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An assessment of acceptance sampling plans as applied to foodstuffs

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Summary

The application of acceptance sampling plans, based on criteria given in two U.S. Military Standards, to evaluate the sulphur dioxide content in the sausage meat portion of sausage rolls produced by a batch process, has been investigated.

It was found that acceptance sampling plans could be successfully drawn up using values given in the Military Standards and that the most economical plans which are statistically sound would probably be based on variables sampling with unknown standard deviations (s.d.). The critical requirement, that of normality of distribution of the criterion being considered throughout the batch, is shown to be complied with in this trial.

Introduction

The European Community (E.C.) has embarked on a programme of harmonization of food standards throughout the Member States. When agreement on standards for any particular commodity is obtained the Community adopts a Council Directive, appropriate to that commodity, which is then published in the Official Journal of the European Communities. There is a requirement in each of the Foodstuffs Directives thus far adopted for methods of both sampling and analysis to be elaborated to enforce the commodity standards. Methods of analysis Directives have been proposed and adopted for some commodities such as sugars (EEC, 1979a) and coffee extracts (EEC, 1979b); discussion on methods of sampling for foodstuffs is

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still at an early stage in the Community and no sampling Directives have been adopted since the U.K. accession to the Community.

Most discussion that there has been on sampling has taken place in Commission Methods of Sampling Working Groups. The discussions are becoming clearly separated into the two aspects of sampling: (1) The procedures for physically obtaining samples from a lot; (2) The number of samples to be taken for analysis and the interpretation to be placed on the analytical results obtained.

The incorporation of methods of sampling for foodstuffs in legislation would be requirements new to the United Kingdom. To date there have only been two examples of sampling requirements laid down in Regulations made under the Food and Drugs Acts, these being the procedures for the sampling of flour in mills (SI, 1963) and preservatives on citrus fruit and bananas (SI, 1979). Neither procedure is particularly extensive.

There has been progress made within Commission Working Groups on laying down procedures for obtaining samples and draft Directives specifying such procedures have been prepared for some commodities. These have been based on methods which have been proposed by International Organizations, as in the guide on sampling procedures for milk and milk products by the International Dairy Federation (IDF, 1980). To date no significant progress has been made within any Commission Working Groups on the interpretation to be placed on analytical results. However, in order to proceed in this area, consideration will have to be given to acceptance sampling plans whereby an assessment may be made of the quality of a complete batch as the result of the analysis of a small number of samples from that batch.

Acceptance sampling plans have been discussed for various individual commodities. They have been based on the two U.S. Military Standards: MIL STD 105 (of April 1963): Sampling Procedures and Tables for Inspection by Attributes; and MIL STD 414 (of June 1957): Sampling Procedures and Tables for Inspection by Variables for Percent Defective.

The Ministry of Agriculture, Fisheries and Food (MAFF) decided to apply the various types of acceptance sampling plans to a food manufacturing process to test practically the efficiencies of each type of plan and to assess the difficulties that might be encountered should any of the plans be prescribed by legislation. The types of plan tested were: (1) Sampling by variables with known standard deviation, (2) sampling by variables with unknown standard deviation, (3) samples by attributes.

It was decided to carry out analyses for one criterion on 300 individual items taken from a production run. This number was chosen as being large compared to the sample sizes (five and twenty) that it was proposed to adopt in the sampling plans being tested. Each of the plans was then applied to a random selection from the 300 measurements.

A product was selected for the trial such that a statutory limit was prescribed for the chosen variable in the product and that the value of the particular variable would probably change throughout the production run.

In addition to the statutory limit it was necessary to set artificial 'project' limits for the purpose of the statistical analysis of the samples so that the production run could be designated 'good' or 'bad'.

The criterion sulphur dioxide in the sausage meat portion of uncooked sausage rolls was selected as being suitable—both practically (a factory producing sausage rolls was willing to provide samples to help the project and the analyses for sulphur dioxide content would be relatively quick) and theoretically (variation should occur throughout the lot as the chemical was being incorporated into the bulk mix *via* a pre-mix). There is a statutory limit (450 mg/kg) and 'project limits' could be readily set.

Materials and methods

The samples were taken from the sausage roll factory of Kraft Foods Ltd, Bristol.

The production of sausage rolls was carried out by a batch process but with each batch being made up of several tubs—each of which may, though should not, have its own characteristics.

A preliminary analysis of fifty samples from one tub was carried out to assess the practicability of the procedure (method of analysis to be used, sample preparation and storage, homogeneity of tub).

The laboratory undertaking the analysis for sulphur dioxide content received two whole uncooked frozen sausage rolls as a 'sample' packaged together in a plastic bag. On receipt by the laboratory they were allowed to thaw for about 45 min, thus enabling the casing to be removed cleanly. After removal the two centre portions were combined and weighed.

The weight was recorded (normally in the region of 50 g) and the two centres were placed in a plastic bag and re-frozen. The whole frozen centres were used as the analysis portion.

A 'simple' method of analysis, with a repeatability of about 3 mg/kg, was used for the determination of the sulphur dioxide content. It involved the distillation of sulphur dioxide from all the (about 50 g) sausage meat sample in a hydrochloric acid medium into 50 ml N/50 iodine followed by back titration of excess iodine against N/50 sodium thiosulphate. Losses of iodine were minimized by not completing the water seal until boiling had begun, and by using a heat screen to protect the N/50 iodine.

The samples were taken in 'clusters' of five at times throughout the use of the tub. Two 'identical' sets of clusters were taken as follows: (1) 5×5 'clusters' taken throughout the time of use of the tub, including one set at 'full' tub and one set at 'empty'; and (2) 5×5 'clusters' taken at the same time as (1) above. Set (2) was deep frozen and analysed at intervals throughout a week. Set (1) was analysed on receipt of samples at the start of the week.

Examination of the results showed that there was variation between the sulphur dioxide content of each cluster in each set, varying from 232.8 to

290.6 mg/kg in Set (1) and 228.7 to 292.6 mg/kg in Set (2). The sulphur dioxide content increased as more material was used from the tub, suggesting that the sausage meat mix in the tub was not homogeneous.

There was little difference between the mean values of the sulphur dioxide content of the corresponding clusters in Sets (1) and (2), indicating that there was no significant loss of sulphur dioxide on storage of the sample in the deep frozen state and that samples could therefore be safely kept deep frozen between being sampled and analysed.

The second part of the trial consisted of the taking and analysis of 300 samples from six tubs of sausage meat mix, all of which originated from one production run and, therefore, from one batch. The samples were again taken in 'clusters' as for the first part of the trial. An adequate number of tubs, clusters and samples was required to allow the components of variation to be estimated with sufficient precision and from past experience it was decided to use five clusters per tub and ten samples per cluster. Because of the large number of samples involved and the practical limit on the number of analyses which could be carried out each day, the analytical work was spread over five days with sixty samples being analysed each day. Part of each tub was analysed each day to ensure that any variation occurring on a day-to-day basis was not attributed to compositional differences between tubs. Additionally samples from within each cluster were analysed on separate days. The order of analysis for each day was randomized.

Results and discussion

The concentration of SO_2 (mg/kg) found in the 300 samples ranged from 211 to 350, with mean value 285 and s.d. 21. The distribution was examined for normality and Fig. 1 shows concentration plotted against cumulative frequency on probability paper. The points lay close to a straight line except at the lower end of the distribution. The reason for the non-normal distribution of the sulphur dioxide concentration of the lower concentration was not ascertained.

The fact that the distribution probably conformed approximately to normal was confirmed by applying the χ^2 test to the expected and observed frequencies after subdividing the data into groups at 10 mg/kg intervals ($\chi^2=9.92$, 8 d.f.).

An analysis of variance was carried out to examine the variation between tubs, between clusters within tubs, and residual within clusters. The latter included analytical errors and day-to-day variation. The result is given in Table 1.

The variation between tubs and between clusters were both highly significant ($P<0.001$). In sampling sausages to take account of these variations, therefore, as many tubs as possible should be withdrawn and one or more samples taken from each at random.

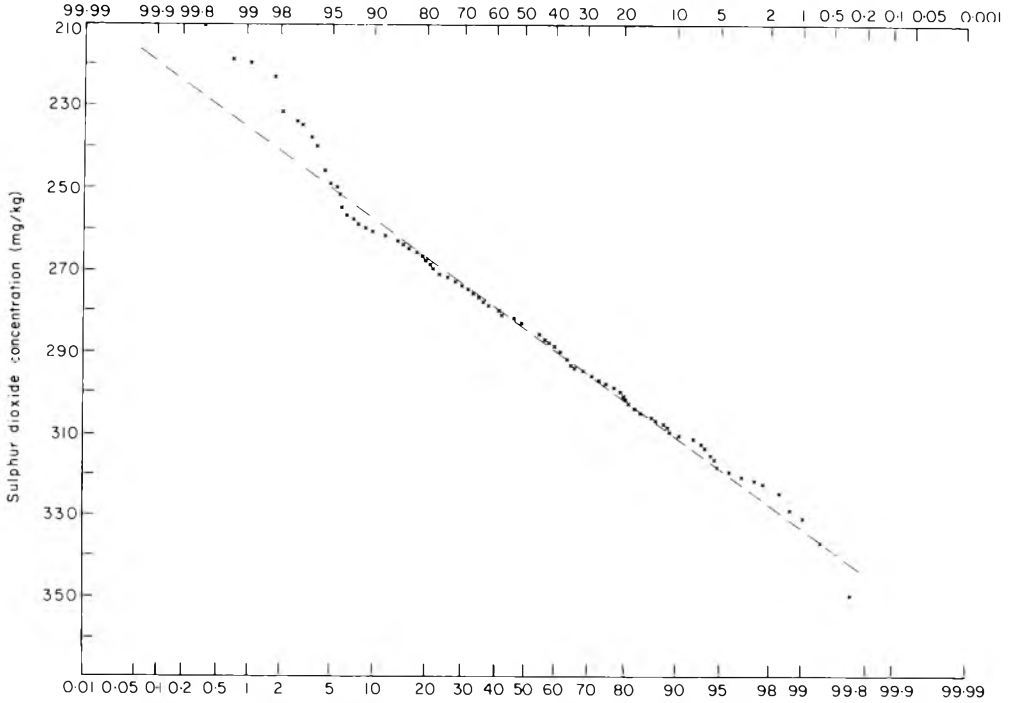

Figure 1. Plot of cumulative frequencies on normal probability paper.

United States' Military plans for sampling by attributes (MIL STD 105) and by variables (MIL STD 414), both known and unknown standard deviation, were tested for their effectiveness in separating good and bad batches of sausages. The particular plans selected for sampling by attributes are shown in Table 2. This gives the MIL STD code letters, the acceptable quality level (AQL) values, sample sizes and maximum number of defective items permitted in the sample for acceptance of the batch. The table also gives the percentage of defectives in batches which the plans would pass 95% and 10% of the occasions on which such batches are examined.

Variable sampling plans were selected for the same sample sizes, namely five and twenty, and the MIL STD code letters, AQL values and acceptance criteria are shown in Table 3. Also given are the proportion of occasions on

Table 1. Analysis of variance of data from the sulphur dioxide content of sausages

Source of variation	Sum of squares	Degree of freedom	Mean square	Variance ratio
Between tubs	62 670.9	5	12 534.2 (M_T)	$M_T/M_E = 10.55$
Between clusters within tubs	28 511.2	24	1 188.0 (M_E)	$M_E/M_R = 7.71$
Residual	41 624.2	270	154.2 (M_R)	—

Table 2. U.S. Military Standard MIL STD 105D criteria for single sampling attribute plans

MIL STD code letter	AQL (%)	Sample size	No. of defectives in sample for acceptance of batch	Operating characteristics: Defectives in batch (%)	Operating characteristics: Chance of acceptance (%)
C	2.5	5	0	1.0	95
				36.9	10
F	0.65	20	0	0.26	95
				10.9	10
F	2.5	20	1	1.8	95
				18.1	10

which the plans would pass batches with the same percentage of defectiveness as those accepted 95% and 10% of the time by the attribute plans.

For the plans with known standard deviation the value of 21 mg/kg, previously established in the experiment, was used in the acceptance criteria. Sample sizes of twenty were not taken because of the high efficiency predicted for plans with sample size of five.

To test the validity of applying the plans to the present data forty lots of five measurements and forty lots of twenty measurements were abstracted randomly from the main bulk of 300. Each abstraction of five or twenty was made from the whole 300 values. Arbitrary 'specifications' for sulphur dioxide content were set so that the percentage of 'defectives' in the batch conformed as nearly as possible to the nominal figures shown in Tables 2 and 3. The appropriate 'specification' limits were obtained by inspection of the original data and are shown in Table 4.

The actual number of occasions on which a 'good' batch (low percentage of defectives) and a 'bad' batch were accepted by the three types of plan applied

Table 3. U.S. Military Standard MIL STD 414 criteria for variables sampling plans

Type of plan	MIL STD code letter	AQL (%)	Sample size	k Value*	Operating characteristics: Defectives in batch (%)	Operating characteristics: Chance of acceptance (%)
Unknown standard deviation	D	2.5	5	1.24	1.0	97
					36.9	9
,,	H	4.0	20	1.33	1.8	99.5
					18.1	9.5
,,	H	2.5	20	1.51	18.1	4
Known standard deviation	F	2.5	5	1.39	1.0	98
					36.9	1

* Batch is accepted if $\bar{x} \leqslant U - ks$ or $\leqslant U - k\sigma$, where $\bar{x}$ = mean value of measurements in sample; U = upper specification; s = estimated standard deviation in sample; σ = known standard deviation of batch (21 mg/kg).

Table 4. 'Specification' limits for sulphur dioxide to give percentage 'defectives' in batch conforming to Tables 3 and 4

Required 'defectives' in batch (%)	Arbitrary 'specification' for SO_2 (mg/kg)	Actual number of measurements in excess of 'specification'
0.26	349	1 (0.33%)
1.0	329	3 (1.0%)
1.8	324	6 (2.0%)
10.9	310	33 (11.0%)
18.1	303	57 (19.0%)
36.9	291	109 (36.3%)

Table 5. Numbers of occasions out of forty on which the sampling plans indicated acceptance of the batch

Size of sample	Type of plan	Code letter	AQL (%)	Actual 'defectives' in batch (%)	Number of occasions on which batch was accepted by plan	
					No.	%
5	Attributes	C	2.5	1.0	37	92.5
				36.3	3	7.5
	Variables (s.d. unknown)	D	2.5	1.0	38	95
				36.3	2	5
	Variables (s.d. known)	F	2.5	1.0	40	100
				36.3	0	0
20	Attributes	F	0.65	0.33	38	95
				11.0	6	15
	Attributes	F	2.5	2.0	37	92.5
				19.0	4	10
	Variables (s.d. unknown)	H	4.0	2.0	39	97.5
				19.0	5	12.5
	Variables (s.d. unknown)	H	2.5	19.0	2	5

to forty sets of five or twenty random measurements are given in Table 5. It can be seen by comparison of the percentage figures with the chances of acceptance shown in Tables 2 and 3 that agreement with the MIL STD predictions was reasonably close. The superiority of variables over attributes sampling is also demonstrated.

Conclusions

The results of the trial indicate that, as and when acceptance sampling plans are applied to foodstuffs, considerable care must be taken to define the batch

that is to be sampled. In this trial a batch is defined as consisting of six 'equivalent' tubs of sausage meat mix. However, the results indicated that not only are the tubs significantly different from one to another in their sulphur dioxide content, but that each tub is itself heterogeneous. It would be reasonable to assume that if this is the case with sulphur dioxide, then the same situation regarding heterogeneity may arise with other parameters in the sausage meat mix, notably the meat content. In such cases the values obtained may be closer to any statutory limits than in the present case (no sample exceeded, or even approached that limit).

Because food materials are not homogeneous the number of samples to be drawn must be sufficiently large to ensure, with high probability, that any statutory limit is not contravened in more than a small proportion of individual items of the product.

The application of the acceptance sampling plan criteria and values laid down in the appropriate Military Standards was successful in that the number of times a batch would be accepted in practice was very close to that which is predicted theoretically.

The order of the powers of discrimination of each type of sampling plan was also as predicted, that is variables sampling with known standard deviation is the most discriminatory whereas attribute sampling is the least. As the application of variables sampling with known standard deviation is unlikely to occur in practical enforcement sampling—the standard deviation always being 'unknown' in that situation—the more powerful of the other two types of sampling for compositional standards, variables sampling with unknown standard deviation, is most likely to be used. The critical requirement for the application of this type of sampling, the distribution of the parameter to be studied being 'normal' throughout the batch, is fulfilled in the example considered in this study.

The use of variables sampling with unknown standard deviation for compositional standards is being advocated by the Codex Alimentarius Commission. The results of this trial endorse that view and the use of the two U.S. Military Standards to provide the acceptance criteria in any future statutory acceptance sampling plans is justified.

Acknowledgments

The authors wish to thank Kraft Foods Ltd, Bristol for the provision of samples used in this trial, Roger Wood, Avon County Council, for arranging the collection of the samples and Anthony Harrison for arranging for the chemical analysis of the samples in the Avon County Council County Laboratory.

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Post-harvest physiology and storage of the white oyster mushroom *Pleurotus flabellatus*

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Summary

Changes in the post-harvest storage of the mushroom *Pleurotus flabellatus* at ambient temperature were studied. There was a decrease in respiratory rate and soluble carbohydrates along with loss in water content. With progress in period of storage, the fresh mushrooms showed an increase in the activities of O-diphenoloxidases and proteases accompanied with decrease of total phenols and increase in free amino acids respectively. The degree of discoloration increased with the lapse of storage time.

Fresh mushrooms (200 g) packed in 25 μm thick polyethylene bags (16×25 cm) with one pin hole on either side stored up to a period of 24 hr at ambient temperature and up to a period of 6 days at 5±2°C in the intact polyethylene bags.

Introduction

Fresh mushrooms have a high rate of metabolic activity which quickly declines and leads to deterioration. Although there are a number of studies aimed at extending the storage life of fresh mushrooms (Tomkins, 1966; Gormley & MacCanna, 1967; Cameron & Chappel, 1970; Nichols & Hammond, 1973, 1974, 1976; Gormley, 1976) relatively little work has been done on the physiological processes underlying post-harvest changes. Gormley & MacCanna (1967) defined a 'Quality spectrum' for *Agaricus bisporus* in which degree of growth (stem elongation and cap opening), discoloration and changes in texture were itemized as the most important factors in consumer acceptance. *Pleurotus* mushrooms are morphologically different and therefore the same quality spectrum cannot apply. The degree of whiteness, the texture and the nature of fruit body margin are taken here as the major characteristics to define the 'Quality spectrum' of *P. flabellatus*.

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Pleurotus flabellatus is a white oyster mushroom which is cultivated for its edible spatulate fruit bodies which have a pleasant flavour. Requirements for the commercial cultivation of this species have been standardized (Bano *et al.*, 1978) and is currently gaining the interest of commercial mushroom growers. The cultivation of *P. flabellatus* is suited to rural development programmes (Bano *et al.*, 1979) and it is necessary in a country like India to preserve the freshness of harvested mushrooms for many days so that markets can be developed for this mushroom. This study was an attempt to quantify some of the physiological changes occurring in fresh mushrooms at ambient temperature and to study the storage life of fresh mushrooms packed in polyethylene bags at ambient and under refrigerated conditions.

Materials and methods

Post-harvest changes

Mushroom production. Fresh mushrooms raised on a substrate of wet chopped rice straw as described by Rajarathnam, Wankhede & Patwardhan (1979) and harvested on the third day following primordia formation (when the margin of the fruit bodies becomes flat (see 3 in Fig. 1) and just when the spores start falling) were used in these studies. Batches of 50 g fresh mushrooms were kept exposed at ambient temperature which ranged from 22–28°C at a relative humidity which ranged from 50–70% and analysed successively at 0, 6, 12, 18 and 24 hr storage. For each stage of analysis during the storage period of 24 hr, there were eight replicates, each weighing 50 g fresh.

Respiration rate. Carbon-dioxide produced by the fresh mushrooms exposed to open atmosphere was measured by the gas stream method

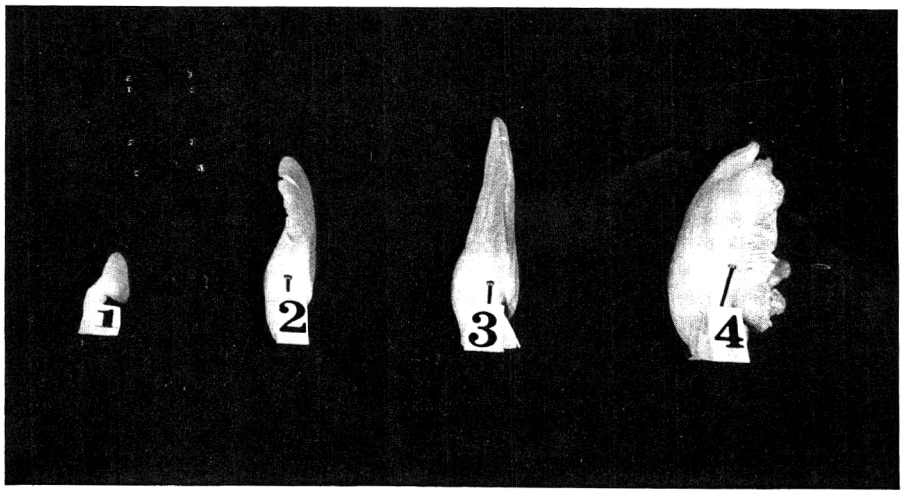

Figure 1. Growth stages of the mushroom *P. flabellatus*. 1, Pinhead; 2, growing fruit body with margin incurved; 3, growing fruit body with margin flat (stage of harvest); 4, over grown fruit body with margin upturned.

(Srivastava & Narasimham, 1967) and the results are expressed as milligrammes of CO_2 produced per grammes of dry weight per hour.

Analytical extractions. Fifty grammes of fresh mushroom sample or weight equivalent to 50 g fresh during storage of 24 hr was homogenized in 400 ml of 70% ethanol, extracted over a boiling water bath for 1 hr and filtered. The residue was re-extracted with 70% ethanol. The filtrates were pooled and clarified by centrifugation at 4000 ***g*** for 15 min. The clear supernatant was concentrated under reduced pressure below 40°C to a final volume of 100 ml and this was taken for further analyses. Three replicate samples were taken individually for extraction for each treatment as above.

Soluble carbohydrates, free amino acids and phenols were estimated according to Dubois *et al.* (1956), Yemm & Cocking (1955) and Swain & Hillis (1959) respectively.

Enzyme assays. Acetone powders of mushroom samples at different lengths of storage period were prepared as described by Yamaguchi, Hwang & Campbell (1970) with slight modifications. An equivalent of 25 g fresh weight mushrooms was homogenized in minimum amount of 50 mM sodium phosphate buffer pH 6.5 to obtain a slurry. This was suspended in 500 ml of acetone at −20°C and the precipitate obtained was filtered immediately through a Buchner funnel. The residue was washed thoroughly with cold acetone, dried under vacuum at ambient temperature, weighed and stored under refrigeration.

One hundred milligrammes of acetone powder was extracted in 20 ml of 100 mM phosphate buffer pH 6.0 and centrifuged at 6000 ***g*** for 20 min. The clear supernatant was used as the enzyme source for assaying O-diphenoloxidase (Murr & Morris, 1975) and protease (Kunitz, 1947) activities. Enzyme protein was estimated using the folin-phenol reagent (Lowry *et al.*, 1951). Both the enzyme activities were expressed as Δ OD per minute per milligram of protein.

Estimation of degree of whiteness. This was done using a photoelectric reflectometer (Photovolt) having a tristimulus green filter and results are expressed as percentage of reflectance shown by the direct meter reading. The reflectometer cell was calibrated using a standard block of magnesium carbonate which gave a dial reading of 100 in the reflectometer. Reflectance of both dorsal and ventral surfaces of the mushroom was recorded separately.

Texture measurement. Texture was measured with a Zenken Texturometer, Japan, using a molar tooth shaped plunger and expressed as kilogrammes per volt. Pieces, 2 cm^2 of mushroom, sliced from the centre of the cap placed one above the other (so that gills faced outwards), were used for texture measurement.

Storage studies

Storage experiments were carried out in the polyethylene bags separately at (1) ambient (22−28°C) and (2) at low (5±2°C) temperatures.

(1) Storage at ambient temperature. (a) Thickness of polyethylene bags: 100 g of fresh mushrooms were packed in 25 and 50 μm thick polyethylene bags (16×25 cm) with and without five pinholes (each 1 mm in diameter) on either side of the lower half. These holes were made with a hot pin and were positioned as corners of a rectangle (measuring 6×8 cm) and the fifth hole at the centre. The open end of the bag was tied with a piece of thread after introducing a rubber tube fitted with a glass rod. This arrangement was made to connect the rubber tube to the Hartman & Brown CO_2 analyser. These mushrooms were stored at ambient temperature. (b) Ventilation of polyethylene bags: Storage studies were carried out by packing 100 g fresh mushrooms in 25 μm thick polythylene bags (16×25 cm) having one to five pinholes at the lower half on either side. (c) Package density: 50, 100, 150, 200 and 250 g fresh mushrooms were packed in 25 μm thick polyethylene bags (16×25 cm) having one pinhole on either side and storage life studied at the ambient temperature.

(2) Storage at low temperature. Two hundred grammes of fresh mushrooms contained in 25 μm thick polyethylene bags (16×25 cm) with and without one pinhole were stored at 5±2°C and studied for acceptability with lapse of time.

In all the experiments of storage, eight replicates were taken for each treatment assorted in a completely randomized design. Degree of whiteness, texture, CO_2 content inside the pack, morphology (appearance) and taste of cooked mushrooms were studied. A panel of eight trained tasters scored cooked mushrooms according to the scale given below:

Acceptability score	Description of cooked mushrooms
1	The fresh mushrooms showed morphological symptoms of deterioration and hence were not cooked
2	
3	
4	Unacceptable
5	Acceptable
6	Fair
7	Good
8	Very good
9	Excellent (like fresh)

Results and discussion

Fresh mushrooms stored at ambient temperature lost approximately 32% of their water content within 24 hr and their respiration rate also declined (Fig. 2). A slight increase in the respiration rate observed at 6 hr of storage might be due to the increased rate of metabolism associated with the shedding of

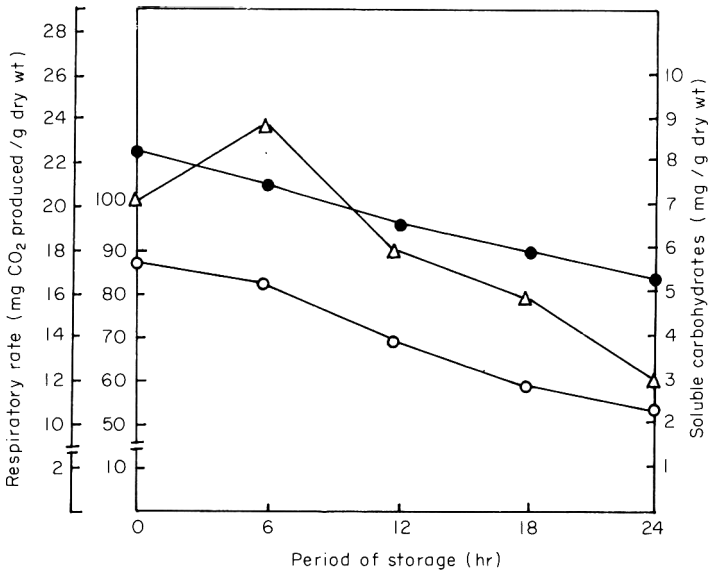

Figure 2. Changes in water content (○——○), soluble carbohydrates (●——●) and respiratory rate (△——△) of the fresh fruit bodies of *P. flabellatus*.

spores. A similar observation was made by Hammond & Nichols (1975) in *A. bisporus*. Soluble carbohydrates decreased during the post-harvest life indicating their probable utilization as respiratory substrate during the storage life. Protease activity increased throughout the 24 hr storage time with a concomitant rise in the free amino acids (Fig. 3). Such a liberation of low molecular weight nitrogenous compounds is thought to support the post-harvest development (Murr & Morris, 1975). O-diphenoloxidase activity

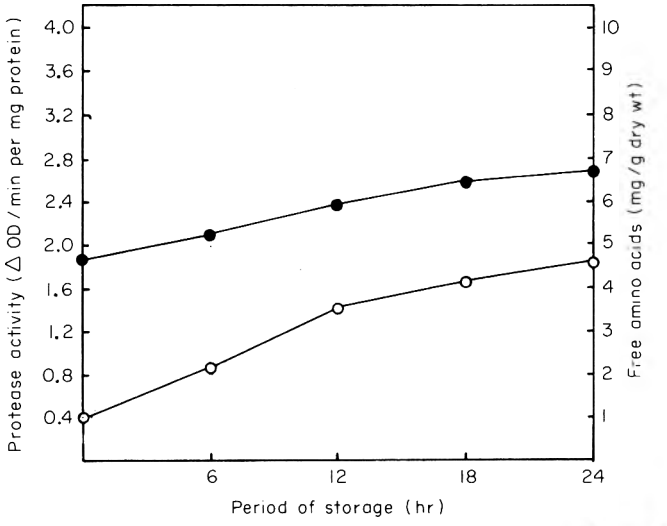

Figure 3. Changes in protease activity (○——○) and free amino acids (●——●) in fresh fruit bodies of *P. flabellatus* during storage at ambient temperature.

