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Journal of Food Technology (1984) **19**, 653–658

Changes in certain free fatty acids as an aid for the detection of spoiled mutton used for processing meat curry

K. V. KUMUDAVALLY, T. S. VASUNDHARA*, JOYCE D'SOUZA AND T. R. SHARMA

Summary

Sometimes canned curried meats do not show any gross can defects but on opening give off-odours. This may be due to the poor quality meat that has been used for processing. At present no reliable method exists for determining the quality of meat that has been used for processing. A chromatographic procedure employing thin layer and gas liquid chromatography is described for the isolation, identification and quantification of free fatty acids from canned meat curries prepared from fresh and spoiled mutton and admixtures of the two. Analysis of the free fatty acid fraction revealed a 10–12-fold increase in palmitic, stearic and oleic acids when spoiled meat was used for canning. The method described is objective and can be used as an aid for quality control of canned mutton.

Introduction

Commercial canned mutton sometimes gives off-odours immediately upon opening. Microscopic examination of the can contents generally do not reveal any alterations. In the absence of microbiological evidence or gross can defects, viz. bulging or faulty seaming, this may be attributed to the poor quality meat used for processing. Sensory methods are the most commonly used techniques for judging meat freshness. Other physicochemical conditions such as water holding capacity (WHC), extract release volume (ERV), pH and other chemical methods which are generally used for assessing the quality of fresh meat are not found suitable for canned meat curries as these are complex mixtures of lean meat, adipose, spices, vegetable fat, etc. (Strange *et. al*, 1977). Though microbiological methods have found wide applicability for assessing the bacteriological quality of raw meat, they are not applicable to processed meats. Hence for more objective and reliable assessment, chemical indices should prove more useful for evaluating the quality of processed meat.

Because of its extreme perishability, occasionally meat of poor bacterial quality or samples on the threshold of spoilage—due to lack of refrigeration facilities coupled with high ambient temperature—find their way into market channels and ultimately into the processing line. Our previous studies on lean meat (mutton) lipids (Vasundhara, Kumudavally & Sharma, 1983) have shown that the concentration of certain fatty acids present in the free fatty acid (FFA) fraction of neutral lipids increased 15 times when spoiled meat with high microbial counts (10^{9-10} organisms/g) had been

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used for processing. These fatty acids were detectable even in fresh meat adulterated with spoiled meat at 10 and 20% levels. In the present paper we report the results of an investigation on the FFA profile of curried canned mutton which is a complex mixture of lean meat, adipose tissue, several condiments and spices and hydrogenated vegetable fat. Advantages of the present method over the titrimetric method of determination of FFA are also discussed.

Materials and methods

Reagents and apparatus

The following chemicals were used for the analytical work: (a) chromatographically pure fatty acids and their methyl esters (Sigma Chemical Company, USA), (b) solvents (S. Merck, India), (c) silical gel G (E. Merck, West Germany), and (d) CIC gas chromatograph (Chromatography Instruments Co., Baroda, India) equipped with a hydrogen flame ionization detector.

Meat sampling

Experiments were carried out on three batches of mutton obtained on three different occasions. Each time fresh mutton was procured from the local market. Age, sex and health status of the animals slaughtered were unknown. Estimated age *post mortem* of the meat used was 3–5 hr. Dressed minced meat was used throughout the analysis. Spoilage of meat was carried out under controlled conditions (37°C/18 hr). From every batch four different samples were used for the preparation of meat curry.

Sample I: fresh meat (control);
Sample II: spoiled meat;
Sample III: 10% of this consisted of spoiled meat (10 g of spoiled meat mixed with 90 g fresh meat); and
Sample IV: 20% of this consisted of spoiled meat (20 g of spoiled meat mixed with 80 g fresh meat).

For every sample, curry was prepared from the required quantities of mutton, spices, vegetable fat (composition is given in Table 1, as per ASC specifications) maintaining meat to gravy ratio of 55:45 on wet-weight basis and then equally distributed in four cans (301 × 309). The weight of contents in each can was adjusted with water to 349 g. From each can duplicate samples 87.5 g curry as used for extraction of fat and further analysis.

Preparation of meat curry

Meat curry was prepared according to ASC specifications (1980), the composition of which is given in Table 1. To obtain a uniform distribution of spices and fat in all the samples, the following procedure was adapted. Seven hundred and seventy grams of mutton (wet wt) and a proportionately equivalent amount of spices and fat were used. Throughout the experiment lacquered cans (301 × 309 size) were used. The required quantities of all the dry spices were finely powdered and blended well with chopped onions, garlic, ginger and salt. The paste thus obtained was fried in 100 g fat for 10 min and cooled. The entire quantity was weighed and distributed equally in four cans. Mutton was cooked with 54 g fat over low fire for 10 min and distributed equally over spice mixture contained in four cans. The weight of the contents in each can was adjusted to 349 g by the addition of water, mixed well, vacuum exhausted and sealed.

Table 1. Composition of meat curry

Minced meat	10 kg
Onions (fresh) (*Allium cepa*)	2.8 kg
Ginger (fresh) (*Zingiber officinale* Linn.)	0.55 kg
Garlic (*Allium sativum*)	0.22 kg
Coriander seeds (*Coriandrum sativum*)	66 g
Chilli powder (*Capsicum annum*)	65 g
Turmeric powder (*Curcuma longa* Linn.)	80 g
Black pepper powder (*Piper nigrum* Linn.)	25 g
Cumin seed powder (*Cuminum cyminum*)	52 g
Cardamom powder (*Aframomum augustifolium*)	65 g
Cinnamon (*Cinnamomum zeylanicum*)	52 g
Cloves (*Eugenia caryophyllus*)	25 g
Salt	200 g
Hydrogenated vegetable fat	1.225 kg

The cans were processed at 15 psig for 60 min which gave a F_0 value of 7. Four cans from each group of sample were analysed.

Microbiological analysis

Standard methods were followed for the microbiological analysis of fresh mutton and meat curry (Vijaya Rao, Bhagirathi & Sharma, 1983).

Raw mutton. Ten grams of minced meat was macerated with 90 ml of 0.1% peptone water. One millilitre aliquots of 10-fold serial dilutions were plated on the media shown in Table 2. Lipolytic and proteolytic bacteria were enumerated on tributyrin agar (Mourey & Kilbertus, 1976) and nutrient agar with 10% skimmed milk (Harrigan & McCance, 1976) respectively.

Bacteriological status of canned meat curry

Ten grams of homogenized sample was used for enumeration of aerobic and anaerobic, mesophilic and thermophilic spores on dextrose tryptone agar.

Lipid extraction

Lipids were extracted from 87.5 g meat curry (Folch, Lees & Stanley, 1957). The solvent extracts after salt washing were dried, concentrated and weighed. The extracted lipids were dissolved in 50 ml chloroform and used for chromatograpy.

Thin layer chromatography (t.l.c.)

The neutral lipids present in the lipid extracts were separated on a preactivated silica gel plate (20 × 20 cm) using petroleum ether : diethyl ether : acetic acid (80 : 20 : 1) as the

Table 2. Microbial analysis of raw mutton

Test	Medium	Incubation	
		Temp. (°C)	Duration (hr)
1. Standard plate count	Plate count agar	30	48
2. Coliforms	Violet-red bile agar	37	24
3. Staphylococcus	Baird–Parker agar	37	48
4. Gram-negative count	Crystal violet tetrazolium agar	37	48

solvent system for the ascending development (Mangold & Malins, 1960). The separated compounds were detected with iodine and 2,7-dichloro fluorescein. The FFA band was marked, eluted with chloroform, dried and concentrated. The residue was weighed and redissolved in 1 ml chloroform.

Esterification of fatty acids

Aliquots of FFA solution containing 4–5 mg of the acids were converted to methyl esters using methanol-sulphuric acid (Chalvardjian, 1964). The esters were extracted, dried, concentrated and dissolved in a known volume of chloroform; 0.8 μl of this solution was used for gas liquid chromatography analysis.

Gas liquid chromatography (g.l.c.)

The fatty acid esters were separated on a 10′ × 1/8″ O.D., S.S. column packed with 5% DEGS on AW-DMCS chromosorb. The column was operated under isothermal conditions at 200°C, injector and detector temperature being 240°C. Flow rates of carrier nitrogen and hydrogen were adjusted to 45 and 30 ml/min respectively. The separated peaks were identified by comparing their retention times with those of the authentic compounds run simultaneously. The quantities were calculated by peak area measurements.

Results and discussion

The levels of standard plate counts of fresh mutton generally ranged from 10^{3-4} organisms/g. In minced meat the SPC was 10^{4-5} organisms/g. in spoiled meat 10^{9-10} organisms/g and in the spice–fat mixture prior to cooking the SPC was 10^{3-4} organisms/g. The reasons for the high initial microbial load on the fresh mutton and in minced meat was due to lack of refrigeration facilities in the slaughterhouses and at the retail outlets coupled with high ambient temperature (on an average during summer, temperature during day time will be 33–35°C). Generally, the type of microflora found on fresh mutton and also mutton undergoing aerobic spoilage include both proteolytic and lipolytic organisms such as *Staphylococcus*, *Micrococcus*, *Bacillus*, *Enterobacteria*, *Acinetobacter pseudomonads* and *Moraxella*. When meat was spoiled under controlled conditions (37°C for 18 hr), there was a considerable increase in *Staphyloccus*, total Gram-negative bacteria and coliforms, whereas when meat was stored at 5°C there was a gradual increase in the total number of organisms. It generally required 10 days to attain 10^{9-10} organisms/g. At this stage the dominant microorganisms was found to be pseudomonads (Vijaya Rao *et al.*, 1983). Cooking and canning of meat rendered the product commercially sterile. Immediately upon opening off-odours were perceivable

in Samples II and IV but not in control sample (Sample I) or in Sample III (10% adulterated)

Lipid content of the cans varied from 14–20%. Fractionation of the lipid extract on t.l.c. plate gave eight distinct bands of which the seventh band from bottom was the major component, corresponding to triglycerides. Of the several bands, the 5th band from bottom showed a significant increase in Samples III and IV and an enormous increase in Sample II compared to the control (Sample I). This was identified as free fatty acid. The quantities of total FFA in Samples I–IV are presented in Fig. 1.

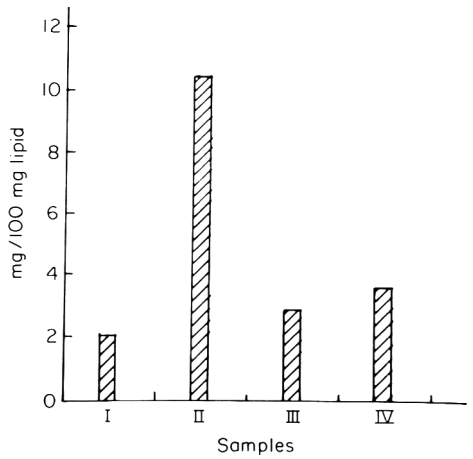

Figure 1. Quantity of total free fatty acid present in Samples I–IV.

The GLC separation of the FFA fraction revealed the presence of several fatty acids, of which only palmitic, stearic and oleic acids showed significant increases in Samples II–IV compared to Sample I. The quantities of these important fatty acids are given in Table 3. Increases in palmitic and oleic to a large extent and stearic to a small extent were clearly identifiable even at 10% adulteration level. But when the curry was prepared using only spoiled meat, there was a sharp increase in all three fatty acids. It appears that there is a preferential hydrolytic breakdown by glycerides containing palmitic, stearic and oleic acids by the microflora. A similar trend had been noticed even in our earlier studies on lean meat (mutton) (Vasundhara *et al.*, 1983). Addition of

Table 3. Quantities of important fatty acids found in the FFA fraction of canned meat curries prepared from fresh and spoiled meats

Sample description	Fatty acids mg/100 g curry (wet wt)*		
	Palmitic acid	Stearic	Oleic
I. Fresh meat—curry	8.29 ± 2.1	5.79 ± 2.4	8.76 ± 2.33
II. Spoiled meat—curry	95.75 ± 13.8	64.38 ± 14.98	119.89 ± 16.09
III. 10% mixture (10 g spoiled + 90 g fresh meat)—curry	25.4 ± 1.99	5.95 ± 1.48	17.44 ± 0.38
IV. 20% mixture (20 g spoiled + 80 g fresh meat)—curry	31.79 ± 3.67	8.86 ± 3.67	21.27 ± 6.01

*Values are means ± standard deviations for four determinations.

spices, adipose and vegetable fat did not interfere in the separation of free fatty acids by chromatography. A comparison of the present chromatographic method with that of the titrimetric method (Pearson, 1968) for the determination of free fatty acids revealed that the former is more objective and specific than the latter, which involves difficulty in the accurate detection of the end-point (which is not sharp due to interference by spices) thus introducing errors due to subjectivity. Using the titrimetric method, it was not possible to detect 10 and 20% adulteration in meat curries although at higher adulteration levels it was detectable. Whereas by the chromatographic method there was a significant increase in these fatty acids even at the 10% level and also at the 20% level. Hence the t.l.c. combined with g.l.c. yields more reliable results which will clearly detect if spoiled meat has been used for processing.

Acknowledgments

Thanks are due to Dr D. Vijaya Rao for the help rendered in carrying out microbiological analysis of the samples. Technical help rendered by Shri C. H. Siddiah while processing mutton curry is also acknowledged.

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Effect of colour on the assessment of ports

ANTHONY A. WILLIAMS, STEVEN P. LANGRON*, COLIN F. TIMBERLAKE AND JOHANNA BAKKER

Summary

Free choice profiling assessment of the appearance, aroma and flavour of ports in red and clear glasses by a panel of assessors of varying experience, coupled with generalized Procrustes analysis, pairwise Procrustes analysis and individual difference scaling (INDSCAL) has been used to investigate the relative information obtained from assessing appearance, aroma and flavour and to determine the influence appearance has on the assessment of aroma and flavour in this beverage. The assessment of aroma and flavour for the panel as a whole was shown to be influenced by ability to see the product. Exploring individual responses, however, indicated that the assessors varied in the importance they attached to appearance when differentiating the ports and also in the degree to which their assessments of aroma and flavour were influenced by appearance when assessing samples in clear glasses. It was also evident from the results that some assessors were showing similar relationships irrespective of whether assessing by aroma or by flavour. No obvious relationships were apparent between the experience of the assessor and his or her performance.

Introduction

When assessing wines, colour and appearance play an important role. In buying and quality control, these criteria are often heavily relied upon in making judgements because of the number of samples which have to be assessed at one session and the consequent fatigue effects on the other senses. One needs to know, however, how such assessments generally reflect the other sensory attributes, namely aroma and mouth flavour, which are possibly more important from the point of view of the consumer. It is also important to know how assessments of aroma and mouth flavour are influenced by preconceived ideas gleaned from a wine's appearance and colour.

Hedonically, the colour and appearance of wines have been shown to be important to overall acceptability, but their relative importance seems to vary depending on the assessor and the publication being consulted (Timberlake & Bridle, 1983; Tromp & Van Wyk, 1977; Williams, Baines & Arnold, 1980). Williams *et al.* (1980) showed colour to be important to acceptability, but by no means to have the same importance as mouth flavour, particularly where the consumer is concerned. Tromp & Van Wyk (1977) obtained an enhancement in quality with increase in colour and believed that, provided the wine did not vary greatly from an assessor's expectations, colour appeared to be the most important factor. Somers & Evans (1974) and Jackson *et al.* (1978) showed that colour density and pigment parameters provided objective guides to red wine quality. Timberlake (unpublished results) found that although the aroma and flavour scores of some wines were influenced by colour, with other wines the effects

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were not significant (Timberlake, 1976); he concluded that further work was desirable in this area.

Colour has also been shown to influence scores given by wine experts for sweetness in wines, but not to have the same effect on scores given by non-experts (Pangborn, Berg & Henser, 1963). Because of their experience, the experts presumably have preconceived associations which bias their assessments. Recent work on Bordeaux wines (Williams, Langron & Noble, 1984) has also shown that trained but non-expert panellists were able to remove bias due to colour when assessing aroma if explicitly instructed to do so. Under normal drinking conditions, however, there was evidence that some aroma attributes were being assessed differently if considered in association with visual attributes. The following experiment provides data on the relative information obtained by visual assessment and assessment of aroma and flavour in ports. It also endeavours to quantify the influence of appearance on the assessment of the other two attributes.

Materials and methods

Materials

Ten 1978 ports that varied in colour, aroma and flavour character were made available from the Long Ashton experimental programme. To respect confidentiality their detailed nature cannot be disclosed.

Assessors

A panel of eight men and two women assessed the wines. Two of these were very experienced port tasters involved in port production, a third was in a similar category but with less experience, and two were concerned with the buying and distribution of ports. These, together with two others, were experienced in the sensory profiling of ports. The remaining three were scientists involved in port research but who had not had any formal training in port evaluation.

Assessment procedure

The assessment procedure was the free choice profiling procedure previously described (Williams & Langron, 1984). Participants were the same as those used in that experiment, and their individual terminology was identical. All ten samples were presented at one session, the order for each assessor being that laid down by a 10×10 latin square (Cochran & Cox, 1957). Samples were assessed both in clear wine glasses (BS 5586) and in straight sided red coloured glasses (120 ml capacity). Both assessments were performed in duplicate and, as the examination formed an integral part of a larger assessment programme lasting 4 days, assessors, prior to carrying out assessments, were unable to deduce any association between repeats and between clear and coloured glass assessments of the same ports. Appearance, aroma and mouth flavour attributes were assessed when samples were presented in the clear glasses and aroma and mouth flavour only when samples were presented in the coloured glasses.

Analysis of data

Each assessor's data for each attribute from each of the replicated experiments were averaged. These mean scores, for appearance, aroma and flavour by mouth in the clear glass and for aroma and flavour in the coloured glass, were then examined in two ways.

In Procedure 1 the response of the panel as a whole was examined; in Procedure 2 individual variation in response was explored. The two are summarized in Fig. 1.

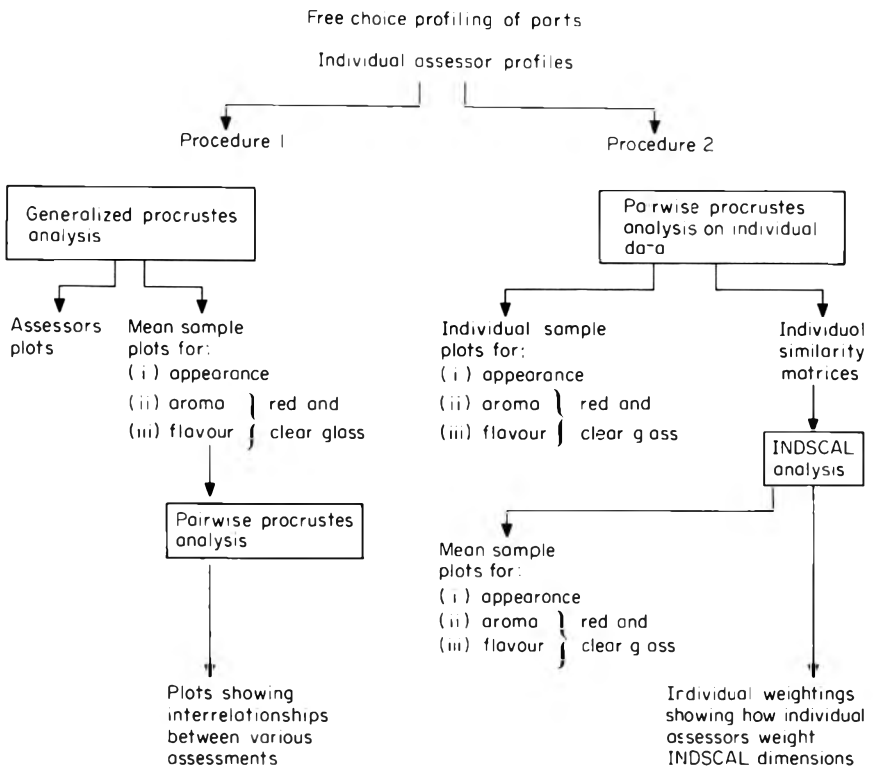

Figure 1. Survey of procedures used for the examination of ports following the assessment of appearance and of aroma and flavour in clear and red glasses.

Procedure 1. The individual mean scores were subjected to a generalized Procrustes analysis (Gower, 1975, 1976; Ten Berge, 1977; Langron, Williams & Collins, 1984) to determine the consensus configuration for the ten ports for aroma and flavour for the two experiments, and for appearance in the case of the assessments carried out in the clear glasses. Both sample plots and assessor plots were obtained for each of the five assessments.

To determine the interrelationships of these five spaces—i.e. how assessments based on appearance related to those based on aroma and flavour when the participants were both able and not able to see the wine—a pairwise Procrustes analysis was then performed (Gower, 1976; Langron & Collins, in press; Krzanowski, 1971; Banfield & Harries, 1975; Harries & MacFie, 1976) on the five sample configurations for appearance, aroma in a clear and coloured glass, and flavour in a clear and coloured glass. In the pairwise Procrustes analysis the five sample spaces are transformed, rotated and scaled as in generalized Procrustes to minimize the overall distances between the same samples in each of the various assessments, resulting in ten satellite configurations each consisting of five sample points (one for each of the assessments). Following this treatment the distances between the five sample points for each of the ten samples are calculated. Taking the mean of these distances provides a mean similarity matrix showing the similarities between the five assessments. This can be entered into a

principal co-ordinate analysis enabling a pictorial representation of the relationship between various sets of observations to be produced. Such an analysis gave a picture of the interrelationships between the five assessments and how the panel as a whole was being influenced by appearance when making judgements about aroma and flavour when appearance attributes were made available to them.

Procedure 2. To discover more about individuals, a pairwise Procrustes analysis was performed on each individual assessor's mean scores for the ten samples in each of the five assessments. Principal co-ordinate analysis on the residual distances after stretching, shrinking and rotating allowed plots to be derived enabling the similarity in the interrelationships between the samples when assessed under the five different experimental conditions to be summarized for the ten individual assessors rather than for the panel as a whole. To find out how these various individual experimental configurations relate, a three-way INDSCAL analysis was performed (Williams & Langron, 1984; Carrol & Chang, 1970; Schiffman, Reynolds & Young, 1981) on each individual's similarity matrix resulting from the pairwise Procrustes analyses. This analysis allows an overall plot of the relationship between the various assessments to be obtained similar to that from the original pairwise Procrustes on the five experimental consensus configurations resulting from the generalized Procrustes analyses. However, in an INDSCAL analysis, data points for individual assessors can be given different weightings on the resultant dimensions in order to minimize the residual between the final concensus configuration and that of individuals. Examination of the weightings which have been applied to the consensus configuration to reproduce individual sample spaces makes it possible to discover just how the individual assessors are performing in relation to one another in terms of the importance they are attaching to the dimensions separating the configurations produced from the various assessments.

Results and discussion

Results of generalized Procrustes analysis

The first two dimensions, following Procrustes analysis of the assessors' data for the five assessments, appearance, aroma in a clear and coloured glass, and flavour in a clear and coloured glass, accounted for 97, 91.3, 85.3, 35.2 and 74.9% of the variation of the final consensus plots, respectively. Examination of the sample plots on these first two dimensions showed little obvious agreement between the results of the various evaluations, in particular between those relating to the two aroma and the two mouth flavour assessments, where one might have expected some agreement if assessors were not being influenced by visual aspects when scoring these attributes in clear glasses. Inspection of the assessor plots showed that the first two dimensions accounted for 47% of their variation in respect to scores given to appearance, 51.4 and 43.3% in respect to aroma and mouth flavour in clear glasses and 49.7 and 45.5% in respect to aroma and mouth flavour in coloured glasses. The two experienced port assessors (1 and 2), when assessing appearance, were scoring differently from the remaining eight, who all behaved very similarly (Fig. 2). When it came to aroma and flavour, however, no grouping could be obtained, all assessors behaving either equally similarly or equally differently from each other.

Results of pairwise Procrustes analysis on sample consensus configurations

The experimental plot over the first two dimensions following pairwise Procrustes

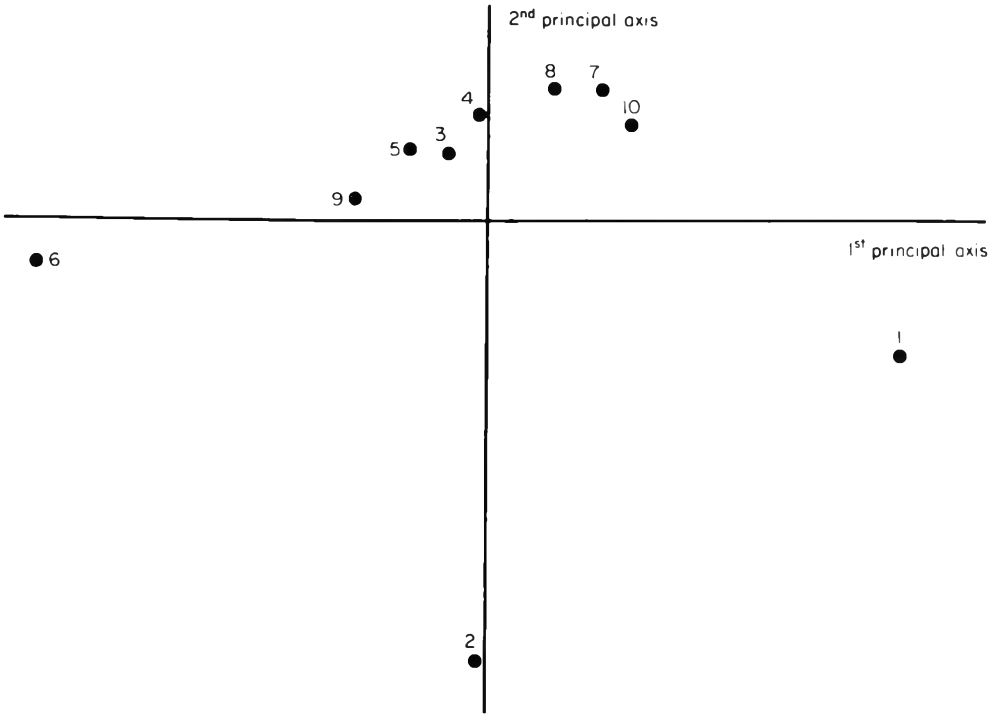

Figure 2. Assessor plot following generalized Procrustes analysis of appearance data.

analysis of the various consensus sample configurations is given in Fig. 3, these two dimensions accounting for 82% of the variation. Overall, there is some indication that the assessment of flavour in a clear glass and the two aroma assessments are grouping together. More interesting is the information conveyed by axis 1: the configuration produced on the basis of appearance is away from the others, but that produced from aroma and mouth flavour in the clear glass is closer to that for appearance than the respective assessments in the coloured glass. This seems to indicate that when assessors are able to see the ports, it influences, but by no means dominates, their assessment of aroma and mouth flavour.

Examination of the similarity matrix from which the actual plot was derived (Table 1) showed that the assessments of aroma and flavour were in general closer to those for appearance than they were to each other, irrespective of whether samples were in coloured or clear glasses. Inspection of the residuals (distance from centroid) for each point indicated that this was largely due to sample 10 having a substantially different appearance from the remainder. Removing this from the analyses produced the similarity matrix given in Table 2 in which the effect of appearance is reduced, whereas other similarities, with the exception of that between flavour assessment in coloured glasses compared to aroma assessment in coloured glasses, are only minimally affected. The relationship between the various assessments produced by principal co-ordinate analysis on this reduced similarity matrix gave the plot illustrated in Fig. 4, in which the influence of appearance on the assessments in the clear glasses is much more pronounced. In this particular examination the first two axes account for 89.9% of the variation in the data. Not only are the configurations produced by assessments in the clear glasses closer to the appearance configuration than those in the coloured glasses, but they are very similar to one another, whereas the two assessments in which

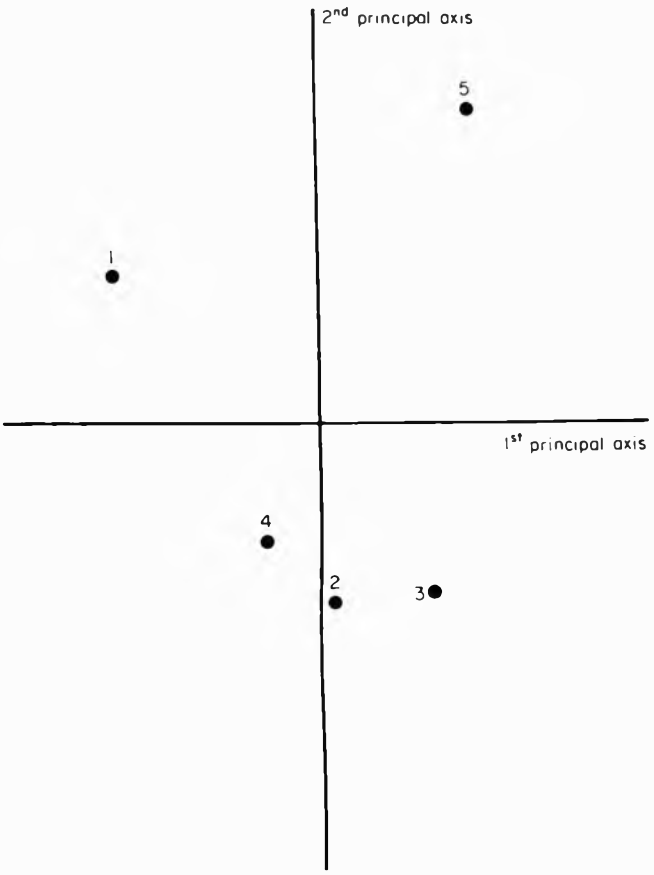

Figure 3. Experimental plot from pairwise Procrustes analysis of consensus configuration following generalized Procrustes analysis of appearance (clear glass [1], aroma (clear [2] and red glass [3]), and mouth flavour (clear [4] and red glass [5]) data (full panel).

Table 1. Similarity matrix following pairwise Procrustes analysis (all ten ports)

Assessment	Appearance	Aroma (clear glass)	Flavour (clear glass)	Aroma (coloured glass)	Flavour (coloured glass)
Flavour (coloured glass)	0.5216	0.5303	0.3012	0.3915	1.00
Aroma (coloured glass)	0.2171	0.0978	0.2118	1.00	
Flavour (clear glass)	0.5324	0.196	1.00		
Aroma (clear glass)	0.3471	1.00			
Appearance	1.00				

appearance had no effect are completely different. When allowed to see the ports, assessors' judgements or aroma and flavour are influenced by appearance to the extent that they are similar, whereas when the visual cue was removed, they assessed the aroma and flavour attributes more independently.

Influence of glass shape. The fact that the clear glasses and red glasses used in this experiment were of different shape must not be overlooked as possibly causing the effects described above, particularly as it is strongly believed that aromas are more

Table 2. Similarity matrix following pairwise Procrustes analysis (less port 13)

Assessment	Appearance	Aroma (clear glass)	Flavour (clear glass)	Aroma (coloured glass)	Flavour (coloured glass)
Flavour (coloured glass)	0.3559	0.3258	0.1357	0.3792	1.00
Aroma (coloured glass)	0.1107	0.0772	0.2053	1.00	
Flavour (clear glass)	0.3648	0.5746	1.00		
Aroma (clear glass)	0.1319	1.00			
Appearance	1.00				

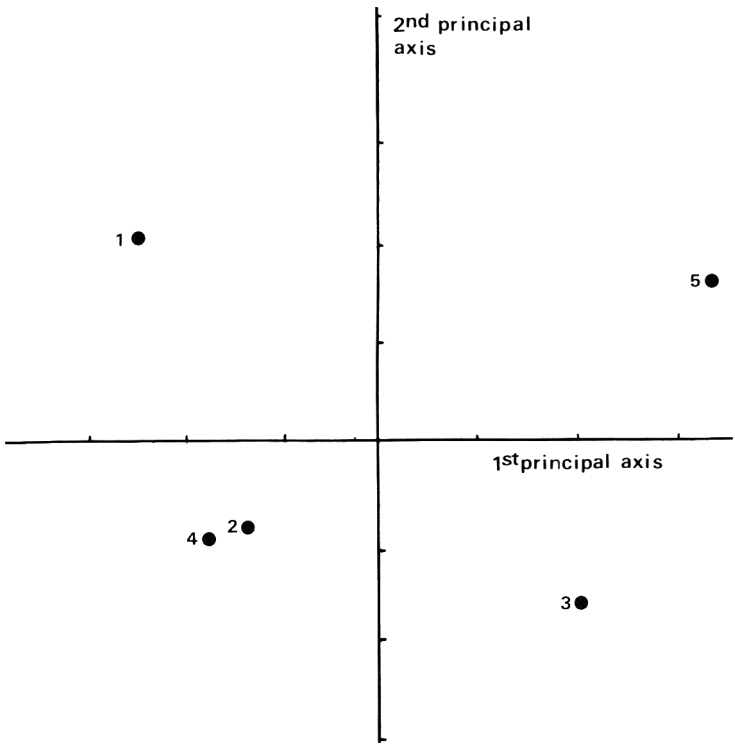

Figure 4. Experimental plot from pairwise Procrustes analysis of consensus configurations following generalized Procrustes analysis of appearance (clear glass [1], aroma (clear [2] and red glass [3]) and mouth flavour (clear [4] and red glass [5]) data (reduced panel).

easily perceived in tulip-shaped glasses. A previous experiment (Williams *et al.* 1984) examining the aromas of red wines, has already indicated that the shape of the glass had minimal effect when assessing sample interrelationships based on aroma. One would also expect the shape of the glass to have only minimal influence on the relative assessments of the appearance of a set of wines. In this experiment assessments based on aroma and flavour in the clear glasses are closer to that based on appearance and to each other than they are to the assessments in the red glasses. If glass shape was an important factor, differences might be expected between clear and coloured glasses, but there is no reason why assessment in the clear glasses should bring the sample configuration closer to that derived by appearance; it is most likely that the dominating

factor in making this shift is appearance and not glass shape. Also, if the shape of the glasses was important, one would expect the assessment of aroma in the clear and red glasses to show greater differences than that of flavour in the red and clear glasses where the influence of glass shape would be less. The contrary is the case in this experiment again leading to the conclusion that the ability to make use of visual cues is the dominant factor and not glass shape.

