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Intermediate moisture tropical fruit products for developing countries*. I. Technological data on papaya

A. LEVI, S. GAGEL and B. JŪVEN

Summary

The effects of short heat (boiling temperature) treatments, consisting of 'blanching' in concentrated sucrose (70%) solution (I), or plain water (II); or osmotic dehydration in cold (room temperature) (III) or hot (boiling) sucrose (70%) solution (IV); and a combination of (III) following (I) or (II), on the drying behaviour of papaya, dehydrated to the intermediate moisture (IM) range, were studied. Significant increases were observed in the dry matter content of papaya treated as above (I, III, IV or III following I or II), with the obvious increase in the expected production yields and probable reduction of the heat energy needed for the drying process.

The drying behaviour of papaya as a raw material for IM products, during and after the above treatments, as well as following hot-air, (cabinet) or direct-sun drying, was studied. The drying time needed for cabinet or solar drying following osmotic treatments of papaya was considerably shortened, and therefore a significant saving in the heat energy needed for drying is to be expected. The optimal treatments to achieve a considerable shortening in the drying time, without affecting negatively the quality of the IM papaya, seemed to be syrup 'dipping', or a combination of water or syrup blanching and cold osmotic dehydration.

The above methods can be applied in both small, non-sophisticated fruit dehydration plants (mainly solar drying), or in larger and more sophisticated ones (mainly forced hot-air drying).

Introduction

Local and overseas markets throughout the world are filled with a great variety of temperate-zone fruit products, preserved by various methods. However, even if a great surplus of fresh subtropical and tropical fruits exists in most

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developing countries, only a limited variety and quantity of products from such fruits (TFP) is produced and marketed. This situation is typical mainly in countries with an undernourished, fast growing, and underemployed population. There is a growing quantity of unutilized, good quality, generally liked and acceptable fresh tropical fruits available during the harvest seasons, whereas there are practically no TFP during the rest of the year, even on the local markets. One of the main reasons for the very low level of production and commercialization of TFP, and in particular of the more sophisticated ones, is the lack of specific know-how of production methods and adequate equipment for their processing and preservation. Another, relatively recent, obstacle, slowing down the industrial development of tropical (developing) countries, is the high cost of the conventional energy sources needed for modern industry. On the other hand, solar energy is an inexpensive, available, and much cheaper source of energy in subtropical and tropical regions. Manual labour, which has become so expensive in the developed countries, is cheap and abundant in most developing countries.

In other words, non-economic, manpower-demanding technologies for developed countries can be not only economically feasible, but even more desirable for the large unemployed population of the developing countries.

Papaya, mango and banana are abundant and representative TF crops in most subtropical and tropical developing countries. Therefore, development of adequate methods for production of TFP from these fruits would serve also for the development of other TFP in the future.

Dehydration of fruits—a classic food preservation method—might become even more prevalent, as the transportation costs of fresh fruits continue to rise (Wagner *et al.*, 1978). However, a relatively large amount of energy is needed to remove moisture from foods and the energy need increases in proportion to the amount of water removed from fruits due to the so-called 'case-hardening' effect of the sugars.

Intermediate moisture (IM) food products in general, and IM fruit products (IMFP) in particular, are developed with the intention to preserve their quality characteristics, such as texture and flavour as close as possible to those of the fresh fruits. Significantly less energy is needed to dry fruits to the IM level, one of the main reasons being the reduced possibility of case hardening. However, preservatives must be added in order to ensure the IMFP's storage stability (Potter, 1970; Brockman, 1973; Levi, Ramirez-Martinez & Padua, 1980); IMFP also possess better rehydration ability than do completely dehydrated fruits, and they can also be consumed directly.

The relatively recent increase in development and commercialization of IMFP is based on the fact that under certain water-activity (a_w) values, and with the addition of some food preservatives such as benzoates, sorbates and sulphur dioxide, the possibility of microbial growth, multiplication, contamination and recontamination is prevented (Brockman, 1973; Levi *et al.*, 1980). Benzoates and sorbates are bacteriostatic, while SO_2—in addition to its microbicidal properties—also prevents enzymatic (Embs & Markakis, 1965; Park *et al.*,

1980) as well as non-enzymatic (McWeeney *et al.*, 1974) darkening, which are responsible for quality deterioration of dried fruits, especially bananas (Weaver & Charley, 1974). Harel *et al.* (1978) showed that the quality of IM apricots, containing a relatively low level of chemical preservatives, did not deteriorate during 80 weeks of storage at 25°C.

'Blanching', a widely practiced, relatively short-time heat treatment for inactivation of undesirable enzymes and quality stabilization of fruit and vegetable products, is not always advisable for tropical fruits because of its negative effects on their delicate flavour and texture (Czyrinciw, 1969). Shorter or longer heat treatments sometimes have a detrimental effect on the product's quality, e.g. purple or pink discoloration of canned banana (Ranganna and Parpia, 1974), astringent off-flavour in IM banana (Ramirez-Martinez *et al.*, 1977), ascorbic acid losses in papaya (Chan *et al.*, 1975).

Conventional (forced hot-air) drying as well as osmotic dehydration were studied as methods for preparation of fully dried and IM fruit products (Ponting *et al.*, 1960; Brekke & Ponting, 1970; Garcia, Menchu & Rolz, 1974; Luh & Palmer, 1975; Moy, Lon & Dollar, 1978). Moy *et al.* (1978) found that the amount of water removed during osmotic dehydration of some tropical fruits increased with increasing sucrose concentration in the dipping solution, and increased further after acidification of a relatively concentrated (60%) sucrose syrup. These workers presumed that the combination of acid and sucrose increased the moisture removal and the sugar penetration from (and to) papaya, by inhibiting gelation of pectins. Levi *et al.* (1980) found that short heat—and SO_2—treatments caused an increase in the rate of water removal (drying velocity) during cabinet drying of banana.

Direct or indirect solar drying have lately been studied extensively, as a result of the high cost of conventional energy sources. Use of solar energy for water heating (Robe, 1980), or drying of agricultural produce (Rossi & Roa, 1980), seems to be already economically feasible, but it has different dynamics and problems from conventional drying methods. Fruit drying by solar energy has also been investigated extensively (Bolin, Huxcoll & Salunkhe, 1980). Altemani (1976) studied sun-drying of banana by direct drying and by indirect air heating (solar energy collector). The drying process took at least 2 days for banana halves and 4–5 days for whole peeled banana. Pablo (1979) found that efficiency of solar drying of papaya, mango and banana was higher and the product quality superior, when drying under transparent polyethylene tents, than with direct sun drying. Wagner *et al.* (1978) reported that after dipping in sodium bisulphite solutions, hot-air-dried samples of tropical fruits retained more SO_2 than solar-dried fruit.

In order to shorten the drying time and thus save energy, as well as to reduce the possibility of deterioration during the drying process of papaya, mango and banana for IMFP, we studied the effects of combined blanching and/or osmotic dehydration on process yields, drying velocity and quality characteristics, during direct sun—or cabinet—drying. A study of the processing yields and the dynamics of the drying process of papaya are reported below.

Materials and methods

Batches of 5–50 kg of fresh, sound, mature papaya fruits, obtained from commercial plantations, were used in these experiments. Fruits of different varieties (e.g. 'Secy', 'Solo') were used, due to the short harvest seasons, but fruits of the same variety were used for all the experimental conditions of the same experiment, at an equal degree of ripeness, in order to ensure maximum uniformity of the raw material. Only for the study of the effect of ripeness, was the papaya selected according to the total area of yellow-orange peel coloration percentage, as described in Results and discussion. The ripening conditions (when needed) were: storage for several days at constant temperature (21°±1°C) and humidity (90±5% r.h.) until the desired peel colour was achieved, or the maximum soluble solids content (°Bx) was reached (see Results and discussion).

Peeling

Papaya fruits were weighed and peeled manually, or in boiling, 10% lye-solution for 6–7 min, except in the experiment in which the lye-peeling conditions were evaluated. In the latter, five to seven whole papaya fruits for each experimental condition were subjected to lye-peeling in a boiling solution of 10 or 20% NaOH for different lengths of time between 2 and 4 min (20% NaOH), or 4 and 7 min (10% NaOH). The lye-treated papayas were washed immediately under a water spray until the adhering NaOH and peel were removed and also by gentle rubbing.

The peeled whole papayas were cut in half and the seeds, as well as the liquid of the central cavity, were completely removed. The edible part was weighed and treated as described below.

Preparation, blanching and osmotic treatment

The peeled papaya halves were cut in slices of uniform thickness (~15 mm) with sharp knives or a bread slicer. When the slices were to be blanched, they were dipped in boiling water or sucrose (70%) solution for 5 min and then cooled immediately by dipping them for about 2–3 min in water or sugar solution, respectively, at room temperature. Unblanched or blanched papaya slices were subjected to direct drying or to osmotic dehydration in hot (boiling) or cold (room temperature) sucrose (70%) solution. The ratio followed was papaya:syrup=1:4. When the 'cold' dipping syrup reached about 50°Bx, sucrose was added to make 70°Bx of the sugar syrup. The dipping syrup was frequently stirred in order to ensure uniform concentration.

Drying and storage

Unblanched or blanched papaya slices, with or without osmotic treatment, were subjected to cabinet (forced hot-air) or direct solar drying. The slices were

placed carefully in a single layer on perforated aluminium trays. The cabinet-drying conditions were 3 h at 70°±5°C+3–6 hr at 50°±5°C, at constant (flow-through) air velocity and at ambient r.h. conditions. The drying continued until the desired moisture content was reached, as judged by the reduction of the original sample weight and dry matter (DM) content.

The osmotically treated slices were subjected to drying at 50°±5°C, in order to avoid as much as possible their quality deterioration, due to the higher sugar content, after the osmotic treatment.

The conditions for solar drying were as follows: the aluminium trays were placed at ~30° inclination toward the sun during the day, and covered with transparent polyethylene sheets (40–50 cm distance between the sheet and trays) during the night, to prevent dew precipitation. The temperatures measured directly on the tray surface, during the daytime, were between 22° and 47°C.

The dried fruit slices were packed loosely in open polyethylene bags and stored in a refrigerator (5°±2°C), or at room temperature, for 24–72 hr, until quality evaluations were made.

Analytical methods

Representative samples of 0.5–1.0 kg of fresh or 250–300 g of osmotically dehydrated or dried, papaya slices, were thoroughly blended in a Waring blender and analysed for total soluble solids (TSS) content, acidity, pH, and dry matter (DM) content as described by Levi *et al.* (1980). The rate of water removal was calculated according to the differences in weight and DM content (Levi *et al.*, 1980).

The dry matter (or TSS) gains or losses after the treatments were calculated by the following equation:

$$\text{DM}^* \text{ gain} = \frac{\text{weight} \times \text{DM}^* \text{ of treated sample} - \text{weight} \times \text{DM}^* \text{ of fresh sample}}{\text{Weight} \times \text{DM}^* \text{ of fresh sample}} \quad (1)$$

The rate of water removal was calculated on the basis of the moisture content of the fresh papaya (100%) and the corresponding percentage after the treatment.

Preliminary sensory evaluation of the general appearance, flavour texture and colour was made by a small trained test panel (of four or five people).

Results and discussion

Effect of ripeness on quality and yield

Approximately 50 kg of sound, mature papaya fruits (*circa* 1 kg±200 g each) of var. 'Secy' were stored for up to 7 days, manually peeled and sliced as

* Or TSS respectively.

Table 1. Effect of degree of ripeness* on yield and quality characteristics of fresh papaya fruits†

Coloration†						
Peel* (%)	Pulp	TSS‡ (°Bx)	Ascorbic acid (mg/100 g)	Peeling losses (%)	Edible§ part (%)	Flavour (fresh)
0–10	Greenish	7.1–9.2	30–45	28–32	40–50	Poor
10–25	Greenish	10.7–11.4		18–20	50–52	Poor
25–50	Yellow	11.2–12.5	72–101	20–22	50–55	Regular
50–75	Yellow-orange	12.0–12.5		18–21	50–55	Regular
80–90	Orange	12.5–13.5	110–123	15–17	60–62	Good
100	(Firm orange)	12.5–14.0	110–150	15–18	54–65	Excellent

* As judged by the yellow-orange area of the peel.
† Range of five similar experiments.
‡ TSS—total soluble solids content.
§ On a whole-fruit basis.

described in Materials and methods. When they reached the desired peel coloration, slices of three or four fruits were analysed (Table 1).

Variable storage time periods (±24 hr), were needed to obtain the desired peel coloration in each experiment. The most suitable stage of maturity, from the aspect of yield, is that with peel coloration >80%, while the best quality characteristics (higher TSS and ascorbic acid contents, best flavour) were of the 100% peel coloration, firm papaya fruits (Table 1). Because it is quite laborious and difficult to judge the exact area of colouration on an industrial scale, use of fruits with peel coloration of >80% would probably give satisfactory yields and quality of the papaya as a raw material for drying.

Texture and quality

Papaya fruits (20 kg, as above) were ripened to 100% yellow-orange peel coloration, divided into firmer and softer fruits, and analysed as above (Table 2).

A slight and non-significant difference in the quality characteristics of firmer *versus* softer fully ripe papaya fruits was observed. The yield (% edible part) of

Table 2. Effect of firmness of fresh, fully ripe (100% orange-yellow coloured) papaya on yield and quality characteristics of the edible portion

Texture*	TSS (°Bx)	DM (%)	Ascorbic acid (mg/100g)	Edible portion (%)	Flavour
Firm	12.4	14.7	123	56.7	Full, typical
Soft	12.5	14.5	119	63.0	Full, typical

* As judged by gentle pressure with the fingers.

the soft fruits was higher than that of the firmer fruits, probably because of the easier hand peeling of the softer fruits. On the other hand, peeling losses were significantly higher during lye peeling and other treatments (such as blanching and osmotic dehydration) of the softer papaya fruits. The total production losses of the papayas were at least 10% higher for the softer fruits. Hence, firm, strongly coloured fruits (>80% peel coloration) are better raw material for drying, and they were used in the following experiments.

Lye peeling

In order to facilitate the peeling and to obtain a smoother surface, five firm, fully ripe papaya fruits (var. Solo) for each experimental condition, were lye-peeled, immediately washed, and sliced as described in Materials and methods. The results are presented in Table 3.

Lye peeling for 6 or 7 min in 10% NaOH was adequate for papaya. The yield (edible portion) after a 6-min dip was higher, but a 7-min dip, significantly facilitated the peel removal. Higher peeling losses were observed after dipping in 20% lye solution. Faster penetration of the more concentrated (and hotter) NaOH was probably the cause of more disintegration of the papaya flesh. No significant differences were observed in the quality characteristics (colour, texture, flavour, TSS, DM, etc.) between the hand- and lye-peeled papaya.

These results are representative of the particular NaOH concentration and respective dipping time, and different results would probably be observed with different varieties due to the natural variations in the texture and structure of different papayas, their degree of ripeness, the peel thickness, etc.

When comparing the yield of lye peeling (Table 3) with that of hand peeling (Tables 1 and 2), it is obvious that lye peeling gives better yields. Only lye peeling was used for the following experiments.

Table 3. Effect of lye peeling* of papaya fruits on the yield and on peeling losses

Dipping time (min)	Peeling losses (%)	Edible portion (%)	Peeling facility
	10% NaOH solution		
4	10.0	60.8	Poor
5	5.5	70.0	Regular
6	3.5	75.2	Good
7	7.1	71.5	Excellent
	20% NaOH solution		
2	12.0		Very poor
3	8.8	69.0	Regular
4	10.0	66.0	Good–excellent

* Dipping each fruit separately in about 20 litres of boiling lye solution.

Osmotic dehydration

Lye-peeled papaya (var. Solo) slices (3–5 kg) were dipped in 70% boiling sucrose solution for time periods of 15–120 min. The influence of the hot osmotic treatment on the TSS increase, and on the weight loss of the papaya, representing the trends of three similar experiments is shown in Figure 1.

From the point of view of water and sugar movement, mainly two phenomena occur during the hot osmotic treatment. At the very beginning, there is an osmotic transfer of water from the fruit slices to the concentrated sugar syrup caused by the difference in their osmotic pressure and facilitated by the turbulence of the boiling syrup. After a relatively short time the selective permeability of the plant membranes and the cell wall is destroyed by the heat-treatment and sugar penetrates by diffusion, causing also an impregnation

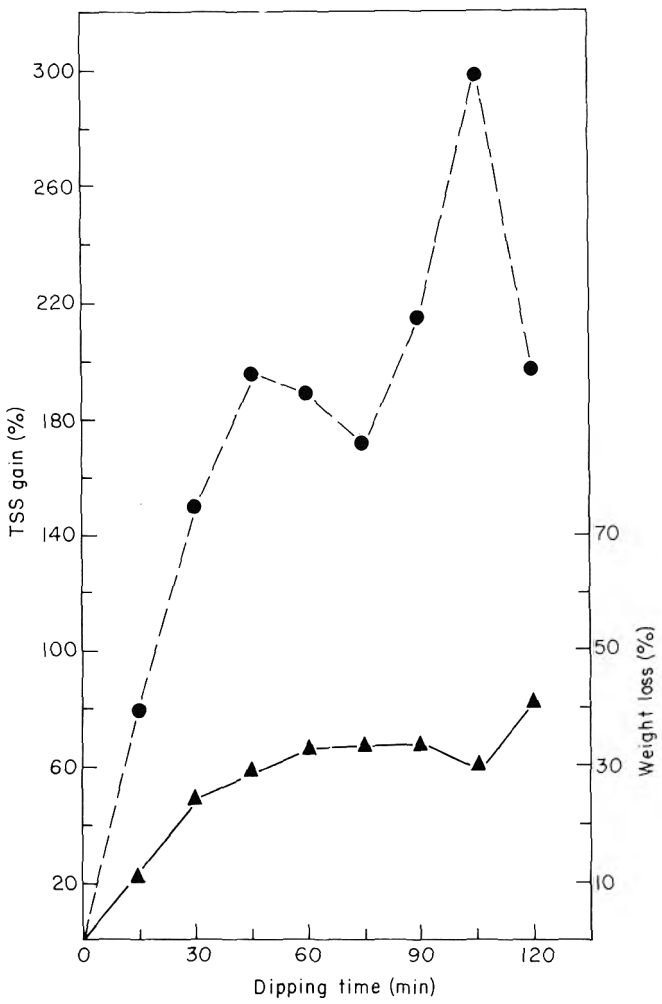

Figure 1. Effect of boiling sucrose (70%) treatment on total soluble solids (TSS) gain and weight losses (WL) of papaya slices. – – –, TSS gain; ——, WL loss.

of the fruit tissue. The sugar penetration and impregnation are seemingly faster than the water removal, and therefore parallel increases in TSS content and in DM are observed. Some disintegration of the fruit tissue was also observed and it also is one of the causes for weight loss. The total effect was in favour of the sugar penetration and impregnation of the fruit tissue, therefore significant increases in total TSS and DM content were observed with increasing time of the osmotic treatment (Fig. 1).

There was slight caramelization of the sugar syrup, and slight browning of the papaya slices, with the 45 min treatment, significant browning at 75–90 min, and dark brown syrup and slices in treatments longer than 90 min. There was significant loss of the typical flavour of the papaya slices with treatments longer than 30 min, and the papaya flavour disappeared completely after 60 min of treatment.

Even if the longer hot osmotic treatment seems inadequate to produce a high-quality IM papaya product, its effects are described herein as possible help for the development of a different type of product—sugar-impregnated papaya—since a variety of sugar-impregnated fruit products are produced commercially by the confectionery industry.

The hot osmotic dehydration of papaya removes the moisture more rapidly than does forced hot-air drying. Therefore, a parallel saving in the energy needed for drying is expected. In order to have an estimate of the quantity of water to be removed after the hot osmotic treatment, the calculated volumes of water to be evaporated, in order to obtain 1 kg of IM papaya with 70% DM content are given in Figure 2. A proper reduction of the actual weight loss

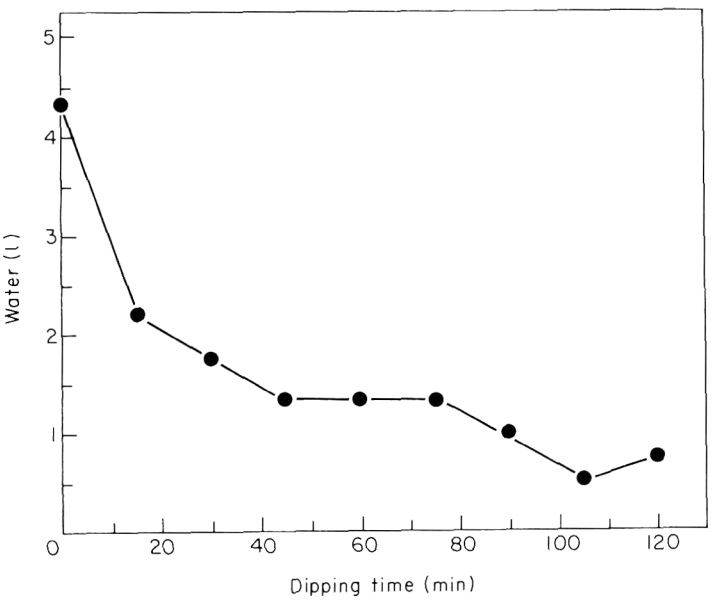

Figure 2. Volume of water to be evaporated in order to obtain 1 kg of IM (70% DM) papaya slices, after hot osmotic treatment.

during the osmotic treatment (Fig. 1) was made, thus making the figures as close as possible to those expected during industrial dehydration.

This evaluation is only an indication, and different practical results are to be expected, because of the natural variability of the papaya fruits (variety, maturity, ripeness, etc.). In addition, the correlation between the volume of water to be evaporated and the energy needed for it is not linear, due to the case-hardening effect, which slows down water evaporation.

The results given in Figures 1 and 2 are averages of three experiments, although some significant differences in individual values were observed in the DM and TSS contents of the fresh (and in the TSS of the osmotically treated) samples. Small differences in weight losses were also observed. All this was because of natural differences in the quality characteristics of the fresh papaya, as raw material, for dehydration. The results obtained can give only an indication of the WL and DM (TSS) gains for osmotic treatments at lower-than-boiling temperature.

Blanching

Blanching, a heat treatment for inactivation of undesirable enzymes and for product stabilization, would probably also affect the dynamics of water and sugar movement from and to the fruit tissue, during the blanching itself, as well as during subsequent dehydration (or drying) processes. If the blanching is performed in a concentrated sugar solution, some water removal can occur also. Therefore, the effect of the blanching in boiling plain water or concentrated sucrose (70%) solution, was also studied.

The effects of blanching papaya slices, on factors affecting the yield (DM, TSS, WL) and the quality (ascorbic acid) of the resulting product are presented in Table 4. For each experiment, batches of ~10 kg of papaya slices, prepared from firm ripe papaya, were blanched. The variability of the results is probably due to the natural differences even at the same TSS content. Even so, when

Table 4 Effect of TSS content of fresh papaya slices subjected to blanching (5 min) in boiling water or sucrose (70%) solution, on the yield and ascorbic-acid content of papaya for IM products (firm ripe papaya)

TSS content of fresh fruit (°Bx)	Blanching gains* (+) or losses (−)							
	TSS (%)		Dry matter (%)		Weight (%)		Ascorbic acid (%)	
	Water	Syrup	Water	Syrup	Water	Syrup	Water	Syrup
9.7	−18.5		−11.2		−11.2		−32.0	
10.2	−21.6	+88.0	−11.4	+87.0	−9.8	−4.3	−22.0	−5.0
10.6		+107.0		+97.0		−4.0		−10.0
11.2	−16.0	+96.0	−14.4	+110.0	−9.4	−3.2	−21.5	−5.0
12.0	−13.3		−18.4		−9.9		−20.0	

* Taking into account the weight losses by calculating the total gain or loss as a percentage of the total content (see Materials and methods).

observing the results of the water and the syrup blanching, the advantages of the latter are obvious. There were differences of about 100% in the total DM and TSS in favour of the syrup blanching. The weight losses (affecting also the total DM and TSS) of the syrup-blanched papaya slices were ~3–4%, as compared with the 9.5–11% of the water-blanched slices. Only 5–10% of the original ascorbic-acid content was lost during the syrup blanching, as compared with 20–32% during the water blanching.

In calculating the quantity of moisture to be removed after the blanching, there is a decrease of ~50% of the initial water content after syrup blanching, while there is an increase of ~10–20% after water blanching.

Cold osmotic dehydration

Heat energy (steam or other means) is needed to heat not only the air—for forced-air drying—but also the sugar syrup for the hot osmotic treatment. Special relatively sophisticated equipment is needed for steam generation, etc., and this could be a serious disadvantage for small plants in developing countries, where many regions lack electricity. On the other hand, sugar and manpower may be abundant and cheap; therefore, a combination of cold syrup osmotic treatment and direct sun-drying would be a simple and economically feasible means for fruit dehydration. Also, better quality characteristics of the final product would be expected with such treatments, since they avoid heat damage to the fruit tissue. The effects of cold (room temperature) osmotic dehydration, as studied and recommended for other fruits (Brekke & Ponting, 1970; Garcia *et al.*, 1974; Moy *et al.*, 1978), were studied for papaya designated for IM products.

Table 5. Effect of osmotic treatment in sucrose (70%) syrup at room temperature on factors affecting the dehydration yield of papaya for IM product (ripe firm fruit slices)

	TSS content (°Bx)		Weight losses (%)		TSS gain† (%)		
Dipping time (hr)	I*	II	I	II	I	II	Remarks
0	11.4	13.0	0	0	0	0	Fresh
1		19.6		9.0		37.0	
3	26.4	27.2	14.0	26.3	68.0	54.0	Sugar added in treatment I. up to 70 °Bx
22	50.0	40.0	34.0	36.5	189.0	59.0	Sugar added (as above) in treatment II
24		48.2		22.2		188.0	
44	60.0	58.0	27.7	25.3	228.0	233.0	

* I and II indicate experiments utilizing firm, fresh papaya fruits of different initial total soluble solids content.

† Calculated after substracting the weight loss.

Five to 10 kg of fresh, firm, ripe papaya slices, divided into batches of ~1 kg, were simultaneously dipped in sucrose (70%) syrup at room temperature. After predetermined periods of time, a 1 kg batch was removed from the cold syrup, the adhering syrup left to drain, and then weighed, blended and analysed. Results of two similar experiments with fresh papaya of different TSS content, are presented in Table 5.

The weight (mainly water) loss during the cold osmotic treatment is probably caused by the difference in osmotic pressure between the concentrated sucrose syrup and the papaya slices. Insignificant disintegration (very few floating fruit pieces) of the papaya tissue was observed. The sugar syrup was frequently stirred, in order to achieve uniformity of concentration of the sugar syrup. Because of the water movement from the fruit slices to the surrounding syrup, the syrup sugar content decreased fast, and in Exp. I it reached about 45°Bx after 3 hr; therefore, sugar was added to bring its soluble solids' content back to 70°Bx. The same adjustment was made in Exp. II, only after 22 hr. As a consequence, the osmotic dehydration in Exp. I was faster and after 22 hr the observed TSS content and gain were higher in Exp. I than in Exp. II. After the sugar concentration readjustment in Exp. II, slight differences were observed between the TSS content and gain of both, probably because of the difference in weight loss. The faster rate of increase in DM (gain), with a parallel decrease in weight loss (a relative increase in weight) during the latter stages of the osmotic dehydration (compare weight losses at 22, 24 and 44 hr), indicate probable faster sugar penetration (by diffusion) and impregnation of the fruit tissue.

The main quality advantage of the cold osmotic treatment was that it preserved the natural (even if diluted by the sugar) flavour, colour and appearance of the papaya slices, as determined by the taste panel. Even after cabinet drying to about 70% DM, the IM papaya slices had a light, typical papaya colour as well as typical flavour and agreeable texture.

The main (expected) disadvantages could be need of relatively large quantities of sugar, and microbial contamination of the syrup and product. The first problem could be minimized by re-use of the sugar syrup, and the second by acidification and addition of preservatives to the sugar syrup.

Another disadvantage of the cold osmotic treatment might be the product's deterioration during prolonged storage at ambient temperature, because of undesirable enzymic activity. In order to prevent this, and to stabilize the storage ability of the product and facilitate water removal and sugar penetration—mostly by diffusion, instead of controlled osmosis—combinations of short heat-treatment (blanching) and cold osmotic dehydration were studied.

Blanching and cold osmotic dehydration

Combinations of water or syrup blanching and cold osmotic treatment were studied with batches of 5–10 kg each of firm, ripe papaya slices, prepared and treated as described above.

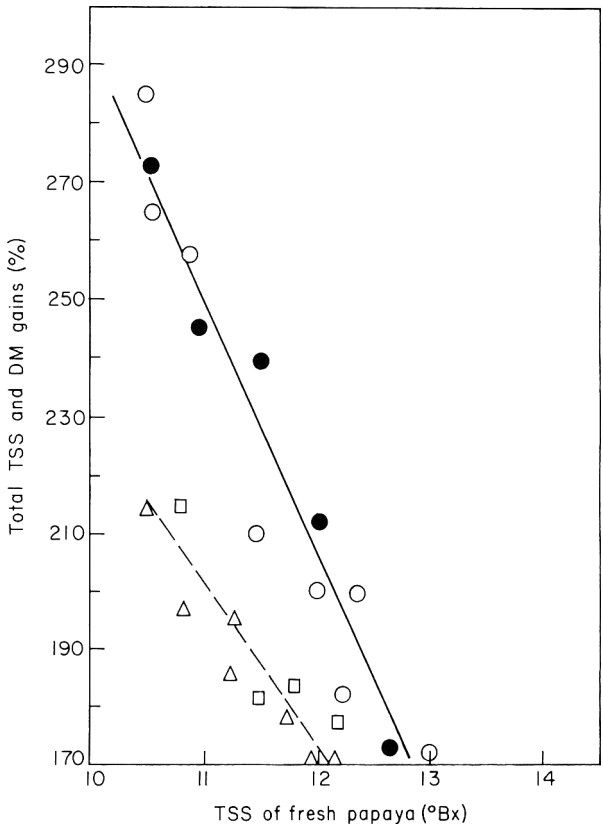

Figure 3. Effect of combined boiling (5 min) water or sugar (70%) syrup blanching and cold syrup (70% sucrose) treatment (24 hr), on TSS and DM gain of papaya slices. ——, water blanching; – – –, syrup blanching; △ ●, TSS; ○ □, DM).

Figure 3 shows the influence of the TSS content (°Bx) of the fresh papaya slices on the TSS and DM gain, after the above treatment. The conditions for blanching consisted of dipping in boiling sucrose (70%) or plain water for 5 min, followed immediately by dipping in cold (ambient temperature) sucrose (70%) syrup for 24 hr.

An inverse correlation was observed between the TSS and DM contents of the fresh papaya slices and the resultant DM and TSS gains, due to the osmotic treatment. The gains were calculated according to the formula given in Materials and methods, discounting the DM and TSS losses caused by the combined heat-osmotic treatment. Some variability was observed between results with papaya of the same (or very near) TSS content, probably because of natural variation in the quality characteristics of the fresh papaya—texture, structure, composition, etc. The observed weight losses after either syrup or water blanching, and the following cold syrup treatment, were irregular between 21 and 40%, with an arithmetic average value of ~32%.

One of the main reasons for the increasing TSS (and DM) losses, with increasing TSS (and DM) content of the fresh slices, is probably the parallel

increase in TSS losses after water blanching (Table 4). The higher values of DM (and TSS) gains of the water-blanched, as compared with the syrup-blanched, slices, is probably due to the greater effect of water blanching facilitating sugar penetration and impregnation by diffusion, caused by its destructive effect on the selective permeability of the cell wall and membranes. The smaller gain in DM (and TSS) content of the syrup-blanched slices could be explained by loss of the capacity of the papaya tissue to absorb more sugars and water due to the different pectin gelation effect of the sugar blanching—similar to that observed by Moy *et al.* (1978).

The overall yield advantage of the combined 'water-blanching'—'cold-syrup' dehydration treatment (I), over the 'cold-syrup' (II) or 'syrup-blanching'—'cold-syrup' treatment (III), is obvious, when comparing the parallel TSS gain of treated papaya with initial TSS content of 11.4°Bx (see also Table 5). The observed TSS gain after treatment I was ~230% and only ~190% after treatments II or III. There was no significant difference, in other quality characteristics (flavour, colour, texture) of the dried papaya slices after these treatments. Therefore, a combination of 'water-blanching' and cold osmotic treatment should be considered as one of the methods which shorten considerably the air-drying time, and also reduce the energy requirements for conventional and solar drying.

Drying behaviour

The air-drying behaviour of fruits and vegetables is affected by their initial DM (including sugar) content, as well as by the natural variability in their

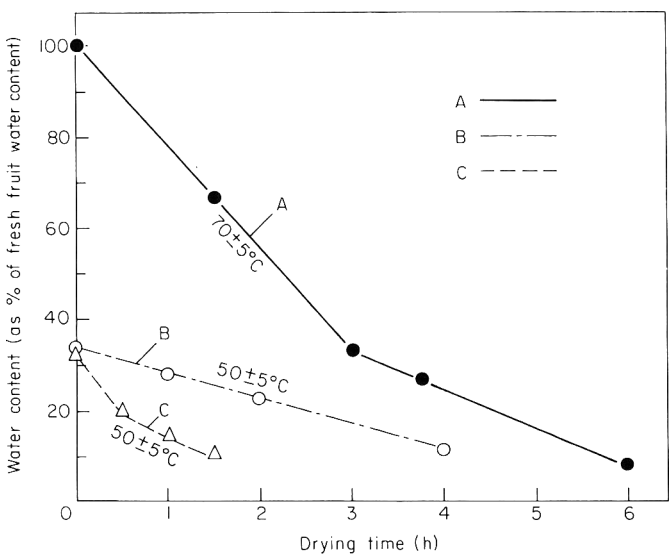

Figure 4. Drying behaviour of cabinet-dried papaya (A, direct drying; B, after 75 min in boiling sugar (70%) syrup; C, after 22 hr. in cold sugar (70%) syrup).

composition, texture and structure. Heat treatments affect the selective permeability of the cellular membranes (Rotstein & Cornish, 1978) and the water-binding capacity of plant fibrous materials (Mathee & Appledorf, 1978). The increase in (added) sugars (DM) content in papaya has also an additional effect—an increased probability of 'case hardening'. All these factors affect significantly the rate of water evaporation during the drying process, as already shown by Levi *et al.* (1980) in banana.

Figure 4 shows the drying behaviour of cabinet-dried papaya slices—dried directly or after dipping in 'hot' (75 min) or 'cold' (24 hr) sugar syrup. The results given here represent similar trends observed in three to five experiments for each type of product, starting with batches of 5–10 kg of firm, ripe, fresh papaya slices for each experimental condition. The slices were subjected to direct drying and to drying after osmotic treatment ('hot' or 'cold'), as described before.

During the first stage of the direct drying (3 hr at 70°±5°C), the drying rate—calculated as % of water removed/hr of the total water content of the fresh slices (100%)—was 22%/hr. The rate was slowed down during the second stage (50°±5°C) to 9.1%/hr. The main reason for the decreased rate of evaporation is apparently the lower drying temperature, even if some case hardening was observed. Altogether it took 6 hr to dry the fresh untreated papaya slices from 13.6% DM to ~72% DM.

After the 'hot' osmotic treatment, the moisture content of the papaya slices was greatly reduced, from 86.4% in the fresh material to 44.5%. The air-drying rate was 3.5%/hr, the reduction in the drying velocity (compare with above treatment) was probably caused by case hardening as well as by the slowed water and heat transfer, due to the impregnation of the papaya tissue with polymerized sugar (caramelization) during the 'hot' osmotic treatment.

After the 'cold' treatment the slices had 44.2% moisture content, and evaporation rate was 20.4%/hr during the first 30 min of the cabinet drying. Afterwards the rate decreased to 9.5%/hr, probably because of the case hardening on the surface of the sugar-enriched papaya slices.

These results (Fig. 4) indicate the advantage of the 'cold' osmotic treatment over the 'direct' or after the 'hot' treatment drying, from the drying velocity point of view. This treatment was significantly better also for general quality as it resulted in produce of natural papaya colour, flavour, texture and appearance.

The treatment—which will shorten considerably the drying time, reduce the danger of quality deterioration, and not need a higher level of technological know-how or expensive and sophisticated equipment—is probably a combination of the 'cold' osmotic treatment and sun drying. Solar drying also would save the expensive energy required for cabinet drying.

A batch of ~15 kg of firm, fresh, ripe papaya slices was subjected to 'cold' osmotic treatment (for 20 hr), and then divided into two equal batches and subjected to cabinet (50°±5°C) or to direct sun drying. The resulting rate of water removal by drying, on a DM basis, is given in Fig. 5.

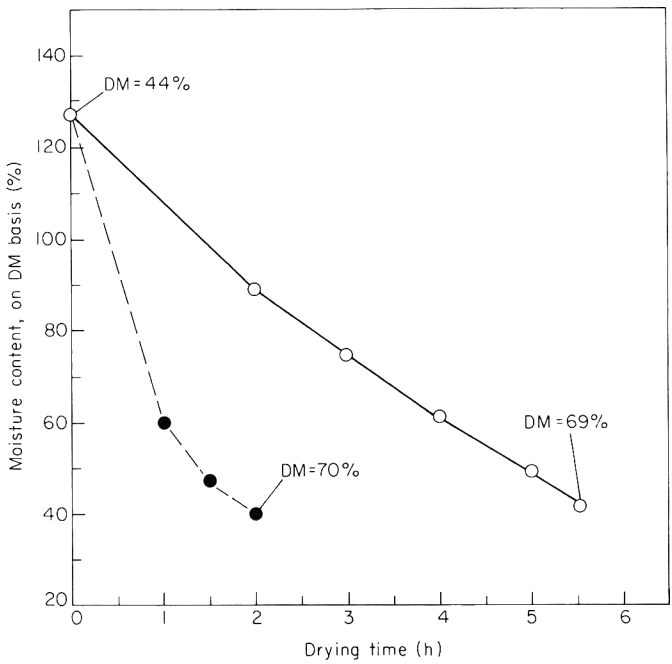

Figure 5. Drying behaviour of cabinet *versus* sun-dried papaya, after cold osmotic (70% sugar) treatment (for 22 hr) – – –, cabinet; ——, sun.

After the osmotic treatment, the resulting DM content of the papaya slices was about 44% (parallel moisture content of 127%) on a DM basis in this case. The rates of water removal (on a DM basis) during the cabinet drying were: 64%/hr during the first hr (to 62% DM) and 25%/hr during the second hr (to ~70% DM).

As expected, the solar drying was slower, and three stages of drying rates were observed: during the first 2 hr the rate was ~20%/hr, during the second 2 hr it was 14%/hr (to ~62% DM), and during the final stage it was 10%/hr.

