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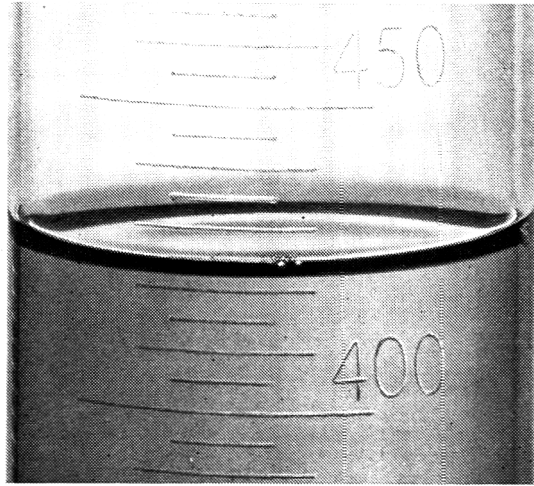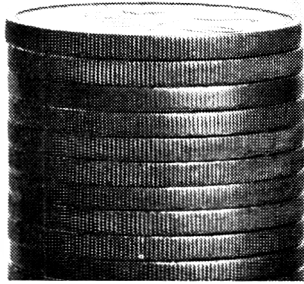
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A technique for comparing judges' performance in sensory tests

C. F. BANFIELD AND J. M. HARRIES

Summary

The scores awarded by a judge when assessing several sensory characteristics of a food sample may be considered as the co-ordinates of the sample in multi-dimensional space. When several samples are scored, each judge produces his own spatial configuration. A method of fitting several such configurations together, by centralization, dilation and rotation, resulting in a measure of residual inconsistency, is described and applied to a set of data obtained during the course of investigations into the carcass quality of beef. The results are discussed from the standpoint of the application of the technique in choosing and training potential members of sensory panels.

Introduction

Expert sensory assessment of a variety of quality characteristics is widely used in the food industry, both for research purposes and for practical grading and classification. Amerine, Pangborn & Roessler (1965) and Harper (1972) for example describe numerous applications. It is accepted that panels of judges are necessary, because of the fallibility of individuals, but methods of selecting and training potential members of 'expert' panels are highly variable, and not adequately reported in the literature. The elementary statistical methods commonly used in screening judges, assessing progress during training, and determining the internal consistency of the final panel, are unsatisfactory from some points of view. Thus, for example, Amerine *et al.* (1965) state that 'in some cases a failure to find differences between trained and untrained panels in ability to discriminate has had its origin in methodological or statistical deficiencies'. This paper describes a technique developed by Gower (1971) for more general purposes and illustrates its use with data from a series of experiments concerned with the grading and classification of meat carcasses. This subject has received little attention in the scientific literature though a series of papers by Gatherum, Harrington & Pomeroy (e.g. 1961) described the use of visual appraisal of sides of bacon. Classification and

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grading of beef carcasses depends in practice on expert appraisal, but research workers have tended to avoid this method, possibly because many of the characteristics normally assessed can be determined objectively by detailed dissection, which is an expensive process since it usually involves destruction of the carcass. However, some of the characteristics of interest cannot so be measured. The results and implications of the experiments, in so far as they relate to practical meat technology, have been reported elsewhere (Williams *et al.*, 1974). The present paper is concerned more with people as judges than with beef as such; it is limited to visual appraisal, but the technique can be applied to any similar set of data derived from sensory assessments.

Computation of a statistic of inconsistency between judges

The scores each judge gives to each sample are considered as the co-ordinates of that sample in an n-dimensional (characteristic) space. Each of the judges is therefore considered to have his own spatial arrangement of the samples in the same characteristic space. Gower (1971) has developed a technique for fitting together a pair of configurations of points in a multidimensional space to obtain a statistic of likeness between them. As a simple illustration consider just two judges who could give scores from 1 to 7 for two characteristics of three samples. The scores are treated as the co-ordinates of the samples in a two-dimensional space. If judge J1 gave 5 and 1 as the scores for the two characteristics of sample A, then A_1 is located at (5, 1) in the two-dimensional space. If he then gave 6 and 4 for sample B and 4 and 3 for sample C, then B_1 and C_1 would be located with co-ordinates (6, 4) and (4, 3) respectively. Similarly the scores given by judge J2 would give his location of the same three samples in the same two-dimensional space, A_2, B_2 and C_2, located at say (6, 6) (4, 7) and (5, 5) respectively. The two judges would then have the three point configurations shown in Fig. 1a and these can be fitted together. The fitting technique firstly centres these configurations and superimposes their centres as illustrated in Fig. 1b by removing the mean score of each characteristic over all samples from the scores awarded for that characteristic. This has the effect of removing any difference in location of the distributions of scores given by the judges, i.e. any difference between judges' concepts of an average for a characteristic. In the example judge J2 was scoring consistently higher for characteristic 2 than judge J1, so this difference is removed by superimposition of the centres. Secondly, each judges' configuration is dilated to be of the same 'size' as the other (Fig. 1c) by scaling the sums of squares of both configurations to be unity. This effectively removes any differences in spread of the distributions of scores given by the judges, i.e. any difference between the judge's abilities to use the full extent of the scoring scale. In the example judge J1 used scores from 1 to 6 but judge J2 only used score 4–7 and this difference is removed by the dilation. Finally one configuration is rotated to fit the other in such a way as to minimize the sum of squares of the distances between the corresponding samples (Fig. 1d). The residual sum of squares, M^2 after applying these three processes is the statistic required

and is a measure of the two judges' inconsistency. In the example it is the sum of squared distances between A_1 and A_2, B_1 and B_2, and C_1 and C_2, after rotating one configuration to fit the other in an optimum way. The rotational fitting technique may expand this idea to as many dimensions (characteristics) as necessary, and to any number of samples. It should not be confused with the rotation of factors in the well-known method of factor analysis.
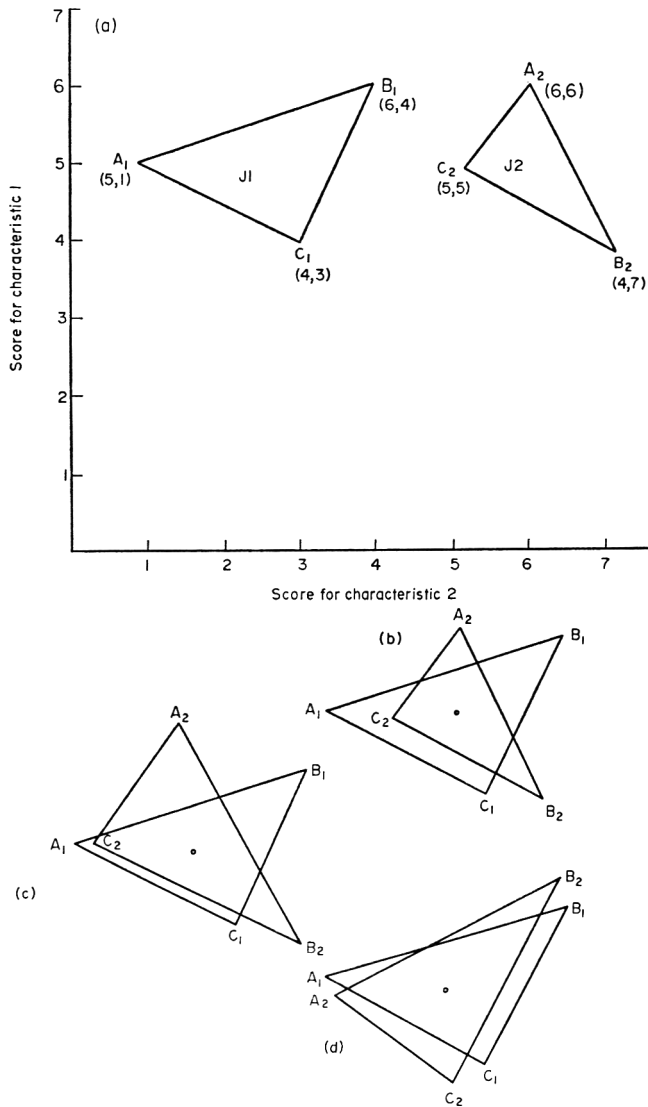
FIG. 1. Simplified illustration of rotational fitting procedure. (a) Plot of original scores, (b) after superimposition of centres, (c) after dilation, (d) after rotation.

Application to scores given to sides of beef

Over thirty judges took part at some stage of the investigations into the visual appraisal of sides of beef, most of them Meat Research Institute staff taking part in trials to select and train those who would subsequently form a panel for future work. Five Fatstock Officers of the Meat and Livestock Commission served throughout as an expert standard for comparison. Their expertise derived mainly from training and experience in operating the subsidy certification scheme, which is based on weight, fatness and conformation. Reported in this paper are the results for those five 'experts' (numbered 1–5) five relatively inexperienced trainees who were present for most of the sessions (numbered 6–10) and two (numbered 11 and 12) who had specialist butchering experience. Each of these twelve judges, working independently, assessed 180 sides of beef during the course of a series of sessions, the arrangement of which has been described by Harries, Pomeroy & Williams (1974). Assessments were made on arbitrary seven-point scales in which increasing scores reflected increasing development or improvement of the following characteristics:

C1 subcutaneous fat (as proportion of side weight);
C2 volume of kidney knob and channel fat;
C3 'feathering' (i.e. fat intermingled with lean between the ribs);
C4 muscle (as proportion of side weight);
C5 muscle to bone ratio;
C6 conformation of buttock;
C7 conformation of rump;
C8 conformation of loin;
C9 conformation of forerib;
C10 overall conformation.

Results for all characteristics

The M^2 values given in Table 1 are the minimum sums of squares of distances obtained from fitting each of the twelve judges' scores to each of the other judges' scores in turn. They were computed using the ROTATE facility in the GENSTAT system, available on the Rothamsted computer (see Nelder *et al.*, 1973). Certain patterns are immediately seen in this table, from which the following inferences about the judges' relative abilities can be made.

The uppermost triangle in the table gives the measures of inconsistency between the five fatstock officers (nos 1–5). The M^2 values here are relatively low, implying that these judges were relatively consistent one with another. By contrast, the values for the five trainees (nos 6–10) were much higher, which indicates that these judges were less consistent in their assessments both between themselves and contrasted with the experts. The final two rows of the table gives the M^2 values for the two butchers (nos 11 and 12)

TABLE 1. M^2 values for each pair of judges (ten characteristics)

	Expert judges					Trainee judges					Butcher
	Judge 1	Judge 2	Judge 3	Judge 4	Judge 5	Judge 6	Judge 7	Judge 8	Judge 9	Judge 10	Judge 11
Expert judges											
Judge 2	0·444										
Judge 3	0·517	0·420									
Judge 4	0·507	0·478	0·500								
Judge 5	0·435	0·369	0·448	0·420							
Trainee judges											
Judge 6	0·745	0·667	0·734	0·762	0·679						
Judge 7	0·908	0·765	0·866	0·859	0·838	0·798					
Judge 8	1·108	1·005	1·075	1·076	1·063	1·037	1·041				
Judge 9	1·095	1·058	1·107	1·104	1·009	1·139	1·215	1·198			
Judge 10	0·986	0·974	0·965	1·018	0·957	0·976	1·026	1·148	1·203		
Butchers											
Judge 11	0·680	0·580	0·601	0·633	0·583	0·730	0·834	1·043	1·070	0·926	
Judge 12	0·571	0·518	0·594	0·553	0·490	0·760	0·888	1·042	1·073	0·956	0·587

who were more consistent with the five fatstock officers than they were with the five trainees, and were more consistent with each other than the trainees but not as consistent with each other as the five experts.

As these M^2 statistics are in effect an overall squared distance between judges in a ten-dimensional space, the technique of Principal Co-ordinate Analysis (see Gower, 1966), can be used to produce the co-ordinates of each judge relative to the principal axes so as to reproduce exactly the given inter-judge distances. By plotting the co-
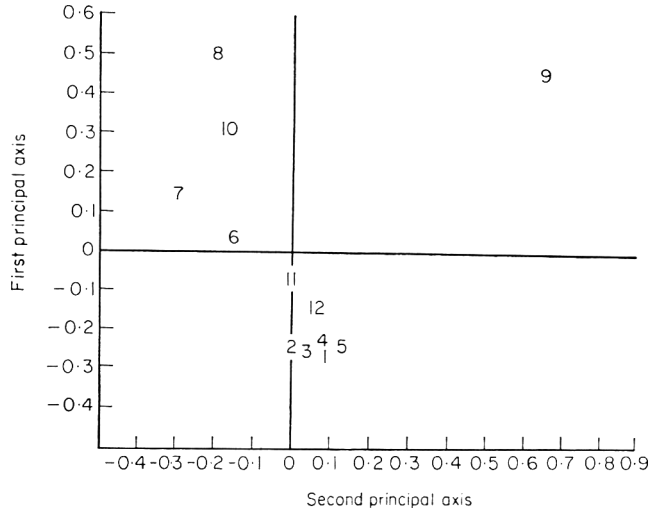
FIG. 2. Co-ordinates of judges relative to the first two principal axes (ten characteristics).

ordinates of each judge relative to the first two principal axes only, a graphical display of the judges' relative positions—distance in this context being synonymous with inconsistency—was obtained using the principal co-ordinate analysis facility in GENSTAT and this is shown in Fig. 2. The picture is as one would expect from the M^2 values. The five fatstock officers are positioned close together, indicating their high measure of consistency. The two butchers are relatively close to one another and are located closer to the fatstock officers than the trainees. The trainee No. 6 is more consistent with the fatstock officers than No. 9 who is just as inconsistent with the other trainees as he is with the butchers and fatstock officers. If the fatstock officers are 'experts' at assessing the characteristics of beef carcasses, how distant a trainee is from the group of fatstock officers at any particular stage indicates how his training is progressing. Trainee No. 6 is consequently considered to be more advanced than No. 9.

TABLE 2. M^2 values for each pair of judges (five conformation characteristics)

| | Expert judges | | | | | Trainee judges | | | | | Butcher |
	Judge 1	Judge 2	Judge 3	Judge 4	Judge 5	Judge 6	Judge 7	Judge 8	Judge 9	Judge 10	Judge 11
Expert judges											
Judge 2	0·513										
Judge 3	0·566	0·552									
Judge 4	0·591	0·569	0·514								
Judge 5	0·502	0·390	0·624	0·546							
Trainee judges											
Judge 6	0·747	0·764	0·810	0·776	0·752						
Judge 7	1·149	1·085	1·088	1·104	1·161	1·153					
Judge 8	1·412	1·449	1·483	1·517	1·513	1·589	1·608				
Judge 9	1·184	1·169	1·253	1·296	1·173	1·318	1·536	1·504			
Judge 10	1·078	0·987	1·041	1·065	1·065	1·113	1·289	1·486	1·569		
Butchers											
Judge 11	0·709	0·682	0·660	0·737	0·727	0·927	1·095	1·576	1·425	1·073	
Judge 12	0·591	0·518	0·620	0·621	0·506	0·861	1·145	1·466	1·310	1·021	0·731

Results for conformation characteristics alone

Five of the ten characteristics assessed by the judges were of conformation (C6–C10). A quantitative method of measuring conformation has yet to be defined. Indeed conformation is probably metathetic rather than prothetic (Stevens, 1959). Metathetic sensations involve some forms of recoding in the nervous system as a means of differentiating between qualities (e.g. pitch) whereas prothetic sensations involve an increase in neural activity as the stimulus is increased (e.g. most intensity variables such as
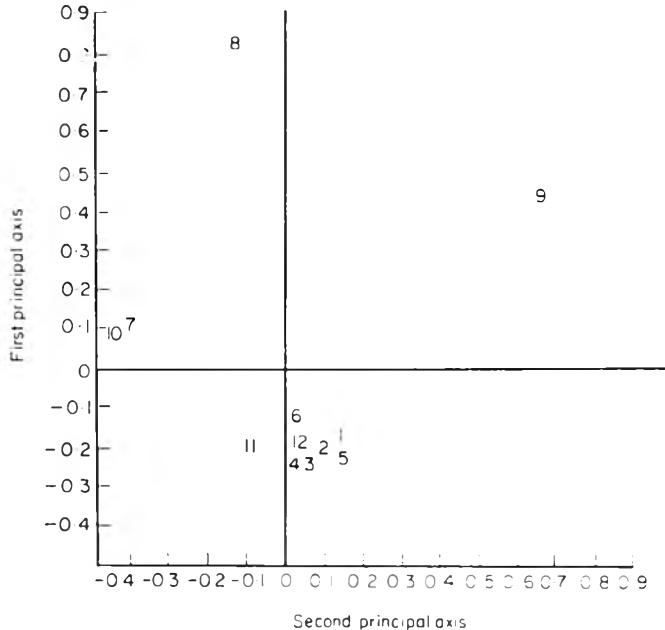
FIG. 3. Co-ordinates of judges relative to the first two principal axes (five conformation characteristics).

perceived strength). So it is of interest to see how consistent these judges were within scores for conformation characteristics only and the values of M^2 for C6–C10 are presented in Table 2. The plot of the judges' co-ordinates relative to the first two principal axes is given in Fig. 3.

The relative positions of the judges are very similar to those in the graph formed when all ten characteristics were used. Again the fatstock officers form a group, although it is not as compact as in Fig. 2. The butchers and trainee No. 6 have moved closer to the fatstock officers, whereas the remaining trainees are just as widely dispersed as before. As these relative positions were arrived at using scores for conformation characteristics only, one must conclude that the judges consistency or lack of consistency is just as apparent when assessing such characteristics. Even if conformation is undefinable

quantitatively the fatstock officers and butchers are obviously scoring the same feature in a consistent way.

Discussion

We must emphasize that the M^2 statistic would *not* be a measure of how good any judge was at assessing the quality of a sample, even if some absolute measure of quality existed. It is purely a measure of how inconsistent a pair of judges is in their assessment of the same characteristics of the same samples allowing for scales that may differ in spread and for differing locations of means. Only if an additional assumption is made, such as that the fatstock officers are 'experts' in the presented illustration, does the M^2 statistic convey anything about a judges' ability to recognize a good side of beef when one is presented to him. However, in this event, it provides a useful method of choosing potential trainees, and of following the course of training. It supplements the more usual methods which depend heavily upon means, variances, and correlation coefficients. When several characteristics are assessed, confusion can result if examination by standard bivariate methods indicates differing courses of action for the separate characteristics; the method described here provides a truly multivariate solution to this problem.

The special case of one judge

Earlier analyses of these data by traditional methods (Harries *et al.*, 1974) showed one interesting contrast amongst the five expert judges in that one of them (No. 4) differed from the others in his scoring of characteristic C4—proportion of lean. The other four expert judges showed positive correlations between this and the characteristics related to fat development. Judge 4 was more rational in achieving a negative correlation. In the earlier paper, it was suggested that the other four experts were conditioned to thinking in terms of an optimum for all characteristics—a traditional method of scoring in the meat industry—and therefore gave positive correlations between all the characteristics. It is noteworthy that the present results show few differences between the five expert judges. The lack of any remarkable differences in the means or the variances of their scores implies that the phenomenon noted above was eliminated during the rotational step. It seems likely that judge No. 4 scored C4 differently from the other judges, and because this difference vanishes with rotation, it is inferred that his scores are largely explained by scale-inversion. This difference would disappear in the reflection process (Gower, 1971) during rotation. It is clear, therefore, that the discrepancy between the five experts is a minor one compared with the gap that exists between the experts as a group and the trainees. Standard methods of screening (see e.g. Amerine *et al.*, 1965) applied to this set of judges suggested the removal of judge No. 4 from the panel, in the interests of consistency amongst the judges. It has been postulated on a number of occasions by those responsible for sensory

assessment that only one judge out of a number may be right and the others wrong, but this situation is normally impossible to recognise because the stimuli are unknown, and the investigator is trying to study them by using the responses of the (human) organisms to the sensations they arouse. In the present application to carcass quality, it was possible to measure some of the stimuli directly after the sensory tests and such measurements confirmed that judge No. 4 was the only correct one in that his scores for proportion of lean agreed better with the carcass analysis of tissues than those of the other experts. But most applications of sensory assessment are intended to solve the problem of how to assess stimuli that cannot be measured, and it is noteworthy that the rotation technique described here, in contrast to the usual bivariate methods, indicates that judge No. 4 does not materially differ from his colleagues in terms of consistency.

The conformation variables

The finding that an analysis of the conformation variables gives very similar results to that of all characteristics indicates that 'conformation' means something real to the expert judges, upon which they largely agree. A search for objective measurements that would explain the scores was unsuccessful (Harries *et al.*, 1974) and the conclusion might have been drawn that 'conformation' was another of those mystical trade terms that have little real foundation. However, the degree of agreement indicated by the present analysis between the experienced judges means that conformation must be some real feature of beef carcasses warranting further study.

Acknowledgment

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Retention of volatile organic compounds in a complex freeze-dried food gel

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Summary

The retention of ^{14}C-labelled ethyl acetate, *n*-propanol and acetone in freeze-dried food gels is studied. Fractional retention of the initially present volatiles increases with increasing solids concentration and decreases with increasing initial volatile content. Retention also decreases with increasing sample thickness in fast-frozen samples, and is lower in rapidly frozen than in slowly frozen samples.

Humidification causes release of retained volatiles, the new level of retention depending upon relative humidity. The results indicate that the predominant retention mechanism is entrapment in microregions, with a small contribution due to adsorption.

Introduction

The quality of a food product depends in part on its flavour constituents. The retention of these compounds during dehydration and other food processing operations is therefore of considerable significance.

Retention of organic volatiles in freeze-dried systems is determined by properties of non-volatile solutes which often form an amorphous matrix of the freeze-dried solid. Retention presumably results from an entrapment mechanism which immobilizes the volatile compounds within that amorphous solute matrix (Flink & Karel, 1970a; Thijssen, 1971).

At present there are two satisfactory mechanistic descriptions for observed volatile retention phenomena. These are 'selective diffusion' (Thijssen, 1971) and 'microregion entrapment' theories (Flink & Karel, 1972). While these are based on different approaches, they may represent macro and micro views of the same basic phenomenon (Chirife, Karel & Flink, 1973).

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Most of the research on volatile retention has been concerned with interactions between carbohydrates and various volatiles. Recently, Chirife, Karel & Flink (1973) were able to extend the applicability of the microregion theory to a system other than carbohydrates. Model systems of a water-soluble polymer, poly(vinylpyrrolidone) (PVP), virtually duplicate the behaviour of carbohydrates, except that a small fraction of volatiles may be retained by adsorption phenomena (Chirife & Karel, 1973a). Other polymer systems studied by Chirife & Karel included cellulose and starch (Chirife & Karel, 1973b, 1974).

Data on retention of flavours in gel systems are limited. Saravacos & Moyer (1968) studied retention of volatiles in different freeze-dried food gels. Sauvageot *et al.* (1969) and Voiley, Sauvageot & Simatos (1973) studied retention of alcohols in a mixed system containing pectin and sugars, as well as in fruit juices. Kayaert (1973) studied the effects of different experimental parameters on the retention of alcohols, aldehydes, 2-ketones and 3-ketones in a complex gel system used as a replacement for pectin. All of the above investigators observed substantial retentions of organic volatiles. The present study was undertaken in an attempt to establish whether the mechanism by which organic volatiles are retained in a freeze-dried multicomponent food gel is the same as that observed in several polymeric single-component systems studied previously (proteins, PVP, starch, cellulose).

Materials and methods

Model system preparation

The model systems consisted of the gel solids described below, ^{14}C-labelled volatile organic compounds, and water. The systems were prepared by dissolving the desired amount of solids in a mixture of water and the organic volatile. Concentrations of volatile are expressed in ppm on a volumetric basis. The gel concentration is given on a weight basis. Aliquots of 1·5 ml of the solution were weighed into standard glass screw-capped scintillation vials, frozen in a cold room at $-40°C$ and then freeze-dried for 48 hr in a Virtis freeze-drier. The drying took place at room temperature and low chamber pressures ($< 0·1$ Torr). Temperatures were not measured during freeze-drying. These conditions of freeze drying were identical to those that we previously found to give high volatile retentions in low molecular weight carbohydrate-volatile systems.

Non-volatile solute

The non-volatile solute (comprising the solids fraction of the gel) consisted of a mixture of four carbohydrate gums. This mixture is used commercially as a substitute for pectin in the production of fruit jellies in Europe. The composition of this mixture is 35% locust bean gum, 20% guar gum, 15% carageenan gum and 30% agar gum. The mixture was provided by Pectinfabrik Herman Herbstreib, Germany.

Volatile compounds

[14]C-labelled acetone, ethylacetate, and *n*-propanol were used (International Chemical and Nuclear Corporation, Irving, California). The radioactive materials were diluted with reagent grade nonradioactive volatiles to give the desired specific radioactivity.

Volatile analysis

Volatile content was determined by measuring the radioactivity of the samples with a liquid scintillation counter. Since the counting was conducted in the same vials in which the samples were freeze-dried, no transfer of aliquots was necessary. The following procedure was found satisfactory, as it avoided potential difficulties in complete dissolution of the freeze-dried gel. Samples were rehydrated in the vials by adding about 1·5 ml of water to restore the original sample weight. Fifteen ml of a water-miscible scintillator solution (2,5-diphenyloxazole 1 g, naphthalene 100 g, dioxane to 1000 ml volume) were then added and the resulting mixture was counted with a liquid scintillation counter (Beckman CPM 100).

The volatile retention was calculated by multiplying the original volatile content by the ratio of the counts after freeze-drying to those in the control solution, and is expressed as a percentage of the initial volatile content.

Humidification

Freeze-dried systems were humidified to the desired water activity by placing them in vacuum desiccators containing saturated salt solutions, which maintained the desired constant relative humidities.

Layer experiments

In some experiments, layers of different composition were prepared as follows. Fifteen ml of a solution containing 1% solids and 500 ppm of acetone were frozen in a 50-ml beaker by immersion in liquid nitrogen. On top of this layer were added and frozen, in turn, two additional 15-ml layers of 1% gel solution without acetone. After freeze-drying, the layers were separated by prying them apart with a spatula along the readily observable interface and analysed individually for their volatile content.