Results of the INDSCAL analysis on similarity matrix derived from pairwise Procrustes analysis of individual assessors' configurations

Comparison of the correlation coefficients for the mean residuals from the pairwise Procrustes analysis with those from the one, two and three dimensional solution, which gives an approximate estimate of the contribution made by each of the axes in the three dimensional solution, is given in Table 3.

Table 3. Correlation coefficients of mean intersample distance with the mean intersample distances from the first three INDSCAL solutions

		Difference
One dimensional solution	0.5747	0.5747
Two dimensional solution	0.7448	0.1701
Three dimensional solution	0.8275	0.0827

Examination of the relationship between the five sets of assessments (Figs 5 and 6) gave similar groupings to those obtained by pairwise Procrustes analysis (Figs 2 and 3) and provided some indication of the meaning of the INDSCAL dimensions. On axis 1, assessment in clear glasses appears to coincide with assessment by appearance. This dimension also contrasts assessments in clear glasses with assessment in coloured glasses and, as a consequence, brings out the influence of appearance on aroma and mouth flavour assessments, those assessors weighting heavily on this dimension being influenced by appearance. Axis 2 contrasts the configuration produced by appearance with those obtained from the chemical senses irrespective of whether made in clear or coloured glasses. Axis 3 contrasts flavour and aroma assessments, particularly those assessed in coloured glasses.

The assessor plots over the three INDSCAL dimensions are given in Figs 7 and 8. They show that assessors divided into five groups. Using the information from the sample plots to interpret these groupings, we find that group 1 (assessors 2 and 3) readily differentiated appearance assessments from those derived from aroma and taste irrespective of whether the latter were considered in clear or coloured glasses (high weighting on axis 2). Because of their low weighting on axis 1, they also appear to give little weighting to the difference between coloured or clear glass assessments—i.e. showing they are not influenced by appearance aspects when assessing aroma and flavour. They also gave little weighting to the dimension which separated aroma configuration from flavour by mouth configuration (axis 3). In fact, all assessments, apart from appearance, are the same; assessments in clear glasses are uninfluenced by appearance, but they are unable to distinguish between aroma and flavour.

Group 2 assessors (1, 7, 8 and 10) produced different spaces when assessing samples in coloured and clear glasses (high weighting on axis 1), the latter being similar to the

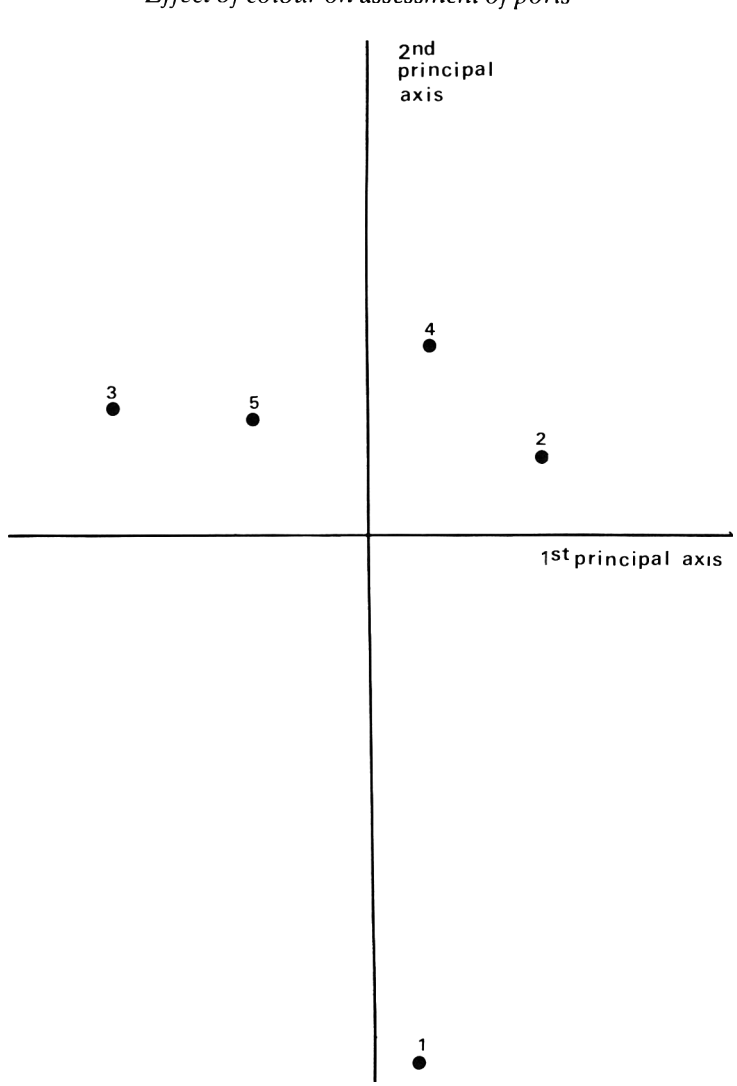

Figure 5. Experimental plot following INDSCAL analysis (axes 1 and 2). 1 = appearance in clear glass; 2 = aroma in clear glass; 3 = aroma in coloured glass; 4 = mouth flavour in clear glass; 5 = mouth flavour in coloured glass.

appearance space. This is confirmed by a low weighting on axis 1. They also assess samples differently by aroma than they do by flavour (medium weighting on axis 3). To summarize, they can distinguish aroma from taste, but their scores are biased by appearance.

Group 3 (assessors 4 and 6) did not appear to distinguish markedly between any of the assessments (i.e. low weighting on all axes).

Group 4 (assessor 9) distinguished between aroma and flavour assessments (high weighting on axis 3) and did not distinguish between samples in clear and coloured glasses—i.e. was uninfluenced by appearance (low weighting on axis 1). Assessments based on appearance were only marginally different from the rest (medium weighting on axis 2).

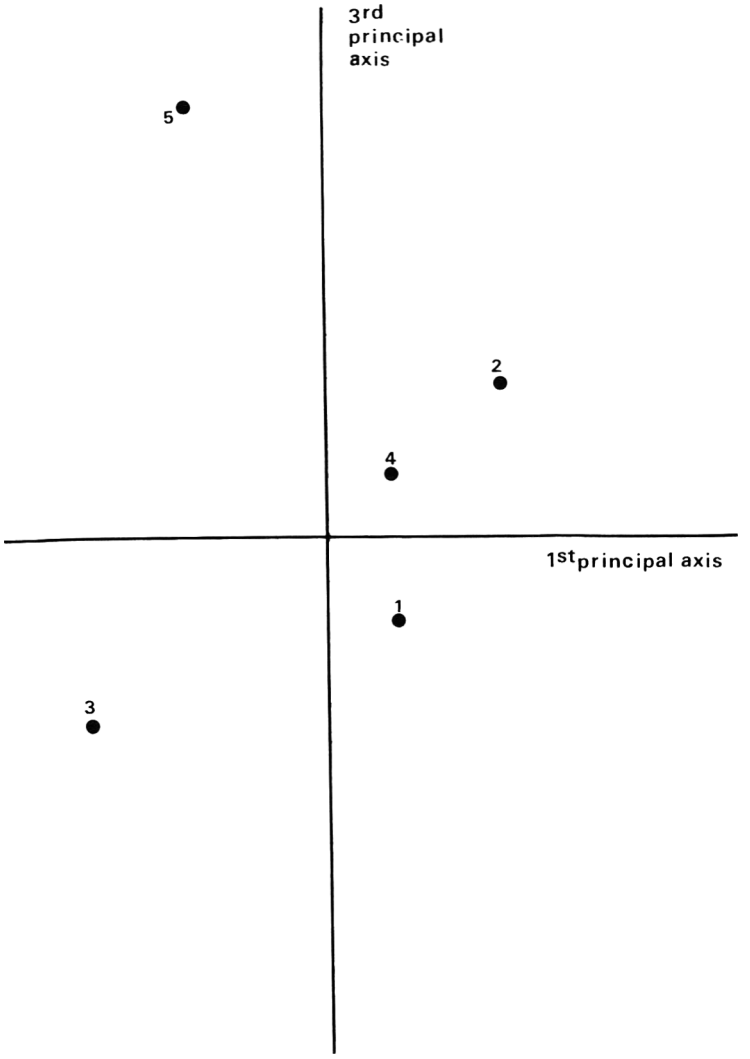

Figure 6. Experimental plot following INDSCAL analysis (axes 1 and 3). 1 = appearance in clear glass; 2 = aroma in clear glass; 3 = aroma in coloured glass; 4 = mouth flavour in clear glass; 5 = mouth flavour in coloured glass.

Group 5 (assessor 5) distinguished coloured and clear glass assessments—i.e. was influenced by appearance (high weighing on axis 1), but did not distinguish either those based on appearance from the rest or those based on aroma from flavour assessments (low weighting on axis 2 and 3). He, like those in group 2, appears to have his assessment of aroma and mouth flavour influenced by appearance, but unlike that group, did not distinguish assessments by aroma from those by flavour.

Similar examinations, with sample 10 omitted which improved results as far as the pairwise Procrustes examination of the consensus configuration was concerned, were similar in respect to the plots showing the relative position of the ports assessed by the five procedures. Axis 3, however, was now dominated by the difference between assessments of flavour and aroma in clear glasses rather than in coloured glasses, as was the case of the examination of the complete data.

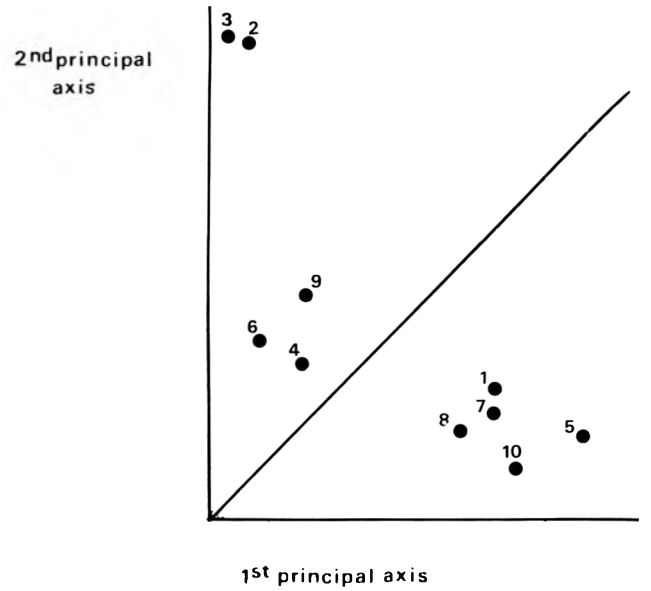

Figure 7. Assessor plot from INDSCAL analysis of similarity matrix following pairwise Procrustes analysis of assessor' raw data (axes 1 and 2).

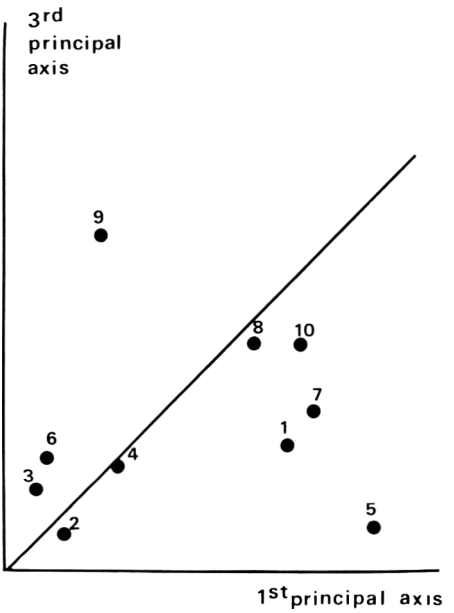

Figure 8. Assessor plot from INDSCAL analysis of similarity matrix following pairwise Procrustes analysis of assessor' raw data (axes 1 and 3).

Examination of the assessor plots again gave similar groupings of assessors. In this examination, however, those assessors in group 3 (assessors 4 and 6) were applying much higher weightings to axis 3 which largely discriminated assessments of aroma and flavour in clear glasses rather than in the coloured glasses. as was the case in the first examination. One can only conclude that the assessments of aroma and flavour are influenced by appearance, but rather than becoming similar to the appearance assess-

ment they are more different from it and from one another than when assessed in coloured glasses.

None of the assessors in this experiment was perfect. The ideal person would have had high scores on dimensions 3 and low on 1. His or her scores on dimension 2 would relate to the relative assessment by appearance in relation to aroma and flavour, but whether this should be high or low cannot be judged. On these criteria, assessor 9 would appear to be the most objective person taking part in this exercise. Assessors 2 and 3 evaluated samples by appearance differently from the rest and did not differentiate between aroma and flavour when the samples were presented in coloured and clear glasses. On this basis they were not unduly biased in their assessment of aroma and flavour by appearance. Unfortunately, a low weighting on 3 meant that neither were they discriminating between aroma and flavour assessments. Assessors 1, 7, 8 and 10 gave vastly different scores to assessments in coloured and clear glasses. Appearance assessments were not significantly different from those for aroma and flavour in clear glasses; therefore one must conclude that they were highly influenced by appearance. Their one redeeming feature was their ability to produce different response patterns when assessing the ports by aroma and taste. Assessor 5's assessments were greatly influenced by appearance, and he was unable to distinguish between aroma and flavour assessments. Assessors 4 and 6 are the most difficult to interpret. On the whole, they do not appear to be good assessors, giving similar information irrespective of whether scoring appearance, aroma or mouth flavour. There is, however, a hint that their assessments of aroma and mouth flavour were influenced by appearance, but in a different way from the other assessors. For these assessors the shape of the glass may have had an influential effect.

Conclusion

The results of examination of consensus data clearly indicated that the panel as a whole assessed appearance, aroma and mouth flavour differently when the latter two attributes were not confused by visual cues. The panel was, however, influenced by appearance when scoring aroma and mouth flavour if this attribute was made available to them during assessment. From the fact that overall aroma and flavour by mouth assessments in the clear glasses are similar on the first two dimensions, accounting for 82% of the variation, one may also conclude that appearance attributes, when available, will dominate the assessment of the other two attributes.

Examination of the data obtained from the analysis of the relationship between the various assessments by individual assessors was more difficult to interpret. The different assessment by appearance can largely be attributed to two judges who were obviously much more certain in their use of visual cues than they were in using the chemical senses. Half the panel appeared to be greatly influenced by appearance aspects when assessing aroma and mouth flavour, whereas the remainder were unable to dissociate these stimuli. Inspection of the third axis from the INDSCAL analyses also indicated that some of the assessors were producing different sample spaces when assessing by aroma than when assessing by mouth, whereas others were not.

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Approaches to the effective utilization of *Haplochromis* spp. from Lake Victoria. I. Chemical composition in relation to utilization

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Summary

There are over 150 species of the cichlid genus *Haplochromis* in Lake Victoria constituting a major underexploited food resource. As an aid to the processing of the deepwater stock, chemical composition data were obtained for the whole fish (separated into weight groups) and for the head, viscera, flesh and residual portions separately. Data are reported for lipid content, fatty acid composition, crude protein, true protein, amino acid composition, ash and moisture content.

Introduction

Large stocks of *Haplochromis* spp. (family Cichlidae) are present in many lakes in Africa. In Lake Victoria there are over 150 species of *Haplochromis* forming a species flock and in any commercial catch a large number of species are present. Fish stock assessment studies of Lake Victoria carried out in the early 1970s indicated that *Haplochromis* constituted about 80% by weight of the fish stock with a potential annual yield of about 200000 tonnes. Although the *Haplochromis* stock has since declined in shallow waters, the deepwater stock (below 20 m) is thought to be large enough to permit a much higher *Haplochromis* catch than the present total annual catch of about 50000 tonnes (CIFA, 1982).

In Uganda, the main method of processing *Haplochromis* s sun-drying, although some large fish are sold fresh or hot-smoked. In general, demand for *Haplochromis* is low compared with other commercially exploited fish, such as *Tilapia* spp. and *Bagrus* spp., and this is in part due to the small size of *Haplochromis*—i.e. typically 70–110 mm in length.

Proposed increases in landings of *Haplochromis* in Uganda necessitate the development of alternative methods of preservation and processing as a means of creating demand and extending its consumption to the population in the inland areas (CIFA, 1982; Dhatemwa, Hanson & Knowles, in press). Information about the chemical composition of *Haplochromis*, including distribution of components and variation in chemical composition with fish size and with season, is important in determining the most effective methods for utilizing this resource.

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Materials and methods

Fish samples

Four batches of *Haplochromis* were caught by bottom trawling (depth 25–45 m) from the Kitubulu-Nsazi, Uganda, inshore fishing ground of Lake Victoria during May, October and November 1980, and February 1981. The fish were frozen, packed into insulated boxes and flown to the U.K. They were kept in cold store (−25°C) until analysed. Most of the analytical work was carried out on the February batch.

Grouping and portioning of Haplochromis

Thawed fish were weighed (to the nearest 0.1 g) and the total length (including caudal fin) of each fish was measured (to the nearest mm). The fish were sorted into four weight groups: Group I: 5.9 g and less; Group II: 6.0–8.9 g; Group III: 9.0–13.9 g; and Group IV: 14 g and above. These four weight groups corresponded approximately to total lengths of: 80 mm and less, 81–89, 90–99 and 100 mm and above, respectively.

Analyses were carried out either on the whole fish or on samples taken after dividing the fish into head, viscera, flesh (skinless) and residual portions. The head portion was removed by a single cut immediately behind the pelvic and pectoral fins. Any visceral material cut off with the head portion was removed and included with the viscera.

Representative samples for each weight group were taken after passing the whole fish (fifty to 500 fish depending on the weight group) or the portions (from fifty to 500 fish) several times through a mincer.

Proximate analysis

Lipid content was determined by a modified Bligh and Dyer technique (Hanson & Olley, 1963). Crude protein content (total N×6.25) was determined by the Kjeldahl technique. Ash content was determined to 500°C. Moisture content was determined by drying samples to constant weight at 105±2°C. Analyses were carried out in triplicate.

Fatty acid composition

Lipid was extracted by the modified Bligh and Dyer method using chloroform containing 0.01% BHT and the solvent as evaporated using a rotary evaporator and vacuum pump at room temperature.

Methylation. One hundred to 150 mg of lipid was saponified by adding 2 ml toluene and 4 ml sodium hydroxide in methanol (1.5 : 228 w/v) in a 50 ml round-bottomed flask and refluxing for 30 min. After cooling, 5 ml boron trifluoride/methanol (12–14% w/v) was added and the mixture was refluxed for a further 30 min. The methyl esters were extracted 3 times with 35 ml portions of hexane, dried with anhydrous sodium sulphate and concentrated using a rotary evaporator.

Gas chromatography. Chromatographic analysis was carried out using a 2 m column packed with 10% SP 2330 on Chromosorb WAW 80-100 operated at 155–230°C. The methyl esters were identified by comparison with two standards: 'PUFA I' supplied by Chromatography Services, Hoylake, and GLC Reference Mixture No. 3 (No. 32246) supplied by Chrompack U.K., Ltd. Analyses were carried out in triplicate.

True protein and non-protein nitrogen

True protein was determined by the method outlined by Cutting (1969). Approxi-

mately 2 g of sample was accurately weighed and ground (pestle and mortar) with 10 ml of 15% trichloroacetic acid solution (TCA). The TCA soluble material was recovered by filtration and the solid residue was washed with four 10 ml portions of 15% TCA. The nitrogen content of the residue (true protein nitrogen) was determined by the Kjeldahl technique. Non-protein nitrogen was calculated as the difference between the total nitrogen of the sample and the true protein nitrogen. True protein content was calculated as true protein nitrogen × 6.25. Determinations were carried out in triplicate.

Amino acid composition

The samples were hydrolysed using the standard 6 M hydrochloric acid method. The amino acids were separated and the amounts determined using an LKB amino acid analyser with norleucine as an internal standard.

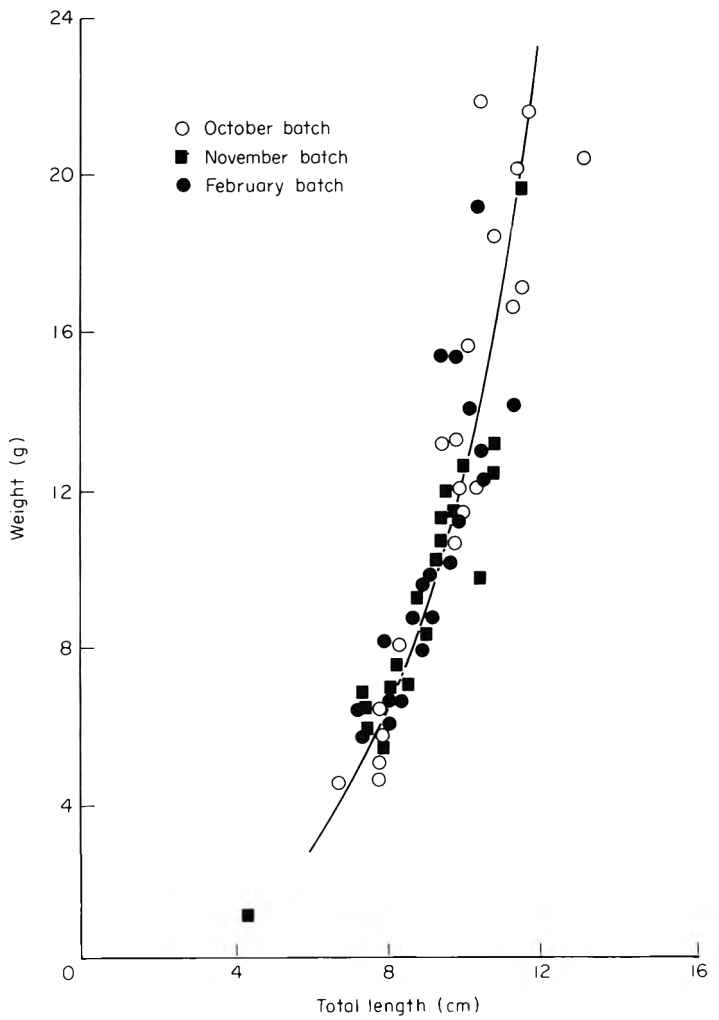

Figure 1. Relationship between weight and total length for *Haplochromis* taken randomly from the October, November and February batches.

Table 1. Weight group distribution and weight composition (February batch)

	Weight group distribution*		Weight composition (% of whole fish)‡				
	Number of fish (%)	Weight of fish (%)	Head	Viscera	Flesh	Residue	Flesh + residue
Group I (5.9 g and less)	8.6	4.5	36.2	10.5	—	—	53.3
Group II (6.0–8.9 g)	61.9	50.5	36.7	9.0	28.1	26.2	54.3
Group III (9.0–13.9 g)	19.7	21.7	36.0	10.4	25.4	28.2	53.6
Group IV (14 g and above)	9.8	23.3	36.9	9.6	—	—	53.5

*For a sample of 1000 fish taken randomly from the whole batch.

‡For eighty-six fish in Group I, 619 fish in Group II, 197 fish in Group III and ninety-eight fish in Group IV.

Table 2. Proximate analysis data (February batch)*

Sample	Lipid (%)	Crude protein (%)	Ash (%)	Moisture (%)
Whole fish				
Group I	5.0 (0.6)	16.0 (0.9)	5.1 (0.4)	70 (4.0)
Group II	6.0 (0.2)	16.4 (0.3)	5.4 (0.1)	71 (0.4)
Group III	5.7 (0.6)	17.1 (0.7)	5.8 (0.6)	74 (1.7)
Group IV	4.4 (1.1)	16.8 (0.7)	5.1 (1.1)	71 (0.4)
Average‡	5.5	16.6	5.4	72
Group II				
Head	8.9 (0.6)	15.0 (0.5)	8.7 (0.1)	69 (0.2)
Viscera	12.7 (0.7)	10.8 (0.4)	1.6 (0.1)	79 (0.4)
Flesh	1.6 (0.8)	19.2 (0.7)	1.1 (0.1)	77 (0.1)
Residue	4.2 (0.3)	17.2 (0.7)	7.6 (0.6)	70 (1.0)

*The percentages are the averages of three determinations; standard deviations are given in parentheses.

‡Weighted average calculated using the weight group distribution data in Table 1.

Results and discussion

No attempt was made to divide the batches of *Haplochromis* according to species or groups of species since members of the *Haplochromis* spp. flock are very similar in appearance and cannot easily be distinguished (Greenwood, 1974). However, it was possible to show by gel electrophoresis (Mackie, 1980) that the batches did contain large numbers of species (Ssali, 1981). Rather than analyse according to species, a size parameter was used. Dividing catches according to size before processing could be commercially viable if the chemical composition of the fish varied significantly with size. Weight was chosen as the grouping parameter since it could be measured rapidly and since it correlated well with other size parameters—e.g. length. A plot of weight against length gave a smooth curve typical of those found for single species (Fig. 1).

The smallest fish (5.9 g and less) were placed in Group I. The majority of the fish, present in the relatively narrow weight range of 6.0–8.9 g, were placed in Group II. The remaining fish, which varied widely in weight, were placed in Group III (9.0–13.9 g) and Group IV (14 g and above). The weight group distribution data given in Table 1 for

Table 3. Percentage lipid content and lipid distribution for the February batch (separated into weight groups)

Sample	Group I	Group II	Group III	Group IV
Lipid content (%)				
Head	7.8 (1.1)	8.9 (0.6)	8.1 (1.6)	6.0 (0.8)
Viscera	9.8 (4.2)	12.7 (0.7)	12.7 (1.6)	9.0 (7.4)
Flesh	—	1.6 (0.8)	1.3 (0.1)	—
Residue	—	4.2 (0.3)	4.1 (0.7)	—
Flesh + residue	2.2 (0.4)	2.9 (0.2)	2.7 (0.3)	2.6 (1.5)
Whole fish	5.0 (0.6)	6.0 (0.2)	5.7 (0.6)	4.4 (1.1)
Lipid distribution (%)				
Head	56	55	51	50
Viscera	21	19	23	19
Flesh	—	8	6	—
Residue	—	18	20	—
Flesh + residue	23	26	26	31

*The percentages are the averages of three determinations; standard deviations are given in parentheses.

Table 4. Percentage lipid content and lipid distribution for four batches (not separated into weight groups)

	Lipid content (%)*				Lipid distribution (%)		
Batch	Head	Viscera	Flesh + residue	Whole fish	Head	Viscera	Flesh + residue
May	—	—	—	4.7 (0.6)	—	—	—
October	10.0 (0.5)	16.0 (1.3)	3.3 (0.2)	6.6 (0.2)	55	18	27
November	8.4 (0.5)	13.3 (0.8)	2.9 (0.2)	5.8 (0.2)	54	19	27
February	8.0 (0.5)	11.7 (1.8)	2.8 (0.4)	5.5 (0.3)	53	20	27

*Averages of twelve determinations, i.e. triplicates for each weight group; standard deviations are given in parentheses.

the February batch are typical of those found for the other batches. On average, Group I constituted about 5–10% by weight of each batch, Group II about 50% and Groups III and IV about 20% each.

From the data given in Table 1 for the proportions of head, viscera, flesh and residue, it can be seen that for all four weight groups the head and viscera together represented almost half of the total weight of the fish, and the flesh was less than 30% of the total weight.

Proximate analysis data for the February batch are given in Table 2 for whole fish for the four weight groups, and for head, viscera, flesh and residue for Group II. The data for the four weight groups do not differ significantly ($P > 0.05$) for any of the constituents. The average values for crude protein and moisture (16.6 and 71% respectively) are close to the values (16.0 and 69.9%) reported by Meynell (1979) for chisawasawa, a mixture of *Haplochromis* and *Lethrinops*, from Lake Malawi. The average lipid content (5.5%) is lower than the 8.0% reported by Meynell and 8.7% by Disney (1974) for chisawasawa, but higher than the 3.15% obtained for *Haplochromis* from Lake Victoria (Tanzanian sector) (C.M. Dhatemwa, personal communication). However these lipid results cannot be compared directly because of the different

Table 5. Percentage fatty acid composition (February batch)*

Fatty acids	Whole fish				Group II			
	Group I	Group II†	Group III	Group IV	Head	Viscera	Flesh	Residue
C14:0	5.0	5.2	4.6	3.2	5.9	3.1	3.0	6.0
	(0.2)		(0.1)	(0.1)	(0.3)	(0.1)	(0.4)	(0.3)
C16:0	23.3	22.4	20.8	21.7	21.6	25.8	21.9	21.5
	(0.4)		(0.1)	(0.6)	(0.6)	(0.1)	(0.6)	(0.6)
C18:0	5.1	5.6	5.3	5.4	5.1	5.8	7.9	5.8
	(0.2)		(0.2)	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)
ΣSaturates	33	33	31	30	33	35	33	33
C16:1	16.6	18.9	18.8	17.6	19.9	17.9	12.8	19.6
	(0.2)		(0.5)	(0.2)	(0.1)	(0.5)	(0.1)	(0.4)
C18:1	10.0	12.7	13.1	19.8	10.3	22.4	9.8	11.0
	(0.2)		(0.3)	(0.7)	(0.1)	(0.3)	(0.2)	(0.4)
C20:1	3.9	3.1	2.9	1.4	3.7	1.4	1.7	3.0
	(0.9)		(0.1)	(0.1)	(0.1)	(0.1)	(0)	(0.2)
C24:1	0.8	1.0	0.6	0.5	1.3	0.5	0.8	0.7
	(0.1)		(0.1)	(0.1)	(0.6)	(0)	(0.1)	(0.1)
ΣMonoenes	31	36	35	39	35	42	25	34
C18:2	2.1	2.2	2.4	2.9	2.4	2.0	1.5	2.3
	(0.1)		(0.1)	(0.2)	(0.1)	(0.2)	(0.1)	(0.3)
C20:5	3.9	2.9	3.4	1.7	3.1	1.8	3.2	3.1
	(0.1)		(0.1)	(0.1)	(0.4)	(0.1)	(0)	(0.2)
C22:5	4.0	3.3	4.0	2.4	3.7	2.1	3.5	3.4
	(0.1)		(0.3)	(0.3)	(0.1)	(0.1)	(0.1)	(0.1)
C22:6	9.1	5.6	6.8	6.0	5.2	3.6	11.4	6.3
	(0.3)		(0.2)	(0.4)	(0.5)	(0)	(0.3)	(0)
ΣPolyenes	19	14	17	13	14	10	20	15
C20:4/C22:1‡	3.6	2.5	3.4	2.6	2.8	2.2	5.2	3.2
	(0)		(0.1)	(0.1)	(0)	(0)	(0.1)	(0.1)
Unidentified GC peaks	6.2	7.0	6.1	7.9	6.3	5.9	10.1	8.9

*The percentages are averages of three determinations; standard deviations are given in parentheses.
†Calculated from the data obtained for the head, viscera, flesh and residue.
‡Overlapping GC peaks.

species and fishing grounds involved and because the lipid content of *Haplochromis* appears to show some seasonal variation, as mentioned below.

The low lipid levels in the flesh, and relatively high levels in the head and viscera, were confirmed by the lipid content and distribution data given in Table 3 for the different weight groups (February batch) and in Table 4 averaged over the four weight groups for the different batches. For all four weight groups, 50% or more of the lipid was found in the head with an average for the batch of 53%. Similar results were obtained for the October and November batches. The uneven distribution of lipid within the fish has important processing implications, as discussed below. The lipid content of the whole fish showed a variation between batches of only about 2%, with the highest value being obtained for the October batch (6.6%) and lowest value for the May batch (4.7%).

The fatty acid composition of the whole fish lipid (Table 5) did not vary significantly

Table 6. Crude protein, true protein and non-protein nitrogen content (February batch)

Sample	Crude protein* (%)	True protein* (%)	Non-protein nitrogen† (%)	Non-protein nitrogen† % of total N
Whole fish				
Group I	16.0 (0.9)	13.5 (0.3)	0.40	16
Group II	16.4 (0.3)	13.9 (0.7)	0.40	15
Group III	17.1 (0.7)	13.5 (1.1)	0.58	21
Group IV	16.8 (0.7)	13.3 (1.6)	0.56	21
Average‡	16.6 (0.3)	13.7 (0.6)	0.46	17
Group II				
Head	15.0 (0.5)	12.6 (0.4)	0.38	16
Viscera	10.8 (0.4)	6.9 (0.8)	0.62	36
Flesh	19.2 (0.7)	16.8 (0.7)	0.38	13
Residue	17.2 (0.7)	14.8 (1.7)	0.38	14

*The percentages are the means of three determinations; standard deviations are given in parentheses.

†Calculated from the crude protein and true protein analytical data.

‡Weighted average calculated using the weight group distribution data in Table 1.

between weight groups. For Group II, the flesh was found to contain a higher proportion of polyenes and lower of monoenes than the whole fish, presumably reflecting the higher unsaturation normally found in phospholipid in fish compared with triglyceride (Ackman, 1974). The fatty acid composition of the whole fish lipid does not differ appreciably from those of commercial fish oils (Windsor & Barlow, 1981).