The above results indicate the advantage of the combination between the 'cold' osmotic treatment and the cabinet or sun drying. The latter (solar drying) could be completed in 1 day, thus greatly diminishing the danger of microbial contamination and insect infestation during long (2–3 days) direct sun drying, and eliminating the need to cover the drying papaya (or other fruit) during the night, in order to prevent dew precipitation on it.

The results given in Figure 5 represent the general drying trend of five similar experiments, being the differences in the drying times observed: about ±0.5 hr for the cabinet and ±1.5 hr for the solar drying. The main reason for the observed differences seems to be the initial DM (and sugar) content of the fresh fruit. The quality characteristics of the sun-dried papaya slices will be described in a separate publication.

The effects of the 'blanching' on the sun-drying velocity of the papaya slices are presented in Figure 6.

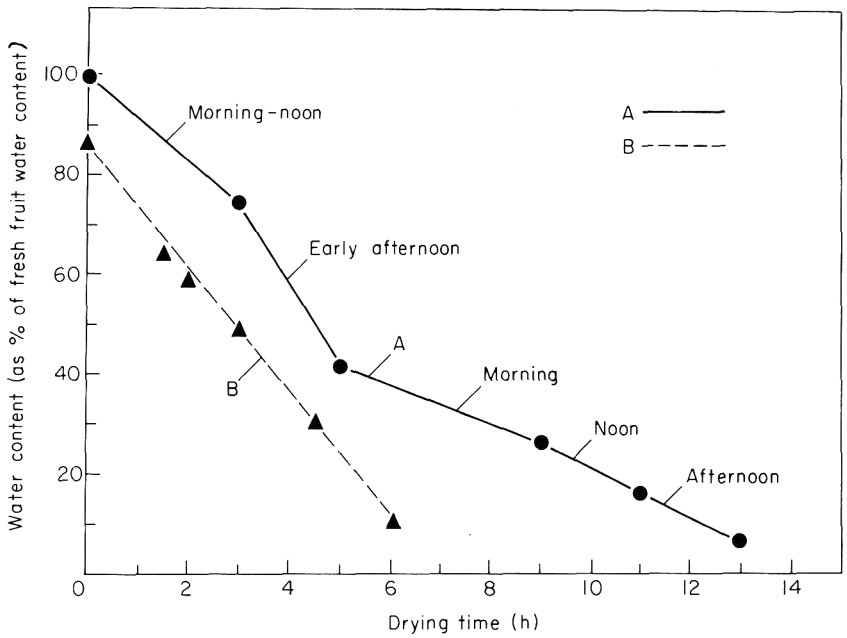

Figure 6. Effect of blanching on sun-drying behaviour of papaya A, direct; B, after syrup (70% sucrose) blanching.

A batch of ~25 kg of firm, fresh papaya slices was divided into two parts, one of which was subjected to direct sun drying while the other was first syrup-blanched and then sun dried. The drying velocity was calculated according to the water content of the fresh papaya slices expressed as 100% (see above), which really was 87.6% of the fresh papaya. The drying rates of the directly dried papaya varied according to the drying period of the day, with ~13 hr needed to dry the papaya to about 70% DM. The 'blanching' probably affected the selective permeability of the cell membranes, as well as the water-binding capacity of the papaya fibrous materials, reducing the drying time to only 6 hr for the same degree of dryness. The 'blanched' dry papaya slices were of good eating quality and translucent in appearance, with more natural orange colour than the directly sun-dried papaya.

The effect of combinations of water or syrup blanching and 'cold' sugar syrup dipping was also studied, in order to observe the effects of the facilitated water removal by the blanching, combined with a reduction in the amount of moisture to be removed (by the facilitated sugar penetration), during the osmotic treatment and the air drying. Table 6 presents the results of five experiments, each with a batch of 10–15 kg of fresh, firm, ripe papaya slices. As a result of the combined treatments, the cabinet drying time was between 1.5 and 2.75 hr, while the solar drying took between 3.5 and 4 hr. It is obvious that the combined blanching-osmotic treatment shortens considerably both the cabinet and solar-drying times, while product stabilization during storage can also be expected, because of the inactivation of the undesirable enzymes. The general

Table 6. Effect of combined 'blanching' and 'osmotic' treatments on the drying rate of papaya slices

Treatments			Dry matter (%)			
Blanching*	Dip syrup† (hr)	Drying method	Fresh	Osmotic	Final	Drying time
Water	22	Cabinet	13.7	55.7	71.5	1 hr 30 min
Water	20	Cabinet	12.6	46.3	70.0	2 hr 45 min
Water	22	Cabinet	12.8	65.6	76.6	2 hr
Water	21	Solar	13.0	59.7	74.0	3 hr 30 min
Syrup	22	Solar	12.7	54.3	78.7	4 hr

* 5-min dip in boiling water or syrup.
† Dip in 70% sucrose syrup at room temperature.

appearance and eating quality characteristics of the product were similar to these after 'cold' osmotic treatment.

Conclusions

The IM papaya products and production methods developed on a pilot-plant scale and described above, could probably be applied in most developing countries on a small and simple or more sophisticated industrial scale, after economic evaluation of their feasibility. The combination of 'osmotic' treatment and conventional (cabinet) or sun drying would shorten considerably the drying time and reduce the costs of the energy required for drying.

Acknowledgments

We wish to express our gratitude to Paulette Shalom, Gita Popel and W. Meirowitch for their invaluable technical help.

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Hygroscopic behaviour of freeze dried bananas

A. W. O. LIMA* and J. CAL-VIDAL†‡

Summary

The hygroscopic behaviour of freeze dried bananas at several temperatures is presented. The adsorption isotherms were of sigmoid shape and the particle size of the freeze dried materials did not affect the general isothermal behaviour. The limits for caking were found at a water activity (a_w) of about 0.33 at 38°C. The results fit the Henderson equation quite well at the four temperatures under test.

Introduction

In Brazil, bananas are produced in very large amounts and several food companies are interested in developing byproducts from this important commodity, mainly dried components in powder and granular form. The understanding of the hygroscopic phenomena of such products is essential in order to establish optimum conditions for packaging and stability during storage, use and distribution. The literature reports a considerable amount of data dealing with the moisture equilibria of dried foods (Chirife & Iglesias, 1978; Iglesias & Chirife, 1976a, 1978; Labuza, 1980; LLadser & Piñaga, 1975; Mazza, 1982; Cal-Vidal, 1982). Several authors have attempted to use their findings to select appropriate flexible packaging material for such products (Quast & Gil, 1973; Salwin & Slawson, 1959; Quast & Teixeira Neto, 1976; Lima, 1981). Others have tried to develop basic equations from the available experimental data (Iglesias & Chirife, 1976a, b, c, 1978; Labuza, 1975; Chung & Pfost, 1967a, b; Mizrahi, Labuza & Karel, 1970).

For freeze dried foods it is well known that the residual moisture content is of critical importance for the extension of their shelf life (Maloney *et al.*, 1966; Martinez & Labuza, 1968; Stevens & Thompson, 1948; Salwin, 1963). The temperature also plays a very important role, because it is well documented that

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the hygroscopic equilibrium is greatly influenced by the temperature (Rockland, 1969; Audu, Loncin & Weisser, 1978; Rasekh, Stillings & Dubrow, 1971; Palnitkar & Heldman, 1971; Landrock & Proctor, 1951; De Gois & Cal-Vidal, 1981). In general, the sorption phenomena obey the Clausius Clapeyron equation, given by:

$$\frac{d(\ln a_w)}{d(1/T)} = \frac{-Q_s}{R}, \quad (1)$$

where a_w=water activity (fractional number), T=absolute temperature (°K), Q_s=sorption heat (Kcal/mol), and R=constant for gases (atm. l/mol °K). Thus, it is expected that an increase in the temperature of the environment will diminish the amount of water absorbed by the food for a given constant water activity.

Another factor affecting the isothermal equilibrium of a dried food would be the particle size, although some researchers have found that this is of little significance (Rasekh, Stillings & Dubrow, 1971; LLadser & Piñaga, 1975; Cal-Vidal & De Gois, 1982). Finally, the chemical composition deserves some consideration mainly when crystalline substances are present—as is the case with dried fruits—and structural modifications of sugars from the amorphous to the crystalline state may favour the release of water to form undesirable hard cakes (Pisecky, 1973; Junk, Harry & Pancoast, 1973; Simatos & Blond, 1975; De Gois, 1981; Cal-Vidal, 1982).

Among the equations proposed to express isothermal relations of dried foods, there is the one presented by Henderson (1952), with the following parameters:

$$1-a_w = e^{-cTU_e^n}, \quad (2)$$

where c and n=constants (dependent on the material), T=absolute temperature (°K), and U_e=equilibrium moisture content (%).

This can be written in the logarithmic form:

$$\ln\left[\ln\left(\frac{1}{1-a_w}\right)\right] = \ln(cT) + n\ln(U_e), \quad (3)$$

which corresponds to the equation of a straight line. This equation makes it possible to extrapolate data for other water activities and water contents, to determine temperature effects and to construct isothermal curves from relatively few experimental observations. Singh & Ojha (1974) tested the validity of this equation on milled nuts and dried pepper and found good correlation. Rockland (1957) tested the validity of the equation for several foods, LLadser & Piñaga (1975), found that the logarithmic equation yields two straight line portions, instead of one, when checking their sorption data of freeze dried avocado, while Iglesias & Chirife (1976b) found the Henderson's

equation applicable to a series of experimental data. More recently (Igbeka & Blaisdell, 1982) found the equation also applicable to a processed meat product. In this paper, the hygroscopic behaviour of freeze dried bananas in powder and granular form is presented, including the fitting of the experimental data in the Henderson's equation for several temperatures.

Materials and methods

Freeze dried bananas

The freeze dried bananas of the 'nanica' variety (*Musa cavendish*, Lambert), with particle sizes between 2.0 and 5.7 mm were produced by Lioval Alimentos Liofilizados, S.A. (Morretes, PR, Brazil). A part of the granular material was passed through a Thomas-Willey Jr. mill (Arthur H. Thomas Co., Philadelphia, Pa, U.S.A.), with screens of 20 mesh (opening=0.84 mm) to obtain the powdered form. These materials were kept in stoppered glass flasks until their use in the isothermal analyses.

Water absorption determinations

Following water content determinations (AOAC, 1970) and the complete drying of the samples in a vacuum oven at 60°C during 7 hr, moisture sorption isotherms were determined gravimetrically by placing the freeze dried samples in vacuum desiccators containing saturated salt solutions (Wink & Sears, 1950; Rockland, 1960). The desiccators were kept in constant temperature cabinets for a period of approximately 14 days. All results are expressed on a dry matter (DM) basis. For each equilibrium moisture determination, a visual inspection of the samples was made to establish the caking point. In addition, to determine the temperature effect on the hygroscopic properties of the freeze dried material and the fitting of Henderson's equation, the corresponding c and n constants were calculated using the method of least squares.

Results and discussion

Sorption isotherms

The water absorption kinetics for powder (Fig. 1) and granules (Fig. 2) indicate, that for freeze dried bananas, the particle size has practically no effect on the water absorption properties of the product, confirming the findings of LLadser & Piñaga (1975) for freeze dried avocado and of Palnitkar & Heldman (1971) for freeze dried beef. This can be seen in Figure 3 which shows the isotherm at 38°C for different particle sizes. This graph also indicates the critical values of water activity favouring caking condition (0.33) and growth of fungi (0.75). The isotherms at different temperatures for the granular product are

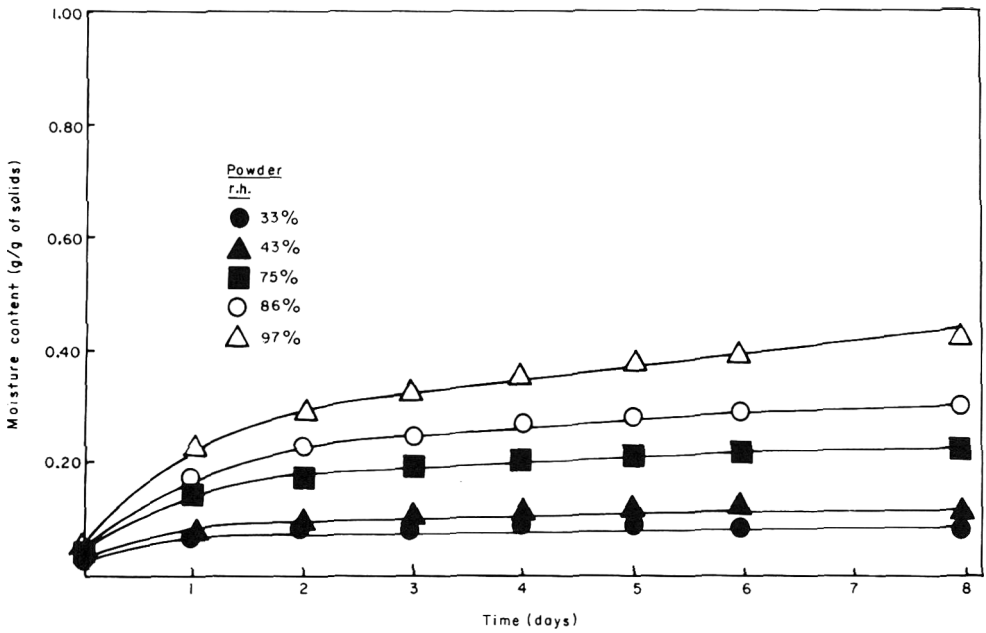


Figure 1. Water absorption kinetics of powdered freeze dried bananas at 22°C.

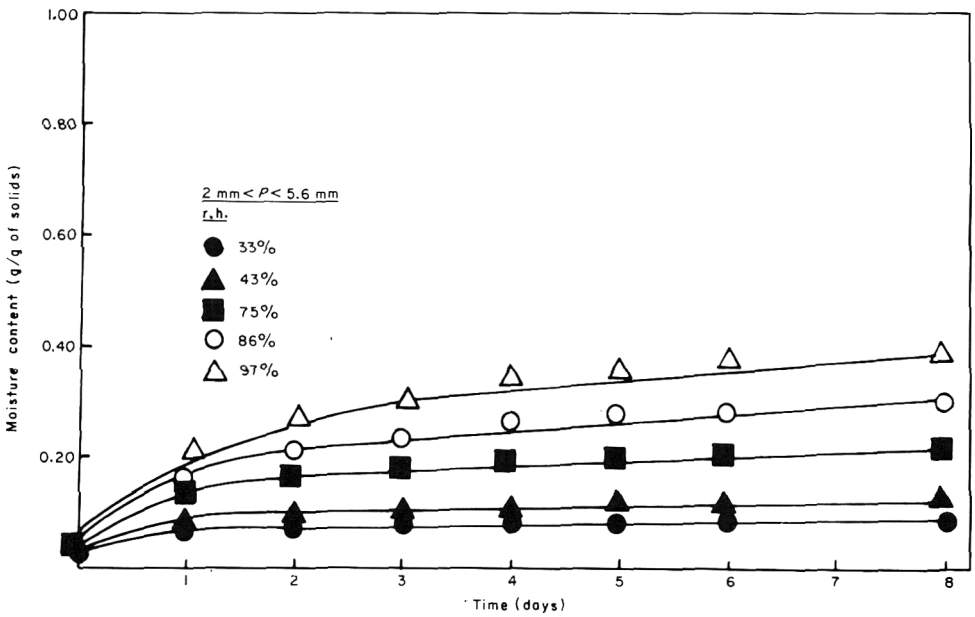


Figure 2. Water absorption kinetics of granular freeze dried bananas at 22°C.

presented in Figure 4. The reasons for the shift of the isotherm to the right as the temperature increases is well documented in the literature (Iglesias & Chirife, 1976b; Igbeka & Blaisdell, 1982). The observed caking phenomenon, of critical significance in freeze dried products, can be considered as an important index to establish the shelf life of freeze dried bananas.

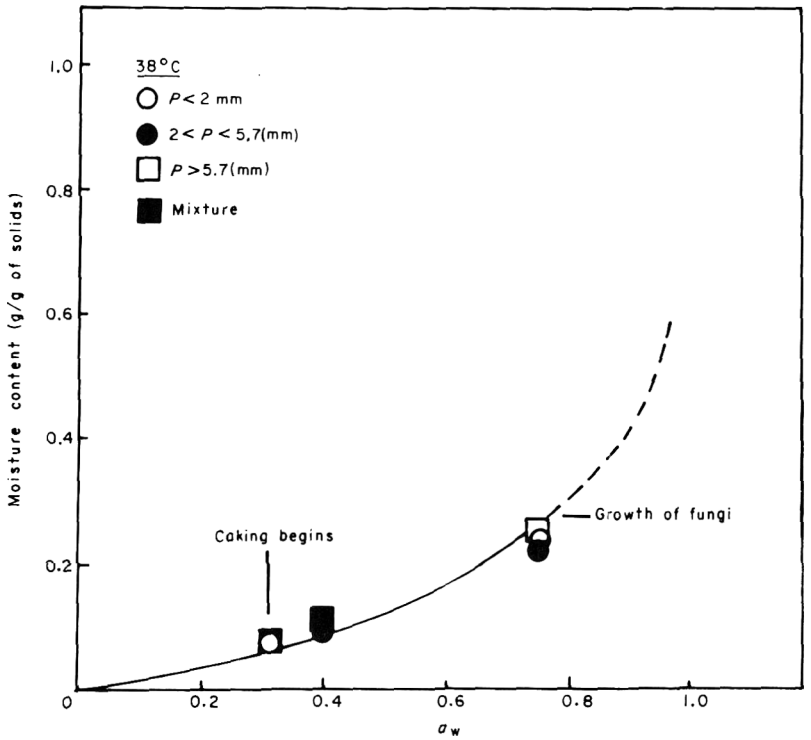


Figure 3. Typical isotherm of freeze dried bananas in powder and granular form at 38°C.

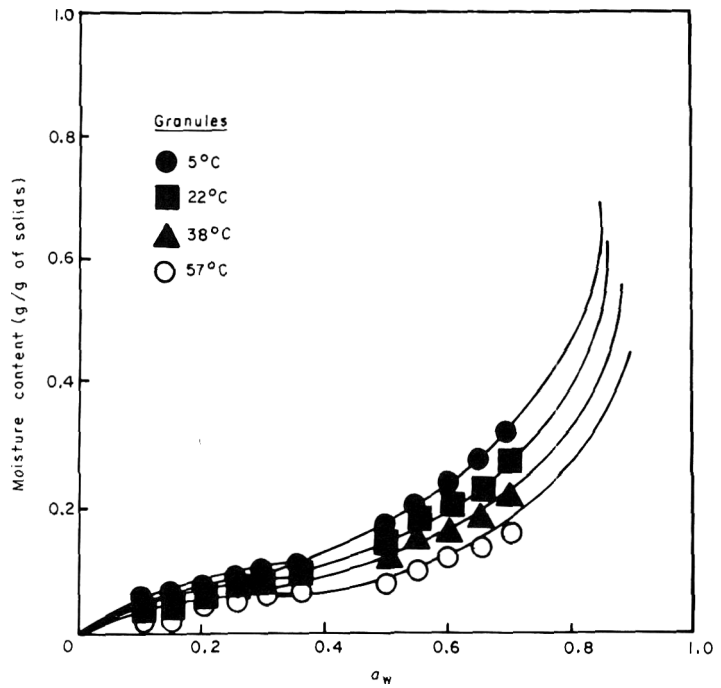
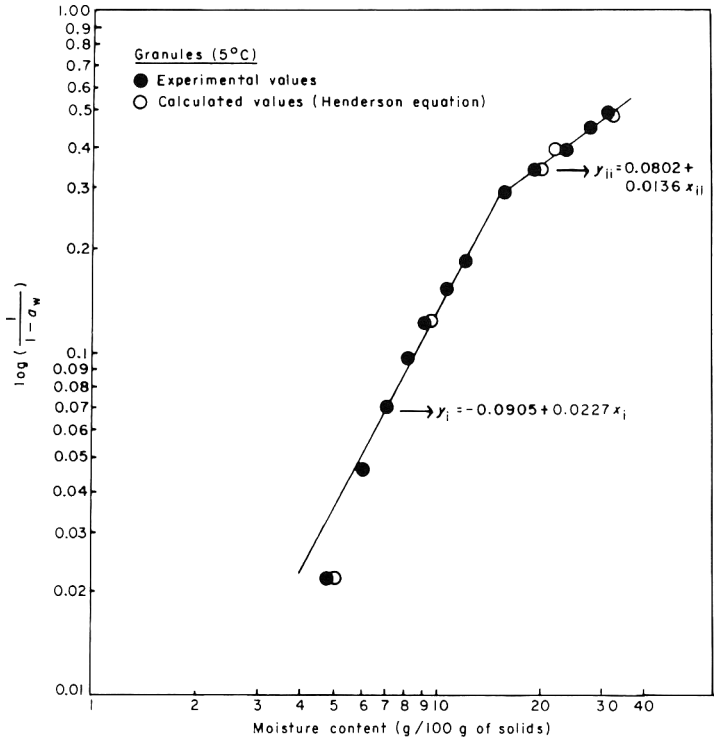


Figure 4. Sorption isotherms for freeze dried bananas in granular form at several temperatures.



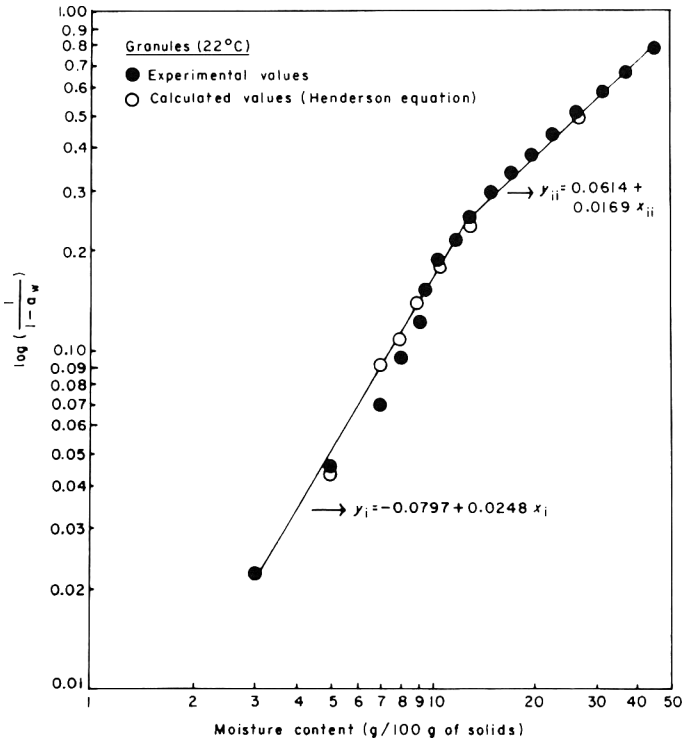


Figure 6. Graphic representation to estimate the values of constants c and n of the Henderson equation (22°C).

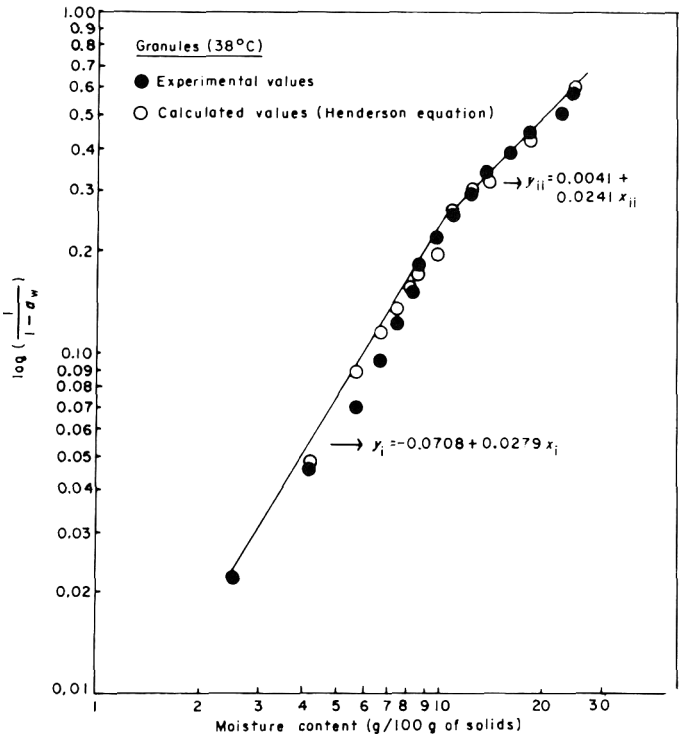


Figure 7. Graphic representation to estimate the values of constants c and n of the Henderson equation (38°C).

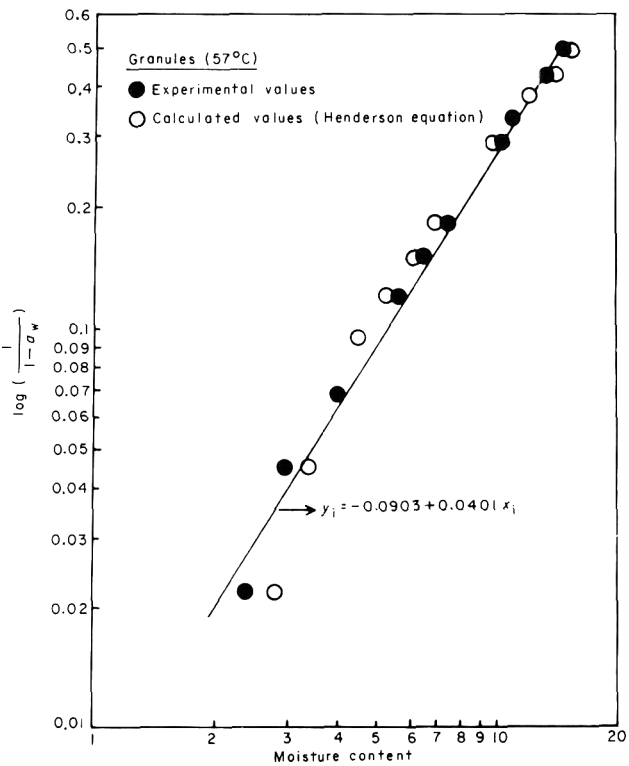


Figure 8. Graphic representation to estimate the values of constants c and n of the Henderson equation (58°C).

Table 1. Values for constants c and n in Henderson plots

Temperature (°C)	Relative humidity (r.h.) interval (%)	Constant values	
		c	n
5	5<UR<35	2.29×10^{-3}	2.27×10^{-2}
	50<UR<70	4.33×10^{-3}	1.36×10^{-2}
22	5<UR<45	2.82×10^{-3}	2.48×10^{-2}
	45<UR<85	3.90×10^{-3}	1.69×10^{-2}
38	5<UR<40	2.73×10^{-3}	2.79×10^{-2}
	40<UR<75	3.25×10^{-3}	2.41×10^{-2}
57	5<UR<70	2.46×10^{-3}	4.01×10^{-2}

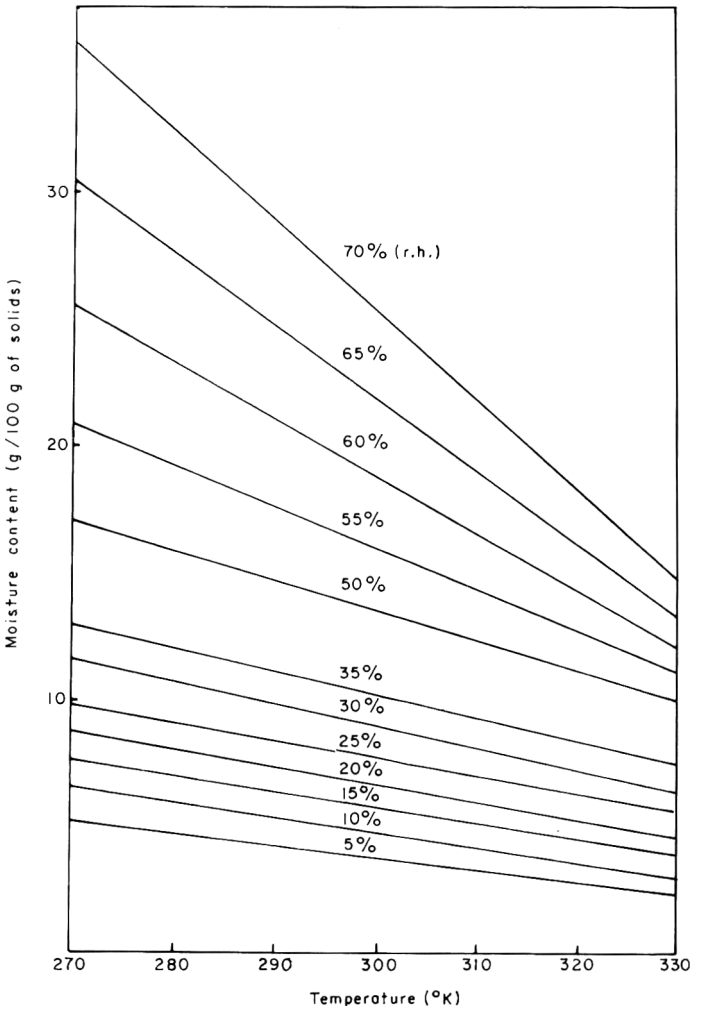


Figure 9. Temperature effect on the equilibrium moisture content of freeze dried bananas at several relative humidities.

Fitting the Henderson equation

Taking equation (3) and the experimental values, the graphs of Figures 5 to 8 were constructed. Two orders of dependence were observed, with two well defined straight lines. This suggests, as Igbeka & Blaisdell (1982) discussed for the case of a meat product, that the bound moisture is, in the present case, also dependent on the temperature, with the discontinuity indicating a change in the nature of the bond. Such a discontinuity, however, was not observed for the temperature of 57°C, where only one straight line was obtained.

The values for the constants c and n of the Henderson's equation are presented in Table 1, and were used to predict the equilibrium water content of the freeze dried bananas at different temperatures. To calculate these constants, the method of least squares was used, considering the slope and intercept of the straight lines.

The effect of temperature on the change of moisture content for a given relative humidity (r.h.) is more clearly seen in Figure 9, where the sorption data for the freeze dried banana were plotted according to the Henderson equation. A change in the gradient of the straight line was observed as the relative humidity varied from 35 to 50%. This may be due to a critical change of the moisture absorption capacity of the product as the relative humidity increases.

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The evaluation of the apple cultivars Bramley's Seedling, Suntan and East Malling A 3022 for the production of apple slices

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Summary

Comparison of slices of the apple cultivars Bramley's Seedling, Suntan and East Malling A 3022 showed that both objective and subjective assessments were considerably influenced by: (1) preparatory procedure (blanching time, sugar or malic-acid additions), and (2) storage (whether as whole fruit or as previously prepared frozen slices). The addition of sugar and acid and method of storage produced a more marked effect than differences due to cultivar. Although Bramley's Seedling could be distinguished objectively from the other two cultivars, primarily on the basis of appearance, this had little influence when it came to making hedonic assessments. Products from Suntan and East Malling A 3022 were considered at least as good in a number of respects as those from Bramley's Seedling.

Introduction

The Bramley's Seedling apple is the accepted standard for the production of solid packs and canned apple products against which other cultivars tend to be compared. It does have a number of disadvantages, its irregular shape and variable size, for example, make it difficult to process. Over recent years, a number of new cultivars have been developed which offer potential as processing fruit. Two such cultivars are Suntan (a cross between Cox's Orange Pippin and Court Pendu Plat) and East Malling A 3022 (a cross between Cox's Orange Pippin and Northern Spy); previous studies on these cultivars have indicated that they are potentially as acceptable if not better than Bramley's Seedling when used for culinary purposes, particularly when dealing with frozen sliced packs (Williams, Warrington & Arnold, 1982a). These comparisons were made using standard preparation procedures, but during the preparation and production of apple products there are a number of variables

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which could be altered, the ideal combination for one cultivar possibly not being the same as for another.

Gormley (1975) has already demonstrated that the addition of malic acid during the preparation of solid packs from Golden Delicious apples improved texture, flavour and overall acceptability. This paper explores further the effect of production variables such as blanching time, and addition of sugar and/or malic acid on the sensory properties and acceptability of apple slices made from three cultivars: Bramley's Seedling, Suntan and East Malling A 3022. Whilst some of the effects due to these factors as well as varietal differences can be minimized by the addition of accompaniments, as is normally done in the meal situation (Williams, Warrington & Arnold, 1982b), it is important to know to what extent one can counteract differences in sensory properties and acceptability by adjusting production procedures.

Materials and methods

Material

Fruit (75 kg) of the cultivars Bramley's Seedling, Suntan and East Malling A 3022 were grown at East Malling Research Station and harvested in late October 1979. They were transported to Long Ashton Research Station for immediate examination and preparation of slices for freezing. The remainder were held under atmospheric storage (3–4°C) until required in January.

Preparation of slices

Fruit was peeled and cut transversely into rings approximately 10 mm thick, cores were removed and edges trimmed, the segments then being cut into halves. Immediately following preparation, sufficient half segments (28) for one assessment were placed in cold water. They were then steam blanched in a fish kettle fitted with a wire mesh trivet. After blanching, slices were cooled in cold water for a time equivalent to that used for blanching, and steeped in the appropriate sugar/acid solution for 5 h prior to assessment, samples being drained before being presented to the panelists.

Blanching time and sugar/acid levels. Following trial cooking tests and sensory assessments, blanching time and sugar/acid levels in the steeping solutions were set as indicated in Table 1. Blanching times varied between the cultivars and were such that they provided adequate enzyme inhibition yet produced a product which did not disintegrate on handling. With Bramley's Seedling it proved difficult to maintain the proper balance particularly as fruit matured, so samples prepared for freezing and from stored fruit in January had to be blanched for a longer time than fruit processed in November.

Samples for freezing. Slices of all three cultivars, following steeping in the appropriate sugar/acid solution, were packed in rigid containers and stored at −18°C.

Table 1. Preparation procedures for apple slices

(a) Blanching times:

Cultivar and source	Code	Blanching time
A 3022 and Suntan throughout	1	2 min
	2	2 min 30 sec
Bramley—November, fresh fruit	1	1 min 20 sec
	2	1 min 30 sec
Bramley—November, fruit for freezing, and January, fruit from store	1	1 min 45 sec
	2	2 min

(b) Steeping solutions:

(i) no addition—water
(ii) acid only—2.5% malic
(iii) sugar only—30% sucrose
(iv) acid and sugar as in (ii) and (iii) together

(c) Sample preparations*:

Code No.	Blanching code	Steeping solution	Source of fruit	
			November	January
1	1	i	fresh	frozen
2	1	ii	fresh	stored
3	1	iii	fresh	stored
4	1	iv	fresh	frozen
5	2	i	fresh	stored
6	2	ii	fresh	frozen
7	2	iii	fresh	frozen
8	2	iv	fresh	stored

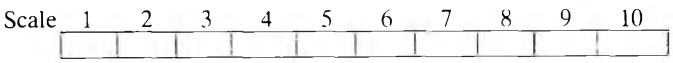
* These were repeated for all three cultivars.

Sensory assessment

Panel. The assessment panel comprised twelve tasters (six men, six women) for the November assessments, and sixteen (seven men, nine women) for the January assessments. Four men and five women were common to both panels. All assessors were members of the staff of Long Ashton Research Station and varied in age from 20–60 years. All were experienced in the sensory analysis of apples.

Assessment sheet. The score sheet comprised the scales illustrated in Figure 1 which are similar to those widely used in sensory analysis (Williams, 1982). Panelists were asked to tick each scale using the appropriate box. Room was left

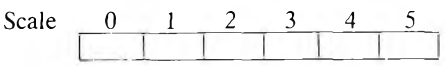
(a) Hedonic assessments



Explanatory description:
1. Very bad (most unacceptable)
2. Bad
3. Poor (unacceptable)
4. Poor (just acceptable)
5. Indifferent (just acceptable)
6. Fair
7. Pleasant
8. Good
9. Very good
10. Excellent (first class)

Attributes assessed:
(a) Appearance
(b) Colour
(c) Flavour
(d) Texture
(e) Overall rating

(b) Objective assessments



Explanatory description:
0. None of character
1. Very weak character
2. Weak character
3. Moderate character
4. Strong character
5. Very strong character

Attributes assessed:
Colour — white/grey
— white/yellow
Flavour — acidity
— sweetness
— apple character
— Cox character
— Bramley character
— wateriness
Texture — firmness
— fibrousness

Figure 1. Scales used in the assessment of apple slices, 1979–80.

on the sheet for any other comments panelists cared to make. Within each session, each sample was asessed using a separate score sheet.

Presentation of samples. For the fresh fruit, all twenty-four combinations of cultivar, blanching time and steeping solution (Table 1) were assessed in randomly selected groups of three. The order of presentation was also randomized for each panelist.

In January, there was an additional variable of storage, with the slices either being prepared from stored fruit or having been prepared and frozen in November, this giving forty-eight possible combinations of variables. As it was not practical for all of these to be prepared and assessed, a half-replicate design was used, again giving twenty-four samples. With this design all second-order interactions between factors other than cultivar are confounded, as are the equivalent three-way interactions including cultivar. The samples were assessed in randomly selected groups of three, in the same manner as before.

In all assessments samples were presented under daylight at room temperature.

Treatment of data

Data from the two assessments carried out in November and January were analysed independently using the GENSTAT package (Nelder, 1977) available on the ARC Rothamsted computer (ICL System 4.470). Inspection of the distribution of the raw data showed no major deviations from normality. The overall hedonic data was therefore subjected to analysis of variance (ANOVA) and any main effects or interactions of significance (established by means of *F*-tests) investigated in more detail.

A multivariate analysis of variance (MANOVA) and a canonical variate analysis (CVA) were carried out on the hedonic and objective data sets separately. This enabled the effects of the various factors involved to be more easily interpreted.

As well as discovering the main factors differentiating the samples, a clue to their relative importance to the overall acceptability of apple slices was obtained by regressing the canonical variate for both objective and hedonic data against overall acceptability.

Results

Objective assessments

In November the MANOVA analysis indicated that the four-factor interaction between cultivar, blanching time and sugar/acid levels was significant. As a consequence, the canonical variate analysis was carried out using each of the twenty-four treatment combinations as separate entities. Using Bartlett's approximation (Chatfield & Collins, 1980; Bartlett, 1947) for the distribution of Wilks Λ statistic and his corresponding χ^2 test for dimensionality, the first three dimensions were found to be significant (Table 2). The first of these is largely the contrast between yellowness and the degree of acidity, the second mainly a summation of the same variates and the third the intensity of sweetness, apple character and, to a lesser extent, acidity.

