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Influence of selected factors on functional properties of textured milk proteins

G. OZIMEK, S. POZNAŃSKI AND R. CICHON

Summary

Studies were made on the influence of two variable factors—fat content and added salt—on the following functional properties of textured milk proteins: water absorption capacity, thermal shrinkage, consistency, viscosity, fat absorption, emulsifying capacity and emulsion stability.

Variation in fat content of the product (11% and 15%) was obtained by introducing various amounts of fat to skim milk in the course of the production of textured milk proteins. The salt concentration of the preparation was modified by adding sodium chloride, Hamine polyphosphate mixture or both those salts jointly.

The results indicate that the functional properties of the preparation were influenced by the two above mentioned factors in a statistically significant manner. The higher fat content of the product (15%) was accompanied by better fat emulsifying capacity and emulsion stability and lower thermal shrinkage. The salts used for these studies were found to influence favourably the functional characteristics of the preparation. The addition of sodium chloride appears to be most expedient, as it improves important technological properties such as fat absorption and emulsifying capacities.

Introduction

As reported by Hermansson (1973), functional properties of protein preparations might be divided into three groups which are formed by molecular interaction of protein and water, protein, water and fat, and protein, water and air.

The molecular interaction determines such properties as protein solubility, water absorption capacity, water binding ability, viscosity, gelation and swelling ability, emulsifying and fat absorption capacity, emulsion stability, foaming

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powers etc. In the case of textured preparations, special importance is attributed to the texture and to the consistency as related to the texture, both those characteristics appearing to determine the utilization of these preparations (Wolf, 1970).

The functional properties of protein preparations depend not only on factors connected with the preparations themselves, such as kind of protein, type of a preparation and the way by which it has been produced, or the presence of non-protein components, but they are also influenced by environmental factors such as salt concentration, pH-value and temperature (Hermansson & Akesson, 1975a, b; Ozimek, 1978).

The object of this study was to determine the influence of variable factors such as fat content and amounts of sodium chloride and polyphosphates added on essential properties of textured milk proteins. Textured milk proteins are used as substitutes for meat in various meat and ready-to-cook products, or are used in products which resemble meat dishes, but with little meat being added.

The textured milk proteins studied in this investigation were produced by the method developed by Poznański *et al.* (1980a, b). Skimmed raw milk is pasteurized at a temperature 75°C for 15 sec. Ninety-five per cent of the processed volume is cooled to 30°C and transferred immediately to the coagulation tank. The remaining 5%, after being cooled to 70°C is mixed with molten beef tallow or pork lard or soya-bean or another vegetable oil in quantities ranging from 0.9 to 1.8% of the whole processed milk, in the presence of 0.1% permitted emulsifier. The mixture of milk with fat is emulsified by agitation and by homogenization and is combined with the remaining milk in the coagulation tank. To the milk with emulsified dispersion of fat, lactic acid is added for a partial decalcification of the particles of calcium phosphocaseinate, to the level of calcium bound with protein of 0.6–0.75 mmol/g of protein. Thereafter, in order to provide a 'meat colour', food colouring is introduced. A solution of rennet is added and after 15–20 min the coagulate of milk proteins is obtained, stirred and separated from the serum. The coagulate should contain 70% water which is obtained by decantation of the serum and by mechanical granulation. The granulated coagulate is subjected to thermal plasticization at 80°C in a heat exchanger with a rotating cylinder. The preliminary plasticized coagulate is textured further by mechanical squeezing and stretching at a temperature of 40–50°C in a special device designed by Poznański *et al.* (1980a, b). The band of textured proteins is cut into pieces approximately $2 \times 2 \times 5$ cm and immersed in water at 12–15°C for 15-20 min and then in a water bath at 2°C for 30 min. Further processing operations such as packing, freezing, drying or sterilizing may then be carried out.

The textured milk proteins obtained in this way are resistant to boiling, frying and sterilization temperature. The method makes possible, with one mechanized production line, a product with good functional properties, high microbiological quality and high biological value. The mechanized production line makes it possible to obtain, with a low consumption level of energy and labour, directly from skimmed milk a product having a fibrous structure.

Materials and methods

The material for the studies was textured milk protein produced by the method described above. It is possible to vary the fat content of the textured protein between 7 and 15% (Poznański *et al.*, 1977). In our experiment it ranged between 11 and 15%. The salt concentration of the preparation was modified by adding 2% (w/w) sodium chloride, 0.5% (w/w) Hamine polyphosphate mixture, or 2% sodium chloride and 0.5% Hamine. Hamine is a commercial polyphosphate mixture made in Holland. This mixture is used by the Polish meat industry for the production of ham. The chemical composition of Hamine is P_2O_5–59%, Na_2O–33%, H_2O–0.6% (Goettlich *et al.*, 1964).

Hamine contains orthophosphates (10.4%, expressed as P_2O_5), pyrophosphates (21.4%, expressed as P_2O_5) and triphosphates (32.9%, expressed as P_2O_5). A 1% solution in distilled water has a pH level of 7.70.

Textured milk protein without added salts was used as a control. Two percent sodium chloride which was added was assumed to be the average salinity of minced meat products (Hamm, 1972, 1973) and the 0.5% of Hamine added was assumed to be the highest polyphosphate level allowed to be added to meat products (Hamm, 1972).

Chemical analysis

Water, protein, fat and ash contents were determined by the methods of AOAC (1975) and the lactose level was determined by the method of Swartling & Mattsson (1953).

Functional properties

The water absorption capacity was determined by the procedure of Janicki & Walczak (1954) modified by Ozimek (1978), and the thermal shrinkage was determined by the method of Townsend *et al.* (1968), as modified by W. Iwański, I. Kwaśniewska & L. Zawadzka (unpublished, 1974).

Consistency was determined using an Allo-Kramer Shear Press, Model S 2HF. Round slices of the products, of 100 g initial weight in which the thermal shrinkage had been determined previously, were used as samples for consistency measurements. Consistency was defined as the peak force required as derived from the chart to shear a sample. Considering that the term 'consistency' is more theoretical than practical, the term 'firmness' is used in the text for describing the rheological characteristics of the product.

Viscosity was determined using a rotation viscometer Rheotest-2, produced in the GDR. After grinding, textured milk proteins were mixed with distilled water in a ratio of 1:1 (w/w). The viscosity was measured 10 min after a sample had been placed in a graduated cylinder. Readings were taken after running the viscometer at the defined shear force for 15 sec. The viscosity was calculated according to the formula given in the directions for use of the Rheotest:

$$\eta = \frac{z \times \alpha \times 100}{D_r} \qquad z \times \alpha = \tau$$

where:

η = viscosity millipoises, m·Pa·S
z = constant value characterizing the definite graduated cylinder
α = angle coefficient, as read during the measurement
D_r = shear force
τ = shear stress

Fat absorption capacity (FAC) was evaluated according to the method of Ozimek (1978). One hundred grams of previously ground product was homogenized with 150 ml (134 g) of soya-bean oil in a homogenizer UNIPAN-302 at 4000 rpm for 3 min. The mixture was held at room temperature (20±2°C) for 30 min and was then centrifuged with a centrifuge Type MPW-6 at 3000 rpm for 3 min (the time to increase the speed up to 3000 rpm was 3 min and the time to reduce speed, 3 min). The separated oil was weighed and values were calculated by using the following equation:

$$FAC = 134\,g - a$$

where:
134 g is the weight of oil added to a sample,
a is the amount of separated oil (g).
Values are reported as grams of absorbed oil/100 g of product.

Emulsifying capacity was determined by the method of Swift *et al.* (1961) as modified by Webb *et al.* (1970) and Kwaśniewska, Jacórzyński & Barylko-Pikielna (1976). Emulsion stability was determined by the method of Inklaar & Fortuin (1969), as modified by Chojnowski *et al.* (1975).

Half an hour after maceration, the pH-value of the textured proteins was determined. Ten grams of ground product were taken and macerated with 10 ml of distilled water.

The effect of varying fat content of textured milk protein, and the effect of sodium chloride, Hamine and sodium chloride and Hamine jointly on the functional properties of the preparation, were determined by analysis of variance. To establish statistical relations between consistency and thermal shrinkage of the product, coefficients of linear correlation were calculated (Elandt, 1964).

Results and discussion

When analysing the basic chemical composition of textured milk proteins it was found (Table 1) that besides assumed different fat contents of 15 and 11%, samples had shown different protein contents of 26.94 and 31.40%, respectively. The values of the remaining components differed very little.

Table 1. Chemical composition of textured milk protein

Variant of textured milk protein	Constituent (%)					
	Moisture	Protein (N × 6.38)	Fat	Lactose	Ash	pH
I	51.83	26.94	15.00	2.40	2.54	6.48
II	51.90	31.40	11.00	2.30	2.30	6.52

When analysing by statistical methods the obtained values of functional properties it was found that they were significantly differentiated at the level of $P < 0.05$ by variation in the fat contents of the product and by the varying concentrations of salt added in the course of this experiment. An inverse relation was found to exist between the level of fat content in the product and the amount of water absorbed (Fig. 1). This indicates that the process of water absorption in the textured proteins was limited and inhibited by fat being hydrophobic. The preparation to which no salt had been added was characterized by the lowest absorption capacity for both levels of fat, 15% and 11%, i.e. 22.8±0.85 g/100 g and 30.1±0.82 g, respectively. That value could be the most effectively increased by adding sodium chloride, the water absorption capacity of the product with 15% fat content increasing upon salt addition up to 34±0.82 g/100 g and that of the product with 11% fat increasing up to 39.2±1.76 g/100 g.

As suggested by Hamm (1972, 1973); Hamm & Deatherage (1960) and Hermansson & Akesson (1975b) concerning muscle proteins, sodium chloride

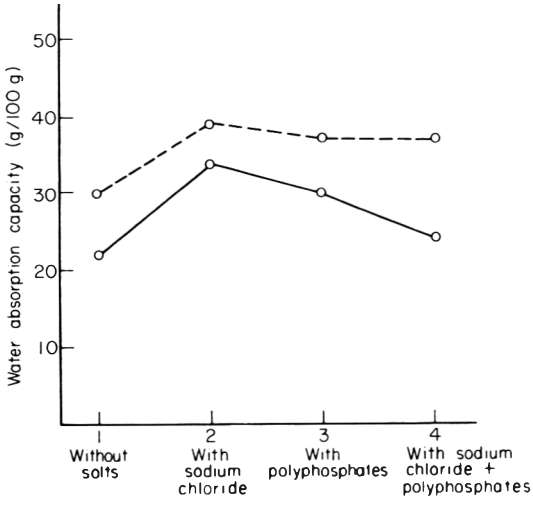

Figure 1. Effect of fat content and addition of salts on water absorption capacity of textured milk protein. Fat content: ——, 15%; – – –, 11%.

ions are bound by polar protein groups with opposite charges. Hamm (1972) emphasizes that as a first step chloride ions are bound to the protein and for this reason 'the net protein charge' of protein molecules increases. This subsequently results in loosening the protein network making it possible for water to enter. It might be assumed that a similar phenomenon could occur in the case of textured milk proteins to which sodium chloride is added.

Lower absorption capacity, as found for the product with the higher fat content, was compensated by lower thermal shrinkages which were found for that preparation (Fig. 2). Only in the case of a preparation to which both kinds of salts were added, was the reverse tendency observed. The salts used in the experiment positively improved the water retention ability of the preparation.

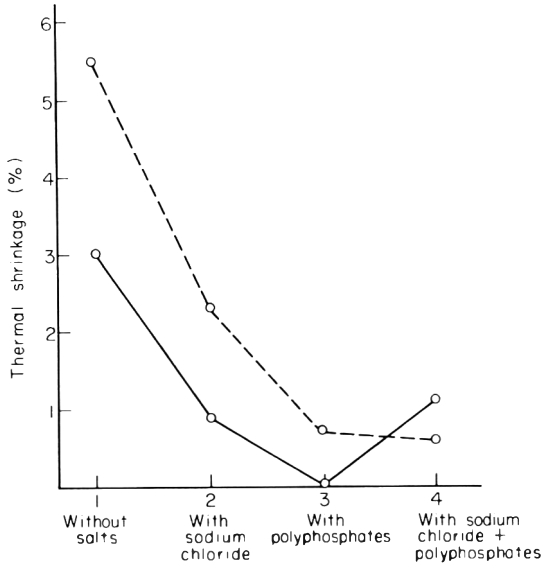

Figure 2. Effect of fat content and addition of salts on thermal shrinkage of textured milk protein. Fat content: ——, 15%; – – –, 11%.

Increased fat content reduced the firmness of the product (Fig. 3). For the preparation with no additives, the shearing force was 729.3±15.09 lb and 568±1.63 lb for 11 and 15% fat content, respectively. The reduced firmness in products with a higher fat content was also observed by Huffman & Powell (1970) who studied rheological properties of beef patties with textured soya-bean protein added. Ozimek *et al.* (1979) found the firmness to decrease and the tenderness to increase in textured milk proteins with higher fat content.

Statistical calculations showed that no correlation exists between thermal shrinkages and consistency for all variants of textured milk proteins under study (Table 2).

Hermansson (1975) and Kwaśniewska (1976) reported the negative relation between thermal shrinkage and consistency for both pure, ground meats and those with protein preparation added. The specific nature of the textured milk

proteins may be responsible for the different behaviours found in this experiment.

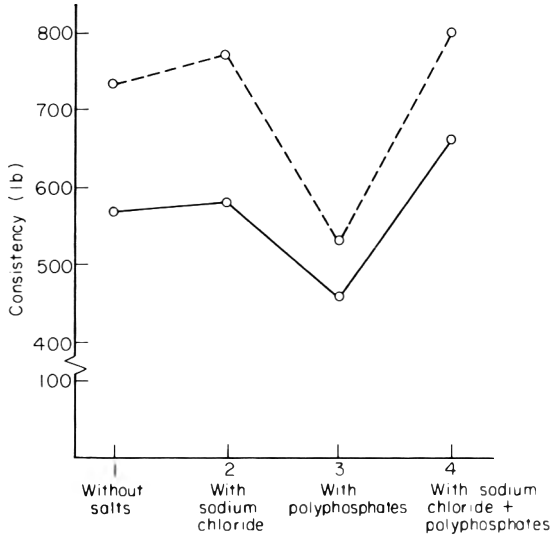

Figure 3. Effect of fat content and addition of salts on consistency of textured milk protein. Fat content: ——, 15%; – – –, 11%.

Table 2. Correlation between thermal shrinkage and consistency of textured milk protein

	Thermal shrinkage of variants of textured milk protein at fat content of							
	15%				11%			
	Without salts	With sodium chloride	With poly-phos-phates	With sodium chloride + polyphos-phates	Without salts	With sodium chloride	With poly-phos-phates	With sodium chloride + polyphos-phates
Con-sistency	−0.93	−0.95*	−0.95*	+0.50	+0.50	+0.94	0.54	—

*Significant at $\alpha = 0.05$.

Viscosity was found to increase with an increased fat content (Fig. 4). The addition of sodium chloride, Hamine, as well as sodium chloride and Hamine jointly resulted in increased viscosity at both levels of fat content. In the case of the product with 15% fat content, the viscosity increased from a value of 252.5±7,22 m·Pa·s for the variant with no salt added up to the value of 849.4±17.34 m·Pa·s for the variant with both salt added. In case of the preparation with 11% fat content, the viscosity increased from the value of 90.1±3.47 m·Pa·s for the variant with no additives up to the value of 513±14.89 m·Pa·s for the variant with sodium chloride and Hamine jointly added. The phenomenon of increased viscosity, as caused by an increased concentration of

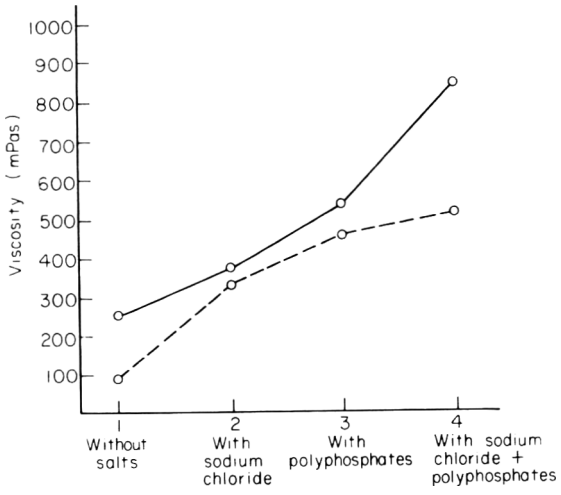

Figure 4. Effect of fat content and addition of salts on viscosity of textured milk protein. Fat content: ——, 15%; – – –, 11%.

sodium chloride, was also observed by Hermansson & Akesson (1975b) and by Kwaśniewska (1976) when studying sodium caseinate and milk protein concentrate obtained at the isoelectric point. In the case of textured milk proteins, the increase of viscosity may have been caused by the influence of salt on the hydration effect of protein, or by changes in the micellar structure of protein which resulted from the interaction between protein and sodium chloride.

Lower fat absorption capacity was found for the product with higher fat content (Fig. 5). It is probable that the preparation with 15% fat content appeared to be more fat 'saturated' and that the most lipophile bonds were

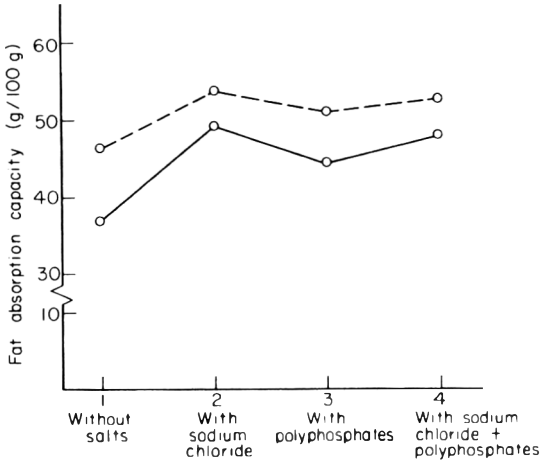

Figure 5. Effect of fat content and addition of salts on fat adsorption capacity of textured milk protein. Fat content: ——, 15%; – – –, 11%.

already occupied by fat which had been introduced during the technological process of production of the preparation (Poznański *et al.*, 1980a, b). At both levels of fat content the addition of sodium chloride increased the absorption capacities of the preparation in the most effective manner. The value of that property increased up to 49.1±0.33 g/100 g, as compared to 37.0±1.10 g/100 g for the sample with no additives at the level of 15% fat content, and up to 53.5±0.41 g/100 g, as compared to 45.90.90 g/100 g at the level of 11% fat content. It is possible that the protein structure was capable of absorbing more oil when being loosened in the presence of Na^+ and Cl^- ions.

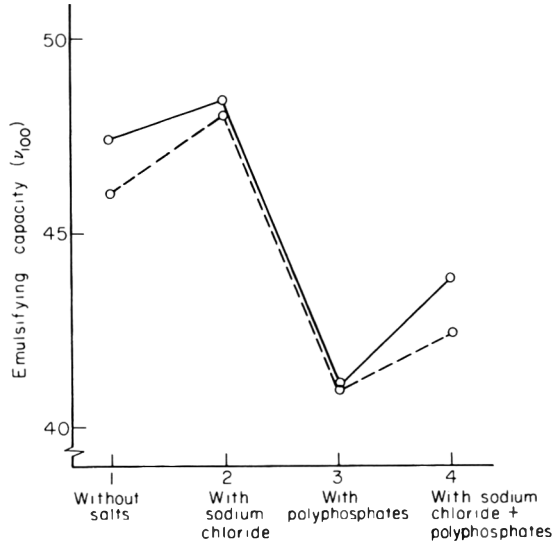

Figure 6. Effect of fat content and addition of salts on emulsifying capacity of textured milk protein. Fat content: ——, 15%; – – –, 11%.

Textured milk proteins with a higher fat content were characterized by similar emulsifying capacities (Fig. 6). The emulsifying capacity of the preparation was influenced greatly by the salts, used in our studies. The most effective improvement of that capacity was attained by the addition of sodium chloride. For the preparation with that additive the value of the emulsifying capacity, expressed as V_{100} (Kwaśniewska *et al.*, 1976) was 48.0±1.18 and 48.3±1.31, the fat content of the texturate being 11% and 15% respectively. The protein molecules were most probably modified by the salts added, so that they could emulsify fat more effectively. A similar tendency was observed by Swift & Sulzbacher (1963) for meat proteins and by Wang & Kinsella (1976) for protein preparation of alfalfa leaves. In the case of the textured milk proteins with a higher fat content, higher emulsion stability was found for preparations both with and without added salts (Fig. 7). The additives as used in this study, improved the stability of the product, the addition of polyphosphates appearing the most effective in this regard.

To summarize this evaluation of the functional properties of the textured milk

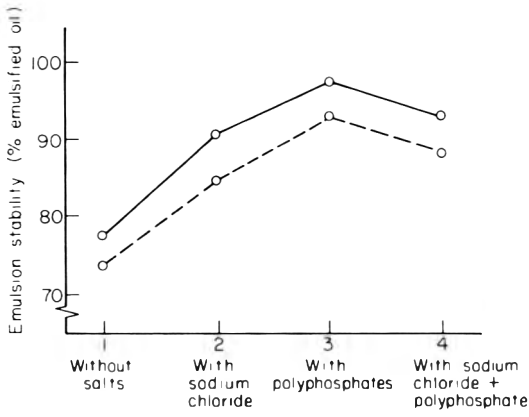

Figure 7. Effect of fat content and addition of salts on emulsifying capacity of textured milk protein. Fat content: ——, 15%; – – –, 11%.

proteins, it must be concluded that these properties are essentially influenced by differences in fat content in the preparation as well as by the addition of sodium chloride, Hamine or sodium chloride and Hamine jointly. The higher content of fat (15%) in the product assured better lipophile properties (emulsifying capacity and emulsion stability) and better water retention in the course of heat treatment. Assuming these properties to be determined by the physico-chemical conditions in the product, it is suggested that the fat acted as a protective factor for protein, thus preventing it from denaturing changes which would be caused by the high temperature (80°C) used in that technological process.

For the most part, the functional properties of the textured milk proteins appeared to be favourably influenced by salts, as used in this investigation. The addition of sodium chloride should probably be considered to be the most desirable, because the preparation to which sodium chloride had been added was characterized by the most favourable values determining technological suitability of the product, i.e. the best water absorption and the highest fat absorption and emulsifying capacities.

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Extension of the shelf life of cream-based liqueurs at high ambient temperatures

W. BANKS, D. D. MUIR* AND AGNES G. WILSON

Summary

The effect of pH adjustment, calcium removal and citrate addition on the stability of a cream liqueur at 45°C was investigated. Evidence is presented to show that by either removal or sequestration of ionic calcium the shelf life of cream liqueurs can be usefully extended. Data is included on the relation between viscosity and composition for a range of liqueurs.

Introduction

Judging from early references to 'Atholl Brose' the organoleptic properties of mixtures of cream and whisky have delighted man for several centuries. 'Atholl Brose' is a home-made Scottish concoction of whisky (previously steeped with coarse oatmeal), fresh cream and heather honey. Although delicious when freshly prepared and yielding as a bonus (from the oatmeal) very fine porridge for accompaniment at breakfast, the product is unsuitable for distribution and retail sale because of the inherent instability of the cream. However, with the advent of modern technology, a wide range of cream liqueurs is now available with adequate stability for many months storage at room temperature. Fat separation is avoided by reduction of the fat globule size—by homogenization—and stability is provided to the newly formed fat surface by the incorporation of additional protein or synthetic emulsifier (Banks, Muir & Wilson, 1981).

Notwithstanding the significant extension of shelf life achieved by such treatments, some problems remain when cream liqueurs are subject to high ambient temperatures for prolonged storage times. Such resistance to high temperature storage is not required when the products are consumed in the temperate climatic zones of the world but assumes great importance when liqueurs are exported to tropical regions. This paper describes some experiments which point to the cause of instability of cream liqueurs at high ambient temperatures and suggests some ways in which stability may be increased.

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Materials and methods

Preparation of cream liqueur

A simple model cream liqueur was prepared from sodium caseinate, sucrose, pasteurized double cream and potable spirit as follows: To water at 85°C (2.57 kg), sodium caseinate (0.47 kg) was added with vigorous stirring. After complete dissolution of the caseinate, sucrose (3.0 kg) was added and the mixture stirred until the sugar dissolved. After the air had dispersed from this mixture, the opalescent syrup was mixed with pasteurized double cream (48% butterfat, 5.0 kg) and the temperature brought to 55°C. The basic mix was then homogenized using either a 'Rannie' single stage homogenizer (540 l/h) or a 'Manton–Gaulin' two-stage homogenizer (55 l/h) to achieve a particle size distribution such that over 98% of the fat globules were less than 0.8 μm. Different homogenization pressures were required for each homogenizer but, typically, two passes at 4000 psig with the 'Manton–Gaulin' homogenizer achieved the desired end-result.

The homogenized base mix was then cooled to 10°–15°C and the appropriate volume of potable spirit added (*ca* 2.58 l of 153° proof) to yield a final proof of 30°. The total solids content of the mixture was then measured and when necessary adjusted to 40% (w/w) by the addition of potable spirit (30° proof). A typical analysis of a product is shown in Table 1.

Table 1. Composition of standard cream liqueur

	Content (%)
Fat	16.0
Sugar	20.0
Protein (N × 6.38)	3.3
Alcohol	14.0
Total solids	40.0

Analysis of product

Protein content. The protein content of the liqueur was estimated by the macro-Kjeldahl technique after preliminary evaporation of ethanol on a boiling water bath.

Fat content. This was estimated by the Röse–Gottlieb method (British Standards Institution, 1963).

Total solids. These were estimated by the hot-plate technique described in Muir & Sweetsur (1978) after a preliminary four-fold dilution with water.

Fat globule size distribution. The fat globule size distribution was measured using a Coulter Counter (Model D-Industrial, Coulter Electronics) and a 30 μm orifice.

Viscosity. The viscosity was measured using a 'Rheolog' viscometer (Brookfield Engineering Laboratories Inc., Stoughton, USA) in a thermostatted small sample adaptor.

Product stability. The stability of the product was evaluated by placing a sample in a sealed container in a constant temperature environment. Preliminary experiments were carried out at 30° and 45°C. At 30°C average shelf lives were too long to allow meaningful progression of the project so 45°C was selected on the grounds that it allowed discrimination between treatments without prolonging shelf life much beyond 100 days (In some export situations, the cream liqueur would be expected to withstand periods of up to 56 days at daytime temperatures of the order of 40°C). The onset of instability was usually accompanied by marked thickening of the liqueur followed by serum separation. The shelf life was taken as the time elapsed before visible separation of serum was observed.

Results and discussion

The effect of pH on shelf life

The pH of many milk-based systems is a critical determinant of stability. For example, when milk is heated the stability may vary by a factor of ten depending on the pH before heating (e.g. Tessier & Rose, 1964). In the case of stability of milk protein to ethanol, similar effects have recently been reported (Horne & Parker, 1980). As a result, it seemed probable that the pH level would have some effect when cream liqueurs are heated. Therefore, a series of sub-samples of a freshly prepared batch of cream liqueur was prepared in which sodium phosphate salts were added (at a final concentration of 0.1 M) to cause a variation in pH. By control of the ratio of sodium dihydrogen phosphate to tri-sodium phosphate, pH values in the range 6.5–7.8 were achieved. The effect of the pH level on shelf life is shown in Fig. 1. It is clear that the pH level plays a dominant role, for at pH 7.8 shelf lives of around 28 days at 45°C were attained whilst at pH 6.5 the corresponding sample became unstable after less than 24 hr storage.

Although this experiment demonstrated the importance of the pH level as a determinant of the stability of cream liqueur no definite conclusions could be reached about the mechanisms involved. Further experiments were then carried out in which the effect of the soluble components of the cream on stability were examined.

The effect of removal of soluble low-molecular weight components of cream on shelf life at 45°C

Cream liqueur was prepared in exactly the same manner as described in the methods section except that anhydrous milk fat (Aberdeen and District Milk

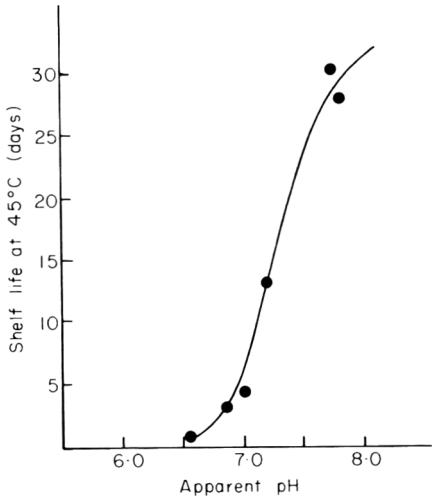

Figure 1. The effect of manipulation of pH by addition of sodium phosphate salts (100 mM) on the shelf life of cream liqueur at 45°C.

Marketing Board, Aberdeen) was substituted for cream, and water and a small additional amount of sucrose were added to restore the original solids balance of the product. The shelf life at 45°C of this liqueur was 63 days compared to an average of 8 days for typical control samples (Table 2). However, the pH of the different liqueurs was not significantly different. This result suggested that pH *per se* is not critical but rather that the effect is due to that of pH on a component of cream which is lost during the manufacture of anhydrous milk fat. The most likely factor is the ionized calcium naturally present in cream (Davies & White, 1958) since the equilibrium between ionized and non-ionized calcium in the complex calcium–citrate–phosphate–caseinate system found in milk is highly pH dependent.

Table 2. The effect of solute removal or citrate addition on the shelf life of cream liqueur at 45°C

Treatment*	Shelf life (days)	Apparent pH level
Control	8	6.72
Anhydrous milk fat	63	6.61
Washed cream	78	6.82
Citrate addition	74	6.80

*Treatment as described in text.

The effect was further investigated by 'washing' cream prior to its incorporation in cream liqueur. The process used was as follows: Three volumes of warm potable water were added to one volume of pasteurized double cream and mixed well. The re-dispersed cream was then separated in a small centrifugal cream-separator. Water was once again added to the fat-rich phase,

the mixture dispersed and re-separated. A fat-rich phase (62% butterfat) depleted of the soluble constituents of milk was obtained by this means. Cream liqueur was then made in the same manner as with anhydrous milk fat and the product stored at 45°C.

The result corroborated the findings with the liqueur made from anhydrous milk fat for the shelf life was 78 days (Table 2). The difference between the liqueurs made from anhydrous milk fat and washed cream was small but may be due to the presence of natural milk surfactants (from the milk fat globule membrane) in the washed cream. Nevertheless, this small difference is overshadowed by the very large extension in shelf life when either treatment is compared to the control sample.

When the results of these experiments and those in which pH was manipulated are considered together the effects are consistent with the observations of Horne & Parker (1980) on the alcohol stability of skim milk. These authors found that calcium ions played a dominant role in determining the ethanol stability of milk protein and that the ethanol stability (at any fixed pH value) could be significantly increased if the ionic calcium in skim milk was sequestered by means of a suitable additive. The sodium salts of ethylene diamine tetra acetic acid were particularly effective in this context but simple anions such as citrate also had a significant effect. Therefore the effect of citrate addition (at the levels naturally found in milk—between 7 and 12 mM) on the stability of cream liqueur was also considered.

The effect of addition of tri-sodium citrate on the stability of cream liqueurs

Cream liqueur was prepared as described in the experimental section and tri-sodium citrate was incorporated at a final concentration of 10 mM. On storage at 45°C, the shelf life was comparable with washed cream and, as in previous experiments with anhydrous milk fat and washed cream, there was no significant change in pH (Table 2). This result strengthens the hypothesis that stability is associated with the interaction of calcium ion and milk protein rather than pH *per se*.

Further experiments with tri-sodium citrate corroborated this initial result and in over 20 separate trials the shelf life of various batches of cream liqueur has been extended by at least the same extent as that shown in Table 2. However, because in all the experiments reported so far the formulation of the cream liqueur was unchanged additional work was carried out to study the effects on stability of changing the fat content and the total solids content.

The effect of variations in fat content on stability at 45°C

The effect of variation in fat content at constant total solids content was investigated by preparing a series of cream liqueurs with the compositions shown in Table 3. The ratio of fat to crude protein was held within practical limits to a constant value (between 4.3 and 4.6) and the solids were adjusted to

Table 3. Analysis of cream liqueurs with varying fat content

Sample	Fat	Crude protein	Sugar	Total solids
A	8.18	1.90	31.0	41.1
B	12.54	2.77	26.0	41.3
C	16.24	3.51	21.7	41.4
D	19.70	4.34	17.4	41.4

41% by the addition of sucrose. The results of the shelf life studies on these samples are shown in Table 4. In the control series—where no additives were present—there was a gradation in stability from less than 24 hr at 19.7% fat content to 34 days when the fat content was reduced to 8.2%. When tri-sodium citrate (10 mM per l) was added, the differences in stability remained but in each case the shelf life of the products was extended by over 90 days at 45°C.