Table 7. Amino acid composition, g amino acid/100 g crude protein (February batch)*

Amino acid	Whole fish		Group II	
	Group II	Group IV	Head + viscera	Flesh + residue
Aspartic acid	9.6	8.6	9.1	9.9
Threonine	4.4	4.1	4.1	4.7
Serine	4.5	4.0	4.6	4.5
Glutamic acid	15.1	14.6	13.0	16.4
Proline	5.1	4.9	5.6	4.8
Glycine	8.9	8.2	10.5	7.9
Alanine	7.5	7.7	7.3	7.7
Valine	4.6	4.1	4.5	4.7
Methionine	2.8	2.6	2.5	3.0
Isoleucine	4.2	3.9	3.6	4.6
Leucine	7.3	6.8	6.4	7.9
Tyrosine	3.3	2.9	3.2	3.3
Phenylalanine	4.0	3.8	3.9	4.1
Histidine	2.1	2.0	2.0	2.3
Lysine	7.6	7.4	6.7	8.2
Arginine	6.9	5.9	6.8	7.0

*Duplicate determinations did not differ for any of the amino acids by more than ±5%.

Table 8. Weight composition and distribution of selected components in Group II fish (February batch)

	Head	Viscera	Head + viscera	Flesh	Residue	Flesh + residue
Weight composition (%)	36.7	9.0	45.7	28.1	26.2	54.3
Component distribution (%)						
Crude protein	34	6	40	33	28	61
True protein	33	4.5	37.5	34	28	62
Lysine	—	—	35	—	—	65
Lipid	55	19	74	8	18	26
Ash	57	2.5	59.5	5.5	35	40.5

The crude protein, true protein and non-protein nitrogen content did not differ significantly between weight groups (Table 6). The average whole fish crude protein content of 16.6% is around the middle of the range of values obtained for whole fish (Meinke, 1974: Windsor & Barlow, 1981). For the Group II fish, the viscera had the highest non-protein nitrogen content and lowest protein content, presumably as a result of protein breakdown by digestive enzymes. The residue contained almost as much protein as the flesh and a similar proportion of non-protein nitrogen. The non-protein nitrogen content of the flesh is within the typical range for teleost fish (Simidu, 1961).

The amino acid composition of the Groups II and IV whole fish (Table 7) did not differ significantly, although slightly lower values were obtained for Group IV reflecting the lower true protein content of that sample. The higher lysine and methionine content of the flesh and residue fraction compared with the head and viscera fraction is due to the high myofibrillar and low connective tissue content of the flesh. This was apparent from analysis of the flesh alone, which gave higher values for lysine and methionine (9.8 and 3.2 g/100 g of crude protein) and lower values for the major constituent amino acids of collagen, proline and glycine (3.2 and 4.9 g/100 g of crude protein).

The uneven distribution of lipid and protein in *Haplochromis* is of importance with regard to processing. For example, for Group II fish (Table 8) removing the head and viscera results in the remaining material (about 54% by weight) having only 26% of the total lipid content of the whole fish, but over 60% of the protein and an even higher proportion of the total lysine content. The lipid-rich head and viscera fraction would be suitable for industrial processing—e.g. oil extraction. The flesh and residue farction, with a relatively low lipid and high protein content, would be more suitable than the whole fish for processing into human food products in which rancidity development could be a problem.

Conclusion

The analytical data obtained in this study on deepwater species of *Haplochromis* from Lake Victoria indicate that: (i) the chemical composition of the fish does not vary significantly with fish size, (ii) the average lipid content of the whole fish is about 5.5% and appears to vary little with season, and (iii) over 70% of the lipid content of the fish is present in the head and viscera.

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Growth of pathogenic bacteria in sliced vacuum packed Bologna-type sausage as influenced by nitrite

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Summary

The influence of sodium nitrite addition on growth of pure cultures of pathogenic bacteria in vacuum-packed Bologna-type sausage was examined. Three different levels of nitrite were studied (0.75 and 150 ppm added nitrite). All bacteria studied—*Staphylococcus aureus*, *Bacillus cereus*, *Yersinia enterocolitica*, *Salmonella enteritidis* and *Salmonella typhimurium*—were influenced by nitrite addition. The inhibition caused by nitrite was strongly temperature dependent for *S. aureus*. This bacterium did not develop in packages with 75 ppm added nitrite, but multiplied without nitrite at 10°C. At 10°C *S. enteritidis* had a long lag phase with 75 ppm added nitrite but not at 12°C. Other bacteria were less affected especially by 75 ppm added nitrite. Recommended refrigeration temperatures of 5°C in combination with high levels of nitrite (150 ppm) was not adequate in preventing growth of *Y. enterocolitica*, and at temperatures of 10–12°C also *B. cereus* and salmonellae developed in spite of nitrite addition.

Introduction

The current pressure for reducing the risk from carcinogens in cured meat products has led to a reduction in the amount of sodium nitrite added to cured, cooked meat products. Most microorganisms are eliminated during cooked sausage production, during heating of the sausage emulsion to *circa* 70°C, only spores of *Clostridium* and *Bacillus* being left intact. During slicing, the product is again contaminated with microorganisms and the bacteria then develop in the vacuum packages during storage when conditions are appropriate. If the concomitant flora be low on account of good hygiene during slicing and packaging, contamination with pathogenic bacteria at this stage of production might be hazardous, if other inhibitive factors such as the nitrite level were to be reduced.

Several studies have been concerned with the influence of sodium nitrite on growth and development of pathogenic bacteria in microbiological media. These studies showed that high levels of nitrite were necessary to prevent growth of pathogenic bacteria. Thus Castellani & Niven (1955) and Cantoni *et al.* (1981) reported that several thousand ppm sodium nitrite were necessary for inhibition of Gram-negative pathogenic bacteria such as salmonellae, *Shigella* and *E. coli* at 30°C.

Temperature was shown to be a very important factor in combination with sodium nitrite in inhibiting pathogenic bacteria in bacteriological media in studies by Shahamat, Seaman & Woodbine (1980) and Albalas & Roberts (1977). Decreasing the temperature made the inhibitive effect of nitrite more pronounced. Thus, while *S. aureus* grew with 400 ppm added nitrite at 15°C growth was only observed in media

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devoid of nitrite at 10°C (Albalas & Roberts) 1977). Likewise nitrite was shown to delay growth of *S. typhinurium* at intermediate temperatures (15.5 and 17°C) but not at higher temperatures (ibid).

Studies with broth cultures have shown that the inhibition caused by nitrite addition was highly influenced by water activity (a_w) as well as pH (Castellani & Niven, 1955; Skjeklvåle & Tjaberg, 1974). Already the old studies (Castellani & Niven, 1955) had shown that the undissociated nitrous acid was the important factor in non-heated broth cultures.

Heating sodium nitrite at high temperatures in microbiological media or in meat products results in the development of several antimicrobial substances (e.g. Perigo & Roberts, 1968; van Roon, 1979). This is, however, of minor relevance to pasteurized meat products under refrigerated storage. In these products, where the temperature during heating hardly exceeds 70°C, it is the residual nitrite concentration which is of importance (Nordin, 1969; Nielsen, 1983). Furthermore, the nitrite concentrations used are, for legislative reasons, much lower than those examined in bacteriological media.

Sporogenic bacteria are the only organisms which can be inoculated into the fresh sausage emulsion and recovered after heating (see above) and in experiments with nitrite and erythorbate only a combination of these substances inhibited *B. cereus* in cooked sausage (200 ppm added nitrite, 500 ppm erythorbate) (Raevuori, 1975). An added nitrite concentration of 100 ppm was unable to inhibit *B. cereus* with any combination of erythorbate (ibid). In contrast to *B. cereus*, *Cl. perfringens* inoculated into the sausage mix during production showed slow death during storage of the finished sausage at refrigerated or abuse temperature irrespective of nitrite addition (Hallerbach & Potter, 1981).

Experiments with frankfurters produced without and with added nitrite (concentration not stated) showed no significant difference in counts of *S. aureus* and salmonellae at high temperature or at low temperatures for *S. aureus* (Bayne & Michener, 1975). Other experiments with frankfurters (Rice & Pierson, 1982) showed that temperature was again, as in studies with culture media, an important factor in the nitrite inhibition of microorganisms. At high temperature (27°C), development of salmonellae was unaffected by 50 or 156 ppm added nitrite, while at low temperature (15°C) the high nitrite addition totally inhibited growth, leaving, however, the salmonellae more or less at their initial level. This is in accordance with the faster depletion of the added nitrite at higher temperatures (Nordin, 1969).

At lower temperature (8°C) slow death of salmonellae was oberved in studies by Leistner, Hechelmann & Uchida (1973b) in Bologna-type sausage produced with nitrite, while growth occurred in the product devoid of nitrite. Further experiments (Leistner *et al.*, 1973a) showed the combined effect of inoculation level and nitrite addition. Thus increases of three and one $\log_{10}$ unit were observed in Bologna-type sausage without nitrite inoculated with 10^4/g and 10^2/g respectively, while the increases in numbers in sausage produced with 42 ppm nitrite were one and zero respectively.

In a study of Bologna-type sausage inoculated with *Shigella* or *E. coli* and stored at 8°C, the temperature was apparently inadequate to support growth of the shigellae, while the numbers of *E. coli* rose one $\log_{10}$ unit in batches without nitrite or with 42 ppm, but not with higher nitrite concentrations (Leistner *et al.*, 1973a).

Relatively few studies have been published concerning the inhibitory effect of nitrite upon pathogenic bacteria in vacuum-packed cured, cooked meat products. The common belief is that several factors act in combination in the vacuum packages and prevent

the development of the pathogens in these products. Today the hygienic level is high in many processing plants. This is of course fortunate, when looking at the organoleptic quality, but accidentally contaminated packages will be devoid of a competing flora, with the result that pathogens will develop under suitable conditions. The trend of reducing the nitrite addition (and the sodium chloride concentration as well) might result in the pathogenic bacteria multiplying under such conditions. In the present study, three levels of nitrite (0, 75 and 150 ppm added nitrite) were examined. The pathogenic bacteria were studied in pure cultures on the Bologna-type sausage in an otherwise sterile product.

Materials and methods

Sausage production and package inoculation

Three batches of Bologna-type sausage (10 kg each) were produced using a standard recipe according to the following composition: beef (25.8%), pork (25.8%), trimmings (20.6%), dried milk (2.0%), potato flour (2.0%), caseinate (2.0%), polyphosphate (0.49%) and water (20.0%).

The meat was pre-salted with sodium chloride, and to two batches were added 75 ppm sodium nitrite the day before processing of the sausages. During production, to one of these two batches was added a further 75 ppm sodium nitrite, resulting in the three batches receiving 0, 75 and 150 ppm nitrite respectively. The sausages were cooked in a steam cabinet until a temperature of 75°C was reached in the centre. The sausages were cooled overnight, the casings removed and the sausages sliced using aseptic procedures. Five slices (50 g) were inserted into pre-made bags consisting of Rilotene, a polyamide polyethylene laminate (Otto Nielsen, Denmark) with an oxygen permeability of 52 ml/m^2/24 hr/1 atm at 25°C and 75% r.h.

0.5 ml of a diluted suspension of the appropriate organism or a mixture of organisms was inoculated onto the slices and the packages were vacuum packed. Packages were stored at appropriate temperatures.

Test organisms

Either single strains or mixtures of strains were used for inoculation. Strains used for experiments with *S. aureus* were Nos 1–5 all from the laboratory culture collection, and the *B. cereus* used (No. 1) and *S. typhimurium* (No. 1) were from the laboratory collection, while *S. enteritidis* and *Y. enterocolitica* serotype 03 (strain No. F5208/82, A, B, 17280 and 17193) were kindly supplied by Dr S. G. Christensen, Royal Veterinary and Agricultural University, Denmark. All bacteria were grown in brain heart infusion broth at 28°C (*Y. enterocolitica* at 25°C).

Sample preparation

At suitable intervals during storage, packages were taken for bacteriological analyses. The whole content of the package was removed after opening, placed in a bag with 150 ml sterile peptone water (0.1%) and homogenized for 1.5 min in a Stomacher. Duplicate packages were examined at each sampling stage. Using sterile peptone water appropriate dilutions of the meat slurry were prepared and plated on to brain heart infusion agar (Difco). Plates were incubated at 25°C.

Chemical analyses

Chemical analyses for sodium chloride, sodium nitrite and moisture were per-

formed as previously described (Nielsen, 1983) and pH was determined on the food homogenates using a combination electrode.

Statistical analyses

Data were analysed by analyses of variance (mean of duplicate packages taken at each sampling stage).

Results

Chemical analyses of the three batches revealed a sodium chloride concentration of 1.6%, a water content of 58.0% (salt/water 2.76), and a nitrite concentration of 1.7, 35.7 and 68.9 ppm in the batches with 0.75 and 150 ppm added nitrite, respectively.

Growth experiment with S. aureus

Growth experiments with *S. aureus* are shown in Figs 1 and 2. *S. aureus* (No. 1) was examined at 8 and 10°C. No growth was observed at 8°C (figure not shown). The mixture of *S. aureus* strains were examined at 10 and 12°C. At 10°C both levels of sodium nitrite were adequate in preventing the staphylococci from developing during 4 weeks. However, the bacteria did not die out during storage. Also in the experiment with the mixture of staphylococci nitrite restricted growth to less than 1.5 $\log_{10}$ unit. Both experiments with *S. aureus* showed that in Bologna-type sausage without added nitrite, storage at 10°C resulted in profound growth to more than 1×10^6/g during 4 weeks. From the 2nd to the 4th week numbers in packages without nitrite and with added nitrite were significantly different ($P < 0.05$). Increasing the temperature to 12°C (Fig. 2) resulted in growth even with 150 ppm added nitrite (75 ppm batch not investigated).

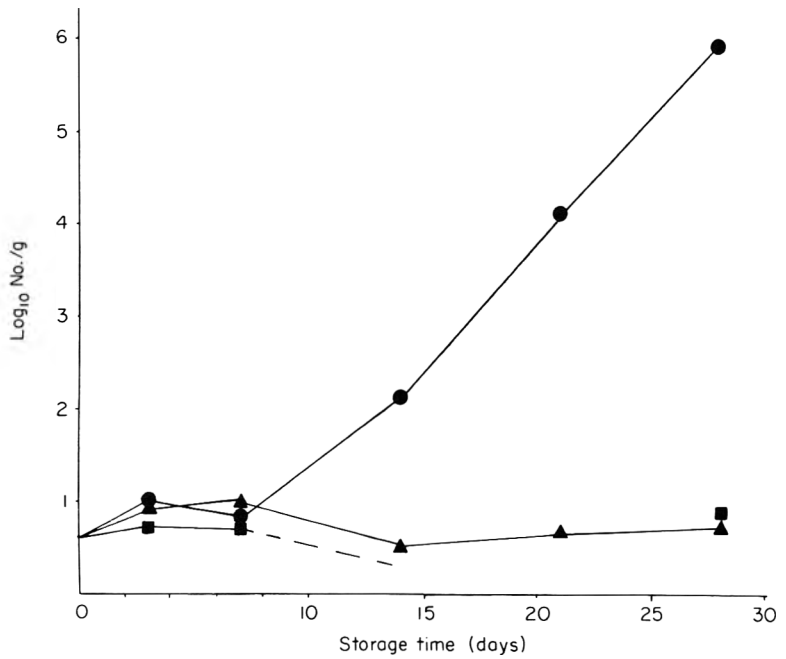

Figure 1. Growth of *S. aureus* (No. 1) at 10°C. O, 0; △, 75; and □, 150 ppm nitrite.

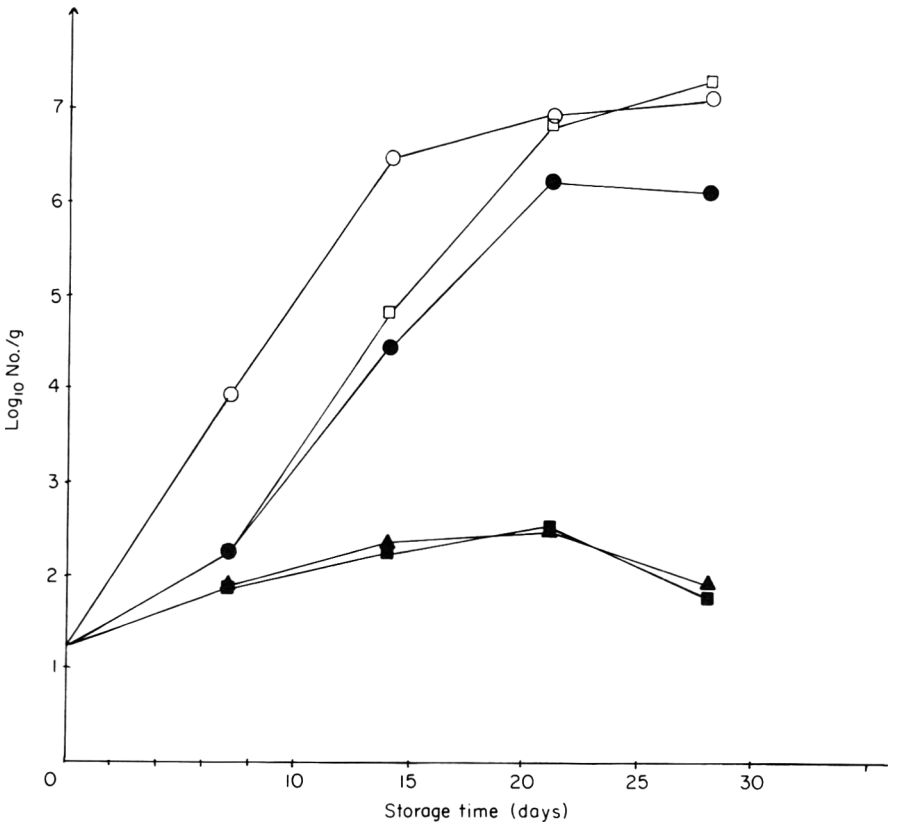

Figure 2. Growth of a mixture of *S. aureus* (Nos 2–5) at 10°C (closed symbols) and 12°C (open symbols). O, 0; △, 75; and □, 150 ppm nitrite.

Growth experiment with B. cereus

Growth experiment with a strain of *B. cereus* was done at 8 and 10°C (Fig. 3). No growth was observed at 8°C (figure not shown), while growth was highly influenced by nitrite at 10°C. Differences in numbers between the product with 150 ppm nitrite and the two other batches (with 0 and 75 ppm added nitrite) were significant during storage ($P < 0.05$).

Growth experiments with Y. enterocolitica

Growth experiment with a mixture of the two strains of *Y. enterocolitica* (Nos A and B) (Fig. 4) were done at 5 and 10°C. A decreasing growth rate with increasing nitrite addition was observed both at 5 and 10°C, maximum numbers being slightly lower in batches with nitrite than without at 5°C (differences in numbers between batches with 0 and 150 ppm nitrite were significant ($P < 0.05$)). Figure 5 shows growth curves for *Y. enterocolitica* (Nos 17193 and 17280) at 5 and 10°C. The same growth pattern was observed for these bacteria as for the former, the inhibition of 150 ppm added nitrite being more distinct at both temperatures. The experiment with *Y. enterocolitica* (No. F5208/82) was performed at 5°C only (Fig. 6) with a slight inhibition at 75 ppm added nitrite and a marked inhibition at 150 ppm. For all experiments with *Y. enterocolitica* pH fell to *circa* 6.0, 6.2 and 6.3 in batches with 0, 75 and 150 ppm added nitrite respectively.

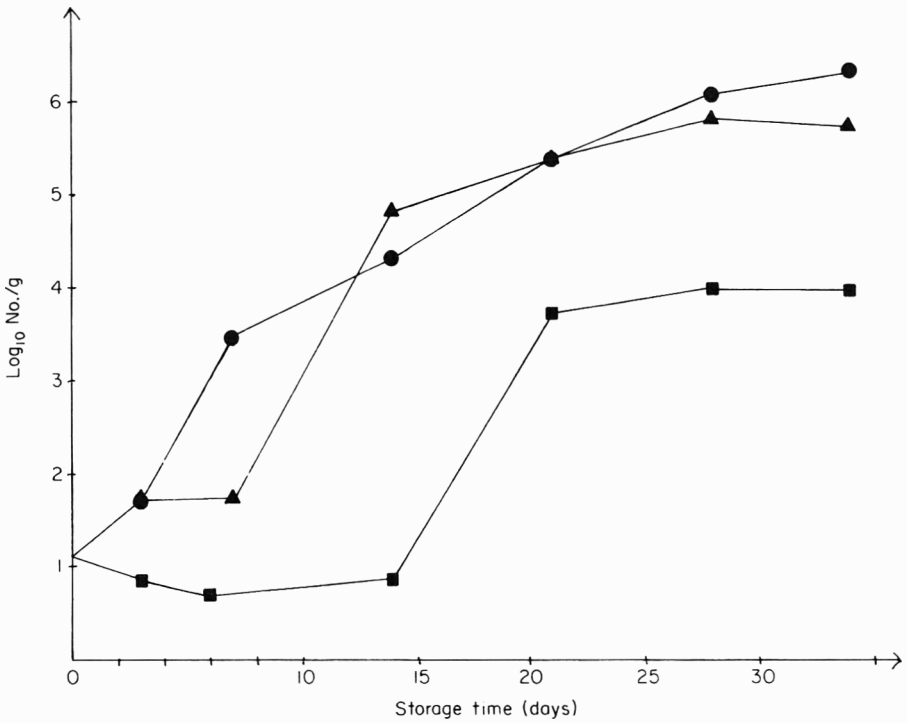

Figure 3. Growth of *B. cereus* (No. 1) at 10°C. O, 0; △, 75; and □, 150 ppm nitrite.

Experiments with salmonellae

Salmonella enteritidis was examined at 12°C during 4 weeks and at 10°C from the 2nd to the 4th week (Fig 7). At both temperatures growth was only slightly inhibited by 75 ppm nitrite. Addition of 150 ppm nitrite strongly inhibited growth at 12°C during the first 2 weeks, at 10°C during all 4 weeks. During these periods, numbers in the packages with 150 ppm added nitrite were lower ($P < 0.025$) than in the other batches.

While pH in packages stayed at their initial level at 10°C, pH fell to 5.8 in packages without added nitrite and to 5.96 in packages with nitrite after 4 weeks at 12°C.

Growth of *S. typhimurium* was examined at 12 and 15°C (Fig. 8). Preliminary experiments showed that these bacteria (mixture of two) did not grow at 10°C in vacuum packed Bologna sausage. At 15°C, growth was slightly inhibited at the 150 ppm level, while only at 12°C a lag phase in the 150 ppm nitrite batch was observed, growth rates in all batches being more or less identical. pH fell in all packages, at 12°C to 5.75, 5.95 and 6.15 in batches with 0, 75 and 150 ppm added nitrite, respectively; at 15°C to 5.85, 5.95 and 6.10, respectively.

Discussion

The inhibitory effect of nitrite is observed throughout the study. It is considerably more pronounced at lower temperatures. This is particularly seen in the experiments with *S. aureus*, but also with *Y. enterocolitica* and *S. enteritidis*. For the single strain of *S. aureus* an almost complete inhibition with 75 ppm added nitrite is seen at 10°C, while 150 ppm added nitrite only delayed growth at 12°C. This temperature dependent effect of nitrite

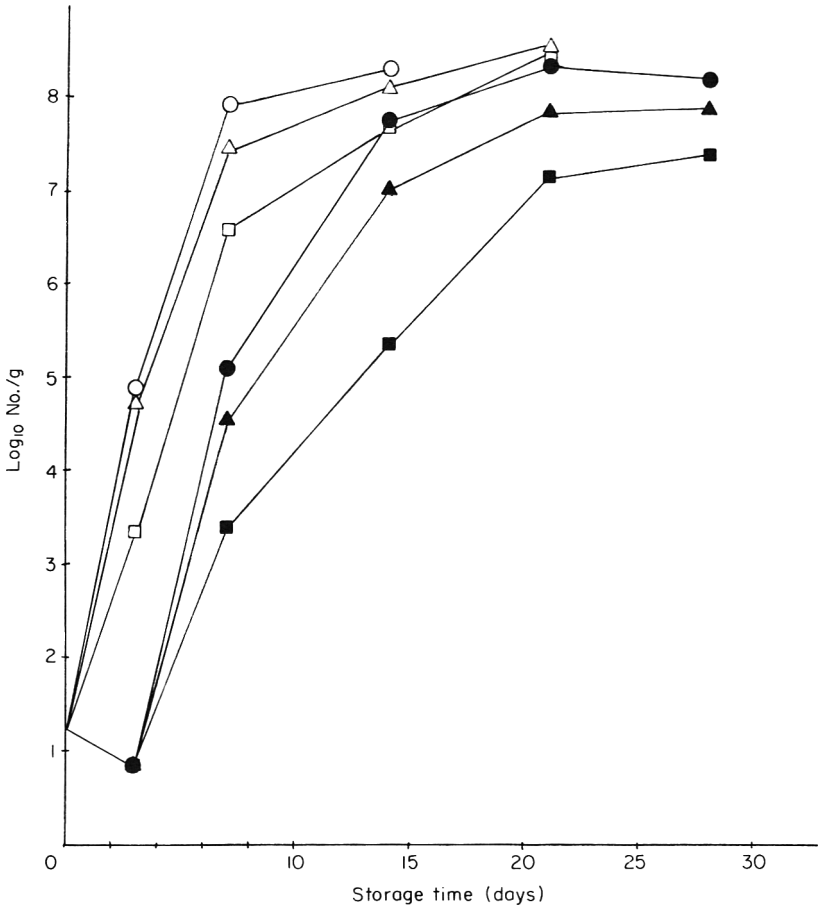

Figure 4. Growth of *Y. enterocolitica* (Nos A and B) at 5°C (closed symbols) and 10°C (open symbols). O, 0; △, 75; and □, 150 ppm nitrite.

is also observed with the normal microbiological flora in vacuum packed cured, cooked meat products (Nielsen, 1983), and is presumably caused by a faster depletion of nitrite at higher temperatures (Nordin, 1969). Whether the growth of *S. aureus* is also accompanied by toxin production, was not determined. Anyway, in packages with added nitrite, no growth was seen and the low levels present during storage will not present a health hazard. At higher temperatures, 15–25°C neither growth nor toxin production was influenced of up to 196 ppm nitrite (after processing) in non-evacuated packages (Crowther *et al.*, 1976). In vacuum packages, growth is seen irrespective of nitrite addition, but enterotoxin was not produced.

B. cereus is apparently not inhibited by addition of 75 ppm nitrite at the temperature examined, while 150 ppm added nitrite reduced the maximum numbers considerably. Raevuori (1975) found no inhibition at 100 ppm, and strong inhibition by 200 ppm added nitrite in whole cooked sausage, not conflicting with the present study.

The results with *S. enteritidis* show resemblance with the study by Rice & Pierson (1982) with respect to the temperature dependence of the nitrite effect but not with the growth pattern at the actual temperatures. In commercial frankfurters with 156 ppm added nitrite (50 ppm after processing) almost complete inhibition was observed at 15°C in the study by Rice & Pierson (1982), while in the present study growth proceeds

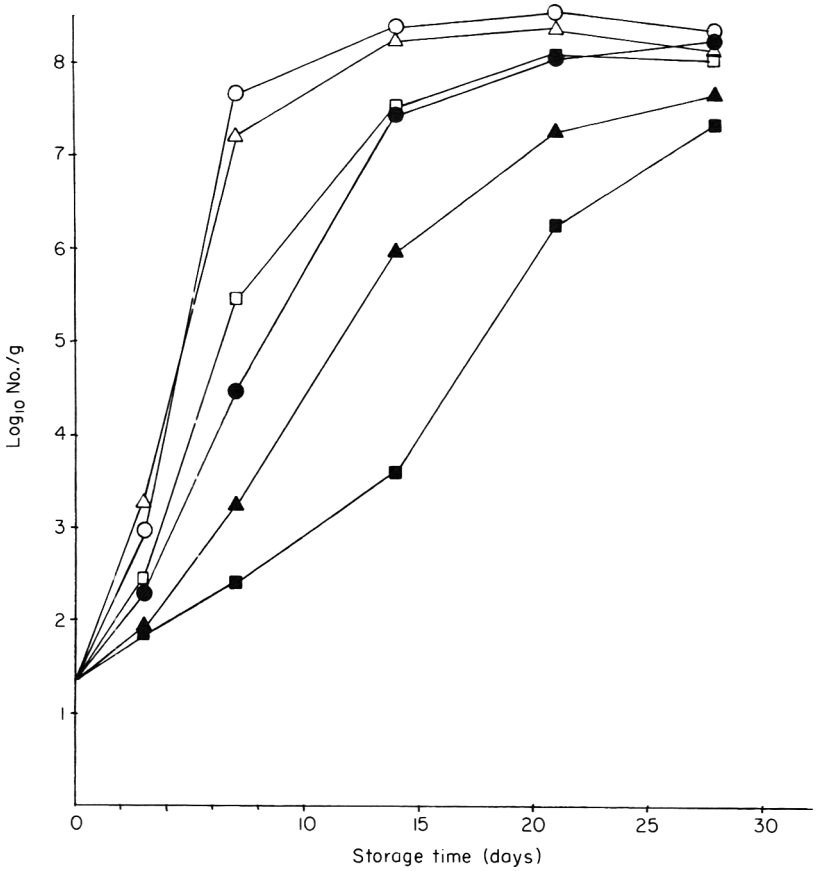

Figure 5. Growth of *Y. enterocolitica* (Nos 17193 and 17280) at 5°C (closed symbols) and 10°C (open symbols). O, ●; △, ▲; and □, ■, 150 ppm nitrite.

to *circa* 10^8/g with 150 ppm added nitrite (69 ppm left after processing). A competing flora was, however, present in the former study, and that probably helped in depressing growth salmonellae. However, some lactics reduce the added nitrite and thereby further reduce the inhibitory effect (Collins-Thompson & Lopez, 1981). At even lower temperature (8°C) salmonellae were completely inhibited by 42 ppm added nitrite at low initial levels and strongly inhibited at high levels (Leistner *et al.*, 1973), while strains used in the present study did not grow at all at 8°C.

The inhibition caused by 75 ppm added nitrite on *Y. enterocolitica* at 10°C is quite small, maximum numbers being high (*circa* 10^8/g) in all batches. Decreasing temperature enhances the inhibition, maximum numbers being about 1 $\log_{10}$ unit lower than without nitrite. Addition of 150 ppm nitrite further reduces growth and maximum counts especially at low temperature. Nevertheless, growth is evident in all batches and at all temperatures, and it is not unthinkable that *Y. enterocolitica* might produce enterotoxin under the given condition. Kapperud & Langeland (1981) found *Y. enterocolitica* produced toxin at 3–6°C after 7 days and Olsvik & Kapperud (1982) reported toxin production by *Y. enterocolitica* at 4°C in tryptone soy broth.

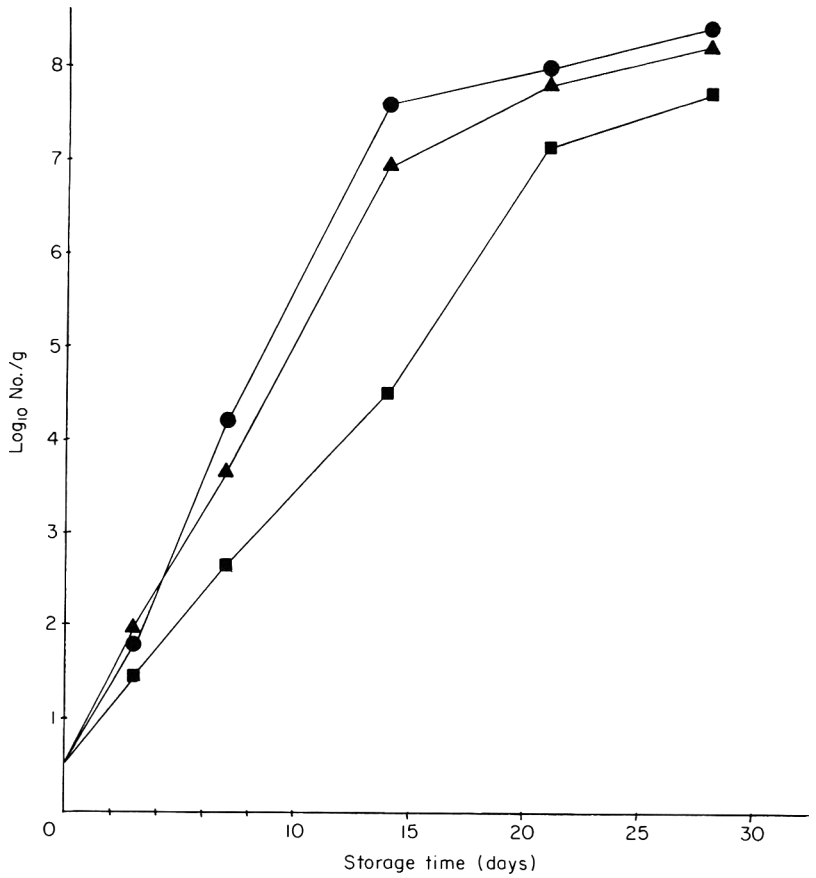

Figure 6. Growth of *Y. enterocolitica* (No. F5208/82) at 5°C. O, 0; △, 75; and □, 150 ppm nitrite.

pH in the packages generally fell during storage, the decrease being dependent on the microbiological development—i.e. strongest decrease in batches with lowest nitrite addition and the highest storage temperatures.

