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Retention of volatile components during freeze drying of substances containing emulsified oils

M. R. ETZEL AND C. JUDSON KING*

Summary

Measurements have been made of the retention of a series of volatile acetates during freeze drying of sucrose solutions containing emulsified vegetable oils. The acetates partition to different extents between the oil and aqueous phases. The observed retentions can be interpreted in terms of a simple model whereby acetate located in oil droplets initially present in regions ultimately occupied by ice crystals are lost completely, whereas acetate in oil droplets present within residual concentrate are fully retained, and acetate in the aqueous phase is lost according to observations for initially homogeneous systems. As a general rule, only a small fraction of the oil droplets are present within the residual concentrate, and extraction of volatiles into an emulsified oil phase reduces volatiles retention during freeze drying.

The results and the model afford a rational interpretation of the results reported by Kayaert, Tobback & Maes (1974) for retention of individual aldehydes and mixtures of aldehydes during freeze drying of a model food system.

Introduction

Freeze drying of liquid foods can exhibit a high degree of retention of volatile flavour and aroma substances, although some freeze-dried materials have low retention. Determination of the mechanism of volatiles retention during freeze drying can help to explain variations in retention, and reveal methods for improving retention.

When a solution of carbohydrate in water is cooled below the freezing point, two phases form, (1) a pure ice phase, and (2) a concentrated amorphous

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solution (CAS) (Massaldi & King, 1974). Since there is a flux of water into the ice phase, the concentration of rejected solutes at the ice-CAS interface must be higher than in the bulk of the CAS. Eventually the high concentration of solute inhibits the diffusion of water to such an extent that ice-crystal growth effectively ends. If volatile substances are originally present, they typically are excluded from the growing ice phase during freezing. During sublimation of the ice, the ice-CAS interface is exposed directly to the low-pressure vapour network, and any volatile material in direct contact with the interface is lost.

Loss of volatiles during freeze drying can occur by any of several mechanisms. First, volatiles which are homogeneously dissolved in the CAS are lost by a selective-diffusion mechanism in the CAS near the ice-CAS interface (Thijssen & Rulkens, 1968; King, 1971). Higher solute (e.g., carbohydrate) contents retard the diffusion of volatiles much more than they retard diffusion of water and thereby promote good volatiles retention.

A second mechanism for volatiles loss during freeze drying results when a separate, volatiles-containing phase is present before the solution is frozen. Volatiles which are present initially at a concentration above their solubility will remain as a separate phase during freezing. Volatiles may also be present, dissolved in another phase. Examples in food systems are citrus juices, where the volatiles themselves form an immiscible organic phase, and milk, where a basically non-volatile fat phase extracts volatile constituents. During freezing, a volatiles phase initially present as droplets would be excluded by the growing ice phase. These droplets would then collect at the retreating CAS-ice interface. Volatiles located at the interface in droplets would be lost during freeze drying because they are exposed to low pressures.

Massaldi & King (1974) developed a theoretical model to describe the retention of volatiles initially present at concentrations above their solubility. In the model, volatile droplets which are originally present within the regions eventually occupied by ice crystals are completely lost during sublimation of the ice. Droplets originally present within regions which remain as CAS during freezing are retained to a high degree, postulated to be 100% retention. Using this model, the percentage of the total droplets located at the interface is simply the volume percentage of ice crystals. Also, it was noted by Massaldi & King (1974) that smaller droplets should be able to enter the CAS by Brownian diffusion and thereby be retained. Microscopic investigations by Gejl-Hansen & Flink (1979) of the location of oil droplets after freezing carbohydrate solutions containing oil emulsions support this model and also show that small droplets are entrapped in the CAS to a higher degree than are large droplets. Some volatiles included in the CAS as droplets may also be lost during freeze drying. When the droplet size is comparable to or exceeds the CAS web thickness, the volatile droplet can easily touch the web surface; it would thereby be exposed to low pressure and lost during sublimation. This situation would most likely occur at high volatiles-to-CAS volume ratios.

A third situation can occur when some volatiles or other volatiles-extracting components exceed their solubility during the freezing process, because of

lower temperatures and/or removal of water as ice. If droplets form, they will probably concentrate in the CAS near the ice-CAS interface, since conditions for insolubility are most extreme there and since the droplets are rejected by growing ice crystals. Microscopic investigations (Flink, *et al.*, 1973) have shown formation of droplets during freezing of carbohydrate solutions. A model for the growth of droplets during freezing has been developed by Massaldi, Newman & King (1975), showing that the maximum droplet diameter is proportional to the square root of the diffusivity of the supersaturating solute in the solution. For common freezing conditions droplet sizes are quite small, which should facilitate Brownian diffusion of droplets into the CAS from the interface, thereby aiding retention of volatiles in the droplets.

Kayaert, Tobback & Maes (1974) measured retentions of various aldehydes and ketones, added individually and as mixtures, during freeze drying of a model food gel. These substances were present at concentrations both above and below their solubilities. The results are complex and have not heretofore been interpreted in terms of the above volatiles-loss mechanisms. Retention of sparingly soluble volatile compounds during freeze drying has also been studied by Smyrl & LeMaguer (1978).

The purpose of this work was to increase understanding of the mechanism of retention of volatiles in freeze drying, when they partition into an immiscible phase. This involved collecting data for a system where such partitioning occurs and developing a quantitative model which describes the data. The system chosen was a series of acetates as volatile compounds, present at high dilution in an emulsion of vegetable oil in aqueous sucrose solution. Kieckbusch & King (1979) have reported phase-equilibrium data for this system, giving the wherewithal for quantitative interpretation. The model developed was then used to interpret the results obtained by Kayaert *et al.*, (1974) for the retention of aldehydes in the presence of an immiscible volatiles phase.

Materials and methods

On the day of an experiment, 500 g of an aqueous 25% w/w sucrose solution were prepared, and put into a conventional blender. The oil solution which was used to prepare emulsions consisted of 2% w/w surfactant (Tween 81, Atlas Chem. Ind., Inc.) and 98% w/w peanut oil, obtained at a local supermarket. Emulsions containing 0.5 or 1% w/w oil were prepared by adding, respectively, either 2.6 or 5.1 g of the oil solution to the sucrose solution with a syringe, agitating at the highest speed for 3 min, and, finally, adding 0.2 ml of the acetate by syringe. For runs where only one acetate was used, 0.2 ml of either ethyl, propyl, butyl, pentyl, or hexyl acetate were added to the sucrose emulsion. In the runs where a mixture of acetates was used, 0.2 ml of an arbitrary mixture of ethyl, propyl, butyl, and pentyl acetates were added to the sucrose emulsion. The emulsion was stirred in the blender for another minute and then poured into a stoppered, 50-ml Erlenmeyer flask and stored in a freezer set at – 10°C.

While the emulsion cooled, sample holders were prepared. These were polyethylene bottle caps, 33 mm in diameter and 4.5 mm deep. A 3-mil iron-constantan, Teflon-coated thermocouple was placed next to the inside bottom of the sample holder, with leads extending laterally along the bottom surface. The sample holders were placed on an aluminum tray, which was on dry ice. In order to provide a suitable nucleation surface, the sample holders were coated with a layer of ice by spraying them with distilled water. The sucrose solution, which was at its freezing point, was poured into four sample holders and frozen for 45 to 60 min. The top surface of the samples to be freeze dried was scraped with a knife. This was done in order to remove the viscous layer of concentrated sucrose solution which formed on the surface of the sample during freezing. Two samples were quickly placed into the freeze dryer and the other two were stored on dry ice. The thickness of a sample was typically 4.5 mm.

The freeze dryer was that described by Massaldi and King (1974). Radiant heat to the sample was supplied from above by a resistance heater covered by an aluminum plate. Sample temperatures were continuously recorded with a multipoint recorder; frozen-zone temperatures were close to −33°C during most of the run. During freeze drying the water vapour partial pressure in the chamber was below 1.5 Pa, as measured by the equilibrium temperature of a thermocouple in a thermally isolated ice block. The sensitivity of the temperature measurement was 0.5°C. This corresponds to a 6% sensitivity in water vapour partial pressure under experimental conditions. Complete sample drying took approximately 5 hr.

Immediately before the end of a run the samples which had been frozen but not dried were scraped and placed into a sample bottle. At the end of the run the freeze-dried samples were weighed and placed into sample bottles. The samples were then rediluted to their original sucrose concentration by adding the necessary amount of water from a pipette. Most of the freeze-dried samples were crisp and showed no signs of collapse. The samples were analysed by means of a flame-ionization gas chromatograph. The volatiles-analysis technique and apparatus were very similar to those described by Kieckbusch & King (1979). The method consists of analysing the equilibrium vapour above a liquid sample. The acetate concentration in the vapour is proportional to that in the liquid at fixed temperature. The frozen, undried samples served as reference points for computing volatiles retentions in the freeze-dried samples. Replication of volatiles retention within 5% could be obtained for repeated runs, if there was complete freeze drying, without collapse.

The drop-size distribution of the oil emulsion was investigated by photographing the suspension under a microscope at ×200 magnification. Droplet sizes ranged from 1 to 10 μm with most between 4 and 6 μm.

Results

Observed volatiles retentions *vs* the total carbon number of the acetate are shown as the individual data points in Fig. 1. Results are shown for 1 and 0.5%

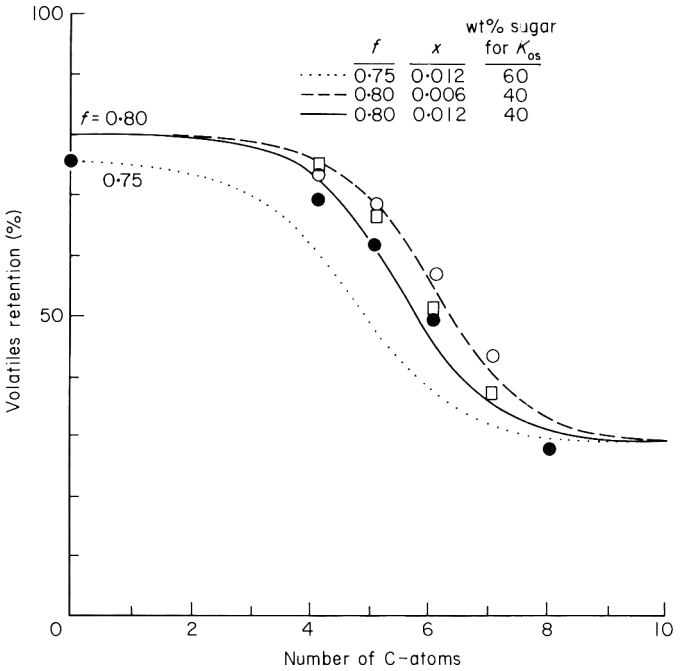

Figure 1. Observed retentions of various acetates during freeze drying, and predictions of model. *Percentage oil:* ●, 1.0 (individual); ○, 0.5 (multiple); □, 1.0 (multiple).

w/w oil emulsions, with the acetates added either individually, or as a mixture of four acetates. In order to determine the retention of a homogeneously dissolved acetate, a run was made using ethyl acetate and no oil. This should be equivalent to the retention of an acetate which does not partition into the oil phase, and hence is designated as a hypothetical zero carbon number in Fig. 1.

Discussion

Theoretical model and present data

The present data can be quantitatively interpreted through a model similar to that of Massaldi & King (1974). If β is the volume fraction of CAS, then $(1-\beta)$ is the fraction of the total number of oil droplets which are forced to the ice-CAS interface. The amount of volatiles at the ice-CAS interface (g/cm³ of total sample) is:

$$x\ (1-\beta)C_{vo}$$

where x is the volume fraction oil in the original solution, and C_{vo} is grams of volatiles per cm³ of oil. Sample-volume changes during freezing and freeze

Table 1. Predicted retention and distribution of volatiles in oil emulsions

Location of volatiles after freezing	Concentration of volatiles	Fraction of volatiles retained	Concentration of retained volatiles
Drops at Interface	$x(1-\beta)\,C_{vo}$	0	0
Drops in CAS	$x\beta C_{vo}$	1	$x\beta C_{vo}$
Homogeneously dissolved in CAS	βC_{vs}	f	$f\beta C_{vs}$

drying are neglected. The amount of homogeneously dissolved volatiles per total sample volume upon freezing is:

$$\beta C_{vs}$$

where C_{vs} is the grams of volatile per cm^3 of CAS. The fractional retention of homogeneously dissolved volatile after freeze-drying is designated f. The droplets located within the CAS are postulated not to lose volatiles during freeze drying. Table 1 summarizes the volatiles location and retention.

Initially the total concentration of volatile is $\beta C_{vs} + xC_{vo}'$, which is the sum of column 2 of Table 1. The fraction volatiles retention (RV) is then:

$$RV = \frac{(x\beta C_{vo} + f\beta C_{vs})}{(xC_{vo} + \beta C_{vs})} \tag{1}$$

Defining $K_{os} = C_{vo}/C_{vs}'$, this can be reduced to:

$$RV = \frac{\beta\,(xK_{os} + f)}{xK_{os} + \beta} \tag{2}$$

K_{os} is the distribution coefficient of the volatile between the oil and CAS phases. The distribution coefficients of acetates between various oil and carbohydrate solutions were measured by Kieckbusch & King (1979). Since peanut oil and coffee oil are similar oils, the distribution coefficients of acetates between peanut oil and sucrose solutions are very close to those between coffee oil and sucrose solutions. Extrapolating the K_{os} data for 60% w/w sucrose solution and coffee oil at 25°C by assuming ln K_{os} linear in the total carbon number of the acetate yields:

$$\ln K_{os} = 1.167\ (\text{Carbon Number}) - 2.293 \tag{3}$$

The volume fraction CAS (β) for 25% w/w sucrose solution cooled to −12°C (60% w/w sucrose in CAS) is 0.29 (Chandrasekaran & King, 1971). The

homogeneous retention of the acetate f is 0.75, based on the observed retention of ethyl acetate in the absence of oil; this is the same as the value of f indicated by the data of Massaldi & King (1974) for homogeneously dissolved volatiles in 25% w/w sucrose solutions. As a first approximation, f will be assumed to be the same for all acetates since their diffusion coefficients in sucrose solutions are not greatly different. The theoretical curve for 1% oil denoted 0.75 in Fig. 1 can now be generated.

Several possibilities can account for the difference between the experimental data and the theoretical curve denoted as $f=0.75$. Partial collapse of a sample was found to cause substantial reductions in the measured acetate retention. When a sample collapses, homogeneously dissolved volatiles can readily escape from the flowing CAS webs, (Bellows & King, 1973), and oil droplets can probably flow to the surface. Gejl-Hansen & Flink (1979) observed high concentrations of oil droplets at the surface of a highly collapsed sample. Partial collapse would result in lower volatiles retention than in non-collapsed samples; this suggests that high retentions are not in error. A small amount of collapse might explain the low data point for ethyl acetate with no oil, and would indicate that a higher value of f should be in the model.

The sucrose concentration at which K_{os} is calculated also is a source of error. As the CAS concentrates during freezing, the diffusion coefficient of water decreases to a level such that ice phase growth effectively stops. The diffusion coefficients of acetates in sucrose solutions are lower than those of water in sucrose solutions (Chandrasekaran & King, 1972). Therefore movement of the acetates into the droplets should be diffusionally cut off before ice-phase growth stops. The correct distribution coefficient would then be the value at which the acetates become diffusionally hindered and can no longer preserve an equilibrium distribution between the oil and aqueous phases. This would occur first for the higher-carbon-number acetates because the diffusion coefficients decrease somewhat with increasing acetate carbon number. This would shift the predicted retention values of the acetates upward, because less than an equilibrium amount of acetate is in the oil phase, and the acetate in the oil phase is lost to the percentwise greatest extent. This effect would be more pronounced for higher carbon number acetates.

Ice-crystal growth effectively stops during freezing when the diffusion coefficient of water drops to its value in approximately 70% w/w solution (Bellows & King, 1973). The same value of diffusion coefficient for acetate in sucrose solutions occurs between 40 and 60% w/w sucrose (Chandrasekaran & King, 1972). In Fig. 1, predictions of the model are plotted for 1 and 0.5% w/w oil, using a K_{os} value for 40% w/w sucrose and an f value of 0.80. The lower-carbon-number acetates might be expected to lie below the 40 wt % sucrose curve, and the higher acetates above the 40 wt % sucrose curve. Using this reasoning, the data follow the model well within experimental error.

It has also been assumed that K_{os} does not change with temperature, so that the value measured at 25°C can be used. This assumption may also lead to error in the model prediction.

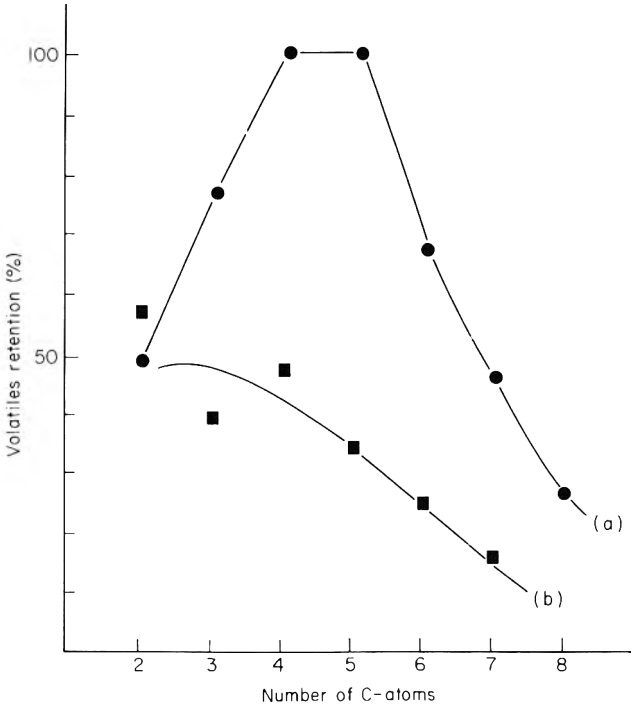

Figure 2. Data of Kayaert *et al.* (1974) for retention of n-aldehydes during freeze drying of a model food gel.

Data of Kayaert, Tobback, and Maes

The model used to interpret the present results can also be employed to rationalize the results of Kayaert *et al.* (1974) for retentions of n-aldehydes and ketones during freeze-drying of a model food gel, composed of a mixture of gums at 2% w/w in water. Figure 2 shows their data for retention of n-aldehydes, individually added in separate experiments at a level of 1000 ppm (curve a). Acetaldehyde is completely miscible with water in all proportions at ambient temperature, but the solubilities of higher adlehydes decrease sharply with increasing chain length. Reported solubilities in water at 20°C are 200 000, 40 000 and 200 ppm for propionaldehyde, n-butyraldehyde and n-heptanal, respectively (Perry, *et al,* 1973). Thus, taking into account the lower temperature and the effect of the gum concentration after freezing, the added aldehydes probably become immiscible during or before freezing for total carbon numbers somewhere in the range of 3 to 5, and above. The rise in curve a below 5 carbon atoms can be explained as a result of decreasing liquid diffusion coefficient with increasing aldehyde molecular weight, giving improved retention by the selective-diffusion mechanism for homogeneously dissolved volatiles. Several previous studies of volatiles retention for homogeneously dissolved substances during freeze-drying have shown that

very low molecular weight volatile components have markedly lower retentions (Bomben *et al.*, 1973). The change in diffusion coefficient within a homologous series affects the retention directly for homogeneously dissolved volatiles, but has much less effect for the case of emulsified droplets, where volatiles retention is more strongly influenced by the locations of the droplets. Any droplets forming for C_4 and C_5 aldehydes are probably very small and show a high retention by the Brownian-diffusion mechanism.

As the aldehyde molecular weight becomes large enough to engender insolubility, the retention mechanism should switch over to that for emulsified volatile droplets. By direct parallel with the results and interpretation of Massaldi & King (1974), this gives a falling retention as the ratio of overall aldehyde concentration to its solubility becomes higher (higher aldehyde molecular weight). Presumably for aldehydes of high enough molecular weight the retention would level off at a value characteristic of the volume fraction of CAS β left after freezing. This would have a very low value because of the low initial gum concentration (2%).

Curve b in Fig. 2 shows the results of Kayaert *et al.* (1974) for the retention of individual aldehydes added at 1000 ppm in separate experiments, each in the presence of 1000 ppm heptanal. The heptanal would serve to create an emulsified organic phase from the start, and thus the results can be interpreted through the model employed in the present work. The equilibrium distribution coefficient for an aldehyde entering the heptanal phase from the aqueous phase should increase sharply as the carbon number increases toward that of heptanal, because of effects of similar polarity. Hence the situation closely parallels that for extraction of acetates into the peanut-oil phase in the present work, and curve b in Fig. 2 resembles the curves and data points in Fig. 1.

Kayaert *et al.* (1974) also report data for retentions of acetaldehyde at 100 ppm in the presence of increasing concentrations (0 to 3000 ppm) of propionaldehyde, n-valeraldehyde and n-octanal, added separately to 1.5% w/w gum gels. These are shown in Fig. 3. Acetaldehyde would partition to only a very small extent into n-octanal, which was found to have very little effect upon the acetaldehyde retention. Acetaldehyde would partition to a greater extent into droplets of n-valeraldehyde, which reduces acetaldehyde retention, because of the high degree of loss of volatiles in these droplets producing a lower, asymptotic retention at levels of n-valeraldehyde above 2000 ppm, where apparently there was nearly complete extraction of acetaldehyde into the droplets of the n-valeraldehyde phase. The behaviour for addition of propionaldehyde suggests that propionaldehyde does indeed become insoluble at sufficiently high concentrations and that above about 1000 ppm propionaldehyde, where the retention levels off, the extraction of acetaldehyde into the propionaldehyde droplets is nearly complete. Since the solubility and diffusivity of propionaldehyde are greater than those of n-valeraldehyde, the analysis of Massaldi *et al.* (1975) for droplet growth indicates that the droplet sizes should be substantially bigger for propionaldehyde. This could give much less Brownian diffusion than for the smaller n-valeraldehyde droplets and might also produce droplets

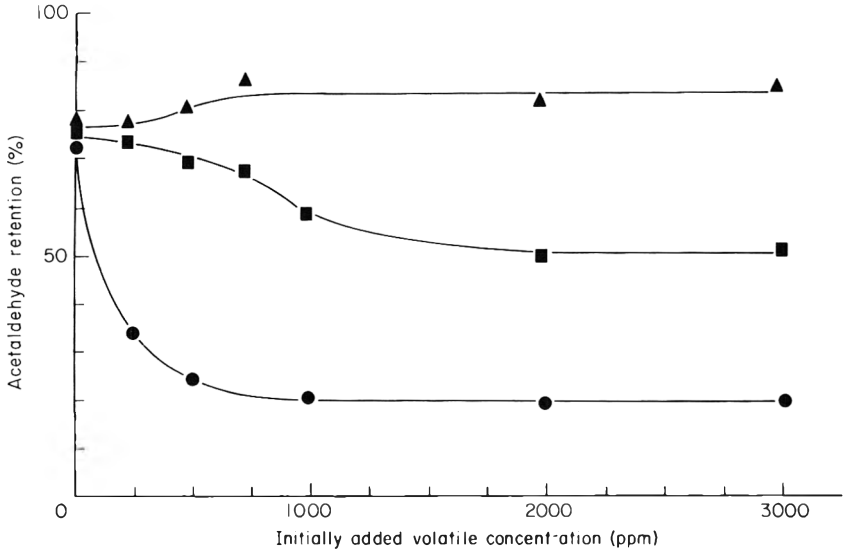

Figure 3. Retention of 100 ppm acetaldehyde in the presence of initially added amounts of other aldehyde volatiles, for freeze drying of 1.5% w/w gum gel (Kayaert *et al.*, 1974). ▲, n-octanal; ■, n-pentanal; ●, propanal.

which have sizes a substantial fraction of the web thickness, thereby giving a lower asymptotic retention for acetaldehyde in the case of added propionaldehyde than for added n-valeraldehyde. Similar results were found by Kayaert, *et al.* (1974) for a 1% w/w gel, but for a 3% gel the retention of 100 ppm acetaldehyde was largely unaffected by the addition of 1000 ppm of other aldehydes. This may possibly reflect chemical interaction of almost all the added aldehyde with the gum molecules through hydrogen bonding and/or dipole interactions, or else much smaller droplets giving considerable Brownian diffusion.