Results and discussion

In several experiments, the effects of composition and of process variables on volatile retention were studied. Figure 1 shows the effect of initial gel concentration on retention of *n*-propanol and acetone. The fractional retention of both volatiles increased linearly with solids concentration . This is very similar to results obtained with cellulose and starch (Chirife & Karel, 1973b). In almost all other systems studied previously, the retention increased in this manner up to a concentration level (in most cases about
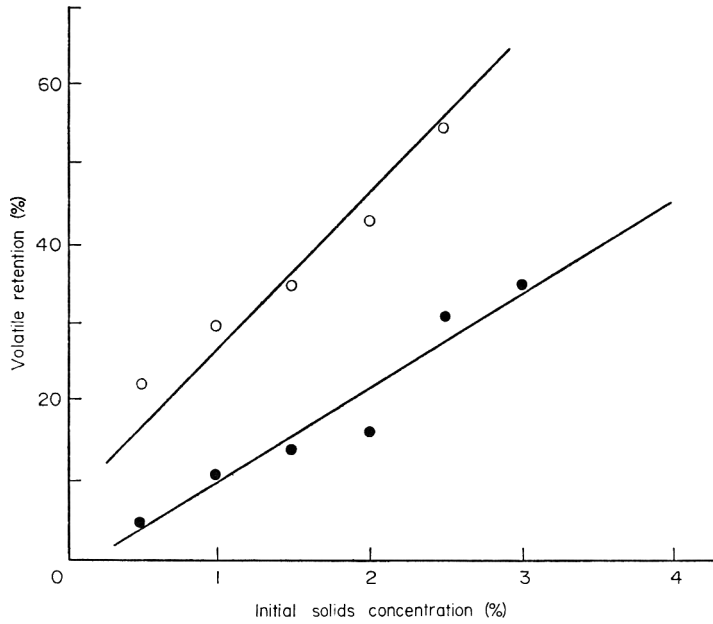
FIG. 1. Effect of gel concentration on retention of (●) acetone and (○) *n*-propanol (initial volatile concentration = 2000 ppm).
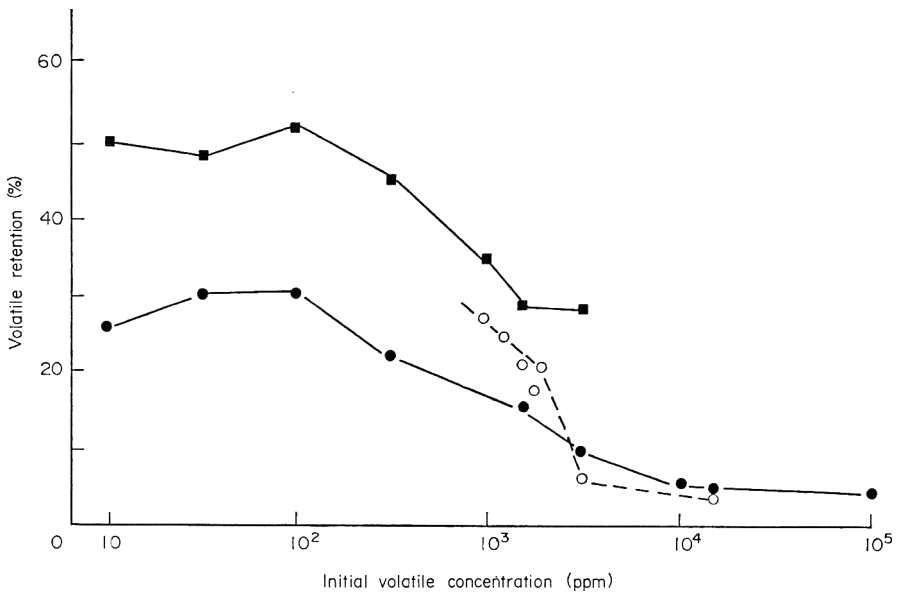
FIG. 2. Effect of initial volatile concentration on volatile retention in freeze-dried 1·5% gels. ●, Acetone; ○, *n*-propanol; ■, ethyl acetate.

20% solids), at which it levels off (Chirife, Karel & Flink, 1973). However, in this study, the gel concentration was limited to 3% because higher concentrations of this poorly soluble material showed erratic behaviour (Kayaert, 1973).

Figure 2 shows the effect of initial volatile concentration on volatile retention in a slowly frozen, 1·5% gel. Previous studies on the effect of initial volatile concentration tended to show that the fractional retention decreases with increasing concentration, but not linearly (Flink & Karel, 1970b; Chirife, Karel & Flink, 1973; Chirife & Karel, 1973a, 1973b). In freeze-dried 20% PVP solutions, for instance, the retention of *n*-propanol decreased from 65% when initial concentration was 50 ppm to about 25% at the 1000 ppm level, and remained constant thereafter. In freeze-dried cellulose and in starch (each initially at a 20% level) initial volatile concentration did *not* change the fractional retention greatly. The behaviour of this complex mixture as shown in Fig. 2 is qualitatively similar to that observed in PVP with *n*-propanol, except for the apparent decrease of retention at levels below 100 ppm for acetone and ethyl acetate.

The 'microregion' theory and 'selective diffusion' theory both predict an effect of thickness, with increased retentions expected in thinner samples. Figure 3 shows the effect of thickness on retention of volatiles in freeze-dried 1·5% gels. The shapes of the curves are similar to those observed by Flink & Karel (1970b) for carbo-hydrates, and by Chirife, Karel & Flink (1973) for PVP, and the behaviour conforms to expectations.
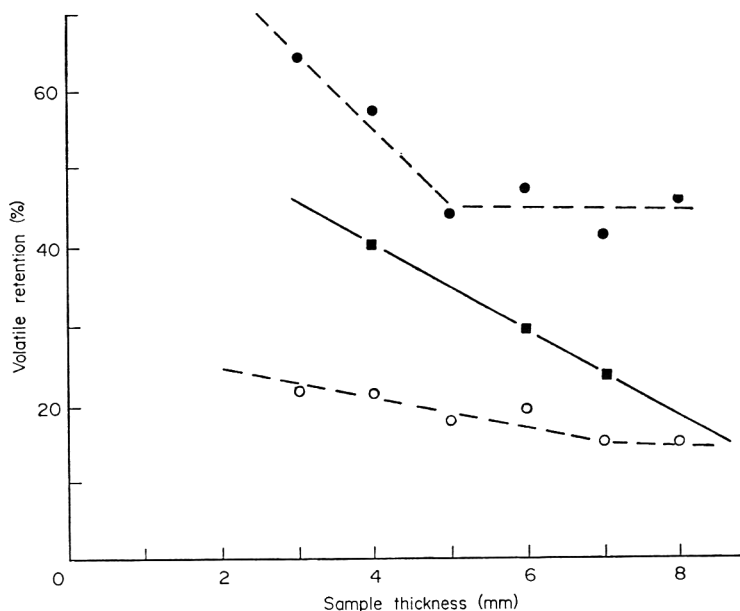
FIG 3. Effect of sample thickness on the retention of volatiles in freeze-dried 1·5% gels. ●, Acetone (initial volatile concentration 100 ppm); ○, *n*-propanol (2500 ppm); ■, ethyl acetate (1000 ppm).

Low molecular weight carbohydrates retain more volatile than polysaccharides, probably because they have a higher mobility prior to freeze-drying and are able to form a more retentive matrix (Flink & Karel, 1972; Chirife, Karel & Flink, 1973). Saravacos & Moyer (1968) observed that addition of sugar to pectin increased retention of volatiles after freeze drying, but not adsorption of volatiles on an already dry system. Table 1 shows the results obtained in the present study with the 2% gel, with 10% sucrose, and with a 2% gel, 10% sucrose mixture. These results are in agreement with the prior work cited above.

TABLE 1. Retention of acetone in freeze-dried solutions of sucrose and of the gel mixture

Initial concentration of acetone (ppm)	Retention of acetone (%) in specified solution		
	2% gel	10% sucrose	2% gel + 10% sucrose
50	19·8	84·8	60·1
100	23·5	77·2	55·8
500	20·3	79·8	57·5

Another process variable expected to affect retention is the rate of freezing. Slow freezing was expected to give higher volatile retention (Karel & Flink, 1973). We observed similar effects with a slowly frozen 1% gel (containing 500 ppm of acetone), which gave a retention of 12·4%, while only 1·5% was retained in an identical sample frozen rapidly.

The results cited above are in agreement with results for other systems, which indicate that most of the retained volatiles are entrapped in microregions. Previous work has shown, however, that in PVP, cellulose, starch and other systems, a relatively small amount of volatile may be retained by adsorption. We performed freeze drying experiments on layered systems and obtained the results shown in Table 2. These results indicate that adsorption may contribute to the total retention, since the acetone in the middle and top layers could not be retained through entrapment. The concentrations of acetone present after freeze-drying in layers originally containing no volatile were a significant fraction of the amount retained in the bottom layer. This behaviour is comparable to that observed in some other systems (Chirife & Karel, 1973a, b). However, most of the retention is apparently due to entrapment.

Previous studies have shown that the microregions entrapping volatiles in freeze-dried systems are sensitive to water and that volatiles are released when water in excess of the B.E.T. monolayer value is adsorbed in humidification experiments (Flink & Karel, 1972; Chirife, Karel & Flink, 1973). Freeze-dried solutions of 1·5% gel and of either acetone, ethyl acetate or *n*-propanol in concentrations of 50, 500 and 5000 ppm,

TABLE 2. Retention of acetone in specified layers
of the freeze-dried gel

	Acetone content (g/100 g solids)	
	Before freeze-drying	After freeze-drying
Top layer	0	0·006
Middle layer	0	0·009
Bottom layer	4	0·031
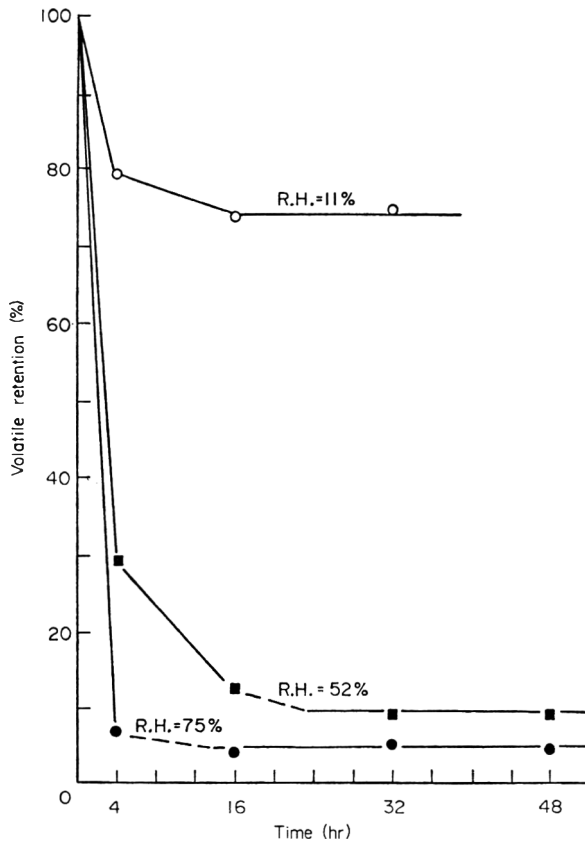
FIG. 4. Loss of acetone from a freeze-dried 1·5% gel humidified to specified relative humidities (initial acetone content prior to freeze-drying = 50 ppm). ○, r.h. = 11%; ■, r.h. = 52%; ●, r.h. = 75%.

were rehumidified over constant humidity solutions and analysed for retention of volatiles. Typical results are shown in Fig. 4, indicating that at each relative humidity there is a new level of retention. Humidification to 11% relative humidity, which is below the monolayer value for water on the dry gel, resulted in a loss of about 20% of the acetone retained after freeze-drying. Similar small losses of propanol from PVP humidified to levels below the monolayer value were explained as being caused by release of adsorbed volatile rather than disruption of microregions (Chirife & Karel, 1973a).

The results presented in this study lead us to the conclusion that entrapment of organic volatiles in the gel mixture is the primary mechanism of retention, with adsorption contributing to a much smaller extent. This complex mixture shows behaviour similar to that observed with model systems, each containing one type of polymer (Chirife, Karel & Flink, 1973; Chirife & Karel, 1973b, 1974). Retention levels are comparable with other polymers at the same solids content. The slight lowering of fractional retention at very low initial volatile concentrations remains unexplained.

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The vitamin C content of quick frozen green beans

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Summary

The effect of maturity, variety, post-harvest delay and various stages in processing, including cleaning, blanching and end-cooking, on the vitamin C content of quick frozen green beans (*Phaseolus vulgaris*) has been studied. The vitamin C content of eight varieties showed considerable variation but no correlation with maturity was observed. Post-harvest delay and processing caused a noticeable reduction in vitamin C content. The overall retention of vitamin C in both whole and sliced frozen beans was approximately 75%. After cooking, fresh manually sliced beans retained 59% of the original vitamin C content compared with 44% for the commercially frozen sliced beans.

Introduction

The same criteria which were investigated previously for frozen peas (Morrison, 1974) have been applied to the present study on the vitamin C content of green beans; thiamin determinations have not been made because of the low concentration in this vegetable and insignificant contribution to the national diet. In this investigation, the total vitamin C activity (L-ascorbic acid + dehydroascorbic acid) has been determined by fluorimetry for some of the parameters and the level of L-ascorbic acid only has been assayed in all samples by indophenol titration; the difference in results between the two methods was taken to be an assessment of the dehydroascorbic acid content.

As was the case with peas, most of the data available for processing losses of vitamin C in frozen beans (Lee, 1958; Voirol, 1972) has been concerned with blanching and the majority of the investigations have been carried out under laboratory conditions. In this study, all samples for analysis were obtained during the normal factory processing of frozen sliced and frozen whole green beans during the summer of 1973.

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Experimental and results

Analytical methods

(i) Total vitamin C content—duplicate 100 g samples of green beans were macerated in 350 ml 5% metaphosphoric acid solution and filtered through cotton wool. Each extract was analysed in duplicate using the microfluorimetric method (A.O.A.C., 1970) which determines ascorbic acid + dehydroascorbic acid.

(ii) Ascorbic acid content—the extracts which were prepared for total vitamin C analyses, as above, were taken for duplicate titrations with indophenol according to the procedure outlined by Mapson (1943).

(iii) Solids content—approximately 100 g samples of green beans were minced and 10 g sub-samples were dried at 80°C for 18–24 hr. The total vitamin C and ascorbic acid contents of the beans have been expressed on a dry weight basis.

Harvesting and processing procedure

The green beans were mechanically stripped from the plants in the field and transported to the factory in either 1/3 ton boxes, or in bulk loads of $4\frac{1}{2}$–6 tons. The delay time between harvesting and the first stage of processing varied from $2\frac{1}{2}$ to 12 hr but the bulk samples were rarely delayed for more than 3 hr; when possible, these short delay beans were used for the investigations.

On arrival at the factory, the beans were emptied into hoppers from which they moved to washers and then to snippers for 'topping and tailing', followed by blanching for approximately 2 min at 93–99°C. The beans were then water and air cooled before slicing and entering the freezing tunnel operating at approximately $-20°C$.

The varieties of beans which were frozen whole had a different texture and were blanched for slightly longer. These beans were water cooled, but not air cooled, before packing into 8 oz or 2 lb cartons which were placed in a plate freezer at $-20°C$ for 3 hr.

Factors investigated

(1) *Maturity.* In order to obtain an approximate objective measurement of the maturity of a bean crop, twenty-five of the longest bean pods were taken from a sub-sample of a bulk load and the centre bean removed. These beans were placed end to end and the length measured. A mean seed size of less than 7 mm signified an immature crop and a value exceeding 14 mm was equivalent to an overmature crop. For processing, the desirable size for the centre beans was 8–12 mm.

Whole plants of variety, Tenderette, were uplifted and brought to the factory and bean pods were selected to give a range of maturities. Each batch was analysed immediately so that the delay time was zero. As the beans matured, the total solids contents of the pods increased progressively from 9·9 to 14·7% but there was no correlation between mean seed size and ascorbic acid content of the whole pod (Table 1a).

TABLE 1. Effect of maturity on the ascorbic acid (AA) content of fresh beans of variety, Tenderette

Mean seed size	Total solids (%)	AA (mg/100 g dry wt)
(a)		
6·5	9·9	179·5
7·5	10·5	172·5
7·75	10·5	184·6
7·75	11·3	157·0
9·0	11·9	161·8
9·5	11·8	186·3
10·0	11·9	178·2
10·5	12·0	188·1
12·0	14·7	160·0
(b)		
12·0	13·6	145·4
12·0	14·6	155·2
12·0	14·7	160·0
12·0	14·9	163·1
12·0	14·7	164·1
12·0	13·4	171·8

TABLE 2. Total vitamin C, ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents of fresh and frozen sliced beans of variety, Tenderette

Sample point	Vitamin C*	AA†	DHAA‡	$\frac{DHAA}{vit. C} \times 100\%$
	mg/100 g dry wt			
1. Hopper	181·8	153·8	28·0	15·4
Freezer	138·2	106·4	31·8	23·0
2. Hopper	211·1	157·0	54·1	25·6
Freezer	153·2	119·0	34·2	22·2
3. Hopper	220·6	164·2	56·4	25·6
Freezer	158·4	122·8	35·6	22·5
4. Hopper	236·5	168·3	68·2	28·7
Freezer	202·9	143·8	59·1	29·1
5. Hopper	249·8	153·8	96·0	38·4
Freezer	177·4	124·0	53·4	30·1

* Vitamin C determined fluorimetrically = ascorbic acid + dehydroascorbic acid.
† Ascorbic acid determined titrimetrically.
‡ Dehydroascorbic acid, by difference.

Similarly, six samples of beans of variety, Tenderette, were selected from handpicked pods to yield a mean seed size of 12 mm. The delay time was zero and the ascorbic acid content ranged from 145 to 172 mg/100 g dry weight (Table 1b).

(2) *Variability within one variety.* On five occasions, beans of variety, Tenderette, with known delay time were taken from the hopper and analysed fresh and after freezing in the sliced form. The total vitamin C content ranged from 182 to 250 mg/100 g dry

TABLE 3. Effect of delay and the method of harvesting on the ascorbic acid (AA) content of fresh beans of variety, Tenderette

Time (hr)	Handpicked	Mechanical
	mg AA/100 g dry wt	
0	190·2	—
5	174·4	168·2
8	159·3	170·1
11	155·0	156·3
14	152·7	155·4
24	133·8	146·3

TABLE 4. Comparison of the total vitamin C and ascorbic acid (AA) contents of various green bean cultivars before and after blanching

Variety	Sample point	Vitamin C		AA	
		mg/100 g dry wt	% retention	mg/100 g dry wt	% retention
Tenderette	Hopper	220·0		159·4	
	Slicer	169·2	77	122·7	77
Provider	Hopper	217·3		163·2	
	Slicer	188·7	87	148·7	91
Tendercrop	Hopper	183·1		145·9	
	Slicer	151·9	83	113·2	78
Gallatin 50	Hopper	221·3		160·1	
	Slicer	174·1	79	121·9	76
U262	Hopper	213·3		123·0	
	Slicer	163·9	77	97·8	80
U248	Hopper	273·8		180·0	
	Slicer	212·4	78	137·1	76
Fortune	Hopper	173·8		137·0	
	Cooler	136·9	79	105·3	77
U266	Hopper	258·2		172·4	
	Cooler	223·6	87	143·5	83

weight for the fresh beans and 138 to 203 mg/100 g dry weight for frozen beans (Table 2). The percentage of ascorbic acid in the dehydro form ranged from 15 to 38% and 22 to 30% in the fresh and frozen beans, respectively.

(3) *Post-harvesting delay.* Whole plants of variety, Tenderette, were brought to the factory from the same field as beans mechanically harvested at a known time. The pods were removed from the plants by hand and stored in a cardboard box in the laboratory alongside a similar box containing the mechanically harvested beans. Samples were taken for analysis at intervals during the following 24 hr period; twenty-five bean pods were selected from both batches to have equivalent maturity at each time interval.

The average of three replicates for the ascorbic acid content at each delay time common to both handpicked and mechanically harvested samples are recorded in Table 3. A decrease of approximately 23% in the ascorbic acid content occurred with both methods of harvesting during the 19 hr period between 5 hr delay and 24 hr delay.

(4) *Various cultivars and the effect of blanching.* Samples of four main slicing varieties (Tenderette, Provider, Tendercrop and Gallatin 50) and two trial varieties (U262 and
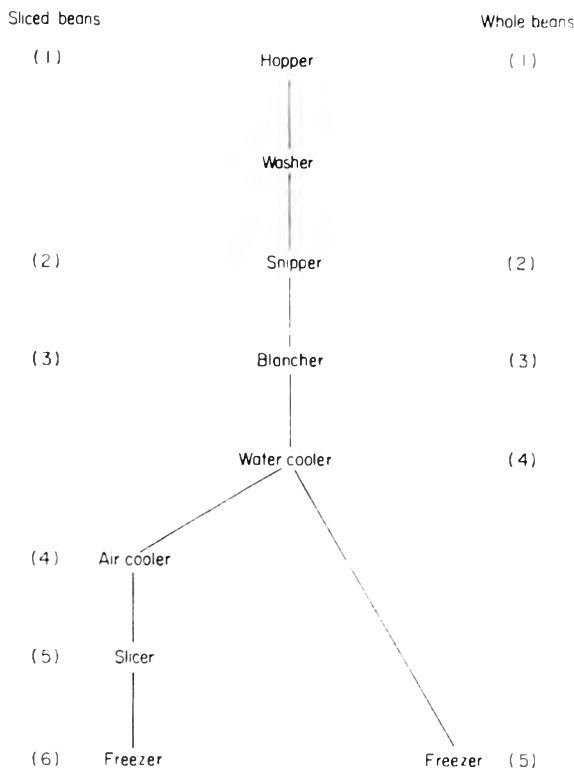
Fig. 1. Exit points of processing line from which samples of green beans were withdrawn for analysis of total vitamin C and ascorbic acid contents.

U248) were taken from the hopper and after slicing. Similarly, the whole bean variety, Fortune, with U266 as a trial variety, were sampled from the hopper and after water cooling. Beans of approximately the same maturity and delay time were used.

The total vitamin C content of the fresh beans of different cultivars ranged from 174 to 274 mg/100 g dry weight (Table 4) with the trial variety, U262, appearing to contain a high proportion of dehydroascorbic acid because of the large difference in results obtained for the fluorimetric and titrimetric methods of analysis. After blanching, but just prior to freezing, the retention of the nutrient for all cultivars came within the range 76–91% whether based on total vitamin C content or ascorbic acid only.

(5) *Other processing variables.* Samples of the slicing variety, Tenderette, and the whole variety, Fortune, were analysed at points along the process line (Fig. 1). The samples were collected in polythene bags and temporarily stored at 4°C; after blanching, the samples were chilled quickly in a mixture of solid CO_2, acetone and water. Beans of approximately the same maturity and delay time were used. The trial with Tenderette was repeated on five separate occasions and on two occasions with Fortune, the averaged results are summarized in Table 5. Retention values for both total vitamin C content

TABLE 5. Total vitamin C and ascorbic acid (AA) contents of sliced beans (Tenderette) and whole beans (Fortune) after various stages of processing

Sample point	Vitamin C		AA	
	mg/100 g dry wt	% retention	mg/100 g dry wt	% retention
Tenderette				
Harvesting	220·0	100	159·4	100
Snipping	210·4	96	154·5	97
Blanching	192·5	88	145·0	91
Cooling	174·8	80	130·2	82
Slicing	169·2	77	122·7	77
Freezing	166·0	76	123·2	77
Fortune				
Harvesting	173·8	100	137·0	100
Snipping	168·4	97	130·2	95
Blanching	151·1	87	112·1	82
Cooling	136·9	79	105·4	77
Freezing	130·2	75	109·0	80

and ascorbic acid only were approximately the same at each stage of the process line and for both types of bean. The loss of nutrient was progressive up to the end of cooling, with a further small loss after slicing in the case of Tenderette, but the freezing operation caused no reduction. The overall retention was 75–77% in terms of total vitamin C or ascorbic acid only.

(6) *Cooking losses.* Whole plants of variety, Tenderette, were brought to the laboratory where the beans were handpicked and sliced manually. The ascorbic acid content was compared with that of commercially frozen beans, of the same variety and maturity and from the same field, after both samples had been cooked. To prepare the frozen/cooked samples, 16 oz of frozen beans were added to 1 pint of slightly salted boiling water which was simmered for 3 min after returning to the boil. The handpicked, sliced beans were cooked in a similar manner but required a longer time to simmer in order to achieve the same texture. The handpicked/cooked beans retained 59% of the original ascorbic acid content whereas the corresponding frozen/cooked beans retained 44% at the point of consumption (Table 6).

TABLE 6. Effect of cooking on the ascorbic acid (AA) content of sliced beans (Tenderette) and whole beans

Sample	Sliced		Whole
	mg AA/100 g dry wt	% retention	mg AA/100 g dry wt
Harvested	154·4		
Frozen	128·8		115·2
Frozen/cooked*	67·6	44	70·3
Handpicked	150·0		
Handpicked/cooked†	88·5	59	

* 3–4 min to return to the boil + 3 min simmering.
† 2–2·5 min to return to the boil + 7–7·5 min simmering.

The retention of ascorbic acid after cooking whole frozen beans was studied on samples with an unknown storage life which were purchased locally. On a dry weight basis, the ascorbic acid contents of the frozen whole and frozen sliced beans were similar after boiling but somewhat less than in the manually sliced fresh beans after cooking.

Discussion

This investigation with frozen green beans has revealed several points of difference when compared with the earlier study of peas.

The difficulty with green beans has been the assessment of the maturity of the pods. The length of the centre seed was taken as the standard for maturity and the results recorded in Table 1 indicate that these measurements corresponded to increasing solids contents but showed no correlation with the ascorbic acid content. Phillippon & Rouet-Mayer (1971) also found that the ascorbic acid content of beans fluctuated during the

growth and development of the pods and seeds. By comparison, tenderometer readings
as a measure of the maturity of peas had shown a good correlation with the ascorbic acid
content within one variety (Morrison, 1974).

Mechanical harvesting of green beans appeared to have no detrimental effect on the
ascorbic acid content when compared with handpicking (Table 3) which was not the
case with peas. However, both green beans and peas were subject to loss of ascorbic acid
when delay occurred between harvesting and the commencement of factory processing.
Zepplin & Elvehjem (1944) reported a 20% decrease in ascorbic acid after storing green
beans for 24 hr at room temperature which was confirmed in this investigation.

Different cultivars of green beans displayed a range of ascorbic acid contents (Table 4)
but, in view of the scatter of values found for the one variety, Tenderette, no claim can
be made that any particular cultivar provides an especially rich or poor source of the
nutrient.

