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The chemistry of smoked foods: a review

J. GILBERT AND M. E. KNOWLES

Introduction

The smoking of meat products, which is usually accompanied by mild heat processing (Draudt, 1963) is one of the oldest forms of food preservation, resulting in a product of desirable colour and flavour acceptable to a large segment of the world population (Gorbatov *et al.*, 1971). Traditionally smoking is carried out by hanging the meat in a kiln through which smoke (generated by the partial combustion of certain hardwoods) is passed. However, with the advent of modern food preservation techniques, meat smoking is now primarily undertaken for its characteristic flavour and colour, although some extension of shelf-life may result from surface-drying and deposition of smoke constituents on the product (Chen & Issenberg, 1972). This preservative action only applies to the primal cut (as smoked) and the product rapidly deteriorates with slicing for retail sale (Bard & Townsend, 1971).

The inherent drawbacks of traditional smoking, such as variability of product 'smokiness' due to changes in wood and/or combustion parameters, uptake of desirable smoke constituents, lack of versatility, etc., have been discussed by Gorbatov *et al.* (1971), Draudt (1963) and Gubler & Vernois (1972). These authors advocate more extensive use of 'liquid' smoke preparations derived from hard or softwoods which they, as well as Booth & Edwards (1971), claim offer the commercial processor uniformity, ease of application, cleanliness and virtual absence of undesirable smoke constituents (e.g. polycyclic hydrocarbons). However, their efficacy in producing smoked products equivalent to those traditionally smoked still has to be proved, as will be seen in the later discussion on smoke flavour.

Smokehouse technology has only significantly advanced over the past thirty years or so since the pioneering work of Pettet & Lane (1940) on woodsmoke composition. These recent technological advances have been recently reviewed by Kersken (1973) and Reuter (1972) and to some extent by Draudt (1963) and it is not proposed to discuss them in this review.

The aim of this paper is to outline the recent advances in the chemistry of the 'smoke' fraction of smoked foods. To accomplish this we have surveyed the constituents which

The opinions expressed in this review are those of the authors and they do not necessarily represent the views of the Ministry of Agriculture, Fisheries and Food.

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have been reported in woodsmoke, briefly considered how they are formed during wood combustion and assessed their significance as far as is known in terms of flavour, colour, preservative effect and food safety. Although the technology of smoke production has not been considered, where necessary the factors which govern the composition and quality of woodsmoke and smoked products, have been included.

The composition of wood and its thermal degradation

Prior to a discussion on woodsmoke constituents, it is worthwhile briefly considering the chemical composition of wood and the important reactions (in terms of formation of smoke-flavour components) which take place during pyrolysis.

The three major constituents of wood are cellulose, hemicellulose and lignin; the essential features of their structures are shown in Fig. 1. The natural abundance of each polymer varies with the type of wood and also with the method of estimation but essentially they can be considered to occur in the ratio of 2 : 1 : 1 respectively. During thermal degradation wood behaves as if it were a mixture of its three major components; yields of the various wood carbonization products are approximately that which would be obtained by pyrolysing proportional amounts of cellulose, hemicellulose and lignin (Goos, 1952). Therefore it seems reasonable to deal with the pyrolysis of each wood component individually.

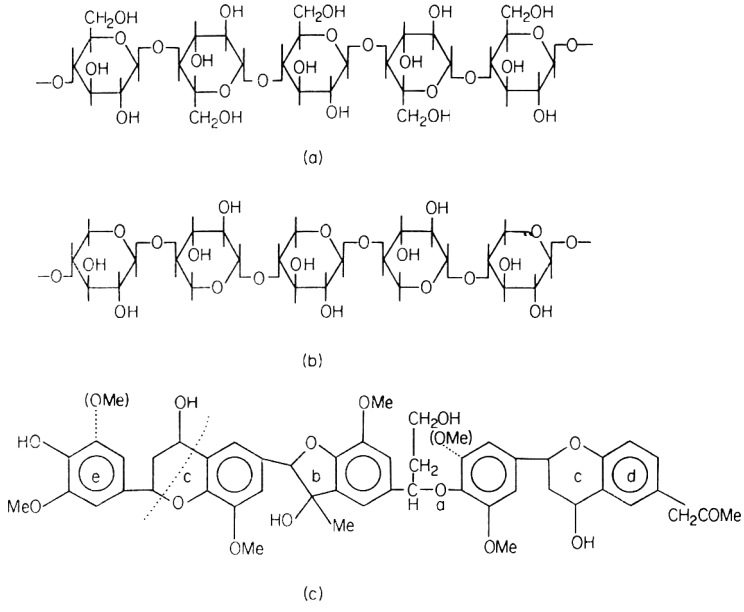

FIG. 1. Major constituents of wood. (a) Cellulose; (b) Hemicellulose; (d) Schematic structural formula for lignin (Goos, 1952).

Pyrolysis of cellulose. The initial reaction appears to be an acid-catalysed hydrolysis to glucose followed by dehydration to 1,6-anhydroglucose (β-glucosan). Secondary pyrolysis occurs immediately producing acetic acid and its homologs, water and occasionally small amounts of furans and phenols (Goos, 1952), although the latter compounds are more characteristic degradation products of hemicellulose and lignin.

Pyrolysis of hemicellulose. The exact polymeric structure assigned to hemicellulose depends on its source; hardwoods are richer in pentosans whilst in softwoods, hexosans may or may not predominate. The hemicelluloses are the least heat-stable of the wood components and readily decompose to yield furan and its derivatives together with a range of aliphatic carboxylic acids. Hardwoods, containing greater quantities of pentosans yield appreciably higher amounts of acids than softwoods. Hexosans decompose in a similar manner to α-cellulose.

It would appear that lower combustion temperatures favour high acid production in the smoke, although the effect of combustion temperature on smoke composition is still in dispute (Wasserman & Fiddler, 1969).

Pyrolysis of lignin. The compounds reputed to be of most importance in smoke flavouring are produced from this fraction. These are phenols and phenolic ethers, typified by guaiacol (2-methoxyphenol) and syringol (2,6-dimethoxyphenol), and their homologs and derivatives. The substituent groups are largely methyl, ethyl, propyl, vinyl, allyl and propenyl and it is generally accepted that the side-chains do not exceed three carbon atoms in length, and occur almost exclusively in the para-position to the phenolic hydroxyl, (Goos, 1952).

From the schematic structure of lignin shown in Fig. 1, it is apparent that under the pyrolytic conditions, the aromatic rings will be relatively stable and fission will be more likely to occur at the heterocyclic furan (b) and pyran (c) rings, and at the ether linkage (a). This in fact appears to happen and (e) possibly yields guaiacol and its homologs, whilst (d) might be expected to yield phenol and cresols. Hardwoods exhibit a slight difference in their lignin structure with the presence of additional methoxy substituents (shown in brackets in Fig. 1). Where such dimethoxy rings occur in the polymeric structure, during combustion they would yield syringol or its *para*-substituted derivatives. Hence softwoods yield predominately guaiacols whilst hardwoods yield a mixture of guaiacols and syringols (Goos, 1952).

Ferulic acid is a known intermediate in the thermal degradation of lignin and the mechanism by which its pyrolysis can yield phenolic products has been studied by Fiddler *et al* (1967). A simplified reaction scheme is shown in Fig. 2; the initial decarboxylation of ferulic acid being an oxygen-catalysed radical reaction. The oxygenated products vanillin, acetovanillone and vanillic acid were found only in a normal air atmosphere whilst only guaiacols were found in nitrogen-enriched air atmospheres.

The composition of woodsmoke, 'smoke flavours' and smoked foods

A knowledge of the composition of woodsmoke is a prerequisite to any study of the

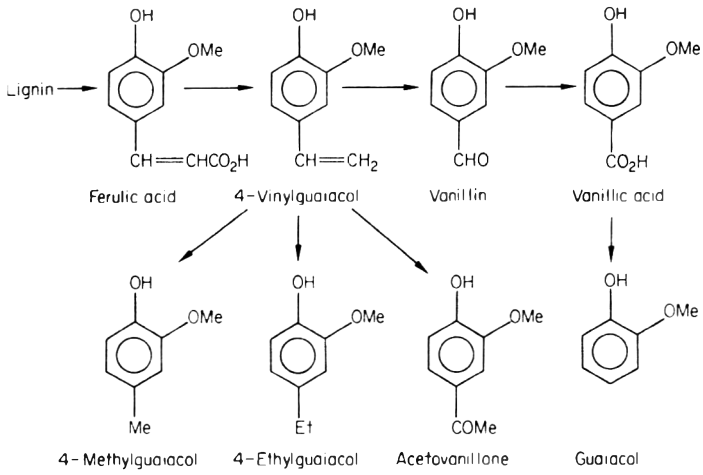

FIG. 2. Thermal decomposition of ferulic acid (Fiddler *et al.*, 1967).

mechanism of flavour and colour development, or to an understanding of the anti-oxidative and bacteriostatic properties of smoked foods. Furthermore this information enables the meat processor to apply his technological expertise to identify and control the critical parameters in woodsmoke production, ensuring uniform products of high quality to be obtained, which incorporate the attributes of smoked foods whilst minimizing the undesirable features.

Woodsmoke. Woodsmoke is a complex system consisting of disperse and particulate phases (Foster & Simpson, 1961). Absorption of the vapours (disperse phase) by the foodstuff results in the characteristic colour, flavour and preservative properties of smoked foods.

Although some deposition of minute light-scattering particles of smoke (particulate phase) occurs during smoking, its contribution to the smoking process is negligible. However, the particles have a secondary role in that they function as a reservoir of smoke constituents in equilibrium with the vapour phase. When the equilibrium is disturbed, e.g. by depletion of the vapours through absorption, the reservoir releases part of its contents to the vapour phase. Since smoke particles contain a relatively low proportion of volatile components and a high proportion of non-volatiles, the effect is to change the composition of the vapour phase and hence that of the smoke deposited in the product (Foster, Simpson & Campbell, 1961).

Many studies have been carried out to identify the components of woodsmoke but only in recent years, with the advent of more sophisticated analytical techniques (e.g. gas chromatography, mass spectrometry) have the identifications become unequivocal and the full complexity of woodsmoke been appreciated. A survey of phenolic compounds and other volatile constituents in woodsmoke and 'liquid smoke' flavours has

TABLE 1. Phenolic components identified in woodsmoke and liquid smokes

Phenol*†‡§¶	2-Methoxy-4-allyphenol*†‡§¶
o-Cresol*†‡§	2-Methoxy-4-*iso*propylphenol*
m-Cresol*†‡§¶	2-Methoxy-4-propylphenol*†‡§
p-Cresol*†‡§	2-Methoxy-4-propenylphenol*†‡§
2,3-Dimethylphenol*	2,6-Dimethoxyphenol*†‡§¶
2,4-Dimethylphenol*§	2,6-Dimethoxy-4-methylphenol*†‡§¶
2,5-Dimethylphenol*§	2,6-Dimethoxy-4-ethylphenol*†‡§¶
2,6-Dimethylphenol*	2,6-Dimethoxy-4-allylphenol*†‡
3,4-Dimethylphenol§	2,6-Dimethoxy-4-propenylphenol*†‡
2-Ethylphenol*	2,6-Dimethoxy-4-*iso*propylphenol*
3-Ethylphenol*	2,6-Dimethoxy-4-propylphenol*§¶
4-Ethylphenol*§	1,3-Dihydroxybenzene*
Vinylphenol*	2-Hydroxybenzaldehyde†
t-Butylphenol*	4-Hydroxy-3-methoxybenzaldehyde†¶
Guaiacol*†‡§¶	4′-Hydroxy-3′-methoxyacetophenone†§
3-Methoxyphenol*	2,6-Dimethoxy-4-vinylphenol†
Trimethylphenol*	4-Hydroxy-3,5-dimethoxybenzaldehyde†
3-Ethyl-5-methylphenol*	3-(4′-Hydroxy-3′-methoxyphenyl)-2-propenal†
2-Methoxy-4-methylphenol*†‡§¶	4′-Hydroxy-3′,5′-dimethoxypropiophenone†
2-Methoxy-4-ethylphenol*†‡§¶	3-(4′-Hydroxy-3′,5′-dimethoxyphenyl)-2-propenal†
2-Methoxy-4-vinylphenol*†‡§¶	

* Tar of hickory woodsmoke 'smoke flavour' (Hruza *et al.*, 1974).
† Laboratory smoke condensate from mixed hardwood sawdust (Lustre & Issenberg, 1969).
‡ Commercial liquid smoke 'Hercosef' (Knowles *et al.*, 1975).
§ 'Charsol'-smoke flavour concentrate (Fiddler *et al.*, 1970a).
¶ Laboratory smoke condensate from hickory wood (Fiddler *et al.*, 1966).

been compiled from the recent literature and is summarized in Tables 1 and 2. Investigations have centred on laboratory generated woodsmoke where careful control of production variables, such as combustion temperature, air flow, etc. has resulted in reproducible condensates, (Doerr, Wasserman & Fiddler, 1966; Lustre & Issenberg, 1969; Fiddler *et al.*, 1966).

In hickory sawdust smoke volatile acids such as acetic, propionic, and butyric have been identified (Hamid & Saffle, 1965) and the functional groups of twenty low-boiling compounds established by subtractive chromatography (syringe reactions) and g.c. retention times (Hoff & Kapsalopoulou, 1964). Acids and carbonyl compounds were fractionated and identified in hard maple sawdust smoke (Porter, Bratzler & Pearson, 1965). Their presence was confirmed in addition to new carbonyl compounds by isolation as 2,4-dinitrophenyl-hydrazone derivatives and identification by g.c. retention times (Love & Bratzler, 1966). Phenols, furans and other low-boiling components have been identified in condensates trapped from hickory sawdust smoke (Fiddler *et al.*, 1966; Doerr *et al.*, 1966) and major differences were observed between the relative

TABLE 2. Volatile compounds identified in woodsmoke and liquid smokes

Acids	Carbonyl compounds	
Formic‡	Acetone*‡¶**††	Pentanal¶**
Acetic†‡§	2-Butanone*¶**	*iso*Pentenal¶**†‡
Propionic*†‡§	2-Pentanone**¶	2-Propenal**
Butyric*†§	3-Pentanone**	2-Butenal¶**††
Pentanoic*†§	2-Hexanone**	2-Methyl-2-butenal*, **
Hexanoic *§	3-Hexanone**	Benzaldehyde*
Octanoic*	3-Methyl-3-buten-2-one*	2-Furfurylmethyl-ketone†
Levulinic†	3-Methyl-2-butanone**	
Tiglic†	4-Methyl-3-pentanone**	2-Cyclohexanone*
Benzoic*	3,3-Dimethyl-2-butanone**	1-Hydroxy-2-propanone†,††
Vanillic‡	Acetophenone*	1-Hydroxy-2-butanone†
Syringic‡	2-Furfural*†‡	3-Methyl-2-cyclopenten-1-one*
2-Methylbutyric*	5-Methyl-2-furfural*†	2-Methyl-2-cyclopentenone†
2-Methylpropenoic*	5-Hydroxymethyl-2-furfural†	2,3-Dimethyl-2-cyclopentanone†
3-Methylpentonic*	2,3-Butanedione*††**	3-Methyl-1,2-cyclopentanedione†
4-Methylpentonic*§	2,3-Pentanedione*	3-Buten-2-one††
2-Methyl-2-butenoic*	2,5-Hexanedione†	3-Hydroxy-2-methyl-4-pyrone†
3-Methylbutyric*§	2,3-Propanedione‡	
	1,2-Cyclopentanedione†	
	3,4-Dimethyl-1,2-cyclopentanedione†	
	3-Ethyl-1,2-cyclopentanedione†	
	2,5-Dimethyl-4-hydroxy-3-(2H)-furanone†	
	Formaldehyde¶**	
	Acetaldehyde*‡¶**††	
	Propionaldehyde*¶**††	
	Butanal¶**	
	*iso*Butanal**	
Furans	Alcohols and esters	
Furan*††	Ethanol*‡†††	Methyl acetate††
2-Methylfuran*††	Methanol††	Methyl formate*††
2-Ethylfuran*	*iso*Propanol††	Methyl acrylate*
2-Acetylfuran*	Acetol acetate†	Ethyl benzoate*
2-Methylbenzofuran*		Cresyl acetate*
Dimethylbenzofuran*		
Lactones	Miscellaneous	
γ-Butyrolactone	1,2-Dimethoxybenzene*	Benzene*
β-Angelicalactone	1,4-Dimethoxybenzene*	Toluene*
γ-Crotonolactone	1,2-Dimethoxy-4-methylbenzene*	Styrene*
	3-Methoxycatechol†	Dimethylindane*
	Indene*	Naphthalene*
	3-Methylindene*	3-Methylnaphthalane*

* Tar of hickory smoke 'smoke flavour' (Hruza *et al.*, 1974).
† 'Charsol' smoke flavour concentrate (Fiddler *et al.*, 1966).
‡ 'Charsol' (Hollenbeck & Marinelli, 1963).
§ Hickory sawdust smoke (Hamid & Saffle, 1965).
¶ Hard maple smoke (Porter *et al.*, 1965).
** Hard maple sawdust smoke (Love & Bratzler, 1966).
†† Hickory sawdust smoke (Doerr *et al.*, 1966).

compositions of the components in whole woodsmoke (examined by direct injection into the g.c.) and in the smoke condensate of the whole smoke (from solid CO_2 traps), (Doerr *et al.*, 1966). Furthermore changes in the condensate were observed on storage, in particular with the formation of esters causing an overall 'mellowing' of the smoke flavour (Doerr *et al.*, 1966; Wasserman & Fiddler, 1969).

Mixed hardwood and hickory sawdust smokes were found to contain the same major thirty-one phenolic constituents but in differing amounts (Lustre & Issenberg, 1969). The identification of coniferaldehyde and sinapaldehyde by these workers has not been reported elsewhere in the woodsmoke literature, but they were unequivocally established by micro-chemical techniques (hydrogenation and ozonolysis) in conjunction with mass spectrometry in the above smokes. These latter compounds are of particular interest in that they produce orange colourations, characteristic of smoked foods, in contact with proteins and become irreversibly bound to the meat matrix (Lustre & Issenberg, 1970; Chen & Issenberg, 1972).

'*Smoke flavours*'. A smaller number of workers have studied the composition of commercial 'liquid smoke' condensates such as 'Charsol' and 'Hercosef'. The major constituents of 'Charsol' were fractionated into acidic, phenolic, carbonyl, alcohol and polycyclic hydrocarbon fractions by Hollenbeck & Marinelli (1963) and several components identified by chromatography. Fiddler, Doerr & Wasserman (1970a) identified many additional phenols and oxygen heterocycles by a combination of g.c.–i.r. These latter compounds are lactones and are of particular interest in that they have been isolated from non-enzymic browning systems (Reynolds, 1970) and identified as potent aroma compounds. The most recent and exhaustive study of a commercial smoke-tar flavour product was that of hickory smoke-tar, by Hruza Sr, van Praag & Heinsohn Jr (1974). Eighty components were identified by g.c.–m.s., many of them for the first time*.

Analysis of smoked foods. Some studies have been carried out on smoked meat products (Lustre & Issenberg, 1970; Knowles, Gilbert & McWeeny, 1975), but difficulties in recovery levels and selectivity in extraction from the meat often means that the results do not necessarily reflect the true composition of the smoke components within a product. Estimates of the smoke phenol content of smoked foods have been based on the 4-aminoantipyrene or the Gibbs colorimetric methods (Tucker, 1942; Procter, Goldblith & Nickerson, 1959; Bratzler *et al.*, 1969) which depend on coupling with the *para*-position of the phenol. From the foregoing survey of phenolic constituents in woodsmoke it is obvious that many of these compounds are already *para*-substituted and hence would not be detected by such methods. Lustre & Issenberg (1970) have reported 280 ppm and 60 ppm levels of phenols in laboratory smoked uncured pork belly and commercial smoked summer sausage by a g.c. method and estimated that only about

*Ninety-eight constituents were identified in an aqueous smoke condensate (Kim, Kurata & Fujimaki, 1974).

half this amount would have been detected by the above colorimetric tests. The method of application of liquid smoke solutions to green bacon was also found to influence the relative composition of the phenols extractable from the smoked product (Knowles *et al.*, 1975). Traces of formaldehyde and phenol in amounts ranging from 0·05 to 0·1 ppm were reported in a variety of continental sausages, hams and bacon (Cantoni, Dragoni & L'Acqua, 1973). An extraction method for phenols bound to a meat matrix (fractionation based on relative acidities) has been evaluated by Issenberg, Kornreich & Lustre, (1971) for phenol guaiacol, cyclotene and 4-methyl guaiacol. Recoveries of 80–90% were reported but a certain selectivity still exists between various phenols when using the above extraction technique (Knowles *et al.*, 1975).

All the above studies on smoke composition have been carried out on smoke as formed (or as supplied as tars) and very few studies have been made of the effect of combustion parameters on smoke composition. However, Wasserman & Fiddler (1969) investigated the effect of varying the type of wood, combustion temperature, moisture and oxygen content of the air, on smoke composition as determined by changes in furans, phenols, cyclotene, etc. Sensory evaluation of each smoke condensate was carried out to correlate combustion parameters with flavour and chemical composition. No statistically significant results were obtained, although the levels of guaiacols present was found important in flavour terms.

Studies on the selective absorption of smoke constituents have been carried out in model systems using water-filled casings as absorbent (Bratzler & Harper, 1968). It was found that increase in temperature led to less guaiacol but proportionately more syringol being absorbed into the sample.

Another method of attempting to obtain a uniform smoke flavour product is to use electrostatically precipitated smoke (Rusz, 1968). This was found to contain lower amounts of acids, carbonyls and phenols than traditional woodsmoke resulting in a milder, less tarry aroma.

Flavour of smoked foods

It is apparent from the above discussion that small changes in the smoke production variables can lead to significant changes in the composition of the final smoke concentrate and hence alter the flavour. Despite numerous studies on the composition of woodsmoke the blend of components responsible for the characteristic flavour of smoked products is still unknown. However, phenols as a class are generally recognized as making a major contribution to smokey flavour (Bratzler *et al.*, 1969; Tilgner *et al.*, 1962). The taste and odour thresholds of guaiacol, 4-methyl guaiacol and syringol have been determined in oil and water systems (Wasserman, 1966) as have the partition ratios of twelve wood smoke phenols between oil and water (Doerr & Fiddler, 1970). Guaiacol, 4-methyl guaiacol and syringol were described by taste panels as 'phenolic' and 'smokey', a smokey odour being most associated with syringol and a smokey taste with guaiacol. However, mixing of these three components in the same proportion

as found in smoke condensate ogave a solution with dour and taste only slightly reminiscent of the original condensate (Wasserman, 1966). Phenols in general were found to give an incomplete 'smoke cured aroma' and it was suggested that for whole smoke aroma a complex mixture of substances was necessary (Daun, 1972). Similarly taste panel evaluation of synthetic mixtures prepared on the basis of g.c. analysis of woodsmoke condensates, were assessed as having aromas and flavours unlike the original preparations (Spanyer, Kevei & Blazovick, 1966). However, efforts to isolate a smokey fraction from 'Charsol' have been more successful (Fiddler, Wasserman & Doerr, 1970b). Concentration was carried out by adsorption on to charcoal, ether and acetone were compared as eluates and the solution was fractionated by preparative g.c. Frankfurters were dipped into water diluted smoke fractions, cooked, and then assessed for 'smokiness' by a trained taste panel. With ether as the eluent the original 'Charsol' was found to be more smokey than any of the trapped fractions or the ether extract. However, the acetone eluate differed in that it contained 'caramel burnt sugar' aroma compounds thought to modify the aroma of the fraction making it more 'smokey' rather than 'harsh phenolic'. Of three fractions isolated by g.c. one containing fifteen phenols, four substituted cyclic pent-ol-ones a hydroxyfuranone, tiglic acid and maltol was thought to be more smokey than the acetone extract from which it was prepared (Fiddler *et al.*, 1970b).

In studies on bologna a good correlation was found between taste panel descriptions of 'smokiness' or 'cured smoked aroma' and the levels of phenols present. Carbonyl compounds had changed little after smoking possibly due to screening effects of the cellulose casing and the analysis of acids showed variable results (Bratzler *et al.*, 1969).

The effect on flavour of using different types of woods has been reviewed elsewhere (Draudt, 1963) and the difference in composition of woodsmoke from hard and softwoods has been discussed in an earlier section. Despite the preference for hardwoods for smoked meat products it appears that for smoked fish softwoods give smoke flavours almost as good as traditional woods (Tilgner & Wierzbicka, 1959) and on examination of twelve different woods for the production of canned smoked fish (Lantz & Vaisey, 1970) found that some softwoods were preferred with desirable salty, sweet, smokey flavour, but hard maple and hickory smoke had only borderline acceptability. The latter two woods are extensively used for smoking meat products when they yield high quality flavoured products.

This difference in the desirability of flavours produced from identical smokes on different types of product possibly indicates that the ultimate desired flavour as consumed is either a complex blend of the smoke and original food flavours and/or reaction of smoke constituents with the surface proteins yielding further flavour components. Hence future work needs to be directed in terms of chemical interaction producing flavours in the same way that the reaction of smoke constituents with meat surfaces has been investigated in terms of colour production.

Colour of smoked foods

Although there has been relatively little research on the colour of smoked foods it is generally agreed that typical colour formation is due to browning involving carbonyl-amino reactions. Development of colour is linked with a quantitative decrease in carbonyl groupings, more marked in the presence of amino compounds (Ziemba, 1969b) the intensity of the colour increases with increase in pH, and with intensity and length of exposure to heat, light and oxygen (Ziemba, 1969a). Phenolic compounds do not participate directly or indirectly in colour formation (Chen & Issenberg, 1972; Ziemba, 1969b), but acidic compounds influence colour formation by surface protein hydrolysis, brown pigments on the surface tissues inhibiting penetration of carbonylic and other smoke components (Ziemba, 1969b).

Most studies on colour formation have involved either smoke absorbates being interacted with proteins or amino acids (Ziemba, 1969a), or specific compounds representative of woodsmoke components, e.g. dicarbonyls being reacted with individual proteins or amino acids (Ruiter, 1969; Kurko & Schmidt, 1969; Chen & Issenberg, 1972), the degree of colour formation being followed spectrophotometrically. Glyoxal, methylglyoxal, diacetyl, furfural and glyceraldehyde as components of woodsmoke have received particular attention (Ruiter, 1970; Kurko & Schmidt, 1969). Whilst as the ϵ-NH_2 groups of lysine are irreversibly bound after smoking, it is assumed these provide the amino function for the browning (Ruiter, 1970). Following the loss of available lysine as a measure of colour formation in meat and model protein systems, coniferaldehyde and sinapaldehyde were found to react at a comparable rate to previously known active aldehydes (formaldehyde, glyoxal, pyruvaldehyde, furfural), and in addition to produce characteristic yellow and orange colours in contact with protein (Chen & Issenberg, 1972). Formaldehyde appears to be the most active of the smoke ingredients in its reaction with the ϵ-NH_2 group of lysine (Dvorak & Vognarova, 1965) but it is unlikely that the reaction product contributes significantly to the colour of the smoked food (Chen & Issenberg, 1972).

Polycyclic aromatic hydrocarbons

As certain polycyclic aromatic hydrocarbons are known to be carcinogenic (Clayson, 1962) the recent public interest in food safety has prompted numerous studies of their occurrence in smoked foods. Amounts of these compounds in foodgrade woodsmoke and smoked foods are surveyed in reviews of the literature up to 1968 (Tilgner & Daun, 1969; Toth, 1969; Malanoski *et al.*, 1968; Lenges, 1972) but more recent reports of the presence of 3,4-benzpyrene in smoked foods and 'smoke flavours' are presented in Table 3. It is worth emphasizing that although 3,4-benzpyrene is the most commonly specifically determined polycyclic hydrocarbon in foodstuffs its presence can only be regarded as an arbitrary indicator of carcinogens (Tilgner, 1968). 3,4-Benzpyrene enjoys prominence among polyaromatics mainly for historical reasons as it was the first

TABLE 3. Polycyclic aromatic hydrocarbons in smoked foods and 'smoke flavours'

Smoked food or 'smoke flavour'	Concn in ppb. of 3,4-benzpyrene		Total number of other hydrocarbons detected	Reference
	Average amounts present	Exceptional samples outside normal range of values		
Smoked ham and belly fat	0·02–0·67	3·8, 3·9, 13·8	11	Toth (1971)
Smoked Yugoslav sausage (15 varieties examined)	0·2–0·9	2·9	14	Filipovic & Toth (1971)
Bologna (a comminuted mixture of beef and pork in a cured sausage)	0·2–0·3	0·6 found in exposed outer layer	—	
Cooked smoked bacon	1·6–4·6	—	—	Rhee & Bratzler (1970)
Fat drippings from bacon	2·8–3·8	—	—	
Frankfurters (cellulose casings)	Average 1·6	—	4	Simon *et al.* (1969)
Frankfurters (animal casings)	Average 13·0	—	4	
Icelandic commercial smoked mutton	Trace 1·0	—	8	Thorsteinsson (1969)
Home smoked lamb	23·0	107 (smoked close to fire)	8	
Herrings: hot smoked	0·6–6·3	—	—	Dikun *et al.*
cold smoked	0–0·19	—	—	(1969)
Various meat products:				
trad. cold smoked	Average 0·6	1·6	18 polycyclics	
rapid cold smoked	Average 0·7	1·4, 2·9	identified	Toth & Blass
black smoking	1 ppb. in 70% of samples	max. 55	from 7–16 in each sample	(1972a)
Water soluble liquid smoke flavours (7 examined)	—	1·7 of methyl derv. of BAP in one sample	6 (in 3 samples)	White *et al.* (1971)
Resinous condensate	25–3800	—	—	
Commercial liquid smoke preparations (16 types examined)	0·05–1·05	8·2, 9·3, 9·5, 15·9	2	Toth & Blass (1972c)

polycyclic hydrocarbon to be identified and shown to be carcinogenic (Tilgner, 1968). However, the total quantity of other carcinogenic polycyclic hydrocarbons in some smoked foods can be five to ten times the content of 3,4-benzpyrene (Toth & Blass, 1972a). About sixty-five other different polyaromatics are believed to be present in curing smoke, some twenty-five of which have been identified (Tilgner & Daun, 1969).

Formation of polyaromatics. In order to understand and control factors governing the formation of polycyclic aromatic hydrocarbons in woodsmoke the mechanism by which these compounds can arise during pyrolysis is of interest. A hypothetical scheme for the

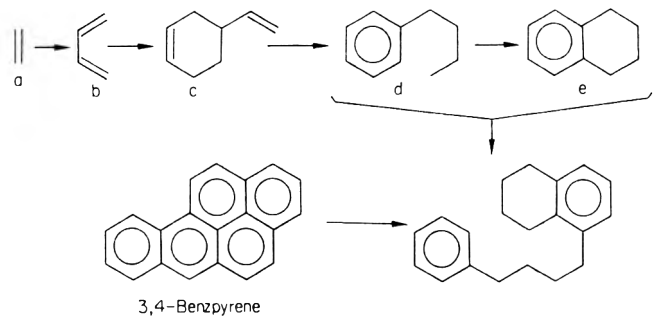

FIG. 3. Schematic view of 3,4-benzpyrene generation (Badger *et al.*, 1960).

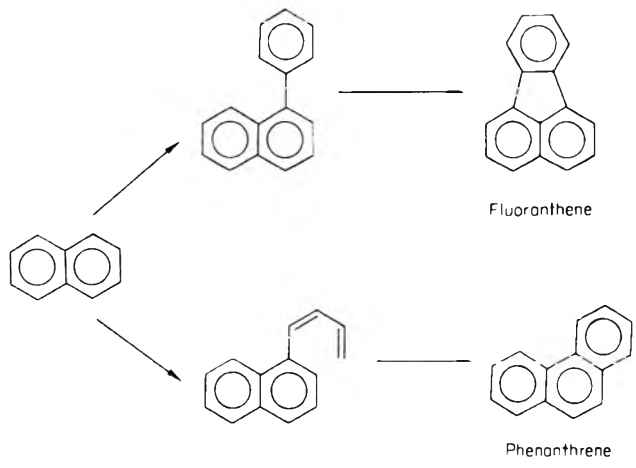

FIG. 4. Polyaromatic generation from naphthalene (Badger *et al.*, 1960).

formation of 3,4-benzpyrene is shown in Fig. 3 (Badger, Kimber & Spotswood, 1960). Initial breakdown of volatile substances under pyrolysis conditions gives rise to methylene radicals and hydrogen. Dimerization of methylene radicals to ethylene and subsequent polymerization, cyclization and dehydrogenation according to the pathway illustrated leads to the formation of the polycyclic hydrocarbons. This postulate can in some way be substantiated by the evidence that individual pyrolysis of intermediates a, b, c, d and e gives rise to 3,4-benzpyrene as a product albeit a minor one in some instances.

The presence of other polycyclic hydrocarbons can be accounted for by extending the hypothesis (Badger *et al.*, 1960) on the basis of attack of the radicals mentioned on naphthalene followed by cyclodehydrogenation as shown in Fig. 4.

Influence of smoke generation conditions. The fact that high temperatures are necessary for

initial generation of the organic radicals means that the wood decomposition temperature is an important factor in determining amounts of polycyclic aromatic hydrocarbons in the smoke. It has been reported that below a wood destruction temperature of 425°C smoke can be produced without 3,4-benzpyrene (Tilgner & Milner, 1963) and that a smouldering sawdust temperature of 350–400°C leads to minimum production of polycyclic hydrocarbons (Rusz, Kopalova & Pelikanova, 1971). A linear increase in the production of amounts of polycyclic hydrocarbons, especially higher four and five ring compounds, is said to occur over the 400–1000°C temperature range (Toth & Blass, 1972b). Hence the smoking of meat products at or below 300°C is recommended with a subsequent tenfold decrease in the level of 3,4-benzpyrene compared with a traditionally smoked product (Toth & Blass, 1972b). Commercial kiln hot-smoked fish had levels of 3,4-benzpyrene similar to those smoked over a log fire, whereas cold-smoking gave much lower levels (Dikun *et al.*, 1969). These lower levels have been confirmed by Toth & Blass (1972a) especially when compared with the high rate of deposition of polycyclic aromatic hydrocarbons which occurs during rapid hot-smoking. This deposition rate is increased even further during black-smoking probably due to the high soot levels, enriched with 3,4-benzpyrene, being deposited on the moist surfaces (Toth & Blass, 1972b).

Penetration into foodstuffs. It appears to be well established that the penetrative power of polycyclic aromatic hydrocarbons in foods is low, possibly because of their high molecular weight. Hence maximum concentrations of these compounds occur in the superficial outer layers, e.g. in smoked mutton 60–75% of the total 3,4-benzpyrene was found in these layers which only constituted 16% of the whole meat (Thorsteinsson, 1969). In smoked ham and belly fat 3,4-benzpyrene occurred chiefly in the outermost exposed layer with more than 95% at a depth < 1 cm (Toth, 1971). In bologna smoked without casings there was practically no penetration of 3,4-benzpyrene beyond the extreme 1·4–1·6 mm outer layer of the meat (Rhee & Bratzler, 1970) and whole smoked bacon showed much less 3,4-benzpyrene than the two outer layers taken from the flesh side (Rhee & Bratzler, 1970).