Table 4. The effect of variation in fat content on shelf life

	Shelf life at 45°C (days)	
Fat content (%)	Control	Citrate (10 mM) added
8.2	34	136
12.5	20	122
16.2	2	101
19.7	< 1	94

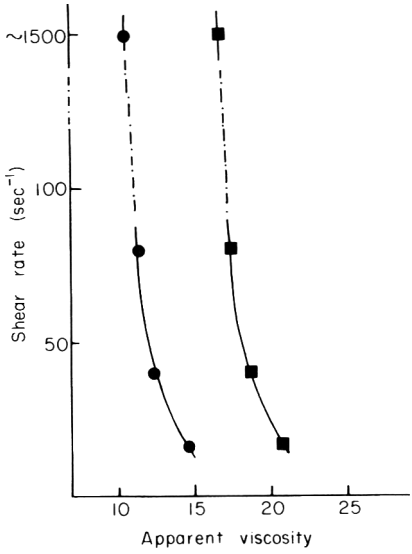

Figure 2. The effect of shear rate on the measured viscosity of two samples of cream liqueur.

The variation in fat content affected not only the shelf life but also the organoleptic properties of the liqueurs. These differences were partially characterized by viscosity measurement. The measured viscosity was found to be related to shear rate and the liqueurs all exhibited thixotropic character. However, once the shear rate exceeded 50 s^{-1} the effect of shear rate on viscosity was of little practical importance (Fig. 2). Viscosity measurements were thus made at a fixed shear rate (79 s^{-1}) for the range of cream liqueurs of varying fat content in the temperature range 4°–440°C and the results are shown in Fig. 3. Between the samples with 8.2% and 19.7% fat content the viscosity doubled although the relative difference was temperature dependent. It is

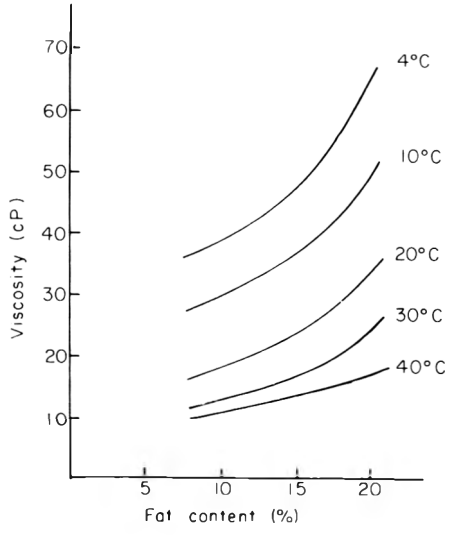

Figure 3. The effect of variation in fat content at 40% total solids on the viscosity of a cream liqueur. Viscosity was measured at a fixed shear rate (79 s^{-1}) throughout.

interesting to note that the viscosity-temperature relation for these liqueurs may be approximated by the relation: log log (viscosity) $\propto \frac{1}{T°C.}$ A relation which has also been observed to apply to concentrated skim milk in a similar temperature range (Muir, 1981).

The effect of variation of total solids content on stability at 45°C

The effect of variations in the total solids content on the stability of cream liqueur was also investigated. In this series of samples, the relative proportions of fat, protein and sucrose were held constant and only the amount of water in the product was varied. The results of the shelf life studies on liqueurs ranging in total solids from 37.5%–45.0% are shown in Table 5. The stability was inversely related to the solids content and at over 40% total solids the liqueur was very unstable. Nevertheless, when citrate (10 mM) was added large extensions to shelf life were achieved even at 45% total solids. The viscosities of the samples

Table 5. The effect of variation in total solids content on shelf life

	Shelf life at 45°C (days)	
Total solids (%)	Control	Citrate (10 mM) added
37.5	18	125
40.0	8	125
42.5	1	88
45.0	<1	81

are shown in Fig. 4 over a range of temperatures. Viscosity was related to total solids content and the temperature dependence of the viscosity was similar to that found for the range of samples in which the fat content was varied.

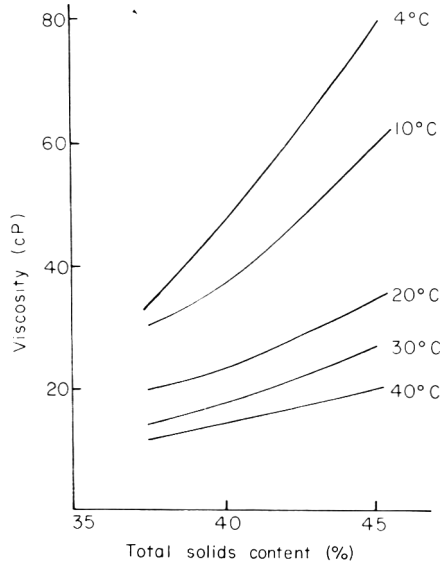

Figure 4. The effect of variation in total solids content on the viscosity of a cream liqueur. Viscosity was measured at a fixed shear rate (79 s^{-1}) throughout.

Conclusions

The results put forward in this paper strongly suggest that the shelf life of cream liqueurs at 45°C is largely determined by components in the serum phase of the cream used for manufacture. The principal component of the serum implicated by these experiments is the soluble (and in particular the ionized) calcium. Any treatment which either depletes the concentration of ionized calcium or effectively sequesters calcium ion is thus likely to extend the shelf life of the liqueurs. A typical calcium sequestrant, tri-sodium citrate, is effective in many

situations and, by its use, cream liqueurs with a wide range of compositional (and hence organoleptic) properties can be manufactured with stabilities significantly greater than are likely to be required even under the most hostile climatic conditions encountered on earth. The level of citrate tested lay within the naturally occuring range of citrate contents found in milk. Consequently stabilization of cream liqueurs with citrate appears to be a practical and safe method of extending shelf life.

Acknowledgment

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The prediction of water activity of some supersaturated non-electrolyte aqueous binary solutions from ternary data

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Summary

Binary data are required for prediction of the water activities of multi-component aqueous solutions by various methods. At high concentrations, these methods would need binary data beyond the solubility limits of individual solutes. Such supersaturated data are usually lacking or very difficult to determine experimentally. The Zdanovskii–Stokes–Robinson equation, which has been shown to be a reliable and accurate method for predicting the water activity of complex solutions, may be applied in reverse to calculate the water activities of supersaturated binary solutions from ternary data. Hitherto unpublished supersaturated binary a_w data of mannitol, sucrose and urea obtained using this method as well as polynomials for their prediction are presented in this paper.

Introduction

The prediction of the water activity (a_w) of multicomponent aqueous solutions is useful in several areas of application such as crystallization, reverse osmosis and intermediate moisture food technology. Methods for predicting a_w require binary data of a_w or related osmotic coefficient or ionic strength. In the case of relatively concentrated complex aqueous solutions, supersaturated binary data which are not usually available or easily determined experimentally may be required. Thus, these predictive methods are restricted in application to concentrations where the solubility limits of individual solutes have not been exceeded. The ability to predict 'virtual' (supersaturated) a_w of individual solutes would yield the necessary binary data required for calculating the a_w of concentrated complex systems by these methods.

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The Zdanovskii–Stokes–Robinson (ZSR) equation, which has been satisfactorily used to predict a_w of multicomponent electrolyte as well as non-electrolyte solutions (Chen *et al.*, 1973) and shown to be more accurate than the Ross (1975) equation (Teng & Seow, 1981), may also be reliably applied in reverse to calculate virtual binary a_w values from ternary data (Sangster, Teng & Lenzi, 1973; Lenzi, Tran & Teng, 1975; An, Teng & Sangster, 1978). In a comparison of methods for the prediction of supersaturated binary a_w, Tran & Lenzi (1974) were of the opinion that the reverse ZSR method can be the most reliable means of calculating supersaturated a_w in the absence of experimentally determined values. Unfortunately, the literature is not replete with the appropriate ternary equilibrium vapour pressure data for use with the reverse ZSR method. As such, only the a_w of supersaturated solutions of NaCl, KCl, K_2SO_4, $NaNO_3$, KNO_3, KH_2PO_4 and mannitol at 25°C have been estimated thus far using this method (Sangster *et al.*, 1973; Tran & Lenzi, 1974; Lenzi *et al.*, 1975; An *et al.*, 1978). Virtual a_w values calculated by the reverse ZSR method were found to agree closely with either experimental values or with those estimated by other methods. In most cases, the unsaturated and calculated supersaturated a_w data for each solute were then fitted to a polynomial of the form proposed by Teng & Lenzi (1974):

$$a_w = 1 + \sum_{i=1}^{k_{\sigma(min)}} A_i m^i$$

Except for mannitol, prediction of virtual binary a_w of non-electrolyte solutions has been largely neglected. Even in the case of mannitol, the fitting of binary data beyond saturation (as predicted using the reverse ZSR method) to a polynomial was omitted (Tran & Lenzi, 1974). Information provided in this paper includes hitherto unpublished supersaturated binary a_w data of mannitol, sucrose and urea obtained using the reverse ZSR method as well as polynomials for their prediction.

The reverse Zdanovskii–Stokes–Robinson method

The ZSR relation of predicting a_w of multicomponent aqueous solutions is generally expressed as:

$$\sum_i \frac{m_i}{m_{oi}} = 1, \text{ at constant } a_w \qquad (1)$$

where m_i is the molality of individual solutes in a mixed solution in isopiestic equilibrium with binary solutions of molality, m_{oi}. For ternary systems, the equation becomes:

$$\frac{m_1}{m_{o1}} + \frac{m_2}{m_{o2}} = 1 \qquad (2)$$

An empirical modification may be applied to take into account deviations from the Zdanovskii linearity (Kirgintsev & Luk'yanov, 1966) and, for ternary systems, may be expressed thus:

$$\frac{m_1}{m_{o1}} + \frac{m_2}{m_{o2}} = 1 - \frac{bm_1m_2}{(m_1 + m_2)} = 1 - by_1y_2m \tag{3}$$

where b is the empirical deviation term representing specific solute-solute interaction and $y_1 = m_1/m$, $y_2 = m_2/m$ and $m = (m_1 + m_2)$. Chen *et al.* (1973) divided ternary systems arbitrarily into three groups according to the magnitude of b. Groups A, B and C systems have average b values of 0–0.02, 0.02–0.10 and > 0.10 respectively.

A prerequisite of the ZSR method for calculating a_w of ternary systems is that $(a_w)_{\text{mixture}} > a_w^*$, where a_w^* is the higher of the two limiting water activities of the binary data as illustrated in Fig. 1. Extrapolation of the a_w versus molality curve beyond a_w^* is fraught with uncertainty (Sangster *et al.*, 1973). For ternary systems whose water activities are lower than the binary a_w^*, equations (2) or (3) can, however, be applied in reverse to determine the a_w of supersaturated solutions of a particular solute if a_w^* represents the solubility limit of that solute. Equation (2) may be used for those systems (Group A) which obey the ZSR relation closely enough, but greater accuracy is achieved with equation (3). In the latter case, however, extrapolated values of the correction factor, b, would

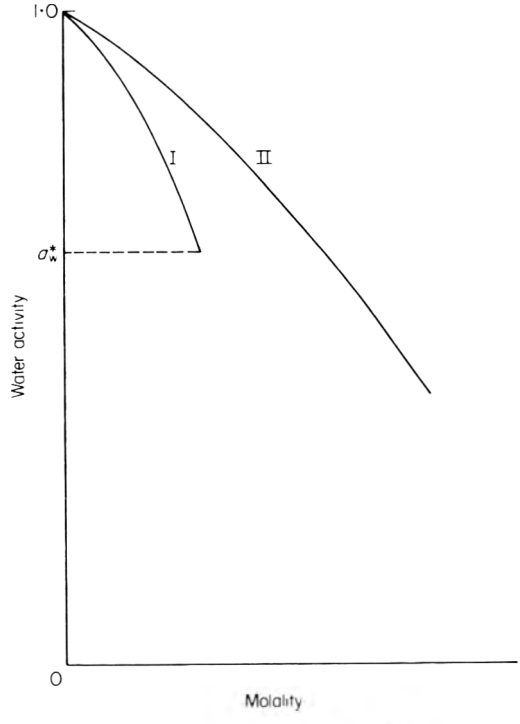

Figure 1. Criterion of applicability for the prediction of a_w by the ZSR method (curve I for a_{w1} vs m_{o1} and curve II for a_{w2} vs m_{o2}).

have to be used since b cannot be found at water activities below a_w^*. This correction term is either a constant b independent of a_w or is a simple function of a_w such as $b = b_0 + b_1 a_w$ or is a multiple function of a_w and molality fraction such as $b = b_0' + b_1' a_w + b_2'/y_1 y_2$ where b_0, b_1, b_0', b_1' and b_2' are constants (Sangster *et al.*, 1973; An *et al.*, 1978). The degree of arbitrariness manifested here may be lessened by calculating the virtual a_w of a particular solute from at least two different ternary systems and this, in fact, is the recommended procedure (Sangster *et al.*, 1973; Tran & Lenzi, 1974). The estimation of virtual a_w from the data of a single ternary system would nevertheless still be valid and acceptable (especially for Group A systems), albeit not so precise.

Results and Discussion

Virtual a_w *of mannitol*

The saturation molality of mannitol in water at 25°C is 1.185, corresponding to a saturation a_w of 0.978 (Robinson & Stokes, 1961). The reverse ZSR method was applied to data from three different ternary systems (i.e. aqueous solutions of mannitol with either sucrose, NaCl or KCl) derived from various literature sources. Since the deviation term for the sucrose (1)—mannitol (2)—H_2O system has previously been found to be negligibly small (Stokes & Robinson, 1966), equation (2) was used directly without correction to obtain the values of m_{o2} (supersaturated molality values of mannitol). The same equation was similarly applied to the other two ternary systems. The calculated m_{o2} and corresponding a_w values are given in Table 1. The results, as illustrated in Fig. 2,

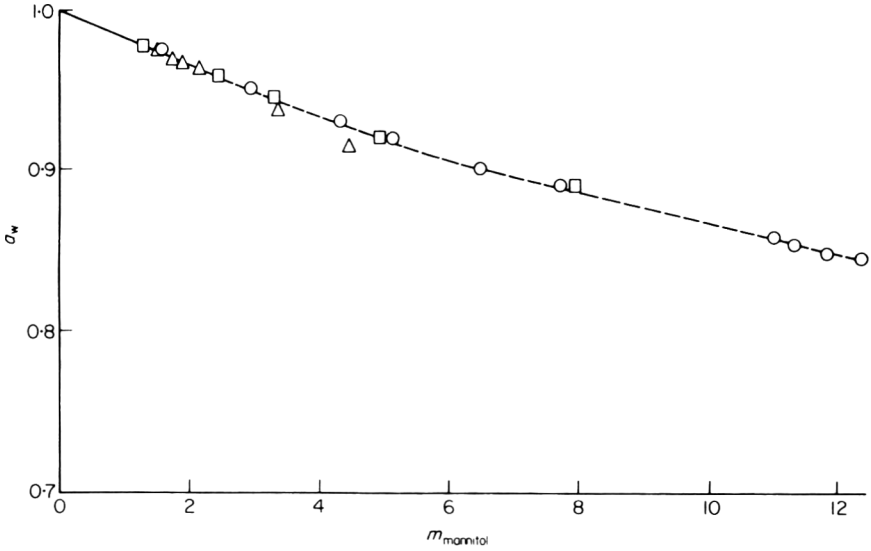

Figure 2. The water activity of supersaturated aqueous solutions of mannitol at 25°C as calculated by the reverse ZSR method from data on the systems sucrose–mannitol–H_2O (△), NaCl–mannitol–H_2O (□), and KCl–mannitol–H_2O (○).

Table 1. Supersaturated a_w and m values for mannitol at 25°C

Aqueous system	Reference	m_1	m_2	$a_{w(mix)}^*$	$m_{mannitol}^{supersat}$
Sucrose (1)—Mannitol (2)	Robinson & Stokes (1961); Stokes & Robinson (1966)	1.1776	0.2528	0.9719	1.5842
		0.9443	0.5105	0.9719	1.5656
		0.7117	0.7682	0.9719	1.5611
		0.5015	1.0008	0.9719	1.5587
		1.2411	0.4342	0.9669	1.8478
		1.0509	0.7692	0.9645	1.9543
		1.1848	0.6191	0.9645	1.9571
		1.6151	0.3172	0.9607	2.1714
		1.3081	0.6653	0.9607	2.1573
		1.0148	1.0012	0.9607	2.1602
		1.5970	0.3594	0.9602	2.1800
		1.4767	0.4971	0.9602	2.1820
		1.1259	0.8979	0.9602	2.1840
		2.5321	0.3874	0.9366	3.4010
		2.2820	0.5756	0.9366	3.3900
		1.9707	1.0494	0.9366	3.3810
		3.4904	0.1760	0.9158	4.4361
		3.3738	0.3224	0.9158	4.4931
		3.2571	0.4637	0.9158	4.4645
		3.0949	0.6557	0.9158	4.4158
NaCl (1)—Mannitol (2)	Kelly, Robinson & Stokes (1961)	0.5700	0.2465	0.9769	1.3273
		0.4400	0.4871	0.9769	1.3114
		0.2324	0.8640	0.9769	1.2934
		1.1483	0.2297	0.9579	2.5577
		1.0259	0.4713	0.9579	2.5227
		0.8993	0.7159	0.9579	2.4929
		0.7702	0.9595	0.9579	2.4634
		1.4870	0.2756	0.9454	3.4087
		1.3621	0.5337	0.9454	3.3767
		1.2592	0.7391	0.9454	3.3344
		1.1740	0.9070	0.9454	3.3063
		2.0923	0.3091	0.9232	5.1717
		2.0541	0.3946	0.9232	5.1291
		2.0342	0.4391	0.9232	5.1132
		1.9316	0.6672	0.9232	5.0552
		1.9013	0.7362	0.9232	5.0564
		1.7129	1.1344	0.9232	4.9266
		2.9900	0.2856	0.8893	8.3645
		2.8905	0.5505	0.8893	8.3050
		2.7872	0.8125	0.8893	8.1532
		2.6857	1.0622	0.8893	8.0201
		3.9097	0.2296	0.8524	13.2204
		3.8348	0.4713	0.8524	13.0223
		3.7631	0.6947	0.8524	12.8144
		3.6835	0.9377	0.8524	12.6343

Table 1 *cont.* Supersaturated a_w and m values for mannitol at 25°C

Aqueous system	Reference	m_1	m_2	$a^*_{w\,(mix)}$	$m^{supersat}_{mannitol}$
KCl (1)—Mannitol (2)	Robinson & Stokes (1962)	0.6177	0.1605	0.9774	1.2757
		0.4792	0.4092	0.9774	1.2715
		0.3441	0.6479	0.9774	1.2629
		0.0618	1.1414	0.9774	1.2508
		0.7565	0.2194	0.9721	1.6083
		0.6535	0.4045	0.9721	1.5925
		0.4290	0.8018	0.9721	1.5713
		0.1821	1.2279	0.9721	1.5501
		1.4161	0.3110	0.9499	3.0129
		1.2041	0.7072	0.9499	2.9780
		1.3174	0.4965	0.9499	2.9959
		1.0783	0.9345	0.9499	2.9466
		2.0010	0.4000	0.9300	4.4241
		1.9055	0.5876	0.9300	4.3908
		1.8136	0.7640	0.9300	4.3508
		1.6679	1.0410	0.9300	4.3047
		2.1665	0.7473	0.9192	5.1830
		2.0379	0.9978	0.9192	5.1174
		2.8746	0.3124	0.9032	6.6232
		2.7630	0.5497	0.9032	6.5317
		2.6157	0.8551	0.9032	6.4301
		2.5355	1.0233	0.9032	6.4129
		3.3214	0.2733	0.8891	7.8315
		3.1947	0.5592	0.8891	7.7977
		3.0617	0.8459	0.8891	7.6650
		2.9367	1.1077	0.8891	7.5518
		4.2142	0.3983	0.8577	11.2625
		4.1411	0.5742	0.8577	11.0216
		4.0223	0.8673	0.8577	10.9381
		3.8824	1.1916	0.8577	10.7048
		4.3879	0.3042	0.8529	11.4368
		4.2782	0.5924	0.8529	11.6307
		4.0626	1.1167	0.8529	11.3069
		3.9376	1.3906	0.8529	10.9936
		4.5328	0.3004	0.8479	11.8301
		4.4182	0.6118	0.8479	12.2278
		4.1973	1.1536	0.8479	11.8282
		4.0715	1.4379	0.8479	11.5422
		4.5879	0.3610	0.8455	12.9177
		4.5261	0.5191	0.8455	12.6487
		4.4658	0.6748	0.8455	12.5391
		4.2921	1.1056	0.8455	12.2006

$^*a_{w\,(mix)} = a^{supersat}_{w\,(mannitol)}$

show that the points obtained from the three different ternary systems all fall on a common curve, a finding which confirms the validity of the approach. The present observations are in agreement with the results obtained by Tran & Lenzi (1974). The experimental unsaturated a_w-molality data and the predicted supersaturated points up to 12.2 m were fitted to the polynomial:

$$a_{w\,(\text{mannitol})} = 1 + \sum_{1}^{7} A_i\, m^i$$

The standard deviation, σ, obtained was 0.00087. The A_i values are given in Table 4.

Virtual a_w *of sucrose*

At 25°C, the saturation limit of sucrose in water is 6.053 m, corresponding to an a_w^{sat} value of 0.849 (Robinson & Stokes, 1961). Due to the lack of appropriate ternary data, the reverse ZSR method has, of necessity, been applied only to data from a single ternary system, i.e. NaCl (1)—sucrose (2)—H_2O obtained from Robinson, Stokes & Marsh (1970). The corrected ZSR equation (3) was used to calculate the virtual a_w s, with $b = -0.044$–$0.0548\, a_w$. This relation for b is based on ternary isopiestic data at $a_w > 0.849$ (Robinson *et al.*, 1970). The results are given in Table 2 and plotted in Fig. 3. The polynomial obtained to 12.8 m (0.6888 a_w) is given by:

$$a_{w\,(\text{sucrose})} = 1 + \sum_{1}^{6} A_i\, m^i$$

with a standard deviation of 0.00097 and with A_i values as listed in Table 4.

Table 2. Supersaturated a_w and m values for sucrose at 25°C

Aqueous system	Reference	m_1	m_2	$a_{w\,(\text{mix})}^{*}$	$m_{\text{sucrose}}^{\text{supersat}}$
NaCl (1)—Sucrose (2)	Robinson *et al.* (1970)	3.3220	2.2943	0.8371	6.4747
		3.3032	2.6779	0.8314	6.8483
		3.9670	2.0520	0.8171	7.1085
		2.9750	3.7160	0.8171	7.1027
		4.2110	2.6570	0.7979	8.0137
		4.0760	3.6780	0.7824	8.5474
		5.0460	2.9580	0.7628	9.4805
		3.9460	4.9130	0.7628	9.3648
		5.7650	2.5780	0.7420	10.2771
		6.1370	3.0620	0.7211	11.0564
		6.1520	4.0440	0.7092	11.9271
		5.6000	5.1250	0.7092	11.7531
		6.0090	5.6680	0.6888	12.8000

$^{*}a_{w\,(\text{mix})} = a_{w\,(\text{sucrose})}^{\text{supersat}}$

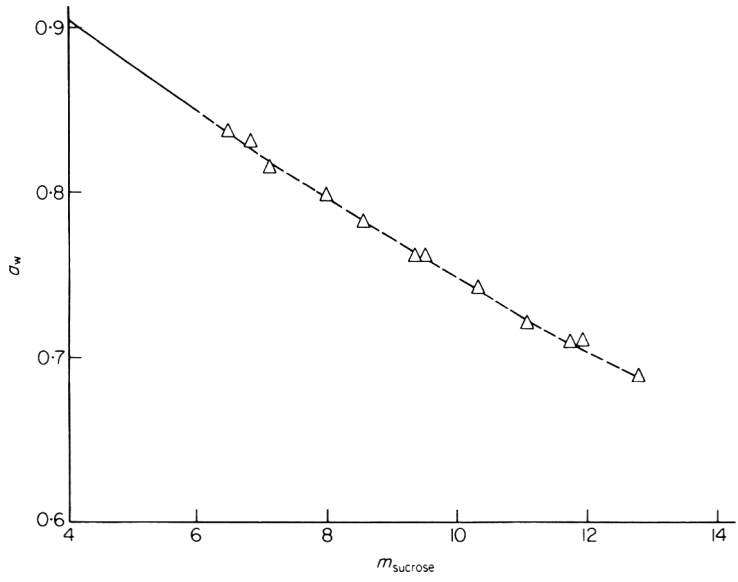

Figure 3. Calculated a_w data for supersaturated aqueous solutions of sucrose at 25°C.

Table 3. Supersaturated a_w and m values for urea at 25°C

Aqueous system	Reference	m_1	m_2	$a^*_{w(mix)}$	$m^{supersat}_{urea}$
NaCl (1)—Urea (2)	Bower & Robinson (1963)	3.7410	9.7975	0.7487	20.4785
		2.8263	12.9670	0.7487	21.0827
		2.5562	15.8330	0.7300	23.1945
		3.1609	16.0470	0.7127	25.4129
		2.5183	18.1070	0.7127	25.4642
		3.6828	18.6970	0.6778	30.0449
		2.9449	21.1760	0.6778	30.0952

$^*a_{w(mix)} = a^{supersat}_{w(urea)}$

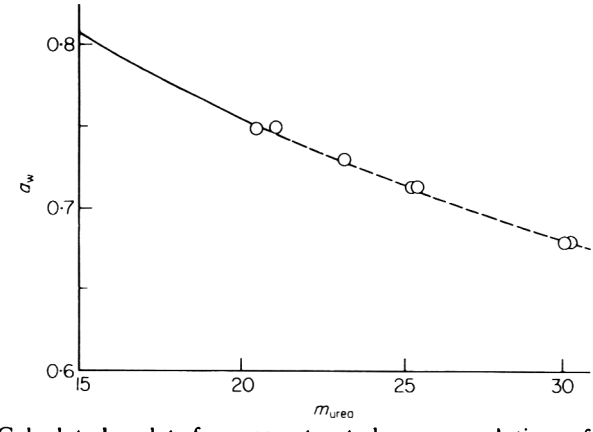

Figure 4. Calculated a_w data for supersaturated aqueous solutions of urea at 25°C.

Virtual a_w *of urea*

For aqueous urea at 25°C, $a_w^{sat} = 0.756$ and $m^{sat} = 20.027$ (Ellerton & Dunlop, 1966). As in the case of sucrose, virtual a_w s of urea were calculated from data of a single ternary system, NaCl (1)—Urea (2)—H_2O, because of the dearth of suitable data from other ternary systems. The virtual a_w s were obtained using the corrected ZSR relation, with $b = -0.0538 + 0.0364\ a_w - 0.0003/(y_1\ y_2)$ where $y_1 = m_1/(m_1 + m_2)$ and $y_2 = m_2/(m_1 + m_2)$. The ternary isopiestic data of Bower & Robinson (1963) were employed in this case.

The results are presented in Table 3 and Fig. 4. A polynomial fitted to the observed unsaturated as well as predicted supersaturated a_w -molality data up to 30.1 m (0.6778 a_w) gives the expression:

$$a_{w\,(urea)} = 1 + \sum_{1}^{9} A_i m^i$$

with a standard deviation of 0.00029. The A_i parameters are given in Table 4.

The results for all three solutes are summarized in Table 5 which lists virtual a_w values at rounded molalities, calculated via the respective polynomials as given earlier.

Table 4. A_i parameters for the equation $a_w = 1 + \sum_i A_i m^i$ for determining the water activity of aqueous solutions of mannitol, sucrose and urea at 25°C

	A_i		
i	Mannitol	Sucrose	Urea
1	-1.61702×10^{-2}	-1.76372×10^{-2}	-1.78126×10^{-2}
2	-4.18817×10^{-3}	-1.60089×10^{-3}	7.26437×10^{-4}
3	2.96922×10^{-3}	-1.18930×10^{-4}	-4.77520×10^{-5}
4	-8.17081×10^{-4}	5.15105×10^{-5}	2.02578×10^{-6}
5	1.10900×10^{-4}	-4.31552×10^{-6}	-5.04309×10^{-8}
6	-7.26757×10^{-6}	1.19737×10^{-7}	7.86507×10^{-10}
7	1.83091×10^{-7}	—	-1.85695×10^{-11}
8	—	—	9.55517×10^{-13}
9	—	—	-1.83499×10^{-14}

Conclusions

The prediction of virtual water activities of individual solutes is useful in that the calculated supersaturated binary a_w data could then be employed for prediction of a_w of concentrated multicomponent systems by those methods which require such binary data. The major disadvantage of the reverse ZSR method is its requirement for aqueous ternary data at water activities beyond the solubility limit of one of the solutes. At present, very little data available in the literature meet this requirement.

Table 5. Predicted a_w of supersaturated binary solutions of mannitol, sucrose and urea at 25°C at rounded molalities

	a_w		
Molality	Mannitol	Sucrose	Urea
1.2	0.978		
2.0	0.965		
3.0	0.950		
4.0	0.936		
5.0	0.922		
6.0	0.909	0.849	
7.0	0.898	0.823	
8.0	0.889	0.797	
9.0	0.881	0.772	
10.0	0.871	0.748	
11.0	0.858	0.725	
12.0	0.846	0.704	
12.2	0.845	—	
12.8		0.689	
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20.0			0.756
21.0			0.748
22.0			0.739
23.0			0.730
24.0			0.723
25.0			0.716
26.0			0.709
27.0			0.702
28.0			0.695
29.0			0.687
30.0			0.679

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A study of water activity lowering in meat during immersion-cooking in sodium chloride-glycerol solutions. I. Equilibrium considerations and diffusional analysis of solute uptake

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Summary

Beef slices were dehydrated by immersion-cooking in a sodium chloride-glycerol solution of lower water activity (0.74) in the range of temperatures 30 to 85°C.

Equilibrium studies of sodium chloride-glycerol concentration in beef and the external solution were conducted. Results showed that the equilibrium condition between beef and external solution is given by the equality of sodium chloride and glycerol concentration in the water of the solution and in the muscle tissue water.

The variation of water content, sodium chloride and glycerol content in beef has been determined as a function of immersion time. Solute uptake data by beef slabs were handled according to standard techniques used for obtaining diffusion coefficients assuming unsteady state Fickian diffusion.

Introduction

One of the techniques for the production of intermediate moisture foods (IMF) is the 'moist infusion' process—also called osmosis dehydration—in which solid pieces of fresh food are soaked and/or cooked in an appropriate solution to result in a final product having the desired water activity (a_w) (Brockmann, 1970; Kaplow, 1970; Heidelbaugh & Karel, 1975). Differences in water activity between the food and the solution cause migration of water out of the food into the surrounding solution. However, there is a simultaneous diffusion of the surrounding solution solute(s) into the food. Unless the rate of water migration

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is much greater than that for solute(s), the food will become impregnated with the solute(s).

Several solutes, also called humectants, may be incorporated in intermediate moisture foods to lower the water activity to the desired range, e.g., 0.65 to 0.90. These solutes include polyols, sugars and salts (Kaplow, 1970; Sloan & Labuza, 1975). The selection of the proper humectant for the stabilization of an IMF involves considerations about its a_w lowering capacity, flavour impact, texture, cost and safety. As a result of the above, a combination of sodium chloride and glycerol has been frequently utilized to depress a_w in a variety of meats, including beef, chicken, pork and fish (Johnson *et al.*, 1972: Kaplow & Halik, 1972; Collins *et al.*, 1972; Obanu, Ledward & Lawrie, 1975; Brockman, 1970).

In order to design properly an optimum process for production of IM meat by the 'moist-infusion' process, it will be necessary to understand the process which occurs in the meat when it is soaked/cooked in a solution of lower a_w. However, fundamental knowledge of the process is slight. Some related studies have been published on penetration of salt in fish (Del Valle & Nickerson, 1967 a, b; Zugarramurdi & Lupín, 1977) or of salt in cheese (Geurts, Walstra & Mulder, 1974) but these studies were performed at or below room temperature. Kinetic data of glycerol uptake in foods are even more scarce.

This paper aims to study the characteristics of sodium chloride and glycerol uptake and water removal—and consequently of a_w lowering—during the 'moist-infusion' process. For this purpose to main aspects are taken into account: one referring to the sodium chloride-glycerol equilibrium in the muscle, and the other to the rate at which this equilibrium is approached.

Experimental part

Equipment

Figure 1 shows a scheme of the equipment used for the studies. It consists of a 4 litre stainless steel container filled with the salt-glycerol solution and immersed in a thermostatic bath for adequate temperature control (± 0.2°C). The cover minimizes evaporative water losses from the solution, particularly during the

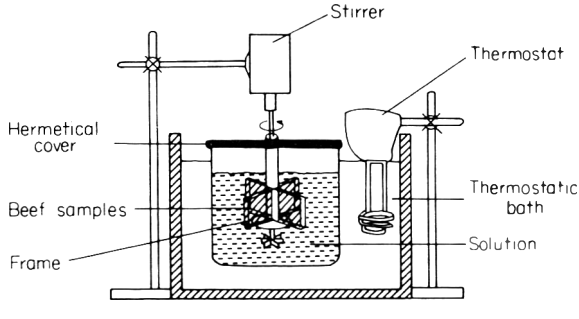

Figure 1. Equipment utilized.

runs at elevated temperatures. A special device consisting of various spaced aluminium frames serves to hold the beef samples submerged in the solution. A stirrer permits vigorous agitation of solution when desired.