The level of vitamin C during the processing of green beans (Table 5) showed small
decreases at each stage indicating that the surrounding water was continually leaching
the ascorbic acid. With peas, the major loss of nutrient occurred during blanching
where the destructive effect of temperature was more noticeable than with green beans;
after blanching, beans retained at least 87% of the original vitamin C content which
confirmed the earlier findings of Adam, Horner & Stanworth (1942) and Lee (1958).
A further and final loss of vitamin C content was noted after cooling, also observed by
Hard & Ross (1956), but the freezing operation caused no reduction in the nutrient
content. The 75–80% retention of ascorbic acid in green beans by the end of the
freezing process was of the same order as that found for peas and in keeping with the
standards of the International Institute of Refrigeration (1971) for good commercial
practice in the freezing of vegetables. Similar values for the overall retention of vitamin
C in beans during processing have been reported by Porter *et al.* (1946) and Hard &
Ross (1956). Lower values have been found for beans which were sliced before blanch-
ing (Adam *et al.*, 1942; Gordon & Noble, 1959) but this was not the commercial practice
employed during the present investigation.

The effect of cooking on the ascorbic acid content of boiled handpicked, sliced beans
indicated a superior retention compared with the frozen product boiled for a shorter
time. However, beans available to the housewife would probably be at least 24 hr old
and the loss of approximately 23% ascorbic acid due to delay would result in both
fresh/cooked and frozen/cooked sliced beans having similar levels of the vitamin. It is of
interest that beans in the sliced form retain ascorbic acid to the same extent after
freezing and cooking as whole beans (Table 6).

The fluorimeter was not available for the whole period of this investigation. The
studies on the effect of maturity, post-harvest delay and cooking are based on the
determination of ascorbic acid by indophenol titration whereas the total vitamin C
content, which includes dehydroascorbic acid, was also determined in the investigation
of variability within and between cultivars, and on the effect of processing.

Since the results reported for the dehydroascorbic acid content depend on the differences obtained between two methods, precision cannot be claimed because analytical errors can combine to increase or decrease the figure. However, from the many analyses (Tables 1, 4 and 5), it appears that an additional 25% vitamin C activity may be present in beans as dehydroascorbic acid. Analysis of peas had shown only 2–3% of the ascorbic acid present in the dehydro form which is in agreement with the level generally accepted for vegetable material (Mapson, 1961). Similarly, Eheart & Odland (1972) reported that the total ascorbic acid content of two varieties of fresh green beans contained 1·8 and 3·3% dehydroascorbic acid. On the contrary, Wells, Tichenor & Martin (1963) analysed ten varieties of beans which had been frozen and found that dehydroascorbic acid accounted for 9·7–28·4% of the total ascorbic acid, eight of the samples having over 20% which is comparable with the findings in this investigation. The dehydroascorbic acid content appeared to be proportional to the ascorbic acid level. Further studies are needed to establish whether the vitamin C activity of some vegetables may have been underestimated in the past by failing to determine the contribution of dehydroascorbic acid.

On a dry weight basis, green beans have a somewhat higher vitamin C content than peas but the solids content of the two vegetables differs so that, on a wet weight basis, the beans contain less of this nutrient than peas. In addition, cooking losses appear to be more severe for beans so that only half as much ascorbic acid is provided by an equivalent weight of beans when compared with peas. Nevertheless, 100 g frozen cooked beans can contribute approximately 1/3 of the recommended daily intake of vitamin C.

Conclusion

In addition to post-harvest delay, leaching during the wet stages of the process appears to be the major factor for loss of vitamin C in the freezing of green beans; blanching is not as detrimental to the nutrient content in green beans as in peas. The overall retention of vitamin C in green beans after freezing is at least 75% which is comparable with peas but cooking is likely to cause greater loss from frozen beans than from frozen peas. Dehydroascorbic acid is present at an appreciable level in green beans.

Acknowledgments

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Pasteurization conditions for juices and comminuted products of Israeli citrus fruits*

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ANNA KARSENTY

Summary

Time-temperature curves were established for heat inactivation of pectin-esterase in citrus juices and comminuted materials of different citrus varieties, pH, and enzyme activities; a highly sensitive analytical method was used as criterion for adequacy of pasteurization. Inactivation temperatures were lower in materials of lower pH, ranging from 75 to 90° for juices and 80 to 95° for comminuted products. But, in order to exploit the 'low pH–low temperature effect, longer holding times had to be applied to the more acid test materials. Fifteen sec were sufficient for orange juice, and longer holding times had no effect on the inactivation temperature required for this variety (90°). However, for grapefruit juice, an increase in holding time, e.g. from 15 to 30 sec resulted in an inactivation temperature change from 90° to 85° and from 90° to 80° for lemon juice.

Introduction

The aim of the study was to establish appropriate pasteurization conditions for juices and comminuted products of the different Israeli citrus varieties, i.e. to achieve destruction of micro-organisms and inactivation of the pectinesterase enzyme, while employing minimal heat treatment. An extensive literature exists on heat treatment of citrus juices which was reviewed for orange juice by Joslyn & Pilnik (1961), for grapefruit juice by Burdick (1961) and by Veldhuis (1971), for lemon and lime juice by Swisher & Higby (1961), and Swisher & Swisher (1971), and for orange and tangerine juice by Veldhuis (1971). In the numerous studies reported, effectivity of heat treatment was assessed on the basis of keeping qualities, i.e. cloud retention, or by quantitative estimation of pectinesterase activity, according to the method devised by Kertesz (1937) (or one of its adaptations by Lineweaver & Ballou (1945), MacDonnell, Jansen & Lineweaver (1945), McColloch & Kertesz (1947), Rouse & Atkins (1952, 1953, 1955), Keller *et al.* (1954)), or by a qualitative test for residual pectinesterase activity (Stevens,

1941; Kew & Veldhuis, 1951; Patron & Swinzow, 1956). Since it is known that inactivation of pectinesterase necessitates higher temperatures than the destruction of microorganisms, adequacy of pasteurization can be verified by checking for absence of pectinesterase activity. This was established by Mannheim & Ziv (1962) who used the method of Kew & Veldhuis (1951) and the Jelly-Test of Pilnik & Rothschild (1960) as an index of sufficient heat treatment for bacteriological preservation of citrus juices. Pilnik & Rothschild (1960) reported that a surplus of cations reduces the sensitivity of the reaction and found that the sensitivity of Kew & Veldhuis' method (1951) could be considerably increased by using a pectin substrate which did not contain NaCl. Mannheim & Ziv (1962) compared reaction sensitivities and observed a five-fold increase in the sensitivity of Kew & Veldhuis' method (1951) when a pectin substrate without NaCl was employed, but noted that the Jelly-Test of Pilnik & Rothschild was ten times more sensitive. In our current work, a modification by Rothschild of the Jelly-Test further enhanced the sensitivity, permitting the detection of minute traces of residual pectinesterase activity. In a comprehensive study on long term storage of juices of the Israeli shamouti and valencia orange, grapefruit and lemon varieties at varying concentrations (Gerda Rothschild & Anna Karsenty, 1974), it was shown, that when negative reactions were obtained by the modified Jelly-Test, no clarification due to residual traces of activity occurred within the 15–24 month observation period.

Materials and methods

Test materials and experiments

Samples used were factory-line or laboratory prepared lemon, grapefruit, shamouti, and valencia juices, or comminuted products having different natural or adjusted pH levels and different pectinesterase activities. The samples were kept at different temperatures, holding times, and combinations of both.

Factory samples were pasteurized in an APV Plate Pasteurizer and cooled either in the cooling section of the plate pasteurizer, i.e. by the regular factory procedure, or by bypassing the hot material through a Liebig glass condenser, permitting additional variations in holding time than usually applied to the different types of products.

Laboratory samples were obtained by adding unpasteurized fruit cells to reconstituted juice from sterile concentrates. The cells were taken from a Sveco Vibrator Screen, adjusted to pH 3·6 (in order to stabilize pectinesterase activity), stored in a deep freeze, and thawed just before use to room temperature. The enzyme activity could then be concentrated by straining through nylon gauze and discarding part or all of the drained-off serum. The reconstituted juice (A) (90 g, PE u. = 0) was heated quickly to various temperatures on an open bunsen flame, and the unpasteurized cells (B) (10 g) at selected enzyme activity levels were added by means of a plastic syringe with a cut-off tip. This test material (C) was kept for the desired holding time in an electrically heated water bath at the temperature reached upon addition of the cells. Heat treatment was inter-

rupted by passing the sample (C) through a Liebig glass condenser. Thus enzyme activity, pasteurization temperature and holding time during heat treatment could be controlled, while coming-up time was eliminated and quick cooling achieved.

After cooling, those samples which had been pasteurized at low temperatures were adjusted to pH 3·6, in order to preserve any residual enzyme activity.

Analytical methods

(1) To determine pectinesterase activity in the unpasteurized materials, or to establish the extent of inactivation which occurred at different temperatures, the quantitative method of Rouse & Atkins (1955) was applied. However, in order to obtain reproducible results, enzyme activity of the more acid test materials had to be stabilized by raising pH levels to 3·5–3·6 (Rothschild, Moyal & Karsenty, 1974). Results obtained were expressed in terms of pectinesterase units (PE. u.), or as percent inactivation, expressed as the difference in PE. u. values before and after heat treatment. According to the sensitivity limits of the method (Gerda Rothschild, unpublished) '100% inactivation' implies a residual pectinesterase activity of less than 1×10^{-4} PE. u. per gram of sample corresponding to approximately 5% unpasteurized juice content.

(2) The fully pasteurized samples were checked for absence of pectinesterase activity by a modification by Rothschild of the Jelly-Test method of Pilnik & Rothschild (1960), which permits the detection of 0·1% unpasteurized juice content, i.e. a residual pectinesterase activity of about $0·02 \times 10^{-4}$ PE. u. per gram of sample (according to the sensitivity limits of the method (Gerda Rothschild, unpublished)).

The 'Sensitized Jelly-Test' was carried out as follows. Samples of test material containing 0·30–0·35 citric acid were weighed into beakers and neutralized against phenolpthalein using 1 N NaOH solution. CaCl$_2$ (5 ml of a 1 N solution) was added and 100 ml of a 1% pectin solution prepared from a 55% esterified apple pectin. After thorough mixing using a magnetic stirrer, the pH of the sample was adjusted to pH 7 with 0·1 N NaOH, and distilled water was added to a total amount of 200 g. The reaction mixture was transferred into previously sterilized small bottles, a few drops of toluene added as preservative, and the closed bottles were kept at room temperature in the laboratory. Daily examinations for increased viscosity or gel-formation were performed by turning an opened bottle upside down and comparing the outflow to that from a control sample. Positive and negative reactions indicated by ' + ' and ' − ' respectively designate insufficient or adequate pasteurization, i.e. if after 3 days, the reaction mixture remained free-flowing, (−), the test material was considered to be adequately pasteurized.

Results

The extent of inactivation which occurred at different temperatures is shown for grapefruit juice at varying pH in Table 1. Inactivation started and was completed at lower temperatures in the more acid test samples. Differences of only 5° had a con-

siderable effect on the degree of inactivation up to pasteurization temperatures of 50° at pH 2·8, 55° at pH 3·1–3·4, and 60° at pH 3·6, and again at temperatures above 70°, or 75° respectively. However, in the intermediate ranges (50–70°, 55–75°, and 60–75°) no changes occurred.

TABLE 1. Pectinesterase inactivation (%) in grapefruit juices at various temperatures and pH (holding time 45 sec)

	Temperature (°C)												
pH of sample	30	35	40	45	50	55	60	65	70	75	80	85	90
2·8 adjusted	0	—	24	61	80	80	80	80	80	86	100		
3·1 natural		0	18	48	74	80	80	80	80	80	95	100	
3·4 adjusted				0	25	80	80	80	80	80	83	95	100
3·6 adjusted					0	54	72	71	72	72	82	92	100

TABLE 2. The influence of different pectinesterase activities on required pasteurization temperatures (°C) (holding time 60 sec)

Test material (juice)	pH	Pectinesterase activity (PE. u. × 10⁴/g)						
		6	12	24	36	48	60	80
Lemon	2·60	80	80	80	80			
Grapefruit	2·80	85	85	85	85	85	85	
Grapefruit	3·00	85	85	85	85	85	85	
Orange (valencia)	3·40	90	90	90	90	90	90	90
Orange (valencia)	3·60	90	90	90	90	90	90	90

TABLE 3. The influence of duration of holding times on required pasteurization temperatures (°C)

Test material (juice)	Activity (PE. u. × 10⁴/g)	pH	Holding times (sec)						
			15	30	45	60	75	90	120
Orange (valencia)	25	3·6	90	90	90	90	90	90	
Orange (valencia)	25	3·4	90	90	90	90	90	90	
Grapefruit	28	3·0	90	85	85	85	85	85	
Grapefruit	28	2·8	90	85	85	85	80	80	
Lemon	23	2·6	90	80	80	80	75	75	75
Lemon	23	2·4	90	80	80	75	75	75	75

TABLE 4. Jelly test reactions for pasteurized lemon products

Holding times (sec)			Test material*	Activity (PE. u. × 10⁴/g)	pH	Jelly test reactions after 3 days; at pasteurization temperatures (°C)				
15	45	90				70	75	80	85	90
	×		(1)	33	2·40	+	−			
	×		(1)	29	2·40	+	−			
	×		(1)	22	2·40	+	−			
	×		(1)	22	2·40	+	−			
	×		(2)	24	2·40	+	−			
	×		(1)	28	2·50	+	−			
	×		(1)	22	2·50	+	−			
	×		(1)	21	2·50	+	−			
	×		(1)	19	2·50	+	−			
	×		(1)	19	2·50	+	−			
	×		(1)	15	2·50	+	−			
	×		(2)	22	2·60		+	−		
		×	(3)	61	2·65		+	−		
		×	(3)	49	2·70		+	−		
		×	(3)	20	2·75		+	−		
		×	(3)	60	2·80		+	−		
		×	(3)	44	2·80		+	−		
		×	(3)	40	2·80		+	−		
		×	(3)	23	2·80		+	−		
		×	(3)	32	2·90		+	−		
		×	(3)	27	2·75			+	−	
		×	(3)	23	2·85			+	−	
		×	(3)	—	2·90			+	−	
×			(2)	22	2·60				+	−

* (1) = Factory pasteurized juice; (2) = laboratory pasteurized juice; (3) = comminuted, factory pasteurized.

Although the requisite pasteurization temperature increased with increasing pH in the lemon, grapefruit and orange (valencia) juice varieties, even very extensive variations in the level of enzyme activity are seen not to influence pasteurization temperature —for example, the same temperature was needed for pasteurization when the pectinesterase activities were PE. u. × 10⁴ g = 6 or 60 (Table 2). The lower pasteurization temperatures required at lower pH depend on the duration of holding time. For example, with 15 sec holding time, the same pasteurization temperature of 90° was needed for lemon, grapefruit and orange juices at pH 2·4–3·6; at 30 sec holding time the temperatures required decreased to 85° for grapefruit and 80° for lemon, while at 60–75 sec

TABLE 5. Jelly test reactions for pasteurized grapefruit products

Holding times (sec)			Test material*	Activity (PE. u. $\times 10^3$/g)	pH	Jelly test reactions after 3 days; at pasteurization temperatures (°C)			
15	45	90				75	80	85	90
	×	×	(2)	27	2·60 (adj.)	+	−		
		×	(2)	27	2·80	+	−		
	×		(1)	26	2·85	+	−		
	×		(1)	19	2·90	+	−		
	×		(1)	17	2·90	+	−		
	×		(1)	17	2·95	+	−		
	×		(1)	16	2·95	+	−		
	×		(1)	23	2·80		+	−	
	×		(2)	27	2·80		+	−	
	×		(1)	20	2·90		+	−	
	×		(1)	—	2·90		+	−	
	×		(1)	19	2·90		+	−	
×	×		(1)	22	2·90		+	−	
×	×		(1)	21	2·90		+	−	
×	×		(1)	—	2·95		+	−	
	×		(1)	19	2·95		+	−	
	×		(1)	14	2·95		+	−	
	×		(1)	17	3·00		+	−	
	×		(1)	13	3·00		+	−	
×	×		(1)	—	3·00		+	−	
	×	×	(2)	27	3·00		+	−	
	×	×	(3)	7	3·40		+	−	
	×	×	(3)	4	3·45		+	−	
	×	×	(3)	3	3·45		+	−	
	×	×	(3)	7	3·50		+	−	
	×	×	(3)	5	3·50		+	−	
×			(1)	23	2·85			+	−
×			(1)	47	2·90			+	−
×			(1)	24	2·90			+	−
×			(1)	25	2·90			+	−

* (1) = Factory pasteurized juice; (2) = laboratory pasteurized juice; (3) = comminuted, factory pasteurized.

holding time, the pasteurization temperature was further reduced to 75° for lemon (Table 3).

Results of tests in which a difference of 5° in pasteurization temperature resulted in a change from a positive to a negative Jelly-Test reaction are presented in Tables 4–6. The temperatures at which the negative reaction appeared were affected by the pH

Table 6. Jelly test reactions for pasteurized orange products

Holding times (sec)			Test material*	Activity (PE. u. × 10⁴/g)	pH	Jelly test reactions after 3 days; at pasteurization temperatures (°C)				
15	45	90				75	80	85	90	95
	×	×	V (2)	24	2·60 (adj.)	+	−			
		×	V (2)	36	2·80 (adj.)	+	−			
	×		V (2)	36	2·80 (adj.)		+	−		
	×	×	V (2)	24	3·00 (adj.)		+	−		
	×		S (1)	26	3·20		+	−		
×	×		S (1)	25	3·25		+	−		
×	×		S (1)	28	3·30		+	−		
	×		S (1)	26	3·35		+	−		
	×		S (1)	29	3·40		+	−		
	×		S (1)	26	3·45		+	−		
	×		V (1)	32	3·50		+	−		
	×		V (1)	—	3·55		+	−		
		×	V (3)	79	3·55		+	−		
		×	V (3)	52	3·60		+	−		
		×	S (3)	112	3·60		+	−		
		×	S (3)	33	3·60		+	−		
		×	V (3)	37	3·70		+	−		
		×	V (3)	32	3·80		+	−		
	×	×	S (3)	5	3·80		+	−		
	×		S (1)	28	3·20			+	−	
	×		S (1)	26	3·20			+	−	
	×		S (1)	31	3·20			+	−	
	×		S (1)	23	3·25			+	−	
	×		S (1)	26	3·30			+	−	
×	×		S (1)	27	3·30			+	−	
	×		S (1)	26	3·35			+	−	
	×		S (1)	24	3·35			+	−	
	×		S (1)	26	3·35			+	−	
	×		S (1)	23	3·35			+	−	
	×		S (1)	24	3·40			+	−	
	×		S (1)	20	3·40			+	−	
	×		S (1)	26	3·40			+	−	
	×		S (1)	25	3·40			+	−	
	×	×	V (2)	36	3·40			+	−	
	×		V (1)	28	3·40			+	−	
	×		V (1)	27	3·40			+	−	
	×	×	V (2)	36	3·60			+	−	
		×	V (3)	58	3·60			+	−	
		×	V (3)	57	3·60			+	−	
		×	S (3)	62	3·60			+	−	

TABLE 6 *(continued)*

Holding times (sec)			Test material*	Activity (PE. u. × 10⁴/g)	pH	Jelly test reactions after 3 days; at pasteurization temperatures (°C)				
15	45	90				75	80	85	90	95
		×	S (3)	57	3·60			+	−	
		×	S (3)	56	3·60			+	−	
		×	S (3)	53	3·60			+	−	
		×	S (3)	40	3·60			+	−	
	×	×	S (3)	30	3·70			+	−	
		×	S (3)	50	3·70			+	−	
	×	×	S (3)	35	3·70			+	−	
		×	S (3)	115	3·90			+	−	
		×	S (3)	90	3·60				+	−
		×	S (3)	45	3·65				+	−

* Factory pasteurized juice; (2) laboratory pasteurized juice; (3) comminuted, factory pasteurized. V = Valencia, S = Shamouti.

of the test materials, but not by mode of pasteurization, enzyme content or prolongation of holding times from 45 to 90 sec.

It was found that the pasteurization temperatures needed for lemon, grapefruit, and orange products range from 75–90° for juices of pH 2·40–3·55, and from 80 to 95° for comminuted materials of pH 2·65–3·90 (Fig. 1).

Discussion

Temperatures at which inactivation occurs, and the extent of inactivation at different temperatures, depends on the pH of the various products. Inactivation commences at temperatures as low as 40° for samples at pH 2·8–3·1. The rate of inactivation increases with increasing temperature up to 50–60° (depending on pH), and again above 70 or 75°, but not between 50 and 70°, 55 and 75°, or 60 and 75° respectively for different pH levels. Similar observations were made by Stevens, Pritchett & Baier (1950) who reported a decrease in the rate of flocculation (due to pectinesterase activity) at temperatures as low as 50°, and an increasing effect of heat treatment with increasing temperature up to 70° and above 80°, but not between 70 and 80°. Also Bisset, Veldhuis & Rushing (1953) noticed a sharp progressive decrease in pectinesterase activity between 49 and 71°, and again between 82 and 93°, whereas only slight changes occurred at pasteurization temperatures between 71 and 82°. Further observations by Stevens *et al.* (1950) that pasteurization temperatures necessary for commercially
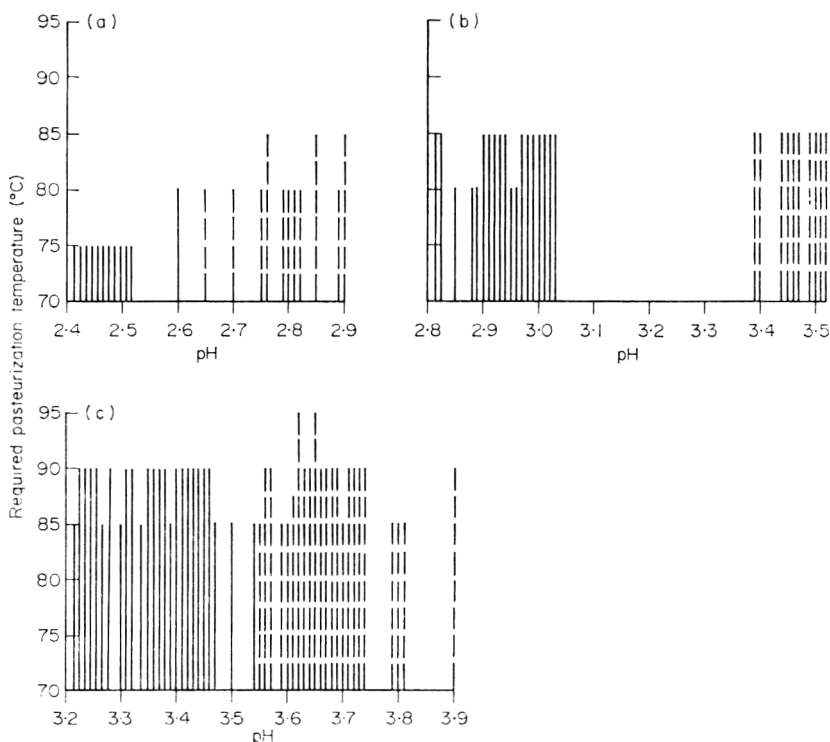
FIG. 1. Influence of pH on required pasteurization temperatures. (a) Lemon, (b) grapefruit, (c) orange. Solid lines; juice, 45 sec holding time. Broken lines; comminuted, 45 or 90 sec holding time.

satisfactory juices decreased as the pH decreased are confirmed by our results; however, in order to benefit from this effect, longer holding times are required for juices of low pH.

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The analysis of strawberries as a means of determining the fruit content of manufactured products

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Summary

Multivariate statistical analysis was examined as a means of obtaining more reliable assessments of fruit contents from analytical determinations than are possible by the current use of average values of insoluble solids, nitrogen, phosphorus and potassium contents. The major portion of the investigation dealt with the fruit itself.

Twenty-three parameters were determined on fifty-four samples of strawberries, culled during three successive seasons, and representing a wide span of cultivars, their origins and growing areas. To derive the weighting factors to be applied to the analytical determinations in order to obtain the optimum estimate of fruit content, the analytical results were submitted to multivariate statistical analysis. This approach led to a marked improvement in the estimations of fruit content when compared with conventional techniques.

Wide variations were found in the levels of all of the parameters determined, but there were highly significant correlations between many pairs, in which the free amino acids figured largely. A limited study to assess the usefullness of these parameters for determining the fruit content of manufactured products showed that the amount of free amino acids recovered from fresh fruit jams and from frozen fruit were very similar. However, the amounts recovered from sulphited pulp and sulphited fruit jams did not fall into any clear pattern. Further investigation of the effect of sulphiting on the free amino acids is therefore necessary before the statistical approach can be applied in these circumstances.

Introduction

The difficulties of characterizing fruits are largely due to the occurrence of wide natural variations in composition (Goodall, 1969). For example, reported nitrogen contents range from at least 77 to 640 mg per 100 g strawberries, likewise potassium contents range from about 78 to 223 mg per 100 g fruit. Thus the assessment of fruit content by determining one or more analytical parameters and comparing these with the average of published values is subject to wide limits of error (Steiner, 1948). The

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widely used practice of assessing fruit content by determining the insoluble solids, potassium, phosphorus and nitrogen contents of fruit products; comparing these results with average published values from authentic material; and then taking the mean of the four figures is more accurate than when only one parameter is used but is still unreliable (see Table 4b).

These difficulties have led several authors (Rolle & Vandercook, 1963; Coffin, 1968; Lifshitz, Stepak & Brown, 1971) to suggest a statistical approach based on the inter-relationships of a number of fruit constituents. For example, Coffin (1968) examined thirty-two samples of commercial orange juice and found that although wide variations existed in the levels of amino acids, betaine, polyphenolics, ash and phosphorus there were significant correlations between pairs of these components, and on this basis he derived multiple regression equations relating amino acid contents to two or more of the other constituents. Workers in this field have, however, restricted their statistical methods to small groups of variants.

In order to assess the value of this type of approach with a wide range of constituents an investigation was undertaken in which fifty-four samples of strawberries were analysed for twenty-three parameters. The results were examined statistically in two ways, firstly to derive an equation relating the parameters—which would give the best estimate of fruit content (this was subsequently extended to determine whether reduced equations from which selected parameters were omitted without losing overall accuracy could be derived)—and secondly, for relationships between pairs of parameters—which could be of value in establishing fruit authenticity.

Materials and methods

A total of fifty-four samples of strawberries representing thirty-three cultivars (ten United Kingdom, eighteen European, five American) were received from twelve sources, eight in England and four in Europe (see footnote under Table 1). Nineteen samples were harvested in 1969, thirty-one (including those from the four European sources) in 1970 and four in 1971.