Smoking of products in casings can lead to a significant reduction in levels of 3,4-benzpyrene. Cotton fabric and cellophane have been shown to be effective barriers to polycyclic aromatic hydrocarbon penetration (Thorsteinsson, 1969). It has also been reported (Simon *et al.*, 1969) that in model systems composed of water or frankfurter mixture filled casings, deposition of polycyclic hydrocarbons occurred on the casing with no penetration to the contents. Hence the commercial practice for manufacture of skinless frankfurters where casing removal occurs after processing would leave the product relatively free of polycyclic aromatic hydrocarbons. However, when frankfurters were smoked in sheeps' gut casings they contained significantly higher levels of 3,4-benzpyrene (Simon *et al.*, 1969) and similarly in Yugoslavian sausages cased in natural gut, penetration up to 70–80% of polycyclic aromatic hydrocarbons occurred (Filipovic & Toth, 1971). These workers also found that when the natural gut casings

were replaced by cellulose or synthetic casings only 30% penetration occurred. These differences are explained by the hydrophilic nature of cellulose rendering it essentially impermeable to the hydropholic polycyclic aromatic hydrocarbons. In contrast the sheeps' casings being primarily protein and lipid is more likely to absorb such hydropholic compounds, thus allowing diffusion and hence penetration to take place (Simon *et al.*, 1969).

Another method of reducing the uptake of polycyclic aromatic hydrocarbons in smoked foods is to filter the smoke through cotton wool or steel wool prior to its contact with the product. Significant reduction of polycyclic aromatic hydrocarbon levels in smoked foods are claimed (Toth & Blass, 1972b) whilst still retaining the desirable smokey aroma of the product.

Electrostatic precipitation of the smoke is claimed to eliminate tar and polycyclic hydrocarbons in the smoke, with a resulting 98% reduction in 3,4-benzpyrene levels in the smoked product (Rusz, Kopalova & Pelikanova, 1971).

Polyaromatics in liquid smokes. One of the important attributes claimed for 'liquid smokes' and 'smoke' flavours is their almost total lack of undesirable tars and polycyclic aromatic hydrocarbons which are removed during processing (Gorbatov *et al.*, 1971). Examination of two commercial liquid smoke samples showed them to be free of 3,4-benzpyrene but five other polycyclic aromatic hydrocarbons were present in trace amounts (Lijinsky & Shubik, 1965). Analysis of seven water soluble smoke flavours obtained from three different manufacturers showed traces of anthracene, phenanthrene, pyrene, fluoranthrene and triphenylene in three samples, but no detectable polycyclic aromatic hydrocarbons in the remaining four. 3,4-Benzpyrene was not detected in any of the products although a methyl derivative was tentatively identified in one case (White, Howard & Barnes, 1971). By contrast, 3,4-benzpyrene was detected in all of fifteen commercially available liquid smoke preparations, but mostly at levels of 0·05–1·05 p.p.b. Furthermore in five of the samples pyrene and fluoranthrene were found in large amounts (e.g. one sample contained 196 p.p.b. of fluoranthrene) (Toth & Blass, 1972c).

The variation in the above figures for different liquid smoke preparations reflects the different processing methods and it should be possible, using reliable manufacturing practice, to produce products with nil or acceptably low levels of polycyclic aromatic hydrocarbons. A problem in the manufacture of these polyaromatic-free products is that essential flavour components are simultaneously removed—thus reducing the effectiveness of the preparation in replacing traditional smoking. Presumably, once the important trace flavour components are known, they can be 'added back' to the liquid smoke as pure chemicals thus maintaining the 'safety' of the liquid smoke preparation.

It should also be remembered that the polyaromatic hydrocarbon levels found in foods (Table 3) are not fairly comparable to those found in liquid smokes since the latter will undergo large dilutions when applied to the product to be 'smoked'.

Conclusions

Commercial interests outside food science have provided the impetus for detailed studies on the physical and chemical structure of wood and its thermal degradation. The information produced in these studies have been utilized by the food scientist in the study of woodsmoke composition and its relationship to the quality of the smoke flavour so produced. With the recent developments in sophisticated analytical techniques extensive listings of woodsmoke constituents have been reported in the literature, often with no assessment of their relevance to smoke flavour. Of perhaps more practical significance has been the work concerned with smoke generation parameters and their effect on the smoke composition in terms of the ultimate quality of the smoked product.

Relatively little work has been carried out to assess the contribution of either individual smoke compounds or mixtures of smoke constituents on the colour and flavour of the smoked product, although the phenolic fraction has been recognized as being an important contributor to smoke flavour. However, the effect of other smoke components, when mixed with the phenolic fraction, on the flavour has only received the attention of a few workers and more studies of this nature could result in improved liquid smoke preparations.

The carbonyl compounds present in woodsmoke are thought to undergo non-enzymic browning with the amino-groups of proteins in the meat matrix leading to the characteristic golden-brown colour of smoked foods. Their precise significance in terms of smoke aroma and flavour, however, are not well understood.

An area of woodsmoke chemistry which has probably received a disproportionate amount of attention (except perhaps in countries like Iceland with a high intake of heavily smoked foods and correspondingly high incidence of some cancers) is the analysis of polycyclic aromatic hydrocarbons. 3,4-Benzpyrene is an arbitary indication of polyaromatic content and therefore has been identified (at varying levels) in most classes of smoked foods, although its relative importance in terms of carcinogenesis has not been assessed. Probably of more significance has been the study of the factors which affect the production and deposition of polyaromatics in smoked foods together with the development of methods to control them.

In this context liquid smokes would appear to offer considerable technological advantages in 'smoking' foods thereby producing a uniform, 'safer' product. Their drawbacks lie at the moment in not being able to reproduce fully the required smoked flavour or colour in many products as compared with the traditionally smoked foods. Unfortunately the variability of some liquid smoke preparations, especially in regard to their polyaromatic hydrocarbon contents, has prompted some countries to ban their use (Bluhm, 1973; Henning, 1973) thus causing processors to reformulate their products.

Hence future developments in woodsmoke chemistry should be directed at identifying and assessing the significant flavour and colour components thereby allowing the development of much improved liquid smokes and smoke flavours for increased usage by the food industry.

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The determination of ascorbic acid in foods by reverse sweep cathode ray polarography

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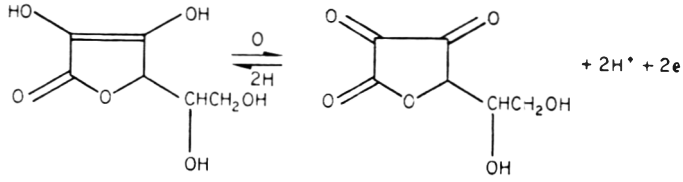
Summary

It has been shown that a variety of mixtures and multivitamin preparations may be analysed for their Vitamin C (ascorbic acid) content using cathode ray reverse sweep polarography. Preliminary results indicate that the method, as described, is flexible and has the apparent advantages of minimal sample preparation involving no separation, large tolerance towards suspended matter and coloured materials, extensive freedom from interference, rapidity and considerable sensitivity. Some difficulties (and their solutions) associated with the use of this method are outlined. Given further refinement the method seems to have considerable potential as a practical analytical technique.

Introduction

Many methods for the determination of ascorbic acid are based on the oxidation/reduction properties and are in practice variations of redox titrations. If reducing substances other than ascorbic acid are present in the assay erroneous results may be obtained. Polarographic techniques, although based on the same properties, are intrinsicly more specific, but only the more sensitive forms of polarography appear to offer possibilities for a procedure giving rapid read-out of results with a minimum of sample preparation. The present paper examines the use of reverse sweep cathode ray polarography for this purpose.

l-ascorbic acid, an unsaturated hydroxylactone is Vitamin C (I). It is oxidized at the dropping mercury electrode (D.M.E.) to dehydroascorbic acid (II).



(I) (II)

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The mercury pool is a satisfactory reference electrode provided that the potential sweep is positive going. If a conventional negative going sweep is used the pool potential is unstable.

Because of the ene-diol system, ascorbic acid is a strong reducing agent and produces an anodic wave which shifts with pH. The anodic oxidation is not completely reversible and the polarographic values are slightly more positive than those obtained from potentiometric measurements. Moreover, the oxidation product, dehydroascorbic acid, is not reducible at the D.M.E. (This irreversibility has also been demonstrated by oscillographic measurements.)

The usefulness of a supporting electrolyte is limited by the available potential range. This range is given by the potential of the dissolution of mercury (at positive potentials) and by the potential of the reduction of the cation (at negative potentials). Solutions containing sulphates, perchlorates, nitrates or acetate and borate buffers are recommended for reaching positive potentials (about +0·3 V. vs. **S.**CE.). Such supporting electrolytes are suitable for the registration of anodic waves with a positive half wave potential (e.g. barbiturates, ascorbic acid, adrenalin).

The basic design, application and advantages of single sweep cathode ray polarography have been described by several investigators (Rooney, 1966; Davis Differential Cathode Ray Polarograph, 1961).

The polarographic behaviour of ascorbic acid has been studied by Kolthoff & Lingane (1952), Zuman (1964) and Milner (1957) principally, but has seen little application. Although, a recent paper by Davidek *et al.* (1972) describes a method for the determination of l-dehydroascorbic acid in foods, based on the polarographic reduction of the condensation product of dehydroascorbic acid with o-phenylenediamine.

This paper presents the application of single reverse sweep cathode ray polarography, combined with minimum sample preparation involving no separation to the analysis of ascorbic acid in a range of dietary foods.

Material and methods

Materials

Vitamin C was obtained from Roche Products Ltd, and was of 99·5% purity.

Sodium acetate and acetic acid were Analar grade.

All other reagents (food grade) were used without further purification.

The vitamin fortified food products (dietary) were provided by Unicliffe Ltd.

The pH 4·7 buffer was a 1 : 1 mixture of 0·2 M sodium acetate and 0·2 M acetic acid.

It is well known that ascorbic acid is a strong reducing agent and at higher pH values reduces even atmospheric oxygen. Therefore, it is preferable to prepare standard ascorbic acid solutions at low pH values. An acetate buffer, pH 4·7, has proved not only useful for this purpose, but also for the preparation of supporting electrolyte used in the

determination of ascorbic acid in biological materials. In the analyses of fruit juices the sample is diluted with acetate buffer. Solids, such as vegetables, may be extracted with the same material.

Methods

Polarography. A twin cell Davis differential cathode ray polarograph (type A 1660, Southern Analytical Ltd), was used. The instrument was equipped with a dropping mercury electrode, which had a natural drop time of 10 sec immersed.

The current record was made from negative to positive potential, versus the mercury pool reference electrode, using a reverse scan. A single cell was used with the Davis differential cathode ray polarograph.

Ten millilitres of sample solution (pH 4·7) were added to a cell and deaerated with high purity nitrogen for 5 min. The dropping mercury electrode was lowered into the cell solution, which was deaerated for another minute and an i.E. curve recorded for the solution. The sweep was over the applied potential range of $-0{\cdot}2$ to $+0{\cdot}3$ v. with a peak potential of $+0{\cdot}15 \pm 0{\cdot}01$ v. The temperature was maintained at 25 ± 2 °C. Sensitivity settings and slope compensation were adjusted for proper wave height and shape.

A standard solution of ascorbic acid was prepared at a concentration of 25 mg% in pH 4·7 acetate buffer.

Each sample extract was prepared to contain approximately 0·5 mg/ml of ascorbic acid. One millilitre of this extract was taken and made up to 20 ml with pH 4·7 buffer. The ascorbic acid content of the sample was then determined using a standard addition technique. One millilitre of the sample extract was taken plus 1 ml of the standard ascorbic acid solution at 0·5 mg/ml and made up to 20 ml with 4·7 buffer. The solutions so prepared were subjected to cathode ray polarography.

Wave heights were measured directly from the calibrated oscilloscope screen as the difference between the top and bottom of the wave on the vertical scale. Sample ascorbic acid concentrations were then calculated from the direct proportion between the wave height produced by the sample alone and the wave height contribution made by the internal standard.

The standard addition method was used throughout the procedures described in this study; it is useful for biological liquids and commercial products, which either cannot be obtained vitamin free or are of unknown composition. It is also useful in that the lengthy preparation of calibration curves is avoided.

Calibration plot. A solution of ascorbic acid was prepared by dissolving 175 mg ascorbic acid in 1 litre of pH 4·7 acetate buffer. Aliquots of this stock solution were taken and diluted with pH 4·7 acetate buffer. These diluted samples were subjected to cathode ray polarography. Peak heights (i.p.) were measured for a number of concentrations and a plot of peak height versus concentration was prepared.

Interferences. Possible interferents with the ascorbic acid peak were tested dissolved in pH 4·7 acetate buffer, in the concentrations and combinations as follows.

Hydrolysed vegetable protein at 5·0, 25·0 and 250·0 mg%.

Sucrose at 5·0, 10·0, 15·0, 20·0, 25·0 and 250·0 mg%.

Fructose at 1·0, 1·5, 2·0, 2·5 and 25·0 mg%.

Vegetable fat (hardened rape seed oil). 10 g of vegetable fat was extracted with 100 ml of pH 4·7 acetate buffer, the qaueous extract was separated off and used to make up ascorbic acid solutions at 2·5 mg%.

Vitamins B_1, B_2, A/D, Nicotinamide and B_6 all at 2·5 mg%.

Sucrose plus vegetable protein both at 250·0 mg% in vegetable fat extract.

Vitamin B_2 at 0·5, 1·0, 1·5, 2·0 and 2·5 mg% after various standing times.

Vitamin premix (including Vitamin C) in the ratio as used in biscuit manufacture.

Fruit juices. The raw fruit pulps were compressed and the juice filtered off. Samples with and without a standard addition were prepared as previously described.

It was taken that the orange, lemon, and grapefruit juices analysed all contained approximately 50 mg ascorbic acid per 100 ml (McCance & Widdowson, 1960).

Using an identical procedure, the ascorbic acid content of a proprietary brand of blackcurrant cordial was determined.

Solid foodstuffs. Using the standard addition technique the ascorbic acid content of potato pellets, vitamin-fortified, chocolate-flavoured powdered milk shake, packet tomato soup, dietary biscuits with chocolate bits, and fruit and nut chocolate were estimated.

In each case the sample was ground in a pestle and mortar, and sufficient to contain 5 mg ascorbic acid (based on label claim) was weighed out. The sample was then extracted by stirring for 1 hr at ambient temperature in 50 ml pH 4·7 acetate buffer. Dilutions were made to give approximately 2·5 mg% and the wave height was recorded.

Results and discussion

It was possible to analyse both standard and sample in the same medium, pH 4·7 acetate buffer was used as the supporting electrolyte throughout.

Pure ascorbic acid. It was demonstrated that at concentrations of ascorbic acid less than 3 mg% the plot of wave height versus concentration (mg%) is linear (see Fig. 1). Plots of wave heights (obtained over a short period of time, e.g. 1 hr) versus concentration, did not always pass through the origin. However, by plotting all results of assays on pure ascorbic acid done over a period of 3 months and by taking the line of best fit, the graph passes through the origin and is linear for concentrations of ascorbic acid below $3{\cdot}0 \times 10^{-3}$ M.

Possible interferents

Proteins commonly found in dietary food formulations are casein and wheat gluten. Both were found to be insoluble in pH 4·7 acetate buffer. Hydrolysed wheat gluten, however, was soluble and was shown to enhance the ascorbic acid peak, this effect

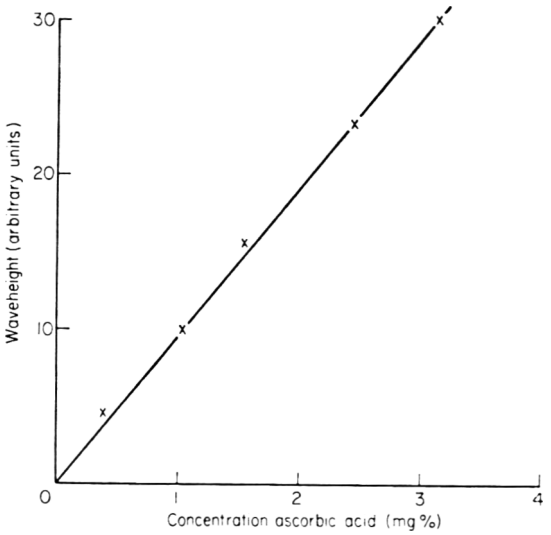

FIG. 1. Plot of peak height (i.p.) vs. concentrations for pure ascorbic acid.

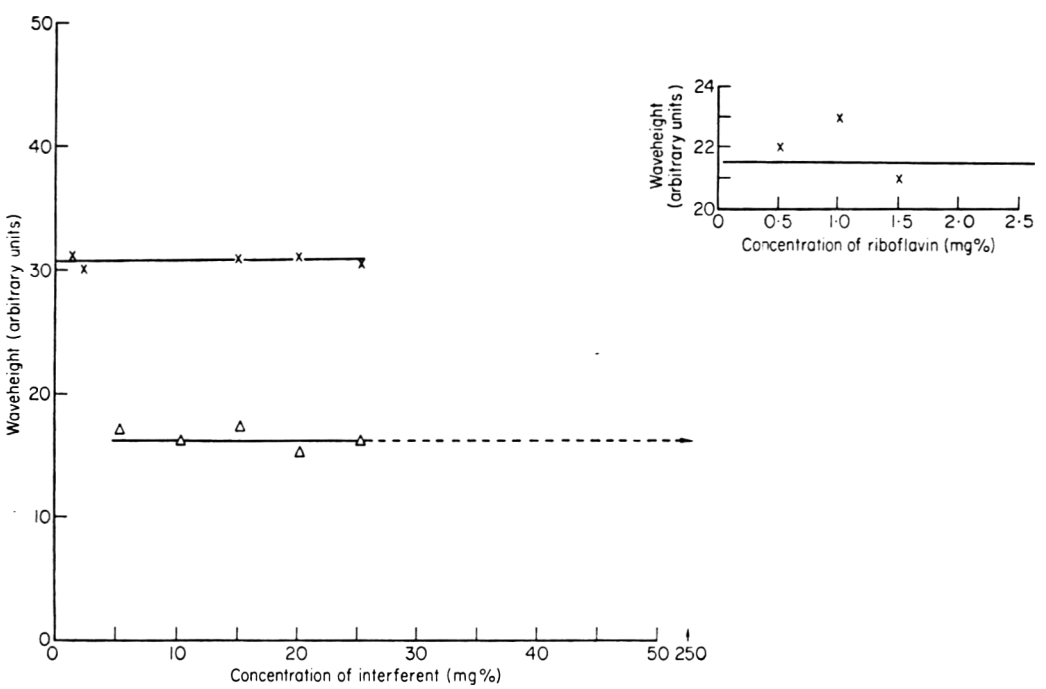

FIG. 2. The effect of varying concentrations of interferents with the i.E. curve of $1{\cdot}43 \times 10^{-3}$ M ascorbic acid. ×, fructose; △, sucrose.

becoming more evident with increasing concentration (Fig. 2), but did not produce any interfering peak(s) over the voltage scanned (Fig. 3).

Sucrose, a carbohydrate source and sweetener, is very commonly used throughout the food industry. In pH 4·7 acetate buffer, it was shown to depress the ascorbic acid peak; however, this depressing effect did not increase with increasing sucrose concentration (see Fig. 2). Sucrose did not give rise to any interfering peak(s) over the voltage scanned. (See Fig. 3.)

Fructose, a carbohydrate source, sweetener and fruit flavour enhancer is commonly used throughout the food industry. Soluble in pH 4·7 acetate buffer, it was shown to slightly enhance the ascorbic acid peak (see Fig. 2), but did not vary its effect with increasing concentration. It too, did not show any interfering peak(s) over the voltage scanned. (See Fig. 3.)

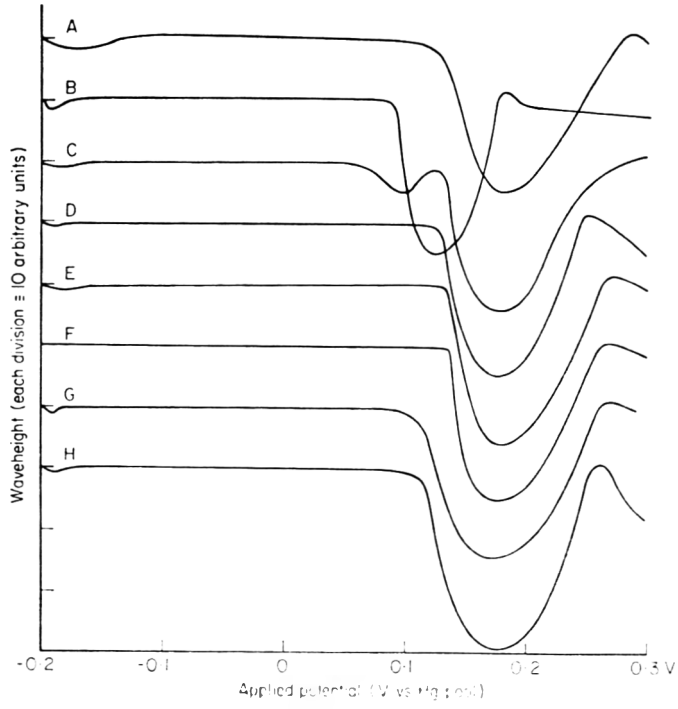

FIG. 3. The effect of some interferents upon the ascorbic acid wave shape.

A, Ascorbic acid at 2·5 mg%;

B,	,,	,,	,,	,,	,,	+	vitamin premix at 2·5 mg%;
C,	,,	,,	,,	,,	,,	+	fat at 2·5 mg%;
D,	,,	,,	,,	,,	,,	+	thiamine at 2·5 mg%;
E,	,,	,,	,,	,,	,,	+	vitamins A–D at 2·5 mg%;
F,	,,	,,	,,	,,	,,	+	nicotinamide at 2·5 mg%;
G,	,,	,,	,,	,,	,,	+	pyridoxine at 2·5 mg%;
H,	,,	,,	,,	,,	,,	+	sucrose at 50 mg% and protein at 50 mg%.

Vegetable fat (hardened rape seed oil with 100 ppm B.H.A. anti-oxidant added) was shown to contain no constituent soluble in pH 4·7 acetate buffer, capable of interfering with the ascorbic acid peak in any way. (See Fig. 3.)

The Vitamins B_1, A, D, B_6 and Nicotinamide, singly, and the following combinations of possible interferents: sucrose plus vegetable protein; sucrose plus vegetable protein in vegetable fat extract; vitamin premix, were all demonstrated to have no detrimental

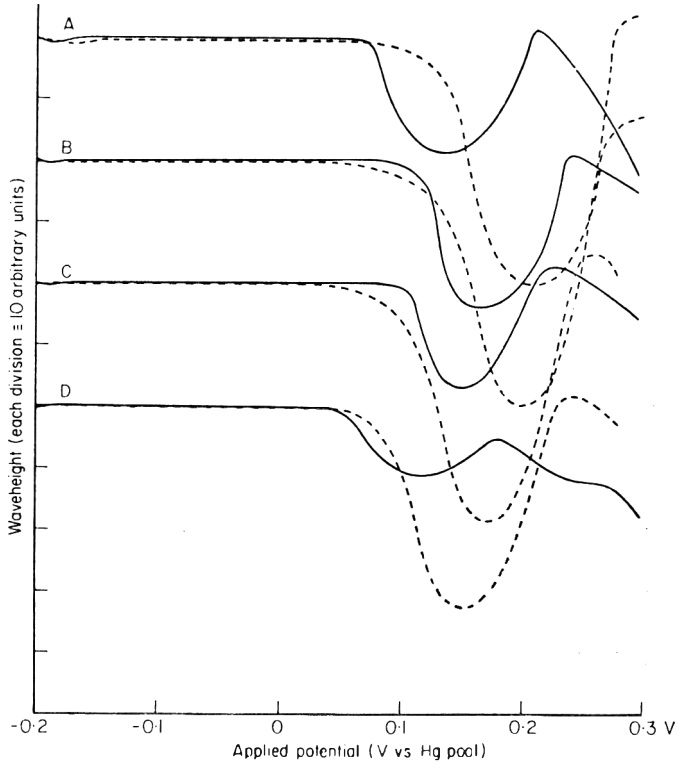

FIG. 4. Current voltage curves for:

A, ———, Orange juice diluted to contain approx. 2·5 mg% ascorbic acid;
– – –, Orange juice diluted to contain approx. 2·5 mg% ascorbic acid + ascorbic acid at 2·5 mg%;
B, ———, Lemon juice diluted to contain approx. 2·5 mg% ascorbic acid,
– – –, Lemon juice diluted to contain approx. 2·5 mg% ascorbic acid + ascorbic acid at 2·5 mg%;
C, ———, Grapefruit juice diluted to contain approx. 2·5 mg% ascorbic acid,
– – –, Grapefruit juice diluted to contain approx. 2·5 mg% ascorbic acid + ascorbic acid at 2·5 mg%;
D, ———, Blackcurrant cordial diluted to contain approx. 2·5 mg% ascorbic acid,
– – –, Blackcurrant cordial diluted to contain approx. 2·5 mg% ascorbic acid + ascorbic acid at 2·5 mg%.

effect upon the ascorbic acid peak that was not adequately overcome by use of the standard addition technique (Fig. 3).

Vitamin B_2 (riboflavin) was, however, shown to have a strong and, at higher concentrations (2·5 mg%), almost totally depressing effect upon the ascorbic acid peak. However, it was found that if an assay was carried out quickly, that is within 1 hr of

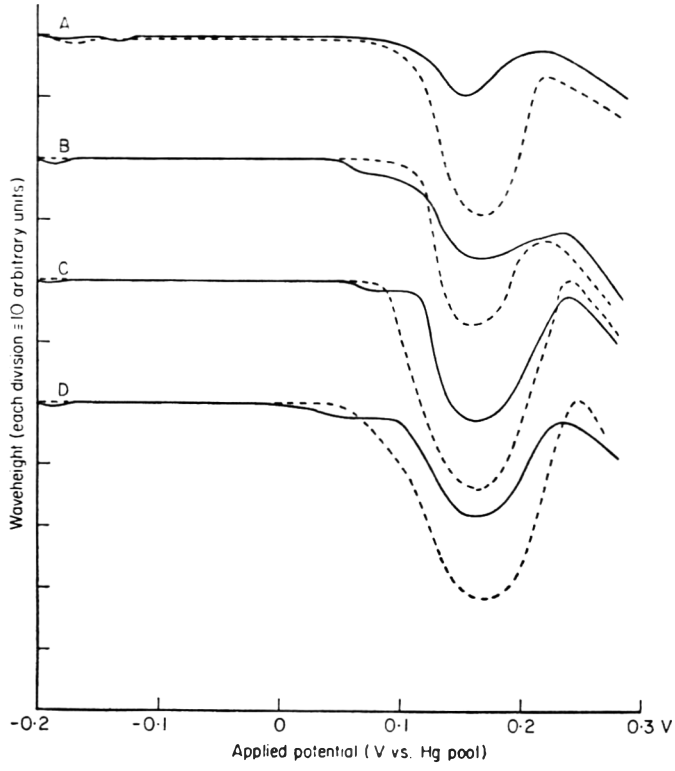

FIG. 5. Current voltage curves of some foods after dissolution in pH 4·7 supporting electrolyte, together with the waveform after addition of standard ascorbic acid.

A, ———, Tomato packet soup diluted to contain approx. 2·5 mg% ascorbic acid,
– – –, Tomato packet soup diluted to contain approx. 2·5 mg% ascorbic acid + ascorbic acid at 2·5 mg%;

B, ———, Chocolate milk shake diluted to contain approx. 2·5 mg% ascorbic acid,
– – –, Chocolate milk shake diluted to contain approx. 2·5 mg% ascorbic acid + ascorbic acid at 2·5 mg%;

C, ———, Potato pellets diluted to contain approx. 2·5 mg% ascorbic acid,
– – –, Potato pellets diluted to contain approx. 2·5 mg% ascorbic acid + ascorbic acid at 2·5 mg%;

D, ———, Biscuit with chocolate diluted to contain approx. 2·5 mg% ascorbic acid,
– – –, Biscuit with chocolate diluted to contain approx. 2·5 mg% ascorbic acid
– – –, Biscuit with chocolate diluted to contain approx. 2·5 mg% ascorbic acid + ascorbic acid at 2·5 mg%.

TABLE 1. The determination of ascorbic acid in foods by reverse sweep cathode ray polarography

Sample	Sample content claimed mg/100 g	Sample content estimated mg/100 g	No. of assays performed at one time	No. of assays performed over period of time	Mean of assay results mg/100 g	Mean of wave height	Standard deviation of assay results	Relative standard deviation
Pure strained grapefruit juice	—	50	7	—	45·11	19·86	±0·8585	1·903
Pure strained orange juice	—	50	7	—	44·70	20·57	±1·1576	2·590
Pure strained lemon juice	—	50	7	—	68·27	21·857	±1·179	1·727
Blackcurrant cordial	200	—	7	—	137·50	15·14	±5·29	3·847
Potato pellets	75	100*	7	—	92·40	7·0	±10·46	11·32
Choc. flavour powder milk shake	60	90*	7	—	82·40	76·70	±1·33	1·614
Packet tomato soup	21	100*	7	—	135·77	11·70	±9·268	6·826
Dietary biscuits	23	48*	7	—	51·40	43·7	±0·9571	1·86
Vitamin C standard	2·5	2·5	10	—	—	26·60	±0·5813	2·185
Vitamin C standard	2·5	2·5	—	12	—	26·83	±0·4032	5·230

* Estimated colorimetrically using 2·6 Dichlorophenol indophenol.

sample preparation, the depressing influence of Vitamin B_2 was virtually eliminated. Further (and provided that the assay was quickly carried out) increasing concentration of riboflavin did not have an increasingly depressing effect. At inclusion levels of B_2 usually recommended for foods, coupled with speedy analysis, the interference due to Vitamin B_2 may be overcome. (It was thought that riboflavin was only slowly soluble in pH 4·7 acetate buffer.)

Well-formed waves were obtained for the ascorbic acid contained in various fruit juices and a blackcurrant cordial. (See Fig. 4.)

Equally well-formed waves were produced with potato pellets, vitamin-fortified, chocolate-flavoured powdered milk shake, packet tomato soup and dietary biscuits with chocolate bits. (See Fig. 5.)

Attempts to analyse Vitamin C fortified fruit and nut chocolate were unsuccessful, no usable peak formation due to Vitamin C could be obtained.

Standard deviations and relative standard deviations found for some of the repetitive assays performed were quite high (see Table 1), and this was due to the way in which wave height was measured on the oscilloscope screen. No reading could be taken with any confidence to any smaller part of the arbitrary units used (in the screen calibration) than 1. Therefore, a small wave height, measured with the same degree of accuracy as a large wave height, was prone to a proportionately larger error. Statistical analysis of all results showed this indeed to be the case (see Table 1). The use of a camera attachment for the oscilloscope screen, or the use of a sensitive pen recorder (neither of which were available to the author), could go some way towards solving this problem.

Acknowledgments

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The effect of plating technique and incubation temperature on bacterial counts

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Summary

A comparison of plating methods and incubation temperatures for aerobic bacterial counts, carried out on samples from lamb, beef and meat processing equipment, showed that the spread plate counts were generally higher than those by the pour plate procedure. This was particularly so for samples which had been exposed to low temperatures for long periods. Incubation of plates at 25 and 30 °C gave similar results but the 37 °C counts were usually lower and more variable. Replication of dilutions or plates was found to give little or no increase in accuracy.

Introduction

Increasing attention is being paid to microbiological quality control in food processing establishments throughout the world. The International Committee on Microbiological Specifications for Foods (ICMSF) was formed in 1962 to establish microbiological criteria and standard methods for the examination of foods. This committee reviewed current methods for the microbiological testing of foods and published interim recommendations for their use (Thatcher & Clark, 1968).

Two procedures in common use for enumerating aerobic micro-organisms are the pour plate and spread plate methods. The ICMSF recommended the pour plate procedure with incubation at 35 °C. The spread plate technique with incubation at 30 °C is favoured by an ISO technical committee which proposed that this method should be adopted as the standard method for total aerobic counts for bacteria in meat and meat products (Barraud *et al.*, 1967).

A comparison of the pour and spread plate methods was undertaken to establish their reliability for use in microbiological quality control of meat processing. The effect of incubating plates at the three temperatures, 37, 30 and 25 °C was also studied.

Materials and methods

Sample preparation

The results analysed in this paper were obtained during studies of boning room hygiene, lamb conditioning and aging, the storage life of chilled lamb cuts and beef dressing

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hygiene (Nottingham, Penney & Harrison, 1974). The boning room samples were obtained by swabbing boning tables and conveyor belts used for carrying meat. The samples were usually taken from the cleaned surface before re-use. The lamb and beef samples were also obtained by swabbing. Sterile metal guides with an exposed inner area of 5 cm^2 were used to define the sampling position and each sampling area was swabbed twice with a sterile cotton tipped applicator. The first swab was moistened with sterile 0·1% peptone water; the second was used dry. Both swabs were placed in a McCartney bottle containing 3–4 glass beads. Immediately before making dilutions and plating out samples, 10 ml of 0·1% peptone water dilution medium were added and the bottles shaken for 2 min.

Dilution and plating

Two series of three decimal dilutions in 0·1% peptone water were prepared from each swab suspension. Six pour plates and six spread plates were inoculated from each dilution. Duplicate spread and pour plates from each dilution were incubated at 25 ± 1, 30 ± 1 and 37 ± 1 °C for 5, 3 and 3 days respectively before counting. In the pour method, plate count agar (DIFCO) was melted, tempered to about 45 °C and mixed with 1 ml of the appropriate dilution in glass Petri dishes. The spread plates were prepared the previous day and dried at 37 °C to evaporate free water from the surface. These plates were inoculated by spreading 0·1 ml of the dilutions over the surface with a sterile glass 'hockey stick'.

Results and discussion

The counts from the four replicate plates (duplicate plates × duplicate dilutions) for each sample were transformed into $\log_{10}$ values before analysis. To assess the significance of differences between plating methods, incubation temperatures and their interaction, an overall analysis of variance was carried out for each of the five sample types. Within each type, for each method and temperature, variance components were calculated to measure variation between plates, between dilutions and between samples.

Plating method

Table 1 shows mean counts ($\log_{10}$ values) for the pour and the spread techniques with incubation at 37, 30 and 25 °C, together with the overall differences ($\pm$ SE) between techniques, and the equivalent ratio of actual counts. Mean counts ranged from 650 for beef carcass samples to more than 100 000 for lamb carcasses after aging. The consistently higher count obtained by the spread technique was evident for each type of sample, and applied at each temperature. It appears that the pour plate count is affected by the sensitivity of certain psychrotrophic organisms to the hot agar used in the procedure (Clark, 1967). The 21% higher count for freshly dressed beef carcasses, where much of the contamination was likely to have been of recent animal origin,

TABLE 1. Effect of plating method and incubation temperature on bacterial counts in five sample types (Mean values of $\log_{10}$ count)

Sample type	No. samples	Temperature (°C)	Plating method		Difference between	
			Pour	Spread	Spread and pour ± SE	37 and 30/25 °C ± SE
Beef carcass	24	37	1·66	1·75	***	***
		30	1·84	1·90	0·082 ± 0·023	0·157 ± 0·024
		25	1·81	1·91	(1·21)†	(1·44)
Boning room equipment	26	37	3·14	3·30	***	***
		30	3·71	3·82	0·118 ± 0·036	0·553 ± 0·039
		25	3·74	3·82	(1·31)	(3·58)
Lamb before conditioning	36	37	3·96	4·16	***	***
		30	4·37	4·56	0·179 ± 0·018	0·422 ± 0·019
		25	4·43	4·57	(1·51)	(2·64)
Lamb after aging	36	37	4·66	4·98	***	***
		30	5·09	5·19	0·197 ± 0·027	0·348 ± 0·028
		25	5·10	5·27	(1·57)	(2·23)
Chilled lamb	36	37	3·30	3·60	***	***
		30	4·08	4·33	0·243 ± 0·058	0·770 ± 0·061
		25	4·14	4·32	(1·75)	(5·89)

***Highly significant ($P < 0·001$).

† Ratio of mean counts.

contrasts with the greater difference where there had been some opportunity for the growth of cold tolerant organisms. With chilled lamb held at —1°C for 6–9 weeks the spread counts were approximately 75% higher than the pour plate counts.