In yet another result, Kayaert, *et al.* (1974) also measured retentions of 1000 ppm n-heptanal in the presence of 1000 ppm of other added aldehydes for freeze drying of 2% gels. Here an extra 1000 ppm of n-heptanal served to reduce the retention from 46 to 14%, which could be the result of larger droplets and less Brownian diffusion and/or loss due to droplets with sizes comparable to the web thickness. As the molecular weight of the added aldehyde decreased, giving substantially more polarity, the retention of n-heptanal increased, presumably reflecting less extraction of the added aldehyde and consequent smaller droplets and more Brownian diffusion and/or loss from droplets large in comparison with the web thickness.

Finally, Kayaert *et al.* (1974) reported retention for acetone and 3-pentanone in the presence of added amounts of various members of the corresponding homologous series. These results are subject to the same interpretation as for the retentions of acetaldehyde and n-heptanal.

Olfactory response

When the ultimate goal is preservation of flavour and aroma characteristics due to various volatile compounds, it is important to take into account the suppression of the equilibrium partial pressure, and hence of the olfactory response, caused by the presence of an organic phase in the reconstituted product. The suppression due to the organic phase is greater when the equilibrium distribution coefficient of the volatile solute into the organic phase is larger. This effect has been analysed by King & Massaldi (1974).

Conclusions

Retention of volatile components partitioning into an emulsified oil phase during freeze drying of solutions appears to be quantitatively describable by a simple model which postulates complete loss of volatiles in droplets located at ice-crystal surfaces and no loss of volatiles located within the residual concentrate. This model predicts a smooth transition between two limiting conditions as partitioning of the volatiles between the oil and aqueous phases changes. One limit corresponds to the fractional retention f for homogeneously dissolved volatiles in the absence of emulsified oil, and the other corresponds to a fractional retention equal to the volume fraction β of concentrated amorphous solution left after freezing. Since β is usually considerably less than f for typical 'good' freeze drying conditions, the presence of an emulsified oil phase generally reduces the fraction retention of a partitioning volatile substance. For small enough oil droplets, there can be a significant migration of droplets into the concentrated solution by Brownian diffusion; this should improve retention over the prediction of the simple model.

The simple model appears to describe quantitatively the experimental results obtained in the present work for retention of various acetates during freeze drying of sucrose solutions containing emulsified peanut oil. It offers a rational, mechanistic interpretation of the results of Kayaert *et al.* (1974) for retention of n-aldehydes and ketones during freeze drying of a model food gel.

It is important to recognize that the presence of an emulsified oil phase serves to depress the equilibrium partial pressure, and hence the olfactory response, for volatiles in a reconstituted product.

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Ice morphology in frozen beef

A. E. BEVILACQUA AND N. E. ZARITZKY

Summary

A histological study was made of pieces of meat which had been frozen from one side, in conditions where the direction of the heat flux was approximately unidirectional and perpendicular to the muscle fibres. The morphology of the ice crystals in tissue frozen under various conditions was studied, and the range of freezing velocities over which no intracellular ice existed, was established.

The average diameter D, of ice crystals, measured in different experiments and at different levels in the same piece of meat was related to the characteristic freezing time t_c, by the equation:

$$D = a + b \log t_c$$

Introduction

In the previous work (Bevilacqua, Zaritzsky & Calvelo, 1979) a study was made of the ice morphology in beef muscle, frozen under conditions where the direction of the heat flux was approximately parallel to the disposition of the tissue fibres. We will refer to this situation as L freezing (Fig. 1a). In that paper, zones were observed in which the ice adopted different morphologies at different levels from the refrigerated border; the percentage of frozen water and the interfacial area were measured and related to the characteristic freezing time (t_c).

In the present work similar experiments were carried out on large pieces of beef but in this case, the heat flux was unidirectional and perpendicular to the muscle fibres (T freezing, Fig. 1b). In this way, the industrial process of beef freezing in plate freezers was simulated in order to: (1) determine in which form

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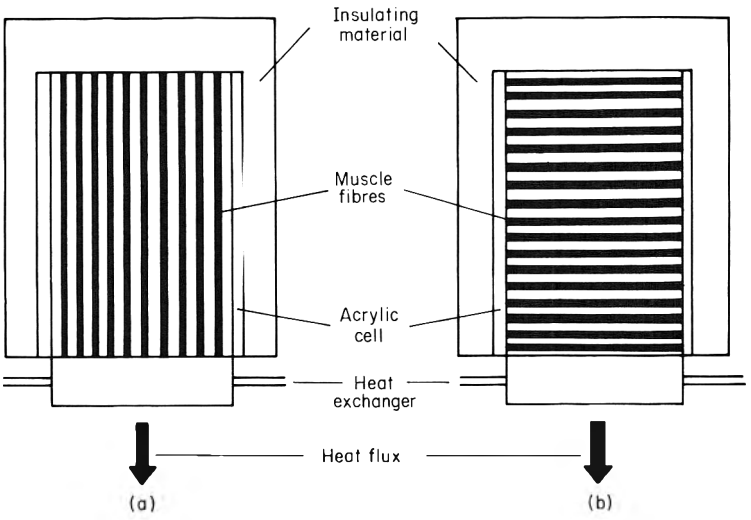

Figure 1. (a) *L* freezing arrangement. The direction of the heat flux is parallel to the muscle fibres. (b) *T* freezing arrangement. The direction of the heat flux is perpendicular to the muscle fibres.

the ice crystals grow through the meat when the direction in which the freezing boundary advances is perpendicular to the muscle fibres, (2) define the range of characteristic freezing times over which intracellular ice exists, and (3) establish the relation between the size of the intra- and extracellular ice crystals and the characteristic freezing time.

Materials and methods

Freezing method

Cylinders of beef (Semitendinosus muscle, post rigor) of approximately 5 cm in diameter and 5 cm in length were cut with the principal axis perpendicular to the muscle fibres. The pieces of beef were frozen unidirectionally on a metallic heat exchanger through which alcohol from a Cryostat, Lauda UK 50-DW, was made to circulate with a temperature control of ± 0.1°C. Each cylinder was placed into an acrylic cell of 2.4 mm wall thickness, insulated with expanded polyestyrene, 5 cm thick (Fig. 1b).

The freezing rate was regulated by modifying the temperature of the refrigerant and by using acrylic slabs interposed between the meat and the surface of the heat exchanger. These slabs simulated the insulating materials normally used in industrial procedures.

In each experiment the thermal history of the meat was registered during its freezing by thermocouples inserted at predetermined points in the samples so that a characteristic freezing time could be assigned to each section. The

characteristic freezing time of any point in the sample, was defined as the time taken for it to pass from −1 to −7°C (Bevilacqua, Zaritzky & Calvelo, 1979). These temperatures were selected because they represent: −1°C the initial freezing point of meat and −7°C, the temperature at which 80% of the water in meat is frozen.

The freezing of the samples was continued on the same heat exchanger until all points of the cylinder had attained a uniform temperature. Then, the cylinders were sectioned in slices of approximately 5 mm thickness in a chamber at −20°C from which small samples of tissue were taken for histological examination. The characteristic freezing time corresponding to the geometric centre of the slide was assigned to each sample.

Histological method

The histological technique employed was the modification of the classical method of freeze-substitution (Cerrella & Zaritzky, 1975) used in a previous work (Bevilacqua, Zaritzky & Calvelo, 1979) whereby observations were made of the holes in the tissue left by the ice crystals. The tissue structure was fixed at the final freezing temperature using Carnoy fluid that quickly diffuses and has a low freezing point and sections were cut perpendicular to the direction of the heat flux to show longitudinal sections of the tissue. Simultaneously histological cuts of non-frozen beef were obtained to compare the results (Fig. 2a).

Determination of the crystal diameter

The size of the minor axis of the holes left by the ice was measured to avoid the errors that could appear if the sections were not perpendicular to the axis of the crystals. In histological cuts which showed intra-and extracellular ice, the average diameter was defined by the size of the intracellular ice crystals. When the ice was located exclusively in the extracellular spaces, the resultant diameter was obtained from the respective measurements of all the orifices that appeared in the micrographs.

Results

Histological zones

The analysis showed the following different zones: Near the refrigerated border (which corresponds to short characteristic freezing times, lower than 4 min) the ice was located in the interior of the fibres and was shaped like needles (Fig. 2b). Zones that were more distant showed a smaller number of intracellular ice crystals, but these were larger, as can be seen in Figs. 2c and 2d. Sections corresponding to values greater than 4 min showed the presence of ice only in the extracellular spaces. The ice was formed in columns that grew towards the

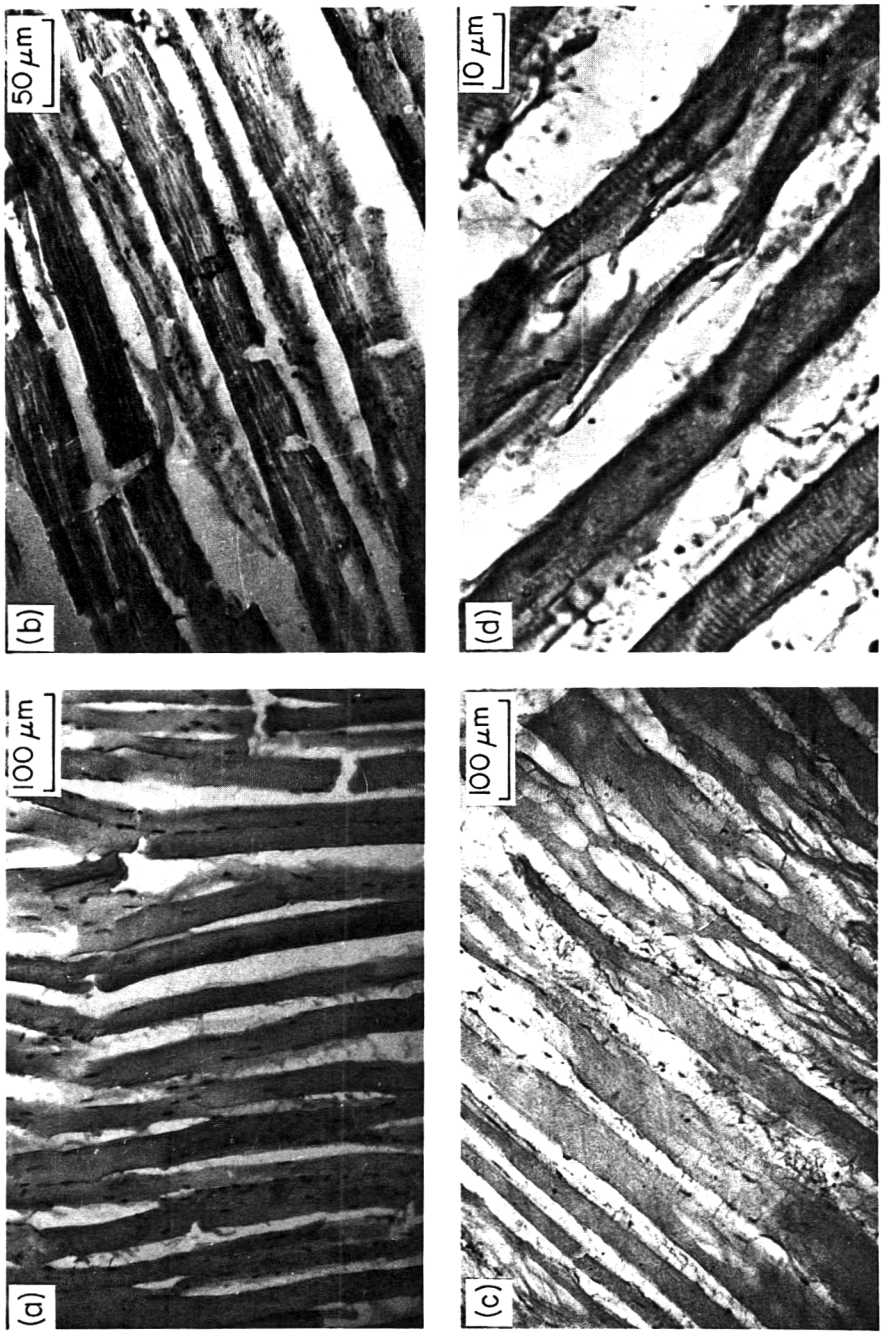

Figure 2. (a) Longitudinal section of unfrozen beef. Longitudinal sections of beef in V freezing: (b) t_c = 1 min; (c) t_c = 2.5 min; (d) t_c = 2.5 min.

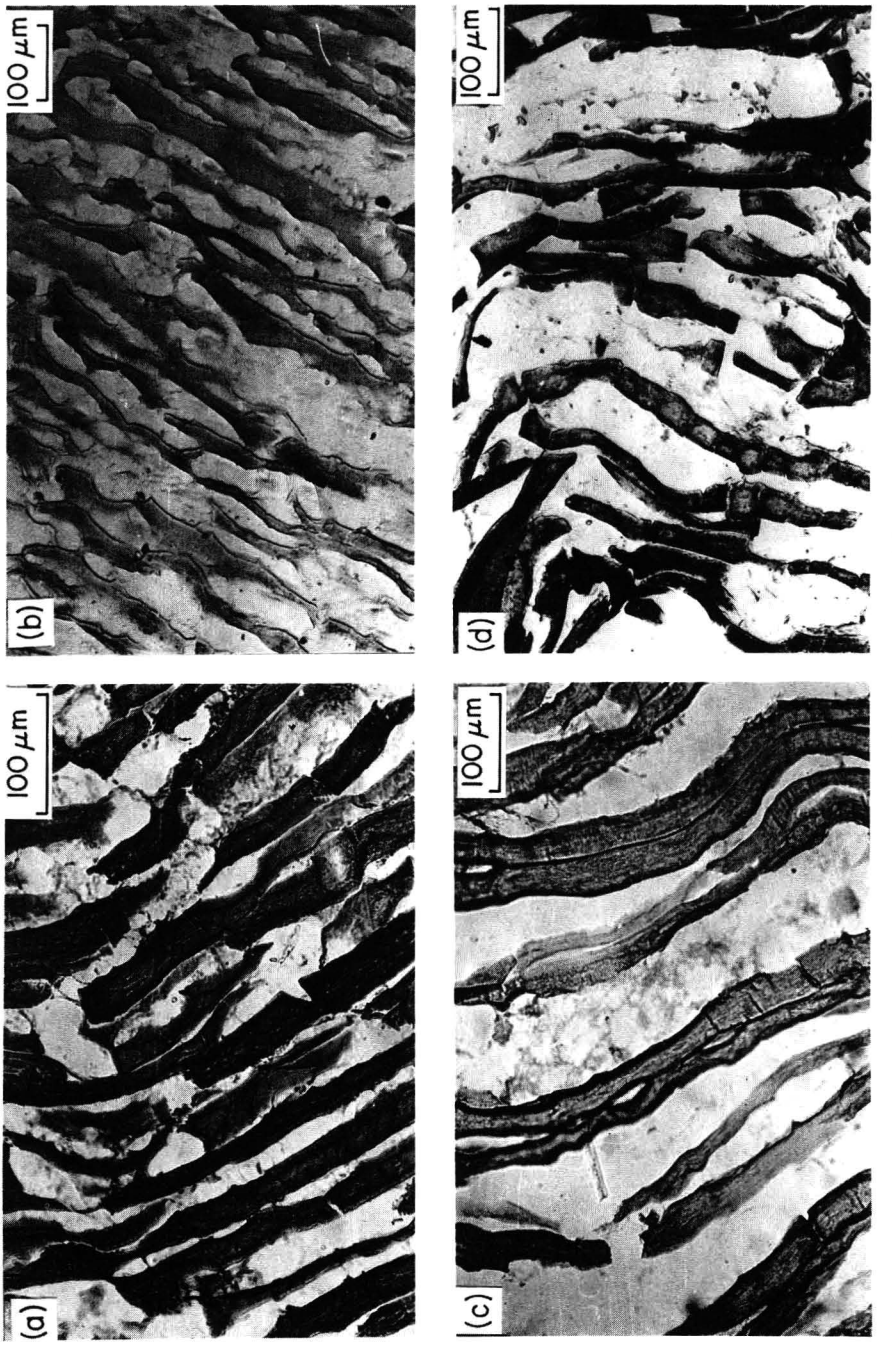

Figure 3. Longitudinal sections of beef in *T* freezing: (a) t_c = 12 min; (b) t_c = 13 min; (c) t_c = 24 min; (d) t_c = 68 min.

interior of the meat in the opposite direction to the heat flux. (Fig. 3a). For t_c = 12 min, the extracellular ice columns increased in size and were distributed among the fibres causing distortion and displacement in a direction perpendicular to that of the crystal growth (Fig. 3b). Figure 3c shows the effects produced by the ice columns in the tissue; histological cuts from sections of higher t_c showed the fibres grouped and dehydrated by the enlarged ice columns (Fig. 3d).

Average diameter of intra- and extracellular ice crystals

The average diameter D, of the ice crystals (Table 1) was related to the characteristic freezing time t_c by the equation:

$$D = a + b \log t_c$$

where t_c is expressed in min, D in μm, log is the decimal logarithm and a, b are constants that were found from the regression of the experimental data to be:

$a = 8.56 \pm 3.34$ μm
$b = 22.61 \pm 3.36$ μm

(with 95% of confidence), and the correlation coefficient of the regression was $r = 0.98$ (Fig. 4).

Discussion and conclusions

Post rigor beef muscle frozen under conditions similar to those operating in industrial plate freezers contained zones of different ice morphology, not only in L freezing (Bevilacqua, Zaritzky & Calvelo, 1979) but also in T freezing (present work). The ice nucleation, in both cases, ocurred in the region in contact with the refrigerated border and was controlled by the magnitude of the supercooling reached in this zone.

In general, the ice nucleation in muscle started in the extracellular spaces. However, when the freezing velocity was high enough, it was possible to extract from the system more heat than that produced by the formation of the extracellular nuclei, allowing the temperature in the interior of the fibre to descend sufficiently for the production of intracellular nucleation. Therefore, the intracellular ice was located in a zone near the refrigerated border.

In contrast to L freezing of meat, where the presence of intracellular ice was observed up to values of t_c = 20 min, in T freezing, the disappearance of the intracellular ice was more abrupt and occurred at lower values of t_c (t_c = 4 min). This difference between the two patterns of freezing can be explained as follows: in L freezing, the intracellular ice that nucleated in the border advanced like needles in the fibres, increasing in diameter, and diminishing in number, owing to the preferential development of ice columns whose axis of

Table 1. Average diameter of ice crystals in samples frozen under different freezing conditions.

Freezing Conditions	x/L	t_c (min)	D (μm)
T_i = 9°C T_f = −30°C L = 6 cm B_i > 100	0.06	6.5	28.5
	0.14	13	35.1
	0.16	16	30.1
	0.23	24	46.2
	0.27	29	43.0
T_i = 9°C T_f = −27°C L = 5 cm B_i = 20.8	0.06	4	20.6
	0.13	9	33.9
	0.43	68	54.5
T_i = 8°C T_f = −38°C L = 5 cm B_i = 20.8	0.05	1	5.9
	0.15	3	15.3
	0.25	8	33.4
	0.35	8.5	25.8
T_i = 23°C T_f = −36°C L = 8 cm B_i = 33.2	0.05	4	21.1
	0.28	11	31.1
T_i = 6°C T_f = −35°C L = 6 cm B_i > 100	0.01	1.5	10.0
	0.08	2.5	8.2
	0.05	2	11.6
	0.15	6	24.8
	0.28	10	36.0
	0.34	12	30.9
	0.42	15	33.3
	0.44	18	38.4
	0.53	20	30.4

T_i : Initial temperature of the piece of meat.

T_f : Temperature of the refrigerant medium.

L : Total length of the piece

x : Position of each histologically analyzed sample measured from the refrigerated border.

B_i : Biot number defined as $B_i = hL/k_c$ where k_c is the thermal conductivity of unfrozen meat (k_c = 0.50 W/m °C) and h is the heat transfer coefficient at the meat-heat exchanger or meat-acrylic slab interface. If the latter, h was calculated from $h = k_a/e$ where k_a is the thermal conductivity of the acrylic slab (0.21 W/m °C) and e is its thickness.

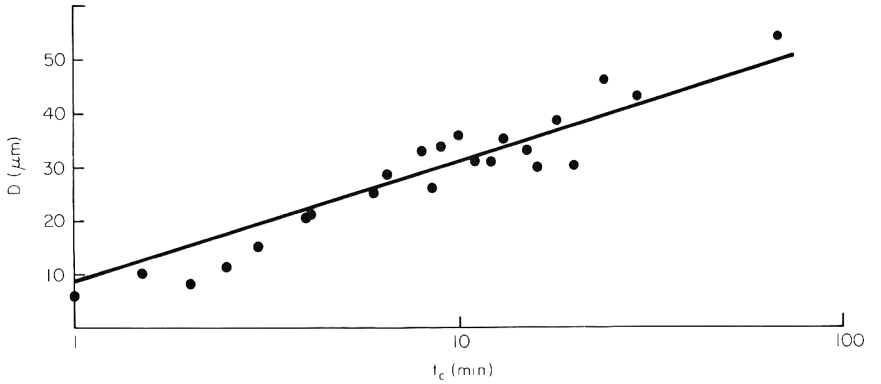

Figure 4. Average diameters of the ice crystals D (μm) as a function of the characteristic freezing time t_c (min).

quick growing coincided with the heat flux direction. While there was coexistence of intra- and extracellular ice, the water within the fibre would not leave the cell. However, when the extracellular columns grew more rapidly than the intracellular ones (Fig. 5), the extracellular solution increased in solute concentration and the fibre began to dehydrate, depressing the intracellular freezing point and enlarging the extracellular crystals. Under these conditions intracellular ice was absent.

On the other hand, for T freezing, intracellular ice was observed over a shorter range of characteristic freezing times, in the zone where it has originally nucleated because it could not grow in the direction of the heat flux across the cellular membranes (Lussena & Cook, 1954). The ice inside the fibres therefore grew in a narrow zone that practically coincided with the nucleation zone.

The analysis presented in this work can be summarized in the following conclusions:

In post rigor beef muscle frozen with a unidirectional heat flux which is

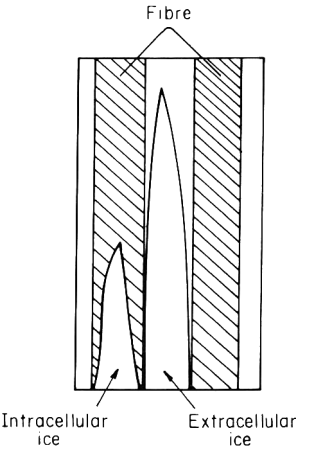

Figure 5. Schematic representation of the ice growth in muscle fibres during L freezing.

parallel (L freezing) or perpendicular (T freezing) to the fibre orientation, the intracellular ice was located only in the zone which was adjacent to the refrigerated border.

In T freezing the disappearance of the intracellular ice corresponded to $t_c > 4$ min, while in L freezing this occurred at $t_c > 20$ min.

In T freezing the presence of intracellular ice over a narrow range of t_c can be attributed to the fact that the ice within the fibres only grew in the places where it had originally nucleated, since growth across the cellular membranes was impossible.

The disappearance of the intracellular ice in L freezing can be explained if the extracellular crystals grew more rapidly than intracellular ones, producing an increase in the solute concentration of the extracellular fluid. This caused dehydration of the muscle fibre with the consequent depression of the intracellular freezing point and absence of intracellular ice.

An equation $D = a + b \log t_c$, which related the observed variation in ice crystal diameters (D) to the characteristic freezing time (t_c) also predicted the observed increase in crystals size with distance from the refrigerated border.

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Effects of freezing on the objective and subjective properties of processed cheese

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Summary

The effects of freezing before processing on the objective and subjective properties of processed cheese were studied. Results indicate that it is possible to freeze the cheese to retard the onset of over-maturity.

Introduction

Australia exports large quantities of cheese, with a large proportion of this cheese being blended, flavoured and then processed to various tastes. The amount of time in storage before shipment, transport, loading and unloading of the cheese and the fact that a large amount of cheese has to be available in the storage rooms of a processed cheese factory for correct blending may result in some cheese being over-mature. This affects both the flavour and texture, so that the cheese may be unacceptable to the importer.

This paper examines the possibility of freezing the cheese to retard the onset of over-maturity by considering the effects of freezing and emulsifying salts on the objective and subjective properties of processed cheese.

