SDS characteristic of porcine stomach protein present in (a) isolate at pH 4·0, (b) supernatant at pH 4·0, (c) isolate at pH 4·5, (d) supernatant at pH 4·5, (e) isolate at pH 5·0 and (f) supernatant at pH 5·0.

Corresponding densitometer traces for porcine stomach protein are reproduced in Fig. 4. Again, the most noticeable feature was the presence of component x (70 000) exclusively in the supernatant. The relative concentration of component h (115 000) in the isolate was considerably lower than in the lung protein isolate and in this instance was detected to a very small extent in the supernatants at pH 4·0 and 5·0. Varying the pH of isolation between 4·0 and 5·0 had a definite effect on the distribution of components in the isolates and supernatants from the stomach protein extracts. At pH 4·0, the concentration of the low molecular weight component (l) in the isolate was very high relative to the components within the range 36 000–43 000 which were present to some

degree also in the soluble protein. However, the concentration of these latter subunits increased in the isolated fraction and decreased in the soluble fraction as the pH of isolation was raised to 5·0. Thus, it appears that for stomach protein variation of the pH between 4·0 and 5·0 could be a critical factor in determining the composition of the isoelectric precipitates.

The nature of protein in spun fibres

Densitometer traces obtained from spun bovine serum and lung proteins are shown in Fig. 5. The most obvious feature characteristic of the spun proteins was the presence of a dense component (*p*) in close proximity to the origin. This component consistently appeared in electrophoretograms from spun proteins and its molecular weight was estimated at about 130 000. The low molecular weight components, typical of bovine

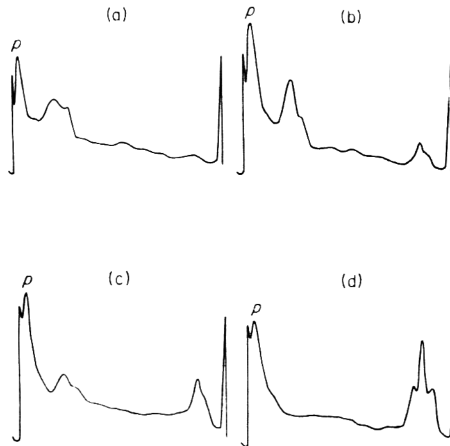

Fig. 5. Densitometric tracings (by laser beam) of polyacrylamide electrophoretograms in SDS of spun proteins. (a) Plasma protein, (b) 80% plasma/20% lung protein, (c) 50% plasma/50% lung protein and (d) lung protein. Note the high molecular weight subunit (*p*) common only to the spun proteins.

lung protein, were clearly resolved in the spun form and were also readily detectable in fibres spun from mixtures of lung and blood plasma protein. However, the other major components of the alkaline protein extract from lung were absent in these electrophoretograms. The pattern for spun plasma protein exhibited close similarity to that for normal plasma protein apart from the presence of the high molecular weight component (*p*).

Discussion

The results of the electrophoretical analysis of offal carried out in the present study differed in some respects from those initially established (Young & Lawrie, 1974a). In particular, with regard to the lung protein extracts, several slowly-migrating bands,

not apparent in the 8% gel, were clearly resolved in the 10% gel incorporating a higher voltage. In the main, the molecular weight values determined for the lung and stomach protein components were in agreement with those previously estimated. However, at the extreme limits of the molecular weight range there were some deviations. These differences probably reflect the inefficiency of resolution of proteins and molecular weight determination using the original electrophoretical procedure.

SDS-electrophoresis established that compositional differences occurred between soluble proteins recovered from the different offal tissues under investigation although little interspecies variation was encountered. It appears that, in general, three distinctly different types of soluble protein extract—lung, stomach and plasma—may be recovered and that they show characteristic electrophoretical patterns.

Although the majority of the components for the lung and stomach protein extracts may be characteristic of smooth muscle, there is a possibility that a quantity of collagen would be recovered in the alkaline extracts. Allowing for experimental error in the determination of molecular weight which may be of the order of ± 10% (Weber & Osborn, 1969), it seems feasible that the subunit of molecular weight 114 000–119 000, common to all protein extracts at pH 10, may represent an α-chain of collagen. The molecular weight of the α-class of denatured collagen has been estimated at 100 000 (Nagai, Gross & Piez, 1964), and alkali-soluble collagen has been shown to comprise a high proportion of α-subunits (Kemp & Tristram, 1971). The extent to which collagen is present in soluble protein extracts is important by virtue of its possible effect on nutritional and functional properties.

The finding that the compositions of the protein isolates (the raw material for fibre spinning) from lung and stomach differed from those of the original extracts may also have some significance with regard to their texturization. The fact that the major subunit of molecular weight 70 000 was completely excluded from the isolates suggests that the necessity for the isolation stage is a disadvantage in the preparation of texturized proteins from these sources. This further emphasizes the advantages of plasma as a raw material for fibre formation since the isolation step prior to spinning is avoided (Young & Lawrie, 1974b).

The electrophoretical patterns obtained for the spun proteins provided some interesting information regarding the nature of protein–protein interactions in the fibres. The dense high molecular weight component (*p*) appears to represent a protein aggregate formed as a consequence of the spinning process since it was totally absent from electrophoretograms obtained from proteins prior to processing. Any association involving electrostatic, hydrogen or disulphide linkages should have been disrupted by the treatment with SDS and β-mercaptoethanol releasing the components for separation by electrophoresis. Hence, it may be suggested that covalent links, other than disulphides, may occur between proteins subjected to the spinning procedure. Indeed, interactions between ϵ-amino groups of lysine residues and modified sulphur groups provided by cystine (Speakman, 1933; Bohak, 1964; Zeigler, 1964) would appear to be feasible

under the conditions existing during spinning, i.e. alkali treatment of proteins followed by coagulation in acid and salt. The extent to which these covalent links may be formed could have a significant bearing on the gel dimensions and strengths of protein fibres used as a base for simulated meat products. A deeper knowledge of the kind of protein–protein interactions involved in spun products may assist in controlling the texture of such products to more specific requirements.

Acknowledgment

This work was supported by a grant from the Meat and Livestock Commission which is gratefully acknowledged.

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(*Received* 20 *December* 1974)

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Nutritional and chemical changes in heated casein

III. A comparison of the alkali-soluble and insoluble fractions

R. C. OSNER

Summary

The NPU, Biological Values, Digestibilities and RNV of the alkali soluble and insoluble fractions of casein were similar both to each other and to the corresponding samples of total heated casein. There were differences in amino acid composition between the soluble fractions, which contained progressively lower concentrations of aspartic acid, threonine, serine, glutamic acid, histidine and lysine as severity of heat treatment increased, and their corresponding insoluble fractions, the latter tending to have a similar composition to their total heated samples.

After the removal of both fractions, a clear yellow solution remained, which must have contained certain as yet unidentified but soluble breakdown products. It is postulated that differences in solubility between the two fractions may have resulted from differences in molecular weight.

Introduction

When dry casein is heated at 120° or above it is partially denatured, and becomes insoluble (Mecham & Olcott, 1947). This is accompanied by the destruction of certain amino acids (Osner & Johnson, 1974.) Furthermore the availability of some amino acids may be partly lost, causing a decrease in the nutritional value of the product (Osner & Johnson, 1975). In this study, the amino acid compositions and nutritional values of the soluble and insoluble fractions of heated commercial casein have been examined in relation to the corresponding total samples in a study of the process of denaturation in this protein system.

Experimental

Analytical techniques

The solubility of casein, moisture, total nitrogen and total amino acid content were

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determined as before (Osner & Johnson, 1974, 1975). Chemically 'available' lysine content was determined by difference between total lysine content and 'residual' lysine (Roach, Sanderson & Williams, 1967) left after the reaction of the casein with fluoro-2, 4-dinitrobenzene (FDNB).

Nutritional techniques

Net Protein Utilization (NPU), Biological Value (BV), Digestibility (D), Relative Nutritive Value (RNV), and microbiological availability of some amino acids were determined as before (Osner & Johnson, 1975).

Preparation of samples

Samples of dry casein* (6·3% moisture) were prepared and heat-treated as described previously (Osner & Johnson, 1974), before separation into alkali-soluble and insoluble fractions. The heated casein (425 g, equivalent to 400 g moisture-free casein) was dispersed in sodium hydroxide (4 litres, 0·1 N) using a 'Silverson' mixer and stirred at room temperature for 1 hr before the soluble fraction was separated from the insoluble fraction using a 'Sharples' continuous centrifuge. The insoluble fraction was washed with deionized water (1 litre) and adjusted to pH 7 before separation, the washings being added to the soluble fraction. The insoluble fraction was washed twice more with deionized water (1-litre portions) before being filled into aluminium trays. The washings were added to the soluble fraction which was then precipitated by the slow addition, over a period of about 1 hr, of concentrated hydrochloric acid (2 N) until pH 4·6 was reached. The mixture was stirred continuously during the addition of hydrochloric acid and subsequently for a further hour before filtration. The precipitate was carefully washed twice using deionized water before being placed in aluminium trays. Both fractions were frozen at −40°, freeze-dried (Edwards High Vacuum Ltd) and then ground mechanically in a mill (Pascall Engineering Ltd) to a fine powder.

Results and discussion

The results from Table 1 show that the NPU of the soluble and insoluble fractions of the samples heated at 120° for 8 hr and 130° for 4 and 8 hr were significantly lower than control casein and this was also true of their Digestibilities and Biological Values except for the soluble fraction heated at 130° for 4 hr which had a similar Biological Value to control casein. Furthermore, the NPU, Biological Values, Digestibilities and RNV of the soluble and insoluble fractions of casein were similar both to each other and to the corresponding samples of total heated casein previously processed (Osner & Johnson, 1975). The similarity of the Digestibilities implies that the two fractions would be equally susceptible to hydrolysis in the gut and that similar quantities of the products

* The commercial casein used for the experiments was a gift from Swift Ltd and came from New Zealand.

TABLE 1. Net protein utilization, Biological Value, Digestibility and relative nutritive value of the soluble and insoluble fractions of heat treated casein

Biological or microbiological test	Control* casein (mean and corrected standard deviation	Soluble fraction					Insoluble fraction				
		120°		130°			120°		130°		
		4 hr	8 hr	2 hr	4 hr	8 hr	4 hr	8 hr	2 hr	4 hr	8 hr
NPU	72 ± 3·2	73	59	77	63	54	70	57	69	60	50
BV	76 ± 3·2	82	69	82	72	62	79	65	76	67	60
D	94 ± 3·0	89	85	94	87	87	89	88	91	89	84
RNV	100 ± 5·1	88	86	88	88	81	88	85	89	88	82

* Values taken from Osner & Johnson (1975).

of digestion were absorbed. The similarity of the Biological Values of the two fractions implies that the availabilities of the limiting amino acids should be similar.

There were differences between the total amino acid content of the alkali-soluble and insoluble fractions of casein (Table 2). As processing becomes more severe, there is

TABLE 2. Total amino acids in soluble and insoluble fractions of heated casein (μmol/g protein)

Amino acid	Soluble fraction					Insoluble fraction				
	120°		130°			120°		130°		
	4 hr	8 hr	2 hr	4 hr	8 hr	4 hr	8 hr	2 hr	4 hr	8 hr
Aspartic acid	500	500	480	470	420	530	520	520	490	470
Threonine	340	340	330	300	260	360	340	340	340	320
Serine	470	490	500	460	400	510	500	500	480	430
Glutamic acid	1480	1470	1460	1370	1240	1550	1530	1560	1520	1550
Proline	860	960	900	930	870	810	810	890	890	840
Glycine	230	260	240	260	260	260	250	240	250	280
Alanine	330	350	350	350	350	340	330	310	310	310
Valine	500	500	530	510	520	540	530	550	530	540
Methionine	180	170	190	170	160	190	180	190	190	180
Isoleucine	370	340	370	350	350	380	370	370	360	360
Leucine	670	670	650	650	660	660	660	660	660	640
Tyrosine	340	340	340	340	350	360	340	330	330	340
Phenylalanine	310	310	290	300	280	320	320	310	320	290
Histidine	160	150	150	150	130	180	180	180	170	160
Lysine	480	420	500	420	330	530	520	540	540	500
Arginine	200	200	210	200	200	200	210	220	210	200

a comparatively greater fall in the aspartic acid, threonine, serine, glutamic acid, histidine and lysine contents of the soluble fractions compared with the insoluble fractions, this being particularly apparent in the most severely processed sample (Table 3). The total amino acid composition of the insoluble fraction bore a closer

TABLE 3. Comparison of the true total amino acid content of casein heated at 130° for 8 hr with the results obtained from the soluble and insoluble fractions (μmol/g)

Amino acid	Soluble fraction		Insoluble fraction		Calculated total	True total*
	Total	28%	Total	72%		
Cystine	25	7·0	18	13	20	25
Aspartic acid	420	117·6	470	338·4	456	480
Threonine	260	72·8	320	230·4	303	330
Serine	400	112·0	430	309·6	422	470
Glutamic acid	1240	347·2	1550	1116·0	1463	1580
Proline	870	243·6	840	604·8	848	890
Glycine	260	72·8	280	201·6	274	280
Alanine	350	98·0	310	223·2	321	330
Valine	520	145·6	540	388·8	534	500
Methionine	160	44·8	180	129·6	174	180
Isoleucine	350	98·0	360	259·2	357	350
Leucine	660	184·8	640	460·8	646	640
Tyrosine	350	98·0	340	244·8	343	310
Phenylalanine	280	78·4	290	208·8	287	280
Histidine	130	36·4	160	115·2	152	160
Lysine	330	92·4	500	360·0	452	440
Arginine	200	56·0	200	144·0	200	220

* Osner & Johnson (1974).

resemblance to that of its corresponding total sample than the soluble fraction. In previous work (Osner & Johnson, 1975), it was found that only 28% of the casein remained soluble after processing at 130° for 8 hr. If it is assumed that the soluble fraction contributed 28% and the insoluble fraction contributed 72% of the amino acids in the total sample, the contributions may be summed and compared with the observed amino acid composition of the total sample (Table 3). There is apparently a discrepancy for the following five amino acids: cystine, aspartic acid, threonine, serine and glutamic acid, the calculated total concentrations of these amino acids being lower than the corresponding true determined totals. It is possible that if peptide links in the casein molecules are broken during severe heat treatment, as appears to occur with bovine serum albumin (Bjarnason & Carpenter, 1970), peptides containing these

five amino acids may be formed. If the peptides were small (Osner & Johnson, 1974), they would not be precipitated at pH 4·6 at the same time as the alkali-soluble fraction was precipitated and so would not contribute to the amino acid content of either fraction. It was noted that after the removal of both fractions, the remaining solution, although clear, had a yellow colour and distinctive odour; an analysis of this solution might yield useful results pertaining to the breakdown products of heated casein.