Incubation temperature

The results obtained for 25 and 30°C were similar for all sample types, but were higher than those for 37°C. The differences and ratios in Table 1 show that the lower temperatures produced 44% higher counts for beef carcasses and counts approximately six times higher for chilled lamb cuts. The ratio of the 25 to 37°C counts for the boning room samples (Table 1) reflects the low temperature conditions (10°C), under which this contamination accumulated. However, the organisms from the boning room samples displayed a lower sensitivity to the lethal effects of pour plating than might have been expected from this ratio. It is possible that this might be due to elimination of many of the heat sensitive organisms during washing of the equipment with hot water.

Many different incubation temperatures have been proposed for the bacteriological examination of food (Ingram, 1966). Body temperature (37°C) is the classical temperature for the recovery of mesophilic organisms. Mossel & van Diepen (1956) recommended incubation at 30–32°C to include both mesophiles and psychrophiles in the counts. A temperature range of 20–25°C is often used for counging cold tolerant organisms from soil, water and foods (Clark, 1967) while temperatures as low as 1°C have been suggested for counts of psychrotrophic organisms growing on refrigerated foods (Barnes & Impey, 1968).

The similarity between the 25 and 30°C counts observed in this study suggests that mesophilic organisms growing at 30°C grow also at 25°C Ayres (1960) and Kitchell, Ingram & Hudson (1973) have shown that 25°C is also a suitable incubation temperature for the enumeration of psychrophilic organisms from meat. It appears, therefore, that if only one incubation temperature is used, 25°C is as suitable as 30°C. As the 25°C count includes both mesophiles and psychrophiles and the 37°C count consists mainly of mesophiles, a comparison of the 25 and 37°C counts gives a measure of the relative proportions of each type of organism. This information can indicate the extent of bacterial growth on chilled or frozen meat during storage.

Sources of variation in counts

Measures of the variation in counts ascribable to differences between replicated plates, between replicated dilutions and between the different carcass samples are given in Table 2. These are the variance components calculated for each sample type and preparation method, pooling incubation temperatures. The large difference between the 'laboratory' sources of variation, plating and dilution, and the inherent differences between samples is indicated by the magnitudes of the respective components. The spread method gave a slightly greater variation in results from replicated

plates than the pour method. The effect of preparation method on the dilutions variance component was not consistent throughout the data. The apparently high dilutions component for chilled lamb was not typical of the other results and resulted from a few samples showing poor agreement between duplicate dilutions. This group of samples was therefore omitted in the calculation of components for each temperature (combining sample types and methods), and shown in Table 3. Even with these samples

TABLE 2. Components of variance ($\log_{10}$ count^2) for each sample type and each method

Sample type	Preparation method	Variance components		
		Plates	Dilutions	Samples
Beef carcass	Pour	0·007	0·021	0·218
	Spread	0·013	0·002	0·236
Boning room equipment	Pour	0·011	0·012	1·605
	Spread	0·032	0·042	1·459
Lamb before conditioning	Pour	0·006	0·002	0·375
	Spread	0·017	0·010	0·379
Lamb after aging	Pour	0·014	0·008	1·710
	Spread	0·045	0·023	1·362
Chilled lamb	Pour	0·028	0·285	1·889
	Spread	0·060	0·095	1·285

TABLE 3. Effect of incubation temperature on laboratory variance components

Incubation temperature (°C)	Variance component ($\log_{10}$ count^2)	
	Plates	Dilutions
37	0·0267	0·0198
30	0·0150	0·0127
25	0·0148	0·0082

Note: chilled lamb samples omitted.

omitted the variance was slightly higher for 37 °C than that for the lower temperatures. The variation between samples, where there had been some opportunity for bacterial growth was much higher than between samples from freshly dressed lamb and beef carcasses. This difference may have been due to natural variation of samples or to non-reproducible recovery of organisms from the surfaces sampled after growth. The consistent variation between replicate plates and dilutions suggests that a part of the difference arose from the breakdown of clumps of bacteria during dilution and plating.

To demonstrate the effect of different laboratory procedures on the accuracy of determining counts, variance components were used to calculate errors of observations shown in Table 4. The 'laboratory' error for a given sample was calculated as:

$$\left(\frac{\sigma_d^2}{s}+\frac{\sigma_p^2}{ns}\right)^{1/2}$$

where σ_p^2 is plate variance component, σ_d^2 is dilutions variance component and n counts are made on each of s dilutions. The 'total' error, applicable to determining sampling schemes to estimate mean values over several samples, includes the samples variance component and is

$$\left(\sigma_s^2+\frac{\sigma_d^2}{s}+\frac{\sigma_p^2}{ns}\right)^{1/2}.$$

The laboratory error shows that improved accuracy results from duplicating dilutions rather than plates and supports the recommendation of the ISO committee (Barraud *et al.*, 1967). However, the dominance of variation between samples is evident (Tables 3 and 4) and for comparing groups, replication of dilutions or plates has little effect on accuracy.

In practice the number of bacterial counts that can be carried out on a particular product is usually limited by the laboratory resources available. Accordingly, to obtain maximum accuracy these resources should be utilized in examining as many samples as possible. However, errors can arise through accidental contamination of media and it may be advisable to duplicate plates, a procedure which would not add as much to the overall work load as replication of dilutions.

TABLE 4. Errors of observations ($\log_{10}$ count) for different laboratory systems

Sample type	No. dilutions	Plates per dilution	Laboratory error*		Total error†	
			Pour	Spread	Pour	Spread
Beef carcass	1	1	0·17	0·12	0·50	0·50
	1	2	0·16	0·09	0·49	0·49
	2	1	0·12	0·09	0·48	0·49
	2	2	0·11	0·08	0·48	0·49
Lamb after aging	1	1	0·15	0·26	1·32	1·19
	1	2	0·12	0·21	1·31	1·19
	2	1	0·10	0·18	1·31	1·18
	2	2	0·09	0·15	1·31	1·18

* Includes only plating and dilution variation.

† Includes sampling variation.

Conclusions

For aerobic plate counts of mesophilic and psychrophilic organisms spread plates are preferable to pour plates; incubation at 25°C gives a higher and slightly more reproducible count than incubation at 37°C; a comparison of counts at 25 and 37°C gives a measure of the relative proportion of psychrophiles and mesophiles; replication of dilutions or plates gives little increase in accuracy.

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Blanching by electro-conductive heating

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Summary

The common method for blanching is heating by water or steam whereby the mode of heat transfer is by conduction. In cases where the object is fairly large, such as corn on the cob conduction heating will require long heating time and might have adverse effects on the product's quality. This paper presents a method for electro-conductive heating of the product immersed in a liquid whose electric conductivity is close to that of the product. The temperature rise at the geometric centre of corn on the cob was found to be 2–3° C/sec when 380 Volt was applied to 7 cm apart electrodes with no effect on flavour. In that case complete peroxidase inactivation was achieved in less than 3 min of current passage as compared to 17 min that was required in the case of boiling water blanching.

Introduction

Blanching is an important process for improving quality of frozen or dehydrated vegetables. Product quality and shelf life depends on the extent of enzymes inactivation.

The most commonly used method for thermal inactivation is heating by steam or hot water whereby the mechanism of heat penetration is by conduction. In such systems the resistance to heat transfer at the surface is negligible compared to the internal resistance to heat transfer. It was shown that in such systems the rate of heat penetration is proportional to the square of the characteristic dimension of the object (Kopelman & Pflug, 1966). As a result the blanching time for small size products, such as peas are 1–2 min, while the blanching time for larger products, such as corn on the cob is 11 min (Feinberg, Winter & Roth, 1968). In the latter case a complete inactivation of the enzymes is not achieved.

The long time required for the temperature rise at the slowest heating point, normally the geometric centre, of a relatively large object such as corn on the cob could damage the quality of the kernels. On the other hand shortening the blanching time could reduce the degree of enzyme inactivation and thus result in shorter shelf-life. As a result the practical blanching time of such a product is quite often a compromise.

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Dietrich *et al.* (1970) and Huxsoll, Dietrich & Morgan (1970) tried to reduce the blanching time by using microwave heating. In that case the uniformity of heating of the corn on the cob by the microwave reduced the enzymes inactivation time to 6 min as compared to 20 min when steam or hot water was used. However, microwave blanching is commercially impractical due to the large capital investment required and high operating cost.

A much less expensive way of uniform heating can be achieved by electro-conductive heating. Such method was used in the past for heating liquid such as pasteurization of milk (Hall & Trout, 1968) as well as for various types of steam generators. The common approach of electro-conductive heating of solid food is by contacting electrodes with the object such as the home appliance for heating sausages (Presto Hot Dogger). Large continuous operation of direct contact of electrodes with the food product is unknown. In any case such system in order to be operational must possess a very high degree of control of electrodes having good contact with each of the food particles.

The aim of this work is to propose and to study the feasibility of uniform heating by electro-conductive system without the electrodes having a direct contact with the heated object.

Methods and materials

Product electrical conductivity measurement

The cell shown in Fig. 1 was filled with distilled water and connected to a bridge type conductometer (Type E382 by Metrohm Herisau AG CN-9100 Henson, Switzerland). The difference in conductivity of the system before and after the product was

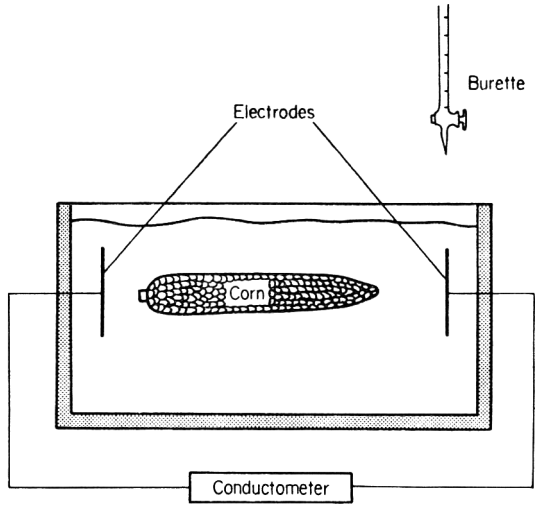

FIG. 1. Electric conductivity measuring cell.

immersed in the water was taken. The product was lifted from the cell, and the conductivity of the water was increased by adding a small amount of 1% NaCl solution. The product was then immersed again, and this process was repeated until no change of conductivity due to immersion of the product was noticed. At that point the conductivity of the product was equal to the conductivity of the liquid which in turn was determined accurately with a calibrated conductivity cell.

Electro-conductive heating procedure

The electro-conductive cell consisted of a bath having two 6 × 14-cm aluminium plates electrodes spaced 7 cm apart. The cell was filled with tap water. A few drops of 0·1% diluted NaCl solution was added until conductivity of about 1000 μ MHO/cm was reached. A fresh raw corn on the cob was placed in a desiccator filled with diluted NaCl solution as mentioned above. A vacuum was applied for a few minutes and then released. The corn was immersed in the electro-conductive cell which was connected to 380 volt A.C. source.

Temperature measurement

Twenty-four gauge copper constantan thermocouple wire was placed at the geometric centre of the cob. The temperature was monitored on a continuous single pen recorder. When temperature reading was taken the voltage was disconnected momentarily so as to avoid electrical interference in the temperature measuring system.

Enzyme activity

Fresh corn on the cob samples were used for each prescribed blanching time (electro-conductive heating as well as boiling water method). Following the blanching the samples were transferred into a cold water bath. Peroxidase activity was determined according to the information sheet AIC-43 of the U.S. Dept. Agr. (Anon 1943).

Design considerations

When heating by the electro-conductive method the electrical conductivity of the product and the immersion liquid are of prime importance for the total electrical efficiency, the uniformity of heating and the ease of operation. There are two possible conditions to be considered:

(1) immersion liquid conductivity different than product conductivity;
(2) immersion liquid conductivity equal to product conductivity.

In the first case, where the immersion liquid conductivity is different from that of the product, the current density through the product, and thus the heat generated within, will be dependent upon various factors such as a product shape, size, orientation, volumetric ratio between product and immersion liquid and their specific conductivity and product load distribution. In the second case where the immersion liquid conduc-

tivity is approximately the same as the product, the current densities in the product and the immersion liquid will have similar values. Therefore, the heating rate of the product, in such case, will be independent of its size, shape, orientation or the system product load. Obviously, the equal conductivity system is much easier to operate and to control than the unequal conductivity system, and was therefore selected in carrying out the investigation.

Results and discussion

In an equal conductivity system the energy dissipated, E, in any unit volume of the system is given in eqn (1):

$$E = \left(\frac{V}{L}\right)^2 kt \tag{1}$$

where E is energy disspated, V is voltage drop between the electrodes, L is distance between electrodes, k is specific electrical conductivity and t is time.

When product with initial uniform temperature T_i is heated by electro-conductive heating the time t_c ('come up time') required to reach the blanching temperature, T_B can be calculated by:

$$t_c = \rho \frac{C_p(T_B - T_i)}{(V/L)^2 k} \tag{2}$$

where C_p is specific heat of the product, t_c is come up time, T_B is blanching temperature, T_i is product initial temperature and ρ is density of the product.

Heating by electro-conductive method is uniform throughout the product whereas heat transfer in conduction heating is by temperature gradient. Therefore, the temperature response at the centre of a large solid product such as corn on the cob can be much faster in electro-conductive heating as compared to that of conduction type heating—resulting in shorter required blanching time.

The usefulness and the advantages of electro-conductive heating is demonstrated on corn on the cob, one of the most difficult products to blanch in the frozen food industry. Table 1 lists typical electrical conductivity values of corn on the cob under various conditions. The fresh raw product has the lowest conductivity due to air spaces in the cob pores and in between the spaces of the kernels. The variation of the electrical conductivity within the samples (Table 1) is probably due to the different amount of air present in the tissues. Removal and replacement of air by the liquid as a result of boiling or vacuum increases the product conductivity. Furthermore, it seems that the efficiency of air removal from tissues, as reflected in electrical conductivity is higher in the case of boiled water than in case of vacuum treatment. Based on the above it is clear that in order to operate in a system having equal conductivity, the immersion liquid should have an electrical conductivity value close to that of a boiled corn on the cob. In addition, in order to increase electrical efficiency and to facilitate immersion,

TABLE 1. Electrical conductivity of corn on the cob

Sample	Conductivity* MHO/cm
Fresh corn on the cob	45–65
Fresh cob	100–150
Boiled corn on the cob	1000–1400
Boiled cob	800–2000
Vacuum treated corn on the cob	250–500

* The given range is for five replicates.

air should be removed prior to blanching. This can be done efficiently by applying vacuum for few minutes and breaking back to atmospheric pressure while the corn is submerged in the immersion liquid.

A typical experimental temperature/time relationship at the geometric centre of the corn on the cob heated by electro-conductive and boiling water is shown in Figs 2 and 3, respectively. For five experiments carried out under 380 V drop and 7 cm electrodes spacing, the temperature rise varied between 2 and 3°C/sec with top temperature of 100°C at the centre being reached within come up time of 25–40 sec. This come up time can be predicted by using eqn (2) as follows. Assuming after vacuum air removal $\rho = 1{\cdot}0$ g/cm³ (actually it was found to be 1·04 g/cm³) and the value of C_p to be 0·9 cal/g°C, calculated on basis of 20% T.S. The specific conductivity required for eqn (2) can be estimated to be the conductivity of the immersion solution at the average temperature of T_i and T_B, (average temperature $= (25 + 100)/2 = 62{\cdot}5$°C). Knowing that the immersion solution concentration was about 10 mEq/litre (specific conductivity at 25°C = 1000 μmho/cm), the specific conductivity at 62·5°C was calculated based upon equivalent conductance of separate ions to be $k = 2370$ μmho/cm. Therefore,

$$t_c = \frac{1 \times 0{\cdot}9\ (100 - 25)}{(380/7)^2\ 2370 \times 10^{-6}} 4{\cdot}2 \approx 40 \text{ sec.}$$

The come-up time, t_c, can be shortened by increasing the voltage drop (V) and/or reducing the electrodes spacing (L).

On the other hand, Fig. 3 shows typical temperature response of similar size corn on the cob heated in boiling water, where the heating mechanism is by conduction. The temperature response parameter, f†, is approximately 9 min and the time required to reach 98°C exceeds 20 min.

The effect of the difference in heating rate of the electro-conductive process as compared to that of conduction heating on the peroxidase inactivation is shown in

† f is the time required for the curve to cross one logarithmic cycle when the temperature difference is plotted versus time on semi-logarithmic paper.

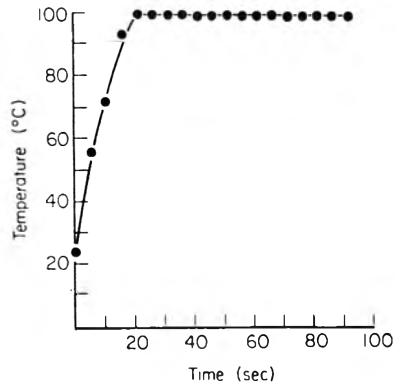

FIG. 2. Corn centre temperature as function of electro-conductive blanching time.

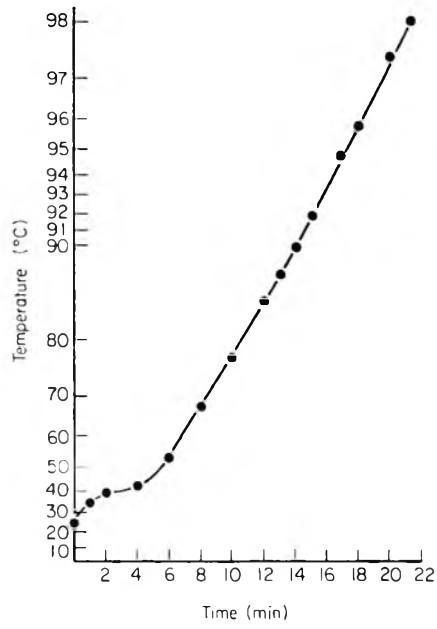

FIG. 3. Corn centre temperature as function of boiling water blanching time.

Table 2. In the case of the electro-conductive process of corn on the cob no peroxidase activation was observed after 3 min as compared to 17 min that was required to reach the same effect under the conduction type heating.

A taste panel of twelve was not able to detect any difference between corn on the cob blanched by the two different methods.

The energy efficiency of the electro-conductive heating system is the ratio between the energy input to the product to raise its temperature from its initial temperature,

TABLE 2. Effect of blanching by electro-conductive heating and by boiling water on peroxidase inactivation in corn on the cob

Electro-conductive heating time (min)	Peroxidase activity	Boiling time (min)	Peroxidase activity
2·0	+	4	+
2·25	+	8	+
2·50	±	12	+
2·75	±	13	±
3·0	−	15	±
3·5	−	16	±
4·0	−	17	−

T_i to its blanching temperature, T_B and the total energy input to the system (eqn 3).

$$\eta = \frac{G \rho C_p (T_B - T_i)}{(V/L)^2 k (V_s + G t_R)} \tag{3}$$

where η is energy efficiency—the ratio between energy input into the product and the energy input into the whole system, G is product volumetric flow rate through the system, t_R is residence time of product through the system and V_s is volume of immersion solution.

Combining eqn (2) with (3) yields:

$$\eta = \frac{G t_c}{V_s + G t_R}. \tag{4}$$

The residence time, t_R, of the product can be decreased (thus improving η) by dividing the blanching process into two sections:

(a) the electro-conductive heating section where the product residence time will equal to the come-up time; and
(b) the holding section consisting of water bath or steam chamber maintained at blanching temperature.

In this way the centre of the product will reach its blanching temperature at the electro-conductive heating section, and the completion of the enzymes deactivation is taking place in the constant temperature holding section.

In this two-section arrangement the electrical efficiency is given by:

$$\eta = \frac{G t_c}{V_s + G t_c}. \tag{5}$$

The maximum electrical energy efficiency that can be obtained in the electro-

conductive blanching cell is determined by the ratio of the product volume and the total cell volume. In a system where the volumetric ratio of the product and the immersion solution is 2 : 3 the electrical energy efficiency calculated from eqn (5) is $2/(2+3)=0{\cdot}4$.

Further work is planned to scale up the electro-conductive blanching process and to find additional uses of heating solid objects by this method.

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An equation for correlating equilibrium moisture content in foods

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Summary

The object of this study was to show that a multilayer adsorption equation, originally developed for physical adsorption on nonuniform surfaces, can be used to describe reasonably well the water sorption isotherms of a great variety of foods and food components. Apparently, the proposed equation has not been applied to the food area before.

Characteristics parameters of the adsorption equation for each of the foods tested were computed and a statistical analysis of its applicability was made.

Introduction

Several mathematical equations have been reported in the literature for describing water sorption isotherms of food materials (Henderson, 1952; Becker & Sallans, 1956; Day & Nelson, 1965; Strohman & Yoerger, 1967; Labuza, 1968; Ngoddy & Bakker-Arkema, 1970; Chen, 1971; Chen & Clayton, 1971). Each of the models reported, empirical, semi-empirical or theoretical, have had some success in reproducing equilibrium moisture content data. However, none of these have been able to give accurate results throughout the whole range of water activity and for different types of foods. This is mainly due to the fact that moisture sorption isotherms of food products represent the integrated hygroscopic properties of numerous constituents, and that the depression of water activity is due to a combination of factors, each of which may be predominant in a given range of water activity (Karel, 1973) in a given food.

Equations fitting water sorption isotherms in food materials are of special interest for the prediction of equilibrium conditions after mixing products with different water activities (Salwin & Slawson, 1959). An analytical expression for the isotherm is also required to predict the shelf life of a dried product in a packaging material of known permeability (Karel, Mizrahi & Labuza, 1971; Labuza, Mizrahi & Karel, 1972), or in predicting drying times of food materials (King, 1968). Labuza (1968) has pointed out the need of mathematical models available for the whole part of the isotherm to be used along with computer techniques to solve problems as those mentioned above.

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TABLE 1. Constants r and a' in Halsey's equation for foods and food components

Product	Specifications	Temperature (°C)	X_m g/100 g (dry basis)	r	a'	Reference
Apple	Desorption	19·5	4·17	0·76432	1·7100	Taylor (1961)
Bean	Freeze-dried Adsorption	25	5·40	1·2461	1·4443	Lafuente & Piñaga (1966)
Beef	Freeze-dried Adsorption	room	5·51	1·3015	1·4215	MacKenzie & Luyet (1967)
Cabbage	Freeze-dried Adsorption	37	4·21	0·68467	1·3069	Mizrahi, Labuza & Karel (1970)
Carrots	Desorption	19·5	4·52	0·91633	1·6631	Taylor (1961)
Cellulose (microcry)	Desorption Added 7% oil	37	4·41	1·5938	1·3393	Labuza & Rutman (1968)
Champignon	Freeze-dried Adsorption	20	4·71	0·95166	1·5141	Iglesias (1973)
Chicken	Desorption cooked	19·5	6·96	1·5918	1·7200	Taylor (1961)
Cod	Adsorption	30	7·68	1·2398	1·3490	Jason (1958)
Corn	Desorption	4·5	8·30	2·2345	1·9748	Chen & Clayton (1971)
	Desorption	15·5	7·68	2·4862	2·0949	Chen & Clayton (1971)
	Desorption	30	7·30	2·5663	1·7950	Chen & Clayton (1971)
	Desorption	38	6·35	2·3711	1·8618	Chen & Clayton (1971)
	Desorption	50	5·89	2·1203	1·5936	Chen & Clayton (1971)
	Desorption	60	5·11	2·2185	1·7430	Chen & Clayton (1971)
Egg, whole	Desorption	19·5	4·10	1·5341	1·6208	Taylor (1961)
Egg, albumin	Adsorption heat coagul.	25	6·25	1·4549	1·2675	Benson & Richardson (1955)
Fish protein conc.	Adsorption	25	5·35	1·9604	1·7898	Raseklı, Stillings & Dubrow (1971)

Fish protein conc.	Adsorption	35	5·08	1·9043	1·6709	Rasekh *et al.* (1971)
	Adsorption	42	4·50	1·7776	1·7470	Rasekh *et al.* (1971)
Gelatin	Adsorption	25	8·48	1·8227	1·9056	Bull (1944)
Green pea	Desorption	19·5	5·03	1·4091	2·0600	Taylor (1961)
Maltose	Freeze-dried Adsorption	23	5·00	0·92244	1·4367	Flink & Karel (1972)
Orgeat	Freeze-dried Adsorption	25	5·16	1·4199	1·1521	Piñaga & Lafuente (1965)
Pea	Freeze-dried Adsorption	25	5·39	1·2366	1·4001	Lafuente & Piñaga (1966)
Potato	Desorption	19·5	7·54	1·7704	1·7597	Taylor (1961)
	Adsorption	25	5·16	1·0912	1·3247	Iglesias (1973)
	Adsorption	30	5·71	1·1344	1·0764	Saravacos (1967)
Pork	Desorption	19·5	6·85	1·4506	1·6969	Taylor (1961)
Rice	Desorption cooked	19·5	8·09	1·9850	1·9937	Taylor (1961)
Salmin	Adsorption	25	5·94	1·1316	1·9686	Bull (1944)
	Adsorption	40	5·53	1·1007	1·9896	Bull (1944)
Salmon	Freeze-dried Adsorption	37	6·06	1·2150	1·1761	Martinez & Labuza (1968)
Serum albumin horse	Adsorption	25	6·52	1·7450	1·5950	Bull (1944)
Sorghum	Adsorption	21·1	6·56	2·1063	2·4249	Fenton (1941)
Soybean	Adsorption	30	2·58	1·0406	1·3103	Saravacos (1969)
Spinach	Adsorption	37	4·32	1·0947	1·6631	Makower & Dehority (1943)
Sucrose	Freeze-dried Adsorption	47	5·68	0·85983	1·1973	Iglesias (1973)
Sugar beet root	Desorption	20	5·55	0·95578	1·4359	Iglesias (1973)
Walnut kernels shelled	Adsorption	22·5	0·74	2·6815	42·8696	Rockland (1957)
Wheat	Desorption	50	6·02	1·9334	1·7784	Becker & Sallans (1956)
Wheat flour	Adsorption	20·2	5·95	2·2566	3·0618	Bushuk & Winkler (1957)
	Adsorption	30·1	5·93	2·2561	2·7158	Bushuk & Winkler (1957)
	Adsorption	40·8	5·56	2·1543	2·6333	Bushuk & Winkler (1957)
	Adsorption	50·2	5·23	2·0168	2·3517	Bushuk & Winkler (1957)

TABLE 2. Statistical analysis on the application of Halsey's equation

Product	Range of A_w	χ^2	Probability level	Variance of regression	Correlation coefficient	$\%(\text{Error})_{av.}$	%Error at A_w 0·30	0·50	0·70
Apple	0·05–0·70	0·2080	<0·0005	0·0060	0·9970	5·51	8·98	1·91	1·70
Bean	0·05–0·80	0·0832	<0·0005	0·0021	0·9984	1·79	1·75	2·67	0·85
Beef	0·10–0·85	0·1244	<0·0005	0·0012	0·9989	2·67	0·63	3·35	4·84
Cabbage	0·05–0·60	0·0795	<0·0005	0·0067	0·9976	2·45	3·56	1·02	——
Carrots	0·05–0·70	0·1613	<0·0005	0·0042	0·9973	4·26	8·93	2·16	0·59
Cellulose microcrys.	0·10–0·80	0·1564	<0·0005	0·0042	0·9929	4·95	9·73	2·45	0·88
Champignon	0·05–0·80	0·3109	<0·0005	0·0061	0·9957	5·80	1·61	2·50	2·14
Chicken (cooked)	0·10–0·80	0·1550	<0·0005	0·0015	0·9979	3·34	4·36	3·82	1·63
Cod	0·10–0·75	0·2613	<0·0005	0·0034	0·9949	5·12	5·00	4·71	3·60
Corn (1)	0·10–0·80	0·2607	<0·0005	0·0038	0·9879	3·07	4·52	0·30	2·08
(2)	0·10–0·90	0·5940	<0·0010	0·0097	0·9775	3·67	1·34	5·59	4·75
(3)	0·10–0·90	0·7002	<0·0050	0·0143	0·9665	3·86	3·27	4·77	4·92
(4)	0·20–0·80	0·2444	<0·0050	0·0039	0·9912	4·92	2·12	0·65	6·52
(5)	0·20–0·80	0·3210	<0·0050	0·0059	0·9881	6·07	2·04	2·47	7·95
(6)	0·20–0·80	0·2252	<0·0010	0·0045	0·9913	5·47	0·15	6·22	5·94
Egg, whole	0·10–0·80	0·2559	<0·0005	0·0056	0·9905	5·79	7·13	6·49	0·27
Egg, albumin	0·20–0·80	0·4086	<0·0050	0·0062	0·9958	6·49	7·83	9·24	1·39
Fish prot. conc. (1)	0·10–0·80	0·1752	<0·0005	0·0027	0·9925	4·33	7·03	2·75	0·16
(2)	0·10–0·80	0·2158	<0·0005	0·0036	0·9901	5·03	8·30	2·74	0·26
(3)	0·10–0·80	0·3227	<0·0010	0·0075	0·9823	7·09	9·69	3·03	0·99

Gelatin	0·20–0·90	0·4417	<0·0050	0·0040	0·9934	5·11	3·11	9·45	1·62
Green Pea	0·05–0·80	0·3159	<0·0005	0·0050	0·9931	7·78	5·22	9·00	0·46
Maltose	0·10–0·70	0·0440	<0·0005	0·0012	0·9991	2·45	0·93	3·33	1·98
Orgeat	0·10–0·80	0·2147	<0·0005	0·0051	0·9925	6·15	8·22	7·98	4·25
Pea	0·05–0·80	0·0977	<0·0005	0·0026	0·9973	3·46	4·64	1·79	1·18
Potato (1)	0·10–0·80	0·2459	<0·0005	0·0025	0·9939	4·38	5·92	1·54	2·31
(2)	0·10–0·70	0·0874	<0·0005	0·0018	0·9974	3·65	4·50	1·62	4·13
(3)	0·20–0·75	0·1687	<0·0005	0·0024	0·9967	4·15	4·71	6·79	1·11
Pork	0·05–0·75	0·1895	<0·0005	0·0033	0·9959	3·92	2·95	3·72	0·22
Rice, cooked	0·10–0·80	0·4815	<0·0005	0·0040	0·9871	5·18	2·80	7·02	1·38
Salmin (1)	0·05–0·80	0·3969	<0·0005	0·0050	0·9959	5·84	12·46	2·92	2·57
(2)	0·05–0·80	0·5088	<0·0010	0·0067	0·9947	6·80	14·45	3·93	3·16
Salmon	0·10–0·80	0·3083	<0·0005	0·0076	0·9902	6·38	11·21	1·28	1·51
Serum albumin horse	0·20–0·90	0·5002	<0·0050	0·0049	0·9934	5·83	5·41	2·69	5·23
Sorghum	0·035·0·84	0·4842	<0·0010	0·0054	0·9889	5·31	0·31	9·88	0·37
Soybean	0·10–0·80	0·1498	<0·0005	0·0050	0·9963	5·58	8·09	1·66	6·01
Spinach	0·05–0·75	0·1094	<0·0005	0·0024	0·9978	3·96	5·45	3·83	0·45
Sucrose	0·20–0·80	0·8609	<0·0005	0·0053	0·9959	5·82	0·95	9·83	4·44
Sugar beet root	0·05–0·70	0·1708	<0·0005	0·0028	0·9971	3·91	1·14	2·10	2·75
Walnut kernels shelled	0·10–0·90	0·0228	<0·0005	0·0008	0·9968	2·32	2·90	0·87	3·33
Wheat	0·13–0·88	0·4721	<0·0005	0·0068	0·9896	5·02	3·42	6·71	2·42
Wheat flour (1)	0·12–0·89	0·0258	<0·0005	0·0004	0·9991	1·62	3·68	0·38	0·40
(2)	0·13–0·90	0·0523	<0·0005	0·0010	0·9982	2·53	1·34	3·09	0·58
(3)	0·13–0·90	0·0754	<0·0010	0·0014	0·9975	3·05	1·54	2·78	1·13
(4)	0·15–0·90	0·0861	<0·0010	0·0019	0·9968	3·25	0·60	4·25	0·68

The object of this study was to show that a multilayer adsorption equation, originally developed by Halsey (1948) for physical adsorption on non-uniform surfaces, may be used to describe reasonably well the water sorption behaviour of a great variety of foods and food components. It was found that this equation is applicable to the range of water activity, $0{\cdot}10 \leqslant A_w \leqslant 0{\cdot}80$, which, as a matter of fact, is the one of most practical applications.

Results and discussion

Halsey (1948) developed the following adsorption equation to provide an expression for condensation of multilayers at a relatively large distance from the surface,

$$p/p_0 = \exp(-a/RT\theta^r) \qquad (1)$$

where $p/p_0 = A_w$ = water activity; a,r = parameters; $\theta = X/X_m$; X = equilibrium coverage, in g adsorbed/g solid; and X_m = monolayer value, same units as X.

For purposes of curve fitting, equation (1) may be put in the form:

$$\ln \ln p_0/p = -r \ln \theta + \ln a'$$

where $a' = a/RT$.

A plot of $\ln p_0/p$ vs. $\ln \theta$ should be a straight line from which the parameters r and a' may be calculated.

Equation (1) was developed by Halsey (1948) on theoretical ground as a criticism to B.E.T. theory. As this equation was shown by Halsey (1948) to be a good representation of adsorption data that conform to the B.E.T. type I, II or III shapes (Gregg & Sing, 1967) we tried to apply it to food products. As far as we know, equation (1) has not been used to describe water sorption isotherms of food materials.

The data for a wide variety of foods and food components reported in the literature were used to test the usefulness of the proposed equation. Although the monolayer value is not essential for fitting purposes, it was used in order to follow B.E.T.'s notation as it was done by Halsey (1948). If X_m is not desired to be used, equation (1) will also fit the experimental data yielding a different value for parameter a'. Monolayer values were in some cases reported by the authors, and when not, they were calculated by applying B.E.T. equation to the experimental data reported (Labuza, 1968).

The parameters r and a' were calculated using a lineal regression program in an IBM 360/50, 128 K computer. Table 1 shows the calculated values. In order to evaluate the goodness of fit for Halsey's equation as applied to the experimental data, it is necessary to have quantitative information. On Table 2 a statistical analysis of all the values obtained is reported. It is worth noticing that the variance of regression was calculated with the ln values of the calculated and experimental data (Williams, 1959), as:

$$\text{variance of regression} = \sum \frac{(\ln y_1 - \ln VT_1)^2}{n-1}$$

where y_i = experimental value; VT_i = calculated value; and n = number of determinations.

The $\%(\text{error})_{av.}$ means an average of the % errors at several equally spaced water activities over the range examined.