Approximately 1 kg of each of the British-grown samples was plugged and then stored in a polythene bag at approximately −30°C until required for analysis. From these bulk samples, portions were drawn as required and allowed to thaw at room temperature before being macerated in a liquidizer. The European samples were plugged and deep-frozen before dispatch by air, and immediately put into cold store as above on receipt.

The samples of fruit were analysed as follows.

Insoluble solids

By extracting the soluble material with hot water followed by drying to constant weight (Chatt, 1957).

Total solids

By drying with sand, first on a waterbath and then in a vacuum oven at 70°C to constant weight.

Total sugars

By the method of Jones (1955) but using standard dextrose solution instead of standard invert solution.

Soluble solids

By refractometer (without correction) at room temperature.

Ash

By drying first on a waterbath then in an oven at 100°C before ashing in a muffle furnace at 500°C to constant weight.

Minerals

The ash was dissolved in 10 ml of 1 : 1 concentrated hydrochloric acid solution, the solution made to 100 ml, and filtered if necessary.

Sodium and potassium. Sodium was determined directly on the ash solution, and potassium after diluting 1 : 100, by means of an E.E.L. flame photometer.

Calcium and magnesium. By titration of the ash solution with EDTA (A.O.A.C., 1970).

Phosphorus. On the ash solution by the molybdenum blue method (Vogel, 1961).

Nitrogen. By Kjeldahl's method (Vogel, 1961).

Formol number

On the strained 50% juice (A.O.A.C., 1970). Reported as mEq/100 g fruit.

Absorptions 515 nm and 275 nm (anthocyanins and flavonoids)

Thirty grams of frozen fruit was thawed under 45 ml of absolute ethanol, macerated in a liquidizer and centrifuged for 10 min at approximately 800 *g*. Absorptions of the clear supernatant and of a diluted solution (1 part + 40 parts 60% ethanol) were measured in the visible and u.v. regions respectively, in a 1 cm cell in a Unicam S.P. 800 spectrophotometer. Traces in the visible region showed peaks with maximum absorptions at 515 nm, and in the u.v. region, peaks, or inflections levelling off, at 275 nm. The absorptions at these wavelengths were taken as indicative of anthocyanins and flavonoids, respectively, and recorded as absorption units of 40% and 1% fruit preparations, respectively.

Amino acids

The thawed fruit was thoroughly macerated with an equal amount of water, and strained through nylon gauze (approximately 100 μm aperture was suitable.) Ten grams of the clear filtrate (after the first runnings) was put on to a column containing

TABLE 1. Analytical results on the individual samples of strawberries

(a) Non-amino acid parameters

Source	Growing season	Cultivar	Insoluble solids (% W/W)	Total solids (% W/W)	Total sugars (% W/W) (as invert)	Soluble solids (%) (R.I.)	Ash (% W/W)	mg %						Formol no. (mEq/100 g)	Absorption 515 nm (40% soln)	Absorptio 275 nm (1% soln)
								Na	K	Ca	Mg	P	N			
K	1970	Ananas	1·62	10·4	6·42	8·1	0·38	0·82	139	26·6	11·1	18·5	73	0·2	1·20	0·92
G	1970	Belrubi	1·78	11·9	5·80	9·8	0·43	0·94	152	26·2	10·5	16·6	123	1·2	1·63	0·70
G	1970	Bemanil	2·08	12·35	7·30	9·8	0·50	0·65	202	26·9	11·0	22·4	121	0·76	1·12	0·65
A	1969	Cambridge Favourite	1·75	10·45	7·25	8·2	0·40	1·47	162	22·4	9·2	17·4	120	1·28	0·78	0·87
C	1969	Cambridge Favourite	1·75	8·4	5·87	6·1	0·38	1·44	126	20·2	9·0	18·8	80	0·83	0·65	0·64
F	1969	Cambridge Favourite	2·20	9·65	6·49	7·4	0·42	1·57	168	23·8	9·0	21·0	129	1·68	0·51	0·71
F	1970	Cambridge Favourite	2·13	10·0	5·45	7·6	0·42	1·05	176	22·2	7·6	24·2	149	1·74	0·56	0·62
G	1970	Cambridge Favourite	1·68	10·5	5·31	8·3	0·40	0·62	172	20·9	8·9	16·8	110	0·93	0·51	0·57
H	1970	Cambridge Favourite	1·59	10·4	6·08	8·3	0·43	1·41	199	18·6	10·4	20·6	103	0·76	0·37	0·72
C	1969	Cambridge Sentry	2·3	11·4	7·70	9·1	0·45	1·41	173	26·8	12·8	28·7	118	1·41	0·57	0·71
C	1969	Cambridge 456	2·45	10·2	6·63	7·7	0·46	1·36	164	29·5	12·5	22·4	95	0·54	0·93	1·07
B	1969	Chanil	1·80	10·9	7·95	8·5	0·53	0·78	140	29·8	9·2	29·8	115	1·31	0·57	0·73
B	1970	Chanil	1·73	10·9	6·09	8·8	0·51	2·20	183	30·5	13·8	23·0	138	0·82	0·86	0·72
B	1971	Chanil	1·66	9·69	7·83	8·8	0·37	0·83	140	31·0	11·5	23·2	59	0·23	0·57	0·44
C	1969	Crusader	1·6	10·1	6·95	7·9	0·49	1·4	137	23·0	10·2	17·0	74	0·50	0·96	0·66
B	1969	Domanil (1)	1·4	11·9	9·40	10·4	0·50	0·88	222	19·7	9·6	24·2	96	1·65	0·52	0·85
B	1969	Domanil (2)	1·5	14·4	10·59	11·3	0·52	1·35	173	23·2	12·4	20·7	67	1·10	0·65	0·84
B	1970	Domanil	1·4	11·3	7·98	9·4	0·41	1·71	164	24·5	8·5	18·5	91	0·49	0·71	0·79
B	1971	Domanil	1·33	11·76	9·72	11·4	0·38	0·80	146	30·0	11·1	24·7	56	0·40	0·63	0·81
G	1970	Domanil	1·42	11·0	7·29	9·5	0·42	0·87	173	15·5	9·5	17·2	95	0·92	0·61	0·50

B	1969	Elista	1·8	10·4	8·07	8·5	0·43	0·68	174	24·0	9·6	22·4	86	1·01	1·07	1·06
C	1969	Elista	1·7	9·7	6·82	8·0	0·36	1·96	148	20·4	10·9	21·5	100	0·98	1·04	0·96
B	1970	Elista	1·93	9·4	5·17	7·4	0·43	2·29	157	26·5	11·1	19·7	89	0·42	2·55*	1·11
G	1970	Fanil	2·1	10·6	5·27	8·0	0·40	1·40	153	25·4	11·6	19·9	129	0·70	0·55	0·57
B	1969	Fructana	1·55	11·5	8·51	9·3	0·44	0·96	118	21·5	10·9	20·8	82	0·77	1·06	0·88
B	1971	Fructana	1·75	9·87	7·28	8·0	0·36	0·57	136	30·5	10·4	22·8	63	0·35	0·83	0·48
B	1970	Geneva	1·31	10·0	5·69	8·2	0·49	1·58	157	24·6	10·2	18·4	103	0·62	1·66	0·70
B	1969	Grundi	1·4	8·7	5·50	7·4	0·52	1·02	180	26·0	10·3	28·5	123	0·85	0·82	0·77
E	1970	Hood	1·46	11·0	7·02	9·0	0·37	1·63	157	17·6	7·6	16·9	86	0·58	2·25*	0·72
G	1970	Jurica	1·53	11·2	6·19	9·2	0·46	1·05	199	20·2	9·3	22·3	134	0·74	0·98	0·58
B	1970	J. 1274	1·33	10·36	5·73	8·6	0·40	2·38	169	23·4	11·1	20·5	98	0·75	0·74	0·62
G	1970	J.I. 1800	1·69	10·67	5·82	9·5	0·52	1·21	185	24·5	12·0	22·2	134	1·52	1·22	0·59
G	1970	J.I. 1805	2·06	9·77	5·47	7·4	0·44	1·26	171	25·7	12·3	23·8	129	0·93	0·48	0·39
C	1969	Late Pine	1·6	12·0	7·12	9·9	0·49	1·61	207	24·6	13·7	30·8	127	1·85	1·52	0·87
E	1970	Mollala	2·08	10·8	5·26	8·4	0·39	2·16	170	18·3	10·3	21·5	118	0·48	1·84	1·17
E	1970	North West	1·14	9·6	5·93	8·3	0·34	0·99	145	14·5	8·0	15·6	74	0·37	1·24	0·64
G	1970	Pocohontas	2·01	11·2	5·45	9·1	0·46	1·39	193	28·5	11·2	24·8	122	0·97	0·67	0·60
F	1969	Red Gauntlet	1·7	7·9	6·06	6·0	0·34	1·75	111	25·7	7·8	14·2	74	0·65	0·92	0·75
G	1970	Robinson	1·87	11·2	7·24	8·9	0·46	0·62	188	26·6	8·9	21·6	114	0·82	0·60	0·69
E	1969	Royal Sovereign	3·3	11·4	7·12	8·6	0·56	1·67	191	28·5	11·3	21·0	145	1·54	0·91	0·79
J	1970	Senga Sengana (1)	2·04	9·9	6·76	7·6	0·40	0·62	151	24·3	10·0	26·3	89	0·47	0·31	0·46
J	1970	Senga Sengana (2)	1·94	10·1	6·53	7·9	0·38	0·79	153	25·1	12·2	24·7	110	0·70	1·30	0·52
K	1970	Senga Sengana	1·71	13·8	9·36	11·6	0·51	0·92	211	31·6	10·8	22·0	111	0·72	1·14	0·80
M	1970	Senga Sengana	1·42	8·9	4·30	7·2	0·39	0·67	140	27·7	12·2	14·8	93	0·42	1·40	0·74
C	1969	Siletz	2·5	10·4	5·81	7·4	0·45	2·22	178	27·6	13·8	26·6	109	1·25	1·25	0·70
E	1970	Siletz	1·9	9·85	4·84	7·7	0·40	1·57	165	19·4	11·0	20·1	87	0·50	1·33	0·56
M	1970	Sirpris	1·82	10·0	5·35	8·1	0·48	0·75	174	26·4	14·1	23·8	153	1·37	0·36	0·64
E	1970	Talisman	2·41	13·6	8·56	11·1	0·45	1·78	176	35·05	11·4	19·15	122	0·65	1·20	1·05
B	1969	Tigaiga	1·8	9·45	8·08	8·6	0·58	0·93	199	28·6	14·4	21·6	84	0·70	0·68	0·78
B	1970	Tigaiga	1·86	9·75	4·79	7·7	0·49	2·08	155	37·2	10·6	17·9	109	0·62	1·12	0·78
B	1971	Tigaiga	1·20	10·27	6·90	8·6	0·43	1·19	169	24·6	12·4	26·7	67	0·44	0·79	0·90
B	1969	1818	2·1	12·05	9·07	9·5	0·43	0·80	107	33·7	9·4	22·0	78	0·50	1·67	0·96
B	1970	1819	1·91	10·3	4·82	7·6	0·56	2·38	177	41·1	14·0	22·0	137	1·13	1·66	0·81
L	1970	Not known	1·77	13·5	9·16	11·0	0·48	0·76	194	24·5	10·3	23·2	103	0·50	0·62	0·94

* Estimated.

(b) Amino acids

μmol per 100 g fruit

Source	Growing season	Cultivar	Aspartic acid	Threonine + serine	Glutamic acid	Glycine	Alanine	Valine	iso-Leucine	Leucine	γ amino n-butyric acid
K	1970	Ananas	2·5	16·4	15·5	2·5	15·3	0·4	0·5	1·0	9·0
G	1970	Belrubi	23·8	196	58·6	2·3	39·4	Trace	Trace	1·0	52·1
G	1970	Bemanil	57·1	210	71·3	5·9	73·4	8·2	2·5	5·4	38·4
A	1969	Cambridge Favourite	170·5	769	71·7	11·7	99·2	8·6	4·8	5·1	18·3
C	1969	Cambridge Favourite	46·9	491	46·5	9·5	96·1	7·6	4·3	4·0	8·8
F	1969	Cambridge Favourite	63·1	959	50·2	7·5	118	13·9	5·1	4·4	15·7
F	1970	Cambridge Favourite	54·7	477	47·3	8·3	104	9·3	3·7	5·9	23·7
G	1970	Cambridge Favourite	27·0	158	48·9	0·9	53·6	3·3	1·0	1·6	21·6
H	1970	Cambridge Favourite	8·8	132	31·9	5·8	60·6	3·7	2·6	6·0	20·2
C	1969	Cambridge Sentry	38·8	578	57·7	6·1	93·2	11·3	7·6	45·7	12·9
C	1969	Cambridge 456	12·8	113	26·3	7·9	30·4	2·1	2·4	2·4	7·0
B	1969	Chanil	37·0	336	77·0	14·5	108	4·0	5·1	5·1	9·3
B	1970	Chanil	10·6	140	59·3	7·9	62·6	1·8	1·6	5·2	35·8
B	1971	Chanil	3·8	31·0	16·5	3·9	21·9	0·8	0·6	1·3	10·0
C	1969	Crusader	11·3	121	20·8	6·7	42·7	3·7	1·8	2·6	7·2
B	1969	Domanil (1)	82·8	790	76·5	15·0	236	14·3	7·4	7·3	7·7
B	1969	Domanil (2)	17·3	151	43·0	6·4	47·4	2·1	1·6	2·9	8·8
B	1970	Domanil	25·9	87	32·3	5·5	62·7	1·8	0·8	4·3	19·6
B	1971	Domanil	13·4	22·3	26·3	3·1	19·5	0·5	0·6	1·2	12·9
G	1970	Domanil	24·2	168	9·9	3·3	31·6	1·9	1·0	1·6	2·79
B	1969	Elista	25·6	279	37·8	6·0	54·5	4·3	1·4	3·4	9·3
C	1969	Elista	35·4	384	73·0	8·0	94·5	4·2	2·6	3·3	11·6
B	1970	Elista	5·2	67·7	9·8	5·0	21·1	1·6	1·0	3·2	20·4
G	1970	Fanil	19·4	118	68·7	4·0	55·9	1·1	1·0	1·8	31·7
B	1969	Fructana	10·1	178	31·9	12·0	27·4	1·2	2·1	3·4	4·6

B	1971	Fructana	12·7	28·9	23·7	2·2	13·8	0·7	0·4	3·1	25·3
B	1970	Geneva	18·0	88·4	36·9	4·6	37·3	1·5	0·9	5·4	20·1
B	1969	Grundi	49·3	500	64·3	14·6	69·4	8·3	4·1	4·3	7·2
E	1970	Hood	7·3	92·4	18·9	3·6	41·9	4·1	2·6	3·6	50·6
G	1970	Jurica	63·2	221	65·8	6·4	50·6	4·5	1·9	4·1	41·1
B	1970	J. 1274	32·9	118	36·4	6·0	38·3	1·2	1·3	6·0	32·8
G	1970	J.I. 1800	27·7	274	36·2	5·5	64·1	6·5	2·9	7·0	51·1
G	1970	J.I. 1805	31·7	295	28·5	4·2	39·4	5·4	2·3	4·1	45·4
C	1969	Late Pine	141	466	167	12·6	154	8·1	2·9	4·3	14·2
E	1970	Mollala	25·6	68·0	27·3	4·8	43·0	2·4	0·9	1·7	24·2
E	1970	North West	9·6	68·0	24·3	5·1	16·3	1·6	1·2	3·1	35·3
G	1970	Pocohontas	29·6	149	59·9	5·0	74·3	2·3	1·1	5·1	33·7
F	1969	Red Gauntlet	22·0	298	27·1	5·7	61·5	3·6	1·9	1·0	10·1
G	1970	Robinson	37·3	198	31·3	2·1	35·4	1·8	0·6	1·2	32·1
E	1969	Royal Sovereign	39·4	624	30·8	9·2	95·4	5·5	3·5	2·6	15·2
J	1970	Senga Sengana (1)	11·9	63·2	22·6	3·8	33·8	2·2	0·7	1·6	21·6
J	1970	Senga Sengana (2)	15·8	122	17·8	5·4	61·3	1·8	0·8	1·8	74·3
K	1970	Senga Sengana	19·0	164	44·2	9·9	78·8	1·8	1·5	4·0	29·5
M	1970	Senga Sengana	31·2	164	49·5	6·3	65·0	3·5	1·3	1·8	14·4
C	1969	Siletz	58·4	675	45·2	9·0	60·9	4·1	3·0	2·8	13·7
E	1970	Siletz	13·0	44·8	15·9	2·6	17·2	1·4	1·0	2·0	11·1
M	1970	Sirpris	15·1	121	20·8	3·4	31·0	3·0	1·4	1·8	21·9
E	1970	Talisman	18·9	142	17·4	4·7	54·6	2·7	1·3	2·6	20·4
B	1969	Tigaiga	16·3	145	37·8	4·9	34·6	1·8	1·5	2·4	12·1
B	1970	Tigaiga	14·4	171	29·4	4·7	53·5	2·3	0·9	1·8	31·7
B	1971	Tigaiga	5·0	30·3	18·2	6·0	0·2	Trace	0·8	1·0	15·3
B	1969	1818	5·9	58·9	28·1	5·7	38·3	2·4	2·1	3·1	7·4
B	1970	1819	45·2	241	25·6	8·2	86·6	2·5	2·1	3·0	33·0
L	1970	Not known	7·9	90·7	25·4	2·7	26·0	3·1	1·2	2·6	7·2

Source: A, Essex, Area 1; B, Essex, Area 2; C, Hampshire; D, Herefordshire; E, Kent, Area 1; F, Kent, Area 2; G, Kent, Area 3; H, Warwickshire; J, Bulgaria; K, Poland; L, Rumania; M, Yugoslavia.

TABLE 2. Range and mean values of analytical determinations

	1969 fruit			1970 fruit			1971 fruit			Combined 1969, 1970 and 1971 fruit		
	Range	Mean	Coefficient of variation	Range	Mean	Coefficient of variation	Range	Mean	Coefficient of variation	Range	Mean	Coefficient of variation
Insoluble solids (%)	1·4-3·3	1·9	24·83	1·14-2·41	1·77	16·59	1·20-1·75	1·48	17·64	1·1-3·3	1·8	20·09
Total solids (%)	7·9-14·4	10·6	14·39	8·9-13·8	10·8	11·06	9·7-11·8	10·4	9·11	7·9-14·4	10·7	12·06
Total sugars (%) (as invert)	5·4-10·3	7·2	17·94	4·3-9·2	6·2	20·44	6·9-9·7	7·9	15·77	4·3-10·3	6·8	21·12
Soluble solids (%)	6·0-11·3	8·4	15·75	7·2-11·5	8·7	12·85	8·0-11·4	9·2	16·36	6·0-11·6	8·6	14·12
Ash (%)	0·34-0·58	0·46	14·32	0·34-0·56	0·44	11·60	0·36-0·43	0·38	7·79	0·34-0·58	0·44	13·17
Na (mg %)	0·68-2·22	1·33	32·02	0·62-2·38	1·31	44·43	0·57-1·19	0·85	30·21	0·57-2·38	1·28	40·71
K (mg %)	107-222	162	20·06	118-211	169	12·36	136-169	148	9·98	107-222	166	15·00
Ca (mg %)	19·7-33·7	25·2	14·96	14·5-41·1	25·2	23·40	24·6-31·0	29·0	10·26	14·5-41·1	25·5	20·02
Mg (mg %)	7·8-14·4	10·8	17·68	7·6-14·1	10·7	15·78	10·4-12·4	11·3	7·47	7·6-14·4	10·8	15·96
P (mg %)	14·2-30·8	22·6	20·00	14·8-26·3	20·6	14·45	22·8-26·7	24·4	7·25	14·2-30·8	21·6	17·10
N (mg %)	67-145	100	22·70	73-153	111·2	18·85	56-67	61	7·81	56-153	104	23·62
Formol number	0·5-1·85	1·07	39·51	0·2-1·74	0·77	44·66	0·23-0·44	0·35	25·66	0·2-1·85	0·84	48·60
Absorption 515 nm	0·51-1·67	0·90	36·02	0·31-2·55	1·07	52·35	0·57-0·83	0·7	17·64	0·31-2·55	0·98	48·57
Absorption 275 nm	0·64-1·07	0·82	15·29	0·39-1·17	0·71	25·78	0·44-0·90	0·66	35·20	0·39-1·17	0·74	23·62
Aspartic acid (μM/100 g)	5·9-170·5	46·5	94·20	2·5-63·2	23·7	64·62	3·8-13·4	8·7	57·60	2·5-170·5	30·6	100·19
Threonine+serine (μM/100 g)	58·9-959	416·6	63·44	16-447	149	57·61	22·3-31·0	28·1	14·15	16·4-959	234·4	92·77
Glutamic acid (μM/100 g)	21-167	53·3	62·02	10-71	35·1	50·73	16·5-26·3	21·2	21·70	9·8-167	40·4	63·28
Glycine (μM/100 g)	4·9-15	9·1	36·56	0·9-9·9	4·9	41·27	2·2-6·0	3·8	42·70	0·9-15	6·3	52·17
Alanine (μM/100 g)	27-236	82·2	61·58	15-104	49·3	43·67	0·2-21·9	13·8	70·13	10·2-236	58·4	66·70
Valine (μM/100 g)	1·2-14·3	5·85	69·29	0·4-9·3	3·0	69·38	0·5-0·8	0·7	22·89	0·4-14·3	3·7	87·61
iso-Leucine (μM/100 g)	1·4-7·6	3·4	54·85	0·5-3·7	1·5	53·53	0·4-0·8	0·6	29·43	0·4-7·6	2·1	79·71
Leucine (μM/100 g)	1·0-45·7	5·8	168·40	1·0-7·0	3·3	54·11	1·0-3·1	1·6	59·06	1·0-45·7	4·0	148·70
γ amino n-butyric acid (μM/100 g)	4·6-18·3	10·6	34·38	7·2-74·3	30·1	47·77	10·0-25·3	15·9	41·86	4·6-74·3	17·9	46·85

10–15 ml of Amberlite I.R. 120 (H) resin in the acid form, and the sugars washed through with about 500 ml water. The amino acids were eluted from the column with 150 ml of approximately N ammonia solution. As the anthocyanins appeared to be eluted somewhat slowly by the ammonia, the column was then allowed to stand for about 1 hr before washing with 150 ml water. The combined ammoniacal eluate and washings were boiled down* to a final volume of 20 ml, and the amino acids in 1 ml of this solution were determined by the standard 20 hr Autoanalyser technique (Technicon). After use the Amberlite column was regenerated with 2N HCl and washed until the washings were neutral. Not more than three samples were put through a column before the resin was renewed.

Results and discussion

General observations

A wide variation in the level of all of the parameters determined was found in the investigation. The individual results for all the samples examined are given in Table 1 and the range, mean and coefficient of variation of the different parameters are shown in Table 2.

Ten peaks appeared consistently on the Autoanalyser traces of the amino acids. The threonine and serine peaks frequently ran together and were therefore estimated together, and several other peaks appeared in some of the fruit samples.

Table 2 shows that the amino acids were much more variable than the other constituents. The range and coefficient of variation for the sugars, solids and minerals were broadly similar for all the three years, and although the ratios of the lowest to the highest values of the amino acids were generally similar in all three years of the investigation, the values obtained in 1970 were approximately half those obtained in 1969, and those obtained in 1971 were lower still. With the exception of the isolated high value of 45·7 μmol per 100 g for leucine in the 1969 fruit, all of the other fifty-three samples fell within the range of 1·0–7·0 μmol per 100 g, and it is therefore highly probable that this high result was spurious.

The stability to boiling of the amino acids in aqueous solution prompted a limited investigation into their stability during jam making. The amounts of amino acids found in four samples of fresh fruit jam (1969 season) were in close agreement (Table 3a) with those found in the corresponding frozen fruit after allowance was made for the fruit content of the jams. However, the amounts found in four sulphited fruits(1970 season) which had been stored for 13 months (Table 3b) and in jam made from one sample (Table 3c) of sulphited fruit (1969 season) were anomalous. The levels of several of the amino acids were substantially higher than those in the corresponding

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* This method of concentrating the solution was checked by boiling the standard amino acid mixture, used for characterizing and quantifying the amino acids, with ammonia in the same way. The amino acids were shown to be virtually unaffected by the process.

samples of frozen fruit, but the differences were not consistent either within samples or within amino acids.

Calculation of fruit content

Steiner (1949) described a technique for obtaining the best estimate of the content of an ingredient from several analytical constituents by applying a series of factors such that:

$$\text{ingredient content}_1 = a_1 X_{11} + a_2 X_{12} + a_3 X_{13}$$
$$\text{ingredient content}_2 = a_1 X_{21} + a_2 X_{22} + a_3 X_{23}, \text{ etc.}$$

where X_{1j} were the analytical determinations and a_j were the appropriate factors. These equations can be solved exactly when the number of samples analysed is equal to the number of parameters measured. However, as fruit is a variable material, the analytical results are only estimates of the true values of the whole sample, and under normal techniques the analysis would be replicated. Since in this investigation we were interested in obtaining factors applicable to the whole global strawberry population, better estimates of the values of the factors were obtained by analysing further independent samples of strawberries such that the number of independent samples substantially exceeded the number of parameters measured. The values of the factors were then determined by minimizing the residual variance after solution of the equations:

$$\text{ingredient content}_1 = a_1 X_{11} + a_2 X_{12} + a_3 X_{13} + \text{Var}_1$$
$$\text{ingredient content}_2 = a_1 X_{21} + a_2 X_{22} + a_3 X_{23} + \text{Var}_2, \text{ etc.}$$

i.e. by minimizing $(\text{Var}_1{}^2 + \text{Var}_2{}^2 + \text{Var}_3{}^2 \ldots)$.