The microbiological availabilities of each of the amino acids were similar for the soluble and insoluble fractions of casein processed under a particular set of conditions (Table 4). The availability of the most limiting amino acid, methionine, in each soluble

TABLE 4. Microbiologically available amino acids in soluble and insoluble fractions of heated casein (μmol/g protein)

Amino acid	Soluble fraction					Insoluble fraction				
	120°		130°			120°		130°		
	4 hr	8 hr	2 hr	4 hr	8 hr	4 hr	8 hr	2 hr	4 hr	8 hr
Valine	540	520	570	520	500	540	530	570	540	520
Methionine	180	170	180	170	160	180	170	180	170	170
Isoleucine	420	400	420	380	370	420	400	420	380	380
Leucine	680	650	670	660	650	680	680	670	670	650
Histidine	150	140	160	140	110	150	150	160	140	130
Arginine	180	170	190	170	160	190	160	190	170	160
Tryptophan	50	48	48	48	47	48	47	48	48	46

fraction was similar to that in each corresponding insoluble fraction and total casein sample, resulting in similar Net Protein Utilisation and Relative Nutritive Values. The reaction of fluoro-2,4-dinitrobenzene with lysine, histidine and tyrosine residues went to completion under the conditions employed, although there was also a partial reaction with arginine, threonine and serine residues (Table 5). With tyrosine, threonine and serine, the reaction is probably with the hydroxyl group. The amount of chemically 'bound' lysine was similar for both fractions of casein processed at 130° for 8 hr (Table 6), although little histidine or tyrosine appeared to be involved in cross-linking reactions. However, chemically 'available' histidine fell from 160 μmol/g casein in control to 140 μmol/g after heat treatment (Table 7), since some histidine was destroyed. The chemically 'available' lysine contents of the two-fractions were not similar, but this was due to the initial differences in the total lysine content of the two fractions. Since both chemical binding and microbiological availability of the amino acids in the two fractions appear to be similar, it may only be a difference in molecular weight (Osner & Johnson, 1974) that causes a difference in solubility between the two fractions.

TABLE 5. Some total, residual 'bound'* and available amino acids in casein (μmol/g protein)

Amino acid	Control casein		Residual amino acids in casein heated at 130° for 8 hr*			Chemically 'available' amino acids in casein heated at 130° for 8 hr‡			
	Total	Residual	Unseparated	Soluble fraction	Insoluble fraction	Control	Total	Soluble	Insoluble
Threonine	370	270	†	†	†	†	†	†	†
Serine	580	240	†	†	†	†	†	†	†
Tyrosine	330	4	2	4	4	330 (100%)	310 (100%)	350 (100%)	340 (100%)
Histidine	180	22	18	20	9	160 (89%)	140 (87%)	110 (85%)	150 (94%)
Lysine	520	18	73	66	77	500 (96%)	370 (84%)	260 (79%)	420 (84%)
Arginine	210	99	†	†	†	†	†	†	†

* After reaction with FDNB.

† Not measured as reaction with FDNB had not gone to completion.

‡ The percentage availability compared to total chemically determined result is given in brackets after the corresponding concentration.

TABLE 6. Some residual 'bound' amino acids after reaction of FDNB with casein (μmol/g protein)

Amino acid	Control casein	Casein heated at 130° for 8 hr		
		Total	Soluble	Insoluble
Histidine	22	18	20	9
Lysine	18	73	66	77
Tyrosine	4	2	4	4

TABLE 7. Some chemically available amino acids in case in (μmol/g protein)

Amino acid	Control casein	Casein heated at 130° for 8 hr		
		Total	Soluble	Insoluble
Histidine	160	140	110	150
Lysine	500	370	260	420
Tyrosine	330	310	350	340

Acknowledgments

The author would like to thank both Professor A. E. Bender, with whom helpful discussions were held, and particularly Dr R. M. Johnson for helpful advice and for reading the manuscript. Grateful acknowledgment is also made to Miss J. Wilson and Mr D. Cortis for technical assistance.

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(*Received* 10 *October* 1974)

J. Fd Technol. (1975) **10**, 541–546.

Properties of protein isolates prepared from ground seeds

I. Development and evaluation of a dye binding procedure for the measurement of protein solubility

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Summary

A dye binding procedure has been developed for the estimation of protein in seed extracts and solutions of seed protein isolates. The method was evaluated using extracts of five seed materials, viz. field beans (*Phaseolus vulgaris*), rapeseed (*Brassica napus*), sesame seed (*Sesame indicum*), cowpeas (*Vigna unguiculata*) and cottonseed (*Gossypium hirsutum* L.). A high correlation between the dye binding measurements and analyses obtained by the Kjeldahl procedure has been demonstrated and the suitability of the dye binding method for routine protein estimations in this work has been confirmed.

Introduction

The seeds of many crops have potential as rich sources of protein but their nutritional and technological value is limited because of the presence of toxic factors, fibrous constituents, soluble carbohydrates, pigments, etc. Before these proteins can be widely utilized for human nutrition, therefore, it is necessary to extract them from the seeds. The functional properties of the resultant isolates must be determined subsequently in order to assess their usefulness and potential both as foodstuffs and as ingredients in manufactured foods.

Seed protein isolates are usually prepared by extracting the ground seeds with an aqueous medium and separating the proteins from the extract by precipitation (Sims & Nunes, 1970). Accordingly, it is desirable to employ conditions which are optimal for the extraction of the proteins and so the acquisition of solubility data is an important aspect of preliminary work. For example, it is necessary to investigate the effects on protein extraction of processing parameters such as the mesh size employed in milling, extraction temperature, meal-to-water ratio, pH and ionic strength of the extractant, etc. (Cogan *et al.*, 1967; Lawton & Cater, 1971; Flink & Christiansen, 1973). Solubility

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is also an important functional property of seed protein isolates because it will indicate the conditions which are compatible with the technological use of a given preparation (Mattil, 1971), e.g. protein isolates which are sufficiently soluble at about pH 6 to 7 could be used in beverages such as 'vegetable milks'.

The Kjeldahl procedure was found to be too time consuming for the large number of protein determinations associated with these investigations. Of the possible alternatives considered, dye binding procedures were selected as being the most appropriate because of their convenience, high precision and low cost, and also because large numbers of samples could be rapidly analysed by these techniques (Cole, 1969; Lakin, 1973). In addition results can be obtained in a few minutes with semi-automatic equipment which is commercially available and it was thought that this equipment could be used to monitor the protein content of seed extracts during process control.

The procedure finally adopted was based on a method developed by Dolby (1961) which employed C.I. Acid Black 1. An important modification was the replacement of this dye with C.I. Acid Orange 12 because it is available in a pure state suitable for use as an analytical reagent (Sherbon, 1967). By keeping the experimental procedure as simple as possible a large number of analyses could be performed by a single operator.

The development of the method was largely concerned with ascertaining the correct dye concentration for the dye-buffer reagent which must be sufficient to ensure complete precipitation of the proteins (Dolby, 1961; Alais, Ribadeau-Dumas & Saint-Lebe, 1961) but not too excessive because this results in considerable experimental error.

Materials and methods

Reagents

C.I. Acid Orange 12. Pure dye was obtained from Udy Analizer Co. (P.O. Box 148, Boulder, Colorado 80302, USA). Less pure products containing sodium chloride are acceptable alternatives, e.g. commercial brands of C.I. Food Orange I, C.I. No. 15970. To facilitate the use of such preparations, the experimental data relating to solutions of C.I. Acid Orange 12 have been given as absorbance values rather than concentrations of dye.

Dye-buffer Reagent. This contained 0·35 g pure C.I. Orange 12 dissolved in 1·1–0·3 M citric acid. Because the concentration of dye employed was dependant on the purity of the dye sample and the quantity of protein being estimated, instructions for the derivation of an appropriate dye concentration have been given in the Appendix.

Methods

Extraction of protein from seed. Three grams of crushed field beans (*Phaseolus vulgaris*) were shaken with aliquots of aqueous extractants of different composition (in order to study the effect on protein solubility) and the volume adjusted to 100 ml.

The aqueous mixture was centrifuged at 1100 g for 20 min and filtered through Whatman No. 41 filter paper to provide a clear filtrate of soluble protein.

Estimation of soluble protein

(a) *Reference method.* The soluble protein in 20 ml of filtrate was determined by a semi-micro Kjeldahl procedure (Pearson, 1973).

(b) *Dye binding method.* The filtrate was diluted with water so as to contain not more than 0·15% w/v crude protein (N + 6·25). Five millilitres of the dilute protein solution were mixed with 10 ml dye-buffer reagent by gentle inversion in a centrifuge tube.

After standing overnight to reach equilibrium, the contents of the tube were again

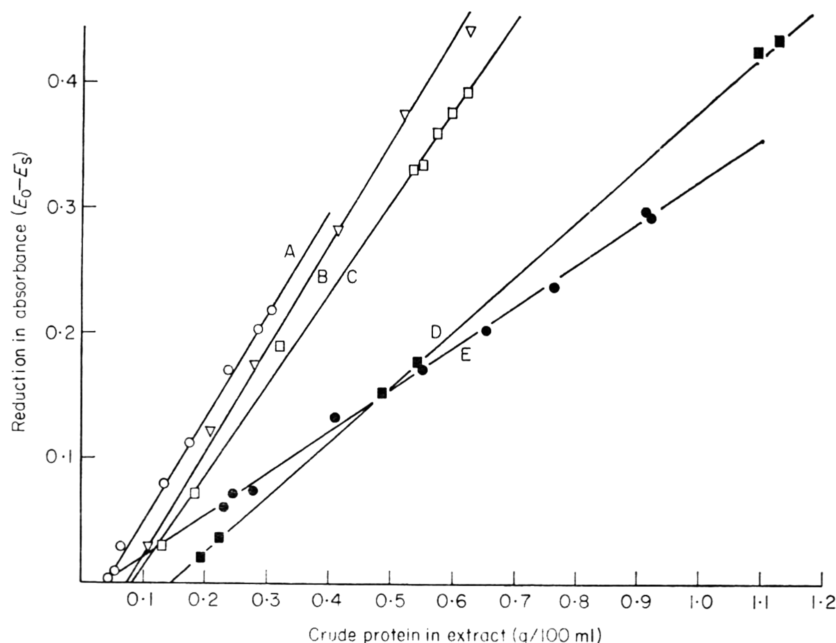

Fig. 1. Relationships between the crude protein contents of five seed extracts and the reductions in absorbance measured in the dye binding procedure. A, Cottonseed (*Gossypium hirsutum* L.); B, Cowpea (*Vigna unguiculata*); C, Field bean (*Phaseolus vulgaris*); D, Sesame seed (*Sesame indicum*); Rapeseed (*Brassica napus*).

mixed, centrifuged at 1100 g for 10 min and the supernatant filtered through a glass fibre filter paper (Whatman GF/A, 9 cm) to provide the test filtrate. A blank was prepared replacing the 5 ml of protein isolate by distilled water.

For each protein extraction procedure it is essential to compare the pH of the test filtrate with that of the blank (normally pH 1·97). If they differ by more than ±0·1 pH unit the protein isolate must be further diluted, and the estimation repeated, until this criterion is satisfied.

After suitable dilution (normally 5 ml up to 100 ml), the absorbances of the test filtrates and of the blank were measured at 482 nm using a 1-cm cuvette. Dilute solutions of C.I. Orange 12 conformed to Beer's Law up to an absorbance of 0·8 and dilution factors were adjusted to yield readings below this value. It was also important to ensure that the calculated absorbance of the test filtrate was not below 4 and when this occurred the determination was repeated using a more dilute solution of protein. Lakin (1973) has shown that a linear relationship is obtained when estimating milk proteins provided the calculated absorbance of the dye-protein equilibrium mixture remained above 4. A similar result has been confirmed in the case of protein extracts from beans and other seed materials.

In the construction of calibration curves, the absorbance of the diluted test filtrate (E_S) was substracted from the diluted blank determination (E_0) which gives a value for the reduction in absorbance due to the added protein. This difference value, ($E_0 - E_S$), was then plotted against the quantity of protein as estimated by the Kjeldahl method (N + 6·25).

Evaluation of the method

By varying the pH of the extractants, a series of eight solutions containing different concentrations of protein was prepared using 3 g of ground field beans to 100 ml of extractant. The crude protein was determined by the semi-micro Kjeldahl method and the above procedure was used to measure the reduction in absorbance ($E_0 - E_S$) due to protein. The results are plotted in graphical form in Fig. 1 and the regression equation was as follows, where *KP* is the crude protein content of the extract in g/100 ml.

$$KP = 1{\cdot}363\ (E_0 - E_S) + 0{\cdot}031$$

(Standard error of estimate = 0·007; $r = 0{\cdot}999$).

Similar experiments were undertaken with four other seed materials. The above procedure for beans was used without modification for cowpeas (*Vigna unguiculata*) but, because of their higher content of protein, extracts were diluted ten times before using the procedure for rapeseed (*Brassica napus*), sesame seed (*Sesame indicum*) and cottonseed (*Gossypium hirsutum* L.).

These results have also been plotted in Fig. 1 and the regression equations were as follows:

Cowpea

$$KP = 1{\cdot}197\ (E_0 - E_S) + 0{\cdot}074$$

(Standard error of estimate = 0·004; $r = 0{\cdot}099$);

Rapeseed

$$KP = 2{\cdot}976\ (E_0 - E_S) + 0{\cdot}037$$

(Standard error of estimate = 0·005; $r = 0{\cdot}998$);

Sesame seed

$$KP = 2{\cdot}254\ (E_0 - E_s) + 0{\cdot}143$$

(Standard error of estimate = 0·010; $r = 0{\cdot}995$);

Cottonseed

$$KP = 1{\cdot}203\ (E_0 - E_s) + 0{\cdot}040$$

(Standard error of estimate = 0·004; $r = 0{\cdot}998$).

Although the regression equations differed according to the nature of the seed material, in each case the dye binding measurements were highly correlated with the crude protein contents of the seed extracts. Furthermore the standard errors of the estimate were very low. The dye binding procedure can be used therefore as a satisfactory alternative to the Kjeldahl procedure for the determination of the protein content of seed extracts.

Discussion

Investigations concerning the extraction of seed proteins and the solubility of seed protein isolates involve the routine determination of protein in large numbers of samples. These analyses are normally done by means of the Kjeldahl procedure which is expensive, inconvenient and time-consuming. This problem is common to many studies associated with foods and other biological materials but in some instances it has been largely eliminated by use of dye binding procedures (Cole, 1969). When adapting these methods to a new circumstance, however, it is necessary to adjust the experimental parameters accordingly and particular attention must be given to the composition of the dye-buffer reagent: the dye concentration must be sufficient to ensure complete precipitation of the protein but not excessive, otherwise accurate results can not be obtained.

This study was undertaken specifically for the present investigation and the reported dye binding method gave measurements which showed a high correlation with analyses obtained by the Kjeldahl procedure. It was found that each seed material gave a different regression equation relating absorbance with protein content but the reasons for these differences were not investigated. Possible explanations are that the total nitrogen contents of the extracts contained different proportions of non-protein nitrogen and that there were inter-species variations in the composition of the seed proteins, particularly with respect to their contents of basic amino acids (Lakin, 1973).