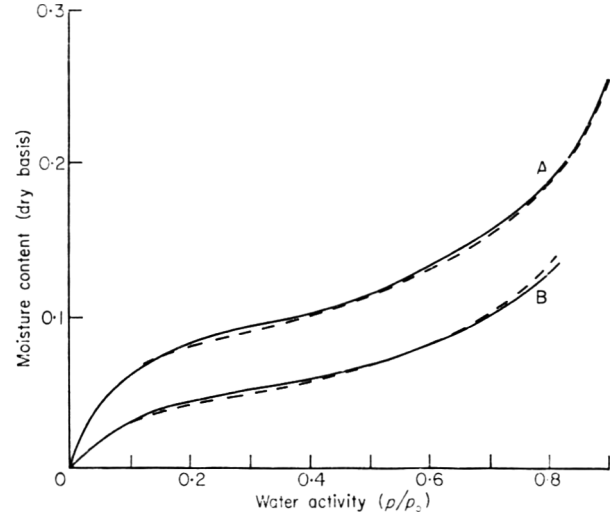

FIG. 1. Comparison of experimental (———) and calculated (– – –) data. A, wheat flour, 20·2°C (Bushuk & Winkler, 1957). B, cellulose microcrystalline, 37°C (Labuza & Rutman, 1968).

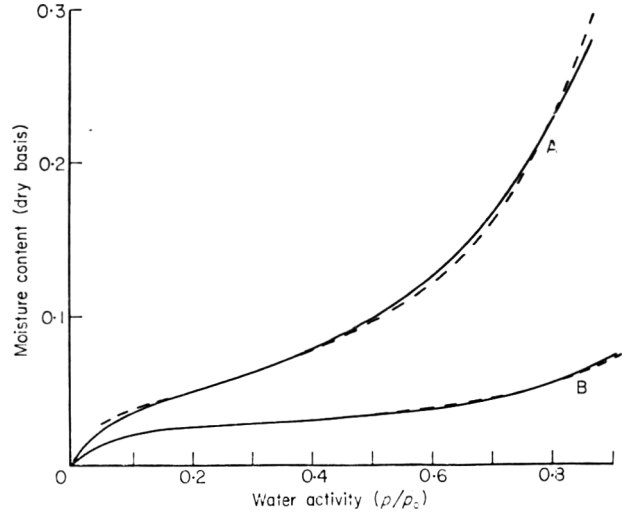

FIG. 2. Comparison of experimental (———) and calculated (– – –) data. A, beef, room temp. (MacKenzie & Luyet, 1967). B, shelled walnut kernels, 22·5°C (Rockland, 1957).

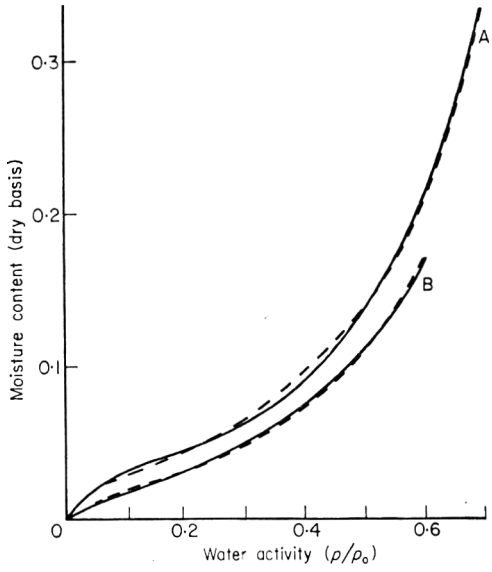

FIG. 3. Comparison of experimental (———) and calculated (– – –)data. A, apple, 19·5°C (Taylor, 1961). B, cabbage, 37°C (Mizrahi *et al.*, 1970).

Calculated and experimental water sorption isotherms for several food materials are plotted in Figs 1–3 and show the degree of applicability of the proposed equation.

Conclusions

It was found that equation (1) describes reasonably well equilibrium moisture contents for over thirty different food materials in a wide range of water activity. The foods tested have moisture sorption isotherms which are representative of nearly all dehydrated foods, i.e. starchy foods, protein foods, high-sugar foods, oilseeds, etc.

Although Halsey's equation was theoretically developed, it must be kept in mind that though the experimental data fit equation (1) this fitness is not a proof of the correctness of the theory, since some food isotherm curves may be described by many different equations (Labuza, 1968; Chen & Clayton, 1971). The main advantage of the use of Halsey's equation in the food area is its simplicity and its wide range of application to both type of food and water activity. When equation (1) was applied to isotherms of some pure proteins reported by Bull (1944) and to starch gel reported by Fish (1958) and Saravacos & Stinchfield (1965), no satisfactory agreement was observed.

Acknowledgment

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Water sorption isotherms in sugar beet root

HÉCTOR A. IGLESIAS, JORGE CHIRIFE AND JOSÉ L. LOMBARDI

Summary

The water sorption isotherms of raw sugar beet root and its water insoluble components, at various temperatures, were determined. An attempt is made to describe the experimental equilibrium moisture content data using some existing theories on physical adsorption.

Introduction

An important characteristic of food products, which influences several aspects of drying and storage is their equilibrium moisture content. There is a well-established relationship between water sorption isotherm and chemical, physical and stability characteristics of dehydrated food products (Loncin, Bimbenet & Lenges, 1968; Labuza, Tannenbaum & Karel, 1970; Labuza, 1970). Sorption isotherms of food products constitute an essential part of the theory of drying (King, 1968) and also provide information directly useful in the design of drying equipment.

Although drying is sometimes a preliminary step to sugar extraction from sugar beet root and the extracted cosettes are dried for use as animal foodstuffs, very limited information is available in the literature on the water sorption characteristics of sugar beet root. In the present study, the sorption isotherms of raw sugar beet root and its water insoluble components, at various temperatures, were determined. An attempt was also made to describe the experimental equilibrium moisture content data using some existing theories on physical adsorption.

Material and methods

Materials

Medium size sugar beet roots from Miramar, Argentina were kept refrigerated at 2–3°C until use; mean value of sugar content was about 18–19% on wet basis. Samples were prepared by cutting discs 1·5 cm in diameter and about 0·15 cm thick. Samples to be used for adsorption experiments were freeze-dried at a pressure less than 100 μ and a platen temperature of 37 °C. Other samples were leached with distilled water to

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remove its soluble-solids content (mainly sucrose) prior to the determination of the water sorption isotherms. The experimental conditions to insure an adequate extraction of the soluble solids had been established previously.

Isotherms

Vacuum desiccators were prepared with saturated salt solutions (Young, 1967) or aqueous sulfuric acid solutions of known concentration (Wilson, 1921; Greenwalt, 1925) to give different constant relative humidities. The desiccators were placed in constant temperature baths ($\pm 0{\cdot}1\,^{\circ}C$). The samples were placed in the desiccators at the various relative humidities and a vacuum was pulled in order to speed up the equilibrium. The samples either lost or gained water until equilibrium moisture content was reached. The moisture content of the equilibrated samples was determined by placing the sample in a vacuum oven at room temperature over concentrate sulfuric acid for 24 hr, and then at 70 °C over magnesium perchlorate for 48 hr. This was done

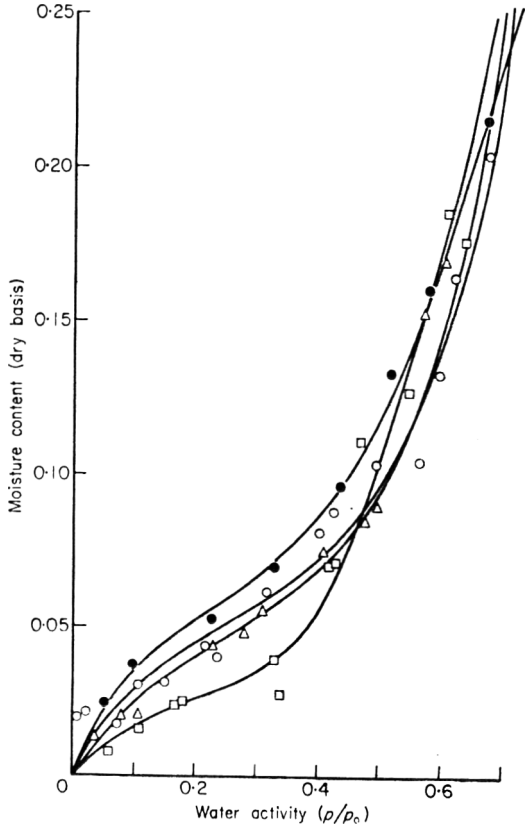

FIG. 1. Desorption isotherms of raw sugar beet root at different temperatures. ●, 20 °C; ○, 35° C; △, 47 °C; □, 65 °C.

in order to avoid the formation of an impervious crust of sugar on the surfaces of the sample.

Results and discussion

The desorption isotherms of sugar beet root at different temperatures are shown in Fig. 1. At low and intermediate water activity, temperature had the normal effect predicted by the theory of physical adsorption, i.e. the quantity of sorbed water at a given relative humidity increased as the temperature was decreased. At high relative humidities an opposite effect of temperature is observed. This is due to the dominant effect of sucrose dissolution (Salwin & Slawson, 1959; Saravacos & Stinchfield, 1965; Loncin *et al.*, 1968) which comprises about 80% of the dry matter. Fig. 2 shows the adsorption isotherms of the water insoluble components of sugar beet root. As it may be observed, temperature has the normal effect over the whole range of water activity.

The desorption isotherms of raw sugar beet root and the adsorption isotherms of its water insoluble components have the typical sigmoide shape of type II isotherms in

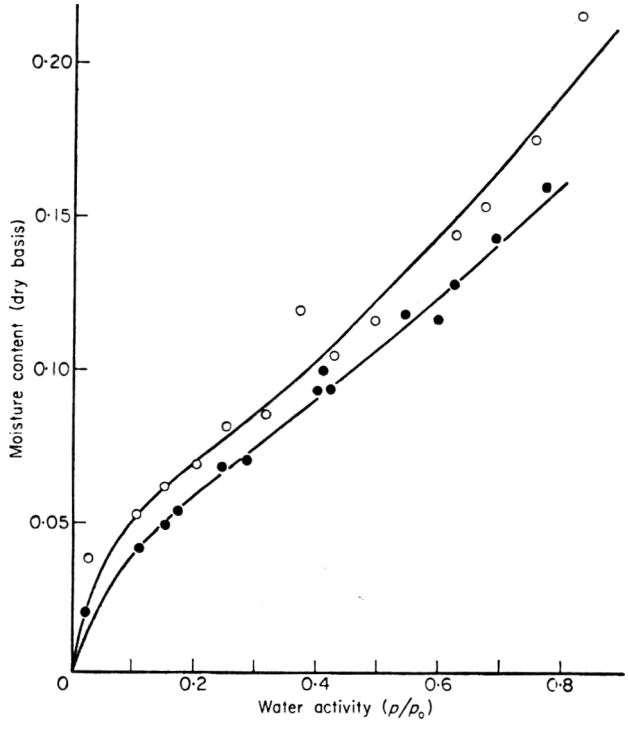

FIG. 2. Adsorption isotherms of the water insoluble components of sugar beet root and comparison with B.E.T. theory (———). ○, 35°C ($n=6$); ●, 47°C ($=n5$).

accordance with B.E.T. classification (Labuza, 1968). Consequently, they were analysed using the familiar B.E.T. equation:

$$\frac{X}{X_m} = \frac{C(A_w)}{1-A_w} \cdot \frac{1-(n+1)\ (A_w)^n + n(A_w)^{n+1}}{1+(C-1)\ (A_w) - C(A_w)^{n+1}} \qquad (1)$$

The analysis of the data was done by an IBM 360/50. 128 K computer. The computer was fed with the experimental equilibrium moisture content values up to $A_w = 0{\cdot}35$ for raw sugar beet root, and over the whole range of A_w for its water insoluble components. A non-lineal regression program of minimization was used and the B.E.T. constants, namely, X_m (monolayer value), C (from which the heat of sorption, Q_s, was calculated), and n (number of layers adsorbed), were obtained. The results are shown in Table I and Fig. 2. It can be seen that the adsorption isotherms of the water insoluble components can be represented by the B.E.T. equation with a finite number of layers for the whole range of water activity investigated. However, for raw sugar beet root, as it will be shown later, the B.E.T. equation was able to reproduce the experimental behaviour only up to $A_w = 0{\cdot}35$. The B.E.T. constants shown in Table I are in accordance with the usual values obtained in food materials, both for X_m and Q_s (Karel, 1973).

TABLE 1. B.E.T. constants and heat effects on raw sugar beet root and its water insoluble components.

Temperature (°C)	X_m g/100 g (dry basis)	C	Q_s (B.E.T.) kcal/g mol	Q_{isos}* kcal/g mol
Raw sugar beet root				
20	5·55	12·02	12·00	14·10
35	4·95	10·00	11·81	
47	4·99	7·00	11·51	
65	3·85	4·39	10·99	
Water insoluble components				
35	6·93	16·21	12·04	17·80
47	6·40	10·88	11·86	

*Average up to the monolayer.

It is also observed a small but significant decrease of the monolayer value with temperature, for both raw sugar beet root and its water insoluble components. An analysis of literature data on monolayer values at different temperatures shows the same behaviour, that is, the monolayer value decreases with increasing temperature. This can be seen in Fig. 3 which was drawn using literature data. This behaviour may be attributed to a reduction of active sites as a result of chemical or physical changes induced by temperature.

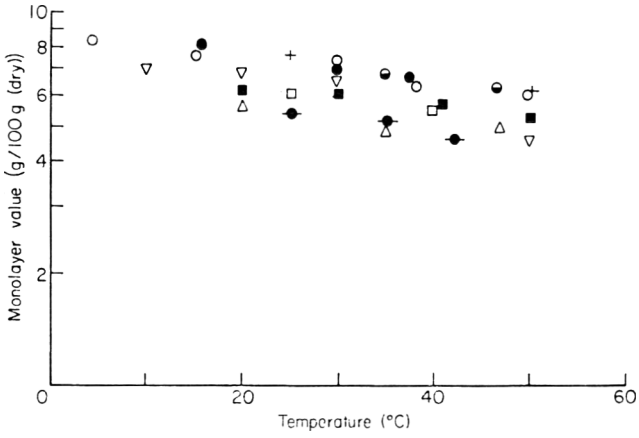

FIG. 3. Effect of temperature on the monolayer value of food materials. ○, Corn (Chen & Clayton, 1971); ●, F.P.C. (Raskh, Stillings & Dubrow, 1971); ●, non-fat milk (Heldman, Hall & Hendrick, 1965); ●, sugar beet root, W.I.C. (this work); △, sugar beet root (this work); ▽, beef (Saravacos & Stinchfield, 1965); ■, wheat, flour (Bushuk & Winkler, 1957); □, Salmin (Bull, 1944); wheat (Becker & Sallans, 1956).

Table 2 shows the statistical values obtained upon application of B.E.T. equation. The data for raw sugar beet root at 65 °C were omitted because of difficulties in the computational procedure.

The isosteric heat of sorption for both raw sugar beet root and its water insoluble components, was calculated using the Clausius-Clayperon equation; an average latent heat of vaporization for the range of temperature employed was utilized. The isosteric heat vs. the moisture content is shown in Fig. 4. In the same figure are also plotted the heats of sorption obtained upon application of B.E.T.'s theory for purposes of comparison. This comparison is also shown on Table 1. It can be seen that there is a notorious discrepancy between the isosteric and B.E.T. heats of sorption. This has been

TABLE 2. Statistical values obtained upon application of B.E.T. equation

Temperature (°C)	Range of A_w	χ^2	Probability level	Regression variance	% Error
Raw sugar beet root					
20	0·05–0·35	0·1876	<0·10	0·3039	7·1
35	0·05–0·35	0·8313	<0·010	0·5789	16·8
47	0·05–0·35	0·2200	<0·010	0·1840	7·1
Water insoluble components					
35	0·05–0·83	0·8174	<0·0005	0·0059	5·8
47	0·05–0·775	0·1885	<0·0005	0·0016	3·3

shown to be the rule rather than an exception in most foods and bio-polymers (McLaren & Rowen, 1951). As has been pointed out (McLaren & Rowen, 1951; Amberg, 1957), this discrepancy might be the logical consequence of the several assumptions made in the development of B.E.T.'s theory. On the other hand, the heat of sorption calculated through the constant C is not a reliable value unless C is larger than 50 (Le Maguer, 1972), which is not the present case.

As was mentioned previously, Fig. 5 shows that for raw sugar beet root, B.E.T. equation represents adequately the experimental data only up to $A_w = 0{\cdot}35$.

Recently, Iglesias, Chirife & Lombardi (1975) showed that a multilayer adsorption equation, originally developed by Halsey (1948), could be used to describe the water sorption behaviour of a great variety of food materials. Halsey's equation is:

$$p/p_0 = \exp\ (-a/RT\theta^r) \tag{2}$$

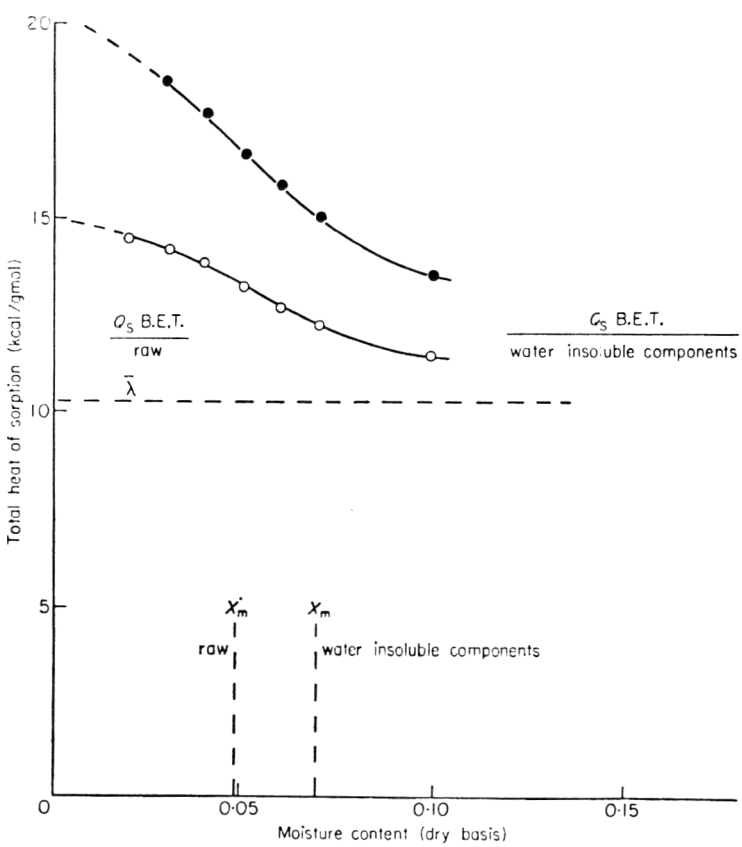

FIG. 4. Variation of isosteric heat of sorption with increasing water sorption. ○, Raw; ●, water insoluble components.

where $p/p_0 = A_w$ = water activity; a, r = parameters; $\theta = X/X_m$; X = equilibrium coverage, in g adsorbed/g solid; and X_m = monolayer value, same units as X. Equation (2) may be put in the form:

$$\ln \ln p_0/p = -r \ln \theta + \ln a'$$

where $a' = a/RT$.

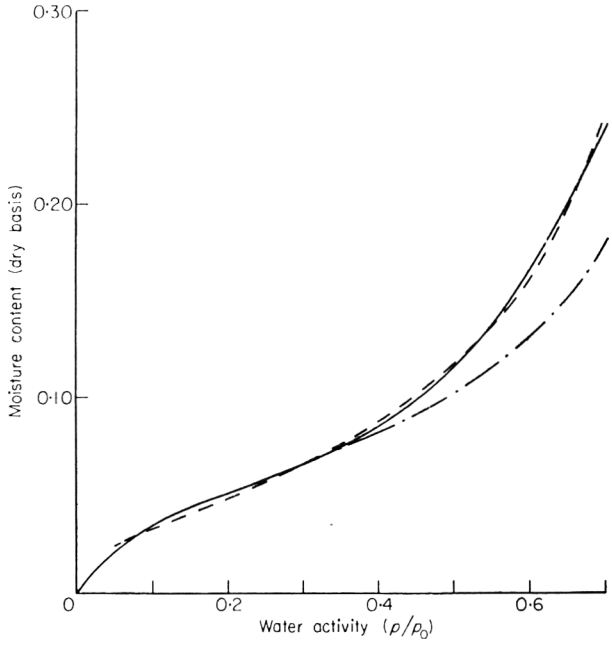

FIG. 5. Desorption isotherms of raw sugar beet root at 20 °C and comparison of experimental and predicted values. ———, Experimental; – – –, Halsey's equation; –·–·–, B.E.T., $n = \infty$.

TABLE 3. Constants r and a' in Halsey's equation and statistical analysis of its application to raw sugar beet root and its water insoluble components

Temperature (°C)	r	a'	χ^2	Probability level	Correlation coefficient	Regression variance	%(Error)$_{av.}$
Raw sugar beet root (range of A_w, 0·05–0·70)							
20	0·9558	1·4359	0·1708	<0·0005	0·9971	0·0028	3·91
35	0·89772	1·3335	0·3705	<0·0005	0·9954	0·0049	5·76
47	0·76711	1·2219	0·8790	<0·0005	0·9945	0·0099	8·77
65	0·63305	1·1597	1·1399	<0·0100	0·9971	0·0108	6·55
Water insoluble components (range of A_w, 0·11–0·80)							
35	1·9403	1·8309	0·8775	<0·0005	0·9821	0·0081	7·17
47	1·7446	1·5045	0·8133	<0·0005	0·9780	0·0091	7·65

A plot of $\ln \ln p_0/p$ vs. $\ln \theta$ should be a straight line from which the parameters r and a' may be calculated. The data for raw sugar beet root and its water insoluble components, at different temperatures, were used to test the usefulness of the proposed equation. The parameters r and a' were calculated by computer techniques; Table 3 shows the calculated values. In order to evaluate the goodness of fit it is necessary to have quantitative information; on the same Table 3 a statistical analysis of all values obtained is reported. The $\%$ $(\text{error})_{av.}$ means an average of the $\%$ errors at several equally spaced water activities over the whole range of A_w. It can be seen that Halsey's equation represents reasonably well the water sorption behaviour of the materials tested over the whole range of water activity.

Calculated and experimental water sorption isotherms for raw sugar beet root at one temperature are plotted in Fig. 5.

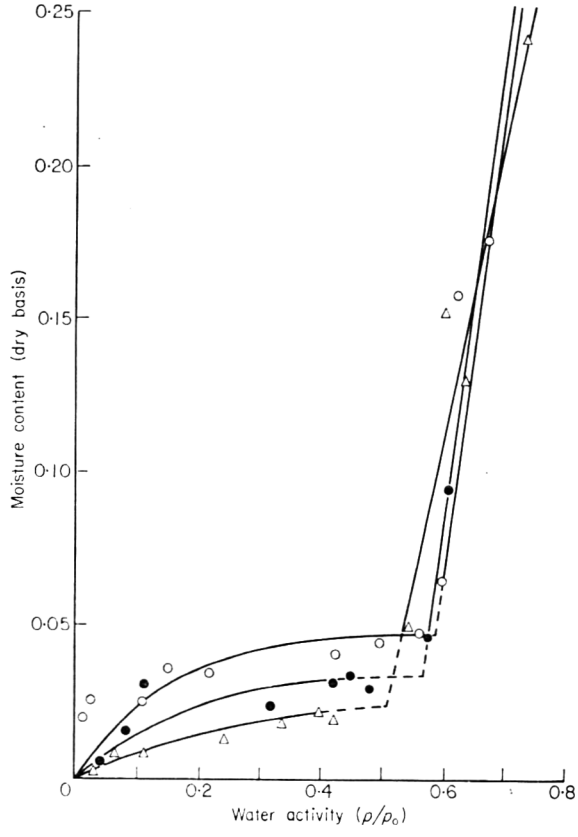

FIG. 6. Adsorption isotherms of freeze-dried raw sugar beet root at different temperatures. ○, 35 °C; ●, 47 °C; △, 65 °C.

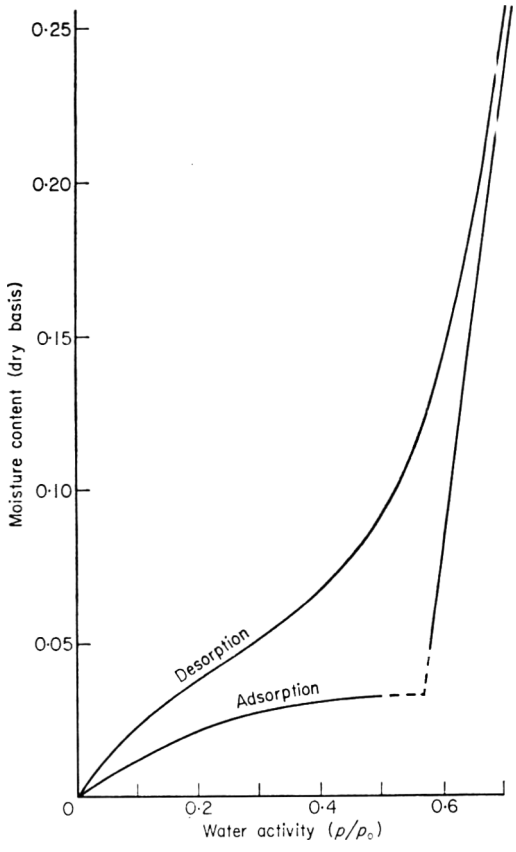

FIG. 7. Comparison of adsorption and desorption branches of raw sugar beet root at 47 °C.

'*Hysteresis*'

The adsorption isotherms of freeze-dried raw sugar beet root at three different temperatures are shown in Fig. 6. The adsorption and desorption branches at 47 °C are shown in Fig. 7.

The adsorption isotherms (Fig. 6) resemble the adsorption of water on crystalline sugars, with dissolution effect at high relative humidity (Loncin *et al.*, 1968). It is commonly accepted that freeze-drying produces amorphous sugars (White & Cakebread, 1966), so in this case the sucrose contained in the sugar beet root probably left the freeze-drier in an amorphous state. However, the sorption of water in the desiccators may result in the sucrose transformation from the metastable amorphous state to the more stable crystalline state (Makower & Dye, 1956; Karel, 1973). In this way, it may be explained the poor sorption of water vapour up to a relative humidity of about 55%.

It is clear that we cannot ascribe the description 'hysteresis', at least as it is commonly used in most cases (Labuza, 1968), to the effect here shown. We must realize that we

are in the presence of a different kind of effect, mainly attributed to phase transformations of the sucrose. Further research is being carried on to clarify this point.

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Ultrastructural changes during frozen storage of cod (*Gadus morhua* L.)

II. Structure of extracted myofibrillar proteins and myofibril residues

L. JARENBÄCK AND A. LILJEMARK

Summary

Myofibrillar proteins from post-rigor unfrozen and stored frozen cod muscle have been studied by electron microscopy. Changes in extracted proteins and corresponding myofibril residues were followed by negative staining and ultrathin sectioning throughout a frozen storage period of 120 weeks.

In myofibrillar extracts a decrease in the number of actomyosin filaments and an increase in the number and size of large aggregates was found. A decrease in the length and amount of attached myosin was noticed in actomyosin filaments. The changes observed were most extensive at −10°C, less pronounced at −20°C and hardly noticeable at −30°C.

According to the appearance of the myofibril residues thick myofilaments had been almost completely extracted from unfrozen muscle and were increasingly resistant towards extraction the higher the temperature during frozen storage.

Introduction

The development of tough and dry texture during frozen storage of certain species of fish has been attributed to changes within their myofibrillar proteins. As a measure of the extent of such changes, differences in the amount of protein extractable from the muscle with neutral salt solutions has often been used. The wealth of literature on this subject has been thoroughly reviewed by, for example, Dyer (1967), Connell (1968) and Powrie (1973).

The protein extracted with salt solutions has been considered as 'actomyosin' while later work has shown it to be a heterogeneous mixture of most of the myofibrillar proteins (Connell, 1968). Typical in such extracts from cod muscle is the presence of the composite complex actomyosin, free myosin, actin and tropomyosin (King, 1966). The presence of small amounts of minor myofibrillar proteins, found in similar extracts from other species (Ebashi, 1963; Ebashi & Ebashi, 1964, 1965; Maruyama, 1965; Rampton *et al.*, 1971), is also possible. Furthermore, phospholipids have been found in fish myofibrillar extracts (Sheltawy & Olley, 1966; Taguchi & Ikeda, 1968).

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The extraction mechanism by which actomyosin is obtained and the thin filaments (F-actin) are released from the myofibrils is not fully understood. Haga, Maruyama & Noda (1965) and Mihalyi & Rowe (1966) extracted pre-rigor rabbit muscle with Weber-Edsall solution (0·6 M KCl, 0·04 M $KHCO_3$, 0·01 M K_2CO_3) and found that myosin was released first, actomyosin appearing in the solution only after the residual supply of ATP had disappeared (5–8 hr). Judging from the uniform length of the actomyosin particles, roughly corresponding to the length of the thin filaments *in situ*, Haga *et al.* (1965) concluded that actin is probably released intact. Later Haga *et al.* (1966) found that myosin must be present in the extracting solution and possibly also bind to F-actin before actomyosin is released in the form of filaments. On the other hand, Maruyama (1966) has shown that F-actin alone can be extracted with 0·6 M KCl when myosin has first been exhaustively extracted.

In post-rigor cod muscle, with a depleted supply of ATP, actomyosin is thought to be derived from actin and myosin already combined in the muscle but may also form in solution from separately extracted F-actin and myosin molecules (Connell, 1968).

Changes in the composition of cod myofibrillar protein extracts in relation to frozen storage time and temperature have been studied by King (1966) and Anderson & Ravesi (1969, 1970a, b). These authors, using ultracentrifugation, found a progressive decrease in the amount of actomyosin, a less rapid decrease of myosin and an increased frequency of large aggregates in the protein extracts with increasing storage time. Similar results have been obtained for other species using gel-filtration (Umemoto & Kanna, 1970).

In studying the separate myofibrillar proteins in cod muscle, especially myosin, extensive work has been carried out by Connell (reviewed 1968). In relation to frozen storage no major changes were found in separately extracted cod actin (Connell, 1960b) while the selective extractability of myosin was found to decrease (Connell, 1962) and its ATP-ase activity to decline (Connell, 1960c). Cod myosin molecules aggregate rapidly in a stepwise manner upon standing in solution and even more rapidly after freezing the solution (Connell, 1959, 1963).

An increased rate of aggregation was also found upon freezing and storing solutions of trout myosin being most rapid at the highest storage temperature (Buttkus, 1970). This author suggested that the aggregation of myosin in frozen solution can take place via sulfhydryl–disulfide interchange without a decrease in the total number of free sulfhydryl groups. This may explain why Connell (1960c) found no change in the amount of free sulfhydryl groups in frozen and stored cod.

Structural changes following extraction of proteins from frozen and stored cod have been studied with light microscopy by Anderson & Ravesi (1969, 1970a, b). The general conclusions from their work on muscle residues, after extraction of protein, were that a disappearance of banding pattern in unfrozen muscle and an increased cohesion of myofibrils in frozen and stored muscle could be detected.

So far no report on the ultrastructural appearance of extracted cod myofibrillar

proteins in relation to frozen storage has been found. Therefore, in connection with work on intact fibres (Jarenbäck & Liljemark, 1975) this investigation was undertaken as an attempt to relate the loss of protein solubility and toughening of the muscle during frozen storage to any structural changes there might be in the extracted myofibrillar proteins or in the residual material.

Materials and methods

For the storage study a batch of frozen cod (*Gadus morhua* L.) was obtained commercially. The fish, ranging in size from 60 to 80 cm, were caught in December in the southern Baltic south of Karlskrona. After gutting and storage in ice for slightly more than 24 hr the fish were put into plastic pouches and frozen in an air blast at −40°C. This material was stored at −30, −20 and −10°C. The fish were thawed in air at +4°C. Unfrozen samples of similar size were also obtained commercially. These fish were caught at different localities off the west coast of Sweden (mainly in the Kattegat) and had been stored in ice for about 4 days.

Texture estimation

As an indication of texture changes in the frozen and stored material the cell-fragility technique of Love & Mackay (1962) was applied at intervals during the first 60 weeks of storage. Due to slight modifications in technique and equipment, turbidity values are expressed on a relative scale. Values given are the mean of eight measurements on the same fish.

Extraction of myofibrillar proteins

All preparative operations and extractions were carried out at temperatures between 0 and 4°C. Muscle samples, free from connective tissue, were taken from the largest lateral muscle bundle adjacent to the backbone and below the first dorsal fin. Myofibrillar proteins were extracted by slight modifications of the method of King (1966). The buffer for washing of myofibrils (3·38 mM KH_2PO_4, 15·5 mM Na_2HPO_4, $I = 0{\cdot}05$, pH 7·6) and the extractant (0·45 M KCl, phosphate buffer, $I = 0{\cdot}5$, pH 7·2) were those suggested by Connell (1958). Myotomes (8·5 g) were mixed with 200 ml phosphate buffer ($I = 0{\cdot}05$, pH 7·6), homogenized for 30 sec at half speed in an Atomix Blendor equipped with a baffle to prevent frothing, and centrifuged for 30 min at 1000 *g* to remove sarcoplasmic proteins. The myofibril pellet was resuspended in further 200 ml phosphate buffer and the washing procedure repeated twice.

Washed myofibrils were blended for 30 sec with 170 ml 0·45 M KCl, phosphate buffer, $I = 0{\cdot}5$, pH 7·2, giving an original muscle-solvent ratio 1 : 20. The solution was then set aside and extraction continued, without stirring, for 20 hr at +2°C. Follow-

ing centrifugation at 2200 *g* for 30 min the supernatant and the muscle residue were collected.

For comparison extraction of myosin according to Connell (1962) was occasionally carried out on similarly washed myofibrils, using extractant containing pyrophosphate and $MgCl_2$ (0·47 M KCl, 65 mM phosphate buffer, 10 mM pyrophosphate, 5 mM $MgCl_2$, $I = 0{\cdot}68$, pH 6·4).

Protein concentration determination

Protein concentrations were measured by the Lowry procedure (Lowry *et al.*, 1951), using crystalline bovine serum albumin as a standard. Myofibrillar protein solutions were diluted with 0·5 M NaCl before assay, in order to prevent the precipitation between KCl and the Folin-Ciocalteu reagent.

Modification of myofibrillar extracts

Extracts of myofibrillar proteins were rapidly diluted with ice-cold glass-distilled water to a final ionic strength of 0·1, checked by conductivity measurement.

Specimen preparation for electron microscopy

Protein solutions were negatively stained with 1% uranyl-acetate in water (Huxley & Zubay, 1960) essentially by the method of Huxley (1963). Prior to staining, the solutions of extracted proteins were always diluted to contain 0·15 mg protein/ml, using 0·45 M KCl, 0·05 M Tris-HCl buffer, pH 7·2. This buffer reduced the formation of precipitates between stain and phosphate present in the extracting solution.

A 10-μl drop of diluted protein solution was placed on a Formvar-carbon coated grid. When the protein had set for 1 min, excess solution was removed with folded lens-tissue and 10 μl of 1% uranylacetate applied immediately. After 20 sec excess stain was removed with a lens tissue and the specimen air dried. More than five specimens were prepared from each extract.

Small fragments of muscle residue were taken from the central part of the pellet formed upon centrifuging the extracted myofibrils. The fragments were put directly into cold 3% glutaraldehyde in 0·1 M phosphate buffer pH 7·2 without prior washing out of the extraction solution. After fixation for 3 hr at +2°C pieces of muscle residue were rinsed and trimmed in 0·2 M phosphate buffer, postfixed for 2 hr in 1% osmiumtetroxide buffered with veronal-acetate pH 7·2, dehydrated in a graded series of ethanol-water mixtures followed by propylene oxide and finally embedded in Epon. Thin sections were cut with a diamond knife in a LKB ultramicrotome. Sections were mounted on uncoated grids, stained with 2% uranyl acetate in methanol for 50 min followed by lead citrate for 12 min (Reynolds, 1963).