Scores for the first two canonical variates for the twenty-four samples are plotted in Figure 2. Clear separations of cultivar and steeping solution are apparent. The Bramley samples appear to be distinct from the other two cultivars mainly on the basis of colour, with East Malling A 3022 and Suntan being a lot yellower than Bramley. The other clear separation, according to differing steeping solutions, appears to be mainly on the basis of perceived acidity. For East Malling A 3022 and Suntan samples, the division depends on whether or not acid was added to the steeping solution, those with acid added tasting more acid. For Bramley, however, there is an interaction with the sugar content of the steeping solution. Those samples steeped in acid only appear the most acid, and those steeped in sugar only the least acid. Using neither or both

Table 2. Canonical variate loadings for objective assessment of apple slices (November 1979)

	Loadings ($\times 10^2$)		
Objective character	1st	2nd	3rd
White/grey	0.46	−1.66	−1.05
White/yellow	−4.72	3.87	−0.58
Acidity	3.85	5.98	−2.72
Sweetness	0.28	−2.10	−5.45
Apple character	1.27	−1.37	−3.97
Cox character	−0.22	1.20	0.72
Bramley character	1.69	0.09	0.85
Wateriness	−0.28	0.88	0.56
Firmness	−1.12	2.76	−0.78
Fibrousness	0.28	−0.56	1.74
Bartletts χ^2 approx.	1037.72	645.60	335.04
df	230	198	168
Significance level	P=0.001	P=0.001	P=0.001
% variance accounted for (based on latent roots)	47.9	31.3	9.4

seems to have much the same effect on acidity perception. This different behaviour between the cultivars could be due to the naturally greater acidity of Bramley's Seedling over the other two cultivars. Making comparisons across cultivars, the perceived acidity appears to be lowest for the A 3022 and Suntan

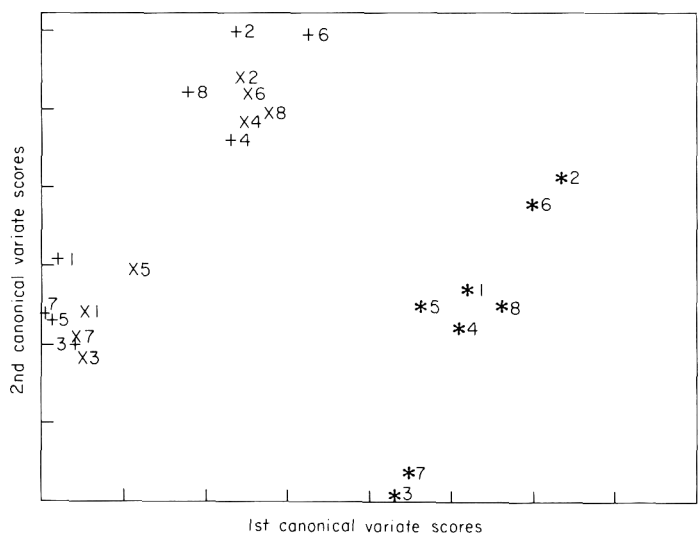


Figure 2. Canonical variate sample scores based on objective evaluation. November 1979 (1st and 2nd canonical variates). *, Bramley's Seedling; +, East Malling A 3022; and ×, Suntan. Nos 1–8. Sample preparation procedure, see Table 1.

samples without added acid, followed by the Bramley samples with added sugar only. The Bramley samples with neither or both additions, and the A 3022 and Suntan samples with added acid are similar in perceived acidity, the Bramley samples with only added acid having the highest level. There does not appear to be any overall effect due to blanching, with textural parameters contributing little to the significant canonical variates.

There was a significant interaction between all the experimental factors in January, so the results were again treated as twenty-four separate samples. Here four canonical variates showed significant differentiation over the samples (Table 3). The first of these variates is comprised mainly of yellowness and firmness, contrasted to a lesser extent with acidity. The second is mainly the contrast of acidity with sweetness and, to a lesser extent, wateriness. The third appears to be mainly yellowness and wateriness contrasted with firmness, and the fourth the combined flavour intensity of acidity and sweetness.

The scores for these canonical variates are plotted in Figures 3 and 4. For convenience, variate 3 is plotted against variate 1 as they both include textural attributes as appreciable contributions, and variate 4 is plotted against variate 2 as both appear to be mainly flavour vectors.

In Figure 3, two different separations are apparent, one separating Bramley samples from those of the other two cultivars and the other differentiating between the two modes of storage. The cultivar separation again seems to be mainly on the basis of colour, with Bramley being whiter than the rest. The storage difference appeared to be mainly on the basis of texture, with the

Table 3. Canonical variate loadings for objective assessment of apple slices (January 1980)

	Loadings ($\times 10^2$)			
Objective character	1st	2nd	3rd	4th
White/grey	1.03	−0.71	0.92	0.93
White/yellow	−4.49	1.67	−3.43	−0.39
Acidity	2.37	3.33	−1.69	−5.00
Sweetness	−0.09	−4.15	0.19	−4.40
Apple character	0.01	−0.68	0.92	−0.72
Cox character	−0.72	−0.65	−0.32	−1.08
Bramley character	0.46	−0.19	0.72	−1.04
Wateriness	0.68	−2.43	−3.38	−0.90
Firmness	−3.27	1.05	2.84	−1.32
Fibrousness	0.94	0.77	−1.37	0.54
Bartletts χ^2 approx.	1636.05	1084.37	683.74	344.01
df	230	198	168	140
Significance level	P=0.001	P=0.001	P=0.001	P=0.001
% variance accounted for (based on latent roots)	43.6	24.4	18.7	7.9

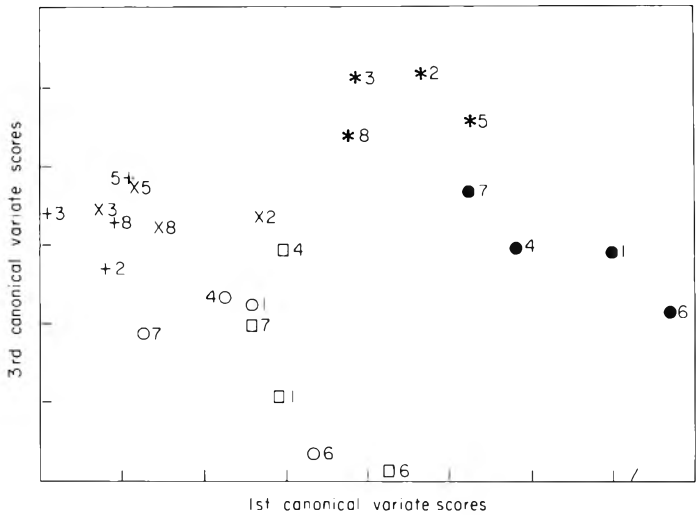


Figure 3. Canonical variate sample scores based on objective evaluation. January 1980 (1st and 3rd canonical variates). Bramley's Seedling: *, stored; ●, frozen. East Malling A 3022: +, stored; ○, frozen. Suntan: ×, stored; □, frozen. Nos 1–8. Sample preparation procedure, see Table 1.

samples from stored apples being a lot firmer than frozen ones. Within the frozen samples, Bramley slices and slices with no added sugar (1 and 6) were considered less firm than the others.

In Figure 4, apart from Sample 6 for Bramley, there are generally clear separations on account of different steeping solutions, based mainly on the perceived acidity and sweetness of the samples. The fourth canonical variate

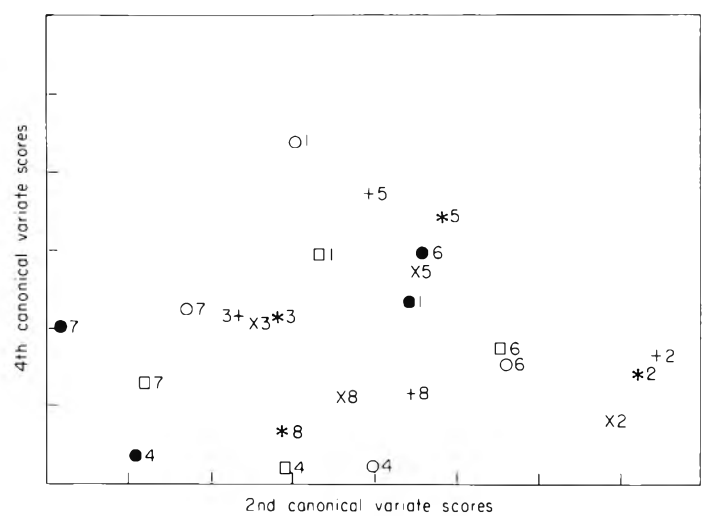


Figure 4. Canonical variate sample scores based on objective evaluation. January 1980 (2nd and 4th canonical variates). Bramley's Seedling: *, stored; ●, frozen. East Malling A 3022: +, stored; ○, frozen. Suntan: ×, stored; □, frozen. Nos 1–8. Sample preparation procedure, see Table 1.

can be thought of as giving the flavour intensity; flavour intensity increased from water treatment through either individual steeping addition, to the greatest intensity when segments were steeped in both acid and sugar. The differentiation between additions is given by the second canonical variate where those samples with added acid only are perceived as having a high acidity and low sweetness and those with only sugar added appearing to be low in acidity and high in sweetness. There is also a negative component in the second variate of wateriness and this is possibly reflected in the frozen samples generally appearing lower on that axis than the equivalent stored samples. There is a slight indication too of the Bramley samples with no additions being perceived as more acid and less sweet than the other two cultivars, attributable to the higher natural acidity of this cultivar.

Hedonic assessments

As with the objective data, examination of the hedonic rating given to specific attributes both in November and January showed interactive effects between treatments; hence the data were treated as twenty-four individual observations in both cases. The first two canonical variates were significant in November and the first three in January (Table 4). In November the first variate is largely the acceptability of flavour and the second that of texture and appearance. In January, the first variate is again the acceptability of flavour, but this time the acceptability of texture is equally important. The second variate is the contrast between the acceptability of appearance and flavour and the third variate the contrast in acceptability of appearance and texture.

Individual sample scores for the first two canonical variates for November and January are separately plotted in Figures 5 and 6 respectively. Apart from

Table 4. Canonical variate loadings for hedonic assessment of general attributes of apple slices

	Loadings ($\times 10^2$)				
	November 1979		January 1980		
Hedonic character	1st	2nd	1st	2nd	3rd
Appearance	−0.83	4.91	1.07	−3.60	4.91
Colour	−1.51	−2.62	−0.03	0.73	−2.31
Flavour	4.81	−0.78	2.13	3.22	2.00
Texture	−0.25	4.06	2.04	−1.95	−4.01
Bartletts χ^2 approx.	276.01	130.17	456.21	239.66	115.76
df	92	66	92	66	42
Significance level	P=0.001	P=0.001	P=0.001	P=0.001	
% variance accounted for (based on latent roots)	57.1	27.4	52.0	25.8	15.7

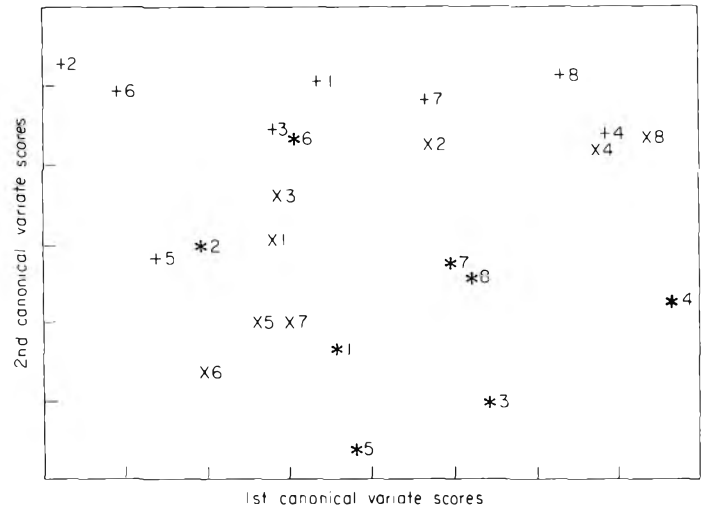


Figure 5. *Canonical variate sample scores based on hedonic data. November* 1979 (1st and 2nd canonical variates). *, Bramley's Seedling; +, East Malling A 3022; and ×, Suntan. Nos 1–8. Sample preparation procedure, see Table 1.

those samples to which both sugar and acid had been added, which appear to have the most acceptable flavour both in November and January, none of the experimental variables has a dominating effect on the acceptability of specific aspects of the slices, although previous storage history does show an effect in January.

Examination of the overall ratings in November shows again that, apart from the addition of sugar and/or acid, none of the variables investigated dominates acceptability. A number of interactive effects were apparent (Table 5).

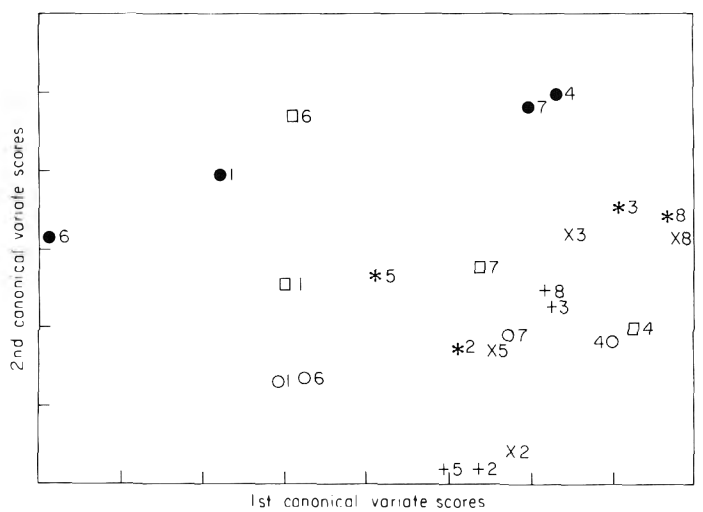


Figure 6. Canonical variate sample scores based on hedonic data. January 1980 (1st and 2nd canonical variates). Bramley's Seedling *, stored; ●, frozen. East Malling A 3022 +, stored; ○, frozen. Suntan: ×, stored; □, frozen. Nos 1–8. Sample preparation procedure, see Table 1.

Table 5. Overall hedonic scores. Tables of means for significant interaction effects (November 1979)

	Blanching code	
Cultivar	1	2
Bramley's Seedling	5.29	5.79
A 3022	5.51	5.08
Suntan	5.68	5.17
s.e.d.	0.261	

	Sugar addition	
Acid addition	Without	With
Without	4.75	5.36
With	4.81	6.79
s.e.d.	0.213	

The addition of sugar appears to be desirable, especially if acid is also added; adding acid without sugar, however, makes no obvious change. With respect to blanching, the longer time gives a preferred product for Bramley and the shorter time a preferred product for Suntan. This could be an effect of the variable times used in blanching the three cultivars (Table 1(a)). It appears that Bramley was preferred after blanching for 1.5 min, and Suntan and East Malling A 3022 after blanching for 2 min.

In January, differences in acceptability were more pronounced. In general, main effects of significance were storage, slices from stored fruit being much preferred to frozen ones, and sugar where its addition increased preference. A number of interactive effects were also significant; cultivar × sugar level, sugar level × method of storage (confounded with blanching time × acid level) and acid level × sugar level (confounded with blanching time × method of storage). Since the two factor interactions not including cultivar are completely confounded, interpretations of those of significance are ambiguous. However, as in November the blanching time × acid level interaction was insignificant and the sugar level × acid level significant, it is assumed that a similar effect is present in January and that those interactions not in brackets are the most plausible. The means for these interactions are given in Table 6. In January, without sugar addition, Suntan is rated higher than East Malling A 3022 or Bramley's Seedling, whereas with the addition of sugar Bramley's Seedling is rated higher than East Malling A 3022. Without sugar, stored fruit slices were rated higher than frozen ones, but the difference between the two methods was not significant when sugar was added. The addition of acid also does not significantly alter ratings without sugar, but the combination of both sugar and acid is rated higher than the others.

As with the November assessments, cultivar, although showing large objective differences, does not seem very important in hedonic assessments, the

Table 6. Overall hedonic scores. Tables of means of significant interaction effects (January 1980)

	Sugar addition	
Cultivar	Without	With
Bramley's Seedling	3.95	6.39
A 3022	4.28	5.81
Suntan	4.77	6.08
s.e.d.*	0.230	
Storage	Without	With
Stored	4.94	6.28
Frozen	3.73	5.91
s.e.d.*	0.188	
Acid addition	Without	With
Without	4.41	5.76
With	4.26	6.43
s.e.d.*	0.188	

* Standard error of the difference of two means.

combination of added sugar and acid having the most effect. Following storage, slices prepared freshly from stored fruit rather than prepared early in the season and frozen, also rated higher.

Relative importance of attributes to overall acceptability of slices

In order to get an indication of the relative overall significance panelists were attaching to both hedonic and specific objective attributes, the canonical variate scores from both the objective assessments and general attribute ratings were correlated with overall ratings. With respect to the objective scores obtained in November, overall ratings correlated highly with the third canonical variate (−0.870) but not with the first and second. The third canonical variate is composed mainly of sweetness and apple character with some contribution from acidity but very little from visual or textural attributes (Table 2). This would seem to indicate that it is flavour which is most important, samples with both additions being most preferred.

In the case of the hedonic variates, only variate 1 was highly correlated with overall rating (+0.890). This again is largely a flavour component (Table 3), stressing the importance paid by the panelists to this property when evaluating the acceptability of the slices.

In January the first hedonic canonical variate correlated highly (+0.969) with overall rating. As in November assessments, this variate has little contribution from appearance and is largely a combination of flavour and textural ratings. Within the January data set overall quality does not correlate particularly highly

with any of the objective canonical variates, the highest being the fourth variate (+0.630). Multiple linear regression on the first two canonical variates, however, accounted for 87% of the variance. Repeating this run on original objective attributes, 92% of the variance was accounted for by the three attributes of sweetness, apple character and firmness. These three attributes were relatively uncorrelated. The addition of acidity, which was highly correlated with sweetness, did not significantly improve the relationship.

The conclusions to be drawn here are similar to those for November. Flavour is of more importance to acceptability than appearance, but in the January sample texture is equally important, there being a far greater range under consideration.

Conclusions

Whether dealing with objective or hedonic data, the results of the sensory assessment showed that the experimental factors interacted with each other, a change in one factor altering the effect of another.

Sugar and acid additions had a marked effect on all cultivars, especially when both were added. For overall acceptability, adding sugar only had more effect than adding acid alone, but for the assessments in November–January, adding both had the most effect. The method of storage of the apples had a significant effect on sensory characteristics in January irrespective of cultivar, slices prepared from stored fruit being firmer and less watery than frozen slices prepared in November. Acceptability of the slices was greater for stored fruit than frozen, although this is rather more apparent when sugar has not been added to the steeping solution.

In general, Bramley's Seedling, being whiter in colour and naturally more acid, appeared to be distinguishable from the other two cultivars irrespective of sugar/acid levels or blanching time. East Malling A 3022 and Suntan were similar in their sensory characteristics, the effect due to sugar and acid additions swamping any cultivar differences. In January, frozen slices from Bramley's Seedling, as well as being distinguishable on the basis of appearance and acidity, were less firm than slices similarly prepared from East Malling A 3022 and Suntan. Of the experimental variables investigated in January, variation in blanching time had the least effect on the sensory characteristics.

The hedonic information reported in this paper must be treated with caution as it is based only on the opinions of a relatively small number of people who cannot be considered as representative of the population as a whole. Although the samples presented to the assessors represented a wide range of sensory characteristics with respect to the appearance, flavour and texture of the apples, there also exists the possibility that conclusions drawn are dependent on this variation and hence care should be taken when extrapolating outside the sample set examined. Despite these reservations the results indicate that preferences were exhibited for the samples to which both sugar and acid had been added and, in the case of slices examined in January, for those prepared

freshly from stored fruit rather than prepared in November and then frozen, especially if sugar had not been added. It would appear too that although a clear objective difference can be detected between cultivars on the grounds of appearance, this is not significant when it comes to making a judgement on the acceptability of the slices, flavour and texture attributes being far more important. Both these experiments also indicated a desire for high levels of acidity and sweetness, this being more important to acceptability than any inherent flavour character of the cultivar.

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A new type of semi-hard cheese from recombined milk

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Summary

Technological aspects for achieving a suitable technique for making a new semi-hard cheese from pasteurized recombined milk were studied. The manufacturing procedure was basically set on setting temperature of 35°C, rennet powder quantity of 8 g/100 kg milk, curd cubes size of 1¼ in, scalding for 15 min at 40°C and pressing at 150 lb/in^2 for 3 hr. The resulting cheese is named 'Ain Shams' and is characterized by a mild and slightly acidic flavour, tender body and very slightly open texture. The new cheese contains 40% moisture and 40% fat/dry matter.

Introduction

Many developing countries are suffering from a great shortage in fluid milk supply and dairy products. About 75% of the world's population lives in underdeveloped and developing countries and those with limited milk supplies (Upadhyay, Dave & Vyas, 1973). In most of those countries, recombined milk is useful to cover needs of growing dairy industries. The difficulties encountered during curdling of recombined milk can be overcome by the use of a large amount of rennet, adding calcium chloride, higher setting and/or scalding temperatures as well as longer processing time (Kemeny, 1959; Peters & Williams, 1962; Robertson, Dixon & Nowers, 1973).

In Egypt, the national milk supply is assigned to markets as liquid milk and fermented milks, and a relatively low percentage is left for cheese manufacture; therefore extensive studies have been lately devoted to that industry. The present work was planned to investigate the technological requirements for making a national type of semi-hard cheese from recombined milk, characterized by slightly acidic taste, mild flavour, tender body and very slightly open texture.

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Materials and methods

Materials

Milk. Recombined milk was prepared by mixing spray dried skimmilk, made in Ireland, and butteroil (France) with fresh water at 45°C, then homogenized using a two-stage homogenizer (2000 lb/in^2 and 500 lb/in^2), followed by heat treatment at 82°C for 15 sec, using an HTST pasteurization unit (Table 1). The pasteurized recombined milk was aged at 5°C for 16–18 hr. Aging was a result of several trials to attain a curd containing the most suitable quantity of moisture.

Starter cultures. Pure cultures of *Streptococcus lactis* and *Lactobacillus bulgaricus* (Hansen Lab., Denmark) were propagated separately in sterilized reconstituted skimmilk at 30°C (*S. lactis*) and 38°C (*L. bulgaricus*) for 16–18 hr, and well mixed at the rate of 1:1 just prior to adding to cheese milk.

Rennet, annatto and salts. Animal rennet powder (Ha-La, Hansen Lab.) of 3.2 standard strength; annatto colour (Hansen Lab.); commercial fine grade salt (NaCl) and pure fine calcium chloride were used.

Experimental procedures

Five factors affecting the physical and chemical properties of the resulting curd and hence the cheese were studied separately and successively. The factors studied were:

(a) *Setting temperature.* Four setting temperatures (30, 32, 35 and 37°C) were studied. Starter cultures, calcium chloride and annatto were added at the rates of 0.5% and 0.002%, respectively. As the milk acidity became 0.19, rennet was added at the rate of 4 g/100 kg milk. The milk was thoroughly mixed, and 100 ml were immediately transferred into a beaker and examined for coagulation time (Fahmi, 1952), curd tension (Chandrasekara *et al.*, 1975) and syneresis (Lawrence, 1959). Sixty min after adding rennet, curd tension and syneresis were determined. The whey collected for syneresis test was measured after 60 min.

(b) *Rennet quantity.* The previous experiment was carried out with setting temperature of 35°C and five different concentrations of rennet, which were 4, 5, 6, 7 and 8 g/100 kg milk.

Table 1. Gross composition of pasteurized recombined milk

Component	Minimum (%)	Maximum (%)	Average (%)
Milk fat	2.90	3.20	3.05
Solids not fat	8.40	8.70	8.55
Casein	2.04	2.30	2.17
Casein/fat ratio	0.70	0.72	0.71
Titratable acidity	0.12	0.14	0.13

(c) *Cutting procedure.* Two trials for cheesemaking from pasteurized recombined milk were carried out at 35°C with the addition of 0.002% annatto colour, 0.02% $CaCl_2$, 0.5% starter culture and 7 g rennet powder/100 kg milk. Coagulation time was approximately 50 min. The resulting curd was cut with American curd knives, to give cubes of two sizes. In the first vat, the cube size was ¾ in, and 1¼ in in the second. Scalding was done by raising the temperature gradually to 40°C within 1 hr, with continuous stirring, thereafter the acidity of whey reached 0.12%. The curd was then drawn to one end of the vat and the whey was drawn off. A slight pressure was applied to the curd to help the whey to run off easily and for matting the curd cubes together. The curd was then filled in perforated aluminium moulds which were covered and put into a press for 4 hr with direct pressure of 150 lb/in^2. The moulds were inverted, hourly. Thereafter, the green cheese was salted in a 20% brine for 40 hr at 10°C. The cheese was then placed for 2 days in a preripening room at 12°C with 90% relative humidity (r.h.). Samples of the resulting fresh cheese were taken and examined for moisture, fat and titratable acidity (British Standard, 1951). The appearance, body and texture were also examined.

(d) *Scalding technique.* A semi-hard cheese was made, applying the preferable results (setting temperature of 35°C, rennet quantity of 8 g/100 kg milk and curd size of 1¼ in) achieved during the previous experiments, and subjected to three different scalding techniques which were continuous moderate stirring accompanied by: (i) raising the temperature gradually during 60 min to 40°C (regular technique); (ii) raising the temperature gradually within 30 min to 40°C; and (iii) raising the temperature gradually during 15 min to 40°C, followed by mild stirring for another 15 min at the same temperature (40°C). Thereafter, the whey was drawn off with gathering the curd cubes. The remaining procedures were applied as before. Samples of fresh cheese were examined for appearance, body, texture, moisture, fat and titratable acidity.

(e) *Pressing time.* The cheese was made according to the preferable procedures (setting temperature of 35°C, rennet quantity of 8 g/100 kg milk, cube size of 1¼ in and the scalding technique (iii) (see above) previously accomplished. The resulting curd was filled in moulds and pressed for 4, 3.5 and 3 hr under pressure of 150 lb/in^2. Samples were examined for appearance, body, texture, moisture, fat and titratable acidity.

The resulting cheese was examined for moisture, fat, protein, salt, and titratable acidity (British Standard, 1951).

Also, essential minerals by flame photometer and titration methods (Black *et al.*, 1965), essential amino acids (Moore *et al.*, 1958) and essential fatty acids by G.L.C. type 'Varian 3700', were determined. Finally, costs of production were calculated.

Results and discussion

Data in Table 2 show that high setting temperature increased the rate of coagulation reaction, i.e. reduced the coagulation time of the milk. Also, the

curd tension increased with raising the setting temperature. The curd resulting from recombined milk at 30°C was weak and tended to break. However, raising the setting temperature to 35°C increased the curd tension which became nearly similar to that recorded for fresh cow's milk. On the other hand, increasing the setting temperature over 35°C rendered the curd firmer than the control. Furthermore, syneresis of the curd resulting from recombined milk at 30°C was less than that produced from fresh cow's milk. Increasing the setting temperature to 35°C produced a curd from the recombined milk with firmness resembling that of the control. However, raising the setting temperature to 37°C caused further increase in the curd tension and syneresis. These results proved that using the setting temperature of 35°C to coagulate the pasteurized recombined milk tended to achieve coagulation time, curd tension and syneresis nearly similar to those recorded for the fresh cow's milk at 30°C. Therefore, the setting temperature of 35°C was selected for coagulating the pasteurized recombined milk.

Results in Table 3 show that increasing the amount of the rennet added to the recombined milk markedly decreased the coagulation time as well as increasing the curd tension and the syneresis. However, adding rennet at 7 g/100 kg milk formed a curd possessing firmness and a syneresis property nearly similar to those of the fresh milk. Accordingly, 7 g of rennet powder was the preferable amount to coagulate 100 kg of pasteurized recombined milk and to obtain a desirable curd. These results coincide with Collins (1951), Kemeny (1959) and Peters & Williams (1962) who reported that added rennet must be increased in the case of using reconstituted milk for making cottage, cheddar and semi-hard cheeses.

Table 4 represents the moisture, fat in dry matter and titratable acidity of the cheese made with applying two different cubes sizes. These results show that these measurements in the cheese made from curd cubes with size of 1¼ in were higher than those in the cheese made from cubes of ¾ in. This agrees with Davis (1965). Besides, treatment II tended to give a semi-hard cheese with better body and texture as well as it being slightly more compact than in treatment I. Therefore, cutting the curd into cubes of 1¼ in was chosen to obtain green cheese with good properties.

In addition, both moisture and acidity of the fresh cheese were higher in the cheese made through the scalding technique (iii) than the two others (see Table 5). However, the fat content in the cheese of the technique (iii) was slightly less than those of the two others. This could be attributed to the increase occurring in the moisture content of the cheese as the scalding time was reduced. In fact, the fat in dry matter in the third cheese was slightly higher than that in the two other cheeses. This agrees with the observations of Davis (1965) who stated that the rapid formation of a protein film surrounding the curd cubes during scalding decreased the loss of fat and moisture out of the curd cubes. This was almost done during the third scalding technique (iii). Moreover, that technique gave fresh semi-hard cheese with proper moisture and fat contents as well as good body and texture.

Table 2. Effect of setting temperature on curdling of pasteurized recombined milk

Setting temperature (°C)	Coagulation time (min)	Curd tension (g)	Syneresis (%)
30*	30.08	11.82	31.6
30	37.27	5.10	23.6
32	35.45	6.93	26.0
35	29.12	9.78	28.2
37	27.25	13.65	33.6

* Pasteurized cow milk.

Table 3. Effect of rennet quantity on curdling of pasteurized recombined milk

Rennet Quantity (g/100 kg)	Coagulation time (min)	Curd tension (g)	Syneresis (%)
4	29.12	9.78	28.2
5	24.42	10.21	29.1
6	19.33	10.91	30.6
7	17.50	12.00	31.9
8	15.42	13.50	36.0

Table 4. Effect of curd cutting procedure on some properties of fresh semi-hard cheese made from pasteurized recombined milk

Curd cubes Size (in)	Titratable acidity (%)	Moisture content (%)	Fat content (%)	Fat/dry matter (%)	Body and texture
¾	0.80	40.7	23.6	39.3	Slightly tough and compact
1¼	0.86	43.4	22.9	40.5	Slightly hard and slightly compact

Table 5. Effect of scalding technique on some properties of fresh semi-hard cheese made from pasteurized recombined milk

Scalding technique	Titratable acidity (%)	Moisture content (%)	Fat content (%)	Fat/dry matter (%)	Body and texture
i	0.86	43.4	22.9	40.5	Slightly hard and slightly compact
ii	0.89	45.3	22.4	41.0	Better than (i)
iii	0.96	47.3	21.8	41.4	Semi-hard and slightly compact

Table 6. Effect of pressing time on some properties of fresh semi-hard cheese made from pasteurized recombined milk

Pressing time (hr)	Titratable acidity (%)	Moisture content (%)	Fat content (%)	Fat/dry matter (%)	Body and texture
4	0.96	47.3	21.8	41.4	Semi-hard and slightly compact
3.5	1.02	48.9	21.0	41.1	Better than 4 hr
3	1.06	50.6	20.3	41.1	Elastic, tender and very slightly open

Moreover, the results indicated that the moisture content and titratable acidity of the fresh cheese increased as the pressing time was decreased (Table 6). However, the fat content showed a slight decrease with decreasing the pressing time. This may be due to the higher moisture content retained in the cheese pressed for a short time. Nevertheless, fat in dry matter did not alter with changes in the pressing time. These results are in accordance with those mentioned by Robertson *et al.* (1973) for Gouda cheese as well as Dvorak, Minarik & Dolezalek, (1974) and Kurmann, Gehriger & Kaufman, (1975) for Emmental cheese.

In addition, cheese pressed for 4 hr had a slightly hard body and compact texture. However, decreasing the pressing time to 3 hr tended to give cheese with elastic body and slightly compact texture and characterized by a desirable tenderness. This may be attributed to the decrease noticed for drainage of whey during pressing for a short time. These results revealed that the preferable pressing time for this cheese type was 3 hr, under pressure of 150 lb/in^2.

Table 7. The gross composition of full ripened cheese aged 3 months at 12°C, with relative humidity of 90%

Major constituents (%)		Essential minerals (%)		Essential amino acids (g/100 g protein)		Essential fatty acids (%)	
Moisture	40.0±0.5	Calcium	1.100	Leucine	9.60	Linoleic	2.53
Protein	28.0±0.5	Phosphorus	0.325	Lysine	8.08	Linolenic	0.86
Fat	24.0±0.5	Sodium	0.966	Valine	6.42	Arachidonic	
Fat/dry matter	40.0±0.5	Potassium	0.663	Phenylalanine	5.15	not	
Salt	2.0±0.1			Histidine	2.78	detected.	
Acidity	1.8±0.05			Isoleucine	5.44		
				Methionine	2.01		
				Threonine	4.17		
				Arginine	3.57		
				Tryptophan	4.63		

Conclusion

A manufacturing procedure was established for making a new type of semi-hard cheese from pasteurized recombined milk. The resulting cheese is named 'Ain Shams'. Both manufacturing procedure and product name are registered as a patent. Ain Shams semi-hard cheese contains 40% moisture and 40% fat in dry matter, and is characterized by a mild and slightly acidic flavour with desirable

Table 8. Value of direct materials and wages needed for producing 1 ton of recombined milk, and to manufacture into cheese

Item of direct costs	Quantity (kg)	Unit price (LE)	Total price (LE)
Materials:			
Skimmilk powder	85.00	1.05	89.25
Butteroil	30.00	2.70	81.00
Rennet powder	0.07	50.0	3.50
Starter culture	5.00	0.30	1.50
Salt (NaCl)	20.00	0.03	0.60
Annatto colour	0.02	10.00	0.20
Calcium chloride	0.20	1.00	0.20
Total value			176.25
Wages:			
Time required=8 hr			
Rate of wage/day=LE 2.80 (7 hr/day)			
Rate of wage/hr=LE 0.40			
Wage value for 8 hr=LE 3.20			

Table 9. Costs value of 1 kg of Ain Shams cheese*

Description	Value (LE)
Direct costs	
Materials	176.25
Wages	3.20
Indirect costs	
Overhead charges (200% of direct wages)	6.40
Total Costs	185.85
Costs value for 1 kg of cheese	1.4868

* One ton of pasteurized recombined milk gave 150 kg of fresh semi-hard cheese, which became 125 after 3 months of ripening, i.e. 1 ton of recombined milk yielded 125 kg of cheese aged 3 months.

tender body and very slightly open texture. The gross composition of Ain Shams cheese is presented in Table 7. According to the international prices of ingredients, costs of production of 1 kg of Ain Shams cheese aged 3 months are LE 1.4868 (Tables 8 and 9).

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Extrusion of defatted soy flour-hydrocolloid mixtures

Effect of operating parameters on selected textural and physical properties

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Summary

Defatted soy flour containing low levels of added hydrocolloid (either sodium alginate or methylcellulose) was thermoextruded in a laboratory single-screw extruder, and the effect of operating parameters on selected textural properties (maximum peak force, determined in an Ottawa Measuring (OTMS) cell, and chewiness) and physical properties (bulk density and water absorption capacity) were studied. Adding sodium alginate generally increased OTMS peak force, chewiness and water absorption capacity and increased bulk density. Extruding at a feed moisture content of 22% w/w resulted in the best properties, regardless of the levels of other independent variables. Maximum force and chewiness were positively correlated while bulk density and water absorption capacity were negatively correlated to each other.

Introduction

Textured vegetable protein products are expected to assume greater importance and acceptance as a means of lowering the cost and improving the availability of protein. One promising route seems to be the development of simulated meat products or substitutes from oilseeds such as soybeans. In order to produce a chewy gel whose mouthfeel simulates meat after cooking, these meat analogues or extenders must be adequately texturized with a fibrous structure. Extrusion seems to be the method of choice in the food industry for texturization of soy proteins. Extrusion cooking of defatted soy flour or concentrates produces a chewy, structured extrudate which has been found suitable as a meat extender for ground meats. These chewy bits are used (after rehydration) by the school lunch programme in the United States and is believed to save $30 million/year (Smith & Ben-Gera, 1980).

It may be necessary sometimes to include texture modifiers such as gums, egg albumin and wheat gluten to help 'bind' protein filaments together to enhance

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chewability and strength of the rehydrated and cooked extender. Among the binders used to date, very little has been published on the use of hydrocolloids such as sodium alginate and methylcellulose for the production of soy protein analogues. Hydrocolloids are water-soluble or water-dispersible polysaccharides that function essentially by providing water control (Igoe, 1982) although some interaction with other food components, such as proteins, should occur during processing. This paper describes a study of the effect of low levels of these hydrocolloids and extruder operating parameters on selected textural properties of an extruded meat extender produced from defatted soy flour. For comparison purposes, textural properties of some commercial soy meat extenders and ground beef have also been determined.

Materials and methods

Materials

Defatted soy flour (Soyafluff-200W) was obtained from Central Soya Company, Fort Wayne, Indiana, U.S.A. Moisture content was 9.53% and protein content (N×6.25) was 53.0% (dry basis).

Hydrocolloids studied were an alginate and methylcellulose. The alginate was Kelgin MV (medium viscosity) neutral sodium alginate manufactured by Kelco, a division of Merck & Co., Inc., Chicago, Illinois. It was in the form of creamy-tan coloured free-flowing granules with a bland taste and no aroma. It contained 1.2% residual calcium. A 2% w/v solution of this hydrocolloid has a viscosity of 600 cp at 25°C.

Methylcellulose (Methocel A4M) was obtained from Dow Chemical Co., Midland, Michigan, U.S.A. It contains 27–32% methoxyl groups and has a viscosity of 4000 cp at 20°C at 2% w/v concentration.

Table 1. Properties of commercial texturized soy flour products and ground beef*

	Mira-Tex 210	TVP 240	Bontrae	Supro 50-2	Ground beef
Manufacturer	A. E. Staley Co. Decatur, IL	ADM Co., Decatur, IL	Central Soya Chicago, IL	Ralston-Purina St Louis, MO	
Protein† (Nx6.25)%	50.0	52.0	50.0	55.0	18.6
Carbohydrate† (%)	32.0	31.5	32.0	28.5	
Moisture (%)	5.5^{d}	8.2^{b}	7.4^{c}	4.9^{d}	60.2^{a}
Max. force (kg)	19.4^{b}	18.5^{c}	17.8^{d}	17.7^{d}	43.8^{a}
Chewiness (cm^2)	25.4^{b}	25.2^{b}	19.6^{c}	19.3^{c}	62.3^{a}
Bulk density (g/L)	332.1^{b}	390.2^{a}	286.2^{c}	254.7^{d}	
WAC (%)	293.1^{c}	243.1^{d}	311.4^{b}	381.1^{a}	

* Values in the same row with the same letter are not significantly different at the 5% level.
† Manufacturer's specifications.

Commercial samples used in this study for comparative purposes are listed in Table 1. All were sieved to −5/+8 mesh size (U.S. Standard) before being used in our study.