It is evident that nitrite addition had an inhibitory effect upon all pathogenic bacteria tested. The reduced growth rate/maximum numbers were more pronounced at the lower temperatures used. nevertheless, temperatures in refrigeration cabinets are not always in accordance with regulation, studies have shown temperatures of over 10°C in such cabinets (Bøgh-Sørensen, 1980). In view of the current level of nitrite addition, which is often 50–100 ppm nitrite, the practical value of the inhibitory effect is quite small, considering the length of the storage period. The period of sale for vacuum packed, cured, cooked meat products may be anywhere from 2 to 6 weeks. With long sales periods (4 weeks) the value of nitrite addition becomes negligible unless the temperature is low, and even at 5°C, an often recommended temperature, the effect upon *Y. enterocolitica* is insignificant. Only at a temperature of 5°C and below as often used during bulk storage, together with a nitrite addition of 150 ppm and a sales period of less than 2 weeks, is a reasonable safety against health hazards attained. Other means

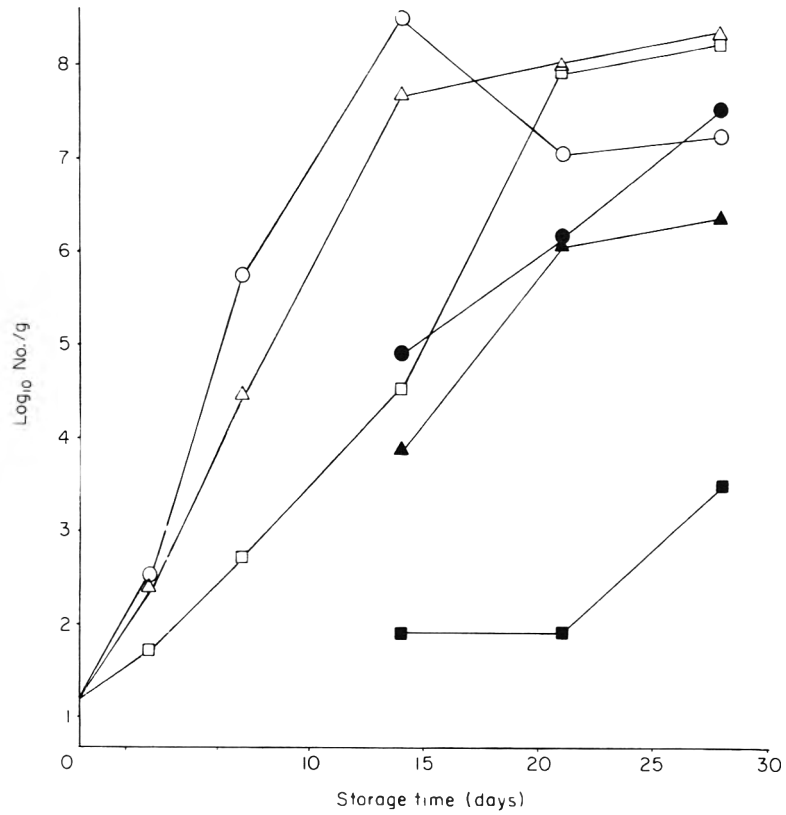

Figure 7. Growth of *S. enteritidis* at 10°C (closed symbols) and 12°C (open symbols). ○, ●: △, ▲, 75; and □, ■, 150 ppm nitrite.

of increasing the safety might be an increasing sodium chloride concentration, which was not examined in the present study. The current trend though is to decrease the sodium chloride concentration in cured meat products. In must, however, be emphasized, that the inoculation levels used in the present study are higher than counts normally observed in vacuum packed cured meat products. With lower counts, a more pronounced influence of nitrite might be found (cf. Leistner *et al.*, 1973a). Normally a competing flora consisting of lactic acid bacteria, *B. thermosphacta* etc., will be present in the packages (Nielsen, 1983) and this flora will inhibit the pathogens to some extent (Leistner *et al.*, 1973a). Serious problems arise if accidental contamination with pathogenic bacteria happens under otherwise hygienic conditions. In such a case no competing flora will be available to influence growth of the pathogenic bacteria.

Acknowledgments

We thank Ms B. Jensen and Ms A.-M. Nepper for their technical assistance. The investigation was supported by the Danish Technical Research Council.

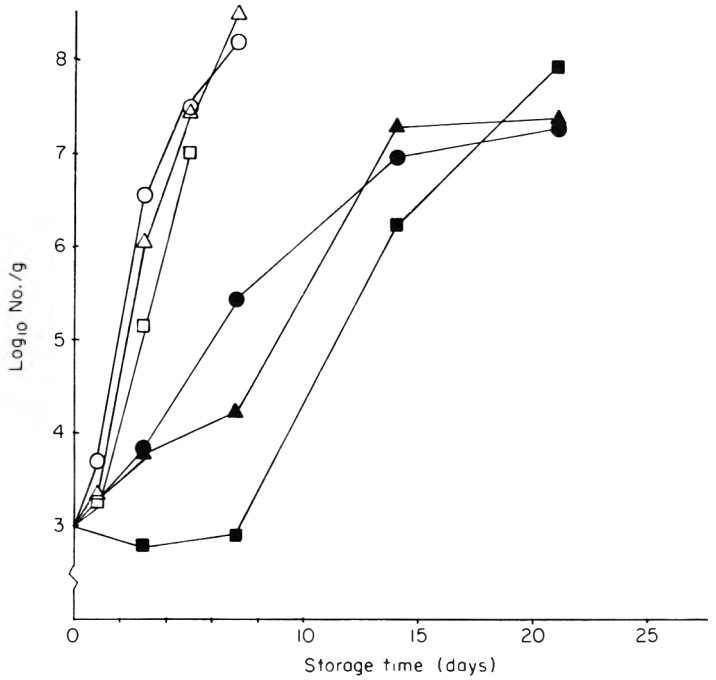

Figure 8. Growth of *S. typhimurium* (No. 1) at 12°C (closed symbols) and 15°C (open symbols). ○, 0; △, 75; and □, 150 ppm nitrite.

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Comparison of the resistance to hydrogen peroxide of wet and dry spores of *Bacillus subtilis* SA22

S. LEAPER

Summary

The sporicidal action of hydrogen peroxide over the concentration range 11.8–41.3% (w/v) was studied at temperatures of 20–65°C, using both wet and dry spores of *Bacillus subtilis* SA22 (NCA 72-52). Wet spores were tested as a suspension in the chemical whereas dry spores were tested by flooding filter paper strips with a spore suspension, allowing them to dry followed by immersion into the chemical. In contrast to earlier reports dry spores were up to 2 times more resistant to the sporicidal effects of hydrogen peroxide than wet spores, although the factor was not constant over the concentration and temperature ranges tested. The resistance of spores on filter paper strips was investigated as this could provide a suitable method for monitoring the efficiency of sterilizing aseptic packaging material. The data have allowed a model to be postulated whereby the sporicidal action of hydrogen peroxide can be determined at any concentration and temperature.

Introduction

Hydrogen peroxide is used in many aseptic systems for the sterilization of packaging material (von Bockelmann, 1973) which is thought to be contaminated with low numbers, approximately 1–5/100 cm^2 of bacteria (Swartling & Lindgren, 1968; Lisiecki, 1971; von Bockelmann, 1973; Buchner, 1978). Existing sterilization indicators are not suitable for use with aseptic packaging. Steiger (1976) has suggested the use of a bacterial suspension dried in a plastic tube together with a chemical indicator as a method of monitoring a sterilization process. Halleck (1982) also utilized a chemical indicator together with a bacterial culture to give evidence of achievement of sterilization. Kereluk's (1973) design included seven spore impregnated strips of filter paper sealed in glassine envelopes incorporating 10^3–10^9 spores of *Bacillus stearothermophilus*, when steam was the sterilizing agent, or spores of *B. subtilis* var. *niger* when dry heat or ethylene oxide was the method of sterilization. *B. subtilis* var. *globigii* (ATCC 9372) on filter strips has also been suggested (Grundy *et al.*, 1957) when ethylene oxide was used. Contaminated aluminium foil on the bottom of cups has been used to assess the effects of accelerated electrons (Sturm & Gilliand, 1974) whilst Martin (1948, 1950) inoculated cans and can covers with spores of known heat resistance and determined their survival after passing through an aseptic canning machine. Similarly Atherton & Brown (1973) and Denny, Shafer & Ito (1979) dried spores onto containers and lid material to assess the sterilization process in Dole aseptic canning systems. In an aseptic bottling system bottles were inoculated with spores prior to the heat treatment to determine efficiency of sterilization (Franklin, 1970).

Most studies have looked at the resistance of wet spores to hydrogen peroxide (Curren, Evans & Leviton, 1940; Swartling & Lindgren, 1968; Cerf & Hermier, 1972;

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Ito *et al.*, 1973; Cerny, 1976; Cerf & Metro, 1977; Bayliss & Waites, 1979; Bayliss, Waites & King, 1981) and only in a few cases has the chemical resistance of dry spores been assessed (Swartling & Lindgren, 1962; Ito *et al.* 1973; Toledo, Escher & Ayres, 1973; Neal & Walker, 1977; Smith & Brown, 1980).

However, contaminating bacterial spores on the packaging material are more probably in the dry state so it is necessary to assess the resistance of dry spores to chemical sterilization.

In this study spores of *B. subtilis* SA22 (NCA 72-52) previously shown to be more resistant than other *Bacillus* strains to chemicals (Toledo *et al.*, 1973; Mottishaw, Brown & Leaper, 1982) were used. The resistance of dry spores is compared to that of wet spores and the suitability of dry spore strips for assessing the sterilization efficiency of packaging material in aseptic lines is considered. A model is postulated whereby the decimal reduction at different temperature and hydrogen peroxide concentrations can be predicted.

Materials and methods

Spore preparation

Bacillus subtilis SA22 (NCA 72-52) was grown at 30°C for 5 days on plates of nutrient agar and spores harvested as described previously (Leaper, 1984). The concentration was adjusted to approximately 3×10^8 spores/ml with sterile distilled water and spore suspensions were stored at 4°C until required.

Preparation of dry spores

Sterile strips (5 × 1 cm) of various grades of filter paper (Whatman) were arranged so that they were not in contact with each other in sterile Petri dishes. Aliquots (0.1 ml) of the spore suspension were pipetted, using a 100 μl pipette (Eppendorf 4700), on to each strip and allowed to dry at 37°C overnight. In order to estimate the release of spores from the strip when in contact with liquids the dried strips were immersed in separate bottles of sterile distilled water for up to 4 min, after which each strip was aseptically transferred to 10 ml sterile distilled water, vigorously shaken, serially diluted and the viable spores recovered using pour plates of yeast dextrose tryptone agar plus starch (YDTAS) and overlayered with YDTAS according to Leaper (1984). In addition, 1 ml of the water into which the strip containing spores had been immersed was also plated out in duplicate. The plates were incubated aerobically at 37°C for 48 hr before the colonies were counted.

Determination of resistance to hydrogen peroxide

Dry spores. Filter paper strips of Whatman No. 2 grade onto which spores had been dried, were immersed one at a time in a closed Universal bottle containing 20 ml of the desired concentration of hydrogen peroxide (BDH) held in a waterbath of the desired temperature. After a timed interval the strip was aseptically withdrawn, the end touched on to the edge of the bottle to allow excess liquid to drain off, transferred within 5 sec to 10 ml sterile distilled water containing catalase (BDH) to give a final concentration of 200 EU/ml in a sterile Universal bottle and vigorously shaken until the strip disintegrated. Appropriate dilutions were plated out in duplicate, as described above, to determine spore survival. This procedure was repeated at 20, 30, 40, 45, 50, 55, 60 and 65°C.

Wet spores. Measured volumes of hydrogen peroxide and distilled water were added to sterile 150 ml conical flasks and allowed to equilibrate at the required temperature in a shaking water bath (Grant Instrument, Cambridge; 120 strokes/min) for approximately 15 min. Spore suspension (0.5 ml) was added to give a total volume of 25 ml containing approximately 6×10^6 spores/ml. Aliquots (1 ml) of the mixture were aseptically removed at timed intervals and mixed with 1 ml water containing 2000 EU catalase in a sterile bottle. The first diluent also contained 200 EU/ml catalase. The first 10-fold dilution was carried out within 1 min of sampling and duplicate pour plates of appropriate dilutions were prepared as described above.

Recovery and enumeration

Treated spores were serially diluted in sterile distilled water and recovered using pour plates of YDTAS, as described previously (Leaper, 1984).

Results

The sporicidal effect of hydrogen peroxide on wet spores of *B. subtilis* SA22 increased as the concentration was increased from 11.8 to 41.3% (w/v) demonstrated by the decrease in the decimal reduction time (D value) from 16.3 min to 1.8 min over this range of hydrogen peroxide concentrations at 20°C (Table 1). The D values were calculated from all the points (average 22) obtained from the survival curves, thus where 'shouldering' was present this was taken into account. Similar trends were seen at higher temperatures. D values obtained using 35.4% hydrogen peroxide at 40°C, 23.6% hydrogen peroxide at 50°C or 11.8% hydrogen peroxide at 60°C were similar. For any one hydrogen peroxide concentration tested the D value also decreased as the temperature was increased. For example, the D value for 17.7% hydrogen peroxide at 20°C using wet spores was 9.4 min whereas at 65°C the D value was less than 0.1 min.

Table 1. Comparison of calculated values and experimental values (in brackets) of decimal reduction times (min) for the destruction of wet spores of *B. subtilis* SA22 by hydrogen peroxide at 20–65°C

Temperature (°C)	% Hydrogen peroxide (w/v)					
	11.8	17.7	23.6	29.5	35.4	41.3
20	14.6 (16.3)	9.7 (9.4)	6.4 (5.6)	4.2 (3.6)	2.8 (2.3)	1.9 (1.8)
30	4.5 (5.1)	3.1 (2.8)	2.1 (2.0)	1.4 (1.9)	0.96 (1.2)	0.65 (0.77)
40	1.5 (1.6)	1.0 (0.93)	0.73 (0.72)	0.50 (0.44)	0.35 (0.24)	0.24 (0.23)
45	0.90 (0.85)	0.63 (0.53)	0.44 (0.35)	0.31 (0.35)	0.22 (0.19)	0.15 (0.17)
50	0.54 (0.57)	0.38 (0.29)	0.27 (0.29)	0.19 (0.16)	0.14 (—)	0.096 (—)
55	0.33 (0.36)	0.24 (0.17)	0.17 (0.17)	0.12 (—)	0.086 (—)	0.061 (—)
60	0.21 (0.23)	0.15 (0.19)	0.11 (—)	0.077 (—)	0.055 (—)	0.040 (—)
65	0.13 (0.16)	0.094 (0.097)	0.069 (—)	0.050 (—)	0.036 (—)	0.026 (—)

(—) not determined.

An empirical model is proposed based on these results in order to predict both D values at hydrogen peroxide concentrations and temperatures which cannot be tested with the methods already described and also to interpolate the results obtained experimentally. It has been assumed that the kinetics of death of spores can be described by the Arrhenius equation (Brown & Ayres, 1982):

$$k_D = A_c^{-\Delta E/RT}. \quad (1)$$

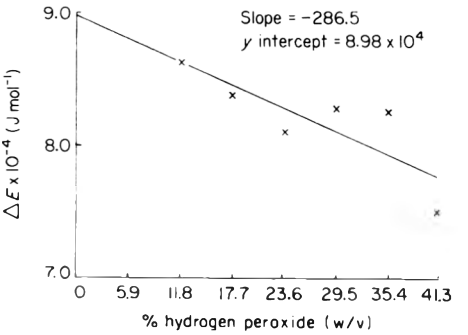

Figure 1. Effect of hydrogen peroxide concentration on activation energy (△E).

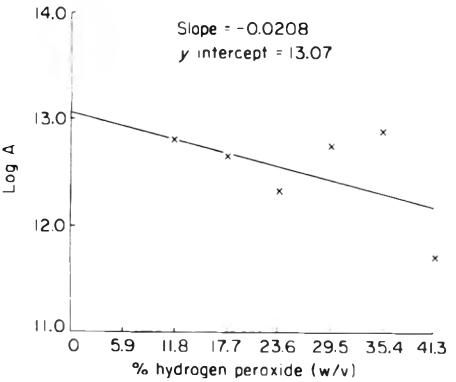

Figure 2. Effect of hydrogen peroxide concentration on the Arrhenius constant (A).

where R is the gas constant (8.31431 J°C $^{-1}$ mole $^{-1}$). The death rate constant (k_D) can be related to the D value as given by Warren (1973) and Brown & Thorpe (1978) using the following equation:

$$k_D = \log_e 10/60D. \tag{2}$$

The change in k_D with absolute temperature (T) is shown for wet and dry spores of *B. subtilis* SA22 in Fig. 3, where $\log k_D$ is plotted against $1/T$. From the data for wet spores, in which the Arrhenius equation can be applied, activation energies and Arrhenius constants have been determined for each hydrogen peroxide concentration using the Arrhenius equation. Both the Arrhenius constant (A) and activation energy (ΔE) are dependent on the hydrogen peroxide concentration (Figs 1 and 2). Substituting for A and ΔE:

$$\log k_D = \log(\text{slope}_1 \times C + \text{intercept}_1) - \frac{\text{slope}_2 \times C + \text{intercept}_2}{2.303\,RT}, \tag{3}$$

where C is the hydrogen peroxide concentration. Slope_1 and intercept_1 were determined from the plot of log A *versus* hydrogen peroxide concentration (Fig. 1), and slope_2 and intercept_2 were determined from the plot of ΔE *versus* hydrogen peroxide concentration (Fig. 2). Rearrangement and substitution for k_D in equation (1) gives:

$$D = \text{antilog}\left[(\text{slope}_1 \times C + \text{intercept}_1) - \frac{(\text{slope}_2 \times C + \text{intercept}_2)}{2.303\,RT} - \log 2.303 + \log 60\right]. \tag{4}$$

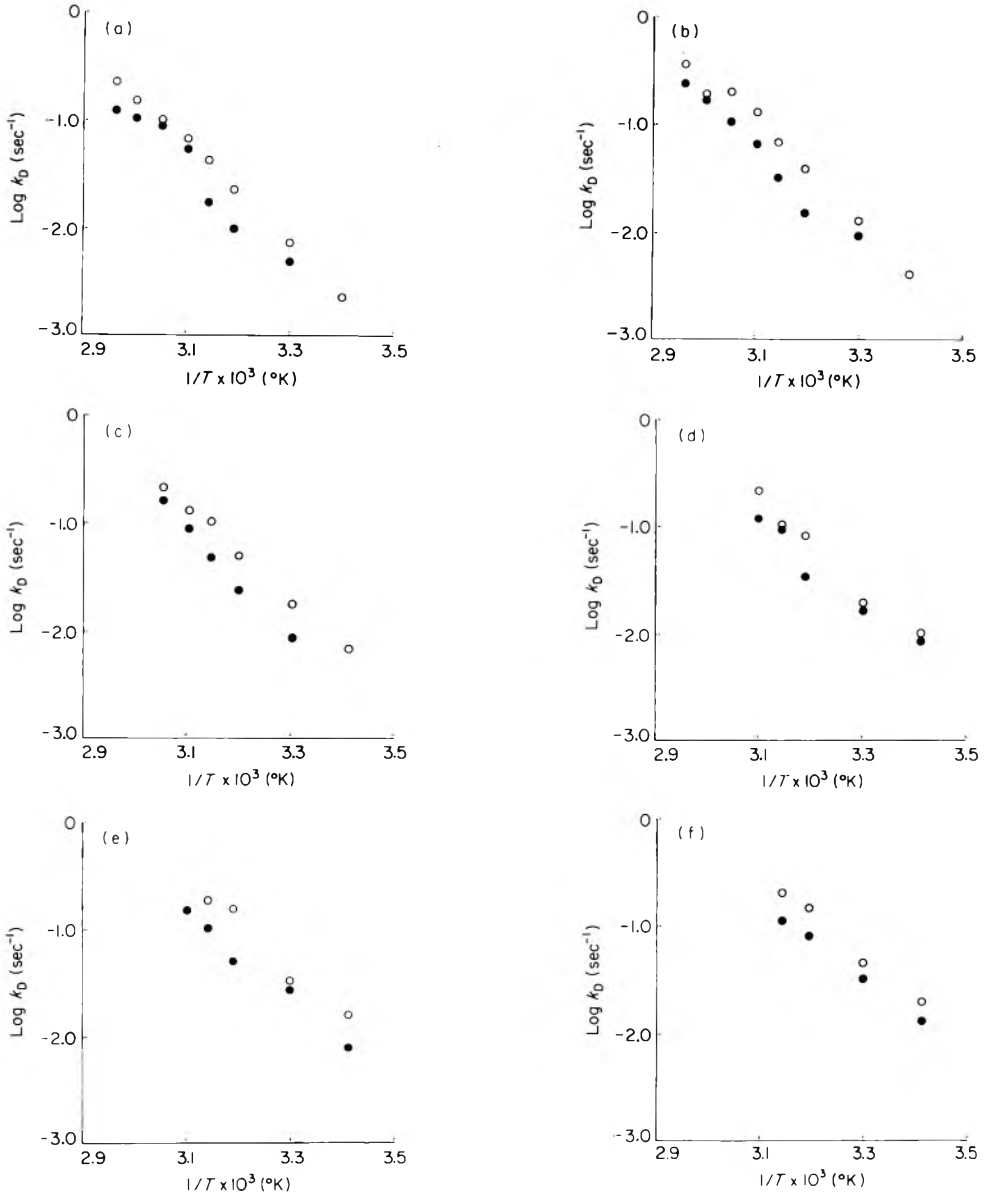

Figure 3. Effect of temperature on the death rate (k_D) of wet spores (O) and of dry spores (●) of *Bacillus subtilis* SA22 exposed to (a) 11.8%, (b) 17.7%, (c) 23.6%, (d) 29.5%, (e) 35.4% and (f) 41.3% hydrogen peroxide.

Using this relationship (equation 4) D values were calculated for all the temperatures and hydrogen peroxide concentrations used experimentally as well as the higher hydrogen peroxide concentrations and higher temperatures which could not be determined by the above experimental methods (Table 1). There was good agreement between the calculated and experimentally determined D values.

Less than 1% of the spores of *B. subtilis* SA22 flooded onto the various grades of

Whatman paper became detached when the spore impregnated strips were dipped into water. This loss was not taken into account in the determination of survival. Whatman No. 2 paper was chosen for study as it had the best overall properties with respect to retention of spores and it was possible to break up the filter strip when vigorously agitated in water. Similar trends in survival to those of wet spores were found with the spores dried onto filter strips but the dry spores were more resistant to the sporicidal action of hydrogen peroxide (Fig. 3). In general as the concentration of hydrogen peroxide was increased at each temperature tested there was a decrease in the *D* value. Similarly for any one hydrogen peroxide concentration as the temperature was increased, the *D* value decreased. Dry spores had similar *D* values to wet spores at 30°C to 29.5 and 35.4% (w/v) hydrogen peroxide whereas at 40°C dry spores were at least twice as resistant to these concentrations of hydrogen peroxide as wet spores. At 45°C the difference in resistance between wet and dry spores to 29.5% (w/v) hydrogen peroxide was minimal. The factor by which the spores differed in resistance at any concentration or temperature was not the same.

Discussion

Spores of *B. subtilis* SA22 dried on to filter paper appear to be more resistant than wet spores to the sporicidal effects of hydrogen peroxide over the concentration and temperature ranges tested. Similarly Ito *et al.* (1973) showed that wet spores of *Clostridium botulinum* 169B were less resistant than dry spores to 20% hydrogen peroxide at 25°C. These findings are in contrast to other published data. For example, Smith & Brown (1980) concluded that their dry spores of *B. subtilis* var. *globigii* (NCIB 8058) were less resistant than the wet spores used in the study by Toledo *et al.* (1973). Toledo *et al.* (1973) had also shown that spores of *B. subtilis* SA22 dried on to the inner surface of a culture tube were less resistant than wet spores in 25.8% hydrogen peroxide at 24°C. However, in a system in which the hydrogen peroxide was not agitated continuously they reported considerable data scatter. The difference between Toledo *et al.*'s (1973) results and those reported here for *B. subtilis* SA22 may be due to the difference in test methods.

Filter paper strips impregnated with spores of known chemical resistance, when attached to the inner surface of a roll of packaging material could provide a method for assessing the efficiency of sterilization in aseptic systems of the packaging material. The packaging material together with the attached spore strips could be passed through the sterilizing areas and retrieved at the end of the line following package formation but without a product fill. Aseptic removal of the strips from the packaged and the culture of surviving spores on the strips should enable the level of sterility with respect to *B. subtilis* SA22 to be determined by the number of log reductions in survivors that are achieved.

The empirical model proposed, based on the kinetics of spore death following the Arrhenius equation, allows extrapolation of results outside the range of conditions that can be tested experimentally so that *D* values can be predicted at other temperatures and hydrogen peroxide concentrations. Sterilization times with respect to spores of *B. subtilis* SA22 would be achieved. von Bockelmann (1973) reported that the packaging material in the Tetra Brik system passes through a 23–35% hydrogen peroxide bath at 60–80°C, this passage taking approximately 0.13–0.15 min. Using the formula suggested above treatment with 29.5% hydrogen peroxide at 65°C would require 0.05 min to achieve one decimal reduction in the surviving number of spores of *B. subtilis* SA22.

However the Tetra Pak process, as reported by von Bockelmann (1973) operates at room temperature with a 15–20% hydrogen peroxide bath, the passage of packaging material taking 0.05–0.07 min. The calculated *D* value for 17.7% hydrogen peroxide at 20°C is 9.7 min and at 30°C it is 3.1 min using spores of *B. subtilis* SA22.

Tetra Pak claim one to two decimal reductions by the chemical bath, as measured with spores of *B. subtilis* (strain not given), in their aseptic systems (von Bockelmann, 1980). Clearly spores of *B. subtilis* SA22 used in this study would not achieve one to two decimal reductions in a 15–20% hydrogen peroxide bath in 0.05–0.07 min.

The chemical resistance of *B. subtilis* is known to differ widely between strains and is dependent upon the conditions under which the spores have been produced (Bayliss *et al.*, 1981; Mottishaw *et al.*, 1982). It is suggested that the use of a strip method containing named spores of known chemical resistance would allow the efficiency of a sterilization system to be assessed. If the relationship of the chemical resistance of these spores with that of *C. botulinum* was known then the sterilization efficiency of a system could be quoted in terms of numbers log reductions in survivors of *C. botulinum* spores.

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Effects of sun drying on a concrete floor and oven drying on trays on the elimination of cyanide from cassava whole-root chips

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Summary

The effects of sun drying on a concrete floor at a loading rate of 10 kg/m² and oven drying of trays at 60°C at a loading of 20 kg/m² on the cyanide elimination from cassava whole-root chips were evaluated, using four cultivars (M Col 113, M Col 22, M Col 1684 and CM 342-170) at four plant ages (6, 8, 10 and 12 months). Fresh whole-root chips of the four cultivars varied considerably in their total cyanide content, ranging from 1031 ± 137 mg/kg on a DM basis for cv. M Col 1684 to 296 ± 48 mg/kg for M Col 113 (a Colombian local cultivar); the other two cultivars showed intermediate levels of around 500–550 mg/kg. Between 20 and 38% of the total cyanide in fresh chips was free cyanide. Sun drying eliminated 86–94% of the initial total cyanide and 93–98% of the bound cyanide, whereas these losses were of the order of 77–80% and 81–85%, respectively, for oven drying at 60°C. Most of the total cyanide in sun dried chips was found as free cyanide (59–76%), whereas in oven dried chips the largest proportion of the total cyanide was still present as bound cyanide (59–69%). The cyanide elimination found in this study is greater than that reported by earlier workers who used chips from peeled roots and different drying procedures.

Introduction

Cassava (*Manihot esculenta* Crantz) cultivars are generally classified as sweet or bitter according to the low or high cyanide concentration in their root parenchymal tissue (Bolhuis, 1954), which is the edible part of the root for human consumption. A broad range of cyanide concentration has been found among several cassava cultivars (Gómez *et al.*, 1980; Kawano, De La Cuesta & Gómez, 1984), and most of them fall within the intermediate range between the sweet and bitter cultivars. Root cyanide content appears to be a highly stable character, and a broad sense heritability of 0.98 has been reported (Kawano *et al.*, 1984).

In most cassava producing areas local cultivars normally produce low-cyanide-containing roots which are used in a wide variety of forms as human food (Lancaster *et al.*, 1982). Peeled roots are traditionally processed by simple methods which reduce their cyanide content (Joachim & Panditteseker, 1944; Cooke & Maduagwu, 1978) and improve their palatability. For animal feeding whole roots including the peel or root cortex are processed and usually sun dried to be subsequently incorporated as cassava root meal in compound feeds (Gómez, Santos & Valdivieso, 1982). In all cassava cultivars analysed the root peel had similar or higher cyanide concentrations

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than the corresponding parenchymal tissue (Joachim & Panditteseker, 1944; de Bruijn, 1971; Gómez *et al.*, 980; Cooke & De La Cruz, 1982; Gómez & Valdivieso, 1983a), however the peel was only around 10–20% of the root total fresh weight (Gómez *et al.*, 1980; Cooke & De La Cruz, 1982; Gómez & Valdivieso, 1983a).

Sun drying on concrete patios is the simplest way of drying whole-root chips for the animal feed market (Thanh *et al.*, 1979; Ospina, Gómez & Best, 1983) and it is also an efficient means of eliminating cyanide (Gómez & Valdivieso, 1983b; Gómez *et al.*, 1983). Although sun drying on trays allows faster drying (Best, 1979; Gómez & Valdivieso, 1983b), the method has not been adopted under practical processing conditions; in addition, sun drying on trays usually eliminates less cyanide than drying on a concrete floor (Gómez & Valdivieso, 1983b; Gómez *et al.*, 1983). However, natural sun drying, either on concrete patios or on trays, is totally dependent on climatic conditions and hence limited to the dry seasons, which imposes a constraint to a year-round continuous processing and supply of dried chips. Artificial drying could be a solution to this problem.

Limited information exists on the effect on cyanide elimination of artificial drying of peeled-root chips (Joachim & Panditteseker, 1944; Cooke & Maduagwu, 1978) and even less with respect to whole-root chips. The present study was undertaken to evaluate the effects on the cyanide elimination of sun drying whole-root chips of four cassava cultivars, each at four plant ages (6, 8, 10 and 12 months) on a concrete floor and of oven drying on trays at 60°C.

Materials and methods

Cassava growth conditions

Three high yielding (cv. M Col 22, M Col 1684 and the hybrid CM 342-170) and one Colombian local (cv. M Col 113 or Valluna) culivars were planted at 1 × 1 m spacing in adjacent fields, each field of about 0.4 ha, at CIAT in October 1981. M Col 1684 is a high cyanide containing variety and the others are considered intermediate and low (M Col 113) cyanide cultivars (Gómez *et al.*, 1980; 1983). The soil has a pH range of 6.2–6.8, a phosphorus availability level of approximately 72 mg/kg (Bray II) and, in general, good characteristics for cassava production (Kawano *et al.*, 1978). Mean temperature from October 1981 to October 1982 was 23.7°C and total rainfall during the year was 1196 mm, with two dry periods (December–February and June–August).

Cassava root harvesting, processing and drying

Starting in April 1982 (6 months of plant age) and ending in October 1982 (12 months) a 650 m^2 plot of each cultivar was harvested at 2 month intervals. Roots were harvested late in the afternoon of the day before processing, stored overnight in a shaded area and processed between 0800 and 1000 hr the next morning. Unwashed roots were processed through a Thai-type chipping machine (Thanh *et al.*, 1979), and the fresh whole-root chips were immediately weighed and spread on either concrete floor platforms (22 m^2 each) or metallic trays (0.54 m^2 each) with wired bottoms. Thai chippers use a circular iron plate into which cutting edges are chiselled and the chips produced are very irregular, with a thickness often greater than 0.3 cm (Thanh *et al.*, 1979). The loading rates (quantities of fresh chips spread over unit surface area) were 10 kg/m^2 for sun drying on the concrete floor and 20 kg/m^2 for oven drying on traps, except for M Col 22 at 6 months of plant age in which the loading for oven drying was 10 kg/m^2 due to the limited quantity of roots. In the case of the other cultivars at that age,

however, when total root weight was not enough for five platforms of 22 m^2 each, the area of each platform was reduced to 12 m^2. Five concrete platforms and ten oven trays for each variety at each plant age were tested at each sampling. Five fresh chip samples in each treatment were analysed: one from each concrete platform and one from every two trays.

Immediately after the fresh chips were spread on the experimental drying surface (floor or tray) a sample as taken and a weighed amount (~60 g) was immersed in phosphoric acid; it was homogenized, filtered, and the homogenate used for cyanide analyses by the enzymic assay (Cooke, 1978); total and free cyanide were determined, in duplicate, by this assay, and bound cyanide (mainly linamarin) was obtained by difference. Chipping and sampling were coordinated so as to keep the time elapsed between the two operations to a minimum; the immersion of the sample in phosphoric acid took place approximately 15 min after the whole roots were chipped.

The chips on the floor were periodically turned over during the drying period using a wooden rake; from 1700 to 0800 hr chips were piled and covered with a plastic sheet; the next day they were spread on the same initial drying area. Meteorological conditions throughout the studied period and the duration of each sun drying were recorded. A forced air, electrically heated oven (Despatch Model V-31, Despatch Industries, Inc., Box 1320, Minneapolis, Minnesota 55440) was used for oven drying and the whole-root chips. A temperature control thermostat was standardized and set at 60°C as the operating temperature. The oven was equipped with two dampers, one on the lower front to control the entrance of fresh air and the other on the exhaust stack on top of the oven to control the amount of air expelled. These dampers were adjusted to reach the operating tmperature in the shortest time after the initiation of the drying period and they were maintained at fixed positions for all the drying batches performed. Oven drying at 60°C at a loading rate of 20 kg/m^2 lasted for approximately 24 hr.