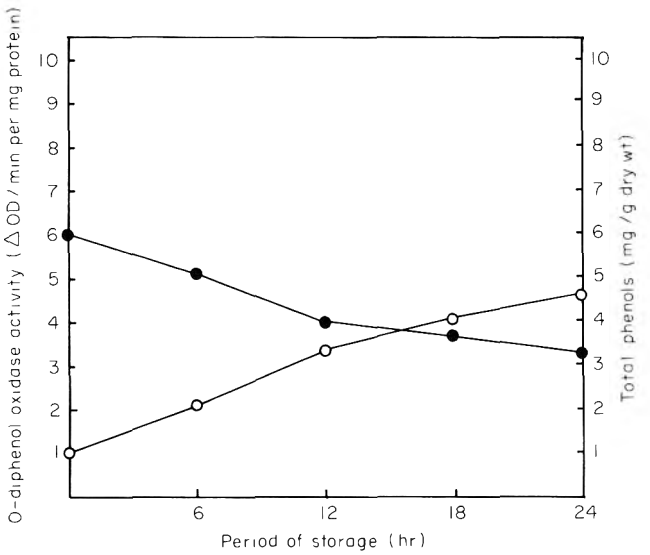

Figure 4. Changes in O-diphenoloxidase activity (○——○) and total phenols (●——●) in fresh fruit bodies of *P. flabellatus* during storage at ambient temperature.

increased with a parallel decrease in total phenolic content (Fig. 4). The role of O-diphenoloxidase activity in browning of mushrooms is well known (Hughes, 1958, 1960; Markakis & Embs, 1966; Long & Alben, 1969). The loss in whiteness as measured by a photoelectric reflectometer agreed with the visual observation of brown colour developed with an increase in storage time (Table 1). The discoloration was more on the ventral gill bearing side than on

Table 1. Discoloration of fresh mushrooms when stored* exposed

Period of storage (hr)	Reflectances†		Reflectance of control (%)	
	D	V	D	V
0 (fresh)	74a‡	62a	100	100
6	68b	54b	91.9	87.1
12	64c	48c	86.5	77.4
18	59d	42d	79.7	67.7
24	53e	33e	71.6	53.2
s.e. mean (35 d.f.)	±1.0	±0.8		

* At ambient temperature (22–28°C) and relative humidity (50–70%).

† Direct meter reading; D, dorsal and V, ventral surface of the mushroom.

‡ Means within each column not followed by the same letter are significantly different according to Duncan's new multiple range test ($P<0.05$).

Table 2. Quality of mushrooms stored* in 25 and 50 μm thick polyethylene bags—intact and perforated (having five pinholes) for 24 hr

Package material	CO_2 content inside the pack (%)	Texture (kg/V)	Reflectance†		Morphology	Acceptability score
			D	V		
Fresh mushrooms	0	5.5a‡	73a	62a	White	9
25 μm thick, intact	7a	4.5b	63b	50b	Like fresh, slight fermented smell	7
25 μm thick, perforated	0	4.0c	58c	46c	More soggy, no fermented smell	3
50 μm thick, intact	9b	4.0c	60c	45c	Less soggy, fermented smell	4
50 μm thick, perforated	0	2.5d	53d	40d	More soggy, discoloured	3
Exposed (control)	0	3.5e	52d	37e	Desiccated, discoloured	2
s.e. mean	(14 d.f.) ±0.3	(42 d.f.) ±0.2	(42 d.f.) ±0.9	±1.1		

Key as for Table 1.

the dorsal surface and this supports the fact that phenol oxidases are concentrated in the gills.

By storing fresh mushrooms in 25 and 50 μm thick polyethylene bags with and without fine pinholes, it was observed that CO_2 accumulated in the intact bags, while in the perforated bags, there was a softening of the mushrooms, development of sogginess, fast discoloration and the mushrooms were not fit for consumption even up to a period of 24 hr of storage at ambient temperature (Table 2). Intact polyethylene bags, 25 μm thick, were found to extend the storage life over a period of 24 hr with no appreciable loss of whiteness and texture, although a slight fermented smell was apparent which disappeared after cooking.

Studies on the effect of the number of pinholes on the keeping quality of the fresh produce revealed a gradual decrease of accumulated levels of CO_2 inside the pack, increased discoloration, increased tenderness and more sogginess with an increase in the number of pinholes. Mushrooms packed in 25 μm thick polyethylene bags with a single pinhole on either side had no fermented smell and were found to be acceptable for over a storage period of 24 hr at ambient temperature (Table 3).

By packing a minimum of 50 g mushrooms in 25 μm thick polyethylene bags (16×25 cm) with a single pinhole on either side, mushrooms became more soggy, discoloured and unmarketable on storage at an ambient temperature for 24 hr. By gradual increasing the package density from 50 to 250 g there was an improvement in the storage quality with respect to colour, firmness, texture and general appearance. Mushrooms packed at densities of 200 and 250 g/package showed features almost akin to the fresh produce. The

Table 3. Effect of number of pinholes of 25 μm thick polyethylene bags on the storage* quality of fresh mushrooms for a period of 24 hr

No. of pinholes	CO_2 content inside the pack (%)	Texture (kg/V)	Reflectance† D	Reflectance† V	Morphology	Acceptability score
Fresh mushrooms	—	4.4a‡	68a	55a	White	9
0	7a	4.0ab	62b	52b	Not soggy, slight fermented smell	7
1	2b	3.7b	60b	50bc	Margins soggy, no fermented smell	8
2	1.5bc	2.5c	52c	48c	Entire fruit bodies soggy, discoloured	5
3	1.0c	2.1cd	45d	40d	Soggy, discoloured with stale smell	4
5	0	1.7e	42d	35e	Discoloured with stale smell	2
s.e. mean	(28 d.f.) ±0.2	(42 d.f.) ±0.2	(42 d.f.) ±0.9	±0.9		

Key as in Table 1.

optimum package density for a polyethylene bag of 16×25 cm size was 200, since 250 g caused tearing at the margins of the packed mushrooms (Table 4). With lower package densities of 50 g there was a lower concentration of CO_2 and more discoloration; with the highest package density studied there was

Table 4. Effect of different package densities on the storage* quality of fresh mushrooms

Package density (g)	CO_2 content inside the pack (%)	Texture (kg/V)	Reflectance† D	Reflectance† V	Morphology	Acceptability score
Fresh mushrooms	—	5.5a‡	69a	58a	White	9
50	1.0a	1.5b	52b	42b	More soggy, discoloured	4
100	2.0b	2.7c	63c	48c	Soggy, discoloured	5
150	3.5c	4.3d	65c	52d	Less soggy, less discoloured	7
200	4.5d	5.0a	65c	55e	Not soggy, not discoloured	8
250	5.5e	5.2a	64c	53de	Not soggy, not discoloured, margins split	8
s.e. mean	(35 d.f.) ±0.2	(42 d.f.) ±0.2	(42 d.f.) ±0.8	±0.9		

Key as in Table 1.

Table 5. Storage of fresh mushrooms in 25 μm thick intact polyethylene bags at 5±2°C

Duration of storage (days)	CO_2 content inside the pack (%)	Texture (kg/V)	Reflectance*		Morphology	Acceptability score
			D	V		
Fresh mushrooms	—	6a	70a	55a	White	9
2	6.0a	6a	65b	53a	Like fresh	8
4	5.0b	6a	62c	48b	Slightly soggy	8
6	4.5bc	4b	58d	40c	Soggy	7
8	4.0c	3c	55e	36d	More soggy, and discoloured	5
s.e. mean	(28 d.f.) ±0.3	(35 d.f.) ±0.2	(35 d.f.) ±0.8	±1.2		

Key as in Table 1.

least browning and a greater accumulation of CO_2. These results imply an indirect relationship between the accumulated concentration of CO_2 and the degree of discoloration. It is well known that phenoloxidases (responsible for discoloration) require oxygen for their activity (Pugh & Raper, 1927; Pugh, 1930; Dawson & Tarpley, 1951).

Mushrooms packed in polyethylene bags with one pinhole on either side stored at 5°C became very soggy within 2 to 3 days and hence the data collected on storage of mushrooms in intact polyethylene bags only is presented in Table 5. An optimum shelf life of 6 days was noticed with refrigerated storage of fresh mushrooms. After 6 days of storage, there was a decrease in the accumulation of CO_2 inside the bag (possibly due to the fall in respiration rate) development of more sogginess, increased discoloration and loss of texture. Development of more sogginess in mushrooms packed in polyethylene bags with one pinhole over those in intact polyethylene bags might be due to the condensation of higher levels of moisture produced due to fast respiration rate leading to fast deterioration in quality. The increased shelf life of the fresh mushrooms under refrigerated conditions over that of the ambient temperature indicates the role of low temperature in retarding the metabolic activity and the importance of refrigeration in extending the shelf life of fresh mushrooms is well known (Hughes, 1959; Dredge, 1964; Sveine, Klougart & Rasmussen, 1967).

Conclusions

The post-harvest storage life of fresh mushrooms of *P. flabellatus* was characterized by an initial high rate of respiration which then declined quickly, heavy loss in water content followed by increased toughness. There was an increase in O-diphenoloxidases, accompanied by a decrease in total phenols and progressive browning and also an increase in protease activity, followed

by an increase in free amino acids. Fresh mushrooms packed in polyethylene bags (25 μm thick) with a single pinhole stored at ambient temperature were of acceptable quality up to 24 hr only, while mushrooms stored at refrigerated temperature in polyethylene bags (25 μm thick) without any pinhole were of acceptable quality up to 6 days. Further studies are required to extend their storage life at ambient temperature. This would be possible by adapting methods to decrease their respiration rate and loss in water content without altering the acceptability characteristics.

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The preparation of protein hydrolysate from defatted coconut and soybean meals

II. Quality and sensory evaluation of products

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Summary

Protein hydrolysate prepared from defatted soybean meal and coconut meal using acid hydrolysis under different conditions were analysed and subjected to sensory evaluation. Analysis of amino acid after hydrolysis showed a 10.32% reduction in the soybean product and a 31.93% reduction in coconut product. Chemical analyses showed similarities in pH, viscosity, salt content and reducing sugar between the hydrolysates and the commercial soy sauce sample. Both amino and total nitrogen and acidity were higher in the hydrolysate than the commercial sample.

Sensory evaluation showed that hydrolysed soybean products gave better scores in terms of colour, aroma, flavour and general acceptability than coconut products. Addition of soybean to coconut meal resulted in improved hydrolysed product.

Introduction

Protein hydrolysate has been used in China and Japan in the ancient times and the process which produced protein hydrolysate used the traditional fermentative procedure (Lockwood & Smith, 1950–51). For the past 60 years, defatted soybean had been used to replace whole beans in the production of soyhydrolysate (Hesseltine & Wang, 1968). Whole wheat or wheat flour have also been used. Japanese and Chinese shoyu are made from equal amounts of soybeans and wheat (Smith, 1949). The working ratio of soybean to wheat has been studied to find out conditions favourable for maximum mould growth, but unfavourable for bacterial growth (Ohara & Moriguchi, 1955). The substitute soybean products were prepared using corn, wheat and soybean (Oda, Ikeda & Tanimoto, 1949). Rice as substitute was tried by Yenko &

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Baens-Arcega (1940) in the Philippines. As a cheap source of proteinaceous material, Baens-Arcega (1966) claimed successful use of a 50:50 mixture of copra meal and soybean in the mould process of manufacturing soysauce. This process however, was slow and took from 5–8 months to accomplish. The protein hydrolysate products made from acid hydrolysis appeared in western countries and in the U.S. in the 19th century, but the technical procedures were not available. There was a growing tendency to make protein hydrolysate by acid hydrolysis in order to increase the yield and reduce cost. This study was carried out to devise a process of protein hydrolysate production, including quality assessment and sensory evaluation of products.

Materials and methods

Materials

Soybean and coconut meals were prepared as described in a previous paper (Pham & del Rosario, 1983). A sample of commercial soy souce was purchased from the market and used for comparison.

Hydrolysis process

Sample meals or mixture of meals were weighed. A pre-mix of the protein meal with water was made in a dough mixer. Water was added until a pasty consistency had been achieved, then the mass was transferred to the glass hydrolyser and 32% concentrated hydrochloric acid (HCl) was added to the mass. Heat was applied for 36 hr at 95°C for soybean meal, while that for coconut meal and mixture meals was at 103°C. Hydrolysate product was neutralised gradually with 50% sodium hydroxide (NaOH) until pH 3. The batch was removed from the hydrolyser and filtered. Filtration was carried out under vacuum. Subsequent to filtration, the hydrolysate was adjusted to pH 4–5, in a storage tank. Hydrolysate product was tempered at 45–50°C, for 2–3 days and aged at 20°C for 2 weeks.

Hydrolysate product formulation

The product of 100% soybean meal; 100% coconut meal; 50–50% weight ratio of soybean meal to coconut meal (50:50 w/w); 25:75; 50:50; and 75:25% volume ratio of hydrolysed soybean to hydrolysed coconut (25:75 v/v; 50:50 v/v; 75:25 v/v) were formulated coming close to the soysauce formulation in terms of soluble solids. The products were pasteurized and then bottled. After 1 week, the products were subjected to sensory evaluation and chemical analysis.

Method of analysis

Chemical composition of the raw materials and total nitrogen of hydrolysed products were determined by the method of AOAC (1975).

Sodium chloride was determined by titration with silver nitrate using potassium chromate as an indicator (Sakasai & Yokotsuka, 1957).

The pure extract which is the difference between percentage of total extract and percentage of sodium chloride was determined by the evaporation of the sample (Sakasai & Yokotsuka, 1956).

Amino nitrogen which is the difference between the formol titration and ammonium values was determined as described in the preceding paper (Pham & Del Rosario, 1983).

Reducing sugar content of the hydrolysed products was evaluated by the colorimetric method, using 3,5-dinitrosalicylic acid for samples diluted fifty to a hundred times (Borel, Hostettler & Deuel, 1952).

Total acidity of the hydrolysed products was titrated with N/10 sodium hydroxide solution until pH 8.3. The titration value was equivalent to total acidity (Sakasai & Yokotsuka, 1956).

Amino acid composition of the raw material meals and hydrolysed products were determined by ion exchange column chromatography in an automated amino acid analyser (Durrum 500). Samples of the raw material containing 100 mg protein were hydrolysed using 6 N hydrochloric acid at 110°C for 24 hr under nitrogen. Tryptophan was determined by a modified procedure based on the method of Spies & Chambers (1949). Cystine was converted to cysteic acid as described by Moore (1963).

Sensory evaluation

The hydrolysed products obtained were coded and served to a taste panel consisting of eleven people by simple random sampling method (Hansen, Hurwitz & Madow, 1962). A maximum of five different samples were served at each sensory evaluation. Three replications were performed with randomized taste panels.

Products were evaluated for colour, flavour, aroma, salt, texture and general acceptability using a 9-point hedonic scale. The scale for colour, aroma and salt was 9, very desirable; 7, moderately desirable; 5, desirable; 3, slightly undesirable; 1, very undesirable. The scale for flavour, texture, general acceptability was 9, very good; 7, good; 5, fair; 3, poor; 1, very poor.

Results and discussion

Amino acid composition

Protein content of coconut and soybean meals were given in Table 1 The amino acid composition found in the coconut and soybean proteins used is closely similar to those published by Lachance & Molina (1974) for coconut and Rackis *et al.* (1961) for soybean. The total amino acids recovered from soybean protein (913.07 mg/g protein) were slightly higher than those recovered from coconut protein (902.01 mg/g protein). The total amount of

Table 1. Percentages of chemical composition of defatted coconut and soybean meals

Flour	Protein (N × 6.25)	Moisture	Crude fibre	Fat	Ash	NFE*
Defatted coconut	21.85	4.62	10.82	3.96	5.63	54.12
Coconut†	17.30	7.61	9.06	7.85	5.34	52.84
Defatted soybean	48.14	8.62	1.65	1.96	5.14	34.76
Defatted soybean‡	31.75	8.97	3.20	1.67	4.99	49.42

* Non-fraction extracted.
† Melo (1969).
‡ Isabel & Iluminada (1961).

essential amino acids of coconut protein (456.57 mg/g protein) was higher than that of soybean (407.39 mg/g protein). Soybean protein is characterized by its low content of sulphur amino acids and high levels of lysine. The lysine content of coconut is 24.78% lower than that of soybean and could be the limiting amino acids as suggested by Curtin (1958). The high lysine content of soybean protein makes it a useful supplement for cereal proteins which are normally deficient in lysine. The first two limiting amino acids in soybean protein are methionine and tryptophan. Methionine content of soybean is 57.50% lower than coconut. This deficiency needs to be considered when the

Table 2. Amino acid composition of coconut and soybean proteins (mg/g protein)

Amino acid	Defatted coconut	Coconut meal*	Defatted soybean	Soybean meal†
Alanine	34.26	34.66	37.49	45.10
Aspartic acid	60.27	60.27	108.37	120.10
Half-cystine	8.84	ND	6.28	ND
Glutamic acid	220.74	141.55	195.02	210.00
Glycine	43.60	27.17	43.54	45.20
Proline	31.06	21.46	49.85	62.80
Serine	39.27	31.23	51.67	55.70
Tyrosine	7.00	16.12	13.46	39.00
Arginine‡	129.19	105.94	61.12	84.20
Histidine‡	23.47	11.10	24.08	25.50
Isoleucine‡	40.09	23.01	58.95	57.00
Leucine‡	63.41	45.11	55.95	77.20
Lysine‡	42.12	24.93	56.00	68.50
Methionine‡	19.34	14.11	8.22	15.60
Phenylalanine‡	39.83	27.40	45.69	50.10
Valine‡	60.02	35.43	53.23	53.80
Threonine‡	36.60	21.69	40.22	43.10
Tryptophan‡	2.50	6.30	3.95	12.80

* Lachance & Molina (1974).
† Rackis *et al.* (1961).
‡ Ten essential amino acids.

protein is used for the nutritional purposes. Arginine, leucine and valine values of coconut protein were higher than those found in soybean protein whereas values for isoleucine, histidine, threonine, phenylalanine and tryptophan were lower in coconut protein.

The available amino acid decreased after hydrolysis for both soybean and coconut meal as well as the mixtures (Table 3). The same result was also exhibited with the essential amino acids for the individual raw materials as well as its mixtures. After hydrolysis, the essential amino acids of the raw materials decreased by 15.21% for soybean and 38.98% for coconut. This indicates that some of the amino acids were destroyed more rapidly under reflux conditions than under inert atmosphere. There was a greater degree of destruction of amino acids in coconut than in soybean due to the higher available carbohydrate in coconut. This was also confirmed by the observation that when a mixture of coconut and soybean was hydrolysed, amino acid recovery was lower than when coconut and soybean were hydrolysed separately. Alanine and leucine values changed only slightly in soybean products, whereas values for glycine, proline, isoleucine and phenylalanine decreased. The same result was obtained with coconut. The basic amino acids: histidine, arginine, glutamic acid value decreased in soybean, and coconut

Table 3. Amino acid composition of hydrolysed products (mg/g protein)

	Products						
Amino acid	Soybean	Coconut	50–50 (w/w)*	50–50 (v/v)†	75–25 (v/v)†	25–75 (v/v)†	Commercial
Alanine	42.12	38.18	34.43	36.00	39.25	33.66	64.57
Aspartic acid	108.35	60.00	85.57	91.83	100.25	80.24	17.57
Cystine	ND	ND	ND	ND	ND	ND	ND
Glutamic acid	168.59	142.27	142.95	151.66	157.87	146.34	276.43
Glycine	39.65	32.27	32.62	34.67	38.00	32.68	9.11
Proline	40.24	20.00	27.38	27.50	35.25	16.59	4.29
Serine	53.53	30.91	38.03	41.83	45.62	36.83	11.25
Tyrosine	20.94	11.82	15.41	16.83	19.25	14.63	Traces
Arginine‡	40.82	64.41	37.70	42.50	38.50	47.07	Traces
Histidine‡	21.18	11.82	13.44	17.83	19.75	15.61	Traces
Isoleucine‡	35.76	23.18	24.10	30.33	33.62	27.56	12.64
Leucine‡	66.12	44.09	49.18	56.67	61.12	51.46	22.93
Lysine‡	57.41	25.45	34.10	42.67	47.62	37.07	Traces
Methionine‡	7.18	12.27	8.52	7.17	7.25	8.05	10.64
Phenylalanine‡	42.94	30.09	31.13	37.50	40.37	32.93	Traces
Valine‡	36.24	45.45	27.87	34.33	35.25	33.17	16.32
Threonine‡	37.76	21.82	27.54	30.50	33.37	28.07	8.07
Tryptophan‡	00.00	00.00	00.00	00.00	00.00	00.00	00.00

* Ratio by weight of soybean to coconut product.

† Ratio by volume of hydrolysed soybean to coconut product.

‡ Ten essential amino acids.

Table 4. Mean taste panel scores of colour, aroma, flavour, salt, texture and general acceptability of hydrolysed products

	Mean score*					
Product	Colour	Aroma	Flavour	Salt	Texture	General acceptability
Soybean	8.00[a]	7.46[a]	7.86[a]	8.18[a]	7.52[a]	8.09[a]
Coconut	6.12[b]	6.18[b]	6.09[b]	7.76[a]	6.43[a]	5.91[b]
50–50 w/w†	6.82[b]	7.07[ab]	6.73[ab]	7.73[a]	6.94[a]	7.03[c]
50–50 v/v‡	7.27[ab]	6.97[ab]	6.70[ab]	8.30[a]	6.64[a]	6.55[bcd]
Commercial	7.12[ab]	6.03[b]	6.64[b]	7.85[a]	6.88[a]	6.79[cde]

* Figures followed by the same letter are not significantly different at $P=0.05$.
† 50–50 ratio by weight of soybean to coconut product.
‡ 50–50 ratio by volume of hydrolysed soybean to coconut product.

products in acid solution and in the presence of a large excess of carbohydrate, whereas aspartic acid remained stable. The sulphur-containing amino acids: cystine and methionine decreased during hydrolysis. Cystine was partially converted to cysteic acid, while methionine was partially oxidized. Tryptophan was particularly unstable and was readily destroyed under acid conditions and presence of carbohydrates, oxygen and other amino acids. Tyrosine was stable in hot acid solution even in the presence of carbohydrate. Serine and threonine were decomposed only slightly in acid conditions.

Sensory evaluation of hydrolysed products

The soybean product had the highest mean score for colour, aroma, flavour and general acceptability while coconut product had the lowest (Table 4). The

Table 5. Chemical composition of hydrolysed products

Product	pH	Viscosity	Pure extract (g/100 ml)	Sodium chloride (g/100 ml)	Total nitrogen (g/100 ml)	Amino nitrogen (g/100 ml)	Reducing sugars (g/100 ml)	Total activity (ml sodium hydroxide/ 100 ml)
Soybean	4.64	16.80	15.86	18.25	1.49	0.71	6.82	24.30
Coconut	4.68	15.20	7.62	17.98	0.95	0.45	4.60	7.90
50–50 w/w*	4.68	16.00	8.98	18.10	1.18	0.57	5.16	15.80
50–50 v/v†	4.65	16.00	11.81	17.89	1.22	0.58	5.73	16.20
75–25 v/v†	4.65	16.40	13.84	18.18	1.34	0.64	6.34	20.60
25–75 v/v†	4.67	15.60	9.62	18.05	1.08	0.52	5.26	12.50
Commercial	4.70	17.10	15.92	18.30	0.83	0.39	6.98	7.10

* Ratio by weight of soybean to coconut product.
† Ratio by volume of hydrolysed soybean to coconut product.

score for colour obtained for the 50:50 w/w product varied significantly with that of soybean product. This indicates that colour development of product during hydrolysis was affected by the presence of coconut meal. The mean scores of aroma, flavour, salt and texture showed that 50:50 w/w product is not significantly different from 50:50 v/v products. The lowest general acceptability was that of the coconut product with a mean score of 5.91 (Table 4). Comparing the commercial soy sauce product with mixed formulations resulted in improved general acceptability of the products (Table 4). The mixing of the raw materials prior to hydrolysis, gave better scores than those mixed after hydrolysis.

Chemical composition of hydrolysed products

Products with high viscosity were also high in soluble solids, both of which contribute to the body of hydrolysed products (Table 5). The sodium chloride content varied between 17.89 and 18.25 g/100 ml of sample which is close to the standard for soy sauce. Total nitrogen of the soy product (1.49 g/100 ml of sample) was higher than that obtained for coconut product (0.95 g/100 ml of sample). This is due to the difference in the protein content of the raw material. Reducing sugars varied from 4.60 to 6.98 g/100 ml of product for the hydrolysates and commercial soy sauce product. Interfering substances in the products such as protein, amino acids and other reducing compounds may cause error in the analysis of sugars, especially when changes in sugar content during hydrolysis are being studied. Total acidity varied between 24.3 to 7.1 ml sodium hydroxide (N/10)/100 ml of product. The acidic substances in hydrolysed products are important to the aroma, flavour and storage quality of the products. Total titratable acidity was measured by titrating to pH 8.3. At this pH, 100% of monocarboxylic acids and phosphoric acid were titrated (Sakasai & Yokotsuka, 1956). The acidity is effective in ameliorating the salty taste and in promoting the stability of hydrolysed products. The pH of hydrolysed products varied from 4.64 to 4.68. The low pH values affect the stability of the mixture as well as its flavour. At pH 4 to 5, Udo (1932) found that arginine, histidine, lysine, putrescine, cadaverine and ammonia conjugated with glutamic acid and choline, the salt of acetic, lactic and phosphoric acids conjugated with succinic acid and these compounds exhibited good taste thus contributing to the taste of the sauce. It is also possible that the flavour development could be affected by ageing. Studies on the changes during ageing or storage would provide the necessary information needed to control the reactions. Another area that has to be studied is the determination of ways and means of deriving flavouring mixtures from these hydrolysed products.

Conclusion

Determination of the individual amino acid composition of raw materials and products showed that the amino acids from the hydrolysed coconut were

decomposed more rapidly than the amino acids from the hydrolysed soybean because of the larger amount of carbohydrate in coconut. The organoleptic test results were compared with the chemical composition of the products. Results showed that hydrolysed soybean products were significantly different and better than the hydrolysed coconut products, but not significantly different from the 50:50 v/v and 50:50 w/w products. Sodium chloride, total acidity and amino acid content are important factors in influencing flavour acceptance.

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Electroconductive thawing by liquid contact

D. NAVEH, I. J. KOPELMAN and S. MIZRAHI

Summary

A method of rapid thawing, especially suitable for large frozen products is proposed. The method is based on electroconductive heating of the frozen product immersed in a liquid and positioned between two electrodes having no direct contact with the piece. The electrical current passing through the product generates internal heat, thus considerably shortening thawing time. Furthermore, in this process a low surface temperature can be maintained at any desired level, by circulating cooling water over the product, without affecting thawing rates. Local overheating within the product is avoided by either controlling current density or by programming various combination of pulse duration and frequency. For example, it took 160 min to thaw a −20°C frozen cylindrical (17 cm ∅ × 33 cm) meat chunk where the temperature at the surface and at the thawed part were maintained below 7 and 20°C, respectively. In comparison, 450 min were needed to thaw a similar frozen sample immersed in a 20°C stirred water.

Introduction

Fast and controlled thawing of frozen foods is highly advantageous from an economical and microbiological point of view. This is especially true in commercial practice when large food products, such as meat and fish, are involved. The different approaches to the acceleration of thawing were reviewed by Everington (1971).

One of the approaches to thawing is by electrical resistance heating of a product which is sandwiched between two electrodes. This method has been proposed for use in thawing of fish (Sanders, 1967) and meat (Kalbert, 1960). A similar approach was used also in thawing and heating of pre-cooked frozen

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casserole (Segars & Kapsalis, 1976). Satisfactory performance of this thawing method is dependent on assuring a good electrical contact between the electrodes and the product, for otherwise sparks and scorching may occur. Oddly shaped products, in particular, may present a practical problem in achieving such a good contact.

The aim of this work is to propose a method of rapid electrical resistance thawing in which the electrodes have no direct contact with the product. The approach is an extension of the concept developed by Mizrahi, Kopelman & Perlman (1975) for electroconductive blanching.

Materials and methods

The experimental set-up of the electroconductive thawing system (Fig. 1) consists of four major parts: (1) A 380 V, 50 Hz electrical system controlled by a timer and by an on–off switch; (2) A cooling system whereby product surface temperature is controlled by recirculating ice cold water; (3) A thawing cell that consists of a round water bath 50 cm ∅ having two 15×22 cm stainless steel plate electrodes spaced 30 cm apart. The cell was filled with tap water (~1000 mmho/cm). The water level was maintained by an overflow; (4) Temperature measurement system. Temperatures were measured with two multi-thermocouple rods. Each rod (6 mm ∅) consists of seven 24-gauge copper-constantan thermocouples with the junctions placed in-line, spaced

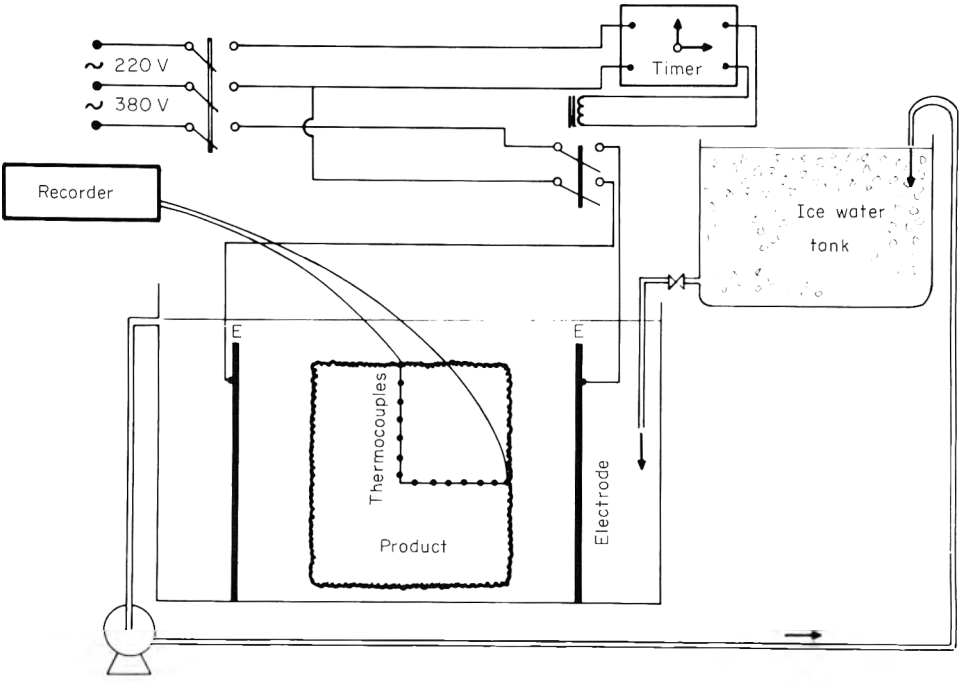

Figure 1. Electroconductive thawing experimental setup.