Manufacture of extruded soy flour-hydrocolloid meat analogue

(a) Preparation of mix. Five pounds of defatted soy flour were weighed into a Blakeslee mixer. The required amount of hydrocolloid was added and the mixture dry-blended for 5 min at speed 1. The appropriate amount of water needed to bring the final moisture content to the required value was added slowly while mixing continuously. Care was taken to avoid lumping. Mixing continued for another 5 min. Each lot of mix was then bagged and stored overnight at 4°C for equilibration prior to extrusion the next day.

(b) Thermoextrusion. A Wenger X-5 laboratory extruder (Wenger Manufacturing Company, Sabetha, Kansa, U.S.A.) was used for the manufacture of the products. It has a single screw (2.5 cm in diameter and 39 cm in length) and a die of 0.36 cm diameter. Feed flow rate was approximately 10.8 kg/hr. The extrudates were allowed to cool to room temperature, bagged and stored at 4°C before grinding in a Burr mill to a size that would pass a No. 5 sieve and remain on a No. 8 sieve. The mix was equilibrated to 28°C before extrusion.

Experimental design

Definition of the five variables studied and their levels used is shown in Table 2. The selection of these variables and their levels were based on preliminary experiments and on the basis of previous work by Harper (1979), Park (1976) and Aguilera & Kosikowski (1976), as being most likely to affect physical and textural properties of the extrudate. Since data from small laboratory extruders such as the Wenger X-5 are difficult to scale up (Harper, 1979), no attempt was made in this study to 'optimize' these variables. At each level of the hydrocolloids, there are twelve possible combinations of steam pressure, screw

Table 2. Definition and levels of experimental variables

		Levels		
Variable	Units	1	2	3
Steam pressure	kPa	268	435	536
Screw speed	RPM	650	800	
Feed moisture	% w/w	22	25	
Sodium alginate	% w/w	0	0.5	1.0
Methylcellulose	% w/w	0	0.5	1.0

speed and feed moisture. Samples containing the hydrocolloid were run in duplicate. There were twelve runs with no hydrocolloids that served as controls.

Analytical methods

Moisture content of the extrudates were determined by the AOAC (1980) method. Bulk density was determined by tap packing the extrudate granules to the 100 ml mark of a graduated measuring cylinder. Ten taps each were given at the 20, 40, 60, 80 and 100 ml of the cylinder. The volume was noted and its weight determined. Bulk density is reported as weight per unit volume (g/l).

Water absorption capacity (WAC) was determined as follows: Twenty gram of extrudate granules were hydrated with 300 gm tap water at 28°C for 1 hr in a covered beaker. The slurry was manually stirred at 15 min intervals. The slurry was then drained on a No. 8 sieve for 3 min and the rehydrated granules weighed. Water absorption capacity is expressed as:

$$\text{WAC } (\%) = (\text{weight gain/dry weight}) \times 100. \quad (1)$$

Texture measurements

Samples for texture measurements were prepared according to the Taranto *et al.* (1975) with some modifications. One hundred gram of the extrudate granules (sieved to $-5/+8$ mesh particles size) were hydrated in 300 gm tap water at 28°C for 1 hr with stirring at 15 min intervals. After hydration, the samples were placed in a plain tin can, sealed and retorted at 121°C for 30 min. The cans were cooled and allowed to equilibrate to room temperature prior to texture measurement. Ground beef samples were not hydrated but were given the same heat treatment.

Texture measurements were made in a Instron Universal Testing Machine (Model 100 kg CTM) using a 20 cm^2 Ottawa Texture Measuring System test cell. Ten 22 gm aliquots of each retorted extrudate sample were compressed-extruded. Data reported are means of twenty readings (ten for each of the two replicates) for hydrocolloid-containing samples and means of ten readings for the control samples. The following test parameters gave well-defined force-deformation curves: full-scale load=20 kg, chart speed=10 cm/min, and loading rate=5 cm/min. The compression plunger was 4.3 cm in diameter (flat head). The compression head was stopped 1.5 mm from the metal grid, as suggested by Taranto *et al.* (1975). The depth of the cell was 13.5 mm, thus giving a 88.9% deformation.

Of the seven textural parameters used by Breene & Barker (1975) (hardness, cohesivness, extrudability, chewiness, maximum force, average maximum force and packability), only two (maximum force and chewiness) were found useful in our work. By definition, maximum force is the greatest force in kg obtained during extrusion and is measued as the maximum height of the

force-deformation curve. Chewiness is the energy required to masticate a solid product to a state ready for swallowing. It is expressed as the area (cm^2) under the force-deformation curve and represents the energy used to compress, shear and extrude the sample. Frazier *et al.* (1980) also claimed to obtain the most reliable data on rehydrated and retorted extrudates with the OTMS test cell used for peak force measurement.

Statistical analysis of data

Data were subjected to analysis of variance and *F*-tests were applied to test for significant differences using the SAS program at the University of Illinois Computing Services (Boison, 1982). Differences between means were evaluated using Duncan's multiple range test.

Results and discussion

Moisture content

Table 3 shows the residual moisture content for the extruded granules. In general, increasing steam pressure, decreasing feed moisture and increasing hydrocolloid concentration significantly decreased residual moisture. Screw speed had no effect with Methocel-containing TVP but did affect residual moisture of alginate-TVP. Thus those conditions resulting in more intense heat treatment resulted in lower residual moisture. Molina *et al.* (1978) came to a similar conclusion. The effect of the hydrocolloid is not clearly understood. The commercial TVP samples were observed to be much lower in moisture content (Table 1), but that is probably due to a post-extrusion drying step prior to packaging rather than due to extruder conditions *per se*.

Maximum force

Maximum force gives an indication of a sample's resistance to compression and extrusion through the cell. The higher the maximum force, the higher the

Table 3. Effect of steam pressure, screw speed, feed moisture content and hydrocolloid concentration on residual moisture content (%) of extruded TVP samples containing either alginate or Methocel as the hydrocolloid*

Variable	Steam pressure		Screw speed		Feed moisture		Hydrocolloid	
level†	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel
1	12.13[a]	12.32[a]	11.38[b]	11.70[a]	11.82[b]	11.13[b]	12.36[a]	12.36[a]
2	11.54[b]	11.68[b]	12.47[a]	11.60[a]	12.02[a]	12.14[a]	11.50[b]	11.73[b]
3	10.89[c]	10.90[c]					11.13[c]	11.18[c]

* Means in the same column with the same letter are not significantly different at the 5% level.
† Levels of variables given in Table 2.

Table 4. Maximum force (kg) of TVP samples*

Variable level†	Steam pressure		Screw speed		Feed moisture		Hydrocolloid	
	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel
1	12.65^{c}	11.05^{b}	14.80^{a}	11.47^{a}	14.60^{a}	12.47^{a}	12.41^{c}	12.41^{a}
2	14.06^{b}	10.69^{c}	12.58^{b}	10.87^{b}	12.78^{b}	9.88^{b}	12.78^{b}	11.03^{b}
3	14.36^{a}	11.78^{a}					15.24^{a}	10.69^{c}

* Means in the same column with the same letter are not significantly different at the 5% level.

† Levels of variables given in Table 2.

resistance and the more 'texturized' or 'tougher' the material. Table 4 is a tabulation of the mean maximum force values for the experimental TVP extrudates. As far as the alginate-TVP samples are concerned, increasing steam pressure, decreasing screw speed, decreasing feed moisture and increasing alginate concentration resulted in higher maximum force, at least, within the range of parameters studied here. The effect of steam pressure (which affects processing temperature) and screw speed (which affects residence time) could be related to the degree of heat treatment and thus to the extent of soy protein denaturation. This in turn apparently results in more 'twisting' of protein strands and increased fibrillation in the protein matrix which results in a 'tougher' sample. These effects are in general agreement with the work of Cegla *et al.* (1978) and Aguilera & Kosikowski (1976).

Increasing feed moisture significantly reduced maximum force. In fact, most of the Methocel-TVP samples containing 25% moisture disintegrated during Instron testing, especially at the highest Methocel concentration. Park (1976) and Aguilera & Kosikowski (1976) reported that feed moisture significantly reduced texturization as a result of less moisture flashoff as the sample exits from the die.

The effect of hydrocolloid concentration is different for alginate and for Methocel (Table 4). The linear high-molecular-weight sodium alginate

Table 5. Chewiness (cm^2) of TVP samples*

Variable level†	Steam pressure		Screw speed		Feed moisture		Hydrocolloid	
	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel
1	11.75^{c}	11.22^{b}	12.94^{a}	11.95^{a}	14.21^{a}	12.95^{a}	12.65^{b}	12.65^{a}
2	12.10^{b}	11.42^{b}	12.90^{a}	11.90^{b}	11.63^{b}	10.09^{b}	11.88^{c}	11.72^{b}
3	14.92^{a}	11.90^{a}					14.09^{a}	10.71^{c}

* Means in the same column with the same letter are not significantly different at the 5% level.

† Levels of variables given in Table 2.

probably interacts with the unfolded soy proteins in the extruder to form protein-alginate strands which on autoclaving formed a more compact structure which resisted disintegration. Smith, Mitchell & Ledward (1982), suggested that hydrogen, hydrophobic and other weak interactions are involved in the alginate-soy flour system. With Methocel, on the other hand, the opposite could be happening. Aqueous solutions of Methocel gel on heating to as low as 50–55°C (Sarkar, 1979). If the Methocel itself is gelling inside the extruder, it could disrupt the continuous protein matrix necessary for good fibrillation, resulting in lower maximum force values with increasing Methocel concentration.

It is to be noted that, as a group, the alginate-TVP samples resulted in higher maximum force readings than Methocel-TVP samples, but almost none of the experimental samples (Table 4) were as good as the commercial TVP samples (Table 1); all TVP samples were much lower than ground beef.

Chewiness

Table 5 shows the mean chewiness values for the experimental samples. Increasing steam pressure and decreasing feed moisture increased chewiness, similar to the maximum force trends discussed earlier. Due to the nature of their definitions, it is not surprising that there should be a good correlation between maximum force and chewiness, as shown in Figure. 1. Note that the

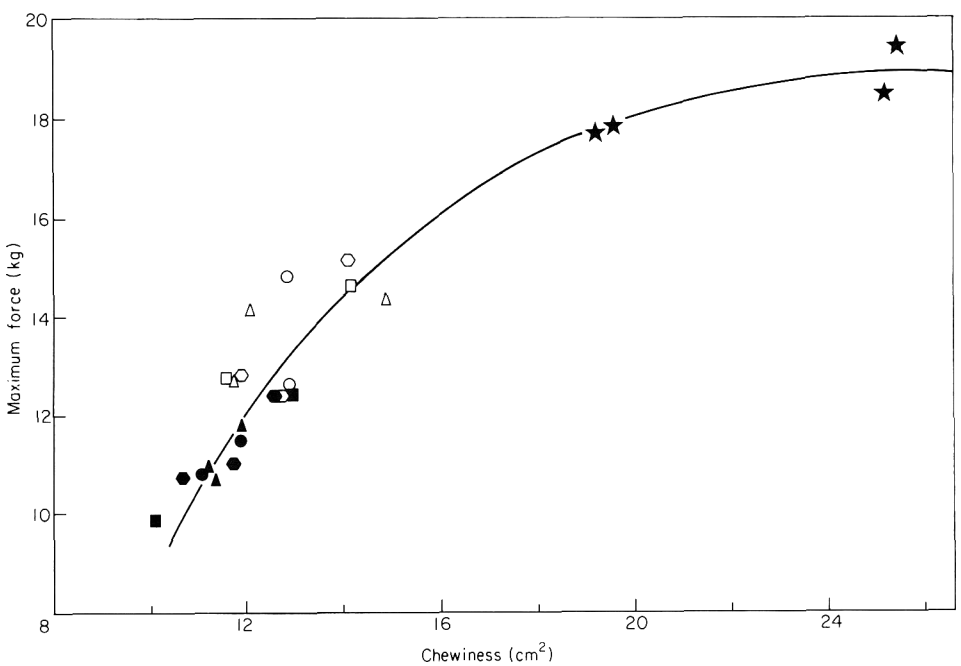

Figure 1. Correlation between chewiness and maximum force for commercial samples (★) and for experimental samples containing either alginate (△ ○ □ ⬡) or Methocel (▲ ● ■ ⬢) as the hydrocolloid.

Methocel-containing samples are grouped near the bottom of the graph while the commercial samples are on the higher end. Thus it appears that any operational adjustment that improves one property will improve the other, although the asymptotic nature of the curve indicates some limits to this concept.

Bulk density

Bulk density is a measure of how much expansion has occurred as a result of extrusion. The heat developed during extrusion can increase the temperature of the moisture above the boiling point so that when the extrudate exits from the die, a part of the moisture would quickly flash-off as steam and result in an expanded structure with large alveoli and low bulk density. On the other hand, if not enough heat is generated to flash-off enough of the moisture (either through low process temperature or high feed moisture), less expansion occurs giving a high bulk density product with collapsed cells which usually disintegrates on cooling. This extrudate structure is characterized by the presence of small sized cells.

According to Kinsella (1978), bulk density of an expanded porous TVP ranges from 160 to 240 g/l (10 to 15 lb/ft^3) and a more dense product is about 256 to 385 g/l (16 to 24 lb/ft^3). By this classification, the commercial samples (Table 1) are within the range of a 'more dense expanded TVP'. High bulk density is an indication of a more uniform and continuous protein matrix and, therefore, the extrudate is dense with parallel layers, no air pockets and is not spongy upon hydration (Taranto *et al.*, 1978). On the other hand, the experimental TVP samples could be characterized as expanded and porous (Table 6) due to the much lower bulk density values. High steam pressure increased moisture flash-off, thus increasing expansion and lowering bulk density. Similar effects of extrusion temperature on bulk density were reported by Molina *et al.* (1978).

Adding 1.0% sodium alginate to the defatted soy flour before extrusion significantly increased the bulk density, an effect similar to that obtained by Smith *et al.* (1982). A good correlation between bulk density (Table 6) and

Table 6. Bulk density (g/l) of TVP samples*

Variable	Steam pressure		Screw speed		Feed moisture		Hydrocolloid	
level†	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel
1	240.63^a	215.49^b	250.47^a	225.0^a	207.85^b	199.31^b	224.25^b	224.25^b
2	242.01^a	231.90^a	220.55^b	227.0^a	263.65^a	254.0^a	218.58^b	233.21^a
3	224.25^b	232.50^a					258.05^a	220.87^b

* Means in the same column with the same letter are not significantly different at the 5% level.

† Levels of variables given in Table 2.

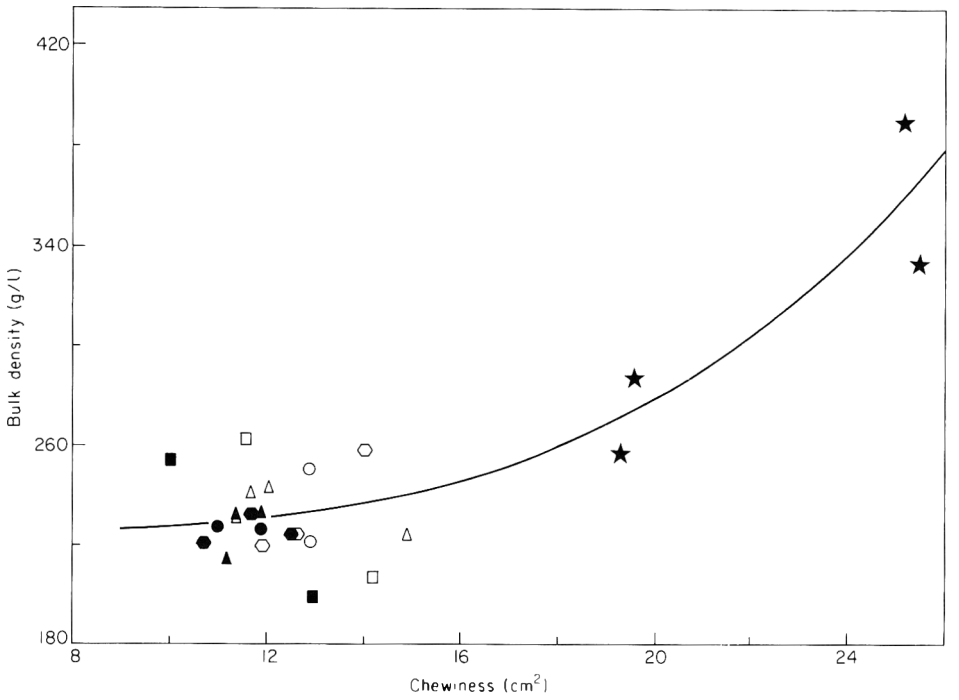

Figure 2. Correlation between chewiness and bulk density for commercial and experimental samples. Symbols have same meaning as in Figure 1.

maximum force (Table 4) can be seen, and since maximum force and chewiness are interrelated (Fig. 1), the correlation between bulk density and chewiness, as shown in Figure 2, is not surprising.

Feed moisture appears to have a tremendous influence on bulk density. A small change from 22% to 25% moisture dramatically increased bulk density of both alginate-TVP and Methocel-TVP (Table 6). Seiler, Weipert & Seibel (1980) also noted that a small change in feed moisture had a much larger effect on bulk density of extruded corn than changes in barrel temperature.

Water absorption capacity (WAC)

Water absorption capacity of TVP relates the amount of hydration to the internal porosity. It could be used as an index of the potential 'juiciness' of the cooked meat-soy blend. In general, the lower the bulk density, the higher the WAC because of the porosity of the structure. For the commercial samples (Table 1), the most dense (TVP-240) absorbed the least amount of water, while Supro 50-2 granules (the lowest bulk density) absorbed the most water. For our experimental samples (Table 7), adjusting steam pressure or screw speed did not significantly change WAC values for either alginate-TVP or Methocel-TVP, although we would have expected greater water uptake at higher steam pressure due to greater protein denaturation. Feed moisture and level of

Table 7. Water absorption capacity (%) of TVP samples*

Variable level†	Steam pressure		Screw speed		Feed moisture		Hydrocolloid	
	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel	Alginate	Methocel
1	300.31[a]	292.94[a]	295.03[a]	290.70[a]	324.33[a]	304.6[a]	273.88[b]	273.88[b]
2	298.31[a]	281.16[a]	309.75[a]	281.15[a]	280.45[b]	267.3[b]	315.41[a]	277.25[b]
3	308.55[a]	283.68[a]					303.63[a]	300.63[a]

* Means in the same column with the same letter are not significantly different at the 5% level.

† Levels of variables given in Table 2.

hydrocolloid does have an effect. The interrelationship between bulk density and water absorption capacity is shown in Figure 3. Similar relationships can be seen in the data of Molina *et al.* (1978).

Conclusions

Incorporating sodium alginate into the defatted soy flour before extrusion generally increased maximum force, chewiness and WAC and increased bulk density. However, adding methylcellulose in the form of Methocel-A4M

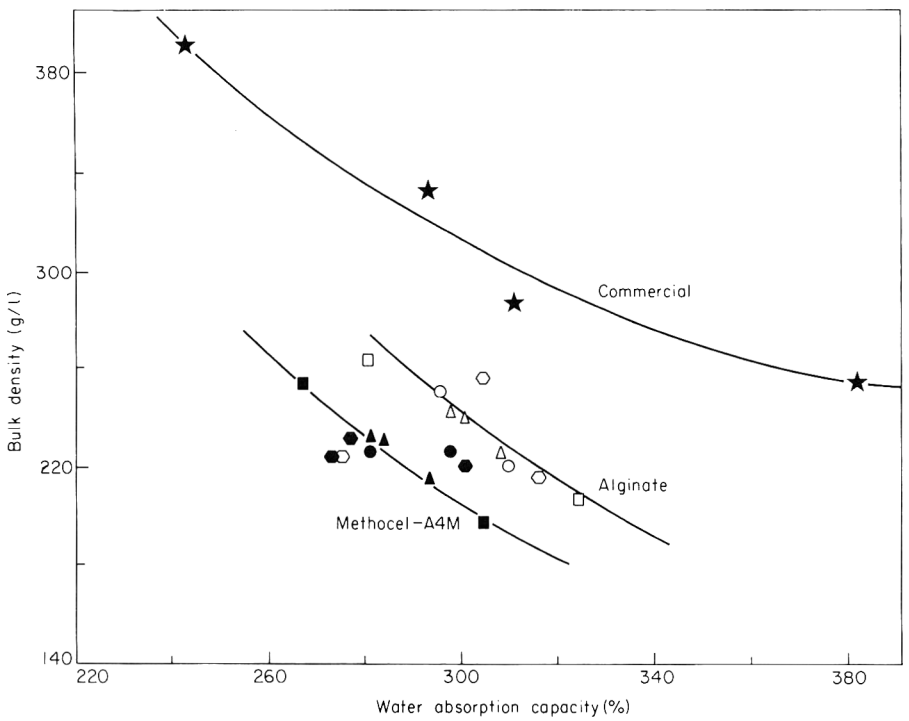

Figure 3. Correlation between WAC and bulk density for experimental and commercial samples. Symbols have same meaning as in Figure 1.

decreased maximum force and chewiness, indicating some structural weakening of the extrudate. It is possible that adding much higher than the 0.5–1.0% used in this study would have improved properties further. Higher steam pressures resulted in higher maximum force and chewiness, probably due to increased fibrillation of the protein matrix. Although our data appear to indicate that screw speed had little or no effect on most properties measured, this could be a reflection of the narrow range of screw speed used in our study (650 and 850 rev/min). Since screw speed affects residence time, it is possible that levels wider apart would have revealed more dramatic effects. Extruding at the lower feed moisture (22%) instead of 25% generally resulted in more desirable properties.

Some interesting correlations between the dependent variables were also observed:

—maximum force and chewiness were positively related;
—maximum force and residual moisture were negatively correlated;
—bulk density and chewiness were positively correlated;
—WAC and residual moisture were negatively correlated;
—WAC and bulk density were negatively correlated.

It is recognized that differences in extruder design and type, rather than process variables and feed formulation as studied here, could account for a substantial part of the differences between our samples and the commercial TVP. Harper (1979) and Kinsella (1978) have indicated that twin-screw or Uni-Tex extruders produce more dense, chewy products than would a single screw extruder such as the Wenger X-5. Nevertheless our data appears to suggest that incorporating hydrocolloids with the appropriate properties may be beneficial to the textural properties of thermo-extruded meat analogues made from oilseed flours.

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A study of the effects of frozen storage on certain functional properties of meat and fish protein

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Summary

This paper presents a study of the effects of frozen storage on certain functional properties, namely protein solubility, emulsifying capacity, and viscosity, both in meat and in fish.

Correlations between these three functional properties were examined in an attempt to find the easiest and most sensitive method of analysis.

High correlation among the different functional properties tested was found only in the case of fish. This, together with the significant variations in the viscosity values found during the storage period, suggests that the analysis of apparent viscosity is a valuable indicator method for determining alterations occurring in the functional characteristics of fish protein.

Introduction

The functional properties of proteins comprise all physico-chemical properties affecting the behaviour of such proteins during food processing.

The following are particularly important functional properties of meat and fish myosystems: protein homogenate solubility, emulsifying capacity and, to a lesser extent, viscosity.

The textural properties will depend largely on protein solubility, especially in the case of fish (Sikorski, 1977), though the emulsifying capacity is important in the case of meat and fish to be processed as sausage. There are many references in the relevant literature discussing the interrelationships between these properties; thus, Grabowska & Sikorski (1974) indicated that emulsifying capacity in fish depends on protein solubility and is more sensitive than solubility as a source of information on structural changes that take place in the protein molecules.

On the other hand, Carpenter & Saffle (1965) pointed to a close correlation between viscosity and emulsifying capacity. Froning (1976) likewise reported

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that viscosity in chicken can be considered indicative of the emulsifying properties.

However, it is generally acknowledged that the salt soluble protein fraction (basically the myofibrillar proteins) shows the strongest emulsifying properties. Gaska & Regenstein (1982) stated that the insoluble protein fraction (connective tissue and unextracted myofibrillar protein) in the presence of salts also plays an important role in the formation of emulsions, because the effect of salt on the hydration state of the protein and on viscosity may be stronger than its effect on solubility. Anyway, there exist discrepancies in this area that are undoubtedly due to the fact that foods are very complex chemical systems.

The purpose of the present study is to investigate the effect of frozen storage and thawing on certain functional properties, namely protein solubility, emulsifying capacity, and viscosity, in both meat and fish. An attempt is also made to establish correlations between these three functional properties, in order to be able to choose the simplest and most sensitive method of analysis for determining any changes that have taken place in the product.

Materials and methods

The food samples were purchased at a local market in September. The meat used was pork and chicken (breast) and the fish was blue whiting (*Micromesistius poutassou*, Risso) and horse mackerel (*Trachurus trachurus*, L.).

These types of muscle tissue were selected in order to investigate the different properties of myosystems exhibiting different behaviour in response to frozen storage.

After being brought back to the laboratory, the samples were minced in a meat mincing machine with plate orifices of 5 mm. Following this, 300 g lots were vacuum-sealed in plastic bags.

The samples were then frozen in a blast freezer at a temperature of −30°C and an air speed of 5 m/sec and kept in cold store at −20°C for 8 months. The samples were twice subjected to temperature variation once after the first month of storage and again after the seventh month; in both cases, the temperature in the thermal centre of the product was raised at night up to about −5°C, and on the following day it was lowered back down to −10°C. This was done to accelerate the changes in the characteristics of the proteins and thus make the alterations more evident.

The functional properties investigated were protein solubility (PS), emulsifying capacity (EC) and viscosity (V). The soluble protein losses were determined by the method of Ironside & Love (1958). The protein extract obtained by homogenizing 100 g of muscle with 400 ml of a 5% NaCl solution (pH 6.5–7.0, 4°C) for 2 min in an 'Omnimixer' homogenizer were analysed for EC and V.

The EC determination was performed three times using the method described by Jiménez-Colmenero & Garcia-Matamoros (1981), and the results

were expressed in g of oil/g of muscle (EC) and in g of oil/100 mg of soluble protein. The apparent viscosity was determined with a Brookfield rotational viscometer using the No. 2 rotor at a speed of 20 rev/min for fish and chicken muscle and a 1 rev/min for pork muscle. Viscosity readings were taken at least five times over a 20 min period, beginning 3 min after starting the rotor, in a temperature range of 5–7°C.

Each type of analysis was performed at five different times over the storage period (8 months) and repeated three times on each occasion.

The degree of significance between the mean values was determined by analysis of variance with an *F*-test.

Regression curves were calculated to clarify trends in the results of different functional tests, and the corresponding correlation coefficients were established. The degree of significance of the correlations were taken from the tables given by Lamotte (1981).

Results and discussion

The results for protein solubility, emulsifying capacity, and viscosity in the samples analysed through the experimental storage period are shown in Table 1. The initial soluble protein levels were higher in fish than in meat, the highest values being found for fish in horse mackerel and for meat in pork. It can be seen that, throughout the storage period, the myofibrillar protein of meat was more stable than that of fish, as the decrease in solubility is less pronounced, so that in the case of chicken it remained constant and showed no significant difference during the eight months of storage.

Of the fish, horse mackerel generally maintained the largest amounts of soluble protein and, therefore, the greatest stability, whereas blue whiting showed the highest degree of denaturation and protein aggregation. The differences in protein stability between lean and semi-fatty fishes, such as blue whiting and horse mackerel respectively, have been ascribed to the protective effect exerted by neutral lipids in response to the insolubilizing action of free fatty acids (FFA) on the proteins. While in lean fishes (blue whiting) there are no neutral lipids capable of counteracting the action of the FFA, the semi-fatty fishes (horse mackerel) do contain these lipids in sufficient concentration and adequately distributed so that they can act (Labuza, 1971).

Pork muscle initially showed EC values lower than, but very similar to, those in horse mackerel, and chicken showed the highest values, both initially and throughout the storage period (Table 1). In all the samples the EC decreased as the frozen storage time increased, and this phenomenon was more marked in blue whiting and horse mackerel and less evident in pork muscle. The change in the EC could, to a great extent, be due to the loss of protein solubility, as suggested by the fact that storage affects both parameters in the same manner. Grabowska & Sikorski (1974) suggested that the EC decrease is due to denaturation induced by the freezing process.

The emulsifying capacity expressed in g of oil/100 mg of soluble protein

Table 1. Mean results obtained for the different samples over storage

Sample	Analysis	Days in storage 0	30	60	150	240
Horse mackerel	PS	78.9a	54.3b	41.9c	38.3d	30.2e
	EC	48.2a	36.7b	35.5bc	36.9b	33.9c
	V	3926a	1760b	814c	198d	39e
Blue whiting	PS	73.5a	42.9b	40.3c	43.5b	25.5d
	EC	53.0a	34.7b	33.4b	34.9b	30.1c
	V	4533a	1502b	353c	153d	15e
Chicken	PS	57.9a	57.7a	57.8a	58.8a	55.6a
	EC	58.1a	48.9bc	45.7b	49.6c	47.8bc
	V	2979a	3398b	3596b	3408ab	3363b
Pork	PS	60.0a	48.4b	54.9c	43.4d	40.0e
	EC	46.0a	44.1a	41.5b	40.6b	39.4b
	V	76666a	77083a	70300ab	65255b	46750c

The means for each type of analysis marked by the same letter are not significantly different ($P>0.05$).

PS (protein solubility) expresed as percent soluble protein on total protein.

EC (emulsifying capacity) expressed in g of oil/g of muscle.

V (viscosity) expressed in cps.

(Table 2) increased with storage time, the opposite of what happened when it is expressed in g of oil/g of sample, as already seen. The decrease in protein solubility leads to a reduction in the concentration of soluble protein in the medium; this dilution causes an increase in the amount of oil emulsified/unit protein. The interdependence between the protein concentration and the emulsified fat, for which both linear (Carpenter & Saffle, 1964; Bello, Ripoll & Larralde, 1978) and curvilinear (Hegarty, Bratzler & Pearson, 1963; Trautman, 1964) relationships have been found, seemed to resemble, under the test conditions, the latter (curvilinear) relationships in the case of fish and the former (linear) in the case of pork.

This behaviour can be linked to differences in the interface films due to the different soluble protein concentrations in the medium (Graham & Phillips,

Table 2. Emulsifying capacity in g of oil/100 mg of soluble protein

Sample	Days in storage 0	30	60	150	240
Horse mackerel	34.75	38.48	48.27	54.79	63.85
Blue whiting	46.95	52.64	54.04	52.29	76.86
Chicken	52.92	44.61	41.61	44.43	45.25
Pork	42.88	50.94	42.27	52.31	55.17

1976), which, according to Bello *et al.* (1978) determine the magnitude of the emulsifying capacity of the system.

In a biological system, with its highly complex medium, there must necessarily be many factors affecting the relationship between EC and PS; however, in the present instance, when the emulsifying capacity is compared at similar protein concentrations, no important differences were found in the emulsified oil in the different samples once the effect of dilution had been obviated, which seems to suggest that the emulsifying properties of the soluble proteins are similar in horse mackerel, blue whiting, pork and chicken.

The highest viscosity values were found in pork, substantially higher than in the rest of the samples. Except in the case of chicken muscle, in which viscosity remained constant, frozen storage resulted in significant decreases in the viscosity of the protein extracts; this decrease was linear for the pork and exponential for the fish. Although viscosity depends on many parameters, trends in this property can generally be related to changes in protein solubility, as will be seen later.

When the significant differences between the values for the different functional properties obtained over the storage period and given in Table 1 are compared, it is found that the changes undergone by the proteins are most clearly reflected by the trends in protein solubility and viscosity.

Table 3 presents the correlation coefficients between the functional properties investigated. There was a highly significant correlation between PS and EC in fish, but this was not the case for chicken and pork. Grabowska & Sikorski (1974) observed a similar phenomenon in cod minces reporting that emulsifying capacity and protein solubility, which are significantly correlated (r=0.85−0.95), decreased due to denaturation and protein aggregation induced by frozen storage.

In the present experiment no significant correlations were found for these two parameters in chicken and pork, corresponding with the results reported by Ockerman & Crespo (1981) for homogenates of beef.

Table 3. Correlation coefficients between the functional properties evaluated

Analysis	Sample	Emulsif. capacity	Viscosity
Soluble protein	Horse mackerel	0.882*	0.988*
	Blue whiting	0.912*	0.925*
	Chicken	0.036	0.129
	Pork	0.728	0.763*
Emulsifying capacity	Horse mackerel		0.923*
	Blue whiting		0.954*
	Chicken		0.668
	Pork		0.145

* Significant at 99%.

Highly significant correlations between protein solubility and viscosity were detected in the case of fish and pork but not in the case of chicken (Table 3). Although viscosity is determined by a number of factors, such as pH, ionic strength, and temperature, the primary factor affecting it is the protein concentration. Thus, in the relevant literature there are data recording the existence of various direct relationships between these two parameters (Kinsella, 1976; Hutton & Campbell, 1977), as well as the dependence of viscosity on molecular interactions and protein aggregation (Nakayama *et al.*, 1979). Nevertheless, on occasion it has not been possible to establish significant correlations (Ockerman & Crespo, 1981).

Emulsifying capacity and viscosity were significantly correlated in horse mackerel and blue whiting (Table 3). Since changes undergone by these functional properties are to a great extent determined by the same factors, it seems logical that there should be such close correlations between them. Although Carpenter & Saffle (1965) found a strong correlation between these parameters (r=0.97), in the present experiment no such high correlation was found in pork or chicken.

It can generally be said that, while significant correlations can be established between protein solubility, emulsifying capacity, and viscosity in fish, the same does not hold true in the case of meat. Among the different factors conditioning emulsifying capacity and viscosity, the main one is soluble protein concentration, which decreases throughout the frozen storage period as a result of protein denaturation and aggregation. Since soluble protein concentration shows greater variation in fish than in meat due to differences in the stability of the proteins in these products, the decrease in protein solubility might be the determinant of the variations detected in emulsifying capacity and viscosity. The same cannot be said, however, for meat, in which, due to lower levels of protein denaturation and aggregation, the combined action of other factors masks the effect of variations in concentration of soluble proteins.

In the case of fish the strong correlations found between the different functional properties tested (Table 3), as well as the variations in viscosity values during the storage period, which cause the values of apparent viscosity obtained in each control to be significantly different from all the others (Table 1), seem to suggest that, among the functional properties tested, apparent viscosity most clearly reflects the changes undergone by the proteins.

In view of the foregoing discussion, it would appear that further research should be undertaken on this point to apply the apparent viscosity test to other fish species of differing characteristics, in order to confirm its usefulness as a routine analysis for quality control in the case of frozen fish.

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Isohalic sorption isotherms. III. Application to a dried salted tropical fish (*Xenomugil thoburni*)

C. A. CURRAN* and R. G. POULTER

Summary

The sorption behaviour of a dried salted tropical fish, lisa (*Xenomugil thoburni*), produced under semi-commercial conditions was investigated. It was found to be similar to that of freeze-dried cod (*Gadus morrhua*) published earlier. The results confirm that the procedure for predicting the water activity of cod muscle from the physical properties of sodium chloride solution and the sorption characteristics of fish muscle can also be applied to tropical fish processed commercially.

Introduction

The microbial stability of salted and dried food products depends on their water activity (a_w) (Scott, 1957). This is a measure of the free or available water in a food which is able to react chemically or, in spoilage, to support the growth of micro-organisms, such as bacteria and moulds (Waterman, 1976).

The relationship between a_w and moisture content in a food is often expressed as a sorption isotherm but the sodium chloride content must also be taken into consideration when dealing with dried salted products. The first paper in this series (Doe *et al.*, 1982) determined isohalic sorption isotherms for dried salted cod (*Gadus morrhua*) and provided a simple means of calculating the a_w of such a product from its moisture and salt contents. The effects of hysteresis were also discussed in this paper. The isotherms for cod were then used in conjunction with growth data for the dun mould, *Wallemia sebi,* to predict the shelf life of dried salted fish in tropical climates (Poulter, Doe & Olley, 1982). The measured shelf-lives of several fish species processed and stored by traditional methods were found to be in agreement with the predictions.

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However, a direct comparison of the isohalic sorption isotherms of cod with other species of fish and the effect of drying temperature on the isotherms have not been studied. This paper seeks to make such a comparison and presents the sorption behaviour of a tropical species, lisa (*Xenomugil thoburni*), which had been salted by different methods and dried at different temperatures under semi-commercial conditions. It may be noted that in previous work (Poulter, Curran & Disney, 1981) temperate fish such as cod have been found to differ from tropical fish in certain characteristics.

Materials and methods

Fish species

Lisa (*X. thoburni*) are a marine species. The samples used in this study were caught by commercial fishing boats off the Galapagos Islands of Ecuador.

Processing methods

The fish were split along the backbone according to local custom, gutted, carefully washed and the flesh scored. Two methods of salting were used: dry salting and brining. Half of the fish (10 kg) were soaked in 10% brine for 30 min and then packed in dry salt (1 part salt: 3 parts fish) and left overnight (21 hr). The remaining fish were simply immersed in a saturated brine for 1 hr. After salting was completed, the fish were washed and allowed to drain. These are not necessarily the optimum procedures for salting fish but were adopted in order to obtain a traditional type of product.

Two drying methods, sun drying and solar drying, were used for each group of salted lisa and, in each case, drying was continued until the samples had attained constant weight. During sun drying, the fish were dried on racks for approximately 6 days during which time the average ambient temperature was 30°C within a range of 26–33°C. For the solar dried samples, the fish were placed in a solar tent dryer (Doe *et al.*, 1977) for approximately 4 days; the average temperature was 50°C within a range of 27–60°C. This produced four groups of dried salted fish:

1 Dry salted, sun dried lisa;
2 Dry salted, solar dried lisa;
3 Brined, sun dried lisa; and
4 Brined, solar dried lisa.

Proximate analysis

Each group of fish was analysed for moisture and crude fat as described by Poulter *et al.* (1982). Sodium chloride content was determined using

ammonium thiocyanate and silver nitrate with ammonium ferrous sulphate as indicator (AOAC, 1965).

Water activity measurements

The flesh of the dried salted fish was cut into small cubes (5 mm^3) and freeze dried. The samples were then equilibrated over saturated solutions of various salts of a_w in the range 0.07–0.90 at a temperature of 27°C in partially evacuated desiccators (Doe *et al.*, 1982). When the samples reached a constant weight, they were analysed in duplicate for moisture and sodium chloride. The moisture content was determined by the method mentioned earlier and the salt content by the silver nitrate method using potassium chromate as indicator (Pearson, 1970).

Results and discussion

Table 1 gives the results of the initial proximate analysis of the four groups of dried salted lisa. The results of the measured moisture (M_w) and salt (M_s) contents of the equilibrated samples at various a_w are presented in Table 2; the fat (M_f) content at equilibrium (calculated from the results of the initial proximate analysis) and the salt-free, fat-free, dry mass (M_b) are also given.

The data were used to calculate the moisture contents on a dry mass basis (M_w/M_b) for each sample; the sorption isotherms for each group of fish were then constructed as shown in Figure 1. The isotherms for the four groups of fish at a_w below 0.75 are very similar but, above this value, they begin to diverge.