At the end of each drying, samples of dried chips (five sun dried and five oven dried) were taken, and each sample was ground and analysed for cyanide contents (Cooke, 1978). Fresh and dried chip samples were analysed for dry matter (DM) by drying them to constant weight at 60°C. All cyanide contents are expressed on a DM basis. The experimental data were statistically analysed using the analysis of variance procedure (Steel & Torrie, 1960).

Results

Figure 1 shows the DM content of fresh-root chips of the four culivars at the four plant ages studied. The largest variations of DM content, as reflected by the standard error of the mean, among the samples analysed were observed in chips from roots of 8 and 10 month old plants corresponding to the periods of the most drastic changes in cassava plant growth in response to climatic conditions (Gómez, unpublished observation). In general, fresh-root chip DM content increased significantly ($P < 0.05$) from 6 to 8 months of plant age and decreased progressively thereafter (Fig. 1). The four cultivars reached their maximum fresh weight yield per ha when the plants were 1 year old (Gómez, unpublished observation). Under the soil and climatic conditions of the present study, the fresh chips of roots of cv. M Col 113 had consistently lower ($P < 0.05$) DM contents than the other cultivars throughout the entire period studied.

Sun drying of the root chips at each sampling period was performed during 2 consecutive weeks. Average ambient temperature throughout all sun drying periods was 24.1±0.8°C. Relative humidity was in the range of 70–74% for the sampling

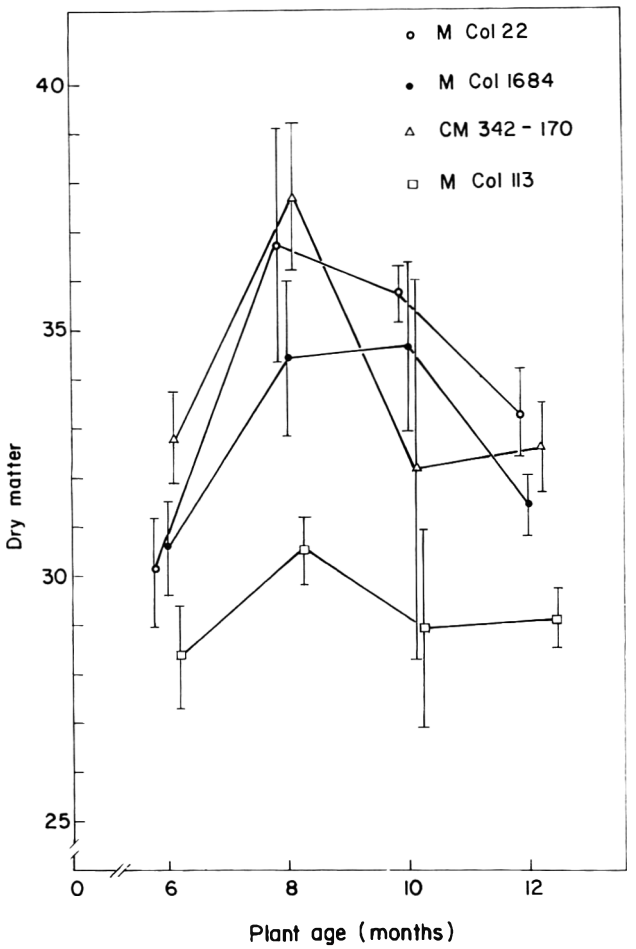

Figure 1. Variation in dry matter contents of whole-root chips of four cassava cultivars as affected by plant age.

periods at the 6th and the 8th month of plant age, then it decreased to a range of 63–58% at the 10th month and reached its highest values of 75–80% in October 1982, at the 12th month. The rainfall recorded during the four drying periods of 2 weeks each were 21, 5, 0 and 79 mm, corresponding to plant ages of 6, 8, 10 and 12 months, respectively. At the drying period corresponding to the 10th month of plant age, when the lowest relative humidity and no rainfall were recorded, the average duration of sun drying was 59 hr compared to 72 hr for the other periods. The shortest and the longest sun drying periods lasted for 53 and 77 hr, respectively.

Figure 2 summarizes the total cyanide concentration in fresh whole-root chips as well as that of the corresponding sun and oven dried chips. Fresh chips of roots of cv. M Col 1684 had the highest ($P < 0.01$) total cyanide content in the range of 900–1200 mg/kg^{1} on a DM basis (overall mean: 1031 ± 137) and the local cultivar (M Col 113) showed the lowest ($P < 0.01$) values (296 ± 48 mg/kg^{1}); the chips of the other two cultivars had intermediate cyanide levels (552 ± 105 and 507 ± 88 mg/kg^{1} for cv. M Col 22 and CM 342-170, respectively). Changes in the cyanide concentration of chips of each cultivar with plant age reflected the combined changes of the cyanide content in separate root peel and parenchyma tissues (Gómez, unpublished observation).

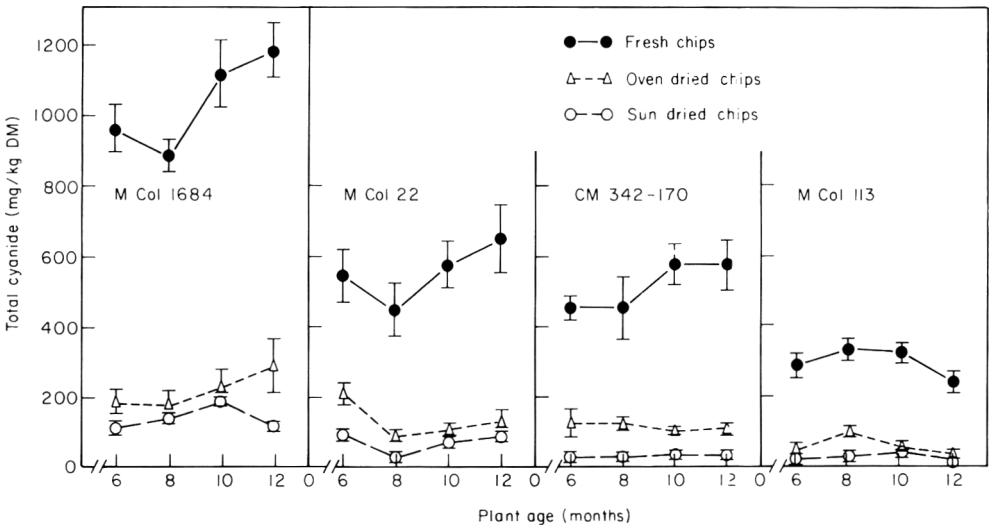

Figure 2. Total cyanide contents in fresh, sun dried and oven dried chips of four cassava cultivars at four plant ages.

Sun drying cassava whole-root chips on a concrete floor led to higher ($P < 0.05$) cyanide elimination than oven drying on trays at 60°C and, therefore, oven dried chips had consistently higher cyanide contents than the corresponding sun dried chips (Fig. 2). Sun drying eliminated between 86 and 94% of the initial total cyanide, expressed on a DM basis, present in fresh chips whereas for oven drying the loss was 77–80% (Table 1).

Table 1. Effects of sun drying on a concrete floor and oven drying at 60°C on cyanide losses and free cyanide content*

Parameter	Cassava cultivar			
	M Col 1684	M Col 22	CM 342-170	M Col 113
Dry matter (%) in				
sun dried chips	94.3 ± 2.3	94.6 ± 1.5	94.8 ± 0.9	93.7 ± 1.4
oven dried chips	95.9 ± 3.0	96.1 ± 2.6	95.2 ± 2.9	97.5 ± 2.1
Total cyanide losses (%) by				
sun drying	86 ± 3	87 ± 5	94 ± 1	92 ± 4
oven drying	79 ± 5	80 ± 4†	77 ± 9	80 ± 7
Bound cyanide losses (%) by				
sun drying	95 ± 2	93 ± 5	98 ± 1	97 ± 3
oven drying	82 ± 5	85 ± 4†	81 ± 10	82 ± 10
Free cyanide (% of total) in				
fresh chips	28 ± 6	20 ± 4	20 ± 4	25 ± 5
sun dried chips	76 ± 12	59 ± 17	74 ± 14	74 ± 15
oven dried chips	41 ± 11	39 ± 7†	31 ± 13	36 ± 15

*Each value is the mean of twenty samples (five per each of the four plant ages: 6, 8, 10 and 12 months) ± s.e.m., and loading rates were 10 and 20 kg/m^2 for sun and oven drying, respectively, unless otherwise indicated.

†Each value is the mean of fifteen samples (five at each plant age of 8, 10 and 12 months) ± s.e.m. The mean values of the samples at 6 months of plant age when the loading rate was 10 kg/m^2 were: 62 ± 5, 67 ± 3 and 29 ± 5 for total and bound cyanide losses and free cyanide, respectively.

Since most of the total cyanide in fresh chips is found in the bound form, cyanide loss is mainly accounted for by the hydrolysis of the cyanogenic glucoside (linamarin) and the release of free cyanide. Under the climatic conditions recorded in this study and at a fresh chip loading of 10 kg/m^2, sun drying allowed the elimination of most (93–98% of the initial level; Table 1) of the bound cyanide present in the fresh chips, whereas the loss resulting from oven drying at 60°C at a loading of 20 kg/m^2 was 81–85% (Table 1). Total and bound cyanide losses obtained by sun drying on a concrete floor were higher ($P < 0.05$) than those obtained by oven drying, for all four cultivars (Table 1) and practically at all plant ages studied.

Fresh chips of cv. M Col 1684 showed higher ($P < 0.05$) levels of free cyanide (28 ± 6% of total cyanide) than chips of the other cultivars, in which free cyanide ranged from 20 to 25% (Table 1). In addition to the quantitative differences in cyanide losses, sun and oven drying also differed ($P < 0.05$) in the final proportions of free and bound cyanide in the dried chips. In sun dried chips most of the residual cyanide was found in the free form; sun dried chips of cv. M Col 22 showed the lowest ($P < 0.05$) free cyanide content (59 ± 17%) as compared to the levels found (74–76%) in the sun dried chips of the other cultivars. Oven dried chips, on the other hand, had only 31–41% of free cyanide, thus most (59–69%) of their residual cyanide was still present as the bound or glucosidic cyanide (Table 1).

Discussion

Previously reported losses of cyanide at drying have varied from 18 to 21% at 80°C and from 26 to 33% at 60°C (Joachim & Panditteseker, 1944) to as high as 85% with oven drying and 56% with sun drying (Paula & Rangel, 1939). A recent study (Cooke & Maduagwu, 1978) has reported that 29% of the bound cyanide was removed by drying at 46.5°C and smaller losses were recorded at the higher temperatures.

A great deal of the discrepancies of these and other reports (Correia, 1947; de Bruijn, 1971; Tewe, Maner & Gómez, 1977) are due to the differences in the experimental processing condition used, which in most cases have not been clearly specified. In several cases (Joachim & Panditteseker, 1944; Cooke & Maduagwu, 1978) chips were prepared from peeled rather than whole roots. Although the root peel constitutes around 10–20% of the total fresh root weight, the root peel usually contains higher, often 5–10 times higher, cyanide levels than the peeled root or parenchymal tissue (Joachim & Panditteseker, 1944; de Bruijn, 1971; Gómez *et al.*, 1980; Gómez & Valdivieso, 1983a). In addition, the root peel or cortex is also a rich source of the enzyme linamarase (Cooke, Blake & Battershill, 1978) and it is used for this reason as the raw material to prepare and purify the enzyme which is used in the enzymatic assay (Cooke, 1978). Therefore, the exclusion or inclusion of the root peel would account for some of the discrepancies found among the reports on cyanide elimination.

Furthermore, in practically all reports there is no mention of the loading rate or the weight of material to be dried per unit of surface area, which is one of the most important processing parameters (Gómez & Valdivieso, 1983b). The loading rate determines the microenvironmental conditions of the biomass being dried, affecting the length of time required to complete drying and hence affecting also the activity of endogenous linamarase. Thus, the differences in loading rates lead to different drying times and cyanide losses (Gómez & Valdivieso, 1983b). For instance, in the present study, when chips of 6 month old cv. M Col 22 were oven dried at 60°C at a loading rate of 10 kg/m^2, the total and bound cyanide losses were 62 ± 5 and 67 ± 3% respectively, as

compared to 80 ± 4 and 84 ± 4% for those parameters, as observed in all other dryings at a loading rate of 20 kg/m² (Table 1). This indicates the importance of loading rate, assuming that plant age is of secondary importance in cyanide losses.

Geometry and size of the chips are also important factors to be considered. Oven drying chips (~0.5 cm thick) of peeled roots allowed greater cyanide elimination than when 1 cm thick slices of parenchyma tissue (Gómez *et al.*, 1984) were used. The shape and size of chips in this study were not uniform, and no attempt was made to control those characters although the same chipping machine was always used.

The results reported confirm previous data (Gómez *et al.*, 1983) showing that the wide range of cyanide concentrations in cassava whole-root chips of several cultivars reflects the different cyanide contents of the root parenchyma and peel tissues (Gómez *et al.*, 1980; Gómez & Valdivieso, 1983a).

Sun drying at a loading rate of 10 kg/m² is a more efficient method for eliminating cyanide than oven drying at 60°C at a loading of 20 kg/m². Furthermore, most of the residual cyanide in sun dried chips is present as free cyanide but in oven dried chips the largest proportion of the residual cyanide is still found at the cyanogenic glucoside, linamarin. This difference emphasizes the need to investigate further the toxicity implications of bound and free cyanide contents in animal feeds based on cassava products and notably on artificially dried whole-root chips.

Apart from the sun dried whole-root chips of cv. M Col 1684, all the others contained total cyanide level lower than the permissible maximum level of hydrocyanic acid (100 mg/kg) set by the Council of the European Community (cited by Ingram, 1975) for cassava products to be used as animal feeds. All sun dried chips of cv. M Col 1684 contained, on the average, more than 100 mg/kg of total cyanide (139 ± 32). Even though the cyanide levels were expressed on a DM basis, their contents expressed as analysed or as fed would be only slightly lower than those reported, since most of the dried chips showed DM contents in the range of 94–96%.

The results of this study confirm the suggestion (Cooke & Maduagwu, 1978) that sun drying would lead to greater losses of cyanide than oven drying because of longer drying times and at moisture contents and temperatures at which endogenous linamarase is active. The magnitude of cyanide losses found are larger than those reported by other workers using different drying conditions, chip sizes and cassava cultivars.

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Relationship between nature of vegetable oil, emulsifier and the stability of w/o emulsion

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Summary

In general, the stability of emulsion (o/w) is dictated by the nature of the emulsifier and the internal phase. However, for water in oil emulsion, in addition to the type of emulsifier, the nature of the continuous phase is of significant importance for providing stability.

The correlation between the degree of saturation of both emulsifiers and vegetable oils has been examined for a variety of water in oil emulsions. Emulsions prepared with unsaturated emulsifiers and highly unsaturated vegetable oils (containing mainly oleic and linoleic acids) were found to be more stable than those prepared with some unsaturated emulsifiers and vegetable oils consisting of less unsaturated fatty acids. Moreover, emulsions prepared with saturated emulsifiers and fully saturated oils have shown better stability than similar emulsions prepared with less saturated oils.

Since rigidity of the emulsifier and the emulsifier-oil interactions on the film are dominant factors in stabilization of w/o emulsions, it is suggested to stabilize water in unsaturated vegetable oil emulsions using specially designed polyglycerol esters of dimerized soybean oil to achieve both requirements.

Introduction

Only few surfactants have been shown to possess the power to stabilize water in oil emulsions (Becher, 1977). Stabilization of such emulsions was claimed to be related to the hydrophilicity of the emulsifiers and several other requirements (Schulman & Cockbain, 1940; Pink, 1941).

1 The soap or the non-ionic emulsifiers must dissolve, in part, in both water and oil phases and should precipitate at the interface.
2 The soap must be wetted by both phases and more easily by the oil and, therefore, lies more into the oil phase.
3 The stabilizing film must have sufficient lateral adhesion.

Other investigators have stressed the need for a rigid or at least a very viscous and uncharged film at the interface (Ford & Formidge, 1966).

Most of the studies carried out on non-ionic surfactants (Sherman, 1950) have emphasized the requirements for the emulsifiers (Lissant, 1974). The relationship between the nature of the continuous phase (the oil) and the emulsifier was examined using the HLB method. Various required HLBs were found and tabulated (Becher, 1977).

The present study examines poassible correlation between the degree of unsatu-

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ration of the oil (vegetable unsaturated oils) and degree of unsaturation of the emulsifiers in view of the requirements for better stability. Therefore, two types of emulsifiers have been considered, sorbitan or polyglycerol esters and the corresponding dimerized surfactants specially dsigned and synthetized to exhibit sufficient rigidity.

Materials and methods

Commercial food grade oils were used without any further purification or treatment. Saturated and unsaturated emulsifiers Spans and Tweens were purchased from Atlas Europol SpA, Italy. Polyglycerol esters, Caprol 6.G.2.0 (hexaglycerol dioleate), were obtained from Capital City Products (Columbus, Ohio, U.S.A.). Polyglycerol esters of dimerized soybean oil were synthesized in our laboratory (Garti, Goubran & Zaidman, in preparation).

Emulsions of water in oil were prepared by dissolving pairs of emulsifiers (5 wt %) in the oil phase followed by dropwise addition of the distilled water (10 wt %) while stirring with a laboratory magnetic stir for 2 min (in some cases heating was necessary). Additional homogenization using a Silverson Laboratory homogenizer, supplied with a square hole high shear screen; for an extra 2 min was carried out to complete emulsification.

Emulsion stability was determined by measuring the degree of creaming and water separation after incubation in 100 ml cylindrical test tubes at 60°C for 24 hr. Particle size distribution was qualitatively determined by observation under the Carl Zeiss optic microscope at 1000 magnification.

Results

Four series of emulsifiers have been used with a variety of saturated and unsaturated vegetable oils for the preparation of 10 wt % water in oil emulsions. Figure 1 demonstrates the stability of the emulsions prepared with a pair of saturated emulsifiers (Span 65 + Tween 60) at the optimal HLB values. It can be seen that the percentage of breaking is directly related to the degree of unsaturation of the oil. Coconut oil consisting of only 9 wt % unsaturated fatty acids formed the most stable emulsion with 30 wt % separation of oil, while olive oil with 89 wt % unsaturated fatty acids was found to form a most unstable emulsion with almost complete separation of the water. Therefore, it is clear that saturated emulsifiers are better for the stabilization of saturated oils. Practically all emulsions were unstable except the emulsion with castor oil (No. 1 in Fig. 1).

It should be noted that castor oil has a high degree of unsaturation combined with the presence of a hydroxyl group at position 12 of the fatty chain.

Similar emulsions were prepared with the same family of emulsifiers (sorbitan esters) except that the hydrophobic chain in the emulsifiers was unsaturated, consisting mainly of oleic acid (Span 85 and Tween 80).

Figure 2 describes the stability of these emulsions. As the percentage of unsaturation in the oil increases, more stable emulsions are formed; for example, emulsions prepared with coconut oil (No. 11 in Fig. 2) revealed 85 wt % separation while safflower and sunflower oils formed more stable emulsions. Here again castor oil emulsions were found to exhibit best stability. The correlation between the emulsifiers and the oil was demonstrated in the two series in which the tendency towards stability is directly related to the degree of saturation of both emulsifiers and oils.

In order to further support these findings, additional series of emulsions were

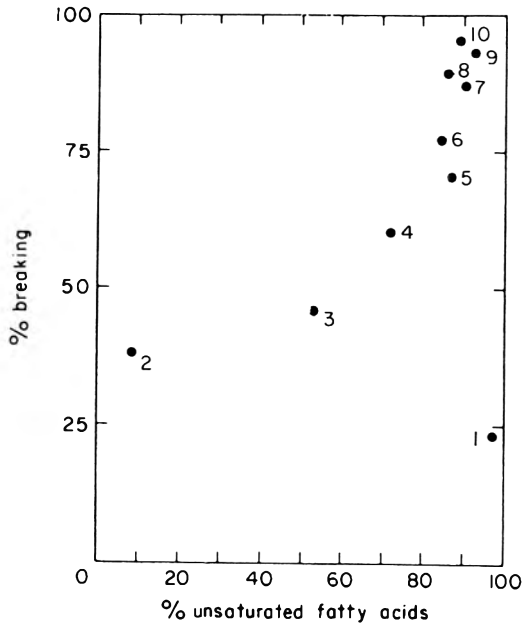

Figure 1. Correlation between stability of w/o emulsions and percentage of unsaturated fatty acids existing in the oil phase ($\phi = 0.10$) prepared with a pair of 5 wt % saturated emulsifiers (Span 65 + Tween 60) at the required optimal HLB of each oil (3–5). (1) Castor, (2) coconut, (3) palm, (4) cotton, (5) soybean, (6) cod liver, (7) corn, (8) sunflower, (9) safflower and (10) olive.

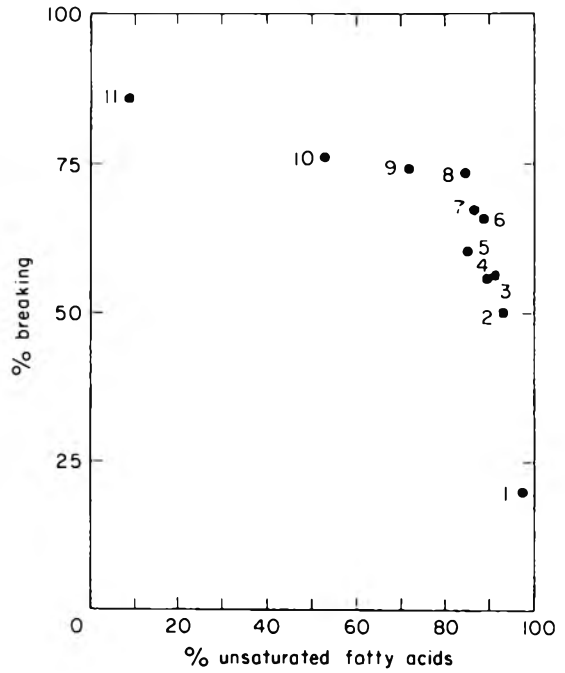

Figure 2. Correlation between stability of w/o emulsions and percentage of unsaturated fatty acids existing in the oil phase in emulsion ($\phi = 0.1$) prepared with 5 wt % unsaturated pair of emulsifiers (Span 85 + Tween 80) at the required optimal HLB of each oil (HLB 3–5). (1) Castor, (2) safflower, (3) sunflower, (4) linseed, (5) cod liver, (6) olive, (7) soybean, (8) corn, (9) cotton, (10) palm and (11) coconut.

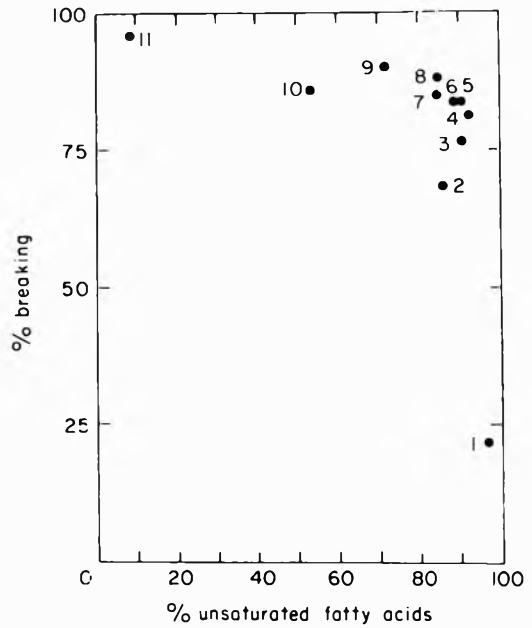

Figure 3. Correlation between stability of w/o emulsions and percentage of unsaturated fatty acids existing in the oil phase ($\phi = 0.1$) prepared with 5 wt % unsaturated pair of emulsifiers (Arlacel 186 + 6.G.2.0) at the required HLB of each oil (HLB 3–5). (1) Castor, (2) soybean, (3) sunflower, (4) safflower, (5) linseed, (6) olive, (7) cod liver, (8) corn, (9) cotton, (10) palm and (11) coconut.

prepared. Figure 3 is a summary of the results obtained from emulsions prepared with the same oils and different pairs of unsaturated emulsifiers. A blend of unsaturated mono-diglycerides (Arlacel 186) and unsaturated polyglycerol ester (6.G.2.0) at similar

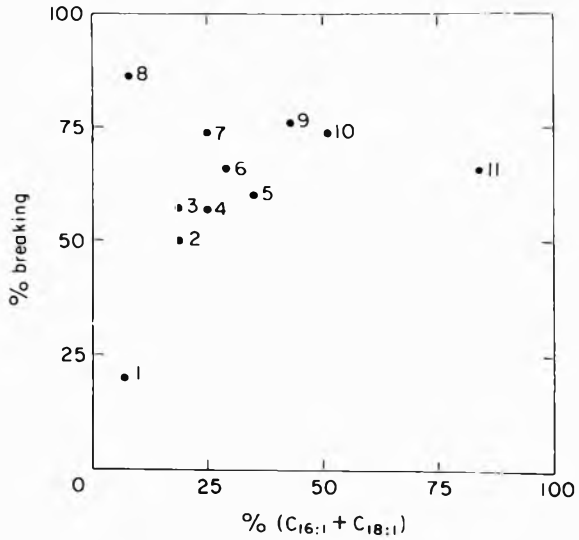

Figure 4. Correlation between percentage of $C_{16:1}$ and $C_{18:1}$ fatty acids in the oil phase and the stability of the w/o emulsion ($\phi = 0.1$) prepared with Span 85 + Tween 80 (5 wt %). (1) Castor, (2) safflower, (3) linseed, (4) sunflower, (5) cod liver, (6) soybean, (7) cotton, (8) coconut, (9) palm, (10) corn and (11) olive.

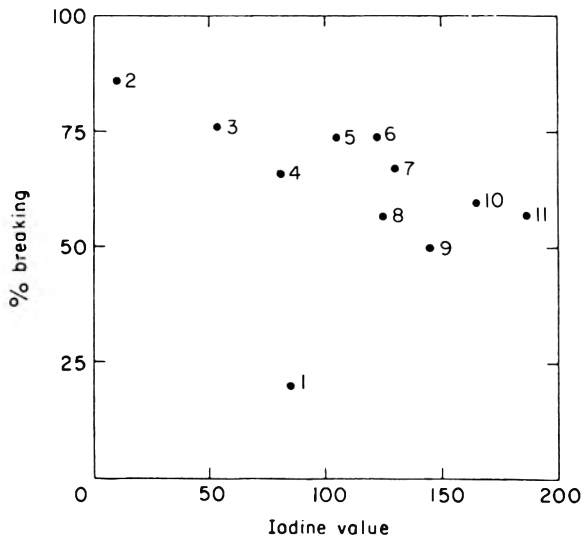

Figure 5. Correlation between iodine value and percentage of water separation in w/o emulsion (ϕ = 0.1) prepared with 5 wt % Span 85 + Tween 80 at the required HLB of each specific oil. (1) Castor, (2) coconut, (3) palm, (4) olive, (5) cotton, (6) corn, (7) soybean, (8) sunflower, (9) safflower, (10) cod liver and (11) linseed.

optimal HLB values was used for the formation of 10 wt % water in vegetable oil emulsions. The trend of stability was as expected; the most stable emulsion was the one with castor oil and the stability decreased with increasing degree of saturation of the oil exactly in the same trend as found with the unsaturated sorbitan esters (Fig. 2).

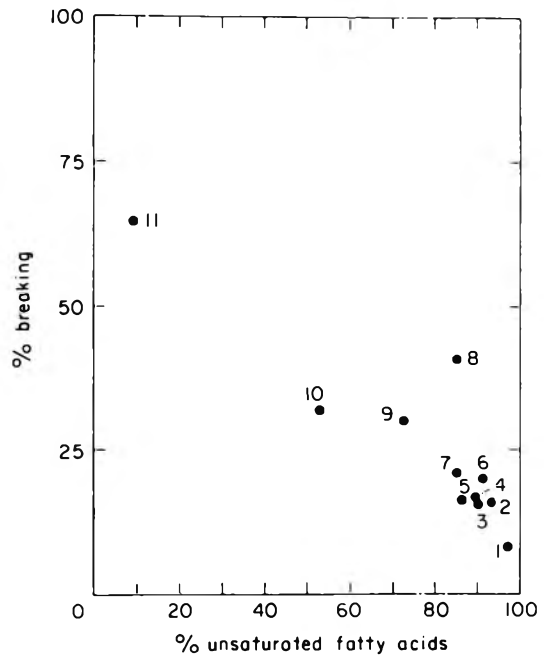

Figure 6. Correlation between stability and degree of unsaturation of the oil phase in w/o emulsion (ϕ = 0.1) prepared with 5 wt % of polyglycerol dimerized soybean oil. (1) Castor, (2) safflower, (3) linseed, (4) olive, (5) soybean, (6) sunflower, (7) corn, (8) cod liver, (9) cotton, (10) palm and (11) coconut.

Attempts to relate stability to the w/o emulsions to a degree of unsaturation based on considering only one of the double bonds in the fatty chain as contributing to stability ($C_{18:1}+C_{16:1}$) is demonstrated in Fig. 4. Good correlation could not be observed.

Similarly, attempts to plot percentage of water separation *versus* the iodine value of the given oils (Fig. 5) revealed a similar decrease in stability with an increase in saturation but not as explicit as found when plotted against the degree of unsaturation (percent of unsaturated acids).

One can notice that all emulsions were found to be relatively unstable and water separation was observed 24 hr after emulsion formation. Several investigations in the past (Schulman & Cockbain, 1940; Pink, 1941; Ford & Formidge, 1966) have shown that stability of water in oil emulsions can be achieved by increasing the rigidity of the film on the interface and adopting its structure to the nature of the emulsified oil. Several commercial materials are offered for stabilization of water in oil emulsions. One of these materials is polyglycerol ester of dimerized soybean oil which serves as a 'pan release' agent.

Since the product does not specify sufficiently the characteristics of the emulsifier it has been decided to prepare such a dimerized emulsifier from pure soybean oil and to use it in our tests.

Using an unsaturated rigid emulsifier with a variety of oils at 10 wt % water in oil emulsion significantly improved the stability of these emulsions. After 24 hr no water separation could be observed and tests were carried out after 7 days' incubation at 60°C (Fig. 6). Only small amounts of water were separated for most emulsions. The emulsions formed with saturated oil (coconut, 91 wt % saturated fatty acids) were again less stable in comparison to the emulsions formed with oils consisting of a high degree of unsaturation.

Since rigidity of the emulsifier plays an important role in the stabilization of water in oil emulsions it has been shown that high stability can be obtained by increasing the concentration of the internal phase of the emulsion. Therefore, emulsions with up to 40 wt % of water in oil ($\phi = 0.4$) were prepared. Table 1 shows that most oils can

Table 1. Summary of stability tests carried out on w/o emulsions ($\phi = 0.4$) prepared with polyglycerol dimerized soybean oil as emulsifier (5 wt %).

Nature of oil	Centrifugation 7500 rev/min (2 min)	Average particle size (μm)*	Incubation at 60°C % breaking† After 24 hr	After 2 weeks
Olive	Creaming	2	21	45
Cod liver	Creaming	2	52	Total separation
Safflower	Creaming	2	0	20
Sunflower	Creaming	1–3	0	10
Soybean	Creaming	4	0	30
Corn	Creaming	1	0	10
Castor	Creaming + breaking	10	80	Total separation
Cotton	Creaming	4	0	37
Coconut	Creaming + breaking	Aggregates	85	Total separation
Palm	Creaming	Aggregates	0	16
Linseed	Creaming	2	0	10

*By microscopic observations.

†No creaming was observed.

Table 2. Summary of stability tests carried out on w/o emulsions (ϕ = 0.4) prepared with polyglycerol dimerized castor oil as emulsifier (5 wt %)

Nature of oil	Centrifugation 7500 rev/min (2 min)	Average particle size (μm)*	Incubation at 60°C		
			After 24 hr	After 2 weeks	
			% breaking†	% creaming	% breaking
Olive	Creaming	3	0	59	0
Cod liver	Creaming	3	0	57	0
Safflower	Creaming	3	0	75	0
Sunflower	Creaming	5	0	54	0
Soybean	Creaming	4	0	81	0
Corn	Creaming	4	0	83	0
Castor	Creaming + breaking	7	66	40	70
Cotton	Creaming	3	0	61	0
Coconut	Creaming + breaking	Aggregates	25	43	52
Palm	Creaming	5	0	58	0
Linseed	Creaming	4	0	57	0

* Using light microscope.
† No creaming was observed.

retain easily 40 wt % water when using the polyglycerol ester of dimerized soybean oil as an emulsifier.

The anomaly found for castor oil emulsions formulated either with saturated or unsaturated emulsifiers and the surprisingly high degree of stability of these emulsions led us to decide to prepare a rigid polyglycerol ester of dimerized castor oil. The nature of the emulsifier and its chemical properties will be discussed elsewhere.

Emulsions consisting of 40 wt % water as inner phase in presence of polyglycerol dimerized castor oil were found to be stable (see Table 2).

The present study emphasizes the need for additional recuirements in preparation of water in oil emulsions that were not mentioned in previous studies: (i) rigidity of the used emulsifier, and (ii) high degree of similarity (structural fitness) between the emulsifiers and the oil used in the system. Therefore, for best stabilization of water in vegetable unsaturated oil emulsions, the formulator should select rigid, specially prepared emulsifiers with a high degree of unsaturation.