1 cm apart. Temperatures were recorded on a 24 point Honeywell (Electronix 15) chart recorder.

Experimental procedure

The two multi-thermocouple rods were placed centrally and perpendicular to each other in a meat chunk. The meat was then frozen to about −20°C. The frozen sample was removed from the freezer and placed in the thawing cell with one thermocouple rod parallel and the other perpendicular to the electrodes (Fig. 1). Current was introduced by applying 380 V pulses to the electrodes. Continuous monitoring of temperatures serves two functions, to follow the retreating ice front in the frozen meat chunk as thawing progresses, and to avoid local overheating by controlling pulse duration. Product surface temperature was maintained at the prescribed temperature (7 or 17°C) by controlled circulation of ice cold water through the cell.

Product electrical conductivity was determined according to the procedure outlined by (Mizrahi, Kopelman & Perlman, 1975). The average specific electric conductivity was 10 mmho/cm for thawed meat (approx. 25°C).

Results and discussion

A comparison between thawing by the electroconductive method to that of the conventional water one is shown in Fig. 2. This comparison is based on

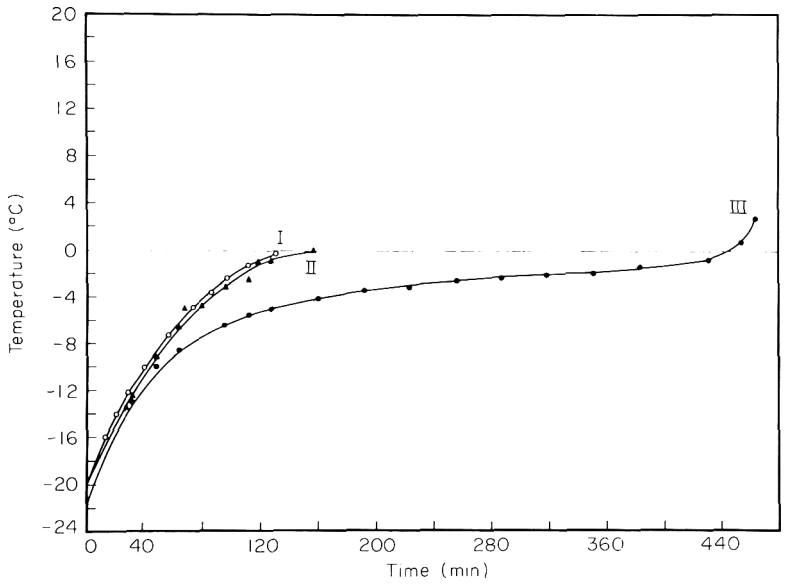

Figure 2. Time–temperature curves during electroconductive and cold water thawing of a meat chunk (17 cm∅ × 33 cm). I, electroconductive—surface temperature 17°C; II, electroconductive—surface temperature 7°C; III, cold water—surface temperature 20°C.

time–temperature data taken at the geometric centre of a meat chunk (a cylinder of approximately 17 cm∅ × 33 cm). Using the electroconductive method, only 160 min were required for a complete thawing of the meat chunk as compared to 450 min required to thaw the same sample in a 20°C constant temperature stirred water bath. The thawing rate in the latter method is governed mainly by the slow conduction heat transfer, thus strongly dependent upon the size of the body. It should be noted that improving total heat transfer by increasing medium (and thus surface) temperature is limited in practice by microbiological and organoleptical considerations. On the other hand thawing rate in the electroconductive method is fast since heat is generated within the product by the passing electric current. This fast thawing rate is almost independent of medium temperature (Fig. 2).

Therefore thawing can be effectively conducted while maintaining a low product surface temperature, thus checking microbial growth.

Since ice has an high electrical resistance, current flows selectively through the already thawed part of the product which thus becomes a heat source. The generated heat is conducted to the retreating ice front. The proximity of the heat source to the ice front and the immediate formation of new heat sources by any melting explain the considerable shortening of thawing time observed. However, excessive continuous input of electrical current may lead to local overheating, especially in thick bodies. Zones most susceptible to overheating are those which are relatively distant from the cold surfaces of the ice front and the cooled product surface.

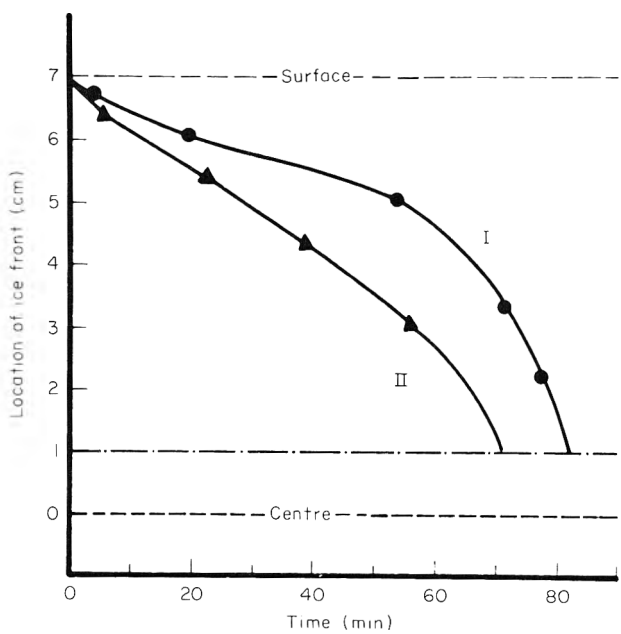

Figure 3. Location of ice front during electroconductive thawing of frozen meat block (14×14×14 cm). I, parallel to electrodes; II, perpendicular to electrodes.

Overheating can be avoided by either controlling current density in a continuous mode or by programming various combinations of pulse duration, pulse frequency and current density. The typical electroconductive thawing curve shown in Fig. 2 was obtained, for example, by applying constant voltage pulses. The duration of the pulses was controlled by surface and body temperature, both pre-set at the desired maximum level of 7 or 17°C at the surface, and 20°C within the thawed portion.

By monitoring two dimensional temperature profiles in the meat block, the position of the retreating ice front as function of time was determined (Fig. 3). Data indicate that the ice front retreats more slowly from the product surface

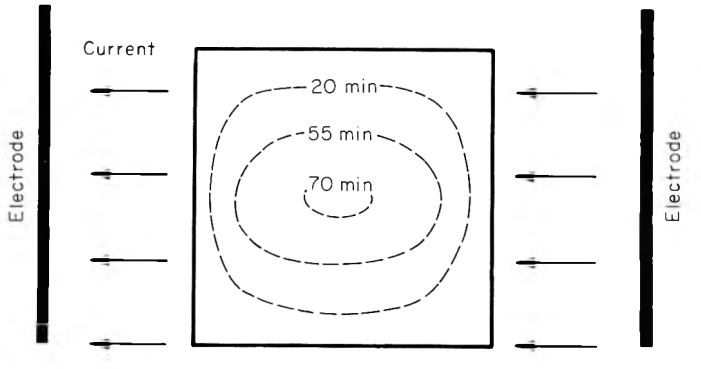

Figure 4. Schematic drawing of ice front retreating pattern during electroconductive thawing of frozen meat block (14×14×14 cm).

facing the electrodes than from that of the perpendicular side. This phenomenon of retreating ice front, illustrated in Fig. 4, is attributed to the high current density in the thawed product layers which are parallel to the current flow. These layers present the lowest electrical resistance path of the system, thus most of the current converges through them. This mechanism suggests that the pattern of the retreating ice front may be affected by electrodes parameters such as size, shape, position and the number of electrode. Those can be advantageously used in optimization of the thawing process. Other parameters that may affect the overall performance and the electrical efficiency of the thawing system would be: the relative values of the thermal conductivity of the thawed food (dynamically changed) and that of the immersion liquid; the anisotropic nature of the product, primarily its skin electrical resistance; the shape of the electrode etc.

In conclusion, a rapid thawing method is proposed. The method is based upon generating heat within the product by passing AC current through it. During the process the product is submerged in an aqueous solution and is placed between two electrodes.

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Stability of carotenoids in freeze dried papaya (*Carica papaya*)

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Summary

Effect of water activity (a_w) and storage temperature on the degradation of carotenoids in relation to keeping quality of freeze dried papaya is reported. Carotenoids were found to be most stable at 0.33 a_w, and both below and above this level their rate of destruction was higher. Above 0.40 a_w browning limited the storage life. Freeze dried papaya has maximum stability between 0.22–0.33 a_w.

Introduction

Papaya (*Carica papaya*) is widely grown in tropical and subtropical regions and is a good source of vitamins A and C. It is mainly consumed as fresh fruit though methods have been developed for processing it into frozen purée, frozen chunks and fruit leather (Brekke, Chan & Caveletto, 1972; Chan & Caveletto, 1978). Conventional drying of papaya has not proved successful mainly because retention of its pleasing colour and delicate flavour is rather difficult. Accelerated freeze dehydration, in general, results in highest retention of colour and flavour and causes least damage to texture of fruits.

The attractive colour of papaya is due to carotenoids (Yamamoto, 1964). In general, carotenoids are highly susceptible to autoxidation during storage of freeze dried foods and their degradation has been reported to be associated with the off-flavour development in dehydrated carrots and sweet potato flakes (Ayers *et al.*, 1964; Walter, Purcell & Cobb, 1970). The present paper describes the effect of water activity and storage temperature on the stability of carotenoids in freeze dried papaya.

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Materials and methods

Freeze dehydration

Good quality ripe yellow papayas were purchased from a local market and washed under running water. The papayas were trimmed, hand peeled, cut into chunks of approximately 1.5×1.5×1 cm dimension and thoroughly mixed. The chunks (10 kg) were frozen in a blast freezer at −25°C and freeze dried by keeping the heater plate at 55°C while the product temperature was allowed to rise from −25°C in the beginning to 50°C at the end of the drying cycle (12 hr). The moisture content in freeze dried papaya varied between 3−4%.

Packing and storage

Freeze dried papaya chunks (50 g) were hermetically sealed in paper–aluminium foil–polyethylene laminate pouches and stored at ambient temperature (16−35°C) and 37°C. For studying the effect of water activity (a_w), 15 g samples of freeze dried papaya powder (15 g) were kept in Petri dishes (8.5 in diameter) over saturated salt solutions in desiccators according to Rockland (1960).

Analysis

Initially and periodically the samples were analysed for total carotenoids, non enzymic browning, TBA value and moisture content according to the method described earlier (Arya *et al.*, 1979b). For sensory evaluation stored samples both before and after reconstitution were given to a panel of six members for detection of perceptible changes in taste and flavour.

Results and discussion

Freshly freeze dried papaya chunks (protein 5.5±0.2%, sugars 70±2%, fat 1.7±0.1%) had 166±4 μg/g total carotenoids and their concentration decreased significantly during storage (Table 1). The destruction of carotenoids was dependent on storage temperature. After 36 weeks of storage, about 80% of total carotenoids were retained at 0°C compared to 22.5 and 12.4% at room temperature and 37°C respectively. Destruction of carotenoids in freeze dried papaya was, however, not accompanied by the violet and hay-like odours reported previously in freeze dried carrots (Falconer *et al.*, 1964). Tomkins, Mapson & Wager (1946) have reported that off-flavour became noticeable in dried carrots when about 20−50% of total carotenoids had been destroyed. In freeze dried papaya, stored at ambient temperature, no off-flavour was detected up to 24 weeks of storage even though 66.6% of the original carotenoids were degraded. In the samples

Table 1. Changes in freeze dried papaya stored at ambient temperature (16–35°C) and 37°C

Storage period (weeks)	Ambient temperature			37°C		
	Total carotenoids (μg/g)	TBA value*	NEB†	Total carotenoids (μg/g)	TBA value*	NEB†
0	166.0	0.09	0.07	—	—	—
8	111.6	0.08	0.09	96.3	0.10	0.11
12	103.7	0.11	0.13	70.3	0.12	0.13
16	68.21	0.14	0.13	37.5	0.18‡	0.14
24	55.3	0.14	0.13	33.0	0.18	0.14
32	43.4	0.16‡	0.13	24.6	0.19	0.14
36	37.3	0.15	0.13	20.5	0.20	0.14

* TBA value, mg malonaldehyde/kg substance.

† NEB, non-enzymatic browning measured as optical density of aqueous extract at 420 nm.

‡ Stage at which off-flavours became noticeable.

stored at 37°C, oily and rancid odours became noticeable after 16 weeks and this was accompanied by a slight but significant increase in TBA value suggesting the involvement of lipid peroxidation in off-flavour development. There was a gradual decrease in the intensity of fresh papaya flavour notes and this along with bleaching of yellow colour were the major contributory

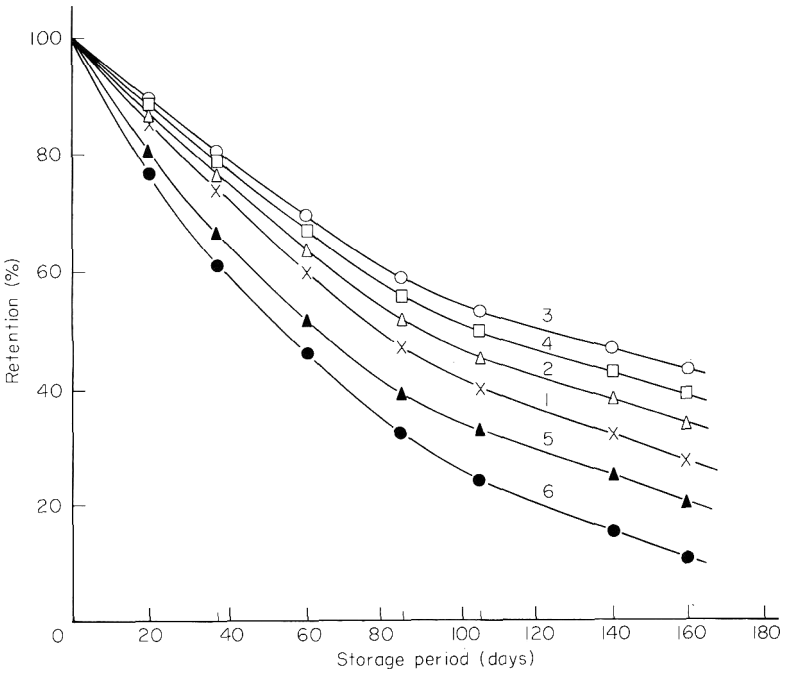

Figure 1. Effect of water activity on the stability of carotenoids in freeze dried papaya. 1, 0.0 a_w; 2, 0.22 a_w; 3, 0.33 a_w; 4, 0.43 a_w; 5, 0.57 a_w; 6, 0.73 a_w.

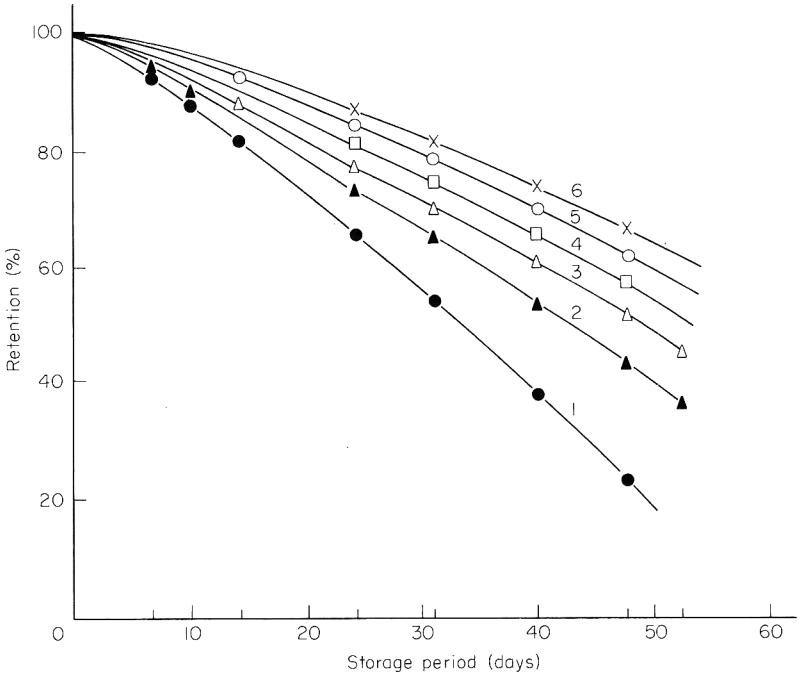

Figure 2. Relative stability of papaya and carrot carotenoids in isolated systems. 1, 2, 3, carrot carotenoids and 5, 6, 4, papaya carotenoids stored at 0.0, 0.33 and 0.73 a_w respectively.

factors in limiting the shelf life of freeze dried papaya. Below 4% moisture browning was not a major problem during storage (Table 1).

Effect of a_w on the rate of carotenoid destruction is shown in Fig. 1. In freeze dried papaya the carotenoids were most stable at 0.33 a_w and both

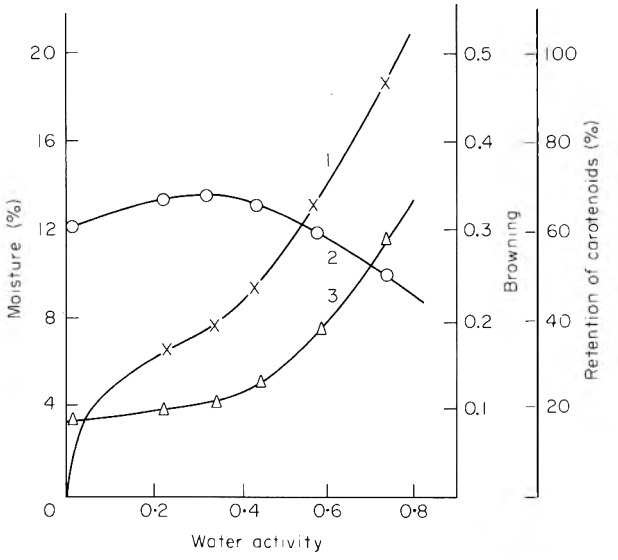

Figure 3. Effect of water activity on browning and retention of carotenoids. 1, Sorption isotherm; 2, percentage retention of carotenoids; 3, browning after 2 months' storage.

below and above this level their rate of destruction increased significantly. This increase was more at higher a_w than at lower a_w. Previously in freeze dried carrots, however, increase in the rate of carotenoid destruction was found to be higher at lower a_w than at higher a_w. It is interesting to observe that more than 95% of total carotenoids were degraded in carrots within 31 days at 0.0 a_w (Arya *et al.*, 1979b) whereas only about 20% were lost in freeze dried papaya under similar conditions. In order to understand this differential behaviour in their stability, carotenoids both from papaya and carrots were extracted with acetone-hexane mixture, brought into the hexane layer by washing with sodium chloride solution, impregnated over microcrystalline cellulose (Arya *et al.*, 1979a) and stored at 3 a_w. The stability of papaya and carrot carotenoids at 0.0, 0.33 and 0.73 a_w is shown in Fig. 2. It may be observed that in an isolated system the rate of degradation is highest at 0.73 a_w in case of papaya carotenoids and at 0.0 a_w in case of carrot carotenoids, thus confirming our previous observations on dehydrated products. Composition of carotenoids of yellow papaya has been reported by Yamamoto (1964). Cryptoxanthin (38.9%), cryptoxanthin-mono-epoxide (15.6%) and β-carotene (24.8%) constitute about 80% of total carotenoids. Apparently, cryptoxanthin and its derivatives seem to be more stable at lower water activities.

Sorption isotherm of freeze dried papaya and the relationship between a_w and browning development are shown in Fig. 3. Freeze dried papaya is highly hygroscopic and equilibrates to about 7.6% moisture at 0.33 a_w and 9.7% moisture at 0.43 a_w. Below 0.40 a_w rate of browning was slow but above this level intense darkening in colour and associated flavour changes due to sugar-amino acids interaction became the major factor in limiting the shelf life of freeze dried papaya. Taking into consideration the carotenoids retention, browning development and flavour changes in freeze dried papaya, the zone between 0.22–0.33 a_w (moisture 6–7%) seems to be most optimum for maximum storage life.

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Chemical characterization of prickly pear pulp, *Opuntia ficus-indica*, and the manufacturing of prickly pear jam

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Summary

Attempts were made to use prickly pear fruits, *Opuntia ficus-indica*, which are locally abundant and relatively inexpensive in the manufacturing of jam.

Physical characterization of the strained pulp showed a value of 14.2° Brix for total soluble solids (TSS), 14.5% total solids and a pH of 5.75. The acidity of the pulp as citric acid was 0.18%. Proximate analysis revealed low amounts of protein (0.21%) as N×6.25, crude fat (0.12%), crude fibre (0.02%), ash (0.44%) and pectin (0.19%). All the sugars were present as reducing sugars (12.8%) and consisting mainly of glucose and fructose (60:40). Vitamin analysis showed only trace amount of vitamin A (β-carotene) and 22.1 mg% of vitamin C. The pulp was rich in potassium, fair in calcium, magnesium and phosphorus and poor in sodium and iron.

Pilot plant studies on the manufacturing of the jam in conjunction with sensory evaluation of the final products showed that blanching in comparison to non-blanching resulted in no significant difference in the sensory quality of the jam. Citric acid and a combination of citric and tartaric acids (1:1) were preferred over several other natural acids used as acidifying agents. The addition of cloves, grapefruit, orange and almond flavours ranked best among several other flavours added in addition to the pulp containing 20% date paste.

Introduction

Cactus plants are documented to have originated in North America. Over the centuries, they have spread throughout the world from their American points of origin to North Africa, Europe, Mediterranean countries, the Middle East and other countries (Hare & Griffiths, 1907). Prickly pears which belong to the

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genus *Opuntia* of the cactus family have various species, most of which grow wild, with only a few commercial plantations that have been established around the world, mainly in Mexico, Chile, Brazil, Argentina and the U.S.A. The bulk of production from these plantations is usually utilized as fresh fruits with only negligible amounts that are incorporated into some speciality confectionaries.

In Saudi Arabia, prickly pears of the *Opuntia ficus-indica*, grow in large quantities in the South-western regions of the country, about 50–100 km inland east of the Red Sea. These regions are mountainous and rocky with an average rainfall of 100 mm/year. The temperature during the year ranges between 16–30°C with an average relative humidity of 20%. The prickly pear plants grow wild and usually are utilized as hedges for gardens. These plants continue to produce edible fruits starting around the second week of July until early October. The country has no accurate estimates about the annual fruit production and the acreage occupied by prickly pear plants. However, it is locally known that prickly pears come only next in quantities to dates and watermelons which are the most abundant locally grown fruits in Saudi Arabia. During the harvest season, prickly pears are very inexpensive in the areas of production and most of the time are given away free.

To date, not much work has been reported in the literature on the industrial use of prickly pear fruits. However, some studies have been carried out on the canning of prickly pear juice (Paredes-Lopez & Rojo, 1973; Villarreal, De Alva & Romero, 1964) and on the compositional changes occurring during development (Lakshminarayana, Sosa & Perez, 1979). Attempts to use prickly pear juice as feed stock and in biomass production have been reported from Mexico (Paredes-Lopez, 1976; Paredes-Lopez, Camargo-Rubio & Ornelas-Vale, 1976). Other investigations were undertaken on the prickly pears mucilage composition (Mindt *et al.*, 1975) in an attempt to study their potential utilization as an alternative source for gum exudates and similar substances. In this study, an attempt was made to use prickly pear pulp of the *Opuntia ficus-indica* grown in Saudi Arabia, in the manufacturing of prickly pear jam.

Materials and methods

Preparation of prickly pear pulp

Prickly pear pulp was prepared from ripe fruits, green to orange-brown in colour, which were purchased from the local market. The fruits were sorted, washed, dried, weighed and hand peeled. They were then passed through a pulper finisher for the separation of the seeds from the pulp. The separated pulp was weighed and was further utilized for both chemical and physical analysis as well as the manufacturing of jam. Part of the fruits were steam blanched for 2 min by a pilot plant blancher (Dixie Canner Equipment Co., U.S.A.) before pulping and the pulp so obtained was used only for the manufacturing of jam.

Preparation of date pulp

Date pulp was prepared from ripe dates of the Sullaj variety at the Tamr stage (dark brown colour). Dates were picked up from the Regional Agriculture and Water Research Center Palm Plantation, sorted, washed, dried and weighed. The dates were then boiled in hot water for about 15 min until they were soft. The cooked dates were pulped through a pulper finisher to separate the seeds and culls. The pulp so obtained was weighed and utilized in the manufacturing of prickly pear jam.

Physical and chemical characterization of the pulp

pH, Acidity and total soluble solids. The pH of the pulp was measured with a pH meter (Beckman, Model 3500). Titratable acidity was determined by titrating the pulp with 0.1 N NaOH according to the standard procedures of the AOAC (1980). Total soluble solids (TSS) were determined by using an Abbe refractometer with temperature adjustment (American Optical, Model 10450).

Chemical composition. Proximate analysis for the moisture content, ether extract, crude fibre, nitrogen and ash as well as vitamin analysis for vitamin C and β-carotene were all determined according to the standard procedures of the AOAC (1980) except for the ash content where samples of the pulp in duplicates (5–10 g) were ashed at a temperature of 550°C instead of the recommended 525°C.

Pectin content was determined according to the method described by Carre & Hames, as reported by Lees (1975). Caloric value was determined on the vacuum dried sample of the pulp by using a Ballistic Bomb calorimeter (Gallenkamp, Model CBA-301) using pure sucrose as a standard.

Total and reducing sugars were determined according to the standard procedures of the AOAC (1980). For sugar monomers, approximately 20 g of the pulp was weighed accurately into a sample cup of Sorvall Omni-mixer/grinder and exactly 200 ml of distilled water was added and the sample ground at 16 000 rev/min for 5 min. The prickly pear pulp/water mixture was shaken for 1 hr to dissolve all sugars in the water. The extract was then filtered through Whatman No. 2 filter paper (discarding the first few millilitres which were cloudy). The clear mixture was analysed on a Waters ALC-201 liquid chromatograph equipped with a differential refractive index detector. The column used for the sugar separations was a Bio-Rad HPX-42 cation exchange column with water as the elution solvent. The area of the recorded peaks from each sample was compared with peaks from a standard water solution of glucose/fructose/sucrose.

Mineral analysis for Na, K, Ca, Mg, P and Fe were determined in the ash with an atomic absorption spectrophotometer (Perkin Elmer, Model 603) by dissolving the ash in 5 ml of 20% HCl and making the volume to 100 ml with deionized water. The final solution for Ca and Mg contained 1% of lanthanum to overcome interferences especially by phosphates. Phosphorus was determined by the molybdovanadate method of the AOAC (1980).

Processing of prickly pears jam

Manufacturing of jams. In the manufacturing of the jam, the variables studied were the sugar/pulp ratio, acidifying agents (type and quantities), per cent of pectin to be added, kind and levels of flavourings, dates/prickly pear pulp ratio and the effect of blanching of the fruits on the quality of the jam.

In all the trials, the pH of prickly pear pulp (5.7–5.8) was adjusted to pH 3.2 by the addition of a 20% solution of the natural acids used. The pulp was then added to a cooking pan or open double jacketed steam kettles (Lee Metal Products Co. Inc., U.S.A.). It was then heated for a few minutes after which the sugar/pectin mixture was added in small increments with continuous mixing. The mixture was left to boil until the total soluble solids value approached 62–63° Brix after which citric acid was added and thoroughly mixed. Cooking was stopped when the final Brix value reached about 65°. Various extracts and natural flavours such as vanilla extract (F-8001), natural almond extract (R-370, natural apricot flavour (R-1078), natural banana flavour (R-10, 75), natural lemon extract (F-8999), natural orange extract (F-1625), that were obtained from Givaudan Corp. (U.S.A.), and clove buds were added at various levels and mixed well with jam after it was finished and before it was filled hot into glass jars. All jam jars were cooled quickly in a chilled water bath. Several trials for the incorporation of date pulp in the prickly pear jam at various levels (10–25%) were also investigated.

Sensory evaluation

Sensory evaluation tests were carried on two batches of jams prepared from the blanched and unblanched fruits. Seven different products were presented to a group of untrained panelists consisting of sixteen to seventeen members. One sample was used as control where citric acid was added as the acidifying agent with no added flavours and the rest consisted of samples having different flavours.