Materials and methods

Results are presented from two experiments:

(1) Three processing procedures and five emulsifying salts (ES) currently used in processed cheese manufacture were examined for their effects on some objective and subjective properties of 6½-month-old processed cheese.
(2) Two processing procedures and five ES currently used in processed cheese manufacture were examined for their effects on some objective and subjective properties of 9-month-old processed cheese.

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Processing procedures

In experiment 1, the following processing procedures were used:
(1) 6½-month-old cheese was processed (processed),
(2) 6½-month-old cheese was frozen at −20°C for 3 months and then processed from the frozen state (frozen-processed)
(3) 3½-month-old cheese was frozen at −20°C for 3 months, matured for 3 months at 10°C and then processed (frozen-matured-processed).

In experiment 2, the following processing procedures were used:
(1) 6½-month-old cheese was frozen at −20°C for 3 months, matured for 2½ months at 10°C and then processed (F-2½M-P)
(2) 8-month-old cheese was frozen at −20°C for 3 months, matured for 1 month at 10°C and then processed (F-1M-P).

Emulsifying salts

Five ES currently used for processed cheese manufacture were used in both experiments; these being Na_2HPO_4 (DSHP), $Na_4P_2O_7$ (TSDP), $Na_5P_3O_{10}$ (STPP), $Na_3C_6H_5O_7$ (TSC) and a mixture of equal quantities of $Na(PO_3)_n$. Na_2O (GPP) and TSDP, the level of each ES being 3%.

Preparation of cheese

The cheddar cheeses used were manufactured in a semi-commercial plant at Hawkesbury Agricultural College and stored at 10°C. A 12 kg block was ground in a cheese grinder, mixed thoroughly and sampled for analysis as 'raw cheese'. The rest of the cheese was processed in a 2 kg capacity Kustner pilot kettle of steam injection type using a steam pressure of 248 kPa with the ES added to these cheeses in dry form. Approximately 5% water was added to the cheese to adjust the moisture content in the final product to 45%. A slow agitation was used for the first 2.5 to 3 min until the temperature reached 85°C and then higher speed was used for a total of 6 min. The melted cheese was poured into 350-ml plastic containers and stored at 5°C.

Objective evaluation

Percentage water soluble nitrogen (WSN) and percentage increase in WSN were calculated using the method of Heide (1966) while relative casein content (RCC) was calculated using the method of Schulz & Mrowetz (1952). Melting index (MI) was determined by the method of Arnott *et al.* (1957), oil separation by the method of Thomas (1973) and penetrometer value (PV) using a modified commercial penetrometer according to the method of Thomas *et al.* (1970). The pH of each cheese was measured using the method of Meyer & Michels (1954).

As shown by Thomas *et al.* (1980), to overcome the difficulty of different pH levels between vats of processed cheese the objective tests were statistically evaluated using analysis of covariance, with pH as the covariate, and the LSD multiple comparison procedure (Steel & Torrie, 1960).

Subjective evaluation

Samples were taken out of the refrigerator at 5°C and placed in a room at 20°C for 2 hr before testing. The samples were graded by professional cheese graders for four characteristics using the following scoring system:

Firmness:	1 (very soft)	– 7 (very firm)
Stickiness:	1 (very sticky)	– 5 (no stickiness)
Sliceability:	1 (poor sliceability)	– 5 (good sliceability)
General quality:	1 (very poor quality)	– 7 (very good quality)

Subjective test scores were statistically evaluated using ANOVA and the LSD multiple comparison procedure.

Results

For experiment 1, the effects of the ES on the objective and subjective properties of 6½-month-old cheese are presented in Tables 1 and 3 respectively, while the effects of the processing procedures on the objective and subjective properties of 6½-month-old cheese are presented in Tables 2 and 4 respectively.

For experiment 2, the effects of the ES on the objective and subjective properties of 9-month-old cheese are presented in Tables 5 and 7, while the effects of the processing procedures after freezing on the objective and subjective properties of 9-month-old cheese are presented in Tables 6 and 8 respectively.

Table 1. Effects of ES on objective properties of 6½-month-old cheese

ES	WSN (%)	WSN increase (%)	RCC	PV	MI(%)	OS
Na_2HPO_4	$39.7_{b\,c}$	$50_{a\,b}$	74.4_a	115_b	98	42_c
$Na_4P_2O_7$	43.3_c	60_b	74.9_a	77_a	39_a	$35_{b\,c}$
$Na_5P_3O_{10}$	$38.7_{a\,b}$	45_a	74.1_a	$102_{a\,b}$	66_b	$30_{b\,c}$
$Na_3C_6H_5O_7$	35.6_a	35_a	71.1	114_b	78_b	$26_{a\,b}$
$Na(PO_3)_n$ + $Na_4P_2O_7$	$35.8_{a\,b}$	35_a	75.5_a	$107_{a\,b}$	38_a	15_a
LSD	4.1	15.4	2.0	31.4	17.7	12.2

Means within each column not followed by the same letter are significantly different ($P<0.05$)

Table 2. Effects of processing procedures on objective properties of 6½-month-old cheese

Processing procedure	WSN (%)	WSN increase (%)	RCC	PV	MI (%)	OS
Processed	45.0	75	75.4	80	77	17
Frozen-processed	37.1	35_a	73.0_a	109_a	55_a	30
Frozen-matured-processed	33.8	25_a	73.7_a	121_a	60_a	43
LSD	3.2	11.9	1.6	24.3	13.7	9.4

Means within each column not followed by the same letter are significantly different ($P<0.05$)

Table 3. Effects of ES on subjective properties of 6½-month-old cheese

ES	Firmness	Stickiness	Sliceability	General Quality
Na_2HPO_4	2.89	2.00	2.00	2.33
$Na_4P_2O_7$	4.28_b	2.89_a	3.22_a	3.44_a
$Na_5P_3O_{10}$	3.67_a	$3.11_{a\,b}$	2.89_a	3.44_a
$Na_3C_6H_5O_7$	3.67_a	$3.11_{a\,b}$	3.06_a	3.61_a
$Na(PO_3)_n$ + $Na_4P_2O_7$	4.78_b	3.56_b	3.11_a	3.89_a
LSD	0.61	0.64	0.50	0.71

Means within each column not followed by the same letter are significantly different ($P<0.05$)

Table 4. Effects of processing procedures on subjective properties of 6½-month-old cheese

Processing procedure	Firmness	Stickiness	Sliceability	General quality
Processed	3.57_a	2.47_a	2.70_a	3.17_a
Frozen-processed	3.73_a	3.60	3.20	3.80
Frozen-matured-processed	4.27	2.73_a	2.67_a	3.07_a
LSD	0.47	0.50	0.39	0.55

Means within each column not followed by the same letter are significantly different ($P<0.05$)

Discussion

For experiment 1, the freezing of the cheese before processing affected both the objective and subjective properties. In Table 2, frozen-processed and frozen-matured-processed cheese showed reduced percentage WSN, percentage increase in WSN, RCC and MI compared to unfrozen-processed cheese. This was accompanied by increased softness as shown by PV and decreased stability as shown by OS. In Table 4, frozen-processed cheese showed a significant improvement in stickiness, sliceability and general quality over unfrozen-processed and frozen-matured-processed cheese.

From Tables 1 and 3, the effects of the ES on the objective and subjective properties can be determined. While no major differences were observed between the ES with respect to the objective properties, the significant factors in terms of the subjective properties were the favourable performance of the mixture of GPP and TSDP and the poor performance of DSHP.

For experiment 2, the effects of the extent of maturation on the objective and subjective properties are presented in Tables 6 and 8. No significant differences were obtained between the maturation periods with respect to the objective properties, except for RCC. However for the subjective properties, the shorter maturation period showed improvements in sliceability and general quality.

From Tables 5 and 7, the effects of the ES on the objective and subjective properties are presented and as in experiment 1, the important results were the favourable performance of the mixture of GPP and TSDP and the poor performance of DSHP.

In this work, older cheese was used in order to simulate some of the existing conditions that would be expected to occur when the cheese arrived at the factories of the importing countries. In normal cheese processing, the average age of the cheese is 3–5 months old, depending on the flavour, texture and keeping quality. This is achieved by blending young cheese of 1–3 months old

Table 5. Effects of ES on objective properties of 9-month-old cheese.

ES	WSN (%)	WSN increase (%)	RCC	PV	MI(%)	OS
Na_2HPO_4	38.5_{a}	$25_{a\,b}$	65.9	191	$81_{a\,b}$	35_{a}
$Na_4P_2O_7$	37.1_{a}	20_{a}	68.1_{a}	132	5	37_{a}
$Na_5P_3O_{10}$	41.6_{a}	$40_{a\,b}$	74.7	69	88_{b}	51_{b}
$Na_3C_6H_5O_7$	39.8_{a}	$35_{a\,b}$	69.8_{a}	97	88_{b}	$44_{a\,b}$
$Na(PO_3)_n$ + $Na_4P_2O_7$	47.1_{a}	60_{b}	78.4	21	70_{a}	$40_{a\,b}$
LSD	10.8	37.1	2.0	21.9	12.9	14.1

Means within each column not followed by the same letter are significantly different ($P<0.05$)

Table 6. Effects of processing procedures on objective properties of 9-month-old cheese

Processing procedures	WSN (%)	WSN increase (%)	RCC	PV	MI(%)	OS
6½-month-old cheese F-2.5M-P	40.3 $_{a}$	40 $_{a}$	70.7	106 $_{a}$	68 $_{a}$	39 $_{a}$
8-month-old cheese F-1M-P	41.4 $_{a}$	35 $_{a}$	72.0	98 $_{a}$	65 $_{a}$	44 $_{a}$
LSD	6.9	23.5	1.2	13.9	8.2	8.9

Means within each column not followed by the same letter are significantly different ($P<0.05$)

Table 7. Effects of ES on subjective properties of 9-month-old cheese

ES	Firmness	Stickiness	Sliceability	General quality
Na_2HPO_4	2.17	2.00	2.33	2.67 $_{a}$
$Na_4P_2O_7$	3.92 $_{b}$	2.83 $_{a}$	3.33 $_{a}$	3.33 $_{a\,b}$
$Na_5P_3O_{10}$	3.50 $_{a\,b}$	3.17 $_{a}$	3.67 $_{a}$	4.50 $_{c}$
$Na_3C_6H_5O_7$	3.25 $_{a}$	3.17 $_{a}$	3.67 $_{a}$	4.50 $_{c}$
$Na(PO_3)_n$ + $Na_4P_2O_7$	4.92	3.50 $_{a}$	3.50 $_{a}$	4.00 $_{b\,c}$
LSD	0.56	0.79	0.82	0.83

Means within each column not followed by the same letter are significantly different ($P<0.05$)

Table 8. Effects of processing procedures on subjective properties of 9-month-old cheese

Processing procedure	Firmness	Stickiness	Sliceability	General quality
6½-month-old cheese F-2.5M-P	3.50 $_{a}$	2.71 $_{a}$	2.94	3.33
8-month-old cheese F-1M-P	3.60 $_{a}$	3.13 $_{a}$	3.67	4.27
LSD	0.36	0.50	0.52	0.52

Means within each column not followed by the same letter are significantly different ($P<0.05$)

with older cheese of 6–9 months old. Young cheese, having a high RCC, can be frozen and used to correct the decrease in RCC brought about by the use of ES such as TSC or other ES in the case of the longer maturation period. Young cheese would also increase the firmness, emulsion stability and percentage WSN increase, thus enabling both body and texture to be controlled.

Conclusion

The results indicate that it is possible to freeze the cheese before processing to retard the onset of over-maturity. In particular, the use of freezing and then processing in conjunction with the use of the mixture of GPP and TSDP has been shown to give the most favourable objective and subjective properties for the processed cheese.

Also, if maturation is required after the freezing of the cheese, the results indicate that the shorter maturation period and the use of the mixture of GPP and TSDP give the most desirable objective and subjective properties for the processed cheese.

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General correlation between the cryoscopic temperature and the soluble solids content of fruit and vegetables

J. GUEGOV

Summary

On the basis of experimental data, the author has established a regression relationship, reflecting the influence of the soluble solids on the cryoscopic temperature value of fresh fruit and vegetables, regardless of their variety and species.

The equation obtained corresponds well with the experimental results of others.

Introduction

A basic principle in modern refrigeration technology is keeping the cooled product at the lowest possible temperature while avoiding ice formation. The development of systems for precise temperature control and maintenance in cold chambers allow us to approach more and more closely these temperature limits. This calls for reliable cryoscopic temperature data and for the elucidation of the role of the factors influencing this temperature.

It is advisable to store most fruit and vegetables at a temperature near the cryoscopic one. Analysis of the literature reveals that the incipient ice formation in fruit and vegetables has been relatively well investigated. There are many factors influencing ice formation, and the interrelations between them are extremely complicated, so no general theory of the process has been developed so far. Neither does the theory of solutions at its present stage allow for the determination of the cryoscopic temperature of multi-component solutions, such as fruit and vegetables (Fikiin, 1962, 1973; Kobulashvili, 1969). The incipient crystallization of these solutions should be studied experimentally in order to obtain experimental data and to obtain empirical or semi-empirical relationships for the influence of some dominating factors.

Numerous data of the cryoscopic temperature of fruit and vegetables can be

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found in the literature, but they represent either averaged results for a specific species or definite values at a given soluble solids content. A limited number of publications give a mathematical relationship between the cryoscopic temperature and the soluble solids content for a specific species or variety (Fikiin & Kuzmanova, 1970; Gegov, 1972; Kondratiuk & Ginsburg, 1974; Popovski, 1975) or are generalized for some species (Gutschmidt, 1968).

It is commonly accepted that the cryoscopic temperature of vegetable tissues is not influenced by cooling conditions (International Institute of Refrigeration, 1972; Gutschmidt, 1968; Lozina-Lozinski, 1972). It has been proved that the extent of precooling does not influence the cryoscopic temperature value (Lozina-Lozinski, 1972). For certain species the temperature of incipient freezing changes insignificantly depending on maturity (Leblond & Paulin, 1968).

A review of the cryoscopic temperature of fruit and vegetables reveals that it depends on various factors of which the soluble solids content is of major importance, whereas other factors such as molecular weight, degree of dissociation of soluble solids, and structure play a minor role (Golovkin, Tschijo & Chkol'nikova, 1955; Fikiin, 1962; International Institute of Refrigeration, 1972; Gutschmidt, 1968; Popovski, 1975). For this reason the present investigation, which is aimed at establishing a general relationship for fruit and vegetables of widely differing chemical composition, structure and type, was confined to the study of the influence of the total solids content on the cryoscopic temperature.

Methods

The cryoscopic temperature t_c was determined by thermal analysis using the isothermal plateau on the cooling thermogramme. The fruits and vegetables placed in the measuring capsule were frozen indirectly in a cryostate with liquid cooling agent at $-11°C \pm 0.05°C$. The soluble solids content ξ was determined by a refractometer to an accuracy of $\pm 0.1\%$. The temperature of the cooled object was measured by low-inertion thermocouples (copper-constantan) using a compensation method to an accuracy of $\pm 0.01°C$. For each species and variety 20 to 65 determinations of the cryoscopic temperature were carried out.

The results were calculated statistically by the least-square method with the aid of a computer. The following parameters were determined: the numerical regression coefficient $t_c = f(\xi)$, the correlation coefficient r, the coefficient for regression adequacy F_{calc} and the confidence interval $\triangle$ a for the cryoscopic temperature values at probability $\alpha = 0.05$.

Results and discussion

Forty fruit and vegetable species were investigated, the major part of which were represented by several varieties.

By processing the thermogrammes of incipient freezing (558 thermogrammes for fruits and 400 for vegetables) the cryoscopic temperature values at a certain soluble solids content were obtained.

It was estimated by regression analysis that a linear dependence described by the equation

$$t_c = 0.36 - 0.175\xi \tag{1}$$

existed between the cryoscopic temperature (in °C) and the soluble solids content.

The correlation coefficient of this regression (eqn 1) was $r = -0.98$ and the regression coefficient $F_{calc} = 898.2$ – much higher than the critical value (F_{cr} 4.08). The confidence interval for the cryoscopic temperature value a calculated at an average soluble solids content $\bar{\xi} - (10.6\%)$ was $\Delta a_{\bar{\xi}} = \pm 0.05$°C.

It was checked whether the mentioned experimental data could be described better by exponential relationships between t_c and ξ of the type:

$$t_c = e^{(A_1 \zeta + A_0)} \tag{2}$$

and

$$t_c = B\xi^D \tag{3}$$

For equation (2) the values of the regression characteristics were: $r = -0.84$, $F_{calc} = 104.8$ and $\Delta a_{\bar{\xi}} = \pm 0.35$°C, and for equation (3), $r = -0.89$, $F_{calc} = 145.9$ and $\Delta a_{\bar{\xi}} = \pm 0.32$°C.

It is obvious that the linear model describes best the correlation between the cryoscopic temperature and the soluble solids content in fruit and vegetables. The regression is very significant and this fact is confirmed by the very high correlation coefficient, and the linear equation describes adequately the existing dependency, demonstrated by the high regression coefficient.

Figure 1 shows the derived linear regression between the cryoscopic temperature and the soluble solids content. The vertical lines represent the values of the confidence interval for the cryoscopic temperature at the respective values of the soluble solids and the dotted lines – the whole confidence interval. In order to illustrate the number and the influence of the investigated vegetable products in deriving the regression equations the intervals of the soluble solids changes are given as an auxiliary diagram for each species.

The equation established is valid in a soluble solids interval of 3.5 – 27%. Between 3.5 and 20% it summarizes the result for the cryoscopic temperature values of 12–17 fruit and vegetable species, and above 20%, for three species. The confidence interval for the cryoscopic temperature is as follows:

ξ (%)	3.5	10.5	18.6	27.0
Δa (±°C)	0.10	0.05	0.11	0.21

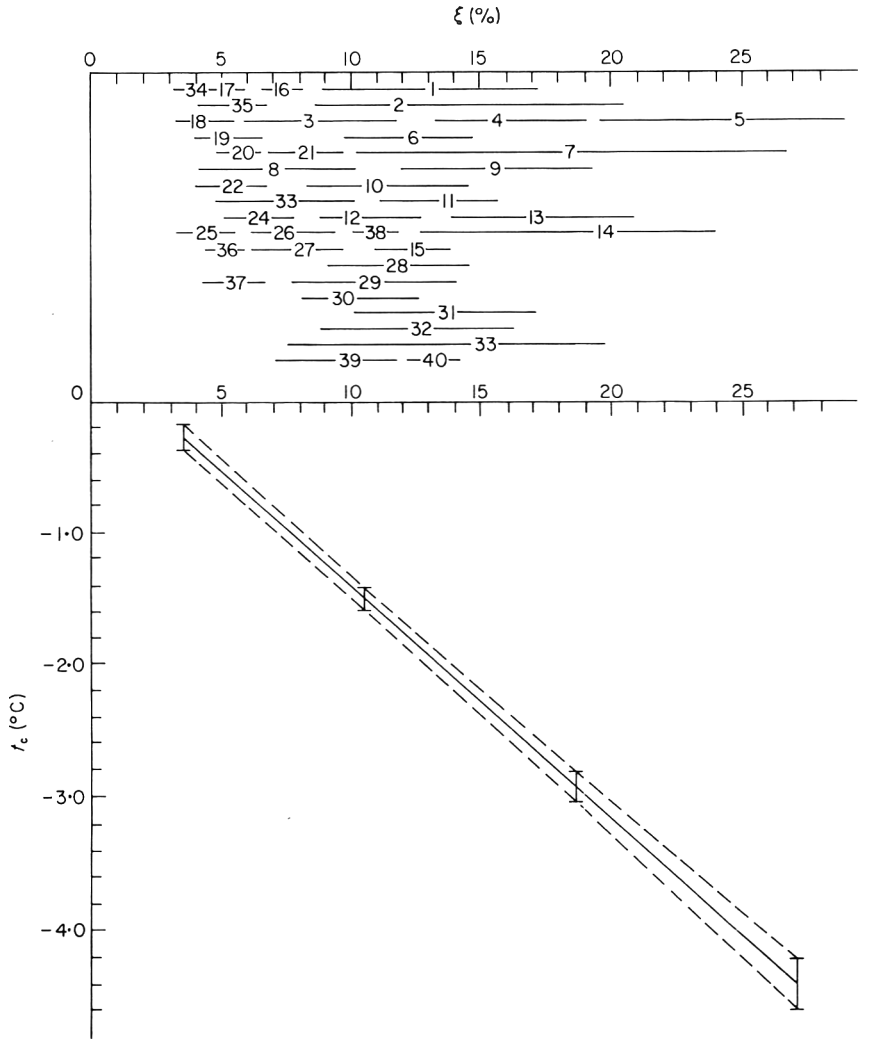

Figure 1. Dependency between the cryoscopic temperature and the soluble solids content of fruit and vegetables: 1, apples; 2, pears; 3, raspberries; 4, quinces; 5, bananas; 6, oranges; 7, cherries; 8, strawberries; 9, sour cherries; 10, peaches; 11, apricots; 12, lemons; 13, plums; 14, grapes; 15, grapefruit; 16, cabbage; 17, small radishes; 18, tomatoes; 19, peppers; 20, green onion; 21, celery; 22, potatoes; 23, water-melon; 24, mushrooms; 25, summer squash; 26, carrots; 27, egg-plant; 28, beetroot; 29, radishes; 30, kohlrabi; 31, onion; 32, leek; 33, sugar-melon; 34, cucumbers; 35, celery-leaves; 36, green beans; 37, spinach; 38, cauliflower; 39, pumpkins; 40, peas.

In the ξ interval from 20 to 27% we have at our disposal 45 experimental results for t_c. Compared with the total number of experimental results (959) this is insignificant and effects only slightly the value of the correlation coefficient and the regression coefficient of the derived equation.

Comparing the obtained analytical dependency with literature data of the cryoscopic temperature of various fruit and vegetable species, in total 109 data (Dickerson, 1968; Fikiin, 1962; Kobulachvili, 1969; Kondratiuk *et al.*, 1974; Riedel, 1950; Riutov, 1976; Tschubik & Maslov, 1970), reveals that it gives on the average slightly lower values −0.17°C, or:
equ (4)

$$\frac{\sum_{i=1}^{n} \hat{t}_{c_i}}{n} - \frac{\sum_{i=1}^{n} t_{c_i}}{n} = 0.17\ (°C) \tag{4}$$

where: t_c = cryoscopic temperature at a certain soluble solids content, from literature data (°C)
$\hat{t}_c$ – cryoscopic temperature by equation (1) at the same soluble solids content (°C)

If we assume that the literature data of the above-mentioned authors have been obtained by measurements of the same accuracy, it would mean that the confidence intervals for the values of t_c, given in the literature would overlap those of the dependency established by us. This allows us to assume that the derived analytical tendency between the cryoscopic temperature and the soluble solids content describes exactly the experimental data also obtained by others.

Acknowledgements

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Physical loss and chemical reactions of SO_2 in strawberry jam production

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Summary

Strawberries for jam production frequently undergo extended storage in sulphite liquor before conversion into jam. Using ^{35}S-labelled sulphite an extensive equilibration of ^{35}S-labelled species between the fruit and the liquor has been demonstrated. Most of the loss of measurable SO_2 was accounted for in terms of (i) the chemical reactions during storage and (ii) the physical losses during jam production. In this work approximately 78% of the liquor sulphite was taken up by the fruit; 98.5% of this either reacted chemically or was boiled off, *viz.* 38% was converted to non-volatile compounds during storage, a further 11% reacted during jam making, 50% was boiled off and 1.5% remained measurable in the jam.

Introduction

Sulphur dioxide is widely used as an additive in food and beverages, e.g. dried fruits, dehydrated vegetables, sausages, wines, soft drinks. It has applications as a preservative and as an antioxidant as well as in controlling enzymic and non-enzymic browning and for modifying the rheology of flour. Its effectiveness in these various applications and in such a wide variety of products implies an ability to participate in reactions with a wide range of food components. It is therefore not surprising that after its incorporation into a food the amount of measurable SO_2 decreases progressively. (Stadtman *et al.*, 1946; Joslyn & Braverman, 1954; Hearne & Tapsfield, 1956).

There is a considerable body of information about the magnitude of post-processing losses of measurable SO_2 but relatively little information about the nature of the products derived from it, thus Mangan & Doak (1947) showed conversion to other sulphur oxyacids to be relatively low in dehydrated veget-

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ables, Ingles (1962), Burton, McWeeny & Biltcliffe (1963) and McWeeny & Burton (1963) implicated the formation of sulphonic acids and more recently Wedzicha & McWeeny (1974a,b, 1975) demonstrated the formation of sulphonated osuloses and estimated their abundance in a few foods. Fragmentary information about the amount of SO_2 which reacts during processing (as opposed to subsequent changes on storage) includes the reports from Thewlis & Wade (1974) on sulphite treatment of flour and from Gilbert & McWeeny (1976) on dehydration of certain vegetables.