Electron microscopy and measurements

Specimens were examined in a JEM 7 electron microscope operated at 80 kV and at fixed lens current settings with careful calibration of the magnification. Photographs of negatively stained specimens were taken at instrumental magnifications in the range of 35 000–75 000 times; for thin sections a range of 5000–35 000 was utilized.

Filament lengths were measured on prints with a final magnification of 100 000 times using a sliding caliper. For studies of details and counting of 'arrowheads' along filaments, micrographs were examined under a dissecting microscope. Mean arrowhead spacing was calculated as the ratio between filament length and the number of arrowheads found along the filament.

Results

Texture changes

Cell-fragility values indicating the texture changes during the first 60 weeks of frozen storage are shown in Fig. 1. The turbidity readings of weighed samples of cod muscle in 1·15% formalin submitted to a fixed amount of homogenization are supposed to reflect the relative disruption of muscle fibres (Love & Mackay, 1962). Although the

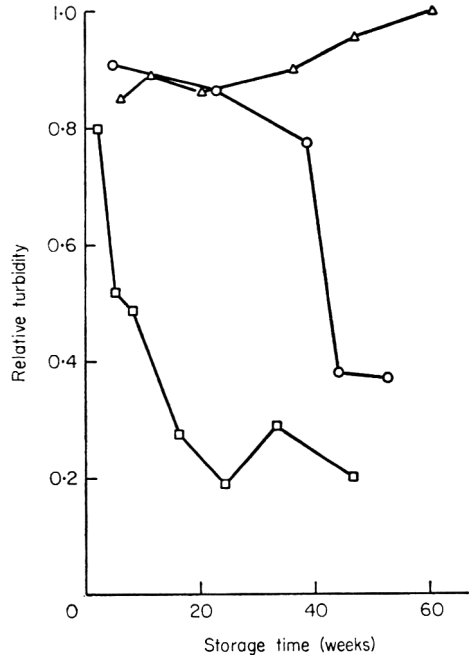

FIG. 1. Changes in texture, as measured by the cell-fragility method, in cod muscle frozen and stored at three different temperatures. □——□, −10°C; ○——○, −20°C; △——△, −30°C.

number of observations is small, the texture characteristics are shown to change rapidly at −10°C, moderately at −20°C and not appreciably at −30°C.

Protein content in myofibrillar extracts

Changes in the amount of protein extracted with KCl–phosphate buffer, from unfrozen and stored frozen cod, are given in Fig. 2. The experimental points show considerable scatter, which is expected, as the number of observations is small. The amount of protein obtained from cod stored at −10°C decreased rapidly and reached a minimum after 30 weeks. A less rapid decrease was found at −20°C, while at −30°C no notable change took place during 120 weeks.

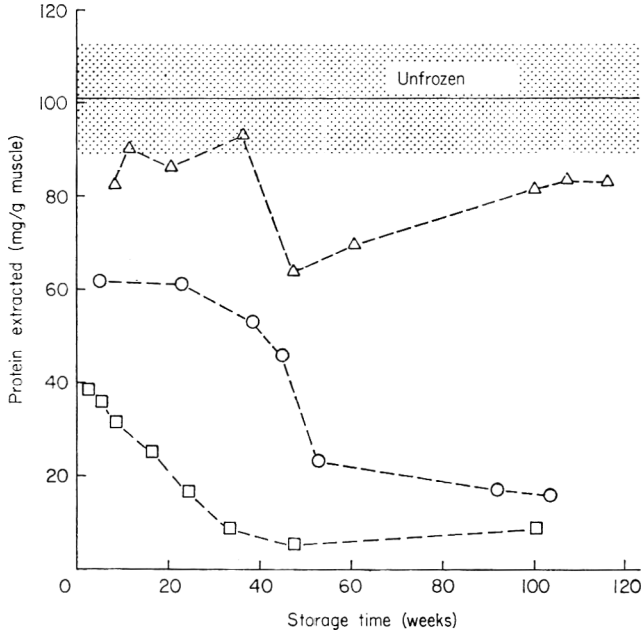

FIG. 2. Changes in the amount of myofibrillar protein extracted from cod frozen and stored at −30°C (△——△), −20°C (○——○) and −10°C (□——□). Straight line and hatched area represent mean ± S.D. of sixteen unfrozen samples analysed during the storage period.

Structure of extracted myofibrillar proteins

Unfrozen muscle. The negatively stained specimens of myofibrillar protein, obtained from unfrozen muscle, contained large numbers of actomyosin filaments or 'decorated' F-actin (Plate 1a). The majority of these filaments show the 'arrowhead' appearance described by Huxley (1963). Arrowheads, formed by myosin molecules bound to F-actin, are distributed at fairly constant intervals of 37 nm along the filaments. They lie at an angle varying between 30 and 80° to the filament axis. The mode of binding

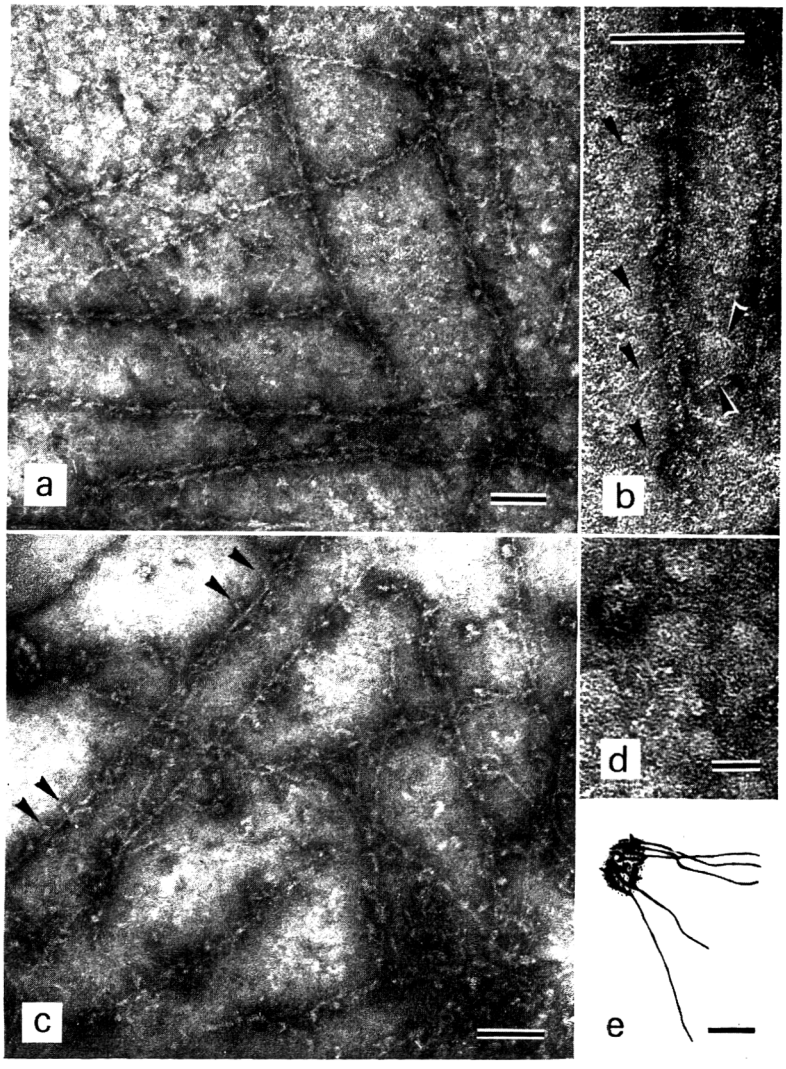

PLATE 1. (a) Actomyosin filaments from an unfrozen muscle. Note regular arrowhead pattern on most filaments. × 70 000. (b) Long extensions originating from the arrowheads are indicated. × 175 000. (c) Actomyosin filaments from a muscle stored at 30°C for 47 weeks. Note curled arrowheads and particles added to the filament surface. × 87 500 In (a), (b) and (c) scales represent 0·1 μm. (d) Enlargement of a 25 × 25 nm particle with tails, possibly representing myosin molecules. × 245 000. (e) Drawing of the same particle. Scales represent 25 nm.

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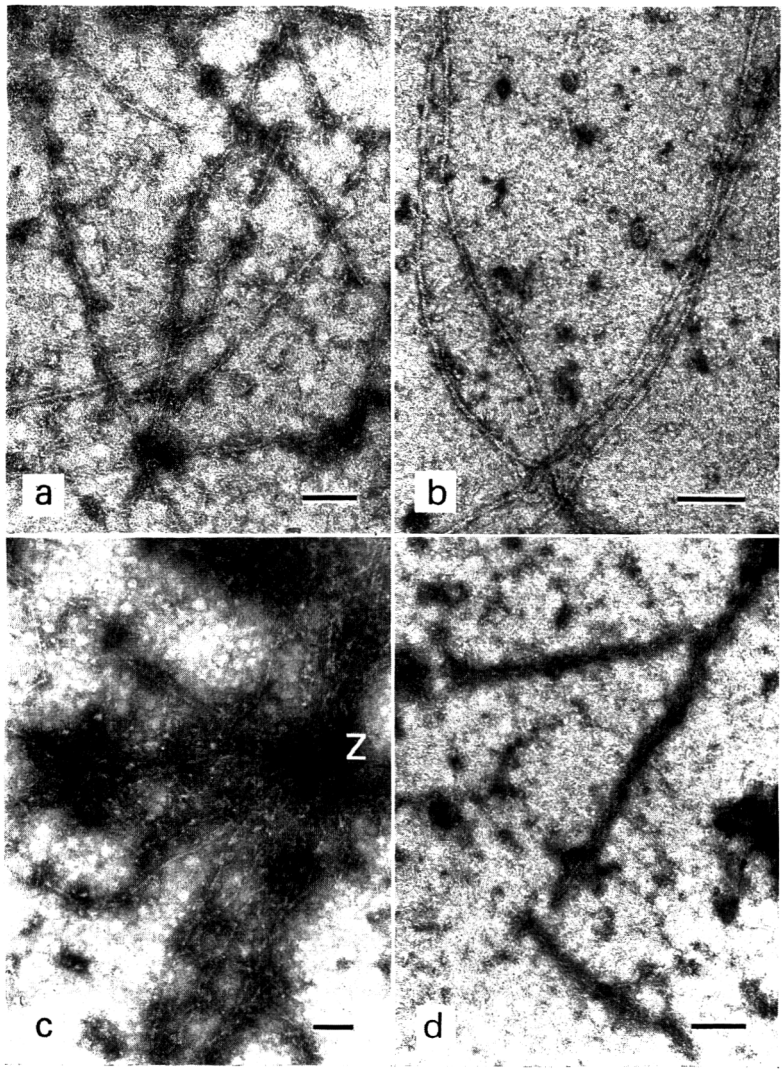

PLATE 2. (a) Actomyosin filaments from a muscle stored at −20°C for 38 weeks. Note irregularly myosin-decorated filaments. × 70 000. (b) Undecorated filaments from a muscle stored at −20°C for 3·5 years. × 87 500. (c) Aggregate of filaments found in a myofibrillar extract from a cod stored at −20°C for 52 weeks. The possible remnant of the Z-disc is indicated (Z). × 52 500. (d) Actomyosin filaments found in a myosin extract from a muscle stored at −20°C for 134 weeks. Note apparently regular myosin-decoration. × 70 000. Scales represent 0·1 μm.

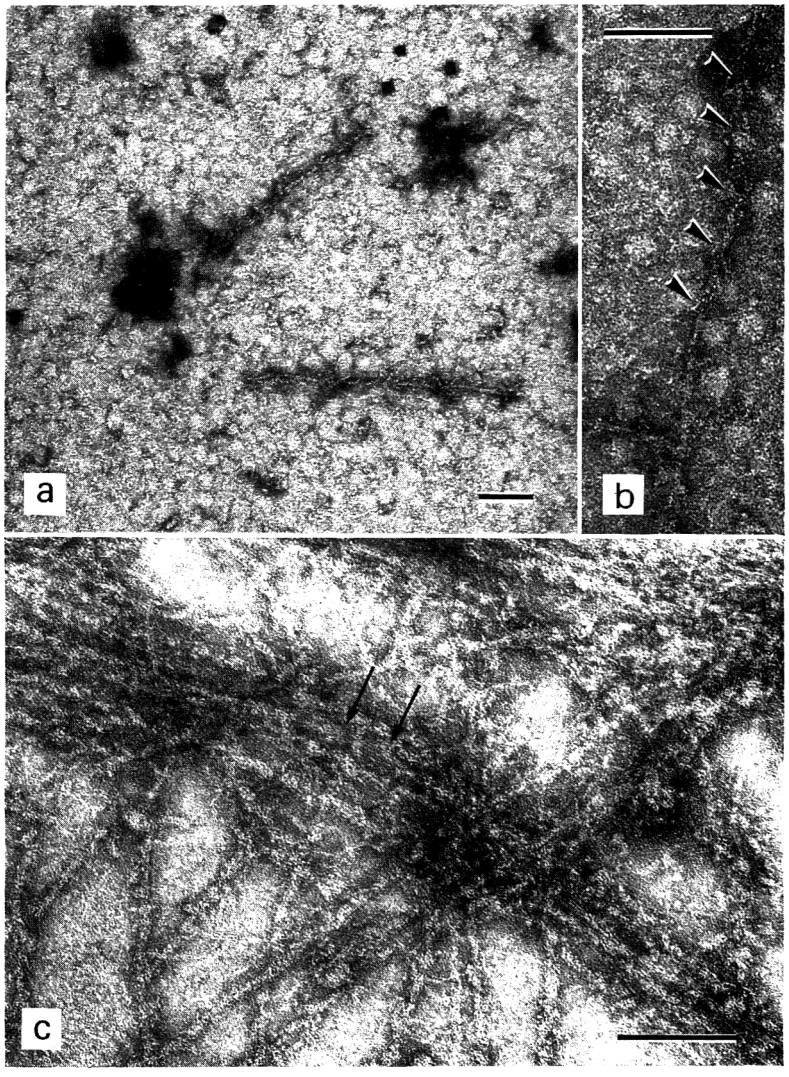

PLATE 3. (a) Actomyosin filaments from a muscle stored at $-10°C$ for 33 weeks. Short filaments irregularly decorated with myosin are shown. × 70 000. (b) An actomyosin filament from the same preparation as (a) showing regular myosin-decoration along portions of the filament. × 140 000. (c) Aggregated actomyosin filaments in a myofibrillar extract from unfrozen muscle at $I = 0{\cdot}1$. Parallel arrangement of substructures is indicated. × 157 500. Scales represent 0·1 μm.

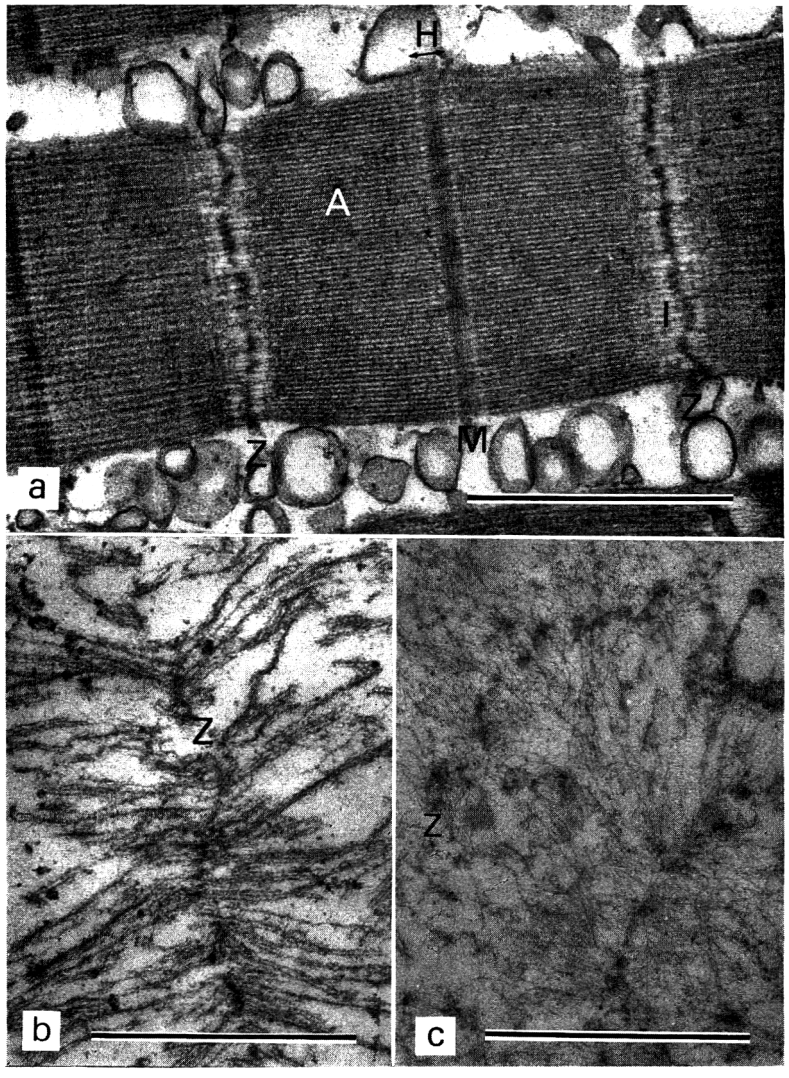

PLATE 4. Longitudinal section of a myofibril from unfrozen post-rigor cod muscle. H-zone, A-, I-, Z- and M-bands are indicated. × 35 000. (b) I-Z-I-brush from unfrozen muscle extracted with 0·45 M KCl, phosphate buffer, $I=0·5$, pH 7·2. Note attachment of knobs along the filaments. × 35 000. (c) I-Z-I-brushes from unfrozen muscle extracted for myosin. Note absence of knobs. × 35 000. Scales represent 1 μm.

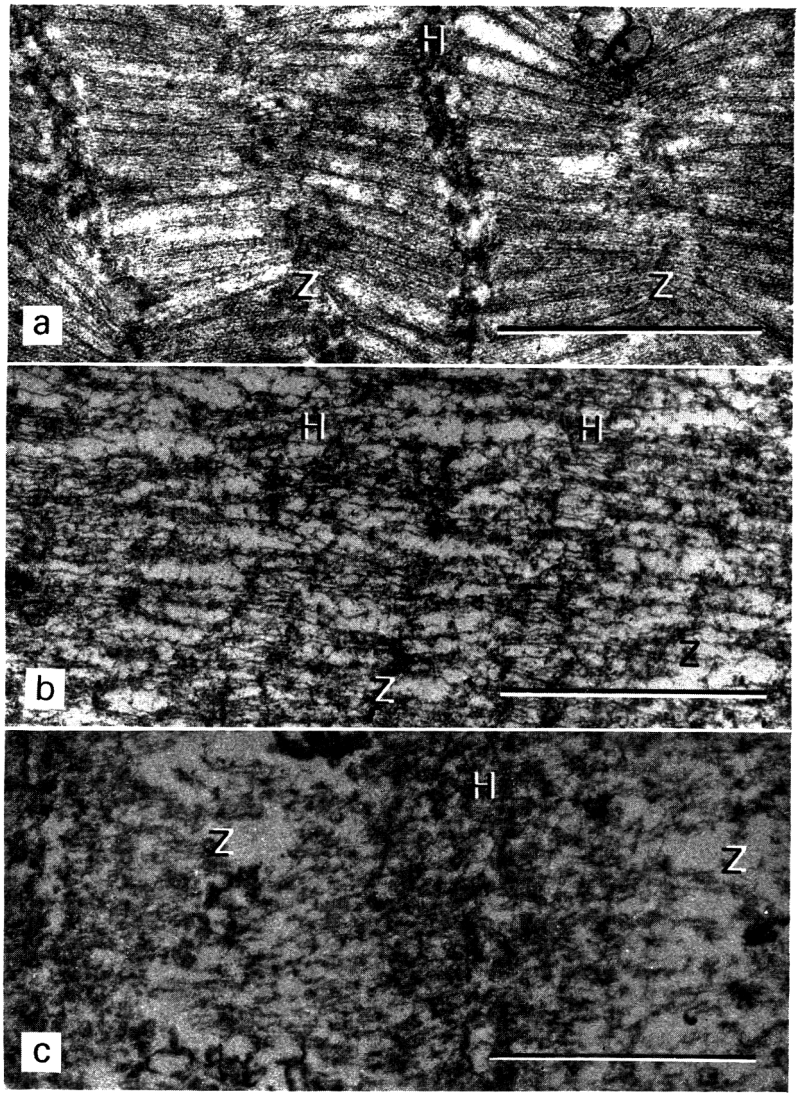

PLATE 5. (a) Longitudinal section of a myofibril residue from unfrozen muscle showing consecutive I-Z-I-brushes. Note precipitated material at the level of the original H-zone. (b) Myofibril residue from a cod stored at −20 °C for 38 weeks. Note overlapping filaments at the level of the original H-zone and clusters of precipitated material along the filaments. (c) Myofibril from the same specimen as (b), but with more material retained in the original A-band. Note in (b) and (c) breaks in the Z-band and loss of thin filaments. (a), (b) and (c) × 35 000. Scales represent 1 μm.

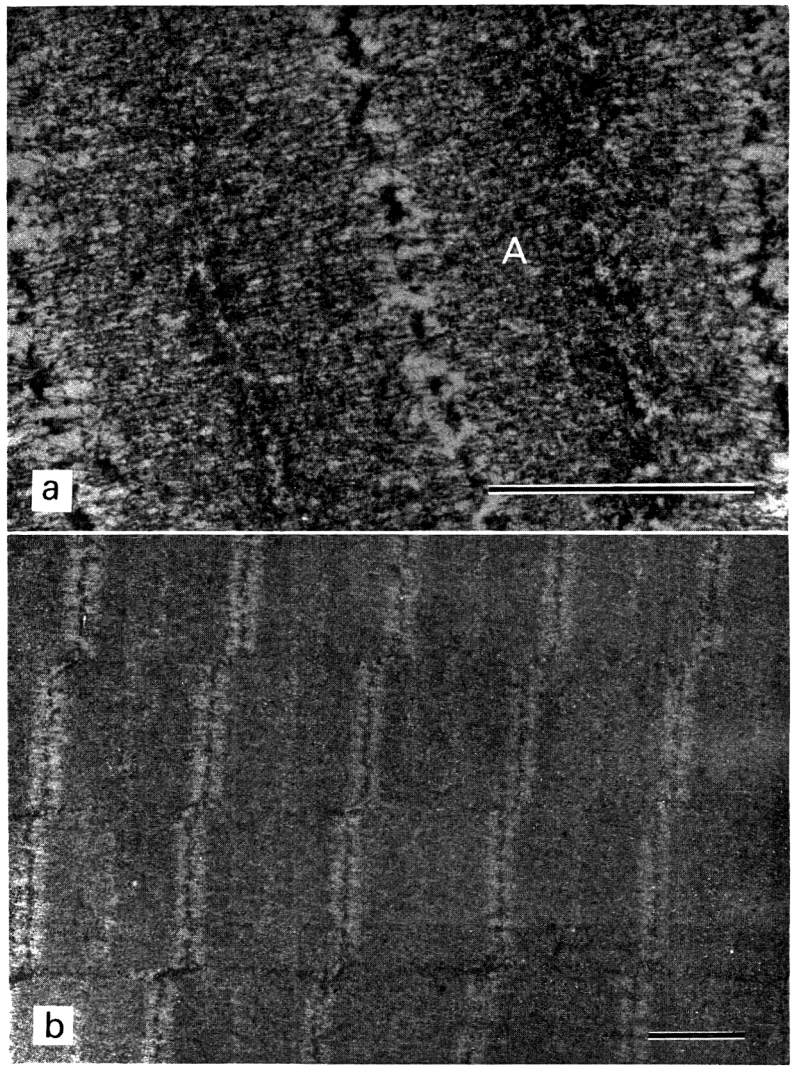

PLATE 6. (a) Myofibril from the same specimen as Plate 5b and c. Note the ordered arrangement of filaments in the A-band, the loss of the M-band and breaks in the Z-band. × 35 000. (b) Myofibril residue from a cod stored at −10°C for 47 weeks showing several closely packed myofibrils, apparently unaffected by the extraction. × 12 600. Scales represent 1 μm.

of the globular heads (or HMM-S1*) of the myosin molecule to subunits in F-actin has been determined by Moore, Huxley & De Rosier (1970) and explains the angular appearance of the arrowheads.

Sometimes long extensions originating from the arrowheads were seen (Plate 1b). Similar structures have been described for rabbit actomyosin by Ikemoto *et al.* (1968) and are thought to represent one or several tail-portions (or LMM) of the myosin molecules. These extended arrowheads were not restricted to actomyosin from unfrozen muscle but were also found in preparations from frozen and stored muscle.

Free myosin, which almost equals the weight of actomyosin in myofibrillar extracts from unfrozen muscle (King, 1966), was not demonstrable in the negatively stained specimens. According to Huxley (1963) and Moore *et al.* (1970) monomeric myosin appears to be destroyed by the negative staining procedure and is only apparent when combined with actin or aggregated in the form of filaments. Any free myosin present in the extracts will therefore manifest itself as an amorphous background in the specimens.

The common characteristic of the extracts obtained from unfrozen muscle was the high amount of well separated actomyosin filaments with a fairly constant length of 0·9 μm. Some aggregates were found consisting of actomyosin filaments interconnected by the remainder of the Z-disc (not illustrated). Thin filaments (undecorated F-actin) were rarely found.

Muscle stored at $-30°C$. Extracts obtained from cod stored at $-30°$C for up to 120 weeks showed no obvious change in separation or length of actomyosin filaments compared with preparations from unfrozen muscle. In addition to the regular arrowhead appearance of actomyosin a different type of myosin decoration pattern along the filaments was found after some frozen storage (Plate 1c). In this case the arrowheads are less regularly spaced and appear to curl up, probably indicating an aggregation of the bound myosin molecules.

Small particles, sometimes added to the surface of the filaments but mostly present as free-lying structures, were found. These measure approximately 25×25 nm and sometimes show tails of considerable length (Plates 1d and e). Particles with tails can tentatively be interpreted as myosin molecules bound to a less polymerized form of actin. Such particles were noticed in all preparations of myofibrillar protein examined, but appeared more frequently in extracts from the frozen and stored material.

Muscle stored at $-20°C$. The extracts obtained from cod stored at $-20°$C were, up the twenty-third week of storage, very similar to those obtained throughout the storage period at $-30°$C. With an increase in storage time, however, the regular arrowhead pattern of actomyosin filaments changed towards a pattern with less regularly aced arrowheads. Such filaments, together with apparently undecorated F-actin,

* Abbreviations: HMM, heavy meromyosin; HMM-S1, heavy meromyosin, subfragment 1; LMM, ght meromyosin.

become progressively more frequent during the storage. The typical appearance of actomyosin filaments found in specimens from cod stored at −20°C for more than 30 weeks is shown in Plate 2a.

During a preliminary study, myofibrillar proteins were obtained from a batch of Kattegat cod stored at −20°C for more than three years. The few actomyosin filaments found in these extracts had very few attachments of myosin and appeared similar to F-actin (Plate 2b).

In extracts from cod stored at −20°C for more than 38 weeks large aggregates with obscure structure were frequent. These were composed of actomyosin and were most heavily stained in positive contrast. Because of their dense appearance these aggregates could not be studied in detail. Less densely stained aggregates were also found, probably representing actomyosin filaments connected by Z-disc material (Plate 2c).

Muscle stored at −10°C. The preparations of myofibrillar protein from cod stored at −10°C showed similar changes in composition as observed in extracts from muscle stored at −20°C, but these appeared much earlier during the storage period. In preparations obtained after 2, 5 and 8 weeks the small particles (Plate 1d and e) were more frequent than in other samples examined.

The changes in the myosin decoration pattern of actomyosin, as described for muscle stored at −20°C, were even more prominent during storage at −10°C. After 33 weeks of storage the majority of filaments had very few arrowheads (Plate 3a) while some filaments showed regular myosin decoration along parts of their length (Plate 3b). On further storage the amount of actomyosin filaments decreased drastically and large aggregates became dominating. After 100 weeks, any free actomyosin filaments were difficult to find.

Myosin extracts

Extraction of myosin was studied only in comparison between unfrozen cod and cod stored for more than two years at −20°C. When extractant containing pyrophosphate is applied to cod muscle the extracted myosin is always contaminated with actomyosin (Connell, 1962) and this was true here. As actomyosin is made visible by negative staining while free myosin is not (Huxley, 1963), actomyosin was the only component readily demonstrable. The actomyosin filaments in myosin extracts from unfrozen muscle appeared similar to actomyosin obtained with KCl-phosphate buffer. The regular arrowhead appearance (Plate 1a) was found on all filaments and their lengths were approximately 0·9 μm.

Negatively stained myosin extracts from a cod stored at −20°C for 134 weeks contained very few actomyosin filaments and showed in addition large amounts of positively stained aggregates. The actomyosin filaments all showed a regular decoration with myosin (Plate 2d). This was in contrast to the appearance of actomyosin obtained by extraction of similar material with KCl-phosphate buffer.

Diluted myofibrillar protein extracts

The purpose of this modification was to get a rough idea about the amount of myosin in the actomyosin filaments. When rabbit actomyosin is diluted from $I=0{\cdot}6$ to $I=0{\cdot}1$, the LMM-parts of the myosin molecules form interstitial filaments between the original arrowhead-bearing actomyosin filaments (Ikemoto *et al.*, 1968).

When solutions of myofibrillar protein from unfrozen or frozen and stored cod were similarly diluted, precipitates formed in all cases examined, indicating the presence of LMM. However, in no case could intermediate LMM-filaments be found. The original actomyosin filaments lost the arrowhead appearance and were, in small domains of the precipitates, oriented in a parallel fashion. Very short filaments and the 25 × 25 nm particles were the only structures found which apparently were not involved in aggregate formation. Only in rare cases could parallel substructures, indicative of myosin aggregation, be found between two or more original actomyosin filaments (Plate 3c).

Length of actomyosin filaments

The impression, that shorter filaments were present in the negatively stained preparations from frozen and stored cod, was verified by length measurements. Only solitary filaments which could be clearly traced were measured. Twisted, curved or otherwise less discernible filaments were avoided. Length measurements on a total of 2120 filaments are presented in Table 1. In extracts from unfrozen muscle, obtained at different times during a year, the mean filament lengths vary somewhat which may reflect an influence by the preparation technique. However, the trend towards shorter filaments in extracts from frozen and stored muscle is obvious.

Distribution curves for filament lengths are given in Fig. 3. The shape and the range of the curves are uniform for the unfrozen muscle samples, indicating negligible differ-

TABLE 1. Measurements of actomyosin filament lengths in negatively stained specimens of three unfrozen muscle and three frozen and stored muscle samples

Storage of muscle	No. of pictures analysed	No. of filaments measured	Mean filament length (μm)
Unfrozen*	15	400	$0{\cdot}718 \pm 0{\cdot}224$†
Unfrozen	13	400	$0{\cdot}665 \pm 0{\cdot}241$
Unfrozen	23	425	$0{\cdot}638 \pm 0{\cdot}244$
−30°C, 60 weeks	24	375	$0{\cdot}576 \pm 0{\cdot}225$
−20°C, 63 weeks	20	250	$0{\cdot}548 \pm 0{\cdot}216$
−10°C, 33 weeks	27	270	$0{\cdot}488 \pm 0{\cdot}230$

* The three muscles were sampled over 1 year.

† Standard deviation of the mean.

ences in this respect. The curves for the frozen and stored muscle samples show different shapes and smaller ranges. In preparations of myofibrillar protein from unfrozen muscle about 50% of the actomyosin filaments had lengths in the range of 0·8–1·0 μm corresponding to the *in situ* length of the thin filaments. In the extract from the muscle stored at −10°C for 33 weeks, filaments having lengths in the range 0·8–1·0 μm amounted to only 17%.

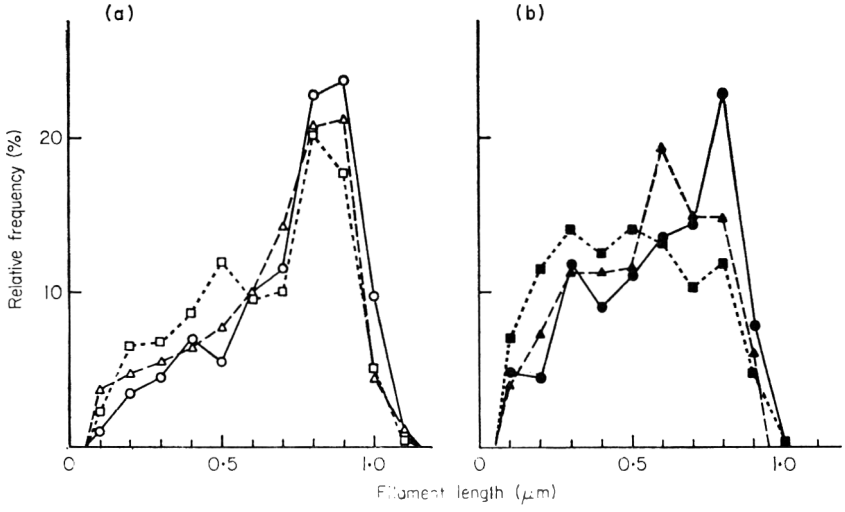

FIG. 3. Frequency distribution curves of actomyosin filament lengths of (*a*) three unfrozen muscles: ○——○, △ – – –△, □·····□, and (b) three frozen and stored muscles: ●——●, −30°C, 60 weeks; ▲– – –▲, −20°C, 53 weeks; ■·····■, −10°C, 33 weeks.

Calculation of the degree of myosin decoration

This calculation supported the subjective observation of a reduced regularity of myosin decoration in actomyosin filaments from frozen and stored muscle. Because of the errors involved the calculation was applied only for a comparison between unfrozen muscle and one frozen and stored muscle. In randomly chosen micrographs, any structure protruding from the central actin-filament was regarded as an arrowhead. In cases where a group of myosin molecules was found only on one side of the filament, this was counted as one arrowhead.

The distributions of mean arrowhead spacing in filaments from three unfrozen muscle samples were not significantly different. Therefore the data from these were pooled into one group. From Fig. 4 it can be seen that the majority of filaments from unfrozen muscle have a narrow spacing of 35–45 nm, the range 35–40 nm corresponding to the repeat in the underlying actin double helix (Huxley, 1963). The frozen and

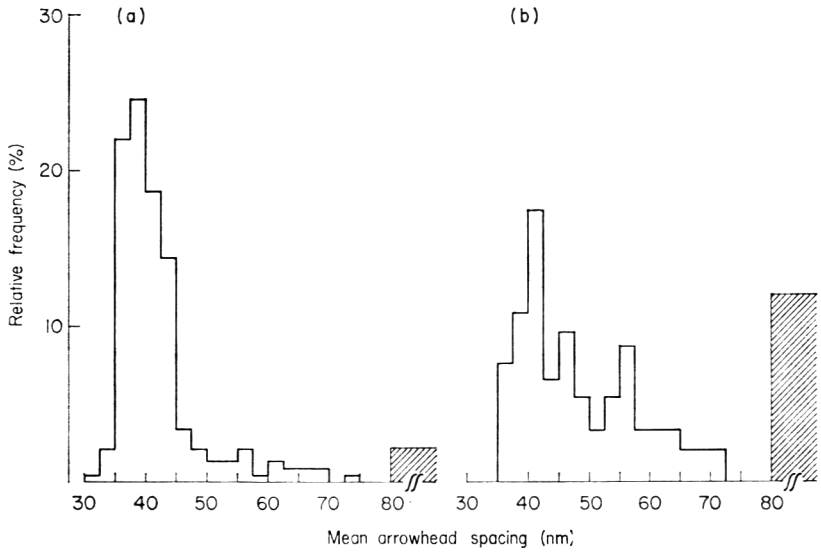

FIG. 4. Frequency distribution of mean arrowhead spacing in actomyosin filaments from: (a) three unfrozen muscles (N=236); (b) one frozen and stored muscle (−10°C, 33 weeks) (N=92). Fifteen randomly selected micrographs from (a) and (b) were analysed. Hatched columns represent filaments showing mean arrowhead spacing exceeding 80 nm. N = number of filaments.

stored muscle (Fig. 4b) still yields a fraction of filaments with 'normal' spacing, but the number of filaments with wider spacing has increased markedly.