The tests carried out consisted of the preference tests, hedonic scales scoring using a scale from 9–1, with 9 being 'Most liked' and 1 'Most disliked' (Larmond, 1977). Panelists were selected randomly from colleagues at the Research Centre including local nationals as well as expatriates. Each product was evaluated for colour, taste and overall acceptability. In addition, a comparison test was conducted between blanched and unblanched jams using again the hedonic scoring system to determine the effect of blanching on the evaluated characteristics (colour, taste and overall acceptability). The samples coding were as follows:

P_1—Prickly pears+citric acid;
P_2—Prickly pears+citric acid: tartaric acid (1:1);
P_3—Prickly pears+citric acid+cloves extract;
P_4—Prickly pears+citric acid+almond flavour;

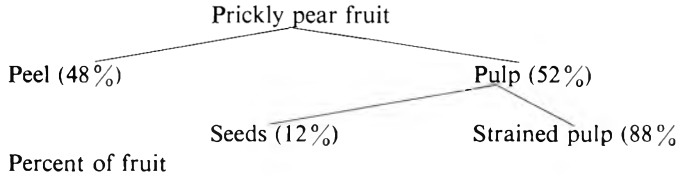
P_5—Prickly pears+citric acid+orange flavour;
P_6—Prickly pears+citric acid+grapefruit flavour;
P_7—Prickly pears+citric acid+20% dates.

Results and discussion

Chemical characterization of the pulp

Table 1 shows the fragmentation of the prickly pears fruit into its various constituents including the peel, pulp and seeds. Even though the peel and seeds together constitute over 50% of the fruit and are considered byproducts, yet they have a potential use as an animal feed. The seeds alone, constituting about 5–10% of the fruit, might also be of certain potential when available in large quantities as a source of edible oil and/or protein for poultry feed (Sawaya & Khan, 1982).

Table 1. Fragmentation of prickly pear fruit



Percent of fruit	
Strained pulp	45%
Peel	48%
Seeds	7%

The results of the characterization of the pulp for pH, acidity and TSS are presented in Table 2. The pH was fairly high (5.75) with a very low acidity, which implies that the pulp will need acidification before its manufacturing into jam. The percentage of TSS (14.2° Brix) was comparable to many of the most commonly consumed fruit pulps such as apricots, apples, cherries and plums and was more than those of strawberries, raspberries and peaches (Jacobs, 1958).

Table 2. Characteristics of prickly pear pulp

pH	5.75
Acidity (expressed as citric acid)	0.18%
Total soluble solids (Brix)	14.2°
Total solids	14.5%

Proximate analysis of the pulp (Table 3) showed that the percentages of protein, ether extract and crude fibre were relatively low. Besides, the pectin content was also very low thus suggesting that additional pectin should be added to the pulp in enough quantities to allow for gelation of the jam. The content of vitamin C in the pulp was fair and represented about half the concentration of vitamin C of some of the vitamin-C-rich fruits such as oranges and lemons, and about one and a half times that of cherries (Pellett & Shadarevian, 1970). However, β-carotene existed only in traces in the pulp. The caloric value was 47.3 kcal/100 g of pulp (3.9 kcals/g on dry weight basis) thus approaching that of pure sucrose.

Table 3. Chemical analyses of prickly pear pulp

Parameter	Fresh weight basis (%)
Moisture	85.60
Crude protein (N × 6.25)	0.21
Crude fat	0.12
Crude fibre	0.02
Ash	0.44
Pectin	0.19
Vitamin C	22 mg/100 g
β-carotene (Vit. A)	Traces
Caloric value	47.30 kcal/100 g

Sugar analysis (Table 4) revealed a 12.8% sugar content in the pulp on fresh weight basis. Sucrose was not detected and all other sugars were of the reducing type. Detailed HPLC analysis of the sugars revealed the presence of only two monosaccharides, glucose and fructose in the ratio of 60:40. Widdowson & McCance as reported by Whitting (1970) cited similar values that are common to various fruits.

Table 4. Sugar content of prickly pear pulp

Parameter	(%) fresh weight basis
Total sugar (TS)	12.8%
Glucose	59.4% TS
Fructose	40.6% TS

Results of the mineral analysis are presented in Table 5. The content of iron in the pulp was relatively low compared to the other tested minerals. The amounts of Ca, Mg, K, Na, P and Fe were comparable to those of other fruit pulps which are commonly utilized in jam production such as apricots, pineapples and strawberries (Paredes-Lopez & Rojo, 1973; Jacobs, 1958).

Table 5. Mineral composition of prickly pear pulp

Mineral	Pulp (mg/100 g)
Ca	27.6
Mg	27.7
Na	0.8
K	161
P	15.4
Fe	1.5

Manufacturing of jam

Before the establishment of the final formula for the jam and the presentation of the final products for sensory evaluation, several trials were done to study the various variables relevant to the establishment of an acceptable formula. Variables included the pulp/sugar ratio, acidifying agents (kind and quantities), per cent of pectin, flavours (kinds and quantities), date/prickly pear pulp ratio and the effect of blanching of the fruits on the quality of the jam.

Preliminary results indicated that among the various pulp/sugar ratios tried, including 40:60, 50:50, 55:45 and 60:40, the last was found most acceptable. In the other three ratios, the taste was dominated by the high sugar content thus producing very sweet products. With respect to the acidifying agents, citric acid as well as a combination of 1:1 citric and tartaric were both superior to either tartaric or malic acid alone and to a combination of 1:1 malic and citric acids.

Variation in the levels of pectin (slow set) added ranging from 0.5–2%, on the basis of the weight of the sugar, showed that a satisfactory gelation of the jam was obtained with a level of 1.25%. The addition of several natural flavours at different levels including orange, lemon, grapefruit, almond, vanilla, clove buds, apricot and banana showed that the most preferable ones were orange and grapefruit (0.045%), almond (0.045%) almond and cloves (0.027%).

The inclusion of various levels of date pulp (5–50%) in the prickly pear pulp for the manufacturing of jams can be of great significance in a country like Saudi Arabia, with an annual date production of over 350 000 tons. Preliminary tests showed that a level of 20% dates was the most preferred. The same level was, therefore, used in the formulation of the final product.

After the establishment of the final formula of the jam, the formulation of the jam was tested on blanched and unblanched prickly pear fruits before pulping. Blanching was included to investigate any improvement on the organoleptic quality of the final product. Hence, two batches of the jam were prepared, blanched and unblanched, and the final products of both batches were presented for the sensory evaluation taste panels.

Sensory evaluation

Based on the above results, a final formula for the preparation of the jam was set and is shown in Table 6. This formula was employed in the preparation of the final products for quality evaluation of both batches, blanched and unblanched. Results of the sensory evaluation of the blanched prickly pear samples (Table 7) showed that there was no significant difference at the 5% level, between the control citric acid (P_1) and the flavoured jams (P_2-P_6) citric/tartaric, orange, grapefruit, almond and cloves, for the three characteristics evaluated, namely, colour, taste and overall acceptability. However, with respect to the inclusion of 20% dates (P_7), there was a significant

Table 6. Prickly pear jam processing formula

Ingredient(s)	Quantity (100 g mix)
Prickly pear pulp	60
Sugar	40
Pectin	0.50
Citric acid	0.55
Citric acid/tartaric acid	0.32/0.32
Flavours:	
Vanilla extract	0.01
Banana flavour	0.042
Lemon extract	0.045
Apricot flavour	0.049
Orange extract	0.045
Grapefruit extract	0.045
Clove buds	0.027
Date pulp*	12

* Date pulp added as 20% of the weight of prickly pear pulp.

Table 7. Sensory evaluation of blanched prickly pear jams by scoring (preference test)

	Criteria evaluated		
Prickly pear jams	Colour*	Taste*	Overall acceptability*
P_1 Citric acid control	6.94	7.06	7.06
P_2 Citric/tartaric	7.34	7.44	7.44
P_3 Clove extract	6.88	6.44	6.56
P_4 Almond flavour	7.63	6.31	6.63
P_5 Orange flavour	7.94	7.25	7.50
P_6 Grapefruit flavour	7.81	7.69	7.69
P_7 20% Dates†	5.00^a	5.50	5.13^b
LSD (5%)	1.27	1.65	1.48

* Values are means of sixteen judgments.

† a, b, (P_7) = Significant difference at 5% level ($P < 0.05$).

difference ($P<0.05$) for both colour and overall acceptability. The colour could have adversely affected the overall acceptability because the ripe date variety used was very dark brown in colour and may have contributed to the darkening of the colour of the final product. Different date varieties of lighter colour can be employed in the future which might eliminate such an effect.

As for the unblanched jams, results in Table 8 revealed that there was a significant difference at the 5% level between the control (P_1) and the almond flavoured jam (P_6) with respect to taste and overall acceptability, and the 20% date-based products (P_7) with respect to colour and overall acceptability. However, there was no significant difference at the 5% level for the orange flavoured, grapefruit and clove jams. The low score obtained for the taste of the unblanched almond jam might have contributed to the low score of the overall acceptability. This could have been due to certain subjective differences among the different panelists which showed only in the unblanched almond jam but not in the blanched one. With respect to the date-based product, the results obtained here agree very well with those obtained in Table 7 for the unblanched product for both colour and overall acceptability.

The results of the comparison of the effect of blanching which were tested on two products, P_2 and P_6, are presented in Table 9. No significant improvement at the 5 and 1% levels between the blanched and unblanched products for both the control and the grapefruit flavoured jam was observed.

After analysing the data obtained from this study, we can conclude that the prickly pear can be utilized successfully for the manufacturing of jams wherever it exists in abundance and as an inexpensive resource, as in Saudi Arabia. Results of the chemical analysis of the pulp revealed that from the nutritional point of view, it is comparable to other fruits that are commonly utilized in the manufacturing of jams and jellies. The sensory evaluation

Table 8. Sensory evaluation of unblanched prickly pear jams by scoring (preference test)

	Criteria evaluated		
Prickly pear jams	Colour*	Taste*	Overall acceptance*
P_1 Citric acid (control)	7.82	7.24	7.35
P_2 Citric/tartaric	7.76	7.65	7.71
P_3 Clove extract	6.94	6.29	6.56
P_4 Almond flavour†	7.35	5.41[b]	5.88[c]
P_5 Orange flavour	6.88	6.94	7.00
P_6 Grapefruit flavour	7.24	7.41	7.41
P_7 20% Dates†	5.88[a]	6.47	6.00[d]
LSD (5%)	1.25	1.53	1.27

* Values are means of seventeen judgments.

† a, b, c, d = Significant at the 5% level ($P<0.05$).

Table 9. Sensory evaluation of blanched *versus* unblanched prickly pear jams

	Criteria evaluated		
Prickly pear jams	Colour*	Taste*	Overall acceptability*
P_2 Unblanched	7.86	7.79	7.64
P_2 Blanched	7.79	7.71	7.79
LSD (5%)	0.73	0.93	0.91
P_6 Unblanched	7.14	7.36	7.00
P_6 Blanched	7.57	7.36	7.29
LSD (5%)	1.07	1.08	0.99

* Values are means of fourteen judgments.

results showed that the jam was acceptable both with and without any flavours added. From an economical point of view, the utilization of prickly pear in the manufacturing of jam can be profitable, especially if efficient mechanical devices are developed that can replace hand peeling of the fruits during processing.

Acknowledgment

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Froth flotation as a means of protein extraction from mussels

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Summary

Batch studies on froth flotation as a means of protein extraction from a milled mussel matrix show that the technique is feasible. High efficiency of separation (>90%) of the mussel flesh and shell, along with high protein recoveries (>90%) are reported, using a combination of a frothing agent, Lissapol, and a flocculent, Difco. Heat pre-treatment of the milled mussel matrix is shown to have little effect on the system.

Introduction

Proteins form a necessary component of an animals diet and as the world population increases, so does the demand for this source of foodstuff. Inevitably this increased demand has led to increased costs for the commodity. The possibility of substantial profits, that could be made by obtaining a cheap source of dry protein rich concentrate, has attracted much attention. Practical feeding trials on poultry (Hopwood, 1978), using mussel protein, have demonstrated that high protein concentrate materials can replace equal weights of herring and soya meal proteins without significant change in live weight gain, feed conversion and mortality.

Due to the fecundity, rate of growth and wide distribution of highly successful cultures of mussels, they may play a role in any assessment of alternative sources of protein. Many countries, such as Spain, Holland, France and Germany have already adopted highly successful culture systems and the reliability of supply, a primary consideration underlying any raw material, seems assured. The developing culture techniques seem to culminate in the use of suspended cultures. Few countries, however, harvest mussels from natural beds alone, an exception being Chile. Around the coastlines of Northern Ireland considerable natural beds of mussels exist. On the map of Northern Ireland (Fig. 1), some of these beds are marked, examples being: Belfast Lough, Carlingford Lough and the Foyle Estuary. From the Foyle

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Figure 1. Mussel locations.

Estuary alone estimates in excess of 11 000 tonnes of full size mussels are given for bed masses (Both, 1978).

Separation of a mixture of particles of protein and shell using froth flotation methods depends on differences in the surface properties and densities of the materials involved. If the mixture of solids is suspended in an aerated liquid, the gas bubbles will tend to adhere preferentially to some of the constituents (in this case proteins), the ones which are more difficult to wet by the liquid, and their effective densities reduced to such an extent that they will rise to the surface. If a suitable frothing agent is added to the liquid, the particles will be held in the surface by means of the froth until they can be discharged over a weir. The separation of protein particle and shell is not 100% as some shell fines are also removed. The shell accounts for less than 1% of the product weight.

The process depends on the existence, or development, of a selective affinity of one of the constituents for the envelopes of the gas bubbles. In general, this affinity must be induced, and the reagents which increase the angle of contact between the liquid and one of the materials are known as promoters and collectors. Promoters are selectively adsorbed on the surface of one material and form a monomolecular layer. Promoters ionize in solution, and the polar end of one of the ions is adsorbed by the particle, the new surface of the particles is therefore made up of the non-polar part of the radicle, so that the contact angle with water is increased. Collectors are materials which form surface films on the particles. These films are thicker than those produced by promoters, and collectors therefore have to be added in higher concentrations. An essential requirement of the process is the production of a froth of sufficient stability to retain the particles in the surface.

Liquid soaps, such as lissapol, not only produce stable froths but in addition act as a collector.

Suspensions of the proteins having micron and sub-micron particle sizes may be more effectively removed if they are caused to aggregate. This may be achieved by 'coagulation' and 'flocculation'. In engineering convention, coagulation is seen as the destabilizing process brought about by the addition of some reagent to a dispersion of particles in a continuous liquid phase in which it is possible for the particles to adhere and form flocs, whilst flocculation is the hydrodynamic process in which these collisions are brought about. In this paper the term 'flocculation' will be used to describe any phenomena which cause aggregation of particles. It has been shown that (Helmholtz, 1879) a potential difference usually exists at a solid–liquid interface. Due to the presence of this surface potential, ions of opposite charge, the counter ions, will be attracted towards the surface giving rise to a high counter ion concentration adjacent to the surface decaying with increasing distance until the solution equilibrium concentrations are attained. An electrical double layer is formed. If the innermost layer of ions is imagined to move with the particle when there is relative motion between the particle and the fluid, a potential will exist at the plane of shear known as the *zeta* potential. It is the reduction of the *zeta* potentials which results in flocculation of the particles. This may be achieved by changing pH and by the addition of counter ions of high charge. As counter ions in aqueous systems are most frequently positively charged this is done in practice by the use of salts containing Al^{3+}, Fe^{3+}, Fe^{2+} and Ca^{2+} ions (Akers, 1978).

In situations (Akers, 1978), where charge neutralization effects could not explain flocculation phenomena, a bridging theory was proposed (Ruehrwein & Ward, 1952) in which polymer molecules are seen as being adsorbed onto more than one particle hence holding them together. The adsorption effect of the polymer to the surface may be charge effects, dispersion forces or hydrogen bonding. These polymeric flocculants may be regarded as crosslinking agents imparting structural rigidity to the floc.

The paper reports research work aimed at developing a commercial method for processing the landed mussels into a suitable high-protein concentrate. The four main crude constituents in mussels are, protein, lipid, shell and water. The process studied employed initial size reduction of the mussels in a suitable mill. Due to the various physical differences of the constituents in the milled product, the technique of froth flotation was investigated to separate the shell from the valuable protein component.

Materials and methods

Materials

Mussel samples were taken from the Foyle Estuary on the northern coast of Northern Ireland (Fig. 1). The mussels were size reduced in a hammer mill

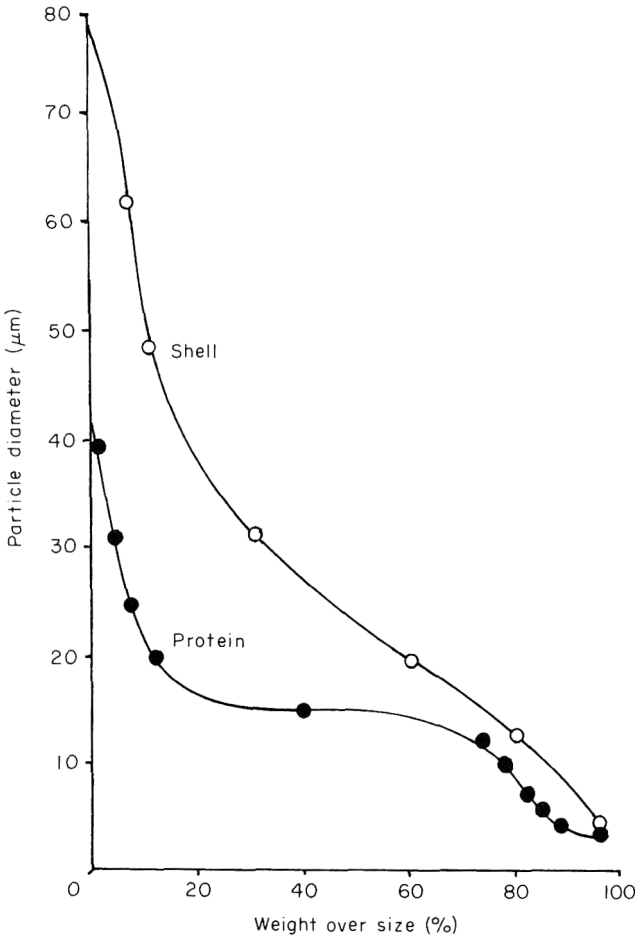

Figure 2. Size distribution of extracted materials.

giving products which fall into three categories. The bulk of the shell was reduced to approximately 3 mm sized particles, and a small proportion of fins, whose size distribution is given in Fig. 2. The flesh was ground very fine, i.e. 80% w/w less than 20 μm, except for some fibrous material which remained in lengths of a few millimetres.

In an attempt to optimize the extraction process various materials were added to the froth flotation column in a series of controlled experiments. A volume of solvent had to be added to the ground mussel bed in the column in order for the frothing process to take place. Two solvents were used, one being fresh water, the other a 5% w/w sodium chloride solution (approximate sea water conditions). The latter solvent was used, as any potential processing plant may be situated on the sea front, to see if any advantages may accure from the use of sea water.

The frothing agent was a liquid soap, lissapol, which not only produced a stable froth but also acted as a collector in the froth flotation system. The influence of a flocculating agent on the system was investigated by using

Difco, a mixture of ferrous and aluminium sulphates in sulphuric acid. Work has been published (Jørgensen, 1968, 1971; Tønseth & Berridge, 1968) on the application of a protein precipitant, lignin sulphonic acid, and tests were undertaken using this material.

Methods

The great difference between the sizes of the milled shell and flesh products, highlighted the possibility of froth flotation as a mode of separation of the products. The basic batch apparatus (Fig. 3) consisted of a vertical Perspex column 67 mm in diameter and 700 mm long. At 30 mm above the base of the column was a fine nylon air distribution plate spanning the whole cross section. Air was supplied, from a compressor to the base of the column, via a gate valve and rotometer. Along the side of the column, at intervals of 100 mm, were side nozzles for sampling or injection purposes. The lowest side nozzle being situated immediately above the air distribution plate. Any

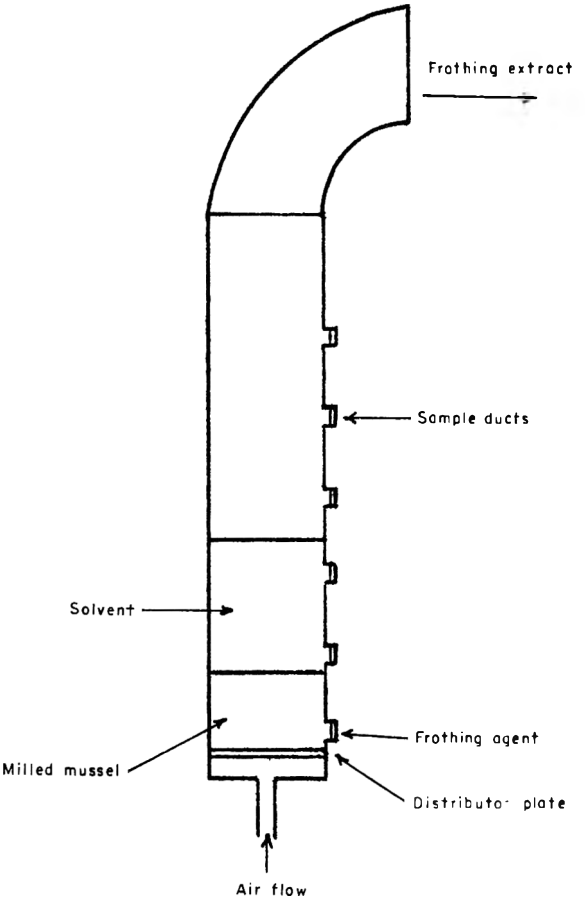

Figure 3. Batch froth flotation apparatus.

additional water, flocculating or frothing agents required could be introduced, in controlled dosages, via this lowest side nozzle, which was plugged using serum stoppers, by use of hypodermic syringes.

The experimental procedure adopted in each test run was the same except for slight variation in mussel pre-treatment and solvents used. The flotation column was charged with a 500 g batch of ground mussels into which was injected 10 ml frothing agent. This was done using an hypodermic syringe through the serum stopper. Following the addition of 1.5 litres of solvent the air flow was adjusted to the required rate. The steadily rising foam was collected at the exit shute. Samples of foam were taken at various times. Each batch sample, collected over 1 min, was broken down using a fine mist spray, centrifuged for 5 min at 3000 rev/min and dried to constant weight at 110°C in a tray dryer.

The protein contents of the original organic charge and all products were obtained by Kjeldahl analysis of dried samples. Drying and incineration tests (800°C for 1 hr) were undertaken on the extracted beds from the column. This was to establish how much organic matter remained unextracted in the bed. In the incineration tests the shell matrix disintegrated and suffered a small weight loss, the magnitude of which was established in a series of controlled experiments. Similar incineration tests were undertaken on samples of the final dry product to establish how much fine shell had been extracted along with the proteinaceous product. This was always less than 1% w/w.

Results and discussion

The protein content of mussels is relatively high, 64 g/100 g dry matter with a good amino acid balance (Table 1). The breakdown shows a wide range of amino acids with good concentrations of lysine and methionine. The figures compare favourably with the Food and Agriculture Organization 'provisional pattern of essential amino acids for human nutrition' (FAO, 1957). It must be emphasized at this point that any product produced would be aimed at animal

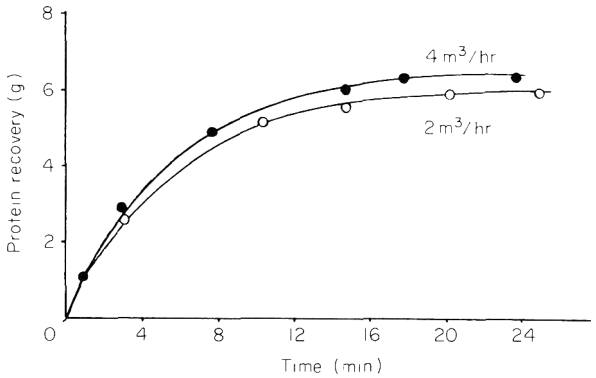

Figure 4. Cumulative extraction of protein using distilled water as solvent.

Table 1. Amino acid analysis of mussel protein and Food and Agriculture Organization provisional pattern of essential amino acids for human nutrition

	Content (%)	
Amino acid	Mussel	FAO
Aspartic acid	11.7	—
Threonine	5.2	2.8
Serine	4.6	—
Glutamic acid	13.0	—
Proline	3.5	—
Glycine	10.5	—
Alanine	8.1	—
Valine	6.0	4.2
Cystine	1.2	—
Methionine	2.0	2.2
Isoleucine	5.2	4.2
Leucine	7.6	4.8
Tyrosine	2.4	2.8
Phenylalanine	3.8	2.8
Lysine	7.4	4.2
Histidine	1.8	—
Arginine	5.9	—

feed and not human consumption. The acceptability of the chemicals used in the extraction process would depend upon the quantities of the product used as supplement to the animal diet.

The results of the extraction of protein from the milled mussels in the flotation column may be conveniently discussed by first considering the basic system, water solvent/lissapol, and then the effects of changing process conditions. The effects of changing the air flow rate are graphically represented in Figs 4 and 5. The initial rates of extraction are higher for the higher flow rates but the values falling with increased time as the protein in the bed was depleted. Greater turbulence of the bed was produced at the higher flows which resulted in higher total protein percentage extractions (Table 2). The percentage recovered of the extracted protein dropped from 30.7 to 27.9%, with increase in air flow rates. The overall separation of the shell and mussel flesh, usually in excess of 90%, was considered satisfactory. The material left in the bed was the strands of fibrous material which was found to be difficult to extract. The percentage recovery of protein was low at 30%, indicating that a high proportion of the protein was still in a soluble form. Coagulation of the proteins was attempted by pre-heating the mixture of water and milled mussels to 70°C and maintained at that temperature for 30 min. The pre-heated bed was then added to the flotation column for extraction. Only a marginal improvement is obtained (Figs 6 and 7). The

Table 2. Extraction and recovery efficiencies

Solvent	Flow rate (m^3/hr)	pH	Frothing agent	Precipitant	Flocculent	Extraction (%)	Recovery (%)
H_2O	2	7	Lissapol	—	—	89.7	30.7
H_2O	4	7	Lissapol	—	—	94.6	27.9
H_2O*	2	7	Lissapol	—	—	80.9	35.6
H_2O*	4	7	Lissapol	—	—	93.6	30.0
H_2O	2	3	Lissapol	—	Difco	76.7	94.0
H_2O	4	3	Lissapol	—	Difco	79.9	95.3
5% w/w NaCl	2	7	Lissapol	—	—	90.7	47.0
5% w/w NaCl	4	6	Lissapol	—	—	97.0	75.1
H_2O	2	3	Lissapol	L.S.A.	—	96.5	19.8

* Solvent and ground mussel bed pre-heated to 70°C.

lower extraction efficiency obtained at the low air flow rate, 80.9% (Table 2) would suggest a greater difficulty of removing the more coagulated material from the bed, but when greater turbulence occurs (4 m^3/hr) no such difficulty is exhibited. The slight increase in percentage recovery obtained would not seem to warrant the added energy costs in heat pre-treatment.

The addition of the Difco flocculating agent produced a significant change. The solvent used in the series of experiments had a concentration of 10 ml Difco in 1000 ml solution and pH 3. The results clearly indicate the advantage

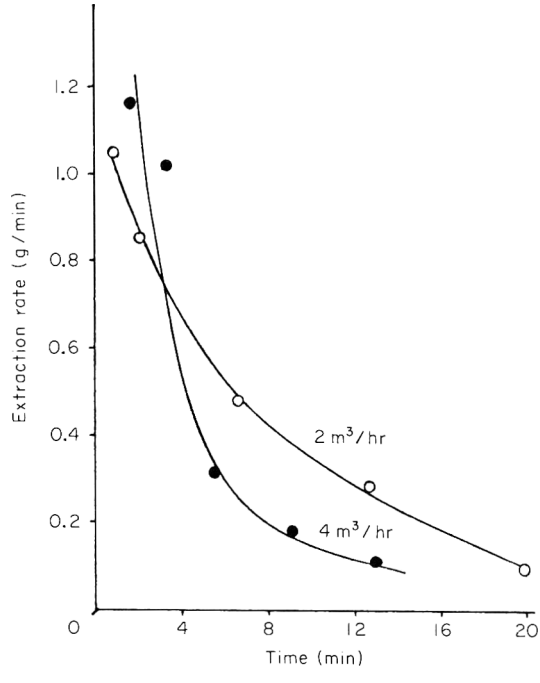

Figure 5. Extraction rates of protein using distilled water as solvent.