Information is now reported on the extent to which SO_2 reacted when incorporated into strawberries stored for 6 months in a sulphite liquor and then made into jam.

Experimental

Strawberries (var. Cambridge Favourite) were sorted, washed and drained. Sound whole berries were stored at room temperature in a liquor containing $^{35}SO_2$ (2 mg/ml) and CaO (6.7 mg/ml); the liquor: fruit ratio was 1:7. After 24 hr the berries softened and settled so that all the fruit was covered by he liquor. After 6 months' storage at room temperature the berries were drained and made into jam using a commercial recipe based on fruit (45 parts), sugar (50 parts), glucose syrup (20 parts) water (5 parts), pectin 0.7% solution (10 parts). The recipe called for heating conditions which would boil off 20 parts of water in approximately 20 min and conditions for achieving this were established in preliminary experiments. In the 'tracer' experiments heat was applied at the same rate but because an enclosed system was employed the weight loss was somewhat less than that observed in the preliminary experiments. Consequently the reaction conditions in the 'tracer' experiment were less severe than would be experienced in an open vessel due to the slightly lower concentration (and hence, temperature) conditions achieved in the closed system.

In the experiment reported here 530 g of berries were stored in 76 ml liquor containing 89.9 $\mu Ci^{35}S$. Measurements of total ^{35}S and of the ^{35}S-activity distillable under the conditions based on the SO_2 determination described by Monier Williams (1928) were made on the fruit and on the jam from it; radioactivity measurements were made by scintillation counting in a 'Triton-X100'-toluene (2:1) scintillation solvent (PPO 4g/l) after appropriate preparation of the sample. All counts were corrected for quenching and radioactive decay and where appropriate the results were expressed in terms of mg SO_2 or 'mg SO_2 equivalent' in the case of 'total ^{35}S'.

Results and discussion

The observations on the extent to which $^{35}SO_2$ from a ^{35}S-sulphite liquor was taken up by strawberries during storage and retained by them when made into

Table 1. Total and acid-distillable ^{35}S in jam prepared from ^{35}S-sulphited strawberries

	Initial liquor	Sulphited fruit	Jam
Total ^{35}S, μCi	89.9	70.7	35.7
μCi/g fruit	–	0.133	0.067
Distillable ^{35}S, μCi	89.9	44.1	1.1
μCi/g fruit	–	0.083	0.002
Non volatile ^{35}S μCi (by difference)	0	26.6	34.6
μCi/g fruit	–	0.050	0.063

jam are summarized in Table 1 in terms of ^{35}S-activity; the SO_2 and SO_2-derived products which this ^{35}S activity represents are in Table 2.

During storage there was extensive migration of ^{35}S from the liquor into the fruit. It was found that of the 89.9 μCi of ^{35}S in the liquor surrounding 530 g berries a total of 70.7 μCi (78%) was located in the fruit after 6 months' storage; at this point the specific activity of the fruit was 0.13 μCi/g. This compares with the figure of 0.15 μCi/g calculated by taking the water content of the fruit to be 89% throughout storage and assuming total equilibration of ^{35}S between the liquor and the water component of the fruit. During jam making there was a major reduction in the amount of ^{35}S retained by the fruit; the total activity dropped from 70.7 μCi to 35.7 μCi and presumably this reflected the physical loss of $^{35}SO_2$ during the boiling stage; the loss of any other volatile ^{35}S compounds was probably very low. The overall effect was that the jam incorporated approximately 40% of the ^{35}S originally present as $^{35}SO_2$ (and related species) in the sulphiting liquor.

However in addition to extensive physical migration and subsequent losses by distillation it is also clear that there were substantial chemical changes. At the end of storage only 62% (44.1 μCi) of the 70.7 μCi ^{35}S taken up by the fruit

Table 2. Concentrations of $^{35}SO_2$ and $^{35}SO_2$-derived products in jam prepared from strawberries stored in a ^{35}S-sulphite liquor*

	Sulphited fruit	Jam
$^{35}SO_2$ (mg/kg fruit)	140	3.5
'total' ^{35}S (mg/kg fruit)†	225	114
$^{35}SO_2$-derived products (mg/kg fruit)	85	110

*initial SO_2 concentration 2000 mg/kg
†expressed in terms of SO_2 equivalent

still existed as SO_2 and SO_2 -releasing compounds; the remaining 38% was present as non-volatile reaction products formed during storage in the sulphite liquor. Extensive chemical changes also took place alongside the physical losses during jam making; thus the 26.6 μCi of non-volatile material after storage increased to 34.6 μCi during jam making. Expressed in terms of SO_2 (Table 2) this means that compounds arising from 85 mg SO_2/kg fruit were formed during storage and a further 25 mg SO_2/kg fruit reacted during jam making – at which time there was also a physical loss of 111 mg SO_2/kg fruit.

In round terms the data indicate that 78% of the liquor sulphite was taken up by the fruit; of this amount 98.5% either underwent an irreversible chemical change or was boiled off, viz 38% was converted to other compounds during storage; during jam making a further 11% reacted, 50% was boiled off and only 1.5% remained reasurable as SO_2 in the jam.

The identity of the compounds formed from SO_2 in strawberries during sulphite liquor storage and during jam making remains to be established although *a priori* it seems likely that inorganic sulphate accounted for part of the SO_2 loss. It also seems probable that the reaction of the glucose syrup and of the fruit components with the absorbed SO_2 will lead to the formation in jam of the sulphonated osuloses isolated previously from (a) sulphited glucose solutions at 100°C (Ingles, 1962), (b) sulphited glucose and glycine solutions at lower temperatures (Knowles, 1977) (c) sulphited ascorbic acid and glycine solutions (Wedzicha & McWeeny, 1974b) and (d) dehydrated cabbage (Wedzicha & McWeeny, 1974a). The extent to which these compounds account for the relatively large amounts of SO_2-derived products formed during sulphite-liquor storage of strawberries remains a matter for future investigation.

Conclusions

During sulphite-liquor storage of strawberries there is extensive equilibration between the liquor and the water content of the strawberries in terms of the ^{35}S-species derived from $Na_2^{35}SO_3$ in the liquor. Under the experimental conditions used in this study about 38% of the ^{35}S in the fruit was converted to non-volatile, non-SO_2-releasing compounds after 6 months' storage. When made into jam 50% of the ^{35}S in the fruit was physically removed, a further 11% was converted into non-volatile, non-SO_2-releasing compounds and only 1.5% was still measurable as SO_2. The jam contained unidentified compounds corresponding to 110 mg SO_2/kg fruit; the measurable SO_2 was only 3.5 mg/kg fruit.

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The stability of *Clostridium botulinum* type E toxin in salty and/or acid environment

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Summary

The stability of preformed *Clostridium botulinum* type E toxin in sterile buffer- and salt-solutions and in some commercial fish products has been examined. It has been found that progenitor toxin is stable for weeks at room temperature in sterile culture filtrate, spoiling fish and in low acid fish products and that it is unaffected by sterile saturated salt (NaCl) solutions and in salted fish. In high acid feed fish (fish silage pH 2–4) some inconsistant increased toxin titres have been observed.

The activated toxin, on the other hand, decreased and increased in titre during several weeks of storage in culture filtrate with added trypsin. In sterile NaCl solutions the titre decreased by a factor of 10 to that of a progenitor toxin, but in spoiling raw and salted fish toxicity was lost when pH exceeded 7.5.

The public health significance of these results is discussed.

Introduction

In some parts of the world, fish are known to be heavily contaminated with *Clostridium botulinum* type E as reviewed by Huss & Pedersen (1979). This contamination is often associated with the intestinal contents of the fish. It has further been shown (Huss *et al.*, 1979) that type E toxin can be demonstrated in the fillet of whole herring, which have been inoculated in the gut with 10^2 spores/100 g fish and stored for only 2 days at +15°C. Within this period the organoleptic spoilage of the fish is advanced, but not necessarily to the point of rejection. This fish may therefore in some cases be used in the production of special types of preserved fish products, particularly heavily salted products, or it may be utilized in the production of animal feedstuff such as fish silage.

The stability of preformed type E toxin under such conditions (high salt content and low pH) has been studied and the results are reported here.

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Materials and methods

Toxin

The toxin was produced by growing *Cl. botulinum* type E strain 4207 (NCIB) (obtained from Torry Research Station, Aberdeen, Scotland) in reinforced clostridial medium (RCM) (Oxoid). RCM (25 ml) was heavily seeded with spores and incubated at 30°C for 24 hr. At this time growth was evident with strong gas production and the whole culture was then transferred to 500 ml freshly prepared RCM. After incubation of this culture at 30°C for 5 days it was centrifuged at 13,200 ***g*** for 30 min. and the supernatant passed through millipore filter with pore size 0.4 μm. The crude toxin solution was tested for sterility on blood agar plates incubated aerobically and anaerobically.

The toxin produced in pure culture is mainly 'activable 12 S toxin' or 'progenitor toxin' (Lamana & Sakaguchi, 1971). This toxin can be activated by trypsin (Duff, Wright & Yarinsky, 1956) or some bacterial proteinases (Sakaguchi & Tokyama, 1955).

In the present work the term 'progenitor toxin' (Pro-Tox) is used when trypsin has not been applied and the term 'trypsin-activated-progenitor toxin' (Try-Act-Pro-Tox) when the material has been treated with 1% trypsin (Difco 1:250) at pH 6 and 30°C for at least 1 hr.

Toxin titration

Sample extracts were prepared as described by Huss, Pedersen & Cann (1974). The crude toxin solutions and sample extracts were diluted five-fold in sterile physiological saline. Each dilution (0.5 ml) was inoculated intra peritoneally into two mice. The dilution giving symptoms of botulism and death in at least one mouse was considered to contain at least one mouse-lethal dose (MLD).

Fish samples

Salted herring- and cod-minces were prepared from fresh fillets using an ordinary household mincer (Braun KM32).

Salt, Pro-Tox and Try-Act-Pro-Tox were added to obtain a final concentration as shown in Figs 1 and 2. All samples containing salt were stored at room temperature. Samples without salt were stored at 0°C in order to avoid toxin production due to a possible naturally-occurring contamination of the fish with *Cl. botulinum* type E.

Marinated herring were prepared from fresh herring fillets. After being inoculated with Pro-Tox the fillets were placed in a marinade consisting of water, vinegar (10%) and salt (1:1:0.3).

After 2 weeks at +10°C the herring fillets were transferred to a second marinade without salt but with 30% sugar and the amount of vinegar reduced to 5%. Storage was continued at +10°C.

Salt-cured and spiced herring were prepared from headed and gutted fresh fish. After inoculation of Pro-Tox the fish were covered with a dry mixture of salt (16 g), sugar (6 g) and allspice (1 g) per 100 g fish.

It was attempted to distribute toxin evenly in fish and fillets by applying multi-stab-inoculation.

Two types of fish silage were prepared by mincing fresh, whole herring and thoroughly mixing with 5% H_2SO_4 (50%) (silage 1) and 1% HCOOH (85%) (silage 2), respectively. Pro-Tox was added immediately after preparation of the silage.

All fish samples except the salted fish minces were prepared following commercial recipes.

Sterile buffer solutions

The stability of Pro-Tox and Try-Act-Pro-Tox was tested when exposed to sterile buffers made up of 0.1 M secondary sodium citrate adjusted to pH 2, 4 and 6 with 0.1 M HCl and 0.1 M NaOH.

Chemical analysis

The proximate composition of fish used in the various experiments was found by estimating protein (Kjeldahl analysis), oil (Bligh & Dyer, 1959), dry matter (based on weight loss during drying at 105°C for 24 h) and salt (Volhard's method).

The levels of pH in toxic material was measured using Acilit indicator paper strips (Merck) while in non-toxic material a pH-meter 28 (Radiometer) was used.

Results

Stability of toxin in a salty environment

Stability in sterile NaCl solutions. One ml of Pro-Tox (100<titre<500) and 1 ml of Try-Act-Pro-Tox (1000<titre<5000) were mixed into 9 ml of sterile solutions containing 0, 10, 20 and 26% NaCl. The mixtures were left in the cold for 48 h and occasionally stirred. No visible precipitation appeared in any of the mixtures. All samples were centrifuged (13 200 ***g*** for 30 min) and toxin assays, carried out on the supernatants, showed that the exposure to NaCl caused no loss in toxicity of Pro-Tox while titres of Try-Act-Pro-Tox had decreased by a factor 10 in all solutions to that of Pro-Tox.

Stability in salted and unsalted fish mince. NaCl was added to freshly prepared mince of herring and cod resulting in three levels of NaCl in water phase as shown in Table 1. The stability of toxin under these conditions is shown in Figs 1 and 2.

Table 1. Composition of herring and cod mince

	Raw material		
	Protein	Oil	NaCl in water phase after salting (%)
Herring	16.72	22.45	9.7
			20.6
			26.3
Cod	18.53	0.53	9.0
			16.6
			26.6

While Pro-Tox was practically stable at all levels of NaCl in herring mince, a minor decrease five–ten-fold) was observed in cod mince after 2–3 weeks of storage. The activated toxin was stable only in herring mince and heavily salted cod mince. After 3 weeks of storage only low levels of Try-Act-Pro-Tox were found in cod mince having 0–16.6% salt in the aqueous phase, and after 6 weeks no toxin could be detected in these samples.

The changes in pH of cod and herring mince are shown in Fig. 3. In cod mince saturated with NaCl in the water phase the pH remained constant at 6.3. In less salted cod mince the pH rose steadily to *ca* 8.5 during 6 weeks of storage. In herring mince, however, the increase in the pH level was much less pronounced and never exceeded pH 7.5.

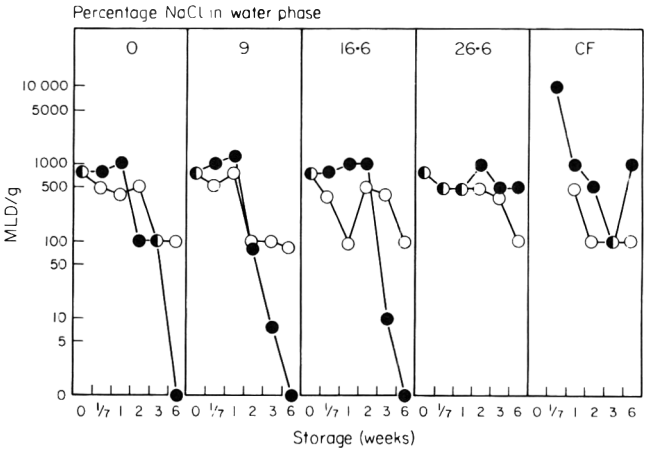

Figure 1. Cod mince. Stability of Pro-Tox (○) and Try-Act-Pro-Tox (●) in culture filtrate (CF) and cod mince containing various concentrations of NaCl in the water phase. Mince without salt was stored at 0°C while mince with salt was stored at room temperature. The zero values (time) represent the calculated titre after introducing crude toxin of known titre into the mince.

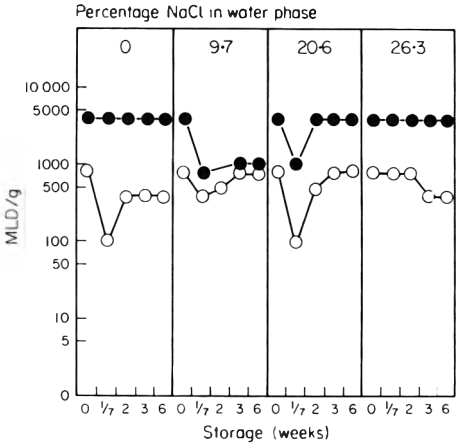

Figure 2. Herring mince. Stability of Pro-Tox (○) and Try-Act-Pro-Tox (●) in herring mince containing various concentrations of NaCl in water phase. Storage conditions as for cod mince.

Stability of toxin in an acid environment

Stability in sterile buffer solutions. Pro-Tox and Try-Act-Pro-Tox were found to be very stable during 3 weeks in sterile buffer solution at pH 2.2, 4.1 and 5.9 as shown in Fig. 4. During further storage the titre of Pro-Tox decreased at pH 2.2 while the toxicity of Try-Act-Pro-Tox was lost at pH 5.9 after 6–8 weeks.

Toxin titres in samples containing Pro-Tox increased 5- to 10-fold upon trypsinization before inoculation.

Stability in fish silage. In one experiment (a) Fig. 5), a ten- to 100-fold increase in toxin titre was observed when Pro-Tox (titre $10<\text{titre}<50$ and $100<\text{titre}<500$) was introduced into herring silage prepared with H_2SO_4 (Silage 1, pH 2.3) and HCOOH (Silage 2, pH 4.1). After 7 weeks of storage, however, toxin titres decreased in some groups but not to the initial level.

In a second experiment (b) this increase, followed by a decrease in titres, was not observed. Pro-Tox was mixed into fish silage prepared as above (pH 2.7 and 4.2) to give an initial titre of $10<\text{titre}<50$ and $100<\text{titre}<500$. In all batches of fish silage the titre remained practically constant during 12 weeks of storage at room temperature.

Stability of toxin in semi-preserved herring

The chemical composition of the semi-preserved herring (raw material and final product) is shown in Table 2. The titre of Pro-Tox inoculated into the herring fillets (2000 MLD/fillet) before processing remained constant during 4 months of storage at room temperature.

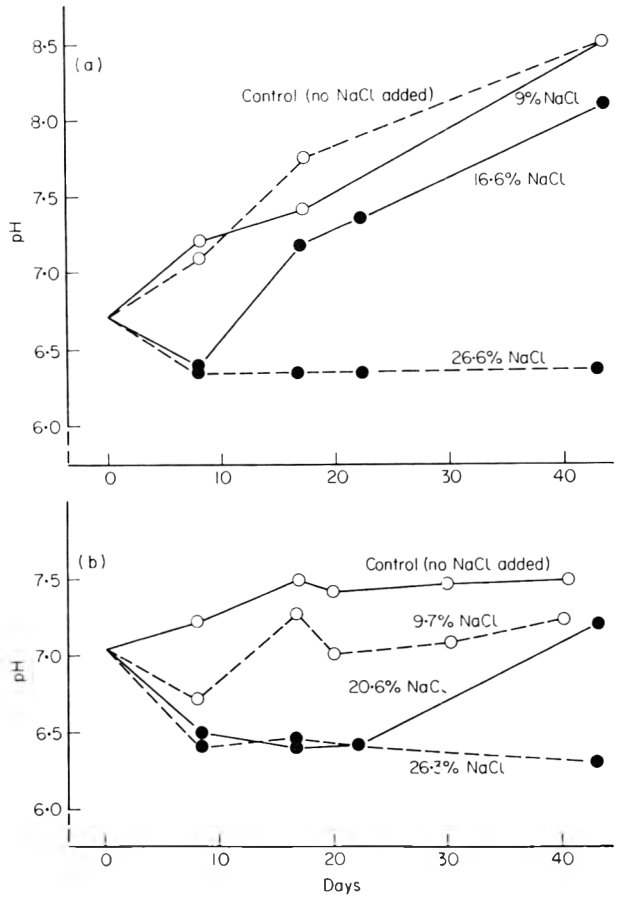

Figure 3. pH-changes in cod (a) cod mince and (b) herring mince containing various amounts of NaCl in the water phase. Storage conditions are as mentioned for cod mince in Fig. 1.

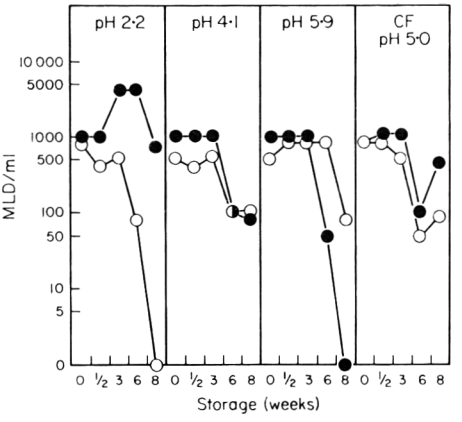

Figure 4. Stability of Pro-Tox (○) and Try-Act-Pro-Tox (●) in culture filtrate (CF) and sterile buffers at various pH and stored at room temperature.

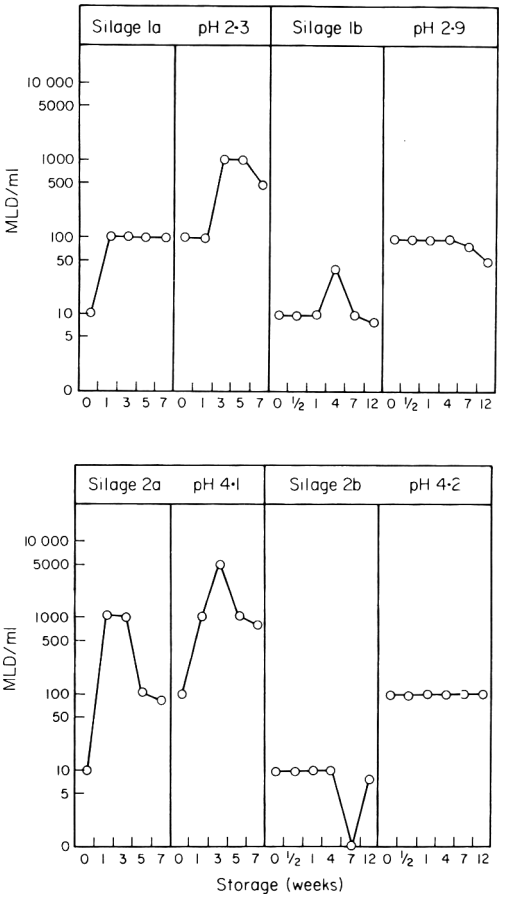

Figure 5. Stability of Pro-Tox in two experiments (a and b) and in two types of fish silage (silage 1 and 2, see text) stored at room temperature. These samples were trypsinized before titration.

Discussion

The toxic substances produced by *Cl. botulinum* are simple proteins or an aggregate of different simple protein components (Lamanna & Sakaguchi, 1971). The purified type E toxin behaves as a homogeneous protein in ultracentrifugation, electrophoresis and chromatography at a pH level below 6 with a sedimentation constant ($s^0_{20\,w}$) of 11.6 and molecular weight of 350 000 (Kitamura *et al.*, 1968). Although the mode of action of botulinal toxins is not fully understood it is currently believed that toxicity depends on a fit of the whole molecule on the target sites at the nerve-endings (Hobbs, 1976). The biological activity (toxicity) may therefore be used as a measure of stability of the molecule.

This work has shown the toxic material (Pro-Tox) in culture filtrate stored at room temperature to be very stable for 3 weeks or more.

Table 2. Chemical composition of semi-preserved herring

	Protein	Oil	Dry matter (%)	Salt in aqueous phase	pH
Raw material	18.74	4.99	25.25	–	–
Marinated	–	–	27.11	6.63	4.3–4.5
Salt cured and spiced	–	–	36.79	13.02	5.7–5.9

The decrease in titre of activated toxin followed by an increase during prolonged storage with trypsin at room temperature is indeed difficult to explain. It has been reported that prolonged exposure to trypsin results in loss of toxicity (Duff *et al.*, 1956, Sakaguchi & Sakaguchi, 1967), but there have never been any suggestions of the activation phenomenon being a reversible process. However, our results seem to indicate that trypsin is not 'digesting' the toxic proteins in a continuous process as a function of time.

Both activated and non-activated toxic proteins were stable for many weeks in a heavily salted environment. Non-activated toxin was furthermore not affected by the strong bacterial and enzymatic activity in spoiling raw or lightly salted cod and herring. Try-Act-Pro-Tox, on the other hand, decreased in toxicity in spoiling raw and lightly salted cod but not in herring. This difference may be due to a difference in spoilage pattern between the two fish species. Thus the very high pH recorded in spoiling cod mince was never seen in herring mince when the pH level did not exceed 7.5. Kitamura, Sakaguchi & Sakaguchi (1969) have shown that at a pH level >7.5 the undissociated 12S molecule splits into the toxic Eα and non-toxic Eβ components, thereby losing its stability completely. However, it is also known that some amounts of formaldehyde are formed in spoiling cod and not in spoiling herring (Savolainen, Kuusi & Nikkila, 1975), which may also affect toxicity.