Materials

For all the experiments reported in this work the composition of the solution was fixed as follows: 40.9% glycerol, 9.4% sodium chloride and 0.5% potassium sorbate. Water activity of this solution was calculated using published data on a_w of sodium chloride and glycerol solutions (Chirife, Ferro Fontán & Benmergui, 1980) and found to be 0.74.

Three kilograms of solution was used for each experiment and ten samples of beef, totalling about 230 g wet weight, were added to this solution. The large excess of osmosis solution limited concentration changes in the solution due to uptake of water from the beef slices and loss of solutes to the slices. Fresh solution was prepared for each run.

Fresh raw beef was purchased in a local supermarket and frozen in a freezer at −20°C during 15 hr (slow freezing, SF). After this period the beef was allowed to warm until it reached a partially frozen state and sliced in this condition using a food cutter. This procedure obtained slabs of a uniform thickness.

Most of the fat and connective tissue was removed from the beef. For all the experiments the beef was cut perpendicularly to the fibre into square pieces of 5 × 5 cm and 0.9 cm thickness. Thickness was measured using a dial micrometer; various measurements were made at different points of the slab and an average value was used.

Procedure

At specified time intervals one sample was removed from the solution and analyzed for moisture, glycerol and sodium chloride content. Samples were drained for about 30 sec and the 'excess' surface moisture was removed using a filter paper. An analysis of the solution was also conducted at the end of each run. The sample was comminuted using a food chopper, homogenized and aliquots taken for analysis.

Glycerol content was determined using the AOCS method (1973); sodium chloride by the AOAC (1970) procedure and moisture content was determined by the Karl-Fischer method (AOCS), and by placing the sample in an oven at 40°C over concentrated sulphuric acid for 76 hr (Favetto, Chirife & Bartholomai, 1979).

Results and discussion

Equilibrium studies

Various studies on fish salting (Del Valle & Nickerson 1967a; Zugarramurdi & Lupín, 1976) have shown that the equilibrium condition between fish muscle

and brine was given by the equality of salt concentration in the brine and in the muscle tissue water. Accordingly, the equilibrium condition between beef and the external solution may be given by the equality of sodium chloride and glycerol concentrations in the water of the solution and in the muscle tissue water. It is assumed that either glycerol or sodium chloride is dissolved in all of the water in the system (muscle tissue water or external solution water). This was verified in the following manner. Slabs of beef were immersed in the solution at 30, 70 and 85°C and allowed to equilibrate. The following determinations were made: (1) sodium chloride in the muscle and the solution, (2) glycerol in the muscle and the solution, (3) moisture content of beef muscle. All analysis were made in duplicate. The equilibrium relation or distribution coefficient is defined as follows:
for sodium chloride,

$$K_S = \frac{\dfrac{\text{moles of sodium chloride}}{\text{mass of water}} \text{ (in beef)}}{\dfrac{\text{moles of sodium chloride}}{\text{mass of water}} \text{ (in external solution)}}$$

for glycerol,

$$K_G = \frac{\dfrac{\text{moles of glycerol}}{\text{mass of water}} \text{ (in beef)}}{\dfrac{\text{moles of glycerol}}{\text{mass of water}} \text{ (in external solution)}} .$$

Table 1. Values of the equilibrium relation for sodium chloride and glycerol at various temperatures

Temperature °C	K_S	K_G
30	0.95	1.02
70	1.06	0.99
85	1.09	0.97

S: sodium chloride.
G: glycerol.

Table 1 shows the results of the equilibrium studies. It may be seen that, within the limits of experimental error, the equality condition did indeed hold for salt and glycerol, so the distribution coefficients calculated on this basis was close to unity. The small deviations observed may be attributed, among others, to one or both of the following factors:

(a) Not all of the water in the beef is capable of acting as solvent for the solutes; a small amount is bound to specific groups of the proteins (Karel, 1973).

(b) Not all of the sodium chloride is in solution; some may be bound to proteins of beef (Karel, 1973).

Diffusional analysis of net solute uptake

During immersion-cooking in a solution of low a_w beef loses water and gains sodium chloride and glycerol. The process is complex since in the range of temperatures used water is removed from beef not only by an a_w driving force, but also due to protein denaturation as a result of cooking. There are various ways to express solute concentration (either sodium chloride or glycerol) in beef. The net solute uptake, however, is best expressed when a constant basis is used, as a beef solids one,

$$\frac{\text{mass of solute}}{\text{(total mass—mass of water—mass of sodium chloride—mass of glycerol)}}$$

Figure 2 shows the net uptake of salt and glycerol and the simultaneous loss of water in beef during immersion-cooking at 85°C in the solution of specified low water activity (0.74). The dramatic influence of temperature on the loss of water is indicated in Fig. 3 which shows the kinetics of water removal from beef at the various temperatures; it shows that at the higher temperatures a much lower water content (on a constant solids basis) is attained which suggests that cooking losses are predominant as compared to diffusional ones. This is verified in the same Fig. 3 by inspecting the curves showing the loss of water from beef pieces cooked in pure water at similar temperatures. It can be seen that water loss through protein denaturation is very significant.

Figure 4 shows the effect of agitation of solution on solute uptake as a function of immersion time at 85°C. It can be seen that agitation has a significant effect on glycerol uptake, for sodium chloride the effect is much less important. On the basis of these results a vigorous agitation of solution was used in all experiments in order to minimize mass transfer resistances in the external solution.

Various studies have been reported on the kinetics of sodium chloride uptake during salting of fish muscle. Del Valle & Nickerson (1967b) and Zugarramurdi & Lupín (1977)—among others—demonstrated that this phenomenon of salt penetration follows the ordinary Fick's law. These studies, however, were made at much lower temperatures (usually below 25°C) than those used in this work. The simple diffusional analysis is not in itself sufficient when protein denaturation and concomitant water loss accompanies solute uptake.

Nevertheless, the penetration of salt and glycerol in beef may be tentatively described according to Fick's law of non-steady state diffusion. When a solute is absorbed by a thin flat sheet of material the process can be considered as one-dimensional, if the ratio thickness/size is about 0.1 or less (Vaccarezza, Lombardi & Chirife, 1974). Owing to practical reasons this ratio was very difficult to achieve with beef slabs (the ratio was 0.18) thus, strictly speaking, the

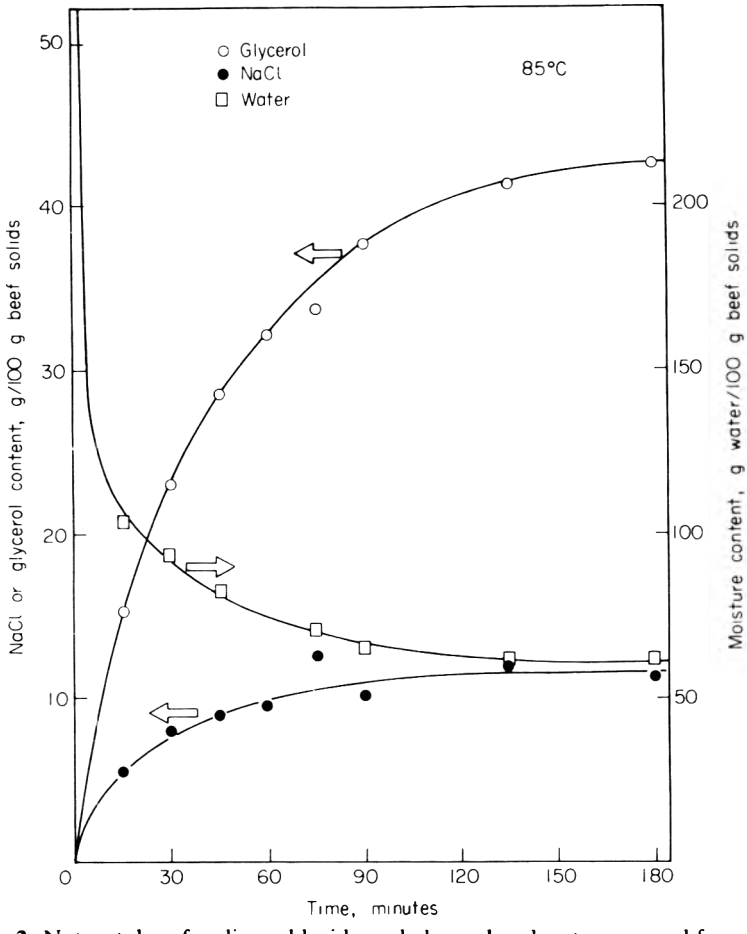

Figure 2. Net uptake of sodium chloride and glycerol and water removal from beef slices (0.9 cm thickness) as a function of immersion time at 85°C.

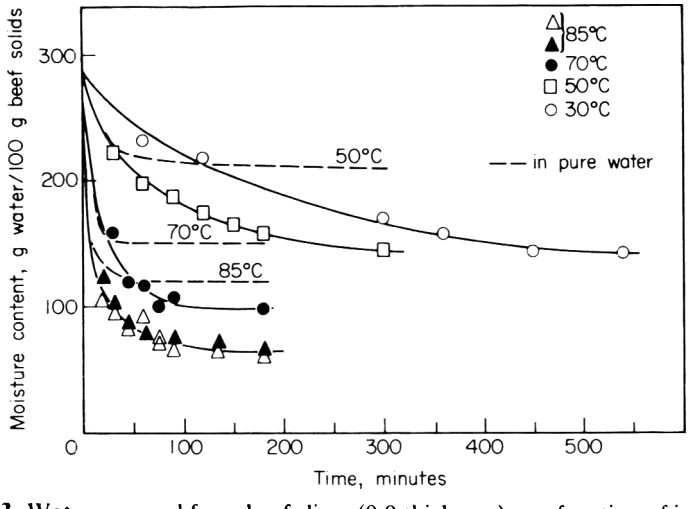

Figure 3. Water removal from beef slices (0.9 thickness) as a function of immersion time in solution of low a_w and pure water at several temperatures.

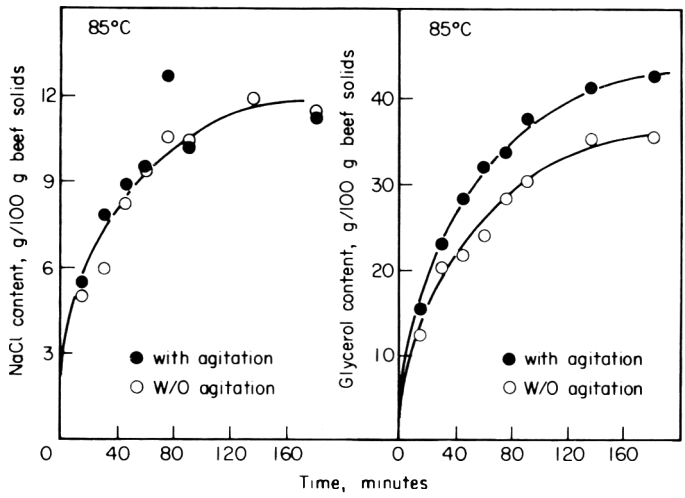

Figure 4. Effect of agitation of solution on solute uptake as a function of immersion time at 85°C.

actual process may be considered approximately as one-dimensional. The equation governing diffusion in one dimension is

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial C}{\partial x}\right) \tag{1}$$

where C is concentration at time t and coordinate x, and D is the diffusion coefficient. If the diffusion coefficient is constant (Zugarramurdi & Lupín, 1977; Fox, 1980; Geurts *et al.*, 1974) the equation to be solved is

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{2}$$

It has been shown (Crank, 1956) that for a considerable period in the initial stages of absorption, the solution of equation (2) for a well-stirred solution may be expressed as

$$\frac{M_t}{M_\infty} = 2\left(\frac{D_t}{\pi l^2}\right)^{1/2} \tag{3}$$

where M_t is the amount sorbed at time t, M_∞ the amount sorbed after an infinite time and l is the half-thickness of the slab. The applicability of this extremely simple form to the early stages of absorption enables D to be determined from a plot of M_t against $t^{1/2}$. At subsequent times the M_t/M_∞ against $t^{1/2}$ plot decreases in slope until equilibrium is attained. It has been shown mathematically (Crank, 1956) that for the early stages of sorption M_t is still proportional to $t^{1/2}$ for a system where D is a function of concentration alone. The value of D obtained using eqn (3) is then an average value for the range of concentrations experienced in the diffusion process. Note that the large ratio of solution to beef slices limited concentration changes in the external solution, as is required to solve eqn (2) in the above manner.

Figures 5, 6, 7 and 8 show the application of eqn (3) to the simultaneous sodium chloride and glycerol uptake by beef slabs at 30, 50, 70 and 85°C. It can be seen that the $t^{1/2}$ law only applies satisfactorily to the data at 30°C and with somewhat more difficulty at 50°C; at the higher temperatures (70 and 85°C) the data deviate from the straight line relationship, a sigmoid character being apparent.

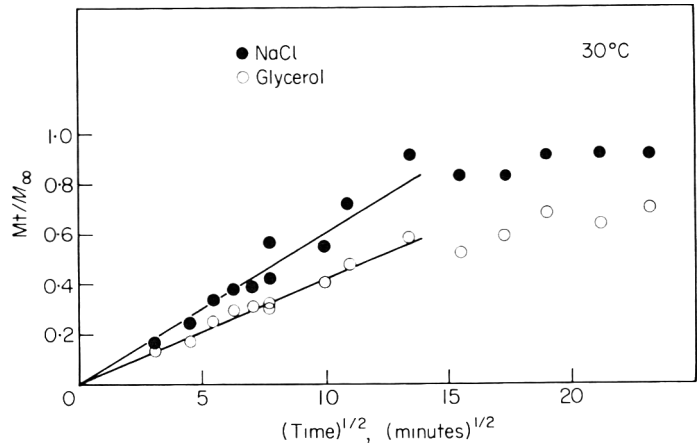

Figure 5. Uptake of sodium chloride and glycerol by beef slabs (0.9 cm thickness) at 30°C.

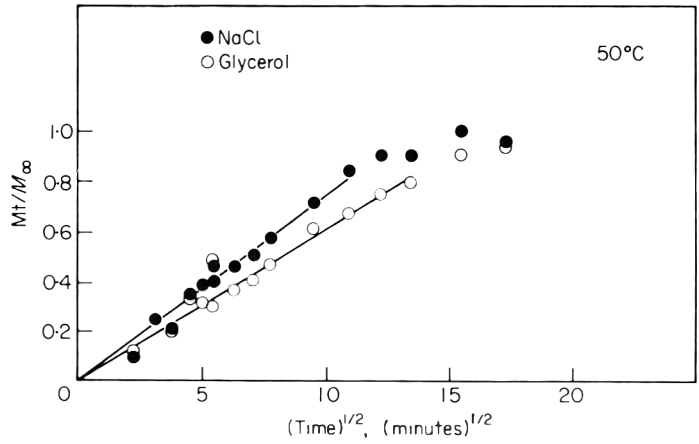

Figure 6. Uptake of sodium chloride and glycerol by beef slabs (0.9 cm thickness) at 50°C.

This apparent 'non-fickian' behaviour can be attributed to protein denaturation (with resultant conformational changes) and concomitant water loss which occur in the initial period of beef samples immersion. This behaviour, however, may be also partially attributed to the transient sample temperature during the initial stages of immersion. For instance it has been calculated, on the basis of certain assumptions (i.e. constancy of density and thermal conductivity of beef), that it takes about 9 min for a beef sample to reach thermal equilibrium with the bath at 85°C.

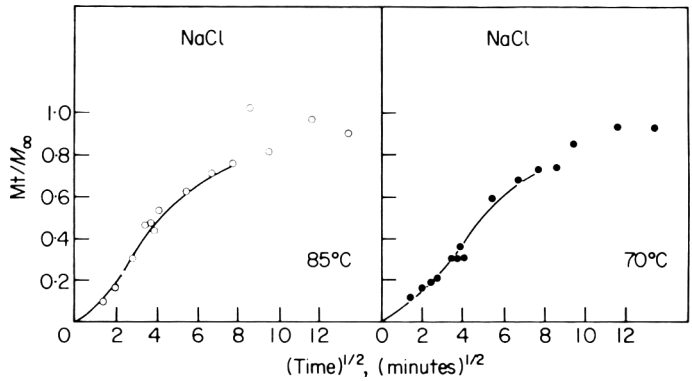

Figure 7. Uptake of sodium chloride by beef slabs (0.9 cm thickness) at 70 and 85°C.

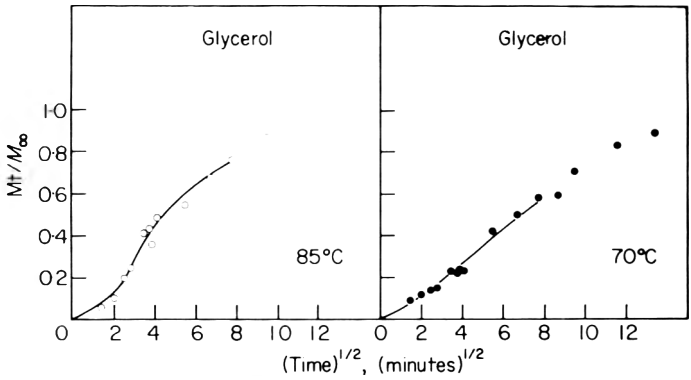

Figure 8. Uptake of glycerol by beef slabs (0.9 cm thickness) at 70 and 85°C.

From the straight lines shown in Fig 5 a value of the mean initial stage diffusion coefficient at 30°C may be obtained.

Table 2 compares values for sodium chloride and glycerol diffusivity in beef with literature data for diffusion in other foods and model systems. Note that

Table 2. Diffusion data for sodium chloride and glycerol in beef and related food systems

Solute	System	Temperature (°C)	$D \times 10^5$ cm^2 s^{-1}	Reference
NaCl	Water	25	1.48–1.6*	Robinson & Stokes (1955)
NaCl	Beef	30	0.94†	This work
NaCl	Fish muscle (swordfish)	25	0.95-1.45*	Del Valle & Nickerson (1967b)
NaCl	Pickles	21.1	0.84–1.19‡	Pflug, Fellers & Gurevitz (1967)
Glycerol	Water	25	0.94	Brown & Chitumbo (1975)
Glycerol	Beef	30	0.47†	This work
Glycerol	Cellulose gel	25	0.24	Brown & Chitumbo (1975)

*Depending on concentration; †mean initial stage diffusion coefficients: ‡values for desalting.

values reported in the present work were obtained from simultaneous uptake data of sodium chloride and glycerol. It can be seen that values obtained in this work compare well with others, in spite of the assumptions made for calculations (constant diffusivity, non-dimensional diffusion, simultaneous fluxes of glycerol and sodium chloride).

The data of diffusion coefficients for sodium chloride and glycerol at 30 and 50°C may be tentatively used to estimate a value of the activation energy for the diffusion process in beef,

$$D = D_0 \cdot \exp^{-E_a/RT} \tag{4}$$

where E_a is the activation energy, R is the ideal gas content and T is the absolute temperature. Assuming that eqn (4) applies the activation energies for sodium chloride and glycerol diffusion in beef were calculated to be approximately 4.4 and 7.2 kcal/mole respectively. In spite of the limitations of the above calculations, the value for sodium chloride compares quite well with that of 4.7 kcal/mole reported by Del Valle & Nickerson (1967b) for sodium chloride diffusion in fish muscle.

As was expected by virtue of its larger molecule size, the activation energy for glycerol diffusion was found to be significatively larger than that for sodium chloride. Note that Brown & Chitumbo (1973) reported an activation energy of 7.6 kcal/mole for glucose diffusion in a cellulose-water gel.

Acknowledgments

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A study of water activity lowering in meat during immersion-cooking in sodium chloride-glycerol solution. II. Kinetics of a_w lowering and effect of some process variables

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Summary

Beef slabs were dehydrated by immersion-cooking in a sodium chloride-glycerol solution of lower water activity (a_w) in the range of temperatures 30 to 85°C. The present work studies the kinetics of a_w lowering in beef as a function of various processing variables (solution temperature, sample thickness) as well as the effect of various pretreatments applied to the beef.

Introduction

In the previous paper (Favetto *et al.*, 1981) solute uptake data by beef slabs immersed in a sodium chloride-glycerol solution of low water activity ($a_w = 0.74$), have been analysed in terms of apparent Fickian diffusion. It has also been shown that the equilibrium condition between meat and external solution was given by the equality of salt and glycerol concentration in the water of external solution and in the muscle tissue water.

As has been discussed previously, beef loses water and gains solutes during immersion-cooking in a solution of low a_w. This process is complex since at the temperatures used here, water is removed from beef not only by a water activity driving force but also due to denaturation. From the point of view of water activity lowering one is interested not only in the net solute uptake, but in the resultant concentration of solute/s in the muscle tissue water, that is in a 'molality' in beef (Chirife, 1978). This work attempts to study the kinetics of 'molality' increase in beef as a function of various processing variables. The predicted behaviour in terms of water activity-time relationships is also experimentally determined and compared with the predictions.

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Experimental procedure

The equipment used as well as the experimental procedure have been described in a previous paper (Favetto *et al.*, 1981).

For one experiment freezing of beef was accomplished by immersion in a acetone dry ice bath and this is referred to as fast freezing (FF).

Some samples were also cut parallel to the fibres when directional effects were investigated.

A few experiments were also made using the flesh of tuna fish and pork.

In one experiment citric acid was added to the solution to lower the pH to 3.75.

Determination of water activity

The water activity of IM beef samples was determined using the 'a_w-Wert Messer' manufactured by firma Lufft, Stuttgart, West Germany. The instrument was carefully checked against various saturated salt solution in the a_w range of interest and a calibration curve was obtained at a constant temperature of 25°C. The instrument was operated following the procedure described in detail by Chirife & Ferro Fontán (1980).

The water activity of the expressible juice of fresh beef and beef samples not subjected to dehydration in salt-glycerol solutions but previously leached in pure water, was also determined. In this case, however, water activity determinations were derived from measured freezing points of beef juices. For this purpose the juice of beef samples (fresh or previously leached in pure water at predetermined temperatures and times) was extracted in a hand-operated juice extractor, centrifuged, filtered on filter paper and the clarified juice used for freezing point determination. Freezing points were determined on an Advanced Instruments Milk Cryoscope 4 L which was calibrated against sodium chloride standard solutions.

Results and discussion

The water activity of intermediate moisture beef (IMB) formulated with sodium chloride and glycerol, may be expressed (Ross, 1975; Chirife, 1978; Chirife *et al.*, 1980)

$$(a_w)_{IMB} = (a_w^0)_S \cdot (a_w^0)_G \cdot (a_w^0)_{SS} \quad (1)$$

where $(a_w^0)_S$ is the water activity value of an aqueous solution of sodium chloride at the same molality as in the water phase of beef, $(a_w^0)_G$ is the water activity value of an aqueous solution of glycerol at the same molality as in the water phase of beef, and $(a_w^0)_{SS}$ is a water activity term due to soluble solids naturally present in beef. Although it may be anticipated that this contribution to a_w lowering is small, it is considered here.

Non-solute solids (beef solids) may also contribute to a_w lowering. This

contribution, however, may be neglected above certain ratios of water/non-solute solids (Chirife, 1978).

The water activity is related to molality as follows,

$$a_w = \exp(-0.018\, m\varphi\nu) \tag{2}$$

where φ is the osmotic coefficient, m is the molal concentration and ν is the number of ions generated by each molecule of solute (in the case of glycerol $\nu = 1$). As osmotic coefficients are readily available in the literature (Robinson & Stokes, 1965; Chirife *et al.*, 1980) the $(a_w^0)_S$ and $(a_w^0)_G$ may be readily calculated if one knows the molal concentration of sodium chloride and glycerol in beef. For this purpose the molal concentration of each solute is expressed as

$$\frac{\text{moles of solute}}{\text{1000 g muscle tissue water}}.$$

This 'molality in beef' is directly related to the water activity but it is difficult to interpret since it is determined by two simultaneous transfer processes, namely the net uptake of solute and the diffusional and cooking losses of water.

Nevertheless the mass transport data in terms of 'molality' in beef can be adequately handled by the same standard techniques used for obtaining diffusion coefficients assuming unsteady state Fickian diffusion. It was found that molality in beef (either sodium chloride or glycerol) at different immersion times may be correlated using the simple expression derived from Fickian analysis of data (Favetto *et al.*, 1981)

$$m = kt^{1/2} \tag{3}$$
(NaCl or glycerol)

where m is the molal concentration in the muscle tissue water and k is an overall mass transport coefficient reflecting the rate of molality increase as a result of both solute uptake and water removal (diffusional and cooking losses). We have observed that this simple expression is able to correlate molality data for most of the time period investigated and not only in the initial stages as it occurs when analysing diffusional-controlled net solute uptake. Some deviations from eqn (3) were observed, however, at short immersion times (i.e. below about 20 minutes) for the higher temperature runs.

Figs 1 and 2 show the degree of applicability of eqn (3) to the experimental data at all temperatures studied and for most of the immersion time of interest. It can be seen that after the few initial minutes (data not plotted) straight lines may be drawn which permit evaluation of k, the overall mass transfer coefficient, which is of direct use for water activity prediction.

The effect of temperature on k, the overall mass transfer coefficient, can be expressed according to an Arrhenius type expression, as it is shown in Fig. 3. Resulting expressions for the effect of temperature are as follows,

$$k_{NaCl} = 15.4 \exp^{-1449/T} \tag{4}$$

$$k_{glycerol} = 56.4 \exp^{-1635/T} \tag{5}$$

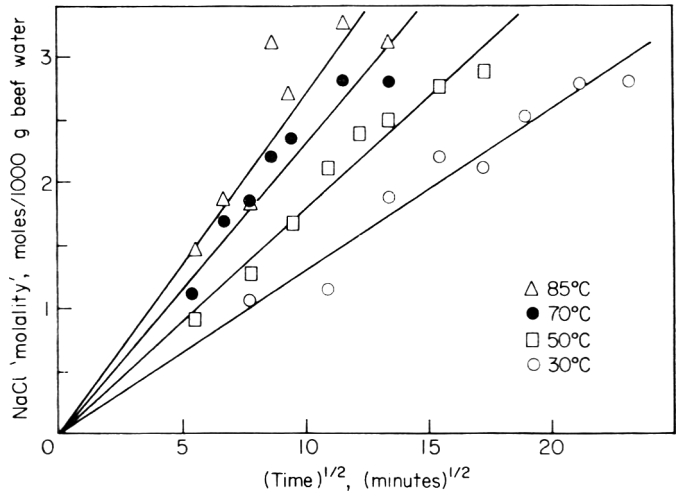

Figure 1. Correlation of sodium chloride 'molality' in beef (0.9 cm) with immersion time at various solution temperatures.

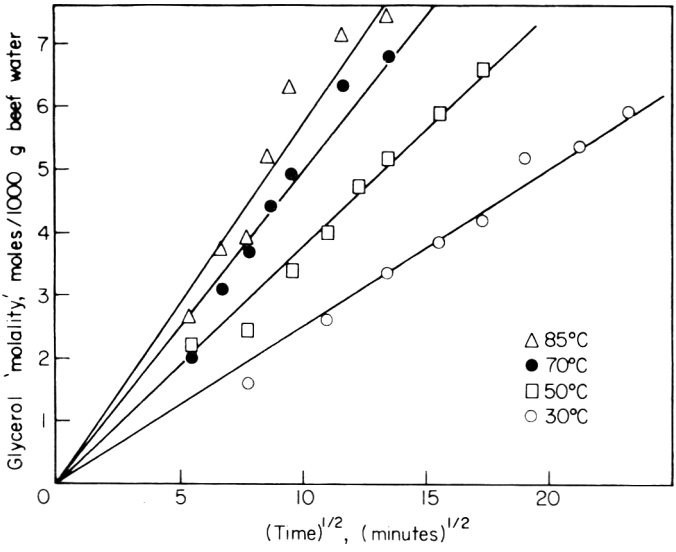

Figure 2. Correlation of glycerol 'molality' in beef (0.9 cm) with immersion time at various solution temperatures.

It is worth mentioning that one should not confuse the activation energy for a pure diffusion process, with some apparent 'activation' value which may be derived from the slopes of the straight lines shown in Fig. 3. The effect of temperature on the empirical coefficient merely reflects a summation of independent effects. Among them may be mentioned, (a) effect on the specific rate of solute diffusion, (b) effect on the water removal (cooking and diffusional effects) and (c) conformational changes in the beef affecting specific diffusion rates.

The effect of various pretreatments such as rate of freezing (prior to

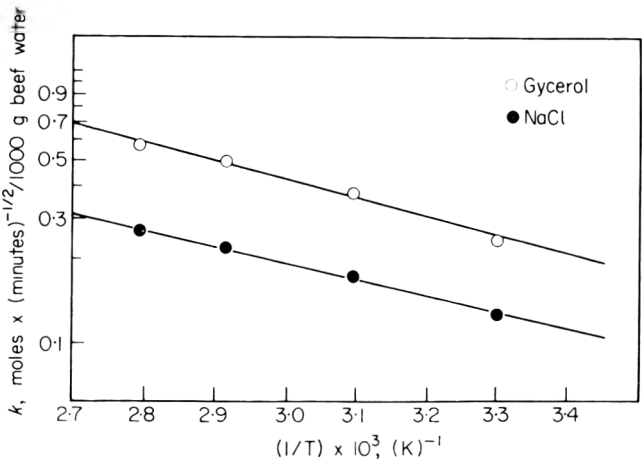

Figure 3. Effect of temperature on overall mass transfer coefficients *(k)* for sodium chloride and glycerol in beef (0.9 cm).

Table 1. Effect of various pretreatments and type of meat on overall mass transfer coefficients *(k)* for NaCl and glycerol in meats immersed in a solution of specified a_w at 30°C

	k_{NaCl} $\frac{\text{moles NaCl}}{1000\text{ g water}} \cdot \text{min}^{-1/2}$	$k_{glycerol}$ $\frac{\text{moles glycerol}}{1000\text{ g water}} \cdot \text{min}^{-1/2}$
Beef, slow frozen, ⊥	0.130	0.253
Beef, slow frozen, =	0.124	0.258
Beef, fast frozen, ⊥	0.130	0.262
Beef, slow frozen, ⊥ pH lowered	0.137	0.280
Pork, slow frozen, ⊥	0.130	0.253
Tuna fish, slow frozen, ⊥	0.136	0.289

⊥ : flow perpendicular to protein fibres.
= : flow parrallel to protein fibres.

immersion), direction of flow (parallel or perpendicular to protein fibres) and pH lowering on rate of molality increase was also studied. Furthermore, the influence of type of meat (beef, pork and tuna fish) on the kinetics of the process was also the subject of investigation.

For this purpose the experimental data were also plotted according to eqn (3) and straight lines were obtained in all cases. Least-squares analysis was used to calculate the values of k for each situation and the results are shown in Table 1.

It can be seen that the different pretreatments do not influence in a substantial manner the value of k. Regarding the type of meat it appears that the process is favoured in tuna fish as compared to the other meats, which can be explained by the less compact structure of fish muscle.

Fig. 4 shows that molality data for slabs of different thickness may be adequately correlated if one plots the molality as a function of $t^{1/2}/2l$.

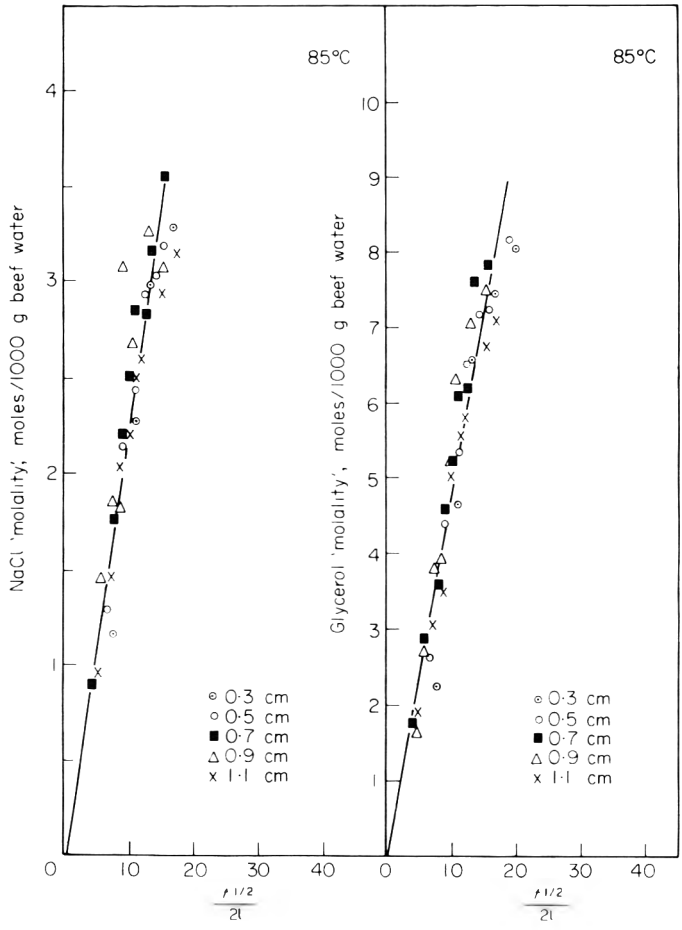

Figure 4. Correlation of sodium chloride and glycerol molality in beef with sample thickness.