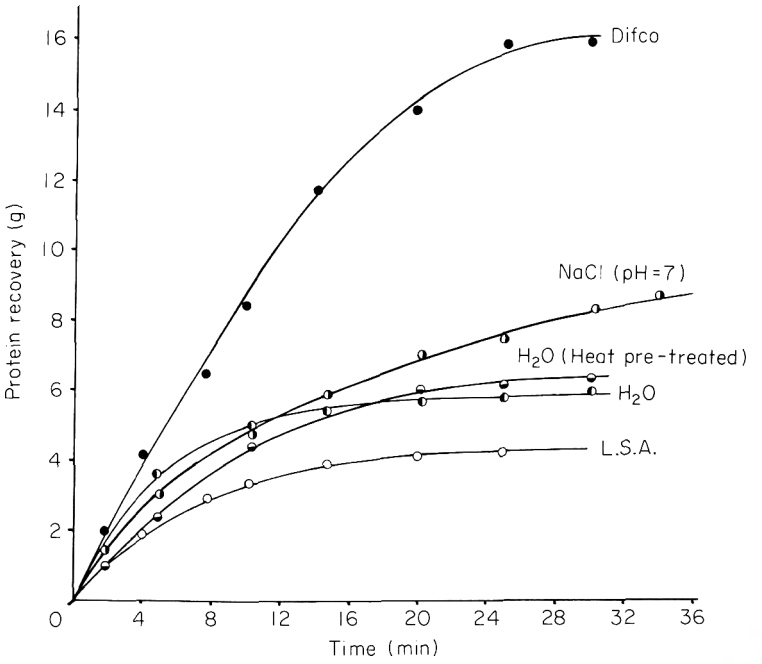

Figure 6. Cumulative protein recovery using various solvents, air flow rate=2 m^3/hr.

of the flocculating agent in the percentage product recovery of the extracted protein, efficiencies in excess of 94%. The percentage extractions are much lower than for the previous process conditions. Flocs are formed within the milled bed and may have a binding effect and also become entrapped within the shell bed cavities. The presence of the Al^{3+} and Fe^{3+} ions and change in pH to an acidic value (pH 3) will have produced a reduction in the *zeta* potentials facilitating coagulation of the proteins. An additional mechanism may be the hydrolysing effect of salts of the polyvalent cations Fe^{3+} and Al^{3+}. The rate of coagulation is enhanced by the presence of hydrolysis products of the salts. The complex ions formed may be regarded as an ion exchange medium capable of adsorbing anionic groups in the proteins. The ability of acid hydrolysing metal salts to act as flocculating agents has been utilized, on an industrial scale, for the recovery of proteins and also fats from colloidal systems (Khlyn, 1979, Hopwood, 1975).

The tests using simulated sea water conditions (5% w/w NaCl) and neutral pH produced only small improvements as compared to the basic system of distilled water with no flocculant. The slight increase in percentage recovery (30.7–47.0%) may be due to the salting out effect. The recovery was still considered too small and, because of more favourable avenues using flocculants, was not pursued. Although work at Aktiebolag Perac in Belgium (1965) has shown the successful application of precipitation with a neutral salt (NaCl) as part of a more complex process. The adjustment of the NaCl solution to pH 6 improved the percentage recovery but it was still less than that obtained using an acidic flocculant.

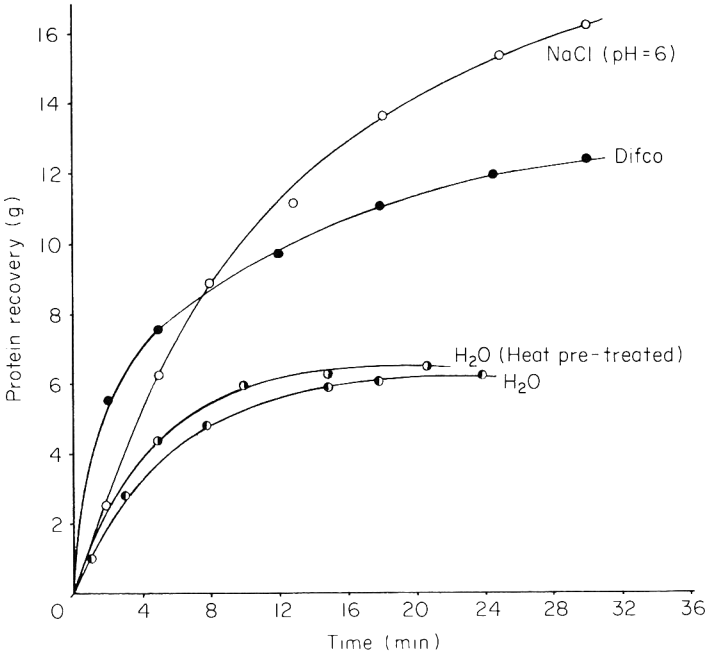

Figure 7. Cumulative protein recovery using various solvents, air flow rate=4 m^3/hr.

Much literature (Jørgensen, 1968, 1971; Tønseth & Berridge, 1968) has been published on the precipitation of protein using lignin sulphonic acid, a byproduct from the sulphite wood pulping industry. The precipitation of soluble protein with soluble lignin sulphonic acid in an acidic aqueous system is believed to be a nearly instantaneous reaction involving the negatively charged lignin sulphonate groups and the positively charged amino groups on the protein molecules. The complexing of these large molecules results in a gelatinous suspended material. The pH of the solutions must be below the isoelectric point of the proteins to ensure a net positive charge on the proteins. Acidification to pH 3.5 or less usually ensures this condition. The cumulative protein extraction, Fig. 6, and the percentage recovery were very low using this precipitant. The major reason for these low values is that the density of the complexes formed is very similar to that of water making centrifugation of the product very inefficient. To improve product recovery other physical separation techniques would have to be employed.

Conclusion

The batch studies, on froth flotation as a means of protein extraction from mussels, have shown that the technique is feasible. The results have shown the importance of the pH of the system and the use of flocculating agents on product recovery. The increase in world demand for protein and the

encouraging feeding trials using mussel protein would indicate a need for commercial development of the investigated extraction system.

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Isolation and characterization of branched chain fatty acids (other than those derived from phytol) in cod liver oil

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Summary

A group of mono- and di-unsaturated branched chain fatty acids were isolated and characterized by GC-MS. A GC-MS analysis of the perhydroderivatives of these acids revealed the presence of the following acids: 5-methyltetradecanoic, 5,7-dimethyltridecanoic, 7-methylhexadecanoic, 5,7-dimethyltetradecanoic, 7,9-dimethylhexadecanoic, 9-methyloctadecanoic, 7,9- and 9,11-dimethyloctadecanoic acids. Of these, only the 7,9-dimethylhexadecanoic acid was fully characterized in its unhydrogenated form as 7,9-dimethylhexadecadienoate (Δ 6,8). The presence of the branched methyl groups on alternate *odd* carbon atoms is uncommon.

Introduction

Cod, *Gadus morhua*, and herring, *Clupea harengus*, are among the greatest weight of fish landed in the world. European production of fish liver oil however is on the decline but the major part of the production is cod liver and halibut liver oils. Cod liver oil was produced and sold mainly for its content of the fat soluble vitamins A and D. Cod liver oil now attracts new interest on account of its content of polyunsaturated fatty acids (PUFA) believed to be important in human nutrition, utilized mainly as a source of essential fatty acids. The branched chain fatty acids present in cod liver oil contribute to the lower melting point of the oil. When esterified to cholesterol, branched chain fatty acids are capable of stimulating protein synthesis. It has been shown that the branched chain ester influences some ribosomal functions which are necessary for peptide elongation (Hradec, Dusek & Mack, 1974). Children in most developing countries are regularly given cod liver oil, presumably due to

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the above reason. Branched chain fatty acids have been identified as growth factors in certain species of rumen bacteria (Allison *et al.*, 1962).

Cod liver oil maintains dermal integrity and its essential fatty acid and possibly, the branched chain ones ensures proper utilization of saturated fat. The PUFA and branched chain acids play a major role in the lowering of cholesterol levels, provide energy and form an integral part of biomembranes. The production of margarines from the hydrogenated oil is an important industrial aspect of the fish oil industry; and in this regard, branched chain acids because of their high temperature stability play an important role in the finished product.

The discovery and characterization of an unsaturated branched chain fatty acid in fish lipids was first reported by Sano (1967), who isolated 7-methylhexadec-6-enoic acid from whale oil. Ackman *et al.* (1973), found the same acid to be a minor component in the lipids of marine animals and also identified 7-methylhexadec-7-enoic and 5-methyl-tetradec-4-enoic acids in the sperm whale (Pascal & Ackman 1975). Pearce & Stillway (1976), isolated and identified 7-methylhexadec-7-enoic acid from the spadefish liver oil.

The occurrence of these unusual fatty acids is not confined to aquatic sources. Gerson, Patel & Nixon (1975) recognized 11-methyloctadec-11-enoic acid among other branched chain acids from the lipid of rhizobium cultured under controlled conditions. The origin of these unusual acids in aquatic animals has been suggested to be dietary (Ackman *et al.*, 1973), while Karlsson, Leffler & Samuelsson (1979) felt the origin may arise from an unusual diene sphingosine derivative by metabolic conversion.

The presence of these unusual acids in many aquatic sources prompted the search for similar acids in cod liver oil.

Materials and methods

Samples

The cod liver oil used in this study was supplied by the Marfleet Refining Company and this was supplemented by purchase of cod liver oil containing high polyunsaturated fatty acids from a local pharmacist. The two samples were co-mingled prior to the analytical procedure.

Transesterification

Cod liver oil (1400 g) dissolved in benzene (1500 ml) was refluxed for 3 hr with methanolic sodium methoxide solution (0.5%, 2 litres). After addition of acetic acid (200 ml) and brine (1.5 litres), the esters were extracted with distilled petroleum ether (b.p. 40–60°C, 2×1 litre), and the petrol extracts were washed first with brine (1×2 litres) and then with distilled water (2×1.5 litres). Removal of solvent furnished cod liver methyl esters (1450 g).

Urea fractionation

Cod liver methyl esters (1450 g) were crystallized at 0°C from methanol (3.6 litres) containing urea (2900 g). The esters (266 g) remaining in the liquor, treated in the same way (urea, 1330 g; methanol, 1330 g) gave a second liquor which contained 111 g esters.

Silver ion column chromatography

A column of silica (52×9.5 cm) containing 30% silver nitrate wrapped in aluminium foil to exclude light, was equilibrated with PE5 prior to addition of the second mother liquor (100 g out of the 111 g esters obtained). Abbreviations such as PE5 indicate mixtures of petroleum and diethyl ether in a volume ratio of 95:5. Material was eluted with PE5 1.5 litres of eluent and yielded 3.0 g of ester from AgccF1 (silver ion column chromatography fraction 1), followed by PE10 1.5 litres of eluent which yielded 10.5 g ester from AgccF2 (silver ion column chromatography fraction 2) and ether 1.5 litres which yielded 85.2 g esters.

Column chromatography (silica)

Sorbsil silica gel (100 g) packed into a glass column (105×120 cm) was equilibrated with distilled petroleum ether (b.p. 40–60°C). AgccF2, (2.5 g) dissolved in petroleum ether was applied to the column and eluted with 100 ml portions of PE1, PE2, PE5, PE10, PE20 and PE50, followed by ether. The progress of elution was monitored by thin layer chromatography (t.l.c.) (silica) using phosphomolybdic acid as the detecting agent. Methyl esters were eluted with PE5 and PE10 after non-ester impurities had been eluted with PE1 and PE2.

Thin layer chromatography (t.l.c.)

Analytical t.l.c. was carried out on glass plates (20×20 cm) coated with a layer of silica gel G (0.25 mm wet thickness) or with silica gel G containing 10% silver nitrate. Preparative separations were carried out on glass plates (20 × 20 cm) coated with silica gel G (0.5 or 1 mm wet thickness) containing 20% silver nitrate. Development of plates were carried out in PE7.5.

Components separated on analytical plates were made visible by spraying with an ethanolic solution of phosphomolybdic acid (10%, v/v) followed by heating at 110–120°C for 10 min. Preparative t.l.c. plates were sprayed with an ethanolic solution of 2′,7′-dichlorofluorescein (0.2%, v/v) and viewed under u.v. light.

Hydrogenation

The unsaturated ester (5–10 mg) was dissolved in methanol (2–3 ml) in a round bottomed flask (25 ml) and palladium on charcoal (10%, 1–2 mg) and a small magnetic paddle were added. The flask fitted with a two-way tap, was alternatively evacuated and flushed with hydrogen several times to remove air, and finally opened to the hydrogen atmosphere.

The reaction mixture was vigorously stirred for 1 hr at room temperature. The catalyst was removed by filtration and the residue washed with warm ether, and the material recovered by evaporation under nitrogen.

GC-MS analysis

Gas chromatography linked mass spectrometry was carried out using a 25 ml silar 5CP capillary column. Spectra were recorded at 70 eV electron impact ionization and linked to the computer data system.

Results and discussion

Isolation of unsaturated branched chain esters was carried out employing urea fractionation, silver ion column chromatography and argentation thin layer chromatography. Details of the isolation procedures are outlined in Scheme I. The mother liquor (AgccF2) was submitted to a series of silver ion t.l.c. separations to obtain the band B4.2.2 which contains some unsaturated branched chain esters and furanoid esters as shown in Table 1. Notable amongst these is a series of esters of unfamiliar ECL values which differ by one ECL unit. On a polar column (SP 2340) esters of ECL 18.8, 19.8 and 20.8 predominated over other branched chain esters. Attempts to free this band of the furanoid esters were unsuccessful.

General comments on the mass spectra of branched chain‹acids

In general a branched-chain ester undergoes cleavage on

$$CH_3(CH_2)_m-\underset{\underset{b}{}}{\overset{\overset{CH_3}{|}}{CH}}-(CH_2)_nCO_2Me$$

$a,\ a+1,\ a+2$

either side of the branched chain carbon atom to give fragments *a* and *b*. With a methyl branch, these differ by 28 atomic mass units (a.m.u.) and the peaks at *a* and *b* are much larger than the peak at $a+14$ which would be obtained in the absence of branching at that point. In addition, fragment *a* is accompanied

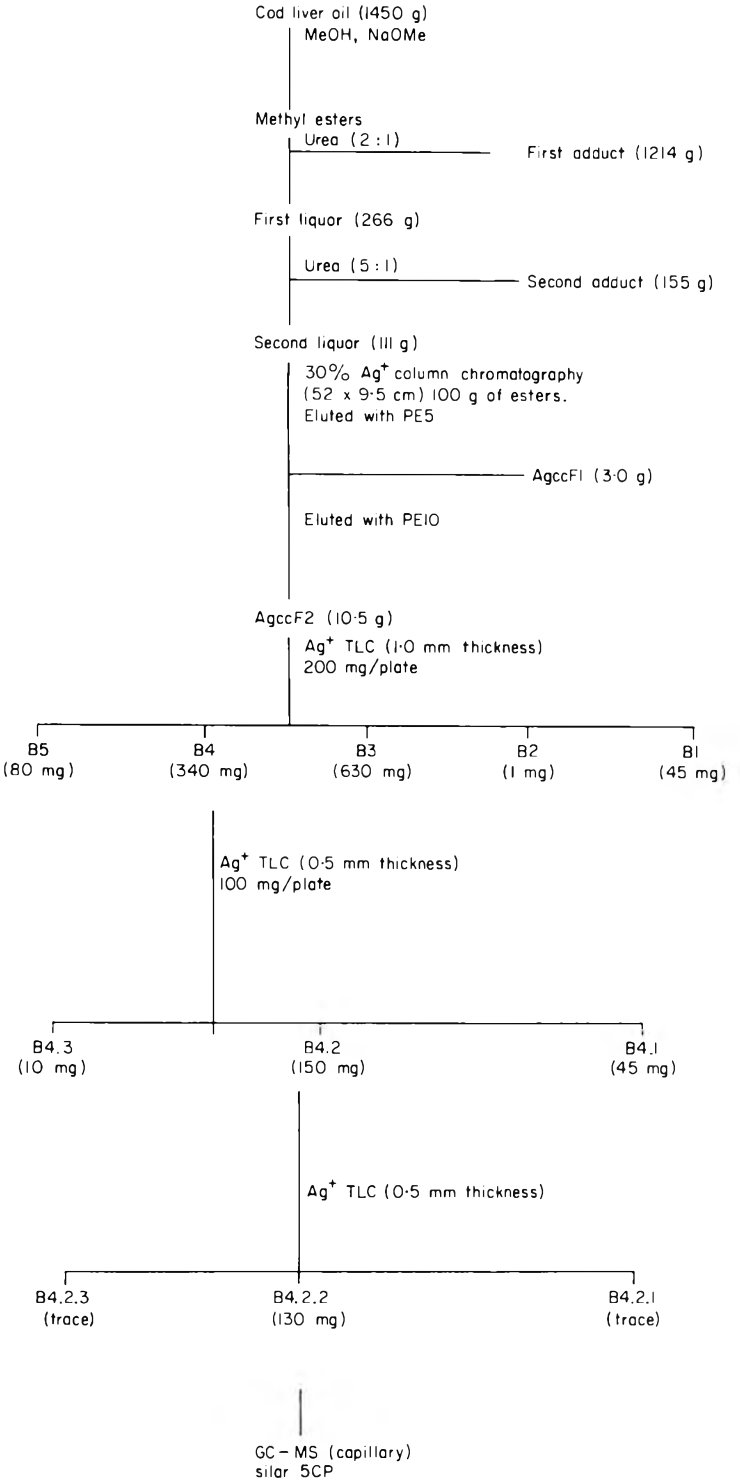

Figure 1. Scheme I: flow diagram for the isolation of branched chain fatty acids.

Table 1. Component acids (% wt) of the concentrate (B4.2 and B4.2.2) and perhydro derivatives of B4.2.2 (SP2340, 185°)

Assignment	B4.2	B4.2.2	B4.2 (perhydro derivative)
12 : 0	—	trace	trace
P16	2.7	1.9	4.3
P17	—	0.5	—
C15 br.	0.4	0.8	—
P19	0.7	—	0.2
16 : 0 br.	—	1.0	28.2
C16 br.	0.9	1.6	—
16 : 1/C16 unsat. br.	1.5	3.6	—
P20/17 : 0 br.	4.7	—	—
C17 br.	0.9	0.4	—
C18 br.	—	—	2.9
18 : 2 br.	23.1	22.2	—
C19 br.	—	—	0.8
19 : 2 br.	3.0	1.4	—
20 : 0 br.	1.5	0.5	3.5
20 : 2 br.	11.7	4.5	—
C20 unsat. br.	—	—	7.3
F1	—	—	1.9
F2	23.3	21.6	22.7
F3	1.6	2.7	6.0
F4	12.4	13.4	15.7
F5	6.9	7.0	0.6
F6	4.7	15.1	5.8
F7?	—	1.1	—
F8?	—	0.7	—
F9?	trace	trace	—

by smaller peaks at $a+1$ and $a+2$, and fragments a and b, may each lose methanol and water sequentially.

A different kind of fragmentation produces peaks at M-29 through loss of the C-2 and C-3 methylene groups and hydrogen, and at M-43 through loss of the C-2 to C-4 methylene groups and hydrogen. These figures will be modified if any of the methylene groups in these positions carry a branched methyl group. A significant peak is also expected at m/e 74 due to McLafferty re-arrangement. This will have a higher m/e value if the ester is substituted at C-2.

GC-MS of hydrogenated concentrate (B4.2.2)

A GC-MS study of the concentrate (B4.2.2.) after hydrogenation was carried out using a 25 m silar 5CP capillary column linked to the mass spectrometer. The study provided spectra of sixteen components. The first

seven were found to be mono- or di-methylalkanoates and the remainder proved to be hydrogenated furan esters. Of the seven spectra relating to the open-chain compounds, the largest peaks corresponded to methyl esters of 7-methylhexadecanoic and 7,9-dimethylhexadecanoic acids. These were preceded by methyl 4,8,12-trimethyltridecanoate, 5-methyl tetradecanoate, 5,7-dimethyltridecanoate and 5,7-dimethyltetradecanoate and followed by methyl 9-methyloctadecanoate, 7,9-dimethyloctadecanoate and 9,11-dimethyloctadecanoate.

Mass spectrum 1

This shows a major molecular ion at m/e 256 and smaller signal at m/e 270. The spectrum relates mainly to methyl 5-methyltetradecanoate along with 5,7-dimethyltridecanoate. The structure of the major component is based on the molecular ion (m/e 256), the base peak at m/e 74 and significant ions of m/e 241 (M-15), 227 (M-29), 213 (M-43), 206 (M-50) and 180 (M-76).

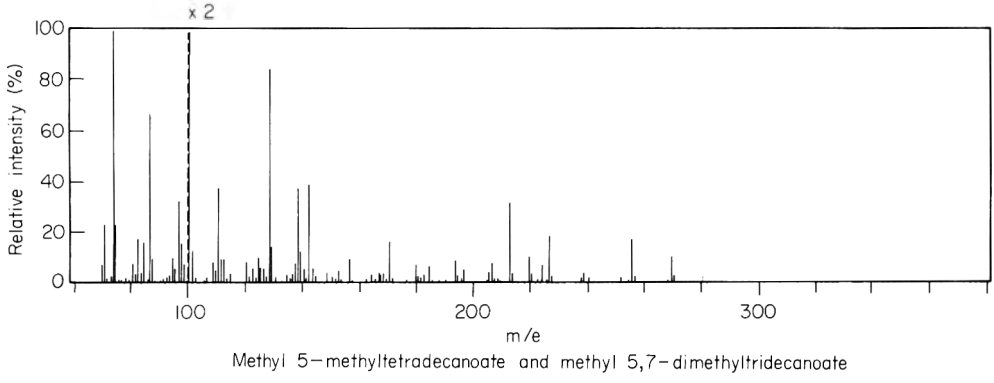

Figure 2. Mass spectrum 1.

Fragment ions of m/e 101 and 129 (which loses 32 a.m.u. to give an ion at m/e 97 indicate the presence of a methyl side chain at C-5.

Mass spectrum 2

This spectrum shows a molecular ion at m/e 270 and a minor signal at m/e 256. The spectrum relates mainly to methyl 5,7-dimethyltetradecanoate. The structure is based on the molecular ion m/e (270), the base peak at m/e 74, and significant ions at m/e 255 (M-15) 227 (M-43), 220 (M-50) and 194 (M-76). Fragment ions at m/e 101, 129, 143 and 171 each of which loses 32 atomic mass units to give ions at m/e 69, 97, 111 and 139 respectively, indicate the presence of methyl side chains at C-5 and C-7. This is confirmed by the ketene ions at m/e 121 and 139.

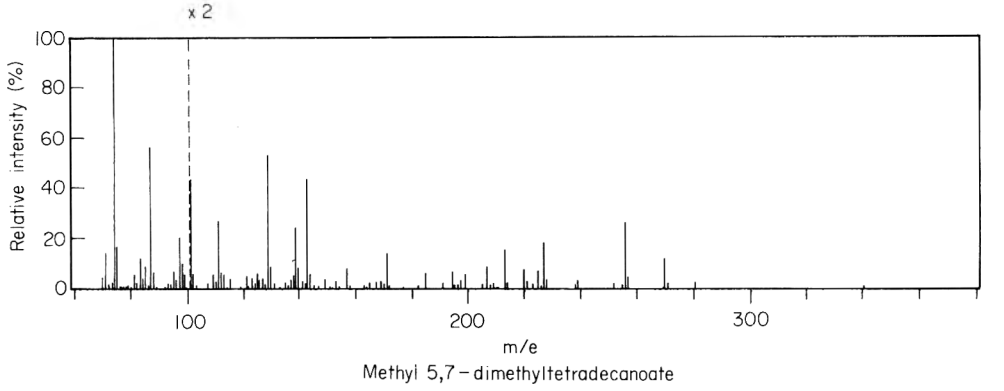

Figure 3. Mass spectrum 2.

Mass spectrum 3

This shows molecular ions at m/e 284 (major) and m/e 298 (minor). This spectrum is mainly 7-methylhexadecanoate based on the molecular ion (m/e 284), base peak (m/e 74) the second largest peak (m/e 87) and fragment ions of m/e 129 and 157 each of which loses 32 a.m.u. to give ions of m/e 97 and 125 respectively.

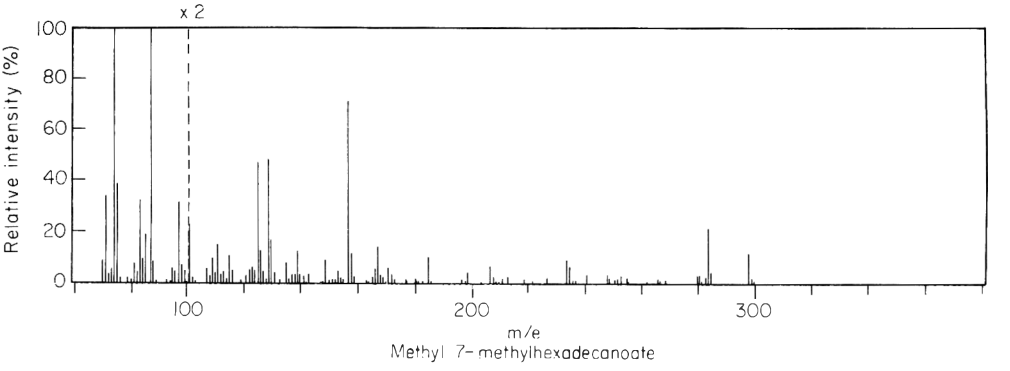

Figure 4. Mass spectrum 3.

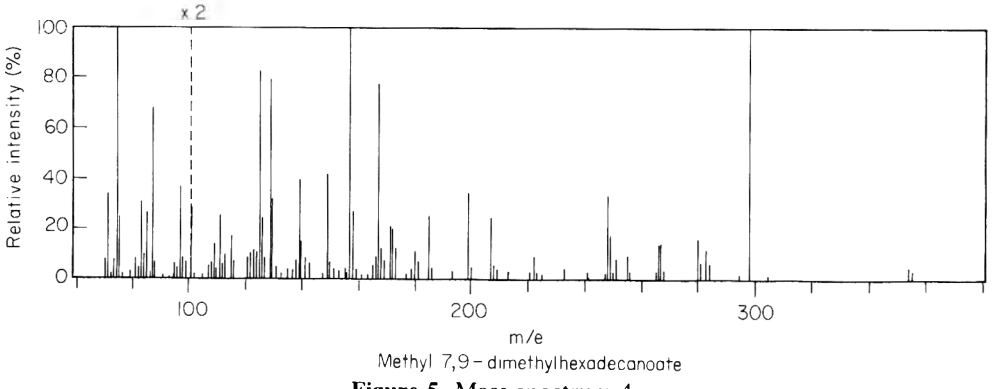

Figure 5. Mass spectrum 4.

Mass spectrum 4

This spectrum displayed a molecular ion at m/e 298. The fragment ions at m/e 129 and 157 each of which loses 32 atomic mass units to give ions at m/e 97 and 125 respectively indicate a methyl side chain at C-7. Other fragment ions at m/e 171 and 199 which in turn loses 32 a.m.u. to give ions at m/e 139 and 167 respectively, indicate a C-9 methyl side chain. The spectrum is that expected of a methyl 7,9-dimethylhexadecanoate.

Mass spectrum 5

The spectrum shows molecular ions of m/e 312 and 326 with the later being the major component. The minor component produces fragment ions which show it to be 9-methyloctadecanoate. The 9-methyl substituent is indicated by fragment ions at m/e 157, 158 and 159 and at m/e 185, 153 (185−32) and 135 (153−18). The major component (M^+ 326) with methyl side chains at C-7 and C-9 is confirmed by characteristic ketene ions at m/e 135 and 153 and is therefore a methyl 7,9-dimethyloctadecanoate.

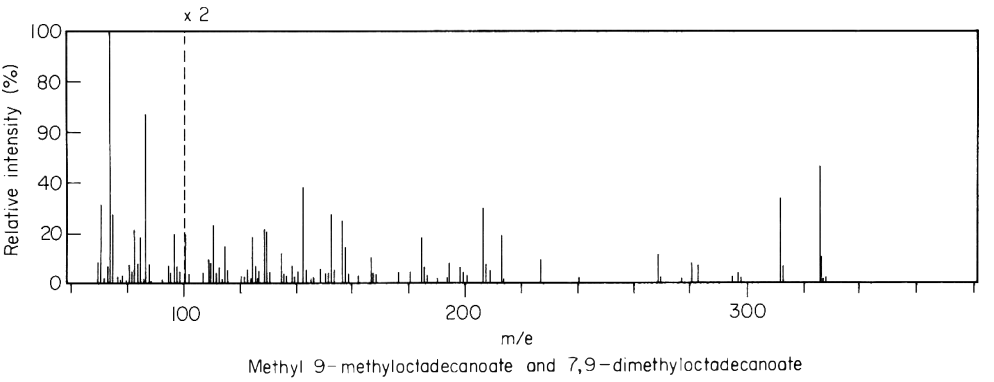

Figure 6. Mass spectrum 5.

Mass spectrum 6

This spectrum shows a molecular ion of m/e 326 and relates to methyl 9,11-dimethyloctadecanoate. The base peak occurred at m/e 74 and significant ions were observed at m/e 87, 101, 115, 129, 130 and 143. Characteristic fragments at m/e 157, 185, 199 and 227 each of which loses 32 a.m.u. to give ions of m/e 125, 153, 177 and 195 respectively, indicate the presence of methyl side chains at C-9 and C-11.

The separation of these methyl-substituted isomers on the 5CP capillary column has not been complete. In most cases the spectrum indicates the presence of two components with molecular weights differing by 14 a.m.u. suggesting that esters with one methyl substituent are eluted coincidently with those with two methyl substituents. However, the detailed g.l.c. behaviour of

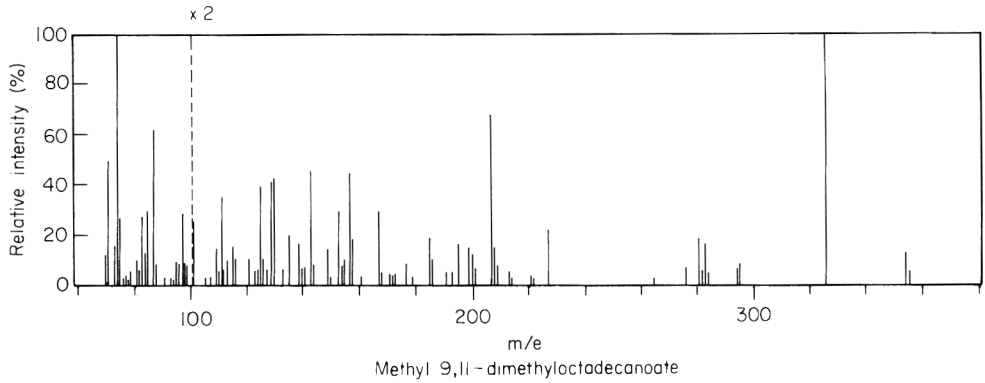

Figure 7. Mass spectrum 6.

monomethyl and dimethyl substituted esters on silar 5CP column is not known.