It is well known that botulinum toxins are most stable at slightly acid conditions (Duff *et al.* 1956; Kitamura *et al.* 1969; Sugii, Ohishi & Sakaguchi, 1977). In our work, the effect of introducing toxic material into an environment of low pH levels was inconsistent. In sterile solutions Pro-Tox appears to be most stable at pH levels of 4–6 while greatest stability of Try-Act-Pro-Tox is at a much lower level (pH 2.2). In good agreement with this we found the progenitor toxin to be very stable in low acid fish products. However, we are unable to offer any explanation of the inconsistent increase in toxin titre noted in fish silage at pH levels of 2–4.

The epidemiology of botulism due to processed food is normally considered to be dependent on presence of the organism and suitable growth conditions during storage of the processed food article. This work has shown that correctly

processed fish products may still be highly toxic as any preformed toxin present in the raw material will be preserved as well as the fish. In analyzing outbreaks of botulism this type of epidemiology should not be overlooked, and strict control with all raw materials used in fish processing is essential in any preventive programme.

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The effect of sex, breed and initial carcass pH on the quality of cure in bacon

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Summary

The relationship between the pH_{45} measured in the *M. longissimus dorsi* and the subsequent appearance of Wiltshire style bacon was examined in 110 pig carcasses. Except for extremely PSE and DFD carcasses, which produced bacon of poor appearance, there was an inverse relationship between pH_{45} and the quality of appearance of bacon. There were no differences between hogs and gilts for either pH or bacon quality. The appearance of bacon was slightly better in breeds with lower mean pH_{45} values but the differences were not significant.

Introduction

Pale, soft, exudative (PSE) pork has two main disadvantages compared with meat of normal quality. Firstly, the excessive drip formation leads to greater weight loss in storage (Kauffman *et al.*, 1978) and secondly, its less desirable appearance in the fresh state affects saleability (Wachholz *et al.*, 1978). The greater weight loss is reflected in the yield of product when PSE meat is cured. Pig carcasses in which the musculature exhibits the PSE conditions give about 1% less yield of bacon compared with those in which the meat is normal in colour and texture (Clark, 1973; Taylor, Dant & French, 1973). The effect of the PSE condition on the appearance of cured bacon is less clear. According to Wismer-Pedersen (1968), meat with a reduced water binding capacity absorbs more pickle than normal meat when cured and there is a more thorough equilibriation of the curing salts and muscle proteins; this meat therefore cures satisfactorily and usually produces Wiltshire bacon of normal quality. Taylor *et al.* (1973) found that bacon produced by the Wiltshire process from PSE carcasses was paler than that from normal carcasses but the difference was not easily discernible to the eye in the *M. longissimus dorsi* (LD). In the *M.*

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semitendinosus however the increased paleness of the light portion accentuated the typical two-toned appearance of this muscle. Overall the authors concluded that there were no relevant differences in the appearance of bacon from PSE or normal carcasses. On the other hand, Vahlun (1973) stated that cured products made from PSE meat had an undesirable pale colour which tended to be unstable.

In contrast to the effect of initial carcass pH, the effect of high ultimate pH on bacon quality is well established. Bacon made from meat with a high ultimate pH has a dark, fiery colour and sticky consistency and is more susceptible to bacterial spoilage (Lawrie, 1979).

As part of a wider assessment of the economic consequences of pre-slaughter stress and the PSE condition in pigs and, since there is some disagreement about the effects of the qualities of fresh meat on the appearance of the cured product, this study investigated the relationship between the appearance of bacon in the LD and the pH in the carcass measured 45 min post-mortem.

Because it is difficult to monitor reliably the quality of cure in bacon sides manufactured under normal commercial conditions we took advantage of the opportunity to use material exhibited at one of the premier British agricultural shows and which therefore was produced under well regulated conditions with adequate documentation of the carcasses.

Materials and methods

Bacon exhibited at the show in 1978 and 1979 was studied. All the pigs slaughtered in each year were processed in one group and in both years the animals were killed and the carcasses cured at the same bacon factory. There were fifty pigs in 1978 and sixty in 1979. Forty-two were hogs and sixty-eight were gilts. The carcasses were manufactured into smoked, Wiltshire style bacon.

Measurement of pH

The pH at 45 min postmortem (pH_{45}) in the left LD between the ninth and tenth ribs was measured using a Radiometer PHM29 meter and combined glass electrode (GK 2321C). The measurements were made just as the carcasses entered the chiller. Ultimate pH values (pH_u) were measured next day (20 hr postmortem) in the same muscle between the tenth and eleventh ribs. The pH meter was standardized against buffers of pH 7.00, 6.00 and 4.01 at the start of readings and was checked after every six to ten carcasses with buffer of pH 6.00.

Assessment of bacon appearance

The quality of curing in the LD was assessed by an experienced bacon judge when the bacon was exhibited. A different judge made the assessment in each

Table 1. Criteria used in judging bacon

Cure score	Characteristics of bacon
1	Poor cure, colour poor and uneven
2	Inadequate cure, some deficiency in colour and evenness
3	Adequate cure, acceptable and fairly even colour
4	Good cure, good and even colour
5	Excellent cure, excellent even colour

year. The quality of cure, judged on the intensity and evenness of colour, was scored for each carcass on a five point hedonic scale (1 = poor cure, 5 = excellent cure, Table 1). Differential staining due to smoking was discounted. The judges had no knowledge of the previous measurements of pH.

Analysis of results

The results for the two years were comparable although the mean pH_{45} in 1978 (6.04±0.04 (s.e. mean)) was significantly ($P<0.01$) lower than that in 1979 (6.22±0.05 (s.e.mean)). This was a reflection of the slightly different numbers of pigs of each breed in the 2 years. In 1979 there were relatively more animals from breeds with a higher mean pH_{45} (see Table 3). There were no

Table 2. The effect of sex on pH and cure score (mean ± s.e.mean)

	Hogs (n = 42)	Gilts (n = 58)
pH_{45}	6.14±0.05	6.14±0.04
pH_u	5.64±0.02	5.62±0.01
Cure score	3.3 ±0.1	3.1 ±0.1

There were no significant differences between hogs and gilts.

Table 3. Carcass pH and bacon cure scores for three breed groups

Breed	n	pH_{45}	pH_u	cure score
LW	31	6.29^a±0.05	5.62^a±0.01	3.1±0.16
LR	50	6.08^b±0.05	5.58^b±0.01	3.2±0.13
W	28	6.06^b±0.06	5.71^c±0.03	3.4±0.20

Means with different superscripts are significantly different at the 5% probability level.

significant differences in carcass weight, pH_u or cure score between years and for the analysis of the effects of sex or breed the data were combined. The results were analysed using Students' t-test and simple regression analysis.

Results

Differences between hogs and gilts

A comparison of the two sexes is shown in Table 2. There were no significant differences between hogs and gilts for any of the variables measured and the data were combined for all subsequent analysis.

Differences between breeds

Of the 110 pigs studied, thirty-one were Large White (LW) or LW crosses, fifty were Landrace (LR) or LR crosses and twenty-eight were Welsh (W) or W crosses. One pig was a Hampshire cross. The average pH values and cure scores for the three major breed groups are given in Table. 3. The LW group had a significantly ($P<0.01$) higher mean pH_{45} than either LR or W pigs. The pH_u was significantly ($P<0.05$) different for all three groups. Cure score, while not significantly different between breeds, increased with decreasing mean carcass pH_{45}.

The relationship between pH and cure score

Table 4 lists the average pH_{45} associated with each cure score (1–5). Except

Table 4 Average pH_{45} values associated with each cure score (means ± s.e.mean, numbers of carcassess in each group are given in brackets).

	Cure score				
Year	1	2	3	4	5
1978	(6.50)*	6.25^{a} ±0.07 (11)	6.05^{ac} ±0.07 (14)	5.92^{bc} ±0.08 (18)	5.93^{bc} ±0.07 (6)
1979	(5.50)*	6.40^{a} ±0.09 (13)	6.30^{a} ±0.05 (27)	6.06^{b} ±0.09 (17)	5.85 — (2)

*1 value only

Means in the same line with different superscripts are significantly different at the 5% probability level.

Table 5. Average pH_u in each cure score group (means ± s.e.mean, numbers of carcasses in each group are given in brackets)

	Cure Score				
Year	1	2	3	4	5
1978	(6.40)*	5.55^a ±0.02 (11)	5.65^b ±0.04 (14)	5.56^a ±0.02 (18)	5.65^b ±0.04 (6)
1979	(5.45)*	5.63 ±0.02 (13)	5.64 ±0.01 (27)	5.64 ±0.03 (16)	5.65 — (2)

*1 value only

Means in the same line with different superscripts are significantly different ($P<0.05$).

for the group given a cure score of 1, in which there were only two carcasses, a higher score, representing a better quality of cure, was associated with lower pH_{45} values in the carcass. This was so for carcasses from both years although the mean pH_{45} values associated with each cure score differed slightly in the two years. This probably indicates a small variation in subjective scoring between the two judges.

Of the two carcasses given a cure score of 1, one was extremely PSE with a pH_{45} of 5.50 and the other would be classed as dark, firm and dry (DFD) with a pH_u of 6.40. The relationship between pH_{45} and cure score was reflected in a significant negative correlation in both 1978 ($r = -0.40, P<0.01$) and 1979 ($r = -0.42, P<0.01$). In calculating these values the two carcasses with scores of 1 were omitted but their inclusion did not affect the significance or conclusions to be drawn, merely reducing slightly the size of the correlation coefficients.

The effect of pH_u on cure score is shown in Table 5. There were small differences between the mean pH_u values associated with different curing scores in 1978 but no differences in 1979. No overall trend in pH_u was apparent and there was no significant correlation between pH_u and cure score in either year.

In both years the proportion of pigs with low initial pH values was high when compared with average values found in recent surveys of pH_{45} in British pigs (Evans, Kempster & Steane, 1978). In 1978 43% and in 1979 25%, of the carcasses had pH_{45} values less than 6.0. This was probably because of the selected nature of the sample, rather than any difference in preslaughter handling given to the show pigs. Kempster and Cuthbertson (1975) found low but significant positive correlations between pH_{45} and backfat thickness in commercial pigs and Evans *et al.* (1978) showed that pigs with good conformation and blockier carcasses had a greater propensity for producing PSE meat.

Table 6. Cure scores and pH_{45} values in PSE (pH_{45} <6.0) and normal (pH_{45} ≥6.0) carcasses (means ± s.e.mean)

	PSE			Normal			Significance of differences between means	
Year	*n*	pH_{45}	cure score	*n*	pH_{45}	Cure score	pH_{45}	Cure score
1978	21	5.74 ±0.03	3.76 ±0.17	28	6.25 ±0.03	3.11 ±0.19	$P<0.001$	$P<0.05$
1979	15	5.78 ±0.03	3.67 ±0.23	44	6.39 ±0.04	2.95 ±0.10	$P<0.001$	$P<0.01$

Because many of the carcasses would be classed as PSE based on their pH_{45} values (Evans *et al.*, 1978), in Table 6 all carcasses, except the two with cure scores of 1, have been grouped into PSE ($pH_{45}<6.0$) or Normal ($pH_{45}\geq6.0$) categories. In both years the PSE groups had significantly higher cure scores.

Discussion

The results give strong evidence for the existence of an inverse relationship between the quality of appearance of bacon and the pH_{45} of carcasses. The only exception was an extremely PSE carcass whose appearance remained poor after curing. The single carcass which exhibited the DFD condition also showed the typical undesirable cured appearance (Lawrie, 1979). Pork with a low initial pH, representing a rapid rate of glycolysis, is paler than normal meat (MacDougall & Disney, 1967) and this paleness is carried through in the bacon made from it (MacDougall, 1970). The judges may have preferred the bacon from carcasses with lower pH_{45} mainly because of this. However, Taylor *et al*, (1973) found that, while PSE bacon was paler than normal bacon when measured by reflectometer the difference in the LD was so small as to be not easily discernible by eye. Only in the *M. semitendinosus*, which shows two-toning, was any real difference in colour observed. It is also possible that the evenness of cure was better in meat with lower pH_{45} which would tend to absorb more pickle during curing because of its reduced water binding capacity (Wismer-Pedersen, 1968). More work is needed to define the reasons for the judges preference of bacon from carcasses with lower pH_{45} values and to examine whether their preference is a reflection of a general one.

Because of the relationship between pH and water holding capacity (Hamm, 1961) pH_{45} values of less than 6.0 have been used in many surveys of meat quality in pigs to indicate PSE meat (McLoughlin, 1965; Bendall, Cuthbertson & Gatherum, 1966; Taylor, 1966; Kempster and Cuthbertson, 1975). However, as pointed out first by Taylor (1966), this criterion considerably overesti-

mates the incidence of PSE. Therefore the indication from Table 6 that carcasses identified as PSE on this basis give, on average, bacon of more desirable appearance, could be misleading. It is possible that, if judged directly by observation rather than indirectly by pH_{45}, many carcasses classed as PSE would appear normal and the apparent paradox of carcasses potentially producing poor quality meat, but in practice giving bacon of improved appearance, would be resolved. Barton (1977), in a comparison of the meat quality of Danish Landrace and Large White pigs, found that while the Large White pigs gave meat of lighter colour, a characteristic generally accepted to be an indication of poorer muscle structure, the meat was in fact less exudative in this breed. This author (Barton-Gade, 1979) has also shown that measurements carried out on the slaughter line, including pH_{45}, are not accurate predictors of meat quality in pigs. It is probable therefore that a low pH_{45} is beneficial to the subsequent appearance of bacon unless it is associated with the PSE condition when appearance will be poor particularly in the ham muscles (Taylor *et al.*, 1973) and the yield of bacon will moreover be lower (Clark, 1973; Taylor *et al.*, 1973). To clarify this issue further work is required using a more accurate method than pH_{45} for detecting PSE meat.

The lack of influence of sex on pH agrees with the work of Smith, Wilson & Burr (1976) and is reflected in a lack of any difference in the appearance of bacon made from the carcasses of hogs and gilts. Differences in average pH_{45} between the three major breed groups represented in the study are in agreement with the results of Bendall *et al.* (1966) and recent Meat in Livestock Commission (1975) findings on the incidence of PSE meat in pigs passing through test stations. Although no significant differences attributable to breed were found in curing quality, cure score was higher in breeds with lower mean pH_{45} values and the relationship between cure quality and breed needs more investigation. Except in so far that typically DFD carcasses have high ultimate pH values and are unsuitable for curing (Vahlun, 1973) pH_u did not appear to influence materially the appearance of bacon over the range of values encountered.

The main conclusion from this work is that, except for extremely PSE carcasses which, like those which are DFD, produce bacon of poor appearance, there is an inverse relationship between pH_{45} and bacon quality. In both years, and assessed by different judges, carcasses with lower pH_{45} values gave bacon which was preferred. This relationship needs confirming over a larger sample of commercially-produced bacon assessed by a wider panel of judges.

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The effect of enzymic modification on the foaming, water absorption and baking quality of defatted soya flour

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Summary

Defatted soya flour slurries were treated for 0,5,10,15 and 30 min with papain at 50°C and tested for whipability, water absorption and baking quality at replacement levels in wheat flour of 10,20 and 30% (w/w). When whipped, all modified suspensions showed volume increases exceeding 250% with lower stability ratings than the unmodified samples. The 30 min enzyme treated product exhibited excellent water absorption. Use in bread resulted in a depression of loaf volume, development of a granular texture, off-colour and flavour. As was expected, all favourable characteristics decreased upon increasing the percentage of soya product in the dough formulation. Loaf volumes of breads containing 10% modified soya flour (MSF) ranged from 444 to 527 cc; with 20% MSF between 374 and 428 cc, and with 30% MSF between 383 and 409 cc. Loaf volume of all wheat flour bread was 861cc. Hunter colour difference meter readings indicated samples at all replacement levels, regardless of the enzyme modification time, exhibited higher levels of visual lightness than all wheat flour bread. All experimental breads exhibited a higher level of yellow tones and lower level of green tones compared to the all wheat flour bread.

Introduction

Enzymic hydrolysis of food proteins generally results in profound changes in their functional properties. Protein hydrolysates may, therefore, be expected to fulfill certain of the food industry demand for proteins with particular, well-defined, functional properties. A wide-spread use of protein hydrolysates in food requires a careful control of the taste and functionality during its hydrolysis and subsequent processing to obtain a reproducible product quality (Adler-Nissen & Olsen, 1979).

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Many studies dealing with the enzymic modification of proteins to improve their functionality have been reported in the literature. Horiuchi *et al.* (1978) studied the foaming capacity and stability of hydrolyzed proteins and their molecular structures and found that foam stability relates to the extent of the absorption of a molecule to the air-liquid interface. Gunther (1979) reported that protein derivatives exhibiting aerating and whipping properties are produced by the action of proteolytic enzymes on a suitable protein substrate. Such findings are meaningful in terms of gas incorporation in protein fortified bread doughs and its eventual effect of loaf volume and general baking properties. Pomeranz, Shogren & Finney (1977) reported that fortified breads with acceptable loaf volume, crumb grain and crumb colour could be produced from wheat flour substituted with 10% (w/w) soya flour produced from germinated soybeans.

The degree of water retention by soya flour is also characteristic considered to be an indication of performance in several food formulations. Water uptake by soya products is attributed primarily to the protein content, but it is also affected by a number of other factors including pH (Wolf & Cowan, 1971). Beuchat, Cherry & Quinn (1975) reported that defatted peanut flour treated with proteolytic enzymes exhibited an increased water absorption. Puski (1975) reported that enzymic modification of soya proteins caused a slight increase in their water absorption. Kulman (1938) stated that the amount of water absorbed during dough formation was related to baking quality. Pomeranz (1966) found that high protein solubility, generally, was detrimental to end-use properties in bread-making.

Despite the increase in nitrogen solubility, Pomeranz *et al.*, (1977) reported an improvement in the bread baking quality of soya flour produced from germinated soybeans (*in vivo* enzymic modification) over the control soya flour. Enzymic modification (*in vitro*) of soya proteins isolated from a defatted soya flour has been shown to improve the surface active properties of the protein isolate (Puski, 1975; Rackis, 1977). In light of the above data, the objective of this present study was to evaluate the effect of *in vitro* enzymic modification of defatted soya flour on its foam capacity and stability, water absorption and baking quality.

Materials and methods

Preparation of enzyme modified soya flour

Defatted soya flour (Protein Dispersibility Index [PDI] =90%) was purchased from A. E. Staley, Mfg, Co., Decatur, IL. Enzyme (papain) hydrolyzed and control soya flours were produced according to the procedure described by Kuo, Taranto & Rhee (1978). Samples were hydrolyzed for 0, 5, 10, 15 and 30 min. Any residual enzyme activity is destroyed by heating the samples at 82°C for 10 min prior to freezing. After freeze drying, all samples were stored at 2°C for subsequent analysis.

Wheat flour

An all-purpose wheat flour (bleached) was purchased at a local supermarket and used in all experimental formulations. The milling extraction was assumed to be approximately 72%. The flour contained 11% protein on a 14% moisture basis.

Foaming properties

Foam capacity. Five-hundred ml of a 4% (w/v) slurry were adjusted to pH 6.5 with either 0.1N NaOH or 0.1N HCl. The slurry was then placed in a Kitchen-Aide mixer and whipped with a wire whisk for 3 min at a speed setting of 2. The resultant foam was transferred to a graduate cylinder and any product which cleaved to the mixing bowl was removed by the use of a rubber scraper. Percentage volume increase was determined as described by Lawhon, Cater & Mattil (1972):

$$\text{Percentage vol. increase} = \frac{(\text{vol. after whipping} - \text{vol. before whipping})}{(\text{vol. before whipping})} \times 100$$

Foam stability. Stability was defined by measuring the leakage of liquid in ml from a fixed foam volume over a 30 min period.

Water absorption.

Water absorption was determined by the procedure of Smith & Circle (1978). Water absorption was calculated as follows:

(1) ml of H_2O absorbed = 40 − ml of supernatant

(2)
$$\text{Percentage } H_2O \text{ absorbed} = \frac{(\text{ml } H_2O \text{ absorbed})}{(\text{weight of initial sample})} \times 100$$

Bread baking

The modified 100% sponge dough method described by Marnett, Tenney & Barry (1973) was used to prepare experimental doughs. Doughs were prepared from wheat-soya mixtures subsituted at 0, 10, 20, and 30% (w/w) replacement levels. All of the enzyme-modified soya flours and control were tested at each replacement level. All sample mixtures contained dough conditioners at the levels prescribed by Tsen, Hoover & Philips (1971) and Marnett *et al.*, (1973). Baking absorptions for experimental mixtures were estimated from the dough farinograms. Duplicate loaves were baked from each dough mixture with 300 g dough/loaf.

Loaf volumes were measured by the rapeseed displacement test. Crumb colour was evaluated on slices with a Hunter Colour Difference Meter.

Results

Foaming properties

Differences in the foam capacity among samples were readily apparent (Fig. 1). The modified samples exhibited larger capacities (ranging from 250–268% volume increase) than the control sample. All foams, whether produced from modified or control samples, possessed at medium-thick egg white-like structure. Hydrolyzed samples, although exhibiting increased foam capacity over the control, showed a decreased foam stability (Fig. 2).

Water absorption

The control soya flour exhibited a larger water absorption than the 5, 10, and 15 min hydrolyzed samples (Fig. 3). The 30 min hydrolyzed sample exhibited a large increase in water absorption when compared to the control (Fig. 3).

Baking tests

All loaves made with soya flour modified for 5, 10 and 15 min at the 10, 20

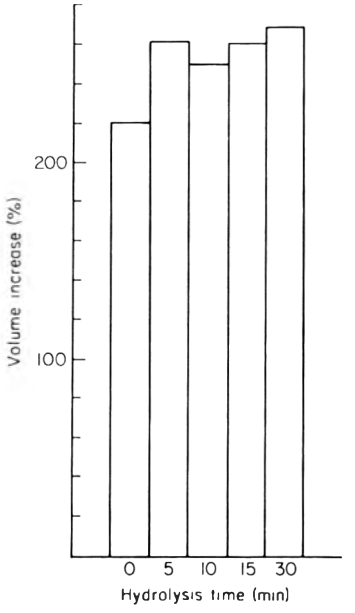

Figure 1. Foam capacity of control and enzyme hydrolyzed soya flours. Foam capacity is expressed as the percentage volume increase after whipping.

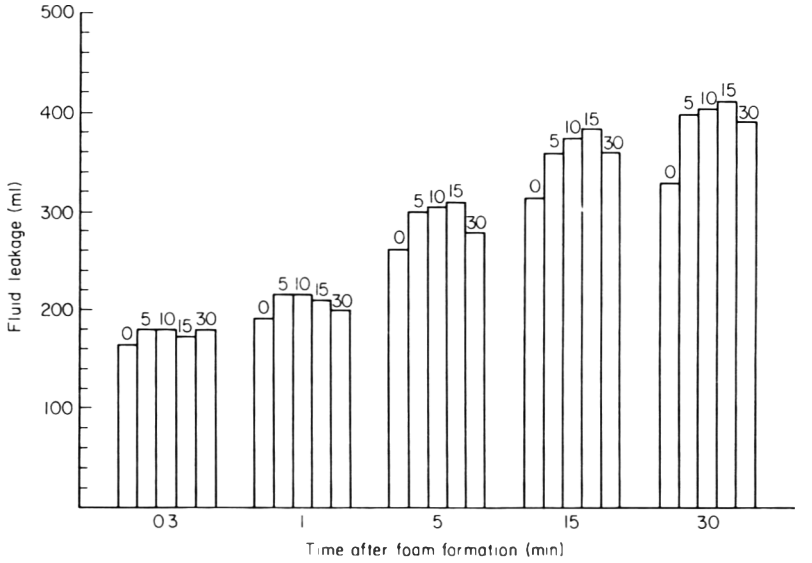

Figure 2. Foam stability of control and enzyme hydrolyzed soya flours. Foam stability is expressed in terms of fluid leakage after foam formation.