Appendix

Derivation of the concentration of C.I. Orange 12 for the dye-buffer reagent

The concentration of dye in the reagent must be sufficient to ensure complete precipitation of the protein present in the sample being analysed, plus sufficient excess

to ensure a linear relationship between the reduction in dye concentration and the amount of protein added (Dolby, 1961; Alais *et al.*, 1961) plus a small 'safety margin'. Spectrophotometric inaccuracies can occur if a large excess of the dye is present.

A dye-buffer reagent was prepared containing approximately 0·5 g dye, accurately weighed in 1 : 1 of 0·3 M citric acid.

The procedure 2(b) described above was followed and the absorbances calculated. With the particular dye used in this work the following results were obtained:

Calculated absorbance of blank = 18·69
Calculated absorbance of test filtrate = 10·23
Reduction in absorbance due to protein = 8·46.

To this reduction in absorbance caused by protein (8·46) the absorbance corresponding to the minimal permissible concentration of excess dye (4·00) was added plus a small 'safety margin' (say 0·5). Hence the required absorbance of the blank was

$$8{\cdot}46 + 4{\cdot}00 + 0{\cdot}50 = 13{\cdot}00 \text{ approximately.}$$

Therefore the appropriate concentration of C.I. Orange 12 in the dye-buffer reagent $= 0{\cdot}5 \times 13{\cdot}0/18{\cdot}7 = 0{\cdot}35$ g/l.

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(*Received* 11 *December* 1974)

J. Fd Technol. (1975) **10**, 547–556.

The effect of selected plastic films and chemical dips on the shelflife of Marmande tomatoes

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Summary

The effect of different chemical dips and plastic films on the shelflife of mature Marmande tomatoes was studied. Respiration gases within packages, weight loss and decay during storage at 25°C of wrapped tomatoes were determined concurrently with respiration rate and colour changes of unwrapped tomatoes. The quality of the packed fruit was assessed by sensory testing, and the combination of treatments and packaging films giving optimum results was established.

Introduction

Controlled atmosphere (CA) storage is a promising method for extending the marketable life of tomatoes (Parsons, Anderson & Penney, 1970). However, the reports have not been consistent in regard to optimal CA conditions. Tomkins (1963, 1966) reported that exposing tomatoes to more than 5% CO_2 delayed ripening but simultaneously increased rotting. Eaves & Lockhart (1961) found lowest decay rate in tomatoes stored in a mixture of 2·5% O_2 and 2·5% CO_2 at 55°F (12·8°C). Parsons *et al.* (1970) reported carbon dioxide injuries in fruit stored in 3% oxygen and 3–5% carbon dioxide, whereas the best storage results were achieved in 3% O_2 and zero CO_2 at 55°F (12·8°C). Critical CO_2 concentration for tomatoes causing injury was reported to be 10% (Tomkins, 1961). Parsons, Gates & Spalding (1964) noticed off flavours in tomatoes stored at 1% O_2.

The use of plastic films to prolong shelflife of tomatoes has been investigated by several workers. Hardenburg (1954), Ayres & Peirce (1960), Anon (1966) and Badran, Woodruff & Willson (1971) used polyethylene. The use of other films was reported by Ayres & Peirce (1960), Lowry (1963) and Gilbert, Henig & Daun (1971). Gilbert *et al.* (1971) measured the O_2 and CO_2 concentration of tomatoes at different stages of maturity, during four days of storage at 23°C.

Domenico, Rahman & Westcott (1972) investigated the effects of fungicides in combination with hot water and wax on the shelflife of tomato. They found that Benomyl

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[methyl 1-(butylcarbamoyl)-2 benzimidazole-carbamate] prevented growth of the most damaging pathogens and thus considered it as a promising fungicide to be used with others.

The use of sodium hypochlorite (300 ppm Cl_2) in washing tomatoes before waxing was reported by Laenzina *et al.* (1973) who found low total counts on treated and stored fruits. The use of chemical dips in combination with other storage practices such as controlled atmosphere was not established.

The object of this study was to evaluate the suitability of prestorage treatments with chemical dips and five plastic films for prolonging shelflife of tomatoes at ambient temperature (25°C). Relationships between fruit respiration, wrapping materials, off flavour formation during storage of Marmande tomatoes were studied.

Materials and methods

Respiration rate

Respiration rate studies of tomatoes were carried out according to a method described by Jurin & Karel (1963). The effect of O_2 on respiration rates and respiratory quotients (RQ) was investigated. The respiratory quotient was determined by the volume ratio of CO_2 produced to O_2 consumed.

Gas analysis

The composition of gas samples from respiration chambers or from packages was determined by gas chromatography using a thermal conductivity detector according to a method described previously by Saguy & Mannheim (1973). Relative humidity calculation was based on water vapour concentration within package, temperature and psychrometric tables (Zimmerman & Lavine, 1945). Ethylene was analysed with a hydrogen flame ionization GLC detector (Varian Aerograph 1400-2), using two copper columns (12 ft long and $\frac{1}{8}$ in. o.d.) filled with chromsorb P 80/100 mesh DMCS (acid washed), coated with Carbowax 20 M 10% (John Manville, USA). The carrier gas used was nitrogen at a flow rate of 15 ml/min. The injection port and detector temperatures were maintained at 50°C. Sample size was 0·5–1·0 ml. Gas mixtures of known composition were used for calibration. Retention time was 39 sec.

Titratable acidity, pH and soluble solids

Titratable acidity (as anhydrous citric acid) and pH of juice were determined using a Beckman Zeromatic pH meter. Soluble solids were determined with a hand refractometer.

Colour measurement

The colour of the tomato was determined with a Hunter Colour Difference Meter (D-25). The instrument calibrated with a standard plate ($a_L = 27{\cdot}5$, $b_L = 12{\cdot}8$ and

$L = 25{\cdot}3$). A rank colour scale was used to aid in the evaluation of colour and maturity of the fruit, according to the method described by Ayres & Peirce (1960) and Meyer (1965). Arbitrary values were assigned ranging from 0 to 10 based on the following criteria: (0), 100% mature green; (1), turning yellow; (2), 10% yellow, 90% green; (3), half of surface red coloured (green <33·3%); (4), changing to pink; (5), turning from pink to red; (6), light red (>33·3% and <66·6%); (7), red (>66·6 and <90%); (8), full red; (9), dark red; (10), over-ripe, soft or spoiled.

Organoleptic evaluation

Sensory comparisons were made after eighteen and twenty-one days of storage using the triangle test method with untrained tasters, and making twenty-four judgments per comparison. In each evaluation the wrapped dipped fruits and the unwrapped undipped and dipped samples (serving as control) were presented an equal number of times and the order of presentation was randomized. All comparisons were conducted in individual ventilated booths equipped with dim lights to mask colour differences between the samples.

Experimental

Freshly harvested Marmande tomatoes were sorted for size and uniformity of colour (colour No. 2 on the rank scale, corresponding to Hunter $a_L/b_L = 1{\cdot}03$). Fruits were dipped for 3 min in either warm water (40°C) or in the following aqueous solutions (at 20°C):

(a) Nipacide* (Alkyl ester of p-hydroxy benzoic acid) in concentration of 100, 500 and 1000 ppm;
(b) Nipagin-M* (Sodium salt of p-hydroxy benzoic acid) in concentration of 100, 500 and 1000 ppm;
(c) chlorine (sodium hypochlorite 3·5%) in concentration of 5, 11, 13 and 25 ppm (calculated as available chlorine).

After dipping, the fruits were dried in an air blast at 35°C for 2 min.

Tomatoes (about 500 g) were placed on an individual tray (expanded polystyrene 13·8 × 22·5 × 2·5 cm) which was then wrapped in the appropriate film. Eight trays were wrapped for each film type and the experiment was replicated three times. Ratio of fruit weight to diffusive film area was 0·992 g/cm^2 TPM-87, VF-71, RMF-61 and 0·930 g/cm^2 for PE-25. Description of films used and their permeabilities are given in Table 1. PVC films were sealed by means of a hot plate, while a conventional heat-impulse sealing machine was used for polyethylene. The fruit was stored at 25°C for up to twenty-eight days. Respiratory gases (O_2, CO_2 and ethylene), relative humidity within packages, and weight losses were determined periodically during storage.

* Nipa Laboratories, UK.

TABLE 1. Wrapping film characteristics

Film	Material	Form	Thickness (mm)	Permeability		
				O_2[a]	CO_2[a]	H_2O[b]
TPM-87	PVC resinite[v]	Film	0·019	114	850	22
VF-71	PVC resinite[v]	Film	0·015	490	4800	60
RMF-61	PVC resinite[v]	Film	0·012	1280	10600	120
PE-25	Polyethylene[w]	Sleeve	0·025	745	5850	5
PE-35	Polyethylene[w]	Sleeve	0·035	258	1435	4

(a) ml (STP)/100 in ^{2}/24 hr/atm.
(b) g-H_2O/m^2/24 hr, at 25°C and 50% R.H.
(v) PVC films were manufactured by 'Borden' Inc., USA.
(w) Polyethylene sleeves were manufactured by 'Plastophyl-Hazorea', Israel.

Colour changes were assessed using the rank scale; initial and final colour were determined using the Hunter meter (a_L/b_L ratio). Tomatoes with visible decay were considered as total waste. Untreated and unwrapped fruit were used as control.

Results and discussion

Respiration

The relationship between colour changes, respiration and ethylene production was determined, to establish the physiological state of the tomato fruits. Results, shown in Fig. 1, indicated similar climacteric patterns for respiration and ethylene production. Colours changes were accelerated as the climacteric stage started. Colour score changed from No. 2 to No. 8, (corresponding to Hunter a_L/b_L ratio of 1·03 and 1·76 respectively)

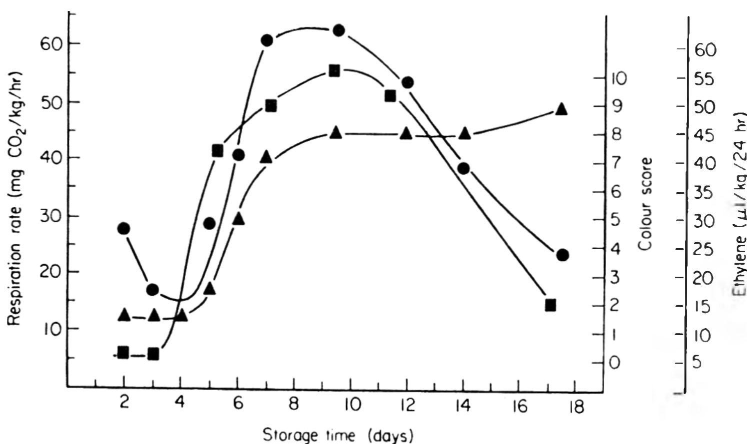

FIG. 1. Respiration rate (●), colour changes (▲) and ethylene production (■) by Marmande tomatoes stored at 25°C.

within the climacteric peak. A modest decrease in respiration was observed as ethylene production increased. This decrease towards the climacteric minimum agrees with the report of Lyons & Pratt (1964). Based on these results, fruit which was within the criteria of No. 2 on colour scale rank was taken for further experiments.

The effect of O_2 concentration on the respiration rate of the tomatoes, was studied in order to simulate conditions within packages. The results indicate a sharp and continuous decrease in the respiration rate as oxygen concentration approached 3% (Fig. 2). The effect of O_2 on the respiratory quotient (RQ) is shown in Fig. 3. RQ value was 1 and remained relatively constant for O_2 concentration of 21 to 3%. A sharp rise in RQ values was observed below the critical oxygen concentration of 3% which

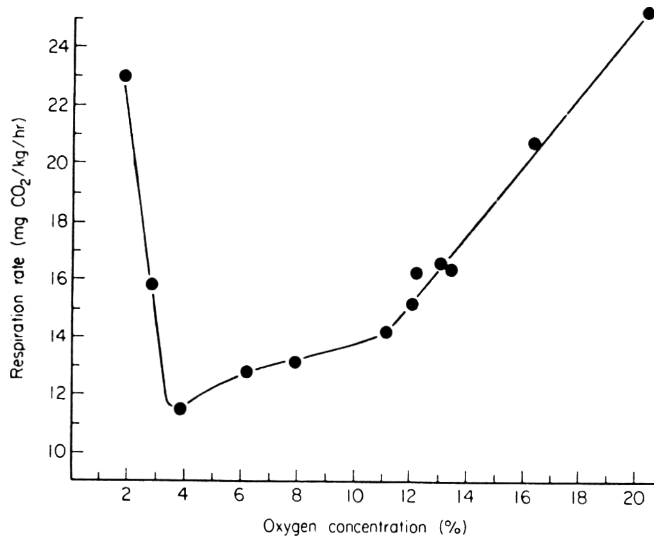

FIG. 2. Effect of oxygen on respiration rate of Marmande tomatoes stored at 25°C.

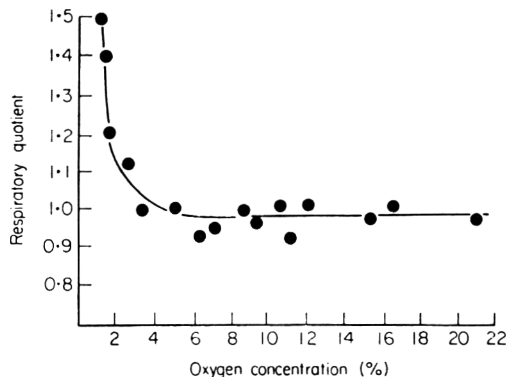

FIG. 3. Effect of oxygen concentration on RQ values of Marmande tomatoes stored at 25°C.

indicated the change from aerobic to anaerobic respiration. The decrease in the respiration rate could be explained by the influence of the modified atmosphere on respiration processes. The use of 3% O_2 for storing O_2 tomatoes was also reported by Parsons *et al.* (1970), as the critical O_2 concentration below which off flavours were produced.

Humidity in packages

Relative humidity within the tomato packages wrapped with different films is shown in Table 2. Results indicate that in VF-71, TMP-87 and PE-25 packages a high humidity was created, however, no condensation was observed. These humidities were achieved despite the low humidity in the storage room. The humidities created within these films were close to those recommended for storage of tomatoes (Lutz & Hardenburg 1966).

TABLE 2. Average relative humidity (and standard deviation) within packages of Marmande tomatoes stored at 25°C

Film	Relative humidity (%)
RMF-61	75 ± 5
VF-71	86 ± 7
TPM-87	90 ± 7
PE-25	91 ± 7
PE-35	96 ± 7*
Storage room	50 ± 7

* In some cases condensation was observed.

Extreme humidity values caused conditions which resulted in either high rate of weight loss (RMF-61, Fig. 4) and thus desiccation, or condensation (PE-35, Table 2) accompanied by a significant increase of decay.

Fruit weight loss as affected by film material is shown in Fig. 4. The high correlation coefficients indicated that water vapour transmission rate (WVTR) depends on the difference in humidities within the package and external surroundings. Consequently PE-35 film was found to be unsuitable for wrapping due to its low WVTR causing condensation which increased decay. This suggests that, when storing wrapped tomatoes at 25°C the water vapour transmission rate of the film must be above 4 g $H_2O/m^2/24$ hr (Table 1). This value should be higher as humidity in the storage room increases.