GC-MS of the concentrate (B4.2.2) before hydrogenation

As expected, it was not possible to identify the position of the double bond(s) in these esters because, the unsaturated centres are labile under electron bombardment (Hallgren, Rhyage & Stenhagen, 1959; Rohwedder, Mabronk & Selks, 1965; Grof, Rakoff & Holman, 1968). It is however possible to determine the degree of unsaturation from the molecular ion and to correlate this with the results obtained on the hydrogenated sample (Table 2).

Table 2. GC-MS results of the concentrate (B4.2.2) before and after hydrogenation

	Hydrogenated sample		Unhydrogenated sample	
Total no. of carbon atoms in the acid	Molecular ion (M^+)	Assignment	Molecular ion (M^+)	No. of double bonds
15	256	5-methyltetradecanoate	—	—
15	256	5,7-dimethyltridecanoate	254	1
16	270	5,7-dimethyltetradecanoate	254	1
17	284	7-methylhexadecanoate	268	1
18	298	7,9-dimethylhexadecanoate	266	2
19	312	9-methyloctadecanoate	282	1
20	326	7,9-dimethyloctadecanoate	280	2
	326	9,11-dimethyloctadecanoate	294	2

Of these branched chain esters those based on 7-methyl hexadecanoate and 7,9-dimethyl hexadecanoate are the major components. In a later study, the full characterization of the latter including the location of the double bonds was undertaken.

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A pulsed low resolution NMR study of water binding to powdered milk

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Summary

Pulsed low resolution NMR was used to study the hydration mechanism of powdered milk samples. The non-exponentiality of the spin-echo decay curves of water was exploited to determine the water mobility, as well as the relative abundance of bound and free water in the different samples. The analysis of the experimental curves was performed by assuming a chemical exchange between bound and free water.

Introduction

Water in foodstuffs is generally referred to as free and bound water. The term bound water (BW) refers to those water molecules which are adsorbed on a solid matrix and which show physical properties different from those of free or bulk water (e.g. lower freezing point).

The technique of nuclear magnetic resonance (NMR) has been used both for the quantitative determination of total water in foods (Miller & Kaslow, 1963; Conway, Conee & Smith, 1957; Hester & Quine, 1976; Brosio *et al.*, 1978), and for studying the amount and the properties of the bound water.

Wide line NMR studies have been based on two different techniques: the first is based on the different freezing point of free and bound water so that at 0°C the signal due to free water disappears and the residual signal can be related to the amount of bound water (Toledo, Steinberg & Nelson, 1968). The second technique takes into account the different relaxation times of free and bound water: at high power of radiofrequency, hydrogen nuclei from free water give a negligible signal and the measured signal is due to bound water (Shanbhang, Steinberg & Nelson, 1970; Mousseri *et al.*, 1974).

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The difference in the relaxation times between free and bound water has also been exploited in pulsed NMR studies for measuring the relative amount of the two types of water as well as for measuring their mobility. If the exchange rate between free and bound water is slow in respect to NMR times, the longitudinal and/or transverse magnetization decays do not show single phase behaviour. The analysis of the decay curves allows one to determine the amount of free and bound water (Leung *et al.*, 1976; Leung, Magnusson & Bruinsma, 1979). However if the exchange rate is fast the magnetization decay curves show a single exponential behaviour; in this case, the amount and the mobility of the different types of water can be derived from the dependence of the relaxation time values on the moisture content of the sample (Zimmerman & Brittin, 1957; Woessner & Zimmerman, 1963; Derbyshire & Duff, 1974; Ablett *et al.*, 1978). The results so far obtained show that the pulsed NMR technique is very suitable for determining the amount and the mobility of free and bound water.

In many cases, however, the results obtained by the pulsed technique do not coincide with wide line NMR and/or with calorimetric determinations. Assuming an exchange process between bound and free water a method to evaluate the experimental data that allows the resolution of these discrepancies has been reported (Di Nola & Brosio, 1982).

In this paper a pulsed NMR study on the hydration mechanism of powdered milk samples has been reported.

Materials and methods

Basic method

The transverse magnetization decay curve of water can be exploited for studying water binding to a solid matrix. A non-exponentiality of the spin-echo decay curve can be accounted for the presence of two types of water components according to:

$$A(2t)=A_0\,[a_b \exp(-\mu_b t)+a_f \exp(-\mu_f t)], \tag{1}$$

where the extrapolated values at the time $t=0$, a_b, a_f coincide with the relative abundance of bound and free water P_b and P_f respectively and μ_b, μ_f are the relaxation rate $1/T_{2b}$ and $1/T_{2f}$ of the two types of water.

Even in the case of chemical exchange between bound and free water (if the exchange rate is lower or near the relaxation rate) the spin-echo curve can be fitted by the sum of two exponentials according to Equation (1). In this case however the measured values of a_b, a_f, μ_b and μ_f do not correspond to the values of P_b, P_f, $1/T_{2b}$, and $1/T_{2f}$ respectively. The apparent (a_i, μ_i) and the real values (P_i, T_{2i}) are related according to:

$$P_b/T_{2b}+P_f/T_{2f}=a_b\mu_b+a_f\mu_f. \tag{2}$$

The problem, whenever the spin-echo curve do not show a single phase behaviour, is to ascertain whether the apparent quantities can be identified with the real ones. In many cases it is possible to solve the question: in fact if an exchange process is present, the apparent relaxation rates may vary with the moisture content of the sample and their values may differ from those of bound and free water.

Whenever a variation of one, or both, relaxation rates is observed and an exchange process is hypothesized the real quantities P_i may be obtained, provided that some conditions are met. Starting from Equation (2); with $P_b+P_f=1$, we obtain:

$$P_b=(a_b+\mu_b+a_f+\mu_f-1/T_{2f})\ (1/T_{2b}+1/T_{2f}) \tag{3}$$

In most cases the transverse relaxation time of free water T_{2f}, even if reduced because of macromolecular interactions, is at least one order of magnitude greater than T_{2b}, $1/\mu_b$ and $1/\mu_f$, so that it can be neglected in Equation (3), which becomes

$$P_b=T_{2b}(a_b\mu_b+a_f\mu_f). \tag{4}$$

Generally in water binding study progressive amounts of water are added to the dried material and for the first additions no free water component is detected.

The measured relaxation time of water in these samples can be then assumed as the relaxation time of bound water and the amount of bound water can be derived from Equation (4).

Experimental

The measurements were made by a pulsed low resolution NMR spectrometer Minispec P20 of Bruker Spectrospin, operating at 20 MHz for protons. The instrument is equipped with an analogue computer B-AC5 of the same company that allows measurements of the spin-echo amplitudes obtained at time $2t$ by a 90°/t/180° pulse sequence. The 90° and 180° pulses were empirically adjusted by varying their respective widths (about 9 μsec and 18 μsec on our instrument). The time delay t between the pulses was varied from 0.1 to 40 msec. Each reported value is the average of thirty measurements. The time delay between two consecutive measurements was 3 sec to allow the nuclear magnetization to return to its equilibrium value.

The samples were prepared by weighing 1 g powdered milk and by adding and thoroughly mixing the desired amount of water (from 0.1 to 2 ml) directly in the NMR tubes (10 mm o.d.).

The powdered milk samples were supplied by Sammontana, Industria Gelati Sammontana (Italy).

The probe temperature was 25±0.5°C.

Results and discussion

Figure 1 shows, as an example, the spin-echo decay curve of water in a sample obtained by adding 0.6 ml water to 1 g of powdered milk. As can be seen the experimental curve (top line in Fig. 1) shows a marked non-exponentiality. This can be accounted for by the presence of three well recognizable components that will be denoted as s (solid), b (bound water) and f (free water) with different relaxation times T_{2s}, T_{2b} and T_{2f}, respectively. The non-exponentiality of the spin-echo curve was resolved by subtracting from the experimental curve the slowly decaying Component f so that Curve b (Fig. 1) was obtained. Curve b once again does not show a single phase behaviour and Curve s can be finally obtained by subtracting from Curve b the slowly decaying part of the curve.

The analysis of the experimental curves previously described enables one to obtain the relaxation time value as well as the relative abundance of the three components.

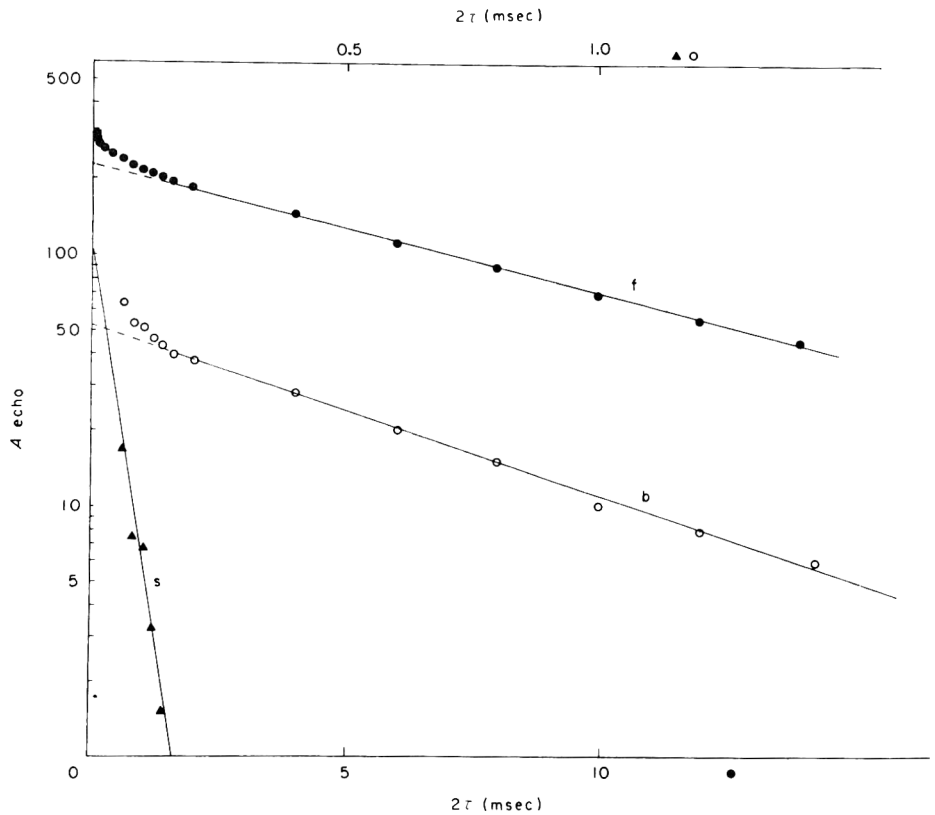

Figure 1. Curve f (●): spin-echo decay curve of water in a sample obtained by adding 0.6 ml water to 1 g powdered milk. Curve b (○) was obtained by subtracting from the experimental curve the slowly decaying part of the curve. Curve s (▲) was obtained in the same manner from Curve b by subtracting the slowly decaying component.

Table 1. Water additions and transverse relaxation times (msec) of the different components

Water H_2O				
ml/g DM	mmol/g DM	T_{2s}	T_{2b}	T_{2f}
0.10	5.5	0.034	0.646	—*
0.15	8.3	0.031	0.635	—*
0.25	13.9	0.036	0.576	2.80
0.30	16.6	0.034	0.675	3.24
0.40	22.2	0.033	0.676	4.43
0.50	27.7	0.033	0.635	6.11
0.60	33.3	0.035	0.644	8.19
0.70	38.8	0.038	0.630	9.23
0.80	44.4	0.036	0.603	11.10
0.90	50.0	0.043	0.592	11.40
1.20	66.6	—	1.230	16.94
1.35	75.0	—	1.690	22.97
1.80	100.0	—	1.870	30.30

* No free component has been observed.

In Table 1 the T_2 values of the three components of the different samples obtained by adding increasing amounts of water to 1 g powdered milk are summarized. From Table 1 it can be seen that the T_{2s} value is almost the same in all the examined samples. The average T_{2s} value of 0.035 msec is typical of solids so that the s component of the spin-echo curve can be assigned to the magnetization decay of the solid components in powdered milk.

Regarding Component b Table 1 shows that the order of magnitude of T_{2b} is about 1 msec—a characteristic value of relaxation time for bound water (Leung *et al.*, 1976). Table 1 also shows that up to water additions of 0.9 ml to 1 g of dry matter (DM), the T_{2b} value does not vary considerably—the average T_{2b} value is 0.630 msec. When the water additions are over 0.9 ml/g DM, the T_{2b} starts to increase indicating a weaker interaction between the solid matrix and bound water. The dependence of the T_{2b} value on the number of millimoles of water per gram of DM is reported in Fig. 2. The shape of the dependence of the T_{2b} value on the amount of water (Fig. 2) seems to indicate a transition in the bound water mobility, so that the bound water molecules measured at water concentrations over 50 mmol H_2O/g DM cannot be compared with those measured before this critical concentration.

Concerning Component f, Fig. 3 shows that the measured T_{2f} values increase with the water content of the samples showing the same break point at 50 mmol/g DM as T_{2b} in the dependence of T_{2f} on the amount of water added. This indicates that also the free water molecules experience, although in a less sensitive way, a different interaction with the solid matrix and/or a change in the solid phase structure.

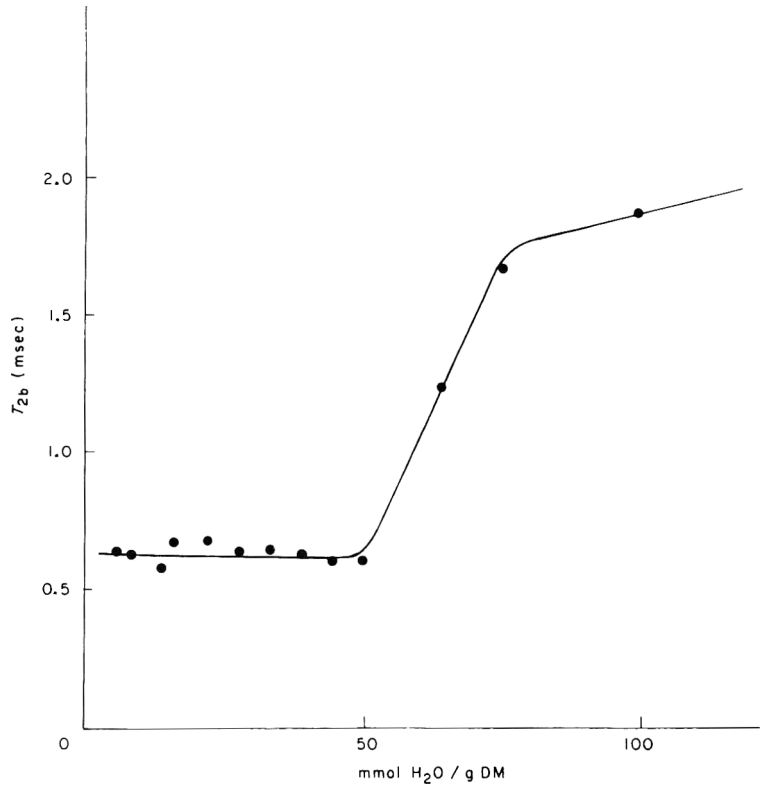

Figure 2. Dependence of bound water relaxation time T_{2b} on water additions (mmol) to 1 g powdered milk.

In order to calculate the relative amounts of bound and free water the 'apparent' concentration of the two types of water, a_b and a_f were first measured by extrapolating at time zero the slowly decaying part of Curves b and f of Fig. 1. Then the 'real' relative abundance of bound and free water, P_b and P_f, were calculated according to Equation (4) assuming a chemical exchange between the two types of water at a rate lower, or comparable to the relaxation rates. Finally, by combining the P_b and P_f values with the total amount of water in the samples the amounts in grams of bound and free water were derived. The results obtained are summarized in Table 2.

From Table 2 it can be seen that, by adding water to powdered milk samples, the amount of bound water (characterized by the relaxation time value T_{2b}=0.630 msec), increases up to the value of 0.20 g bound water/g dry matter (BW/DM). For water additions over 50 mmol H_2O/g DM, the amount of bound water continues to increase but the measured values cannot be compared with those measured before the transition point because the bound water mobility varies considerably indicating that the bound water molecules are quite different.

At this point, we would like to emphasize that it has little or no sense to speak in general terms of 'bound water', while a complete definition of 'bound

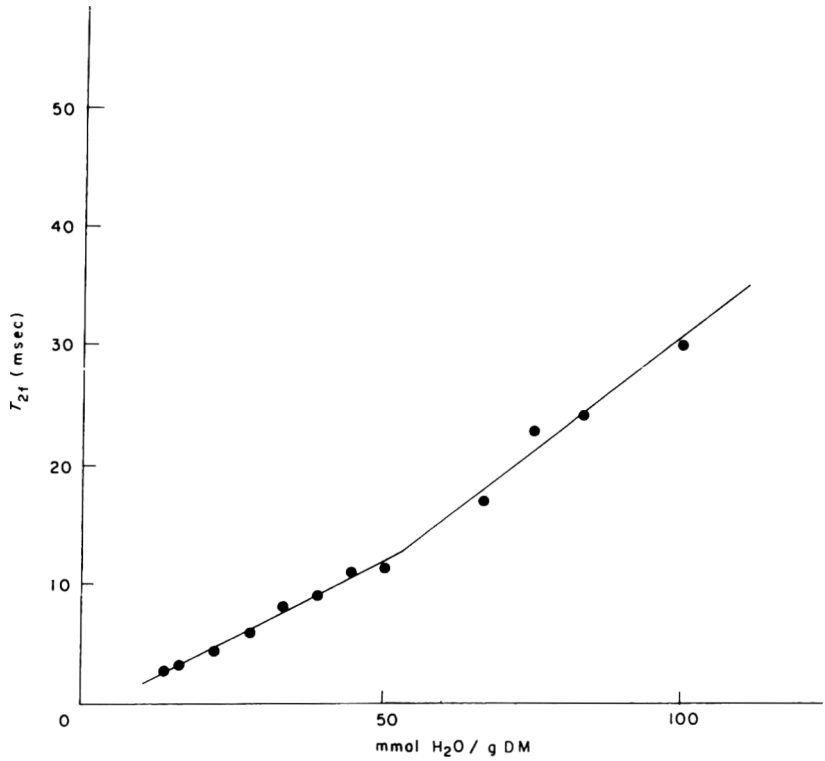

Figure 3. Dependence of free water relaxation time T_{2f} on water additions (mmol) to 1 g powdered milk.

water' should involve a transverse relaxation time value which is a true measurement of the actual water mobility.

Regarding the free water component, Table 2 shows that, by adding water to powdered milk the amount of free component increases considerably faster

Table 2. Apparent (a_i) and real (P_i) relative concentrations of bound and free water; grammes of bound (BW) and free water (FW) per gramme of DM in the different samples

Water H_2O (mmol/g DM)	a_b	a_f	P_b	P_f	BW/DM	FW/DM
13.9	29.5	70.5	44.0	56.0	0.11	0.14
16.6	19.3	80.7	36.1	63.9	0.11	0.19
22.2	18.9	81.1	31.3	68.7	0.13	0.27
27.7	26.5	73.5	34.1	65.9	0.17	0.33
33.3	18.1	81.9	24.6	75.4	0.15	0.45
38.8	18.4	81.6	24.0	76.0	0.17	0.53
44.4	15.2	84.8	19.8	80.2	0.16	0.64
50.0	18.6	81.4	22.8	77.2	0.20	0.70
66.6	13.3	86.7	19.6	80.4	0.23	0.97
75.0	10.4	89.6	17.0	83.0	0.23	1.12
100.0	12.4	87.6	17.0	83.0	0.30	1.50

than that of bound water. This indicates that, apart from the first additions, almost all the water added to the samples contributes to increase the amount of the free component. However we want to point out that the free water component measured in the examined powdered milk samples is quite different from bulk water that can be measured in an aqueous solution of the same ionic strength. In fact, due to the inter-molecular interactions between water and the solid matrix as well as to the chemical exchange between the two types of water, the mobility of the free water component in the examined samples is considerably reduced so that the relaxation time and the freezing point of this component is considerably lower than that of bulk water.

In conclusion, not only the bound water (whose abundance is relatively small), by mainly this 'free water' component has to be taken into account in describing the hydration mecahanisms of powdered milk.

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Sensory assessment of the shelf life of carbonated soft drinks

R. L. McBRIDE and K. C. RICHARDSON

Summary

Six commercial soft drinks, in glass bottles and aluminium cans, were stored at 0.6°C and ambient temperature and assessed by a consumer-type sensory panel every 3 months for 2 years. While there was evidence of deterioration with storage, more so at ambient temperature than at 0.6°C, all drinks were still considered satisfactory at the conclusion of the trial.

Introduction

In 1978, the State of New South Wales introduced mandatory date coding for those food and beverage products with an expected shelf life of less than 2 years. Although soft drinks were exempt from date coding requirements, there is still some uncertainty about their shelf life. Richardson (1976) had suggested that the shelf life of soft drinks may, in some instances, be less than 2 years; Tilley (1978) considered soft drinks to have a shelf life of up to 12 months; and Kieninger, Köberlein & Boeck (1979) found deterioration in the flavour of commercial cola drinks stored for 4 weeks at 40°C. The packaging of soft drinks in two-piece aluminium cans, instead of tinplate, has further complicated the issue, since shelf life can be limited by container breakdown as well as product degradation (Alderson, 1970).

Those previous studies which have included investigation of the shelf life of soft drinks have tended to focus on new product formulation (e.g. Lime & Cruse, 1972; Saeed & Ahmed, 1977), or microbiological aspects (Panezai, 1978; Jährig & Schade, 1979). The present study is specifically concerned with sensory quality.

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Materials and methods

Materials

Six carbonated soft drinks, typical of commercial production in Australia, were included in the study: cola, low calorie cola, lemon, low calorie lemon, lemonade, and orange. All drinks contained added flavour, and the lemon drinks contained 5% lemon juice. Both low calorie drinks were sweetened with a mixture of sodium saccharin and calcium cyclamate; the others were sweetened with cane sugar and varied from 10.5 to 12° Brix. The degree of carbonation (in volumes) was: cola, 3.5–4.0; low calorie cola, 3.5–4.0; lemon, 2.6; low calorie lemon, 2.6; lemonade, 3.7; and orange, 2.7.

Three soft drink manufacturers each supplied two products. All products were processed locally and delivered immediately to the Food Research Laboratory in brown cardboard cartons. Samples of each product came from the same production batch. Half the bulk quantity of each drink was packaged in 375-ml 'ring-pull' aluminium cans, the other half in 1 litre screw-cap glass bottles.

Methods

Some of the bottles and cans of each product were placed in a covered storage bay at ambient temperature, to simulate non-insulated warehouse conditions. One side of the storage bay was open to the air, but products were protected from direct sunlight. The remaining samples were stored in a constant temperature room at 0.6°C. Thus there were effectively twenty-four treatments: six drinks × two container types × two storage temperatures.

Each treatment was assessed by a sensory panel every 3 months over a 2 year period (nine assessments in all). There were twelve sessions at each 3-monthly assessment (two sessions a day on 6 days over a 2 week period), and two drinks were evaluated at each session. The order of evaluation of drinks over the twelve sessions was random, except that no two drinks of the same type (e.g. cola and low calorie cola) were presented at the same session. Twenty-four hours before each session, the appropriate drinks were removed from their respective storage conditions and kept at 5°C.

The sensory panel comprised men and women employees of the CSIRO Food Research Laboratory (age range 22–58 years). Composition of the panel varied between assessments, but most assessors had some previous experience in the sensory evaluation of food. The number of assessors at each session ranged from forty-one to seventy-two, with a median of fifty-one. At every session, assessors were each presented with a chilled (approximately 8°C) 100 ml sample of soft drink in a clear glass tumbler. They were required to drink the sample and to rate their overall impression on a 7-point hedonic scale: Extremely good (7), Very good (6), Good (5), Satisfactory (4), Poor (3), Very poor (2), and Extremely poor (1). Only the verbal descriptors were attached to the response scale.

On completion of this task the assessor returned the response scale and tumbler, and was then presented with another drink to evaluate (except at the first assessment where absence of the storage variable meant there were only twelve treatments). This 'single presentation' design more closely simulates normal consumer assessment than comparative evaluation (McBride & Richardson, 1979; McBride, 1980), and has been shown to be less prone to methodological bias (McBride, 1982). Assessors were not informed of the purpose of the test, nor were they given any information on the history of any sample. The sensory laboratory in which the testing was conducted has been described elsewhere (Christie, 1966).

Results

The 10300 responses were subjected to an analysis of variance using the GENSTAT statistical package on Cyber 76 computer. The results are summarized in Fig. 1.

There were significant differences between the hedonic scores for the six types of drink ($F=60.25$, d.f.$=1/10213$, $P<0.001$). Overall mean scores were: cola, 4.63; low calorie cola, 4.46; lemon, 4.80; low calorie lemon, 4.23; lemonade, 4.43; and orange, 4.30. The low calorie drinks were rated lower than their sugar-sweetened counterparts.

There was a significant drop in scores over the eight storage times ($F=13.63$, d.f.$=7/10213$, $P<0.001$). The overall mean score at 0 months was 4.80; at 24 months, 4.34.

The effect of storage temperature was significant ($F=85.51$, d.f.$=1/10213$, $P<0.001$). The mean score for drinks stored at 0.6°C was 4.56, compared with the lower value of 4.34 for those stored at ambient temperature.

Overall, the mean score for drinks stored in bottles (4.50) was marginally higher than for those in cans (4.45), ($F=6.03$, d.f.$=1/10213$, $P<0.05$). However, this container-type main effect was found to be due only to the lower scores for low calorie lemon in cans; for the other drinks the scores for bottles and cans were almost identical.

Interactions

There was a significant drink type × container type interaction ($F=12.88$, d.f.$=5/10213$, $P<0.001$) but, as for the container main effect, this was due entirely to the lower scores for low calorie lemon in cans.

The storage time × storage temperature interaction was also significant ($F=4.65$, d.f.$=7/10213$, $P<0.001$), indicating that the scores for samples stored at ambient temperature dropped more than those for samples stored at 0.6°C. This is evident in Fig. 1.

A significant storage temperature × drink type interaction ($F=4.54$,

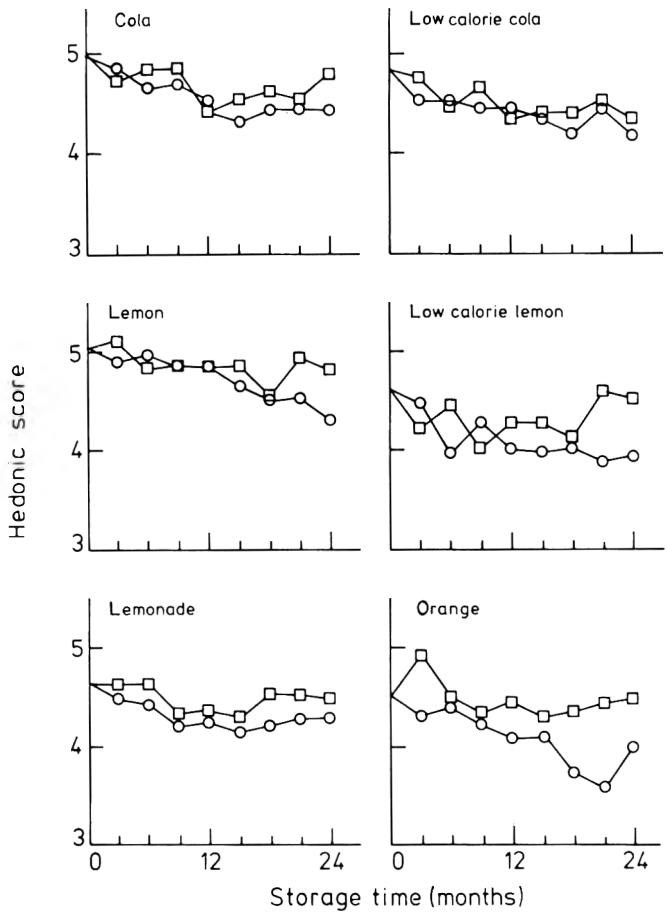

Figure 1. Mean hedonic scores for six types of carbonated soft drink (in glass bottles and aluminium cans) stored at either 0.6°C (□) or ambient temperature (○) and assessed every 3 months for 2 years. The scores 3, 4, and 5 on the hedonic scale denote 'Poor', 'Satisfactory' and 'Good' respectively.

d.f.=5/10213, $P<0.001$), implies that storage temperature affected the six drink types differentially. Inspection showed least difference between the scores for low calorie cola stored at 0.6°C and ambient temperature (4.46 and 4.37 respectively), and the largest discrepancy for orange (4.49 at 0.6°C, 4.07 at ambient).

A small but significant storage time × container type interaction ($F=2.47$, d.f.=7/10213, $P<0.05$) revealed a slight cross-over effect: scores for drinks in bottles were marginally higher at the first six assessments, but the trend reversed at the last three assessments.

There was no storage time × drink type interaction, suggesting all drinks deteriorated to the same extent during storage, nor was there any storage temperature × container type interaction.