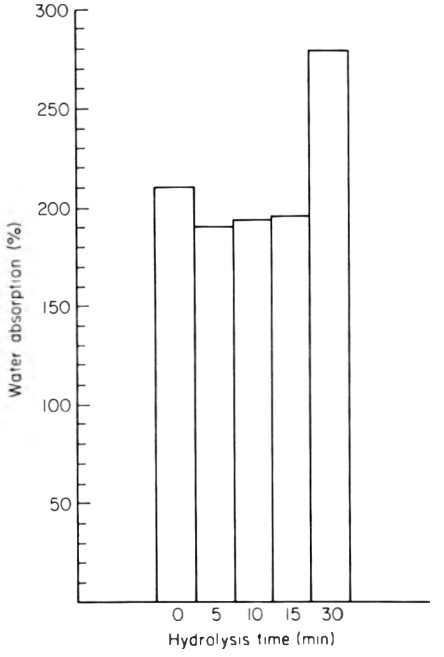

Figure 3. Water absorption of control and enzyme hydrolyzed soya flours. Water absorption is expressed in terms of the percentage water absorbed.

and 30% (w/w) replacement levels had similar quality characteristics compared to the control and all wheat flour breads. The breads made with the 30 min modified soya flour at the 20 and 30% (w/w) replacement levels were cylindrical in shape and more compact (lower loaf volume) than the other bread samples. Doughs at the 10% (w/w) replacement level for all modified flours were highly extensible and resembled toffee. The doughs had little resistance, were pale white in colour with no objectionable odour. The 20% (w/w) replacement level doughs were more gummy than the 10% soya doughs and were less elastic. The colour and odour were similar to the 10% soya doughs. The 30% (w/w) replacement level doughs were yellowish in colour, had a beany odour, exhibited a severely reduced elasticity and were extremely tacky. This high degree of tackiness made it difficult to roll the dough properly to form the loaves.

Loaf volumes of the control, enzyme-modified flours and all wheat flour are presented in Table 1. The addition of either unmodified or modified soya flour reduced the loaf volume when compared to the all wheat flour bread. However,

Table 1. Loaf volumes of all wheat flour and experimental breads

Sample	Bread loaf volume (cc)
Control	
10%	444
20%	428
30%	409
5 min (MSF)	
10%	527
20%	374
30%	318
10 min (MSF)	
10%	521
20%	396
30%	381
15 min (MSF)	
10%	447
20%	425
30%	411
30 min (MSF)	
10%	439
20%	419
30%	384
All wheat	861

Table 2. Colour difference meter tristimulus readings

Samples	R_d	L^*	a (−)	b (+)
Standard	85.8		0.8	1.2
wheat	37.1	26.3	0.30	0.85
control				
10%	28.3	31.1	0.29	0.85
20%	30.8	29.8	0.14	1.13
30%	34.8	29.6	0.13	1.32
5 min (MSF)				
10%	28.6	30.9	0.34	1.08
20%	31.0	29.6	0.32	1.08
30%	32.0	29.1	0.21	1.16
10 min (MSF)				
10%	27.8	31.4	0.28	1.07
20%	27.8	31.4	0.28	1.08
30%	32.9	28.6	0.25	1.16
15 min (MSF)				
10%	31.1	29.6	0.21	1.08
20%	31.6	29.3	0.20	1.10
30%	32.6	28.8	0.13	1.18
30 min (MSF)				
10%	29.0	30.7	0.20	1.18
20%	30.1	30.1	0.19	1.18
30%	31.1	29.6	0.20	1.18

*L values calculated according to the following equation: $L = R_d$ (dl/dr$_d$) where (dl/dr$_d$) = 0.541.

compared to the 10% control soya bread, the 10%, 5 and 10 min modified soya breads had slightly larger loaf volumes (Table 1). As expected, the loaf volume decreased as the replacement level increased from 10 to 30% for the control and all modified soy flours.

Tristimulus values for the experimental breads are presented in Table 2. Negative a values indicate greenness and positive b values indicate yellowness. The L values signify visual lightness: 100=all white, 0=all black. All soya-fortified breads had higher L values than the all wheat flour bread, while the green tones decreased and the yellow tones increased.

Discussion

The trends observed in the data caused by enzymic modification (increase in foam capacity, decrease in stability and ultimate increase in water absorption)

have been reported by other workers. Puski (1975) working with enzyme-modified soya protein isolate reported increased water absorption and foaming capacity and a decrease in foam stability when compared to the control. Kuehler & Stine (1974) working with enzyme-modified milk whey proteins reported increased foaming and emulsification capacities, but decreased stabilities when compared to the control. Zakaria & McFeeters (1978) reported that soya protein isolate exposed to limited pepsin hydrolysis exhibited an increased emulsification activity and decreased emulsification stability. These authors noted a decrease in emulsification activity as the enzyme incubation period increased beyond 2 hr. Beuchat *et al.*, (1975) working with enzyme-hydrolyzed peanut flour were the only workers who reported no initial increase in emulsification capacity with limited hydrolysis. These authors did find the usual reduction in emulsification stability and increase in water absorption after enzyme treatment.

In our work, the foams produced from the control and enzyme modified soya flours exhibited a medium-thick egg white-like structure. McWatters & Cherry (1977) reported that soya flours formed very-thick egg white-type foams at pH 6.5 and medium-thick foams at pH 4.0. The reason for the differences between our results and those of McWatters & Cherry (1977) deserve some comment. Many factors influence the whipping properties of proteins. One in particular is the type of mixer and form of agitation. Others include moisture, temperature, pH level, concentration, whipping speed and time (Huffman, Lee & Burns, 1975). The differing results obtained in various studies stem from the many different methods used to measure foaming properties. We agree with Pour-El (1979) and recommend the development of a standard method for preparing foams with experimental variations being directed towards manipulation of the raw materials and not the method for forming the foam.

The structure of wheat-flour doughs and the development of the doughs into bread have been studied by the use of transmission electron microscopy (TEM) by Simmonds (1975) and Khoo, Christianson & Inglett (1975). Simmonds (1975) described changes that occurred during the conversion of flour to dough and suggested that two types of inclusions occurred in the protein phase of the dough: type I were irregular, densely staining and were assumed to be formed from the endoplasmic reticulum; type II were spherical, had not been formed in doughs from defatted flours and were, therefore, assumed to be lipid-rich. Khoo *et al.*, (1975) describe the stages of breadmaking as: (1) freshly mixed dough; (2) fermented and proofed dough and (3) fully baked bread. During baking, the protein fraction changed little (in microscopically visible structures), but the starch granules, particularly the large ones, became gelatinized. These data indicate that during the dough formation the wheat gluten is hydrated and its film forming (surface active) properties are 'activated'. With adequate mixing, the gluten is formed into strands or sheets which form a matrix in which starch and other components are embedded and dispersed (Bechtel, Pomeranz & de Francisco, 1978). In baked bread, most of the starch was

gelatinized into fibrous strands interwoven with the thin protein strands (Bechtel *et al.*, 1978). This indicates that during the baking process, the starch granules imbibed water released from the protein network as it was heat denatured.

When wheat flour is fortified with foreign proteins and subsequently baked into bread, the resultant products exhibit lower loaf volumes, compact or coarse crumb grains and firm textures (Fleming & Sosulski, 1978). The supplemental proteins were shown to disrupt the well defined protein-starch complex observed in wheat flour bread (Fleming & Sosulski, 1978). Small pores were observed in the thick cell walls of supplemental breads and these pores may have allowed gases to escape during the baking process (Fleming & Sosulski, 1978).

Knorr & Betschart (1978) showed that the reduction in loaf volume in protein supplemented bread was not due to a simple dilution of the wheat gluten. These authors postulated an interaction between the wheat gluten and foreign protein which alters the surface active properties of the gluten. It turns out that Hyder *et al.* (1974) demonstrated several interactions between various protein components of soya flour and wheat flour during dough formation. These authors attributed the loaf volume reduction in supplemented breads to a decrease in the gas retention property of the dough.

Based on the above data, one would predict that the loaf volume of supplemented breads could be increased by improving the surface active properties of the foreign protein. As shown in this and other studies, enzymic modification is one way to achieve this goal. Our experiments showed a small increase in loaf volume with short enzyme incubation times. We attribute this increase to the improvement in the surface active properties of these enzyme-modified samples. Our foaming properties data are corroborated by Zakaria & McFeeters (1978) who reported an increase in the surface active properties of soya protein treated for short incubation periods with pepsin. The concept of limited enzyme hydrolysis has been studied in some detail by Adler-Nissen & Olsen (1979). These authors also demonstrated that the surface active properties of soya protein were improved by limited hydrolysis and greater degrees of hydrolysis were detrimental to these functional properties.

We are presently studying the effect of limited hydrolysis of soya protein isolate with enzymes other than papain on the dough rheology and baking characteristics of soya protein supplemented wheat flour. Results of this work will be reported at a later date.

Acknowledgments

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The development of a soya-based yoghurt

I. Acid production by lactic acid bacteria

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Summary

The development of a fermented yoghurt-like food from soya is described. Acid produced by the lactic acid bacteria, *St. thermophilus* and *L. bulgaricus,* singly and combined, was determined and the effects of supplementation with glucose and yeast extract quantified. A product with acceptable acidity was produced using *L. bulgaricus* in soya milk supplemented with 1% glucose and 0.1% yeast extract.

Introduction

The inadequate supply of protein-rich foods is a major factor in the occurrence of malnutrition in developing countries. Protein derived from animal sources, such as meat, eggs, fish, chicken and milk products, are expensive and are not readily available in sufficient quantities. One source of cheap protein that is still under-exploited in Thailand is soya. Foods presently produced from soyabeans are mainly fermented products, used for seasoning purposes, and hence only consumed in small amounts.

The main problems limiting the widespread consumption of soyabeans is their objectionable flavour, a green/grassy or beany note (Rackis *et al.,* 1972). Additionally the high levels of oligosaccharides present lead to flatulence, caused by the breakdown of these indigestible sugars to carbon dioxide, hydrogen and methane in the lower intestine by the natural microflora present (Cristofaro, Mottu & Wuhrmann, 1974). If these problems could be overcome and soya processed to yield acceptable products, a large source of highly nutritious food could be realized.

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The fermentation of soya, in the form of 'milk', could theoretically lead to a solution of these problems, namely a reduction in the objectionable flavour and a decrease in the levels of oligosaccharides present. The use of lactic acid bacteria could also lead to products with sufficient acidity (low pH) for good keeping properties. The production of a reasonable level of acidity would also help to improve the flavour.

In the present study, described here, and in subsequent papers, attempts to grow various lactic acid bacteria in soya milk were made, together with investigations of the effects on acid production, volatile compounds which influence organoleptic quality, and levels of oligosaccharides.

Materials and methods

Soya milk was prepared by the addition of full fat soya flour, obtained from British Arkady, (110g) to almost-boiling water (1 litre), 85–90°C, and mixing the resulting suspension thoroughly with a Silverson blender for 2 min. The mixture was cooled to *ca* 70°C and centrifuged at 3000 ***g*** for 5 min. Samples of the milk (50 ml) were autoclaved at 15 psi for 5 min and stored at 5°C until required.

Yeast extract (Oxoid code 21), as a 5% (m/v) solution, and D-glucose (BDH, Analar), as a 50% (m/v) solution, were used to supplement media. Both solutions were sterilized prior to use.

Yeast glucose soya milk was prepared by the addition of yeast extract (3 g) and glucose (10 g) to soya milk (1 litre).

L. bulgaricus and *St. thermophilus* in yeast glucose soya milk were used to innoculate soya milk, at 2% when used singly or at 1% of each culture when used in combination. Temperatures for incubation were 43°C for *L. bulgaricus,* 37°C for *St. thermophilus* and 37°C for the combination.

Acidity, expressed as percentage lactic acid, was determined on 10 g samples by titration with N/9 sodium hydroxide using phenolphthalein as indicator (0.5% m/v) in 50% aqueous ethanol (v/v)).

Total solids, protein and fat determinations were carried out as described by A.O.A.C. (1970)

The soya milk used in these experiments contained 3.84% protein (N × 6.2), 2.35% lipid with total solids 7.8%.

Results and discussion

Many workers have attempted to produce fermented soya products with a range of lactic acid bacteria, e.g. *St. lactis, St. citrovorus, St. thermophilus, L. delbrueckii* and *L. pentosus,* with the addition of fermentable supplements (Gaddi, 1970; Mital & Steinkraus, 1974, 1976; Wang *et al.,* 1974; Kanda *et al.,* 1976; Mital 1977).

Among these *St. thermophilus* was found to produce the most acid but the flavour of the product was still unsatisfactory (Mital & Steinkraus, 1976). *L. bulgaricus,* in conjunction with *St. thermophilus*, is commonly used as a yoghurt starter, where, in addition to acid production significant flavour modification is obtained (Shankar, 1977). Consequently these two micro-organisms, singly and together were extensively studied as possible starters for a soya-based yoghurt.

The acid produced by three strains of *St. thermophilus* at various temperatures (30, 35 and 43°C) was determined after the starters were subcultured three times in soya milk. Of these strains, St_1 obtained from The National Institute of Research in Dairying, produced the highest acidity at 35°C (0.64% T.A., pH 4.28).

L. bulgaricus in the absence of supplements does not grow in soya milk. Mital & Steinkraus (1974) reported 0.21% T.A. and a pH 6.32 after fermentation of soya milk with this organism. These findings were verified in the present work.

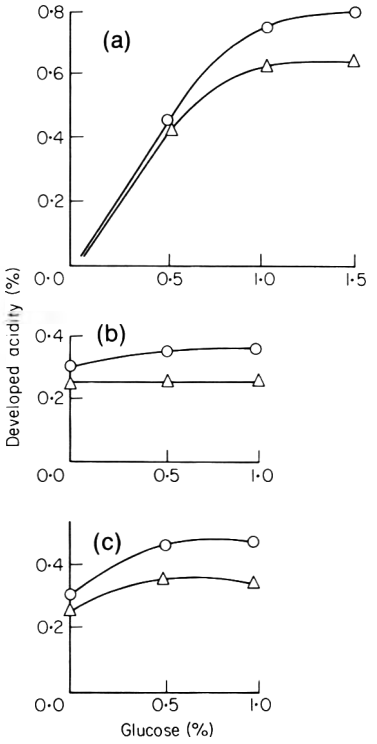

Figure 1. Acid production by *L. bulgaricus* and *St. thermophilus* singly and combined in soya milk with the addition of varying levels of glucose and yeast extract. (a) *L. bulgaricus* (incubated at 43°C for 24 hr): ○, 0.1 and 0.15% yeast extract; △, no yeast extract. (b) *St. thermophilus* (incubated at 37°C for 24 hr): ○, 0.1 and 0.15% yeast extract; △, no yeast extract. (c) *St. thermophilus* and *L. bulgaricus* (incubated at 43°C for 24 hr): ○, 0.1 and 0.15% yeast extract; △ no yeast extract.

During the isolation of micro-organisms from a rice wine starter, obtained from Thailand, it was observed that lactobacilli from the starter grew in the presence of yeast. This confirmed that yeast was supplying essential nutrients required by the lactobacilli.

Consequently yeast extract was used to stimulate the growth of *L. bulgaricus* in soya milk. Various levels of glucose, the most readily available carbohydrate for glycolysis, were also added to encourage acid production. The effect of addition of various levels of yeast extract and glucose on acid production with *L. bulgaricus* and *St. thermophilus,* singly and combined in soya milk is shown in Fig. 1.

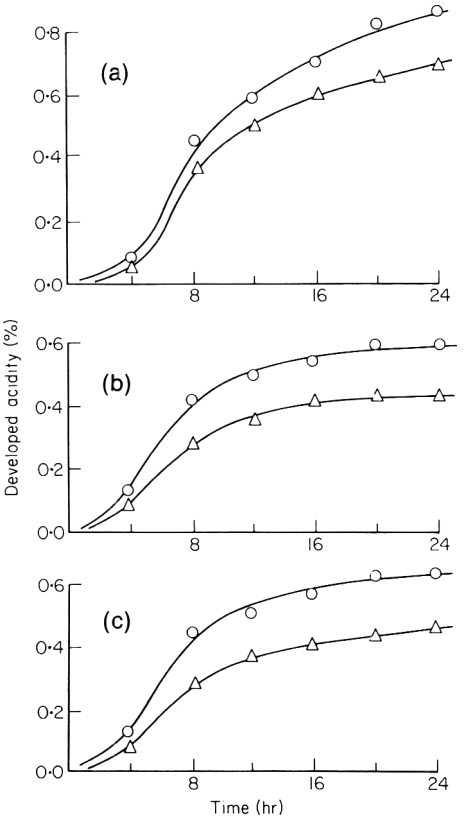

Figure 2. Rate of acid production of *L. bulgaricus* and *St. thermophilus* singly and combined in soya milk with varying amounts of glucose and yeast extract. (a) *L. bulgaricus* at 43°C: ○, 1% glucose + 0.1% yeast extract; △, 1% glucose. (b) *St. thermophilus* at 37°C: ○, 0.5% glucose + 0.1% yeast extract; △, no glucose or yeast extract. (c) *St. thermophilus* and *L. bulgaricus* at 43°C: ○, 0.5% glucose + 0.1% yeast extract; △, no glucose or yeast extract.

In the case of *St. thermophilus* addition of glucose alone had little influence on acid production but supplementation with yeast extract and glucose led to a significant increase in acidity. *L. bulgaricus* does not possess invertase activity and cannot utilize the natural sugars, sucrose, raffinose and stachyose. Thus the

addition of glucose dramatically increased the amount of acid produced. Supplementation above 1% (m/v) produced no further effect. The presence of yeast extract, as in the case with *St. thermophilus* led to a significant increase in acidity.

The combination of the two micro-organisms produced only slightly higher acidity than *St. thermophilus* alone and considerably less than *L. bulgaricus* alone, the addition of glucose in this case having only little effect. The reason for this effect, although reproducible, is not known.

The optimum conditions for *L. bulgaricus* were 0.1% (m/v) yeast extract and 1% (m/v) glucose, and for *St. thermophilus* singly and the combination of the two organisms 0.1% (m/v) yeast extract and 0.5% (m/v) glucose. The developed acidity under these conditions was 0.84%, 0.56% and 0.68% for *L. bulgaricus, St. thermophilus* and their combination respectively after 24 hr incubation.

In order to obtain further data on the effect of supplementation on the rate of acid production, the acidity, with and without supplementation was determined for 24 hr after inoculation with *St. thermophilus* and *L. bulgaricus* both singly and in combination. A short fermentation is obviously desirable for food production and it was anticipated that supplementation would increase the rate of acid production in the early stages of fermentation, as well as increasing the final levels obtained. The results are shown in Fig. 2, each data point is the average of 4–6 replicates. In the case of *L. bulgaricus* alone no acidity was produced without supplementation. In all the experiments the exponential phase of acid production was between 4 and 8 hr. The increase in rate of acid production, as a result of supplementation, appears to be proportional to the final increase in acidity in all cases, i.e., the general shape of the curves was not altered. With *L. bulgaricus* alone acid was still being produced at an appreciable rate after 24 hr and developed acidities in excess of 1.0% can be realized.

Conclusion

In terms of acid production there is little advantage to be gained in using a combination of *St. thermophilus* and *L. bulgaricus* as compared with the latter alone. Supplementation with 1% (m/v) glucose and 0.1% (m/v) yeast extract produces a developed acidity in excess of 0.8% (pH 3.8) after incubation at 43°C for 24 hr with *L. bulgaricus*. Acidities in excess of 1.2% can lead to products with unpleasant acid tastes, and a developed acidity of *ca* 1.0% (titratable acidity *ca* 1.15%) is considered to be in the optimum range. The resulting pH of 3.8 should lead to good keeping properties as microbial growth is severely limited at such a low pH.

The product, after fermentation, had a firm homogeneous curd and there was little separation of whey. The organoleptic properties of these fermented products, together with the analysis of volatile compounds and oligosaccharides, are presented in subsequent papers (Pinthong *et al.*, 1980a,b).

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The development of a soya-based yoghurt

II. Sensory evaluation and analysis of volatiles

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Summary

Soya milk samples fermented with *L. bulgaricus* and *St. thermophilus,* both singly and combined, were tested organoleptically. The volatile compounds responsible for the undesirable flavour in both fermented and unfermented samples were analysed and a correlation between organoleptic quality and the levels of certain compounds demonstrated.

Introduction

In part I of this study (Pinthong, Macrae & Rothwell, 1980) the production of fermented soya products, using *L. bulgaricus* and *St. thermophilus,* was described. Supplementation with glucose and yeast extract was required to produce a reasonable level of acidity (developed acidity *ca* 0.8%). The amount of acid present is not a sufficient criterion alone for quality and so these fermented products were subjected to organoleptic assessment. Both natural and flavoured samples were tested.

The objectionable flavour in soya products is caused by the presence of a small number of simple compounds, mainly aldehydes and alcohols (Fujimaki, Arai & Kiri, 1965; Fujimaki *et al.,* 1970). In order to understand the role of fermentation in flavour modification in soya milk products the levels of these flavour compounds in fermented and unfermented samples were studied. Low molecular weight aldehydes are derived from the unsaturated fatty acids present in soyabeans. Lipoxygenase enzymes catalyse the formation of hydroperoxy unsaturated fatty acids which can be degraded enzymatically or non-enzymatically. The former pathway, involving peroxidases and lipoxidases, is

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more selective, resulting in the narrow range of compounds formed (Eriksson, 1975). For example the degradation products of linoleic hydroperoxide are *n*-hexanal and *n*-pentanal.

Materials and methods

Samples for ranking trials (Herschdoerfer, 1967) were distributed into small plastic cups and tested by twenty judges under red light to minimize the influence of colour differences.

Samples for acceptability trials (Herschdoerfer. 1967) were similarly presented to thirty-six judges in random order to reduce bias derived from the order in which the samples were tested.

Volatile compounds were collected from the fermented and unfermented samples with the distillation train shown in Fig. 1. The sample was held at 37°C and the coolants were tap water, 12°C, salt in ice-water, – 10°C and liquid nitrogen, – 196°C. During distillation a pressure of 40 mmHg was maintained with a water pump, while a stream of nitrogen was bubbled through the sample. *n*-Butanol (20 μl) was added to each sample (1 kg), as an internal standard, prior to distillation. After distillation for 3 hr 150 μl of distillate was collected in trap D, of which 10 μl samples were used directly for gas chromatography, immediately after thawing. A 1.5m glass column, 5 mm i.d., packed with 10% polyethylene glycol on 100 – 200 mesh Diatomite MAW was used in a Pye 104 gas chromatograph with flame ionization detection. Nitrogen flow rate was 20 ml min^{-1} and that of hydrogen 30 ml min^{-1}. The column oven was operated isothermally at 70°C. Peak areas were recorded with an electronic integrator, Pye DP80.

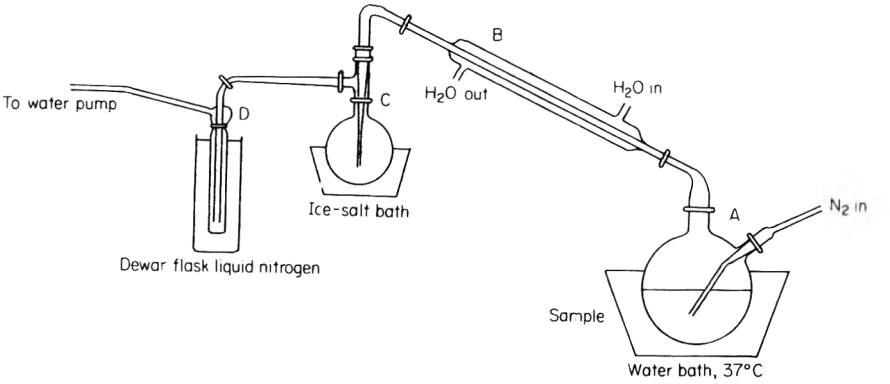

Figure 1. Distillation train

Results and discussion

A ranking trial was carried out to ascertain whether there was any significant difference between the fermented samples and between the original soya milk and the fermented samples. A cow's milk yoghurt and a sample of soya milk, acidified with lactic acid to give a similar pH to that of the fermented samples, were used as standards.

The samples used were: (1) Soya milk, supplemented with 0.1% (m/v) yeast extract and 1% (m/v) glucose, fermented with *L. bulgaricus* for 10 hr at 43°C and stored for 18 hr at 5°C prior to testing, resulting pH 4.02; (2) soya milk, supplemented with 0.1% (m/v) yeast extract and 0.5% (m/v) glucose, fermented with *St. thermophilus* for 16 hr at 37°C, resulting pH 4.10; (3) soya milk, supplemented with 0.1% (m/v) yeast extract and 0.5% (m/v) glucose, fermented with *L. bulgaricus* and *St. thermophilus* for 16 hr at 37°C, resulting pH 4.16; (4) cows' milk yoghurt (14% total solids) prepared by fermentation with *L. bulgaricus* and *St. thermophilus* at 37°C for 16 hr, resulting pH 4.38; (5) unfermented soya-milk acidified to pH 4.1 with lactic acid.