Doe *et al.* (1982) suggested that the a_w of dried salted fish can be expressed as:

$$a_w = a_{wo} \cdot a_{wn} \qquad (1)$$

where a_{wn} is the water activity of the sodium chloride solution present in the fish sample and a_{wo} is the water activity of the fish muscle (i.e. salt-free, fat-free, solid fraction). A saturated sodium chloride solution has an a_w of 0.75 and this explains why the isotherms in Figure 1 fall into two parts. As shown in Table 3, below an a_w of 0.75, the a_{wn} of the samples is constant and the reduction in a_w as M_w/M_b decreases is due to a reduction in a_{wo}. Above an a_w of 0.75, however, a_{wo} is constant at about 1.0 and the a_{wn} increases as M_w/M_b increases.

Table 1. Proximate analyses of dried salted lisa

Group	% Moisture	% Salt	% Fat
1 Dry salted, sun dried lisa	10.80	32.32	1.07
2. Dry salted, solar dried lisa	11.28	28.07	1.59
3 Brined, sun dried lisa	15.70	9.66	2.54
4 Brined, solar dried lisa	9.56	16.58	3.99

Table 2. Sorption data for dried salted lisa

	Group 1 Dry salted, sun dried lisa				Group 2 Dry salted, solar dried lisa				Group 3 Brined, sun dried lisa				Group 4 Brined, solar dried lisa			
a_w	M_w	M_s	M_f	M_b	M_w	M_s	M_f	M_b	M_w	M_s	M_f	M_b	M_w	M_s	M_f	M_b
0.07	1.64	40.79	1.18	56.39	3.06	38.93	1.74	56.27	4.81	16.89	2.87	75.43	4.19	16.64	4.23	74.94
0.11	2.51	40.71	1.17	55.61	3.07	32.21	1.74	62.98	5.12	15.11	2.86	76.91	5.11	16.63	4.19	74.07
0.22	4.83	38.40	1.14	55.63	4.30	30.91	1.72	63.07	6.58	15.14	2.82	75.46	6.24	18.47	4.14	71.15
0.33	6.11	32.58	1.13	60.18	5.37	35.86	1.70	57.07	7.95	15.50	2.77	73.78	7.33	17.77	4.09	70.81
0.44	7.20	33.88	1.11	57.81	6.56	32.08	1.68	59.68	9.46	14.00	2.73	73.81	8.73	17.71	4.03	69.53
0.54	6.66	41.17	1.12	51.05	7.42	28.90	1.66	62.02	11.53	16.81	2.67	68.99	10.24	15.38	3.96	70.42
0.57	12.03	36.84	1.06	50.07	9.03	34.12	1.63	55.22	12.63	14.59	2.63	70.15	11.80	18.36	3.89	69.95
0.62	9.34	41.08	1.09	48.49	12.34	30.12	1.57	55.97	14.55	14.16	2.58	68.71	13.14	15.99	3.83	67.04
0.65	10.05	40.32	1.08	48.55	10.79	30.12	1.60	57.49	15.33	14.64	2.55	67.48	13.24	11.62	3.83	71.31
0.68	12.84	37.80	1.05	48.31	14.85	31.03	1.53	52.59	18.35	15.27	2.46	63.92	15.85	13.87	3.71	66.57
0.71	17.01	31.55	1.00	50.44	14.89	28.66	1.53	54.92	24.79	13.74	2.27	59.20	23.96	14.31	3.36	58.37
0.73/4	22.35	33.09	0.93	43.63	26.04	28.29	1.33	44.34	30.42	11.93	2.10	55.55	29.93	11.79	3.09	55.19
0.75	36.95	22.05	0.76	40.24	39.41	29.48	1.09	30.02	32.10	11.64	2.05	54.21	32.16	12.83	2.99	52.02
0.80	50.45	17.77	0.59	31.19	51.24	16.60	0.87	31.29	38.76	10.94	1.85	48.45	40.42	11.50	2.63	45.45
0.85	57.19	18.16	0.51	24.14	54.34	15.30	0.82	29.53	41.50	10.18	1.76	46.56	41.81	10.62	2.57	45.00
0.90	61.28	13.57	0.47	24.68	58.97	12.58	0.74	27.71	47.37	8.88	1.59	42.16	55.58	10.15	1.96	32.31

M_w: moisture content.
M_s: salt content.
M_f: fat content.
M_b: salt-free, fat-free, dry mass.

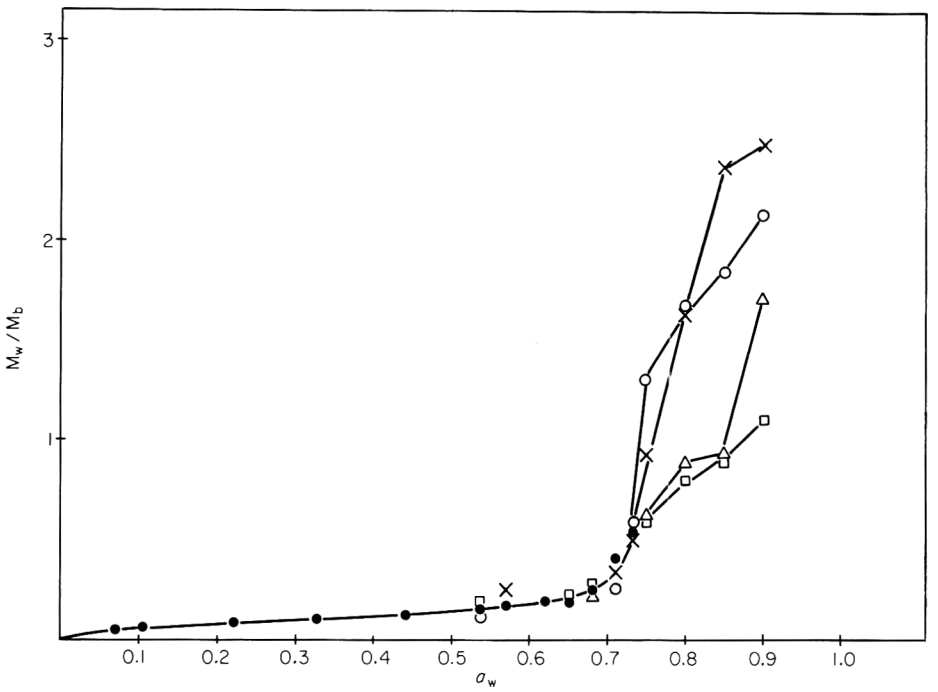

Figure 1. Isohalic sorption isotherms for dried salted lisa. ×, Group 1, ○, Group 2, □, Group 3, △, Group 4. For sake of clarity, the black symbol ● denotes where the symbols *not shown* hit either directly on, or very close to, the same co-ordinate.

The deviation from a smooth curve for any one treatment in the points above a_w 0.75 is explained by variations in the measured salt contents (M_s/M_b). In theory, these values should be constant for each sample and, hence, the curve in Figure 1 should represent the isohalic isotherms for that salt level; Doe *et al.* (1982) give theoretical curves for cod. However, when dried salted fish are produced under semi-commercial conditions, as was the case during this study, there is a degree of uneven salt penetration and distribution, particularly in dry salted samples, and this leads to variation in measured salt content and hence a_{wn}. At high a_w, particularly for the heavily salted samples (1 and 2), excess surface water created sampling problems resulting in apparent a_{wo} values of 1.1.

Since it is known that in some cases the characteristics of tropical fish differ from those of temperate species (Poulter *et al.*, 1981), part of the aim of this study was to compare the sorption isotherms obtained for dried salted lisa, a tropical fish, processed under semi-commercial conditions with those obtained previously for unsalted cod, a temperate water fish, freeze dried in the laboratory. In order to do this, it is advantageous to remove the effect of the salt in the sample, i.e. to calculate and compare the a_{wo} of the samples. The a_{wo} data for lisa given in Table 3 have been plotted on the curve for the a_{wo} values of unsalted cod muscle (Figure 2) obtained by Doe *et al.* (1982)

Table 3. Calculation of a_{wo} for dried salted lisa

a_w		0.07	0.11	0.22	0.33	0.44	0.54	0.57	0.62	0.65	0.68	0.71	0.73/4	0.75	0.80	0.85	0.90
Group 1:	M_w/M_b	0.03	0.05	0.09	0.10	0.12	0.13	0.24	0.19	0.21	0.27	0.34	0.51	0.92	1.62	2.37	2.48
	a_{wn}	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.76	0.78	0.86
	a_{wo}	0.09	0.15	0.29	0.44	0.59	0.72	0.76	0.82	0.86	0.91	0.95	0.97	1.0	1.1	1.1	1.1
Group 2:	M_w/M_b	0.05	0.05	0.07	0.09	0.11	0.12	0.16	0.22	0.19	0.28	0.27	0.59	1.31	1.64	1.84	2.13
	a_{wn}	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.78	0.81	0.86
	a_{wo}	0.09	0.15	0.29	0.44	0.59	0.72	0.76	0.82	0.86	0.91	0.95	0.97	1.0	1.1	1.1	1.1
Group 3:	M_w/M_b	0.06	0.07	0.09	0.11	0.13	0.17	0.18	0.21	0.23	0.29	0.42	0.55	0.59	0.80	0.89	1.12
	a_{wn}	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.81	0.84	0.89
	a_{wo}	0.09	0.15	0.29	0.44	0.59	0.72	0.76	0.82	0.86	0.91	0.95	0.97	1.0	0.99	1.0	1.0
Group 4:	M_w/M_b	0.06	0.07	0.09	0.10	0.13	0.15	0.18	0.20	0.19	0.24	0.41	0.54	0.62	0.89	0.93	1.72
	a_{wn}	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.81	0.84	0.89
	a_{wo}	0.09	0.15	0.29	0.44	0.59	0.72	0.76	0.82	0.86	0.91	0.95	0.97	1.0	0.99	1.0	1.0

M_w/M_b: moisture content, dry mass basis.
a_{wn}: water activity due to salt content.
a_{wo}: water activity of lisa muscle (salt-free, fat-free solid matter).

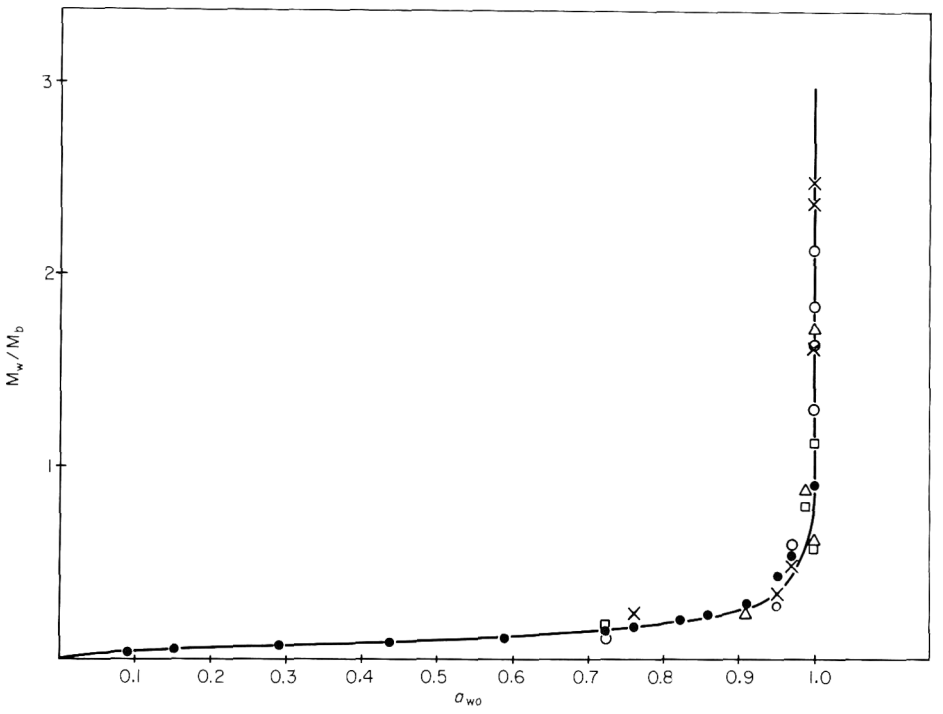

Figure 2. Sorption isotherm for unsalted, freeze dried cod with a_{wo} data for lisa. ×, Group 1, ○, Group 2, □, Group 3, △, Group 4. For sake of clarity, the black symbol ● denotes where the symbols *not shown* hit either directly or, or very close to, the same co-ordinate.

and it may be seen that there is excellent agreement between the four sets of lisa samples and the cod muscle samples.

These results suggest that the sorption characteristics of the muscle from tropical fish species are very similar to that of cod and that the salt concentration and drying temperatures which would be found under commercial processing conditions in the tropics do not affect the results. However, previous work by Iglesias & Chirife (1976) on precooked beef and Tarr (1945), Shewan (1953), and Cutting, Reay & Shewan (1956) on cooked fish has shown that the temperature of the samples during drying affected the isotherms obtained. It may be that, under commercial sun or solar drying conditions, the fish are not subject to time/temperature regimes which are severe enough to cause changes. Work is continuing on this aspect at the Tropical Development and Research Institute.

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Functional aspects of blood plasma proteins. I. Separation and characterization

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Summary

Porcine plasma proteins were fractionated by ion-exchange chromatography on DEAE-Sephadex using continuous ionic gradient elution. The separation was scaled up to produce gram quantities of three fractions, by stepwise elution. Subsequent diafiltration resulted in fractions of high protein content. The whole plasma, serum and the resultant fractions, and commercially available dried plasma, were characterized by polyacrylamide gel electrophoresis.

Introduction

With the rise in world population it is logical to make the maximum use of the byproducts of the meat industry. Moreover a number of these cause serious pollution problems.

Byproducts, such as blood, are generally nutritionally sound (Delaney, 1975) although it is apparent that whole blood has limited use because of the red colour, and strong metallic flavour, imparted by the haemoglobin of the red cells. In recent years methods to separate blood into plasma and red cell fractions by centrifugation have been established commercially (Akers, 1973; Halliday, 1973). Plasma thus recovered has been incorporated successfully in meat products (Ranken, 1977; Schut, 1968) and certain bakery items (Brooks & Ratcliff, 1959; Khan, Rooney & Dill, 1979; Johnson, Havel & Hoseney, 1979).

Plasma comprises a diverse range of molecular protein species. These exhibit various physico-chemical properties which presumably would be reflected in correspondingly different functional properties. Fractionation of porcine plasma proteins was attempted in the present study to ascertain whether the functionality of certain components was superior or inferior to that of others.

In selecting a separation method, the current commercial procedures for plasma fractionation, and those attempted in laboratories by other workers, were considered. Although variations of the Cohn fractionation method (Cohn

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et al., 1946; Sandberg, 1977) are used worldwide to produce plasma fractions for use in clinical medicine, the need for low temperatures and solvents such as ethanol make the method prohibitively expensive for the manufacture of food proteins and it is not always practicable. Again, gel permeation chromatography, which is a useful method for the separation of proteins on a small scale (Donnelly & Delaney, 1977) cannot be scaled up without an unacceptable increase in the time required for the operation. In contrast, ion-exchange chromatography can be usefully applied to the separation of large quantities of protein components.

In the present investigation, the ion exchanger DEAE-Sephadex was selected because of its high binding capacity, especially at high ionic strength. The latter resulted from the presence of citrate, required to prevent the precipitation of the plasma protein fibrinogen. (Due to the problem of the precipitation of fibrinogen most studies hitherto have been carried out on blood serum and not on plasma.)

Materials and methods

Materials

Porcine plasma was obtained from the Meat Research Institute (MRI), Langford. Blood was collected from Landrace or Large White pigs using clean bleeding tubes and sodium citrate anticoagulant (added at a level of 4 g/l). Plasma was separated from the red cells by centrifuging the blood at 14 000 *g* at 4°C in a Sharpless continuous centrifuge previously washed with an isotonic solution, 0.85% (w/v) sodium chloride, to prevent haemolysis. The resultant plasma fraction was stored at −18°C for subsequent tests and used either in a thawed liquid form or freeze dried.

Laboratory freeze dried porcine serum was made by dialysing liquid porcine plasma in Visking tubing against distilled water overnight. Serum was separated from the precipitated fibrinogen by centrifugation at 700 *g* for 5 min and freeze dried.

Commercially dried porcine plasma (Regalbumin 26) and bovine plasma (Regalbumin A24) powders were obtained from Regal Food Ltd, Dublin.

The protein content of the following powders are given in parentheses (see also Table 1): freeze dried porcine plasma (MRI) (69%), commercial Regalbumin porcine plasma (70%), commercial Regalbumin bovine plasma (70%), commercial Regalbumin bovine plasma (70%). From Miles Laboratories, U.S.A., the following standards (protein content figures supplied by manufacturer): albumin fraction V (95%); porcine γ-globulin fraction IV (98%); bovine β-globulins (88%); bovine α-globulins (73%); bovine fibrinogen (77%). Selected fractions from the ion-exchange chromatography of plasma and plasma fractions I, II and III. The following marker proteins for sodium dodecyl sulphate (SDS) electrophoresis from Sigma London Chemical Company Ltd: cytochrome-*c*, ovalbumin, catalase, bovine serum albumin, pepsin and phosphorylase-*a*; from BDH Chemicals Ltd, lysozyme.

The following buffers:

1 ion-exchange chromatography buffer: 0.1 M tris-citrate, pH 8.0;
2 stock buffer for SDS-electrophoresis: 0.25 M sodium barbitone and 0.1% (w/v) SDS, pH 8.6;
3 stock buffer for electrophoresis on 7% polyacrylamide gel rods: 0.062 M trisglycine, pH 8.6; and
4 tank buffer for electrophoresis on 7% polyacrylamide gel rods: 0.0062 M tris glycine, pH 8.6.

Compositional analysis of plasma proteins

The following properties were determined on five samples of liquid plasma (MRI) and of freeze dried plasma (MRI) and on two samples each of Regalbumin porcine and of bovine dried plasma. AOAC methods (Horwitz, 1970) were used to determine moisture, crude protein, ash and fats.

Moisture. Three gram samples were dried in a vacuum oven at 100°C for 18 hr.

Crude protein. 3.5 g (liquid plasma) or 0.5 g (dried plasma) were digested by micro-kjeldahl and protein calculated using a protein conversion factor of N × 6.25 (Delaney, 1975).

Ash. Twenty gram (liquid plasma) or 10 g (dried plasma) samples were heated at 550°C in an electric muffle furnace.

Fat. Soxhlet extraction procedure, using petroleum either bp 40–60° (AR) was applied to 10 g of dried sample.

Citrate. This was determined by enzymic and spectrophotometric methods, using a Boehringer Mannheim citrate test kit.

Sodium ions. These were measured on a flame emission atomic absorption spectrometer IL151 at 330.2 nm (flame air/acetylene).

pH. This was measured by glass electrode on 6% plasma solutions in distilled water or on liquid plasma.

Microbiological status. This was assessed by using a serial dilution in 1/4 strength Ringer's solution on plate count agar (Oxoid: 3½ in single vent Petri dishes). After incubation at 37°C for 2 days a total count was made.

Chromatographic separation of porcine plasma

Porcine plasma proteins were separated in a Wrights 60 ml chromatographic column packed with an anion exchanger, DEAE-Sephadex A-50.

To equilibrate, the ion exchanger was swollen in 0.5 M tri-sodium citrate in distilled water for 2 days and then suspended in the ion-exchange-chromatography buffer (0.1 M tris-citrate, pH 8.0). Microbial growth was prevented by adding 0.002% (v/v) Hibitane (chlorohexidine). The binding capacity of the ion exchanger was determined according to the manufacturer's instructions; 1.6 ml of liquid porcine plasma, diluted with 2 ml of the starting

buffer (0.1 M tris-citrate, pH 8.0) was pumped on to the column. A continuous ionic gradient (0–0.3 M NaCl) was achieved using a gradient mixer (Pharmacia). The plasma proteins were eluted at a flow rate of 12 ml/hr and their optical density was recorded on a LKB Unicord detector at 277 nm. Fractions were collected on the LKB fraction collector for subsequent analysis.

Having established the separation pattern by a continuous ionic gradient elution, the process was accelerated by using the following five-step change in ionic strength of the ion-exchange chromatography buffer to elute the desired peaks:

1 buffer;
2 buffer + 0.05 M NaCl;
3 buffer + 0.075 M NaCl;
4 buffer + 0.15 M NaCl;
5 buffer + 0.3 M NaCl.

Macroscale batch segregation

Porcine plasma was fractionated on a large scale by stepwise elution using three steps following binding on DEAE-Sephadex A-50; 500 ml of the swollen ion exchanger was placed in a sintered glass funnel and 150 ml liquid plasma (diluted with 150 ml ion-exchanger-chromatography buffer) was stirred into the ion exchanger. After 1 hr, and after each consecutive hr, three fractions (designated I, II and III) were eluted using buffer, buffer + 0.05 M NaCl, and buffer + 0.3 M NaCl respectively.

Each fraction (1 litre) was diafiltered in an Amicon Thin Channel Ultrafiltration Unit, against 18 litres of distilled water at a flow rate of 1 l/hr (Amicon membrane PM 10, outlet air pressure, 30 psi; inlet nitrogen pressure, 10 psi). Approximately 1 litre of the desalted, concentrated protein fraction was obtained and freeze dried. Compositional analysis and electrophoretic characterization of the resultant fractions were carried out.

Electrophoretic characterization of plasma protein

Electrophoresis on 8% polyacrylamide gel (PAGE) slabs with sodium dodecyl sulphate (SDS). Liquid porcine plasma (MRI) (25% w/v), dried plasma samples and the plasma protein standards (1% w/v) were dissolved in the extracting buffer containing 3% (w/v) SDS and 1% (w/v) β-mercaptoethanol and incubated at 30°C overnight. Sucrose (20% w/v) was added prior to electrophoresis.

A thin layer vertical slab polyacrylamide gel was used similar to that described by Parsons *et al.* (1969). The apparatus was constructed from a design by Akroyd (1967).

The gel slab was composed of two types of gel, an 8% (w/v) running gel, making up 60 mm of the gel, and 3% (w/v) stacking gel, 15 mm in height,

polymerized on top of the running gel. The composition of the gels was as follows:

8% acrylamide running gel	
Cyanogum 41	6.4 g
1% (v/v) thioglycollic acid in buffer	4.0 ml
deaminopropionitrile (DMAPN)	210 μl
Stock buffer (0.25 M sodium barbitone and 0.1% (w/v) SDS, pH 8.6)	32.0 ml
water	42.0 ml
ammonium persulphate 7% (w/v) in buffer	1.0 ml
3% acrylamide stacking gel	
Cyanogum 41	2.8 g
1% (v/v) thioglycollic acid in buffer	4.0 ml
DMAPN	210 μl
Stock buffer	32.0 ml
water	42.0 ml
ammonium persulphate 7% (w/v) in buffer	0.4 ml

The space above the gel was filled with a layering solution containing 0.1% (w/v) SDS and 0.1% (v/v) β-mercaptoethanol in stock buffer. Both the lower and upper tanks were filled with stock buffer diluted 2.5 times with distilled water.

Pre-electrophoresis was conducted at 30 V for 25 min. Next the samples (10 μl) were applied for electrophoresis was performed at 30 V for 10 min followed by 100 V for 2 hr. The separate protein bands were stained for 16 hr with 0.5% (w/v) Coomassie brilliant blue in 40% (w/v) methanol and 1 M acetic acid in water. Destaining was achieved electrolytically in 7% (v/v) acetic acid for 5 hr. Densitograms of the stained protein bands were obtained using the Joyce Loebl Chromoscan (settings 0–2 OD; Cam A; aperture V; gain 3 and wavelength 550 nm).

The mobilities of the proteins were calculated from the densitometer tracings. Mobility was defined as the distance of the protein peak from the origin divided by the total length of the trace.

The molecular weights (MW) of the plasma proteins were estimated by comparing their electrophoretic mobility on SDS-PAGE to those of the marker proteins of known MW. a calibration graph of mobility versus log MW was constructed (Fig. 1) using the following proteins (their MW are given in parentheses): cytochrome-*c* (12 400), lysozyme (14 300), haemoglobin (15 500), trypsin (23 000), pepsin (35 000), ovalbumin (43 000), catalase (58 000), bovine serum albumin (68 000) and phosphorylase-*a* (100 000).

Electrophoresis on 7% polyacrylamide gel rods. In contrast to SDS-electrophoresis, in which both undenatured and denatured proteins are solubilized by reagents such as SDS and β-mercaptoethanol, the following method characterizes only water-soluble proteins which are undenatured and in

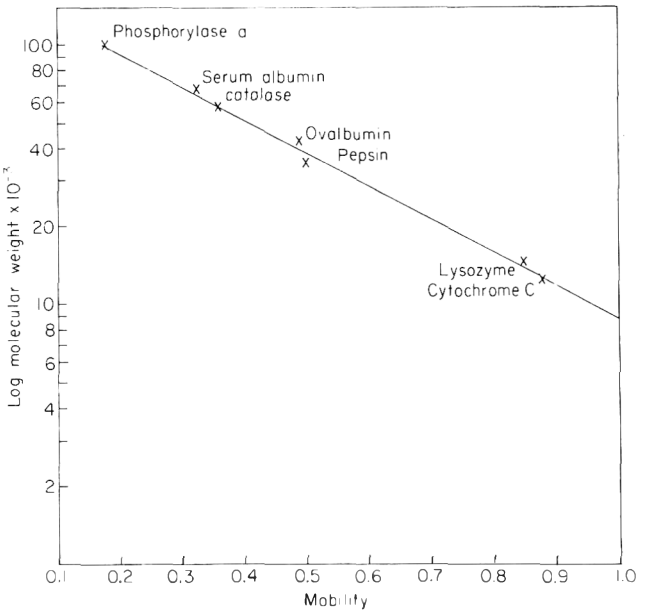

Figure 1. Plot of logarithm of the molecular weights of marker proteins, treated with SDS, against their mobilities on an 8% SDS polyacrylamide gel.

their native state. The soluble protein molecules migrate according to their net charge and molecular size leaving the larger denatured molecule aggregates at the surface of the gel rod. Thus the method permits an assessment of denaturation or change in the native state of the protein which may be perceived either as a reduction in the amount of protein migrating and therefore a reduction in the peak height in the densitogram or as a loss of resolution.

Polyacrylamide gels (7%) in 0.0062 M tris-glycine buffer pH 8.6 were made up as follows:

Cyanogum 41 (a mixture of acrylamide and NN methylene bisacrylamide (95:5 w/w)	3g
0.062 M tris-glycine buffer pH 8.6	20.0 ml
deaminopropionitrile (DMAPN) 2% (v/v) in the above buffer	10.0 ml
ammonium persulphate (w/v) in the above buffer	10.0 ml

Electrophoresis was carried out on gel rods in a Shandon apparatus according to the manufacturer's instructions (tank buffer; 0.0062 M tris-glycine buffer, pH 8.6; voltage 200 V; sample volume 10 μl; stain 1% Naphthalene Black 12B in acetic acid; destaining solution 7% acetic acid). The stained protein bands were scanned at 620 nm.

Results and discussion

Laboratory processed plasma, obtained from the Meat Research Institute, and the commercially produced porcine and bovine plasma were all found to be

Table 1. Compositional analysis of plasma protein samples

Source of plasma	Parameter							
	Protein % (w/w) (N×6.25)	Moisture % (w/w)	Ash % (w/w)	Sodium % (w/w)	Citrate % (w/w)	Lipid % (w/w)	pH of 6% (w/w) protein solution	Total bacterial count at 37°C
Porcine plasma (MRI)	6.8 (±0.1)*	91.0 (±1.0)	1.1 (±0.1)	0.5 (±1)	0.44 (±0.2)	0.15 (±0.01)	8.1 (±0.1)	390/ml to 4×10^3/ml
Freeze dried porcine plasma (MRI)	68.0 (±1.0)	9.2 (±0.4)	11.5 (±0.5)	5.2 (±0.2)	4.6 (±0.1)	2.0 (±0.2)	8.1 (±0.1)	3.8×10^3/g
Dried porcine plasma (Regalbumin)	70.0 (±1.0)	8.9 (±0.4)	11.8 (±0.4)	5.1 (±0.2)	4.1 (± 0.1)	1.5 (±0.5)	8.0 (±0.1)	10×10^3/g
Dried bovine plasma (Regalbumin)	70.0 (±1.0)	9.4 (±0.5)	10.3 (±0.5)	5.0 (±0.1)	4.0 (±0.05)	1.5 (±0.5)	8.0 (±0.1)	10×10^3/g

* Figures in brackets refer to standard deviations based on five replicates.

Table 2. Characterization of porcine plasma proteins by SDS-polyacrylamide gel electrophoresis

Protein	Peak No.	Mobility	Possible substance	Peak height (mm)	Molecular wt $\times 10^3$ Estimated	Published
Whole plasma (cf. Fig. 1a)	1	0.077		10	150	
	2	0.093		35	130	
	3	0.114	α-globulins	15	125	
	4	0.13		20	115	
	5	0.14		23	110	
	6	0.16		30	105	
	7	0.20	β-globulins	10	92	
	8	0.26	β-, γ-globulins	50	78	
	9	0.28	β-globulin (transferrin), α-globulin	63	76	76.5
	10	0.325	albumin	148	68	65–69
	11	0.35	fibrinogen, α-, β-globulins	97	60	
	12	0.37	γ-, α-globulins	90	56	
	13	0.40	β-globulins	18	52	
	14	0.43	β-globulins	8	48	
	15	0.51	fibrinogen	12	40	
	16	0.57	γ-globulins	50	33	
	17	0.62		22	27	
	18	0.83	β-globulins	3	15	
	19	0.90		10	12	
	20	0.93		5	11	
Albumin (cf. Fig. 1b)	1	0.325	albumin	148	68	65–69
γ-globulins (cf. Fig. 1c)	1	0.16		55	110	
	2	0.37		102	58	160
	3	0.57		82	33	
α-globulins (cf. Fig. 1d)	1	0.07		5	160	
	2	0.11		10	120	
	3	0.16		14	100	
	4	0.21		35	90	
	5	0.28		110	76	
	6	0.32		40	67	
	7	0.36	α_1-antitrypsin	40	58	54
	8	0.53		38	35	
	9	0.60		15	29	
	10	0.84		10	14	
	11	0.96		15	9	
	12	0.99		10	7	
β-globulins (cf. Fig. 1e)	1	0.17		17	100	
	2	0.22		24	89	
	3	0.24	plasminogen	33	85	87
	4	0.28	transferrin	32	76	76.5
	5	0.33		22	66	
	6	0.36	hemopexin	28	58	57
	7	0.40		10	52	
	8	0.43		22	48	

Table 2.—(*continued*)

Protein	Peak No.	Mobility	Possible substance	Peak height (mm)	Molecular wt × 10^3 Estimated	Published
	9	0.46		36	45	
	10	0.52		45	36	
	11	0.57		14	32	
	12	0.59		8	30	
	13	0.83		64	15	
	14	0.93	β_2-microglobulin	27	11	11.8
Fibrinogen	1	0.29		86	70	
(cf. Fig. 1f)	2	0.33		88	65	340
	3	0.375		100	56	
	4	0.51		22	40	

* The published MW figures refer to those of human plasma proteins (Putnam, 1975).

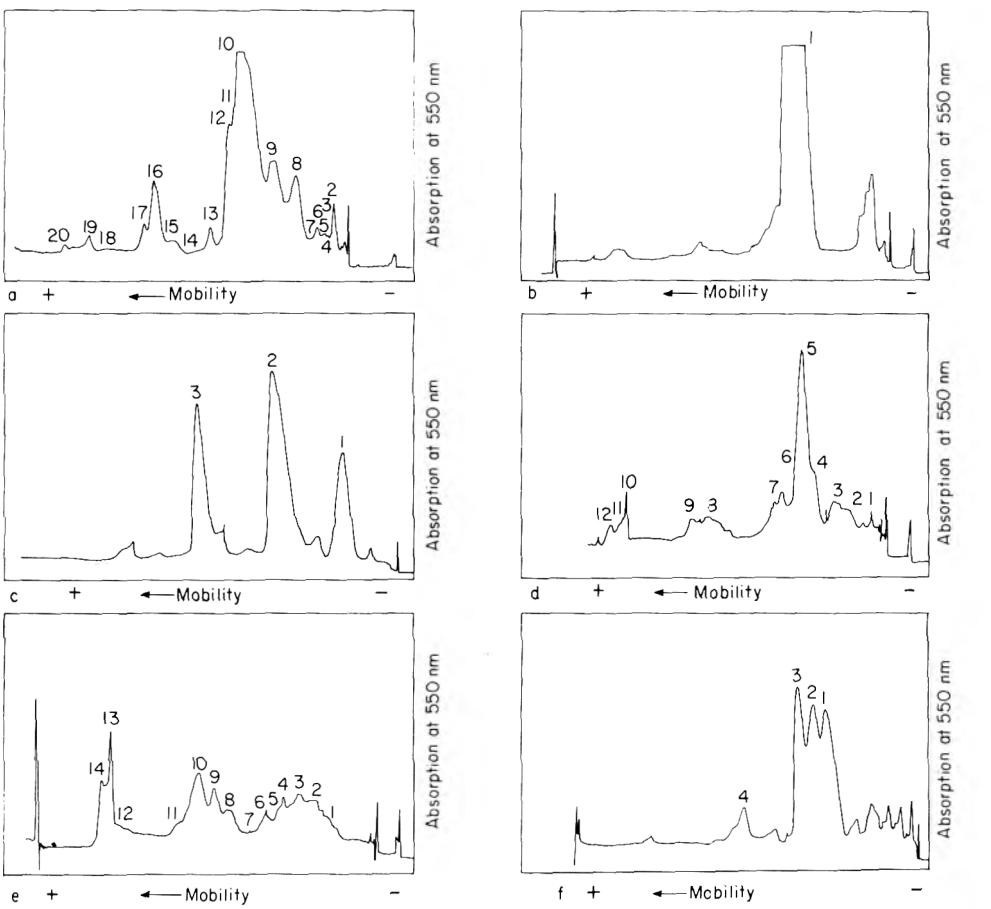

Figure 2. Densitograms of plasma proteins separated by 8% sodiumdodecyl sulphate-polyacrylamide gel electrophoresis. (a) porcine plasma; (b) porcine albumin standard; (c) porcine γ-globulin standard; (d) bovine α-globulin standard; (e) bovine β-globulin standard; (f) bovine fibrinogen standard.

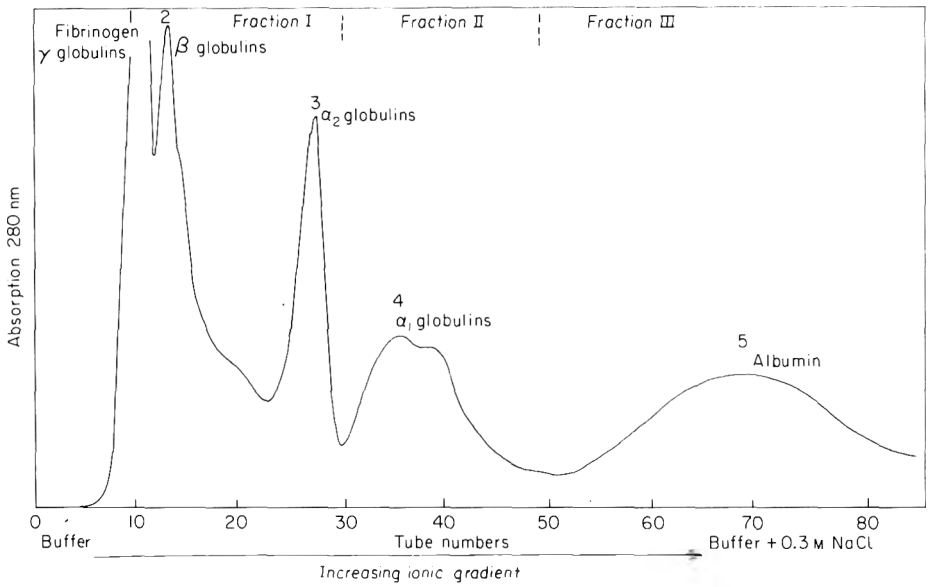

Figure 3. Large scale separation of porcine plasma proteins on DEAE-Sephadex A-50, using 0.1 M tris-citrate buffer, pH 8.0; and a continuous ionic gradient elution.

similar in composition (Table 1). The protein content of the porcine plasma (in laboratory freeze dried form), ranged from about 67% to 69% by weight; whereas the commercially dried plasmas both contained about 70% protein by weight (Table 1). This agrees well with the findings of Donnelly & Delaney (1977) who analysed porcine and bovine plasma which has been collected over 3 months from a local abattoir. They also found slightly higher values for protein (about 72% by weight) in both porcine and bovine plasma powders than in laboratory-produced material. On the other hand a lower protein content (65% by weight of dry powder) was reported by Delaney (1975). The variation in the protein contents reported is probably due to the differing methods employed in each case for the separation of plasma and red cell fractions. The bacterial count at 37°C was considered satisfactory for plasma proteins (Table 1), based on private standards used for meat quality control at Lyons Central Laboratories. There are no legal microbiological standards for plasma, meat or meat products in the U.K.