Discussion

It is important in this experiment to distinguish between *statistical* and *practical* significance. Although most of the main effects and first-order interactions are highly significant statistically, it does not necessarily follow that they are highly significant in practice. For example, Fig. 1 shows that, irrespective of treatment condition, almost all scores lie between 4 (Satisfactory) and 5 (Good) on the hedonic scale: despite the deterioration with storage time, highly significant statistically, only five of the 102 scores in Fig. 1 lie below 'Satisfactory' on the hedonic scale. Figure 1 also shows that the highly significant storage temperature effect becomes noticeable only after 12 months' storage: in the first 12 months the pattern is not consistent.

The design of this study was such that assessors were not able to make direct comparisons between samples. McBride & Richardson (1979) and McBride (1980) argue that, in the sensory laboratory, this provides a more valid estimate of practical shelf life, since the only comparison possible is that which is also available to the consumer, i.e. a comparison against a remembered level of quality. The use of large panels of assessors, as in the present experiment, also helps to simulate consumer assessment.

The experimental design used here, with assessments at periodic intervals and with no reference standards, can always be criticized on the grounds that it is susceptible to 'panel drift'; that the drop in scores may reflect assessor satiety rather than a genuine deterioration in the product. On this point it is pertinent to note that participation in the sensory panel was entirely voluntary; that co-operation and motivation were good throughout; and that assessments were conducted only every 3 months. Besides, there can be considerable methodological problems when reference standards are employed (see Wolfe, 1979).

In conclusion, this experiment suggests that, provided they are protected from light, carbonated soft drinks packed in glass or aluminium containers have a practical shelf life of at least 2 years. Even those drinks stored at ambient temperature, and which experienced seasonal fluctuations of more than 30°C, were still considered satisfactory at the end of testing.

Acknowledgment

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Determination of molecular and free sulphur dioxide in foods by headspace gas chromatography

E. G. DAVIS, D. BARNETT and P. M. MOY

Summary

A study was made of the equilibria between SO_2 in packaged liquid foods and gaseous SO_2 in their headspace atmospheres as a basis for a new analytical method for the determination of molecular and free SO_2. Using a gas-chromatographic method developed previously to determine SO_2 at the 1×10^{-3} mg/l level in atmospheres saturated with water vapour, studies with aqueous solutions showed that headspace SO_2 was directly proportional to molecular SO_2 in accordance with Henry's law.

Using the Henry's law constant for the SO_2/H_2O system, determinations of molecular SO_2 were made on a white and a red wine and a fruit juice, each adjusted to five levels of pH to achieve different concentrations of molecular SO_2. The results observed by the headspace method showed good agreement with those determined by a reference analytical method with white wine and fruit juice, but with red wine the reference method gave values which were more than 45% higher. Free SO_2 was determined in a range of white wines by the headspace and reference methods, and the results showed high correlations.

The headspace method is rapid, precise and non-destructive. A major advantage is that, during the analysis, it does not change the complicated equilibria between SO_2 and its ionised species, hence complexes of SO_2 and food constituents are not disturbed.

Introduction

Sulphur dioxide (SO_2) is used as a preservative in a wide range of foods (Roberts & McWeeny, 1972; Green, 1976). Part of the added SO_2 combines with food constituents and part remains as free SO_2. For legislative purposes

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the amounts of total SO_2 are required, but in food science it is important to distinguish between the amounts present in the free and combined forms.

Free SO_2 comprises the various species described in the following equilibria which exist in aqueous solutions of SO_2:

$$SO_2\,(gas) + H_2O \rightleftharpoons SO_2 \cdot H_2O\,(solution) \quad (1)$$

$$SO_2 \cdot H_2O \overset{pK_1}{\rightleftharpoons} H^+ + HSO_3^- \quad (2)$$

$$HSO_3^- \overset{pK_2}{\rightleftharpoons} H^+ + SO_3^{=}. \quad (3)$$

In foods, however, the above species can react with a range of food constituents to form complexes, adducts, salts, and other reaction products. Some of these are equilibrium reactions and may be reversed by varying the pH, temperature, or a combination of both; the SO_2 released is then determined as combined SO_2. In other cases, some reactions are irreversible and the SO_2 involved cannot be determined by the analytical procedures commonly used.

An important function of SO_2 in foods is to inhibit the growth of spoilage organisms. Ingram (1948) was one of the first to show that microbial inhibition is related to free rather than to combined SO_2. More recently, Macris & Markakis (1974) and Carr, Davies & Sparkes (1976) showed that molecular SO_2, defined as $SO_2 \cdot H_2O$ (Equation (1)), is the active species.

Molecular SO_2 cannot, at present, be determined directly in foods, and it is necessary to rely on values calculated from measured pH and free SO_2, using published pK values for sulphurous acid. In aqueous solution, molecular SO_2 accounts for only 0.06% of the free SO_2 at pH 5.0 and 6.06% at pH 3.0; the major amount (>93%) being present as HSO_3^-. These calculations emphasize that pH markedly influences the amount of molecular SO_2 present, and that the concentration of this species is small relative to the overall amount of free SO_2 at pH levels which exist in foods.

Terraglio & Manganelli (1967) have shown that, over the gas concentration range 0.81×10^{-3} to 8.73×10^{-3} mg/l, the solubility of SO_2 in water follows the law of partial pressure (Henry's law) when related to the concentration of molecular SO_2 but not when based on the total amount of SO_2 dissolved. This finding has potential applications in food science since it introduces the possibility of determining the concentration of molecular SO_2 in food from measurement of the concentration of gaseous SO_2 in an atmosphere in equilibrium with the food.

The present work shows that a new procedure developed in this laboratory for determining headspace SO_2 (Barnett & Davis, 1983) may be used to measure both molecular and free SO_2 in liquid foods.

Materials and methods

All measurements were carried out at 25°C. Headspace analyses were made by the gas-chromatographic procedure described by Barnett & Davis (1983). The instrument was calibrated daily using a mix of SO_2 in nitrogen at 92% relative humidity (r.h.) from a permeation tube assembly.

Measurements of pH were made with a Type M28 Radiometer pH meter. The Rankine method (Rankine & Pocock, 1970) as modified by Fujita *et al.* (1979) was used as the reference method for the estimation of free SO_2.

Henry's constant (H) determination

A Quickfit test tube (50 ml) containing deionized distilled water (30 ml) and fitted with a Dreschel head was connected to the effluent line from a permeation tube assembly. A controlled concentration of SO_2 in nitrogen at 92% r.h. was then bubbled into the water and the concentrations of SO_2 in the gas stream were monitored from ports in the line before and after the sorption tube. To ensure equilibrium, the gas flow was continued for 24 hr after the two concentrations of SO_2 in the stream were found to be the same. Determinations of free SO_2 were then made in duplicate on aliquots (10 ml) of the solution, and the concentrations of molecular SO_2 were calculated by the procedure described by Terraglio & Manganelli (1967). Seventeen determinations were made at gas concentrations ranging between 8×10^{-3} and 100×10^{-3} mg/l, and the data were analysed by linear regression to estimate the value of H.

Adjusted pH trials

Bulk quantities (2 litres) of orange juice and a white (moselle) and a red (claret) wine were each divided into 5×400 ml lots and filled into pouches made from a laminate of PVDC/nylon/polyethylene to which silicone rubber sampling septa had been fitted. The samples of each product were adjusted to five pH levels (Table 1) by the addition of NaOH (25% w/v) or H_3PO_4 (25% v/v). The pouches were then sealed and a headspace of nitrogen (100 ml) injected through the sampling ports. After an equilibration period of 30 min, the samples were analysed for free and headspace SO_2, and molecular SO_2 was calculated from these data.

Free SO_2 in wine

Five batches of white wine packaged in winecasks (4.5 litres) were obtained from two wineries; moselle, riesling and sauterne from Winery A and moselle and riesling from Winery B. The Fattori valve caps on the casks were replaced with caps containing two holes (3 mm) which had been filled with polydimethyl siloxane to act as septa for sampling the headspace. The air from

Table 1. Molecular SO_2 levels determined in three products, each adjusted to five pH levels, by headspace and modified Rankine methods

Product	pH	Molecular SO_2 (mg/l)	
		Headspace method	Rankine method
Orange juice	2.95	0.84	0.80
	3.14	0.57	0.56
	3.48	0.23	0.26
	3.76	0.13	0.14
	3.94	0.09	0.09
White wine (moselle)	3.06	1.84	1.83
	3.38	1.05	0.95
	3.66	0.48	0.49
	3.97	0.25	0.23
	4.27	0.12	0.12
Red wine (claret)	2.97	1.76	2.55
	3.31	0.79	1.26
	3.59	0.52	0.73
	3.91	0.23	0.33
	4.21	0.11	0.17

each cask was removed and nitrogen (150 ml) injected into the headspace using a syringe needle inserted through the sampling port.

Samples for headspace analyses were taken from the casks lying with their valves facing upwards, then the casks were stood upright for withdrawal of wine samples through the valve for analyses of free SO_2. Examinations were continued on three to four casks of each type of wine over a period of about 6 months to obtain a range of concentrations of free SO_2 down to approximately 2 mg/l.

Calculations

Estimations of the concentrations of molecular SO_2 based on the levels of free SO_2 were calculated from the equilibria given in Equations (2) and (3). Considering Equation (2) and ignoring Equation (3), the distribution of the two species involved at specific pH may be expressed by the Henderson–Hasselbalch equation

$$\mathrm{pH} = \mathrm{pK} + \log \frac{[\mathrm{base}]}{[\mathrm{acid}]}, \tag{4}$$

where HSO_3^- is the base and $SO_2{\cdot}H_2O$ is the acid. Taking antilogs and re-arranging, Equation (4) reduces to

$$F = \frac{1}{[10^{(\mathrm{pH}-\mathrm{pK}_1)}+1]}, \tag{5}$$

where F is the fraction of free SO_2 present in the molecular form. Products in which SO_2 is used as a preservative have pH values less than 4.5. At these levels, using values of $pK_1=1.81$ and $pK_2=6.91$ for sulphurous acid (Weast 1975), the contribution of pK_2 for Equation (3) is negligible, hence it has been ignored in the present work. Thus molecular SO_2 was calculated from the relation

$$\text{Mol } [SO_2] = \text{Free } [SO_2] \times F \tag{6}$$

using $pK_1=1.81$.

The headspace method for the determination of molecular SO_2 is based on the work of Terraglio & Manganelli (1967) who showed that Henry's law applies to the solution of SO_2 in water (Equation (1)) so that

$$\text{Mol } [SO_2] = \text{gas } [SO_2] \times H, \tag{7}$$

where H is the Henry's law constant. If the concentrations of both molecular and gaseous SO_2 are expressed as mg/l, H is dimensionless. In the present work, therefore, concentrations of molecular SO_2 were determined by means of Equation (7) from measured concentrations of headspace SO_2 and a value of 27.5 for H. Free SO_2 levels were then calculated from these data using Equation (6).

Results

Regression analysis of the Henry's law data showed a significant linear relation between gas concentration and molecular SO_2 in solution ($r=0.99$, 33 d.f.). The value calculated for H was 27.5 (s.e.$=\pm0.29$, 33 d.f.).

Table 1 shows a comparison of the concentrations of molecular SO_2 determined by the headspace method and those calculated from the levels of free SO_2 determined by the modified Rankine method. With each product, the concentrations observed by the headspace method decreased with increases in pH in a manner which was consistent with the calculated values. At each pH, the headspace values were similar to the calculated results with orange juice and white wine, but with red wine the calculated values were, on average, over 45% higher.

For each of the five batches of white wine, linear regressions were calculated on the free SO_2 data determined by the headspace method against those determined by the modified Rankine method. The data from all batches were then combined and examined similarly. Highly significant correlations ($r\geq0.982$ on at least 7 d.f.) between the two methods on each batch of wine and on the pooled data for all batches were found (Table 2). The slopes of the five regression lines were tested for homogeneity and the differences were not significant ($F=0.50$ on 4 and 64 d.f.).

Table 2. Coefficients for the regression equation $Y=a+bx$, where Y is the free SO_2 determined by the headspace method and x is the free SO_2 determined by the modified Rankine method on five batches of white wine

Wine	Manufacturer	a	b	Correlation coefficient (r)	d.f.
Moselle	A	−1.220	0.970	0.985	18
Riesling	A	−0.763	1.016	0.989	16
Sauterne	A	−1.357	0.940	0.990	11
Moselle	B	−1.057	0.964	0.982	7
Riesling	B	−1.594	1.001	0.984	12
Combined data		−1.431	1.002	0.986	64

Discussion

There are difficulties associated with existing methods for the determination of SO_2 in foods. This is reflected by the large number of published methods which have been reviewed by Carswell (B.F.M.I.R.A., 1977) who concluded that as recently as 1977 that no single method was available for the determination of the true level of SO_2 in all types of foods. Since that time, work has continued on modifications to existing methods and on the development of a new method based on glc using a flame photometric detector (Hamano *et al.*, 1979). Mitsuhashi *et al.* (1979) claim that the gas liquid chromatographic method and the Rankine method as modified by Fujita *et al.* (1979) are reliable procedures for the determination of free and combined SO_2 in foods. However, with all these methods there are uncertainties which arise from two major sources; interference by other constituents in the food and disturbance of the complicated equilibria between free and combined SO_2 during the analyses.

The headspace method offers a new approach for the determination of molecular and free SO_2 since it is based on the direct relation between gaseous and molecular SO_2 as defined by Henry's law. Since the concentrations of headspace SO_2 associated with foods are generally higher than the range studied by Terraglio & Manganelli (1967), their work was extended using our method to cover the concentration range 8.2×10^{-3} to 100×10^{-3} mg/l. The results show that Henry's law is applicable over the extended gas concentration range, and our observed value for H of 27.5 agrees, within experimental error, with Terraglio & Manganelli's (1967) value of 29.1, recalculated using $pK_1=1.81$.

As pointed out by Macris & Markakis (1974), the proportion of free SO_2 present as the molecular species in aqueous solution is strongly dependent on pH. This effect has been used empirically in food science and it is well recognized that the effectiveness of the preservative is enhanced by decreases in pH. The results set out in Table 1 show that the relations between pH and

molecular SO_2 determined in three products by the headspace method were consistent with the theoretically expected relations.

With white wine and orange juice, concentrations of molecular SO_2 determined by the headspace method agreed closely with those calculated from the levels of free SO_2. The large differences observed between the concentrations of molecular SO_2 in red wine at each pH by the two methods suggest that the anthocyanins present in red wine were responsible. Anthocyanins are known to complex strongly with SO_2 at food pH levels (Timberlake & Bridle, 1967; Somers & Evans, 1977; Brouillard & El Hage Chahine, 1980). With methods such as the modified Rankine method, which depend upon a pH reduction of the sample to around 1.0, these complexes are almost completely destroyed (Timberlake & Bridle, 1967) and the SO_2 liberated is determined as free SO_2.

The results observed on white wine (Table 2) show that the headspace method is a satisfactory alternative procedure to conventional methods for the determination of free SO_2 in liquid foods.

The products included in the present work fall within the category of liquid foods which have high water activities. Such foods include beverages and fruit juices, and the data reported support the claim that the new method is applicable to foods of this type. However, further work is necessary to determine the suitability of the headspace method for the determination of molecular and free SO_2 in foods having water activities lower than those examined in the present studies.

The headspace method requires no pH or temperature adjustment to the sample, hence equilibria between SO_2 and food constituents, including anthocyanins, are not disturbed during the analysis. Furthermore, the removal of samples of headspace gas for analyses does not change the concentration of free SO_2 in the sample, hence disturbance to the various equilibria from this source is avoided. These considerations suggest that the new method has important advantages over other methods, and that it has potential applications for studying problems such as the formation and stability of complexes between SO_2 and food constituents, and the resistance of micro-organisms to SO_2.

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Factors affecting the development of 'finger drop' in bananas after ripening

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Summary

A simple mechanical method has been developed to measure the force required to break banana fruit peel in the region of the pedicel. Values obtained for peel breaking force correlate well with measured differences in susceptibility to 'finger drop'.

Longer periods for fruit ripening, obtained by a reduction of the conventional ripening temperature, favour maintenance of a high peel breaking force and extend the shelf life of ripe fruit but reduce the degree of coloration. Finger drop in bananas of tetraploid clones, which are highly susceptible to its development, could be minimized by ripening at temperatures about 2°C lower than those optimal for 'Valery', a major commercial triploid cultivar.

The peel breaking forces of different tetraploid clones were compared under controlled ripening and shelf life conditions and several clones with significantly reduced susceptibilities to finger drop were identified. Clones with a low susceptibility to finger drop were generally also characterized by a long pre-climacteric period.

Introduction

Banana fruits are highly sensitive to temperature during ripening; as temperature increases, fruits ripen faster to a fully ripe, yellow condition and this rapid ripening is generally associated with a decreased shelf life (Loesecke, 1949; Simmonds, 1966). For cultivars of the Cavendish sub-group, treatment with ethylene is necessary to accelerate and synchronize the onset of ripening (Anon, 1964). The fruit is sealed for 24 hr in a purpose-built

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ripening room maintained at 18°C and 90–95% r.h. containing an ethylene-rich (1000 p.p.m.) atmosphere. This treatment initiates the climacteric phase and ripening is allowed to proceed at a temperature of 16–19°C with restricted ventilation for about 5 days when the fruit has reached the yellow-green stage appropriate for distribution. The ripening period can be conveniently manipulated to allow several days flexibility by slight changes in temperature (Anon, 1964; Rippon & Trochoulias, 1976).

The shelf life of the cultivar Valery (Cavendish sub-group) is limited by development of senescent spotting whereas that of cultivars Gros Michel, Highgate and its tetraploid progeny is terminated by weakening of the fruit peel around the pedicel causing 'finger drop', the tendency of fingers to break easily from the crown during handling (New & Marriott, 1974b; New *et al.*, 1976). The differences between cultivars are probably related to the known difference in rates of endogenous ethylene production (New & Marriott, 1974a) and senescent spotting in Valery can be inhibited by treatment with exogenous ethylene (Liu, 1976). During trials in which tetraploid and Valery fruit were ripened together, tetraploids invariably exhibited limited shelf life under commercial conditions in which Valery fruit ripened satisfactorily (New & Marriott, 1974a). This investigation was carried out to determine the optimum conditions for ripening tetraploid clones separately from Cavendish clones and to develop a method for screening new clones for susceptibility to finger drop.

Materials and methods

Conditions for storage, ripening and shelf life assessment

All studies were carried out using cv Valery, clone A, a reference tetraploid, and other tetraploids as they became available. Agronomic practices, harvesting and handling methods have been described elsewhere in a parallel study on preclimacteric period and commercial storage potential (Marriott *et al.*, 1979). For storage and ripening trials fruits were packed in commercial cartons, weighing about 15 kg when fully packed, with each carton containing fruit from a single bunch. Cartons containing tetraploid fruit were unlined but, to increase inter-clonal synchronization of ripening, Valery fruit was loosely wrapped in perforated polyethylene film. All fruit was stored at a pulp temperature of 13.5°C in refrigerated air-tight rooms (capacity 30 000 litres). After a storage period the room temperature was raised and ethylene introduced at an atmospheric concentration of 1000 p.p.m., to initiate ripening. The room was sealed for 24 hr then thoroughly ventilated with ambient air. During subsequent ripening, room temperature and ventilation were adjusted in accordance with the schedule being tested (see Results section) and each box was removed from store when the fruits were at Colour stage 4 (yellow-green). For shelf life studies fruit was placed on an open table in an air-conditioned room maintained at 19°C and 30–40% r.h.

Fruit pulp temperatures were recorded twice daily through the experiment. Any deviations from these procedures are noted in legends to figures and tables.

Measurement of clusterability

Prior to the sale of bananas, either at the wholesale or retail stage, complete hands are broken into clusters of three to five fruit by applying a leverage from the flower ends of the fruit cluster (Fig. 1). The procedure depends upon individual fruit pedicels and peel having greater strength than the crown. This ensures that the crown splits preferentially, leaving both the cluster and the remaining portion of the hand with fruit intact and attached at the crown region. The term 'clusterability' was introduced to describe the capacity of complete hands of bananas to be broken into saleable clusters according to trade procedures. Five hands were taken for each test. An attempt was made to break each hand into several clusters of five fingers by gripping the distal ends of the fruit of the prospective cluster, holding the remainder of the hand firmly and applying a leverage to split through the crown (Fig. 1). Intact clusters were those in which no breaking or splitting of fruit peel occurred during clustering. Clusterability was defined as:

$$\frac{\text{Number of intact clusters}}{\text{Total number of clusters attempted}} \times 100\%.$$

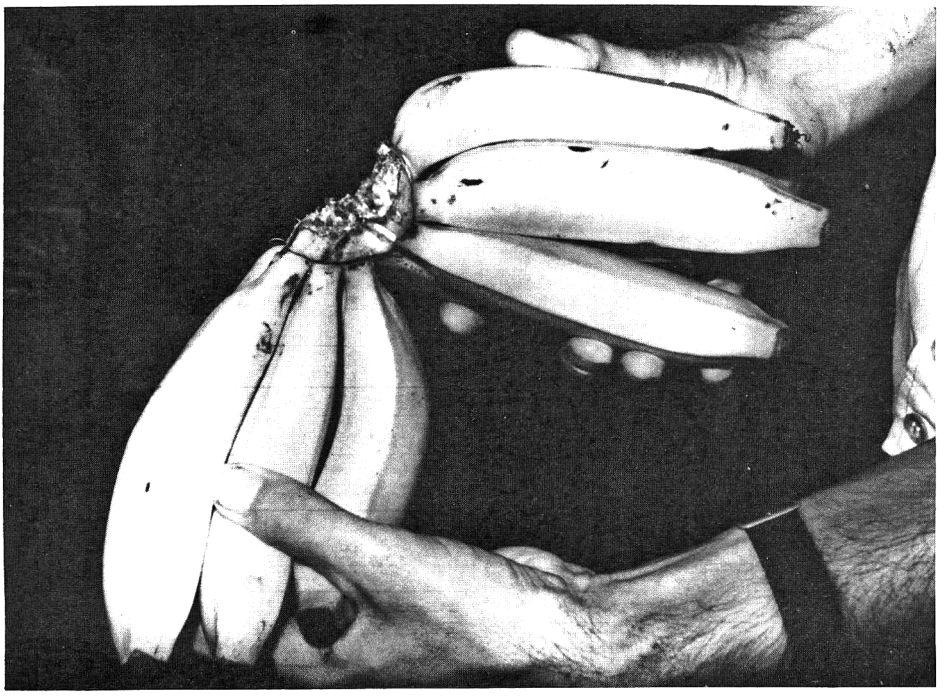

Figure 1. Measurement of clusterability.

Measurement of finger drop and shelf life

Finger drop occurs in bananas when the strength of the pedicel or peel deteriorates to such a degree that individual fruit drop from the crown during normal handling (Fig. 2). To quantify finger drop, one cluster of five fruit was cut from each of five hands and subjected to consecutive 5 sec periods of 'soft', 'medium' and 'hard' manual shaking. Scores were awarded as follows:

6, All fruit dropped from the cluster during a soft shake;
5, One or more fruit dropped during a soft shake;
4, All fruit dropped during a medium shake;
3, One or more fruit dropped during a medium shake;
2, All fruit dropped during a hard shake;
1, One or more fruit dropped during a hard shake;
0, No fruit dropped during a hard shake.

Measurement of peel breaking force (pbf)

The force required to break fruit peel directly at the point where breakage resulted in finger drop as measured directly using a Chatillon DPP 10 force gauge. The banana was hooked to the force gauge from a V-shaped notch (1–2 mm deep) cut in the pedicel 1 cm from the junction of pedicel and fruit, where breakage normally occurred. The bulk of the fruit, held firmly against

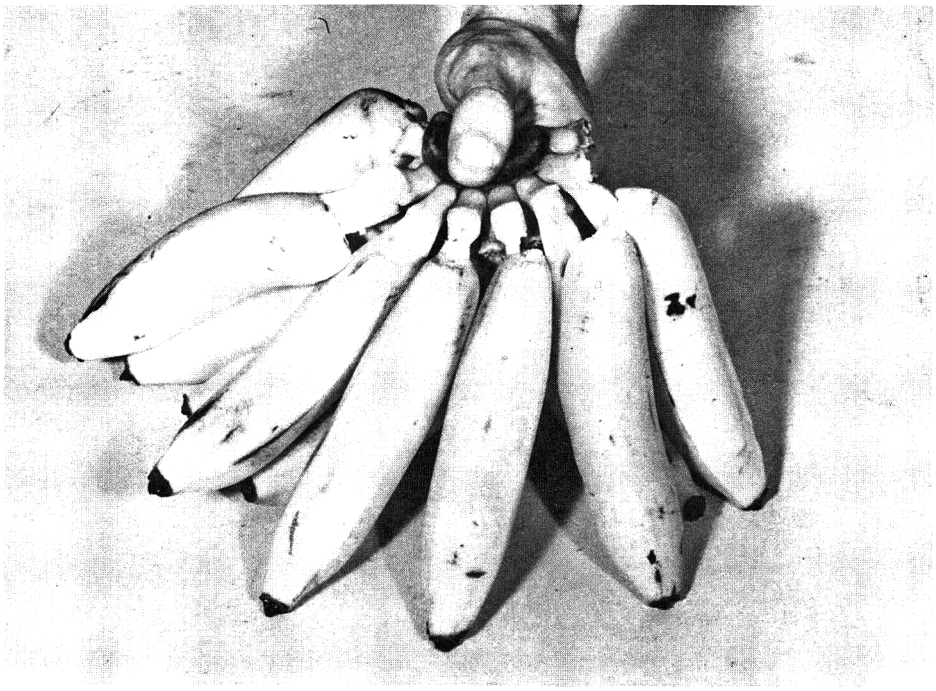

Figure 2. Finger drop in Clone A.

the platform of a test stand by hand, was moved downwards at 160 mm/min applying a pivotal force to the pedicel which remained fixed to the force gauge. The applied force which caused the peel to break and finger drop to occur was read directly from the gauge.

Results

Ripening trials

Table 1 is a summary of ripening rates and subsequent shelf life from seven ripening trials. Trials in which room temperature was raised but no ethylene added did not give uniform ripening and the results are not given here. However, exposure to 0.1 p.p.m. ethylene gas caused all fruit to ripen uniformly and rapidly in 3 days with a subsequent shelf life to 3.2–3.4 days (Trial 1). In Trials 2–4, using commercial ripening conditions employed for Valery (1000 ppm ethylene), all fruit ripened to Stage 4 in 4–5 days. The shelf life of Clone A was 2.0–2.6 days. When ethylene concentration was reduced to 1 p.p.m. in Trial 5, a lower maximum pulp temperature was reached and the shelf life of Clone A was extended to 4.4 days. When ripening temperature was reduced to 14–15°C in Trial 6, shelf life was extended to 5.1 days but ripening was undesirably slow (8 days). At ripening temperatures lower than 14.5°C shelf life was reduced and fruit were slightly chilled (Trial 7). Valery fruit, packed with polyethylene liners, ripened at about the same rate but not as uniformly as tetraploid fruit in these trials or as Valery fruit ripened under optimum conditions. In all trials, development of senescent spotting preceded finger drop and determined the shelf life of Valery fruit. The shelf life of tetraploid fruit was comparable with that of Valery fruit in the two trials using low concentrations of ethylene (Trials 1 and 5) and in Trial 6 where the maximum temperature during ripening was 15°C.

Correlation of peel breaking force with finger drop and clusterability

The relationship between clusterability and finger drop score is shown in Table 2. Fruit with a clusterability of 100% invariably had a finger drop score

Table 1. Shelf life of Valery and Clone A under various ripening conditions

Trial no.	Initial ethylene treatment (ppm)	Ripening temperature (°C)		Time to colour Stage 4 (days)	Shelf life (days)	
		Initial	Final		A	Valery
1	0.1	22.5	16.7	3	3.2	3.4
2	1000	18.4	19.6	4	2.6	3.7
3	1000	17.2	18.3	4	2.0	3.5
4	1000	16.0	18.6	5	2.5	3.5
5	1.0	16.0	16.7	6	4.4	4.5
6	1000	14.0	15.0	8	5.1	4.0
7	1000	14.3	14.3	7	3.7	4.5

Table 2. Variation of finger drop score with clusterability in Clone A

Clusterability (%)	Finger drop score	Standard error
100	0.00	—
90–99	0.45	0.12
80–89	1.37	0.31
60–79	2.56	0.18
40–59	2.44	0.21
20–39	3.43	0.21
0.19	4.83	0.36

Table 3. Variation of peel breaking force with finger drop score in Clone A

Finger drop score	Peel breaking force* of outer whorl (kg force)	Peel breaking force* of inner whorl (kg force)
0	4.60a	5.25a
1	3.32a	3.91a
2	2.48b	3.07b
3	1.84c	2.68b
4	1.43d	1.91c

* Within each column, values followed by a different letter are significantly different ($P=0.05$).

of zero. Observations of trade procedures indicated that the lowest tolerable clusterability score was 95% which corresponded to a finger drop score of 0.5. Observations of point-of-sale handling indicated that, after clustering, fruit could be safely handled up to a finger drop score of 2.5.

The relationship between peel breaking force (pbf) and finger drop score is shown in Table 3. Differences between pbf values for each finger drop score were all significant ($P=0.05$). Values of pbf for fruit from the inner whorl were less consistent than those from the outer whorl. No significant differences in pbf values for each finger drop score were obtained between experiments conducted on separate occasions.