Decay

Statistical analysis of results indicated that treatment with 25 ppm chlorine, 1000 ppm Nipagin-M, 1000 ppm Nipacide and wrapping with VF-71 and TPM-87 decreased decay significantly (Table 3).

The best results were achieved when the fruit was treated with 25 ppm chlorine and wrapped in VF-71 and TPM-87 (Table 3). In these packages decay was 0–5% after twenty-one days of storage at 25°C, while deterioration of control fruit was 40%.

Dipped fruit which was not wrapped showed a higher rate of deterioration as compared to the control, thus emphasizing the benefits of the modified atmosphere. Experiments with chlorine concentrations above 25 ppm results in off flavours. The use of warm water (40°C) increased decay.

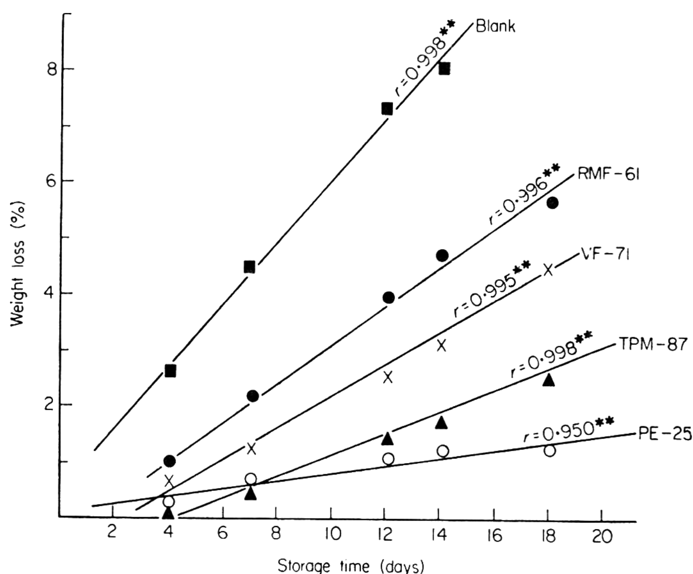

Fig. 4. Weight loss of the tomato fruits as effected by storage (at 25°C) and wrapping material. ** Correlation coefficient significant at 1% level.

Changes in gas concentration, colour, acidity and total solids during storage

Results of gas analysis within packages indicated that relatively constant levels of gas concentration were obtained after one day and were maintained during the subsequent twenty-one days of storage.

The range of gas levels were: 2·5–3·8% O_2 and 11·9–13·4% CO_2 in TPM-87; 5·1–7·2% O_2 and 2·1–4·3% CO_2 in VF-71; 9·3–13·6% O_2 and 1·3–3·1% CO_2 in RMF-61; 4·1–7·7% O_2 and 3·1–4·4% CO_2 in PE-25.

The concentration of respiratory gases in the packages depended on the nature of the film. The lower the permeability of the film to gases, the higher CO_2 and lower O_2 concentrations were found within the packages.

There was a slight increase in CO_2 after twelve days and this could probably be explained by the climacteric rise. At the same time ethylene concentration increased to

TABLE 3. Effect of prestorage treatment, type of film and storage time on the average percent decay of Marmande tomatoes stored at 25°C; average of three experiments, eight packages per film

Treatment	Untreated				Nipagin-M 1000 ppm				Nipacide 1000 ppm				Chlorine 25 ppm				
Film	Time (days)																
	7	14	21	28	7	14	21	28	7	14	21	28	7	14	21	28	Average film*
Unwrapped	10	25	40	†	15	†	—	—	18	†	—	—	15	40	†	—	† c
PE-25	15	†	—	—	0	15	40	†	0	0	15	30	0	0	15	30	35·0 b
RMF-61	15	†	—	—	0	0	10	40	0	10	25	†	0	5	15	40	34·7 b
VF-71	15	40	†	—	0	0	10	30	0	0	10	30	0	0	5	25	22·8 a
TPM-87	15	30	†	—	0	0	10	30	0	0	10	30	0	0	0	20	21·9 a
Average** treatments			†z				30·0 y				28·9 y				20·2 x		

* L.S.D.$_{0\cdot01}$ = 6·1.

** Films and treatments were tested by Duncan's M.R.T. Different letter shows difference at 1% level.

† Decay greater than 50%.

TABLE 4. Effect of wrapping film on changes of colour, total soluble solids and titratable acidity in tomatoes stored at 25°C for twenty-eight days

Film	a_L/b_L*		Rank colour scale		Bx°		Titratable acidity (%)†	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Unwrapped‡	1·03	1·96	2	9	3·5	4·0	0·50	0·32
PE-25	1·03	1·90	2	9	3·5	3·8	0·50	0·35
RMF-61	1·03	1·88	2	8	3·5	3·8	0·50	0·34
VF-71	1·03	1·76	2	7	3·5	3·7	0·50	0·38
TPM-87	1·03	1·51	2	6	3·5	3·6	0·50	0·47

* Hunter ratio.

† Expressed as anhydrous citric acid.

‡ Final tests were carried out after eighteen days.

maximum of 3·00, 3·35, 4·45 and 4·43 ppm in RMF-61, VF-71, TPM-87 and PE-25, respectively. This low level of ethylene and increased level of CO_2 created in the packages at this time is believed to be the cause of delaying and decreasing the climacteric rise, thus prolonging fruit shelflife. Burg & Burg (1965) indicated that retardation of fruit

ripening depends on CO_2 concentration which acts as a competitive inhibitor to the stimulatory action of ethylene.

Changes in colour, total solids and acidity are given in Table 4. The results indicated that the wrapping film with lowest permeability to gases minimized these changes and kept the best quality of the stored fruit.

Organoleptic evaluation

Tomatoes dipped in aqueous solutions of Nipacide, Nipagin-M (1000 ppm) and chlorine (25 ppm) and wrapped in RMF-61, VF-71, TPM-87 and PE-25 were evaluated organoleptically after eighteen and twenty-one days of storage at 25°C. The panel did not find any difference in triangular tests and odour comparisons between fruit dipped and wrapped in RMF-61, VF-71 and PE-25 against the unwrapped dipped and undipped controls. Only fruit wrapped in TPM-87 was found to be significantly worse than control due to off flavours. This was probably caused by the presence of more than 5% CO_2 as also reported by Tomkins (1963).

In conclusion, it was found possible to obtain a shelflife of twenty-one days at 25°C for dipped and wrapped Marmande tomatoes as compared with a shelflife of less than seven days for unwrapped, dipped or undipped fruit. The most suitable treatment found in these experiments was due to the combined effect of dipping fruit, before storage in 25 ppm chlorine solution and wrapping in VF-71 film.

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(*Received* 24 *October* 1974)

J. Fd Technol. (1975) **10**, 557–563.

Effect of orange juice parameters on gelation in hot-pack concentrate

S. MIZRAHI AND R. FIRSTENBERG

Summary

The effect of the pulp content of shamouti orange juice (S), the specific viscosity of the serum (η_{sp}) and the pH level, on the degree of gelation (DG) of hot-pack six-fold concentrate, was studied on samples prepared with a single factor varied at a time. The degree of gelation was found to be controlled by three main factors, namely:

(1) formation, under favourable pH conditions, of a pectin-sugar-acid-type gel by the soluble pectin;
(2) interference of pulp particles with the formation of the gel, mainly at low pulp concentrations (up to $S=4\%$);
(3) strengthening of the gel by the rigidity of the suspended particles, their mutual interaction and possibly their coupling ability.

The following relationship was established between the degree of gelation (4-point scale) on the one hand, and S, pH and η_{sp} on the other, and confirmed on commercial samples:

$$DG=\frac{12{\cdot}9\,\eta_{sp}}{1+10^{(pH-3{\cdot}945)}}-0{\cdot}59S+0{\cdot}136S^2-0{\cdot}006S^3-1{\cdot}0.$$

Introduction

Orange juice can be marketed or stored in the form of hot-pack pasteurized six-fold concentrate. This mode of processing can entail a serious problem of gelation, the concentrate becoming unpourable and difficult to reconstitute.

One well known form of this effect, observed in frozen four-fold concentrate, and discussed in detail by Joslyn & Pilnik (1961), is caused by the formation of a calcium pectate-type gel through pectin esterase (PE) activity; in this case the degree of gelation was found to depend on the pulp content and on that of total and low-methoxyl pectin

This project was supported by the Israeli Ministry of Commerce and Industry and Citrus Products Export Board.

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(Kohen Mosse & Royo Iranzo, 1959). This mechanism, however, is not valid for the hot-pack concentrate where PE is completely inactivated. The latter was found (Mizrahi, 1968) to contain, normally, pectin of 8–13% methoxyl content, and the process was tentatively claimed to depend on the content and molecular weight of the soluble pectin as well as on the pH of the system, with the pulp acting as strengthening agent in the evolving structure. This hypothesis was unsubstantiated at the time in the absence of means for separating the effects of simultaneously changing variables; the present work was undertaken with a view to remedying this situation, using samples prepared with a single factor varied at a time.

Experimental

Shamouti oranges of uniform size, provided by the canning industry, were extracted on an FMC in-line juice extractor model 718, at two different machine settings (standard and bored upper cup). The juice thus obtained was screened in a Sweco-Vibro separator model S-18 (0·6-mm and 0·2-mm screens) and pasteurized for 30 sec at 90°C in an Alfa-Laval plate heat-exchanger model PL-1-HB. Each variant of the juice was divided into two portions, from one of which the pulp was removed in a De Laval Centrifuge model E 519 and added to the other. The four resulting samples were concentrated to 60°Bx in an Alfa-Laval Centritherm evaporator model CT-1B and blended into a balanced matrix of 100 concentrates with pulp content S-1·5–11% and specific viscosity of the serum $\eta_{sp} = 0{\cdot}15–0{\cdot}35$. The specific viscosity was found to be practically the same for original and reconstituted juice. The samples, adjusted with citric acid to pH 2·8–3·5, were heated to 80°C in a hot water bath, canned in 100-g containers, and stored for two weeks at room temperature. The degree of gelation was then evaluated on the following scale, according to FMC (1964): 0, free flowing; 1, flowing but small gel particles present; 2, flowing but contains large gel particles or lumps; 3, gelled to extent that when removed from the can, a portion of the concentrate retains the shape of the can; 4, solid gel holding shape of can when removed.

Serum is defined as the supernatant of 11°Bx juice, centrifuged for 10 min at 18 400 × **g** in a Servall centrifuge model SS-34.

Pectin content in the serum was determined according to McComb & McCready (1952). The method is based on the precipitation of pectin substances by alcohol and subsequent treatment of the precipitate with sulphuric acid and carbazol. The colour developed was read in a Klett Summerson colorimeter using a No. 54 filter. The standard curve was established with anhydrous galacturonic acid, analytical grade.

The pulp content was measured by centrifuging reconstituted juice in conical 50-ml tubes in an M.S.E. centrifuge for 10 min at 360 × **g** (1300 rpm).

Viscosity at 30°C was determined in No. 50 Ostwald-Cannon-Fenske capillary viscometer and converted into specific viscosity, namely:

$\eta_{sp} = 1 - \eta_{serum}/\eta_{reference}$ the reference being an 11°Bx sugar solution.

Curve fitting was carried out on an IBM 370/165 computer at the Technion computer center, using the BMDX85 program for the non linear functions and the BMD02R for the linear functions (Dixon, 1971).

Results and discussion

In the commercial samples tested, the specific viscosity of the serum—the most readily measurable and reproducible of the parameters involved—is in linear correlation with the soluble-pectin content (Fig. 1), with the variation about the regression line mostly

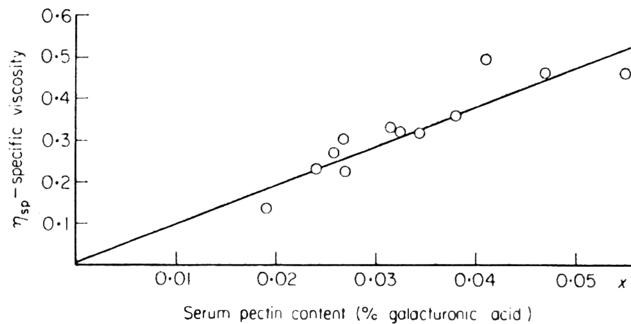

Fig. 1. Correlation between specific viscosity and pectin content of serum. $\eta_{sp} = 9 \cdot 39x + 0 \cdot 008$; $r = 0 \cdot 905$.

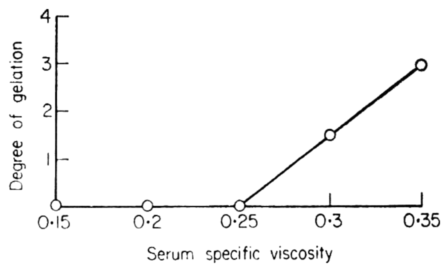

Fig. 2. Effect of specific viscosity of serum on degree of gelation of pulpless concentrate. pH = 3·2; pulp concentration = 1·5%.

attributable to molecular weight. In these circumstances it is the best available indicator for the amount and gel forming power of the soluble pectin in the concentrate (Mizrahi, 1968).

Figure 2 indicates that at a low pulp level (1·5%) considerable gelation takes place in a concentrate with a soluble-pectin content of about 0·32%, i.e. within the normal range for formation of a typical pectin-sugar-acid gel. On the other hand, at the commercial levels, pulp lacks such gel forming power when suspended in depectinized serum (Fig. 3). A very weak gel is formed only at higher pulp levels (about 15%). When present together, the soluble pectin and the pulp exert an additive effect in

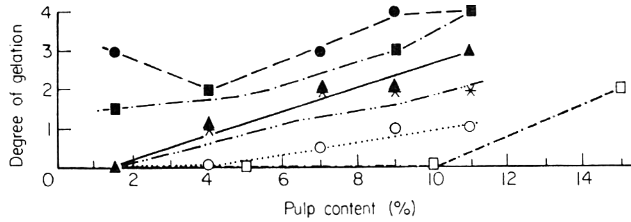

FIG. 3. Effect of pulp content and specific viscosity of serum on degree of gelation. pH = 3·2. Serum specific viscosity: 0·35, ●——; 0·3, ■—·—; 0·25, ▲——; 0·2, * —··—; 0·15, ○····; 0, □·——.

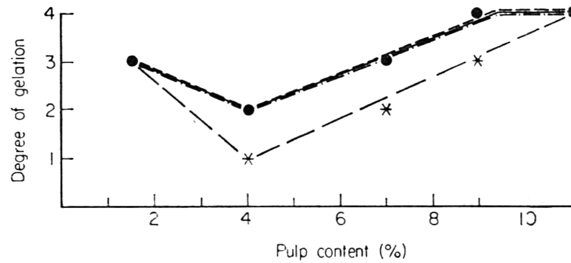

FIG. 4. Effect of pH and pulp content on degree of gelation. pH: ●, 2·8,---; 3·0——; 3·2—·—·—; 3·5*——. Serum specific viscosity = 0·35.