Prediction of water activity

Having a model for correlating salt and glycerol 'molality' in beef as a function of immersion time one may now predict the course of a_w lowering by applying eqns (1) and (2), and simply neglect the small contribution of naturally present soluble solids. These soluble solids mainly consist of soluble proteins and amino acids, carbohydrates and inorganic salts.

This contribution to a_w lowering, however, can be also handled by simply measuring the freezing point of beef juice. It has been shown that a_w values of solutions having very high water activities (i.e. >0.98) may be accurately calculated from measured freezing points (Chirife *et al.*, 1981; Miracco *et al.*, 1981). Milk cryoscopes are available at present which permit a high degree of accuracy (Zarb & Hourigan, 1979; Nijpels *et al.*, 1980) in freezing point (and thus a_w) determinations. The relationship between a_w and freezing point

depression (θ_F) may be expressed by the following equation (Ferro Fontán & Chirife, 1981).

$$\ln a_w = 9.6934\times10^{-3}\theta_F + 4.761\times10^{-6}\theta_F^2. \quad (6)$$

Miracco *et al.* (1980) have shown that a_w values derived from freezing point measurements using a milk cryoscope may be safely expressed to four decimal places. Fig. 5 shows the variation of a_w in beef slabs as a function of immersion time in pure water at 30 and 85°C. It can be seen that the water activity of fresh beef is close to 0.991 and it increases during immersion due to leaching of soluble

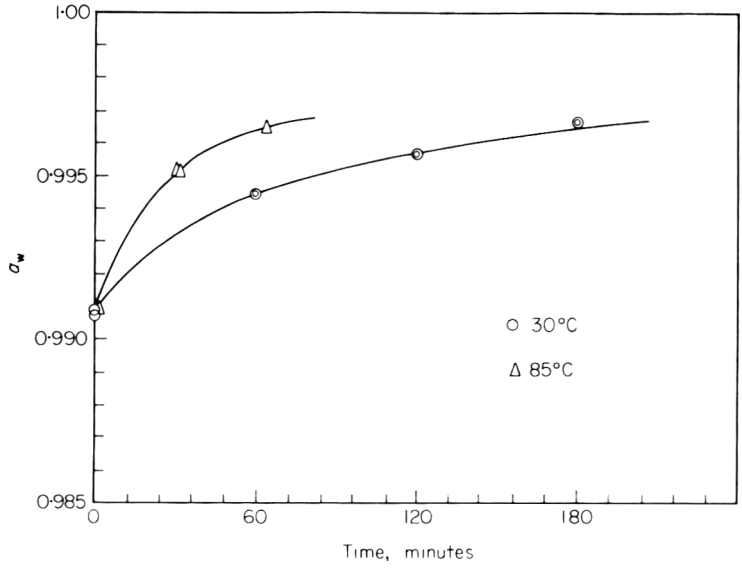

Figure 5. Evolution of water activity of beef slabs (0.9 cm thickness) during immersion in pure water at 30 and 85°C.

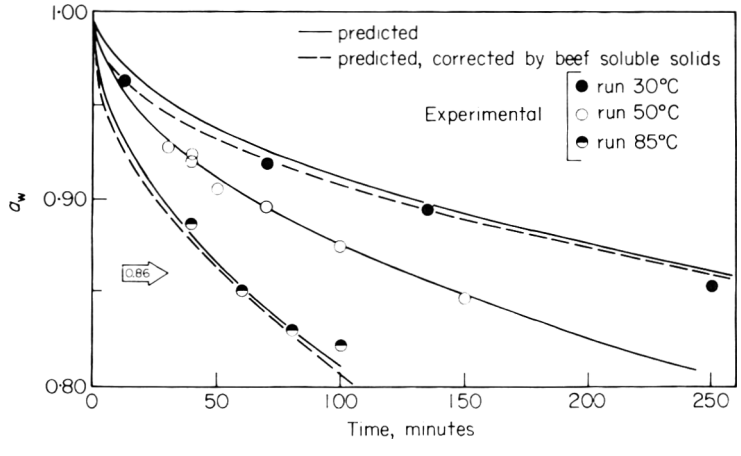

Figure 6. Comparison of predicted and measured water activity of beef slabs (0.9 cm thickness) during immersion in solution of low water activity at various temperatures.

solids. As was to be expected samples soaked in water at 85°C show higher water activities than those soaked at 30°C.

These results now permit a more exact prediction of a_w because the $(a_w^0)_{SS}$ term in equation (1) may be also approximately calculated from the curves displayed in Fig. 6.

Fig. 6 shows predicted and experimental a_w data in beef slabs (0.9 cm thickness) as a function of immersion time. Full lines represent values predicted without taking into account the small contribution of beef soluble solids. It can be seen that the agreement between predictions and reality is fairly good. It is noteworthy that a_w measurements of the IM beef slabs were performed at 25°C and not at the solution temperatures (30, 50 and 85°C). However, this is not important since it has been shown by Ferro Fontán & Chirife (1981) that the a_w's of aqueous solutions of sodium chloride and glycerol do not change significatively (at least for practical purposes) between 25 and 85°C.

Acknowledgments

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Simultaneous determination of oil and water content in olive husk by pulsed low resolution nuclear magnetic resonance

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Summary

A pulsed low-resolution NMR method for the oil and water determination in olive husk samples is reported. It is based on the determination of the solid to liquid ratio of the sample and on the detection of the spin-echo decay curve. This method is very fast and can be fully self-contained, as it does not require either to weigh and dry the sample or any correlation curve. It seems suitable for other oil containing samples, such as seeds. The way to simplify and speed up the procedure is also discussed.

Introduction

Nuclear magnetic resonance spectroscopy (NMR) has been used extensively for the determination of oil content in seeds. The technique is rapid and non-destructive, thus being of great importance in oilcrop improvement programmes. Several methods have been developed in the past years based on wide-line (Conway & Earle, 1963; Alexander *et al.*, 1967; Robertson & Morrison, 1979) and pulsed low-resolution NMR (Tiwari, Gambir & Rajan, 1974; Tiwari & Burk, 1980; Srinivasan, 1979). However, several problems still remain in the application of the technique to oil content determination. For a moisture content greater than 5% the presence of water in the sample usually affects the oil signal. In most methods the sample must be weighed and dried which reduces the rapidity of the measurement. The different hydrogen contents of the liquid and solid phases in the sample does not allow the direct correlation of the NMR result, given in terms of hydrogen content, with the oil content in terms of weight. Finally a correlation curve between the NMR response and the oil content is required. In a recent paper (Tiwari & Burk, 1980)

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a method that overcomes the need to weigh and dry the sample was developed which seems suitable for samples with low moisture content. A comparison of the different pulsed low-resolution NMR methods for the estimation of the oil content in seeds has recently appeared (Srinivasan, 1979).

The authors are now developing a new application of pulsed low-resolution NMR to oil determination. The method presented can be fully self-contained, as it is not necessary to weigh and dry the sample, nor construct a correlation curve; moreover the simultaneous determination of oil and water contents is obtained. In this paper the basis of the method and the results obtained in olive husk samples which contain a higher amount of water than seeds, are reported. Olive husk is the residue from the last expression of olives (*Olea europea*) in the industrial process. Besides a solid phase (predominantly cellulose), it consists of a liquid phase of oil and water. Olive husk is treated in order to obtain the so called olive husk oil. Evaluating large samples of different crops as well as comparisons with other techniques are now in progress and will be reported in a separate paper.

Method

In the following paragraphs we make a distinction between the content of a component (or a phase) in the sample in terms of hydrogen abundance (ha) and the content in terms of weight (g). Moreover, with 'specific hydrogen abundance' of a component (or a phase) we mean the grams of hydrogen per gram of component (or phase).

The present method is based on: (i) the measurement of the liquid content (ha) in the sample from the solid/liquid ratio (S/L) determination obtained by the free induction decay signal (FID); (ii) the measurement of the water and oil content (ha) in the liquid phase by the detection of the spin-echo (SE) decay curve. By inference of the FID and SE results the oil and water contents (ha) in the sample are obtained; (iii) the conversion of the (ha) contents to the (g) contents, by the 'standard addition method' that gives the ratio between the specific hydrogen abundance of liquid and solid phases.

(i) *S/L determination*

Due to the interaction between a static magnetic field and the magnetic moments of protons within the sample a net macroscopic magnetization, along the field axis, is built up. If the proton magnetic moments are excited by a short pulse of radiofrequency, a transverse component of the magnetization is originated. The decay of this component, called the free induction decay (FID), gives rise to a signal in a receiver coil wound around the sample. The magnetization decay is exponential and depends on a time constant T_2^* given by $1/T_2^* = 1/T_2 + \gamma \Delta H/2$ where T_2 is the transverse relaxation time, ΔH is the magnetic field inhomogeneity and γ is the proton magnetogyric ratio.

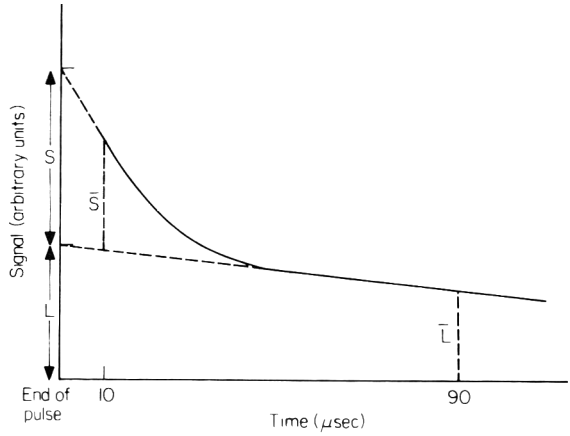

Figure 1. Schematic representation of the free induction decay (FID) of an olive husk sample.

In the present case T_2^* is the order of μsec for solid and of msec for oil and water protons respectively. A FID curve of an olive husk sample is given in a schematic way in Fig. 1. The fast decaying component is due to the total protons, while the slowly decaying component is due to the liquid protons only. The difference between the total curve and the extrapolated values of the liquid decay corresponds to the solid phase decay. The extrapolated values of the liquid and solid decays just at the end of the pulse (L,S) are proportional to the content (ha) of the two phases. Unfortunately, for technical reason, it is not possible to measure the signal at precisely the end of the pulse. The usual procedure is to measure the signal ($\bar{L}$, $\bar{S}$) at two different times, ~ 90 μsec and few μsec from the end of the pulse respectively, and multiply the $\bar{L}$ and $\bar{S}$ values by two coefficients, f_l and f_s respectively, in order to obtain the L and S value. The two coefficients account for the magnetization decay of the two phases between the end of the pulse and the time chosen for the measurement. Their values obviously depend on the time of the measurement and on the transverse relaxation times, T_{2L}^* and T_{2S}^*, of the two phases. f_l and f_s can be obtained by a fully digitized FID. The liquid content (ha) of the sample can then be expressed as:

$$X_l = 100 f_l \bar{L}/(f_l \bar{L} + f_s \bar{S}) \tag{1}$$

The technique of the solid to liquid ratio determination was first introduced by Van Putte (Van Putte & Van den Enden, 1973, 1974) and has been applied for the solid (or liquid) content determination of different foodstuffs (Brosio *et al.*, 1978, 1980). In most applications the liquid phase magnetization decay is so slow that, at the time of the measurement (~ 100 μsec), it is negligible and the f_l coefficient can be set equal to unity.

(ii) *SE measurement*

In a SE measurement a two pulse sequence 90°/τ/180°, separated by a time delay τ, is applied to the sample. The two pulses rotate the magnetization by 90°

and 180° respectively around an axis (x axis) normal to the static magnetic field (z axis); at a time 2τ an echo signal along the y axis is detected. As the solid proton signal is not detected in our SE measurement, the SE signal only depends on the liquid components in the sample (oil and water in the present case). The decay of the echo signal is not affected by the inhomogeneity of the magnetic field and depends on the transverse relaxation times T_2, of the different components in the sample, being of the order of 10–100 msec for oil and a few msec for water.

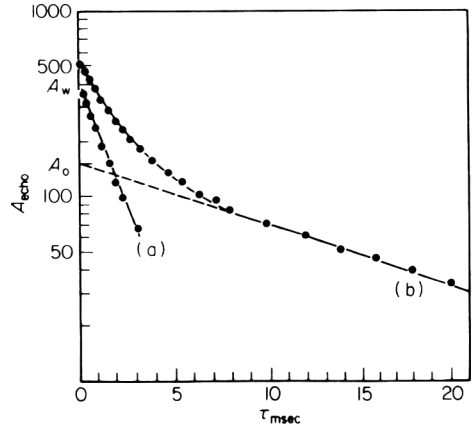

Figure 2. Spin echo (SE) decay curves of an olive husk sample (curve b). Curve (a) which represents the SE decay of the water component, was obtained by subtracting from the experimental curve (b) the spin-echo decay of the oil component (dotted line).

A semilogarithmic plot of the echo amplitude versus the time 2τ for an olive husk sample is reported in Fig. 2 (line (b)). The curve can be fitted by the sum of two exponentials. The slowly decaying component is due to the oil protons, while the fast decaying component (line (a) in Fig. 2), obtained by subtracting from the total curve the extrapolated values of the oil decay, is due to the water. The extrapolated values of the two exponentials at time $\tau = 0 (A_o$ and $A_w)$ are proportional to the oil and water content (ha) in the liquid phase. The oil (or water) percentages (ha) in the liquid phase is therefore given by

$$X_{o(w)} = 100A_{o(w)}/(A_o + A_w)$$

(iii) *Determination of the liquid content by weight*

By combining the results of the S/L and the SE measurements, the oil and water content in the sample (ha) can be obtained. Obviously the most important quantities are the oil and water contents by weight, W_o and W_w. The conversion between the (ha) and (g) values can be obtained by using the 'standard addition' method reported in detail in a previous paper (Brosio *et al.*, 1978). Suppose two phases A (liquid) and B (solid) are present in the sample (the symbols A and B will indicate the quantity in grams) and let α and β be the specific hydrogen

abundance of each phase, then the (ha) and the (g) contents of phase A in the sample, denoted as X and W in the following equation, will be related by:

$$W = X/[X + k(1 - X)] \tag{2}$$

where $k = \alpha/\beta$

The knowledge of the constant k allows conversion of the X to the W value. If k is not *a priori* known it can be determined by adding a grams of phase A to the total quantity (A + B) of the sample and measuring the new value of X, called X'. This leads to:

$$k = \frac{1}{m}\left[\frac{1}{1 - X'} - \frac{1 + mX}{1 - X}\right] \tag{3}$$

where $m = \dfrac{a}{A + B}$

The present case seems somewhat more complicated, as in the liquid phase two components, oil and water, are present. However the method described above could be generalized, provided that two separate adjoints of oil and water are added to the sample and the corresponding S/L and SE measurements are performed.

Luckily this more complicated procedure is not necessary in the present case, as the specific hydrogen abundance of oil and water is very similar being ~ 0.111. For such a reason the previous procedure is still applicable and a simple adjoint of one of the two liquid components is necessary. In this work the m quantity of eqn (3) was obtained by measuring the weight loss of the sample after desiccation in the oven for 90 min at 130°C.

Experimental

(i) *NMR measurements*

The measurements were made with a pulsed low-resolution NMR spectrometer operating at 20 MHz, model Minispec P20 of Bruker Spectrospin. The instrument is equipped with an analogue computer B-AC5 of the same company, that allows measurement of the FID values after a 90° pulse and the spin-echo values obtained by a $90°/\tau/180°$ pulse sequence. The 90° and 180° pulses were empirically determined by varying their respective widths (about 9 μsec and 18 μsec on our instrument). The time delay τ between the pulses was varied from 0.5 to 20 msec until the echo amplitude represented 10% of the initial signal. Each reported value is the average of 100 measurements. The time delay between two consecutive measurements was 3 sec to allow the nuclear magnetization to return to its equilibrium value. The olive husk samples were measured without any pretreatment in sample tubes of 10 mm external diameter. The probe temperature was 41 ± 1°C.

(ii) *Desiccation of the sample*

For desiccation the sample was reduced by mixing and successive division; the reduced sample was milled in order to obtain particles 0.335 mm of diameter. The desiccation was carried out on about 10 g in a previously tared dish by heating in the oven for 90 min at 130°C (± 1°C).

The olive husk samples were kindly supplied by Stotani-Meconi, Faleria (Viterbo) Italy (sample P) and Cooperativa Agricola Olearia Sannicolese A.r.l., Sannicola (Lecce) Italy (sample C).

Results and discussion

(i) f_s *and* f_l *values*

The f_s and f_l coefficients of eqn (1) were determined by measuring the FID height at different times, from the end of the pulse. The f_s and f_l values at 8.3 μsec and 91.0 μsec from the end of the pulse were 1.90 and 1.23 for pressed olive husk (P) and 1.79 and 1.23 for centrifugated olive husk (C).

In order to investigate the influence of the water content in the f_s and f_l values, the samples were dried in an oven at 130°C for 90 min, so that they were almost completely dried, and the new coefficients were determined. The results show that the f_s values do not appreciably change, while the two f_l values vary slightly being both 1.45. As the f_s and f_l coefficients, at a fixed time, only depend on the T_2^* relaxation time of the solid and liquid components, these results indicate that the T_2^* of the solid matrix is not affected by the moisture content, while the T_2^* of the liquid component depends on the amount of water in the sample.

(ii) *S/L determination*

The liquid percentage (ha) was determined by measuring the $\bar{L}$ and $\bar{S}$ values of eqn (1). The obtained results for the liquid percentage are $X_l = 56.7\%$ for the pressed olive husk sample and $X_l = 63.0\%$ for the centrifugated sample. In order to convert the liquid content X_l to the (g) content W_l the 'standard addition method' was applied. The samples were partially desiccated, the new liquid content was measured, the ratio (k) between the specific hydrogen abundance of the liquid and the solid phases was calculated by eqn. (3) and finally the liquid content by weight of the samples was calculated by eqn (2).

The results give $m = 0.2844$, k = 2.83, $W_l = 31.6\%$ for pressed olive husk sample and m = 0.4919, k = 2.41, $W_l = 41.4\%$ for centrifugated sample.

(iii) *Oil and water content in the sample*

By applying the SE method the oil and water content (ha) in the liquid phase, X_o and X_w, were obtained. As previously described, due to the comparable

hydrogen content of the two components, these quantities also represent the oil and water content (g) in the liquid phase. The oil and water content (g) in the sample can be calculated by $W_{o(w)} = W_l . X_{o(w)}$.

The results for pressed and centrifugated olive husk samples are reported in Table 1.

Table 1. Liquid content (W_l) by weight in the sample: oil (X_o) and water (X_w) content in the liquid phase; oil (W_o) and water (W_w) content by weight in the sample for pressed and centrifugated olive husk

	W_l	X_o	X_w	W_o	W_w
P	31.6	20.2	79.8	6.9	25.2
C	41.4	28.0	72.0	11.6	29.8

Conclusions

The method reported in the present paper seems suitable for the oil and water determination in different samples. So far it has been applied to olive husk, but in principle is valid for any other sample, like seeds.

It overcomes the need to weigh and dry the sample and to have a correlation curve between the NMR response and the oil content. We want to point out that the present method, in a routine experiment, is very fast; in fact only the S/L determination and the SE measurement are necessary; the other parameters involved in the computation namely f_1, f_2 and k can be accurately calculated for sets of samples of different crops and set at fixed values in the routine experiment. We are now performing a set of measurements to determine the influence of different crops on such parameters.

The S/L value can be accurately determined from the mean of ten measurements, requiring a total of 30 sec. In principle the SE measurement needs the full SE curve and extrapolation of the oil and water signal. That needs a longer time but it can be reduced by detecting only a few points, at least six, in order to fit the straight lines of the oil and water echo signals (lines b and a in Fig. 2). Actually this problem can be completely solved by using instead of a SE experiment a Carr–Purcell pulse sequence (Carr & Purcell, 1954) not available on our instrument. This experiment needs only one measurement to detect the whole curve and, if the NMR instrument is equipped with a computer, the whole procedure, namely the S/L determination, the conversion of the (ha) to (g) content, the Carr–Purcell experiment and the calculation of the oil and water content, requires a few minutes.

In some samples, like seeds, the orientation of the sample in the NMR tube is an important error source in the S/L determination. However it has been shown (Tiwari & Gambir, 1974) that, if the measurement is made at times less than 150

μsec, the signal is independent of the orientation. The S/L determination applied in the present paper needs measurements at times of ~ 90 μsec, well below 150 μsec; and so this method seems suitable for these samples.

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Cycled pressure and near-optimal pressure policies for a freeze dryer

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Summary

The performance of a freeze dryer operating under either cyclical pressure or near-optimal pressure control has been evaluated by numerical solution of a model describing the process.

The particular situation considered was where an interface temperature constraint prevented operation at a pressure which would minimize drying time. Comparison of the two processes showed that over the entire drying period the cycled process was marginally inferior to the near optimal scheme. Hence it appears that in industrial practice the near optimal pressure process, which requires a minimum of additional equipment, would be preferred.

Introduction

Freeze drying is an important industrial process for the drying of foodstuffs, surgical spare parts, pharmaceuticals and other materials whose internal structure or chemical composition are subject to thermal degradation.

The process consists of several stages, including initial freezing to a low temperature, followed by the freeze drying process proper which involves applying heat either by conduction or radiation to the frozen object and removing by sublimation the vapour (usually under a high vacuum), which then desublimes on to a frozen surface. Energy must therefore be supplied to freeze the material, sublime the ice, desublime the ice and also to maintain the vacuum. When compared to a conventional drying process, which removes the water in a single stage, it is clear that freeze drying is inherently more expensive, and that only a limited—but important—range of products can economically be produced in this way. Foodstuffs are, in terms of quantity, the most significant product produced by freeze drying.

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One of the major problems in freeze drying, which limits its potential, is the prolonged drying times encountered (Mellor, 1978, reports drying times as high as 30 hr). These long drying times mean that expensive equipment, such as vacuum chambers, pumps, etc., are monopolized for long periods and throughput is consequently low. The reason that drying times are prolonged is because, as drying proceeds, the ice layer is surrounded by a highly insulating dried layer, which severely limits the transmission of heat, especially under conditions of high vacuum. The insulating layer also offers resistance to mass transport, but often the rate limiting factor is the transfer of heat.

The thermal conductivities of several freeze dried materials have been determined experimentally by Harper (1962), and found to be a function of chamber pressure, conductivity increasing non-linearly with pressure in the range 10–1000 N/m^2. As chamber pressure increases, however, resistance to mass transfer increases, so that the process may be heat transfer limited at one pressure but mass transfer limited at another, higher, pressure. These conflicting effects have been shown experimentally by Sandall, King & Wilke (1967) to produce a pressure dependent drying rate which exhibits a maximum. Liapis & Litchfield (1979a, b) and Litchfield & Liapis (1980) and Litchfield, Liapis & Farhadpour (1980) have developed a rather rigorous mathematical model of the process, and used the calculus of variations to determine a control policy that minimizes overall drying time (rather than an instantaneous drying rate). This optimization was with respect to the control variables energy input and chamber pressure and is valid for the first 70–95% of water removal. Feedback control schemes have also been suggested (Liapis & Litchfield, 1980) to implement these strategies.

An alternative policy for reducing drying times has been suggested by Mellor (1967, 1978); Greenfield (1974) and Greenfield & Mellor (1974). This policy involves cycling the pressure between a high limit, where the thermal conductivity is high but mass transfer rates are low, and a low limit where heat transfer rates are low but mass transfer is high. During the high pressure period, the increased heat transmission and reduced mass transport serve to raise the ice layer temperature, although not to such a degree that melting would occur. If the melting temperature T_m were exceeded, the quality of the product would deteriorate, so T_m forms a constraint which must not be violated. Cyclical pressure freeze drying requires expensive equipment, but the advantages are believed to be sufficiently great to justify the additional cost. Mellor (1978) reports that drying times are reduced by up to 30% and CSIRO (Australia) has built several semi-industrial and pilot scale plants to implement cycled-pressure freeze drying.

Recently, Litchfield *et al.* (1980) have investigated the relative merits of cycled pressure and near-optimal constant pressure control policies. They found by numerical simulation studies that, although the cycled pressure system improved drying times when compared to a typical industrial low chamber pressure process, the system was marginally inferior to their near-optimal constant pressure process. They were also able to show from a simplified

analysis that, where the drying rate exhibits an attainable maximum with respect to pressure, the cyclical process is always inferior. As the cycled pressure process requires a higher capital investment, it was therefore concluded that in the case where drying times exhibited a minimum with respect to pressure, the constant pressure process would be preferred.

The purpose of this present work is to investigate the efficacy of the cycled pressure process when operation at a minimum is not possible, either because the minimum does not exist, or because the interface temperature constraint prevents operation at the pressure which would minimize the drying time. In the latter case, a constant pressure policy would cause the interface temperature to be controlled at the constraint, whereas the cycled pressure policy would cause the interface temperature to cycle (although at all times below the constraint). Since in this situation the higher pressure part of the wave will increase the heat flux passing through the dried layer, the possibility of an improvement in the overall drying time when compared to the near-optimal policy exists.

Mathematical development

Fig. 1 is a schematic of the material undergoing freeze drying. Radiant energy enters the surface at $x = 0$ and travels through an insulating dried layer to the ice interface. The energy then provides the heat of sublimation for the water vapour, or may accumulate in the ice layer. The vapour must flow through the dried layer to the surface countercurrent to the heat flow. The following assumptions have been made in the development of the mathematical model:

(1) Only one-dimensional heat and mass flows, normal to the interface and surface are considered.

(2) Sublimation occurs at an interface parallel to, and at distance X from, the surface of the sample.

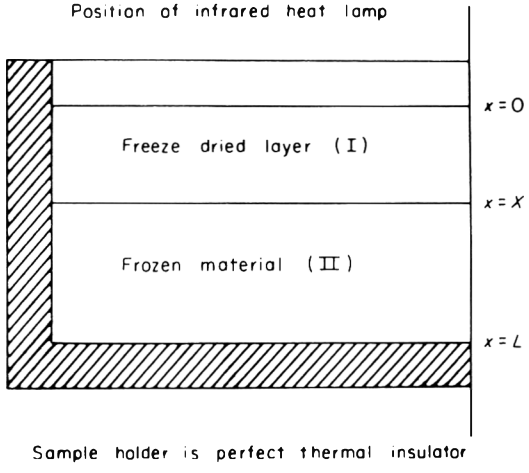

Figure 1. Schematic of material undergoing freeze drying.

(3) The thickness of the interface is taken to be infinitesimal.
(4) A binary mixture of water vapour and inert gas flows through the dried layer.
(5) At the ice interface, the concentration of water vapour is in equilibrium with the ice.
(6) In the porous region, the solid matrix and the enclosed gas are in thermal equilibrium.
(7) The frozen region is considered to be homogeneous, of uniform thermal conductivity, density and specific heat, and to contain as insignificant proportion of dissolved gases.
(8) The sides and the bottom of the slab are assumed to be perfectly insulated against the transfer of mass and heat.

An unsteady state energy balance in the dried region (I) yields:

$$\frac{\partial T_I}{\partial t} = -N_t \left(\frac{C_{pg}}{\rho_{Ie} C_{pIe}} \right) \frac{\partial T_I}{\partial x} + \alpha_{Ie} \frac{\partial^2 T_I}{\partial x^2}, \quad 0 \le x \le X. \tag{1}$$

The frozen region has a high thermal conductivity, and therefore only small temperature differences will exist within the ice layer. Under these circumstances, a lumped parameter model for the frozen region (II) can be employed:

$$\frac{d}{dt}\left((L - X) T_{II} \right) = \frac{\Delta H N_t}{\rho_{II} C_{pII}} + \frac{T_{II} N_t (C_{pg} + R_I)}{\rho_{II} C_{pII}} - \frac{k_{Ie}}{\rho_{II} C_{pII}} \frac{\partial T_I}{\partial x} \tag{2}$$

where $R_I = \left(\frac{\rho_{II} C_{pII} - \rho_I C_{pI}}{\rho_{II} - \rho_I} \right)$.

Although the time constants of eqn (1) are over an order of magnitude greater than those of eqn (2), eqn (2) was nevertheless retained because it is thought that the thermal capacity of the ice layer plays an important role in cycled pressure freeze drying (7, 8).

Using the assumptions in (4), the molar water vapour flux is given by

$$N_t = N_w = \frac{-C_2 D^0_{w,in} K_w (P_w|_{x=X} - P_{wc})}{(C_2 D^0_{w,in} + P_0 K_w) RTX + \frac{C_2 D^0_{w,in} K_w}{K_{ge}}} \tag{3}$$

Finally, the velocity of the interface is given by:

$$V = \frac{dX}{dt} = \frac{-N_t}{\rho_{II} - \rho_I}. \tag{4}$$

The boundary conditions for eqn (1) are:

$$q = -k_{Ie} \left. \frac{\partial T_I}{\partial x} \right|_{x=0} \text{ at } x = 0, \ 0 < t \le t_f \tag{5}$$

$$T_I = T_{II} \qquad \text{at } x = X, \ 0 < t \le t_f. \tag{6}$$

The initial conditions for eqns (1) and (2) are:

$$T_I = T_{II} = T^0 \qquad \text{at } t = 0,\ 0 \le x \le X \tag{7}$$

and for eqn (4) are:

$$X = X^0 \qquad \text{at } t = 0. \tag{8}$$

Computational

The mathematical model, eqns (1)–(4) and the boundary and initial conditions, eqns (5)–(8) are non-linear and require a numerical solution. Such a solution can be obtained by the method of orthogonal collocation (Villadsen & Michelson, 1978), which is known to be superior to finite difference techniques (Liapis, 1977). However, the moving boundary must first be immobilized by substitution of

$$\xi = x/X \tag{9}$$

and by the use of the relationships

$$\left.\frac{\partial T_I}{\partial x}\right|_t = \frac{1}{X}\left.\frac{\partial T_I}{\partial \xi}\right|_t;\quad \left.\frac{\partial^2 T_I}{\partial x^2}\right|_t = \frac{1}{X^2}\left.\frac{\partial^2 T_I}{\partial \xi^2}\right| \tag{10}$$

and

$$\left.\frac{\partial T_I}{\partial t}\right|_x = \left.\frac{\partial T_I}{\partial t}\right|_\xi - \frac{\xi}{X}\frac{dX}{dt}\left.\frac{\partial T}{\partial \xi}\right|_t. \tag{11}$$

Eqns (1) and (2), when transformed using eqns (9)–(11) and applying the method of orthogonal collocation become, respectively:

$$\frac{dT_{I,i}}{dt} = \frac{\alpha_{Ie}}{X^2}\sum_{j=1}^{N+2} B_{ij}T_{ij} - \frac{N_t}{X}\left[\frac{\xi_i}{\rho_{II}-\rho_I} + \frac{C_{pg}}{\rho_{Ie}C_{pIe}}\right]\sum_{j=1}^{N+2} A_{ij}T_{ij}\ i = 2, \ldots N+1 \tag{12}$$

and

$$q = -k_{Ie}\sum_{j=1}^{N-2} A_{1j}T_{1j}. \tag{13}$$

$T_{I,N+2}$ is calculated using eqns (2) and (6). Jacobi polynomials of degree 6 were chosen, with the two boundaries as external collocation points. The resulting six ordinary differential equations, together with the ice layer equation, were integrated by a third order semi-implicit Runge-Kutta method developed by Michelson (1976). The energy input policy was the optimal one developed by Liapis & Litchfield (1979a), and the chamber pressure could be close to the optimal, or could cycle, as the calculation proceeded. Computation times varied between 5 and 15 minutes on the powerful Surrey multi-PRIME 750 multi-access computer network.

Results and discussion

The model equations were solved for turkey meat slabs, with an interface melting constraint, T_m, of −20°C and a surface temperature constraint of 60°C (see table 1 for a full list of data used). With this value of T_m, the optimal pressure and energy policies hold the interface and surface temperatures at their constraints after an initial transient period. The pressure required to maintain these conditions is, however, below the value that would minimize the drying time if the constraint were not imposed. Therefore the cyclical pressure policy can be examined in a situation where an increase in pressure would lead to an enhanced transfer of heat, and the possibility of a reduction of drying time arises. Such pressures can only be introduced in a cyclical fashion, however, because a non-cyclical policy would cause the interface temperature constraint to be exceeded.