TABLE 3a. Free amino acids in strawberries and strawberry jams (1969 fruit) (results expressed as μmol per 100 g original fruit)

Cultivar Source Sample	Cambridge Favourite F		Cambridge Sentry C		Crusader C		Grundi B	
	Fruit	Jam	Fruit	Jam	Fruit	Jam	Fruit	Jam
Aspartic acid	63	60	39	35	6	7	43	14
Threonine + serine	960	1019	580	500	98	103	440	388
Glutamic acid	50	26	58	52	19	14	56	29
Glycine	7	10	6	10	5	6	13	11
Alanine	118	123	93	94	41	25	61	59
Valine	14	12	11	8	4	3	7	5
iso-Leucine	5	5	8	4	2	Trace	4	2
Leucine	4	5	46	3	2	2	4	5
γ amino *n*-butyric acid	16	17	13	12	7	6	7	6

TABLE 3b. Free amino acids in strawberries preserved by freezing and sulphiting (1970 fruit) (results expressed as μmol per 100 g fruit)

Cultivar Source	Talisman E		JI 1800 G		JI 1805 G		Cambridge Favourite H	
Storage method	Freezing	Sulphiting	Freezing	Sulphiting	Freezing	Sulphiting	Freezing	Sulphiting
Aspartic acid	19	62	28	82	32	129	9	129
Threonine + serine	142	136	274	264	295	200	132	168
Glutamic acid	17	68	36	78	29	84	32	92
Glycine	5	18	6	13	4	12	6	21
Alanine	55	58	64	50	39	49	61	11
Valine	3	23	7	17	5	16	4	21
iso-Leucine	1	12	3	8	2	7	3	10
Leucine	3	49	7	30	4	32	6	47
γ amino *n*-butyric acid	20	6	51	23	45	8	20	17

TABLE 3c. Free amino acids in frozen fruit and in jams prepared from fresh and sulphited fruit, Cambridge Favourite (Source F) (results expressed as μmol per 100 g fruit)

Amino acid	Jam prepared from			Frozen fruit
	Fresh fruit	Sulphited pulp 8 months old	Sulphited pulp 24 months old	
Aspartic acid	68	200	376	63
Threonine + serine	1019 ⎤	476	⎰ 598	959
Glutamic acid	26 ⎦		⎱ 123	50
Glycine	10	22	26	8
Alanine	123	109	165	118
Valine	13	2	1	14
iso-Leucine	5	6	14	5
Leucine	5	29	42	4
γ amino *n*-butyric acid	18	28	17	16

This process was carried out by the least squares analysis of the linear model (Seal, 1964) obtained from the analytical values.

The factors were then used to calculate the 'fruit content' of each sample of strawberries from its own analytical results. The coefficient of variation and 95% confidence limits (i.e. $\pm$ the product of the standard deviation and t_α ($P = 0.05$)) of the calculated 'fruit contents' were also calculated (Table 4b).

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TABLE 4a. Factors to be applied to analytical parameters for calculating fruit content

Parameter	Using all twenty-three parameters	Omitting parameters marked with —	
Insoluble solids	−0·078	—	3·54
Total solids	3·03	2·71	1·75
Total sugars	1·13	1·42	3·15
Soluble solids	0·236	—	−0·625
Ash	45·7	37·1	21·2
Sodium	3·85	3·53	3·38
Potassium	−0·044	—	0·0580
Calcium	−0·105	—	−0·0665
Magnesium	1·47	1·37	1·11
Phosphorus	0·947	0·906	0·593
Nitrogen	0·088	0·090	0·155
Formol number	−13·9	−14·0	−8·86
Absorption 275 nm	5·53	4·26	−1·58
Absorption 515 nm	1·76	2·52	4·58
Aspartic acid	−0·0156	—	—
Threonine + serine	0·0159	0·0136	—
Glutamic acid	0·0038	—	—
Glycine	−0·997	−1·13	—
Alanine	−0·0267	—	—
Valine	0·790	0·402	—
iso-Leucine	1·822	2·265	—
Leucine	−0·585	−0·576	—
γ amino *n*-butyric	0·132	0·109	—

TABLE 4b. Calculated fruit content of fifty-four samples of strawberries

		Range	Coefficient of variation	95% confidence limits
(1)	Statistical analysis of results using all 23 parameters	79·1–115·3	7·76	15·81
(2)	As 1, but omitting insol. solids, sol. solids, K, Ca, aspartic acid, glutamic acid, alanine	79·4–115·8	7·81	15·81
(3)	As 1, but omitting all amino acids	78·9–116·9	8·41	17·00
(4)	By use of average insol. solids	61·1–183·5	20·09	40·30
(5)	By use of average K	64·3–133·5	15·00	30·09
(6)	By use of average P	65·8–143·7	17·10	34·30
(7)	By use of average N	53·8–146·5	23·62	47·38
(8)	By averaging results of 4–7	66·1–120·2	15·40	30·92

It is desirable that an estimate of the fruit content of a product should be based on the minimum number of analytical parameters consistent with acceptable accuracy. In order to assess the desirability of each parameter, they were removed in turn from the matrix of data and new sets of factors were calculated. By comparing the standard deviations and 95% confidence limits of 'fruit contents' computed with the new factors against the original calculated fruit contents the desirability of the omitted parameter was assessed. In this investigation the criterion was adopted that a parameter was desirable if its removal increased the 95% confidence limits of the calculated fruit contents. The removal of seven parameters (insoluble solids, soluble solids, potassium, calcium, aspartic acid, glutamic acid and alanine) individually or in groups did not increase the 95% confidence limits of the calculated fruit contents (Table 4a). The factors to be applied to the remaining sixteen parameters (total solids, total sugars, ash, sodium, magnesium, phosphorus, nitrogen, formol number, absorption at 515 nm and 275 nm, threonine+serine, glycine, valine, *iso*-leucine, leucine and γ amino *n*-butyric acid) in order to obtain the best estimate of fruit content, and the range, coefficient of variation and 95% confidence limits of the fruit contents based on these factors are shown in Tables 4a and b. Since all the free amino acid determinations are carried out in one operation, the effect of removing all of these parameters was tested (Table 4b, line 3), the results showed a slight decrease in the accuracy of the calculated fruit contents. Therefore, before making a decision on whether to include amino acids in any analytical procedure it would be necessary to assess the relative importance of the slightly reduced accuracy against dispensing with amino acid determinations.

Significantly correlated parameters

Calculation of the correlation coefficients for each of the parameters with all of the others revealed a large number of highly significant relationships (Table 5) all but one of which (sodium : total sugars) were positive. Most of the relationships at 0·1% significance were between pairs of amino acids, or between these parameters and formol number, but there were several correlations at 1% significance between amino acids and nitrogen, phosphorus, potassium or ash. There were also highly significant correlations between several pairs of the non amino acid parameters.

These correlations can be used as a basis for determining the authenticity of samples of strawberry products. Limits may be placed upon the ratios of significantly related parameters to each other (see Table 6) and if the ratio of two parameters from an unknown sample fell outside the corresponding limits, then the sample would be unlikely to be based on strawberries as the sole fruit. However, it is not possible to use conventional statistical techniques to determine confidence limits of these ratios since the distribution of ratios of individual samples may or may not be normal (e.g. potassium : ash is approximately normal; *iso*-leucine : formol number is not).

Bearing in mind the wide ranges of some of the ratios, it would be unwise to base a judgement on one pair of parameters, and the overall pattern of ratios would give a

TABLE 5. Significantly related parameters

All correlations were positive except Na/total sugars.
* 5% significance; ** 1% significance; *** 0·1% significance.

better assessment of fruit authenticity. In addition the extent to which these relationships involving free amino acids may be useful for detecting adulteration will only become apparent when the effect of preservation with sulphur dioxide on the free amino acids (see p. 47) has been further investigated, and when similar information is available for other fruits.

Conclusions

This investigation of fifty-four samples of strawberries demonstrated that, by the correct selection and use of the most important parameters for the purpose, marked improvements can be made in the accuracy with which fruit content is estimated from analytical determinations.

TABLE 6. Relationships of significantly related parameters (0·1% significance) to each other

	Mean	Range	95% confidence limits*
Total sugars : total solids	0·63	0·47–0·86	0·20
Soluble solids : total solids	0·81	0·71–0·97	0·093
Soluble solids : total sugars	1·30	1·00–1·69	0·39
Potassium : ash	377·5	248·8–462·8	95·2
Nitrogen : potassium	0·63	0·38–0·88	0·25
Formol no. : nitrogen	0·080	0·027–0·17	0·063
Aspartic acid : Formol no.	33·8	11·0–133	—
Threonine + serine : Formol no.	245	55·8–601	289
Threonine + serine : aspartic acid	8·36	1·66–17·6	7·76
Glutamic acid : Formol no.	51·1	10·8–118	44·9
Glutamic acid : aspartic acid	1·94	0·41–6·20	—
Glutamic acid : threonine + serine	0·28	0·049–1·18	—
Glycine : Formol no.	8·3	0·97–17·2	7·9
Glycine : aspartic acid	0·35	0·033–1·20	—
Glycine : threonine + serine	0·046	0·0057–0·198	—
Glycine : glutamic acid	0·18	0·018 –0·51	—
Alanine : Formol no.	69·8	22·6–155	56·4
Alanine : aspartic acid	2·62	0·58–6·89	3·04
Alanine : threonine + serine	0·35	0·090–0·93	0·37
Alanine : glutamic acid	1·55	0·56–3·44	1·51
Alanine : glycine	10·3	1·70–59·6	—
Valine : Formol no.	4·2	0·0084–10·8	5·0
Valine : aspartic acid	0·15	0·00042–0·56	0·22
Valine : threonine + serine	0·019	0·000051–0·044	0·019
Valine : glutamic acid	0·098	0·00017–0·28	0·13
Valine : glycine	0·64	0·0017–3·67	—
Valine : alanine	0·062	0·00026–0·14	0·064
iso-Leucine : Formol no.	2·4	0·0084–5·4	—
iso-Leucine : aspartic acid	0·098	0·00042–0·36	—
iso-Leucine : threonine + serine	0·012	0·000051–0·036	0·015
iso-Leucine : glycine	0·33	0·0043–1·25	—
iso-Leucine : alanine	0·037	0·00025–0·082	0·038
iso-Leucine : valine	2·11	0·30–80·0	—
Leucine : *iso*-Leucine	3·99	0·53–100	—

* Calculated on assumption that data distributed normally, no value given when this was markedly not so.

This is shown by comparison of line 2 with lines 4–8 in Table 4b where the range of calculated fruit contents based on the sixteen parameters, 79·4–115·8%, compares with at best a range of 66·1–120·2% based on insoluble solids, N, P, K.

Likewise the large number of highly significant correlations between pairs of parameters indicate that the ratios between the pairs of parameters might be used in determining fruit authenticity more reliably than is at present possible.

There is no reason to suppose that the same approach applied to other fruits would not yield similar improvements.

However, the application of the findings for authentic samples of strawberries to manufactured products is not straightforward. For example, some of the parameters found to be important for the calculations of fruit content (e.g. total solids, total sugars) are obviously useless for assessing the fruit content of jams or similar manufactured products. There is also the question of the effect of storage with sulphur dioxide on the free amino acids.

Further work on the effect of sulphur dioxide is therefore necessary, not only in the above context, but also with a view to the determination of fruit authenticity by means of correlations between parameters, as it was shown that, for authentic strawberries, the amino acids figured significantly in these relationships.

Acknowledgments

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The production of polyunsaturated tallows and their utilization in margarine manufacture

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Summary

Offal materials were collected from sheep and cattle, which had been slaughtered after a period of feeding with a protected polyunsaturated oil supplement. Tallows produced from these offals by methods simulating batch dry rendering and low temperature wet rendering processes contained about 19% linoleic acid and had a saturated/unsaturated ratio lower than in normal tallows. Pilot scale refining and margarine manufacturing processes were used to convert the tallows to margarines. No unusual or unexpected processing or quality effects are expected in commercial production using standard 'good practice' rendering, refining and margarine manufacturing procedures.

Introduction

It is widely thought that human health may benefit by an increase in the ratio of polyunsaturated to saturated fatty acid in the diet (Anon, 1972; American Medical Association, 1972). There are obvious advantages in presenting foods of high polyunsaturated fat content in traditional and well accepted forms such as meat, milk and butter. The possibility of manipulating the composition of the fat components of monogastric animals by supplemental feeding has long been recognized, but only recently have techniques been developed by Scott *et al.* (1970) to allow such supplements to be fed in 'protected' form to ruminants so that they are not substantially altered by the processes occurring in rumen digestion. They showed that droplets of sunflower seed oil, protected by encapsulation in a thin sheath of protein hardened by formalin treatment, remain intact during passage through the rumen but are released and absorbed into the body as a result of digestive processes in the abomasum and small intestine. Cook *et al.* (1970) found that animals fed on protected supplements for 6 or 7 weeks had linoleic acid contents in peri-renal and subcutaneous fat of around 20%, some ten times greater than in the fat of control animals fed on diets not containing the supplement.

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In late 1972, the Commonwealth Scientific and Industrial Research Organization (CSIRO) entered into a joint venture with Alta Lipids Australia Pty Ltd, a subsidiary of Dalgety Agrilines Pty Ltd, to develop the production of meat and dairy products with a high content of polyunsaturated fatty acid using the method of protected supplement feeding patented by CSIRO (1970). Such products are generally referred to as Alta-products, e.g. Alta-beef, Alta-cheese, and this convention is used in the present report.

Meat production results in substantial quantities of non-meat materials such as bones and fat, which must be converted into by-products by rendering processes. Batch dry rendering is generally used where the materials contain bone, giving tallow and a dry meat-and-bone meal. Continuous low temperature wet rendering is commonly used on fat and meat trimmings, to produce edible tallow and a high grade wet protein residue, suitable for incorporation into small goods. Since rendering is an integral and important facet of abattoir operations, it was considered advisable to investigate the processing of Alta bones and offals by standard rendering procedures.

Alta-tallow has obvious attractions as a starting material for the manufacture of margarine with a significant content of polyunsaturated fat particularly in Australia, where legislation severely restricts the amount of non-animal fats that can be used in margarine manufacture.

This report describes the rendering procedures used to produce two experimental batches of Alta-tallow, and the salient features of the refining and manufacturing procedures used to produce experimental Alta-margarine therefrom. Results of standard chemical and physical tests on the tallows and margarines are also reported.

Procedures

Preparation of sheep tallow

Edible offal (mainly caul and kidney fats and trimmed bones) was collected from the carcasses of five lambs and hoggetts slaughtered after 39 days of feeding with supplement containing protected polyunsaturated lipid, and a further fifteen slaughtered after 55 days of feeding with the same supplement. The offal was packed into cartons frozen within 24 hr of collection and stored for about 6 weeks at below 20°C until required for rendering.

A small batch dry rendering cooker, of geometrical arrangement similar to the cooker described by Herbert & Norgate (1971) but scaled down to 0·3 m internal diameter and 1·2 m long was used to render the offal. A total of 58 kg of offal, comprising 34 kg of fatty materials and 24 kg of bones was charged into the cooker, 3 g butylated hydroxyanisole (BHA) and 6 g of Silicone Antifoam A (Dow Corning) were added and the materials rendered under atmospheric pressure throughout to an end point temperature of 122°C in just under 1 hr.

The tallow was separated from the meal in a perforated bowl centrifuge during the

2 hr following discharge of rendered materials from the cooker. Attempts made to prevent contact of air with the tallow by nitrogen blanketing were probably ineffective during the separation stage. The tallow was stored at below −20°C for 2 weeks before being processed into margarine.

The tallow was of poor quality compared with the crude mutton tallows normally received by margarine manufacturers (see Table 1) and so was washed three times with water before neutralization and twice after neutralization. Fractional crystallization produced an oleo fraction (shot melting point—SMP—36°C, 77% yield) and a stearine fraction (SMP 50·6°C, 23% yield).

Preparation of beef tallow

About 20 kg of caul and kidney fats were collected from the carcasses of four young cattle (average carcass weight 58 kg) which had been fed with protected polyunsaturated lipid supplement for about 10 weeks before slaughter. The fats were stored at about

TABLE 1. Chemical and physical analysis of mutton tallow

Analysis		Crude Alta mutton tallow	Crude normal mutton tallow
Colour (Lovibond)		30 R, 79 Y, 3 B	10 R, 40 Y
Free fatty acid (%)		1·26	0·5
Initial peroxide value (mEq/kg)		0·9	0–1·4
16 hr AOM (mEq/kg)		7·9	4·0
Iodine value		62·8	47·0
Shot melting point (°C)		42·6	41–45
Solid set indices (A.O.C.S.)			
10°C		24·2	31·5
21·1°C		16·8	22·0
26·7°C		15·2	20·0
33·3°C		12·3	15·0
37·8°C		8·7	10·0
40°C		6·8	7·5
Fatty acid composition (%)			
Myristic	C14	1·5	3·8
Myristoleic	C14′	Trace	Trace
Palmitic	C16	16·6	25·5
Palmitoleic	C16′	1·6	4·7
Margaric	C17	0·9	1·9
Stearic	C18	24·3	22·0
Oleic	C18′	33·7	38·3
Linoleic	C18″	19·2	2·2
Linolenic	C18‴ ⎫	1·2	1·6
Arachidic	C20 ⎭		

0°C for 4 days, and were then passed through a meat mincing machine; 2 g BHA were added to the mince.

The rendering procedure was intended to simulate low temperature wet rendering and involved adding about 10 kg of the minced fat rapidly and with violent agitation to about 20 kg of boiling water in a steam jacketed vessel. The mixture temperature was maintained at about 80°C for 1 hr with occasional stirring; the tallow was then separated from the mixture by passing it through a small clarifier (Disc Centrifuge, Alfa Laval B1300). Feed rate was adjusted to give a clear tallow output from the clarifier, and about 2 hr was required to produce about 7 kg of tallow. The balance of the fat mince was rendered and separated by the same procedure.

The tallow was stored for 4 weeks at below $-20°C$ and was then used for margarine manufacture. The tallow was of high quality, and required only neutralization and water washing to remove the sodium soaps. Fractionation produced an oleo fraction, SMP 37·8°C, 88% yield, and a stearine fraction, SMP 50°C, yield 12%.

Preparation of margarine samples

The oleo fractions of the mutton and beef tallows were bleached and deodorized then emulsified with skim milk solids, water and milk, together with small quantities of colouring, flavouring and anti-oxidant materials, to give the experimental batches of Alta-margarine.

Analytical procedures

Chemical and physical analyses were carried out in accordance with the methods laid down by the AOCS (1972). Fatty acid compositions were obtained by the gas chromatographic techniques outlined in Ce 1–62 (revised 1970) of the AOCS Methods manual (1972).

Results and discussion

Analytical results for the crude Alta-mutton tallow are listed in Table 1, and for the crude Alta-beef tallow in Table 2. For comparison, typical results for the corresponding normal tallows are given in the same tables. The results of similar analyses carried out on the stearine and oleo fractions are given in Table 3.

The colour and free fatty acid (FFA) content (Table 1) of the Alta-mutton tallow were high compared to the normal crude tallow values; the tallow also had a burnt appearance and a 'gutty' odour. The high FFA value is probably linked with deterioration of materials during the long period of storage before rendering. The high colour rating is typical of tallows containing fine protein particles, and in this case, indicates that the tallow/meal separation step using the perforated bowl centrifuge was not effective in removing all the fine particles from the tallow. Unexpectedly, the 16 hr Active Oxygen Method (AOM) value was only slightly higher than for normal tallow (cf. value for Alta-beef tallow, Table 2), possibly as a result of the inhibiting effect of finely divided protein.

The Alta-beef tallow colour rating (Table 2) was lower than for conventional crude beef tallow, as might be expected in fat obtained from animals fed on dry rations for some weeks before slaughter and rendered by the low temperature wet-rendering process which is well-known for producing high grade tallow. Clarification of the tallow in a high speed disc centrifuge removed all fine protein, and the 16 hr AOM was very high compared with normal tallow, reflecting the high unsaturated fatty acid content.

TABLE 2. Chemical and physical analysis of beef tallow

Analysis		Crude Alta beef tallow	Crude normal beef tallow
FAC Colour		2 F.A.C.	7 F.A.C.
Free fatty acid (%)		0·57	0·62
Initial peroxide value (mEq/kg)		2·6	1·1
16 hr AOM (mEq/kg)		330	3
Iodine value		61·3	45
Shot melting point (°C)		41·7	43–44
Solid set indices (A.O.C.S.)			
10°C		22·5	31·5
21·1°C		16·0	22·1
26·7°C		14·1	19·8
33·3°C		10·9	15·1
37·8°C		7·6	10·1
40°C		5·3	7·3
Fatty acid composition (%)			
Myristic	C14	2·2	3·8
Myristoleic	C14′	Trace	Trace
Palmitic	C16	10·1	26·0
Palmitoleic	C16′	1·8	4·8
Margaric	C17	0·4	Trace
Stearic	C18	24·3	22·5
Oleic	C18′	30·1	38·0
Linoleic	C18″	19·8	2·2
Linolenic	C18‴ }	1·4	1·6
Arachidic	C20		

It appears that a satisfactory margarine grade tallow could be produced from batch dry rendering or low temperature wet rendering processes of the type commonly used in Australian abattoirs. Present 'good' rendering practices, including rendering offals within 4 hr of slaughter, and clarification of the tallow, should be adequate. However, even with anti-oxidant added at the concentration used in these experiments—about 150 g BHA per tonne tallow—stability appears to be low and the tallow should be sent for margarine manufacture as soon as possible after rendering.

TABLE 3. Chemical and physical analysis; Alta-tallow fractions

Analysis		Oleo fraction (deodorized)		Stearine fraction (Neutralized)	
		Mutton	Beef	Mutton	Beef
Colour (Lovibond)		4·4 R	4 R	—	—
		30 Y	30 Y	—	—
Free fatty acid (%)		0·22	0·14	—	—
Initial peroxide value (mEq/kg)		0·96	Nil	—	—
Iodine value		64·7	63·7	48·1	49·9
Shot melting point (°C)		36·0	37·8	50·6	50·0
Solid set indices (A.O.C.S.)					
10°C		18·1	21·6	41·0	39·6
21·1°C		11·6	13·1	35·9	35·8
26·7°C		8·9	10·5	35·2	35·4
33·3°C		5·2	6·7	32·6	31·7
37·8°C		2·2	3·4	29·2	27·5
40°C		0·6	2·0	26·4	24·2
Fatty acid composition (%)					
Myristic	C14	2·0	2·2	2·3	2·5
Myristoleic	C14′	0·1	Trace	0·3	Trace
Palmitic	C16	17·3	20·1	21·4	24·0
Palmitoleic	C16′	2·2	2·0	1·7	1·4
Margaric	C17	1·3	Trace	1·9	1·1
Stearic	C18	21·9	24·0	30·4	29·9
Oleic	C18′	35·2	31·6	27·7	25·0
Linoleic	C18″	17·7	20·1	12·6	16·0
Linolenic	C18‴ ⎫	2·2	—	1·3	Trace
Arachidic	C20 ⎭				

The increase in unsaturation is indicated by the increased iodine values, the lower shot melting points, and the lower dilation values of both crude Alta-tallows (Tables 1 and 2). Their linoleic acid content is about nine times higher than conventional tallows (over 19% as compared to 2·2% in normal tallow). It appears that the linoleic acid content has increased mainly at the expense of oleic and palmitic acids; on balance, there is a slightly reduced saturated fatty acid content in the Alta-tallows. The linoleic acid content is considerably lower than the 40% laid down by the Australian National Health and Medical Research Council (NHMRC) as the minimum content necessary to permit description of the product as polyunsaturated. In those Australian States which have legislated in line with NHMRC requirements, therefore, the experimental margarines could not be sold as polyunsaturated margarines.

The Alta-margarines were of satisfactory appearance and were softer and easier to

spread than margarines made from normal tallows. Taste panel evaluations were carried out to establish whether flavour and aroma simliar to those observed in meats from animals fed on these supplements were present. The Alta-margarine samples and samples of two commercially available tallow based margarines were spread on slices of bread and panel members were asked only to report whether they could detect any unusual aroma and flavour. Results given in Table 4 show that over half the panel members detected it in both Alta-margarines, although it was more apparent in the mutton product. Since all panel members were involved in work on Alta-meats and trained to detect their characteristic aroma and flavour it is perhaps significant that approximately one-third of the panel were unable to detect either. It appears that acceptable margarines have been produced from the Alta-tallows, even from the mutton tallow, which was initially of poor quality. The characteristic flavour and aroma associated with the fat of Alta-meat, were detectable in the margarines but appear not to be at an unacceptably high level, and could probably be further reduced or if necessary masked in commercial production.

TABLE 4. Taste panel evaluation of Alta-margarines

| | Number of panel members | Percentage detecting | | | |
Source of margarine		Unusual aroma	Unusual flavour	Both	Neither
Alta mutton tallow	32	56	69	56	31
Alta beef tallow	20	55	55	40	30

The procedures used to prepare the Alta-margarine samples in general simulated production procedures, except that there was a much longer holding period between individual refining steps, particularly between bleaching and deodorizing than would be the case in full scale production. No unusual or unexpected processing characteristics were evident at any stage of experimental margarine production. Since much shorter holding periods are involved between the stages of manufacture in full scale production plants, it is unlikely that any problems would be encountered in commercial production of Alta-margarines.

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Fat cooking losses from non-emulsified meat products

G. G. EVANS AND M. D. RANKEN

Summary

The fat (lipid) losses which occur on cooking non-emulsified meat products are largely determined by the properties of the fat (fatty tissue) used. Soft fatty tissue such as pork jowl fat lost *c.* 20% of its lipid on heating to 80° or 100° whereas hard fatty tissue such as pork flare fat lost as much as *c.* 80%. A similar pattern of losses was observed in meat mixtures containing these fatty tissues. Damage to the fatty tissue, especially that caused by mincing or chopping in the frozen condition, increased the lipid loss both from fatty tissue alone and from sausages made with it. Similar effects were demonstrated in a limited number of beef fatty tissue samples. The differences in lipid loss appear to be due to differences in the connective tissue present and to the extent to which fat cells are broken during comminution. In sausages and in certain meat mixtures the loss of lipid was less than that expected from the behaviour of the fatty tissue alone: this suggests the existence of another mechanism, possibly involving emulsification, to retain lipid in the mixture after its release from the tissue.

Introduction

In the course of a general research project conducted in this laboratory into cooking losses from non-emulsified meat systems (Evans & Ranken, 1972) it was observed that in these systems the lipid loss on cooking could be very variable but appeared to be determined almost entirely by the fatty tissue used in the recipe. It also appeared that the non-lipid part of the fatty tissue was of particular importance. There is an extensive literature on meat 'emulsions', on the composition of the lipids of animal fats and on the cooking behaviour of joints of meat containing fat, but little appears to have been published concerning the cooking properties of the fatty tissue itself. The work described here was undertaken to explore this subject further.

Materials and methods

Fatty tissue

Fatty tissue of the kinds normally used by meat products manufacturers were employed. Pork jowl fat was taken as a typical example of a very soft fat, flare as a hard fat and

back fat as intermediate in softness between them. Each specimen was usually a mixture of tissues from different animals, and in the case of back fats also from different anatomical regions. Some experiments were also done on samples of beef fatty tissue selected from the crop region of two different animals and differing in their degree of hardness.