Structure of muscle residues

Plate 4a shows for comparison structures in a thin section of a myofibril from unfrozen post-rigor cod muscle. The ultrastructure of thin sectioned cod muscle has been described previously (Bishop & Odense, 1967; Liljemark, 1969).

In thin sections of unfrozen muscle residues, remaining after the extraction with KCl-phosphate buffer, the 'I-Z-I brush' structure (Huxley, 1963) is prominent. As shown by Plate 4b this consistes of a Z-disc with thin filaments (I-filaments) attached on both sides. The thin filaments have knobs attached regularly on their surface and may represent actomyosin filaments connected to the Z-disc. Similarly interconnected actomyosin filaments were found in the myofibrillar extracts as previously mentioned.

The residue from unfrozen muscle remaining after myosin extraction with pyrophosphate-containing extractant was mainly composed of I-Z-I brushes with no sign of myosin decoration along the thin filaments (Plate 4c). This result is to be expected as pyrophosphate is known to dissociate actin-myosin bonds and inhibit their reformation (Hasselbach & Schneider, 1951).

The amount of residue from unfrozen muscle was generally very small, but in cases where the myofibril alignment was still retained, consecutive I-Z-I brushes could be found (Plate 5a). The homogenization process had certainly caused displacements in the myofibrils, as thin filaments both overlapped and touched each other at the level of the original H-zone. The presence of precipitated myosin between I-Z-I brushes is not excluded either. Most thick filaments, however, seem to be almost completely dissolved.

Residues after extraction of myofibrillar protein from muscle stored at −30°C were studied only until 8 weeks of storage and showed similar structural character as found in residues from unfrozen muscle.

In residues from muscle stored at −20°C, a number of intermediate states of myofibril breakdown could be found. Early during the storage period residues were similar to those from unfrozen muscle (Plate 5a) but at the end of the storage the fibrils appeared almost unaffected by extraction.

Within a single preparation different degrees of extraction could be seen, illustrated in Plates 5b and c and 6a taken from a muscle stored at −20°C for 38 weeks. Plate 5b shows a compressed myofibril in which thin filaments overlap in the original H-zones. Dense clusters can be seen along the filaments, probably representing aggregates of myosin. The diameter of the filaments found in the H-zone is 9–15 nm. This does not exclude the presence of thick filaments, but they were not recognized here. Typical also are breaks in the Z-band and missing thin filaments, which probably is consistent with the greater number of aggregates found in corresponding protein solutions (cf. Plate 2c). Plate 5c shows an intermediate state with more material remaining in the original A-band, which indicates that less myosin has been extracted. The loss of Z-band material is still prominent. The fibril in Plate 6a shows a degree of order closest to that of the original A-band. The extraction has caused some blurring but individual thick filaments can be traced. A common character of similar myofibrils was the loss of the M-band. Discontinuities in the Z-band and loss of thin filaments from this type of fibril can be consistent with the presence of apparently undecorated thin filaments in corresponding protein solutions. As a thin section represents only a very small fraction of the total composition of the residues any statement about relative amounts of myofibrils shown in Plates 5b and c and 6a, is uncertain. However, after more than 90 weeks' storage at −20°C most myofibrils present in the residues looked like the one shown in Plate 6a.

Muscle stored at −10°C showed similar changes in extraction residues as described for muscle stored at −20°C. These changes, however, occurred at a much greater rate, and after a period of only 24 weeks in storage at −10°C most myofibrils found were similar to the one shown in Plate 6a. Furthermore, myofibrils tended to stick together and large areas with non-separated myofibrils of almost full fibre width could be found (Plate 6b). Signs of uneven extraction of protein within the single myofibrils were seen only at the ends of larger fragments of joined myofibrils.

When a muscle stored at −20°C for 134 weeks was extracted for myosin, the residue appeared essentially as the one shown in Plate 6b, indicating that the selective extraction of myosin is seriously impaired after this time.

Discussion

This investigation, although based on a somewhat subjective impression of structures seen with the electron microscope, shows a quantitative change in the composition of myofibrillar protein extracts from frozen cod with increasing storage time. The changes are most obvious in extracts from cod stored at −10°C, where the number of free actomyosin filaments rapidly decreases and large aggregates become dominating when storage proceeds. This is in accord with earlier results obtained with analytical ultracentrifugation (King, 1966; Anderson & Ravesi, 1969, 1970a, b). In addition, a progressive change in the structure of actomyosin is suggested.

The difference in amounts of protein extracted from unfrozen cod and cod stored at −30°C (Fig. 2) may be due to freezing and thawing rather than to storage of the latter. As shown by Ironside & Love (1958) and Love & Ironside (1958) the mere act of freezing and immediate thawing, without storage, will reduce the amount of extractable protein in cod muscle to a similar extent. A further cause of the discrepancy in amounts of extractable proteins may be that fish were obtained from different localities. Nutritional state of cod, different fishing grounds and catch at various times of the year are known to affect the amount of extractable protein (Ironside & Love, 1958; Castell & Bishop, 1973) and cell-fragility measurements (Love, 1969). However, the variation between individuals from the same locality, can be as great as any of the above mentioned factors (Love, 1965).

To interpret changes in the structure of actomyosin obtained from frozen and stored cod one has to consider the mechanism of its extraction, possible interactions between actin and myosin taking place in solution, the effect of frozen storage on its separate components, specific effects of the extraction procedure and the preparation technique.

The occurrence of shorter actomyosin filaments in extracts from frozen and stored cod (Fig. 3) may be explained in several ways. A progressive fixation of myosin in the thick filaments (indicated by the structure of muscle residues) may retain thin filaments in the myofibrils and liberate only portions of the thin filaments during extraction. Moreover, actomyosin filaments may be further degraded during the 20-hr extraction period.

King (1966), using ultracentrifugation, suggested that actomyosin obtained from unfrozen cod dissociates into a globular form (G-actomyosin) during prolonged storage in the extracted state. The ultracentrifuge pictures of actomyosin prepared from frozen and stored cod resembled those obtained by storing actomyosin from unfrozen cod. Based on the similarities of ultracentrifuge pictures, King proposed that a globular form of actomyosin could be an intermediate in the reaction which in frozen

muscle leads to inextractability of the myofibrillar proteins. However, no ultrastructural changes, indicating dissociation of the myofilaments in frozen and stored cod muscle have so far been found (Liljemark, 1969; Jarenbäck & Liljemark, 1975). Actomyosin from frozen and stored cod is possibly more susceptible to dissociation in the extraction solution than actomyosin from unfrozen cod. An indication of filament dissociation could be the presence of small particles in the extracts (Plate 1d and e) which because of their dimensions and details might be dissociation products. These structures can possibly bear some resemblance to the G-actomyosin suggested by King (1966).

Furthermore, actomyosin may be degraded by the staining procedure. Synthetic acto-(S1)-filaments, obtained by mixing actin and the globular heads of the myosin molecules (S1) at high ionic strength, are considered to be sensitive to negative staining (Moore *et al.*, 1970). Synthetic acto-HMM filaments have actually been shown to break when negative staining has been applied (Kawamura & Maruyama, 1972). In this study, it is therefore possible that filaments broken during staining contributes to the groups of filaments showing the shortest lengths in Fig. 3.

The apparent lower content of myosin along actomyosin filaments (Fig. 4 and Plate 2b) found in extracts from frozen and stored muscle may reflect changes in the properties of myosin as a result of frozen storage. If myosin is not readily released from its site in the thick filaments, pre-existing actin-myosin bonds may have been broken during the extraction procedure, and few arrowheads are observed. The actin combining power of myosin may also have changed.

Myosin with unusual properties and no actin combining power can be extracted from cod stored for a long time at −29°C (Connell, 1968). Contrarily, the myosin combining capacity of selectively extracted actin has been shown not to change during frozen storage (Connell, 1960b). To what extent the tropomyosin-troponin system, or changes in its physical state, is responsible for the observed changes in myosin decoration is so far unknown.

The calculation of mean arrowhead spacing along actomyosin filaments, as given in Fig. 4, is subject to many errors and should be taken as an approximation only. Differences in the stain distribution and density of negatively stained specimens may cause variations of filament structure detail. Further, the specimen support film, free myosin and other cell constituents present in solution can give an interfering background structure. Troponin, appearing with a periodicity of 38·5 nm along thin filaments (Ohtsuki *et al.*, 1967; Spudich, Huxley & Finch, 1972), may show up and be mistaken for myosin.

The actomyosin filaments found in pyrophosphate extracts from a frozen and stored muscle (Plate 2d) all showed a 'normal' arrowhead appearance in contrast to actomyosin obtained with extractant not containing pyrophosphate (Plate 2a and b). As the preparation of myosin in this case involves a precipitation step and redissolving in KCl-phosphate buffer, the pyrophosphate is partly washed out. Myosin extracts obtained at any time during the frozen storage contains some native myosin (Connell,

1962), and in a system where myosin is in excess over F-actin, native myosin molecules may interact with F-actin and form the 'fully decorated' filaments seen in Plate 2d.

The failure of actin-bound myosin to form visible filaments interstitial between original actomyosin filaments, upon dilution of myofibrillar extracts, can indicate special properties of cod myosin. As reported by Mackie & Connell (1964), cod myosin does not precipitate in the same way as rabbit myosin when the ionic strength is reduced to 0·1–0·05, but remains in solution as a polydisperse aggregate. A reduction of pH from 7 to 6 increases the aggregation rate for cod myosin (Connell, 1960a). Filament formation in rabbit-myosin solutions occurs more rapidly at pH 6 than at higher pH when the ionic strength is reduced (Kaminer & Bell, 1966). In the present study, the pH of the extracting solution was kept buffered at 7·2. A reduction in pH could possibly have given a different form of precipitate than the one shown in Plate 3c.

The increasing amount of large aggregates found in myofibrillar extracts, especially towards the end of the storage period at −20°C and earlier during storage at −10°C, needs some comment. Thin sections of the muscle residue after extraction from cod stored at −20°C (Plates 5b and c and 6a) show breaks in the Z-band and loss of thin filaments. Thin filaments, rather than being solubilized as single filaments, are kept together by portions of the Z-disc and probably form the basis for the large aggregates found in corresponding protein solutions (cf. Plate 2c). The discontinuous Z-bands could also indicate that a specific change in the Z-disc has taken place during frozen storage.

The I-Z-I aggregates described above may be related to the approx. 20% gel-fraction found in cod myofibrillar extracts analysed with ultracentrifugation (Ellis & Winchester, 1959; King, 1966). The gel-fraction has been shown, in species other than cod, to consist of aggregated actomyosin (Umemoto & Taguchi, 1972).

Aggregated material in KCl-extracts of frozen and stored cod were also found in ultracentrifuge studies by King (1966) and Anderson & Ravesi (1969, 1970a, b). Large aggregates were especially noticeable when the amount of intrinsic free fatty acids had been increased by storage of cod in ice for several days prior to frozen storage (Anderson & Ravesi, 1970a). Apart from the possible effects of fatty acids formed *in situ* during storage (for review, see Powrie, 1973) those released during the extraction procedure could also interact with actomyosin filaments giving rise to large aggregates. Oxidation of phospholipids and free fatty acids present in the myofibrillar extracts can take place during the 20-hr extraction period (Sheltawy & Olley, 1966) and the peroxides thus formed may cause further aggregation of the filaments.

The changes in actomyosin filament structure, shown here, may indirectly reflect a gradual fixation of myosin in the thick myofilaments with increasing time of frozen storage. A more direct demonstration of this is the retention of A-band material in corresponding myofibril residues. These structural changes may demonstrate a progressive cross-linking of molecules within the thick filaments which could increase the toughness of the muscle, as discussed by Connell (1968).

An increased cohesion between myofibrils was found after prolonged frozen storage (cf. Plate 6b). A contribution to this may be the collapse of the sarcoplasmic reticulum also seen in intact fibres (Jarenbäck & Liljemark, 1975). The joining of myofibrils will in turn reduce the amount of protein obtainable by extraction with salt solutions as this is in part influenced by the degree of subdivision of the muscle fibres (Anderson & Ravesi, 1970a).

In muscle residues from cod stored at $-10°C$ and showing several joined myofibrils (cf. Plate 6b) no tendency towards a radial gradient of uneven extraction through myofibrils was ever observed. The filaments adjacent to the remnants of the sarcoplasmic reticulum would have been expected to be more resistant if diffusion of fatty acids from the membrane systems, as suggested by Anderson & Ravesi (1970b), causes the proteins to be inextractable. The influence of a fatty acid on myofibrils and extracted proteins will be described in a forthcoming report.

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Effect of sodium chloride and sodium nitrite on the heat resistance of *Staphylococcus aureus* NCTC 10652 in buffer and meat macerate

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Summary

The heat resistance of *Staphylococcus aureus* NCTC 10652 in tris-maleate buffer pH 7·0 was increased by 4 or 8% w/v NaCl ($P = 0{\cdot}001$). At pH 6·5 the $D_{60°C}$ in both buffer and meat macerate was increased by 8% w/v NaCl. Addition of $NaNO_2$ had little effect on the heat resistance.

Introduction

Outbreaks of food poisoning sometimes occur after the ingestion of cured meat on which *Staphylococcus aureus* has grown. Large hams ($> c.$ 2 lb) are usually only lightly heated, to a centre temperature of approximately 60–70°C, and, since meat is known to protect bacterial cells against the lethal effects of heating, it seems conceivable that *Staphylococcus* spp. contaminating the raw material might survive the pasteurization process. Jensen (1942) reported that meat micrococci were protected against heat by sodium chloride, and Calhoun & Frazier (1966) showed that the heat resistance of *Staph. aureus* at 60°C was more than trebled in phosphate buffer at pH 7·0 when adjusted to a water activity of 0·95 with NaCl (about 8·5% w/v). Gross & Vinton (1957) concluded that the heat resistance of *Staph. aureus* was greater in cured meat than in uncured meat, although the two meat samples used in that study differed in pH (cured, pH 6·84; uncured, pH 7·87) and this factor was not taken into account.

While there is general agreement that bacterial cells are more resistant to heat in cured meat than when suspended in buffer, the exact roles of NaCl and $NaNO_2$ have not been clearly elucidated, and this was the purpose of the present study. An enterotoxin A producing strain of *Staph. aureus* was used as this group is more commonly implicated in food poisoning cases in the UK (Šimkovičová & Gilbert, 1971), while reports in the literature on heat resistance have more often used enterotoxin B-producing strains.

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Material and methods

Culture

Staphylococcus aureus NCTC 10652 (often referred to in the literature as 196-E), which produces enterotoxin A was grown in 2 × 15 ml of Heart Infusion broth (HI) (Difco) at 37°C for 22 hr to the stationary phase of growth. After agitation with sterile glass beads on a Whirlimixer (Fisons Scientific Instruments Ltd) to disaggregate clumps, cells were harvested by centrifugation at 4500 rpm, washed three times in distilled water and finally resuspended in distilled water at about 5×10^9 cells/ml.

Heating substrates

The following substrates were used.

(a) 0·05M tris-maleate buffer, pH 5·0–8·6 (Gomori, 1955) containing combinations of NaCl and $NaNO_2$.

(b) A macerate of porcine muscle in water prepared as follows. One hundred grams of muscle were sterilized in a closed vessel by autoclaving at 115°C for 20 min, cooled and homogenized in an Atomix (M.S.E.) for 30 sec at half-speed and 2 min at full-speed with the exuded juices and 200 g of appropriate sterile salt solution, prepared assuming 80% water in the muscle. The macerate was adjusted to pH 6·5 with sterile 1N NaOH using a model 38B pH meter (E.I.L.) and a filter-sterilized solution of $NaNO_2$ was added as required. After aseptic distribution into 1 oz wide-necked screw-capped bottles, the macerate was held overnight at 1° to permit equilibration of the salts. Possible loss of $NaNO_2$ during heating was monitored, using the official Society of Analytical Chemistry (1974) method, by determinations on uninoculated samples before heating and after the longest heating period (50 min).

Heating procedures

All resistance studies were carried out at 60 ± 0·1°C in a water bath fitted with a mercury contact control thermometer (Model SB 2X, Grant Instruments, Cambridge).

In buffer. One millilitre of cell suspension was transferred to 9 ml of 0, 4 or 8% w/v NaCl solution and mixed. From this suspension, 1 ml aliquots were added to 9 ml of buffer solution previously equilibrated to 60°C and containing the same concentration of NaCl, thus maintaining the NaCl concentration on addition of inoculum. Where appropriate, $NaNO_2$ was added to the buffer solution immediately prior to the inoculum as 0·1 ml of a filter-sterilized solution. Inoculated bottles were immediately resubmerged and 20 sec allowed for the contents to regain 60°C. Bottles were removed at intervals and cooled by immersion in ice/water. Decimal dilutions in maintenance medium (MM) (0·85% NaCl + 0·1% peptone) were prepared from cooled samples for viable counting. Three replicates were heated at each of four time intervals.

In meat macerate. Procedure (a) was followed except that each bottle contained 20 ml

of macerate to which was added 0·2 ml of a × 10 concentrated inoculum. After cooling, decimal dilutions were prepared in MM, the initial dilution being 10 ml of the heated macerate with 90 ml of MM. Resistance was determined in macerates prepared on two separate occasions using two replicates at each of six times of heating.

Viable counts

Duplicate 0·02 ml drops of decimal dilutions in MM delivered from 20 SWG stainless steel cannulae (Astell Ltd) were spread on quarters of the surface of plates of Blood Agar Base (Oxoid No. 2) to which 5% oxalated horse blood (Wellcome Reagents Ltd) had been added. Colonies were counted after incubation for 48 hr at 35°C and viable counts calculated as described by Farmiloe *et al.* (1954).

Calculation of $D_{60°C}$ values

In preliminary experiments, the linearity of the regression of $\log_{10}$ survivors against time of heating in buffer was established, by the method of least squares (Bailey, 1959), for a number of combinations of NaCl, $NaNO_2$ and pH value, correlation coefficients ranging from 0·87 to 0·96 ($P < 0·001$). $\log_{10}$ survivor counts from heating in buffer in this study were subjected to the same regression analysis.

In experiments where cells were heated in buffer, $\log_{10}$ viable counts on three replicate samples at four heating times were used to calculate a single regression equation. When cells were heated in meat macerate, $\log_{10}$ viable counts from duplicate samples at six heating times were used to calculate a single regression equation. The negative reciprocal of the regression coefficient is equivalent to the $D_{60°C}$ value.

Measurement of water activity

Water activity (a_w) determinations of buffer solutions at pH 6·5 containing NaCl and/or $NaNO_2$ were made at 30°C using a Sina Equihygroscope Type SMT 9 (Sina AG, Zurich, Switzerland), which had been calibrated against a range of salt solution using a_w values quoted by Robinson & Stokes (1955).

Results

Heat resistance in buffer

The heat resistance of *Staph. aureus* NCTC 10652 was determined at 60°C in 0·05M tris-maleate buffer in all combinations of the following: NaCl—0, 4, 8% w/v; $NaNO_2$—0, 200, 400 ppm; pH—5·0, 5·5, 6·0, 6·5, 7·0.

The regression $\log_{10}$ surviving viable cells against duration of heating was always linear ($P < 0·001$). Heat resistance was increased by 4 and 8% NaCl ($a_w = 0·978$ and 0·957 respectively) at pH 6·0–7·0 ($P = 0·05$–$0·001$). In the presence of NaCl, $D_{60°C}$ values increased progressively with increasing pH (Table 1). The results in Table 1 were subjected to multivariate analysis and the resultant information is incorporated

TABLE 1. The effect of NaCl, $NaNO_2$ and pH on the heat resistance of *Staph. aureus* NCTC 10652 in tris-maleate buffer

pH	$NaNO_2$ (ppm)	$D_{60°C}$ (min) NaCl (% w/v) in buffer		
		0	4	8
5·0	0	1·54[a]	0·95[a]	1·18[a]
	200	1·20[a]	1·83[a]	1·36[a]
	400	0·99[a]	0·93[a]	0·95[a]
5·5	0	1·01[b]	1·19[b]	1·19[b]
	200	0·85[b]	1·17[b]	1·40[b]
	400	1·00[b]	1·06[b]	1·60[b]
6·0	0	1·67[c]	2·00[c]	4·57[e]
	200	1··3[d]	1·60[c]	6·71[e]
	400	1·20[d]	1·80[c]	6·06[e]
6·5	0	1·64[f]	2·67[g]	5·22[h]
	200	1·57[f]	1·85[f]	7·63[h]
	400	1·67[f]	2·65[g]	8·62[h]
7·0	0	0·86[i]	2·99[k]	6·33[m]
	200	1·83[j]	4·44[e]	8·33[m]
	400	1·22[i]	7·63[m]	11·49[m]

[a–m]: at any pH value. $D_{60°C}$ values with the same superscript are not significantly different at the 5% level.

into the table, each pH value being considered separately. At pH values 5·0 and 5·5 there were no significant differences between the $D_{60°C}$ values. At pH values 6·0, 6·5 and 7·0, 8% NaCl always increased heat resistance significantly. At pH values 6·5 and 7·0, 4% NaCl increased heat resistance significantly in five of six cases. In most instances the addition of $NaNO_2$ did not increase heat resistance significantly. Although progressively larger $D_{60°C}$ values were obtained with increasing $NaNO_2$ concentration at pH 6·5 and 7·0 in the presence of 8% NaCl, differences were not significant. At pH 7·0 with 4% NaCl a similar increase in heat resistance with increasing $NaNO_2$ concentration was evident, and the differences were significant.

Heat resistance in meat macerate

Heat resistance at 60°C was also determined in a macerate of porcine muscle at pH 6·5 and the effect of NaCl added at about 4 and 8% (g/100 g H_2O), and $NaNO_2$ added at about 500 ppm was studied. Heating at 60°C for 50 min (the longest heating period) had no effect on the level of NaCl, but $NaNO_2$ was reduced by 24–32 ppm

TABLE 2. The effect of NaCl and $NaNO_2$ on the heat resistance of *Staph. aureus* NCTC 10652 in meat macerate at pH 6·5

	$D_{60°C}$ (min)			
$NaNO_2$ (ppm)	NaCl (% w/v)			
	0	3·27	4·15	8·4–8·5
0	4·61	—	4·47	18·62
500	4·51	—	6·45	28·49
0	9·62	5·49	—	26·38
500	5·67	6·55	—	26·81

from initial levels of 501 and 493 (both referred to as '500 ppm'). The regression of $\log_{10}$ surviving viable cells against duration of heating was always linear ($P < 0{\cdot}001$) and calculated $D_{60°C}$ values are listed in Table 2. Two separate macerates were prepared and these varied slightly in NaCl content. Despite this difference it is evident that heat resistance was increased by the presence of 8·4–8·5% NaCl. The effect of adding $NaNO_2$ was not consistent. Once, when added alone, $NaNO_2$ slightly reduced heat resistance, and, once, in the presence of 8% NaCl, $NaNO_2$ increased heat resistance. These findings paralleled those obtained on heating in buffer solution.

Discussion

The results show a specific protective effect of NaCl against the lethal action of heat in buffer solution which, although absent at pH 5·0 and 5·5, was evident at pH 6·0, 6·5 and 7·0 and most marked at pH 7·0. The influence of pH alone on heat resistance (chloride and nitrite both absent) was small ($D_{60°C}$ 0·86–1·67 min), but the protective effect of 8% w/v NaCl was more marked at pH 7·0 ($D_{60°C}$ 6·33 min) than at pH 6·0 ($D_{60°C}$ 4·57 min), and no protection was evident at pH 5·0 and 5·5. The additional protective effect of $NaNO_2$ when added with NaCl was only evident at pH 7·0, the largest $D_{60°C}$ being × 13 greater than that in the absence of both salts.

In a meat macerate, adjusted to pH 6·5 to reflect the pH trend in modern pasteurized hams, the effect of 4% NaCl alone or with $NaNO_2$ was small (Table 2) and not significant at the 5% level. The increase in resistance afforded by 8% NaCl which increased $D_{60°C}$ four-fold and in combination with $NaNO_2$ six- to eight-fold, was significant at the 5% level.

Many pasteurized cooked meat products receive only minimal pasteurization to retain the flavour and texture of the product which suffer deterioration from over-cooking. Such products are stable with respect to spoilage by spore-forming bacteria. The

results of this study indicate that *Staph. aureus* are capable of surviving for long periods at 60°C in a cured meat macerate (36–69 min would be required to destroy 1000 organisms in macerate containing 8·4 g NaCl/100 g H_2O). Even greater resistance to heat is to be expected in whole pork because of the presence of fat. The possible survival of *Staph. aureus* after processing is important since inhibition of growth and enterotoxin production cannot be achieved by commercially realistic concentrations of NaCl and $NaNO_2$ unless both the pH and incubation temperature are relatively low (Bean & Roberts, in preparation). Thatcher & Robinson (1962) observed that, in foods inoculated with *Staph. aureus*, 48 hr was commonly required for enterotoxin production, whereas in outbreaks of food poisoning it apparently took place within a few hours of the handling thought to be the means of recontamination with the organism. The heat resistance of *Staph. aureus* in cured meat reported here approaches that required to survive a pasteurizing process. Hence, it seems possible that the use of relatively heavily contaminated raw materials could result in survival of *Staph. aureus* and enterotoxin production where storage temperatures allow growth to take place. Data should be obtained to show the extent of *Staph. aureus* survival under commercial processing conditions.

To minimize the food poisoning risk from growth of *Staph. aureus* surviving pasteurization, storage below 10°C, recommended by Eddy & Ingram (1962) for uncooked bacon, should also be recommended for all pasteurized cured meats.

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Cooking shortening and the toughening of beef

C. L. DAVEY AND K. V. GILBERT

Summary

The relationship between cold-shortening and toughness has been determined for the sternomandibularis muscles of young ox cooked at both 60 and 80 °C, and therefore taken through the recently established first and second stages of cooking toughness development respectively.

At both cooking temperatures, toughness increases approximately three-fold on shortening to 35–40%, and declines steeply at higher shortenings. At a given shortening, the toughness of meat taken through the first cooking stage is only half that of meat taken through both stages. The pronounced cooking shortening accompanying the second stage contributes to toughness to virtually the same degree as cold-shortening in pre-rigor muscle.

On the basis of this observation toughness has been related in general terms to muscle structure and function. The mechanics of cleaving cooked meat across the grain and of how shearing force increases with shortening are also discussed.

Introduction

Locker (1960) first demonstrated that shortening induces toughness in beef. Following from this, the relationship between shortening and toughening was put into quantitative terms by Marsh & Leet (1966a). They observed a two- to three-fold toughening in muscle shortened by about 40% of the resting excised length. At still higher shortenings the muscle tenderized again, and by 60% had returned to its unshortened tenderness. The form of the shortening–toughening relationship was essentially unaffected by the method of pre-rigor stimulation used.

The peaked relationship is so distinct that it has prompted attempts to explain it in terms of muscle structure and function (Marsh & Leet, 1966b). There seems little doubt from microscopic studies that the declining side of the peak at shortenings beyond 40% is due to gross disruption of tissue, with nodes of shortening stretching adjacent regions sometimes to breaking point (Marsh, Leet & Dickson, 1974). It is imagined that tenderness is caused through the development of such planes of easy fracture. Although of considerable scientific interest, this declining phase at high

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shortenings is probably not encountered in practical meat treatment. On the other hand, the rising phase of the relationship at lower shortenings is of greater importance. It has been explained in terms of the sliding filament theory of contraction. In one proposal, the peak at 40% is claimed to be reached when the thin filaments of the sarcomere are pulled fully into the spaces between the thick filaments so that the Z-lines abut the A-bands (Davey, Kuttle & Gilbert, 1967). The toughness develops as the percentage of sarcomeres in this condition increases with shortening (Voyle, 1969). In another proposal, the progressive disappearance of I-bands with shortening has been mathematically related to increasing toughness (Marsh & Carse, 1974).

Forms of toughening and shortening not previously considered are those occurring on cooking rigor meat. In contrast to earlier studies (Sanderson & Vail, 1963), cooking toughness has been shown to develop in two distinct stages—the first at temperatures between 40 and 50 °C and the second between 65 and 75 °C (Davey & Gilbert, 1974). It is this latter stage which is accompanied by cooking shortening. Coarse-textured bull sternomandibularis muscles were used. These are structurally very uniform, with fibres lying parallel to the muscle axis. Their tenderness can be precisely measured by a tenderometer after cooking at very low temperatures (< 40 °C), an essential requirement in identifying the two stages of toughening.

Actomyosin is denatured in the first stage, and this, together with the fact that the meat is in rigor mortis at time of cooking, weighs against cooking shortening being due to a sliding of filaments. Rather it appears that such shortening is due to the shrinkage of connective tissue (Davey & Gilbert, 1974).

Since there seems little doubt that pre-rigor cold shortening and cooking shortening arise from entirely different structural events, it is to be expected that they would also contribute to toughness to different degrees. This paper compares their contributions to toughness and describes the shortening–toughening relationship in terms of whole muscle structure, rather than solely in terms of the fine structure of the sarcomere.

Experimental

Meat

Beef sternomandibularis muscles (neck muscles) of Angus ox (15–20 months of age) were excised and trimmed within 30 min after slaughter. They were cut into oblong portions and set to varying degrees of cold shortening (S) as previously described (Marsh & Leet, 1966a, b).

Only those muscles with ultimate pH values below 5·7 were used. Thus the muscles were from a single breed of young ox, were set in rigor mortis at various degrees of shortening, and had normal, low ultimate pH values.

For experimentation the muscle portions, shortened to differing degrees up to S, 0·50, were cut into small oblong samples (4–5 cm along the fibre × 1·0 cm × 1·5 cm). The variations in the lateral dimensions were not more than 0·1 cm. With practice, samples

could be cut with the required accuracy to give as many as twenty samples from a pair of neck muscles.

In this respect, standard-sized samples for tenderometer evaluation are normally cut from cooked meat. The fibre numbers cleaved are not constant, but depend on the degree of cooking shrinkage across the grain. It was for this reason that the standard samples were cut from raw meat so that fibre numbers at a given S value were constant and independent of transverse cooking shrinkage.

Cooking shrinkage and weight loss

After measuring weights and lengths, each oblong sample was placed horizontally in a polyethylene bag containing a stainless steel rod as a weight, and heated in a water-bath at either 60 or 80°C for 40 min. The cooked samples were chilled to 2°C, and the lengths measured again. The degree of cooking shrinkage (S_1) in the fibre direction was then determined. Thus the total cold and cooking shortening was given by $S+S_1$.

Tenderness and ultimate pH determinations

Tenderness was measured using the MIRINZ tenderometer described by Macfarlane & Marer (1966). The tenderometer wedge is forced through the sample at right-angles to the fibre axis, the sections for biting being 1·5 cm wide by 0·7 cm thick. Shear force (*SF*) values are given in arbitrary units which are approximately four times those given by the Warner Bratzler tenderometer (1·26 cm cores). For ultimate pH, one of the muscle portions from each animal was used after it had been held for the 48 hr post-mortem period to enter rigor mortis. Estimations of PH were made on homogenates of 1–2 g muscle in 10–15 ml neutralized sodium iodo-acetate solution (2 mM).

Results

The shortening-toughness relationship for neck muscles from young ox is given in Fig. 1. In accordance with previous convention (Marsh & Leet, 1966a; Davey *et al.*, 1967), *SF* values were related to pre-rigor muscle shortening alone, despite the fact that cooking shortening occurs on heating meat above 60°C. Curve 1 is typical of meat which has undergone such shortening through being heated at 80°C. Toughness increased sharply to peak at S, 0·42 and declined just as sharply at still higher cold shortenings. Curve 2 describes meat heated at 60°C and taken through the first stage of cooking toughening only. As expected, cooking shortening did not occur. The *SF* values at each S value were about half of those for meat cooked to 80°C. The characteristic cold shortening–toughness relationship was therefore still obtained. It reached its maximum at the somewhat lower cold shortening of S, 0·36.

Curve 3 relates pre-rigor cold shortening to the additional cooking shortening on taking rigor meat through the second cooking-toughening stage by heating at 80°C.

Fig. 1 supplies all the data for determining whether lengthwise cooking shortening

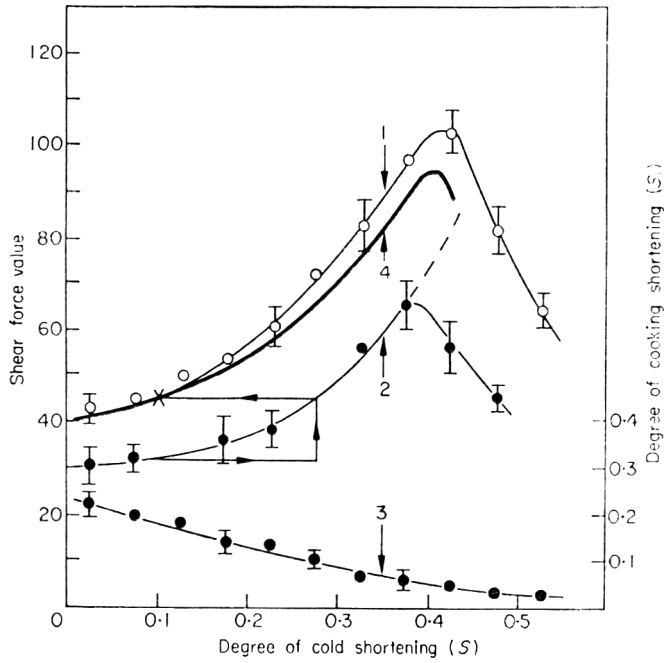

FIG. 1. Shear force and cooking-shortening values for beef neck muscle relative to the degree of cold shortening. Curve 1: meat cooked for 40 min at 80 °C; Curve 2: meat cooked for 40 min at 60 °C; Broken line: theoretical extension of the rising phase of Curve 2 (see text); Curve 3: the additional degree of shortening along the direction of the fibre induced by cooking for 40 min at 80 °C; Curve 4: The theoretical shortening–toughness curve for meat cooked for 40 min at 80 °C and obtained from combining the information of Curves 2 and 3. The arrowed lines linking Curves 2 and 4, and the cross on Curve 4 relate to an example of how the information of Curves 2 and 3 was combined (see text). The values for the various cooking changes have been separately summated within each interval of 0·05 S units and the means obtained. Mean values (closed circles) have been plotted against the central S values within the appropriate shortening intervals. Each mean has been determined from up to thirty separate determinations from the neck muscles of twenty animals. Standard deviations, vertical lines. For clarity, not all standard deviations are shown.

contributes to toughness to the same degree as cold shortening induced pre-rigor. This can be tested by determining whether the additional shortening produced on raising the cooking temperature from 60 to 80 °C will lift *SF* values sufficiently to convert Curve 2 to Curve 1.