Table 1. In the freeze-drying calculations the following parameter values were used

Parameter	
P_{wo} N/m^2	5.2668
L m	6×10^{-3}
q Kw/m^2	10.107
X^0 m	6×10^{-5}
ρ_I kg/m^3	333
ρ_{II} kg/m^3	1133
ΔH kJ/kg	2791.2
C_{pI} kJ/kg K	2.93076
C_{p2} kJ/kg K	1.9678
C_{pg} kJ/kg K	1.6747
T_m K	263.84
T_s K	333.15
C_2	0.6
$D^0_{w,in}$ kg m/s^3	$8.729 \times 10^{-7} (T_1 + T_x)^{2.334}$
K_w m^2/s	$1.6395 \times 10^{-4} (T_1 + T_x)^{0.5}$
T^0 K	241.8
K_{ge} gmol/Ns	0.0524
$k_{le} = 85 \times 10^{-6} (1 - 0.325 \exp(-5 \times 10^{-3} P))$ kW/m K	

The cyclical pressure policy was introduced as a triangular wave, with a peak of 250 N/m^2 and a period of 60 secs, as shown in Fig. 2. This particular wave was chosen because it just avoided violating the interface temperature constraint during the early part of the drying sequence. This variation caused the interface temperature and the heat and mass fluxes to oscillate. The interface temperature cycled over about 1.5°C, creating interactively changes in the temperature driving force for heat transfer, and in the interface water vapour partial pressure and hence the mass flux driving force. The pressure wave, of course, caused changes in the resistances to both heat and mass transfer.

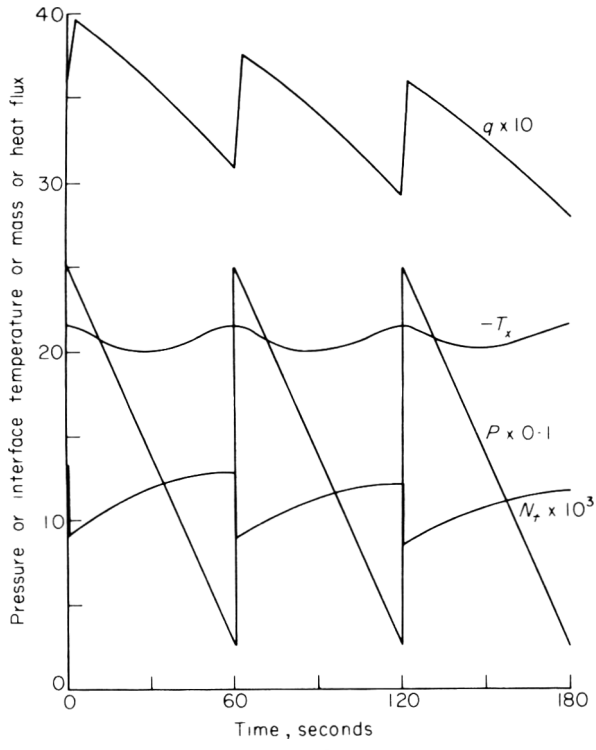

Figure 2. Showing the responses of the process undergoing cyclical pressure freeze drying. The units are: P, N/m^2; q, kW/m^2; T_x, °C; N_t, kg/sm^2.

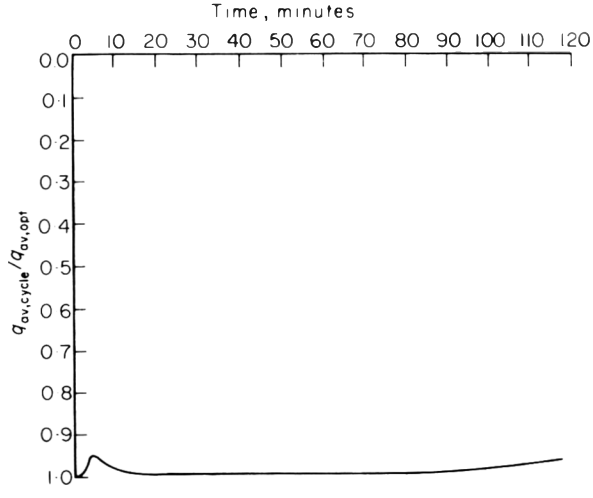

Figure 3. Ratio of heat input during cyclical pressure freeze drying to heat input during near-optimal pressure freeze drying.

However, as is shown in Fig. 3, cycling the pressure does not, in fact, improve upon a near optimal policy; initially, the heat input using cycled pressure drops to about 0.96 of the optimal, later recovers to 1.0, and then falls again. However, the apparent improvement in the middle of the run is due to the fact that the graph shows the cyclical policy in an optimistic light, as time is the

independent variable and the interface has receded further with the optimal policy. The fall in the relative heat input towards the end of the run is caused by the increasing resistance to heat transfer in the dried layer, so that the ice temperature cycles somewhat below the interface constraint: this would indicate that, if a cyclical policy were employed, its amplitude and duration should change as drying proceeds.

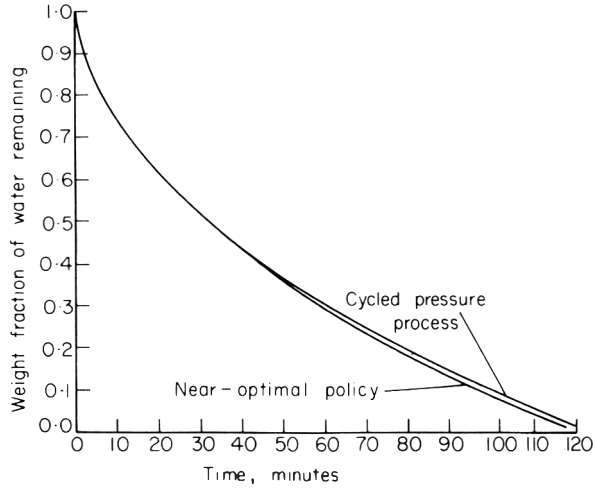

Figure 4. Drying curves for cyclical pressure and near-optimal pressure freeze drying.

Finally, in Fig. 4, the drying curves for the two processes are shown. Here, the optimal policy slowly but surely dries the material faster than the cycled pressure process. Final drying times are 118.2 and 121.4 min for the optimal and cycled processes respectively.

Conclusion

Cyclical pressure and near-optimal pressure freeze drying processes have been compared in a situation where operation at an optimal point was not possible due to an interface temperature constraint.

Calculations of average energy input and of drying times showed that the cycled process was marginally inferior to the near-optimal process; at no time during the entire run did the cycled pressure process prove superior. Hence it is concluded that a near-optimal pressure process would be preferable industrially to the more capital intensive cycled pressure process.

Nomenclature

$A_{i,j}$: elements of the discretization matrix A of the differential operator $\partial T_I/\partial \xi$

$B_{i,j}$: elements of the discretization matric B of the differential operator $\partial^2 T_1/\partial \xi^2$

C_{pI}, C_{pII}, C_{pIc}: heat capacities

C_1: constant dependent only upon structure of porous medium and giving relative Knudsen flow permeability

C_2: constant dependent only upon structure of porous medium and giving the ratio of bulk diffusivity within the porous medium to the free gas bulk diffusivity, dimensionless

$D_{w,in}$: free gas mutual diffusivity in a binary mixture of water vapour and insert gas

$D^0_{w,in}$: $D_{w,in} P$

k: thermal conductivity

K_{ge}: external mass transfer coefficient $= \dfrac{D_{w,in}}{RT|Z_c|}$

K_w: Knudsen diffusivity, $(K_w = C_1 \sqrt{RT/M_w})$

L: sample thickness

M: molecular weight

N: number of internal collocation points

N_t: total molar flux $(N_t = N_w + N_{in})$

N_w: water vapour flux

N_{in}: insert gas flux

P: total pressure in dried layer

P_T: total pressure in drying chamber

P_o: drying chamber pressure at surface of dried layer

P_w: partial pressure of water vapour

P_{wc}: partial pressure of water vapour at the condenser

P_{wx}: partial pressure of water vapour at interface, 133.32 exp $(23.9936 - 6138.198/T_x)$, N/m^2

q: radiant energy flux

R: universal gas constant

$T°$: sample temperature at t = 0

T: temperature

t: time

V: velocity of interface

X: position of frozen interface

Greek symbols

α: thermal diffusivity

ξ: dimensionless distance $\dfrac{x}{X(t)}$

ΔH: enthalpy of sublimation

ρ: density

Superscripts

^{0}:	initial value at time zero
Λ:	time averaged value

Subscripts

av, cycle:	average value over period of cycle
av, opt:	average value over same period of time
e:	effective value
f:	final value
g:	gas
m:	melting temperature
w:	water vapour
X:	interfacial value
0:	surface value
I:	dried region
II:	frozen region

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Microwave attenuation of frozen *Nephrops norvegicus*

M. KENT AND G. D. STROUD

Summary

The effects of chilled storage on the microwave attenuation of frozen *Nephrops norvegicus* are studied in relation to changes in various constituents of the flesh. Correlations are sought between attenuation and nitrogen content as well as solids and fat contents. The study is unable to detect regular seasonal variations in these constituents or in microwave attenuation but significant correlations are obtained with changes occurring during chilled storage due to uptake of water.

Introduction

When a plane polarized electromagnetic wave at microwave frequencies is passed through blocks of frozen fish it has been shown that a linear relationship exists between the attentuation of the wave and the unfrozen water content of the block (Kent, 1977). This relationship has been exploited both in the study of enthalpy changes in frozen systems (Kent, 1977) and in the study of changes with time of unfrozen water content (Kent, 1975). It was considered that yet another application might be in the investigation, and possible measurement, of the variations of moisture content occurring in frozen foods.

At the time the experiment was mooted a general study of the seasonal and storage variation of the composition of scampi flesh *(Nephrops norvegicus)* had begun. It was decided therefore to use the same samples as a model system and to study the results of both seasonal changes and conditions of storage prior to freezing. It has been pointed out by Kuprianoff (1958) that the mass of unfreezable water per unit mass of non-aqueous material is always constant for many foods whatever concentration of water is present in the initially unfrozen tissue. At temperatures just below the freezing point minor variations in the unfrozen water content would be expected due to fluctuations in salt content from sample to sample and consequent changes in eutectic behaviour but this

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would not be the case for unfreezable water at lower temperatures. Any dilution of the protein fraction by, for example, the uptake of water from melting ice during chilled storage, would be expected to reduce the total fraction of unfreezable water accordingly. This in turn would result in a lower attenuation per unit mass being observed at temperatures low enough to have only unfreezable water present. The study to be described took place over a period of 2 years and parallel measurements were made of total water content, fat, total nitrogen and non protein nitrogen.

Materials and methods

Material

The samples of *Nephrops norvegicus* were taken at approximately monthly intervals and were obtained from landings made at Pittenweem, Fife in Scotland. The tails were separated and stored, unpeeled, in ice after which measurements were made at 1, 4, 7, and 11 days of chilled storage.

Microwave attenuation measurements

Samples consisting of fifteen shelled *Nephrops* tails were minced in a Moulinex mincer and approximately 15 g was packed into a polyethylene sample cell as shown in Fig. 1. The quantity used was weighed after each run being removed conveniently in the frozen state from the cell. After filling the cell it was inserted into a suitable length of *X*-band (10 GHz) waveguide (Fig 1). Power was coupled into and out of the waveguide by coaxial line through coaxial-to-waveguide transformers at each end. These being watertight allowed the immersion of the whole assembly in a low temperature bath maintained at $-20°C \pm 0.5°C$. The coaxial line allowed flexibility in positioning the system. It was expected that freezing at $-20°C$ would require a long equilibration time as the amount of 'unfreezable' water has been observed to be time dependent and some in fact eventually freezes (Kent, 1975). To avoid this problem the samples were 'annealed' by freezing first at $-40°C$ then rewarming to $-20°C$. In this way it was expected that the unfrozen water corresponding to the higher temperature would be stable and equilibrated. The attenuation or 'insertion loss' of the waveguide plus sample was then measured using the classic vector-balance null method of Buchanan (1952). This is a bridge technique in which phase and amplitude matching of the wave through the sample with that of a reference wave through another arm of the bridge is achieved by adjustment of suitably calibrated variable components. The components, a variable phase shifter and a variable attenuator, are manipulated to yield a null output from the bridge. The technique is well known in the field of dielectrics and needs no further elaboration here since no innovations were made. Normally such a method would be used for elucidating ϵ' and ϵ'', the real and imaginary components of

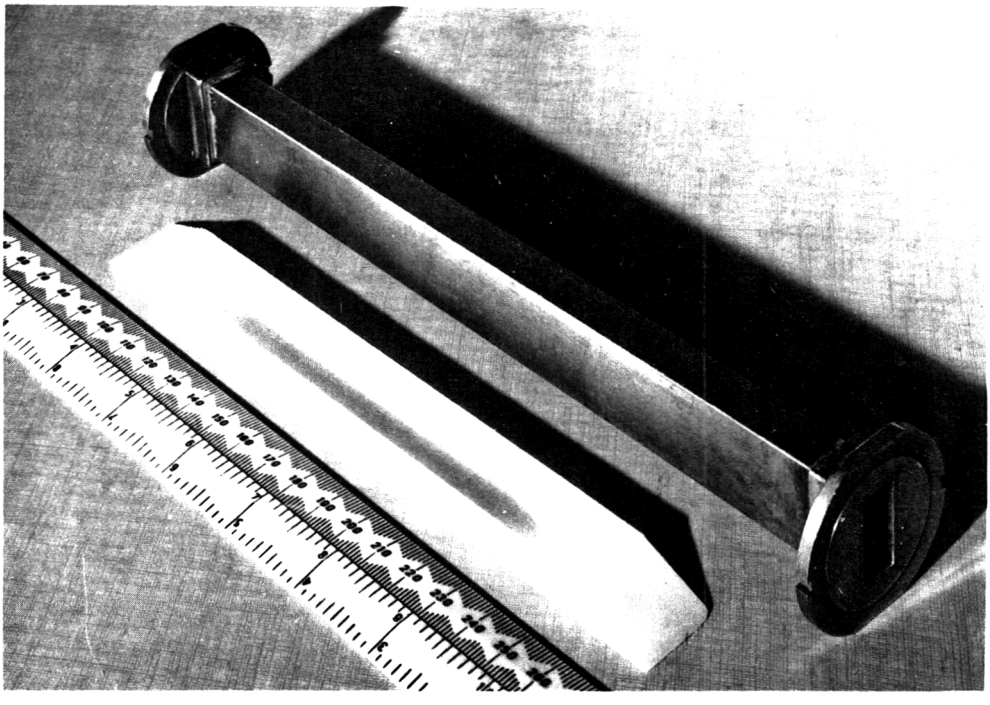

Figure 1. *X*-band waveguide and sample cell.

the complex dielectric permittivity of the sample. However the conditions of this experiment with the sample held within the polyethylene cell do not allow such a calculation to be made simply and in any case only the attenuation is of interest. When the sample had been weighed, this attenuation was then expressed as an attenuation per unit mass in units of decibels per gram after subtraction of the attenuation of the empty cell. The assumption was made that the measured attenuation was a linear function of mass within the limits of variation expected. The accuracy of these measurements was estimated to be ± 1.0 dB or typically $\pm 4\%$ of the measured attenuation.

Moisture

Moisture contents were determined by vacuum drying three weighed samples of approximately 4 g each for 24 hr at 70°C. These were cooled in a desiccator over P_2O_5 prior to weighing.

Fat

Fat contents were determined on duplicate samples by the method of Bligh & Dyer (1959) as modified by Hanson & Olley (1963).

Nitrogen

Total nitrogen (TN) determinations were carried out by the micro-Kjeldahl procedure, the digestion stage following the method established by MacKenzie & Wallace (1954).

Non-protein nitrogen (NPN) was determined after precipitation of the protein by homogenizing the sample (~ 0.8 g) with 10% trichloroacetic acid (80 ml) and carrying out the digestion on an aliquot (10 ml) of the protein-free filtrate.

Determinations were carried out on triplicate samples of the mince for both the TN and NPN. Protein nitrogen (PN) was established by simple subtraction.

Results and discussion

The seasonal fluctuations observed in the various measurements are shown in Figs 2 and 3. The results in Fig. 2 refer only to fresh samples kept 1 day on ice. In Fig. 3 the results are presented for both 1 and 7 days in ice. Similar parallel fluctuations occur for the other periods of storage but these are not shown for the sake of clarity. Other aspects of the composition and behaviour of *Nephrops* will be found in Stroud & Early (unpublished).

Figure 4 shows the attenuation per unit mass plotted versus total solids content. Total solids are of course derived by subtracting the moisture content from 100. The data for both seasonal and storage variation are combined in this

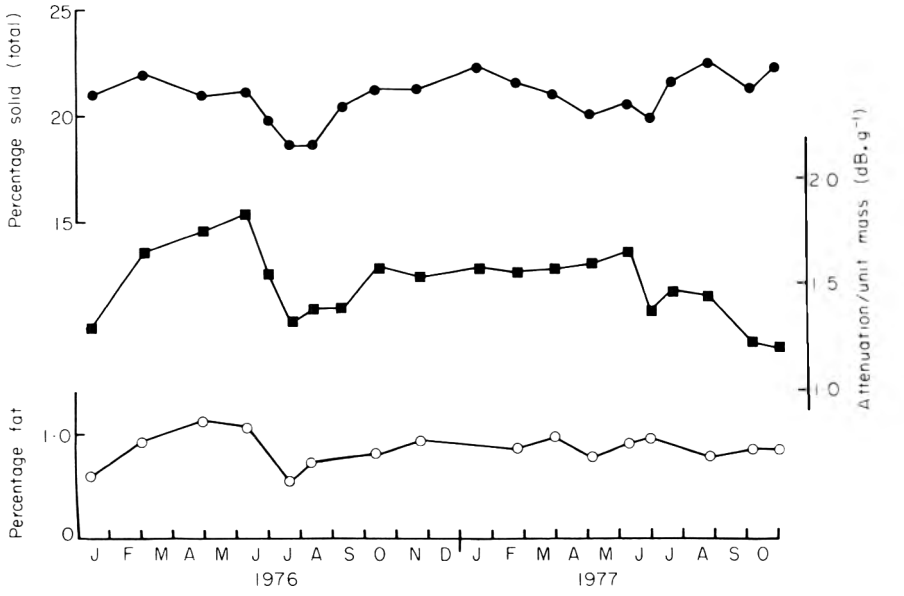

Figure 2. Seasonal variation in *Nephrops norvegicus* of (a) solids content, (●) 1 day on ice: (b) fat content, (○) 1 day on ice; (c) attenuation per unit mass, (■) 1 day on ice. Percentages refer to wet basis.

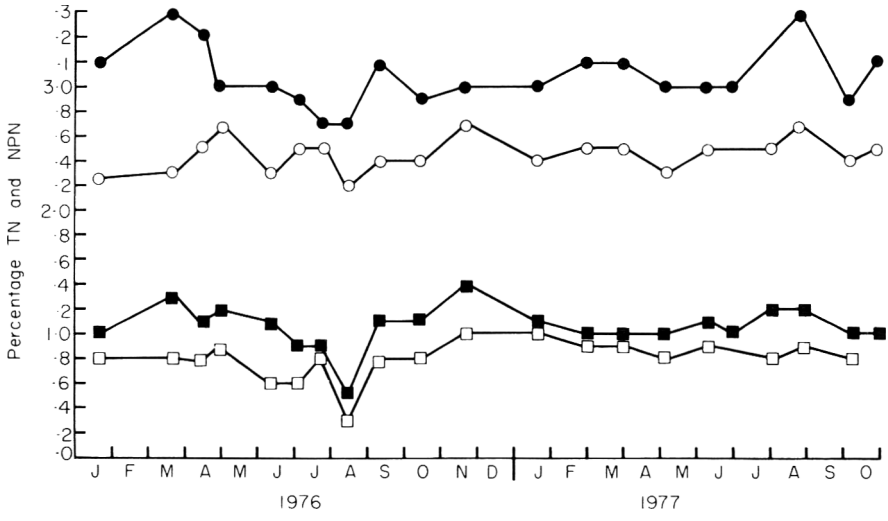

Figure 3. Seasonal variation in *Nephrops norvegicus* of (a) Total nitrogen (TN) content, (●) 1 day on ice and (○) 7 days; (b) Non-protein nitrogen (NPN) content, (■) 1 day on ice and (□) 7 days.

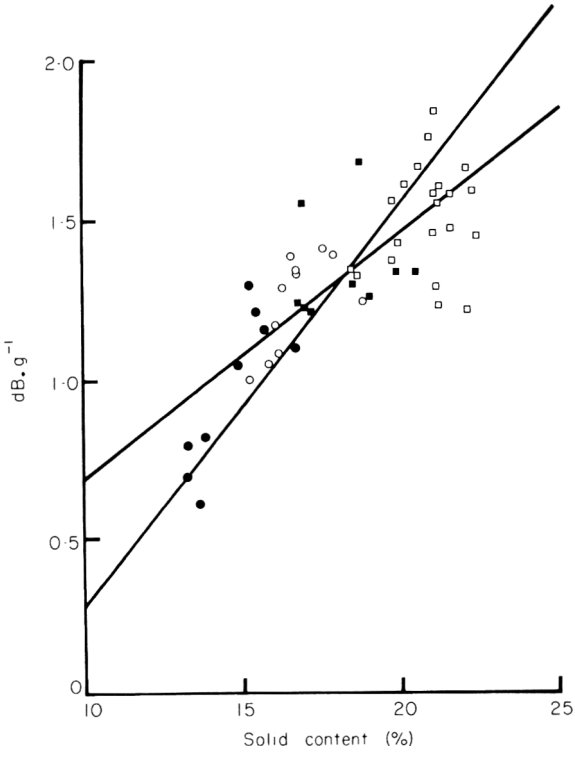

Figure 4. Attenuation per unit mass versus solids content (% wet basis) for various storage times in ice, (□) 1 day, (■) 4 days, (○) 7 days and (●) 11 days. (a) Regression of attenuation on solids (dB . g^{-1}) = (0.0759±0.009) (%) −0.0725±0.167; (b) regression of solids on attenuation (%) = (7.926±0.939) (dB . g^{-1})+7.874±1.266.

figure but are identified as indicated. The two lines shown are for the alternative regressions of attenuation on solids or of solids on attenuation. The latter is of course to be used for the estimation of the solids content from an attenuation measurement. Statistical analysis shows that this would only be possible to within ±3.4% solid or water content but this is a pessimistic result arising from the large scatter at the higher solids content.

Regression analysis of all these data is summarized in Tables 1 and 2. In general, with the exception of fat content, there is no significant correlation between attenuation and the other variables for freshly caught samples i.e. no correlation of seasonal variations. This is probably due to the small range over which all of these components seem to vary throughout the period of the experiment. The exception to this seems to have been during the summer of 1976 when a very large increase in water content led to obvious dilution of all the constituents with a consequent diminution of attenuation in the frozen state. For both TN and PN, significant correlation is only obtained when all the data are pooled. For fat, as for solid content, significance of correlation increases when all the data are combined to give a wider variation of each constituent.

Table 1. Regression analysis of attenuation per unit mass ($\alpha \cdot g^{-1}$) versus (a) Protein nitrogen, (b) Total nitrogen, (c) fat, (d) solids for different times of storage at 0°C

	Protein nitrogen	Total nitrogen	Fat	Solid
$\alpha \cdot g^{-1}$ 1d	n.s. (19)	n.s. (19)	* (16)	n.s. (21)
4d	n.s. (1)	n.s. (1)		n.s. (9)
7d	n.s. (11)	n.s. (11)	n.s. (8)	* (11)
11d	n.s. (1)	n.s. (1)		** (9)
All data	* (32)	*** (32)	** (24)	*** (50)

n.s. no significant correlation.
* significant at 5% level.
** significant at 1% level.
*** significant at 0.1% level.
No. of data points indicated in brackets.

Table 2. Regression parameters for attenuation per unit mass versus (a) Protein nitrogen (PN), (b) total nitrogen (TN), (c) fat, (d) solids

Regression	Slope	s.e.	Const.	s.e.	Correlation coefficient
PN (all data)	0.475	0.183	0.511	0.337	0.428
TN (all data)	0.537	0.093	−0.105	0.258	0.732
Fat (1 day)	0.933	0.329	0.689	0.292	0.633
(all data)	0.867	0.230	0.721	0.190	0.644
Solid (7 days)	0.0873	0.0374	−0.213	0.626	0.614
(11 days)	0.169	0.0425	−1.51	0.626	0.832
(all data)	0.0759	0.009	−0.0725	0.167	0.776

This means that the dilution occurring due to uptake of water from the melting ice, i.e. storage variation, is easier to observe by the method of microwave attenuation than the seasonal variation. This is because such water is readily frozen leaving a smaller fraction of unfrozen water. The supposition that this 'absorbed' water is not tightly bound and is available for freezing is supported by measurements made by Stroud & Early (unpublished) which show an increase in drip-loss with increasing storage time. Also the increasing significance of correlation with seasonal variation as the storage time increases (Table 1) is probably due again to the fact that the changes in water content seen for a given storage time are changes mostly in this 'absorbed' water. Otherwise the nature of the seasonal variations is not clear and no light is thrown upon it by these measurements.

The other highly significant correlation observed is between attenuation per unit mass and fat content. However no physiological explanation can be attached to this since it is largely a result of dilution of all the solid matter although the correlation remains significant at the 5% probability level for freshly caught samples i.e. there is a correlation with the seasonal fluctuations.

Conclusions

Attempts to determine moisture or solid content of frozen scampi flesh by a microwave attenuation method yields only an absolute accuracy of $\pm 3.4\%$ constituent content (95% confidence). For typical solids content around 20% (wet basis) the relative accuracy is $\pm 17\%$ though for samples stored for longer than 1 day on ice this is an overestimate of the size of the error. The observation that seasonal changes of moisture content lie well within this band means that even if the unfreezable water-to-solids ratio remains the same it would not be possible to use microwave attenuation to measure these seasonal changes. On the other hand the changes due to uptake of water during chilled storage are more easily seen due to the much larger quantities involved.

The hypothesis that the unfreezable water would be a constant fraction of the protein or solids content could not be verified from seasonal variations alone due to the large scatter of the results. As noted only the addition of water produced significant effects and this may be regarded simply as a result of dilution.

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A comparative study of consumer preference for blue whiting *(Micromesistius poutassou)* and whiting *(Merlangius marlangus)* before and after incorporation into products

MORAG HAMILTON AND R. BENNETT*

Summary

Good quality blue whiting and whiting fillets were compared by taste panels before and after incorporation into five products. In the plain cooked form, blue whiting was liked by assessors but whiting was preferred marginally. In product form, both fish were well liked and there was no significant preference for either species.

Introduction

During the past decade the quantities of the more popular white and fatty fish landed at UK ports have decreased considerably and the need to utilize the less accepted species for direct human consumption has become increasingly important. A considerable amount of attention has been focused on the blue whiting, a gadoid which is abundant in the North-East Atlantic. The estimated spawning stock of 5–15 million metric tonnes would allow a yearly catch of at least one million metric tonnes without depletion. (Torry Research Station, 1980).

Blue whiting is closely related to whiting and it is similar to whiting and cod in taste and chemical composition (Dagbjartsson, 1975). It is small (*ca* 30 cm) and has a rather unattractive appearance and is unlikely to appeal to the consumer if supplied directly as whole fish or fillets. The size and soft body of the fish cause technical problems in machine filleting, it spoils relatively quickly and can only be harvested in large quantities when it shoals for spawning between March and May. The majority of present catches go for fishmeal and if it is to be exploited for direct human consumption the most probable method would be by the supply of deep frozen fillet or mince blocks to the processing industry.

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The White Fish Authority has conducted a number of consumer trials with blue whiting and has shown it to be generally acceptable in most cooked forms but not fresh on the fishmongers slab (Wray, 1977). The authors have carried out a number of investigations with a variety of white fish species which indicated that consumers could distinguish between species by taste (Hamilton, 1980). Whiting rated particularly well in these tests for flavour, texture and appearance and was very acceptable to assessors. It is often marketed as a relatively small fish and has an obvious similarity in name to this new species, blue whiting. It was, therefore, decided to carry out a series of direct comparisons between the two species and to investigate the effects of incorporating them into products.

Materials and methods

Blue whiting and whiting were supplied as frozen fillets by the Torry Research Station, Aberdeen. The blue whiting had been caught and handled under experimental, not commercial, conditions. It had been caught in February 1980 and was frozen at sea as 50 kg blocks within hours of catching. It had been stored at −30°C for 6–8 months before use and was thawed at ambient temperature and hand-filleted immediately before using in the experiments.

Storage under these conditions for this period cause no detectable loss of quality. The whiting had been selected for quality and freshness using the raw odour scale devised by Shewan *et al.* (1953). The fish had all been scored at 8 or 9 on the scale which in effect means that they were the equivalent of high quality fish which had been stored in ice for 2–4 days. The comparisons were therefore between fish of excellent quality for both species. The fish were used in sensory tests and were incorporated in five different recipes chosen as being those most frequently used in the home (steamed in parsley sauce, fried in batter or crumbs, fish cakes and fish pies).

Both species were prepared and cooked by identical methods. After thawing, the top and tail ends and the side edges of the fillets were removed. For fish cakes and fish pies the whole middle section was steamed between two plates and then flaked to the same degree. The samples for steaming in sauce and frying in batter and crumbs were trimmed down from the centre section to produce two samples from each fillet. The methods of cooking varied with different recipes but were standardized so that both fish were cooked identically. Steamed samples were cooked without added condiment on covered plates held over steam and were cooked to the same degree. Frying was carried out for the same time at the same temperature (185°C) in thermostatically controlled deep-fat friers. The fish pies were baked in the same fan-assisted oven at 150°C for the same length of time.

Standard recipes were used for incorporating the fish into products (Hammond, 1975). Where samples included sauce, batter or mashed potato, one quantity was prepared and was divided between the samples. Assessors were not provided with extra seasoning for use during tasting.

The paired preference and identification (or matching) tests were carried out on unseasoned, unmashed plain steamed samples.

Taste panel methods

In the rating tests the assessors awarded ratings to each sample using a seven-point hedonic rating scale. The numerical scores corresponded to degrees of liking (7, like very much; 4, neither like nor dislike; 1, dislike very much). Only unit marks were allowed.

In the paired preference test two plain samples were presented simultaneously to assessors who were asked to state which sample they preferred. They were not allowed to make a 'no preference' response.

In the identification test, panellists were presented with three named and different samples of plain steamed fish and two coded samples which they had to match with the named samples. The coded samples could be two different species or two samples of one species

Taste panels were conducted in booths and assessors were allowed to rinse their mouths freely with tepid water. None of the assessors were trained in fish tasting.

Presentation of samples

The cooked samples were served immediately in identical disposable white polystyrene containers, except for fish pies which were presented in the individual 5-cm foil containers in which they were cooked. The samples were coded with two-digit random numbers taken from random number tables.

The paired preference test was balanced so that each sample appeared in each test position an equal number of times. The identification test was partially balanced so that the nine possible arrangements of coded pairs were used as near to an equal number of times as was possible.

In the rating tests samples of each product were presented successively in random order (i.e. the pairs of each product were presented randomly). No attempt was made to treat the five pairs of products as ten individual samples.

Results

The results of the paired preference test between whiting and blue whiting showed fifteen out of a total of twenty-four assessors preferred whiting to blue whiting but the result is not significant statistically (significance level 21%).

Table 1 shows that the assessors were able to identify the fish species with considerable accuracy.

Table 2 summarizes the results obtained when fifty-eight assessors were asked

Table 1. Results of an identification test using thirty-six panellists

	Species		
	Whiting	Haddock	Blue whiting
Number of coded samples	20	28	20
Number of correct matches	17	25	17
Percentage of correct matches	85	89	85
Significance of results	**	**	**

**Significant at 0.1% level.

Table 2. Results of a rating test carried out by fifty-eight assessors on five products using a seven-point hedonic rating scale

Product	Species	Mean score	Standard deviation	Paired Student's *t*-test	Significance	Mean product score
Steamed in Parsley Sauce	Whiting	5.9	1.2	1.92	N.S.	5.7
	Blue Whiting	5.5	1.2			
Deep fried in batter	Whiting	5.5	1.4	1.69	N.S.	5.35
	Blue Whiting	5.2	1.4			
Deep fried in crumbs	Whiting	5.8	1.1	1.16	N.S.	5.7
	Blue Whiting	5.6	1.3			
Fish Cakes	Whiting	5.4	1.4	1.37	N.S.	5.5
	Blue Whiting	5.6	1.2			
Fish Pie	Whiting	5.6	1.5	1.02	N.S.	5.5
	Blue Whiting	5.4	1.7			

NS = not significant at 5% level.

to rate the five products into which whiting and blue whiting had been incorporated.

Discussion

The results of the paired preference test and those shown in Table 1 were produced under conditions which were optimal for discrimination since the fish were presented as unseasoned plain samples. It is clear that although the fish species have sufficiently distinctive sensory characteristics for assessors to readily distinguish between them, the different qualities were valued at varying levels so that comparative preferences were much less clear cut.