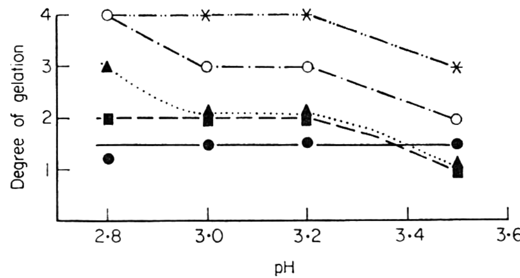

FIG. 5. Effect of pH on degree of gelation at different pulp contents. Pulp content: 1·5, ●——; 4, ■---; 7, ▲,·······; 9, ○—·—·, 11, * —..—. Serum specific viscosity = 0·30.

increasing gelation—except at high η_{sp}(0·35), in which case a minimum was observed at 4% pulp content (Figs 3 and 4). Apparently, the pulp is involved in two mutually counteractive processes: (a) bulk type interference with intermolecular crosslinking in the soluble pectin; and (b) strengthening of the gel through mutual interaction of the particles, their rigidity and probably their coupling ability. When a dense gel matrix is expected, the interference effect of the low content of pulp predominates and the resulting gel is weaker compared with the no-pulp case; by contrast, with a higher pulp content in a gel where crosslinking density is already low, due to the pulp or to a

low soluble pectin content, the strengthening effect predominates and the typical additive pattern is observed. It should be noted that in all cases practically no pectin was extracted from the pulp into the serum. This is supported by the fact that only negligible changes in serum specific viscosity were observed.

The results in Figs 4 and 5 indicate that the degree of gelation depends also on the pH of the concentrate; as expected, the lower the pH the higher the gelation. At $\eta_{sp}=0{\cdot}35$, where a strong gel was formed, the pH level had a considerable effect in the 3·2–3·5 interval. At the lower η_{sp} level of 0·30, the degree of gelation was strongly dependent on the pulp content and so was the contribution of the pH, the effective interval being 3·2–3·5 and 2·8–3·5 for pulp contents of 11% and 7% respectively. The upward shift in the lower limit of the interval is due to the fact that once a strong gel has been formed, practically no further noticeable strengthening of the matrix is obtainable by reducing the pH; a weak gel, on the other hand, is sensitive over a wider interval.

Quantitative evaluation (by curve fitting) of the effect of the variables η_{sp}, S and pH on the degree of gelation yielded the following equations:

$$DG=P_1\times S+P_2\eta_{sp}+P_3\times \mathrm{pH}+P_4 \quad (1)$$

$$DG=\frac{P_1\eta_{sp}}{1+10^{(\mathrm{pH}-pK)}}+P_2\times S+P_3 \quad (2)$$

$$DG=\frac{P_1\eta_{sp}}{1+10^{(\mathrm{pH}-pK)}}+P_2\times S+P_3\times S^2+P_4 \quad (3)$$

$$DG=\frac{P_1\eta_{sp}}{1+10^{(\mathrm{pH}-pK)}}+P_2\times S+P_3\times S^2+P_4\times S^3+P_5 \quad (4)$$

$$DG=\frac{P_1\eta_{sp}}{1+10^{(\mathrm{pH}-pK)}}+P_2\times S+P_3\times S^2+P_4\times S^3+P_5\times S^4+P_6 \quad (5)$$

where pK and $P_1 \ldots P_6$ are the equation parameters (see Table 1 below).

Equation (1) is based on two-dimensional plots drawn for each variable with the other two kept constant; the effects of all three on the degree cf gelation were roughly linear and additive, as though there were no interaction between them.

Equation (2) is based on a modification of a model suggested by Mizrahi (1968), whereby gelation is dependent on the amount of non-ionized pectin (Hinton, 1940). The constant of equilibrium (K) between the ionic and non-ionic groups of the pectin (contents denoted by IP and NIP respectively) is given by:

$$K=\frac{(IP)(H^+)}{(NIP)}. \quad (6)$$

Recalling that, with sufficient accuracy (Fig. 1),

$$\eta_{sp}=C[(IP)+(NIP)] \quad (7)$$

where C is a constant, we have by equations (6) and (7):

$$(NIP) = \frac{\eta_{sp}/C}{1+\{(H^+)/K\}} \tag{8}$$

or

$$(NIP) = \frac{\eta_{sp}/C}{1+10^{(\mathrm{pH}-pK)}} \tag{9}$$

Assuming linearity between degree of gelation and NIP, the first term in equations (2)–(5) is of the type 'proportionality factor/C', while the remaining terms are a polynomial representing the contribution of the suspended pulp.

TABLE 1. Equation parameters determined by least-square curve fitting*

Equation	P_1	pK	P_2	P_3	P_4	P_5	P_6	Correlation coefficient R	Standard error of estimate
(1)	0·24	—	11·03	−0·864	+0·47	—	—	0·886	0·587
(2)	12·92	3·945	0·237	−2·23	—	—	—	0·889	0·578
(3)	12·92	3·945	0·049	0·015	−1·83	—	—	0·896	0·563
(4)	12·92	3·945	−0·591	0·136	−0·006	−1·0	—	0·9035	0·545
(5)	12·92	3·945	−0·410	0·081	0·0	−0·00025	−1·19	0·9031	0·546

* Based on $n = 100$ experimental values.

TABLE 2. Prediction of degree of gelation in hot-pack six-fold orange juice concentrate

Sample no.	Pulp content of juice	Specific viscosity of serum	pH	Degree of gelation	
				Observed	Computed
1	7·0	0·227	3·0	2	2·1
2	7·5	0·302	3·0	3	3·1
3	1·7	0·404	3·0	4	3·0
4	8·8	0·457	3·2	4	4·0
5	9·6	0·316	3·1	4	4·0
6	7·5	0·358	3·0	3	3·8
7	8·8	0·269	3·0	1	3·3
8	6·4	0·128	3·1	0	0·6
9	10·8	0·464	3·0	3	4·0

The equation parameters were determined by a least squares technique and are summarized in Table 1.

The correlation coefficient of 0·886 obtained for equation (1) indicates that the model based on linearity and additivity alone is satisfactory. Equations (2)–(5), based on a combination of soluble-pectin gelation and gel strengthening, are even better, especially equation (4), where the correlation coefficient is 0·9035. This equation was used successfully in predicting gelation in commercial concentrate samples, as shown in Table 2. Except for sample 7, the deviation from the observed *DG* was less than one point, i.e. approximately within the limits of the experimental error. It should be noted that the prediction is based on data obtained under a standardized set of conditions, while the actual value may be affected by differences in handling (e.g. breakdown of the gel as a result of spinning or shaking).

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(*Received* 19 *November* 1974)

J. Fd Technol. (1975) **10**, 565–574.

Preservation of comminuted orange products

ANNICE C. LLOYD

Summary

The yeast commonly responsible for spoilage problems in comminuted orange base and the cordial made from this base, was found to be *Saccharomyces bailii* Linder var. *bailii*. This organism was resistant to benzoic acid as a preservative at the maximum allowable level in the cordial (800 ppm), but was more sensitive to the action of sorbic acid, 400 ppm preventing fermentation in cordial and 800 ppm preventing fermentation in base. The only other allowable preservative under local regulations, sulphur dioxide, at a maximum level of 230 ppm appears to be the most suitable preservative for the comminuted products in view of the colour deterioration associated with the use of sorbic acid. Comminuted orange cordial inoculated with *S. bailii* at a level of approximately 2/ml and preserved with 230 ppm total initial SO_2 was stored in PVC bottles for six months at 30°C without spoilage. Sulphur dioxide is much less effective as a preservative in base than in cordial, but by increasing the acidity of the base, the preservative action may be greatly enhanced.

Introduction

Comminuted citrus beverages in which varying proportions of the whole fruit, including juice, peel, pith and seed may be used, were first introduced overseas some twenty years ago. Relatively little technical information has been published on this type of product (Charley, 1963; Rao *et al.*, 1969), but it is clear that comminuted bases and the beverages made from them vary considerably in their content of pulp and peel depending on the country of origin and the mode of manufacture.

When these products were first introduced British manufacturers chose to use sodium benzoate as a preservative in preference to SO_2, the traditional preservative for soft drinks (Charley, 1963). However, Rao *et al.* (1969) claimed that in Indian comminuted products, SO_2 was more suitable as a preservative than benzoic acid as it gave better retention of colour and aroma. The application of overseas processes and formulations to the manufacture of Australian comminuted citrus drinks has been

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discussed in detail by Casimir & Chandler (1970), but no mention was given to preservative treatments.

Little information is available in the literature on spoilage yeasts found in comminuted products or on the comparative effectiveness of preservatives against these organisms. This investigation was instigated to study these features using locally produced comminuted citrus products which were sometimes found to present spoilage problems. Under Queensland regulations the maximum allowable concentrations of preservative which can be used in any fruit juice cordial are sulphur dioxide 230 ppm, or sorbic acid 800 ppm, or benzoic acid 800 ppm, or any combination of these three provided the sum of the proportional fractions of each does not exceed unity.

Materials and methods

Comminuted products

Unpreserved, pasteurized, comminuted orange base and cordial prepared commercially from the same batch of fruit were used throughout this investigation. Under normal commercial operations the comminuted base may be stored in bulk for several months before being incorporated into the final cordial as sold to the consumer. For the purposes of this investigation, however, unpreservatized cordial was made immediately from a batch of unpreservatized base and the required quantities of both freshly prepared products were frozen in plastic containers at −20°C until required.

The base [pH 3·7, 11° Brix, titratable acidity 0·7% citrus oil (w/v) 0·4%] consisted of juice to which had been added comminuted flavedo in the approximate ratio (3 : 1). The final cordial [pH 3·0, 46° Brix titratable acidity 1·75%, citrus oil (w/v) 0·1%] was prepared by diluting the base approximately one in four with water and then adding sucrose, citric acid and colouring.

Preservatives

As the base is diluted one in four in the final product, approximately four times the maximum allowable concentration of cordial preservative may be used in the base. In the course of this work three preservatives, SO_2, sorbic acid and benzoic acid were added respectively as the following aqueous solutions, sodium metabisulphite, potassium sorbate and sodium benzoate.

Physical and chemical estimations

Titratable acidity (as percent citric acid) was determined by the recommended A.O.A.C. method for fruit products (A.O.A.C., 1970). Free, combined and total SO_2 in base and cordial were measured by the method of Rankine & Pocock (1970). It was considered unnecessary to measure sorbic acid and benzoic acid as these preservatives

remain stable during storage. Colour of the comminuted products was evaluated visually.

Microbiological technique and preservative treatments

Spoilage yeasts in comminuted products which had fermented under factory conditions were isolated and maintained on Wickerham's glucose–yeast extract–malt extract agar containing 10% glucose (10 GY) (Wickerham, 1951). Three such isolates obtained from fermented comminuted products at different times were identified by the methods of Lodder (1971) as *Saccharomyces bailii* var. *bailii*. One of these isolates (designated FC) was used throughout the following investigations.

Sugar tolerance of the typical spoilage yeast was tested on 40°, 50°, 60° Brix glucose agar as described by Lodder (1971) as well as in unpreservatized cordial (initially 46° Brix) adjusted with sucrose in 2° Brix intervals up to 62° Brix. The effect of pH on growth of the spoilage yeast was tested in unpreservatized cordial (initially pH 3·0) adjusted to pH values covering the range 2·75–1·75.

Yeast inocula for all experiments were grown in unpreservatized base or cordial as required for approximately three days at 30°C. The level of inoculation varied slightly in different experiments as specified later, but was always low (<100/ml) in keeping with practical levels of contamination known to be attainable with responsible care in commercial practice. For most experiments, cordial or base, inoculated and preservatized as required, was stored in 26-oz PVC screw-cap bottles as used commercially for this particular product. Experiments to test the effect of three initial total levels of SO_2 (115, 230 and 460 ppm) and four levels of benzoic acid and sorbic acid (200, 400, 600 and 800 ppm) on yeast growth in base and in cordial respectively were replicated twice.

A long-term storage trial designed to test the effectiveness of various maximum allowable combinations of SO_2 and benzoic acid as preservatives involved three replications of six preservative treatments (SO_2 ppm/benzoic acid ppm as follows: 0/0, 0/800, 57/600, 115/400, 172/200, 230/0), and six sampling times (0, 1, 4, 8, 16 and 24 weeks). Three bulk samples of cordial were each inoculated with the spoilage yeast at an average level of 2 yeasts/ml and then each batch was distributed in 6×1 gal lots. Each 1-gal sample was preservatized as specified above by adding the appropriate volumes of sodium metabisulphite or sodium benzoate solutions and then distributed into 6×26 oz PVC bottles (one for each sampling time). All bottles of cordial were stored at 30°C until the required sampling time had elapsed, when three bottles (one from each replication) for each preservative treatment were analysed for free, combined and total sulphur dioxide and number of viable yeasts/ml.

Yeast counts throughout this work were carried out using a membrane filtration technique. Plastic membrane filtration units (Sartorius S, 16510) and 0·8 μ gridded membranes were used. Ten millilitres of an appropriate dilution of cordial or base was washed through a membrane with 2×20 ml lots of sterile distilled water. Due to the

particulate nature of the comminuted product 10 ml of 10^{-1} dilution was the most concentrated amount able to be filtered so yeast counts of less than 1 per ml were not detected. All filters were plated on poured 10 GY plates and incubated three to four days at 30°C before counting.

Results and discussion

Spoilage yeasts

The typical spoilage yeast isolated from fermented comminuted products agreed closely with Lodder's description of *Saccharomyces bailii* var. *bailii* (Lodder, 1971). Glucose and sucrose were fermented rapidly and raffinose weakly and slowly. Evaginated cells which conjugated and sporulated readily were formed on 10 GY streak plates as well as on sporulation media such as Gorodkowa and malt extract agars. The yeasts grew on 40° Brix and 50° Brix glucose agar but not on 60° Brix glucose agar. In cordial, the maximum sucrose concentration and the minimum pH level at which active fermentation occurred were 62° Brix and pH 2·0 respectively. Fermentation at pH 1·75 was extremely slow.

Growth of yeast in unpreservatized cordial and base

When *S. bailii* var. *bailii* was grown in broth media (2% glucose yeast-extract malt extract) prior to inoculation into either comminuted product, a marked lag phase occurred before fermentation actively commenced (Fig. 1). After this initial lag period fermentation in comminuted base was more rapid than in comminuted cordial. This could be accounted for partly by the higher Brix level in the cordial, for when sucrose was added to the base to bring it to the same Brix level as the cordial (46°), the onset of active fermentation was delayed by several days. When the spoilage yeast was grown initially in base or cordial prior to inoculation into fresh base or cordial the lag periods observed in Fig. 1 were completely eliminated.