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Rheological properties of comminuted meat batters and the relationship to constituent interactions

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Summary

Comminuted meat batters were evaluated for effects of fat level (11, 18 and 26%), endpoint chopping temperature (4, 10 and 16°C), and viscometric measurement temperature (2, 7.3, 12.6 and 18°C) on rheological properties. A capillary extrusion viscometer was utilized with data fitted to the power law model. Batters exhibited pseudoplastic behaviour with apparent viscosities increasing as fat level decreased. Batter fat level and endpoint chopping temperature did not significantly affect the coefficient of shear rate or flow index. Yield stress significantly decreased with an increase of fat level from 11 to 18% but was not affected by chopping temperature. When viscometric measurement temperatures were adjusted from 2 to 18°C, significant flow property changes occurred in the midpoint temperature region (7.3 and 12.6°C), indicating a reversal of the control of flow behaviour by the fat phase at lower temperatures to the aqueous phase at higher temperatures. Transitions of flow behaviour did fit known relationships of water-binding and fat encapsulation by meat protein, fat dispersion and fat melting zones during temperature increases from 2 to 18°C, and protein–protein interactions in the 7.3–18°C temperature range.

Introduction

The flow properties of raw meat batters prior to heat processing have been previously examined in an attempt to link protein functionality with changes that occur in meat microstructure upon comminution. Hamm (1975) studied muscle homogenates and suggested that flow properties were related to water binding capacity of muscle proteins. He reported parallels between minimum yield stress values and apparent viscosity at pH values between 4.8 and 5.4 where protein swelling due to water binding is at a minimum. Toledo, Cabot & Brown (1977) tested seven formulations of varying composition (moisture, fat, protein) using the power law model and there appeared to be no relationship between rheological parameters and comminuted batter stability when batters were heat processed. The major functional property changes in water and fat binding by proteins during batter preparation (chopping) were reported by Brown & Toledo (1975) and the interactions between muscle proteins, fat, water and temperature were discussed in the review of Acton, Ziegler & Burge (1983). These reports did not relate functional properties to flow behaviour during comminution.

Flow properties of meat batters are established during comminution in the temperature region of 0 (initial) and 16–22°C (final) with transformation to a non-flowing solid product during heat processing. During comminution of meats, the temperature

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increases as a result of frictional forces necessary to create the dispersion of meat and fat particles. However, in studies from available literature (Mayfield *et al.*, 1978; Toledo *et al.*, 1977), flow properties with the power law model were evaluated at only one temperature.

The objective of this study was to evaluate the effects of batter fat level, temperature attained during chopping, and viscometric measurement temperature on batter flow properties using the power law model and to relate findings to the known interactions of muscle proteins, water and fat that occur during comminution.

Materials and methods

Ingredients and batter processing

Frankfurter batters were prepared from mixtures of lean boneless beef (4.9% fat) and pork trimmings (55.3% fat) calculated to yield final batter fat levels of 11, 18 and 26%. Total individual batter weights were 3.9 kg and each batter contained 0.156 g $NaNO_2$, 0.55 g sodium erythorbate, 30.0 g NaCl and 200 g added water/kg of meat. Protein and moisture contents (AOAC, 1980) ranged from 13.3 to 15.6% and 56.6 to 68.3%, respectively.

The lean beef was added to a Hobart bowl chopper (model 84181D) and chopped for 1 min. One-half of the water (as ice) was then added along with the $NaNO_2$, sodium erythorbate and NaCl. After chopping for an additional 3 min, the pork trimmings and remaining ice were added and chopping continued until an endpoint temperature of 16°C was attained. Total chopping time was approximately 20 min. At the time of addition of the final ingredients, the batter temperature was near 0°C and as the temperature increased during chopping, samples of batter were removed at 4, 10 and 16°C for rheological analyses.

Rheological evaluation

Batter samples removed during chopping were placed in closed containers and held at 4°C for 24–48 hr. Flow properties were determined by extrusion through a capillary viscometer attached to an Instron (Model 1122). The capillary viscometer was temperature controlled throughout the study by means of a copper coil jacket attached to a circulating water bath (Masterline 2095) containing a 50% ethanol solution. This allowed adjustment of the initial sample to temperatures as low as 0°C with movement to higher temperatures as required.

The capillary extrusion viscometer and viscometric data analyses were previously described and outlined by Mayfield *et al.* (1978). Data were fitted to the power law model of a fluid having a finite yield stress:

$$\tau - \tau_0 = K\gamma^n,$$

where: τ = shear stress, τ_0 = yield stress, K = coefficient of shear rate, γ = shear rate, and n = flow index.

Power law measures of τ_0, K and n were obtained using the iterative computer program adapted from Rao (1974). The apparent viscosity, η_{app}, was also determined at shear rates of 2.95, 11.81 and 59.06/sec using the ratio (slope) of shear stress (τ) to shear rate (γ).

In addition to the effect of fat level on batter flow properties, the effect of viscometric measurement temperature on each batter was determined by evaluation at 2.0, 7.3, 12.6 and 18°C.

Statistical analysis

The experiment was a three factorial arrangement of batter fat level, chopping temperature and replicates, with three replicates performed at each level of batter fat content in a split–split plot design (Steel & Torrie, 1980). Duncan's new multiple range test was used to determine significant differences ($P < 0.05$) among means of rheological measures.

Results and discussion

Apparent viscosity

The effects of fat level and shear rate on apparent viscosity of frankfurter batters chopped to 16°C and measured at 18°C are given in Table 1. Since the apparent viscosity decreased with increase of shear rate, the batters exhibited pseudoplastic behaviour, irrespective of their fat content. The pseudoplastic behaviour also occurred for batters chopped to 4 and 10°C (not shown) and is in agreement with previous findings for comminuted beef, pork and poultry meat batters (Hamm, 1975; Toledo *et al.*, 1977; Mayfield *et al.*, 1978). From the apparent viscosity data it is clear that at 18°C, the aqueous phase of the batter system is more the controlling factor of viscosity than the fat phase.

Table 1. Effect of fat level and shear rate on apparent viscosity (η_{app}) of frankfurter batters*

Fat content of batter (%)	Shear rate per sec		
	2.95	11.81	59.06
11	554.6 ± 176.0	141 ± 46.5	35.5 ± 13.1
18	302.4 ± 3.9	86.4 ± 9.3	27.0 ± 0.6
26	168.3 ± 82.7	57 ± 21.8	18.1 ± 7.7

*Apparent viscosity units are N sec/m^2. Standard error of means are given.

Apparent viscosities at each shear rate increased with decreasing fat level. These results are in general agreement with those of Toledo *et al.* (1977) who reported that at 15°C, lower concentrations of fat in batters increased their resistance to flow. However, Townsend *et al.* (1971) had reported no significant statistical effect for apparent viscosity due to fat content for batters measured at temperatures of 7.2, 12.7 or 18.3°C. Exactly opposite results to those of the current study and those of Toledo *et al.* (1977) were reported by Mayfield *et al.* (1978) for batters of mechanically deboned poultry meat containing 16 and 21% fat. Their results may have been due to evaluating apparent viscosity at 5°C. At that temperature the poultry fat would be expected to exhibit greater viscosity control as compared to evaluation at higher temperatures. Thermal transitions of fat from the solid-to-liquid state are known to influence flow behaviour. Hamm (1975) reported that at 24°C, pork fat and lean beef homogenates independently exhibit similar flow properties.

The temperature range of 15–21°C has several significant aspects in comminuted meat batter preparation: (i) this range is at the maximum level of water binding and dispersed fat stabilization by the meat proteins in the comminuted batter (Toledo *et al.*, 1977); and (ii) this range is at the initial point of the second zone of pork and beef fat

fraction melting, as determined by differential thermal analysis, where release of entrapped fat begins to occur (Townsend *et al.*, 1968) if further mechanical disturbance is placed upon the batter (Brown & Toledo, 1975). Based upon these observations and the current results for apparent viscosity (Table 1), it is evident that apparent viscosity of meat batters is dependent upon batter composition (fat and moisture) and the temperature at which flow behaviour is measured. Temperature effects were explored further in this study using the power law.

Flow behaviour from the power law model

Batter fat level was found to have a significant ($P < 0.05$) effect only on yield stress (τ_0), which decreased as the fat level increased from 11 to 18% (Table 2). The coefficient of shear rate (K) and flow index (n) were not significantly altered although both decreased in magnitude as batter fat content increased.

Table 2. Effect of fat level and endpoint chopping temperature of frankfurter batters on power law measures*

Meat batter variable	Rheological parameter		
	τ_0 (N/m^2)	K ($N\ sec/m^2$)	n (dimensionless)
Fat level			
11%	587.5[a]	441.7[a]	0.42[a]
18%	239.1[b]	367.2[a]	0.40[a]
26%	148.5[b]	244.8[a]	0.37[a]
Endpoint temperature			
4°C	294.8[a]	336.8[a]	0.43[a]
10°C	309.1[a]	385.6[a]	0.38[a]
16°C	371.2[a]	331.1[a]	0.38[a]

*Means within each column for fat level and for endpoint chopping temperature having the same letter are not significantly different ($P > 0.05$).

Yield stress theory (VanWazer *et al.*, 1963) has been described in terms of structuring of particles through attraction of charged surfaces. An analogous description may be attributed for these batters in that charged proteinaceous membranes surrounding the dispersed fat particles are amphoteric and the attractive and repulsive forces of the membrane would impart resistance to flow. Increasing the dispersed fat content would conceivably reduce the resistance to flow due to a larger surface area with less adhering protein and less attraction or resistance to flow.

When temperature effects related to batter chopping temperatures were examined with the power law (Table 2), there were no significant changes in batter flow properties. This implies that during batter development which involves meat particle disruption and protein hydration, flow behaviour is balanced by additional factors related to fat dispersion and its entrapment by the meat protein–water matrix. The temperature effect due to chopping is thus not a factor during batter preparation for the power law components of τ_0, K and n.

The temperature selected to evaluate the batter system by viscometry definitely has an effect on flow behaviour from application of the power law as given in Table 3. No significant ($P > 0.05$) differences were observed for τ_0, K or n when comparing 2 to

18°C. This is probaby due to the reversal of fat and aqueous phase roles in controlling flow behaviour due to the more solid-like characteristics of the fat at 2°C and its fluid-type characteristic at 18°C such that protein–water (aqueous phase) influences predominate at the higher temperature. Both of these factors were discussed earlier as related to apparent viscosity and batter fat level effects on yield stress.

In the midpoint temperature range of 7.3–12.6°C (Table 3), shifts of τ_0, K and n are most likely affected by transitions involving fat melting (Townsend *et al.*, 1968), increase of protein water binding (Brown & Toledo, 1975) and initiation of the heat induced aggregation of protein in forming a protein–protein–water matrix (Acton *et al.*, 1983). Support for these possibilities is present since fat melting is initiated near 7–8°C (Townsend *et al.*, 1968) and the yield stress decreased between 2 and 7.3°C. Also the flow index (n) decreased, indicating development of structural resistance to flow (probably due to protein–protein interactions) between 2 and 7.3–12.6°C. The fact that the flow index increased between 12.6 and 18°C currently cannot be explained by any of these interactions known to occur during comminution.

Table 3. Effect of temperature selected for viscometric measurement on power law parameters for frankfurter batters*

Rheological parameter	Temperature of measurement (°C)			
	2.0	7.3	12.6	18.0
τ_0 (N/m^2)	402.2^a	187.8^b	302.5^{ab}	410.6^a
K (N sec/m^2)	343.4^{ab}	442.4^a	442.5^a	196.6^b
n (dimensionless)	0.425^{ab}	0.337^b	0.314^b	0.505^a

* Means within rows having the same or one of the same letters are not significantly different ($P > 0.05$).

In summary, it is clear that: (i) the composition (fat and aqueous phase levels) of frankfurter batters significantly affected the apparent viscosity and yield stress; and (ii) the temperature selected for measuring power law parameters is important and needs further study to clarify the observed shifts with protein, water and fat interactions known to occur in meat batters.

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Guidelines for the desirable operation of batch freeze driers during the removal of free water

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Summary

A sublimation model of the freeze drying process, which accounts for the removal of free water, is presented and is used to study the operation and design of freeze driers whose plates are heated by controlled means. The analysis is applied to driers wherein heat can be transferred either through the dried layer or through both the frozen and dried layers of the material being dried. In the latter case and for the material chosen for evaluation (non-fat milk), it is shown that most (up to 95%) of the heat used during drying is transported through the frozen layer.

It was found that the shortest drying times are obtained when the condenser temperature and chamber pressure are kept at their lowest values, and the plate temperatures are controlled independently so that the scorch and melting constraints are both held throughout the drying period. The effect of sample thickness on the drying time is significant.

Introduction

The freeze drying process is used to dry certain pharmaceuticals, biological materials, and foodstuffs, which may not be heated even to moderate temperatures in ordinary drying (Goldblith, Rey & Rothmayr (1975); Mellor (1978); Liapis & Marchello (1984)). The substance to be dried is frozen, and the water is removed as a vapour by sublimation from the frozen material in a vacuum chamber (Fig. 1). After the moisture sublimes to a vapour, it is usually removed by an appropriate condenser, and sometimes by steam jet ejectors or mechanical vacuum pumps.

Freeze drying requires a very high vacuum because the vapour pressure of ice is very small. If the water were in a pure state, freeze drying could be performed at near 273 K at a pressure of 4.58 mmHg absolute. However, since the water usually exists in a solution or a combined state, the material must be cooled below 263 K to keep the water in the solid phase. Most freeze drying is usually done at 263 K or lower at chamber pressures of about 2.00 mmHg absolute or less (Goldblith *et al.*, 1975; Mellor, 1978; Liapis & Marchello, 1984).

As a rule, freeze drying produces the highest quality food product obtainable by any drying method. However, freeze drying is an expensive form of dehydration of foods because of the slow drying rate and the use of vacuum. Thus, the major disadvantage of freeze drying relates to energy costs and the lengthy drying times encountered.

Energy costs are high because the material to be dried must first be frozen, followed by freeze drying proper where heat must be supplied to sublime the ice and remove bound water. Thus, the latent heat of fusion must first be removed and then supplied.

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Energy must also be supplied during the freeze drying process to refrigerate a moisture condensing plate, which provides the driving force for water vapour mass transfer (Fig. 1). While it may be possible to recover some of the heat—e.g. by rejecting the condenser heat to the heating surfaces in order to provide the sublimation energy—it is obvious that the process will be inherently energy and capital intensive. Also, a significant amount of energy is used to power the vacuum pump.

The lengthy drying times are caused by resistances to heat and mass transfer and other factors, which have been investigated extensively (Mellor, 1978; Flink & To, 1978; Liapis & Litchfield, 1979a; Litchfield, Liapis & Farhadpour, 1981; Liapis & Marchello, 1984; Flink & Karel, 1972; Flink & Modelina, 1982). A reduction in the drying time can result in reduced energy and labour costs, since throughput may be increased proportionately. Many different designs and operating procedures of freeze driers have been proposed (Goldblith *et al.*, 1975; Mellor, 1978) not all of which are optimal (Liapis & Marchello, 1984). It should be noted that the total drying time must be long enough so that the final moisture content is below about 5 weight percent (wt %) to prevent degradation of the final material during storage.

This paper presents a sublimation mathematical model for the freeze drying process, and this model is used to study the operation and design of freeze driers (Fig. 1) whose plates are heated by controlled means and in which the heat can be transferred through both the frozen and dry layers of the material being freeze dried (Fig. 2). Only batch freeze driers are considered, but the results may have general qualitative applicability to other designs currently in use or contemplated. It should be noted that the sublimation model in this work accounts for the removal of free water only. Free water usually accounts for 75–95% of the total water in the material before drying (Mellor, 1978). Liapis & Marchello (1984), Liapis (1980), and Millman (1984) present models that account for sorbed water and discuss operational policies for the removal of sorbed water during the terminal drying phase.

Freeze drying process

In the freeze drying system shown in Fig. 1 (Keey, 1972), material either frozen or to be frozen *in situ* by passing refrigerant through the plates, is loaded on to the trays. The

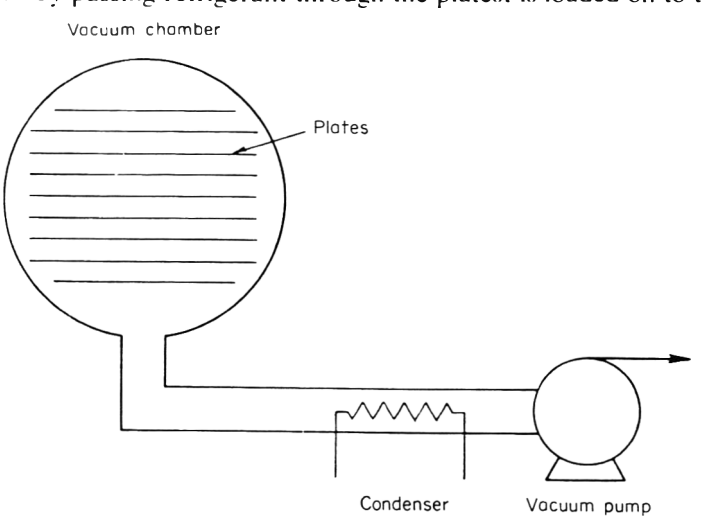

Figure 1. Schematic of a freeze drier whose plates may be heated by controlled means.

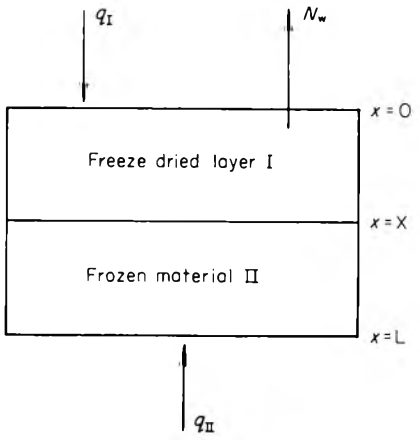

Figure 2. Schematic of sample during freeze drying.

chamber is evacuated, ice starts to sublime, and the vapour flows through the chamber to the condenser plate. Heat is supplied through the plates to provide the heat of sublimation.

The initial drying rates are fast because there is little resistance either to heat transport from the plate to the material, or to mass flux from the material to the condenser. However, as drying proceeds a porous, dried and highly resistant layer builds up around the material. This dried layer is highly insulating to heat flux, but is usually less resistant at low pressures to mass transfer. Since heat and mass transfer resistances are functions of pressure, increasing the pressure may enhance the heat transfer at the expense of an increased resistance to mass transport. However, the net effect may still be an increase in the drying rate, since the water vapour driving force will also increase (Mellor, 1978). It is therefore clear that the chamber pressure is a major control variable of the freeze drying process. The temperature of the plates is another important variable, because it affects the rate of heat transmission to the surfaces of the material being dried, and also the energy reaching the interface between the dried and frozen layers. The condenser temperature is also a control variable because it affects the water vapour pressure driving force for mass transfer (Mellor, 1978).

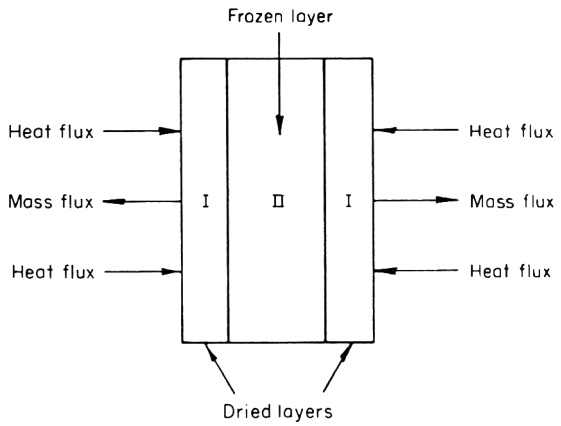

Figure 3. Symmetrical feeze drying.

The maximum temperatures reached in the dried layer and reached in the frozen layer must be low enough to keep degradation to a minimum. Therefore the control variables must always be set in such a way as to prevent a surface temperature higher than the scorch temperature of the drier layer, $T_0 > T_s$, or an interface temperature which exceeds the melting temperature of the interface, $T_X > T_m$.

The most widely used freeze drying process is based upon the heat of sublimation being supplied from the surrounding gases to the sample surface (Fig. 3). Then the heat is transferred by conduction through the dried material to the ice surface. Previous work (Litchfield & Liapis, 1982; Litchfield *et al.*, 1981) with symmetrical solid freeze drying (Fig. 3) has shown that considerable reductions in drying time can be obtained by choosing a slightly elevated pressure (e.g. 2–5 mmHg) and simultaneously maintaining the dried surface temperature at its constraint. The optimum pressure, in fact, also maintains the ice interface at its constraint so that the driving force for mass transfer is maximized.

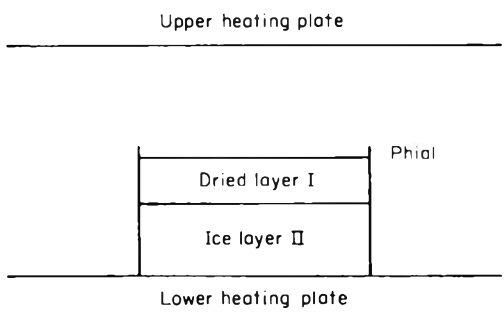

Figure 4. Freeze drying of a liquid frozen in a phial.

In the present work, various cases are studied in which heat is transferred to the interface separating the frozen and dried layers, by conduction through the dried material and/or through the frozen layer (Fig. 2). A typical case of freeze drying by conduction of heat through both the frozen and dried layers is shown in Fig. 4, in which a frozen liquid in a phial is being freeze dried (Liapis & Marchello, 1983). The system in

Table 1. Heat transfer mechanisms and plate conditions

Case	Heat transfer mechanisms	Plate conditions
A	Radiation only to both upper and bottom surfaces	Upper and lower plates at the same temperature, $T_{UP} = T_{LP}$, with a maximum permissible value of 30°C
B	Radiation to upper dried surface; conduction through a film layer at $x = L$	Upper and lower plates are at the same temperature, $T_{UP} = T_{LP}$, and the plate temperatures are controlled subject to the constraints that $T_I \leq T_s$ for $0 \leq x < X$, and $T_{II} \leq T_m$ for $X \leq x \leq L$
C	Same as in B	Upper and lower plates operate at different temperatures, and the plate temperatures are controlled subject to the constraints that $T_I \leq T_s$ for $0 \leq x < X$, and $T_{II} \leq T_m$ for $X \leq x \leq L$
D	No radiation to upper dried surface; otherwise as B	Lower plate temperature, T_{LP}, is controlled subject to the constraint that $T_{II} \leq T_m$ for $X \leq x \leq L$

Fig. 4 is extensively used in the production of drugs by the pharmaceutical industry, while the arrangements presented in Figs 2 and 3 are representative of those used in the food industry. For one dimensional heat and mass transfer, and when the phial offers insignificant resistance to the transfer of heat, the systems in Figs 2 and 4 may be described by the same mathematical model. Previous work (Goldblith *et al.*, 1975; Mellor, 1978; Liapis & Marchello, 1984) has shown that most freeze drying systems may be represented accurately by one dimensional heat and mass transfer models, and the phial introduces, usually, a negligible (Goldblith *et al.*, 1975; Mellor, 1978; Cise, 1982) resistance to heat transfer. In the present study, the systems in Figs 2 and 4 are taken to be equivalent with respect to the mathematical model representation, and the heat transfer mechanisms and plate conditions of the operational policies are shown in Table 1. Various modes of operation can be represented by cases A, B, C, and D in Table 1 and with varying chamber pressures and condenser temperatures.

Model formulation

The one dimensional system considered is shown in Fig. 2. A heat source (e.g. the upper heating plate in Fig. 4) is positioned above a slab of material of thickness L, and provides the heat flux q_{I}. The slab has a dried, porous but resistive later, and a non-porous but conductive ice layer. The base of the slab ($x = L$) may either receive heat by radiation only, or be in perfect thermal contact, or have a thin film layer between its surface and the heat source that provides the heat flux q_{II} (e.g. the lower heating plate in Fig. 4).

The following assumptions are made in the development of the mathematical model:

(1) Only one dimensional heat and mass flows, normal to the interface and the surfaces, are considered.
(2) Sublimation occurs at an interface parallel to, and at a distance X from, the sample surface.
(3) The thickness of the interface is taken to be infinitesimal (Mellor, 1978).
(4) A binary mixture of water vapour and inert gas flows through the dried layer.
(5) At the ice interface, the water vapour is in equilibrium with the ice.
(6) In the porous medium, the solid matrix and the enclosed gas are in thermal equilibrium.
(7) The frozen region is homogeneous, of uniform physical properties, and contains an insignificant proportion of the dissolved gases.
(8) For the system shown in Fig. 4, the thickness of the phial is considered to be infinitesimal when it is compared to the thickness of the sample, and the phial offers insignificant resistance to the transfer of heat (Goldblith *et al.*, 1975; Mellor, 1978; Liapis & Marchello, 1983; Cise, 1982).

Energy and material balances can be made in the dried (I) and frozen (II) layers:

$$\frac{\partial T_{\mathrm{I}}}{\partial t} = \alpha_{\mathrm{Ie}} \frac{\partial^2 T_{\mathrm{I}}}{\partial x^2} - \frac{N_{\mathrm{t}} C_{\mathrm{pg}}}{\rho_{\mathrm{Ie}} C_{\mathrm{pIe}}} \frac{\partial T_{\mathrm{I}}}{\partial x} \quad 0 \leq x \leq X. \tag{1}$$

$$\frac{\partial T_{\mathrm{II}}}{\partial t} = \alpha_{\mathrm{II}} \frac{\partial^2 T_{\mathrm{II}}}{\partial x^2} \quad X \leq x \leq L. \tag{2}$$

In the dried layer, effective parameters are used which include the physical properties of both the gas and solid, which have been assumed to be independent of position. The initial and boundary conditions are:

$$T_{\mathrm{I}} = T_{\mathrm{II}} = T^{\circ} \text{ at } t = 0, 0 \leq x \leq L, \tag{3}$$

$$q_{\mathrm{I}} = -k_{\mathrm{Ie}} \frac{\partial T_{\mathrm{I}}}{\partial x} \text{ at } x = 0, t > 0, \tag{4a}$$

$$q_{\mathrm{II}} = k_{\mathrm{II}} \frac{\partial T_{\mathrm{II}}}{\partial x} \text{ at } x = L, t > 0, \tag{4b}$$

$$T_{\mathrm{I}} = T_X = T_{\mathrm{II}} \text{ at } x = X, t > 0, \tag{5a}$$

$$k_{\mathrm{II}} \frac{\partial T_{\mathrm{II}}}{\partial x} - k_{\mathrm{Ie}} \frac{\partial T_{\mathrm{I}}}{\partial x} + V(\rho_{\mathrm{II}} C_{\mathrm{pII}} T_{\mathrm{II}} - \rho_{\mathrm{I}} C_{\mathrm{pI}} T_{\mathrm{I}}) + N_{\mathrm{t}} C_{\mathrm{pg}} T_{\mathrm{I}} = -\Delta H N_{\mathrm{t}} \text{ at}$$

$$x = X, t > 0. \tag{5b}$$

also,

$$q_{\mathrm{I}} = \sigma F(T_{\mathrm{UP}}^4 - T_{\mathrm{I},0}^4) \text{ at } x = 0, t > 0. \tag{6}$$

The boundary condition at $x = L$ depends upon the circumstances chosen. For radiation only,

$$q_{\mathrm{II}} = \sigma F(T_{\mathrm{LP}}^4 - T_{\mathrm{II},L}^4). \tag{7}$$

For perfect thermal contact,

$$T_{\mathrm{II},L} = T_{\mathrm{LP}}. \tag{8}$$

For a thin film between the frozen material and the lower plate,

$$q_{\mathrm{II}} = k_{\mathrm{f}}(T_{\mathrm{LP}} - T_{\mathrm{II},L}). \tag{9}$$

The value of the film thermal conductivity, k_{f}, was estimated from Carslaw & Jaeger (1976), and suitably adjusted to account for the lowered pressure.

In Table 1 and for case A, the temperatures T_{UP} and T_{LP} are simply fixed temperatures with a maximum permissible value of 30°C. In case B, $T_{\mathrm{UP}} = T_{\mathrm{LP}}$ and the plate temperatures are controlled subject to the constraints that $T_{\mathrm{I}} \leq T_{\mathrm{s}}$ for $0 \leq x < X$, and $T_{\mathrm{II}} \leq T_{\mathrm{m}}$ for $X \leq x \leq L$. For case C, T_{UP} and T_{LP} have different values, and the plate temperatures are controlled subject to the constraints that $T_{\mathrm{I}} \leq T_{\mathrm{s}}$ for $0 \leq x < X$, and $T_{\mathrm{II}} \leq T_{\mathrm{m}}$ for $X \leq x \leq L$. Finally for case D, the lower plate temperature, T_{LP}, is controlled subject to the constraint that $T_{\mathrm{II}} \leq T_{\mathrm{m}}$ for $X \leq x \leq L$.

The mass flux of water vapour through the dried layer, neglecting the mass transfer resistance of the chamber, is given by a simplified version of the dusty gas model (Liapis & Litchfield, 1979a):

$$N_{\mathrm{w}} = -\frac{C_2 D_{\mathrm{w,in}}^0 K_{\mathrm{w}} M_{\mathrm{w}}}{(C_2 D_{\mathrm{w,in}}^0 + K_{\mathrm{w}} P_0) RTX}(P_{\mathrm{wX}} - P_{\mathrm{w0}}), \tag{10}$$

where $P_{\mathrm{wX}} = \mathrm{f}(T_{\mathrm{X}})$ is a function of the interface temperature only. It is sufficient to use the average of the surface and interface temperatures to evaluate with good accuracy the Knudsen coefficient, K_{w}, and the diffusivity, $D_{\mathrm{w,in}}^0$, in eqn (10). The velocity of the

interface is given by,

$$V = \frac{dX}{dt} = -\frac{N_w}{\rho_{II} - \rho_I}, \tag{11}$$

with the initial condition:

$$X = X^0 \text{ at } t = 0. \tag{12}$$

Equations 1–12 represent the sublimation model which has been shown by comparison with experimental data (Liapis & Litchfield, 1979b) to predict accurately the first 75–95% of water removal; this model accounts only for the removal of the free water. Two other models developed by Litchfield & Liapis (1979) and by Liapis & Marchello (1982, 1984) which account for the desorption of bound water and the effect of pore size and tortuosity distributions, predict the experimental data of the freeze drying process more accurately than the sublimation model, but they require many more data from various independent experiments in order to estimate their parameters. These data are not usually available for the foodstuffs and biological materials of interest, and are moveover very difficult to obtain experimentally. The sublimation model requires fewer data, it is easily solved numerically, and when coupled with actual freeze drying data (either pilot or production), can offer the user a means of performing quick 'magnitude' studies in control, design and operation of freeze driers. Recent work (Millman, 1984) indicates that the sublimation and sorption-sublimation models (Liapis & Marchello, 1983; Millman, 1984) provide similar general guidelines for the design and operation of batch freeze driers.

External transport resistances can be easily incorporated into the sublimation model by including the transport equations presented by Liapis & Litchfield (1979a). In a well designed freeze drier, the external mass and heat transfer resistances are made to be insignificant (Mellor, 1978), therefore, they are not considered in the model presented above.

The time constant of eqn (2) has been estimated by Liapis & Litchfield (1979a), and Millman (1984) to be over an order of magnitude smaller than that of eqn (1). Thus, the time derivative in eqn (2) may be set equal to zero, and eqn (2b) becomes:

$$q_{II} = k_{II}\left(\frac{T_{II,L} - T_X}{L - X}\right). \tag{13}$$

The sublimation model involves a moving boundary, the position of the interface, being $X(t)$. This boundary is immobilized by the following transformation:

$$\xi = \frac{x}{X(t)} \quad 0 \le x \le X, \tag{14}$$

which leads to the following relationships:

$$\left(\frac{\partial T_I}{\partial x}\right)_t = \frac{1}{X}\left(\frac{\partial T_I}{\partial \xi}\right)_t, \tag{15}$$

$$\left(\frac{\partial^2 T_I}{\partial x^2}\right)_t = \left(\frac{1}{X^2}\right)\left(\frac{\partial^2 T_I}{\partial \xi^2}\right)_t, \tag{16}$$

$$\left(\frac{\partial T_I}{\partial t}\right)_x = \left(\frac{\partial T_I}{\partial t}\right)_\xi - \frac{\xi}{X}\frac{dX}{dt}\left(\frac{\partial T_I}{\partial \xi}\right)_t \tag{17}$$

Substitution into eqn (1), (4a) and (5b) yields:

$$\frac{\partial T_I}{\partial t} = \frac{\alpha_{Ie}}{X^2}\left(\frac{\partial^2 T_I}{\partial \xi^2}\right) + \frac{1}{X}\left(\xi \frac{dX}{dt} - \frac{N_t C_{pg}}{\rho_{Ie} C_{pIe}}\right)\left(\frac{\partial T_I}{\partial \xi}\right) \quad 0 \le \xi \le 1, \tag{18}$$

$$q_I = -\frac{k_{Ie}}{X}\left(\frac{\partial T_I}{\partial \xi}\right) \text{ at } \xi = 0 \tag{19}$$

and

$$k_{II}\left(\frac{T_{II,L} - T_X}{L - X}\right) - \frac{k_{Ie}}{X}\frac{\partial T_I}{\partial \xi} + V(\rho_{II} - \rho_I C_{pI})T_X + N_t C_{pg} T_X = -\Delta H N_t \text{ at } \xi = 1, t \ge 0. \tag{20}$$

The domain of the differential eqn (18) is now fixed since the boundary is always at $\xi = 1$, and the numerical method of orthogonal collocation (Holland & Liapis, 1983) can be applied in a straightforward way to effect a solution. Jacobi polynomials of sixth order were chosen, and the resulting ordinary differential equations were solved using Gear's (Holland & Liapis, 1983) integration method for stiff differential equations.