Cooking shortening (S_1) corresponding to a particular degree of cold shortening (S) was read from the right-hand ordinate of Fig. 1. Combined, these shortenings would give a total shortening ($S+S_1$). The *SF* value from this total shortening was read from Curve 2, presuming that both shortenings contribute equally to toughening. For direct comparison with Curve 1, the *SF* values obtained were then plotted against the degree of cold shortening to give Curve 4. As an example, at S, 0·10, S_1 is 0·18, giving a total

cold plus cooking shortening of 0·28. This corresponds to *SF*45 on Curve 2, and is marked at *S*, 0·10 in Fig. 1 as a cross on Curve 4.

Since Curve 1 reached its maximum at *S*, 0·36, it cannot be used to determine theoretical *SF* values at shortenings higher than *S*, 0·36. To achieve this, a single basic assumption has had to be made. As already discussed, the decline in toughness above *S*, 0·36 (Curve 2) is due to marked structural damage of the meat. The assumption is that such damage is caused only by pre-rigor shortening and not by cooking shortening. On this basis, whereas cold shortening above *S*, 0·36 tenderizes meat, cooking shortening at greater than *S*, 0·36 would toughen it. Arising from this assumption, Curve 2 has been extended and used for analysis above *S*, 0·36. To obtain the extrapolation, the curve must first be described mathematically. Between zero and *S*, 0·35 it is closely exponential and has the form $SF = 2 \cdot 5\, e^{7 \cdot 3S} + 27$. The extrapolation to *S*, 0·45 can then be made from this formula. The broken line extending Curve 3 was determined in this way.

Fig. 1 shows that the correspondence of Curves 1 and 4 was good at low shortenings (up to *S*, 0·2). With shortenings beyond *S*, 0·2 the curves diverged, with Curve 4 falling below Curve 1. However, even at *S*, 0·35 correspondence was still reasonable, the theoretical *SF* value (82) being 90% of the experimentally found one on Curve 1.

Discussion

This study extends our understanding of why shortening causes toughness. It shows that the rise in toughness to a peak at approximately *S*, 0·4, and the fall at higher shortening, are characteristic of meat cooked not only to 80 °C but also to 60 °C. Thus the peaked relationship exists in meat which has passed through either one or both of the toughening phases of cooking.

The shortening–toughening relationship obtained at 80°C can be derived theoretically from that at 60 °C by regarding longitudinal cooking shrinkage occurring on raising the cooking temperature from 60 to 80 °C as an extra shortening, contributing to toughness. This has important implications for our understanding of the development of toughness in term of structure. Attempts to explain toughness from the sliding filament theory of contraction are oversimplified in that they disregard other complex events in muscle; the decline in fibre numbers per unit of muscle cross-section with shortening, the shortening from cooking rigor muscle, changes in the characteristics of connective tissue and sarcolemma with shortening and cooking. We have sought a less specific explanation of the rising phase of toughness than can be derived solely from fine changes in the structure of the sarcomere.

The relationship of structure to toughness cannot be assessed adequately in isolation from a consideration of the mechanics of cleaving meat. A variety of tenderometers are used to determine meat tenderness. They measure the force to shear a standard section of cooked meat across the fibres. The Warner Bratzler machine cleaves with a thin metal plate, whereas the tenderometer of Macfarlane & Marer (1966), used in this study,

bites through the meat with a blunt wedge. Despite such wide design differences, we consider that the mechanical events occurring on cleaving are essentially the same and can be visualized as follows.

The wedge compresses the meat and the fibres are stretched, especially at the leading edge, where they reach their yield point and break. This occurs at a certain deformation related to wedge loading and to the degree to which the fibres are able to stretch before yielding. However, fibres do not react independently to the biting wedge. They are linked by lateral adhesions, such as the epimysium or perimysium, which will transmit an applied load to adjacent fibres and disperse it through the sample away from the biting wedge. Lateral transmission of forces within the fibres is also important. The myofibrils are linked by shared sarcoplasmic reticulum and the transverse tubular system (Bennett, 1960). Together with the inherent strength of the longitudinal myofilaments, they will determine overall fibre tensile strength.

For a given fibre tensile strength it is reasonable to suppose that the more widely the wedge loading is dispersed amongst fibres, the greater the load will have to be to bring fibres to their yield point. In other words, toughness will depend both on fibre tensile strength and on the capacity of meat to disperse the wedge load through the sample. In these terms, how does shortening produce toughness? It is visualized that it will do so through an enhancement of the tensile strength of fibres from increased overlapping of thick and thin filaments in the sarcomere, as has previously been suggested (Marsh *et al.*, 1974); through increasing the degree of stretch necessary to extend the shortened fibres to the yield point; through developing frictional contact between fibres and filaments with the tighter packing of protein in the shortened cooked meat fibre; through increasing the number of possible links between fibres per unit muscle length. In this latter respect inter-fibre adhesion has been shown to increase with shortening (Bouton *et al.*, 1973). This is only to be expected. Assuming a fibre to be a cylinder, it can be calculated that shortening to S, 0·5 would increase adhesion or links per unit surface area by 40%.

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Colour retention in fresh meat stored in oxygen—a commercial scale trial

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Summary

Aged fresh beef was conventionally packaged at a central cutting plant and transported in oxygen filled containers to a retail outlet where it was compared in a refrigerated display cabinet with similar meat held in air. Colour deterioration during transport and storage was successfully delayed by holding in oxygen and the process was most effective in muscles prone to rapid discolouration. Even a small rise in temperature accelerated discolouration so that good refrigeration during display was still essential.

Introduction

Fresh meat is selected by the consumer on the basis of its attractiveness, especially colour of the lean, and its rate of sale is directly related to the degree of discolouration (Hood & Riordan, 1973). Rate of discolouration is the major obstacle to centralized prepacking and distribution because prepacked fresh meat remains bright red for only 1–2 days. By the time centrally packed meat reaches the retail outlet it may have already lost much of its attractiveness so that its display life is considerably less than meat prepared within the store. If redness could be maintained until the package was delivered to the store, that is for a period of about one day, a successful centralized operation competitive with in-store preparation would be possible.

The red colour of beef can be prolonged for several days by contact with high concentrations of oxygen and carbon dioxide (Brody, 1970; Georgala & Davidson, 1970; Schweisfurth und Kalle, 1970). Redness is maintained because the surface layer of oxymyoglobin formed in high oxygen concentrations is thicker than that formed in air; the increased thickness masks the layer of brown metmyoglobin formed at the limiting depth of oxygen penetration into the meat and delays the emergence of metymyoglobin to the surface (Taylor & MacDougall, 1973). Meat can be held for as long as 8 days in a mixture of 85% oxygen and 15% carbon dioxide and still be sufficiently attractive for a further 2 days' display in air (Clark & Lentz, 1973). Carbon dioxide is used to suppress bacterial growth for such prolonged holding periods, but the substitution of 1 day in air with 1 day in oxygen before display in the store should

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not require the incorporation of carbon dioxide. Daun *et al.* (1971) found that meat stored in air or 90% oxygen supported equivalent microbial growth at 4°C.

The concept of packing meat in oxygen has not yet been applied commercially. This paper describes experiments designed to test a relatively simple method of holding prepacked beef in oxygen until required for display.

Experimental

Experiment 1: preliminary trial

Meat packing. One beef rump (*M. gluteus medius*), aged anaerobically in Cryovac for two weeks at +1°C was cut into 2·5 cm thick slices which were overwrapped on white expanded polystyrene trays with highly oxygen-permeable polyvinyl chloride film. The packages were placed in two plastic baskets (65 × 40 × 15 cm), one kept as control and the other treated with oxygen.

Oxygen treatment. The basket was placed in a polyethylene bag (500 gauge) and air displaced by flushing thoroughly with oxygen introduced by a tube before sealing the bag.

Storage and display. After 1 day at +2°C the basket was removed from the polyethylene bag and both sets of packages transferred to a fan assisted display cabinet at +4°C. The packages were displayed for 6 days during which their colour was measured and bacterial counts taken.

Bacteriological sampling. 50 cm^2 areas were swabbed first with wet and then dry cotton wool. The swabs were taken into 10 ml of I.S.O. maintenance medium (0·85% NaCl + 0·1% peptone) from which counts were prepared using the Colworth agar droplet technique (Sharpe & Kilsby, 1971). Incubation was at 25°C for 2 days in Oxoid plate count agar + 1% NaCl.

Experiment 2: commercial scale trial

Meat packing and oxygen treatment. This trial was designed to simulate commercial conditions of meat handling, transport and store display although during the experiment the store was closed. The stages in the process and meat and air temperatures are given in Table 1.

Three fillets (*M. psoas*), three rumps (*M. gluteus medius*) and three sirloins (*M. longissimus dorsi*), of normal pH (5·4–5·6), aged in Cryovac for 2 weeks at −2°C at a packing plant some distance from the store, were sliced and packed as in Experiment 1. Eighteen packages were placed in each basket and half were treated with oxygen; the ratio of oxygen to meat volume was approximately 10 : 1. Transportation to the refrigerated warehouse and to the store's cold room was by refrigerated vehicle.

Display. Thirty hours elapsed between packing and the start of display. The overpacked baskets were then removed from the cold room, the polyethylene bags opened and the

TABLE 1. Meat and air temperatures throughout centralized beef packaging and distribution

Time		Stage	Temperature (°C)	
Day	Hour		Meat	Air
Two weeks prior to packing		Primal cuts of beef held in Cryovac	−2	−3 to −1
1	12.00	Meat sliced; overwrapped and packed in baskets transferred to chill	−2 to +3	Cutting room +10
1	14.00	Half of samples treated with oxygen	+3	Chill room +1
1	18.00 to 20.00	Transported to depot	+3	Refrigerated vehicle −2 to +2
1	20.00 to	Held in depot chill	+3	Chill room +3
2	04.00			
2	0.400 to 06.00	Transported to store	+4	Refrigerated vehicle +4
2	06.00 to 18.00	Held in store chill	+3	Store chill −1 to +1
2	18.00 to	Meat displayed at two positions in cabinet:		
4	16.00	(a) centre of cabinet	+4	+3
		(b) end of cabinet	+7	+6 (Store air temperature 12 to 14)

treated and corresponding untreated samples laid out on two areas (the centre and one end) of a 'gondola' type display cabinet. Additional samples were placed among them to load the cabinet completely. Air and meat temperatures were measured at the two locations in the cabinet and the store lights and heating left on during display.

Gas composition. The gaseous atmosphere in the polyethylene bags was sampled during the oxygen treatment with a hypodermic syringe through a rubber patch cemented on to the bag. The atmosphere in individual overwrapped trays was sampled periodically during display by inserting a syringe needle through the side wall of the foam plastic tray; the hole was then sealed with plastic tape. The composition of the gas atmosphere was estimated using a Fisher Gas Partitioner.

Colour measurement. Each sample was measured over three 19-mm diameter spots on

a Gardner Automatic Colour Difference Meter model XL10 scaled to read in uniform lightness (L), redness (a_L) and yellowness (b_L) from which the hue ($H = \tan^{-1} b_L/a_L$) and saturation ($S = (a_L^2 + b_L^2)^{1/2}$) were calculated. The angle H describes the attribute of colour denoted by purple to red to orange to yellow, etc. and S describes purity or lack of greyness.

Oxygen penetration. The thickness of the oxygenated surface layer was measured by a technique based on that of Brooks (1929). Thin slices of meat, one edge of which was covered with polyvinyl chloride film, were pressed and held between two Perspex sheets and the depth of the red boundary was measured using a magnifying glass with an integral 0·1 mm scale.

Pigment concentration and pH. Total pigment (myoglobin plus haemoglobin) was determined by the method of Wierbicki *et al.* (1955) on samples taken before packing and expressed as myoglobin. pH was measured in a 1 : 9 distilled water macerate.

Results

Experiment 1: preliminary trial

Meat colour. The oxygen concentration in the polyethylene bag exceeded 90% throughout the 1 day holding period, after which time the oxygen treated meat had a brighter red colour than the meat held in air. By the start of display the saturation (S) of the samples held in air had decreased from a maximum of 27·9 to 27·1 units, but in the corresponding samples held in oxygen it had increased to 29·4 units. This difference of 2 units of S represents a distinct difference in colour. After 4 hr display the S of the samples which had been held in air decreased to 24 units whereas in those samples which had been treated with oxygen it remained above 28 units. S then decreased at a similar rate in both sets of samples maintaining the 4 unit difference in S for 3 days. Beef with $S > 25$ was bright red and with $S < 20$ was brown. The improvement in colour produced by oxygen treatment compensated for 1 day's colour deterioration at 4°C.

The change in hue (H) relative to change in S was the same as that reported by Taylor & MacDougall (1973); as S decreased and became grey H increased and became brown.

Bacteriology. There were no differences in total bacterial counts which could be attributed to the oxygen treatment, either during the holding period or during the 2 days' display during which the oxygen treated meat remained attractive (Table 2). After 3 days' display bacterial growth had entered the logarithmic phase but by this time all samples were unacceptably brown.

Experiment 2: commercial scale trial

Temperature. Before display the temperature of the meat did not exceed 4°C, and during display the temperature of the meat was approximately 4°C in the centre of the cabinet and 7°C at the end of the cabinet (Table 1).

TABLE 2. Bacterial counts of surface of prepacked beef, treated with oxygen or held in air for 1 day at +2°C, and then displayed in air at +4°C

	Log_{10} bacterial counts/cm² at 25°C	
	Oxygen treated	Air control
Initial		4·3–5·6
Held for 1 day	5·7	5·1
Displayed for 1 day	4·7	4·9
Displayed for 2 days	4·9	4·6
Displayed for 3 days	8·6	6·6
Displayed for 6 days	8·4	8·0

Gas changes. Immediately after gas flushing the oxygen concentration in the polyethylene bags was 97% and after transportation and storage was still above 90%. Individual packages were sampled after 1 hr display by which time the oxygen concentration inside was between 50 and 70%; after 20 hr it had dropped to approximately 30%. The concentration of carbon dioxide in packages was 2–3% after 1 hr display and after 20 hr was less than 1%. In the packages which had been held in air oxygen concentration remained between 19 and 21%. Carbon dioxide was 1–2% after 1 hr display and was less than 1% after 20 hr.

TABLE 3. Changes in thickness of oxymyoglobin layer in oxygen treated meat and air controls during display at 4°C

Display period (hr)	Thickness of oxymyoglobin layer (mm)					
	Fillet		Rump		Sirloin	
	Oxygen treated	Air control	Oxygen treated	Air control	Oxygen treated	Air control
1	3	2*	9	5	10	5·5
4	3	0	8	4	8	4
12	2	0	4·5	3	4·5	3
20	2*	0	4·5	3	4·5	3
36	1·5	0	3·5	2·5	3·5	2·5*

* Metmyoglobin visibly present at the surface.

Meat colour. After 30 hr contact with oxygen, observation of the thin slices pressed between plates showed the surface layer of oxymyoglobin to be nearly twice as thick as in the corresponding samples held in air (Table 3). The effect of the oxygen treatment was less pronounced in fillet than in rump and sirloin. At the start of display, metmyoglobin was barely visible at the interface between oxymyoglobin and myoglobin in rump and sirloin but in fillet a band of metmyoglobin had already formed in both the oxygen treated and air control samples. During display subsurface metmyoglobin diluted and displaced oxymyoglobin on the surface faster in fillet than in rump and sirloin. The boundary between oxymyoglobin and metmyoglobin quickly became diffuse in rump and fillet but remained distinct in sirloin. The predisplay oxygen treatment delayed the emergence of metmyoglobin by at least 1 day in rump and sirloin and 12 hr in fillet.

Lightness (L) of the samples was related to concentration of muscle pigment; rumps were darker than fillets with sirloins intermediate (Table 4). Mean ($\pm$ se) myoglobin concentrations (mg/g wet weight) were 6·4 (0·7) for rumps, 5·1 (0·6) for sirloins and 4·7 (0·3) for fillets. No important change occurred in L during transportation and display; all the important changes were in S and H and the relationship of decrease in S to increase in H was the same as in Experiment 1. During the holding period, oxygen treated fillets and sirloins increased in redness and rumps remained as at the start of the oxygen treatment. Some of the air controls showed obvious deterioration by the end of the holding period; fillets were considerably less red, and rumps showed a smaller but definite deterioration (Table 4).

The improvements in colour saturation gained by oxygen treatment were maintained

TABLE 4. Changes in colour of beef held in oxygen or air before display (three samples from each of three animals, means $\pm$ se)

Treatment	Muscle	Colour values		
		Lightness (L)	Hue (H)	Saturation (S)
2 hr after packing, before oxygen treatment	Fillet	30·0 (0·7)	20·8 (0·2)	27·3 (0·4)
	Rump	26·3 (0·3)	19·7 (0·2)	28·3 (0·4)
	Sirloin	28·0 (0·5)	20·5 (0·2)	27·1 (0·3)
Start of display, oxygen treated	Fillet	30·6 (0·5)	20·9 (0·2)	28·7 (0·5)
	Rump	26·4 (0·3)	19·7 (0·8)	27·8 (0·4)
	Sirloin	28·6 (0·4)	20·2 (0·1)	28·2 (0·4)
Start of display, air controls	Fillet	30·4 (0·4)	22·5 (0·5)	24·6 (0·7)
	Rump	26·6 (0·5)	20·2 (0·5)	26·7 (0·4)
	Sirloin	28·7 (0·6)	20·6 (0·2)	27·4 (0·3)

for at least a further 36 hr (Fig. 1). Colour improvement and rate of deterioration were different for each muscle and the 3°C difference in temperature between the centre and the end of the display cabinet doubled the rate in each case. The times on display that each muscle remained very attractive ($S > 25$) and the times to become unacceptably brown ($S < 20$) are given in Table 5 where the interactions of muscle type, display temperature and oxygen treatment are clearly seen. Fillet, which showed the greatest improvement, deteriorated fastest whereas sirloin, which showed least improvement deteriorated slowest, and in every case there was a distinct advantage in displaying the meat at the lower of the two temperatures.

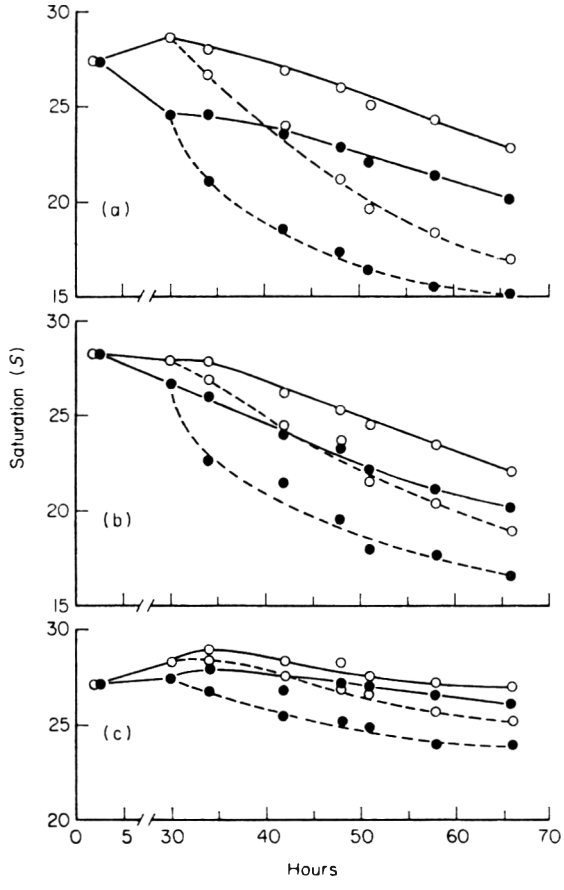

FIG. 1. Change in saturation (S) of (a) fillet, (b) rump and (c) sirloin held in oxygen or air for 30 hr and then displayed for 36 hr at 4°C or 7°C. ——○——, Oxygen treated, 4°C; - - -○- - -, oxygen treated, 7°C; ——●——, air control, 4°C; - - -●- - -, air control, 7°C.

TABLE 5. Time on display that oxygen treated and air control packages of beef remained bright red ($S > 25$) and became brown ($S < 20$)

	Time (hr) to			
	$S > 25$ at		$S < 20$ at	
	4°C	7°C	4°C	7°C
Fillet				
Oxygen treated	25	8	> 36	20
Air control	< 3	< 1	36	7
Rump				
Oxygen treated	18	10	> 36	30
Air control	10	3	36	15
Sirloin				
Oxygen treated	> 36	36	> 36	> 36
Air control	> 36	18	> 36	> 36

Discussion

The preliminary trial clearly demonstrated the benefit of holding conventionally wrapped packages of beef in an oxygen-enriched atmosphere for one day prior to display, and it has since been demonstrated (D. B. MacDougall, unpublished) that as little as 12–16 hr oxygen treatment is sufficient to produce the necessary increase of 3–5 mm in the thickness of the oxymyoglobin layer. The commercial scale trial indicated that there should be no difficulty in applying the technique in practice; oxygen treatment arrested the loss of redness that usually occurs during transportation from centralized packing plant to retail outlet. At the start of display the oxygen treated meat was considerably more attractive than that which had been held in air; thereafter the rate of deterioration was similar in both cases. Oxygen treatment extended the attractive display life by at least 20 hr except in the case of the very unstable fillet at 7°C when the advantage was only 12 hr.

Muscles which exhibit fast rates of pigment oxidation benefit most from packing in high concentrations of oxygen. Of the three muscles used in this investigation, the *M. psoas* and *M. gluteus medius* showed marked improvement while the *M. longissimus dorsi* discoloured so slowly that oxygen treatment was only marginally beneficial. These results are similar to those of Hood (1971) who showed that the rate of metmyoglobin formation was muscle dependent and was *M. psoas* > *M. gluteus medius* > *M. longissimus dorsi*.

The meat used in this investigation had been aged for 2 weeks before cutting and packing. Although the colour of aged meat is initially brighter than unaged meat

(Doty & Pierce, 1961; Tuma *et al.*, 1962, 1963), the red colour has been shown to deteriorate more quickly (MacDougall, 1972). This trial was conducted, therefore, under particularly stringent conditions. Colour retention would have been better with meat packed at an earlier stage post-slaughter.

It is important to realize that oxygen treatment is no substitute for good refrigeration. While oxygen treated meat retained its redness for longer than meat packed in air, the rate of deterioration was governed by the temperature and was doubled by an increase of only 3°C in display temperature. A running temperature of 7°C is typical of many fan assisted convection display cabinets (Malton, 1972), and the results obtained at 4°C in this trial must be regarded as nearly ideal. Combined with good refrigeration practice, oxygen treatment could enable centrally prepacked fresh meat to be displayed as effectively as meat packed at the retail outlet.

Acknowledgments

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The effect of extraction, animal age and post mortem storage on tendon collagen. A differential scanning calorimetric study

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Summary

Differential scanning calorimetry was used to study the collagen → denatured collagen transition in tendon collagen. It was found that purification of collagen by salt (KCl) and trypsin caused a slight, but significant, decrease in the transition temperature, but no significant change in the enthalpy, ΔH_D, of the transition.

Animal age (0–6 years) had no significant effect on either ΔH_D (mean value = 1293 ± 72 cal. amino acid residue^{-1}) or the extrapolated onset temperature, T_0, (334·2 ± 0·3°K) of the transition in untreated tendon. However, the amount of collagen melting after 340°K increased markedly with age (from 0 to about 250 mg/g of tendon) although the amount melting before 340°K was approximately constant, 138 ± 17 mg/g tendon.

Upon storage at 1 ± 1°C for 2 weeks 7 out of 16 tendons became less stable as judged by decreases in T_0 and increases in the amount of collagen melting before 340°K. Two of the tendons became more stable as judged by increases in T_0 and decreases in the amount melting before 340°K. These results are briefly discussed.

Introduction

An important factor affecting the 'toughness' of certain meat cuts is the amount and chemical nature of the connective tissue present (Bailey, 1972; Lawrie, 1974). Collagen is a major component of both connective tissue and tendon and has been extensively studied by various physico-chemical techniques. However, most of these studies by necessity, have been on collagen extracted from the tissue and it is possible that the extracted collagen may differ in nature to that found *in vivo*.

Recently scanning calorimeters have become available with adequate sensitivity and stability to measure the weak heat effects associated with protein denaturation and this technique has been used to study the collagen → denatured collagen transition both in

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solutions of tropocollagen (Privalov & Tiktopulo, 1970; Monaselidze & Bakradze, 1968) and in insoluble collagen fibres (Finch & Ledward, 1972, 1973; Finch *et al.*, 1974). In a tissue rich in collagen it should be possible to measure this transition directly. Thus, analysis of the characteristics of the endothermic transition may be a useful index of any changes undergone by collagen during subsequent extraction.

Even if changes in the nature of collagen do occur during extraction scanning calorimetry offers the possibility of looking at *in vivo* differences between animals as, for some tissues, the collagen concentration is sufficient to enable the collagen → denatured collagen transition to be studied directly.

It is well established that the percentage and nature of collagen in various tissues varies with animal age (Bailey, 1968, 1972; Carmichael & Lawrie, 1967) and in the present study this age relationship for untreated tendons was analysed by differential scanning calorimetry.

It is generally accepted that meat collagen is relatively stable post mortem, although recent work has indicated that a small, but significant breakdown does occur during rigor (Hall & Reed, 1973) and storage (Stanley & Brown, 1973). Since this could be of great importance to the meat industry, the present study also considered the effect of cold storage on the thermal characteristics of untreated sow tendon collagen.

Materials and methods

Treatment of the tendons

All the tendons studied were obtained from animals slaughtered at the School of Agriculture. They were removed from the carcass within 1–2 hr of death. In the case of sows, the samples were the metacarpal portion of the tendon from the flexor digitorum profundus muscle of the thoracic limb. The beef tendons were the metatarsal portions from the flexor digitorum profundus muscle of the pelvic limb. The sow tendons, by time of receipt had been through the scalding process. The tendons were wrapped in polythene, frozen in liquid nitrogen and stored at $-12°C$ until required. In the storage experiments half of each tendon was stored in polythene at $1 \pm 1°C$ for 2 weeks prior to freezing. For DSC analysis 6–15 mg samples of the interior of the thawed samples were accurately weighed on a microbalance.

Extraction of the collagen

For the three tendons used to evaluate the effects of extraction on the thermal properties of collagen the extraction procedure of Mohr & Bendall (1969) was used. DSC analysis in 0·9% NaCl was performed at each purification step after thoroughly washing the material in 0·9% sodium chloride for 4 days. Following purification the solutions used were all dialysed against distilled water, concentrated by freeze-drying to about 25·0 ml and the hydroxyproline—Pro(OH)—content determined as a measure of the percentage collagen solubilized.

Calorimetry

Thermograms of the collagen samples were determined at a scan rate of 5°K/min on a Perkin-Elmer DSC II differential scanning calorimeter. The chart speed was 2 cm/min. Sealed aluminium pans, recommended for volatile materials, were used to encapsulate the samples. The actual calibration and measurement procedure was as described previously (Finch & Ledward, 1972).

Determination of collagen content

After the DSC analysis the hydroxyproline content of each sample was determined (Woessner, 1961). From this the corresponding quantity of collagen was calculated as described by Mohr & Bendall (1969).

Results

Effect of extraction on the melting of tendon collagen

Typical thermograms for untreated and purified beef tendon are shown in Fig. 1. ΔH_D, the enthalpy change associated with the transition, was found from the area of the endothermic peak and T_0, the extrapolated onset temperature, and T_p the peak temperature were determined as shown in Fig. 1. The thermal characteristics of the melting of collagen from the tendon of an 18-month-old heifer are shown in Table 1,

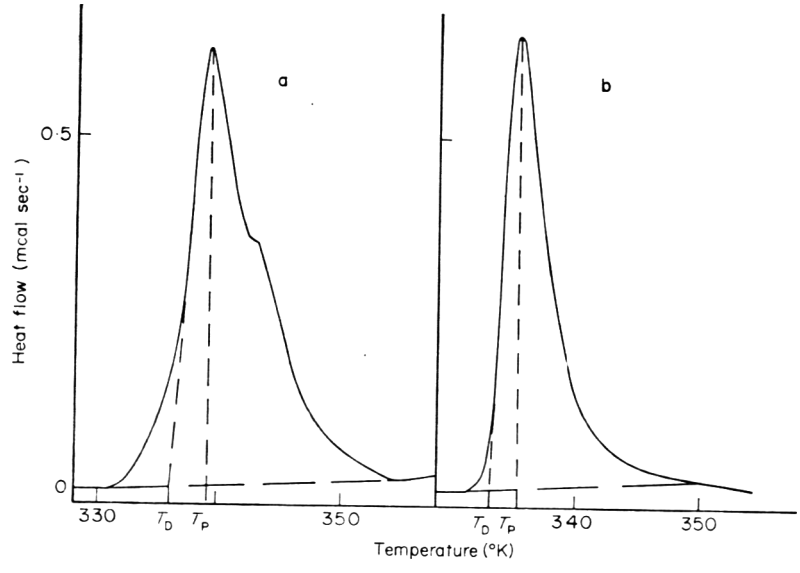

FIG. 1. Thermograms of steer tendon collagen at a scan rate of 5°K/min. A, 7·95 mg of untreated steer tendon (3·27 mg collagen); B, 2·47 mg of purified steer tendon collagen after treatment with M-KCl, trypsin, M-KCl and swelling in 0·9% NaCl.

TABLE 1. The effect of extraction on the thermal characteristics of tendon collagen

Treatment	Animal								
	18-month-old heifer			5-month-old sow			6-month-old sow		
	T_o°K	T_p°K	ΔH_D cal.AA^{-1}	T_o°K	T_p°K	ΔH_D cal.AA^{-1}	T_o°K	T_p°K	ΔH_D cal.AA^{-1}
None	336·4±1·2	340·1±0·7	1504±54	333·8±0·3	339·4±0·4	1436±91	333·4±0·3	338·7±1·4	1459±27
Swollen 0·9% NaCl	335·4±1·0	338·5±0·6	1392±51	332·1±0·2	337·3±0·4	1314±154	332·3±0·3	336·7±1·6	1503±158
1 M KCl—0·9% NaCl	332·6±0·3	336·5±0·7	1314±96	331·6±0·2	336·6±1·0	1338±91	331·4±0·2	336·1±1·0	1434±83
1 M KCl—Trypsin-0·9% NaCl	332·9±0·2	335·7±0·4	1316±52	331·7±0·1	335·1±0·7	1501±79	332·0±0·3	335·1±0·4	1527±182
1 M KCl—Trypsin-1 M KCl—0·9% NaCl	332·5±0·3	335·1±0·3	1326±75	331·0±0·3	334·0±0·4	1500±132	331·1±0·3	334·5±0·6	1444±50

Each value is the mean ± the standard deviation on six determinations.
ΔH_D is expressed as the enthalpy change per amino acid residue, assuming an average molecular weight of 91.

together with the results for the tendons from two female pigs about 5 and 6 months of age.

It is seen that swelling in 0·9% NaCl (pH 6·1) causes significant decreases in both T_o and T_p (Table 1). It is known that both ΔH_D and T_o for collagen fibres, are very salt dependent (Finch & Ledward, 1973), and it is possible that at least some of this change merely represents a change in the aqueous environment. However, in all cases T_o and T_p decrease during the purification ($P < 0{\cdot}001$) (Table 1). This decrease in thermal stability must represent a change in the nature of the collagen or its environment, as the swelling agent is the same in all cases (0·9% NaCl). This cannot be due to a loss of soluble (less mature collagen) during purification as the total amount solubilized is very small ($\sim 1\%$ of the total) and, as this is presumably the less stable material, it would, if anything, increase T_o. However, this loss of less stable collagen may explain the apparent increase in T_o following treatment with trypsin. As T_o is representative of the least stable collagen and T_p is a measure of the 'average' stability, the loss of the less stable collagen would be unlikely to affect T_p (Table 1). Except for the behaviour of T_o following treatment with trypsin it is seen that the collagen becomes progressively less stable, as judged by both T_o and T_p, following each step of the purification (Table 1).

Due to the large standard deviations associated with the enthalpy determinations it is not possible to say if ΔH_D varies with purification.

Effect of animal age on the collagen → denatured collagen transition in sow tendon

As changes do occur during extraction it was decided to study the collagenous material without any purification. This also enabled the concentration of collagen in the tendon to be determined, after each DSC scan, as a percentage of the wet weight of tendon.

From Fig. 2 it is seen that age of the sow does not significantly affect either the extrapolated onset temperature, T_o, or the enthalpy of the collagen → denatured collagen transition in the tendon. Each point on Fig. 2 is the mean of from 6 to 16 determinations plus their standard deviations. The mean values and their standard deviations are $\Delta H_D = 1293 \pm 72$ cal. AA^{-1} and $T_o = 334{\cdot}2 \pm 0{\cdot}3$°K on twenty tendons.

Although T_o was apparently independent of animal age it was seen that in the new-born (1-day old) piglets, a small percentage of the collagen melted before 334°K (Fig. 3), but that the sharp transition occurred at about 334°K and T_o was determined from this peak (Fig. 3).

Examination of the thermograms indicated that a greater proportion of the collagen melted at the lower temperatures, e.g. 333–340°K in the young tendons than in the samples from the older sows. As the total collagen content of each sample was known the amount melting before 340°K was calculated by multiplying the percentage of the area melting before 340°K by the collagen content of that sample. The results are summarized in Fig. 4. It is seen that the amount of collagen melting before 340°K is approximately independent of age whilst that melting after 340°K increases markedly

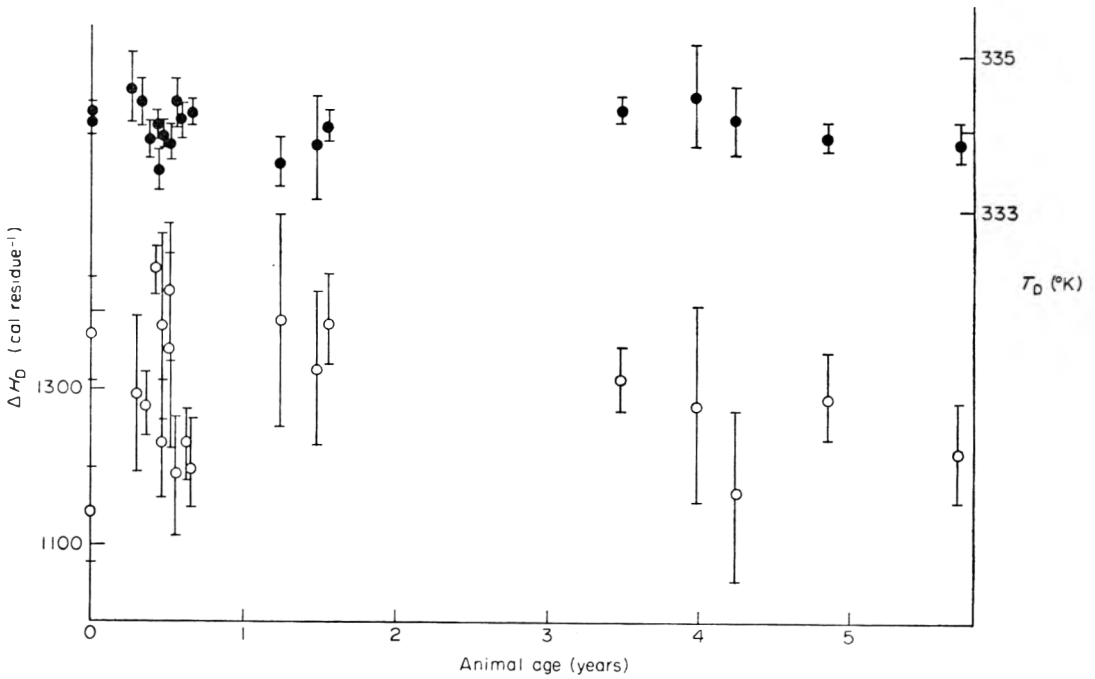

FIG. 2. The effect of animal age on the enthalpy, ΔH_D (○) and extrapolated onset temperature, T_o, (●) of the collagen → denatured collagen transition in sow tendon collagen.