Results of preliminary rating tests in which whiting and blue whiting were scored as plain samples showed the whiting to be approximately 1 unit higher than blue whiting for all 4 characteristics (texture, flavour, appearance, acceptability). Table 2 shows that after the incorporation of the fish into products the scores become quite similar and the products made with either fish

are well liked. It is interesting that this is not a progressive effect which is being influenced by the extent of treatment (e.g. frying in batter) or volume of other material (e.g. potato in fish cakes). There is an indication that in the simplest product (coating plain fish with parsley sauce) whiting is preferred to blue whiting since the Student's *t*-test on the difference between mean scores is significant at about the 6% level. All the products made with both fish were liked by the assessors. Since there is no significant difference in the products made with either species it is possible to calculate a product score by halving the combined means and this score is also shown in Table 2. It can be seen that all the products scored very similarly; this is quite an interesting result since it would not have been unreasonable to expect assessors to like some products more than others.

An analysis of variance was carried out on the results and confirmed that there were no significant species differences within products or between products. Significant differences were found between assessors which is the normal finding.

It should be noted that if the main object of the exercise had been to compare products with one another, a balanced presentation of all ten samples would have been the method of choice in the experimental design rather than the presentation in product pairs used in these experiments.

It is evident from these experiments that products made from blue whiting are liked by consumers and would be a satisfactory alternative to products made with more traditional species, provided that good quality fish can be supplied at an economic price to the processing industry.

Acknowledgments

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Selection and training of panelists for sensory evaluation of meat flavours

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Summary

Despite the necessity to select panelists sensitive to flavour changes and rigorously train them for meat quality evaluations, there is no published methodology to achieve this aim. Meat off-flavours, notably rancidity, are extremely complex. General methods already described for sensory evaluation of foods are inadequate to establish rigorous yet practical procedures for use in studies on meat quality. This paper summarizes the experiences and details the procedures developed for training panelists in the rancid flavour assessment of lamb.

Introduction

For studies involving the early detection of flavour changes in meat it is vital to select panelists sensitive to the flavour change in question and rigorously train them (Lowry, 1979). There are several reports (e.g. Harries *et al.* (1963); Amerine, Pangborn & Roessler (1965); Larmond (1970); Cross, Moen & Stanfield (1978); Ford & Park, (1980)) outlining important procedures to follow and general methods for sensory evaluation of food and meat. Park, Corbett & Furnival (1972) outlined a methodology for lamb flavour evaluation. There are no reports, however, describing in detail the training of panelists specifically for meat flavour analysis. Meat flavours are extremely complex and it is often difficult to collect the wide variety of flavours and extreme range of flavour intensities needed for rigorous training of panelists.

This paper describes in detail the selection and training of panelists for the evaluation of rancid flavour development in lamb during frozen storage. Although descriptions involve lamb, the procedures should be readily

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applicable to other meats. It should also be recognized that the commercial products described in this paper have equivalent counterparts in other countries.

Materials

A variety of commercial products was used in the trial. They included 'Chefade' (hydrogenated Oleo oil, containing antioxidants, Abels Ltd, NZ); 'Maggi Beef Stock' (powdered beef flavoured stock, the Nestlé Company, NZ); sodium caseinate (normal viscosity, first grade, courtesy NZ Dairy Board, Wellington, NZ); 'Fielder's Wheaten Cornflour' (wheat starch, Neill Cropper & Co. Ltd, Auckland, NZ).

A reference sample of fresh (24 hr post mortem) lean lamb mince was prepared from muscles dissected away from bone and subcutaneous fat. The tissue was minced three times through a 13-mm diameter plate and thoroughly mixed after each mincing. The lean mince (7–9% fat content, measured by AOAC (1980) method) was packaged in 500-g lots in polyethylene bags, frozen rapidly to −35°C (2 hr to cool to −10°C) and stored at −35°C until used.

Stock samples of lamb fat were rendered from a variety of carcasses. Some samples were from carcasses stored at about 7°C for 24 hr ('fresh' lamb fat). Others were from carcasses or lamb cuts stored for various time-temperature histories. Times ranged from 1 to 5 years and temperatures from −10 to −20°C. The whole tissue was minced once through a 13-mm diameter plate, then cooked gently in a closed pot until most of the fat had melted. The fat was then decanted into an approximately equal volume of hot (80–90°C) water, mixed quickly and allowed to separate from the water and meat particles. The fat was decanted and stored in covered glass beakers at either 2–4°C for up to 1 week, or −35°C for up to 1 month.

Each panelist used crackers (Bycroft Water Crackers, Aulsebrooks Ltd, NZ) and either Coca Cola (with or without an extra glass of hot water), or apple juice (with or without an extra glass of hot water), or hot water (40–50°C) alone to rinse their mouths of flavours between samples.

Methods

Panelist selection

The triangle test and statistical procedures outlined by Amerine *et al.* (1965) were used for this test. Using the terminology of Amerine *et al.* (1965), the variables selected for the sequential analysis procedure were: $\alpha = 0.10$; $\beta = 0.05$; $P_0 = 0.40$; $P_1 = 0.65$. The fat used for this trial was rendered from a carcass stored for 5 years at −18°C and was extremely rancid. A basic stock solution was prepared as follows: 1 l of a 2% (w/v) starch slurry was boiled for about 1 min and 0.3% (w/v) beef stock added. One half of this hot stock solution was added

to a mixture of 5 g rancid fat and 10 g Chefade. The other half was added to 15 g rancid fat. The two solutions were kept covered with 'Glad Wrap' (polyethylene cling film, Union Carbide NZ Ltd) at 70°C.

Samples (10 to 15 ml) of these solutions were poured into randomly numbered paper portion cups (Lily Products Ltd, NZ) immediately prior to serving to panelists. Each panelist was presented with two triangle tests at each sitting.

Each panelist tasted in an enclosed, individual taste booth, illuminated with 40 W tungsten light bulbs.

The number of correct responses was plotted against the number of triangle tests completed, as described by Amerine *et al.* (1965). Tasting was continued for two weeks after which only panelists exceeding the upper 'accept' limit line were selected for further training.

Training

Stage I. A variety of rendered fats was used for this stage. One 10 kg stock of lean mince was used for the entire trial.

The basic sample recipe was 100 g mince, 20 g fat, 3 g sodium caseinate, 1.5 g starch and 15 ml water. To alter the level of rancidity in any sample, the composition of added fat was modified. For example, 20 g of either 'fresh' lamb fat, or 'Chefade' was used as a non-rancid sample. A range of rancidity was achieved by using differing amounts of rancid fat from one source diluted with suitable amounts of 'Chefade' to make a total of 20 g added fat.

Less than 2 hr prior to a tasting session a 600 g sample of mince was thawed in a water bath at 35 to 40°C and cooked as follows: the fat was melted in a stainless steel pot over a moderately hot element. The pot was then removed from heat and the mince added. While being stirred continuously, the mince was browned thoroughly without burning (about 4–5 min) and then removed from the heat. The caseinate powder was added and mixed in, the mince was cooked until it steamed (about 15–20 sec) and the pot was then removed from the heat. The starch was slurried with the cold water and added to the mince. While being stirred vigorously, the mince was further cooked (about 1 min) until there was no free liquid remaining and the meat was a 'sloppy paste'. The mince was then placed in a beaker, sealed with 'Glad Wrap' and stored at 70°C.

About 15 min prior to the taste session, the cooked mince was placed in portion cups (about 1 teaspoonful per cup) and stored at 70°C in a water-saturated atmosphere until served. For all sessions, the panelists were first presented with an identified reference sample containing 20 g 'Chefade'. An additional 3 to 5 samples were served at any one session.

Rancid flavour was scored on a 150 mm unstructured line for reasons outlined by Stone *et al.* (1974). The results were measured as distance (mm) from the left end of this line. Hence results range from 0 (no rancid flavour) to 150 (extremely rancid). Panelists tasted as a group, sitting together around a table. Once all samples had been tasted, the panelists discussed the results and arrived at a rancid flavour intensity score for each sample.

Stage II. A variety of lamb and mutton carcasses was used in this stage. Some meat was from fresh (24 to 48 hr post mortem) carcasses, some from lamb loins stored at −12°C for about 20 months. The lean and fat portions from each animal were separated, then remixed in the combination 80:20 (w/v) lean : fatty portions. These were then minced as described in the materials section and stored at −35°C until required (maximum 2 weeks).

The mince was thawed at 2 to 4°C overnight. The recipe used was 150 g mince, 4 g sodium caseinate, 2 g starch and 27 to 30 ml water. Cooking was similar to that described under Stage I, except that a small quantity (less than 5 g) of 'Chefade' was melted in the pot to coat the bottom prior to adding the mince.

The panelists were given six samples per session. The first sample was always identified to the panelists as the reference. This was mince from a batch of fresh, non-rancid meat. The remaining five samples were presented as unknowns.

We had found that only marking made by xylene-based felt-tip pens consistently remained visible on the portion cups in the 70°C water-saturated environment used to store samples. The smell of the felt-tip pens (even 24 hr old markings) significantly confused the panelists. Therefore no numbering was used on any portion cups. Panelists recorded their results from 1 to 5 in order of sample presentation.

As well as scoring for rancid flavour intensity according to Stage I, panelists were asked to describe the non-rancid flavours in the samples. This latter function was assisted by a list of flavour descriptors (developed from MacLeod & Coppock, 1978). The tasting was conducted as a group session, each panelist individually scoring and describing each sample followed by a group discussion after all samples had been tasted. A total of about thirty different samples was used in this stage, ensuring the greatest possible flavour variation.

Performance evaluation. One way analysis of variance on panelist consistency at tasting the same sample on successive days, as described by Stone *et al.* (1974) and Cross *et al.* (1978) was used at the beginning and end of the training session. Nine samples were used for the initial evaluation, being prepared according to methods described for Stage I. Five different samples were presented at the final evaluation, with samples prepared according to the methods described for Stage II.

In addition, several samples were repeated during training sessions. A sample would be tasted and described at one session. This same sample would be presented again at one or more later sessions.

Results and discussion

Selection

There is one major problem in devising a suitable selection procedure for any flavour attribute. Any change in one flavour attribute is invariably mimicked to

some degree by other flavour changes. For example, the lamb and meaty flavours were very readily altered by adding rancid fat. While establishing this selection procedure using a broth the following points were noted:

(1) Rancid flavour is associated almost entirely with the fat.

(2) Bland, stable fat rather than 'fresh' lamb fat was required to dilute rancid fat to give two different rancidity intensities (we used 'Chefade').

(3) Meat was an extremely poor carrier of rancid flavour for selection trials. Not only were subtle differences in texture sometimes apparent, but non-rancid flavour changes were unavoidable. It was almost impossible to remove sufficient natural fat from the meat during pre-cook preparation to avoid these interactions. Thus the differences noted by untrained panelists in triangle tests using meat as a rancid flavour carrier were often due to factors other than rancid flavour.

(4) The thin, hot starch gel used as a fat carrier was ideal for uniform distribution of fat throughout a sample, and also suspended the fat adequately for tasting. To overcome the unpalatable nature of the starch gel it was made in a very dilute beef stock. It was very important to ensure that no discernible beef or meaty flavour was present to minimize rancid fat interacting with it.

(5) Irrespective of these precautions, there was some distinct interaction between starch, beef broth and rancid fat, even at extremely low levels of added fat (1 g per litre broth). Thus, two different concentrations of rancid fat were used rather than one rancid and one non-rancid sample. This minimized the effect of these non-rancid flavour changes and was found to be essential in our selection trial. Some panelists were sensitive to these non-rancid flavour changes but quite insensitive to rancid flavour itself!

Of the fifty-three people tested, twenty-eight were selected for further training. The method using broth was very simple to perform and, as subsequent training ultimately showed, a good method for selecting panelists.

Training

For the training stages, lamb mince was used for a variety of reasons:

(1) Mince was a more familiar and realistic method of presenting rancid samples than broths.

(2) Mince was preferred to intact meat since it eliminates texture as a major sensory attribute which could influence panelists.

(3) A simple, reproducible and very effective method of cooking and presentation could be used.

(4) Differences in sample colour were essentially nullified. Each sample could be browned to approximately the same colour.

(5) It was an extremely effective method for ensuring a uniform distribution of fat throughout the sample.

(6) It was found that mince could be prepared in advance, then rapidly frozen and stored at −35°C for at least 10 weeks without any perceptible difference in flavour.

The training procedures were conducted in two stages. Firstly, panelists were subjected to samples where non-rancid flavour variations were minimal and rancid flavours could be identified and quantified. The second stage involved tasting of realistic samples where rancid flavours were only a part of the entire changing flavour profile.

Because the rancid flavour was carried in the fat, its intensity could be modified by the amount of rancid fat added to the sample. The initial stages of training used a standard batch of lean lamb mince to which was added various quantities of rancid and non-rancid fat. The basic meaty flavour of lean meat (Hornstein & Crowe, 1963; Sink & Caporaso, 1977) together with some lamb flavour from the entrapped lamb fat initially gave the same flavour to all samples prepared from this meat. It must be reiterated that addition of rancid fat to this meat base caused some alterations in these basic flavours, although the degree of these changes was not proportional to the amount of fat added. By adjusting the concentration of rancid fat added to the meat, a different intensity of rancid flavour was achieved. The panelists were asked to score these intensities on the unstructured scale and were trained to consistently distinguish small differences in rancid flavour. They were also instructed on the use of the scale. Through persistent repetition and group discussions, panelists were able to adjust their scoring so that all would score any one sample within about 20 mm of the group's mean on the unstructured scale. A highly rancid sample was chosen as being 'very rancid' (about 100 on the 150 scale) and this sample was used to standardize the random scale between 0 and 150. Using this sample as a standard and the reference as zero rancidity, all other samples could be suitably ranked on the scale. Panelists who initially deviated greatly from the group's mean were forced to re-evaluate their scoring to conform.

At the end of this stage in training, fifteen panelists consistently agreed on rancidity levels above threshold, five panelists were usually in agreement, two panelists were inconsistent and six panelists had excused themselves for other reasons.

The second stage in training was designed not only to give the panelists an indication of the variation in flavour amongst lamb, but also to assist in standardizing sample preparation procedures. Lamb loins were used. The tissue was dissected away from the bone, and the fat and lean separated. The lean meat was weighed and a suitable amount of the fatty tissue recombined with the lean. The sample was then minced and the fat and lean tissues intimately mixed. This meant there was no free fat to melt in the pot prior to cooking the meat. As a result, the meat tended to catch and burn before enough fat had melted. To overcome this problem a small amount of 'Chefade' was melted in the pot to coat the bottom before the meat was added. For tasting purposes, samples from a variety of lamb breeds, sexes and animal ages were used to help identify the variety of fresh lamb flavours to be found. Panelists were given these fresh (non-rancid) lamb samples for several sessions and asked to describe the flavours. Individual records were kept by each panelist and they could refer to them at each session. Eventually, rancid samples were interspersed throughout

the fresh samples, so panelists could identify the rancid flavours from the non-rancid ones. Panelists were continually reminded to score rancid flavour only if they were absolutely sure it was present. Initially, many panelists found it hard to distinguish rancid flavours from non-rancid flavours. By the end of this stage, however, most panelists consistently distinguished rancid flavour from other flavours. It was noticeable, however, that the scoring for rancid flavour intensity was more variable than it had been for Stage I. This variability was heightened because panelists would score rancid flavour only if they were sure it was present. Thus, unless the rancid flavour was distinctly above their threshold (mean score about 30 on the scale), many panelists were scoring zeros, while a few would score between 20 and 40. The resulting mean was low (e.g. 10–20), but the variation large. Such variation from panelist to panelist continued, despite efforts to standardize results. In short, when rancid flavour was confounded with strong, non-rancid flavours, panelist sensitivity varied enormously.

The panel mean, however, varied little when identical samples were tasted in different sessions. Table 1 gives the panel means for six samples which were tasted in six successive taste sessions. Very clearly, panelists readily distinguished the non-rancid samples (no panelist scored any rancid flavour intensity for these samples). The means for the other samples are very consistent. Not every panelist tasted in every session, which indicates that the group was in general agreement on rancidity levels irrespective of the panel composition.

As mentioned earlier, with such low rancid flavour intensities as those in Table 1, some panelists had not reached their flavour threshold.

Panelist evaluation

The evaluation procedure using analysis of variance conducted at the beginning of training involved samples ranging from 0 to about 120 on the scale, with at least six samples in between. This was readily achieved because the intensity of rancid flavour in that trial was determined by the concentration of added rancid fat. The stock supply of rancid fat was extremely rancid. The analysis at the end of training, however, involved samples with low levels of rancid flavours (see Table 1). With such low levels of rancid flavour, small inconsistencies (e.g. 20 mm) in scoring a sample from one day to the next caused a dramatic reduction in the panelist's F-value whereas this was not true with higher levels of rancidity. In other words, the analysis of variance method of analysis is more sensitive to low scores than high scores. To successfully use this procedure as a reliable indicator of panelist improvement, identical samples (or at least very similar samples) must be used for every evaluation trial. These samples should extend over the entire range to be measured. It appeared many panelists had not improved with training when their F-value scores were compared. In fact, by comparing the panel and individual panelist performance at identifying and scoring known samples at different tasting sessions

Table 1. Sensory scores for final panelist evaluation trial

		Sample number					
Day	Session*	1	2	3	4	5	6
1	1	0 (0)†	0 (0)	19 (5)	19 (10)	29 (6)	31 (7)
	2	0 (0)	0 (0)	30 (9)	10 (9)	26 (8)	25 (10)
2	1	0 (0)	0 (0)	15 (5)	18 (9)	25 (5)	29 (5)
	2	0 (0)	0 (0)	16 (4)	21 (9)	21 (6)	31 (6)
3	1	0 (0)	0 (0)	17 (4)	14 (5)	18 (6)	27 (6)
	2	0 (0)	0 (0)	15 (5)	–§	27 (8)	31 (8)
Overall‡		0 (0)	0 (0)	17 (3)	16 (2)	24 (2)	29 (1)

*There were 2 sessions per day. One at 10 am (1), the second at 3 pm (2).

†Scores represent means of all panelists tasting. Standard errors given in parentheses. Each score represents the distance (mm) from the origin (no rancidity = 0).

‡Overall mean is the average of the means presented in the table. The standard deviation is given in the parentheses. This represents the standard deviation of the means presented in this table and is not derived from the individual panelists' data.

§This sample was not tasted in that session.

throughout the entire training period, it was obvious the panelists had significantly improved. They were more proficient at distinguishing rancidity. They also became less tolerant or more discerning of rancidity. That is, samples they accepted at the onset of training were considered 'inedible' at the end of training. It was also found that some samples the trained panelists described as 'inedible' were classified as barely perceptibly rancid by some untrained people who tried them. This is further support for the concept that trained panelists must never be asked to rate samples on acceptability (Amerine *et al.*, 1965).

Conclusions

About twenty panelists, selected from over fifty staff members, were progressively trained to distinguish rancid flavour in lamb. They were readily able to identify the rancid from other flavours. They were trained to use an unstructured line scale to score rancid flavour intensity. They were also capable of describing the overall flavour of the sample in simple descriptive terminology.

These techniques were developed over 9 months and were gradually improved with considerable trial and error. The methods are a selection of the best from many less successful procedures.

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Effects of blanching and sulphur dioxide on ascorbic acid and pigments of frozen capsicums

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Summary

The frozen storage of capsicum tissue for 1 year at −12°C resulted in appreciable losses (from 10 to 58%) of ascorbic acid dependent upon the cultivar and the extent of tissue blanching. Ascorbic acid retention was maximal, but retention of chlorophyll *a* was minimal and pH dependent, in frozen blanched tissue which accordingly showed a greater increase in pheophytin formation. Sulphite treatment of unblanched tissue maintained acceptable colour through retardation of degradative interconversion and isomerization reactions of chlorophyll and carotenoid pigments during frozen storage.

Introduction

The quality characteristics of capsicum cultivars grown in Australia are currently under investigation (Rahman, Buckle & Edwards, 1978; Rahman & Buckle, 1980). Although other forms of preserved capsicums are readily available, there appears to be no established technique for the preservation of capsicums by freezing even though it appears likely that capsicums could be an acceptable frozen food. The literature contains conflicting reports on the effectiveness of the blanching of capsicums before freezing, with some processors reported to favour blanching before freezing, while others claim that the unblanched product has an adequate storage stability. Although published results of the organoleptic evaluation of colour, flavour, texture and general acceptability of frozen capsicums showed that unblanched samples were superior to blanched material (Shipton & Last, 1967; Mathews & Hall, 1978), no information is available regarding qualitative or quantitative changes in chlorophyll or

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carotenoid pigment contents of capsicums during extended frozen storage. The present study involved a quantitative analysis of ascorbic acid, chlorophylls and carotenoids of unblanched, blanched and sulphited, frozen capsicums at two stages of maturation stored for 1 year.

Materials and methods

Blanching and freezing of capsicums

Five capsicum cultivars, namely Large Green, Tomato-shaped Green, Tomato-shaped Yellow, Ram Horn and College Gold were grown at Hawkesbury Agricultural College, Richmond, NSW during the summer season. Mature fruits of all five cultivars were hand picked, transported to the Kensington laboratory, and stored overnight at 2–5°C. Fruit of each of the five cultivars (2 kg) were washed, de-seeded, cored, cut into 1 × 1 cm pieces and divided into two parts: one part was blanched for 2 min in an atmospheric steam blancher, immediately cooled in running cold water and drained, while the other part remained unblanched. Both blanched and unblanched samples were frozen rapidly at −27°C.

The optimum blanching time for the inactivation of the peroxidase enzyme system was determined following the method of Masure & Campbell (1944).

Approximately 50-g samples of both blanched and unblanched frozen capsicums were set aside for initial ascorbic acid and gross and individual pigment analyses. The remaining blanched and unblanched frozen samples were packed separately into plain 99 × 118.5 mm tinplate containers, vacuum sealed by steam flow closure and stored for 12 months at −12°C.

Three weeks later, fully ripened red fruits of the Large Green and Ram Horn cultivars were similarly prepared, vacuum-sealed into tinplate cans and stored frozen for further analysis.

Pacific Bell and Ram Horn cultivars were grown under polyethylene cover during the winter season at Hawkesbury Agricultural College, Richmond, NSW. Matured, unblanched and steam blanched (2 min) samples of Pacific Bell and Ram Horn cultivars were prepared as above and then dipped separately for 5 min in sodium bisulphite solution containing 500 and 1500 mg/l of sulphur dioxide for Ram Horn and Pacific Bell cultivars respectively, drained and frozen at −27°C and stored in epoxy-lacquered vacuum-sealed cans for 12 months at −12°C.

Determination of ascorbic acid and sulphur dioxide

Ascorbic acid was determined in frozen capsicums stored at −12°C. Duplicate 10.0 ± 0.1 g samples of frozen tissue were weighed and extracted with 3% metaphosphoric acid, filtered and ascorbic acid determined following the method of Kefford (1957).

The ascorbic acid content of sulphited frozen samples was determined by the method of Mapson (1942) which depends upon the formation of the bisulphite compound of sulphur dioxide with acetone in acid solution and titration of the resultant solution against 2,6-dichloro-phenol indophenol dye.

The sulphur dioxide content of the unfrozen and frozen samples was determined following the method of Shipton (1954).

Extraction, separation and estimation of individual pigments from capsicum tissue

Pigments were extracted from weighed (20 ± 0.1 g) frozen samples and applied as a 16-cm streak on cellulose thin layers using the method of Bacon (1964). Four solvent systems were used for the development of the chromatogram followed by column chromatography of mixed streaks on MgO-Hyflo Super-cel (1:1 w/w) and aluminium oxide as described by Buckle & Rahman (1979). Pigments were eluted from thin layers (Jeffrey, 1968), individual pigments identified (Buckle & Rahman, 1979) and quantitatively estimated (Davies, 1965) using extinction values recorded in the literature (Foppen, 1971). For the estimation of the approximate concentration of unidentified carotenoid pigments, an $E^{1\%}_{1\,cm}$ value of 2500 was assumed.

Results and discussion

Effects of blanching

There was a progressive decrease of ascorbic acid in all cultivars, but considerable differences were observed between some of the blanched and unblanched samples (Table 1). After 12 months' frozen storage, ascorbic acid

Table 1. Ascorbic acid content of frozen blanched and unblanched capsicums stored under vacuum for 12 months at −12°C

		Ascorbic acid content (mg/100 g)			
		Blanched		Unblanched	
Cultivar	Stage of maturity	Initial	Retention (%)	Initial	Retention (%)
Large Green	Mature	76.4	39	87.0	32
Tomato-shaped Green	Mature	112.3	19	131.4	14
Tomato-shaped Yellow	Mature	97.3	16	117.7	16
Ram Horn	Mature	110.0	35	138.0	28
College Gold	Mature	118.6	15	147.7	10
Large Green	Fully ripe	114.0	58	125.6	41
Ram Horn	Fully ripe	162.8	60	189.8	36
College Gold	Fully ripe	146.4	52	162.0	54

retention in the mature College Gold cultivar was as low as 15% and 10% in blanched and unblanched samples respectively. The highest retention was in the mature Large Green cultivar which gave 39% and 32% retention in blanched and unblanched samples respectively. These results can be compared to those of frozen green beans and peas in which, respectively, only 25% and 36% retention of ascorbic acid has been reported (Derse & Teply, 1958).

Ascorbic acid retention after storage of frozen blanched fully ripened samples of Large Green and Ram Horn cultivars (58 and 60% respectively) was significantly higher than that of stored unblanched samples (41 and 36% respectively). However, in the fully ripened College Gold cultivar, there was no significant difference in the ascorbic acid retention between blanched and unblanched frozen samples (52% and 54% respectively).

Retention of ascorbic acid in fully ripened samples was higher than that of mature samples of the same cultivar. The reasons for the higher stability are unknown, but may be related to the increased availability of oxidation inhibitors in the fully ripened material as there is evidence that many fruits contain substances which inhibit the oxidation of ascorbic acid, e.g. tannins from Indian gooseberries (Damodaran & Nair, 1936), anthocyanin and flavonone materials from blackcurrants (Hooper & Ayers, 1950), and phenolic compounds in rose hips (Jackson & Wood, 1959). This point, though of interest, was not pursued further in this study.

The results of total pigment analyses of frozen samples of five cultivars at the mature stage and three cultivars at the fully ripe stage are presented in Table 2. There was a progressive decrease in total pigment content during the frozen storage of all cultivars at both stages of maturity, the decrease becoming greater as the storage time increased.

Table 2. Total pigment content of frozen blanched and unblanched capsicum cultivars stored under vacuum for 12 months at −12°C

		Pigment content of frozen tissue as β-carotene (μg/g)			
		Blanched		Unblanched	
Cultivar	Stage of maturity	Initial	Retention (%)	Initial	Retention (%)
Large Green	Mature	14.7	46	16.6	72
Ram Horn	Mature	7.8	41	8.9	57
College Gold	Mature	2.9	59	3.7	69
Tomato-shaped Yellow	Mature	4.7	47	6.0	50
Tomato-shaped Green	Mature	9.7	39	11.8	66
Large Green	Fully ripe	165.7	91	168.0	93
Ram Horn	Fully ripe	173.0	94	181.5	92
College Gold	Fully ripe	211.4	94	218.4	94

The Large Green and Tomato-shaped Green cultivars at the mature stage showed significantly higher pigment retention in the unblanched samples compared with the blanched samples. The remaining cultivars at the mature stage also showed a higher retention in the unblanched than in the blanched samples.

No appreciable differences in pigment loss were observed between blanched and unblanched samples of any of the three cultivars at the fully ripe stage, with retention in excess of 90% in all cultivars.

Results of individual pigment determinations of mature Large Green and Ram Horn cultivars before and after 12 months' storage at −12°C are shown in Table 3. An examination of the pigment data for blanched and unblanched samples of both Large Green and Ram Horn cultivars shows that the initial blanching process resulted in the formation of pheophytin *a* from chlorophyll *a* and of pheophytin *b* and chlorophyll *b'* from chlorophyll *b*. There was less than

Table 3. Pigments of frozen mature blanched and unblanched Large Green and Ram Horn cultivars stored under vacuum for 12 months at −12°C

	Pigment content (μg/g)							
	Large Green				Ram Horn			
	Unblanched, stored for (months)		Blanched, stored for (months)		Unblanched, stored for (months)		Blanched, stored for (months)	
Pigment	0	12	0	12	0	12	0	12
Chlorophylls								
Chlorophyll *a*	14.6	9.1	11.7	3.1	4.8	2.5	3.9	0.9
Chlorophyll *b*	6.4	4.8	5.5	3.1	2.3	1.6	2.1	1.0
Chlorophyll *b'*	—	—	0.7	0.5	—	—	0.2	0.1
Pheophytin *a*	—	1.6	1.1	6.4	—	1.3	0.4	1.5
Pheophytin *b*	—	0.9	0.1	1.6	—	0.3	0.1	0.5
Pheophorbide *a*	—	1.3	Tr	0.8	—	0.3	—	0.2
Chlorophyllide *a*	—	0.3	—	—	—	0.3	—	—
Carotenoids								
β-Carotene	2.4	2.0	2.4	1.9	1.3	1.1	1.3	0.8
Neo-β-carotene	—	Tr	—	0.3				
5,6-Diepoxy-β-carotene	—	—	—	—	0.6	0.3	0.6	0.3
α-Carotene	0.4	0.3	0.4	0.3				
ζ-Carotene	—	—	—	—	2.8	0.1	0.1	0.1
Lutein	3.2	3.2	3.2	3.1	Tr	2.7	2.7	2.6
Zeaxanthin	0.1	0.2	0.1	0.2	0.6	0.2	0.1	0.2
Violaxanthin	2.1	1.1	2.1	0.1	0.5	0.3	0.5	0.1
Neoxanthin	0.9	0.6	0.9	0.2	—	0.4	0.5	0.1
Luteoxanthin	—	0.6	—	0.8	—	0.1	—	0.4
Auroxanthin	—	0.3	—	0.6	—	0.1	—	0.2
Unidentified	0.2	0.4	0.2	0.5	—	0.1	—	0.2

— = Not detectable; Tr = trace.

1% pheophytin *a* formation in the Large Green and Ram Horn cultivars during blanching, and pheophytin *b* concentrations were significantly low (0.1 μg/g) in each cultivar. The lower stability of chlorophyll *a* compared to chlorophyll *b* during blanching is in agreement with observations in spinach (Clydesdale & Francis, 1968), brussel sprouts (Dietrich & Neumann, 1965) and broccoli (Eheart & Odland, 1973). However, the concentration of chlorophyll *b'* was significantly higher (0.7 μg/g and 0.2 μg/g) than that of pheophytin *b* (0.1 μg/g in each cultivar) during blanching of these two cultivars. The reason for this higher isomerisation of chlorophyll *b* to chlorophyll *b'* is not apparent. Although both chlorophyll *a'* and *b'* were formed in *Herceleum* discs when heated in boiling distilled water for 5 min (Strain, 1954a, b; Strain & Manning, 1942), only chlorophyll *b'* was formed as a result of steam blanching of capsicum for 2 min.

During storage at −12°C, there was an unusual pattern of chlorophyll degradation in blanched samples when compared with the unblanched samples, a difference that was reflected in their visual colour. Visually the unblanched samples of the Large Green cultivar showed no appreciable change of green colour, whereas the blanched sample changed from a green to a brown colour during the same storage period. Blanched samples of the Ram Horn cultivar also showed a similar discolouration during storage compared to the unblanched samples.

In blanched samples of the Large Green cultivar, pheophytin *a* increased from 1.1 μg/g before storage to 6.4 μg/g after 12 months. At the same time, chlorophyll *a* decreased from 11.7 μg/g before storage to 3.1 μg/g after 12 months' storage at −12°C. On the other hand, the increase of pheophytin *a* in the unblanched sample of the same cultivar was significantly lower than in the blanched sample, and only 1.6 μg/g of pheophytin *a* was found after 12 months' storage at −12°C, although no pheophytin *a* was present in the unblanched sample before storage.

Very small amounts of chlorophyllide *a* and pheophorbide *a* were found in unblanched and blanched samples, respectively in both the cultivars. A high conversion of chlorophylls to pheophytins in blanched frozen samples generally indicates either that the sample has not been adequately blanched, i.e. the enzyme system responsible for the conversion of chlorophylls to pheophytins has not been inactivated or retains sufficient activity during storage to induce the change noted above, or that frozen storage temperatures have been excessively high. To examine the inactivation of the peroxidase enzyme system in the blanched samples, peroxidase determinations were carried out on these samples before and after storage for 12 months at −12°C. The test was negative for blanched and positive for unblanched samples, even after storage for 12 months at −12°C, and similar to the results found before storage. The above observations of greater chlorophyll change apparent in blanched samples compared with unblanched samples could be explained by changes in pH of samples during storage, effect of plant acids, liberated from ruptured cells caused by the blanching process, on chlorophylls and (or) overblanching of capsicum samples since enzymic degradation was not involved in blanched samples. Further clarification of these observations is required.