The results of the ranking trial, with twenty judges are shown in Table 1. χ^2-Distribution test showed that the samples were significantly different at $P=0.001$. Fermentation thus does improve soya milk and indeed there are significant differences between the various fermented samples.

The two apparently preferred samples from the ranking trial were then modified with sucrose and some with flavouring to produce, what was hoped would be, acceptable products. The results, shown in Table 2, suggest that the samples fermented with *L. bulgaricus* were likely to be more acceptable than

Table 1. Scores of the rank order of experimental samples

Samples	Scores of ranked order 1	2	3	4	5	Total
Cow's milk fermented with *St. thermophilus* + *L. bulgaricus*	16	6	–	4	–	26
Soya milk fermented with *L. bulgaricus*	2	22	15	8	–	47
Soya milk fermented with *St. thermophilus* + *L. bulgaricus*	1	8	27	20	5	61
Soya milk fermented with *St. thermophilus*	1	2	9	36	30	78
Acidified soya milk	1	1	9	12	65	88
Sum of Total						300

Table 2. Sample distribution in the degree of acceptability

Code	Samples	No. of Judges		
		Like	Indifferent	Dislike
A	Soya milk fermented with *L. bulgaricus* with 5% sugar, 0.4% strawberry flavour, pH 3.92	18	2	16
B	Soya milk fermented with *St. thermophilus* + *L. bulgaricus* with 5% sugar 0.4% strawberry flavour, pH 3.9	15	9	12
C	Soya milk fermented with *L. bulgaricus* with 5% sugar, without flavour, pH 3.92	16	4	16
D	Soya milk fermented with *St. thermophilus* + *L. bulgaricus* with 5% sugar without flavour, pH 3.90	10	2	24

Table 3. Significant differences of acceptability trial between the paired samples

Paired comparison	t – value	P
A/B	0.2569	NS
A/C	0.8036	NS
A/D	2.3593	S at 97% ($P_{0.025,70}$; $t=2.299$)
B/C	0.5945	NS
B/D	2.2634	S at 95% ($P_{0.05,70}$; $t=1.994$)
C/D	1.5295	S at 86% ($P_{0.137,70}$; $t=1.5295$)

those fermented with *L. bulgaricus* and *St. thermophilus*, although the presence of flavouring appeared to mask this difference. The results of paired comparisons are shown in Table 3. Additional smaller scale trials, with alternative flavouring agents, such as banana, pear and pineapple, indicated that these produced more acceptable products than strawberry. Indeed these later samples were considered acceptable.

The volatile compounds from four samples were collected and analysed: (1) soya milk (Pinthong *et al.*, 1980); (2) soya milk, supplemented with 1% (m/v) glucose and 0.1% (m/v) yeast extract and fermented with *L. bulgaricus*; (3) soya milk, supplemented with 0.5% (m/v) glucose and 0.1% (m/v) yeast

Table 4. Relative amounts (μg) of volatile compounds found in 1 kg distilled sample

Volatile* compounds	Repetitive determination	Sample (1 kg) Soya milk	Soya milk + *St. thermophilus*	Soya milk + *L. bulgaricus*	Soya milk + *St. thermophilus* and *L. bulgaricus.*
Acetaldehyde	1	10.05	86.85	56.20	28.1
	2	9.97	87.43	55.37	53.40
Acetone	1	10.65	38.05	25.85	22.68
	2	10.56	38.29	25.53	22.44
Methanol	1	62.43	25.72	86.37	40.7
	2	62.52	25.89	85.39	82.87
Ethanol	1	trace	18.84	trace	42.58
	2	trace	19.00	trace	12.88
n-Pentanal	1	1.45	34.10	2.42	26.99
	2	trace	34.26	2.50	16.73
n-Hexanal	1	10.48	6.25	4.47	6.63
	2	13.88	6.32	4.55	7.56
n-Butanol†		90.45	90.45	90.45	90.45

*Relative results (μg) based on comparison of all data obtained with 90.45 μg *n*-butanol (maximum amount detected from 1 kg distilled sample).

†*n*-Butanol as internal standard with 0.56% recovery.

extract and fermented with *St. thermophilus*; (4) soya milk, supplemented with 0.5% (m/v) glucose and 0.1% (m/v) yeast extract and fermented with *St. thermophilus* and *L. bulgaricus*.

The results of duplicate determinations are shown in Table 4 and a typical chromatogram in Fig. 2. All the data are corrected to a fixed weight of *n*-butanol, added to the sample prior to distillation as an internal standard. The major compounds detected were acetaldehyde, acetone, methanol, ethanol, *n*-pentanal and *n*-hexanal.

St. thermophilus produced more acetaldehyde and acetone than *L. bulgaricus*, both of these compounds were present only at very low levels in soya milk. Methanol was utilized by *St. thermophilus* but not by *L. bulgaricus*, whereas ethanol was produced by *St. thermophilus* to a greater extent than by *L. bulgaricus*. *n*-Pentanal, which is only present in small amounts in soya milk, is produced by *St. thermophilus*. The species of *St. thermophilus* used might possess peroxidase activity, which can decompose preformed fatty acid hydroperoxides to yield *n*-pentanal. Alternatively the *n*-pentanal may arise from α-oxidation of *n*-hexanoic acid (Conn & Stump, 1976).

Soya milk fermented with *St. thermophilus* and that fermented with *L. bulagaricus* contain less *n*-hexanal than the unfermented sample. *n*-Hexanal may be converted to *n*-hexanoic acid by an acid dehydrogenase or to *n*-hexanol by an alcohol dehydrogenase. The former path would seem more probable as

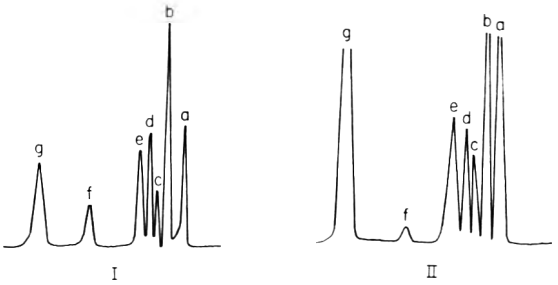

Figure 2. Gas chromatograms of volatile compounds found in soya products. I, Reference mixture; II, soya milk fermented with *St. thermophilus*.
a, Acetaldehyde; b, acetone, c, methanol; d, ethanol; e, pentanal; f, hexanal; g, *n*-butanol.

no *n*-hexanol, was detected in the volatile components. n-Hexanoic acid may further be assimilated through β-oxidation (Lehninger, 1975). The larger assimilation of *n*-hexanal by *L. bulgaricus* may be due to it being able to release protein-bound material more effectively, as it has a stronger proteinase activity than *St. thermophilus* (Kanner & Karel, 1976).

In fermentation with *St. thermophilus* and *L. bulgaricus* together the resulting levels of volatiles reflect the individual characteristics of the bacteria. Replication of data was poor, as shown in Table 4, and this may be accounted for by different relative activities of the starters.

The relative levels of *n*-pentanal and *n*-hexanal in the three fermented samples and in the original soya milk correspond well with the rank order established in the sensory tests. This would suggest that although *n*-hexanal is important in terms of the adverse flavour of soya products, other aldehydes, in particular *n*-pentanal, must be considered in fermented samples. The preferred sample, that is soya milk fermented with *L. bulgaricus* alone, indeed showed the lowest levels of *n*-pentanal and *n*-hexanal.

Conclusion

Fermentation of soya milk with lactic acid bacteria, in particular *L. bulgaricus*, leads to an improvement in flavour. Products of reasonable acceptability can be prepared with *L. bulgaricus* alone. The organoleptic quality of the fermented products is directly related to the levels of *n*-pentanal and *n*-hexanal, the former being produced by *St. thermophilus* and the latter being naturally present in soya milk.

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The development of a soya-based yoghurt

III. Analysis of oligosaccharides

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Summary

The effect of fermentation on the levels of oligosaccharides present in soya milk has been determined for a number of lactic acid bacteria. The sugars in the fermented samples were quantified by high pressure liquid chromatography (HPLC) after extraction with aqueous alcohol and clearing with Carrez solution. A lipid extraction stage was found to improve the quality of the chromatograms. Oligosaccharide utilization by *L. fermenti* was found to be less than in some published reports, when fermentation was carried out under optimum conditions for acid production.

Introduction

The major carbohydrates present in soyabeans are sucrose, raffinose and stachyose. When consumed in large amounts, as in soya products, the last two oligosaccharides can give rise to flatulence (Cristofaro, Mottu & Wuhrmann, 1974), and this severely limits the acceptability of soya-based foods. A considerable amount of work has been carried out on methods to reduce the levels of oligosaccharides in soya-products by leaching (e.g. Wagner *et al.*, 1975) and enzymic hydrolysis (e.g. Sugimoto & van Baren, 1970). However, both of these methods are expensive, either in terms of equipment or reagents. Additionally there is the risk of losing valuable protein during the process. An alternative approach would be to incorporate microorganisms, which possess α-galactosidase activity and hence can hydrolyse the oligosaccharides, directly into the fermentation stage. This would not only reduce the levels of oligosaccharides but would provide simple sugars as substrates for the lactic acid

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bacteria. A number of papers (e.g. Mital & Steinkraus, 1975) have been published showing that various micro-organisms, e.g. *L. fermenti,* can reduce the levels of raffinose and stachyose significantly. In this paper the effects of a wide range of micro-organisms are reported, including combined fermentation with lactic acid bacteria under optimum conditions for acid production (Pinthong, Macrae & Rothwell, 1980).

Many methods have been devised for the determination of the oligosaccharides in soyabeans, including gas chromatography (Delente & Ladenburg, 1972) and, more recently, HPLC (Black & Bagley, 1978). The latter method is to be preferred as no derivatives have to be prepared . However, extraction and removal of proteinaceous material are still a problem and, with soyabeans, this is particularly difficult after fermentation due to the formation of small protein fragments by proteolysis.

Materials and methods

Pediococcus pentosaceus (NIRD–990), *L. fermentum* (NIRD–215), *L. delbrueckii* (NIRD–1744) and *L. bulgaricus* Lb_1) were obtained from the National Institute for Research in Dairying. *L. fermenti* (NRRL–B–585) and *L. acidophilus* (NRRL–B–1910) were obtained from the United States Department of Agriculture.

Soya-milk, with and without supplementation was prepared as described in part I (Pinthong *et al.*, 1980). Starters were used at 2% (v/v) with 24 hr incubation at 37°C, except for *L. bulgaricus* and *L. delbrueckii* which were incubated at 44°C.

Extraction of the oligosaccharides was carried out by a modification of the method of Macrae & Zand-Moghaddam (1978). The sample (60 ml) was treated with ethanol (60 ml) and the resulting mixture centrifuged. The supernatant was collected and the residue washed with 70% (v/v) aqueous ethanol (3 × 25 ml). The extract and washings were combined, treated with chloroform (65 ml) and shaken. The aqueous phase was isolated and evaporated *in vacuo* below 50°C to near dryness. The residue was dissolved in water (10 ml), treated with Carrez solution (2 ml) (Macrae & Zand-Moghaddam, 1978) and finally diluted to 15 ml with water. The precipitated material was removed by centrifugation and the supernatant filtered prior to analysis.

The liquid chromatograph used was an Applied Chromatography Systems model 750 with a differential refractometer. The column used (250 mm × 5 mm i.d.) was packed with Spherisorb-5-amino, in our laboratory, as a slurry in propan-2-ol. Sample injection was achieved by means of a fixed loop (20 μl). A Rheodyne injection valve, model 7120 was used. The eluting solvent was acetonitrile/water (64:36, v/v) with a flow rate of 2.0 ml min^{-1}.

The retention times for the sugars were: glucose 3.3, sucrose, 3.9, raffinose 5.7 and stachyose 8.4 min. Quantification was carried out by peak area comparisons of sample and standards of known concentration.

Results and discussion

The method of extraction employed by Macrae & Zand-Moghaddam (1978), which was devised for defatted samples, gave poor chromatograms in the present study, due to co-extraction of non-carbohydrate material. A typical chromatogram using this method of extraction is shown in Fig. 1. However, modifications of the method, to include a simple lipid extraction, greatly improved the base-line as shown in Fig. 2.

The resulting levels of oligosaccharides after fermentation of soya milk with *L. fermenti, L. fermentum, L. delbrueckii, P. pentosaceas, L. bulgaricus* and *L. acidophilus,* without supplementation are shown in Table 1. Clearly the micro-organisms *L. fermenti* and *L. fermentum* possess relatively greater α-galactosidase activity than invertase activity as compared with the other members of the above group. The former group reduce the levels of stachyose and raffinose whilst the latter utilise sucrose more effectively. The lower pH developed by the latter group simply reflects the ease with which the hydrolysis products of sucrose, fructose and glucose, can pass into the glycolytic pathway, as compared with galactose produced by the hydrolysis of raffinose and stachyose.

L. bulgaricus does not grow readily in soya milk without supplementation (Pinthong *et al.*, 1980). However, it decreases the amount of stachyose slightly

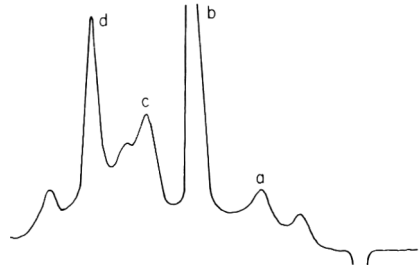

Figure 1. Chromatogram of oligosaccharides extracted from soya milk, following the method of Macrae & Zand Moghaddam (1978)

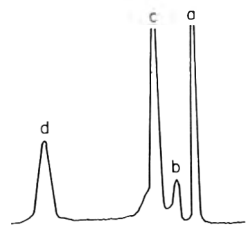

Figure 2. Typical chromatogram of oligosaccharides extracted from soya milk (fermented with *L. fermenti*) following modified procedure.

Table 1. The oligosaccharides and pH in soya milk and fermented soya milk

Sample	Stachyose		Raffinose		Sucrose		pH
	mg/100 ml	Percentage dry wt	mg/100 ml	Percentage dry wt	mg/a00 ml	Percentage dry wt	
Soya milk	437.82	5.61	114.20	1.52	546.17	7.00	6.6
L. fermenti (NRRL-B-585)	350.82	4.50	none	none	435.36	5.58	5.2
L. fermentum (NIRD-215)	335.56	4.30	36.85	0.48	455.15	5.84	5.2
L. delbrueckii (NIRD-1744)	410.60	5.27	93.37	1.19	154.51	1.98	4.2
P. pentosaceus (NIRD-990)	410.14	5.26	93.50	1.20	95.52	1.22	4.2
L. acidophilus (NRRL-B-1910)	442.24	5.67	113.47	1.46	179.96	2.31	4.3

Table 2. The oligosaccharides in soya milk fermented with *L. bulgaricus*

Soya milk treated with	Stachyose		Raffinose		Sucrose		Glucose	pH
	mg/100 ml	Percentage dry wt	mg/100 ml	Percentage dry wt	mg/100 ml	Percentage dry wt		
L. bulgaricus	414.97	5.32	134.96	1.73	580.13	7.44	trace	6.3
L. bulgaricus + 0.1% yeast extract	403.28	5.17	107.73	1.38	563.10	7.22	-Do-	5.8
L. bulgaricus + 1% glucose	425.68	5.45	108.03	1.39	572.68	7.34	-Do-	4.2
L. bulgaricus + 1% Glucose + 0.1% yeast extract	438.35	5.62	104.70	1.34	567.36	7.27	-Do-	4.0

Table 3. The oligosaccharides in soya milk fermented with *L. fermenti* (NRRL-B-585)

Soya milk treated with	Stachyose		Raffinose		Sucrose		Glucose		pH
	mg/100 ml	Percentage dry wt	mg/100 ml	Percentage dry wt	mg/100 ml	Percentage dry wt	mg/100 ml	Percentage dry wt	
L. fermenti (NRRL-B-585)	350.82	4.50	none	none	435.36	5.58	trace	trace	5.2
L. fermenti (NRRL-B-585) + 0.1% yeast extract	331. 20	4.25	trace	trace	85.11	1.09	-Do-	-Do-	4.8
L. fermenti (NRRL-B-585) + 1.0% Glucose	421.05	5.29	65.36	0.84	550.70	7.06	566.63	7.27	4.9
L. fermenti (NRRL-B-585) + 1% Glucose (w/v) + 0.1% yeast extract (w/v)	391.59	5.03	none	none	424.80	5.44	85.81	1.11	4.0
Condition in fermentation									
L. bulgaricus + *L. fermenti* (NRRL-B-585) + 0.1% Yeast Extract + 1% Glucose	385.25	4.70	none		276.59	3.55	111.62	1.43	4.0

with corresponding increase in raffinose and sucrose. Supplementation with yeast extract increases stachyose utilization but addition of glucose has the opposite effect, as would be expected (Table 2).

L. fermenti has been reported to be efficient in oligosaccharide utilization (Mital & Steinkraus, 1975). However, when used to inoculate soya milk under the same conditions of supplementation as required by *L. bulgaricus* to produce optimum acidity the decrease in levels of stachyose and raffinose was relatively modest. In the absence of supplementation a reduction in stachyose of 20% was reached and all the raffinose was removed but acid production was poor.

L. fermenti, when used in conjunction with *L. bulgaricus* under the optimum conditions of supplementation (0.1% yeast extract and 1% glucose) indeed produced only a 16% reduction in the amount of stachyose present, although raffinose was completely removed. There was more utilization of sucrose and less assimilation of glucose than with fermentation with *L. fermenti* alone (Table 3). This would indicate that this combination of organisms could produce an adequately low pH level (*ca* 4.0) with less glucose supplementation.

Mital & Steinkraus (1975) fermented soya milk with *St. thermophilus* and *L. fermenti* with apparently complete utilization of stachyose and raffinose. However, previous work (Pinthong *et al.*, 1980a) has shown that *St. thermophilus* contributes to the unpleasant odour of fermented soya milk, by the production of *n*-pentanal, whereas *L. bulgaricus* reduces the beany odour by partial removal of *n*-hexanal. Thus the combination of *L. bulgaricus* and *L. fermenti* should produce a more acceptable product. Futhermore the latter combination results in a lower pH level of 4.0 than *St. thermophilus* and *L. fermenti* (pH 4.6) and so would possess improved keeping properties.

The level of stachyose could be further reduced by altering the supplementation conditions, in particular by lowering the amount of added glucose. The inoculum used in this study was prepared in soya milk, treated with yeast extract and glucose, whereas that of Mital & Steinkraus was prepared in soya milk alone and this may influence the efficiency of oligosaccharide utilization.

Conclusion

The degree of oligosaccharide removal in the present study was relatively small. The incorporation of a microorganism, such as *L. fermenti* solely to remove raffinose and stachyose would not be justified from these results. However, by altering the levels of supplementation and also the method of starter preparation a greater reduction in the oligosaccharides should be realized.

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Technical note: Vitamin C content and distribution in mangoes during ripening

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Introduction

The mango (*Magnifera indica* L.) is the most popular fruit cultivated in India with an annual production exceeding 7 million tons (Bhatnagar & Subramanyam, 1971). The fruit is considered to be a rich source of vitamin C and provitamin A (Hulme, 1971). Spencer, Morris & Kennard (1956) reported that vitamin C concentration was higher in the peel than in the edible portion. Siddappa & Bhatia (1954) reported that in green but well developed fruit of the cultivar 'Raspuri', the peel contained nearly 1.5 times as much vitamin C as the pulp. Several popular products like pickles and chutneys are prepared from immature and mature unripe as well as ripe fruits. In spite of their nutritional importance, information on the content and distribution of vitamin C and changes in its levels on ripening in important mango cultivars grown in India is scant.

The present study was undertaken to determine the amount and distribution of vitamin C in the peel and pulp tissues of some important mango cultivars at commercial harvest maturity and how the storage temperature affect the vitamin on ripening.

Experimental

Four commercially important cultivars, namely, Alphonso, Dasheri, Langra and Pairi were used for these studies. The cultivars Alphonso, Langra and Pairi were harvested from the Institute's garden. Some batches of Alphonso (grown in Ratnagiri, West Coast of Maharashtra State), Langra and Dasheri (grown in Lucknow and Saharanpur, Uttar Pradesh) were obtained through a local dealer. These fruits were 2 to 4 days old from harvest but in preclimacteric unripe state at the time of their receipt in the laboratory.

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Table 1. Distribution and content of vitamin C in mature, unripe mango fruits

Variety	Source	Vitamin C (mg/100 g fresh tissue)	
		Peel	Pulp
Alphonso	Trombay	219±4.9	102.7±0.4
	Ratnagiri	199±0.9	88.5±1.7
Dasheri	Lucknow	130.7±4.8	30.4±0.9
Langra	Trombay	546.6±4.8	114.0±1.2
	Lucknow	590.7±2.3	125.6±3.4
	Saharanpur	541.8±2.9	143.2±2.9
Pairi	Trombay	250±1.0	40.7±1.3

Fruits were ripened either at ambient temperature (29 to 33°C; r.h. 65–85%) or at 20°C; r.h. 80–85%. Some fruits were treated with ethrel (Cl $CH_2CH_2PO_3H_2$; 10 min dip in a 1000 ppm solution) for acceleration of ripening.

For vitamin C estimation, 10 g of peel or pulp from a single fruit was homogenized with 100 ml of 3% metaphosphoric acid-acetic acid reagent and the vitamin content in the supernatant of centrifuged extract was estimated by visual titration against 2,6-dichlorophenol indophenol dye (Association of Vitamin Chemists, 1966). Values reported are mean and standard deviation of three to four such independent estimations.

Results and discussion

Table 1 shows the vitamin C content in the peel and pulp tissues of mature, unripe fruits. Depending on the cultivars the peel contained two to six times as much vitamin as the pulp, the maximum being 590 mg in 100 g fresh peel in the cultivar 'Langra'. Among the four cultivars, 'Langra', showed higher vitamin C levels in peel and pulp tissues. These high levels were consistently observed irrespective of the source of the fruit. The cultivar 'Dasheri' had the lowest levels, 130 and 30 mg/100 g in peel and pulp respectively.

In mature, unripe fruits, the peel constituted 16 – 20% of the total amount and in the cultivar 'Langra' the peel contributed 50 – 55% of the total vitamin C present in a single fruit.

The influence of ripening temperature on vitamin C levels in peel and pulp tissues of the different cultivars at eating ripe state is shown in Table 2. As in the unripe fruits, the peel of ripe fruits showed higher levels of vitamin than the pulp. In all the cultivars, maximum vitamin C retention was observed in fruits

Table 2. Effect of storage temperature on vitamin C levels in ripe mango fruits

Cultivar	Storage temperature (°C)	Days in storage	Total soluble solids	Vitamin C (mg/100 g fresh tissue)			
				Peel	retention (%)	Pulp	Retention (%)
Alphonso	29–32	10	17.0	199.2±8.0	90.9	52.5	51.0
		13	15.0	112.0±3.5	51.1	38.4±0.9	37.4
		15	13.0	94.4±6.2	43.1	32.4±1.2	31.5
	20	10	16.0	216.2±2.4	98.7	89.9±1.4	86.5
		13	17.0	208.4±0.9	95.1	117.3±0.9	114.2
		15	15.0	209.7±0.9	95.7	88.3±0.9	85.9
Langra	29–32	6*	19.5	422.1±2.5	77.2	78.5±1.5	68.8
		6	20.0	519.0±4.9	94.9	75.4±2.1	66.2
		9	21.0	238.7±1.9	43.6	80.9±1.8	70.9
	20	15*	17.0	431.0±0.9	78.9	98.3±3.1	86.2
		15	17.0	476.8±0.9	87.2	96.9±2.8	85.0
Pairi	29–32	9	18.0	119.3±0.9	47.7	18.9±1.2	46.4
	20	15	19.5	118.0±1.8	47.2	12.1±0.8	29.7
Dasheri	29–32	5	20.5	85.8±2.6	65.6	29.1±1.9	95.7

*Ethrel treated.

ripened at 20°C while those ripened at ambient temperature (29–33°C), as practised commercially, showed higher losses. It is interesting to note that fruits ripened at ambient temperature showed a continued decrease in the vitamin level with advancing storage period while those ripened at 20°C showed minimal losses. This is in conformity with the report of Thomas (1975) that in Alphonso mangoes vitamin C retention on ripening was maximal in fruits ripened at 20°C while prolonged storage at low temperatures caused a net synthesis. Ethrel-treated fruits on ripening showed a decrease in the vitamin levels in peel while no differences were discernible in the pulp tissues. In ripe fruits, the cultivar 'Langra' recorded higher vitamin levels both in peel and pulp while the cultivar 'Dasheri' had the lowest levels.