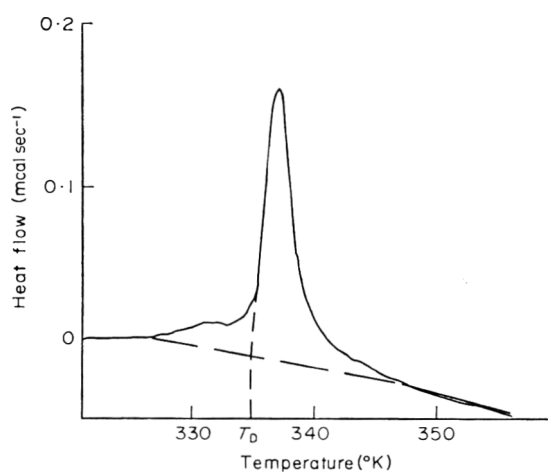

FIG. 3. Thermogram of 7·49 mg of tendon (0·76 mg collagen) from a 1-day-old piglet. Scan rate 5°K/min.

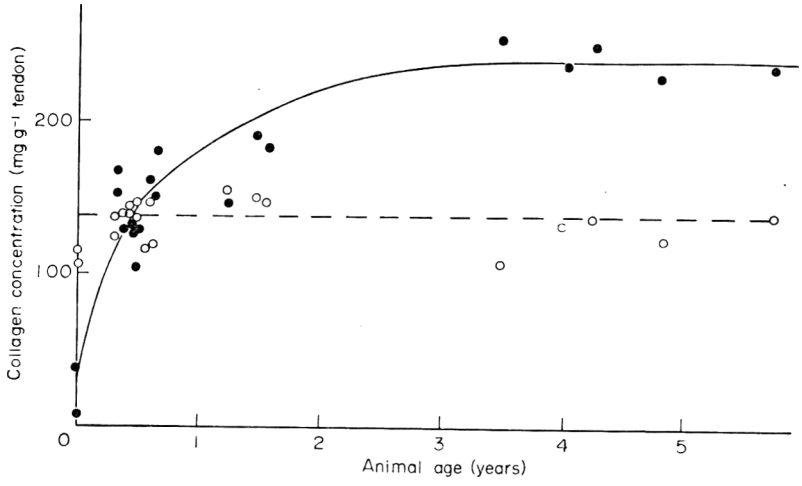

FIG. 4. Effect of animal age on the concentration of collagen melting before 340°K (○ — — ○) and melting after 340°K (●——●) in sow tendon.

with age. After about 3 months of age there is only a slight increase in the temperature at which the transition finishes (1–2°K). However, from 0 to 3 months this temperature increases from about 344°K to about 356°K.

Effect of storage on sow tendons

Most of the tendons used to obtain the data in Fig. 4 were also aged at 1 ± 1°C for 14 days. There was no significant change in the enthalpy of the collagen → denatured collagen transition during this period of storage ($\Delta H_D = 281 \pm 80$ cal. AA^{-1} on sixteen samples), but in some cases T_0 changed (Table 2) and the amount melting before 340°K also altered (Table 2). Age appeared to be a factor in this change in the nature of the collagen as the younger samples tended to exhibit decreases in T_0 and increases in the amount melting before 340°K. However, there were apparent exceptions to this age related generalization (Table 2). In two of the tendons there was a significant increase in T_0 (plus a decrease in the amount melting before 340°K).

Discussion

The only parameter that animal age appeared to affect was the amount 'melting' after 340°K (Fig. 4). Thus, as the collagen melting before 340°K is the less stable collagen, then it is apparent that this is relatively constant (138 ± 17 mg/g of fresh tendon on twenty samples), whilst, with age, there is a marked increase in the concentration of the more stable material (Fig. 4). This would agree with the well established fact that with increasing age collagen crosslinks (Bailey, 1968, 1972; Bailey & Lister, 1968; Carmichael & Lawrie, 1967) although it appears that the formation and stabilization

TABLE 2. Effect of storage for 2 weeks at 1 ± 1°C on the 'melting' of sow tendon (each determination is the mean ± the standard deviation of at least six samples)

Age (months)	0 weeks		2 weeks		Relative stability of the tendon after storage*
	No. milligrams melting before 340°K	T_0°K	No. milligrams melting before 340°K	T_0°K	
0	104 ± 9	334·4 ± 0·9	All melted	329·5 ± 1·2	Decreased
0	109 ± 20	334·1 ± 0·7	by 338°K	329·0 ± 1·3	Decreased
4	136 ± 12	334·6 ± 0·3	115 ± 15	334·5 ± 0·3	N.S. change
4	124 ± 18	334·5 ± 0·1	110 ± 16	334·7 ± 0·2	N.S. change
5½	142 ± 22	334·2 ± 0·6	240 ± 8	330·6 ± 0·3	Decreased
5½	140 ± 11	334·1 ± 0·2	195 ± 30	332·4 ± 0·6	Decreased
6	134 ± 21	333·9 ± 0·4	224 ± 19	330·7 ± 1·0	Decreased
8	146 ± 18	334·3 ± 0·2	123 ± 10	334·4 ± 0·3	N.S. change
8	117 ± 10	334·3 ± 0·2	110 ± 18	334·4 ± 0·1	N.S. change
15	153 ± 19	333·6 ± 0·3	97 ± 24	334·5 ± 0·2	Increased
18	148 ± 11	333·7 ± 0·5	129 ± 30	333·9 ± 0·3	N.S. change
42	104 ± 12	334·3 ± 0·1	110 ± 18	334·4 ± 0·3	N.S. change
48	130 ± 11	334·5 ± 1·0	198 ± 42	331·7 ± 1·0	Decreased
51	144 ± 20	334·2 ± 0·5	191 ± 34	333·3 ± 0·9	Decreased
58	121 ± 12	334·1 ± 0·1	77 ± 10	334·5 ± 0·3	Increased
70	139 ± 17	333·8 ± 0·1	146 ± 13	333·9 ± 0·1	N.S. change

* If the increase in the amount of collagen melting before 340°K and the decrease in T_0 were significant ($P < 0·001$) the stability of the tendon was taken to have decreased during storage. If the decrease in the amount of collagen melting before 340°K and the increase in T_0 were significant ($P < 0·001$) then the tendon was taken to be more stable.

N.S. change = no significant change ($P > 0·05$).

of these crosslinks in sow tendons, at least after about 3 months of age, occurs at about the same rate as uncrosslinked collagen is generated, resulting in a constant concentration of less mature collagen. It must be noted though that the temperature of 340°K was selected as below this temperature most of the collagen in new-born tendon melts although, of course, it is not a critical temperature below which immature collagen melts and above which mature collagen melts. These classes of collagen will both melt over overlapping temperature ranges, the ranges being dependent on the definition of mature and immature collagen and on the shape of the sample, i.e. how rapidly it equilibrates with temperature.

From the results on the stored tendons it is seen that in seven of the samples there is a significant decrease in T_0 and increase in the amount melting before 340°K during storage. In fresh tendon all the samples, independent of animal age, start to melt at the same T_0 and so the changes during storage cannot be 'reverse' of animal ageing, i.e. the breaking of crosslinks, as this would lead to an increase in the amount melting

before 340°K, but no change in T_0. What the nature of this breakdown is, and why it is sample dependent, is difficult to say, but possibilities are (a) the mucopolysaccharides in the ground substance may break down (McIntosh, 1967) and may thus lower the stability of the collagen (Gelman & Blackwell, 1974) and (b) specific enzymes (collagenases) may be present in some samples that may degrade the collagen by, for example, peptide bond hydrolysis.

However, in two of the tendons there was a significant ($P < 0{\cdot}001$) increase in onset temperature, and concomitant decrease in the amount of less stable collagen. Bailey & Lister (1968) described how the labile crosslinks in collagen fibres may be reduced, both *in vivo* and *in vitro*, leading to increased stability and this reduction may occur in the stored tendons. The possibility of both breakdown and/or further stabilization of collagen may, in part, explain the great divergence of opinion regarding the reactivity of collagen during storage. If the breakdown of collagen occurs in some intramuscular connective tissues as well as some tendons, then it obviously would be of great importance to the meat industry as an understanding, and control, of the mechanism would aid the desire for uniformity of texture between carcasses. This study is at present under way in our laboratory.

Whatever the nature of the changes in collagen it does appear that scanning calorimetry may be a very useful technique to monitor *in vivo* changes in stability of this protein.

Acknowledgments

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Technical note: Food texture—modification of the shear press using a strain gauge system

D. KEPPEL AND T. R. GORMLEY

Introduction

The shear press is an instrument for measuring food texture (Kramer, Burkhardt & Rogers, 1951). The original model was introduced in 1950 and it was later modified for electrical indicating and recording (Decker *et al.*, 1957). A new more versatile and precise model was introduced in 1956 (Kramer & Twigg, 1958). In this instrument force is measured by a maximum-reading dial gauge fitted across the vertical diameter of a proving ring. If time-force texture graphs are required, a transducer can be fitted across the ring in place of the dial gauge to give a print-out on a strip chart as the sample is tested.

This note reports an alternative and relatively cheap system for converting a dial reading shear press to a recording model. Strain gauges are attached to the proving ring and the electrical output is amplified and is recorded on a strip chart in the form of a texturegram. The dial gauge remains in place to indicate maximum deflection. The system is simple and easy to construct.

Apparatus

Strain gauges, R1–R4, (Constantan foil type on a flexible polyimide backing) were attached to the proving ring (Fig. 1) by a high performance epoxy adhesive and protected by a thin coat of polyurethane and polysulfide epoxy. The four gauges were connected in a full Wheatstone Bridge configuration (Perry & Lissner, 1962). The output from the bridge E_i was applied to the differential input of an operational amplifier through R_i and R_i (100 kΩ). The amplifier serves as a buffer stage between the strain gauges and the recorder to prevent over-loading of the signal source. It also provides the small voltage gain necessary to drive a recorder (in this experiment a Philips PM 8100 flatbed recorder was used).

The amplifier voltage gain is defined by the ratio R_0/R_i (1 MΩ/100 kΩ) (Greame, 1973). The amplifier used (Burr Brown 3522J) has a moderately low offset and in this particular application a means of externally nulling this offset is provided with an external potentiometer (10 kΩ).

Authors' address: The Agricultural Institute, Kinsealy Research Centre, Dublin 5.

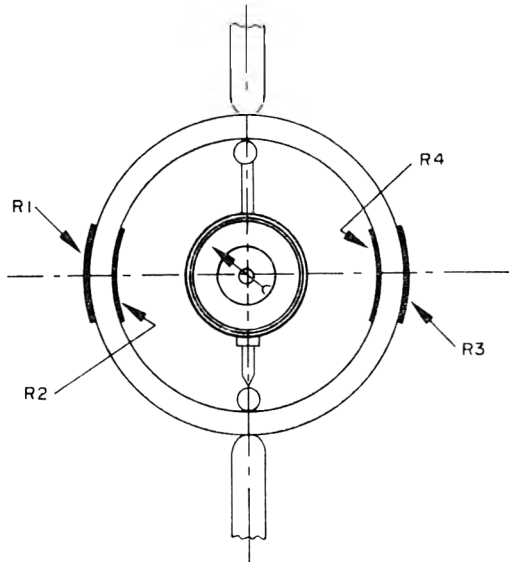

FIG. 1. Arrangement of strain gauges on the proving ring.

Strain gauges were attached to two proving rings, i.e. 2280 kg and 68 kg and a balancing network was provided to facilitate correction of small mismatches between rings. This balancing network can be adjusted to apply a small balancing voltage to point A on the bridge. The value of resistors in this network will depend on the required offset. A range control was also included which provided a means of varying the output voltage E_0 between 80 and 100% of maximum. It consists of a 1 kΩ potentiometer and a 3·9 kΩ resistor. The 2280 kg ring is used for measuring firm foods such as raw diced carrots and the 68 kg ring for soft foods such as strawberries.

The complete system is mains operated and a stabilized power supply was built which provides +5 V (E_S) to the bridge and ± 15 V to power the amplifier. A schematic diagram of strain gauges, amplifier and balancing network is shown in Fig. 2.

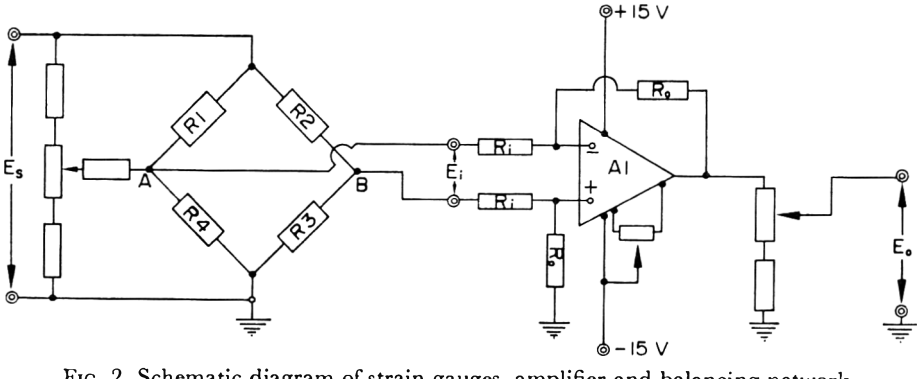

FIG. 2. Schematic diagram of strain gauges, amplifier and balancing network.

Calibration of the system can be checked with any sample tested as the maximum dial gauge reading can be related directly to the peak height on the recorder.

Operation and performance

When carrying out a shearing test a weighed sample of food is placed in the standard shear-test cell and the dial gauge is set at zero. The shearing blade assembly and the strip chart are started simultaneously by the operator. When the shearing system reaches the end of its travel the chart drive is stopped. The maximum dial reading (kg) is then related to the peak height (mm) on the chart. Different chart speeds can be used depending on the detail required from the texturegram. The chart speed can be related to the speed of descent of the shearing blades and the area under the curve is adjusted accordingly to give the work (force-time) required to shear the sample. Adjustments may also have to be made to the area depending on which recorder 'span' setting is selected. The area is measured conveniently by weighing the etched-out curve (Barker & Webbing, 1974). Alternatively, a simple electronic integrator with digital print-out could be attached to the system.

A range of fruit and vegetables was sheared to test the system and texturegrams for an under-ripe tomato, a ripe tomato and a sample of diced carrots are shown in Fig. 3

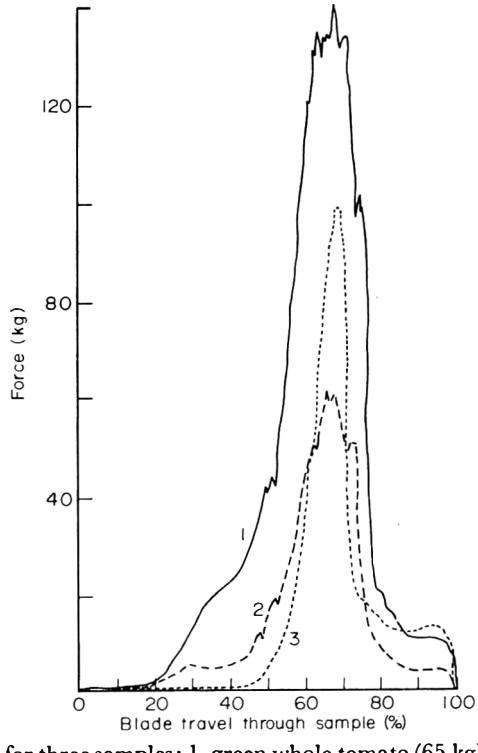

FIG. 3. Texturegrams for three samples: 1, green whole tomato (65 kg); 2, mature whole tomato (60 g); 3, diced carrots (40 g, force reading kg/4).

TABLE 1. Linearity check for dial reading versus recorder peak height (2280 kg ring)

Sample*	Dial reading (kg)	Peak height (mm)	Recorder span (mV)	Ratio kg/mm/10 mV span
1	720	103	200	0·35
2	680	97	200	0·35
3	580	167	100	0·35
4	480	135	100	0·36
5	400	112	100	0·36
6	250	149	50	0·34
7	124	179	20	0·35

* Sample weights from 10–100 g of raw diced carrots were tested.

TABLE 2. Linearity check for dial reading versus recorder peak height (68 kg ring)

Sample*	Dial reading (kg)	Peak height (mm)	Recorder span (mV)	Ratio kg/mm/100 mV span
1	65	167	100	0·39
2	58	151	100	0·39
3	51	132	100	0·39
4	43	110	100	0·39
5	32	82	100	0·39
6	27	68	100	0·40
7	21	52	100	0·40
8	16	81	50	0·40
9	11	55	50	0·40

* Sample weights of 3–15 g cooked cauliflower were tested.

(1, 2 and 3 respectively). The linearity of the ratio between dial reading and peak height was checked over a number of sample weights for both proving rings. The ratios of kg/mm (adjusted for recorder span setting) thus obtained were almost constant (Tables 1 and 2) indicating a degree of linearity well within acceptable limits for practical texture testing.

Conclusions

The strain gauge system reported is simple to construct and is of low cost. The main expense is that of purchasing a flat bed recorder. However, most laboratories already have recorders for other purposes and this system can be connected to one of these when necessary.

Acknowledgment

We thank Dr B. Cunney, Oakpark Research Centre, The Agricultural Institute for his assistance and useful suggestions.

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(*Received* 23 *August* 1974)

J. Fd Technol. (1975) **10,** 365–367.

Technical note: Organic vapour permeation through packaging films

C. NEWTON BLAKESLEY

The deterioration of the flavour and quality of food during storage may partly be related to packaging. This can be due to loss of volatile components from the food to the atmosphere, contamination by foreign chemicals which may be in the storage atmosphere, or contamination by substances in the packaging material itself. It is obviously important that methods should exist for measuring the rates of permeation of various substances through packing materials.

Recently a kinetic method for measuring the permeation of organic vapours through flexible packaging films was presented by Blakesley (1974). This method yields quantitative, reproducible data in the form of first order rate constants for the permeation of individual organic chemicals through a film. Data were presented for the permeation of several organic vapours through a highly permeable polyetheylene film. Further experiments, however, have indicated that the degree of reproducibility of the data decreases as the film becomes a better barrier for the vapours. There are two causes for this, and with a minor modification of the method the data may be significantly improved.

The method is described in detail in the previous paper. In general, the apparatus consists of a glass cell divided into two chambers by the test film. An equal amount of internal standard is injected into each chamber and the cell is placed in an isothermal environment. When equilibrium has been reached, the vapour or vapours to be tested are injected into one chamber. The loss of material from the initial chamber, A, and the corresponding gain of material in the other chamber, B, are followed by gas chromatography. First order rate constants are then determined and potentially interesting parameters such as temperature dependence, energy of permeation, permeability constants, half-lives of retention, etc. may be calculated.

The first difficulty encountered using high barrier films was in balancing the internal standard between chambers A and B. The internal standard is normally injected as approximately 0·5 μl of liquid. It is extremely difficult to make consistent injections of such small amounts, and there can easily be a difference of 5% or more in the amount of internal standard injected into chambers A and B. With a highly permeable film this is no problem since equilibrium will be reached in a relatively short time. This is not the case with a high barrier film.

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To overcome this problem, a right-angle teflon stopcock was attached to each chamber of the cell and the open ends of the stopcocks joined with teflon tubing. The internal standard is injected into each chamber and the cell is equilibrated at 50–75° for 1 day with the stopcocks open. The stopcocks are then closed. The cell is brought to the desired temperature and the compounds to be tested are injected into chamber A. The permeation rate is then measured in the normal manner. A diagram of the modified permeation cell appears in Fig. 1.

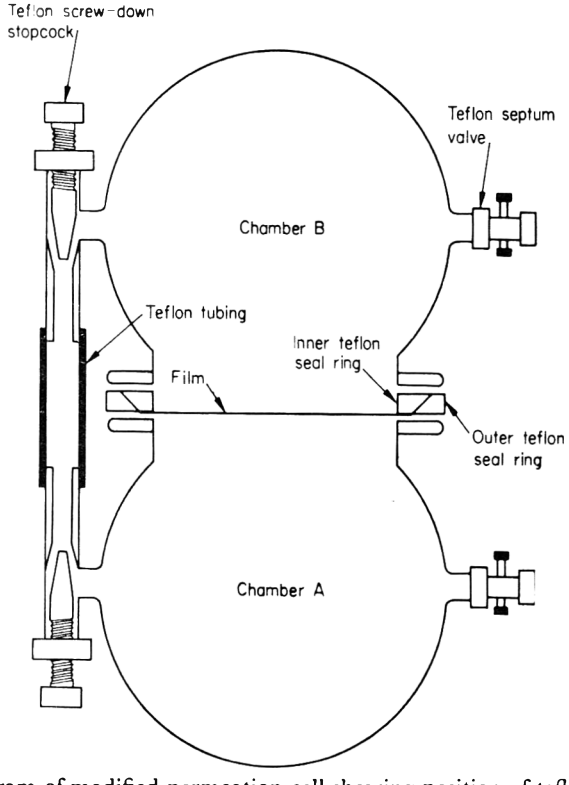

FIG. 1. Diagram of modified permeation cell showing position of teflon stopcocks.

The second difficulty encountered was the day to day variation of response factors for the various organic chemicals to the FID detector of the gas chromatograph. For low barrier films this is not a problem since sufficient data for determining permeability can be gathered in a single day. However, for high barrier films, measurements must be extended over several days. Thus a 1-l. flask was fitted with a teflon septum valve similar to those found on the permeation cell. A mixture of each of the chemicals being tested and the internal standard is injected into the flask. The flask and cell are then placed in the same oven. A daily check on the FID response for the various chemicals in the flask with respect to the internal standard is made and the kinetic data are adjusted accordingly.

If the above modifications to the procedure are followed, the method is useful for evaluating the ability of both low and high barrier permeable films to retain or exclude organic vapours from flexible film packages.

Acknowledgment

The author is grateful to Mr J. Loots for running several of the permeation experiments.

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(*Received* 12 *December* 1974)

J. Fd Technol. (1975) **10**, 369-373.

Book reviews

Dehydration of Natural and Simulated Dairy Products: Food Technology Review No. 15. By M. T. GILLIES.
New Jersey: Noyes Data Corporation, 1974. Pp. xi + 328. U.S. $36.

The Noyes Data Corporation is rendering a significant service to industry by publishing this series of Food Technology Reviews surveying the U.S. patent literature in some selected fields. A search through the lists of patents and the study of patent specifications can be a tedious task and the author must be congratulated on his ability to present technical details in a more readable form.

The subject of this book is 'Dehydration of natural and simulated dairy products' and it includes a chapter on the dehydration of eggs. The first chapter is a review of patents dealing with dehydration processes applicable to a wide range of dairy products. This is followed by chapters dealing with the dehydration of fats containing milk, skim milk, whey and concentrated milk. There is also a very useful chapter on miscellaneous dairy products which include dry dessert mixes, milk drinks, butter milk, sour cream, yogurt and dried cheese. The last chapter surveys the patent literature on simulated dairy products such as coffee whiteners, dessert mixes, artificial sour cream whips, whipped toppings and cheese substitutes.

This review of the U.S. patent literature could have been of greater value to the reader if some critical assessment had been included of the claims made for the inventions. The information contained in this volume is limited to U.S. patents and one has to assume that most patents filed in other countries have also been registered in the U.S.A. Much valuable information is, however, published not in the form of patents, but in other scientific literature. As an example one might quote an article by Maubois & Mocquot (1971) in which they describe a cheese manufacturing method where skim milk is concentrated six to seven times by ultra-filtration before the curd is produced (*Le Lait*, **51** (508), 495–533).

The technologist anxious to keep himself fully informed in this field will therefore have to supplement the valuable information contained in this review by a search of other publications.

S. M. HERSCHDOERFER

Food Additives to Extend Shelf Life. Food Technology Review No 17. By N. D. Pintauro.
New Jersey: Noyes Data Corporation, 1974. Pp. x + 402. U.S. $36.

This book is based on 152 U.S. patents, from 1960 to early 1974, on additives which have an effect on the shelf life of foods or food ingredients. The value of such an ap-

proach is justified in the foreword by the claim that the U.S. patent literature is the largest and most comprehensive collection of technical information in the world and covers much basic commercially useful information not available in the journal literature.

While there is some merit in this claim it must be equally clear that over a limited span of time there have not been inventions in every area although the short introductions to each section go a fair way to fill in the gaps.

The principal part of the book is composed of fairly extensive reproductions of the matter of the individual patents and it is no easy matter to find one's way through it. There is no general index but only an additives index (in addition to indexes of inventors, patent numbers and firms) which does not include such functional references as 'antioxidant', 'preservative', etc. or references to commodities, although the latter omission is less serious because the chapters are each devoted to a particular commodity group. In effect the four-page contents list has to serve as a substitute for a general index.

Because of the structure of the book it is only really useful to experts in the field who have sufficient background knowledge to put the information into context. The first entry, for example, could easily lead a non-expert to believe that the function of antioxidants is to reduce the free fatty acid content of fats and an 'anti-additive' fanatic, attracted by the title, might well be horrified at the vast variety of chemicals added to foods. In fact, a large proportion of the additives are not permitted in foods, and in most cases probably never will be. Some of the substances suggested for use in the text are even specifically prohibited by the U.S. 'prohibited list'. In Appendix III the substances prohibited from direct addition in the U.S. are listed. Five of these could have a function in prolonging shelf life and each of them appears, without comment, in the text at some point as a suggested ingredient in food.

Naturally, the reviewer has not read the whole text but it is perhaps indicative that in examining the numerical list of patents in order to find the range of dates, the first number (or its date) was found to be wrong, and the second entry gave the wrong page number. During a quick look at a patent on the use of aluminium hydroxide gel in tortillas it was found that the additive had become *ammonium* hydroxide gel at one point. A further patent on the use of polycarboxylic acids in tortillas says that the additive may be hydrochloric acid, sulphuric acid or monocalcium phosphate!

In spite of the shortcomings of this book, an expert, capable of a sufficiently critical approach, could find it very useful in suggesting new leads or if he should find a patent that fulfilled his own requirements.

N. R. Jones

Progress in Industrial Microbiology, Vol. 13. By D. J. D. Hockenhull (Ed.). Edinburgh: Churchill Livingstone, 1974. Pp. vii + 285. £9.50.

The latest volume in this series covers a wide range of interests but, as with the earlier volumes, not all are of direct interest to the food scientist or technologist.

The first article (by Jean L. Shennan and J. D. Levi) is concerned with 'Growth of yeasts on hydrocarbons'. After a brief historical introduction the authors consider the ecological and taxonomic aspects of the subject and tabulate the organisms reported in the literature to be capable of growth on hydrocarbons. The nature of the different possible hydrocarbon sources, together with the fundamental aspects of the growth of micro-organisms on them, are followed by a detailed section on developmental aspects. This latter section contains much valuable source material on patents together with consideration of the practical aspects of commercial hydrocarbon fermentations, including suggested standards for microbial contamination of hydrocarbon-grown yeasts intended for animal feeds. Finally, the authors consider briefly the chemical composition of hydrocarbon-grown yeasts and the nutritional and toxicological aspects of their use as animal feed. This is a well conceived article which contains much valuable information for anyone concerned with 'single cell protein', especially hydrocarbon yeasts. The only real omission from the article is an economic appraisal of the priorities in the use of fossil fuels, but in the current economic climate this is perhaps not surprising.

A second article (by E. O. Bennett) on hydrocarbon microbiology considers 'The deterioration of metal cutting fluids'. Whilst this is not a topic for the food microbiologist, or the food technologist, it contains much to interest the specialist in biodeterioration and could be of use to the mechanical engineer who encounters problems in lubricant quality in the machine shop.

'Pectinases and pectic polysaccharides' by W. M. Fogarty and O. P. Ward is essential reading for those concerned with pectins in foods. The authors consider firstly the nature, composition, occurrence and properties of the pectins and provide source data on the occurrence of pectins in plant tissues. They then consider the commercial manufacture of pectins and the methods for determination and characterization of pectic polysaccharides. Following a brief classification of pectinases the authors discuss at some length the various methods available for the qualitative and quantitative assay of the enzymes, together with the occurrence and measurement of pectinases in plant materials. The article then turns to microbial pectinases and considers occurrence, production and purification of the enzymes. A section on properties contains much useful information on pH optima and ranges for pectinases from various sources, together with some data on thermal stability of the enzymes. Finally, the authors consider the commercial manufacture and application of pectinases in the food processing and other (e.g. textile) industries.

The *pièce de resistance* of this book is an article on 'Vaccine production as a unit process' by Paul van Hemert. Whilst this is of little direct interest to food technologists it makes fascinating reading for the applied microbiologist and the fermentation technologist.

As in previous volumes the standard of presentation is excellent: clear printing, few typographic errors and good indexing. Since this is the last volume which will be

edited by Professor Hockenhull, it is appropriate to pay tribute to the admirable manner in which he has edited the series and to thank him for the varied diet which he has produced for the industrial microbiologist during the past thirteen years.

B. JARVIS

Report of the Government Chemist, 1973. Department of Industry. London: H.M.S.O., 1974. Pp. iv + 175. £1.80.

The Laboratory of the Government Chemist provides a chemically based analytical and advisory service to central government departments. Most of its activities are related either to public health and the environment or to the public purse and protection of the revenue. Several chapters in the Report for 1973 are of interest to the food technologist. Not surprisingly, Chapter 2, 'Food and nutrition', covering some seventeen pages shows that much of the work is influenced by E.E.C. membership. During the year there were collaborative studies of methods of analysis on dyes, vegetable fats admixed with cocoa butter, nitrite and nitrate and thiabendazole. Data are presented on the levels of nitrosamines found in various foods and improvements are reported in methods employed for estimating these compounds down to the 1 μg/kg level.

One aspect that will interest almost all workers in the food field is the part being played by the Government Laboratory in the revision of McCance & Widdowson's *The Composition of Foods*. In connection with this compositional survey much needed improvements in our methods for determining starch in meat products have been developed and finally adapted for use with the Autoanalyser. In relation to the Government Laboratory's work for the Armed Services the development of breadmix formulations has continued. One form includes a mixture of flour, salt, diastatic agents and yeasts food, which in conjunction with yeast and sugar, permit bread of good quality to be prepared. Also several combinations of laminates have been examined with a view to improving the wrapping of small packets of service biscuits.

In other chapters reference is made to the development of improved analytical methods, some of which are applicable to foodstuffs. A simple method for calculating alcoholic strength from a combination of the refractive index and specific gravity could well be useful in coping with the larger numbers of samples of wines needing examination due to E.E.C. requirements. More general advances have been made in the use of high pressure liquid chromatography and during the year a project on automated plate readers for gel electrosphoresis and antibiotic assays has been completed. A method for assessing the efficiency of sterilizing agents used in dairying is outlined in the Report.

The title of this booklet may give an impression that it is 'dry' to read, but in practice most will find something of interest. The sections reflect modern thinking in relation to techniques and legal matters that affect the work of most food technologists.

D. PEARSON

Triticale. Proceedings of an international symposium, El Batan, Mexico, 1–3 October 1973. By R. MacIntyre and M. Campbell (Eds)
Ottawa: International Development Research Centre, 1974. Pp. 250. No price.

Just as the term 'wheat' covers a wide range of diploid, tetraploid and hexaploid types, so can the term 'triticale' encompass all the progeny of as many of these wheats as have been coerced into reluctant union with a diversity of rye types. Bearing this in mind, it can be appreciated that generalizations concerning cultivation, food uses and nutritional aspects are made only with difficulty. New varieties rapidly replace old and evaluation inevitably remains at least one step behind breeding successes. It is with some wisdom, then, that the organizers of the El Batan symposium of October 1973 devoted most of the available time to consideration of genetic aspects of triticale. So strong is the emphasis in this direction that the unqualified *Triticale* under which title the proceedings appear is a barely adequate indication of the content. True, there are two papers on diseases of triticale and a further two on nutrition. Agronomic and physiological aspects are also given space in specialized papers, and all these interests receive some attention in the twelve papers which together provide a survey of progress in the triticale programme throughout the world. Apart from the rousing conference finale in which optimistic crystal gazing is indulged, the papers contain a great deal of factual information which allows the reader to assess for himself the progress of triticale breeding and, to some extent, its usefulness as a contribution to world food requirements.

A. D. Evers

Books received

Practical Electrophoresis. By G. J. Moorley and J. D. R. Thomas.
Watford: Merrow Publishing Co. Ltd, 1975. Pp. vii + 104. £2.50.
An introduction to the techniques of electrophoretic separation of molecules on inert support media.

Interaction of Agriculture with Food Science. By R. MacIntyre (Ed.).
Ottawa: International Development Research Centre, 1974. Pp. 166.
Proceedings of an interdisciplinary symposium held in Singapore in 1974 in which Asian workers discussed specific Asian problems of food and agriculture.

Laboratory Tests for the Assessment of Nutritional Status. By H. E. Sauberlich, J. H. Skala and R. P. Dowdy.
Cleveland: C.R.C. Press, 1974. Pp. 136. £7.00.
A critical review of the biochemical methods available for the assessment of nutritional status. Contains extensive lists of literature references.

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Dunedin, New Zealand

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Society for Applied Bacteriology
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SOME METHODS FOR MICROBIOLOGICAL ASSAY

edited by R. G. Board
School of Biological Sciences
University of Bath, England
and D. W. Lovelock
H. J. Heinz Ltd., Hayes
Middlesex, England

December 1974, xiv + 286 pp.
£7.80/$20.75
0.12.108240.7

Assay is a word the microbiologist has taken from the metallurgist to describe the response of micro-organisms to a specific substance in a growth *milieu*. Perhaps through emphasis on antibiotics and growth factors, many microbiologists tend to consider assay in this somewhat narrow context whereas in practice there are many who use the assay technique to study a wide range of materials. Moreover, introductory courses of microbiology tend to impart the view that assay is a labour-intensive method based on wells cut from a nutrient agar in Petri dishes.

This book shows clearly the progress made in automation which diminishes the need for large numbers of skilled technicians. It emphasizes also the wide range of materials which need to be assayed under conditions which attempt to simulate practical and/or industrial conditions. It would be presumptious to expect one book to provide all things to all microbiologists but the editors are of the opinion that the present volume, through complementing well known treatises on special facets of assay, will provide the worker at the bench with methods and ideas upon which to base an assay method for a particular situation. It should be of especial value to microbiologists with interests in medicine, veterinary science, soil microbiology, and paint and paper technology.

Contents

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Journal of the Science of Food and Agriculture

Volume 26, Number 4, April 1975

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Typescripts (two complete copies) should be sent to the Editor, Dr H. Liebmann, c/o Research and Development Department, Metal Box Co. Ltd, Twyford Abbey Road, London NW10 7XQ. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be double-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References.

References. Only papers closely related to the author's work should be included; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s); (b) year of publication in parentheses; (c) title of journal, underlined, abbreviated according to the *World List of Scientific Publications*, 4th edn and supplements; (d) volume number; number of first page of article. References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) publisher; (f) place.

Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Verbs which contain the suffix ize (ise) and their derivatives should be spelt with the z. Statistics and measurements should always be given in figures, i.e. 10 min, 20 hr, 5 ml, except where the number begins the sentence. When the number does *not* refer to a unit of measurement, it is spelt out except where the number is greater than one hundred.

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'.

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in capitals with an Arabic number, e.g. TABLE 2. Each table must have a caption in small letters. Vertical lines should not be used.

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Contents

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