Table 4. Pigment content of frozen fully ripe, blanched and unblanched Large Green and Ram Horn cultivars stored for 12 months at −12°C

	Pigment content (μg/g)							
	Large Green				Ram Horn			
	Unblanched, stored for (months)		Blanched, stored for (months)		Unblanched, stored for (months)		Blanched, stored for (months)	
Pigment	0	12	0	12	0	12	0	12
Phytofluene	0.8	0.8	0.7	0.6	6.2	6.0	6.1	5.8
Phytoene	—	—	—	—	Tr	Tr	Tr	Tr
α-Carotene	1.1	1.0	0.9	0.5	1.3	1.0	1.0	0.6
β-Carotene	22.7	13.8	19.8	9.8	28.0	19.7	26.3	16.2
ζ-Carotene	0.3	0.3	0.4	0.3	3.8	2.5	2.7	2.3
Mutatochrome	1.1	0.9	0.9	0.6	3.2	3.0	3.2	2.9
Hydroxy-α-carotene	—	—	—	—	1.9	1.1	1.6	0.8
Cryptoxanthin	15.8	13.0	13.8	9.4	15.3	12.4	15.1	10.1
Capsolutein	Tr	Tr	Tr	—	3.8	3.7	3.7	3.4
Cryptocapsin	2.0	1.7	2.1	2.0	1.1	1.0	1.1	1.0
Zeaxanthin	5.1	7.1	6.7	8.1	15.0	16.6	15.5	17.1
Antheraxanthin	4.8	5.8	5.4	6.9	3.0	4.4	3.6	4.9
Mutatoxanthin	0.6	1.9	1.2	2.7	2.0	3.1	2.2	3.8
Violaxanthin	15.2	10.3	13.7	9.7	16.7	13.0	15.2	10.4
Luteoxanthin	—	0.7	—	1.3	1.1	2.0	1.7	2.8
Neoxanthin	5.1	4.0	4.8	4.1	—	—	—	—
Capsanthin	50.6	50.0	51.5	51.0	52.0	50.9	52.3	50.2
Capsorubin	21.0	20.0	19.2	19.1	15.3	14.1	16.1	14.0
Capsanthin isomer	16.8	17.8	17.4	18.0	21.0	23.3	21.2	23.8
Capsorubin isomer	1.8	2.4	2.0	3.4	3.1	4.0	3.1	4.6
Unidentified	1.3	2.8	1.3	3.9	3.7	4.3	1.9	2.1

— = Not detectable; Tr = trace.

Carotenoid pigment determinations on frozen samples of the Large Green and Ram Horn cultivars at both the mature and fully ripe stages are presented in Tables 3 and 4. Violaxanthin (zeaxanthin 5,6,5′,6′-diepoxide) was one of the most labile of the xanthophylls and was isomerised to luteoxanthin (5,6,5′,8-diepoxide) and to auroxanthin (zeaxanthin 5,8,5′,8′-diepoxide). The concentration of violaxanthin decreased and that of luteoxanthin and auroxanthin increased in both cultivars, with the degree of conversion higher in the blanched than in the unblanched samples. This pigment isomerization resulted in tissue discolouration, especially in the Ram Horn cultivar, because of the shift of absorbance peak to shorter wavelengths. Neoxanthin was also unstable and its concentration decreased substantially during 12 months' storage.

Lutein and β-carotene were the most stable carotenoids of those found in the mature samples. Among the carotenes, β-carotene 5,6-diepoxide decreased appreciably in the Ram Horn cultivar, while ζ-carotene and α-carotene remained constant during storage.

Light-induced de-epoxidation of violaxanthin to zeaxanthin via antheraxanthin has been reported in leaves (Yamamoto, Nakayama & Chichester, 1962). Hager (1969) found conversion of violaxanthin to zeaxanthin in isolated chloroplast pigment without illumination when the pH level was close to 5. In the dark at pH 5, the de-epoxidation of violaxanthin has also been observed in isolated chloroplasts when ascorbate was added (Yamamoto *et al.*, 1972). The pH of the blanched and unblanched frozen capsicums was close to 5, and since the samples contained high amounts of ascorbic acid, it was possible that de-epoxidation of violaxanthin occurred in a manner similar to that described by the above workers. Although few published reports are available on the isomerization of violaxanthin to luteoxanthin and auroxanthin in frozen vegetables, the observed isomerization of violaxanthin in the present work was in agreement with the studies of Curl & Bailey (1956, 1959) who found complete disappearance of 5,6-epoxides and the appearance of appreciable amounts of 5,8-epoxides in stored orange juice and orange juice powder.

The formation of 5,8-epoxides was higher in blanched samples than in unblanched samples, suggesting a combined effect of pH and blanching on the conversion of 5,6-epoxides in capsicum tissue. Although Strain (1954a) showed that, when treated with acetic acid, violaxanthin yielded two flavoxanthin-like pigments and then auroxanthin after further action of acids, it would be of interest to determine the effect of heat and plant acids on the conversion of 5,6-epoxides to 5,8-epoxides.

The results of pigment determinations on fully ripe fruit revealed no appreciable changes in the predominant red pigments, capsanthin and capsorubin in blanched and unblanched samples of all cultivars (Table 4). Nevertheless, there was a significant loss of β-carotene (approximately 50%) in some blanched samples at the fully ripe stage. However, unlike the frozen mature samples, the fully ripe samples showed only a 20 to 35% decrease of violaxanthin, and a very small increase of zeaxanthin, mutatoxanthin, auroxanthin and luteoxanthin during 12 months' frozen storage. The cause of the higher stability of carotenoids in fully ripe frozen capsicum samples compared to mature samples is not known, but is clearly related to the complex changes in chemical composition and structure undergone by the tissue during transition from one maturity stage to the other.

Effects of sulphur dioxide

There was a decrease of ascorbic acid in both the Pacific Bell and Ram Horn cultivars during storage, the decrease being higher in the Ram Horn cultivar (Table 5). The retention of ascorbic acid in blanched and unblanched Pacific Bell samples (56% and 49% respectively) was signficantly higher than in both blanched and unblanched non-sulphited samples (35% and 23% respectively), sulphur dioxide acting as an antioxidant in preventing the loss of ascorbic acid during frozen storage.

Table 5 also shows an initial difference of sulphur dioxide content in blanched

Table 5. Ascorbic acid, sulphur dioxide and total pigment content of mature, blanched and unblanched frozen capsicums stored under vacuum for 12 months at −12°C

	Ascorbic acid (mg/100 g)				Sulphur dioxide (mg/kg)				Pigment content of frozen tissue calculated as β-carotene (μg/g)			
	Unblanched		Blanched		Unblanched		Blanched		Unblanched		Blanched	
Cultivar	Initial	Retention (%)	Initial	Retention (%)	Initial	Retention (%)	Initial	Retention (%)	Initial	Retention (%)	Initial	Retention (%)
Pacific Bell (non-sulphited)	70.5	35	82.1	23	—	—	—	—	25.1	47	29.8	48
Pacific Bell (sulphited)	70.5	56	82.1	49	1186	73	1092	76	25.1	65	29.8	80
Ram Horn (sulphited)	113.1	25	142.7	21	234	38	159	38	13.7	34	15.1	74

and unblanched samples because of the greater penetration of sulphur dioxide in the blanched tissue. During storage, sulphur dioxide continued to decrease in both blanched and unblanched samples of both cultivars, although the retention in the Ram Horn cultivar was only half that in the Pacific Bell cultivar.

Since the Pacific Bell cultivar produces large fruits having a high flesh content, whereas the Ram Horn cultivar produces smaller fruit having a lower flesh content, the penetration of sulphite was greater in the fleshy Pacific Bell cultivar than in the Ram Horn cultivar where it remained mostly on the surface and from where it was rapidly lost during storage.

From the pigment data (Table 5) it can be seen that total pigment contents decreased during storage in both blanched and unblanched samples of both cultivars, the decrease being significantly higher in the blanched compared to the unblanched samples. Pigment retention was significantly higher in sulphited samples, e.g. the non-sulphited, unblanched Pacific Bell sample showed a

Table 6. Effect of blanching and sulphur dioxide on pigment content of mature Pacific Bell capsicum stored for 12 months at −12°C

	Pigment content (μg/g)							
	Sulphited				Non-sulphited			
	Unblanched, stored for (months)		Blanched, stored for (months)		Unblanched, stored for (months)		Blanched, stored for (months)	
Pigment	0	12	0	12	0	12	0	12
Chlorophylls								
Chlorophyll *a*	28.1	21.7	25.7	13.2	28.1	16.8	25.7	6.5
Chlorophyll *b*	16.2	12.9	15.1	11.2	16.2	12.0	15.1	7.6
Chlorophyll *b'*	—	—	1.1	7.6	—	—	0.3	0.2
Pheophytin *a*	—	1.4	—	—	—	3.0	1.1	7.9
Pheophytin *b*	—	0.7	—	2.1	—	2.2	—	3.1
Pheophorbide *a*	—	0.3	0.3	0.2	—	1.2	—	0.9
Chlorophyllide *a*	—	0.3	—	0.1	—	0.3	—	—
Carotenoids								
β-Carotene	2.9	2.3	2.4	1.9	2.9	1.9	2.4	1.7
Neo-β-carotene	—	Tr	Tr	0.2	—	Tr	Tr	0.1
α-Carotene	0.4	0.4	0.4	0.3	0.4	0.3	0.4	0.3
Lutein	3.5	3.4	3.5	3.1	3.5	3.5	3.5	3.0
Zeaxanthin	—	—	—	—	0.3	0.4	0.2	0.3
Violaxanthin	1.3	0.6	1.2	0.4	1.4	0.6	1.2	0.2
Neoxanthin	0.9	0.3	0.8	0.3	0.9	0.5	0.8	0.1
Luteoxanthin	0.1	0.4	0.1	0.4	—	0.6	Tr	0.8
Auroxanthin	—	0.1	Tr	0.1	—	0.3	—	0.6
Unidentified	—	0.04	—	0.1	—	0.3	—	0.5

— = Not detectable; Tr = trace.

pigment retention of only 48% compared to 80% retention in the sulphited unblanched sample.

Results of chlorophyll pigment determinations on sulphited and non-sulphited samples of Pacific Bell capsicums and sulphited Ram Horn capsicums are presented in Tables 6 and 7. Retention of both chlorophyll *a* and *b* was higher in sulphited, unblanched samples compared to blanched samples of both cultivars. Sulphur dioxide has long been used for its action in inhibiting non-enzymic browning, and also for the inhibition of enzymic browning and other enzyme-catalysed reactions. The role of sulphur dioxide in the present experiments is not clear. It would not appear to be acting only as an enzyme inhibitor since unblanched samples showed less chlorophyll change than blanched samples in which enzyme activity has been destroyed.

As in frozen non-sulphited samples, the most stable carotenoids were lutein and β-carotene, while the most unstable carotenoids in both cultivars were violaxanthin and neoxanthin. The most interesting point is that, unlike non-sulphited samples, there was no significant isomerization of violaxanthin, and no

Table 7. Effect of blanching on sulphited, mature Ram Horn capsicums stored for 12 months at −12°C

	Pigment content (μg/g)			
	Unblanched, stored for (months)		Blanched, stored for (months)	
Pigment	0	12	0	12
Chlorophylls				
Chlorophyll *a*	8.6	4.9	7.3	2.4
Chlorophyll *b*	4.6	2.5	4.4	1.9
Chlorophyll *b'*	—	—	0.4	0.2
Pheophytin *a*	—	2.4	0.9	2.8
Pheophytin *b*	—	0.5	0.1	1.0
Pheophorbide *a*	—	0.3	—	0.2
Chlorophyllide *a*	—	0.3	—	0.1
Carotenoids				
α-Carotene	1.45	1.26	1.38	1.11
ζ-Carotene	0.13	0.13	0.13	1.10
5,6-Diepoxy-β-carotene	0.59	0.31	0.55	0.11
Lutein	3.10	2.8	3.10	2.9
Violaxanthin	0.82	0.45	0.68	0.16
Neoxanthin	0.63	0.43	0.61	0.39
Zeaxanthin	—	0.17	—	0.23
Luteoxanthin	—	0.07	—	0.10
Auroxanthin	—	0.16	—	0.24

— = Not detectable; Tr = trace.

auroxanthin was found in the Pacific Bell cultivar at the end of 8 months' storage. However, at the end of 12 months' storage only 0.1 μg/g of luteoxanthin and a trace amount of auroxanthin were found in this cultivar, while a slightly higher proportion of both luteoxanthin and auroxanthin were found in the Ram Horn cultivar, especially in the blanched sample compared to the Pacific Bell cultivar. These differences in xanthophyll isomerization might be partly due to the low concentration of sulphur dioxide (500 mg/l) used for the Ram Horn cultivar compared to the high concentration (1500 mg/l) used for the Pacific Bell cultivar. Moreover, during storage the rate of loss of sulphur dioxide from the Ram Horn cultivar was high with only 33% sulphur dioxide retained after 12 months' storage compared to 72% retention of sulphur dioxide in the Pacific Bell cultivar giving rise to the higher retention of carotenoid pigments. However, the mechanism of retardation of the isomerization of 5,6-epoxides to 5,8-epoxides by sulphur dioxide is at present unknown. Further work is required to determine the effect of sulphur dioxide on carotenoid degradation, oxidation and isomerization, specially the mechanism of the conversion of 5,6-epoxides to 5,8-epoxides.

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Collaborative trial to examine the commercial application of parts of Council Regulation (EEC) No. 2967/76 laying down common standards for the water content of frozen and deep frozen chickens, hens and cocks

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Summary

Under commercial conditions the techniques described in Annexes I and III of Regulation 2967/76 are practicable. However, in interpreting the water/protein relationships of commercially produced poultry, consideration should be paid to the fact that water pick-up during spray washing and mechanical immersion chilling is inversely related to carcase weight. Also since offal contains a higher physiological water content than chicken carcases and the carcases are always packed with a random set of washed offal their influence on the water/protein relationship of the carcase and offal combined is greater for smaller carcases than for larger ones. (In accordance with UK practice throughout this paper, offal means neck, gizzard, liver and heart.)

Introduction

During the commercial preparation of frozen poultry it is inevitable that the carcases come into contact with water (Thomas, 1977; Erdtsieck, 1978). Some of this water will be retained by the carcases particularly if they are chilled by immersion in water prior to freezing. In an attempt to control the amount of extraneous water present in frozen poultry the Council of the European Community promulgated a Regulation in 1976 (Council Regulation (EEC) No. 2967/76, hereafter called 'The Regulation') (Council, 1976) laying down common standards for the water content of frozen and deep frozen chickens, hens and cocks. Annex I of the Regulation requires the producers of frozen poultry to regularly check and control the amount of water picked up during washing and chilling. (Within the context of this paper 'poultry' means chickens,

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hens and cocks.) The average amount of pick-up over these processes is limited to 5% of the weight of the carcase determined at the end of the evisceration line or such other figure as will allow compliance with a prescribed limit on total extraneous water (i.e. water retained at all stages of the process). The prescribed limit for total extraneous water varies with the method of estimation. Within the UK total extraneous water will be controlled using the method laid down in Annex III of the Regulation. This method relates the average total water content of a sample of carcases to the average protein content. The prescribed limit for extraneous water is expressed in the form of a water/protein regression which is based upon research carried out in a number of laboratories within the Community (Commission, 1976). This paper describes an experiment carried out jointly by members of the British Poultry Meat Association, consumer protection officials, the Food Research Institute and the Ministry of Agriculture, Fisheries and Food to examine the relationship between Annexes I and III of the Regulation when applied to poultry produced under commercial conditions in the UK. Additionally, results are presented to relate the results obtained to estimates of extraneous water content obtained by measuring loss on thawing using both the procedure laid down in Annex II of the Regulation and an alternative (room temperature) technique proposed by the Food Research Institute.

Experimental

Annex I procedure

The Annex I technique used was that subsequently incorporated into the Regulation by Regulation (EEC) No. 2632/80 (Commission, 1980). The technique involves the selection of 25 carcases at random from the evisceration line before the final washing preceding chilling. These carcases are then identified, weighed and rehung on the line to pass through the normal washing and chilling process. The identified carcases are removed at the end of the drip line and the first 20 recovered are individually reweighed to determine the average percentage pick-up. In this experiment, at each of the plants involved, 21 such checks were carried out over a period of five working days. In addition, the individual weights of all the identified carcases recovered were recorded. A set of washed offal was added to the carcases after the second weighing in accordance with normal UK practice and the carcases were frozen and stored until required for analysis.

Annex II and III procedure

Eighty-four sample carcases were taken at random from the frozen carcases recovered from the in-plant phase of the experiment at each plant, for analysis using the procedure laid down in Annex III of the Regulation. This Annex requires the whole frozen carcase including offal to be homogenized and the

homogenate analysed for water and protein content using the weight loss on drying and nitrogen estimation procedures of ISO 1442 and ISO R 937 respectively. Eighty sample carcases were also taken to determine weight loss on thawing both at 42°C using the procedure in Annex II of the Regulation and also at room temperature.

Plant operation

Three poultry processing plants took part in the trial and the birds used were chosen at random from the normal input to the plants over the trial period, and were mainly Ross or Cobb breeds. The birds varied in age from 48–58 days and were of live weights in the range from about 1.7–2.2 kg.

Average weight gains of about 5–6% determined by the Annex I procedure were aimed at during the 21 runs at each plant. This was achieved by adjusting the period spent in the chiller on the basis of another Annex I test carried out prior to the experimental runs. This figure is somewhat above the figure envisaged in the Regulation but allows for the normal commercial practices of grading and packing after the second weighing point and the associated water losses. Such flexibility is allowed for in paragraph 9 of Annex I ('result shall not exceed 5% . . . or any other figure allowing compliance with the total permitted extraneous water content'). Chilling equipment at each plant was operated in accordance with the conditions subsequently laid down in Directive 78/50/EEC (Council, 1978) which supplements, as regards the chilling process, Directive 71/118/EEC (Council, 1971) on health problems affecting trade in fresh poultry meat.

Results and discussion

Annex I tests

During the course of the in-plant tests 525 carcases were weighed at each plant before immersion and all but a few of these were recovered and reweighed after immersion and again after the addition of a set of offal. Table 1 shows the mean results for all of the carcases recovered over the 21 tests at each plant.

Table 1. Mean carcase weights at each plant (g)

Plant		1	2	3
First weighing	Mean	1434.1	1367.3	1324.2
	sd	223.5	189.5	183.7
Second weighing	Mean	1518.3	1443.7	1397.8
	sd	230.0	193.3	192.3
Weight with offal	Mean	1623.5	1578.7	1514.6
	sd	233.3	194.6	197.3
Weight of offal	Mean	105.2	135.0	116.8
	sd	19.1	14.1	19.2

The Annex I result was calculated for each run as was the average pick-up for all the carcases recovered during each run. (The Annex I test is based on the results of the first 20 carcases recovered.) The two results for each run are tabulated in Table 2 together with their means for the 21 runs. The two sets of figures for each plant do not differ significantly indicating that carcases recovered later and excluded from the Annex I test do not pick up more water than those included in the Annex I calculations.

Table 2. Summary of in-plant pick-up figures (% of prechiller weight)

Plant	1		2		3	
Run number	Annex I result	Average pick-up	Annex I result	Average pick up	Annex I result	Average pick-up
1	5.81	6.01	6.66	5.85	4.05	4.06
2	5.66	5.61	5.07	5.12	4.14	4.20
3	5.35	5.89	5.49	5.40	7.04	6.92
4	5.98	6.46	5.18	5.03	6.42	6.43
5	6.97	6.85	5.18	5.01	6.05	5.76
6	6.10	6.16	4.70	4.65	4.98	5.34
7	5.35	5.35	4.97	4.88	5.82	5.94
8	4.30	4.41	4.64	4.57	5.61	5.84
9	5.45	5.43	5.30	5.33	5.23	5.37
10	5.14	5.37	5.25	5.40	5.05	5.33
11	4.90	5.27	6.09	5.81	5.03	5.16
12	5.45	5.38	6.60	6.50	5.40	5.49
13	6.46	6.33	6.36	6.23	4.86	4.96
14	6.13	6.52	6.33	6.55	6.49	6.30
15	6.95	7.08	7.30	7.41	4.71	4.82
16	5.95	6.01	5.78	5.81	6.70	6.89
17	6.47	6.26	5.03	4.85	5.97	6.14
18	6.45	6.63	4.62	4.61	5.63	5.59
19	5.92	6.00	8.68	8.65	5.73	6.49
20	5.53	5.22	5.59	5.48	4.72	4.84
21	5.28	5.34	4.55	4.72	4.92	5.07
Mean	5.790	5.885	5.684	5.517	5.455	5.569
sd	0.662	0.652	1.027	1.132	0.807	0.789

The Annex I result is the mean pick-up by the first 20 carcases recovered from the 25 weighed prior to chilling, the average pick-up is the mean pick-up by all of the carcases recovered during the run.

From the individual carcase figures at each plant the mean figure for individual carcase pick-up was calculated, the results being given in Table 3. These differ from the mean figures given in Table 2 which are the means of the results for each run, which are themselves means. Histograms showing the pick-up of the individual carcases recovered at each plant are given in Figs 1–3. Some

Table 3. Individual carcase pick-up (% of prechiller weight)

Plant	Mean	sd
1	5.97	2.36
2	5.68	2.19
3	5.60	2.17

Table 4. Pick-up for carcases with water pockets (% of prechiller weight)

Plant	Number of carcases	Pick-up (%) Mean	sd
1	36	9.51	3.12
2	48	9.67	2.69
3	8	11.86	3.98

positive tailing occurred and some carcases with uptakes in excess of 12% were recovered. This tailing was particularly noticeable at plant 1. Some carcases were recorded as having readily noticeable pockets of water beneath the skin at the end of the drip line and the mean pick-up of these carcases is shown in Table 4. Histograms for the pick-up for the individual carcases, excluding those with water pockets, are superimposed on Figs 1–3.

Analysis of the factors influencing carcase pick-up indicated that carcase weight was significant. Regressions were calculated to relate percentage weight gain (U) to eviscerated carcase weight (E) in the form $U = aE + b$. Parameters calculated for these regressions are shown in Table 5. The regressions are significant at the $P < 1\%$ level even though the scatter is high.

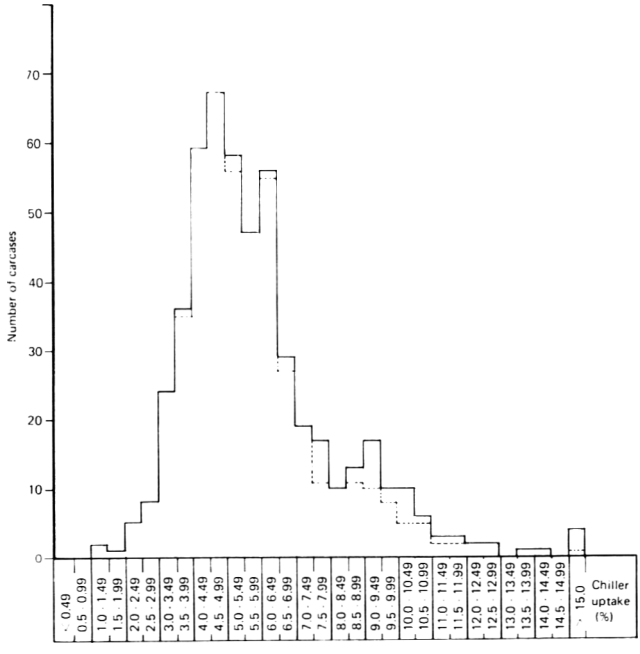

Figure 1. Histogram showing individual carcase pick-up figures at plant 1. (———), all carcases; (— — —), excluding carcases with water pockets.

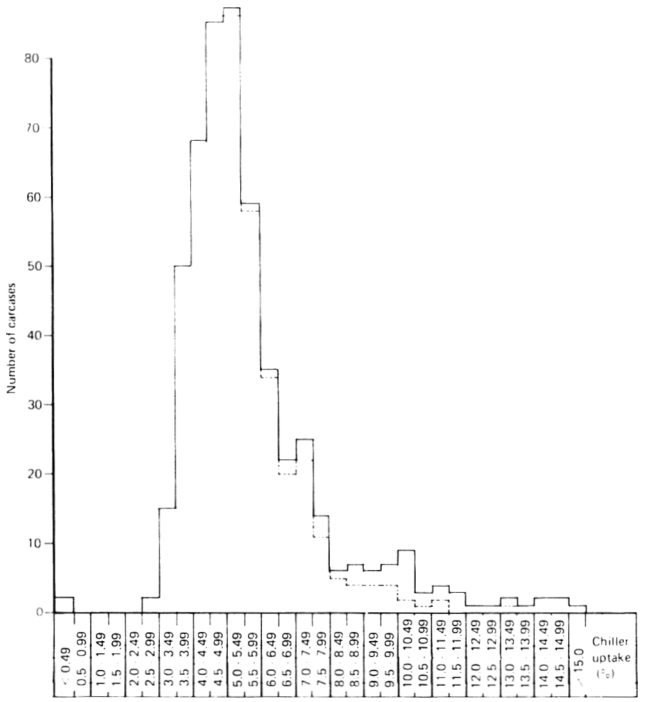

Figure 2. Histogram showing individual carcase pick-up figures at plant 2. (———), all carcases; (— — —), excluding carcases with water pockets.

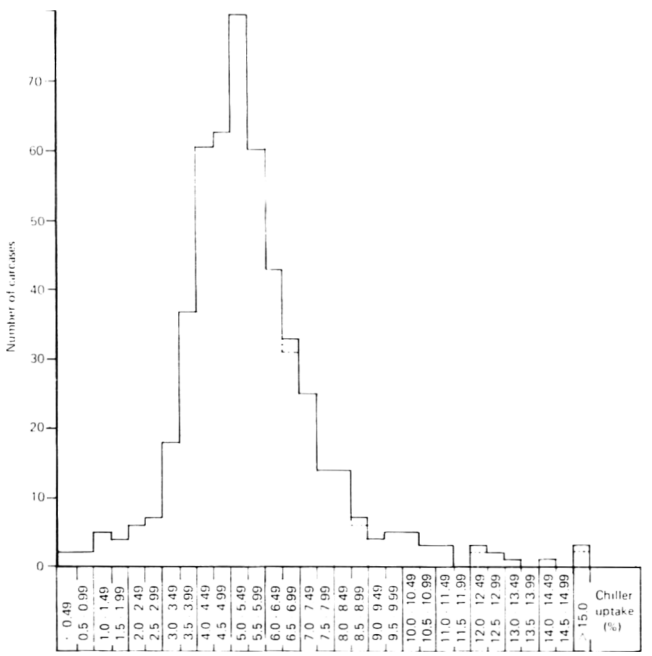

Figure 3. Histogram showing individual carcase pick-up figures at plant 3. (———), all carcases; (— — —), excluding carcases with water pockets.

Table 5. Effect of carcase weight on percentage weight gain ($U = aE + b$)

Plant	a	b	Correlation coefficient
1	−0.0028	10.00	−0.266
2	−0.0035	10.42	−0.299
3	−0.0017	7.85	−0.133
Overall	−0.0024	9.07	−0.215

Annex III tests

Eighty-four carcases from each plant selected randomly from those subjected to in-plant checks were analysed, the analyses for each plant being distributed between six of the nine participating laboratories.

A pre-trial experiment demonstrated that inter-laboratory variations in mean protein content determination could, when applied to a whole carcase, result in a variation of about 5% in the calculated physiological water content. The limit regressions in Annex III of the Regulation have, built into them, confidence limits which take into account inter-laboratory variations observed between the results from eight EEC laboratories. Analytical variations in this trial were no larger than in the EEC experiment used as a basis for the regressions in the Regulation. Inter-laboratory variations in analytical results for more conventional meat product analysis are not a barrier to enforcement. It was therefore concluded that the results of this trial properly reflect the working of the Regulation under practical conditions of enforcement.

For each laboratory in the trial the water and protein contents of the individual carcases were fitted to the linear regression equation $W = aP + b$ where W and P are the water and protein contents (g) respectively. The parameters a and b for each laboratory are given in Table 6 along with the correlation coefficients.

In order to draw conclusions about the overall behaviour of individual plants the analytical figures from all laboratories for each plant were combined to give a single regression line for each plant. The parameters a and b and the correlation coefficients for these three lines are also given in Table 6. In this way conclusions can be drawn concerning the operation of the Regulation under practical conditions. A similar combination of laboratory result was made in the EEC experiment (Commission, 1976).

Prechiller pick-up

The water uptake by the carcases prior to spray washing was estimated for those carcases analysed by the Annex III procedure. The pre-spray wash uptake was expressed as a percentage of the eviscerated carcase weight. It was calculated by subtracting from the estimated water content of the carcase with

Table 6. Water/protein regressions ($W = aP + b$)

Plant	Lab	a	b	Correlation coefficient
1	1	3.30	181.97	0.964
	2	3.77	54.43	0.965
	3	3.82	62.94	0.937
	4	4.01	− 12.16	0.976
	8	3.51	133.04	0.970
	9	3.19	208.13	0.983
	Combined	3.53	122.61	0.959
2	1	3.79	37.41	0.973
	2	3.36	141.84	0.978
	3	3.78	6.69	0.979
	5	3.28	175.55	0.961
	8	3.65	78.97	0.963
	9	3.26	196.46	0.983
	Combined	3.48	115.95	0.962
3	1	3.19	194.31	0.971
	2	3.99	− 11.04	0.973
	3	3.79	25.42	0.988
	6	3.48	139.44	0.936
	8	3.32	150.11	0.965
	9	3.59	83.49	0.984
	Combined	3.54	102.16	0.964

offal the water content of the offal, the pick-up and the physiological water content of the carcase without offal. The last mentioned quantity was not determined due to the practical difficulties and cost of so doing but was estimated as $W = 3.31P + 41.5$ where P is the calculated protein content of the carcase without offal. This equation forms the basis of Community Legislation and was derived in the EEC experiment (Commission, 1976) for the mean physiological water content of dry processed carcases. There must therefore be a considerable degree of uncertainty in the estimate of pre-spray washer uptake and this is reflected in the high standard deviations about the mean values given in Table 7.

Table 7. Pre-spray washer uptake (% of eviscerated weight)

	Uptake %	
Plant	Mean	sd
1	2.16	1.71
2	1.45	2.03
3	1.70	1.77

Offals

All of the carcases analysed in the trial included a washed set of offal, which following UK practice included neck, gizzard, liver and heart. In order to determine the influence of offals in this experiment the average weight and composition of both dry (unprocessed) and wet (washed and processed) offals was determined at each plant and the mean results are given in Table 8.

Table 8. Composition of offals (mean of all plants)

Wet or dry processed offal	Offal	Mean weight (g)	Composition (%)		
			Moisture	Protein	Fat
Dry	Heart	11.1	67.11	12.86	19.02
	Liver	36.2	70.77	19.43	8.19
	Neck	29.9	68.98	17.99	7.61
	Gizzard	46.2	56.52	13.16	28.76
Wet	Heart	11.7	69.21	12.22	17.38
	Liver	36.4	73.35	18.73	6.70
	Neck	25.2	71.65	16.59	6.87
	Gizzard	46.5	74.10	15.78	9.23

From the mean results for dry offals the nitrogen factor (f) was calculated for each plant as $f = 100P/6.25\ (100 - F)$ where $P = \%$ protein (nitrogen $\times$ 6.25) and $F = \%$ fat. Overall values for each offal (which includes bone) calculated by combining the results for each plant are given in Table 9 along with the

Table 9. Offals – nitrogen factors and extraneous water content

Offal	Plant	Calculated nitrogen factor (f)	Calculated extraneous water (%) (H)
Heart	1	2.50	4.45
	2	2.58	7.68
	3	2.54	4.83
	Overall	2.54	5.67
Liver	1	3.26	4.12
	2	3.46	6.94
	3	3.45	3.44
	Overall	3.39	4.79
Neck	1	2.98	5.38
	2	3.25	11.24
	3	3.11	6.96
	Overall	3.12	7.92
Gizzard	1	2.92	5.16
	2	3.01	9.85
	3	2.94	0.90
	Overall	2.96	5.36

extraneous water content *(H)* of the wet offals calculated as $H = 100 - F - 100P/6.25f$. The reason for the higher extraneous water content in the offals from plant 2 is unknown, but is probably related to the harvesting and processing conditions.

Water/protein ratios were calculated for both wet and dry offals using the mean compositional data. For sets of wet and dry offal the ratios are respectively 4.42 and 4.01. The factor 3.82 is used in the EEC limit regression for carcases sold with or without offal, but the above result suggests that the same regression limit might not be equally applicable to carcases sold with and without offal. The sets of offal packed with the carcases did not originate in the same carcases. Thus, the effect of the offal water/protein ratio on the composition of the carcase plus offal will depend upon the relative amount of offal with the carcase and will be proportionally higher with smaller carcases.

In order to estimate the magnitude of the effect of using the same limit regression for carcases sold with and without offal it was assumed that the maximum water which could be associated with the offal is given by $3.82P_o$ where P_o is the protein content (g). This was calculated for each plant and subtracted from the average water found in the wet offals from each plant. The difference (shown as 'excess water in offals' in Table 10) represents the amount of water present in the offal which has to be counted towards the extraneous water content of the carcase. This effect is very significant especially for small carcases and reflects differences between this trial using random sets of wet, chilled offal and the EEC experiment in which each carcase was analysed with the set of dry (unprocessed) offal originating from that carcase.