To ensure that a homogeneous sample of fat was used for each experiment or series of experiments, large batches of fat were diced by hand and minced through a 5 mm plate, then thoroughly mixed by hand.

When it was necessary to use the same batch of fat over a long period of time the batch was frozen at $-32°C$ and held at that temperature until required. Portions were removed and thoroughly thawed before use.

Softness of fat

This was assessed manually and rated on a descriptive scale from 'soft' to 'hard'.

Lean meat

This was pork shoulder meat (fat content *c.* 2–3%, occasionally 5%), delivered 1 day after slaughter, occasionally 2 days, and normally used on the day of delivery. Any obvious excess fat or connective tissue was trimmed off before use. After dicing the meat was minced through a 5 mm plate.

Mixtures of minced meat and fat

These were made by mixing appropriate proportions of minced meat and minced fat by hand, using a kitchen fork.

Chopping

A 20 l Müller bowl chopper (Foodtech Ltd) was used. Three knives were set with 1·5 mm clearance at the periphery of the bowl, knife rotation speed was 1400 rev/min and bowl speed 13 rev/min. All chopping was performed at 5°C. Fat alone or mixtures of minced meat and fat were chopped in batches of not less than 1·5 kg. The degree of chopping would be described as a 'coarse chop' and was not fine enough to produce a 'meat emulsion'.

Freezing and thawing

To confirm the significance of an early observation that the mincing or chopping of frozen material could affect the results, an experiment was conducted using large well mixed diced samples of fatty tissue, in which the factors of comminution and temperature were systematically varied. The procedures and the terms used to describe them, were as follows:

Unchopped unfrozen. Held at $+5°C$ for 48 hr then minced and allowed to reach ambient temperature.

Unchopped thawed. Frozen 24 hr at $-32°C$ then thawed 24 hr at $+20°C$ before mincing.

Unchopped frozen. Frozen 24 hr at $-32°C$ then minced in the frozen state, using a chilled mincer. The minced fat was then allowed to thaw and attain ambient temperature.

Chopped unfrozen. As for unchopped unfrozen, but chopped for 80 sec after mincing.

Chopped thawed. As for unchopped thawed, but chopped 80 sec after mincing.

Chopped frozen. As for unchopped frozen, but chopped for 80 sec after mincing. During chopping the fat became thawed.

Laboratory cooking test

The cooking conditions were those selected for the general research work (Evans & Ranken, 1972). Samples (40 g ± 0.5 g) were placed in boiling tubes fitted with stoppers and air condensers and cooked by immersion for 28 min in a water bath maintained usually at $80°C \pm 0.1°C$. A few experiments were done in a water bath at $100°C$. Inside each tube was a perforated metal plate, shaped to fit the bottom of the tube and attached to threads by which it could be pulled up at the end of the cooking time, so enabling the residue of cooked fat or meat to be raised free of the cooked-out water and fat. By chilling the tube and its contents in ice-water after cooking, the cooked-out fat could be solidified, separated from any aqueous layer and weighed. The fat cooking loss was normally calculated as a percentage of the fatty tissue present in the cooked mixture, so permitting direct comparisons of losses sustained by the fatty tissue under different conditions.

Preparation and cooking of coarse-chopped sausages

Standard methods developed in these laboratories were used. The composition, conforming with the British regulations (S.I. No. 862 : 1967), was:

Lean pork shoulder meat	32·6%
Fat	32·6%
Rusk	12·0%
Water	20·5%
Salt, seasoning and preservative	2·3%.

The lean meat and fatty tissue were diced and separately minced through a 5 mm plate. All the ingredients required for a 2·5 kg batch of sausages were evenly distributed around the bowl of the chopper and the whole mixture was chopped for 80 sec. The final mixture was filled into collagen casings and linked in the usual manner. The sausages were held overnight in polythene bags at 5°C.

Four similar sausages with a known total weight in the range 220 g $\pm$ 2 g were fried in identical flat-bottomed frying pans on a thermostatically controlled heating plate maintained at $163 \pm 2°C$. They were cooked for 20 min, turning every 2 min to ensure

uniform cooking. The fat in each frying pan was poured into a tared beaker and the pan drained for 15 sec. The weight of fat was taken as the fat loss on cooking.

Physical and chemical analysis

Moisture content of fatty tissue. Approximately 5 g of minced fatty tissue were weighed accurately into a dish, and dried to constant weight at 105°C.

Fat content of fatty tissue. Approximately 5 g of fatty tissue were accurately weighed on a dried filter paper and partially dried in a vacuum oven for 4 hr at 40°C under a vacuum at 50 Torr. The filter paper plus contents was then extracted for 12 hr with petroleum ether (B. pt 40–60°C) in a Soxhlet extractor. After removal of the solvent, the extract was dried to constant weight at 40°C in vacuum.

Dried fat free residue (DFFR) of fatty tissue. The residue remaining in the Soxhlet thimble was removed and dried to constant weight at 105°C.

Hydroxyproline content of fatty tissue. This was estimated by the method of Mohler & Antonacopoulos (1957).

Iodine value of extracted fat. This was estimated by Wij's method.

Slip point of extracted fat. The procedure was that described in BS 634 (1953).

Solid/liquid ratio of extracted fat. The wide line nuclear magnetic resonance method described by Evans & Meara (1971) was used, employing a Newport Instruments Quantity Analyser.

In this method the resonance given by a sample of tempered fat is compared with that given by winterized maize oil. The percentage of liquid phase in the sample is calculated by direct proportion, taking the winterized maize oil to be 100% liquid.

Fatty acid composition of extracted fat. The fat was esterified in the usual way. The methyl esters were analysed by glc on steel columns (1·5 m × 6·4 mm, o.d.) packed with 15% DEGS on Chromosorb W (100–120 mesh) and operated at 185°. Peaks were identified by comparison of retention times with known standards and quantified by peak area. The ratio of total saturated fatty acids to total unsaturated fatty acids was calculated.

Results

The results of a series of experiments involving thirty-three samples of pork fat and two samples of beef, are recorded in Tables 1–5.

Cooking losses of pork fatty tissues, laboratory cooking test

The first column in each of Tables 1–5 shows that the cooking losses of the pork fats tested ranged from 11·4% to 86·7% of the original weight. It can also be seen that the cooking losses were consistently related to the hardness or softness of the fatty tissue, as assessed manually, increasing with increasing hardness.

In Tables 2 and 4 it can be seen that the effect of chopping fatty tissue in the unfrozen condition, compared with mincing but not chopping, was to increase the cooking loss

TABLE 1. Pork fatty tissues: relationships between cooking loss and chemical composition

Fatty tissue and softness	Cooking Loss (%) * †	Moisture content (%) *	Fat content (%) *	Dry fat free residue (%) *	Slip point (°C) ‡	Proportion of fat solid at 20°C ‡	Iodine value ‡	Ratio sat./unsat. fatty acids ‡	Hydroxy-proline (%) §
Jowl, soft	13·9	14·1	81·4	4·5	29·1	13·9			2·0
soft	19·9	16·4	78·3	5·3	28·0	14·0			1·5
soft	21·4	17·1	78·0	4·8	27·9	14·6	59·0	0·62	1·4
Back, soft	21·3	8·5	89·1	2·2	29·4	14·7			4·0
medium	34·9	8·6	89·2	2·1	36·3	15·4			4·2
medium	37·8	8·3	90·2	1·8	37·0	15·0	52·8	0·86	4·3
Flare, hard	85·0	7·5	90·5	1·7	45·0	35·0			3·2
hard	85·9	7·1	91·3	1·8	41·0	38·9			3·1
hard	86·7	7·1	90·6	2·0	44·2	36·2	47·6	1·12	3·0

* Fatty tissue; † minced sample; ‡ extracted fat; § dry fat-free residue.

TABLE 2. Pork and beef fatty tissue: effect of freezing and chopping on the fat losses on cooking, as % of fatty tissue

Fatty tissue	Softness	Unchopped unfrozen	Unchopped thawed	Unchopped frozen	Chopped unfrozen	Chopped thawed	Chopped frozen
Pork							
Jowl	Soft	11·4	9·7	71·2	19·6	22·3	77·4
Back	med. soft	21·3	20·6	77·4	45·1	47·3	83·1
Flare	hard	75·9	73·2	75·0	82·6	85·0	82·4
Beef							
Crop	med. hard	27·6		49·2	36·2		81·2
Crop	hard	34·4		48·6	46·5		82·6

in every instance. Freezing the fatty tissue followed by thawing again produced little difference in cooking loss (Tables 2 and 5), whether the tissue was chopped before cooking or not, but mincing or chopping fatty tissue in the frozen condition increased the losses considerably, up to about 80% except in the case of hard fats whose cooking losses were already of this magnitude.

Cooking at 100° instead of 80° (Table 3) made no significant difference to the cooking losses of jowl (soft) or flare (hard) fat but significantly increased the losses from back fat (intermediate). The general relationship of cooking loss to hardness was not altered.

TABLE 3. Pork fatty tissues: effect of cooking
temperature on fat losses on cooking, as per
cent of fatty tissue

Type of fat	Temperature of cooking	
	80°C	100°C
Jowl	15·6	17·1
	26·1	27·1
Back	29·1	47·8
	34·3	49·8
Flare	85·1	82·2
	86·2	87·5

TABLE 4. Mixtures of lean pork and back fat: effects of composition and softness of fatty tissue on
fat loss on cooking, as % of fatty tissue

Composition	100% fat 0% lean		60% fat 40% lean		30% fat 70% lean	
Comminution	Unchopped	Chopped	Unchopped	Chopped	Unchopped	Chopped
Softness						
medium	38·3	48·1	34·5	46·5	26·7	27·6
medium	34·5	59·1	34·4	58·6	28·9	39·8
medium	32·6	54·7	31·1	53·9	21·0	25·6
hard	49·1	64·8	49·7	67·2	52·7	64·1
hard	48·6	64·1	51·0	66·2	50·2	56·6

Cooking losses of beef fatty tissues, laboratory cooking test

The values for the two samples of beef fat quoted in Table 2 show the same relation-
ship between cooking loss and hardness as with pork, though the general level of loss
from unfrozen material was much lower than that from pork flare fat of comparable
hardness. The effect of chopping compared with mincing, in the unfrozen state, was to
increase the cooking loss by about the same amount as in the case of pork, and mincing
or chopping in the frozen state again produced large increases in loss. The losses from
beef fats chopped when frozen were over 80% and similar to those found in pork fats
similarly treated.

Relationship between fatty tissue softness and lipid softness

The figures in Table 1 show that the softness of the fatty tissues, assessed manually

TABLE 5. Comparison of fat losses from sausages with those from
fatty tissue alone

Type of fatty tissue	Condition during preparation	Fat loss on cooking, % of fatty tissue	
		Fatty tissue*	Sausage
Jowl	Unfrozen	11·4	4·6
	Thawed	12·1	4·9
	Frozen	71·4	55·8
Back	Unfrozen	32·6	20·2
	Thawed	33·2	13·2
	Frozen	75·1	61·0
Flare	Unfrozen	81·3	69·3
	Thawed	83·2	70·2
	Frozen	82·2	67·8

* Sample minced and chopped, cooked in laboratory apparatus.

at room temperature or below, was directly associated with those chemical and physical properties which are indices of the softness or degree of unsaturation of lipids—viz. iodine value, slip point, proportion of fat solid at 20°C, ratio of saturated to unsaturated fatty acids.

The non-lipid portion of the fatty tissue also varied with softness, there being two to three times as much dry fat free residue and twice as much water in soft fatty tissues as in hard ones. The hydroxyproline content of the dry residue, which may be taken as an index of its collagen content, varied among the fatty tissues but was not consistently related to their softness.

These results are in agreement with those of other workers, such as Hilditch & Williams (1964) and Ingr (1971).

Cooking losses of fatty tissue in the presence of lean meat

The cooking losses of a number of pork back fats, alone and in admixture with lean meat, cooked by the laboratory test, are given in Table 4. For the hard fat samples, the cooking losses were practically the same in the presence of lean meat as when the fat was cooked alone. As previously, the samples which were minced and chopped had higher losses than those which were minced but not chopped; this increase in loss was also unaffected by the presence of lean meat. With soft fats, however, and in the presence of excess lean meat (70% lean, 30% fat), there were large reductions in the cooking loss compared with the 100% fat controls, by about one-third in the unchopped mixtures and one-half in the chopped mixtures.

Table 5 shows the fat losses sustained on frying sausages in which different pork fats

were used, each fatty tissue having been comminuted either in the unfrozen or the frozen state. These losses followed the same pattern as those of the fatty tissues cooked in the laboratory cooking apparatus, being related to the softness of the fatty tissue and reaching very high values with hard fat or with softer fats comminuted in the frozen state. The losses from the sausages were lower than those of the fatty tissues alone.

Discussion

The fat loss on cooking a meat product may be considered to take place in two stages. Lipid must first be released from the fatty tissue in which it was at first contained and then it must escape from the meat product as a whole.

These experiments show that the first of these stages is strongly influenced by the softness of the fatty tissue and by the conditions under which it is comminuted.

The analysis of the pork fatty tissues confirms the well-known relationship between tissue softness as assessed by the hand and softness of the lipid in the classical chemical sense, but it also shows that both kinds of softness are inversely related to the content of non-lipid material or 'connective' tissue. That is to say, soft fats (of the same animal species) have higher connective tissue contents than hard fats. It is probable that the connective tissue content has a greater influence on cooking loss than the lipid softness. Thus, in tests on roasted beef, Anderson *et al.* (1971) found that the fatty acid composition of the lipids in the fatty tissue was unchanged by the cooking process and their conversion into drip, but they inferred from their data that different fatty tissues contributed in different proportions to the total drip: the seam fat which they considered had contributed most was also shown to be the hardest fat, in agreement with our results.

Van den Oord & Visser (1973) have recently published photomicrographs which demonstrate that even in a so-called 'emulsified' sausage mixture the fat is normally present as intact fatty tissue cells and that significant loss of lipid occurs only when some of the cells are broken. Thus the ease with which fat cells become broken is likely to be of major importance. The observation that softer fats contain more connective tissue—but not necessarily more collagen—suggests that their cell walls may be thicker than those of harder fats. Microscopic studies (to be fully reported later) confirm that this is the case.

Thus the very wide variations in our experimental results may be accounted for on the grounds that harder fats have weaker cell walls which are more easily broken than those of softer fats, leading to higher fat losses. The losses of all kinds of fatty tissue may be expected to increase with increased comminution, as has been observed. The large increases in fat loss caused by mincing or chopping the softer fatty tissues when they are frozen, but not when they are unfrozen, are probably due to mechanical damage occurring when the structure is rigid but not when it is more flaccid. The greatest effect occurred in those tissues which had the highest connective tissue and water contents: the

majority of the water is known to be associated with connective tissue and may be expected to produce such a rigidification on freezing.

When fat has been released from fatty tissue on heating some additional conditions seem to be operating to retain free lipid in the cooked mixture and to prevent it from appearing as a cooking loss. This retention may be enhanced by the presence of lean meat. Thus in the sausage experiments (Table 5), cooking loss from the sausage was in every case lower than that from the fatty tissue alone, both expressed on a fatty tissue basis. This result might of course be influenced by differences in cooking procedure, but when cooking conditions were the same, as in the values in Table 4, the fat loss from mixtures containing soft fatty tissue and 70% lean meat were lower than those from the fatty tissue alone, especially when the materials were chopped. It has long been considered that emulsification is an important factor in reducing fat losses from cooked meat products. Although it now seems probable that true emulsification is not of prime importance in this regard (Van der Oord & Visser, 1973) there might be some emulsification of lipid released from damaged fatty tissue cells, which could account for the observed effects. If this explanation is correct, the absence of any corresponding reduction in fat loss from hard fatty tissue in the presence of lean meat must be because hard fat emulsifies less readily than the softer. Schut (1968) has demonstrated that this is the case with mixtures of fatty tissue, water and casein.

It is particularly interesting that reductions in fat loss were observed in simple mixtures containing minced meat and minced soft fatty tissue, made with only the minimum mixing necessary to ensure homogeneity (Table 4). This effect has been observed a number of times in other experiments (Evans & Ranken, 1972) and therefore does not appear to be accidental. In these mixtures emulsification of fat by the lean meat before cooking must have ben negligible. It is possible that emulsification, or some other fat binding process, may occur during the course of heating and this is being further investigated.

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A national survey of muscle pH values in commercial pig carcasses

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Summary

Measurements of muscle pH at 45 min post mortem (pH_1) and after overnight cooling (pH_2) were taken in a total of 6015 commercial pig carcasses at sixteen bacon factories in Great Britain. Measurements were made in the M. longissimus in the area of the last rib and in the M. semimembranosus.

Differences between muscles in the overall distribution of pH_1 and pH_2 were negligible. The overall mean for pH_1 measured in the M. longissimus was 6.55 ± 0.32 (s.d.) and 5.7% of the values were below pH 6.0. The overall mean for pH_2 in this muscle was 5.81 ± 0.25 and 2.1% of the values were above pH 6.5. Wide differences were recorded between factories in the distribution of pH_1 values; means ranged from 6.98 to 6.20 and the percentage of values below pH 6.0 from 0.3 to 20.6.

pH was independent of muscle temperature and carcass weight. Low and positive correlations were recorded between pH measurements and backfat thickness.

Comparison of the overall results with those of a similar survey carried out in 1964 indicated that the distribution of pH values in commercial British pigs has changed very little in the last decade.

Introduction

Published estimates of genetic parameters for pale, soft, exudative muscle (PSE) suggest that the incidence of this condition will increase as a correlated response to selection for lean meat production (for example, Weniger, Steinhauf & Glodek, 1970; Jonsson, Jensen & Pedersen, 1972). The question of whether an important trend in this direction exists for British pigs commands much speculation among research workers but there are no recent reports of investigations designed to answer it.

PSE is associated with an abnormally rapid fall in muscle pH after slaughter and the proportion of low pH values at 45 min post mortem has been used as a guide to the incidence of the condition; pH 6·0 has tended to be adopted as the level below which

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carcasses are likely to exhibit PSE. A survey of pH values in commercial bacon weight carcasses was carried out by the British Food Manufacturing Industries Research Association (B.F.M.I.R.A.) in 1964 at eight factories in Great Britain (reported by Taylor, 1966). pH values taken in the M. semimembranosus at 35–45 min post mortem formed a single-peaked frequency distribution with a mean of 6·48 and a range of 5·4 to 7·6; 4·5% of the values were less than 6·0. The present report presents the results of a survey carried out to determine whether there has been a significant change in the distribution of pH values since the B.F.M.I.R.A. survey, and to provide a firm base-line for future studies.

Some concern has recently been expressed by the bacon industry over the frequency with which carcasses with dark, firm, dry muscle occur. This condition, which gives rise to 'glazy' bacon with poor keeping quality, is known to be associated with an abnormally small drop in muscle pH post mortem, and, thus, a high ultimate pH (measured after overnight cooling). The distribution of ultimate pH values in commercial British pigs was also examined in the survey reported.

Experimental

A sample of sixteen bacon factories was selected so as to be representative of the various slaughtering procedures used in Great Britain and to provide coverage of the main geographical regions. The size of kill at the selected factories ranged from 300 to 4500 pigs per week.

Measurements were taken on a total of 2810 carcasses at these factories in June/July 1972 and 3205 carcasses in May 1973; the number of carcasses measured per factory in both surveys ranged from 306 to 417.

pH was measured at 45 min post mortem (pH_1) and after overnight cooling (pH_2) both in the M. longissimus in the area of the head of the last rib (LD muscle), and in the M. semimembranosus (SM muscle). In the 1972 survey, a Radiometer pH meter Model 24, which has separate glass (G213C) and calomel (K4111) electrodes designed for direct insertion into muscle, was used. The meter was calibrated against a standard buffer of pH 6·5; it was recalibrated after measurements had been taken on batches of approximately twenty carcasses. The procedure for measuring pH was as follows. Both electrodes were inserted into the LD muscle and the pH reading noted; the glass electrode was then twice transferred to a new position in the muscle, the calomel electrode being left undisturbed, and the readings repeated. The mean of the three readings was then taken as the pH of the muscle being tested. Leaving the calomel electrode in the LD muscle, the same procedure was repeated in the SM muscle. In the 1973 survey, a combined probe (GK2321C) was used instead of separate glass and calomel electrodes. The mean of three readings was similarly taken as the pH of the muscle being tested. Muscle temperatures were recorded when the pH measurements

were taken. In the 1973 survey, backfat thickness, measured by introscope at 6·5 cm from the mid line at the head of the last rib (P_2) and carcass weight were also recorded.

Results

Differences between years

The results of the 1972 and 1973 surveys are compared in Table 1. There was less variation and a smaller proportion of carcasses with extreme values in the 1973 survey. Mean pH values also tended to be lower in the latter survey, the difference between years being significant for pH_2 measured in the LD muscle.

TABLE 1. Distribution of pH values: comparison of 1972 and 1973 surveys

		1972	1973	SE of difference	Level of significance
pH_1 LD muscle:	mean	6·58	6·53	0·051	NS
	s.d.	0·37	0·26		
	%<6·0	6·9	4·6		
SM muscle:	mean	6·59	6·58	0·044	NS
	s.d.	0·37	0·25		
	%<6·0	5·5	3·1		
pH_2 LD muscle:	mean	5·89	5·73	0·055	$P<0·05$
	s.d.	0·32	0·21		
	%>6·5*	3·4	0·7		
SM muscle:	mean	5·90	5·82	0·059	NS
	s.d.	0·32	0·23		
	%>6·5*	3·6	0·9		

* Since there is little experimental evidence to indicate the threshold pH_2 level above which dark, firm, dry muscle is likely to occur, the proportion of carcasses above the arbitrary level of 6·5 is given.

There were no important interactions involving years and the results presented below are for years pooled.

Differences between factories

The distribution of factory means is shown in Table 2. Most factories had pH_1 means within the range 6·4–6·7 and 8% or less carcasses with values below 6·0; the means obtained at Factories O and P were well below average. These two factories exhibited a marked muscle × factory interaction for the proportion of pH_1 values below 6·0: the difference between muscles for Factory O was 1·7% and for Factory P, 10·7%.

TABLE 2. Distribution of factory means

pH$_1$		pH$_2$	
LD muscle	SM muscle	LD muscle	SM muscle

pH$_1$ scale (left, top to bottom): 7·0, 6·9, 6·8, 6·7, 6·6, 6·5, 6·4, 6·3, 6·2, 6·1

pH$_2$ scale (right, top to bottom): 6·2, 6·1, 6·0, 5·9, 5·8, 5·7, 5·6, 5·5

pH$_1$ — LD muscle
- 7·0 — A(0·3)
- 6·9 — B(1·3)
- 6·7 — C(2·0) D(2·8)
- 6·6 — E(1·3) F(3·3) G(3·9) H(3·8) I(4·8)
- 6·5 — J(2·8) K(3·8) L(5·9) M(4·0)
- 6·4 — N(8·7)
- O(19·8)
- 6·2 — P(20·6)

pH$_1$ — SM muscle
- 7·0 — A(0·3)
- 6·9 — B(0·0)
- 6·8 — C(1·2) H(0·4)
- 6·7 — E(1·1)
- 6·6 — I(1·6) L(3·3) G(2·3) J(1·2) M(2·5) F(4·6) D(1·8) K(7·3)
- 6·4 — N(10·8)
- O(18·1) P(9·9)

pH$_2$ — LD muscle
- 6·1 — J(14·2)
- 6·0 — D(2·0) P(1·7) N(3·7)
- 5·9 — G(2·9) I(1·0) C(0·3)
- 5·8 — K(1·0) M(2·3) H(1·1) F(3·4) O(0·5) B(2·6) L(0·0)
- 5·7 — E(0·0)
- 5·6 — A(0·3)

pH$_2$ — SM muscle
- 6·1 — J(13·0) D(3·2)
- 6·0 — H(5·0) M(3·7) P(1·9) G(2·2) N(2·5)
- 5·9 — L(0·4) F(5·5)
- 5·8 — K(1·0) O(0·7) I(0·0) C(0·3) B(0·8)
- 5·7 — E(0·9)
- 5·6 — A(0·3)

The percentage of values < 6·0 and > 6·5 are given in brackets for pH$_1$ and pH$_2$ respectively.
Factories are denoted by letters from A to P.
Standard error of difference between factory means for: pH$_1$ LD muscle = 0·203; pH$_1$ SM muscle = 0·174; pH$_2$ LD muscle = 0·219; pH$_2$ SM muscle = 0·234.

With the exception of Factory J, there was little variation between factories in the proportion of pH$_2$ values above 6·5, all the percentages being within the range 0–5·5.

Differences between muscles

There were neglibile differences between muscles in the overall distribution of pH values (Table 1) and clearly the same conclusions about the national incidence of PSE would be drawn from pH measured in either one of these muscles. The ranking of factories in order of mean pH measurements was similar for both muscles (Table 2), but there were important differences for the proportion of extreme values as indicated by the muscle × factory interaction referred to above.

Inter-relationships of pH and other measurements

The correlations, pooled within factory and year, among pH and other measurements are shown in Table 3. The correlations between muscles for the same pH measurement accounted for less than 50% of the variation, indicating that pH measured in one muscle would not provide a good estimate of pH in the other muscle for an individual pig (the RSD for predicting pH_1 in the LD muscle from that in the SM muscle was 0·24, and the corresponding value for pH_2 was 0·17). A low and positive correlation was recorded between pH_1 and pH_2 measurements. pH measurements were independent of muscle temperature and carcass weight. Significant correlations were recorded between fat thickness and pH_1 but the degree relationship was very low indeed.

TABLE 3. Correlations (pooled within factor and year) between pH and other measurements

	pH_1 SM	pH_2 LD	pH_2 SM	Corresponding muscle temperature	Carcass† weight	P_2†
pH_1 LD	0·575***	0·189***	0·148***	−0·003	−0·021	0·041*
pH_1 SM		0·181***	0·198***	0·020	0·015	0·040*
pH_2 LD			0·688***	−0·011	0·001	0·039*
pH_2 SM				−0·012	0·025	0·056**

The means and pooled standard deviations of the other measurements were as follows. Muscle temperature (°C): pH_1 LD, 36·8 ± 1·23; pH_1 SM, 37·9 ± 0·53; pH_2 LD, 5·1 ± 1·01; pH_2 SM, 5·0 ± 0·73. Carcass weight (kg): 66·9 ± 3·7. P_2 (mm): 17·7 ± 3·4.

*$P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

† Based on the 1973 survey only.