The porcine plasma proteins were successfully characterized by SDS-PAGE. Electrophoretograms and densitograms of the separation of porcine plasma into twenty bands or peaks, and those of plasma protein standards (Fig. 2), were used to characterize the number of peaks, the mobility of each peak and the peak height, which indicated the relative amounts of each protein present (Table 2). Furthermore it proved possible to identify the major protein bands, namely albumin, fibrinogen, γ-globulins (immunoglobulins), α- and β-globulins, by determining the molecular weight of the proteins on the polyacrylamide gel slabs. Estimated molecular weights of the plasma proteins,

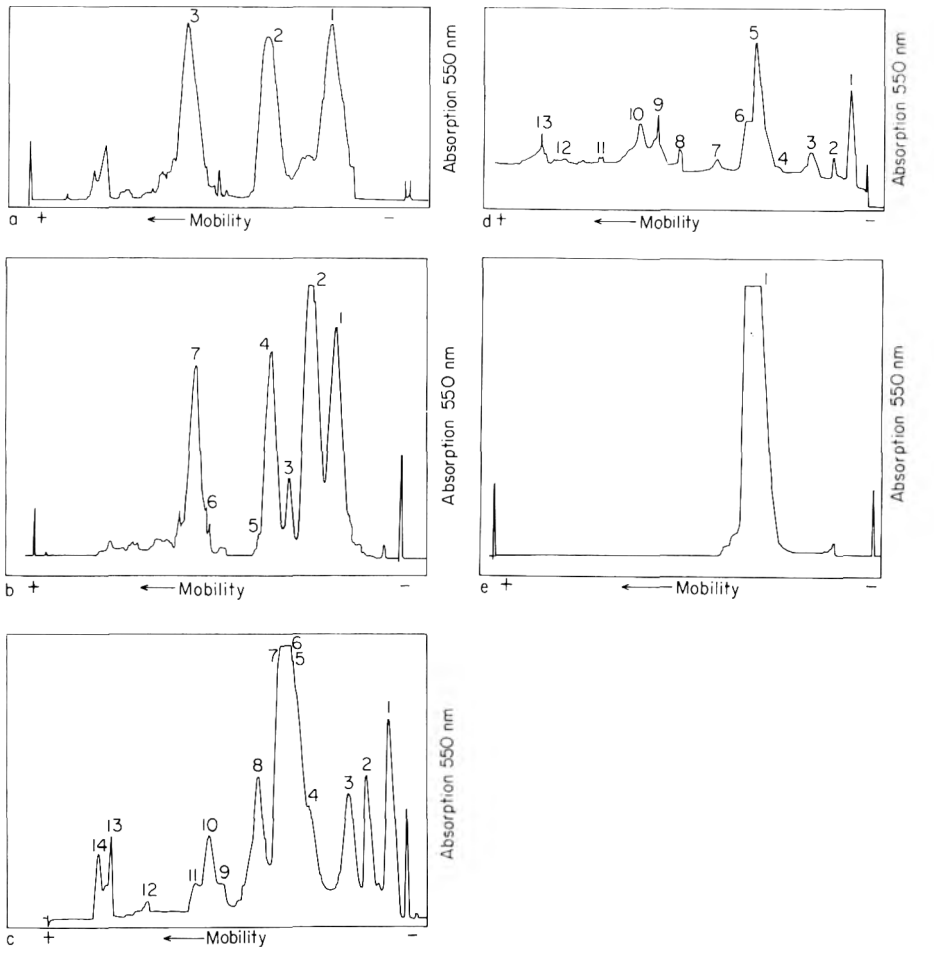

Figure 4. Densitograms of porcine plasma proteins fractions separated by ion-exchange chromatography on DEAE-Sephadex A-50 continuous gradient elution; using polyacrylamide gel electrophoresis incorporating SDS. (a) Peak 1, tube 10; (b) peak 2, tube 12; (c) peak 3, tube 23; (d) peak 4, tube 43; (e) peak 5, tube 75. (The peak and tube numbers referred to are those shown in Fig. 3.)

together with the published molecular weights of likely proteins, are included in Table 2. However, only limited identification of specific proteins, such as transferrin and α-antitrypsin, was possible since standards corresponding to the individual α-, β- and γ-globulins were not available commercially. Nevertheless the electrophoretic separation clearly indicated that the plasma proteins are highly complex entities differing in molecular weight, size and net charge and therefore possibly in functional properties. The separation of the plasma proteins by ion-exchange chromatography into reproducible fractions thus provided an opportunity to examine their functional properties.

On attempting large scale separation of porcine plasma proteins, five major peaks (fractions) were clearly separated using a continuous ionic gradient

Table 3. Characterization of porcine plasma protein fractions obtained by ion-exchange chromatography on DEAE-Sephadex A-50 by PAGE, incorporating SDS (Fig. 2).

Tube No.	Peak No.	Mobility	Height (mm)	Possible substance
10	1	0.14	160	γ-globulin
(Peak 1)	2	0.37	150	γ-globulin, fibrinogen
	3	0.57	150	γ-globulin
12	1	0.14	120	
(Peak 2)	2	0.28	160	transferrin
	3	0.33	45	fibrinogen, β, α-globulins
	4	0.37	110	hemopexin
	5	0.40	10	β-globulin
	6	0.52	25	
	7	0.57	100	
23	1	0.07	110	
(Peak 3)	2	0.14	74	
	3	0.20	63	
	4	0.32	58	
	5	0.36	>160	α-globulin
	6	0.38	>160	
	7	0.41	150	
	8	0.49	78	
	9	0.60	15	
	10	0.65	45	
	11	0.69	15	
	12	0.82	5	
	13	0.96	40	β-globulin
	14	0.99	35	
43	1	0.07	55	
(Peak 4)	2	0.10	18	
	3	0.20	22	
	4	0.28	8	
	5	0.37	80	
	6	0.40	34	
	7	0.45	10	α-globulins
	8	0.65	10	
	9	0.69	15	
	10	0.76	22	
	11	0.94	3	
	12	0.97	3	
	13	0.99	5	
75 (Peak 5)	1	0.33	160	albumin

Table 4. Compositional analysis of porcine plasma fractions and porcine serum.

Fraction	Parameter						
	Protein % (w/w) (N×6.25)	Moisture % (w/w)	Ash % (w/w)	Sodium % (w/w)	Citrate % (w/w)	pH of 6% (w/w) protein solution	Total bacterial count at 37°C
Freeze dried porcine fraction I	78 (±2.0)	11.1 (±0.5)	6.6 (±0.1)	0.313 (±0.02)	0.33 (±0.05)	7.3 (±0.1)	150/g (±10)
Freeze dried porcine fraction II	70.4 (±0.8)	11.6 (±0.5)	13.5 (±0.1)	0.035 (±0.005)	0.04 (±0.01)	7.3 (±0.1)	90/g (±7)
Freeze dried porcine fraction III	75.7 (±0.5)	11.6 (±0.5)	8.6 (±0.1)	0.025 (±0.005)	0.06 (±0.01)	7.4 (±0.1)	80/g (±5)
Freeze dried porcine serum	83.7 (±0.8)	10.8 (±0.4)	2.4 (±0.05)	0.010 (±0.005)	1.0 (±0.1)	7.3 (±0.1)	2.6×10^3/g

* Figures in brackets refer to standard deviations based on five replicates.

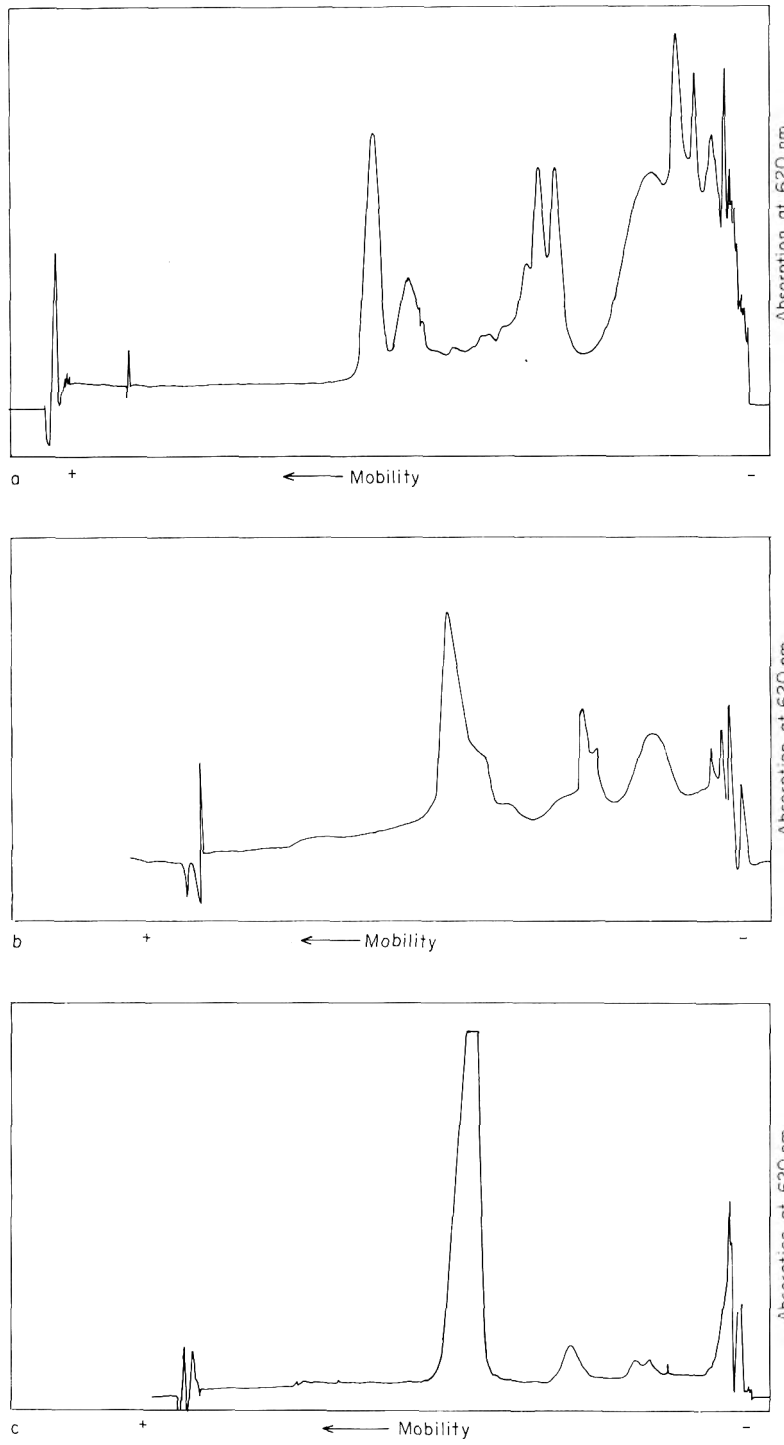

Figure 5. Densitograms of the separation of porcine plasma protein fractions, obtained by ion-exchange chromatography on DEAE-Sephadex A-50, stepwise elution; using electrophoresis on 7% polyacrylamide gel rods. (a) Fraction I; (b) fraction II; (c) fraction III.

elution on DEAE-Sephadex A-50 with 0.1 M tris-citrate buffer at pH 8.0 (Fig. 3). A few tubes containing proteins representing each of the five peaks were selected for characterization by SDS-PAGE. Densitograms of the electrophoretic separation of these peaks are shown in Fig. 4. The mobility and peak height of the separated protein bands are given in Table 3 together with the possible identity of some of the proteins. Peak 1 consisted mainly of fibrinogen and γ-globulins; Peak 2 of β-globulins (and some α-globulins); Peak 3 of α_2-globulins; Peak 4 of α_1-globulins and Peak 5 of albumin.

However, when elution by a stepwise change of ionic strength was adopted to speed up the procedure, it was discovered that the first three peaks were eluted together in the initial step (comprising ion-exchange-chromatography buffer only) (fraction I, Fig. 3). This effect was possible due to the sieving action of the Sephadex ion exchanger since lowering the ionic strength and pH of the buffer (in order to increase protein binding) did not separate the first three peaks. Peaks 4 and 5 (Fractions II and III) were eluted with 0.05 M NaCl and 0.3 M NaCl respectively. Thus three fractions, namely plasma fraction I, II and III, were obtained by stepwise elution by both column and batch methods.

Despite the reduction in the number of fractions from five to three, this procedure was considered satisfactory since whole plasma (including fibrinogen) could be investigated and the three fractions obtained were sufficiently different electrophoretically to suggest that a comparison of their functional properties would be useful. Rapid and efficient production of five plasma fractions on large scale would no doubt be possible by using commercial processing scale columns, ultrafiltration units and driers (which are all currently available on the market).

In the present study, following separation, ultrafiltration and freeze drying, good recovery of the total proteins was obtained. Plasma fractions I, II and III had protein contents of 78%, 70% (w/w) and 76% (w/w) respectively (Table 4). Thus, the process was satisfactory and produced reproducible fractions when characterized by electrophoresis on 7% polyacrylamide gel rods, indicating little denaturation of the protein (Fig. 5). The three plasma fractions so obtained were collected over a period of time, bulked and tested for functional properties which will be reported in subsequent papers.

In order to compare the functional properties of the plasma fractions with those of whole plasma and serum proteins, porcine serum was prepared in the laboratory and analysed (Table 4).

It was noted that none of the plasma fractions (I, II or III) developed a fishy odour on storage, unlike freeze dried plasma or serum or commercially dried plasma. The production of a fishy flavour in plasma has been discussed by Ratcliff & Brooks (1959); and was attributed by them to the oxidation of polyunsaturated fatty acids, phospholipids and proteins. The reason why the plasma fractions produced in this study did not possess a fishy odour may have been the removal of lipids during the ion-exchange chromatography. This aspect required further study as the production of odourless plasma proteins would considerably enhance their applications.

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Compositional variations and sensory acceptability of apple juice drink

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Summary

Apple juice drinks containing 60% of Granny Smith or Jonathan juice, 8–14% soluble solids and Brix:acids ratios of 15:1 to 30:1 were assessed by a panel of twenty-five tasters. Sweetness and sourness increased with increasing levels of soluble solids and acidity respectively. Sourness showed little change with soluble solids at constant Brix:acid ratio but sweetness changed significantly. Intensity of flavour increased with soluble solids but the changes differed in the two types of drinks. Drinks having different soluble solids or Brix:acid ratios sometimes had similar flavour acceptabilities. Sweetness or lack of sourness, and intensity of flavour appeared to account for much of the flavour acceptability of the drinks. Equations for predicting flavour acceptance, based on Brix and Brix:acid ratios, were also developed.

Introduction

Processed products are expected to be essentially uniform in composition and sensory characteristics from one batch to another. In fact, compositional limits for many processed foods are set out in official regulations and purchasing specifications. To obtain the required degree of uniformity and quality in their products, processors often have only one option, i.e. to use carefully selected raw materials. In other instances, different batches of raw materials or product are blended to give a final product with the required attributes. For example, apple juices of different compositions are sometimes blended to obtain products with specified levels of sugar and acid (Smock & Neubert, 1950).

In other cases the desired characteristics in the final product are obtained by adding ingredients such as sugar and food acids. Apple juice drink, which according to Australian food regulations contains a minimum of 50% apple juice, is typical of products whose sensory qualities may be modified by the

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addition of sugar and/or acid. It is therefore a suitable product for investigating the interactions of sugar content and acidity on the perceived intensity of flavour and flavour acceptability. It is also a suitable product for determining how much variation may occur in sugar content and acidity before consumers perceive differences in the sensory properties of the drinks. This information is required before properly based limits can be defined for soluble solids content and acidity.

This paper gives the results of taste tests which were carried out to investigate these points.

Materials and methods

Cloudy apple juice drinks were prepared from Granny Smith and Jonathan juices; both varieties are moderately acidic dessert types with no astringency. The juices were made by passing the washed apples through a hammer mill fitted with a 12.7 mm screen; ascorbic acid (100 mg/kg of apples) was added as an antioxidant during milling. The juices were separated from the mash in a press having a 0.5 mm screen and then filtered through a 100-mesh vibratory screen. The drinks, which contained 60% of juice, were made by adding solutions of sucrose and citric acid to give soluble solids contents of 8, 10 12 and 14°Brix combined with Brix:acid ratios of 15:1, 20:1, 25:1 and 30:1 in a 4×4 factorial design. The drinks were filled into 74×112.5 mm plain tinplate cans which were vacuum closed and processed in steam at atmospheric pressure to ensure the product was commercially sterile.

The sixteen treatments for each variety were assessed by twenty-five tasters who were members of various sections of the laboratory staff and who said they liked apple juice drink. Four treatments were tasted in a balanced incomplete block design at each session and each treatment was assessed on five occasions over twenty sessions during a period of 3 weeks. The tasters were asked to taste the four samples in a specified order, randomized over tasters, and to indicate their opinion of the samples for sweetness, sourness, and intensity of flavour on structured nine point scales. A score of 1 was designated 'not sweet', 'not sour' and 'none' for the three characteristics respectively and a score of 9 was assigned for 'extremely sweet', 'extremely sour' and 'extremely strong'. The scores 3, 5 and 7 were also given appropriate descriptors, e.g. 5 was 'moderately sweet', 'moderately sour' and 'moderate' on the scales for sweetness, sourness and intensity of flavour respectively. Tasters also assessed flavour acceptability on the standard nine point hedonic scale (Peryam & Pilgrim, 1957).

Apart from the descriptors on the score sheets the tasters were given no instructions about what constituted sweetness, sourness or flavour. The tasters' scores, especially for flavour, may therefore be influenced by taste sensations as well as by aroma.

Sweetness and sourness were selected because the primary treatments involved changes in soluble solids and acidity. Intensity of flavour was assessed

because, in the judgment of the authors, it was an important factor in determining the acceptability of apple juice drinks, and flavour acceptability was measured because it is important to the consumer. Moskowitz & von Sydow (1975) assigned values of 4.47, 3.66, 5.32 and 5.45 on a scale of ten for the descriptors sweet, sour, total taste strength and taste pleasantness, respectively for apple juice; the average value for their thirty-two descriptors was 2.54. Thus the factors used in this investigation corresponded to some of those identified by other workers as being of major importance in the flavour profile of apple products.

Each sample for tasting consisted of 80 ml of drink served at about 15°C in a small clear glass under standard conditions in the sensory laboratory. The tasters were members of the staff of the laboratory who were familiar with tasting and who said they liked apple juice drink; they were not specially trained to taste apple juice drink. The Granny Smith drinks were assessed first and the Jonathan drinks were tasted a few weeks later by essentially the same panel.

Results and discussion

Mean panel scores for sweetness, sourness, intensity of flavour and acceptability of flavour for the 32 drinks are given in Table 1; the scores for each attribute were subjected to analysis of variance. Pair-wise significant differences were examined using the LSD technique.

Effect of Brix and Brix:acid ratio on perceived sweetness and sourness

The panel generally responded to changes in soluble solids and Brix:acid ratio, the primary variables used in this investigation, in the expected way. Thus sweetness scores increased as the soluble solids content increased at each level of Brix:acid ratio for both varieties, and the sourness scores increased as the Brix:acid ratio decreased, i.e. as the acidity of the drinks increased, at each level of soluble solids. In addition, the scores for sweetness generally increased as the Brix:acid ratio increased at fixed soluble solids contents. However, the scores for sourness showed little change with increase in soluble solids at fixed Brix:acid ratios.

The drinks with the highest scores for sweetness were those of 14°Brix and 25:1 Brix:acid ratio even though the 14°Brix, 39:1 Brix:acid ratio drink contained less acid. The difference in mean scores for these two drinks was significant at $P<0.05$ for the Granny Smith formulations but not significant for the Jonathan samples.

The soluble solids × Brix:acid ratio interactions were significant for both varieties. One aspect of this interaction, clearly demonstrated by the results for the Jonathan samples, concerns the sweetness perceived at different Brix:acid ratios. Sweetness scores were not significantly different in the 8°Brix drinks

Table 1. Mean panel scores for sweetness, sourness, intensity of flabour and acceptability of flavour for apple juice drinks

Brix:acid ratio	Brix: 8	10	12	14	Brix: 8	10	12	14
Granny Smith drink								
	Sweetness				Sourness			
15	1.88_{j}	2.84_{h}	3.97_{f}	4.60_{e}	4.18_{e}	4.47_{e}	4.38_{e}	4.27_{e}
20	2.31_{i}	3.31_{g}	4.61_{e}	5.63_{c}	3.18_{d}	3.18_{d}	3.00_{d}	3.09_{d}
25	2.45_{i}	3.91_{f}	4.94_{de}	6.83_{a}	2.44_{c}	2.19_{bc}	2.43_{c}	1.73_{a}
30	2.52_{hi}	3.85_{f}	5.24_{d}	6.01_{b}	1.95_{ab}	2.03_{ab}	1.82_{a}	1.76_{a}
	Intensity of flavour				Acceptability of flavour			
15	3.51_{i}	4.32_{fg}	5.15_{cd}	5.59_{a}	4.12_{h}	4.63_{fg}	5.15_{c}	4.84_{ef}
20	3.26_{ij}	4.16_{gh}	4.98_{de}	5.54_{ab}	4.39_{gh}	5.51_{d}	5.86_{bc}	5.92_{b}
25	3.17_{j}	4.51_{f}	4.95_{de}	5.29_{bc}	4.45_{gh}	6.05_{ab}	6.32_{a}	5.54_{cd}
30	3.01_{j}	4.01_{h}	4.81_{e}	5.15_{cd}	4.41_{gh}	5.58_{cd}	6.03_{ab}	6.18_{ab}
Jonathan drink								
	Sweetness				Sourness			
15	2.60_{i}	3.48_{g}	4.23_{f}	4.80_{d}	4.27_{g}	4.54_{g}	5.00_{h}	5.31_{h}
20	2.70_{i}	4.07_{f}	5.22_{c}	5.97_{a}	3.31_{c}	3.39_{cf}	3.70_{f}	3.53_{ef}
25	3.05_{h}	4.34_{ef}	5.50_{bc}	6.24_{a}	2.54_{bc}	2.90_{d}	2.81_{cd}	2.89_{d}
30	3.29_{gh}	4.65_{de}	5.64_{b}	6.17_{a}	1.94_{a}	2.26_{ab}	2.26_{ab}	2.38_{b}
	Intensity of flavour				Acceptability of flavour			
15	3.54_{g}	4.55_{ef}	5.22_{bcd}	5.48_{ab}	4.00_{f}	4.85_{de}	5.12_{d}	4.87_{de}
20	3.19_{h}	4.28_{f}	5.28_{bc}	5.58_{a}	4.15_{f}	5.58_{c}	6.06_{ab}	6.12_{a}
25	3.30_{gh}	4.60_{e}	5.17_{cd}	5.45_{abc}	4.37_{f}	5.74_{bc}	6.29_{a}	6.06_{ab}
30	3.25_{h}	4.38_{ef}	4.97_{d}	5.41_{abc}	4.68_{e}	5.71_{c}	6.27_{a}	6.38_{a}

Figures having the same subscript letter were not significantly different ($P<0.05$) within each set of data.

having Brix:acid ratios of 15:1 and 20:1 but the scores were significantly different in the 10, 12 and 14°Brix drinks; there were no important interactions between sweetness and Brix:acid ratio for the higher ratios.

Variation between tasters was highly significant as were most taster interactions. This indicates there may be differences in individual panelists' perception of sweetness and sourness and/or differences in the way panelists used the scales.

Intensity of flavour

The mean scores for flavour intensity for both varieties of drinks increased ($P<0.001$) with increase in soluble solids content. This indicates that sweetness had a marked influence on the tasters' perception of flavour intensity; there may also be an interaction of individual perception of sweetness with the flavour components from the apples. Scores for sweetness and flavour intensity

were correlated (e.g. $r=0.46$ on 1974 d.f. for the Jonathan drinks). In general, the mean scores for intensity of flavour within each level of Brix tended to increase as the Brix:acid ratio decreased, i.e. as the acidity increased. Within the range of treatments used in this investigation soluble solids had a greater influence on the scores for intensity of flavour than acidity.

Acceptability of flavour

The level of soluble solids and the Brix:acid ratio had highly significant effects on the mean scores for acceptability of flavour. The highest mean score for the Granny Smith samples was for the drink of 12°Brix and 25:1 Brix:acid ratio. This drink was not significantly different in acceptability from those of 10°Brix, 25:1 Brix:acid ratio, and 12 and 14°Brix and 30:1 Brix:acid ratio. The Jonathan drinks having the highest scores which were not significantly different, covered the compositional range of 12 and 14°Brix and 20:1, 25:1 and 30:1 Brix:acid ratios. It therefore appears that drinks having a range of compositions, a range which may extend beyond the limits used in this investigation, would have high flavour acceptabilities which are not significantly different. It is considered that these compositional limits for high flavour acceptability are probably conservative because they are based on the responses of tasters working under conditions which would give better discrimination than the average person consuming the product under ordinary circumstances. Clearly the panel was sensitive to small differences in the drinks as is shown by the several significantly different classes into which each characteristic was divided. Thus a manufacturer might use a range of levels of sugar or acid or both, depending on the characteristics of the base juice, to formulate a product of optimal acceptability.

Factors contributing to acceptability

Although many sensory attributes have been shown to contribute to the flavour of apple products (von Sydow *et al.*, 1974; Moskowitz & von Sydow, 1975; Watada *et al.*, 1981) the present work was restricted to the assessment of sweetness, sourness, intensity of flavour and flavour acceptability. To estimate the contribution of the three sensory attributes to flavour acceptability (y), multiple linear regression equations were calculated relating the scores for sweetness (x_1), sourness (x_2), intensity of flavour (x_3), and combinations of these factors, to flavour acceptability. Trant, Pangborn & Little (1981) discuss possible errors in correlating hedonic responses with intensity scores but the data in Table 1 are approximately linear so this treatment is considered to be sufficiently rigorous for the purpose of the investigation. The main effects are demonstrated by the equations given below; the figures in brackets give the percentage of the variance taken into account by each equation.

Granny Smith

$3.70+0.40x_1$			$=y$	(58.2)
6.39	$-0.37x_2$		$=y$	(19.8)
2.75		$+0.58x_3$	$=y$	(41.7)
$3.62+0.38x_1$		$+0.03x_3$	$=y$	(55.1)
3.78	$-0.41x_2$	$+0.61x_3$	$=y$	(72.2)

Jonathan

$2.64+0.61x_1$			$=y$	(84.7)
6.31	$-0.28x_2$		$=y$	(6.0)
2.04		$+0.73x_3$	$=y$	(57.1)
$3.00+0.73x_1$		$-0.19x_3$	$=y$	(84.5)
2.93	$-0.47x_2$	$+0.88x_3$	$=y$	(91.9)

These equations indicate that sweetness and intensity of flavour, separately and together, account for much of the variance and make important contributions to flavour acceptability. Sourness is a negative factor and alone it accounts for little of the variance.

Prediction of flavour acceptability

The chemical properties Brix (B) and Brix:acid ratio (BA) were examined to see if combinations of them provided a useful predictor of flavour acceptability (F) at least over the range of compositions used in this investigation. The best prediction equations obtained were:

Granny Smith

$$F=-6.58+0.00514\,B\times BA-0.0825\,B^2+1.91B \qquad (85.1\%)$$

Jonathan

$$F=-6.48+0.00604\,B\times BA-0.0780\,B^2+1.84B \qquad (92.2\%)$$

Again the figures in brackets give the percentage of the variance taken into account by the equations.

Both equations have the same form and account for a large percentage of the variance. They were therefore used to calculate the levels of Brix and Brix:acid ratio that corresponded to maximum flavour acceptability. The equations contain linear terms for Brix:acid ratio so the range of values used in the test samples (15:1 to 30:1) did not extend to the point where the tasters might give reduced scores for flavour acceptance because of lack of sourness, or blandness. The value of Brix corresponding to maximum flavour acceptability was found by differentiating the equations with respect to Brix. The values obtained, to the nearest 0.5°Brix, were 12.5 and 13.0°Brix for the Granny Smith and Jonathan formulations respectively. These values differ slightly from the values that might be selected by inspection of the data for flavour acceptability in Table 1.

The linear increase in flavour acceptability with increase in Brix:acid ratio indicates that drinks with ratios greater than 30:1 would probably have a higher acceptability than those used in this investigation. However, the production of such drinks is limited by the high natural acidity of most types of apple juice. The natural acidity of the juice used in a 60% drink of 12.5°Brix and 35:1 Brix:acid ratio would have to be not more than 0.6% and many juices would contain more than this level. In practice therefore it appears that the drink should be prepared from juice from mature fruit which has a high level of fruit sugars and apple flavour and a low acidity.

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Organoleptic assessment of full fat soy flour in various indigenous products

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Summary

Full fat soy flour was blended with cereal, millet or pulse flours to make a variety of Indian traditional products. It was judged by a trained panel that all the products, namely 'chapati, 'puri', 'pakoda', 'sev', 'bread pakoda', 'soy-peanut crisp', 'burfi', 'mysore pak' and 'halwa' were all acceptable with no significant differences among the products.

Introduction

The search for a new unconventional source of protein to meet the requirement of the ever expanding population of India is the dire need of the present day. Soybeans with 40% protein and 20% oil have a great potential in solving the problems of protein and calorie malnutrition. Starting from 1965 efforts are being made to produce soybeans in dry farming areas. Madhya Pradesh is the leading state in India with 0.6 million ha under soybean cultivation. However, soybeans have not reached the diets of rural people. There is an ever growing demand for soy products in the market. Among the various products, full fat soy flours would serve two purposes as they contain an oil rich in calories and can be easily blended in the recipes of indigenous foods. Hence simple methods (Albrecht *et al.*, 1967) were developed for making full fat soy flours at the rural level. This would enable the farmers to process their soybeans and utilize them in their daily diets. These flours are blended at different proportions with cereal, millet or pulse flours to make a wide range of traditional dishes, but organoleptic evaluations of such products are not available at present. Hence, the present investigation was carried out to study the organoleptic evaluation of various indigenous products prepared from soy blends.

Materials and methods

The soybean variety PT49 was obtained from the Farm Section, Central Institute of Agricultural Engineering, Bhopal.

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0022–1163/83/1200–0771 $02.00

The whole sound soybeans were cleaned of dirt, foreign matter, and brokens to improve the flavour of the products. The beans (8% moisture on a wet basis) were cracked with a hand stone grinder or a mini grain mill and the hulls were removed with a hand winnower. The split pulse was filled in cloth bags to one third of their fullest capacity and soaked in water containing 1% (w/v) sodium bicarbonate at ambient temperature for 4 hr. The beans were conditioned to 52% moisture on a wet basis. The beans to water ratio was 1:3. The addition of sodium bicarbonate aids the improvement of beany or greasy flavour. The soaked beans were cooked in boiling water (100°C) for 15–20 min to detoxify the beans. Later the bags were lifted up and allowed to drain for a few minutes. The cooked beans were spread in single layers on black polyethylene surfaces and sun dried for 32–36 hr to bring the moisture level again to 8% on a wet basis. The size reduction was undertaken in a hand stone grinder or a mini grain mill. The product is now ready for use.

Products prepared from soy flour

Salt dishes. 'Chapati' was prepared from: soy flour, 15 g; wheat flour, 100 g; salt to taste; and ghee. The flours were sifted and mixed thoroughly. The salt was added and kneaded into a dough. It was divided into three equal portions and rolled out into chapaties. They were baked on a hot pan for 2 min and spread with the ghee.

'Puri' was prepared from: soy flour, 15 g; wheat flour, 100 g; salt to taste; and fat for frying. The flours were sifted and mixed properly. The salt was added and kneaded into a dough. It was divided into six equal portions and rolled out into puries and fried in hot fat till golden brown.

'Pakoda' was prepared from: soy flour, 25 g; chickpea flour, 100 g; chopped green chillies, 10 g; chopped onions, 20 g; cumin seeds, 1 teaspoon; paprika powder, 1 teaspoon; salt, sufficient to taste; and fat for frying. The soy flour and chickpea flours were sifted and mixed well. The chopped onions, green chillies, cumin seeds, paprika powder and salt were added to the flour to a drop batter consistency and a heaped teaspoon of batter was dropped in the form of 'Pakoda' into the heated oil and fried till deep brown.

'Sev' was prepared from: soy flour, 15 g; chickpea powder, 100 g; salt, 1 teaspoon; cumin seeds, 1 teaspoon; and fat, sufficient for frying. The flours were sifted and mixed well. The salt and cumin seeds were added and kneaded to a hard dough. Then a hand extruder was used to extrude the dough into 'Sev' and deep fried in oil till golden yellow.

'Bread pakoda' was prepared from: Brown sliced bread, 100 g; chickpea powder, 100 g; soy flour, 25 g; paprika powder, 2 teaspoons; salt, to taste; and fat, sufficient for frying. The flours were sifted and mixed properly. The salt and paprika powder were added and a drop batter consistency was made with water. The sliced bread was diced into desirable shapes and dipped in the batter and dropped into the heated oil and fried till golden brown.

Sweet dishes. 'Soybean-peanut crisp' was prepared from: soy flour, 75 g;

refined wheat flour, 150 g; milk, 75 ml; hydrogenated fat, 200 g; cane sugar, 150 g; salt, 1 teaspoon; peanuts, 120 g; and a pinch of baking powder. The flours were sifted and mixed thoroughly along with the salt and baking powder. The sugar was mixed with fat and creamed a while. The peanuts were added to this mixture and the sifted flour was added and stirred continuously till it was kneaded to a dough. It was divided into small equal portions and pressed into biscuits. These were kept on trays smeared with ghee and baked for 10–15 min.

'Burfi' was prepared from: soy flour, 50 g; chickpea flour, 50 g; cane sugar, 50 g; and hydrogenated fat, 50 g. The sifted flours were mixed well and fried in oil till golden yellow. The sugar and sufficient water were added and fried for another 2 min. Finally the contents were poured into a tray spread with ghee and allowed to settle for 2 hr and then diced.

'Mysore Pak' was prepared from: soy flour, 100 g; chickpea flour, 250 g; ghee, 250 g; and cane sugar, 200 g. The flours were sifted and mixed well. They were fried in oil until golden. A sugar syrup of two-thread consistenty (65° Brix) was prepared and the fried flour was added to it with continuous stirring. The melted ghee was added a little at a time until the contents left the sides of the pan. The contents were then removed and poured into a tray spread with ghee and allowed to remain for several hr and cut into pieces.

'Halwa' was prepared from: soy flour, 100 g; green gram (mung) flour, 400 g; ghee, 300 g; khoya, 250 g; cardamom powder, ½ teaspoon; cashew nuts, 50 g; and cane sugar, 300 g. The sifted flours were mixed well and fried in ghee till light brown. The khoya was roasted separately on low heat for 10 min. The sugar syrup was made and the khoya and fried flour mixture were added to it. The material was then cooked till it left the sides of the pan. The product is garnished with cardamom powder and cashew nuts.

Sensory evaluation of soy blends

Sensory evaluation was conducted on different recipes prepared from soy blends by a panel of eight trained judges (ISI Standard, 1971). The various characteristics like taste or flavour, feeling or texture, colour or appearance and general acceptability for each of the products were assessed using a nine-point hedonic scale (Bhat & Vivian, 1980) of excellent=9, very good=8, good=7, below good and above fair=6, fair=5, below fair and above poor=4, poor=3, very poor=2, and extremely poor=1. Analysis of variance was used to test the difference between the treatments (ISI Standard, 1975).

Results and discussion

The products prepared were organoleptically evaluated by a panel of trained judges and the scores awarded were computed. The mean score values of the different soy blends are given in Table 1. The scores are allotted as compared to the original recipes without full fat soy flour, assuming highest acceptability for them (score of nine). The mean scores for all the quality characters and general

Table 1. Mean scores of sensory panel judges for the characteristics of soybean recipes

Character	Products								
	Salt dishes					Sweet dishes			
	Chapati	Puri	Pakoda	Sev	Bread pakoda	Soy-peanut crisp	Burfi	Mysore pak	Halwa
Taste/flavour	7.14	7.0	7.3	7.1	8.0	7.2	8.0	8.4	8.2
Feeling/texture	7.02	6.9	7.2	7.5	8.4	7.1	8.3	8.5	8.0
Colour/ appearance	7.56	7.0	7.7	7.8	8.0	6.5	7.0	7.7	7.4
General acceptability	7.06	7.0	7.1	7.0	8.3	7.0	7.7	7.0	7.3

acceptability were more than the minimum acceptable score of five. The results thus indicate that all the products prepared from soy flour when blended with wheat flour and chickpea flour are well liked. These products are devoid of off flavours and possess acceptable characteristics. The results are in accordance with Vijayalakshmi & Vaidehi (1982) who obtained similar results while evaluating the Tofu blended with chickpea flour at 10% level. A separate analysis of variance was done for each character: taste or flavour, feeling or texture, colour or appearance and general acceptability from every individual score of taste panel. This analysis was carried out to find out the differences among the characteristics and panelists for the soy blends. The results are presented in Table 2. The results reveal that the difference among the treatments is significant for taste and feeling and not significant for the colour and general acceptability at a 5% level of significance. The disagreement among the judges for all the characteristics is not significant at a 5% level of significance. Thus the data indicate that the source of variance among the products is only due to characteristics like taste and feeling and variance due to judges does not exist. This study further indicates that all the soy blend preparations are acceptable.

Table 2. Analysis of variance of the taste panel scores for the characteristics of different soyblend products

Source of variation	Degree of freedom (d.f.)	Mean sum of squares			
		Taste	Feeling	Colour	General acceptability
Products	8	2.4*	3.24*	1.79†	1.33†
Judges	7	0.1†	0.37†	0.17†	1.7†
Error	56	0.18	0.15	0.02	0.27

* Significant at 5% level of significance.
† Not significant at 5% level of significance.

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The relationship of bacterial numbers and types to diamine concentration in fresh and aerobically stored beef, pork and lamb

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Summary

Bacterial numbers and putrescine and cadaverine concentrations were measured in intact beef, pork and lamb and minced beef at retail and during aerobic chill storage at 5°C. Putrescine concentrations increased consistently with 'total' aerobic viable count (TAVC) but cadaverine concentrations increased only when high numbers of presumptive *Enterobacteriaceae* were present. Significant changes in diamine concentration did not occur until the TAVC exceeded $4.2\times10^7/cm^2$ or g when the meat was clearly spoiled. Changes prior to the onset of spoilage were not sufficient for their use as a predictive indicator of the acceptability of the meat.

Introduction

The acceptability of fresh meat for consumption is usually assessed organoleptically and/or by the measurement of bacterial numbers. The possibility of using chemical changes as a rapid and objective alternative, or adjunct, to these methods has long been of interest. Unfortunately the magnitude of most of the changes investigated to date has proved to be insufficient for their reliable use during the incipient stages of spoilage. They appear to serve a confirmatory rather than predictive role in spoilage assessment.

However, it has recently been claimed (Slemr, 1981) that determination of the combined putrescine and cadaverine content of chill-stored meat might serve as a useful index of acceptability. Inoculation of pork muscle with a mixture of *Enterobacteriacae* and *Pseudomonas* strains led to a readily detectable, pre-spoilage increase in diamine content, which correlated with microbial numbers.

Although other data on the diamine content of fresh pork have been published (Spinelli, Lakritz & Wasserman, 1974; Lakritz, Spinelli & Wasserman, 1975; Yamamoto *et al.*, 1982; Nakamura *et al.*, 1979) only the

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latter authors studied diamine concentrations in relation to spoilage development at chill temperatures. Prior to detectable organoleptic change they were unable to find increased levels of either amine in naturally contaminated ground pork muscle stored at 4°C.

In an effort to explain the discrepancies between these findings and those of Slemr (1981) we have (a) surveyed the diamine content of intact pieces of beef, pork and lamb and of minced beef at retail and after storage to obvious spoilage at 5°C and (b) followed the time course of changes in diamine concentration in slices of beef *M. longissimus dorsi* and *M. semitendinosus* inoculated with a mixed, natural flora and in minced beef allowed to develop its own natural flora. In both types of experiment, TAVC and numbers of presumptive *Enterobacteriaceae* were determined in order to investigate possible relationships between diamine concentration and microbial numbers.

Materials and methods

Survey of retail meat

Braising steak, minced beef, leg of lamb and shoulder and leg of pork were obtained on three separate occasions from retail butcher shops. Excess fat and connective tissue were removed from the non-minced samples and slices *circa* 1 cm thick placed in glass dishes and overwrapped with 'clingfilm' (O_2 permeability *circa* 10000 ml/m^2/24 hr/atm. O_2). Mince was distributed in *circa* 1 cm thick layers in similar dishes. All samples were stored at 5°C until visually spoiled.