Termination of shelf life at a finger drop score of 2.5 corresponded to a pbf value of 2.0–2.3 kg force on the outer whorl (Table 3). Consequently the shelf life of Clone A was taken as the time between removal from the ripening room and deterioration of fruit peel strength to a minimum pbf value of 2.0 kg force on the outer whorl of the hand. The pbf value corresponding to lowest tolerable clusterability was 4.0 kg force.

The pbf value, and therefore finger drop score, correlated highly with tensile strength and shearing strength of the peel during shelf life for both Valery and Clone A (New, 1976). However, as screening tools these parameters offer no advantage over the pbf value, which can be measured rapidly and is more representative of the finger drop process. Since pbf is measured on a continuous scale, less variation about the mean is obtained

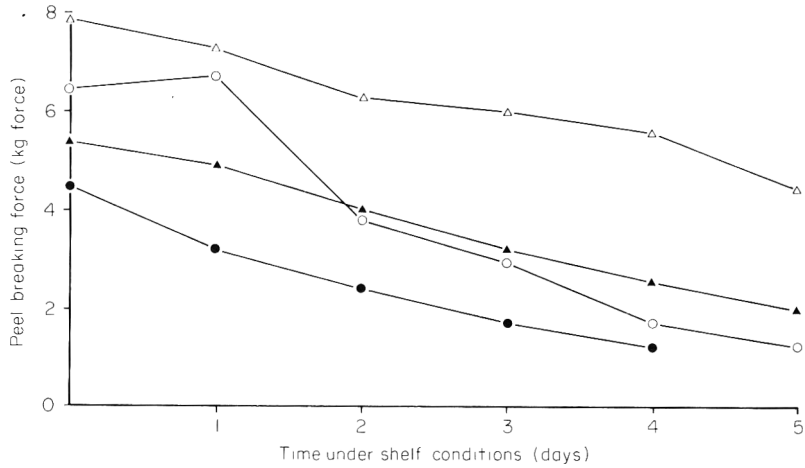

Figure 3. Peel breaking force associated with finger drop in fruit ripened at different temperatures. △, Valery ripened at 13.9°C; ▲, Valery ripened at 19.0°C; ○, Clone A ripened at 14.0°C; ●, Clone A ripened at 19.0°C.

than for finger drop scores measured on a scale of discrete values. Whilst the estimation of finger drop by shaking is useful in commercial trials, it requires six times the amount of fruit necessary for measuring pbf values and is therefore inappropriate for screening single bunches of new clones. It is also to some extent, subjective.

Effects of ripening temperature and ventilation on pbf after ripening

Lower ripening temperatures caused higher values of pbf in freshly ripened fruit of both Valery and Clone A and this difference was maintained over a shelf life of 5 days (Fig. 3). To obtain a high ventilation rate, fruit was stacked directly in front of the refrigeration fan in the ripening room. The room contained 10–15% by volume of fruit and was maintained at 85–90% r.h. by flushing completely with fresh ambient air twice each day. A low ventilation rate was obtained by storing fruit in plastic containers inside the ripening room. Fruit occupied 70–80% of the volume of the containers which were ventilated with air from the ripening room atmosphere at a rate of eight to ten changes per hour, maintaining 90–100% r.h.

The high ventilation treatment during ripening caused retention of a greater pbf after ripening for all clones at both ripening temperatures (Table 4). The pbf values after 3 days under shelf conditions were 30–51% higher for the fruit ripened at 15.7°C. Differences in pbf between ventilation rates and between temperatures were all significant (P=0.05).

Clonal differences in pbf after ripening

Significant clonal variation in pbf existed for fruit ripened at 14–16°C and initiated with 0.1 ppm ethylene (Table 5). All clones tested had stronger peel

Table 4. Effect of ventilation rate during ripening on the peel strength of ripe fruit

Clone	Mean ripening temperature (°C)	Peel breaking force after 3 days shelf life (kg force)		Mean total shelf life (days)	
		High ventilation	Low ventilation	High ventilation	Low ventilation
A	15.7	4.15	1.98	4.5	3.0
Q	15.7	—	3.08	—	4.0
A	17.9	2.05	1.37	3.0	1.5
Q	17.9	2.42	1.62	3.5	1.5
Valery	17.9	2.75	1.52	3.5	2.0

Table 5. Clonal variation in peel breaking force and in pre-climacteric period

Clone	Peel breaking force (kg force)*			Estimated† pre-climacteric period (days)
	Day 3	Day 4	Mean (%)	
Valery	6.45a	5.96a	100	30+
Q	6.22a	5.22ab	92	45.4
P_2	6.41a	5.06b	92	31.9
Q_2	5.46b	4.59bc	81	28.9
R_2	5.11b	4.21c	75	24.3
S_2	4.80b	4.19c	72	23.0
A	3.60c	2.92d	53	8.2

* Within each column values followed by a different letter are significantly different ($P=0.05$).

† From Marriott *et al.* (1979).

than Clone A, the reference tetraploid, and pbf values for Valery were similar to or greater than those obtained for all the tetraploids tested. A highly significant correlation existed ($P=0.01$, $r=0.94$) between clonal pre-climacteric period (at a caliper grade of 35 mm) and the pbf of the ripe fruit (Table 5). Under the optimum ripening conditions prevailing in these trials, all clones except Clone A had pbf values more than double the critical finger drop value after 4 days shelf life.

Discussion

Ripening trials (Table 1) and pbf measurements (Fig. 3 and Table 4) all demonstrated that finger drop can be reduced by lower ripening temperatures than those currently used commercially. This is consistent with early trials by Hicks (1934) and with our previous work (New & Marriott, 1974a). A high rate of ventilation also reduced finger drop (Table 4), perhaps through the

removal of physiologically active volatiles, so that well-ventilated rooms and cartons without polyethylene liners are factors conducive to the reduction of finger drop in susceptible clones. Optimum ripening temperature may vary according to pre-harvest environmental conditions just as for Australian Cavendish bananas between summer and winter seasons (Rippon & Trochoulias, 1976). However, tetraploid Clone A clearly tolerated lower temperatures than are used commercially for Valery (Anon, 1964) and optimum temperatures are about 2°C lower both at initiation (15°C) and maximum (17°C). Our results indicate that use of low concentrations of ethylene, probably about 1 p.p.m., could also reduce finger drop and this merits further investigation. Maintenance of a monitored low ethylene concentration during ripening is standard commercial practice in the trickle degreening of citrus and should be feasible for bananas.

Other changes in the commercial handling of fruits susceptible to finger drop would also be necessary. Fruit peels weaken so that clustering is impracticable at a pbf of about 4 kg force, that is 2–3 days before they develop finger drop at a pbf of 2 kg force (Fig. 3). Thus it would be necessary to cluster fruit either when packing in the producing country or immediately after ripening prior to dispatch from the ripening depots Both procedures are current practice in limited sectors of the trade. Further precautions might also be necessary in handling of boxes since the pulp is softer and more liable to bruising in Clone A and other tetraploids than in Valery (New, 1976; Baldry, Coursey & Howard, 1981).

Clonal differences in susceptibility to finger drop were demonstrated for selected clones (Table 5) and selection of clones for low susceptibility could be considered. These differences may relate to variation in pectic constituents between clones (Coursey *et al.*, 1974). A significant correlation existed between the clonal component of pre-climacteric period and clonal susceptibility to finger drop, so that it may be possible to select simultaneously for storage life and shelf life. This would be consistent with suggestions of Marriott *et al.* (1979) that endogenous ethylene production, control of the fruit climacteric and rate of senescence may all be under the control of an allele which modulates fruit ripening, comparable to that demonstrated in tomato fruits by Ng & Tigchelaar (1977).

The spread of black leaf streak, a virulent form of banana leaf spot, from the Pacific to the Caribbean (I. Muirhead, pers. comm.) emphasizes the need for new banana cultivars with increased genetic diversification and the potential for disease resistance. Our results suggest that commercialization of selected tetraploid clones would be possible although some achievable modifications to trade procedures would be necessary.

Acknowledgments

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Technical note: A test for pro-oxidants in printed packaging films

S. HERNANDEZ-BRIZ and R. CLOTET BALLUS

Introduction

It has been found that chip potatoes and extruded fried snacks, packaged in polypropylene film with outside print developed deterioration by fat oxidation in a short period of time of about 10 days at 20°C, 75% r.h. A simple analytical method has been developed that can provide rapid information about all pro-oxidant interactions specific to the packaging material and the oil or fat used in the process.

Materials and methods

A fixed quantity of an edible oil is brought into contact with a standard area of film for a pre-determined period. The changes in the oil are determined by the Kreiss reaction (Jacobs, 1951) by means of a spectrophotometer.

In order to obtain quantitative and reproducible measurements, a simple device has been developed to maintain the constant oil weight in contact with the film surface.

A square section of the film to be studied is placed loosely over a 90 mm ID glass dish bottom, with the internal face (or the surface in contact with the product) facing up. Next, a 88 mm ID plastic Petri dish bottom is forced down into the glass Petri dish, thereby moulding a tray. The excess film is carefully trimmed off in such a way that when the plastic Petri dish is removed, the tray formed by the film stays in the glass Petri dish bottom in a position whereby the top edges of the foil are recessed below the top surface of the glass Petri dish and are not in contact with the Petri dish cover. Add 15 ml of standard edible oil to each film tray (supported by a Petri dish bottom). Maintaining the Petri dishes in a horizontal position, evenly distribute the oil across the bottom of the film tray. The Petri dishes containing the film samples, together with a

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0022–1163/83/0400–0251 $02.00

control dish without film, but containing 15 ml of oil, are incubated at 60°C for 16 hr.

After the incubation period the changes in the oil are determined as follows: to 5 ml of oil removed from each Petri dish, add 5 ml of concentrated hydrochloric acid and agitate vigorously. Add 5 ml of a 0.1% solution of phloroglucinol in ethyl ether, and agitate a second time. Centrifuge the sample. With a pipette, remove the top layer containing the colour phase and measure the transmittance at 550 mμ, using the incubated oil blank as a reference.

In the present tests, the oil was refined soya oil with the analytical parameters: Acidity (oleic), 0.1% maximum; peroxide value (m.eq./k), 1.0% maximum; iodine value, 130–141; saponification index, 189–193; A.O.M. (ADCS), maximum 35 m.eq./k 8 hr; soaps, negative.

Table 1. Transmittance values grouped in keeping with the sensorial evaluation transmittance (550 mu)

	Flavour strongly oxidated	Flavour slightly oxidated	Normal flavour
	33.75	28.15	53.75
	24.3	24.3	63.3
	13.5	38.05	81.05
	1.3	34.5	78.1
	18.05	33.45	47.7
	12.55	30.1	46.0
	17.1	9.45	54.5
	1.85	16.95	45.25
	6.2	45.45	47.75
		14.65	53.0
		29.15	54.6
			48.0
			59.8
			57.0
			65.0
			63.3
$\bar{x}$	14.3	27.7	79.3
Range	1.3–33.75	9.45–45.45	42.25–81.05
$\bar{x}$	21.64		
Range	1.3 –	45.45	

In order to correlate observed changes in the test oil with sensory changes produced by the packaging film, printed bags formed from the test film being evaluated were filled with chip potatoes and stored at 37°C for 7 days.

The sensory evaluation was carried out by a panel of trained technicians, utilizing the hedonic scale reported for oxidation by Amerine, Pangborn & Roessler (1965).

Results and discussion

Table 1 shows that, if the sensory results are arranged in three groups, there is a correlation with transmittance values. The utilization of this method permits a rapid evaluation, simple and unrelated of any other sensory evaluation of possible induced effect of rancidity due to the film or its printing. The obtained index corresponds exclusively to the face in contact with the product, and evaluates the total pro-oxidant effect. The numerical values shown in Table 1, and the intervals between pro-oxidant and transmittance effect are specific of the oil used. This method evaluates all pro-oxidant effects owing to packaging material and has been running well as a quality control operation; however, it is necessary to calibrate each test as described, with the oil used and product manufactured.

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Book reviews

Preventive Nutrition and Society. Ed. by M. R. Turner. London: Academic Press, 1981. Pp. xii+228. ISBN 0 12 704450 7. £16.40.

Most people who work in the nutrition/food science area have pretty clear ideas about what's wrong with the nation's eating habits. There may not be uniformity of opinion but that's another matter. But when it comes to the methods the so-called educators would use to effect those changes most ideas are based on nothing more than telling people the facts. This book begins by reviewing the role of food in causing and preventing certain health problems and moves easily into the area of motivating people to change. It leaves no one in any doubt that getting the facts right is only the beginning of the communications effort.

There are fifteen chapters, the first of which occupies about a quarter of the entire book. This seemingly disproportionate allocation of space is, perhaps, one of the great benefits of the book. Drs James, Powles and Williams, the authors of the opening chapter on 'The Prevalence of Diet Related Diseases' give an excellent review of the current hot potatoes—heart disease, hypertension, obesity and colon cancer. A little later the role of diet in dental caries is covered and Professor Bender looks specifically at the nutritional health of children in this country.

The remaining chapters cover the cost of diet related diseases, and the influence of social and economic factors on health. Two papers tackle the difficult area of measuring the extent of malnutrition and the final chapters examine the crucial aspects of effecting change.

The book marries the experience and needs of the commercial world of advertising and of nutritionists very successfully. Judith Lannon from J. Walter Thompson points out the similarities between the job of the advertising agency and the nutritionist. Both frequently want either to initiate behaviour change or to reinforce 'good' habits. It really is about time the two 'sides' recognized each other and realized that each can learn a great deal from the other to be more effective in communicating the messages of healthy eating.

At least three contributors to the book are not overly optimistic about the ability of conventional methods of persuasion to bring about changes in eating habits. The power of the media is recognized by almost everyone as being very considerable. Tony Smith from the *British Medical Journal* has a warning that a convincing lunatic is much more likely to be followed than an uncharismatic, albeit factual speaker. We would do well to heed the message that facts aren't always all that powerful!

Overall this book is easy to read, interesting and more important for academic students and practitioners of food science, nutrition and dietetics than many would think. References are good, mainly relating to work done in the 1970s.

Jenny Salmon

Kaffee. By Hans G. Maier (in German).
Berlin: Verlag Paul Parey, 1981. Pp. ix+199. ISBN 3 489 61414 3. DM 78.

This book is the eighteenth in a series of food science monographs entitled *Grundlagen und Fortschitte der Lebensmitteluntersuchung und Lebensmitteltechnologie*. The literature of all aspects of the chemistry of coffee is vast and this volume represents one of the few recent attempts to rationalize this mass of data.

After a very limited historical introduction in Chapter 1 (two pages), Chapter 2 describes the various botanical varieties and the conditions required for cultivation. Chapter 3 covers all aspects of green (or raw) coffee from the technology of processing equipment to a detailed description of the chemical composition. In addition to the major components such as carbohydrates, lipuids, protein, phenolic acids and alkaloids attention is also paid to minor components such as mycotoxins and vitamins. Many of the compositional data are presented in tabular form, which greatly assists the abstraction of required information.

Chapter 4 is of similar format to the preceding chapter but concerned with roast coffee; here again both technological and compositional aspects are covered. There is considerable discussion on changes in chemical composition of roasting, together with a large section on aroma compounds produced during this process. Chapter 5 turns to the preparation and properties of coffee beverage, including an interesting table of the percentage extraction of the various components during preparation. The physiological role of caffeine receives considerable attention from which it is evident that this is still a poorly understood area.

Chapters 6 and 7, which are both short, cover processed coffees (e.g. decaffeinated) and Kaffee-Trockenextrakten (instant coffees). The brief discussion of instant coffees probably reflects their relatively low significance as a proportion of the total coffee consumption in Germany (*c.* 25% whilst in the U.K. it is nearer 80%).

Chapter 8 represents the most important part of the book, that is to say a detailed description of analytical methods for all the components discussed in the previous chapters. For each determination there is a detailed description of the experimental procedure together with the principle of the method. The emphasis is on traditional, or accepted methods, and in general there is only brief mention of modern instrumental methods (e.g. high performance liquid chromatography of caffeine or chlorogenic acid).

Chapter 9 deals briefly with food legislation as applied to coffee and Chapters 10–19 cover all aspects of 'Kaffemittel' (coffee additives and substitutes). This book provides an excellent text on the chemistry of coffee and should be purchased immediately by all those working either on coffee research or in quality control. It is hoped that a translation into English will be forthcoming, possibly with an adaption to cater for the differences in coffee consumption in the U.K. and America.

R. Macrae

Flavor Research. Recent Advances. Ed. by R. Teranishi, R. A. Flath and H. Sugisawa.
New York: Marcel Dekker, 1981. Pp. x+381. ISBN 0 8247 6568 0.
SwFr. 168.00.

This book is the successor to *Flavor Research: Principles and Techniques,* published 10 years earlier. The emphasis has been deliberately shifted to pointing out some of the accomplishments of the applications of the methods developed to a range of foods, but, owing to the weight of the literature, comprehensiveness is not attempted. Thus, about half the book is devoted to methodology, dealing in turn with problems in flavour research, sample preparation (isolation, concentration, separation), identification, and sensory characterization. The second half is concerned with the flavour of vegetables and fruit, meat, tea, coffee, cocoa, and related beverages, taste and flavour enhancers, and, briefly, with dairy and soy products. There are five additional authors, R. G. Buttery, D. A. Forss, I. Katz, A. Kuninaka, and T. Yamanishi. All are internationally known.

The book, like the earlier one, is the result of a workshop; this one was held in Hawaii jointly by a number of national chemical societies. It is thus intended for those actively interested in flavour chemistry and who have a grounding in its principles. A novice would need to have available more basic texts, such as the earlier volume; experts will enjoy the viewpoints presented and benefit from the ensuing consolidation. Unfortunately, production of the book seems to have taken rather long, references virtually ceasing with 1979.

Overall, the book is authoritative and well produced, though not entirely without errors. Tighter editing would have been helpful.

In spite of the advances described, the senior editor can still point out in the preface that, although we can record, store, retrieve, amplify, transmit, duplicate, and describe objectively the sights we see and the sounds we hear, none of these operations can be carried out for a single taste or odour. The study of flavour clearly has yet a long way to go, but a stock-taking, such as is presented in this book, is greatly to be welcomed.

H. E. Nursten

Human Nutrition Research (Bcltsville Symposia in Agricultural Research No. 4). Ed. by G. R. Beecher.
London: Granada, 1982 (New Jersey: Allanheld, Osman, 1981). Pp. xiv+303. ISBN 0 916672 48 4. £20.00.

Human Nutrition Research is made up of a collection of papers which were presented at the Fourth Annual Symposium at the Beltsville Agricultural Research Center in Maryland in 1979. Twenty-nine contributors highlighted specific areas of nutrition research that are of concern to the scientific community as a whole. The book is divided into five broad subject areas: nutrients affecting health; nutrient intake and composition; nutrition research directed toward public health problems; assessment of the nutritive value of foods and of human nutrient requirements; and the implementation of human nutritional knowledge.

Authors of several chapters suggest that there is moderately good evidence to link the excessive consumption of dietary fat and cholesterol with the development of arteriosclerotic disease. Some dietetic information is presented and referenced in sufficient detail to permit a meaningful follow-up by dieticians and the medical profession. Much of the research evidence presented is based on probabilities of the risks or benefits to be derived from modifications in the diet. For example, several authors point to the importance of dietary fibre in disease prevention but warn that fibre makes both positive and negative contributions to the quality of the diet. The complex interrelationships of the factors involved in the degenerative diseases are discussed, and obesity and energy balance are critically reviewed as a major preventive issue. The association between sodium intake, and the more recent evidence of the link between dietary fat intake and hypertension are brought to the reader's attention.

Several chapters emphasize the methodological limitations in nutrition, such as the determination of nutrient levels in foods, the assessment of the nutritional status of the population and the development of recommended daily amounts of nutrients to meet the needs of practically all healthy people.

The book brings together a wide range of research approaches and is broad in scope. The reader really has to be prepared to sift through several of the chapters in detail to gain full insight into the subject matter. A stricter format of chapter structure with clearly defined objectives and concluding statements would have been useful. Many papers did not have adequate summaries. Proceedings of symposia tend to have these inherent weaknesses and this book is no exception. A few chapters were familiar pieces of work diluted down by the same authors from more thoroughly presented expositions elsewhere.

Nevertheless, the type-face—bar one or two typographical errors—and the illustrations are good, and the 303 pages should be useful as an update to dieticians, nutritionists, researchers and policy makers in the agricultural and health professions.

D. P. Richardson

Mixed Culture Fermentations (Special Publications of the Society for General Microbiology No. 5). Ed. by M. E. Bushell and J. H. Slater. London: Academic Press, 1981. Pp. xi+175. ISBN 0 12 147480 1. £12.00.

This book comprises eight chapters based on papers delivered at a Symposium organized by the Fermentation Group of the Society for General Microbiology in December 1980. The first two chapters deal with general aspects of microbial communities and population dynamics while the remaining chapters are concerned with specific mixed culture associations. As is often the case with symposium-based books, some of the chapters constitute useful broad reviews of their subject that can be appreciated by the general reader and the advanced student of microbiology, while others are relatively confined reports of specific studies likely to appeal only to the specialist, who in any case is likely to have met them already in the scientific journals.

The first chapter considers the general nature of microbial communities and provides a very extensive and well referenced survey of studies in this area. The next chapter on mixed culture kinetics is largely mathematical analyses of microbial interactions, but in passing becomes entangled in definitions of competition. Discussion about the meaning of words is often a relatively unprofitable activity, but when the definition of competition is pursued without reference to the work of plant and animal ecologists it does seem that microbial ecologists are assuming a mantle of unwarranted independence.

The outstanding chapters in the book are those dealing with anaerobic waste treatment and yoghurt production. Hobson presents an extremely lucid survey of the microbial ecology of anaerobic waste treatment, with an emphasis on practical aspects and current problems. Driessen's review of the *Lactobacillus–Streptococcus* interaction in yoghurt is also a very clear exposition of what is known about this association. It includes considerable details of studies conducted in pH-stat (turbidostat) culture, and might, in fact, be taken as a model of how the turbidostat can be used as an experimental tool in the unravelling of interrelationships in microbial communities.

The chapters dealing with aerobic waste treatment and with yeast–*Lactobacillus* interactions in beverages and foods are largely descriptive and serve mainly to indicate how little is really known about these associations. The two remaining chapters concentrate on specific problems, one involving *Lactobacillus* contamination in beer yeast and the other the use of algal-bacterial associations for biomass production.

Although the book will appeal mainly to research workers and advanced students in microbiology, the chapter on yoghurt, which is ultimately concerned with the development of a continuous manufacturing process, and the chapter on anaerobic waste treatment, an area of increasing concern to the food industry, will make interesting and profitable reading for many food technologists.

Overall the book is well presented with few misprints and a brief index.

J. D. Owens

Trends in Fish Utilization. By J. J. Connell and R. Hardy. Farnham, Surrey: Fishing News Books, 1982. Pp. x+103. ISBN 0 85238 120 4. £6.00.

There can be little doubt that the fishing industry in the U.K. has suffered considerably during the last decade. The deep-sea fleet is rusting by the quayside, the inshore boats can do little more than survive against the subsidized competition from our European neighbours, and every year seems to bring fresh problems for those involved in the catching and/or processing of fish. The causes of this decline can be traced to various political and economic factors, but one important factor is the reluctance of consumers to buy 'wet' fish in any appreciable quantity. This reservation applies particularly to the more readily available pelagic species, and there is some evidence that this pattern is becoming steadily more pronounced. If these trends could be reversed, or if consumers could be persuaded to purchase more fish in other forms, then clearly the increased revenue might go some way towards easing the plight of the industry. The extent to which this 'hope' could become reality provides the basic theme for this book, and it is to the author's credit that their assessment of the possibilities has remained both rational and objective.

In order to reduce the vast array of available material to useful proportions, the subject has been discussed under three broad headings, namely: (1) 'unused and underused resources', which describes briefly some of the marine species that are, at present, underutilized as human food. The coverage ranges from the much maligned mackerel through to more unusual items like squids and sandeels, and although the discussions are inevitably short, the narrative never becomes trite or repetitive; (2) the future 'development of conventional methods' of presentation to the consumer, with especial emphasis on how the efficiency of handling has been steadily improving; and (3) 'newer methods and products' which considers how flesh recovered from carcasses can be transformed into edible products, such as moulded fillets for direct consumption, or high protein flours for incorporation into bakery or other food products.

A general discussion of marketing and related problems completes the coverage, so rounding-off a most absorbing review of possible future activities in relation to fish and fish products.

It should, therefore, prove a most useful book for college students seeking an insight into likely trends in fish processing and distribution, and indeed any 'student' of the food industry will find it a most enjoyable narrative. Thus it is a book which is both informative and readable, and it is rather a pity that the price is not more conducive to casual purchase. It must be conceded, of course, that the quality of presentation is higher than that often associated with 'soft-back' publications and that it is well illustrated, but even so, many a potential purchaser may well query whether it is an 'essential' for his/her bookshelf.

R. K. Robinson

Books received

Food Science and Technology, 4th ed. By Magnus Pyke, revised by L. Parducci.
London: John Murray, 1981. Pp. xvi+304. ISBN 0 7195 3850 5. £7.50.

This introductory text has been revised to include discussion of extrusion cooking, microwave cooking, sterilizable flexible pouches, aseptic packaging, reformed meat products, leaf protein, TVP, and yeast SCP.

Enzyme Kinetics: The Steady State Approach, 2nd ed. By P. C. Engel.
London: Chapman & Hall, 1981. Pp. vi+96. ISBN 0 412 23970 1.

A short introduction to enzyme kinetics 'aimed at "non-believers", that is to say the 90% or so of biochemistry students, and indeed of practising biochemists, who place enzyme kinetics in the same category as Latin and cold showers, character-building perhaps, but otherwise to be forgotten as quickly as possible'. Algebra is used, but the calculus is avoided. The book pays particular attention to multi-substrate kinetics, and to the differences between the various models for the allosteric control of enzyme activity.

A Guide to Improving Food Hygiene, 2nd ed. By G. Aston and J. Tiffney.
London: Northwood Books, 1981. Pp. 168. ISBN 7198 2864 3.

Aimed at food handlers, the book is written in 'plain language', and contains many apposite illustrations. Intended also to be of value to environmental health technicians, domestic science teachers, architects and kitchen planners, it not only discusses the principal types of food poisoning and hygienic aspects of food production and handling, but also covers such topics as the design and construction of food premises, design of equipment, and problems associated with market stalls and mobile food vending.

Meat, Poultry and Seafood Technology: Recent Developments. By E. Karmas.
Park Ridge, New Jersey: Noyes Data Corporation, 1982. Pp. xv+426. US$45.00.

This 'data-based publication' provides information retrieved from the U.S. patent literature. The patents described, which are not necessarily being utilized commercially, cover topics such as post-mortem operations, meat–bone separation, integral meats, flavourings and colourings, tenderness evaluation, cooking, curing, anti-nitrosamine agents, meat products, meat and bacon analogues, and packaging. Subjects of seafood technology covered include filleting and freezing of fish, fish protein hydrolysates and isolates, kamaboko, roe and shellfish products and shell extracts.

Haemophilus influenzae (Public Health Monograph Series No. 17). By D. C. Turk.
London: HMSO, 1982. Pp. vii+56. ISBN 0 11 887108 0. £3.50.

The topics covered in this short monograph relate to both the clinical significance of the organism and its isolation and identification.

Die Verarbeitung von Krill zu Lebensmitteln. By W. Schreiber *et al.* (in German).
Hamburg: Bundesforschungsanstalt für Fischerei, 1981. Pp. vi+215. US$10 (+$2 for postage by airmail).

Methods are given for processing krill into food, based upon the use of krill mince for production of boiled or cured sausages.

Developments in Food Microbiology. Volume 1. Ed. by R. Davies
London: Applied Science, 1982. Pp. x+219. ISBN 0 85334 999 1. £24.00.

In this inaugural volume of a new *Development Series* the topics covered are microbial spoilage of meats; the control of *Salmonella* in poultry; the bacteriology of fish handling and processing; UHT processed foods; regulation of lactose metabolism in dairy streptococci; and rapid estimation of microbial populations in foods.

Gasohol, a Step to Energy Independence. Ed. by T. P. Lyons.
Lexington, Kentucky: Alltech, 1981 (London: E. & F. N. Spon, 1982). Pp. v+346. £22.50.

This comprehensive guide to the technical aspects of alcohol production provides information on the use of feedstocks such as maize, potatoes, wheat, sorghum, sugarbeet and sugarcane. There is also a chapter on the biology and genetics of *Saccharomyces*.

Advances in Optical and Electron Microscopy, Volume 8. Ed. by R. Barer and V. E. Cosslett.
London: Academic Press, 1982. Pp. xii+281. ISBN 0 12 029908 9. £28.20.

The topics discussed are: spatial filters including Schlieren systems; photoelectric setting microscopes; investigation of chromosome structure; mounting macromolecules for electron microscopy; triode electron guns; and field electron emission systems.

BCC: Beef Cuts Code, 2nd ed. By E. B. Riordan, S. J. Schwer and C. Godson.
Dublin: An Foras Talúntais, 1980. Pp. 37. (First available on sale 1982.) £4.00 from CBF, 26/28 Bedford Row, London WC1R 4HE.

Specifications are provided in English, French and German for continental cutting and trimming of beef.

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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

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

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

SI UNITS

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

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in^2	= 645·16 mm^2
square foot	ft^2	= 0·092903 m^2
cubic inch	in^3	= $1{\cdot}63871 \times 10^4$ mm^2
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·54611
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= $2{\cdot}76799 \times 10^4$ kg m^{-3}
dyne		= 10^{-5} N
calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 T°C + 32

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

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

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