Effect of preservatives on yeast growth in cordial and base

The effectiveness of the preservative SO_2 at three different concentrations in base and in cordial is shown in Fig. 2a and b. Even at the very low level of inoculation used in this experiment (< 1/ml) base preservatized with up to 460 ppm total initial SO_2 still fermented rapidly (within five to ten days) whereas in cordial the same initial levels of SO_2 were much more effective in preventing yeast growth. Cordial preservatized with 115 ppm SO_2 fermented after twenty days, but no viable cells could be detected in the presence of 230 ppm SO_2 or 460 ppm SO_2 after the same time. Later experiments indicated that cordial preservatized at the maximum allowable limit of SO_2 (230 ppm) could be stored in sound condition for up to six months provided the level of yeast contamination was low.

As reported elsewhere for other fruit juice products (Ingram, 1948), a considerable

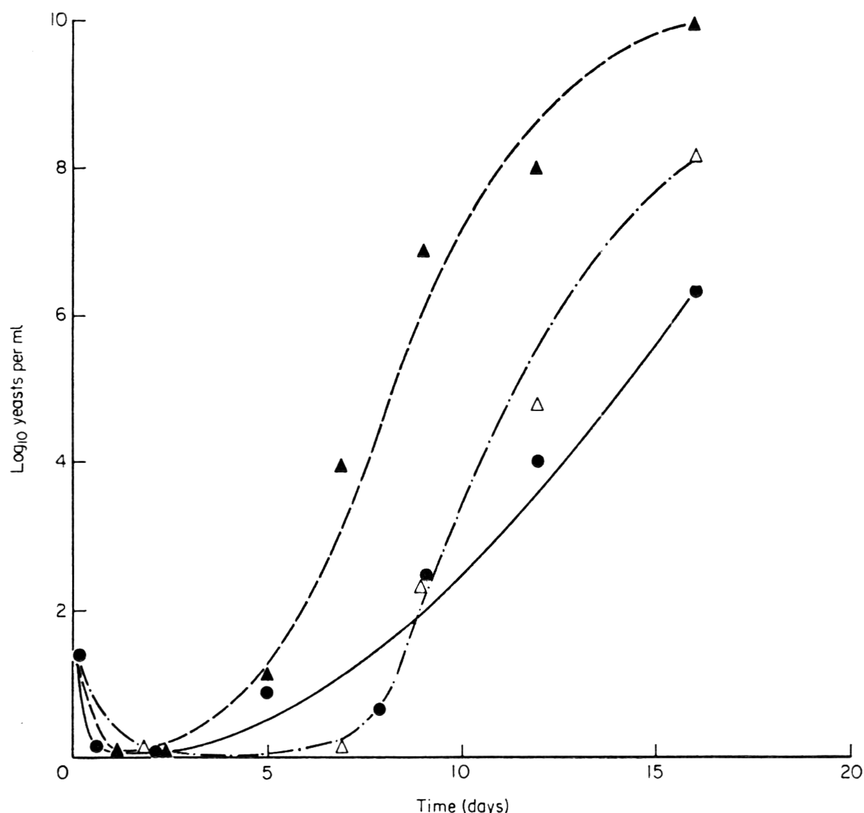

FIG. 1. Growth of *Saccharomyces bailii* var. *bailii* in comminuted orange base and cordial. ●——●, Cordial, 47° Brix; ▲---▲, base, 11° Brix; △—·—△, base, 47° Brix.

proportion of the total initial SO_2 added to comminuted juices becomes bound to constituents in the product after the first two or three days as shown in Fig. 2a. Once fermentation has commenced the free SO_2 level drops even more rapidly as shown by the curves in Fig. 2a for base initially preservatized with 115 or 230 ppm total SO_2. Free SO_2 alone, however, does not determine the effectiveness of the preservative in preventing yeast growth. In base preservatized with 115 or 230 ppm SO_2 levels at this time were approximately 100 and 200 ppm respectively. In base with initially 460 ppm SO_2, yeast growth commenced after five days when the free SO_2 level had fallen to approximately 300 ppm. In contrast, cordial preservatized with only 115 ppm SO_2 initially did not show signs of fermentation until the free SO_2 level dropped below 50 ppm.

To investigate the difference in SO_2 action observed above, samples of base were adjusted to the same Brix and pH levels as cordial, preservatized with approximately 230 ppm SO_2 and inoculated with 60 yeasts/ml. The results in Table 1 show that after two days the free SO_2 levels were approximately the same in all samples, but yeast growth had occurred only in two base samples, one with and one without added

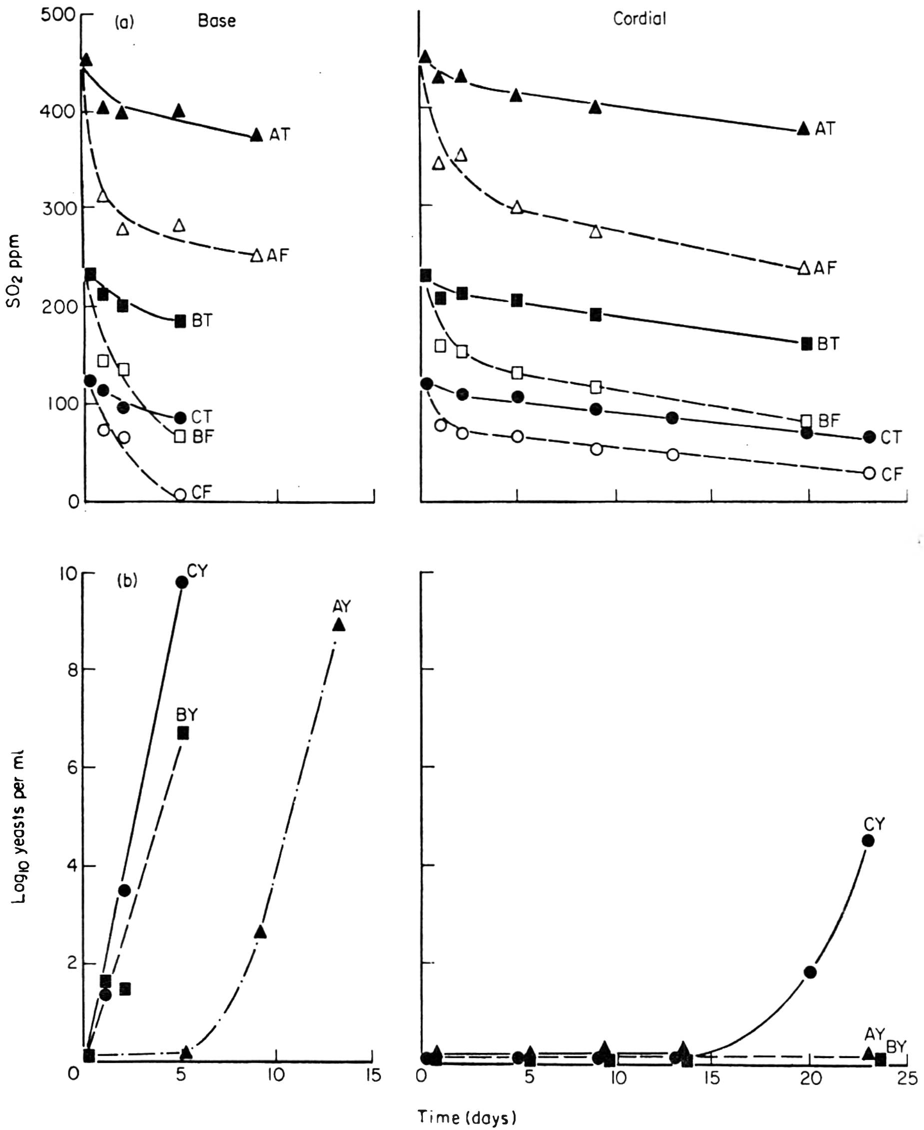

FIG. 2. (a) Free and total SO_2 levels in comminuted base and cordial containing initially 115, 230 and 460 ppm total SO_2. Initial total SO_2 ppm: 460 (Preservative treatment A); 230 (B); 115 (C). ▲——▲, Total SO_2, Treatment A; △——△, Free SO_2, Treatment A; ■———■, Total SO_2, Treatment B; □——□, Free SO_2, Treatment B; ●——●, Total SO_2, Treatment C; ○——○, Free SO_2, Treatment C. (b) Yeast growth in comminuted base and cordial containing initially 115, 230 and 460 ppm total SO_2. Initial total SO_2 ppm: 460 (Preservative treatment A); 230 (B); 115 (C). ▲—-—▲, Treatment A; ■---■, Treatment B; ●——●, Treatment C.

sucrose, but both at the unadjusted pH of the base 3·7. Base at 11° or 46° Brix which had been acidified to pH 3·0 was effectively preserved by the free SO_2 present as was the unadjusted cordial. Other experiments have shown that comminuted orange base preservatized with up to 700 ppm total SO_2, will ferment within two to three weeks if inoculated with *Saccharomyces bailii.* However, by acidifying the base to the same pH as the cordial, the effectiveness of SO_2 as a preservative can be greatly enhanced. By lowering the pH of the base still further to pH 2·6 (4·6% tit. acidity as citric acid) total initial levels of SO_2 as low as 180 ppm have been shown to retard fermentation

TABLE 1. Effect of increasing acidity and sucrose concentration on preservative action of SO_2 in comminuted orange base

Medium	Initial total SO_2 ppm	Initial yeast/ml	Free SO_2 ppm after 2 days	Yeast/ml after 2 days	Days to obvious fermentation
Cordial pH 3·0 unadjusted	238	60	132	0	No fermentation up to 10 days
Base pH 3·7 unadjusted	285	60	157	$8{\cdot}7 \times 10^3$	4
Base pH 3·7 adjusted to 46° Brix	247	60	120	$2{\cdot}7 \times 10^2$	7
Base adjusted to pH 3·0	244	60	126	0	No fermentation up to 10 days
Base adjusted to pH 3·0 and 46° Brix	231	60	117	0	No fermentation up to 10 days

for longer than six weeks even though base at the same level of acidity fermented within three days in the absence of SO_2, as did base at the same level of SO_2 but with no added acid.

These findings are in agreement with other results reporting the effectiveness of SO_2 as a preservative in fruit juice as summarized by Vas & Ingram (1949) who state that as pH is lowered the equilibrium distribution of the various ionic forms present as free SO_2 changes and at pH below 3·0 the proportion of anti-microbially active non-ionized H_2SO_3 increases rapidly. Increasing acidity has also been shown to increase the effectiveness of SO_2 as a preservative by delaying the combination of SO_2 with sugars (Vas, 1949). Vas showed that this latter effect becomes considerable only when the pH is reduced to less than 2·0 which in the case of the comminuted products in question is above the maximum acidity allowable in the base to give the desired acidity in the finished cordial (approximately 1·6% citric acid).

TABLE 2. Resistance of *Saccharomyces bailii* var. *bailii* to sorbic acid and benzoic acid in comminuted orange base and cordial

Preservative	Benzoic acid				Sorbic acid			
Concentration (ppm)	200	400	600	800	200	400	600	800
Incubation time		1–2 weeks				3 months		
Fermentation in cordial	+	+	+	+	+	–	–	–
Fermentation in base	+	+	+	+	+	+	+	–

Inoculation level approximately 100 yeasts/ml.

The effectiveness of the other two allowable preservatives sorbic acid and benzoic acid in preventing fermentation by the spoilage yeast in both base and cordial is shown in Table 2. Benzoic acid used in concentrations up to the maximum allowable 800 ppm did not prevent fermentation occurring within one to two weeks. Although much more effective as a preservative, at least in cordial, sorbic acid was found to be unsuitable for commercial use because of colour deterioration which increased with increasing amounts of the preservative. To maintain an acceptable colour in the final product, it was apparent that considerable amounts of SO_2 were required.

The long-term storage trial designed to assess the preservative value of various combinations of SO_2 and benzoic acid in cordial showed that 230 ppm SO_2 or 172 ppm SO_2 + 200 ppm benzoic acid were the only two treatments which were effective in preventing fermentation during six months storage at 30°C. All other combinations of SO_2 and benzoic acid tested allowed fermentation to develop within three weeks (Table 3). The changes in total and free SO_2 in the two effective treatments over six months are shown in Fig. 3. At the end of the required storage period, free SO_2 levels were negligible in both treatments but no viable yeasts were detected in any of the stored samples.

TABLE 3. Effectiveness of benzoic acid and sulphur dioxide in preserving comminuted orange cordial for six months at 30°C

Preservative treatment		Days to first observed fermentation (averages of three replication)
ppm SO_2	ppm benzoic acid	
230	0	Not observed up to 6 months
172	200	Not observed up to 6 months
115	400	22
57	600	15
0	800	22
0	0	3

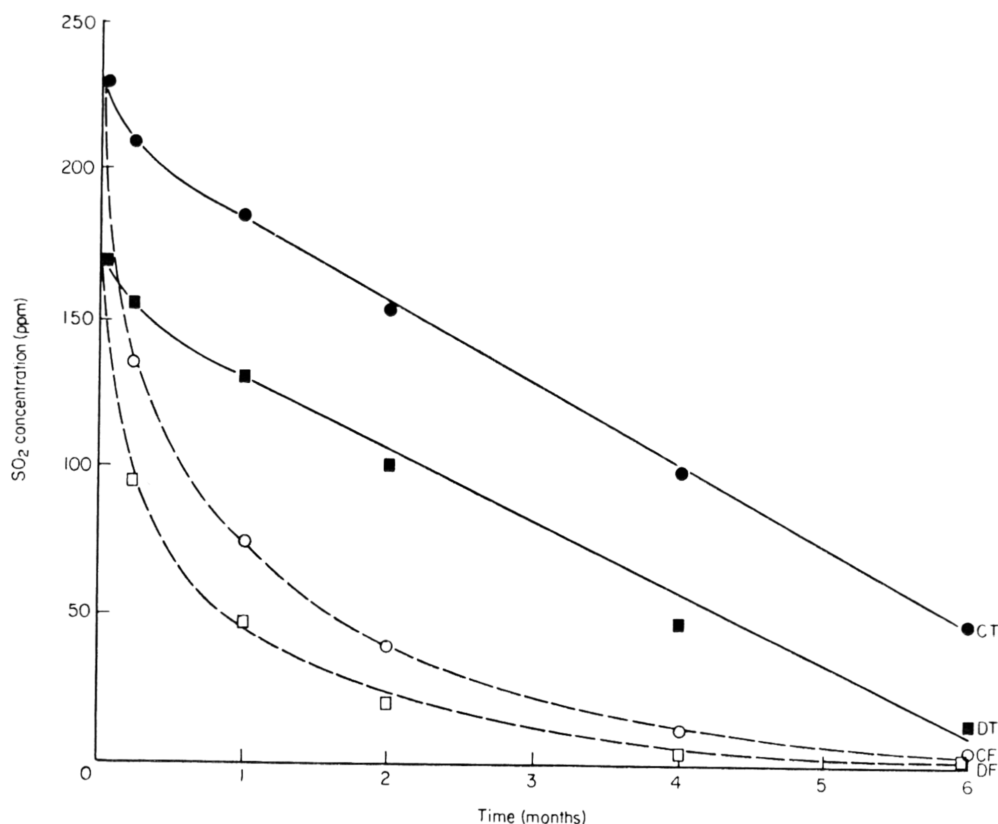

FIG. 3. Changes in free and total SO_2 in cordial during six months storage at 30°C. 230 ppm SO_2 + 0 ppm benzoic acid: ●——●, Total SO_2; ○ — — ○, Free SO_2. 172 ppm SO_2 + 200 ppm benzoic acid: ■——■, Total SO_2; □— — □, Free SO_2.