Discussion

The percentage distribution of pH_1 values in the present survey is compared with that of the 1964 B.F.M.I.R.A. survey in Fig. 1 (both distributions are for the SM muscle). There was more variation in the present survey and the mean was 0·1 pH units higher;

the proportion of values below pH 6·0 was the same in both surveys. If the 1964 and 1972–3 samples are taken as representative of the national distributions of pH_1 values in these two periods, the results indicate that the distribution has changed very little in the last decade. The relative contributions of genetic and environmental factors to this result cannot be determined and it is conceivable that, if any genetic deterioration in pH_1 has occurred, it has been counterbalanced by an improvement in the conditions of transport or in abattoir environment.
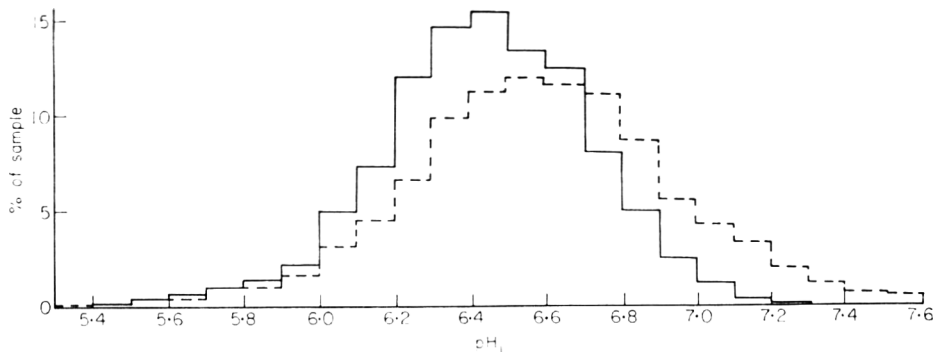
FIG. 1. Overall distributions of pH_1 values in the 1964 (——) and 1972–3 (-----) surveys (SM muscle).

The proportion of pigs carbon dioxide-stunned is one environmental factor which differed between the surveys, and which is known to influence the percentage of low pH_1 values. Bendall, Cuthbertson & Gatherum (1966), for example, found that carbon dioxide stunning reduced mean pH_1 by 0·15 units and increased the percentage of values below pH 6·0 by 3·7% in comparison with electric stunning in a sample of commercial British pigs. Two factories in the 1964 survey, accounting for 34% of the overall sample of carcasses, used carbon dioxide stunning while, in the present survey one factory, accounting for only 7·5% of the sample, used this method. It was not possible to obtain a breakdown of the results by factory for the 1964 survey and a within stunning method comparison could not be made. An approximate adjustment based on the results of Bendall *et al.* (1966) would be to increase the proportion of values below pH 6·0 by 1·5% in the present survey. This adjustment has only a marginal effect and would not obscure an important genetic change in pH distribution. The importance of other possible environmental differences cannot be assessed.

It is also evident that there has been little change in the distribution of pH_2 values during the last decade (Fig. 2).

The relationships among pH measurements found in the present survey are in general agreement with those reported elsewhere. The similarity of the overall distributions of pH_1 measured in the LD and SM muscles agrees with the findings of Bendall *et al.* (1966) and Taylor (1966). These workers also reported substantial variation between
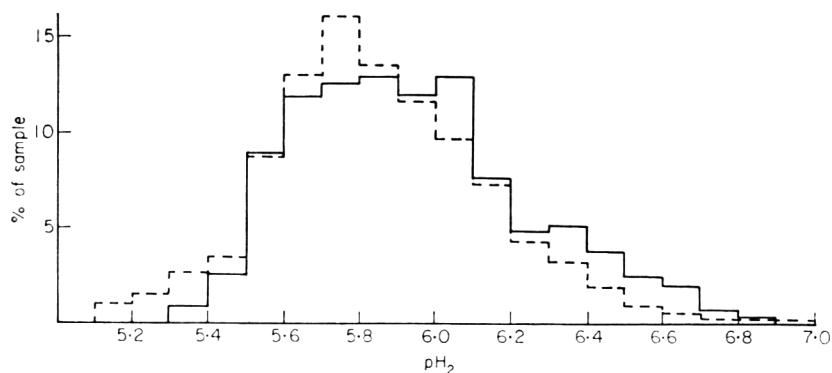
FIG. 2. Overall distributions of pH_2 values in the 1964 (——) and 1972–3 (-----) surveys (SM muscle).

muscles within individual pigs, supporting the contention that pH_1 measured in one muscle will be a poor predictor of pH_1 measured in another muscle; Wismer-Pedersen (1959) reported a correlation of 0·543 between pH_1 measured in the loin and gammon. The correlations of 0·04 to 0·06 recorded between pH measurements and backfat thickness accord with the estimates reported by Jensen, Craig & Robison (1967) and Weniger *et al.* (1970), and also with the estimate for British Combined Test pigs (Cook & Cuthbertson, 1972).

It is important to be aware of the main limitation of a survey of this type. This is that pH does not provide a precise indication of the incidence of muscle quality defects. The proportion of pH_1 values below 6·0 can be regarded only as a guide to the incidence of PSE—the results of Taylor (1966) and our own unpublished work with MLC Combined Test pigs suggest that this criterion overestimates the incidence of PSE. The use of pH_2 to indicate the incidence of dark firm dry muscle is equally imprecise. However, the present survey does not set out to provide an absolute estimate of the incidence of muscle quality defects but rather to compare the present level with that obtaining in 1964. Assuming that the relationship between pH and muscle quality is fairly constant, one may conclude that the level of muscle quality defects in commercial British pigs has not shown an important increase in the last decade.

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Spoilage and spoilage indicators in queen scallops
(*Chlamys opercularis*)
II. Effects of processing

A. B. THOMSON, H. K. DAVIS, J. C. EARLY AND J. R. BURT

Summary

Queen scallops were shucked and held as prepacked meats. Some of the prepacked meats were frozen and held frozen together with some shell on queens. At various times of storage, organoleptic descriptions of the meats were given along with determinations of TMA,* TVB, VRS, glycogen hypoxanthine, ribose fractions and optical density ratios before and after treatment with ion-exchange resin. Of these tests, only hypoxanthine showed any promise as a freshness indicator under all the conditions covered.

Introduction

When queen scallops are landed in the United Kingdom, they are either quick frozen with their shells on or processed more or less immediately. Processing involves shucking the meats, which is removal of the adductor muscle from the shell and the other organs, and then either sealing them in prepacks or quick freezing and packing them. It was decided to investigate the effects of all these types of treatments, together with that of double freezing, on certain factors which may have a value as indicators of deterioration.

No previous work has been done on the spoilage of queen scallops under conditions other than those of keeping them in shell in ice. Hiltz & Dyer (1973) have studied the storage of the adductor muscle of sea scallops (*Placopecten magellanicus*) at 5°C and also the effect of freezing and thawing on these meats.

Of the tests looked at by Hiltz & Dyer (1973), hypoxanthine showed the most promise as a quality index of frozen and thawed meats but they did not undertake a sufficiently comprehensive study to be able to make an adequate assessment of its potential.

Methods

Preparation of prepacks

Each pack contained approximately 160 g of shucked, washed, adductor muscle sealed in air. The meats were placed on an expanded polystyrene tray which was

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placed in an overwrap pouch made from a laminate of orientated polypropylene (60 gauge) and 0·025 mm polythene, and then sealed.

Organoleptic assessments and chemical analyses were carried out as described previously (Thomson *et al.*, 1974).

All frozen samples were thawed in still air for 3–4 hr before extracts were made.

Samples

The queen scallops used for this experiment were caught off the Cock of Arran on the west coast of Scotland and they all measured between 5 and 7 cm across the shell. Some were frozen immediately after washing with sea water and the remainder were stored in ice between layers of polythene to prevent the possibility of leaching. Four different storage conditions (or combination of storage conditions) were used as follows.

(*A*) On the second, sixth and ninth days batches were shucked, the meats packed and held at 4°C for various lengths of time before sampling.

(*B*) After 0, 1, 2, 3 and 4 days batches were frozen and kept at − 28°C for a few days. These were then thawed, shucked, packed and held at 4°C for various lengths of time before sampling.

(*C*) After 0, 1, 2, 3 and 4 days batches were frozen and kept at − 28°C for a few days. These were than thawed, shucked, packed, refrozen and held at − 28°C. Samples were taken after 7 weeks and the remaining packs were transferred to another cold store at − 13°C and sampled after a further 20 weeks.

(*D*) After 0, 2, 4, 6 and 8 days, batches were frozen (still in shells) and kept at − 28°C. Samples were taken after 7 weeks and the remaining shells were then transferred to another cold store at − 13°C and sampled after a further 20 weeks.

Results

Experiment A

Organoleptic. Cooked odours and flavours of the shucked meats after holding in pre-packs at 4°C showed the same overall pattern as they did for iced in shell queen scallops (Thomson *et al.*, 1974). The same stages going from very sweet to sour and then putrid were observed. The results showed that storage in prepacks leads to slightly faster deterioration in eating quality, but as the prepacks were held at 4°C this is to be expected; the limits of edibility were reached after 8 days total (2 or 6 in ice followed by 6 or 2 respectively at 4°C) compared with 8–10 days in ice all the time. Surprisingly the odours (raw) and textures (cooked) of these prepacked meats were not detectably

different from those of meats of comparable ages from whole queen scallops stored in ice.

Chemical. Glycogen values ranged from 1–3·3 g/100 g but showed no relation to time held in prepacks.

VRS values varied from 0·4 to 27·0 μEq per 5 ml press juice; there was an overall increase with time held but values were very scattered.

TMA values could not be obtained for all the samples because interference with some substance in the extract caused anomalous high readings. The results obtained show an increase with holding time, but it is impossible to give an assessment of the relation to time held.

Total ribose values fell with time held in prepacks and gave a good correspondence with days held at 4°C irrespective of time in ice before packing (Fig. 1a).

Barium soluble-alcohol soluble ribose tended to increase then decrease during torage with values ranging from 28 to 64 mg per 100 g.
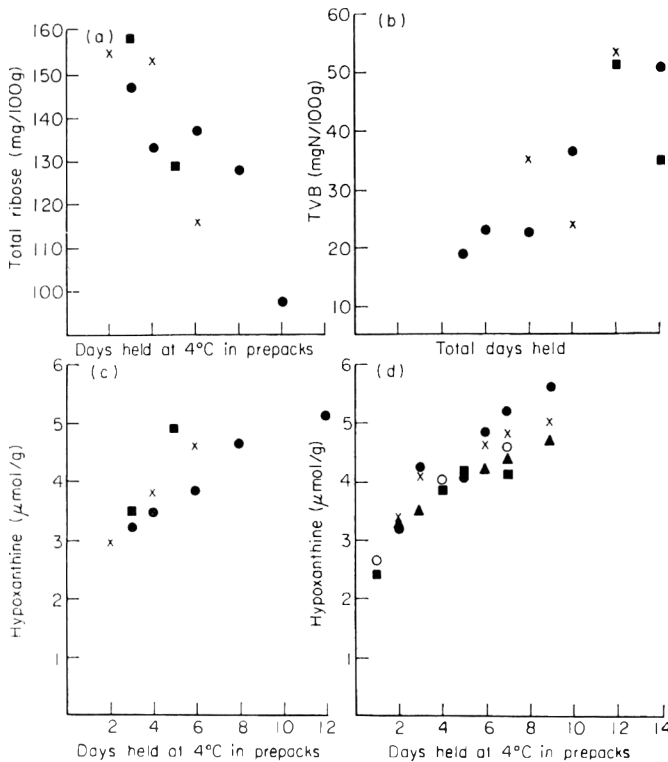
FIG. 1. The effect of storage history on (a) total ribose, (b) TVB and (c) and (d) hypoxanthine concentrations in prepacked queen scallop meats held at 4°C. (a), (b) and (c): ● = 2 days; × = 6 days; ■ = 9 days in ice in shell before shucking and packing. (d): ● = 0 days; × = 1 day; ▲ = 2 days; ■ = 3 days; ○ = 4 days, in ice in shell before freezing. These were subsequently thawed, shucked, packed and held at 4°C.

Ribose ratios also increased then decreased, ranging from 29 to 46%.

TVB values increased with total time held from capture (Fig. 1b). The results agree fairly well with those obtained for iced in shell queen scallops (Thomson *et al.*, 1974) being scattered about the line of increase. Hypoxanthine concentration increases on holding the shucked meats in prepacks and gives a good indication of time held in prepacks, irrespective of icing history before shucking (Fig. 1c).

The optical density ratio at 248 nm increased on holding as prepacks, ranging from 79 to 87%. The very small increase obtained over 12 days in prepack (8%) would limit the potential of this test as a quality index for this product.

Experiment B

Organoleptic. Cooked flavours showed a decreasing degree of sweetness throughout the storage time covered but with a slight sweetness still detectable up to the point when the taste panel refused to taste. Raw odours and cooked textures showed similar sequences of changes to those found in Experiment A. Limits of edibility for the 0, 1 and 2 day pre-iced samples were reached after a further 6 to 7 days at 4°C, for the 3 day iced sample after 4–5 days and for the 4 day iced sample before a further 4 days had elapsed.

Chemical. Glycogen values ranged from zero to 4 g/100 g showing an overall decrease with time but no consistent relationship with days held as prepacks.

VRS values ranged from 0·4 to 8·0 μEq per 5 ml press juice but there was no consistent trend when plotted against days held as prepacks.

TMA values were not obtained from all the extracts because of interference but the results obtained indicate there is no increase on holding the meats in prepacks.

Total ribose values ranged from 200 mg/100 g down to 110 mg/100 g, but there is too great a variation from day to day for this to be a useful quality index.

Barium soluble-alcohol soluble ribose values showed a large variation from day to day as does the ribose ratio thus ruling both values out as possible quality indices.

TVB values ranged from 8 to 30 mg/100 g with no correlation with days held as prepacks.

Hypoxanthine concentration shows a fairly good relationship with days held as prepacks (Fig. 1d). The values to which the individual treatments eventually rose decreased with longer holding times of the queen scallops in ice before shucking and prepacking.

Optical density ratios at 248 nm ranged from 76 to 85% with a tendency to increase on holding as prepack. This increase, however, is too erratic to be a good indicator of quality for this product.

Experiment C

Organoleptic. Flavour and texture were not analysed after 7 weeks at $-28°C$ but the odours of the raw and cooked meats did not change discernibly during this time. A

further 20 weeks at $-13°C$ however did produce a slight deterioration in flavour and odour but no difference in texture.

Chemical. Glycogen values of prepacked meats held in cold storage showed a decrease, but considering the variation found from pack to pack in previous experiments these results should be interpreted with caution.

VRS and TMA concentrations were not determined on all of these samples so it is impossible to draw any conclusions about their possible use as indices of quality change during frozen storage.

Total ribose values decreased on freezing and holding in cold storage, but the values obtained after 7 and 20 weeks were much the same. All values fell between 180 mg/100 g and 230 mg/100 g but showed no relation to prefreezing iced history.

Barium soluble-alcohol soluble ribose values showed a marked increase on freezing and cold storage but again there was no relation to prefreezing iced history. Some of the values obtained (up to 107 mg/100 g) were the highest in any of the experiments so far.

Ribose ratios showed an increase on freezing and cold storage but again there was no correlation with prefreezing history.

None of the ribose indices could be used to assess frozen storage history unless pre-freezing history was known.

TVB values showed no increase after holding the prepacked meats at $-28°C$ for 7 weeks but there was an increase after a further 20 weeks at $-13°C$, when all the values seem to be the same (approx. 20 mg/100 g), irrespective of prefreezing history, suggesting that a maximum value has been obtained for these storage conditions. The concentration of TVB might therefore be used to detect poor cold storage but only over a short length of time.

Hypoxanthine concentrations in the samples thawed after 7 weeks at $-28°C$ were significantly higher than they were before freezing, and those in samples thawed after a further 20 weeks at $-13°C$, with one exception, were higher still (Fig. 2a). On the whole, the fresher (before freezing) samples showed the biggest increase as a result of the processes involved (freezing, thawing, shucking, packing, refreezing and thawing again) in this experiment but the effects of storage in ice are still discernible in the two sets of hypoxanthine values from the frozen end-products.

Optical density ratios at 248 nm show an increase after 7 weeks and after a further 20 weeks in cold storage. After 7 weeks at $-28°C$ the effects of prefreezing storage are still discernible but after a further 20 weeks at $-13°C$, all values lie between 80 and 82% irrespective of prefreezing history (Fig. 2b), suggesting that a maximum has been reached for these storage conditions.

Experiment D

Organoleptic. Odours of raw and cooked meats showed a slight deterioration after 7 weeks at $-28°C$ and a further deterioration after another 20 weeks at $-13°C$. Cooked
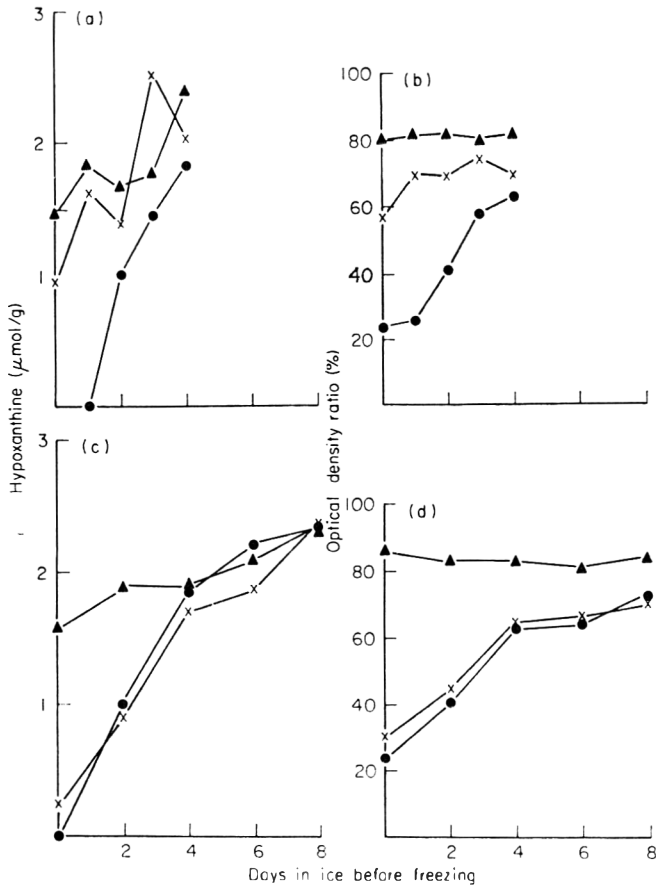
FIG. 2. The effect of cold storage on the production of hypoxanthine in (a) frozen prepacked meats and (c) frozen shell on queen scallops; and the optical density ratio at 248 nm of extracts prepared from (b) frozen prepacked meats and (d) frozen shell on queen scallops. ●——● = unfrozen material, ×——× = frozen and held at −28°C for 7 weeks, ▲——▲ = frozen and held at −28°C for 7 weeks followed by a further 20 weeks at −13°C.

flavours, however, showed no difference after 7 weeks at −28°C but a definite deterioration after another 20 weeks at −13°C. Texture of the cooked meats remained the same throughout the cold storage period covered.

Chemical. Glycogen values showed a great deal of variation within individual treatments but with no consistent trend when plotted against time held in cold storage.

VRS and TMA values were not estimated on all of the samples covered in this experiment.

Total ribose values tended to increase within any treatment on holding in cold storage, but the results obtained ranged from 170 to 270 mg/100 g and showed no

correlation with prefreezing iced history. Barium soluble-alcohol soluble ribose values increased on holding in cold storage, irrespective of prefreezing history, but the final values reached showed no correlation with prefreezing history. Some of the values were amongst the highest obtained (approx. 100 mg/100 g).

Ribose ratios increased during cold storage but again the correlation with days in ice before freezing was very poor after cold storage thus ruling out this value as a possible index of cold storage in this product.

TVB values did not increase during cold storage at $-28°C$ for 7 weeks (if anything there was a slight decrease). However, after a further 20 weeks at $-13°C$ there was a large increase and the values obtained (between 24 and 49 mg/100 g) were amongst the highest obtained and corresponded to a storage time of greater than 9 days in ice unfrozen (Thomson *et al.*, 1974). The increase obtained on holding in cold storage for a total of 27 weeks corresponds to the increase occurring during approximately 6 days in ice.

Hypoxanthine concentrations in the samples thawed after 7 weeks storage at $-28°C$ were very little different in most instances from their levels before freezing (Fig. 2c). However, the levels present in the samples thawed after a further 20 weeks at $-13°C$ showed that significant production of hypoxanthine had occurred in the 1 and 2 days in ice (before freezing) samples while the levels in the staler samples were essentially the same as those in the corresponding unfrozen and 7 weeks frozen storage ones. At each sampling time prefreezing iced history is still reflected in the values obtained although it would appear that prolonged frozen storage has tended to reduce the differences between the fresher and staler (before freezing) samples.

Optical density ratios at 248 nm showed a very slight increase during 7 weeks at $-28°C$ but after a further 20 weeks at $-13°C$, there was a large increase. There was still a gradation with prefreezing history after 7 weeks (Fig. 2d) but after a further 20 weeks, all values were approximately the same irrespective of iced history before freezing. These values (81–86%) were slightly higher than those obtained for comparably stored prepacked meats.

Discussion

Of the various chemical tests investigated, it appears that only hypoxanthine concentration shows potential as a possible quality test for queen scallops under each of the storage conditions covered in these experiments.

A comparison of prepacked and stored meats which were frozen and thawed before packing (Fig. 1d) with meats which had been held in ice only before packing (Fig. 1c) shows that there is little difference between the two patterns obtained. In each case the effects of holding at 4°C in prepacks swamp any differences which may have been present due to initial freshness. One interesting feature exhibited by the data in Fig. 1d, though, is that the hypoxanthine concentration reached after a particular time at 4°C is inversely related to the length of time in ice before freezing and processing. This

is in agreement with the results of Hiltz & Dyer (1973) who found that freezing and thawing sea scallops in the prerigor state increased the enzymatic breakdown of nucleotides to a greater extent than it did for postrigor frozen specimens.

In comparison with iced in shell specimens (Thomson *et al.*, 1974) prepacked meats not only produce hypoxanthine at a higher rate—a consequence of holding at a higher temperature—but the maximum levels obtained were higher. This phenomenon has also been observed in prepacked cod fillets (Stroud & Burt, 1973) where hypoxanthine levels of 8 to 10 μmol/g have been attained. Such values are about 50% in excess of the available potential in terms of nucleotidic precursors and presumably arise from *de novo* purine synthesis by the spoilage microflora.

Correlations of organoleptic quality with hypoxanthine concentration could not readily be obtained since a scoring system and a trained panel of the Shewan *et al.* (1953) type do not exist for queen scallops. However, a rough comparison, using the limits of edibility, could be obtained and this showed some disparity between the different types of material. The limits of edibility corresponded to hypoxanthine concentrations of 2·5–2·8 μmol/g in whole iced queen scallops, of 3·5–4·0 μmol/g in prepacked meats which had been ice stored whole before shucking and of 4·0–5·0 μmol/g in prepacked meats which had been removed after freezing and thawing whole, ice stored queen scallops.

Interpretation of the results obtained from the frozen storage experiments (Fig. 2a, c) is somewhat more difficult. Where the intervening cold storage has been for a short time at a low temperature freezing and thawing lead to little if any change in hypoxanthine concentration (Fig. 2c). However, a longer period at a higher temperature leads to significant increases in concentration in the case of samples held for up to 2 days in ice in the shell before freezing. The fact that similar increases do not occur in staler specimens may be due to the fact that the animals still appeared to be 'alive' after 1 to 2 days in ice and their meats would still be essentially in the prerigor state when processed. This is again in accordance with the findings that enzymatic activity is higher in pre- than post-rigor muscle.

Double freezing and thawing, on the other hand (Fig. 2a), appears to stimulate more change in hypoxanthine concentration than the single cycle does. This may be due in part to the proportionately longer times the samples will have experienced above zero temperatures and also in part to the disruptive effects of freezing and thawing on cellular structures. The samples here differ from those of experiment D in that prolonged periods were available after thawing (albeit in the refrozen state) for the cell contents to react. The higher activities of fresher samples can still be discerned as can (with the exception of one anomalous result) the effects of prolonged cold storage.

It is evident from these results that hypoxanthine is a useful indicator of quality. The levels in frozen products reflect prefreezing history and also the small amounts of deterioration which result from freezing and thawing and from holding for long periods in frozen storage.

Levels found in unfrozen prepacks would need to be interpreted with caution, however, since they appear to be higher than the change in storage conditions would lead one to expect.

Of the other chemical tests investigated, only TVB and the optical density ratio at 248 nm before and after resin treatment show any value as quality indicators for queen scallops and then only in some cases. However, the power of these tests can be increased if more than one is carried out on any one sample. In particular, the value of running a sensory evaluation with a chemical test, such as hypoxanthine, can be readily appreciated since each test responds differently to changes in holding conditions.

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Fish muscle in the frozen state: time dependence of its microwave dielectric properties

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Summary

Microwave attenuation properties of frozen fish muscle exhibit a time dependence, falling in value over periods of 1–3 weeks. This reduction of attenuation is due to gradual accretion of ice in the frozen system at the expense of unfrozen water. More than one exponential component is observed in the overall change and at least two are seen. These are both thermally activated and the activation energies for the characteristic time constants lie in the range 32–49 kJ mol⁻¹. This is similar in value to activation energies for diffusion through dehydrated and frozen systems (after recalculation of the latter published data). An explanation is offered which takes this into account.

Introduction

Some interest centres around the problem of the behaviour of water in frozen foodstuffs. It is well known that basically there exists in these materials at all sub-zero temperatures an amount of unfrozen water (Love, 1966). At very low temperatures this is termed unfreezable since there appears to be a fixed fraction of the total water which will not change phase, presumably due to energetically or entropically preferred states bound to the protein.

Variations in the amount of so-called 'liquid' water at temperatures in the range $-30°C$ to $0°C$ affects considerably many physical properties of the material. Apart from the rheological properties, others such as thermodynamic (specific heat, enthalpy, e.g. Riedel, 1956), paramagnetic (nuclear magnetic resonance, e.g. Sussman & Chin, 1966) and electrical (d.c. conductivity, complex dielectric permittivity, Bengtsson *et al.* 1963) all have very strong relationships to the amount of unfrozen water. The measurement of rates of diffusion of water in frozen fish muscle is also markedly dependent on the water available for diffusion (Storey, 1970). All these properties can be used as a measure of the state of water in the material. With some techniques judicious choice of experimental conditions can make the measurement of a physical property specific to one or the other state of water in the system. The method chosen for this work has been one related to the electromagnetic property of complex dielectric permittivity.