Table 10. Excess water in offals

Plant	Mean wet offal wt (g)	Mean offal protein (%)	Mean offal water (%)	Excess water in offals (g)
1	109.6	15.95	72.19	12.3
2	136.1	16.18	73.03	15.3
3	113.6	17.33	73.36	8.1
Average	119.8	16.50	72.88	11.8

Drip (thaw) tests

Eighty carcases from each plant selected randomly from those subjected to in-plant checks were analysed by the drip test procedure in Annex II of the Regulation and 80 carcases (60 from plant 1) were analysed by a modified procedure in which they were thawed at room temperature for 24 hours. The mean drip loss is compared in Table 11 with the mean measured pick-up for the same carcases.

Table 11. Summary of drip loss results

	Mean drip loss			
	Annex II method		Modified method	
Plant	% of carcase wt inc. offal	% of wt of pick-up	% of carcase wt inc. offal	% of wt of pick-up
1	4.40	92.00	3.52	68.10
2	4.98	102.63	4.00	87.90
3	4.30	91.19	3.68	87.09

Regressions relating drip to pick-up (both in g) were calculated from the results for each plant in the form drip = a (pick-up) + b). The parameters are shown in Tables 12 and 13.

Table 12. Regression parameters – Annex II drip test

Plant	a	b (g)	Correlation coefficient
1	0.503	30.40	0.744
2	0.514	37.46	0.774
3	0.538	23.81	0.873

Table 13. Regression parameters – modified drip test

Plant	a	b (g)	Correlation coefficient
1	0.577	7.55	0.845
2	0.416	31.54	0.651
3	0.348	31.09	0.552

On balance it would appear that the procedure in Annex II gives a better indication of pick-up than the modified procedure. Neither technique however gives more than an indication of pick-up.

Annex II of the Regulation prescribes a limit on drip loss of 5.2% of the frozen carcase weight. From the figures given in Table 11 it is apparent that the risk of samples failing this test is minimal. The Annex I test result therefore provides a more reliable prediction of the probability of failing the Annex III test. In addition in assessing the usefulness of Annex II it must be remembered that drip loss is less sensitive to changes in pick-up than chemical analysis and may be influenced amongst other things by factors such as storage time and temperature.

Statistical analysis of results

The overall plant water/protein regressions and their error variances were used to calculate the probability that one or two samples of seven carcases taken at the end of the drip line under the conditions of the trial would fail the Annex III test

according to which the mean water content of a sample of seven carcases must not exceed $3.82P + 59$ where P is the mean protein content. The plant regressions, given in Table 6, and the EEC limit regression are not parallel as can be seen from Fig. 5. This is not surprising since it has been demonstrated that percentage pick-up is weight dependent and the EEC limit was calculated on the basis of an average pick-up at all weights of 8%. The effect of the higher water content of offals also becomes more significant at lower carcase weights. The mean water content for samples of carcases (including offal) with a mean protein content of 200, 250 or 300 g calculated in accordance with the individual plant regressions is shown in Table 14. For comparison the water content limit calculated in accordance with the EEC limit regression is also included.

Table 14. Calculated mean total water content for carcases as a function of mean protein content

Regression	Water content corresponding to a mean protein content of (g) 200	250	300
Plant 1	828.6	1005.1	1181.6
Plant 2	812.0	986.0	1160.0
Plant 3	810.2	987.2	1164.2
EEC limit	823.0	1014.0	1205.0

The probability that either one or two samples of carcases, taken at random from the end of the drip line, would fail the Annex III test depends on the mean protein content and hence on the average carcase weight of the sample. Table 15 shows, for each plant, the probability that one or two samples of seven carcases would fail the Annex III test if the plant were operating at an actual percentage pick-up equal to the calculated mean pick-up of all the carcases analysed by the Annex III test at that plant.

Table 15. % probability of samples of seven carcases failing the Annex III test when the pick-up is equal to the mean pick-up of carcases analysed by the Annex III procedure

Plant	No. of samples	Probability of failure (%) with a mean sample protein content of (g) 200	250	300
1	1	62	26	6.5
2	1	25	2.1	0.2
3	1	15	1.1	0.2
1	2	38	6.9	0.4
2	2	6.4	0.04	<0.01
3	2	2.1	0.01	<0.01

The raw data for the carcases weighed at each plant were used to calculate, for each plant, the probability that one or two samples of seven carcases would fail the Annex III test if the plant were operating at an actual percentage pick-up equal to the calculated mean pick-up of the carcases examined in the in-plant checks. Results are given in Table 16 (cf. Table 15).

Table 16. % probability of samples of seven carcases failing the Annex III test when the pick-up is equal to the mean pick-up of carcases tested in plant

Plant	Pick-up (%)	No. of samples	Probability of failure (%) with a mean sample protein content (g) 200	250	300
1	5.97	1	34	6	3
2	5.68	1	13	2	0.4
3	5.60	1	2	0.1	<0.1
1	5.97	2	11	0.4	<0.1
2	5.68	2	1.5	<0.1	<0.1
3	5.60	2	0.1	<0.1	<0.1

In order to relate these probabilities to practical enforcement where carcase weight and not protein content is known the protein contents of Table 15 must be translated into carcase + offal weights using the mean protein/carcase weight ratios for each plant (respectively 16.13, 16.39 and 16.36% at plants 1, 2 and 3).

It was also possible to estimate the probability that a sample of seven carcases selected at random from each plant operating at any given mean overall pick-up would fail the Annex III test. The results of this estimation are given in Fig. 4.

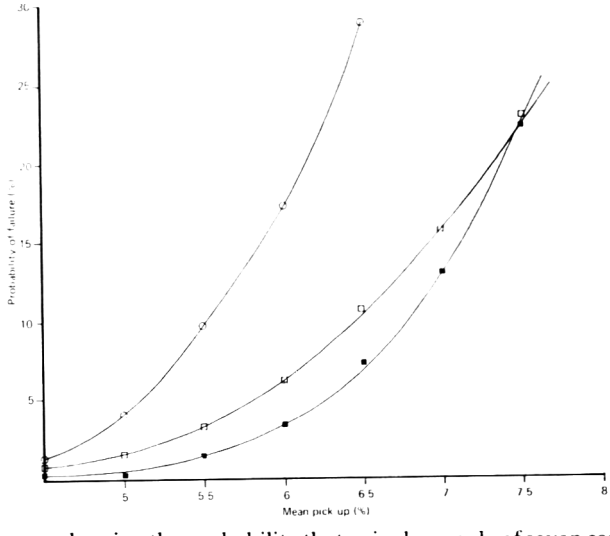

Figure 4. Curves showing the probability that a single sample of seven carcases at each plant would fail the Annex III test, as a function of mean percentage pick-up. (○), plant 1; (□), plant 2; (■), plant 3.

Thus the probability that a random sample from the output of each plant operating under the conditions of the trial would fail the Annex III test was estimated. The results are shown in Table 17.

Table 17. Probability of random samples of seven carcases failing Annex III

Plant	No. of samples	Probability of failure (%)
1	1	17
2	1	4
3	1	1.5
1	2	3
2	2	0.2
3	2	<0.1

Correlation of analytical and in plant tests

Table 18 shows the risk of failing the Annex III test at each plant and the mean Annex I and mean pick-up figures taken from Tables 2 and 3 respectively.

Table 18. Pick-up and risk data

		Pick-up			
	Risk of failure*	Annex I results		Individual results	
Plant	(%)	Mean	sd	Mean	sd
1	7	5.8	0.7	6.0	2.4
2	0.04	5.7	1.0	5.7	2.2
3	0.01	5.5	0.8	5.6	2.2

*Percentage risk of two samples of seven carcases of 250 g average protein content both failing the Annex III test.

The greatest risk of two samples of seven carcases (random or of 250 g mean protein content) failing the Annex III test was at plant 1 where it was more than 100 times greater than at the other plants. This was despite the mean protein content of the carcases examined being lower at plant 1 than at plant 3 and the mean Annex I results being comparable at plants 1 and 2.

It seems likely that the high failure risk at plant 1 results from the greater spread of individual carcase pick-up figures, the skewness of the individual carcase pick-up curve (Table 3, Figs 1 and 2), and the higher pre-chiller uptake although carcase characteristics may also be involved. The greater pick-up spread in the chiller and the higher pre-chiller uptake could be associated with the design and operation of the plant. Calculations indicate that the risk of failure at plant 1 could be reduced to about 1 in 400 by reducing the mean Annex I figure by about 0.5%. Alternatively examination of the processing line might indicate where modifications could reduce the risk of 'rogue' carcases with a

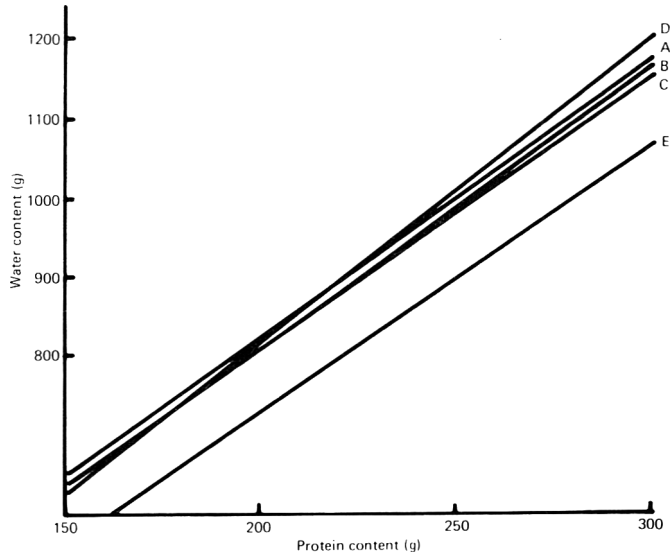

Figure 5. Individual plant and EEC regressions relating total water content to protein content. (A), plant 1; (B), plant 2; (C), plant 3; (D), EEC limit; (E), physiological water content as determined in the EEC experiment.

high water content while remaining within constraints imposed by hygiene requirements and thus reduce the risk of failure whilst keeping a mean Annex I result of 5.5%.

Conclusions

The tests described in this paper were carried out under commercial conditions in three plants in the UK in contrast to earlier EEC studies conducted under purely experimental conditions. The main conclusions are as follows:

The Annex I (in-plant) test measures the average weight gained during washing and chilling of the first twenty carcases to emerge from a random group of 25. In the three plants operating under the conditions of the trial the Annex I result approximates to the true average weight gain of the carcases being chilled and is therefore a reliable and simple method of monitoring weight gain during the washing and chilling process.

Neither the Annex II drip test nor a modified (room temperature) drip test give a reliable estimate of the total extraneous water content of frozen chicken carcases. The probability that samples taken from the carcases examined during the trial would fail the Annex II limit of 5.2% was minimal.

The Annex III test uses a relationship between water and protein content as a method of estimating extraneous water. In interpreting water/protein relationships several factors need consideration including the following:

(1) Under commercial conditions the percentage water pick up during spray washing and chilling is inversely related to carcase weight.

(2) Offals contain a higher physiological water content than chicken carcases and under commercial conditions chickens are unlikely to be packed with their own offal but with a random set of washed offal. The influence of offals on water/protein relationships is greater for smaller carcases than for large ones.

The techniques described in Annexes I and III of the Regulations are practicable under commercial conditions. However in determining the level of pick-up which corresponds to a particular risk of failing the Annex III test the following factors need to be considered.

(1) The chance presence in the sample of carcases with high extraneous water contents, for example carcases with water pockets, will have only a small effect on the Annex I result but can have a considerable effect on the risk of failing Annex III;

(2) Under the conditions of the trial the risk of failing the Annex III test was higher for smaller carcases than for larger, and higher for those packed with offal than without.

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Technical note: Reaction of egg extracts with 3, 3′, 5, 5′ tetramethyl benzidine—a possible method for evaluating the storage history of chicken shell eggs

J. B. MONSEY AND J. M. JONES

Introduction

It has long been known that the internal quality of shell eggs deteriorates on storage, especially at elevated ambient temperature. The viscosity of the egg white is lowered, while the egg yolk membrane weakens leading to rupture. Consequently the baking properties of the egg are adversely affected and foaming agents (e.g. triethyl citrate) are often added to egg white to facilitate increased aeration during whipping, prior to cake making. For these reasons, in some countries there is legislation for the transport and storage of eggs. In such circumstances, a test to determine compliance would be of value.

The sensitive test for the estimation of blood using 3, 3′, 5, 5′ tetramethyl benzidine (TMB) described by Liem *et al.* (1979) was applied to incubated, fertile eggs as a possible means of detecting incubator rejects, i.e. those eggs which fail to develop chicks. In practice, it was found that some batches of incubator rejects contained no blood and thus the TMB test could not be applied (J. B. Monsey & J. M. Jones, unpublished). However it was noted that when a small amount of copper was present in the reaction mixture, a positive reaction was obtained with both fertile and infertile incubated eggs. This reaction of blood free eggs with TMB in the presence of copper suggested a possible means of determining the storage history of shell eggs.

Materials and methods

Materials

The eggs used, less than 24 hr old and purchased locally, were from a brown egg laying flock (Hubbard Comet). TMB was supplied by Aldrich Chemical Co., Milwaukee, U.S.A. or Sigma Chemical Co., St Louis, U.S.A. All other

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chemicals were 'Analar' grade supplied by British Drug Houses Ltd, Poole, U.K.

Storage of eggs

Eggs were placed in constant temperature rooms maintained at 10°, 20° and 25°C. Batches of 2 dozen were withdrawn after 1, 2, 3 and 4 weeks of storage. The eggs from each batch were homogenized together (MSE Ato-mix, ½ speed) before testing. In addition, other eggs were placed in a commercial incubator and sampled at intervals up to 18 days. Eggs containing obvious blood spots were rejected.

Method

Whole egg (10 g) was shaken vigorously with chloroform (20 ml) in a 50-ml stoppered centrifuge tube for 2 min. After centrifugation (MSE minor at 2000 × *g*) for 15 min the clear amber-coloured top layer was removed. To 2.0 ml of this aqueous extract 0.10 ml $CuSO_4$ $5H_2O$ solution (1% w/v) was added and mixed well. One ml of this mixture was added to 1.0 ml TMB solution (0.20% w/v in 10% acetic acid v/v) contained in a stoppered tube. After 2 min, 1.0 ml H_2O_2 solution (4.0 ml 100 volumes diluted to 100 ml) was added and the contents of the tube mixed well. After 5 min 10 ml acetic acid (10% v/v) were added. The absorbance was measured after a further 55 min either at a fixed wavelength of 370 nm using a Unicam SP500 spectrophotometer or scanned (Unicam SP800) between 700 and 325 nm in 10-mm cells with water in the blank cell.

Results and discussion

In agreement with Liem *et al.* (1979), it was found that the green oxidised form of TMB absorbed in three spectral regions, with the highest absorbance at 370 nm: a characteristic absorption spectrum is shown in Fig. 1. With our modification using copper in the test system, solutions from both stored and unstored eggs were green on the addition of H_2O_2, the colour from the stored eggs however remained and even increased in intensity on standing, whereas with unstored eggs the colour faded and was completely gone within 55 min.

Fig. 2 shows the effect of storage conditions on the reaction with TMB. When eggs were stored at 10°C no colour was seen in the test solutions when the eggs were less than 2 weeks old and even at four weeks of storage the absorbance was low. With increase in storage temperature the colour became correspondingly more intense and when eggs were held at hatching temperature (37.2°C) the maximum colour was achieved after only 1 week of storage.

Additionally eggs were purchased from four different local shops and tested after ensuring that they were free of traces of blood. The absorbances at 370 nm were 1.22, 0.54, 1.55 and 0.76. Although only small samples (6 eggs each) were

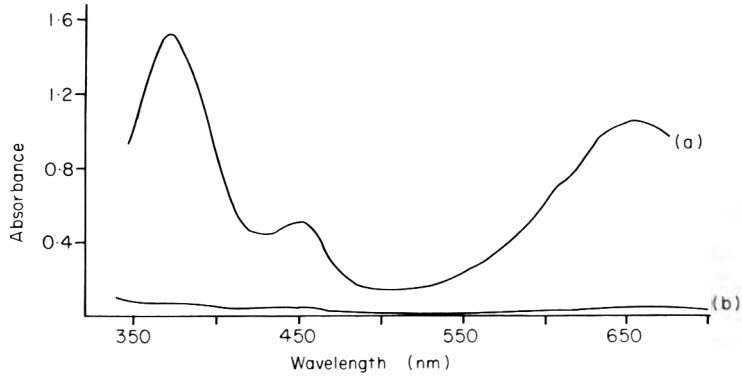

Figure 1. Typical absorbance spectra of the coloured reaction product of TMB with aqueous extracts of eggs. (a) Eggs stored at 20°C for 4 weeks; (b) unstored eggs.

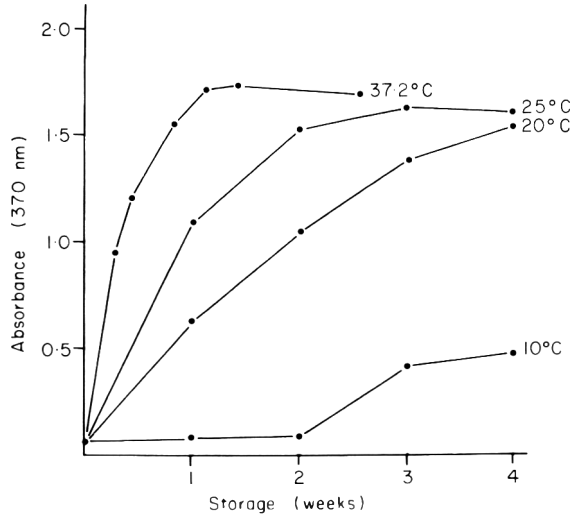

Figure 2. Effect of egg storage time and temperature on the colour reaction of aqueous extracts with TMB.

taken, the very different absorbance values probably reflected the variable storage histories of these eggs.

The nature of the reaction between stored eggs and TMB in the presence of copper remains obscure and clearly needs to be elucidated before the chemical test procedure for the evaluation of shell egg shelf life can be recommended.

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Liem, H.H., Cardenas, F., Tavassoli, M., Poh-Fitzpatrick, M.B. & Muller-Eberhard, U. (1979) *Anal. Biochem.* **98,** 388.

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Book reviews

Fluidization. Ed. by J. R. Grace and J. M. Matsen.
New York: Plenum Press, 1980. Pp. xviii + 605. $69.50.

Fluidization is, of course, widely used in the process industries, including the food industry, where advantage may be taken of its attractive characteristics of high rates of heat transfer, temperature uniformity and solids mobility.

This book of 600 pages has been produced in camera-ready format to speed publication and contains three review papers and 60 refereed research papers presented at the International Fluidization Conference held in the USA in August 1980.

The three review papers provide detailed surveys of the development of fluidized bed technology; flow in vertical pneumatic beds; and mixing patterns in large-scale fluidized beds.

The research papers present an up-to-date picture of international research activity in fluidized bed technology and all the major research centres connected with the field have made submissions. The papers fall into three main groups: fluid bed heat and mass transfer and combustion; mixing, segregation, entrainment and attrition of particles; and papers concerned with the design aspects of fluidized beds. The submissions have been carefully written and edited to present a standard format which makes for easy reading in most cases. The papers are well supported by extensive references; the figures and diagrams, with one or two exceptions, are clear and unambiguous and very few typographical errors are evident.

The research work reported is mainly concerned with small (< 1 mm) particle behaviour and, not surprisingly, is preoccupied with the major applicational fields of coal gasification, combustion and catalysis—areas of activity not of direct interest to the food industry.

Much of the work reported is concerned with the development of correlations to enable predictions of behaviour in fluidized beds but such correlations are generally empirical and specific to a given system. As such it is unlikely that direct extrapolation to any other system could be practised with confidence. Thus direct quantitative application of the data reported to food systems is likely to be unrewarding.

There is little to be found on the important subjects of liquid-solid fluidization or of pneumatic or hydrodynamic transfer and only one paper is directly concerned with foods.

In spite of these comments, this important work represents a valuable addi-

tion to the literature available to students and practitioners of fluidization and many will feel that at a price of $69.50 it is a good buy.

A. E. V. Lilly

Chemistry and the Food System. A Study by the Committee on Chemistry and Public Affairs. American Chemical Society.
Washington, D.C.: American Chemical Society, 1980. Pp. xvi + 138. ISBN 0-8412-0557-4. $15.00.

This report is one of a series of studies of public problems involving chemistry, and as such is aimed at the general public, and in particular, at that section of the public involved in public policy-making: members of the legislature, leaders of industry, activists in environmental and related societies and organizations. The 11 contributors are all distinguished scientists from university departments of Chemistry, Chemical Engineering and Food Science, research institutes of the US Department of Agriculture and of industrial companies with interests in the area of agricultural chemicals. Like many an official report, the book opens with a chapter of summary and recommendations—on fertilizers, on pest control, on crop, animal and fish production, on food additives, on the handling, storage, preservation and processing of foods, on food fortification, dietary foods and unconventional food sources, and on 'the wise use of chemicals'. In such a wide-ranging survey it is perhaps inevitable that the recommendations often seem pretty general and non-specific; a cynic might sum them up as: spend more on chemical research. Bearing in mind that 'chemistry' is here taken to include the many applied disciplines involving chemistry, including agriculture, nutrition, biochemistry, food science—and, possibly, even microbiology—food scientists will have little dispute with these recommendations, but will wonder if they mean very much to the layman.

Each of the topics is developed at greater length in subsequent chapters in a way which serves to show something of the interaction of scientific research, economics and public choice. In each chapter the format is essentially the same, and the treatment of problems of fertilizer supply and usage may serve as an example. Here the chapter reviews the changes in supply, usage and technology over the past two decades, indicates the present and expected future problems of supply and environmental impact, summarizes the alternatives available and indicates the areas where more research information is required.

The surveys are fairly brief, though wide-ranging and well-referenced and interspersed with appropriate figures and tables, and one is left with the feeling that they may be too concentrated for the layman, yet insufficiently detailed and quantitative for the scientist interested in public policy.

The book is obviously intended for the American public, so all the examples and a high proportion of the references are from US sources, with a nod in the direction of FAO and problems of developing countries. The list of chemicals used in American agriculture and food industry for a variety of purposes may come as a surprise to the UK reader—used as he is to 'media' suggestions of a relative lack of control of 'chemicals in food' this side of the Atlantic.

The final chapter—on the wise use of chemicals—gives a highly useful and many-sided presentation and evaluation of the problems, concepts (and economics) involved in the evaluation of safety, with a clear statement of the problems for the legislature arising from the cost/benefit nature of each decision, and the impossibility of achieving 'complete information' or a 'no risk' situation.

As a general conclusion—this little book gives a handy global review of problems in the food area, and of possible solutions worthy of research. Despite its American bias it does provide much 'food for thought' for all English-speaking food scientists.

E. C. Apling

Fundamentals of Food Process Engineering. By R. T. Toledo. Westport, Connecticut: AVI Publishing Company, 1980. Pp. ix + 409. ISBN 0–87055–338–0. $27.00 (paperback).

'Food engineering has traditionally been the least popular course in the food science curriculum', the text begins, 'mainly because of the poor mathematical background of the majority of students following the course'. The author sets out to overcome this problem with an opening chapter, entitled Review of Mathematics. The principles of differentiation, integration, logarithms and curve fitting are covered and well illustrated by a number of worked examples.

Following this are chapters on units and dimensions, material balances, gases and vapours, energy balances, flow of fluids, heat transfer, thermal process calculations, refrigeration, evaporation and dehydration. The principles of mass transfer are covered in the dehydration chapter.

On the whole the chapters are well written and the principles are clearly explained. Each chapter is liberally spiced with examples and the chapter normally ends with some additional problems and suggestions for further readings.

The level at which the material is presented, in my opinion, is suitable for Food Science students and should serve as a sound basis for courses in Food Technology and Engineering. I have found it very difficult to recommend a suitable introductory textbook that adequately covers the basic principles since *Unit Operations in Food Processing* by R. L. Earle, went out of print. Toledo's

book gives a slightly more advanced treatment than Earle's but not as advanced as that offered by Leniger and Beverloo's *Food Process Engineering* or Loncin and Merson's *Food Engineering*. The large number of excellent worked examples should be of benefit both to students and to people teaching in this subject area, for use in tutorial sessions.

One possible drawback to the book is its use of a mixture of Imperial and SI units throughout. Many of the students I have shown it to have been put off by this. It is debatable whether students should be taught entirely in SI units or in both sets of units: students currently arriving at British universities are conversant only with SI units, whereas the food industry still works in degrees Fahrenheit and pounds force per square inch, etc. The compromise I would have made would have been to have a longer chapter on Units and Dimensions, dealing thoroughly with conversions from one system to another, and then to have covered the remainder of the material in SI units. Students are much more critical about the books they buy now, because of their high price; it would be a shame if this book did not appeal to the audience it is suitably and obviously intended for because of its over-indulgence in Imperial units.

There are a number of mistakes, in particular, three on page 364. The book deals very scantily with food processing equipment. Perhaps there are a few omissions—no mention is made of leaching or extraction techniques, none of separation techniques or physical technique, e.g., mixing, size reduction, homogenization, and nothing of membranes.

In dealing with unsteady state heat transfer it covers heat penetration into canned food, but no mention is made of prediction of heating, cooling or freezing times. The section on radiation is brief, but there is a useful outline on heat penetration using microwave radiation. However, most of these are minor points.

I would strongly recommend that all food science practitioners make the effort to familiarize themselves with this book—it is well worth it.

M. J. Lewis

Advances in Catering Technology. Ed. by George Glew.
London: Applied Science Publishers, 1980. Pp. xii + 492. ISBN 0–85334–844–8. £40.00.

This volume records the Second International Symposium on Catering Systems Design held in Harrogate in September 1979. It was organized by the Catering Research Unit of the University of Leeds with the help of an international advisory committee drawn from prominent European participants in the first symposium. The book presents 43 papers grouped into four themes.

Part One is concerned with present trends and future possibilities. George Glew examines the Western European eating habits over the last decade,

considers the influence of technology and changing attitudes towards the relationship between diet and health. Guy Livingstone performs a similar role for the United States and Ronald Brech does a standard piece of futurology and predicts trends that may affect the industry in the year 2000.

The Second Part of the book, subtitled 'Constraints on Food Quality' contains eight papers. Seven of them were commissioned each to give a different perspective on the problem. The most notable are perhaps Hilda Van Oosten's perceptive description of the difficulties in persuading commercial caterers to offer food which is nutritionally sound both itself, and as part of a balanced diet, and Nils Bengtsson's contribution on the role of equipment design.

In sharp contrast to most of the papers Michael Nightingale's short contribution describing the problems of determining quality and reliability standards which can be operationalized by hoteliers and restaurateurs is completely devoid of technological content. An excellent summary of the preoccupations of many managers, it should be read by food technologists if only to learn that two groups of people can talk about the same subject without any apparent common interest, or, more accurately, without a common language.

The bulk of the book, 27 papers, is devoted to catering technology. They contain both invited contributions and papers offered by authors, and most of them are fairly specific in content as against the wider perspectives of the earlier papers in the book. Perhaps the easiest way to give the flavour of this section is to give the title of every fifth paper: 'The Balance between Raw Material, Manpower Utilisation and Technology', 'Reheating of Minced Products in a Convection Oven', 'The Nutrient Content of Meals on Wheels in Leeds', 'A General Systems Theory Approach to Catering', 'Chilled Meals: Sensory Quality' and 'The Reliability of Manufacturers' Nutritional Data'. The range of topics is so wide it would be invidious to comment on any individual paper.

Part Four, perhaps the least satisfying section in the book, has five papers devoted to the needs of consumers. However, it includes what is, in the reviewer's opinion, the best paper in the symposium, Joe Balintfy's account of 'Catering to Consumers' Food Preferences', a truly elegant application of management science to catering. The work described uses quadratic programming to design a menu which maximizes consumers' food preferences subject to cost, production and nutritional constraints.

The volume is an accurate account of the symposium, and its strengths and weaknesses are those of the symposium itself: some of the contributed papers seem frankly makeweight and at least one of the invited papers seems rather flat unless one remembers the accompanying 'son et lumiere'. On balance, it is clearly a seminal contribution to the infant discipline of catering technology, and an indispensible addition to the library of any institution offering undergraduate catering courses. It is a great pity that the ridiculous price (£40) will inhibit most workers in the field, and probably all students, from obtaining personal copies.

G. Wilson

Le Conserve di Pomodoro (Tomato Preserves). By Carlo Leoni and Giancarlo Bellucci.
Technological Monograph No. 7. Parma: Stazione sperimentale per l'industria dell conserve alimentari, 1980. Pp. 175.

This paperback production of the Food Preservation Industries Experimental Station, Parma is extremely well produced and printed, with many illustrations and plant diagrams in full colour—and is useful as a source of such material even for a food technologist who knows no Italian. Coverage from tomato cultivation, through harvesting, to processing methods and quality control is quite comprehensive, and two Appendices list official methods of analysis and Italian legislation controlling tomato products.

E. C. Apling

Seaweeds and their Uses. 3rd edn. By V. J. Chapman and D. J. Chapman.
London: Chapman and Hall, 1980. Pp. x + 334. ISBN 0–412–15740–3. £17.50.

The new edition of the Chapmans' well-known book takes account of the advances in the knowledge of seaweeds and their constituents that have been made in the last two decades and has references to publications as recently as 1979. At the same time the first four chapters of the first edition have been replaced by a new one by D. L. Chapman: 'Occurrence, Distribution and Historical Perspective', so that much less space is occupied by purely historical aspects, and the book is in many respects an up-to-date account of seaweeds and their uses.

In recent years the cultivation of seaweeds has increased so much that probably more is supplied by cultivation in the sea than by collection from natural sources. Information on these important developments can be found in various parts of the book (e.g., cultivation of *Eucheuma* in the Philippines under 'Eucheuman', pp. 137–141, and *Laminaria* in China under 'Looking for Seaweeds', p. 266). The new chapter on 'Mariculture' by D. J. Chapman deals only with experimental cultivation in artificial environments.

Chapters are devoted to 'Seaweed as Animal Fodder, Manure and for Energy', 'Algae as Food for Man', 'Laver or Nori Industry and Carrageen or Irish Moss', 'Agar-Agar' and 'Alginates'.

Food technologists may be most interested in information on agar, carrageenan and alginates and will perhaps be surprised to learn that more seaweed is used as food for man, with little or no modification, than is used for all other purposes. This food use is almost entirely in the Far East, particularly in China and Japan, and the cultivation and preparation of different types is described.

The chemical structure of agar and carrageenan are now known in broad outline and are included in the respective chapters. With this in mind it is rather

confusing to find products treated under names such as, for example, 'Eucheuman'. The user will look in vain for a product with this name on the market, as the extractives from *Eucheuma* species are sold as types of carrageenan, as indeed they are chemically.

Up-to-date information on the structure of alginates is given but some misleading statements are made. On p. 200 it is said that algin from *Ascophyllum* and *Laminaria hyperborea* contain considerable amounts of fucose. This is not the case for the products sold as 'algin' in the USA, and sodium alginate in Britain. The seaweed exported by Kelp Industries Pty from Tasmania to Britain is not *Macrocystis* but *Durvillea potatorum*. Only the very high alginate content of this seaweed makes the operation economic.

Although the book gives a general idea of the way in which seaweeds and their extractives are used, the discussion of many suggested applications which never developed or have become obsolete could well have been omitted and more space given to some of the newer products such as agarose and propylene glycol alginate. The uses of the seaweed colloids in food are summarized in tables taken from other publications and there is little discussion of them in the text.

The final chapter on the world's seaweed resources gives a good summary of the available information, although estimates of natural crops of seaweed are far from satisfactory largely due to the enormous variation from one year to another.

There is an extensive bibliography with over 1000 references but a large number of them are not mentioned in the text. These are hardly useful for further reading as no indication is given of their subject-matter. On the other hand some references in the text are absent from the bibliography. Small errors are too numerous to detail.

This edition of 'Seaweeds and their Uses' is a much more useful reference book than previous ones and provides in one volume a great diversity of information.

R. H. McDowell

Food Technologist

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The Society for Applied Bacteriology, which has just celebrated its 50th anniversary, launched the *Journal of Applied Bacteriology* in 1954. Since that time the *Journal* has grown in size, two volumes per year being published from 1975 onwards, in prestige and in the subject matter covered. It has established an international reputation with readers and authors; indeed each number of the *Journal* contains papers from worldwide sources. The Society's interest in the systematics and ecology of groups of microorganisms is reflected in the *Journal* and a major change in the organization of papers on systematic microbiology is detailed in a revised Instructions to Authors published early in 1982 as a supplement to Volume 52. The Instructions also give details of a novel form of publication, observation papers. These are intended to provide the *Journal's* readers with a succinct up-date of rapidly developing areas of microbiology. Such articles will be of particular interest to young scientists who seek conceptual enlightenment and senior persons in industry, Universities, etc., who need to keep abreast of developments in many areas of microbiology. Comprehensive reviews have become another important feature of the *Journal* and the publication of critical book reviews has attracted enthusiastic comment.

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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 numbers; 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. 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 one hundred or greater.

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	μ = 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	= 4·54611
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 bold 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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