Results and discussion

Instant non-fat milk is chosen as a model material, and the values of the parameters used in the sublimation model are given in Table 2. The heat transfer mechanisms and the four plate conditions are presented in Table 1, and are discussed in the previous section of this paper. In case A, the limit of 30°C is frequently employed in the pharmaceutical industry (Mellow, 1978; Cise, 1982). In Table 3, the calculated drying times of the four cases are shown for various chamber pressures, condenser temperatures, and values of the sample thickness L.

Table 2. Parameter values

Parameter	Value
C_{pg} (kJ/kg K)	1.6747
C_{pI} (kJ/kg K)	2.595
C_{pII} (kJ/kg K)	1.9678
C_2	0.428
k_f (kW/m² K)	0.03
k_{II} (kW/m K)	0.0021
T^0 (K)	241.8
T_m (K)	263.15
T_s (K)	303.15
ΔH (kJ/kg)	2791.2
ρ_I (kg/m³)	145.13
ρ_{II} (kg/m³)	1058.00
$D^0_{w,in}$ (kg·m/sec³)	$8.729 \times 10^{-7}(T_0 + T_X)^{2.334}$
K_w (m²/s)	$1.4298 \times 10^{-4}(T_0 + T_X)^{0.5}$
k_{Ie} (kW/m·K)	$0.68(12.98 \times 10^{-6} P_0 + 39.806 \times 10^{-6})$
$P_{wX} = f(T_X)$ (N/m²)	$133.32 \exp\left(23.9936 - \frac{2.19\Delta H}{T_X}\right)$
P_{w0} (N/m²)	$f(T_{condenser})$

Table 3. Drying times (min)

Case	Chamber pressure (mmHg)	Condenser temperature (K)	Sample thickness (mm)							
			2	3	4	5	6	9	12	15
A	0.1	167.0	128.66	203.89	283.38	367.29	454.83	737.96	1051.97	1383.72
		225.0	134.08	211.51	292.62	378.09	466.88	758.97	1070.87	1405.43
		233.5	139.93	219.41	302.67	390.19	480.94	778.18	1094.78	1434.22
	1.0	167.0	131.65	207.95	288.24	372.38	461.55	751.70	1060.08	1390.15
		225.0	135.36	212.76	295.14	380.70	470.09	763.47	1073.72	1407.03
		233.5	140.02	219.63	304.13	391.05	481.85	778.43	1093.97	1429.72
B	0.1	167.0	9.85	18.73	29.54	42.20	56.87	121.65	218.76	344.24
		225.0	9.93	18.87	29.78	42.60	57.54	124.47	223.79	352.25
		233.5	10.04	19.09	30.15	43.24	58.67	128.97	231.83	364.74
	0.25	167.0	9.62	18.33	29.00	41.67	56.89	127.87	229.94	361.84
		225.0	9.69	18.46	29.24	42.12	57.76	130.80	235.16	370.13
		233.5	9.79	18.66	29.62	42.86	59.31	135.48	243.51	383.11
	0.5	167.0	9.27	17.73	28.31	41.69	60.21	138.39	248.74	391.35
		225.0	9.33	17.86	28.58	42.42	61.58	141.49	254.28	400.07
		233.5	9.42	18.06	29.04	43.77	63.79	146.47	263.15	413.93
	0.75	167.0	8.96	17.24	28.23	44.56	64.93	149.03	267.70	421.35
		225.0	9.01	17.37	28.73	45.57	66.39	152.32	273.57	430.38
		233.5	9.09	17.59	29.70	47.19	68.73	157.60	282.97	445.26
	0.9	167.0	8.79	17.02	29.28	46.52	67.74	155.36	278.94	439.25
		225.0	8.84	17.18	29.95	47.57	69.25	158.76	258.50	448.28
		233.5	8.92	17.46	31.02	49.24	71.67	164.21	294.71	463.97
	1.0	167.0	8.68	16.94	30.16	47.89	69.69	159.76	286.75	451.18
		225.0	8.73	17.15	30.84	48.96	71.25	163.23	293.28	460.73
		233.5	8.81	17.56	31.94	50.67	73.72	168.81	303.21	476.44
C	0.1	167.0	5.82	13.13	23.40	36.57	52.68	118.58	210.82	329.32
		225.0	5.93	13.42	23.87	37.30	53.75	120.98	215.10	336.03
		233.5	6.13	13.83	24.63	38.50	55.47	124.85	221.97	346.75
	0.25	167.0	6.07	13.70	24.38	38.13	54.92	123.62	219.82	343.33
		225.0	6.20	13.97	24.87	38.88	56.03	126.10	224.20	350.33
		233.5	6.38	14.42	25.67	40.12	57.80	130.07	231.25	361.23
	0.5	167.0	6.48	14.62	26.03	40.70	58.60	131.93	234.52	366.37
		225.0	6.62	14.92	26.53	41.50	59.77	134.50	239.15	373.57
		233.5	6.82	15.37	27.35	42.77	61.62	138.65	246.50	385.07
	0.75	167.0	6.88	15.53	27.65	43.22	62.23	140.10	249.05	389.02
		225.0	7.02	15.83	28.18	44.05	63.43	142.80	253.87	396.53
		233.5	7.23	16.32	29.03	45.38	65.37	147.13	261.55	408.53
	0.9	167.0	7.12	16.07	28.60	44.72	64.40	144.95	257.67	402.47
		225.0	7.27	16.38	29.13	45.55	65.63	147.70	262.58	410.17
		233.5	7.48	16.87	30.02	46.95	67.60	152.13	270.45	422.47
	1.0	167.0	7.28	16.43	29.22	45.70	65.83	148.17	263.38	411.37
		225.0	7.42	16.73	29.78	46.58	67.30	150.98	268.28	419.20
		233.5	7.65	17.23	30.70	47.95	69.07	155.47	267.38	431.70
D	0.1	167.0	6.40	14.48	25.82	40.38	58.20	131.12	233.22	364.42
		225.0	6.55	14.80	26.40	41.30	59.52	134.08	238.52	382.72
		233.5	6.77	15.33	27.32	42.75	61.62	138.83	246.97	385.95
	0.25	167.0	6.75	15.27	27.18	42.53	61.30	138.10	245.62	383.80
		225.0	6.90	15.60	27.78	43.48	62.67	141.17	251.10	392.38
		233.5	7.13	16.13	28.75	44.98	64.83	146.08	259.87	406.10
	0.5	167.0	7.32	16.53	29.47	46.08	66.42	149.60	266.07	415.72
		225.0	7.47	16.88	30.10	47.08	67.87	152.85	271.87	424.82
		233.5	7.72	17.47	31.12	48.68	70.17	158.07	218.17	439.35

0.75	167.0	7.87	17.80	31.72	49.60	71.47	160.95	286.28	447.27
	225.0	8.03	18.17	32.38	50.65	72.98	164.40	292.42	456.87
	233.5	8.30	18.78	33.47	52.33	75.43	169.82	302.22	472.23
0.9	167.0	8.20	18.55	33.03	51.68	74.47	167.73	298.32	466.07
	225.0	8.38	18.93	33.75	52.78	76.05	171.30	304.67	475.97
	233.5	8.65	19.57	34.85	54.53	78.57	176.98	314.80	491.83
1.0	167.0	8.43	19.05	33.93	53.08	76.48	172.23	306.32	478.53
	225.0	8.60	19.45	34.65	54.18	78.08	175.87	312.77	488.65
	233.5	8.88	20.08	35.78	55.97	80.67	181.67	323.67	504.85

For case A, the drying times are longer than the corresponding times for cases B, C and D. In the purely radiative case, A, for all sample sizes examined, a decrease in condenser temperature at constant chamber pressure brought about a reduction in drying time varying from 11 min (for a 2 mm thick sample) to 50 min (15 mm sample). The largest reduction in drying time due to a reduction in condenser temperature amounted to approximately 8%. For the two lower condenser temperatures, a decrease in chamber pressure leads to a decrease in drying time. However, for the highest condenser temperature, 233.5 K, the drying time is shortened by operating at an elevated chamber pressure when sample thicknesses equal to or greater than 12 mm are used; this indicates that for fairly thick samples, a higher chamber pressure should be used in order to substantially increase the heat flux through the dried layer so that the interface temperature is raised, and the driving force for water vapour mass transfer is increased. This policy is necessary in order to overcome the reduction in mass transfer imposed by the thicker dried layer (eqn (10)) and the elevated condenser temperature.

The drying times for case A are lengthy, due to the relatively small driving forces, and the plates are at 30°C for the entire drying period. Neither the melting or scorching point is encountered during the drying period, so the driving forces for heat and mass transfer are never at their maximum values. Increasing the temperature of the heating plates will reduce the drying times by increasing both heat and mass transfer in the dried layer. The heat transfer will increase because the difference between the surface and interface temperature rises and increases the water vapour pressure at the interface of the dried and frozen regions. It should be noted at this point that numerous simulations (Millman, 1984) have shown that for case A, the shortest drying times are obtained at the combination of minimum condenser temperature and chamber pressure.

In case B, the simulations show that the melting constraint is always encountered before the scorch point is reached (Millman, 1984). This indicates that in case B the process is at first mass transfer limited, and then progresses to a heat transfer limited operation which causes a decrease in the drying rate since the overall heat transfer into the sample is decreased. It is also found (Millman, 1984) that the scorch constraint is reached rather quickly for the smaller sample thicknesses.

As in case A, a reduction in the condenser temperature always produces a reduction in the drying time for cases B, C and D. However, these reductions are smaller than those encountered in case A. It should be noted that the drying times are up to 15 times shorter for case B than for case A under similar operating conditions. The ratio of the drying time for case A to the drying time for case B decreases as the sample thickness increases due to the reduction of heat transfer through the frozen region. While the melting constraint is held at $x = L$, the heat transfer is reduced due to a decrease in the temperature gradient with thicker samples.

An advantage is noticed when the plates are independently controlled, case *C* Feedback controllers can independently set the plate temperatures so that the scorch and melting constraints are both held throughout the drying period. The advantage of case C over case B occurs because the control in case C allows larger heat fluxes through the frozen and dried layers of the sample. The magnitude of the advantage is appreciable for both large or small samples, and is not very great at intermediate sample thicknesses. For small samples, the advantage is due to an increased heat flux in the frozen region, while for large samples, it is due to an increase in the heat flux through the dried layer. It is also seen in Table 3 that case C always provides lower drying times than the other cases.

The drying times for case D are from 5 to 15% longer than those calculated for case C, indicating that most (up to 95%) of the heat used in drying is transported through the frozen layer. The difference between cases C and D is that case C has the maximum allowable heat being transferred from the upper plate, while case D allows no heat transfer from above. The trends noticed in case D show that the dominant heat transfer is through the frozen region, and since no heat is transferred from above, the chamber pressure and condenser temperature influence predominantly the mass transfer of the freeze drying process. This indicates that when the melting constraint is active throughout the drying period, the process is then mainly limited by the rate at which the water vapour can be transported through the porous dried layer.

The results for the situation where perfect thermal contact between the lower plate

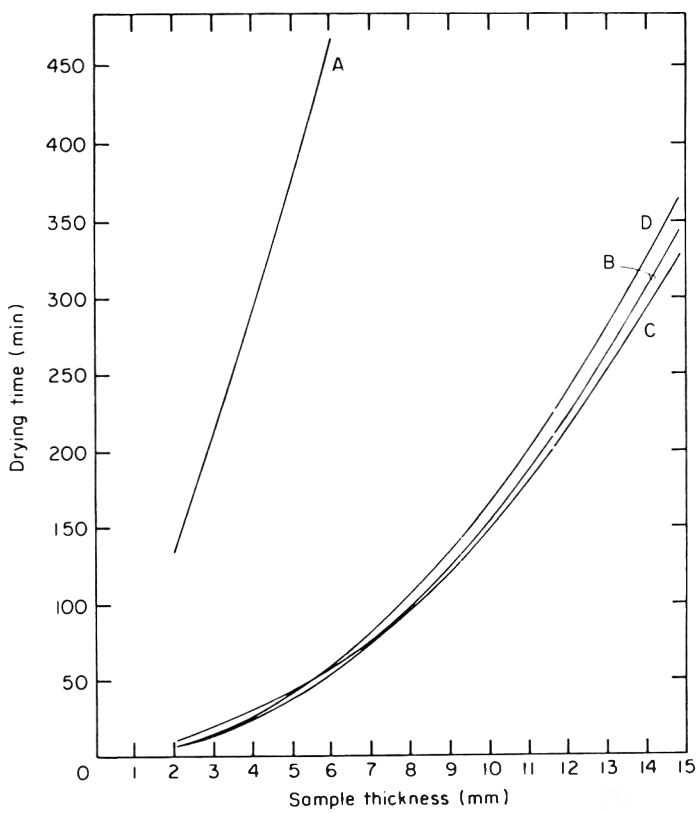

Figure 5. Effect of sample thickness on drying time (chamber pressure = 0.1 mmHg; condenser temperature = 225 K).

and the surface of the frozen layer could be attained, are the same as those obtained in cases C and D, the only difference being that the lower plate temperature is equal to the melting temperature, and the power requirements are less since there is no film heat transfer resistance. However, the cost of designing the drier so that perfect thermal contact is possible may be prohibitive (Goldblith *et al.*, 1975; Mellor, 1978).

In Fig. 5, the drying time *versus* sample thickness data are presented, for a chamber pressure of 0.1 mmHg and a condenser temperature of 225 K. Whereas the drying time increases linearly with sample thickness in case A, a non-linear relationship is observed for cases B, C and D. The increase in sample thickness has a much larger effect on the drying times of case A since the heat flux through the frozen region, for cases B, C and D, is maximized by maintaining $T_{II,I}$ at T_m. The effect of sample thickness is observed to be smaller for case C.

The time variation of the water vapour flux for all four cases, and for a particular set of operating conditions, is shown in Fig. 6. It is observed that the initial mass flux of case A is more than one order of magnitude less than those calculated for cases B, C and D. The flux for case A decreases linearly with time, while the fluxes of the other cases exhibit a non-linear relationship with time. From the data of Fig. 6, the speed of the moving interface can be estimated (eqn (11)).

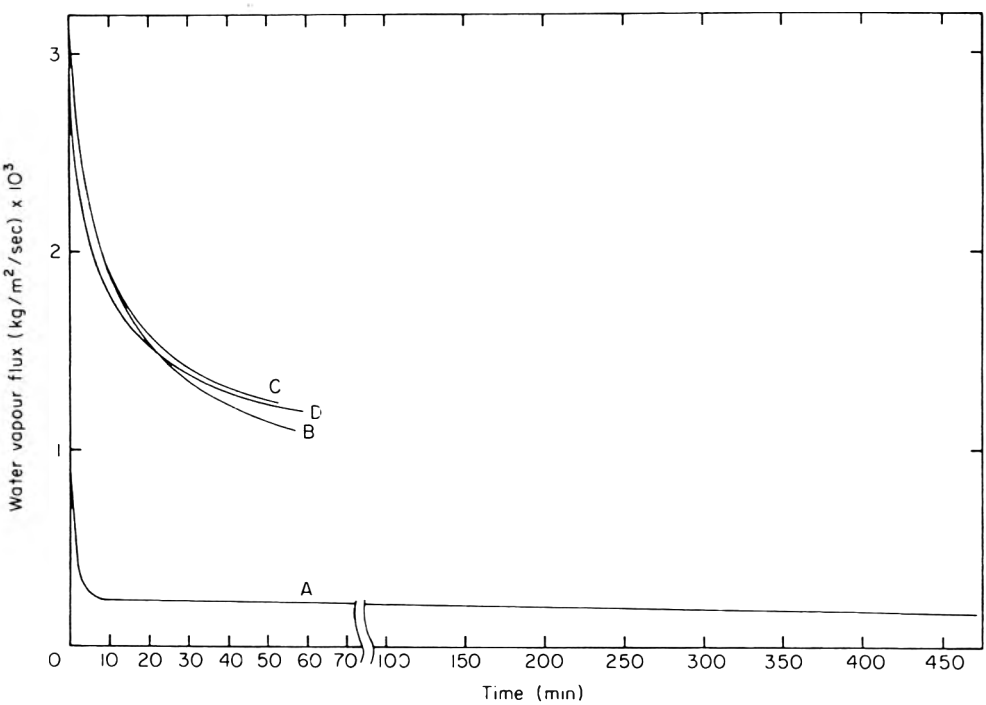

Figure 6. Time variation of mass fluxes (chamber pressure = 0.1 mmHg; sample thickness = 6 mm; condenser temperature = 225 K).

Conclusions and remarks

The results of this work show that the shortest drying times are obtained when the condenser temperature and the chamber pressure are kept at their minimum values, and the plate temperatures are controlled independently so that the scorch and melting

constraints are both active throughout the drying period. They also show that most (up to 95%) of the heat used in the drying phase of the freeze drying process, is transported through the frozen layer.

The purely radiative heat transfer, case A, required the longest drying times and appears to be inferior by comparison to cases B, C and D. Its use may be practical for materials and freeze drying systems for which cases B, C and D are not applicable. For purely radiative transfer, it was also found that for a relatively higher condenser temperature, the drying time is shortened by operating at an elevated chamber pressure when sampies of large thicknesses are used.

Finally, the effect of sample thickness on the drying time is significant for all modes of heat application and transmission, and would be of importance in economic studies dealing with the task of minimizing the cost of the entire freeze drying cycle.

Notation

C_p: heat capacities.
C_2: constant dependent only upon structure of porous medium and giving the ratio of bulk diffusivity within the porous medium to the free gas bulk diffusivity, dimensionless.
$D_{w,in}$: free gas mutual diffusivity in a binary mixture of water vapour and inert gas.
$D^0_{w,in}$: $D_{w,in}P$.
F: Hottel view factor.
$f(T_r)$: water vapour pressure–temperature functional form (Table 1).
k: thermal conductivity.
k_f: film thermal conductivity.
K_w: Knudsen diffusivity (Table 1).
L: sample thickness.
M: molecular weight.
N_t: total flux ($N_t = N_w + N_{in}$).
N_w: water vapour flux.
N_{in}: inert gas flux.
P: total pressure in dried layer.
P_n: drying chamber pressure at surface of dried layer.
P_w: partial pressure of water vapour.
q: energy flux.
R: universal gas constant.
T: temperature.
t: time.
T_{LP}: lower plate temperature.
T_{UP}: upper plate temperature.
V: velocity of interface.
X: position of interface.

Greek symbols

α: thermal diffusivity.
ΔH: enthalpy of sublimation.
ρ: density.
ξ: defined in eqn (14).
σ: Stefan-Boltzmann constant.

Superscripts

0: initial value at time equal zero.

Subscripts

e: effective value.
g: gas.
in: inert.
L: value at $x = L$.
m: melting.
s: scorch.
w: water vapour.
X: value at interface.
0: value at surface.
I: dried region
II: frozen region.

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Technical note: A note on the drying behaviour of cottonseed

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Introduction

The applicability of Fick's law of diffusion to interpret drying curves of more or less 'homogeneous' oilseeds and grains such as soybean, rice, sorghum and wheat has been well demonstrated by various workers (Suárez, Viollaz & Chirife, 1980a; Aguerre, Suárez & Viollaz, 1982; Becker & Sallans, 1955). Cottonseed is a very heterogeneous material in the sense that the hull comprises about 40% of the seed and its chemical composition differs markedly from the endosperm. For this reason the water distribution (in terms of moisture content) inside the seed may not be assumed to be uniform. The purpose of the present communication is to analyse the drying behaviour of this type of material according to Fick's diffusion equation.

Materials and methods

Laboratory drier

The through-circulation laboratory drier has been described in detail in a previous publication (Suárez *et al.*, 1980a). A high constant air velocity (15 m/sec) was used in all experiments in order to minimize external resistances to the moisture loss. This through-flow of air produced a vigorous fluidizing action in the seed sample.

A single layer of seeds (about 10 g) was used for each drying experiment, the progress of which was followed by weighing the sample periodically on a precision balance (±0.0001 g). The reproducibility of drying curves of various cereal grains and oilseeds obtained in this equipment, has been well demonstrated in various publications (Suárez *et al.*, 1980a; Aguerre, 1982).

Materials

A local variety of cottonseed was used in all experiments. Drying runs were performed either with whole seeds or the endosperm; the endosperm was manually separated from the hulls.

It was necessary to add water to the seeds for the drying experiments. This was done by placing the seeds in evacuated vacuum desiccators over pure water (100% r.h.). The samples were kept in a refrigerator at 4°C until 'quasi' equilibrium was obtained. The moisture content of the whole seed (or the endosperm in some experiments) was increased by this procedure to 31 and 24% (dry basis), respectively. These values were taken as the initial moisture content for the drying experiments. Measurements conducted to determine the radius of cottonseeds (whole seed and endosperm) were undertaken by means of a pycnometer, using chlorobenzene as a fluid. The volume of five seeds (by triplicate) was measured, from which the equivalent spherical radius was easily calculated. The measurements were done with both humidified and dried seeds;

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the reduction of the radius for the whole seed and endosperm was, respectively, 13 and 3%.

The moisture content of the seeds was determined gravimetrically using a vacuum oven method at 70°C over magnesium perchlorate.

Determination of equilibrium moisture contents

The details of the experimental technique have been published by Iglesias, Chirife & Lombardi (1975). Equilibrium values at the drying conditions were determined for whole seeds and endosperm. Also the absorption isotherm at 34°C was determined for hull and endosperm separately.

Results and discussion

Table 1 shows the values of the equilibrium moisture content of the whole seed and endosperm for the different drying conditions. These values corresponded to the desorption branch of the isotherm.

From drying runs conducted at 60°C and varying the air velocity (results are not shown here), it was found that operating with air velocities greater than 9 m/sec the drying rate of the seeds was independent of air velocity (i.e. internal control of drying rate). Consequently, an air velocity of 15 m/sec was used in all drying experiments.

Assuming the drying process is totally controlled by water migration within the seed, Fick's second law solution for diffusion out of a sphere will be used to analyse drying kinetic data. Similar analyses were performed by other investigators, with satisfactory results, to interpret drying behaviour of cereal grains and oilseeds (Becker & Sallans, 1955; Suárez, Viollaz & Chirife, 1980b; Steffe & Singh, 1980).

Fick's second law solution expressed in terms of moisture contents is (Luikov, 1968):

$$m^* = \frac{\overline{m} - m_e}{m_o - m_e} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-\frac{n^2 \pi^2 D\theta}{r^2}\right), \tag{1}$$

where m_o and m_e are the initial and equilibrium moisture content, respectively (dry basis); D: the diffusion coefficient (cm^2/sec); r: equivalent radius (cm); and θ: time (sec).

Equation (1) was solved assuming uniform initial moisture content and boundary conditions of the first kind (negligible external resistance to mass transfer).

Although drying is a simultaneous process of heat and mass transfer, it was found for grain drying that the thermal effects can be neglected, taking into account the low values of initial moisture contents usually employed for grain and oilseed drying (Becker & Sallans, 1955; Steffe & Singh, 1980; Suárez *et al.*, 1980a,b). Hence, it can be supposed that the grain temperature of the air and therefore the drying can be analysed as a process controlled by mass transfer, only (Fortes, Okos & Barrett, 1981).

The analysis of the experimental data was performed programming equation (1) in a digital computer. The results for whole seeds are shown in Fig. 1; it can be seen that the experimental data deviate markedly from those predicted by eqn (1). Figure 2 shows similar results for the endosperm; in this case, however, a good agreement between experimental and predicted (Fick's law) data may be observed.

Table 1. Equilibrium moisture content of whole seeds and endosperm at selected r.h.% and temperatures (corresponding to drying conditions)

	Dry bulb temperature (°C)	r.h. (%)	m_e
Whole seed	40	16	0.0480
	60	10	0.0263
Endosperm	50	16.3	0.0236
	60	6.5	0.0120
	70	3.9	0.0105

The values of the diffusion coefficient calculated from the drying curves for the endosperm (Fig. 2), were correlated according to the Arrhenius equation:

$$D = A \exp(-E_a/RT), \tag{2}$$

where E_a is the activation energy and T the absolute temperature. The plot of the values of log D *versus* $1/T$ is shown in Fig. 4, from which the value of E_a calculated by linear regression was 13.8 kcal/mol. For soybean, the activation energy reported by Suárez *et al.* (1980b) as 8.9 kcal/mol.

Cottonseed is a very heterogeneous material composed mainly of the hull and the endosperm, and the hygroscopic nature of both components is expected to be different. This is shown in Fig. 3 which compares the adsorption isotherm (at 34°C) of the hull and the endosperm separately. It can be seen that at any relative humidity, in the range studied, the amount of moisture held by the hull is much larger than that adsorbed by the endosperm. Considering the dry weight proportions of hull and endosperm in the seed, the above means that the hull accounts for most of the total water sorbed by the seed. Thus, the hypothesis of initial uniform moisture distribution used to solve equation (1) may be seriously questioned when applying to the whole seeds. This fact

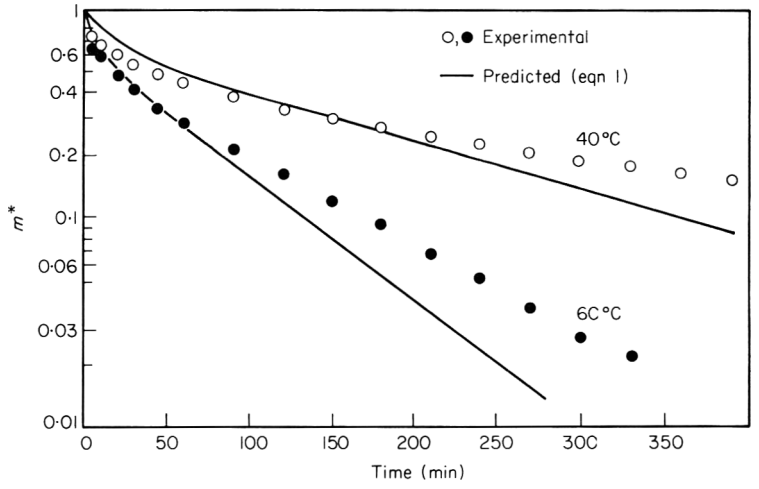

Figure 1. Comparison of experimental and predicted drying data for whole seeds.

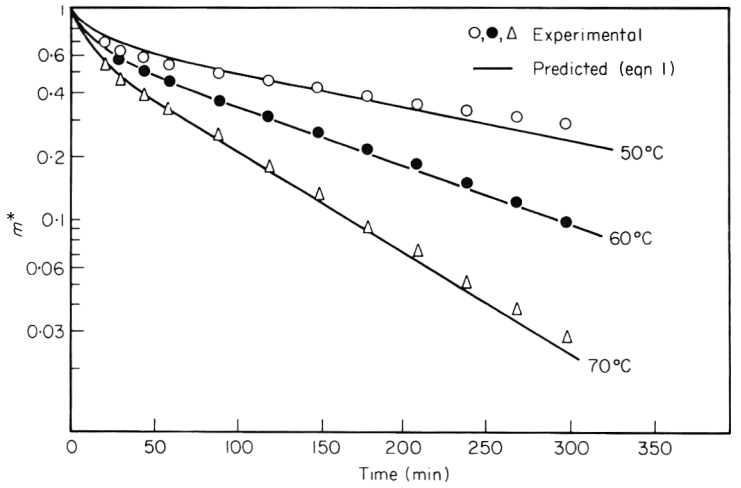

Figure 2. Comparison of experimental and predicted drying data for endosperm.

may explain the lack of agreement between experimental and predicted data, as shown in Fig. 1. Furthermore, it is well known that the rate of moisture diffusion in dried foodstuffs is linked with the slope of the sorption isotherm (King, 1968). The 'anomalous' drying behaviour observed for the whole seed may also be explained by the different characteristics of the sorption isotherm of hull and endosperm. On the other hand, the more homogeneous endosperm shows 'Fickian' behaviour, as it has been usually observed by others for drying of several grains and oilseeds.

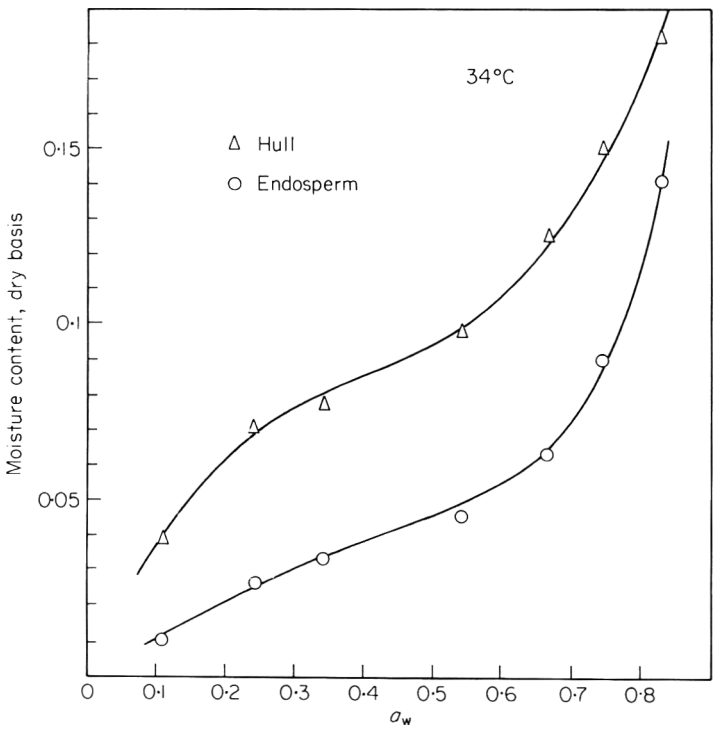

Figure 3. Comparison of the adsorption isotherm of hull and endosperm.

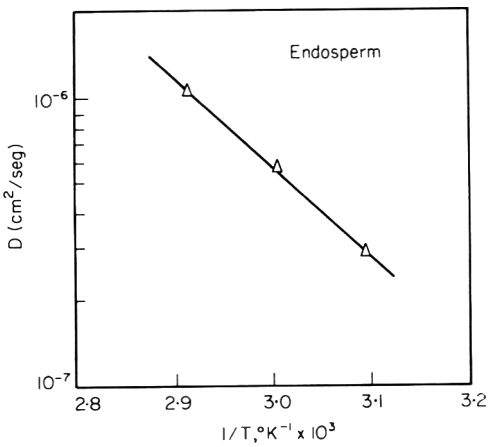

Figure 4. Arrhenius plot for the diffusion coefficients of water in the endosperm.

Acknowledgment

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Technical note: Batch pasteurization of liquid whole egg. II. Baking trials of pasteurized whole egg

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Introduction

Murdoch *et al.* (1960) described the conditions required to establish a satisfactory system for the pasteurization of liquid whole egg. These results are the basis for the Liquid Egg Regulations (S.I. 1963, No. 1503) which uses a high temperature short time (HTST) system.The advantages of a batch system of pasteurization which permits the satisfactory processing of smaller quantities of liquid egg have been discussed by Barclay, Potter & Wiggins (1984).

Since the majority of the commercially pasteurized egg is subsequently either canned or frozen to extend the shelf life, it was considered desirable to test the batch pasteurized egg and compare the baking properties with those of a similar product which had been commercially pasteurized using the HTST system.

Materials and methods

Eggs

Size 4 or 5 infertile eggs were obtained from the Poultry Department of the West of Scotland Agricultural College. These were broken out by hand the day before processing and held at 4°C. Yolks were ruptured but the melange was not otherwise mixed or homogenized before processing.

Later trials used liquid egg which had been broken out and held in frozen storage (−18°C) for variable periods of time. These were allowed to thaw the day before the trial.

In order to determine any change in the baking properties of the liquid egg due to the pasteurization process it was compared with commercially pasteurized liquid egg, which had been stored at −18°C, allowed to thaw as above and used for similar baking trials.

Pasteurization of liquid egg

The equipment used has been described by Barclay *et al.* (1984). The average batch of liquid egg processed weighed 22.5 kg and the processing profile comprised approximately 30 min to achieve pasteurization temperature, 64.4°C, 2.5 min holding, and approximately 30 min to cool the product.

Samples of each batch were examined to confirm the destruction of the enzyme α-amylase by the method described by Shrimpton *et al.* (1962).

The pasteurized product was then filled into plastic pouches for freezing, and refrigerated storage.

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Blast freezing

This was carried out in a Frigoscania multipurpose blast and fluidized bed freezer at a temperature of −30°C. The plastic pouches containing the frozen egg were then stored at a temperature of −18°C until required.

Baking trials

These have been previously described (Barclay *et al.*, 1984). The specific volume (SV) of the baked products was determined by seed displacement (King, Morris & Whiteman, 1936).

Preparation of pasteurized egg samples

Each batch of liquid egg processed was sampled and the samples used in baking trials to compare bakery performance with HTST pasteurized egg both before and after extended frozen storage.

The four series of trials were:

A Held at 4°C and incorporated in different baking recipes within 24 hr of processing.

B Blast frozen at −30°C, stored for 7 days at −18°C before being allowed to thaw and used in baking trials as in A.

C Blast frozen at −30°C, stored between 1 and 3 months at −18°C, thawed and a composite sample used in baking trials as in A.

D Blast frozen at −30°C, stored for different periods from 1 to 6 months at −18°C, thawed and each sample used in baking trials as in A.

Results and discussion

When the bakery trials were carried out on the freshly pasteurized egg (treatment A) the SV ratios obtained by the method of King *et al.* (1936) were test 4.7, control 4.6 for the sponge formulation and test 2.4, control 2.4 for the yellow cake formulation.