Before removing the 'clingfilm' the permanent gas composition of the headspace above the meat was determined by withdrawing a 10 ml sample by syringe and analysing aliquots with a Gow-Mac model 69-552 thermal conductivity gas chromatograph.

For microbiological analysis a 10 cm^2 boring was taken through the slices and the sample macerated for 1 min in a Stomacher (A. J. Seward Lab., London) with 20 ml of maintenance medium (MM) containing (g/l distilled water): bacteriological peptone (Oxoid L37), 1.0; NaCl, 8.5. A 10 g sample of mince was treated similarly. Decimal dilutions were prepared in MM and duplicate 1/50 ml drops spread on plate count agar (PCA, Oxoid CM326) containing 1% (w/v) NaCl for TAVC. Presumptive *Enterobacteriaceae* were enumerated on pour plates of violet red bile glucose agar (VRBG) according to ISO document number 5552. The PCA plates were incubated aerobically for 3 d at 20°C; VRBG plates in H_2 for 1 d at 30°C.

For diamine analysis the meat was sampled as above. An internal standard of 10 μg diaminohexane in 1.0 ml 0.1 N hydrochloric acid was added to duplicate 10 g samples of meat which were extracted using the scheme described by Spinelli *et al.* (1974). Pentafluoropropionyl derivatives of the extracted amine hydrochlorides were prepared and analysed by gas–liquid chromatography (glc) (Staruszkiewicz & Bond, 1981) using a Pye 204 instrument (Pye Unicam

Ltd, Cambridge) fitted with flame ionization and ^{63}Ni electron capture detectors and a 2 m×4 mm i.d. glass column packed with 3% OV-225 on 80–100 mesh Gas-Chrom Q. The operating conditions were: injection port 220°C, detector 250°C, column oven 190°C and nitrogen carrier gas flow 30 ml/min. Quantitation was by peak height measurement normalized against the internal standard.

Positive identification of amine derivatives was carried out on a selection of samples by mass spectrometry using the glc column described above fitted in a Finnigan 4000 mass spectrometer (Finnigan Corporation, Sunnyvale, CA, U.S.A.). This was coupled to an Incos 2100 data system operated in the continuous scan mode with a scan time of 2 sec over 33–420 a.m.u. The ion source energy was 70 eV. Derivative formation was confirmed by monitoring the m/e 176 ion of the $-CH_2NHCOC_2F_5^+$ fragment and the m/e 380, 394 and 408 ions of the M^+ of putrescine, cadaverine and 1,6 diaminohexane respectively.

Storage experiments

Beef *M. semitendinosus* and *M. longissimus dorsi* of normal pH (5.5–5.7) were removed 5–9 days post slaughter from three different animals and sterile 1 cm thick slices of the muscles prepared (Dainty & Hibbard, 1980). One surface of each slice was inoculated with an appropriate volume of a suspension in MM of the natural contaminating flora of braising steak. This was obtained by swabbing the surfaces of retail steak bought on separate occasions for each of the three reported experiments. The inoculated samples were stored in deep polystyrene dishes overwrapped with 'clingfilm' as described above. At 24 hr intervals, seven cork borings (5 cm^2) were taken through a slice of meat. Each cylinder of meat was inverted, i.e. surface exposed during storage downwards, on to a bacteriological filter placed in the base of a 5 cm^2×2 mm deep well cut into a metal die. The surface layers of meat (*circa* 2 mm thick) were obtained by slicing through the lightly compressed cylinder of meat at the metal surface. Six discs of meat in two lots of three (*circa* 3 g meat) were extracted for amine analysis and one for determination of microbial numbers as described above for the survey. Duplicate cork borings taken through similarly stored samples of retail mince were used in their entirety for amine and microbiological analysis also as described in the survey.

Results

Survey

'Total' microbial numbers on the non-minced samples at purchase ranged from 10^4 to $5\times10^5/cm^2$ while with one exception (beef sample 3) the numbers of presumptive *Enterobacteriaceae* were of the order of a few hundred/cm^2 (Table 1). Small but readily detectable concentrations of putrescine and cadaverine, the former 3–10-fold greater than the latter, were detected in each sample.

Table 1. Concentration of diamines and microbial counts for meat at retail and after refrigerated storage

Sample		At retail				After storage at 5°C			
		Putrescine (μg/g)	Cadaverine (μg/g)	*Enterobacteriaceae* ($\log_{10}$ No./cm^2 or g)	TAVC ($\log_{10}$ No./cm^2 or g)	Putrescine (μg/g)	Cadaverine (μg/g)	*Enterobacteriaceae* ($\log_{10}$ No./cm^2 or g)	TAVC ($\log_{10}$ No./cm^2 or g)
Beef:									
braising steak	1	2.3	0.2	2.57	5.38	13.5	6.6	7.00	9.04
braising steak	2	1.3	0.2	2.00	4.72	6.9	4.6	5.79	8.48
braising steak	3	1.5	0.3	4.42	5.79	3.5	14.8	4.54	8.54
Pork:									
shoulder	1	0.6	0.2	2.54	5.79	16.0	2.8	6.00	9.04
leg	2	0.8	0.2	0.30	5.04	13.9	16.6	5.70	8.92
leg	3	1.6	0.2	1.42	4.28	3.0	1.1	4.00	8.81
Lamb:									
leg	1	0.7	0.2	2.11	5.38	14.7	4.6	6.00	9.34
leg	2	1.3	0.2	0.78	4.11	2.8	6.7	4.65	8.54
leg	3	1.3	0.2	1.70	4.18	3.3	1.3	6.04	8.73
Beef:									
mince	E 1	1.0	0.3	3.72	6.58	59.1	26.1	7.51	9.57
mince	2	1.1	0.2	3.08	6.64	35.8	12.6	7.20	9.72
mince	3	0.9	0.2	4.08	7.66	11.6	3.2	N.D.	9.00
mince	F 1	0.9	0.6	4.48	7.04	51.0	37.8	7.18	9.11
mince	2	1.2	0.8	5.08	7.48	78.4	68.7	8.11	9.40
mince	3	0.9	0.6	5.88	7.43	17.7	52.4	N.D.	9.48
mince	H 1	0.7	0.3	4.64	6.64	37.6	24.3	7.78	9.28
mince	2	1.1	0.2	2.80	7.18	38.6	0.9	7.20	10.30
mince	3	0.9	0.2	2.66	6.60	14.2	1.4	N.D.	9.48

Samples were bought on three separate occasions (1, 2, 3) and the mince from three different retail outlets (E, F, H).
N.D. Not done.
Diamine values are the mean of two determinations.

'Total' numbers and *Enterobacteriaceae* counts were substantially higher on the minced beef samples, the three samples obtained from one butcher (F 1, 2, and 3) having the highest numbers of *Enterobacteriaceae* and also the highest concentrations of cadaverine (Table 1).

After storage at 5°C to obvious spoilage (5 d for non-minced samples, 4 d for minced beef) analysis of the headspace above the meat samples for the permanent gases showed that aerobic conditions had been maintained in all samples. 'Total' viable counts on the slices ranged from $10^8-10^9/cm^2$ and on the minced samples they were all $>10^9/g$. The *Enterobacteriaceae* had increased to numbers in excess of $10^7/g$ on the mince while the numbers on the slices were variable and ranged between $10^4-10^7/cm^2$. Increases in both putrescine (2–8-fold) and cadaverine (3–100-fold) concentrations accompanied the microbiological changes with the minced beef samples showing the greatest changes in both diamines. There were no obvious differences between beef, pork and lamb, in either the initial or stored samples.

In general putrescine concentrations in excess of 10 μg/g were associated with a TAVC approaching or exceeding $10^9/cm^2$ or g of meat and an *Enterobacteriaceae* count of *circa* $10^6/cm^2$ or g. The highest cadaverine concentrations were found in samples of minced beef supporting more than 10^7 *Enterobacteriaceae*/g (Table 1) but there did not appear to be a consistent correlation between the numbers of these organisms and cadaverine concentration. For example, mince sample H2 with an *Enterobacteriaceae* count of 1.6×10^7/g contained <1 μg/g of cadaverine. Furthermore sample 3 of braising steak and sample 2 of pork leg with *Enterobacteriaceae* counts of 3.5×10^4 and 5.0×10^5/g respectively contained more cadaverine than sample 1 of braising steak with a count of $10^7/cm^2$. There was clearly no correlation between cadaverine concentration and TAVC (Table 1).

Storage experiments

Use of a natural mixed flora obtained from retail braising steak to inoculate beef *M. semitendinosus* and *M. longissimus dorsi* produced different initial microbial floras in the three experiments reported *viz* (1) high 'total' bacterial numbers with a high (10%) proportion of *Enterobacteriaceae*; (2) high 'total' numbers with a low (0.1%) proportion of *Enterobacteriaceae*; and (3) low 'total' numbers with no *Enterobacteriaceae* (Table 2). In all three experiments the TAVC increased steadily on both types of muscle throughout storage with detectable increases in putrescine concentration once the count exceeded $10^7/cm^2$ (Table 2). Only in experiment 1 did the *Enterobacteriaceae* compete successfully with the other organisms when a progressive increase in their numbers was accompanied by increases in cadaverine concentrations. Although there was growth of *Enterobacteriaceae* in experiment 2, the counts did not exceed $1.8\times10^5/cm^2$ and no cadaverine was produced. Nor was it produced in experiment 3 when no *Enterobacteriaceae* were present.

Putrescine and cadaverine concentrations also increased throughout storage

Table 2. Development of microbial numbers and diamine concentrations on beef muscle inoculated with a mixed natural microbial flora and stored at 5°C

Sample	Storage time (*d*)	*M. semitendinosus* Putrescine (μg/g)	Cadaverine (μg/g)	*Enterobacteriaceae* ($\log_{10}$ No/cm^2)	TAVC	*M. longissimus dorsi* Putrescine (μg/g)	Cadaverine (μg/g)	*Enterobacteriaceae* ($\log_{10}$ No./cm^2)	TAVC
1	1	0.4	1.2	4.43	5.42	0.5	1.0	5.18	5.94
	2	0.6	1.5	4.69	6.23	0.9	2.5	5.46	6.78
	3	1.4	3.6	6.15	7.20	6.8	14.2	6.57	7.28
	4	10.2	9.1	6.58	7.98	25.3	25.6	7.80	8.88
	5	30.5	13.4	7.49	8.67	46.0	33.8	8.26	8.97
	7	39.5	14.8	7.94	9.08	48.6	33.1	8.34	9.26
2	1	0.5	0.8	2.00	5.96	0.6	0.8	2.00	6.00
	2	0.6	0.8	2.30	6.61	0.7	0.8	2.18	6.36
	3	2.8	0.8	2.32	7.08	6.0	0.9	2.96	7.72
	4	2.5	0.7	2.20	7.73	11.4	0.8	2.49	7.97
	5	13.4	0.5	2.38	9.00	15.6	0.6	2.04	9.00
	6	19.8	0.6	3.59	9.32	24.8	0.7	3.59	9.43
	7	21.6	0.7	4.11	9.69	29.9	0.7	4.93	9.80
	8	22.6	0.7	3.74	9.60	38.3	0.7	5.26	9.18
3	2	0.6	1.0	–	1.34	0.7	1.0	–	2.40
	4	0.6	0.3	–	2.23	0.8	t	–	2.95
	5	0.6	0.3	–	3.32	0.8	0.4	–	4.49
	6	0.6	t	–	4.48	0.8	0.4	–	6.54
	7	0.6	t	–	5.97	1.0	t	–	7.62
	8	0.7	t	–	5.40	1.6	t	–	7.60
	9	4.6	t	–	8.20	7.5	t	–	8.49
	10	0.9	t	–	6.94	16.4	t	–	9.62

–, none detected; t, trace amount. Samples 1, 2 and 3 were obtained on separate occasions.
Diamine values are the mean of two determinations.
Diamine concentrations in the sterile meat were putrescine, 0.5–0.8 μg/g; cadaverine 0.9–1.1 μg/g.

Table 3. Development of microbial number and diamine concentrations on naturally contaminated minced beef stored at 5°C

Sample	Storage time (*d*)	Putrescine (μg/g)	Cadaverine (μg/g)	*Enterobacteriaceae* ($\log_{10}$ no./g)	TAVC
E	0	1.2	0.1	3.81	6.29
	1	1.8	0.1	3.56	7.66
	2	4.2	0.5	4.57	8.49
	3	10.0	0.5	5.86	9.48
	4	26.1	0.6	7.54	9.97
F	0	2.3	1.3	6.18	7.49
	1	3.9	4.5	6.23	7.85
	2	12.4	17.9	6.69	8.73
	3	29.9	35.2	7.94	9.69
	4	59.2	40.8	9.00	9.91

Samples E and F were obtained from two different retail outlets. Diamine values are the mean of two determinations.

in the naturally contaminated mince sample F and there were accompanying increases in total and *Enterobacteriaceae* counts (Table 3). In mince sample E a similar picture for putrescine concentration and TAVC was observed but little cadaverine was produced despite *Enterobacteriaceae* counts exceeding 10^7/g.

To define relationships between bacterial numbers and diamine concentrations more accurately all relevant data from the survey and the storage experiments were plotted graphically (Figs 1 and 2) and the data analysed statistically.

The scatter of the observations relating putrescine concentration to TAVC (Fig. 1) suggested either an exponential or a diphasic relationship. In fact neither a single nor double exponential model was able to fit the data adequately and a computer program to detect the breakpoint (d) in the diphasic model:

$P=B$ for log (TAVC)$\leq d$,
$P>B$ for log (TAVC)$>d$,

where P=putrescine concentration, and B=basal concentration of putrescine, was written following Lerman (1980). This gave a better fit and indicated a breakpoint of 6.6×10^7/cm^2 or g and a basal concentration of 1.4 μg/g for putrescine, i.e. only a TAVC$>6.6\times10^7$/cm^2 or g gave a significantly increased level of this amine above those to be expected in meat of the highest bacteriological quality. Apparent from Figure 1 was a wide range of putrescine concentrations for any TAVC$>6.6\times10^7$/cm^2 or g.

Statistical analysis of the data relating *Enterobacteriaceae* count to cadaverine concentration was not feasible because of the generally wider scatter of results and in particular the presence of a few obvious outliers (Fig. 2).

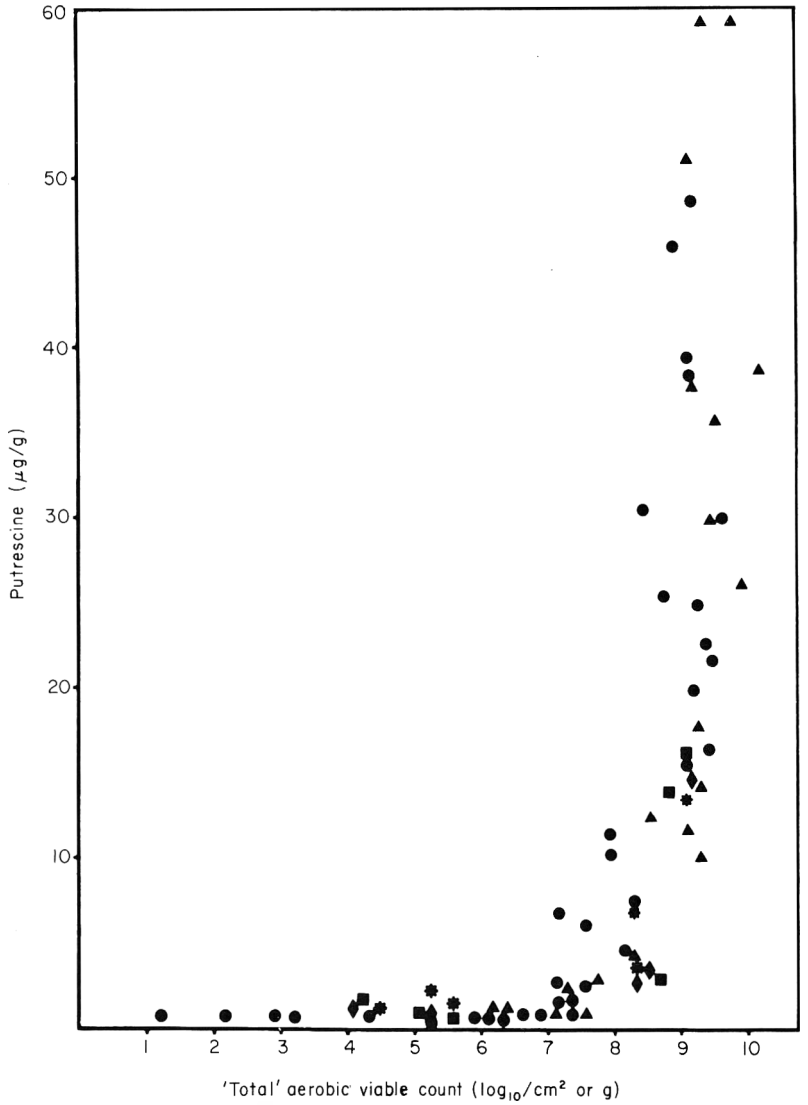

Figure 1. Relationship between 'total' aerobic viable count (TAVC) and putrescine concentration. ●, Beef storage experiments 1, 2 and 3; ▲, minced beef; ■, pork joints; ★, beef joints; ♦, lamb joints. Each point represents the mean of two determinations.

The reason for the latter was not clear but visual interpretation of the data indicated a breakpoint in the region of 10^6/cm^2 or g.

Slemr (1981) suggested that the combined concentration of putrescine and cadaverine could serve as an index of acceptability. Data relating this to TAVC was plotted (Fig. 3) and statistical analysis revealed a similar relationship to that between putrescine and TAVC. The basal concentration was higher (2.1 μg/g) and the breakpoint occurred at 4.2×10^7 organisms/cm^2 or g.

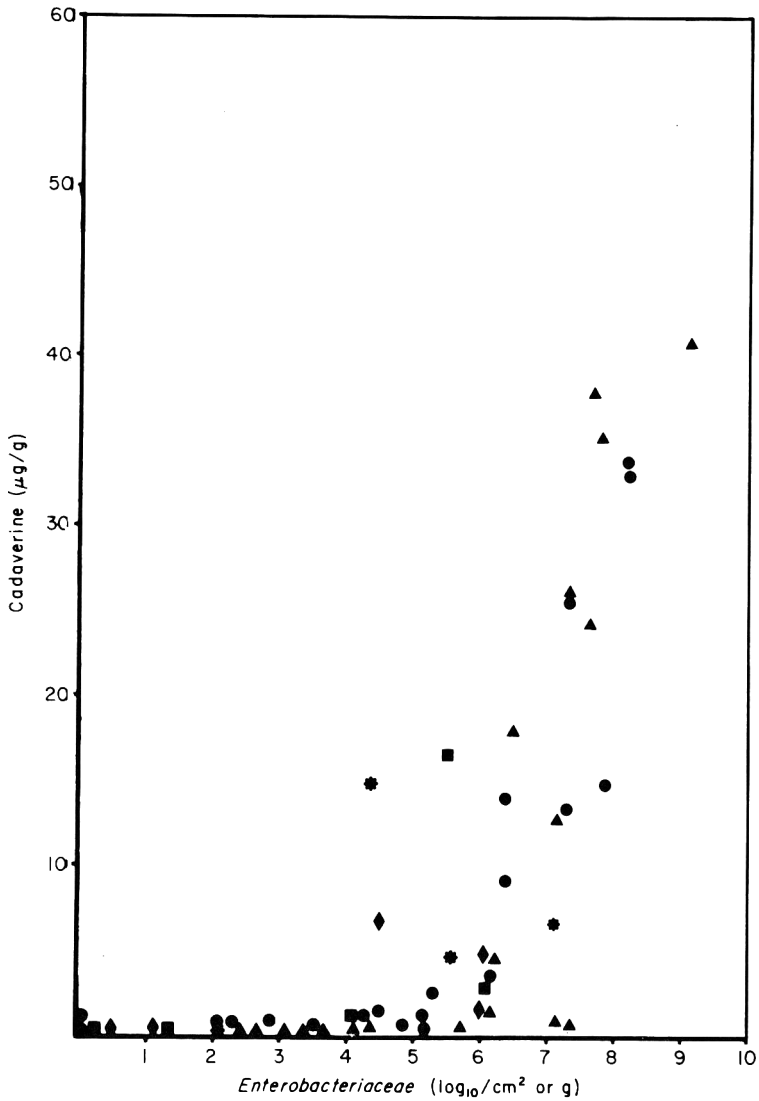

Figure 2. Relationship between *Enterobacteriaceae* counts and cadaverine concentration. Key as Figure 1.

Discussion

The concentrations of putrescine (range 0.4–2.3 μg/g) and cadaverine (0.1–1.3 μg/g) reported above for beef, pork and lamb are similar to those found by several other authors (Nakamura *et al.*, 1979; Yamamoto *et al.*, 1982; Slemr, 1981) and can be regarded as typical basal levels for red meat at retail. The higher concentrations found in 'fresh' pork, bacon and ham by Spinelli *et al.* (1974) and Lakritz *et al.* (1975) may have resulted from undetected microbial growth prior to purchase, as pointed out by the authors, and/or from the tlc method of analysis used (see Nakamura *et al.*, 1979).

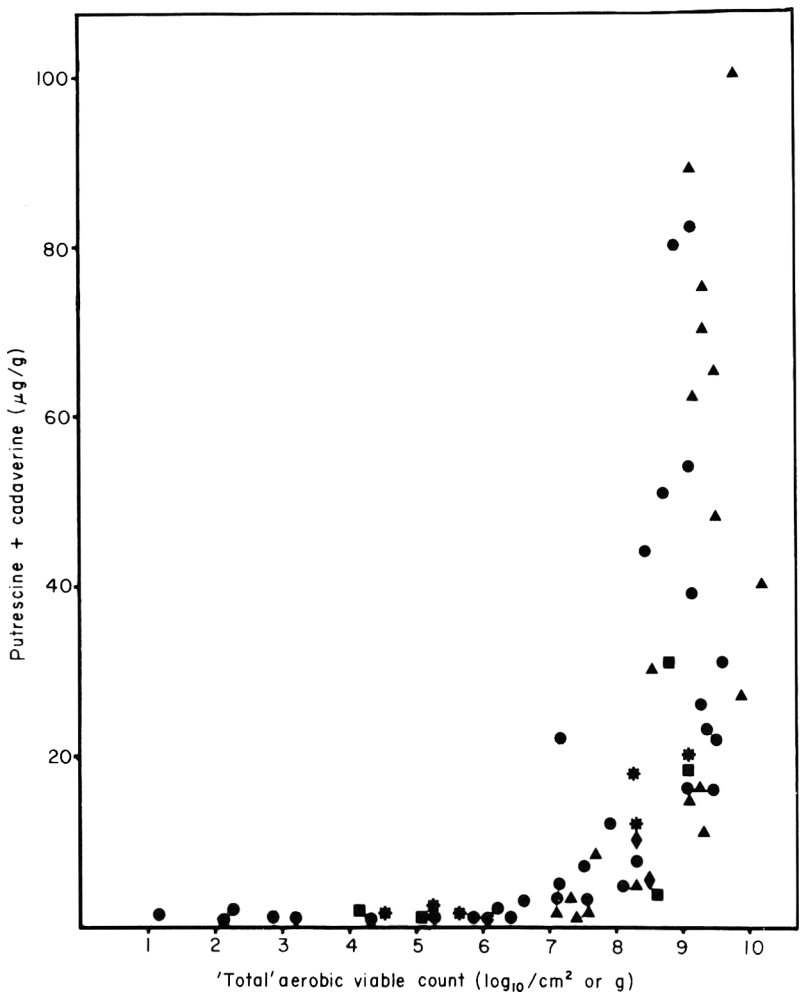

Figure 3. Relationship between 'total' aerobic viable count (TAVC) and combined putrescine and cadaverine concentration. Key as Figure 1.

Substantial increases in the combined putrescine/cadaverine content of our samples were observed after storage to an advanced stage of spoilage at 5°C, with production of both diamines in most cases. Statistical analysis of the results showed however that measurable changes in concentration did not occur until microbial numbers were well in excess of 10^7/cm^2 or g of meat, by which time the majority of samples were beginning to show visible signs of spoilage and/or off-odour development. Using organoleptic criteria alone, Nakamura *et al.* (1979) also concluded that increased diamine levels, in their case predominantly putrescine, coincided with the onset of spoilage. By contrast, Slemr (1981) reported a 10-fold increase in the combined putrescine/cadaverine content of chill-stored pork, *prior to* obvious organoleptic spoilage when microbial numbers were only in the region of 10^6/cm^2. At the onset of spoilage, bacterial

numbers were between $10^7 - 10^8/cm^2$ and a 100-fold increase in diamine content was recorded and as in the earlier sample over 90% of the increase was due to cadaverine.

These apparent anomalies are best explained by considering the probable microbial floras in the three studies. Slemr (1981) inoculated his samples with a 50/50 mixture of *Pseudomonas*/*Enterobacteriaceae* strains, having shown in pure culture studies that putrescine was the major amine produced by the *Pseudomonas* strains, cadaverine by the *Enterobacteriaceae*. High levels of cadaverine in his mixed inoculum samples strongly indicate, therefore, that *Enterobacteriaceae* strains remained a dominant element of the flora throughout storage, although it is not possible to prove this from the published data. Such a situation is atypical of naturally contaminated, chill-stored red meats, the spoilage flora of which is normally dominated by *Pseduomonas* strains, with rarely more than 3% or so of *Enterobacteriaceae* strains (Dainty, Shaw & Roberts, 1983). The fact that putrescine was the major diamine in the samples examined by Nakamura *et al.* (1979) is consistent with the development of a typical flora on their samples but unfortunately microbiological data was not reported. Furthermore, a comparison of the TAVC with the number of *Enterobacteriaceae* in our own samples, taken together with the elevated levels of putrescine in all of the stored samples and its predominance over cadaverine in the large majority of them, provides strong evidence of typical spoilage floras on these samples as well.

It therefore seems reasonable to conclude from our results and those of Nakamura *et al.* (1979) that there is insufficient accumulation of diamines in naturally contaminated red meats prior to obvious signs of spoilage to justify using their concentrations as in index of acceptability of the meat. Although this possibility cannot be entirely ruled out in situations where *Enterobacteriaceae* become dominant, the presence of these organisms in excess of 10^7/g on one of our samples of mince (Table 3, sample E) without any increase in cadaverine concentration suggests otherwise. Furthermore, Nakamura *et al.* (1979) were unable to detect elevated levels of cadaverine in samples of pork stored at 20°C, conditions favouring the growth of *Enterobacteriaceae*, prior to regarding the meat as 'unfit for human consumption'.

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Dissociation constant of sorbic acid in water and water-glycerol mixtures at 25°C from conductance measurements

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Summary

Molar conductivities of dilute solutions of sorbic acid in water, and in the mixed solvents water +10, 20, 30, 40 and 50% glycerol by mass at 25°C are reported. The results have been analysed with the Pitts conductance equation and pK_a values for sorbic acid in these solvent mixtures are tabulated. Similar data for sorbic acid in water+30% glycerol at 10°C are also included. The implications of the results for the role of sorbic acid in food preservation are discussed.

Introduction

Sorbic acid, *trans,trans*-hexa-2,4-dienoic acid, is now a common acid-potentiated food preservative (Sofos & Busta, 1981). Following its early application to low pH foods (Sheneman & Costilow, 1955), sorbic acid is being used increasingly as one of the numerous 'hurdles' employed in the preservation of intermediate moisture foods (Haas *et al.*, 1975; Raevuori, 1976; Roberts, Gibson & Robinson, 1982; Tompkin *et al.*, 1974).

In common with other carboxylic acid food preservatives, the antimicrobial activity of sorbic acid is dependent upon the undissociated molecule (Freese & Levin, 1978). Thus a prerequisite for its use is a knowledge of the pH of the food and pK_a of the acid. However, the pK_a value is dependent upon other properties including water activity, a_w, as dissociation requires the association of the hydrogen ion of the carboxylic group with water to form a hydroxonium ion (Lewin, 1974). Literature values for the pK_a of sorbic acid in water, though varying considerably, appear to originate from the determination by Ostwald (1889) of the dissociation constant as 1.73×10^{-5} mol dm^{-3} (equivalent to pK_a 4.76). There are no literature values for the dissociation constant of sorbic acid in systems in which other solutes are present in the water, for example, in

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water-glycerol mixtures which we have used as model systems of low, known water activity (Ison & Harrigan, 1981).

Recently, Strong, Kinney & Fischer (1979) have shown that the pK_a values for a wide range of substituted benzoic acids ($pK_a \sim 4.2$) can be obtained from conductance measurements on dilute aqueous solutions of the acids alone, without the necessity for making additional measurements on solutions of a salt of the acid. Although sorbic acid is rather weaker than those studied by Strong *et al.* (1979), it is just strong enough for measurements on the acid alone to give useful values of pK_a when the data are fitted in terms of Λ_0 (limiting molar conductance) and K_a simultaneously by the method of Ives (1933). The method is suitable for use with non-aqueous and mixed solvents, provided that no salts are present.

Materials and methods

Sorbic acid (purriss grade, Koch Light), was recrystallized six times from hot water and twice from hot conductivity water and dried over P_2O_5. Before use it was dried overnight at 50°C in a vacuum desiccator. Purity checks indicated 99.7% by titration and a m.p. 133°C and it was assumed to have a density of 1.204 g cm^{-3} (Weast & Astle, 1980). Glycerol (AR grade, Fisons) was purified by vacuum distillation and stored under dried N_2. Purity by refractive index measurement and reference to the tables of Stedman (1928) was determined to be 99.4%. All water was purified by ion exchange of distilled water.

Conductances were measured with a Wayne-Kerr B905 transformer ratio arm bridge with a nominal accuracy of 0.05%. However, when used to determine the cell constant the scatter of individual results about the mean was 0.02%. Measurements were made at 100 Hz, 400 Hz and 1 kHz and extrapolated to infinite frequency to eliminate polarization effects. The thermostat was maintained at 25±0.01°C and was checked against a NPL calibrated thermometer. The conductance cell was a three electrode cell of the Barthel type (Barthel, Wachter & Gores, 1979) with a cell constant of 4 cm^{-1}. The solvent conductance was assumed to be due to residual traces of CO_2 and was therefore ignored as we were measuring acidic solutions.

Sorbic acid is not sufficiently soluble at room temperature to make up a stock solution of adequate concentration and because of its finely crystalline, extremely hydrophobic character, the usual method of adding weighed samples of solid to the conductance cell could not be used as the rate of dissolution was far too slow. Consequently, a fairly concentrated stock solution was prepared at 50°C and a weight burette filled with this stock was kept in an air thermostat at this temperature. With practice it was possible to make an addition and the accompanying weighings without the burette cooling sufficiently to cause precipitation of the acid. Naturally the reproducibility of results obtained in this way is a little worse than that usually found, due to the slight evaporation of the solvent. However, the method was tested by determining the dissociation constant of benzoic acid in water at 25°C which was found to be

6.324×10^{-5} mol dm^{-3} (pK_a 4.1990). This compares favourably with the value of 6.331×10^{-5} mol dm^{-3} (pK_a 4.1985) determined by Strong *et al.* (1979).

Results and discussion

Values for the molar conductivities and concentrations of sorbic acid in water and the various water-glycerol mixtures at 25°C are given in Table 1a. One set of measurements at 10°C is also included in Table 1b. Values of the physical properties of the solvent mixtures used in our analysis are given in Table 2. These were calculated from smoothing functions determined from data in the literature.

Unfortunately, the concentration range which could be studied was severely limited at both the low and high ends. At concentrations below about 1.0×10^{-4} mol dm^{-3} the solvent conductance becomes too large a fraction of the total conductance (up to 20%) for it to be neglected with confidence. High concentrations could not be attained because of solubility limitations on our method. Typically the stoichiometric concentration range studied was from 3 to 10×10^{-4} mol dm^{-3} with a free ion concentration of $0.7-1.3\times10^{-4}$ mol dm^{-3}.

The results were fitted to the scheme:

$$\alpha=\Lambda/\Lambda_i, \tag{1}$$

$$\Lambda_i=\Lambda_0-f(\Lambda_0, \alpha c, R), \tag{2}$$

$$K_a=\alpha^2 cy^2/(1-\alpha), \tag{3}$$

$$\log y=-A(\alpha c)^{1/2}/[1+BR(\alpha c)^{1/2}], \tag{4}$$

where:
α=degree of dissociation,
c=molar concentration,
Λ=molar conductivity,
Λ_0=limiting molar conductivity as $c\rightarrow0$,
Λ_i=molar conductivity of a hypothetical fully dissociated solution at concentration αc,
R=closest distance of approach of free ions,
y=mean molar activity coefficient,
A and B=constants dependent upon solvent properties and temperature.

The ionic concentrations of the solutions are so low that almost any theoretical conductance equation can be used in (2) to give an equally good fit of the data. For the same reason there is no significant variation in the standard deviation of $\Lambda_{obs}-\Lambda_{calc}$ with the value assumed for R, the closest distance of approach of free ions. We have analysed our data with the full Pitts equation (Pitts, 1953; Pitts, Tabor & Daly, 1969), expressed as a power series (Pethybridge & Soltani-Taba, 1980), at a fixed R value of 500 pm using the program Hunter (copies available from ADP), but equations based on the theory of Fuoss & Hsia (1967) or Lee & Wheaton (1978) give identical results.

Table 1a. Molar conductivities of sorbic acid in water and water-glycerol mixtures at 25°C

Water		10.02% glycerol	
10^4 conc. (mol dm^{-3})	Λ (Ω^{-1} cm^2 mol^{-1})	10^4 conc. (mol dm^{-3})	Λ (Ω^{-1} cm^2 mol^{-1})
3.0430	86.801	3.1291	67.060
3.6528	80.166	3.6452	62.637
4.3151	74.478	4.4332	57.405
5.1006	69.129	5.3987	52.500
6.3410	62.681	6.0727	49.761
7.1779	59.247	6.6341	47.779
8.2527	55.602	7.5339	45.062
9.5836	51.934	8.2647	43.179
10.535	49.721	8.8986	41.728

20.00% glycerol		29.80% glycerol	
10^4 conc. (mol dm^{-3})	Λ (Ω^{-1} cm^2 mol^{-1})	10^4 conc. (mol dm^{-3})	Λ (Ω^{-1} cm^2 mol^{-1})
3.2218	49.018	3.4915	33.487
3.9640	44.679	4.2561	30.647
4.8762	40.687	5.1704	28.050
5.5885	38.237	5.9819	26.249
6.2816	36.261	6.7707	24.803
6.9707	34.571	7.4879	23.685
7.6845	33.050	8.3402	22.530
8.5002	31.544	9.0607	21.695
9.1560	30.481	9.8657	20.851

40.03% glycerol		50.02% glycerol	
10^4 conc. (mol dm^{-3})	Λ (Ω^{-1} cm^2 mol^{-1})	10^4 conc. (mol dm^{-3})	Λ (Ω^{-1} cm^2 mol^{-1})
3.5324	21.237	3.5668	12.246
4.2569	19.540	4.3572	11.218
5.1262	17.982	5.1947	10.379
6.1186	16.592	6.1147	9.651
6.8632	15.741	6.9721	9.098
7.5904	15.033	7.7010	8.698
8.3178	14.413	8.5215	8.309
9.2369	13.733	9.4500	7.928
10.019	13.227	10.169	7.667

Table 1b. Molar conductivity of sorbic acid in water-glycerol at 10°C

29.40% Glycerol	
10^4 conc. (mol dm^{-3})	Λ (Ω^{-1} cm^2 mol^{-1})
2.6940	26.271
3.7490	22.763
4.6129	20.760
5.4347	19.292
6.3883	17.927
7.2711	16.905
8.0847	16.120
8.8410	15.464
9.7130	14.818
10.601	14.229

The values of Λ_0 are not as reliable as those usually obtained from conductance measurements, but because the acid is so weak the values of pK_a are not particularly dependent upon the exact value of Λ_0. The value of Λ_0 is quite close to that expected from the limiting conductances of the hydrogen ion, 349.8 Ω^{-1} cm^2 mol^{-1}, (Robinson & Stokes, 1970) and the sorbate ion, 31.3 Ω^{-1} cm^2 mol^{-1} (Pethybridge & Cassford, 1982, unpublished results: measurements on dilute aqueous solutions of potassium sorbate at 25°C whose analysis using the full Pitts equation with $R=500$ pm yielded $\Lambda_0=104.80$ Ω^{-1} cm^2 mol^{-1} and an association constant, $K_A=0.72$ mol^{-1} dm^3).

Table 2. Values of dielectric constant* (ε) viscosity† (η) and density‡ (ρ) of the solvent mixtures used in this work

% Glycerol w.w at 25°C	ε	η poise	ρ (g cm^{-3})
0	78.303	0.008903	0.99707
10.02	75.69	0.01158	1.0204
20.00	72.69	0.01540	1.0460
29.80	69.85	0.02126	1.0707
40.03	66.99	0.03160	1.0969
50.02	64.32	0.05024	1.1241
29.40§	75.76	0.03411	1.0745

* Åkerlöf (1932).
† Segur & Oberstar (1951).
‡ Bosart & Snoddy (1972).
§ At 10°C.

Table 3. Best-fit parameters for sorbic acid solutions at 25°C using the full Pitts equation with R=500 pm

% w.w. glycerol	n	Λ_0 (Ω^{-1} cm^2 mol^{-1})	$\sigma\,\Lambda_0$ (%)	10^5 K_a mol dm^{-3}	σK_a (%)	pK_a	Walden Product $\eta_0\Lambda_0$ (Ω^{-1} cm^2 mol^{-1} poise)
0	9	373.37	0.025	2.12	0.054	4.674	3.33
10.02	9	329.83	0.016	1.60	0.035	4.796	3.82
20.00	9	246.74	0.023	1.56	0.051	4.807	3.80
29.80	9	174.05	0.033	1.58	0.073	4.801	3.70
40.03	9	106.51	0.015	1.73	0.033	4.762	3.37
50.02	9	53.635	0.027	2.37	0.059	4.625	2.69
29.40*	10	108.32	0.037	2.07	0.081	4.684	3.71

* 10°C.

The best-fit values of Λ_0 and K_a for our data in all the solvents studied are given in Table 3. The values were derived from the conductance measurements from individual concentrations of sorbic acid in each solvent mixture, Tables 1a and b. It is clear that whilst the exact value of pK_a is not particularly dependent upon the solvent composition (because the dielectric constant hardly changes), the values of Λ_0 change dramatically. This is due primarily to the change in viscosity as the Walden product, $\eta_0\Lambda_0$ (Table 3), is almost constant in this system, except in the extremely viscous water+50% w.w. glycerol mixture. The pK_a values of sorbic acid at 25°C in water-glycerol mixtures can be fitted with a standard deviation of fit of 0.017 by the equation:

$$pK_a = 4.680 + 0.0123m - 2.66\times10^{-4}m^2, \qquad (5)$$

where m=% glycerol w.w.