Conclusions

The yeasts frequently responsible for spoilage of locally produced comminuted orange base and cordial were found to be *Saccharomyces bailii* var. *bailii*. The organism is resistant to benzoic acid as a preservative at the maximum allowable level (800 ppm) and so contrary to British practices, this preservative is unsuitable for the comminuted product concerned. Sorbic acid is much more effective as a preservative than benzoic acid, 400 ppm preventing fermentation in cordial but 800 ppm being required to prevent fermentation in base.

In view of the deterioration in product colour associated with the use of sorbic acid, it appears that SO_2 is the only acceptable preservative for this comminuted orange product. Used at the maximum allowable level (230 ppm), SO_2 will preserve comminuted cordial for six months at 30°C provided the initial level of contamination is kept low. However, it is much less effective as a preservative in natural comminuted base,

although because of dilution during formulation, four times the maximum allowable cordial SO_2 may be used in base. The antimicrobial action of SO_2 in base may be greatly increased by acidifying the base to the same pH as the cordial or lower. From a commercial point of view, the maximum allowable citric acid could be added to comminuted base during storage instead of to the cordial during final formulation. This would greatly increase the effectiveness of SO_2 as a preservative but difficulties may be experienced under factory conditions of bulk storage in maintaining SO_2 levels in such a highly acid environment.

Acknowledgments

The technical assistance of Mrs J. Leighton and Miss L. L. Hawkins is gratefully acknowledged.

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(*Received* 5 *January* 1975)

J. Fd Technol. (1975) **10**, 575–581.

Osmophilic yeasts in preserved ginger products

ANNICE C. LLOYD

Summary

One hundred and twenty-eight samples of crystallized ginger and forty-one samples of fermented syruped ginger were examined for osmophilic yeasts. Twenty-four of the crystallized ginger samples and all of the syruped samples contained yeast belonging to the species *Saccharomyces rouxii*. These yeasts, when present in syruped ginger, were destroyed by dipping ginger pieces in 80° Brix sucrose syrup at 93° ± 1°C for a period of 2 min by which time the centre of each ginger piece had reached a minimum temperature of approximately 60°C.

Introduction

The brining, syruping and crystallizing techniques employed in processing ginger have been previously described (Leverington, 1969a, b, c; Brown, 1969a, b, c, 1972; Brown & Lloyd, 1972). The microbial changes occurring in salt brined green ginger have been investigated (Brown & Lloyd, 1972) and the yeasts associated with this product have been identified (Lloyd, 1975). However, the yeasts associated with the more osmophilic environment of syruped ginger and crystallized ginger have not previously been reported.

The commercial processing of ginger involves storage of green rhizomes in brine, followed by leaching, boiling and syruping to a final sugar concentration of 72° Brix. Drained syruped ginger is then dipped in hot high Brix sucrose syrup before rolling in sugar to give the final crystallized ginger product.

The aim of this investigation was to study yeasts normally present in syruped and crystallized ginger and also to determine the minimum heat treatment necessary using commercial operations to destroy the yeasts in the syruped product before the final application of crystalline sucrose to give the crystallized ginger confection.

Materials

One hundred and twenty-eight packets of commercially produced crystallized ginger ($\frac{5}{8}$ in. cut) from fifty-one sampling dates over a two-year period were examined. At the time of examination sixty-six of the samples were less than twelve months old (1971 stock) and sixty-two were more than twelve months old (1970 stock).

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At the original time of sampling the average initial composition of syrup expressed from the crystallized ginger was 72° Brix (Range 70·8–74·7°) and 20·5% invert (17·2–23·8%). The equilibrium relative humidity of crystallized ginger stored in cellulose film packets under ambient local conditions had previously been shown to be approximately 73–75% (unpublished departmental data).

Forty-one samples of ($\frac{5}{8}$ in cut) syruped ginger taken directly from drums of syruped ginger which had developed some signs of fermentation during storage were also examined. Larger composite mixtures of some of this fermented ginger were used in the pasteurization experiments. The fermented syrups had an average pH 3·9 (3·7–4·2) and Brix 72° (70–74°).

Methods

Surface contamination of crystallized ginger

Approximately half of each 4-oz sample (four or five pieces weighing about 20 g) was weighed into a sterile screw-cap jar. Sixty millilitres of sterile 10% sucrose solution was added and the jar shaken vigorously for 1–2 min. A drop of wash solution was examined microscopically for viable cells using Wolford's stain (Wolford, 1953). Appropriate dilutions in 10% sucrose (usually 10^0, 10^{-1}, 10^{-2}) were plated with 15 ml of Wickerham's osmophilic (WO) agar (containing 30% glucose) (Wickerham, 1951). Preliminary investigations indicated that if small total numbers of cells were present in the stained preparation, the number of viable yeasts on the ginger was likely to be below the limit of detection in a normal pour-plate technique. Hence, for such samples, duplicate 20-ml samples of wash solution were poured through a membrane filter (47 mm, 0·8 μ) and followed by a 10-ml wash with 10% sucrose solution. Membranes were plated on WO agar and counts on both plates and filters were made after three days at 30°C. If yeasts were present, representative colonies were subcultured for later identification.

Examination of fermented syruped ginger

The numbers of yeasts present in fermenting syrups and in drained fermented ginger pieces from the same samples were determined separately. To remove excess syrup, six pieces of fermented syruped ginger were centrifuged for 1 min, weighed, and then blended with 60 ml of 10% sucrose solution at low speed for 1 min and high speed for 1 min. Appropriate dilutions of syrup or blended ginger were prepared in 10% sucrose solution and plated on WO agar. For each syrup, pH and °Brix were recorded. Yeasts plates were examined after three days at 30°C and representative cultures from ginger pieces were retained on WO slopes for later identification.

Pasteurization of syruped ginger

Crystallized ginger confectionery is prepared by dipping drained syruped ginger in hot high Brix sucrose syrup before application of crystalline sugar. This dipping treat-

ment coats the ginger with an adhesive layer of syrup for attachment of sucrose crystals and also is effective as a pasteurization treatment because spoilage problems rarely occur in the finished crystallized product although fermentation of the syruped ginger prior to sugar coating is common. In commercial batch operations the actual dipping time may vary considerably, but for future continuous line operation the minimum pasteurizing time under such conditions is required to be known.

To determine the required pasteurization process for fermented syruped ginger, comparisons were made of yeast counts on an initial sample of centrifuged syruped ginger with yeast counts after immersion of the ginger for various times (0·5, 1·0, 1·5, 2·0, 2·5, 3·0, 3·5 min) in stirred 80° Brix sucrose syrup maintained at 93 ± 1°C. Eight replicated dipping runs using ginger samples with initial yeast contamination between 800 and 37 000/g were performed. Five thousand grams of 80° Brix syrup were heated in a stainless steel container in an oil bath. The container was fitted with a paddle stirrer passing through a lid under which was suspended an open grid disc. For each pasteurizing treatment, a weighed sample of four pieces of ginger was threaded on nichrome wire suspended under this grid in such a way that when the lid was lowered the ginger was completely immersed in the hot stirred syrup. After the required dipping time the lid and grid were raised and the ginger pieces removed from the wire with sterile tweezers into 60 ml of ice cold 10% sucrose solution in a cold blender jar. The delay in immersing the ginger in the cold solution was approximately 15 sec. Pasteurized samples were cooled in the refrigerator for approximately 15 min before blending and plating with WO medium as above.

To determine the actual heat penetration into the ginger during dipping, thermocouples were inserted into the centre of pieces of ginger and the internal temperatures recorded over 7-min dipping periods. Heat penetration data for eighteen individual pieces of ginger during six separate dipping runs were obtained.

Yeast identification

Twenty yeast isolates from crystallized ginger and twenty-four from fermented syruped ginger were classified according to the methods of Lodder (1971). The following characteristics were investigated: cell morphology and size in liquid and solid media; mycelium formation on Dalmau corn meal agar plates; sporulation on Gorodkowa; McClary's acetate and malt extract agars; fermentation of glucose; sucrose; maltose; lactose; galactose and raffinose; growth in 50° and 60° Brix glucose media.

Results

Microbiology of crystallized ginger

Of 128 packets of crystallized ginger examined, only twenty-four samples were found to contain viable yeasts, although large numbers of dead cells were present on most samples as indicated by visible staining of the wash solutions. Moulds were frequently

present but no bacteria were observed on any of the plates. Twenty-two of the sixty-two samples from 1970 stock contained osmophilic yeasts whereas only two of sixty-six samples of 1971 stock were contaminated. The twenty-two positive samples from 1970 were from fifteen different sampling dates spread throughout the year, so no seasonal influences appeared to be affecting the probability of yeast contamination in the samples examined. Yeast counts on the surface of the crystallized ginger ranged from 3/g to greater than 10^4/g. A few samples were much more moist in appearance than others, at the time of examination, but there was no correlation between visible wetness and yeast growth.

Microbiology of fermented syruped ginger

The average characteristics of the syrups examined were pH 3·9 (3·7–4·2) and 72° Brix (70–74). Yeast counts in the fermented syrups ranged from 200/ml to $3{\cdot}2 \times 10^7$/ml (average $2{\cdot}3 \times 10^6$/ml) while the centrifuged ginger pieces from the same samples contained between 130 and $6{\cdot}9 \times 10^5$ (av. $3{\cdot}6 \times 10^4$) per gram.

Pasteurization of fermented syruped ginger

The effectiveness of 1-min and 2-min pasteurizing dips in reducing yeast counts on eight samples of centrifuged syruped ginger is shown in Table 1. Heat penetration data from six separate dipping runs is shown in Fig. 1 to indicate the range of centre temperatures likely to occur inside a ginger piece during dipping. After 1 min the average internal ginger temperature was less than 55°C and total yeast counts were greatly reduced; after 2 min the centre temperatures of the ginger pieces were in the range 60–74°C and all contaminating yeasts were effectively destroyed. Throughout these pasteurization experiments, it was observed that pieces of fermented ginger when immersed in hot syrup, released streams of gas bubbles (presumably the product of

Table 1. Effect on yeast counts of dipping fermented syruped ginger in 80° Brix-sucrose at 93 ± 1°C

Yeast counts per g of centrifuged syruped ginger		
Initial	After 1 min dip	After 2 min dip
37 400	0	0
37 000	4	0
20 000	89	0
14 300	0	0
4700	209	0
4600	0	0
820	0	0
770	1	0

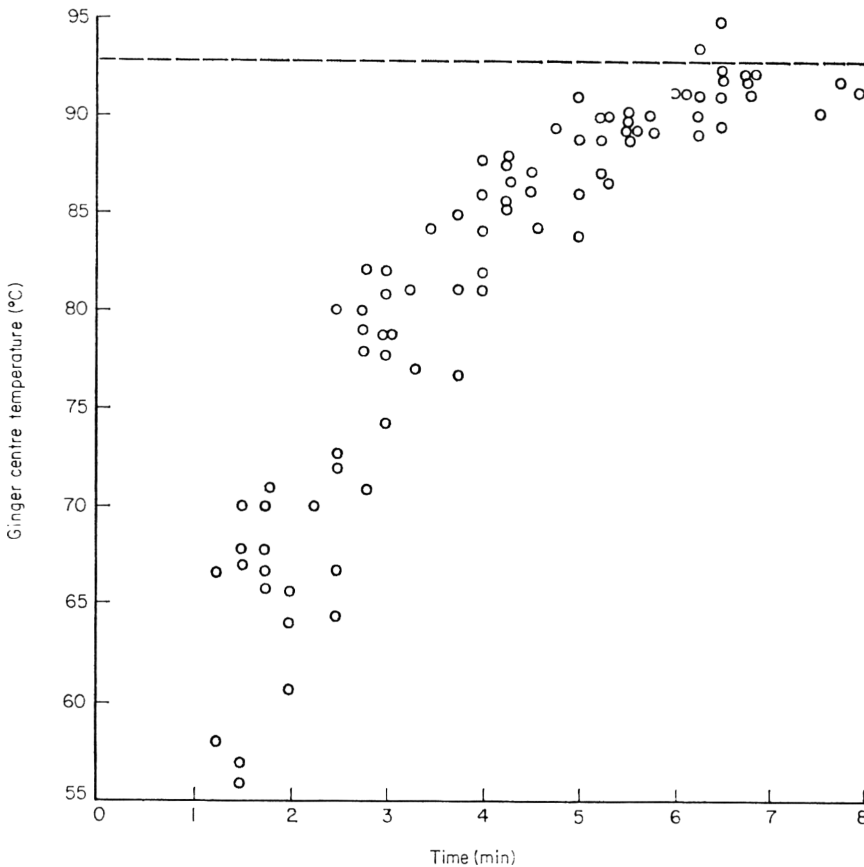

FIG. 1. Heat penetration during pasteurization of syruped ginger. Dotted line represents average syrup temperature (93°C).

internal fermentation) and the colour of the ginger changed from golden brown to pale yellowish white.

Yeast identification

All forty-four yeast isolates from syruped and crystallized ginger were very similar in properties and agreed with Lodder's description of the osmophilic species *Saccharomyces rouxii* (Lodder, 1971). In liquid culture the cells were sphaeroidal to ovoid, occurring in pairs or in small clusters. The average cell size was 4–5 $\mu \times$ 5–6 μ and elongated cells were rarely observed, none of the isolates appearing to fit precisely into either of Lodder's size groupings. All cultures formed a sediment in liquid media and pseudomycelium formation was absent on Dalmau plates. Glucose and maltose were fermented rapidly (one to three days) by all isolates, while sucrose was fermented more slowly (up to ten days). Fermentation of raffinose and galactose was variable, the latter frequently being

very slow and weak. All isolates grew on osmophilic agar (50° and 60° Brix glucose) but not in 70° Brix glucose media, although growth readily occurred in 70–72° Brix sucrose media, presumably due to the lower osmotic pressure of sucrose compared with glucose in solution. None of the isolates was observed to spore when tested on three different sporulation media. This is in agreement with Lodder (1971) and Wickerham & Burton (1960), who found that heterothallism is frequently present in this species and isolates soon lose their ability to spore when kept on laboratory media for any length of time.

Discussion

The osmophilic yeasts isolated from crystallized and syruped ginger belong to the species *Saccharomyces rouxii* which has been shown to be associated with many other similar high sugar environments such as crystallized fruit, dates and figs, as summarized by Scarr (1953). Although osmophilic fermentation is able to proceed actively in stored syruped ginger, only a relatively small percentage (19%) of 128 crystallized ginger samples up to eighteen months old was found to be contaminated with osmophilic yeasts. Samples more than twelve months old showed a much higher incidence of yeast contamination than samples less than twelve months old (33% compared with 3%). It is not known if this reflects different commercial handling procedures in the two seasons concerned, or if the higher numbers of yeasts in the older samples are simply the result of slow growth of osmophilic species which contaminated the ginger during post dipping sugaring and packaging.