The second set of samples (treatment B) which had been frozen provided slightly poorer results, namely SV ratios of test 4.4, control 4.6 for the sponge formulation and test 2.3, control 2.4 for the yellow cake formulation. However, King *et al.* (1936) state that the estimated error of such SV tests in baking trials is ±0.4, so the lower ratios are not significant.

Table 1. Baking trial of stored frozen egg

Duration of storage at −18°C of pasteurized frozen egg (months)	Sponge recipe (SV ratios)		Yellow cake recipe (SV ratios)	
	Test	Control	Test	Control
1	4.3	4.0	2.5	2.5
2	4.0	4.0	2.4	2.4
3	3.8	4.0	2.5	2.5
4	4.1	4.0	2.4	2.4
5	4.0	4.0	2.4	2.5
6	4.1	4.0	2.5	2.5
Average	4.1	4.0	2.5	2.5

In all cases the SV ratios were measured by seed displacement using the method of King *et al.* (1936) who showed the estimated error of such test baking trials as ±0.4.

Treatment C, where the egg had been frozen and stored prior to use, gave SV ratios of test 4.6, control 4.2 for the sponge formulation and test 2.5, control 2.4 for the yellow cake formulation.

Treatment D results are shown in Table 1. Each batch of egg was tested by baking a standard number of cakes using both formulations. The average SV ratios for the sponge recipe were test 4.1, control 4.0 and for the yellow cake recipe test 2.5, control 2.5

It would therefore appear that there is no appreciable change in the quality of the baked product due to batch pasteurization and the additional processing and storage of liquid whole egg.

Conclusion

The baking trials showed that the properties of the liquid whole egg which had been pasteurized by the batch system previously described compare very favourably with liquid egg which had been pasteurized by a HTST system. Similar results were also obtained in baking trials where such egg had been frozen and stored for periods up to 6 months before being allowed to thaw and used for baking.

Acknowledgments

The authors wish to record their sincere appreciation of Mr John Seymour of Rowallan Creamery, Kilmarnock for his valuable contribution in carrying out the extended baking trials and for the directors of his company for the provision of their facilities.

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Book Reviews

Encyclopaedia of Emulsion Technology. Vol. 1. Basic Theory. Ed. by Paul Becher. New York: Marcel Dekker, 1983. Pp. xiv+725. ISBN 0 8247 1876 3. SFr.270.00.

This work consists of nine chapters to which nine experts from five different countries contributed. Chapters 1–7 cover various aspects of emulsion formation and stability in a logical sequence. These are entitled: liquid/liquid interfaces, formation of emulsions, emulsion stability, microemulsions, phase properties of emulsions, emulsion droplet size data and rheological properties of emulsions. Chapter 8 is concerned with the optical properties of emulsions and Chapter 9 comprises an extensive review of the dielectric properties of emulsions and related systems. The book reflects its title—i.e. it is concerned with basic theory. Mathematical expressions, graphs and diagrams abound in the text. Indeed a reader of modest mathematical ability, such as the reviewer, might be, and indeed was, dismayed at first sight of the book. However, on the whole the text is clearly written and most concepts can be grasped in spite of this limitation on the reviewer's part. Nevertheless it is not a book for beginners. Its readership is likely to be confined to those researching in this field and teachers and students studying this subject at postgraduate level.

The book is well presented, the typeface legible, diagrams are clear and helpful. Each chapter has a list of symbols and an extensive reference list which covers the literature comprehensively up to 1982. The book is well indexed.

In spite of the varied backgrounds and nationalities of the authors there is a commendable uniformity of presentation from chapter to chapter, very little unnecessary duplication of material and few typographical errors, all evidence of good editorship. This book represents a useful addition to the literature on emulsion theory and should provide a good basis for Volume 2—Applications.

J. G. Brennan

Introductory Nutrition. 5th edition. By Helen A. Guthrie. St Louis, Missouri: C. V. Mosby, 1983. Pp. viii+675. ISBN 0 8016 1997 1. £19.50.

The first part of the 5th edition of this popular book deals with basic principles of nutrition and covers the main macro and micro nutrients in foods. Part 2 is the applied nutrition section and covers: diet selection, nutritional standards and composition of foods tables; nutritional status; nutrition of different human groups; weight control; alternative diets (such as vegetarian) and national and international problems in nutrition.

The book is intended as an in-depth introduction to nutrition for 'high school graduates' who have had experience of dealing with the concepts of the biological and physical sciences without 'college' training in science. Recent research findings of greatest significance in the application of nutritional principles to the practical task of feeding people are included. The book provides an extremely useful reference text. I thought that one chapter on fat soluble vitamins and another on the evaluation of

nutritional status were particularly good, although all chapters are written to a high standard. One chapter, rarely found in texts of this type, deals with dietary factors which are sometimes given vitamin status and includes such compounds as choline, ubiquinone, lipoic acid and taurine. A few faults have crept in, which is, perhaps, inevitable in a book written by one person. Examples are given below.

In a section on lactose intolerance, the enigmatic phrase 'milk intolerance and lactose intolerance may not be the same thing' is stated without an explanation of milk intolerance. In another chapter on infant feeding, little specific mention is made of infant feeding in less developed countries, in which there may be very great differences from those in the U.S.A. Another point concerns the lack of description of the absorption of bile salts in the enterohepatic circulation. Indeed, the impression given in both the text and the accompanying diagram is that bile salts are not absorbed from the intestine.

There is only a very small section on dietary fibre (about a page in a chapter on carbohydrates). This is disappointing, considering that dietary fibre has been the subject of so much research effort over the last decade. However, lack of emphasis on this aspect of nutrition is common practice in standard nutrition texts. Much more space tends to be devoted to specific vitamin deficiencies which have never been demonstrated in man.

I was puzzled in the section on dietary fibre by one part concerned with disease protection which stated: 'currently the best estimates are that 6 to 8 g/day will afford protection. The intake associated with the typical American diet is 10 to 20 g/day. The recommendation will undoubtedly vary with the source of fiber since the type and amount of the separate fiber components vary greatly from one food to another'. This seems to imply that the average American already has a dietary fibre intake in line with (or in excess of) recommendations. However, as recent recommendations for dietary fibre intakes are around 30 g/day, the value of 6–8 g may have been printed in error. The value of 10–20 g/day given as the average dietary fibre content of the typical American diet corresponds well to that found in recent family surveys in the U.K.

Until a few years ago, a lack of dietary protein was considered to be the primary cause of diet-related malnutrition in the world. This view has changed and most would now agree that if energy needs are fulfilled, then protein intake will be adequate, unless the staple in the diet is exceptionally low in protein (e.g. cassava). In this book, no clear-cut view on the protein/energy controversy is expressed. Perhaps because of this, contradictory statements occur. Thus, 'In kwashiorkor there is a deficiency both in quality and quantity of dietary protein in the presence of adequate calories' is followed later in the same section by 'The most promising attack on the problem of kwashiorkor is prevention, which implies the maintenance of a diet adequate in good quality protein and calories'. It would have been better, from the point of view of future direction of aid programmes if Professor Guthrie had come off the fence, especially in a text read by so many prospective nutritionists. The development of varieties of staples high in certain amino acids (e.g. high lysine maize) has consumed a great deal of time and money which could have been more effectively spent in the relief of malnutrition.

The book is clearly presented on good quality paper (pages: 22 × 28 cm). Each page is divided into three columns, with the outside columns reserved for diagrams and definitions. This is an attractive format, but can lead to wasted space on some pages with no diagrams. At the end of each chapter there is a list of up-to-date references. Where appropriate these are arranged under subject headings. The book is well illustrated with useful tables and figures. However, some diagrams have been simplified

to the extent of leaving out units. There are a number of appendices including a short glossary and a handy list of nutrition prefixes and suffixes with their meanings, recommended nutrient intakes and desirable weights for height and thirty-eight pages of tables of food composition. Finally there is a very comprehensive index. For those in the U.K. who are used to expressing the nutrient composition of foods in units per 100 g, the American use of ounces or servings (cups, glasses or tablespoons) throughout the book may be irksome. However, despite some adverse comments, this is a comprehensive book which is of value to nutritionists at all levels and which nicely complements standard texts of nutrition published in the U.K.

Ann F. Walker

Lipids in Foods: Chemistry, Biochemistry and Technology. By Frank D. Gunstone and Frank A. Norris.
Oxford: Pergamon Press, 1983. Pp. xiv+170. ISBN 0 08 025498 (-3 hardback; -5, flexicover). £14.50 (hardback), £8.75 (flexicover).

This book provides a concise account of the chemical and biochemical properties and technology of lipids in foods. Chapters 1–10, written by Professor Gunstone, describe the chemistry and biochemistry of lipids. The topics covered include chemical structure, separation, analysis, biosynthesis and metabolism, physical properties, hydrogenation, oxidation, reactions and synthesis of lipids. Chapter 11–19, written by Dr Norris, describe technological aspects of fats. Recovery and refining, modification by hydrogenation, fractionation and interesterification, fat products, flavour stability and antioxidants are discussed.

The authors are to be congratulated for writing a book that introduces a wide range of lipid topics whilst, at the same time, the length, and consequently the cost, are maintained at reasonable levels. The book represents a useful introductory text for readers with a sound chemical background. The approach of the authors in writing distinct sections does, however, have significant drawbacks. Discussion of the chemical principles are generally well separated from the technological aspects of a topic. Thus, hydrogenation is covered in Chapter 6, where the emphasis is on pure compounds, and in Chapter 14, where fats are discussed. Also the practical importance of some of the lipid properties and reactions is not adequately indicated in some cases.

The layout of the book is good and there are few errors. The brevity of the coverage requires readers to consult more advanced texts for specific topics of interest, and the authors encourage this by providing a few references at the end of each chapter. The book is completed by a comprehensive index.

This book is recommended as an introductory text for students and others who require a basic knowledge of lipid chemistry and the use of fats in industry.

M. H. Gordon

Instrumental Analysis of Foods: Recent Progress. Vol. 1. Ed. by George Charalambous and George Inglett.
New York: Academic Press, 1983. Pp. xvi+437. ISBN 0 12 168901 8. U.S.$42.00.

This collection of twenty-six papers records the proceedings of a symposium of the 3rd International Flavour Conference held in Corfu, 27–30 July 1983. The conference

covered beverages also, but they are to be dealt with in Vol. 2. The title of the book must be read in this context; on its own it is likely to mislead.

Thus, a number of papers have little direct concern with instrumental analysis: European and International Flavour Regulations (F. Grundschober, 15 pp.); Chemical Senses and Food Flavor: an Overview (J.C. Boudreau, 19 pp.); NBS Standard Reference Materials for Quality and Assurance of Food Analyses (R. Alvarez, 16 pp.); Heterocyclic Compounds in Browning and Browning/Nitrite Model Systems: Occurrence, Formation Mechanisms, Flavor Characteristics, and Mutagenic Activity (T. Shibamoto, 50 pp.); Taste Properties of Amides (H.-D. Belitz, W. Stempfl, H. Wieser, H. Gries, and W. Mützel, 9 pp.); Osmotic Adaptation of *Escherichia coli* B/r/l (G.D. Anagnostopoulos and S. Roller, 14 pp.); Effective Crop Yield Enhancing Formulations Containing Fatty Acids, Fatty Esters, and Ca^{2+} (A.J. Welebir, 17 pp.); and Study of Barrier Properties of Polymeric Films to Various Organic Aromatic Vapors (S.G. Gilbert, E. Hatzidimitriu, C. Lai, and N. Passy, 7 pp.). Most of these are valuable and it is to be hoped that they will not be lost to the literature because of the inappropriateness of the title of the book. In particular, Boudreau reviews well the anatomy and physiology of taste and smell, Alvarez gives an authoritative and timely reminder, and Shibamoto's paper includes extensive tables and 137 references. The paper by Belitz *et al.*, like many others, summarizes work published, or to be published, in full elsewhere. It is most important work, namely, the discovery that 3-substituted 2,4,6-tribromobenzamides can be very sweet, the β-carboxyethyl derivative being one of the sweetest compounds known with a recognition threshold of 1 μmol l^{-1}, about 20 times lower than that of sodium saccharin and 2000 times lower than that of sodium cyclamate.

Papers directly in line with the title of the book are of two kinds: those concerned with a particular technique and its development, and those concerned with recent applications of analytical techniques to foods. The former comprise only three: Mass Spectra Bank of Volatile Compounds Occurring in Food Flavors (M. Petitjean, G. Vernin and J. Metzger, 28 pp.); More Power to Your Analysis—Integrators and Computers in the Flavor Laboratory (G.G. Barberio, 15 pp.); and Near-Infrared Reflectance Analysis of Major Components in Foods (D.L. Wetzel, 20 pp.). Each of these presents interesting views, more as an hors d'oeuvre than a main course.

The latter constitutes the largest part of the book. Many deal with applications of gas chromatography, often combined with mass spectrometry: Relationships between Gas Chromatographic Profiles of Soy Sauce Volatiles and Organoleptic Characteristics Based on Multivariate Analysis (T. Aishima, 20 pp.); Effect of Latitude on the Composition and Content of Aroma Compounds in Dill (R. Huopalahti, 8 pp.); Volatile Components Produced by Callus Tissues from Three Perilla Plants (K. Nabeta and H. Sugisawa, 20 pp.); Application of the Mass Fragmentographic SIM Technique to the Analysis of Volatile Compounds of Berries, especially of the Genera Vaccinium and Fragaria (T. Hirvi and E. Honkanen, 11 pp.); Formation of Esters and Terpenoids in Passion Fruits and Their Importance to Quality Evaluation (R. Tressl and K.-H. Engel, 30 pp.); GC-MS Comparative Analysis of the Triterpene Alcohols of Cocoa Butter and Cocoa Butter Substitutes (K. Staphylakis and D. Gegiou, 10 pp.); and Off-Flavors from Packaging Materials in Food Products: Some Case Studies (N. Passy, 9 pp.). Aishima's paper is a good example of how the relationships between GC profiles and sensory data are currently being investigated by multiple regression analysis, discriminant analysis, principal component analysis and cluster analysis. Nabeta and Sugisawa use simultaneous distillation and adsorption for the isolation of volatiles and

present useful data on recoveries. As far as production of volatiles by callus is concerned, yields are too poor for it to be practical as yet. Hirvi and Honkanen indicate the power of SIM: they claim methyl and ethyl 2- and 3-hydroxy-3-methylbutanoates as character-impact compounds for bilberries, which enable them to distinguish berries, juices and jams of bilberry from those of other Vaccinium berries. Similarly, they use methyl anthranilate and its *N*-formyl derivative to distinguish wild strawberries (berry, juice or jam) from cultivated. Tressl and Engel show that in passionfruit, as in grapes, an important proportion of potential volatiles is locked up in terpenoid glycosides, so isolation procedures must be selected carefully. Four 3,7-dimethyloctane derivatives were found to be important intermediates, the 1-ene-3,7-diol, 1,5-diene-3,7-diol, 1,7-diene-3,6-diol, and 1-ene-3,6,7-triol. The degree to which such compounds are liberated enzymically and changed chemically determines decisively the profile of volatiles and will itself be determined by the processing conditions to which the passionfruit is subjected. In addition, several new esters were identified and their use, as well as that of other components, in the differentiation of passionfruit by variety, origin, date of harvest and processing conditions is described. Staphylakis and Gegiou's work is an impressive example of differentiation achieved by application of argentation TLC followed by GC-MS to acetylated triterpene alcohols, and Passy quotes four cases, including defective soft-drink polyster/Al foil/polyethylene pouches with excessive residual toluene and polystyrene biscuit trays with a monomer concentration within legal limits, yet responsible for a very unpleasant off-flavour.

Other forms of chromatography are exploited in some of the remaining papers. Recent Procedures in the Isolation and Purification of Amadori Compounds—Applications (N. Moll and B. Gross, 23 pp.) is based on semi-preparative reversed phase HPLC for the convenient isolation and purification of Amadori compounds from crude extracts. Purity of the re-crystallized products was tested by TLC and HPLC. Infrared, mass, and ^{13}C NMR spectra were in accord with the expected structures. The Amadori compound from valine and glucose was converted into its *N*-nitroso derivative. Determination of Tylosin in Tissues, Milk, and Blood Serum by Reversed Phase High Performance Liquid Chromatography (W.A. Moats, 9 pp.) is shown to be capable of detecting 0.05 ppm, about 4 times less than bioassay. Analysis of Peanut Oil in Plasticized PVC and Plasticizer in Peanut Oil (M.-P. Petitjean-Jacquet and J.-M. Vergnaud, 12 pp.) employs gel permeation chromatography on μ-Styragel, deliberately to exclude the PVC and thus allow determination of transfer of oil and of plasticizer. The Identification of Taxiphyllin in *Dendrocalamus latiflorus* Munro and Its Heat Degradation Products (C.-M. Wu, W.-L. Liu and C.-C. Chen, 12 pp.) is based on Sephadex LH-20 column chromatography, followed by UV, IR, NMR and mass spec troscopy. Taxiphyllin, β-D-glucopyranosyloxy-D-*p*-hydroxymandelonitrile, is bitter and had been previously found in other species of bamboo. Under canning conditions it is converted into a number of other compounds. Analysis of Polyethylene Glycols and Determination of Their Mean Molecular Weight by Physical Methods (F. Taleb-Bendiab, S.-A. Taleb-Bendiab and J.-M. Vergnaud, 11 pp.) also uses gel permeation, as well as GC, 'reverse GC' (using PEG as the stationary phase, the relative retention times of, e.g., alcohols, are a function of M_n of the PEG), viscometry, vapour pressure osmometry, and IR. Surface Analysis of Plasticized PVC Packagings by Attenuated Total Reflectance (J.-L. Taverdet and J.-M. Vergnaud, 11 pp.) was used to determine the dioctylphthalate concentration from the absorbance ratio 1720 cm^{-1} (due to ester)/ 955 cm^{-1} (due to PVC) by means of a calibration curve constructed from samples of known composition. The kinetics of transfer of plasticizer in a model system (*n*-

heptane) was then followed experimentally and the results compared with those calculated from diffusion equations. Thermal Degradation of PVC by Hot Wire Cutting as Measured by HCl Generation (M.G. Kontominas, E.K. Voudouris and S.G. Gilbert, 14 pp.) was studied because of the widespread use of hot wire cutting in overwrapping meat for retail with plasticizer PVC. The determination of HCl was undertaken by absorption in a known amount of water and measurement of the pH of the resultant solution. The method was shown to be as precise as titration, but able to detect a minimum of 7 as compared with 40 μg HCl. The amount of HCl formed increased with time and temperature of pyrolysis: with the wire at 250°C, 15'' wide, 0.75 mil gauge PVC gave 0.66 mg HCl per cut. No VCM was detected by GC-MS (limit 10 ppb v/v). Instrumental Neutron-Activation Analysis of Trace Elements in Edible Mollusc Species (C. Papadopoulou, 11 pp.) is concerned with Cr, Co, Zn, Se, Ag, Sb, Sc, Cs and V (V was determined by a fast radiochemical technique) and with three species of mollusc from the Saronicos Gulf. Accuracy was checked by analysing standard reference materials (see above). The levels of toxic elements found were considerably lower than those reported for the Mediterranean and did not reach potentially harmful levels.

It is clear from the above that this volume contains much interesting information on a wide range of topics. Libraries will need to buy it, as will those in the flavour field, and they will be encouraged to do so by the reasonable price. The rapidity of manuscript reproduction is very commendable and the style is acceptable, but there is a consequent lack of editorial control, both overall and in detail. The index is brief (3 pp.).

H. E. Nursten

Enzyme Technology: Preparation, Purification, Stabilization, Immobilization—Recent Advances. Ed. by S. Torrey (Biotechnology Review No. 2; Chemical Technology Review No. 222).
Park Ridge, New Jersey: Noyes Data Corporation, 1983. Pp. xi+308. ISBN0 8155 0956 1. U.S.$42.00.

Enzyme Technology is an up-to-date review of U.S. patent literature on the preparation and uses of enzymes in industry. It is primarily aimed at those directly involved in the industrial application of enzymes but should also be of considerable interest to research orientated enzymologists. As the editor points out, patent literature is frequently overlooked by those who rely on periodical journals!

The book is well turned out and designed and remarkably free of typographical errors. Each chapter deals with one aspect of commercial enzymology or class of enzymes. Rather than just listing the patents and describing their contents the authors have described the problems the patents are to be applied to and then brought in the patents as examples. I found this most effective and enough general information is included to make this book legible and useful to those with limited expertise in enzymology.

Only a small section of the book is concerned with enzymes important to the food industry. However, many more examples of interest to food technologists can be found in the chapters dealing with specific classes of enzyme. This brings me to my main complaint against the book: the index is terrible. There is so much useful information in this book that I'm amazed that there is no general index. The company index, inventor

index and patent index will be most useful to industry but the general reader has to plough through the contents to find the enzyme or subject of interest.

Enzyme Technology is an interesting and useful book which does an admirable job in summarizing and introducing the reader to the U.S. patent literature. The book is well produced and reasonably priced. In my opinion it should find a place in the collections of both academic and industrial institutions.

F. F. Morpeth

Scheme for the Examination of Foreign Material Contaminants in Foods. By P.R. Smith
Leatherhead: Leatherhead Food Research Association, 1983. Pp. iii+33. £18.00.

Those, whether in industry or enforcement laboratories, who have risen to the dizzy heights of responsibility for consumer complaint samples will have experienced the frustration from failure to identify foreign material and the exhilaration as the mystery falls away from some refractory query. This type of sample (or specimen as some would have it) has always been a challenge to the analyst that has enabled him to display the mixture of flair and analytical acumen that represents one of the minor peaks of his artform. At last, with the publication of this book, he has a vade-mecum. This is an A4 pamphlet of some thirty-three pages including index and space for the user's notes and supplied in a protective plastic cover. The booklet is a model of how to deal with a disorderly subject in an orderly way. There are a lot of statistics on foreign materials, their possible origins and the foods in which they are most commonly found. This is followed by an excellent protocol on the documentation and laboratory procedures to be followed, including the wheezes and dodges acquired only as the result of long experience. There is a list of advisory services and a good bibliography. I found no typographical errors. Everyone looking at consumer complaints should have a copy of this publication. I am sure in years to come we will wonder how we managed without it. I think the answer will be, with difficulty.

P. G. Martin

ICUMSA—Proceedings of the 18th Session, 1982.
Peterborough: International Commission for Uniform Methods of Sugar Analysis, 1983. Pp. xix+425. ISBN 0 905003 10 1. £16.00.

The book presents the proceedings of the ICUMSA meeting (18th session) held in Dublin, 13–18 June 1982.

Thirty subjects were discussed at the meeting (1, 1A–29) each concerned with a specific aspect of sugar analysis in relation to the properties and production of the commodity. Each subject is dealt with in basically the same manner to provide a degree of uniformity in the text. A referee introduces the subject and describes the problems associated with the analysis in question. Methodology, details and specifications for methods, work carried out since the last report and recommendations regarding the methods in use, in the light of recent developments in that area (references provided) are amongst the topics discussed. Comprehensive details for selected methods are given

in the appendix where appropriate, and data given as necessary. A transcript of the discussion following the referees report is given. This proves useful in some respects as it provides the reader with information other than that set out in the formal text of the report.

Recommendations for adoption of new methods, where appropriate, for the need for further studies in a particular area, and for modification of existing methods are given. Selected references are given to provide further information in specific areas. The book is basically a reference text to consult where latest guidelines or methods concerning sugar analysis are required and is of interest to all scientists working with sugar or in the field of food analysis. It is unlikely, I feel, that the book will have a wide readership but will be purchased by libraries and research establishments rather than by individuals. The book has been produced by a group of workers in a specific area of the food industry for their own use and to this end the book meets their requirements. It is not the type of book to take off the shelf and read for its own sake however.

Reproduced typescript has been used rather than the more usual printing techniques, presumably to save costs and whilst the text is on the small side compared with printed works it is nonetheless quite legible. The few illustrations are clear and well drawn and the index deals adequately with the major subject areas.

Whilst the book is literally packed with information it is of a very specific type and it is thus difficult to recommend it for general reading. As a source of up-to-date methods in sugar analysis it is invaluable.

Malcolm W. Kearsley

The Preservation of Fruit and Vegetable Food Products. By S. D. Holdsworth. London: Macmillan, 1983. Pp. viii + 159. ISBN 0 333 32292 4. £6.95.

The processing and storage of vegetables and fruit is increasingly becoming an important area in the curriculum of students following horticulture courses and this book provides a very useful addition to the Science in Horticulture series of text books. These have been designed for horticulture students at Higher National Diploma level and have also provided very useful texts for undergraduate students. The author has attempted to provide the reader with a comprehensive overview of the theory and practice of commercial preservation of fruit and vegetables. This has been achieved commendably with the production of a very readable book which is not often associated with student texts.

The book begins by introducing the reader to the subject with summaries of the main methods of preservation together with tables classifying fruit and vegetables into their various types and various statistics on canned and frozen vegetables and fruit. The text then goes on to describe the main causes of deterioration of vegetable and fruit products. The next chapter considers the production of the raw materials for preservation, covering such subjects as cultivar selection, harvesting and post-harvest preparation. The main preservation processes, thermal processing, freezing and dehydration are then described in a series of logical and clearly written chapters followed by a rather brief chapter describing techniques for extending shelf-life by chilling and controlled atmosphere storage. The author then goes on to describe other methods of preservation including jam making and fermentation before devoting a chapter to fruit juice production followed by a chapter which considers the nutritional status of processed food

and undesirable constituents of food. Finally, the last chapter considers the food processing factory, its location, design and construction and the economics of running a processing factory.

The resulting text book is readable, informative and could well provide ideal reading for courses on fruit and vegetable preservation and would also be well suited to students who are studying food science and technology. However, it is intended for students studying horticulture and in this respect it falls short in one main area. The book tends to concentrate on the preservation of processed fruits and vegetables, but the storage, both long and short term, of fruit and vegetables is equally important and yet is only considered in a brief thirteen page chapter. Although the book is commendably low priced, I feel that in places this has been at the expense of quality particularly in the illustrative material used in the book. Many of the figures illustrate particular pieces of equipment used in the preservation and processing of fruit and vegetables but on the whole they are not well chosen for these purposes and generally they are poorly reproduced. Overall though, it is a very useful book which should find its place in many undergraduate and diploma courses on the processing and storage of fruit and vegetables.

P. Hadley

Techniques in Visible and Ultraviolet Spectrometry. Vol. 3. Practical Absorption Spectrometry. Ed. by A. Knowles and C. Burgess.
London: Chapman Hall, 1984. Pp. xxii+234. ISBN 0 412 24390 3. £18.00.

Practical Absorption Spectrometry is the third volume that has arisen from the working parties set up by the UV Spectrometry Group in 1977 and 1981 and was conceived as a modern version of Edisbury's book, *Practical Hints*. The first volume published in 1981 covered standards in absorption spectrophotometry followed by a second volume on standards in fluorescence spectrometry also in 1981. This is by far the most substantial of the volumes to date and in the opinion of the present reviewer by far the most useful.

The book starts with a ten page glossary of terms encountered in spectrophotometry and immediately sets the scene for what is a very practical book. Chapter 1 deals with the basis of absorption spectrophotometry in a way that will be intelligible to all students at all levels, explaining concepts such as band-width and stray-light in a delightfully simple manner.

Chapter 2 turns to spectrometer design which is well illustrated with configurations of a number of instruments, although I should not describe an instrument case as 'a box to put the whole lot in'. The next three chapters deal respectively with the major components of the spectrophotometer—i.e. light sources, monochromators and detectors. In general these chapters are excellent and provide much information, which although it may not be of direct concern to the analyst, helps provide some idea as to the limitations of the instruments. Chapters 6 and 7 turn to electronic signal processing and interfacing techniques. Chapter 6 will only be of interest to those with a basic knowledge of electronics, but here again it does help the analyst appreciate the function of the instrument and hence its limitations. The chapter contains a number of circuit diagrams, some of which are perhaps not essential—e.g. that for an early amplifier using valves. Cell holders are discussed in Chapter 8, which should more logically have been placed before Chapters 6 and 7, and at this point the reviewer was struck with a feeling of *déjà vu*. This was confirmed when Fig. 8.1 and the unnumbered figure on the

following page were compared with Figs 2.1 and 2.2 in Vol. 1 of the series, whose reproduction is acknowledged. The remaining chapters are concerned with spectral measurement, numerical methods of analysis, special techniques (e.g. derivative spectroscopy, difference spectroscopy and dual wavelength spectroscopy) automated sample handling (including continuous flow techniques) and finally instrument maintenasnce.

Overall this is an excellent, well written book which provides much useful data for the practising analyst. However, it does seem that the material in Vols 1 and 3 of this series could have been sensibly combined, after all surely standards are an integral part of practical work. The standard of presentation and illustration is good but as this is a 'working book' the binding is likely to disintegrate before the book loses its usefulness. I am pleased to see the quality of paper is an improvement on that of Vol. 2. At £18 this is not an expensive book considering the wealth of information it contains and I should expect that one will soon see copies of this volume appearing on both library and laboratory shelves.

R. Macrae

Upgrading Waste for Feeds and Food. Ed. by D. A. Ledward, A. J. Taylor and R. A. Lawrie.
London: Butterworths, 1983. Pp. ix+321. ISBN 0 408 10837 1. £34.00.

This book contains the written versions of the lectures given at one of the Easter Schools held regularly by the University of Nottingham School of Agriculture. The meeting had the objective of bringing together academic and industrial scientists to discuss ways in which nutrients that are currently being wasted might be recovered and upgraded to provide human food. The scope of the discussions was broadened by allowing the route to human food to include the feeding of animals to provide meat for human consumption; indeed, in view of the social problems of persuading people to consume material which has conventionally been discarded, this path via animal feeding may be the only option with many waste materials. Because the outlet was restricted to food, the meeting necessarily omitted consideration of other attractive options, such as the manufacture from waste of a fuel such as methane, which seems likely to be the sensible option to take in cases where the waste material is difficult or expensive to handle.

Turning to the individual chapters, after introductory accounts of the world food situation and the assessment of the parts of the food chain in which waste arises, five chapters discuss possible methods by which waste—protein, fat, meat and solutes—may be recovered. These are useful and interesting to the large number of food technologists who are not already familiar with these approaches. Six chapters then discuss ways in which food wastes may be manipulated and modified and these encompass such varied agents as enzymes, earthworms and the microbiological processes involved in silage manufacture. Another five authors discuss the utilization of waste in pet food, meat and meat products, beverages, animal feeds and heat processed foods. Finally, the book concludes with summaries of the nutritional and health implications and of economic aspects.

It is the last aspect which is the nub of the topic of the meeting and of this book. Wilson and Brigstocke comment that many processes which are ingenious and scientifically sound were presented at the meeting but that there was little attempt to present

cost : benefit calculations. It may well be that the majority of the proposals are indeed not economically attractive today. An even more fundamental problem relates to the extent to which, in the developed countries, there is a need to recover and utilize *for food* material which is currently wasted. The concensus today seems to be that the developed world is in a surplus situation. The need to increase food supply is enormous in the developing world but there the technologies described are too sophisticated and too demanding of a highly organized infrastructure to be applicable at present. So, my summary of this book is that it gives excellent accounts of methods of waste utilization and upgrading that, for one reason or another, are unlikely to be used commercially except on a very minor scale.

Brian Kirsop

Books received

A Bibliography of Applications of Near Infrared Reflectance Spectroscopy to Food Analysis. Compiled by B. G. Osborne.

Chorleywood, Herts: Flour Milling and Baking Research Association, 1983. Pp. 56. ISBN 0 907503 01 2. £12.00 including postage, from FMBRA, Chorleywood, Herts.

Sustainable Food Systems. Ed. by Dietrich Knorr.
Chichester: Ellis Horwood, 1983. Pp. xiv + 416. ISBN 0 85321 623 2. £22.50.

Aspects of agricultural production, food processing and distribution, quality and quality control covered in this volume include: energy in agricultural production in U.S., Europe, and the Third World; integrated pest management; organic farming; solar dehydration; recycling of nutrients from food wastes; nutritional considerations and food habits; 'alternative' agriculture; and 'appropriate' food technology.

Grain Processing Losses: Bibliography. Supplement 1 to G117. Compiled by Ruth Kasasian.
London: Tropical Development and Research Institute, 1983. Pp. iv + 67. ISBN 0 85954 163 0. £2.20 including postage.

This bibliography covers combine harvesting, threshing, hulling, milling, grinding, etc., but excludes storage.

Methods of Enzymatic Analysis. Vol. 2. Samples, Reagents, Assessment of Results, 3rd edition. Ed. by Hans Ulrich Bergmeyer, Jurgen Bergmeyer and Marianne Grassl.
Weinheim: Verlag Chemie, 1984. Pp. xvi + 539. ISBN 3 527 26042 0. DM 245.00.

Aspects covered include: preparation and processing of samples; cell and tissue disintegration, fractionation techniques; protein determination by UV absorption, Biuret, Lowry, and dye-binding; reagents and reagent kits; transformation and evaluation of experimental data; quality control of the analysis; and statistical treatment and curve fitting.

Development and Control of Dust Explosions. By John Nagy and Harry C. Verakis. (Occupational Safety and Health Series, Vol. 8.)
New York: Marcel Dekker, 1983. Pp. x + 279. ISBN 0 8247 7004 8. SFr. 147.00.

The main chapter headings are: test methods, definitions, and units of measurement; comparison of dust and gas explosions; ignition of dusts; explosion development in a closed vessel; venting principles for unrestricted vents; explosion experiments with unrestricted vents; vent closures and ducts; and explosion prevention.

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

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

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

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

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

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

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

SI UNITS

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

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

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

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

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