With respect to chemistry, the dissociation constant of sorbic acid in water at 25°C determined in this work, 2.12×10^{-5} mol dm^{-3} (pK_a 4.67) differs significantly from Ostwald's original value of 1.73×10^{-5} mol dm^{-3} (pK_a 4.76) and the commonly quoted pK_a value of 4.8 (Baird-Parker, 1980). However, in the context of food preservation the values are not significantly different. Food systems cannot be regarded as homogeneous environments, especially with respect to pH and therefore a small error in the preservative pK_a value used for calculations, such as reported here, would not significantly affect the efficacy of the preservative.

The pK_a values have been plotted against theoretical a_w values for the water-glycerol mixtures investigated in Figure 1. Again, in the context of food preservation, reduction of the a_w to 0.80 by glycerol would not significantly affect the ratio of undissociated : dissociated acid at constant pH. The effect of a reduction of temperature to 10°C in the 30% w/w glycerol mixtures can also be regarded as negligible (Table 3). However, although the dissociation characteristics of sorbic acid would appear to be relatively constant with change in a_w and temperature, such parameters may have a significant effect on the pH of food systems. Therefore should conditions prevail which cause a small

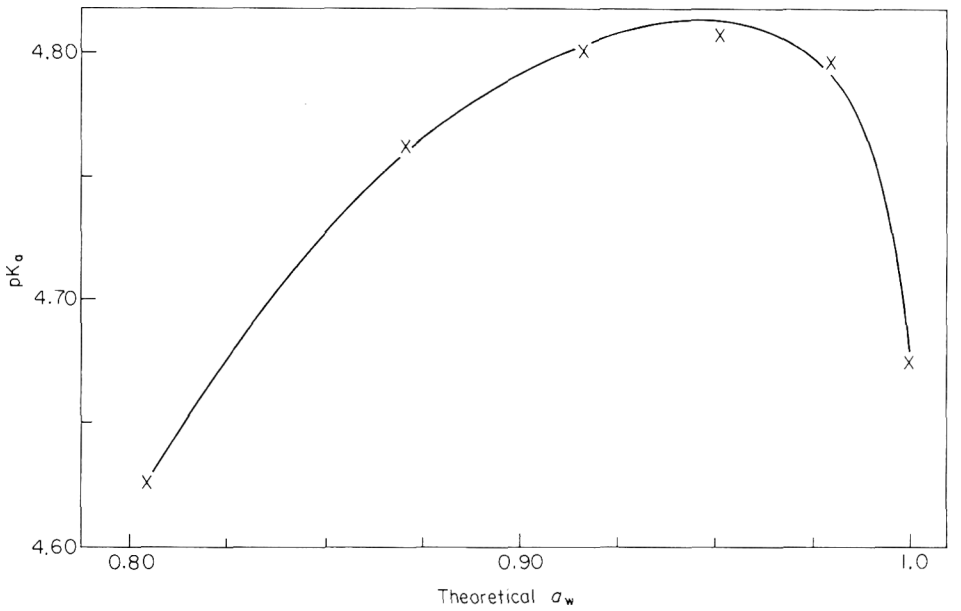

Figure 1. Effects of a_w (by glycerol) on the pK_a of sorbic acid at 25°C. (a_w values determined by reference to tables in Scatchard, Hamer & Wood (1938).)

reduction in the pK_a value and an increase in the pH then it is possible that the efficacy of the food preservative may be dramatically reduced. This emphasises the importance of having an awareness of the environmental conditions which will be encountered by the preservative and, where possible, adjustments made to the levels of preservative added to achieve the desired degree of efficacy.

Other carboxylic acids employed for food preservation have been shown to demonstrate a more predictable response to reduced a_w. Gelsema, De Ligny and Van der Veen (1978) demonstrated by emf studies that the pK_a values of acetic acid (pK_a 4.76) and propionic acid (pK_a 4.87) at 25°C increased by 0.66 and 0.73 units respectively in 60% w/w glycerol, and 0.53 and 0.57 units respectively in 60% w/w sucrose. Thus, at constant pH, the proportion of undissociated to dissociated molecules for both acids increased with increasing concentration of non-aqueous solvent and hence decreasing a_w.

The method employed in this study can be used for any carboxylic acid—e.g. benzoic, citric, etc.—in the presence of any non-ionic solvent—e.g. alcohols and carbohydrates. Such studies would supply useful information to the food industry enabling a better understanding of the interaction of preservatives with the food environments and the identification of possible synergistic systems which would optimize or enhance the preservative efficacy.

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Technical note: The improvement of green colour of green beans by acidified bulk storage

RICHARD M. BASEL

Introduction

Acidified bulk storage of green beans has been successful using hydrochloric acid as the sole acid when acidifying to a pH of ≤ 1.5 (Basel, 1982). This method allows the storage of green vegetables for later processing. It is the first method for economical storage to lengthen the plant operating season beyond harvest. During the storage of green vegetables, it was noted that the colour of the finished products was better than that found in conventionally canned products. The purpose of this study was to verify and quantify how much greener the colour of green beans was upon acidified storage.

Materials and methods

The green bean cultivars ('Tendercrop', 'Stretch' and 'Eagle') were grown in the Ohio State University Horticulture Research Farm located at Columbus, Ohio. Green beans were stored (a) after blanching or (b) after canning into standard 303 tin cans under normal processing with a cover solution (50% by wt) of 30 ml HCl/l distilled water to a final weight of 454 g. The samples were stored for 3 months in vacuum sealed polyethylene bags performed with a Griswold type VNP Vacu-U-Seal machine (Cheslam Corp., Yonkers, NY) and no heat treatment was employed. The pH adjustment to pH 1.3 was sufficient to prevent microbial deterioration. Monthly samples in triplicate were removed for analysis and neutralized to their original pH with 30% sodium hydroxide in three equal steps. Since quality did not change appreciably after a few days of storage, the 3 months' quality results presented are representative of what could be expected after almost any storage time. A post treatment of canning the neutralized blanched green beans (c) was used to demonstrate what colour a

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finished product might have. Colour of the snap beans was determined in plexiglas cup 1½ in deep of product on Hunter Colorimeter D25D3A (Hunter Assoc. Lab., Reston, VA) using a green C2-4308 Hunter tile (L, 64.6, a, 14.2, b, 6.0), where L=visual lightness (100 being the lightest), a=redness, $-a$=greenness, b=yellowness, and $-b$=blueness. All tests were triplicated and statistically treated with Duncan's multiple range test (Ohio Agricultural Research and Development Center Statistics Laboratory, Wooster, OH).

Results

With raw blanched green beans, a dramatic visual change in colour from bright green to olive green occurred upon acidification. Table 1 shows a yellowing by the increase in the b value that corresponds to a chromatic shift from the blue. Changes in the L and a values are presumably a function of this chromatic shift. When green beans were removed from storage, they had a dark green colour with approximately the same green intensity of canned product as shown by the a values which are a measure of the red to greenness. After neutralization and canning, the product becomes lighter coloured (seen as a change in L value) and much greener than conventionally canned product.

This same trend was found with the canned product. Canned product had a typical olive green colour of canned green beans. Upon storage for 5 days, the colour improved statistically. This is seen as a shift to higher $-a$ values and lower b values demonstrating a blue shift. Upon canning, the colour was darker as indicated by a lower L value. There was not any statistical difference in the degree of a value greenness after heat processing.

Table 1. Greening effect of acidified bulk storage on three cultivars of green beans from conventionally blanched product

		Cultivars		
Treatment		Tendercrop	Stretch	Eagle
Before acidified storage	Hunter L*	21.67a†	24.41b	24.40b
	Hunter a	−0.68a†	−0.50a	−1.37b
	Hunter b	7.89a†	8.92b	9.34b
After acidified storage and neutralization	Hunter L	30.42c	31.06c	32.40c
	Hunter a	−1.70c	−1.95cd	−1.71c
	Hunter b	12.70c	13.23d	13.96e

* Hunter Lab. colour values.

† Means followed by the same letter are not significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Table 2. Greening effect of acidified bulk storage on two cultivars of green beans from conventionally canned product

Treatment		Cultivar	
		Stretch	Tendercrop
Conventionally canned	L*	33.04a†	28.72a
	a	0.14a†	−0.09a
	b	17.20a†	14.43b
Acidified storage of above product	L	30.77b	27.61b
	a	−0.92b	−1.27b
	b	15.66c	12.94d
After glass packing reprocessing of above product	L	21.17c	21.72c
	a	−0.45ab	−1.31b
	b	8.40e	8.35e

* Hunter Lab. colour values.

† Means followed by the same letter are not significantly different ($P<0.05$) as determined by Duncan's multiple range test.

Discussion

In this study significant changes were found to occur in green vegetables during bulk storage. This is mainly a shift in green colour (*a* value) during storage and a shift in the *L* value after canning. While shifts in colour are not nearly as dramatic in the storage of conventionally canned beans, it is obvious that chemical changes occur in the pheophytins and pheophorbides just as in the native chlorophyll of freshly blanched green beans. This is an important discovery because it affords the processor a method of storing a green vegetable that has better colour attributes than that of conventional canned green vegetables. Since no end products except common table salt are present after storage this method should not face strict governmental restrictions as other preservation methods have.

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Technical note: The estimation of viable count in the control of quality in food manufacture

R. P. MURPHY

Introduction

Apart from the function of bacteriology in assessing the safety of food from a public health point of view, it can also be used to measure the uniformity and hygiene of a process line. In the case of hygiene, the product is examined for evidence of man-borne contamination (Hall, 1969; APHA, 1958). This is commonly shown by high numbers of *Escherichia coli* or *Streptococcus faecalis* (Collins, 1967) in the product. However it has been recognized that, in the absence of these, there is a general level of bacterial contamination in most food process lines. In canneries, this is kept to a certain minimum above which a given sterilization process in the retorting would be ineffective. In the case of dried and frozen foods, the contamination is very high and some processors are content to ensure a low level of *Escherichia coli* and faecal streptococci. However, it has been found that even in these forms of processing, the contamination level as measured by the so-called total viable count is an accurate measure of the hygiene control of the process. Unfortunately, the methods which use this parameter usually call for a 48 hr incubation period (Collins, 1967 and ICMSF, 1980) except for counts at or over 55°C.

It will be easily appreciated that any analytical method which purports to be a control over a production line must be capable of producing results quickly; otherwise the technique will only serve to show that over a certain period, there has been some malfunctioning in the process. This can be very costly if the line has been producing defective material for 48 hr or more. The value of any saving in time in producing process control results will be directly proportional to the amount of material manufactured in the period in question.

Materials and methods

Over 200 samples were tested. These were made up of fourteen different dried soup mixes in lots of six and ten. Each lot was composed of replicate samples

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taken from 5 lb tins of the soup in question. The remaining samples were from production runs of individual vegetables such as peas and carrots. These were sampled as follows.

Several pounds of each vegetable were taken from the line just after final drying and before packaging. Each primary sample of both vegetables and soups was carefully sub-sampled by dividing into four and taking an aliquot from each quantity.These were then mixed and used to produce the sample analysed.

In the case of powdered soups without large particles, 5 g were suspended in 95 ml, quarter strength Ringers solution and shaken 25 times through a 30 cm arc to mix; 10 ml of this solution were pipetted into 90 ml sterile quarter strength Ringers solution and mixed as above. One ml of each dilution was pipetted into each of two Petri dishes into which Oxoid Nutrient Agar No. 1 was poured. This was allowed to set and overlaid with 2 or 3 ml of water agar.

In the case of samples containing large particles, 10 g were used and treated as above. The plates so prepared were inverted and incubated at 37°C. Plates were counted after 24 hr, then re-incubated and counted again after a further 24 hr.

Results and discussion

Counting was done by the comparator method described by Murphy & Tucker (1970) and checked by counting the colonies of 20% of the plates in the normal

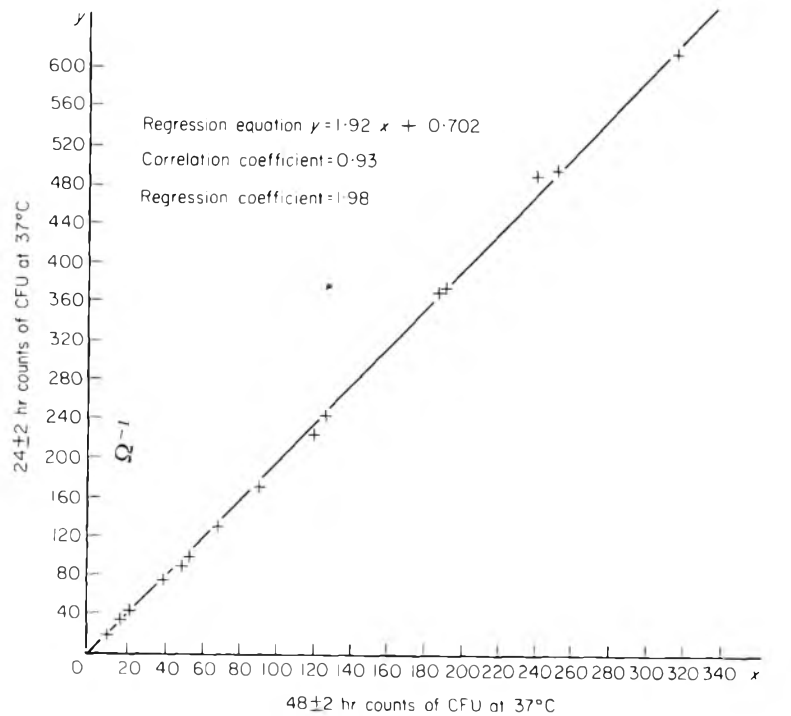

Figure 1.

way. Where there was a significant difference between dilutions, that dilution giving counts of between 30 and 300 colonies per plate was counted. The numbers used to prepare the graph in Figure 1 are actual plate counts. Figure 1 shows the relationship of 24 and 48 hr counts. As it may be thought essential to macerate large particles to get an accurate count of the bacteria present, a comparison was made between the counts found by the above method and those found by a similar method using a maceration technique. In this method, the material was suspended in 90 ml of quarter strength Ringers solution in an Ato-Mix jar and macerated for 2 min. This material was used to prepare working dilutions, aliquots of which were pipetted into Petri dishes as above. No significant difference in counts in either technique was found.

Figure 1 shows the regression line derived by pairing the data points found in the 24 and 48 hr counts. The regression equation is also shown. The regression coefficient found was 1.98, so that an estimate of the 2 day count is obtained by multiplying the 1 day count by 1.98 or without much loss of accuracy by 2.

The results of these analyses confirm that the counts obtained after 24 hr are almost precisely doubled by continuing the incubation for a further 24 hr. Furthermore, it can be demonstrated that there is no further significant increase following any incubation prolonged beyond the 48 hr.

Conclusion

It is shown that counts of CFUs after 48±2 hr incubation at 37°C are almost exactly double those at 24±2 hr. It is not suggested that such a short incubation period would be satisfactory for all purposes; indeed it is obvious that it would be very undesirable if isolation of various specific organisms were required. However, in the control of food processing, it has been found that the level of bacterial contamination of the product may be subject to changes due to the development of faults in the production line. It is therefore, desirable to have the results of bacterial counts as quickly as possible. It is shown that for this purpose, 24 hr counts are just as effective as 48 hr counts.

It has also been shown that mechanical maceration of the particles examined gives no great increase in the counts. In fact, it may even lead to false high counts as it is difficult to distinguish colonies from small food particles.

In the case of dried soup mixes which already contain fine particulate matter, it has been found that at the dilution factor normally necessary (about one in 200) the particles are not plentiful enough to effect the counts. In any event most of the individual particles are too small to be readily visible.

Acknowledgment

I would like to express gratitude to my assistant, Miss Frances Anderson, for all the preparation work involved.

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Book reviews

Handbook of Carcinogens and Hazardous Substances: Chemical and Trace Analysis. Ed. by Malcolm C. Bowman.
New York: Marcel Dekker, 1982. Pp. ix+750. ISBN 0 8247 1683 3. Swiss Francs 284.

This volume provides a comprehensive treatise on the analysis of toxic compounds in food, biological and environmental samples. The amount of material and detailed references is almost overwhelming and the editor is to be congratulated on the compilation of an extremely useful reference book. In particular the attention to experimental detail (including modern instrumental techniques) is most impressive and fundamental problems such as recovery and sample preparation have been by no means ignored. Chapter 1 presents an overview of chemical carcinogens and, although this is extremely well referenced, could perhaps have been extended to include more detail on the biological function of these compounds. The next chapters cover the occurrence and analysis of a wide range of toxic compounds including alkylating agents, aromatic amines, oestrogens, mycotoxins, nitroso-compounds, pesticides, polynuclear aromatics, toxic metals and halogenated contaminants (e.g. dibenzo-p-dioxins). The authors have not adopted a standard format, e.g. in terms of sections on sample preparation, extraction, analysis, etc., but the necessary information is readily accessible. The chapter on toxic metals is rather brief; for example, given the current debate on lead in the environment, a single page devoted to its occurrence and analysis seems rather inadequate.

This book, despite its high price, will be enthusiastically received as a reference book by those people working in this area. Indeed it will be essential reading for those institutions setting up analytical procedures for these toxic substances.

R. Macrae

Food Drying. Ed. by Gordon Yaciuk.
(Proceedings of a Workshop held at Edmonton, Alberta, 1981.) Ottawa: International Development Research Centre, 1982, Pp. 104. ISBN 0 88936 3331. Canadian $8.00.

Contributions to this workshop, and hence this book, were organized to cover four important areas in the design and operation of a drying system, namely: drying requirements, consumer acceptance, heat and mass transfer and heat sources. Topics included under the first of these headings are: drying of fish in India, by solar and hot air methods; solar drying of vegetables, Jew's mallow

and okra, in Egypt; hot air drying of potatoes in Peru and artificial drying of paddy in Indonesia. Under the heading of consumer acceptance are contributions on the nutritional quality and acceptability of: a range of dried foods, leafy vegetables, mango and papaya, in Kenya; cowpea products in Thailand; banana weaning food in Costa Rica and fish in Indonesia. Heat and mass-transfer aspects of the drying of cereal grains and fish in the Philippines, onions in Nigeria and grapes in Chile form a third section. In a fourth section, entitled heat sources, the topics covered are: solar drying of crops in Sierra Leone; solar and natural air drying of rough rice in Korea; a farm grain dryer in Thailand, using a rice-hull furnace to heat the air; an economic appraisal of alternative energy sources, diesel oil, biogas, firewood, coffee pulp and solar energy to dry coffee beans; a description of four different types of dryer, two flat beds, a Lister dryer and a bin dryer, for use in Indonesian co-operatives.

As with most works which are a collection of contributions by different authors, there are considerable variations between them in terms of their length, the depth of the treatment and the style of writing. However all are interesting and read well. About half of the papers include relevant references. The diagrams and plates are of good quality. The book is not indexed.

This volume should be of considerable value to individuals and organizations concerned with food production, processing and storage in developing countries. It should also be of interest to those involved in teaching and research in food dehydration, in both developing and developed countries, and would be a useful addition to libraries concerned with such topics.

J. G. Brennan

Chemistry of Foods and Beverages: Recent Developments. Ed. by George Charalambous and George Inglett.
London: Academic Press, 1982. Pp. x+348. ISBN 0 12 169080 6. £19.60.

This book is the combination of the proceedings of two separate, and, in many ways, quite distinct conferences. The first of these was the Second International Flavour Conference in Athens held in July 1981 and was concerned with the quality of food and beverages. The second conference was titled Formulated Foods and their Ingredients and was held in Anaheim, California in November 1981. It is not obvious from the book which contributions were presented at which conference but it is reasonable to assume that the first nine chapters were derived from the conference in Athens and the remaining eight from that in California.

The topics in the first part cover the most up to date gas chromatographic techniques for aroma compound identification, including the description of a micro-olfactometer. The use of computers to assist quantification is outlined in a chapter on carrot volatiles. Certain sensory techniques are described with

reference to soy protein hydrolysates and there is a good review on the use of flavour nucleotides in food. A chapter on the problems of 'Fast Indigenisation' on the quality of food and beverages in Nigeria seems out of place compared with the more analytical preceding contributions. However, this is obviously a major problem for countries such as Nigeria and deserves exposure.

The remaining chapters, from the conference in California, are more concerned with food technology and as such have little relevance to the title of the book. For example a chapter on the manufacture, use and nutritional aspects of 90% high fructose corn sweeteners can scarcely come under the umbrella of food chemistry. Other topics that are covered include: functionality of corn-derived sweeteners, dairy-based ingredients, low moisture foods, xanthan gum, polydextrose and extrusion cooked foods. It is to be hoped that potential purchasers of this book will not be misguided by its apparently too narrow title.

The chapters are generally of a high standard and do indeed cover a wide range of topics as stated in the preface. Much of the material is original. This is a rapid manuscript-reproduction volume and thus the contributions are typed; this is an annoyance which is becoming unfortunately too common, even in a book of this price. The standard of reproduction of diagrams is acceptable although photoreduction has rendered some legends almost illegible. There appears to have been no attempt to standardize the format with some chapters having abstracts, some literature reviews etc. There are a few minor typographic errors in the text and illustrations.

Many of the contributions will be of interest to research workers but they do not combine to form a text with a common theme and as such it will become a library reference book rather than an addition to personal collections.

R. Macrae

Fragrance Chemistry: The Science of the Sense of Smell. Ed. by E. T. Theimer.
New York: Academic Press, 1982. Pp. xiii+635. ISBN 0 12 685850 0. £59.20.

The book is intended as an introduction to the chemistry of odorous molecules. It is too detailed for an introduction and it is limited to those types of odorous molecules familiar to the perfumer, dealing only incidentally with the interests of the flavourist and omitting altogether types of primary importance to him, such as esters and pyrazines. Off-odours and stenches, surely another essential concern of any science of the sense of smell, are not given due consideration either. The sub-title is thus seriously misleading.

The sixteen chapter titles, together with their authors, illustrate the scope: Physiology of vertebrate olfactory chemoreception (T. V. and M. L. Getchell, 25 pp.); Odor theory and odor classification (J. E. Amoore, 50 pp.); Odor and stimulant structure (M. G. J. Beets, 46 pp.); Acyclic monoterpene alcohols

with a 2,6-dimethyloctane skeleton (H. Boelens, 41 pp.); Advances in the chemistry of some interesting cyclic monoterpene alcohols (P. C. Traas, 55 pp.); Sesquiterpene alcohols (V. Herout, 45 pp.); Benzene-derived cyclic carbinols (E. T. Theimer, 17 pp.); Violet fragrance compounds (P. Z. Bedoukian, 31 pp.); Syntheses of vetiver oil components (H. van den Dool, 32 pp.); The fragrance of jasmine (E. P. Demole, 48 pp.); Chemistry of sandalwood fragrance (E.-J. Brunke and E. Klein, 35 pp.); The chemistry and fragrance of natural musk compounds (B. D. Mookherjee and R. A. Wilson, 62 pp.); Chemistry of synthetic musks: I. Non-benzenoid musks; II. Benzenoid musks (T. F. Wood, 13 and 26 pp.); The fragrance of ambergris (G. Ohloff, 39 pp.); Analysis of fragrance materials (J. P. Walradt, 41 pp.). Most of the authors are recognized experts, some with outstanding reputations.

The first three chapters and the last are general and constitute very good surveys, which most flavourists will find helpful and thought-provoking. The chapters central to the book are written primarily from the point of view of chemical synthesis, though the style differs from chapter to chapter. Clear structural formulae abound. Some indications of industrial significance are given, as are odour assessments, but the editor has not insisted on parallel treatment. Tighter editing in other respects, too, such as uniformity of nomenclature, cross-linking, and clarity of expression, would have been beneficial. Nevertheless, there is no doubt that those concerned with fragrance chemistry will find the book of great value, presenting as it does a very substantial collection of ordered data.

Although by no means free from errors, the volume is well produced and has a subject index (18 pp.). Although fully documented, half the chapters have no references later than 1979; the book has clearly been some time in production. The price is high.

H. E. Nursten

Food Proteins. Ed. by P. F. Fox and J. J. Condon.
London: Applied Science Publishers, 1982. Pp. xi+361. ISBN 0 85334 143 5. £38.00.

This is the book of the symposium which was held to mark the opening of a new Food Science Building at University College, Cork. It has the strengths and the weaknesses of most books of the genre. The strengths are those which result from obliging the very expert speakers at a symposium to put their understanding of their subjects down on paper, having already taken the trouble to organize their thoughts clearly and succinctly for the benefit of a critical audience. In this book this part has been well done. The weaknesses however are those which are difficult to avoid with such an approach. The

chapters stand independently, avoiding repetition certainly, but with some patches of thin or no coverage and not flowing too well from one to another.

The first chapter is an excellent review of the food economy of Ireland (mainly, but far from entirely that of the Republic). Thereafter the Irish dimension almost disappears, to be observed only very faintly in the relative emphasis given to meat and dairy proteins.

There are five chapters which deal with the world supply and availability of protein, questions of nutritional measurement, allergy and the effects of food processes on nutritional quality. The authors of all these chapters appear to be in agreement that the worldwide problems here are primarily those of poverty and *food* shortage and that the problems of protein shortage are derivative from these. There is a review of the 'functional' properties of food proteins as related to what is known of their structures, then the rest of the book deals with the science and technology of particular food proteins. There are four chapters on milk and its products (where, interestingly, the intense research of recent years has been directed to the problems not of shortage but of excess), three chapters on meat, one on general techniques for the isolation of proteins, one on the specific problems of leaf proteins and two concerned with protein recovery from semi-solid or liquid wastes.

Mixed into the middle of all this is a contribution on biological nitrogen fixation, much too brief to do justice to the fundamental production of protein without use of fossil-based fertilisers, yet still this chapter contains almost all that there is to be found here on legume proteins. The treatment of the cereal proteins is similarly deficient—the science underlying gluten behaviour is adequately covered in the chapter on functional properties, but that is all.

Where this book is good it is, like the little girl, very good. The chapters on nutritional matters give a fair summary of current thinking and attitudes, as do those on meat; those on milk give much recent factual material in a well balanced way. But the gaps are unfortunate and must diminish the brave sound of the editors' claim that 'the scope of this symposium is unique'.

Perhaps this highlights another feature of publications of this 'Book-of-the-Symposium' kind: surely all but the most outstanding of them must be regarded not as textbooks, from which a certain completeness must be expected, but as collections of review papers. To extract the maximum benefit from them it is essential that they are indexed in at least the same depth as the issues of a periodical journal, to ensure that all that is good can be found but time is not wasted looking for what is not there. Which is only another way of saying that this book should be purchased and carefully indexed by all libraries whose interests lie in the fields indicated above as being covered by it.

It is well produced, with an adequate (internal) index. This reviewer found few typographical errors, and those he did find amused him. 'Gelatin' instead of 'gelation' (p. 97) is a pity, the 'grass protein value' as a term to be restricted to specialists (p. 123) is hilarious! And there is a curious stiffening piece in the middle of 'the rigor | state' on p. 245.

M. D. Ranken

Colloids in Food. By Eric Dickinson and George Stainsby.
London: Applied Science, 1982. Pp. xiv+533. ISBN 0 85334 153 2. £48.00.

In the now classical, DLVO, theory of colloid stability a (usually) repulsive force arising from the interaction of diffuse double layers is added to a (usually) attractive dispersion force to give the total interaction. In spite of many successes, DLVO theory has failed to describe adequately stability phenomena in certain lyophobic and many lyophilic colloidal dispersions. With the development of concepts of steric stabilization (i.e. stabilization by lyophilic polymer molecules projecting from the colloid into the continuous phase), the range of application of DLVO theory has been extended and bold attempts have been made to describe aspects of the stability of even some complex biological systems. In *Colloids in Food*, Dickinson and Stainsby are predominantly concerned with such problems, and the usefulness of the theories of colloid science in food science provides the raison d'être for this book.

There are three chapters devoted to the DLVO theory and steric stabilization, a chapter on the oil-water interface and an excellent summary of protein adsorption. Sandwiched amongst these is a chapter on experimental methods, providing thumbnail sketches of a wide range of techniques and later in the book a whole chapter on rheology. A notable feature is the chapter on colloidal aspects of milk which, as the authors correctly claim, is the food colloid about which most is known. The book ends with a brief description of some other food colloids. What is not dealt with, for the title may mislead, are the subjects of gels, lipid bilayers, optical spectroscopy, X-ray methods or, more importantly, nuclear magnetic resonance. With the exception of dairy products readers will learn comparatively little about individual food colloids but a great deal about colloid stability.

The treatment is predominantly physico-chemical stressing physical principles and quantitative descriptions, although the authors take care to provide a clear physical feel for equations to guide the less mathematically inclined student through the text. The level of treatment is about that which is appropriate for a first year (U.K.) graduate student in chemistry with a strong background in physical chemistry. Sufficient material is provided to introduce problems at the current frontiers of research and ample references are given up to the first half of 1982. I came across few errors other than in the first half of the chapter on the colloidal aspects of milk, and there were ample, clear, illustrations, many reprinted from the original literature, which were always appropriate and repaid careful study.

Water structure, solvent-solute interactions and the thermodynamics of polymer solutions are subjects that I would have liked to see treated in greater detail. In food colloids adsorbed macromolecules invariably are implicated in stability and the structure of interfacial water has a significant influence on aggregation phenomena. Is it sufficient to state (p. 34) that 'It has become apparent in recent years that the traditional need to invoke additional

"anomalous" forces to explain special effects (e.g. "Stern layer", "structural water", "hydrophobic interaction" etc) is unnecessary if statistical factors are properly included', when the statistical factors cannot be properly included for a variety of reasons? Colloid scientists (and all other scientists) currently use a number of *ad hoc* approaches, only a few of which are outlined in the book, so an eclectic summary would have been most helpful.

Finally, there is a *caveat* to the use of DLVO and related theories which it is appropriate to mention here. Continuous dielectric media and smeared-out charge distributions appear in the DLVO theory as approximations to reality that are most exact at large distances of interaction. Steric stabilization is essentially a short-range force (although its effects may be apparent when the centres of mass of the particles are still far apart) so when it is due to a polypeptide chain, details such as protein conformation, distribution of charged residues and the variety of interactions of amino acids with solvent can become of overriding importance. Smeared-out distributions of charge, mass and 'chemical nature' are not always good approximations in such systems.

Nevertheless, the approach of the colloid scientist is a valid one, at least in the first instance, and one that has been used insufficiently in the past. *Colloids in Food* should do much to correct this imbalance for it is an admirable introduction to an important body of knowledge and theory.

C. Holt

Nutrition Policy Implementation: Issues and Experience. Ed. by Nevin S. Scrimshaw and Mitchel B. Wallerstein.
New York: Plenum, 1982. Pp. xiv+558. ISBN 0 306 40858 9. US$65.00.

The book reviews a variety of intervention programmes which have been developed to alleviate hunger and malnutrition in developing countries. The material has been organized into eight sections beginning with a 'rationale' for investment by national governments in nutrition programming. The other sections include the status of policies and plans with regard to food fortification; supplementary feeding and formulated foods; integrated multisectoral village-level interventions; small farm agricultural systems; food conservation and post-harvest food loss; food price controls and consumer subsidies. The final section attempts to draw together the critical social, economic and political issues of nutrition policy implementation with a series of discussions, summary comments and concluding afterthoughts.

The book stems from a multidisciplinary undertaking between the Department of Nutrition and Food Science and the Centre for International Studies at the Massachusetts Institute of Technology and the Centre for International Health of the Harvard School of Public Health. The resultant

programme is known as the International Food and Nutrition Program (IFNP) which has benefited greatly from the support of several institutions including the Rockefeller and Ford Foundations and the United Nations University World Health Programme.

The various key topics are highlighted by several case studies followed by a number of specific workshop discussions. Herein lies the strength of the book. In the final analyses, perhaps the most cogent points are included in the 'afterthoughts' which look critically at the successes and failures of nutrition policies in the past. A criticism that may well be levelled at the book is that it is too theoretical and that even after 10 years' experience, the authors have found it extremely difficult to identify the components of a successful nutrition programme implementation policy, and how to disseminate the available information in such a way that those in a position to make a difference do something speedily and effectively.

Overall, the text presents a clear account of the critical food problems facing several developing nations and lays down a challenge to the international nutrition community to come up with effective and convincing arguments to promote nutrition policies. The analysis of several nutrition programmes in a number of different national settings should be of interest to nutritionists and food scientists as well as policymakers and health professionals.

David P. Richardson

Books received

Report of the National Institute for Research in Dairying, 1982.
Reading: National Institute for Research in Dairying, 1983. Pp. 190. ISSN 0302 0851. £3.00.

Includes reports on: taxonomic studies of bovine coryneform bacteria, thermoduric coryneform bacteria and group D streptococci; effect of psychrotrophic bacteria in milk; assessment of hygienic quality of milk; keeping quality of HTST milk; stability of milk and creams; genetic modification of streptococci; nutritive value of dairy products and milk replacement formulae; nutrient binders and bio-availability. Two hundred and fifty-one publications are listed.

Temperature Sensing with Thermocouples and Resistance Thermometers: A Practical Handbook.
Hampton, Middlesex: Labfacility Ltd, 1982. Pp. 47. £1.50 (including postage and packing).

Includes: temperature/EMF tables for seven types of thermocouple; tables giving details of widely used thermocouples and their tolerances; thermocouple sheaths; resistance/temperature tables for platinum detectors and tolerances.

The Destruction of Bacterial Spores. By A. D. Russell.
London: Academic Press, 1982. PP. x+333. ISBN 0 12 604060 5. £19.20.

The ten chapters contained within this book cover the topics: structure, formation and germination of the bacterial spore; inactivation of bacterial spores by thermal processes involving moist and dry heat; effects on spores of ionizing radiations, ultraviolet radiation, liquid-phase antibacterial agents, vapour-phase bactericides, hydrostatic pressure, and combined treatments; and recovery and revival of damaged spores.

Dictionary of Nutrition and Food Technology, 5th ed. By Arnold E. Bender.
London: Butterworths, 1982. Pp. vi+309. ISBN 0 408 10855 X. £15.00.

This latest edition contains 250 new entries, and 350 revised entries. In addition to the main dictionary section, there is a bibliography of about 180 books listed under subject headings, and tables of FAO and U.K. recommended intakes, U.S. recommended dietary allowances, and energy and protein contents of some typical foods.

Advances in Food Research, Volume 28, Ed. by C. O. Chichester.
New York: Academic Press, 1982. Pp. ix+409. ISBN 0 12 016428 0.

Includes contributions on: phytates in legumes and cereals; physical, chemical and nutritional properties of *Phaseolus* proteins; porcine stress syndromes; chemical, biochemical, functional and nutritional characteristics of collagen in food systems; and food technological evaluation of xylitol.

Food Irradiation Now. Proceedings of a Symposium held in Ede, The Netherlands, October 1981.
The Hague: Martinus Nijhoff/Dr W. Junk, 1982. Pp. viii+157. ISBN 90 247 2703 0. $22.00.

This book contains contributions on protection of the consumer against enteropathogenic bacteria in fresh meats and poultry; public health aspects of food irradiation; technological aspects of irradiation of foodstuffs; industrial application of food irradiation, international legislative and regulatory aspects; consumer reactions; and wholesomeness of irradiated food.

Report of the Government Chemist, 1981.
London: HMSO, 1982. Pp. 188. ISBN 0 11 513607 X. £8.50.

Includes reports on: organochlorine pesticide residues in Chinese rabbit and human milk; trace element levels in the duplicate diet of Shippam residents; estimation of styrene monomer in glass-reinforced plastic by headspace g.c.; survey of vitamin A in foods; vitamins A and D_3 in hen's eggs; and CUSUM plots for quality assurance.

Microbiology of Foods: The Ecological Essentials of Assurance and Assessment of Safety and Quality, 3rd ed. By D. A. A. Mossel. Utrecht: University of Utrecht, 1982. Pp. 188. ISBN 90 6159 007 8. Dutch fl. 52.

This well known introductory text continues to offer the unusual feature of presenting in ninety-one pages a summary of food microbiology suitable for undergraduates and beginning food scientists, in combination with a comprehensive list of 2553 references so that topics mentioned briefly in the text can be followed up in greater detail. There is also an eleven-page index.

Report of a Seminar on Energy Conservation in Food Processing Industries.
Ottawa, Canada: International Development Research Centre, 1983. Pp. 84. Publication No. IDRC-MR70e.

L'Industrie Alimentaire Halieutique. I. Le Poisson Matière Première. By M. Sainclivier.
Rennes, France: Ecole Nationale Superieure Agronomique de Rennes, 1983. Pp. xxxiv+263. ISSN 0370 8411. FFr. 250.00.

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Contents

Volume 18, Number 1, February 1983

Volume 18, Number 2, April 1983

Volume 18, Number 3, June 1983

Volume 18, Number 4, August 1983

Volume 18, Number 5, October 1983

Volume 18, Number 6, December 1983

SUBJECT INDEX

AUTHOR INDEX

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Typescripts (three complete copies) should be sent to the Editor, *Journal of Food Technology*, c/o Institute of Food Science and Technology (U.K.), 20 Queensberry Place, London SW7 2DR. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be doubled-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References. Authors should consult the current issue in order to ensure that their manuscript conforms to the Journal conventions on such things as subheadings, layout of tables, etc.

References. Only papers closely related to the author's work should be included; exhaustive lists should be avoided. References in the text should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc. should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s) (b) year of publication in parentheses: (c) title of paper; (d) the title of the journal given in full and not abbreviated, set in italics (underlined once in typescript); (e) the volume number; (f) the first and last page numbers of the paper—e.g.

Steiner, E. H. (1966). Sequential procedures for triangular and paired comparison tasting tests. *Journal of Food Technology*, **1**, 41–53.

References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) place of publication and publisher—e.g.

Lawrie, R. A. (1979). *Meat Science*, 3rd edition. Oxford: Pergamon Press.

In the case of edited multi-author monographs, the editor(s) should be indicated in parentheses after the book title—e.g.

Hawthorn, J. (1980). Scientific basis of food control. In *Food Control in Action* (edited by P. O. Dennis, J. R. Blanchfield & A. G. Ward). Pp. 17–33. Barking, Essex: Applied Science.

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

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

SI UNITS

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

Chemical formulae and solutions. The correct molecular formula should be used indicating whether the substance is anhydrous or hydrated. Solutions should be given in terms of molarity. The arithmetic basis of other expressions of concentration must be clearly defined.

Species names. In addition to common or trivial names of plants, animals or microorganisms, the species should normally also be indicated by use of the latin binomial at least once. This should be given in full and italicized (underlined once in typescript). In subsequent use, the generic name may be abbreviated to a single letter provided that this does not result in ambiguity.

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

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

Offprints. Fifty offprints will be issued free with each paper but additional copies may be purchased if ordered on the printed card which will be sent to the senior author with the proofs.

Journal of Food Technology
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Contents

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