To ensure against the possibility of yeast growth and subsequent spoilage under favourable conditions, it is necessary to ensure the complete destruction of all osmophilic yeasts contaminating the syruped product prior to sugaring. This pasteurization can be effected by raising the internal temperature of each ginger piece to at least 60°C. This can be accomplished by dipping the drained syruped ginger in 80° Brix sucrose at 93 ± 1°C for a minimum time of 2 min.

Acknowledgments

Ginger for these investigations was supplied by the Ginger Growers Co-operative Association Ltd, Buderim, Queensland. The technical assistance of Mrs J. Leighton and Miss L. L. Hawkins is gratefully acknowledged.

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(*Received* 5 *January* 1975)

J. Fd Technol. (1975) **10**, 583–587

Book Reviews

Single Cell Protein. Ed. by P. Davis.
London: Academic Press, 1974. Pp. xxii+278. £5.60.

By the end of this year we should be observing the realization of a new phase in the development of man, when he ceases to have to rely on the vagaries of nature for food supplies and continued survival. The advances in the production of organisms (single cells) are fantastic. Who, faced with the problems of growing penicillin in milk bottles, would have believed that in thirty years plant to produce organisms would operate at the 100 000 tons per annum level? One of these plants is that of Liquichimica, who in 1973 sponsored a symposium in Rome on single cell proteins, the papers of which constitute the book. Some forty speakers were involved in the three day symposium and they were able to deal with a wide range of topics. The first session considered single cell proteins as a protein source. Dr B. Nicol (FAO) discussed the recent developments in the status of international food and nutrition, a paper which filled in the background against which the importance of SCP must be recognized. Economic aspects were introduced at an early stage by Dr J. C. Abbott (again of FAO), but it was disappointing that the present price of SCP, which is really determined by external factors, was not justified. There was only one paper on production, that by Dr E. L. Gaden on substrates. Among the tables he presents is one showing the yield varying from 0·008 in the case of sulphite waste liquor to 0·7 with ethanol and 1·0 with *n*-paraffins. Clearly these enormous differences must influence the value of alternative substrates.

Most of the remainder of the conference was devoted to the usual questions of safety in use, the effects of using it in animal feeds. While some of the data were very well known (for instance the B.P. data) other were from Japan and, therefore, were able to provide a useful contrast. As far as direct human consumption is concerned Dr D. H. Calloway's paper flashes all the warning signals that are needed.

Five topics were particularly considered by panels who presented their expert opinions on the questions posed. Again the main emphasis was on the safety questions and how to secure approval.

Probably the most interesting part of the whole book is the ten pages in which Kiyoaki Katoh of the National Food Research Institute in Japan gives an account of the current status of SCP production in Japan. He describes the development of the Japanese culture, the influence of the mass media and the reaction of the public to the polluted environment. One reporter of a leading newspaper was strongly against SCP approval by the Government and started a campaign which was picked up by the opposition. As a result the Government pressurized industry to drop their ideas. While no one wishes to suppress the rights of the individual, it is frightening that

pressure groups can operate so that decisions are made without any logical basis. The problem is aggravated by the behaviour of 'many food industries who have betrayed our reliance'. As professionals of the Institute we must avoid the situation arising in this country.

Overall the book is well worth having as a survey of the present position on single cell protein.

A. W. HOLMES

Food Poisoning and Food Hygiene, 3rd edn. By BETTY C. HOBBS. London: Edward Arnold, 1974. Pp. ix+308. £4.50 (hardback), £2.25 (paperback).

Dr Hobbs has very successfully brought her classic book up to date by including the latest ideas on the spread and incidence of bacterial food poisoning. The book is divided into two parts, the first deals with food poisoning and food borne infections (Ch. 1–8) and the second part with food hygiene in the prevention of food poisoning (Ch. 9–17). There are also two very helpful appendices concerned with lecture material and hints on the prevention of food poisoning abroad.

Always fascinating are the case histories of food poisoning outbreaks (Ch. 6) that illustrate various lapses in personal or product hygiene, and here the author has included recent outbreaks involving *Bacillus cereus* (from fried rice) and *Vibrio parahaemolyticus* (from Far Eastern fish and shellfish). A welcome addition is a chapter on sterilization and disinfection by Isabel M. Maurer (Ch. 13), whilst Ch. 14–16 on kitchen design and equipment, control of infestation and legislation, originally contributed by the late L. Kluth, have been revised by R. J. Govett.

Two minor points which could be revised in the next edition—for I am certain this book will have a continuing life span—concern some of the foods prohibited under Jewish law (p. 4) and the use of quaternary ammonium compounds for washing beer glasses (p. 178). In the first case the author states that ducks and mackerel are prohibited foods; this is not so, although all types of shellfish are prohibited. On the second point, although many QAC preparations can be used for washing beer glasses their effectiveness may be limited by possible loss of beer head retention, and in choosing a sanitizing agent this point should be borne in mind.

My one major criticism, and I make it because I would like to see this very valuable book become an invaluable textbook on the subject, is the lack of references. I realize the need not to break up the clarity of the text with interpolated references, but the key sources could be given at the end of chapters, making the work essential reading for all engaged in the teaching of food hygiene, at all levels. This apart, however, the present edition should prove to be just as popular as its predecessors, and deservedly so!

ARNOLD FOX

The Economics, Marketing and Technology of Fish Protein Concentrate. Ed. by S. R. TANNENBAUM, B. R. STILLINGS and N. S. SCRIMSHAW.
Cambridge, Mass. and London: M.I.T. Press, 1974. Pp. ix + 500. £10.00.

This book is based on papers presented at the International Conference on Fish Protein Concentrate held in Massachusetts Institute of Technology in 1972. The editors point out in the preface that while the book is derived from that Conference, many of the papers are written at greater length and some are omitted. It is intended to be an appraisal of the present status and future outlook for fish protein concentrates (FPC). This is a very useful collection of papers as it brings together the essential arguments for and against FPC and puts into perspective the whole concept of producing a stable, dry tasteless and odourless powder which can be used as a nutritional supplement in foods for human consumption. Since the original concept was introduced in the U.S. in 1962 a great deal of work on processing and on its nutritional evaluation has been done but despite all this effort, twelve years on, the main conclusion is that many of the original questions are still unanswered and that the goal of converting the protein resource of the sea into food for the starving millions is still far off. Three questions raised by Olcott in the Introduction are discussed in various chapters. These are:

(1) Are there enough underutilized species?
(2) Are present technologies really adequate?
(3) Will the cost be too high?

It is clear that there is no unequivocal affirmative to any of these questions but there is general agreement that the original concept of a tasteless and odourless powder was too restrictive and that much greater effort should be put into the production of less expensive stabilized or semi-stabilized products possibly with odour and taste and into the improvement of functionality of fish proteins, a property completely lacking in what is conventionally known as FPC.

The book is divided into five sections: resources, processing, nutrition, utilization and economics. Under each section there is at least one main chapter which is well supplied with useful references. Available resources are extensively reviewed by Arnold and Sprague and others. The various solvent-extracted processes for FPC manufacture are described by Finch and the newer aqueous-solvent processes by Spinelli; the development of a fish protein hydrolysate (not strictly an FPC) by Rutman & Heimlich to feed the Chilean infant population is of special interest because of all the processes it appears to be nearest to commercial production and to be aimed at an undernourished population.

Nutritional aspects of FPC with descriptions of feeding trials are described very fully by Stillings, Scrimshaw & Young and its utilization by Sidwell and others. A very extensive account of economic considerations is given by Crutchfield & Deacon who conclude that Government subsidies will be needed to 'deliver adequate nutrition to target populations'. In this chapter are indeed many of the conclusions made by other

authors. One minor criticism I would make of the book is that it does not have a final conclusion, a section which one would expect in an appraisal of this kind. With twenty-seven chapters and almost as many authors there is almost inevitably a certain amount of repetition in the book which makes for rather tiring reading. Nonetheless I wholeheartedly recommend this book to anyone who wishes to have an up-to-date account of present thinking on fish protein concentrates.

IAN M. MACKIE

Textbook of Meat Hygiene, 6th edn. By H. THORNTON and J. F. GRACEY. London: Bailliere Tindall, 1974. Pp. viii + 599. £8.50.

Publication of the sixth edition of this textbook twenty-five years after its first appearance confirms that it holds its place as a leading work of reference. The change of title from *Textbook of Meat Inspection* was overdue, as successive editions have dealt increasingly with the wider aspects of meat hygiene. Great Britain now feels the impact of EEC legislation, with its requirement for official inspectors to exercise more effective general control of slaughterhouse hygiene, and the new title will help to remind students of this overdue increase in responsibility.

The length of the book is unaltered and its general balance has not greatly changed, although there has been some redistribution of the space given to most major topics and they have been rearranged in a more logical sequence. A substantial increase in the chapter on poultry is noteworthy, particularly as mandatory official inspection of poultry meat destined for the home market must surely be introduced in the United Kingdom before long. The principal innovation is replacement of the chapter 'Bacteriology of meat' by a much expanded chapter on 'Meat hygiene practice'. While the new title is more apposite, the material presented is so varied that it makes difficult reading. Splitting the major subjects into shorter, firmly edited chapters would be beneficial. Here, as elsewhere, greater clarity could be achieved by giving more attention to the typography of the various headings and sub-headings. Although they are systematically arranged, their relative importance is often not immediately apparent.

There has been a marked improvement in the quality of several of the illustrations in the four-colour plates of various pathological conditions. This is a valuable feature, in marked contrast to several of the black and white figures, which, as is all too common with such photographs, add little to the description of the disease condition in the text.

In the chapter on 'Food animals', the authors have apparently been overtaken by the speed of events; referring to the importation of exotic breeds to develop beef production in Britain, they mention only the Charolais and Simmental. The views expressed on desirable qualities of carcasses are not in line with current opinions and there are unfortunate errors in the captions of Figs 15 and 18, in which reference is made to Essex and Wessex Saddlebacks respectively, although these two pig breeds were merged several years ago.

Somewhat surprisingly, there is no reference to the use of young boars for meat production in this chapter and the section on refrigeration in the chapter on 'Abattoirs' omits any mention of the occurrence of cold shortening as a result of excessively fast chilling. Muslim ritual slaughter is incorrectly referred to as 'Mohammedan' and the rather confident illustration of the mechanics of blood splashing is not well supported by experimental evidence. The same can be said of some of the opinions expressed on the effects of transport and lairing in the chapter on 'Meat hygiene practice'.

However, it is not the purpose of this review to provide a catalogue of errors and omissions. Although the text would benefit from more rigorous editing, the book contains a wealth of information, thus maintaining its value as a source of reference to the student and practising inspector. Dr J. F. Gracey, whose assistance was acknowledged in the preface to the fifth edition, has now become co-author. It is to be hoped that this will ensure the continuing revision of future editions of this standard work when the senior author, doyen of English speaking writers on veterinary public health, decides to lay down his pen.

T. M. Leach

Books Received

The Market for Cashew Nut Kernels and Cashew Nut Shell Liquid. By R. J. Wilson.
London: Tropical Products Institute, 1975. Pp. vii + 120. £1.50.
A monograph on the production, processing and marketing of cashew nut kernels and cashew nut shell oil.

The Market for Cloves and Clove Products in the United Kingdom. By A. D. Adamson and S. R. J. Robbins.
London: Tropical Products Institute, 1975. Pp. v + 37. £0.60.
A study of the uses and future prospects of clove buds and clove oils.

Dictionary of Nutrition and Food Technology, 4th edn. By A. E. Bender.
London: Newnes Butterworth, 1975. Pp. 250. £4.75.
This is the fourth enlarged edition of the well established dictionary.

Preservation of Bacteria with Notes on other Microorganisms. Public Health Laboratory Service, Monograph No. 7. By S. P. Lapage and K. F. Redway.
London: H.M.S.O., 1974. Pp. x + 120. £1.20.
A practical manual of methods for the preservation of bacteria and other microorganisms.

Isolation of Salmonellas. Public Health Laboratory Service, Monograph No. 8. By R. W. S. Harvey and T. H. Price.
London: H.M.S.O., 1974. Pp. iv + 52. £1.50.
Describes the principles and practical methods for the isolation of salmonellas from a wide range of samples.

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Soybeans as a Food Source

W. J. Wolf and J. C. Cowan

The recent interest in soybeans as a source of food proteins and as the basis of unconventional foods stimulated the revision of this monograph. New developments in processing and technology are outlined and the growing potential of soybeans is discussed.

Selection from Contents Soybean production; Conversion to edible oil products; Food uses of soybean proteins; Physical and chemical properties; Nutritional properties; Food containing soy proteins; Problem areas.

Second Edition, 1975. 120 pages. £9.80.

CRC Press

Blackwell Scientific Publications

Oxford London Edinburgh Melbourne

The Second Edition

Handbook of Flavour Ingredients

Edited by Thomas E. Furia and Nicolo Bellanca, *Dynapol, Palo Alto, California*

The new data incorporated into this well-known reference is so extensive that the work has been expanded into two volumes. The general format and contents of the first edition have been retained and the information provided is essential to anyone concerned in the use or development of flavour ingredients.

Volume 1, 1975. 526 pages. £24.50

Volume 2, 1975. 926 pages. £32.20

Storage, Processing and Nutritional Quality of Fruits and Vegetables

Edited by D. K. Salunkhe, *Utah State University*

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Needs for future research

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Retention of nutrients and quality in plants produced by chemical treatments

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Chemicals that hasten ripening and senescence
Chemicals that may hasten or delay ripening and senescence
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Controlled atmosphere storage
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Other important and associated considerations to control ripening, senescence and microbial growth

1975. 176 pages, 32 illustrations. £13.20

CRC Press

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Oxford London Edinburgh Melbourne

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Abbreviations. Abbreviations for some commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular. Wherever possible the metric SI units should be used unless they conflict with generally accepted current practice. Conversion factors to SI units are shown where appropriate.

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3} m	hour	hr
micrometre	μm = 10^{-6} m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m^3		

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in^2	= 645·16 mm^2
square foot	ft^2	= 0·092903 m^2
cubic inch	in^3	= 1·63871 × 10^4 mm^3
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·5461 l
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= 2·76799 × 10^4 kg m^{-3}
dyne		= 10^{-5} N
Calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 T°C + 32

Figures. In the text these should be given Arabic numbers, e.g. Fig. 3. They should be marked on the backs with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of a figure the top should be marked with an arrow. Each figure must bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Line diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper and should be about four times the area of the final reproduction. Lines and lettering should be of sufficient thickness and size to stand reduction to one-half or one-third. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs. The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to Figures'.

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Printed by Adlard and Son Ltd, Bartholomew Press, Dorking