The physiological significance of the high vitamin C levels in the peel tissues of the mango fruit is not well understood at present. Eaks (1964) reported that the peel of lemons contained about three times as much ascorbic acid in concentration and in total amount as the juice while the flavedo contained as much as the albedo. In apples the concentration of this vitamin is two to three times as great in peel as in pulp (Zilva, Kidd & West, 1935). Similarly, in persimmons the vitamin level in the non-edible peel of the mature fruit is reported to be considerably more than that of pulp (Ito, 1971).

The results from this study have application in the mango processing industry. At present green mango pickles are prepared with or without the peel. Preparation of pickles with the peel tissues would be more desirable practice on account of the very high vitamin C levels in the peel. Secondly, ripening of fruit under controlled temperatures (20°C) would provide more vitamin in the ripe fruit. However, in some cultivars like Alphonso a lower ripening temperature can result in fruits with low aroma, flavour and carotenoid content (Thomas, 1975).

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Book reviews

Nutrition and the World Food Crisis. By M. A. Caliendo. New York: Macmillan, 1979. Pp. viii + 368. ISBN 0 02 318340 3. £5.95.

Critical Food Issues of the Eighties. Ed. by M. Chou and D. P. Harmon. New York: Pergamon Press, 1979. Pp. xv + 404. ISBN 0 08 024611 7 (0 08 024639 7 pbk). $9.95 pbk.

Food Science and Nutrition: Current Issues and Answers. Ed. by F. Clydesdale. Englewood Cliffs, N.J.: Prentice-Hall, Inc., 1979. Pp xii + 226. ISBN 0 13 323162 3. £9.70.

Over the last two decades many publications have appeared which deal with the demographic questions of population growth and control, primary agricultural production and the Green Revolution, availability of non-renewable resources and the control of environmental pollution. Rechcigl (1975) prepared a useful bibliography of publications concerned with the agricultural and food aspects.

Some neo-Malthusians took a distinctly pessimistic view of the prospects for the human race. Such presentations ranged from the declamatory (e.g. Paddock, 1967) to the careful but ambitious mathematical modelling presented by the Club of Rome study (Forrester, 1971; Meadows *et al.*, 1972, 1974). On the other hand the optimistic view could also be found (Russell & Wright, 1961). Subsequently the World 3 computer model of Meadows and colleagues were assessed as unduly pessimistic (Cole *et al.*, 1974). A good review of the range of technological forecasts has been presented by Miles (1978). The controversy still continues, and debate has been presented in many text books (for example Ehrlich, Ehrlich & Holdren 1977). The political implications of the possible need to restrict population growth have been discussed (for example Parsons, 1977) often with the conclusion that only a global political administration is likely to achieve success (Sauvy, 1975).

The production and consumption of food is one of the cornerstones of human survival, and student texts exist which examine the scientific and technological problems of increasing world food production (e.g. Börgstrom, 1973). The student of food science and technology is required to consider the scientific discipline in the context of society and its needs. Aspects of consumer acceptability; safety; imbalances in production and distribution and inequalities of supply which lead to whole communities, or sectors of communities, being undernourished, malnourished or overfed; economics of national and interna-

tional trade; and food supply and distribution as a political weapon, must all come within the purview of the serious student and the thinking practising professional food scientist and food technologist.

Comparatively few books have been published which deal with these matters in such a way that the nature of the impact on the food industry is revealed or even hinted at. The three books listed here appeared in the same year and have in common the attempt to direct at least part of the discussion at the impact of society on the food industry and/or *vice versa*. The titles of the three books indicate rather similar subject matters. The book by Caliendo is in fact substantially different from the other two in that the emphasis, as the title suggests, is on nutrition, but it is being considered here with the other two in the context of possibly providing the food scientist or food technologist with information which helps to underline the important interaction between the food industry and society. Malnutrition and undernutrition are not phenomena confined to developing countries. The final sentence in Caliendo's book gives the clue to the reason for considering it of interest to food scientists and technologists as well as nutritionists and dieticians – 'If a workable solution of the pervasive problems of malnutrition is to be found, it will inevitably require widespread efforts and changes in national policies to integrate food and nutritional objectives'.

The book by Caliendo is by a single author, whereas the other two books are edited multi-author volumes. The work by Clydesdale *et al.* was inspired by a nutrition education conference held in 1974 by Pennsylvania State University and the Nutrition Foundation. The book by Chou, Harmon and twenty-three colleagues results from the Food, Agriculture & Society Research Programme of the Hudson Institute.

The target readership of Caliendo consists of 'teachers and students engaged in any aspect of nutrition education' with 'the nutritionist, the educator, the health professional or the student concerned about the world hunger crisis' using it as a reference work. The aims and objectives of the book are to discuss 'the subjects of humankind, environment and nutrition in an integrated manner' and 'to provide the concerned individual with information about some of the fundamental issues involved in the problem of chronic malnutrition affecting many of the world's population'. It attempts this by a review of 'some of the currently available knowledge and literature relating to subjects of malnutrition, surveillance and nutritional status, food production and distribution, population and education and policy making'. The 12 chapters after an introduction cover the topics of Nutritional deprivation; Nutrition and mental development; Assessment of nutritional status; Food production; Food distribution systems – trade and marketing; Poverty and its relationship to malnutrition – strategies for increasing the purchasing power of the poor; Food and population – fertility and nutrition; Infant feeding patterns – the bottle feeding problem; Social and cultural values as they affect nutrition; Education and nutrition – modification of food habits; Synthesis – nutrition interventions, planning and policy making.

Clydesdale rather similarly aims his book at the 'health professional' intend-

ing the book to be 'a widescale review of the principles and methods utilized by (food) technology' explaining 'how and why foods are preserved, packaged and distributed' so that 'students in the health professions and the general public will come to recognize the reasons for and the advantages of processing food'. This book contains 8 chapters which, after an introduction, cover the topics of Society and technology; Food processing; Nutritional changes upon processing; Food microbiology; Food ingredients and additives; Food safety and toxicology; and Economics in food and nutrition issues. It can be seen that although Caliendo and Clydesdale have similar individuals in mind as their primary readership, the subjects covered are very different indeed.

The intended readers of Chou and Harmon, however, are members of the food industry, the U.S. government and the public. The object of this book is 'to focus on and provide alternative solutions to current and near-term food and agricultural policy issues likely to have a long term effect' and 'to increase public understanding of the economic and sociopolitical aspects of the food and agricultural sector' by presenting 'a balanced view of the important food and agriculture issues likely to be with us (U.S.A.) throughout the 1980's'. There are 24 chapters in this book arranged into four major topic areas which are identified as: The socio-economic climate likely to face the food industry and the country (U.S.A.) over the next decade; The changing nature of food and nutrition policies; The outlook for technology and the associated impact of regulation for food, agricultural productivity and agricultural chemicals; An examination of food and agricultural issues that will have a profound and continuing impact on U.S. agricultural policy. It would require too much space to list individually all the chapter titles but amongst the chapters which can be singled out as of great interest to the food scientist and technologist are 2, The Preoccupation with Food Safety (M. Chou); 7, Changing Food Policies (M. Chou); 8, The U.S. Quandary: Can we Formulate a Rational Nutrition Policy (R.E. Olson); and 10, Changing Attitudes and Lifestyles: Shaping Food Technology in the 1980's (M. Chou). Food economists should also find much to interest them in 4, Food Price Inflation – A Heretical View (D. Peterson); and 22, The Multinational Corporation: A buffer in the Food-Climate System (D.P. Harmon).

Although the reviewer was excited to receive a book entitled *Food Science and Nutrition – Current Issues and Answers*, he was largely disappointed because many of the contributors avoided discussing problems and controversial issues. On page 2 the editor observes that present day technology produces some products which can 'satisfy the dimension of food that demands pleasure; however they may also lead to overconsumption and obesity'. It is then stated that 'it is not the intent of this chapter or this book to *discuss*, defend, or defame these products; the intent of this book is to present the methodology and principles behind today's methods of preservation' (my italics). Surely it is a necessary part of a health professional's teaching and background to be able to place such products into context in society, balancing the hedonic virtues against the hazards of obesity and malnutrition.

The chapter in Clydesdale on Society and Technology is very disappointing – as the book is not very long, it seems a misuse of space to discuss *Homo erectus*, Neanderthal and Cro-magnon man, and the Neolithic age and the dawn of agriculture at the expense of discussion of aspects of present day society. Readers interested in the historical aspects would do better to consult books such as Tannahill's *Food in History* or the Brothwells' *Food in Antiquity*. The stated intention of Chapter 3 on Food processing is to give the reader 'a better perspective for the following chapters'. The chapter is not well oriented to the title of the book or to the chapter's stated objectives. It appears to be unrelated to most of what follows, and the reader is left to guess at the questions or issues to which the described processing techniques have provided the answers. The reviewer found Chapter 4 on Nutritional Changes upon Processing (by James R. Kirk), Chapter 7 on Food Safety and Toxicology (by Melvin A. Benarde) and Chapter 8 on Economics in Food and Nutrition Issues (by Daniel Melnick) interesting and thought-provoking.

Chou & Harmon and Caliendo present a fascinating contrast. Although both books originate in the U.S.A. they offer very different views. Caliendo suggests (p. 3) that 'neither increased food production alone nor improved production in combination with more equitable methods of food distribution and marketing ensures adequate consumption of nutritious food for every individual', and commenting on the title observes that the Chinese word for 'crisis' has a dual connotation of 'danger' *and* 'opportunity'.

Chou & Harmon's book, on the other hand, presents a domestic and apparently complacent U.S. view of world food production (the book would have been better entitled 'Critical U.S.A. Food Issues of the Eighties') and the first chapter entitled 'Coping with Abundance' amply indicates the consensus of the contributors, or at least of the editors or the sponsoring institute. The careful reader may well care to take issue with this view however, if the argument presented in Chapter 1 by Herman Kahn is accepted. In this Chapter, Kahn, whilst labelling 'anyone who talks about future exponential growth rates for population or resource consumption as either a fool or ignorant', presents a graph showing the world 'population growth rate growth in long-term historical perspective'. The graph shows a spike of rapid growth in the world population growth rate over the years 1750 to 2250 AD, peaking at 1974, with a constant slight positive growth rate of *ca* 0.05% over the years 8000 BC to 1750 AD, and 2250 AD to 8000 AD. This '16,000 year context' is stated to mean that 'if momentum is maintained on food output, the world should do very well in terms of supporting a large population'. In fact, if the reader does a calculation on the basis of Kahn's predictions to 8000 AD with a slight positive growth rate of around 0.05% from 2200 AD, the world population at 2000 AD may by around 6.4×10^9, at 2200 AD around 3×10^{10} and at 8000 AD may be around *5 to* 6×10^{11}! Kahn may well believe that a world population over 100 times greater than it is today will do 'very well', but this reviewer has doubts, and hopes rather that economists and politicians will find peaceful ways of establishing societies which can successfully cope with zero or even, in the short-term, negative growth rates.

Caliendo and Chou & Harmon seem to have few misspellings or errors; on page 161 of the latter the phrase 'By preventative malnourishment, parenteral nutrition has enabled cancer patients to . . .' presumably should read 'By preventing malnourishment . . . .'.

Clydesdale abounds in misspellings (for example: 'Magnus Pike'; 'a dome of tarf' presumably should be 'a dome of turf'; 'phosphorous' should be 'phosphorus' and ungrammatical presentation (for example, on p. 13 'taste' and 'flavour' are used synonymously; Chapter 2 abounds with split infinitives; and in Chapter 5 the only three verbs to occur in two consecutive sentences are all tenses of 'represent' and the meaning of the passage is almost lost). More worrying, however, are the erroneous, inaccurate and misleading statements to be found in certain contributions. Only a few of these can be listed in this review for the purpose of example. An inaccurate description of the 'botulinum cook' on p. 40 leaves the reader with the impression that all thermal destruction processes aim to achieve 12 decimal reductions in the population of all microbial contaminants. On p. 56 the reader is left wondering whether cooking meat at 70–80°C results in it being tougher (because of 'protein hardening') or more tender (because collagen is converted to gelatin). The paragraph on page 96 describing psychrophiles and psychrotrophs is completely muddled, and an implication is made that a bacterium isolated from a frozen food must be either psychrophilic or psychrotrophic! The statement on p. 100 that 'the optimum pH for the growth of most micro-organisms is approximately 7.0, with a general range of 6.6 to 7.5 (see Table 5.2)' is not qualified by an explanation of what the word 'most' refers to and the Table shows minimum and maximum pH values for growth of selected organisms (including *Lactobacillus* and *Streptococcus lactis*) with no indication of their optimum values! A low pH environment will tend to favour both aciduric and acidophilic organisms.

References and bibliographies are presented at the end of each chapter in all three cases. In the case of Caliendo and of Chou & Harmon references are presented in the order in which they occur in the text, in which they are identified by an integer, and the absence of an author index makes it difficult to use the books as sources of references. Clydesdale has permitted each contributor to present references, bibliography, notes and comments in whatever form the contributor wishes making this book even more difficult to use.

Typographically Caliendo and Clydesdale are more pleasing to the eye than is Chou & Harmon, and the frequent photographs, graphs and tables in Caliendo are well laid out and well produced.

To sum up, although Clydesdale contains some good contriubtions, it should not be used for student teaching without adequate academic support to make students aware of the erroneous or misleading statements. Chou & Harmon, and Caliendo, provide useful and contrasting additions to college and university libraries to support courses in food science, food technology, food economics, and nutrition, and also offer interesting and thought-provoking reading for the practising food scientist and technologist.

W. F. Harrigan

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Food Microscopy. Ed. by J. G. Vaughan.
London: Academic Press. 1979. xvi + 651 pp. ISBN 0 12 715350 0 £29.60.

As the first new book in the English language specifically concerned with the microscopy of food for over 40 years, this work is a most timely addition to the series of monographs on Food Science and Technology, published by Academic Press. Its chapters are generally organized on a commodity basis; one is contributed from West Germany, the remaining fifteen are by authors from the laboratories of well-known food firms, research institutes and university departments in the United Kingdom.

The individual chapter titles are: fruits and vegetables; oilseeds; cereals and bakery products; cereal starches and proteins; meat; meat products; milk and its products; the domestic hen's egg; fish; animal feeds – plant constituents; animal feeds – animal constituents; oils and fats; ice cream; novel protein foods; sugar; and, computer-aided identification of food materials. All chapters except the last – which contains a computer program – are well illustrated with photomicrographs, reproduced with excellent clarity, and the occasional diagram (one minor irritation here is that, where a whole page is taken up by illustrations, the page number is omitted – so that in some chapters location of a particular page referred to in the index requires a certain amount of page counting).

In the late nineteenth and early twentieth centuries, the light microscope played a fundamental role in studies of the composition and structure of food and, even more particularly, in identification of food materials and the detection of adulteration. In more recent decades, developments in electron micros-

copy and in other sophisticated instrumentation, coupled with a change in emphasis from identification and detection of adulteration (except in the case of animal feedstuffs and adventitious contaminants) to a need to evaluate raw materials as to their suitability for processing and to rectify processing faults or deterioration problems, have led many food scientists and technologists to regard microscopy as a specialist technique.

A very great merit of this volume is that it shows how light and electron microscopy together – or even the former alone – can be used to investigate problems of food processing, food acceptability (for example, relationships of structure and texture) and deterioration, while at the same time it contributes information on contemporary – and formerly less usual – problems of identification. The authors of several chapters draw attention to a comparative paucity of published information on microscopical changes associated with processing, and it is much to be hoped that this volume will stimulate a revival of interest in microscopical studies of processed foods beyond the more usual areas of fats, baked goods and ice cream.

The references at the end of each chapter give an excellent indication of the extent and scattered nature of the primary literature relevant to food microscopy, and, at the same time, increase the value of each individual chapter.

This is a book by experts for the practising microscopist; the novice may easily find it overwhelming. His starting points should be the editor's own Chapter 10 on animal feeds – plant constituents, and the final Chapter 16 on computer-aided identification. In both these he will find some guidance on choice of simpler techniques and a guide to earlier (mainly out of print) basic handbooks. Thereafter any chapter will disclose a multitude of ways to usefully apply techniques once acquired, and there is every possibility that the enthusiasm of the authors for the application of microscopy will convince an increasing number of young food scientists that the microscope is not merely for specialists but a tool they must learn to apply themselves.

Individuals may be discouraged by the price, but this is such an important and well-produced book that those who purchase now are likely to congratulate themselves in a few years' time for having obtained a bargain.

E. C. Apling

Food Texture and Rheology, Ed. by P. Sherman.
London: Academic Press. 1979. x + 456 pp. ISBN 0 12 639960 3 £20.00.

This volume contains the papers presented at a symposium held under the auspices of the International Union of Food Science and Technology at Queen Elizabeth College (University of London) on 19–22 December 1977. The authors of the twenty-six papers were from twelve different countries, with multiple contributions from U.K., U.S.A., Canada, Japan, Sweden and Switzer-

land and single papers from Finland, France, East and West Germany and the Netherlands, covering a wide range of rheological topics and food commodities.

The symposium, like the subject, was essentially multi-disciplinary, bringing together a spectrum of interests, from those concerned with the relation of texture to molecular structure, through those concerned to develop instrumental methods of assessing the processing properties of particular food commodities, to those whose studies relate to assessing and describing and measuring individual perception of texture in foods.

From the commodity aspect the largest group of papers is concerned with the rheology and microstructure of flour, dough and bread (six papers); others are concerned with dairy products, chocolate, bacon, mashed potato, food gels, and the properties of vegetable protein solutions or dopes.

On the instrumental side, one paper reviews recent advances in texture instrumentation generally, another the theory and application of puncture testing. Two papers advance understanding of the principles of operation of the Instron Universal Testing Machine and the Brabender Amylograph and Viscograph, and another outlines an engineering approach to the subject of food texture.

On the 'personal' aspect, there are papers concerned with the mouth-feel of beverages, crispness and crunchiness of foods, correlations between sensory and instrumental assessments of spreadability of butter and margarine, correlations between sensory and rheological properties of liquids and gels, and a discussion of the use of models in psychorheology.

Some papers are critical reviews, others report particular new studies; most combine elements of both, with an approach varying from the 'literary' to the mathematical, so reflecting the wide variety of outlook and specialization which requires to be brought to bear on this important but 'difficult' area of food research.

Despite the apparent disparity of material involved, this collection of papers produces a volume which is a greater contribution to the development of the subject than the mere sum of its parts. The volume is printed in unjustified typescript, but the reproduction of figures and illustrations is perfectly adequate. It must be recommended to all involved, however peripherally, in the subject of the title; it will, I feel sure, be referred to time and time again in the years ahead.

E. C. Apling

Recommendations for chilled storage of perishable produce.
Paris: International Institute of Refrigeration, 1979. Pp 148. FFr 35.

This brochure is a follow-on to the first two editions of *Recommended Conditions for Cold Storage of Perishable Produce* (1959, 1967). Many experts have

contributed to the detailed information on the optimum chilled storage conditions for a wide variety of foods. In the preface the editor claims that the technical advances and accumulated knowledge gained over the last 10 years make this a truly new brochure.

The brochure is divided into eight chapters. The first gives a general introduction and mentions the practical considerations to be taken into account when designing and operating chilled storage facilities. Subject areas include storage life and quality, cooling down, storage temperature, relative humidity, air circulation, ventilation, packaging and stacking, volatile substances, hygiene, and condensation problems. A chart is provided to predict whether condensation will occur when transferring food from chilled storage to a warmer environment. The second chapter deals with fruits and vegetables. Practical aspects of preparation and controlled atmosphere storage are briefly mentioned. However, most of the space is devoted to detailed storage conditions for fruits, (temperate, citrus, and tropical), nuts and vegetables. Particular attention is paid to apples and pears. The table headings are: 'storage temperatures', 'relative humidities,' 'practical or expected shelf life,' 'origin of information and additional comments.' One slight criticism is that it would be fairly difficult to follow up any of the information because no references, apart from country of origin, are given. The chapter ends with a very useful table giving highest freezing points and heat evolutions (w/mg) for a range of commodities. I feel it would have been useful to have incorporated specific heat data in the same table so that total refrigeration capacity could have been calculated. Chapters 3 and 4 cover meat, poultry, eggs and fish following a similar pattern to Chapter 2, a descriptive section on the practical and biochemical aspects followed by tabulated information on recommended conditions.

Chapter five, on dairy products, deals briefly with hygienic handling on the farm, pasteurization, cream production, fermented milk and cheese. Information on cheese salting, controlled drying, ripening and storage is given. Practical information on storage of cut flowers, seeds and selected miscellaneous items bring the book to a close.

No index or references are provided and the brochure is only half as long as it may appear because of the side by side use of French and English. However, it provides a very useful introduction and data source to anybody concerned with chilled storage.

M. J. Lewis

Food Legumes. Ed. by D. E. Kay. TPI Crop and product digest No. 3. London: Tropical Products Institute, 1979. Pp. xvi + 435. ISBN 0 85954 085 5. £6.50 plus postage. (Free to public bodies in countries eligible for British Aid.)

This digest is the third in the series which so far has included No. 1 *Oils and oilseeds* and No. 2. *Root crops.*

The aim of these digests is 'to provide a ready reference tool, for use particularly by non-specialists, and especially by practical workers in the developing countries concerned with advancing the rural economy'. It is also hoped that they may prove a 'useful starting point . . . for specialists and researchers, working within individual countries, or on individual crops, or their products'.

Entries for twenty-seven crops are arranged alphabetically on the basis of the most widely used English name, with two indices providing access on the basis of the Latin binomial and of a very wide range of trivial names, not only English but in many other languages and including local names. Thus, for example, no fewer than 133 names, all indexed, are given for the chick pea (*Cicer arietinum*).

Each crop has a large amount of information given under standard subheadings, namely:
Common names; botanical name and family; other names; botany; origin and distribution; cultivation conditions; planting procedure; pests and diseases; growth period; harvesting and handling; primary product; yield; main use; subsidiary uses; secondary and waste products; special features; processing; production and trade; major influences; and bibliography.

In the sections on 'Botany' some information is sometimes included on some of the common cultivars and botanical varieties, but not invariably. For example, the food scientist and food technologist reading the entry for the pea (*Pisum sativum*) hoping to see listed the commonest cultivars used for freezing, canning or drying will be disappointed. The entry states 'In view of the very large number of cultivars and strains of peas grown it is not proposed to list or describe any of them in this digest'. Whereas, he or she will find in the entry for the haricot bean (*Phaseolus vulgaris*) a page on botanical varieties and cultivars under 'Botany' and a further page under 'Primary product'.

The sections on 'Pests and diseases' include information on storage pests as well as pests and diseases of the standing crop. The sections on 'Harvesting and handling' also gives information on appropriate treatments to control insect infestation and microbial spoilage of the stored material.

The sections on 'Main use' and 'processing' obviously contain much to interest the food technologist and food scientist, as do the sections on 'Subsidiary uses' and 'Secondary and waste products'. These often contain fascinating items such as the use of lupin seeds as a coffee substitute or mung bean flour as a soap substitute. The sections headed rather cryptically 'Special features' include data on the proximate analyses of the material and on toxic constituents which may be present.

In common with statistical data on most crops, the sections on 'Production and trade' have to concentrate on international trade, as much of the information on local consumption of the crops is unavailable.

The bibliographies are extremely helpful with an emphasis on literature published between 1965 and 1976.

It should be mentioned that the groundnut (*Arachis hypogea*) and the soyabean (*Glycine max*) are not included, having been dealt with in the digest on

Oils and oilseeds, and the yam beans (*Sphenostylis stenocarpa* and *Pachyrrhizus erosus*) are to be found in the volume on *Root crops*.

To sum up, the primary readership of this book as outlined in the preface will be involved in the agricultural and primary production sector of developing rural economies. Consequently treatment of those aspects of leguminous crops which relate to their place in the food processing industries of developed temperate climate countries is necessarily brief. Nevertheless, the food scientist and food technologist will find much of interest in this useful and attractively presented book.

W. F. Harrigan

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

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3} m	hour	hr
micrometre	$\mu = 10^{-6}$ 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^2
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'.

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