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In respect of the states of water in frozen tissues we must make certain generalizations. In this work 'unfreezable' water is considered to be 'bound' but it is borne in mind that the binding is merely one of a degree stronger than the 'freezable' water and that to some extent all the unfrozen water present is bound. Probably the only water in the system which exists in a clearly defined state or phase is the ice. The rest is to varying extents under the influence of polar forces both from sites on the protein matrix and from ions in solution. It is under the influence of these forces that the various states of binding are produced and the result is observed of the continued existence of some 'liquid' states of water at temperatures below the normal freezing point. This is discussed extensively in Fennema, Powrie & Marth (1973).

As stated above, it is possible to choose the measurement conditions with certain techniques, to make them specific, or partly so, to one particular phase or range of states of water. Complex permittivity measurements made at suitably high frequencies are specific to unfrozen water. Briefly, the reason for this is that the dipolar relaxation frequencies, ν_c, of the three phases of water present in protein/water mixtures (three, loosely speaking) are of very different orders of magnitude. In the temperature range $-30°C$ to $0°C$ the ice dipoles relax in the low frequency range 4–100 kHz, the 'bound' water in the range 10 MHz–1 GHz and the 'liquid' water between 100 MHz and 10 GHz. It is not possible to be more categorical since the degree of binding and rotational hindering has a very great effect on the actual relaxation time or distribution of times.

The effect of relaxation on the dielectric relative permittivity is to make it dispersive and thus introduce a loss factor which is frequency dependent. Thus a complex permittivity obtains $\epsilon_r{}^* = \epsilon_r' - i\epsilon_r''$. The loss factor, ϵ_r'', is a maximum at the relaxation frequency or effective relaxation frequency viz. the inverse of the relaxation time. For this complex permittivity, $\epsilon_r{}^*$, to be dominantly affected by liquid water properties, the measurement frequency must lie somewhere around or preferably above the middle of the relaxation band. This places it in the microwave region. There will still be some contribution from the bound water but the ice present will contribute virtually nothing to the loss factor.

The type of measurement made will also be important. Obviously if the whole dielectric relaxation is to be studied then measurements must in some way be made over virtually the whole spectrum of both parameters ϵ_r' and ϵ_r''. Alternatively, if only changes in state are to be observed the absolute determination of both ϵ_r' and ϵ_r'', which necessarily introduces complications, need not be made. It would be sufficient in many cases to measure some property which depends on these parameters. Such a property, easily measured, is the intrinsic attenuation α of a dielectric material. As a function of the complex dielectric permittivity it may be expressed thus,

$$\alpha = \frac{54 \cdot 6}{\lambda_0} \left\{ \frac{\epsilon_r'}{2} \left[(1 + \tan^2 \delta)^{1/2} - 1 \right] \right\}^{1/2} \tag{1}$$

where α is in decibels per metre, $\tan \delta$ is the ratio ϵ_r''/ϵ_r' and λ_0 is the free space wavelength of the measurement frequency. This property tells us nothing about relaxation

times which themselves could indicate the degree of binding or structuring of the water. It is merely an indication of the amount of water present in a given state. State in this sense means having a particular dipolar relaxation frequency, a particular degree of co-operation with surrounding molecules and a particular ordering or structuring. Thus changes in α can be an indication of changes in any or all of these things. However, the most significant effect on α will occur from those changes of phase which result in actual freezing for then the ice formed no longer contributes to the total attenuation and we can roughly ascertain, by subtraction, the amount of ice so formed.

These experiments, therefore, began with the intention of merely relating α, of frozen fish muscle, to temperature. From the outset, however, it became clear that this was no simple relationship since this is not a unique measurement at any given temperature. It is evident, for example, that because of polarization effects, the value of α observed varied, depending on the orientation of the muscle fibres relative to the microwave electric field. In addition α and $\epsilon_r{}^*$ were found to change on storage of the frozen fish muscle. The reasons for this were not clear but bearing in mind the fact that a similar effect had been reported in the properties of ice and frozen water emulsions (Le Petit, 1972) it was decided to investigate this phenomenon further.

Materials and methods

Values for $\epsilon_r{}'$ and $\epsilon_r{}''$ of frozen cod muscle were initially determined using the equipment built by Risman & Bengtsson (1971) and described elsewhere. Those measurements were made at $-30°C$ and at a frequency of 915 MHz. The muscle samples were prepared both by mincing at just above $0°C$ and by taking samples from frozen flesh using a cork borer.

The intrinsic attenuation α at 9·37 GHz (x-band) was determined by filling pieces of x-band waveguide with whole fish flesh. The samples so formed were terminated in tapered PTFE plugs to minimize the effects of standing waves within the sample and the measuring system. Such standing waves can be a source of great error if not limited. Since the absolute value of attenuation was not often required, the measurements were made in arbitrary units using an automatic device (MESL 810; automatic attenuation meter). Such measurements can of course be converted easily to units of dB m^{-1} if required by a simple calibration procedure.

The temperature of the sample was measured by inserting a copper/constantan thermocouple through the narrow wall of the waveguide into the centre of the sample. In this position and with such an orientation relative to the microwave fields in the waveguide the interference with those fields is minimal. Both temperature and attenuation were recorded at regular intervals on paper tape using a data logging system. In this way regular measurements were performed over periods of up to 3 weeks.

Temperature was controlled by complete immersion of the leak-proof waveguide

system in a refrigerated bath containing ethylene glycol. This gave a temperature control to better than $\pm 0.25°C$.

Because the automatic attenuator had some slight sensitivity to temperature the whole experiment was conducted in a thermostatically controlled room. In this way the overall precision of the measurement was improved and very small changes in α could be observed. It was assumed that no drying had occurred since the samples were contained within an airtight enclosure. This was justified by the absence both of any weight change during the experiment and of ice formation inside the small unfilled sections of waveguide.

Errors

Permittivity and loss measurements at 915 MHz had an estimated error of $\pm 5\%$ for ϵ_r' and $\pm 10\%$ for ϵ_r''. However, the precision for a given sample was very much better being $\pm 1\%$ for ϵ_r' and ϵ_r''.

The attenuation measurements made with the attenuation meter had an accuracy of ± 0.1 dB but again for continuous measurement of the same sample the equipment could resolve much smaller changes, e.g. < 0.01 dB. After temperature fluctuations were taken into account the precision was better than ± 0.03 dB.

The temperature itself, measured with an electronic thermometer with a copper/constantan thermocouple, was accurate to $\pm 0.25°C$.

Results and discussion

Permittivity measurements at $-30°C$ and 915 MHz

From the preliminary measurements on frozen cod at 915 MHz it was clear that even while holding the samples at a given temperature, the loss factor changed considerably (Table 1). Some, but by no means all, of this change could be attributed to temperature equilibration. Although slight numerical changes in ϵ_r' were observed, these were not statistically significant. Consequently changes in α reflect largely the changes in ϵ''. For low loss materials equation (1) reduces to

$$\alpha = \frac{27\cdot3}{\lambda_0} \cdot \sqrt{\epsilon_r'} \tan\delta$$

or

$$\alpha = \frac{27\cdot3}{\lambda_0} \frac{\epsilon_r''}{\sqrt{\epsilon_r'}}.$$

ϵ_r' should not be an important component of α at high frequencies for a number of reasons.

(1) At the high frequency end of a dispersion the changes in ϵ_r'' are great for small changes in ϵ_r' and anything which affects ϵ_r' slightly such as changes in ν_c or concentration of relaxing species will considerably affect ϵ_r''.

(2) Also at high frequencies the values of ϵ_r for ice, water and protein are not dissimilar, therefore changes in any of these components affect ϵ_r' only slightly. On the other hand, ϵ_r'' can be affected greatly because the various components at this frequency are very different.

(3) α has only an inverse, square root dependence on ϵ_r' meaning that small changes in ϵ_r' are halved in α.

TABLE 1. Changes in values of complex permittivity of frozen cod muscle at $-30°C$ over a period of 16 hr, measured at 915 MHz

	No. of samples	Mean ϵ_r'	Standard deviation	Mean ϵ_r''	Standard deviation	$\Delta\epsilon_r'$	$\Delta\epsilon_r''$
Initial value	6	3·7	0·16	0·340	0·018		
After 16 hr	6	3·59	0·21	0·268	0·022	0·11	0·072
Initial value	6	3·79	0·18	0·302	0·040		
After 16 hr	6	3·78	0·17	0·214	0·012	0·01	0·088

Attenuation measurements at 9·47 GHz

The recordings of attenuation and temperature were made typically every 30 min. It quickly became clear that it was not necessary to record the temperature; merely to monitor it was sufficient. The course of a typical change in attenuation with time is shown in Fig. 1. This example was for codling at $-15·4°C$. Similar results were also obtained for haddock muscle at other temperatures. The overall change corresponds to approximately 10% of the total attenuation of each sample. The asymptotic values were determined by the method of Mangelsdorf (1959) from the hand smoothed data and in this way the values for the time constants of the decay were extracted. As will be seen in Fig. 2 more than one single exponential decay is involved the longest, τ_2, being the more easily determined. The determination of the value of τ_2 was carried out at various temperatures and combining all the results an Arrhenius plot was made as in Fig. 3. The justification for combining the results in different species is that within the experimental errors noted no differences could be observed. The results shown are due to properties of water in gels, not to anything specific to fish. As can be seen the decay is thermally activated, τ_2 having an activation energy of $32·7 \pm 5·5$ kJ mol^{-1}. The value of τ_1 is less easily determined and is considerably more in error. However, by graphical means this was attempted and an activation energy of $49·5 \pm 10·8$ kJ mol^{-1} was obtained.

It is relevant to note that Jason (1958) has measured activation energies for diffusion in frozen cod muscle of $36·9 \pm 5$ and $30·1 \pm 0·8$ kJ mol^{-1}. Therein perhaps lies part of the explanation of the phenomenon observed here.
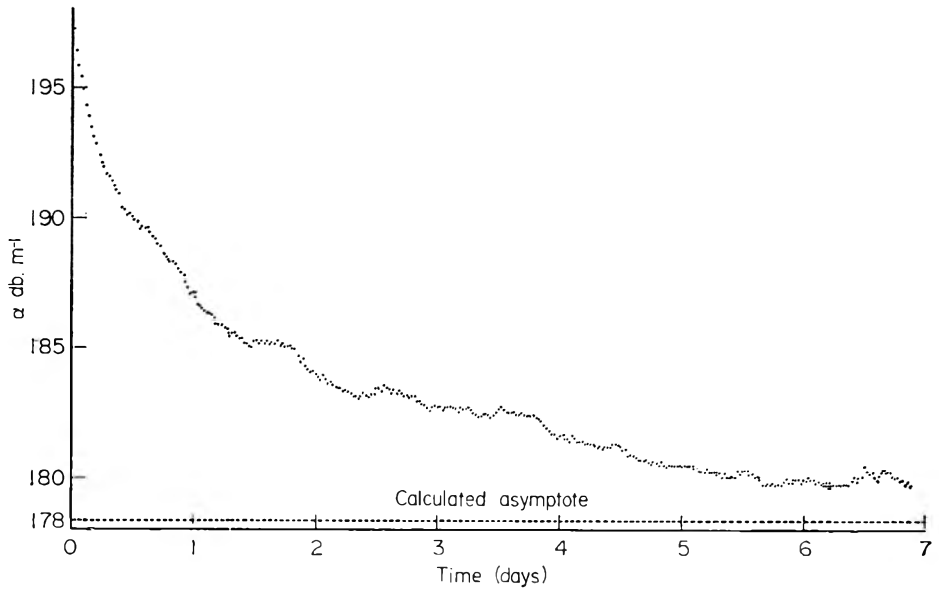
FIG. 1. Change with time of microwave attenuation per unit length at 9·37 GHz and −15·4°C of cod muscle post rigor; muscle fibres arranged along direction of propagation as far as possible.
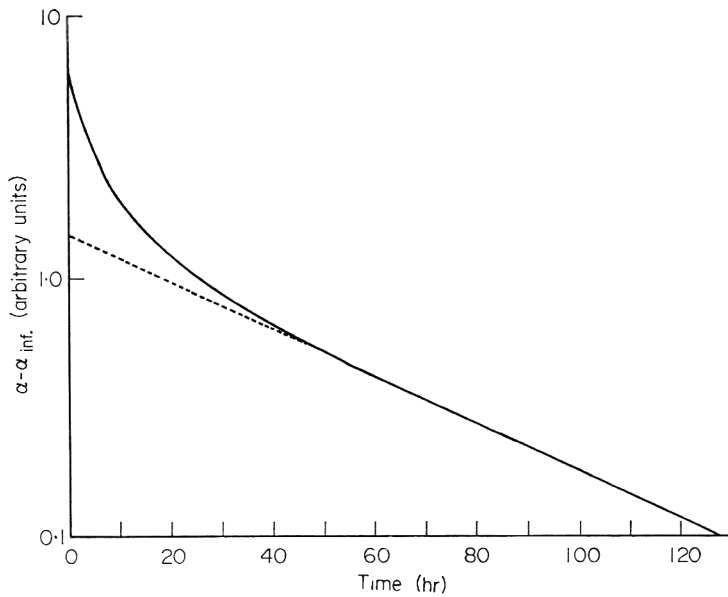

FIG. 2. Difference between actual attenuation and final asymptotic value as calculated plotted on a log scale versus time. The presence of more than one exponential is clearly seen. Haddock, −8·0°C.
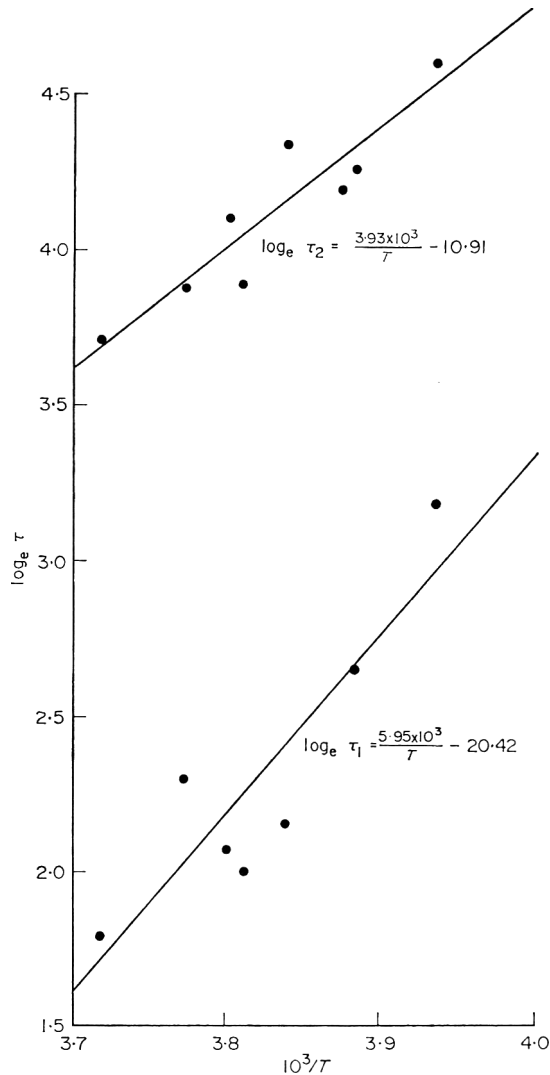
FIG. 3. Arrhenius plot of τ_1 and τ_2. Gradients yield for τ_1, $\Delta E_1 = 49 \cdot 5 \pm 10 \cdot 8$ kJ mol^{-1} and for τ_2 $\Delta E_2 = 32 \cdot 7 \pm 5 \cdot 5$ kJ mol^{-1}. Codling, $-15 \cdot 4$°C.

The fact that changes occurred at low temperatures over such a long period of time (by extrapolation $\tau_2 = 150$ hr at -30°C) and of such a magnitude, being some 10% of the initial values, might invalidate some other measurements made to determine the amount of unfreezable water. It seems from the work described here that if it is accepted that α is proportional to the amount of water unfrozen then a sample measured immediately after freezing has not had sufficient time to equilibrate. It might be expected that the ice crystals formed initially continue to grow by the slow accretion of randomly

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diffusing water molecules and the speed of this process when most of the 'liquid' water has been frozen, will be determined by the rate of diffusion through the more tightly bound water or whatever phase is relevant. Thus the two activation energies measured should be related if not identical to those of diffusion coefficients studied by Jason (1958) in drying muscle and Storey (1970) in frozen muscle. Certainly Storey's (1970) work should have measured the same property as Jason's though it must be pointed out here that a basic assumption made by Storey is probably wrong. In measuring diffusion coefficients he assumed that diffusion took place only through the liquid like states of the water present. Since at temperatures above zero during drying Jason showed the diffusion also to take place through the hydration layers of the protein it seems logical to assume that this also would occur at sub-zero temperatures. Freezing could be likened to drying in as much as water is removed from the diffusion space. By correcting the available area for diffusion by allowing only for unfrozen but freezable water Storey obtained anomalous values for the diffusion coefficient at the higher temperatures. If, however, the total unfrozen water is used in this correction, these anomalies disappear and as the temperature is lowered and more water is 'removed' the corrected diffusion coefficient approaches Jason's values for D_2. This is all shown in Table 2.

TABLE 2. Storey's (1970) data recalculated assuming that the total unfrozen water is available for diffusion

Temp. (°C)	Measured diffn coeff. (A) (cm² sec⁻¹ × 10⁸)	Fraction of freezable water remaining (B)	Fraction of unfrozen water remaining (C)	Corrected diffn coeff. (cm² sec⁻¹ × 10⁷)		Extrapolated diffn coeff. through monolayer after Jason (1958) (cm²sec⁻¹ × 10⁷)
				(A/B)	(A/C)	
− 5	7·0	0·166	0·233	4·22	3·00	1·260
−10	3·8	0·084	0·157	4·54	2·42	0·881
−15	1·95	0·050	0·126	3·90	1·55	0·636
−20	1·0	0·033	0·110	3·07	0·909	0·433
−25	0·5	0·022	0·101	2·34	0·495	0·320
−30	0·25	0·019	0·097	1·37	0·258	0·220

Values in italics represent data used or collected by Storey (1970).

It could be proposed, therefore, that we are observing the slow equilibration of water states within the system. Such equilibration takes the form of gradual accretion of ice to its equilibrium point, a process which must be diffusion controlled, especially at low temperatures where low concentration and mobility obtain. The diffusion is taking place through relatively tightly bound unfrozen water and because of the two distinct

time constants the temptation exists to relate these to the two diffusion coefficients, D_1 and D_2 in Jason's (1958) work on drying. There, D_2 was shown to correspond to diffusion through the actual monolayer on the protein, the other, D_1, through less structured water. The latter reaches equilibrium more quickly. The end product of a diffusion chain in the frozen system is ice and could probably occur when the diffusing water molecule actually reaches an ice crystal or finds a suitable site on which to crystallize.

The problem is to see how previously discussed data on diffusion fits into this picture and what geometric model is most suitable for its description. Let us consider a plane sheet of which the lateral dimensions are very much greater than the thickness. Crank (1956) has shown that the total amount of diffusing material, M_t, which has already entered the sheet (or left it) at time t, given that at all times the surface concentration is constant, is given by

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left(-D(2n+1)^2 \pi^2 t / 4l^2\right) \tag{2}$$

where l is the half thickness of the sheet, M_∞ is the total amount taken up by the sheet at infinite time and D is the diffusion coefficient.

For a cylinder, the corresponding equation is

$$\frac{M_t}{M_\infty} = 1 - \sum_{n-1}^{\infty} \frac{4}{a^2 \alpha_n^2} \exp\left(-D\alpha_n^2 t\right) \tag{3}$$

where a is the radius of the cylinder and α_n are the roots of the Bessel function of zeroth order.

$$J_0(a\alpha_n) = 0.$$

The case of a sphere is similar and

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n-1}^{\infty} \frac{1}{n^2} \exp\left(-Dn^2 \pi^2 t / r^2\right) \tag{4}$$

where r is the radius of the sphere.

Where $n > 0$ for equation (2) and $n > 1$ for equations (3) and (4), the exponential terms become very small and leave us with the following

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \exp\left(-\frac{\pi^2}{4} \frac{Dt}{l^2}\right) \quad \text{(plane)} \tag{5}$$

$$\frac{Mt}{M_\infty} = 1 - 0 \cdot 6917 \exp\left(-5 \cdot 783 \frac{Dt}{a^2}\right) \quad \text{(cylinder)} \tag{6}$$

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \exp\left(-\pi^2 \frac{Dt}{r^2}\right) \quad \text{(sphere)}. \tag{7}$$

With these three basic shapes in mind, we must try and decide which relates most closely to this problem. Let us rewrite the various exponential terms as

$$\exp\left(-t/\tau\right)$$

where for the plane $\tau = 4l^2/\pi^2 D$, for the cylinder $\tau = a^2/5\cdot783D$ and for the sphere $\tau = r^2/\pi^2 D$.

Thus for a given τ with the shape unknown, the characteristic diffusion lengths $r : a : l$ are in the ratio $2 : 1\cdot531 : 1$. We can say with some certainty that the pertinent shape lies somewhere between a sheet and a sphere so these correspond to upper and lower bounds for the diffusion length i.e. the distance on average that a water molecule travels before crystallizing. It might be considered that the muscle topography dictated a more cylindrical form and conveniently it is seen that this lies almost halfway between the two bounds.

However, using various diffusion data as proposed creates some unexpected difficulties. From Table 3 we see that if Jason's (1958) data on D_1 and D_2 are combined with τ_1 and τ_2 respectively as measured here, extraordinarily large values of diffusion length are obtained typically 2–4 mm. The length, l, in this case has been calculated for the plane sheet. Even if we assume that in this case D_2 refers to the fast change, τ_1, the length calculated only reduces by a factor $\sqrt{D_2/D_1} \simeq 4$ becoming some $0\cdot5 \sim 1$ mm. An examination of Love's (1966) work on ice in fish muscle shows that a typical dimension of ice crystals is $\sim 0\cdot2$ mm which at least is of the same order as this last diffusion length calculated from D_2. That leaves us with the longer term changes to explain and here the procedure is clear. If we insert the typical length of $0\cdot2$ mm into the diffusion equations and calculate a corresponding diffusion coefficient, D_3, we find, as Table 4 shows, that D_3 lies somewhere between that of Jason's monolayer diffusion and that of ice itself. Consequently, it is proposed that what is observed is either the diffusion of liquid water through clathrate type ice structures to a point on the surface where crystallization can take place, or diffusion through the surface states of the ice crystals themselves.

TABLE 3. Diffusion lengths l for plane sheet calculated from Jason's (1958) data and the time constants found in this work

Temperature (K)	D_1 ($\times 10^7$)	D_2 ($\times 10^8$)	τ_1	τ_2	l_1 (mm)	l_2 (mm)
268·8	7·65	5·34	6·0	40·86	3·14	2·1
263·0	5·60	3·72	8·0	60·6	3·18	2·1
257·6	4·26	2·61	14·2	71·11	3·5	2·0
254·2	3·53	2·08	24·5	100·5	4·8	2·1
264·4	6·11	4·07	10·1	52·36	3·2	2·1
265·0	6·31	4·22	10·0	48·4	3·6	2·1

TABLE 4. Diffusion coefficient D_3 calculated from τ_1 and τ assuming a diffusion length of 0·2 mm

Temperature (K)	τ_2	D_3 $(\times 10^9)$	D_2 (Jason, 1958) $(\times 10^8)$	D (ice) $(\times 10^{11})$
268·8	40·86	1·102	5·34	2·65
263·0	60·6	0·743	3·72	1·74
257·6	71·11	0·633	2·61	0·93
254·2	100·5	0·448	2·08	0·67
204·4	52·36	0·860	4·07	1·73
265·0	48·40	0·930	4·22	1·78

It should, however, be remembered that D_3 has been derived from an assumption of an arbitrary diffusion length and thus has limited accuracy.

Finally, the fact that Le Petit (1972) and also Taubenberger (1972) have observed similar time dependent properties in pure ice crystals plus the, as yet unpublished, observations made in this laboratory that such changes occur in other aqueous systems show that it is basically a property of water substance and not of the protein or whatever matrix the water is held in.

Conclusions

Clearly the changes that have been observed in this work show that ice and water fractions in a frozen gel such as fish muscle, are not stable. These experiments only observed changes over a period of weeks. The changes that might occur in the 'unfreezable' water content over periods of months or years need some investigation. Longer time constants may be found and the term 'unfreezable' may only apply to a much smaller fraction of water. Further work will be done to investigate the complete permittivity spectrum of frozen muscle from DC to microwave regions. This should elucidate further the processes by which such changes occur.

Acknowledgment

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Technical note: Application of a permittivity method for the rapid determination of water in meat

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Introduction

The conventional method for the determination of moisture in meat involves drying a sample at 105° to constant weight (*c.* 2 hr). The sample is mixed with dry sand and ethanol, details of which are given concisely in BS 4401, part 3, (1970). However, the process is time consuming, and to reduce the time required for each analysis various workers have indicated that comparable and reproducible results may be obtained by drying at more elevated temperatures. Perrin & Ferguson (1957) dried for 15 min at 200°, Krol & Meester (1963) for 15 min at 170° and Pearson (1971) for 35 min at 135°.

Drying methods will always include in their results losses of non aqueous volatiles and probably decomposition products. Removal of water by solvent and subsequent determination of water in the resultant solution would avoid these difficulties. In the present work water extraction from meat with dioxan and the determination of water either by Karl Fischer titration or by permittivity measurement has been examined, and the conditions for a fully automatic analysis procedure have been explored.

Materials and methods

A prototype Technicon SPS II homogenizer was employed with dioxan solvent (B.D.H. technical) and 2 g meat samples. To obtain representative samples 200 g of each type of meat were minced three times through a mincer with 4-mm holes, and analysed the same day. Two 5 g portions were used for the moisture determination by drying and three portions of 2 g were treated in the homogenizer to produce three solutions of water in dioxan, whose water contents were determined by both Karl Fischer titration and permittivity measurements.

The SPS II unit comprises a compartment containing the homogenizer rotor, a tray of sample cups and a reservoir of solvent. At the start of the cycle the rotor compartment is rinsed. The preweighed cup is inverted and the contents washed into the rotor compartment with the selected volume of solvent, in this case 50 ml. It is homogenized

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and then aspirated into the analytical system. Finally the residuum is discarded and the rotor compartment twice rinsed with fresh solvent. The sequence and duration of these operations is controlled by a programme disk on the front of the instrument. In this work a twenty sample/hr programme disc provided by the manufacturers was employed with the homogenization time extended to 2 min, and set at the maximum rate.

The minced meat samples were weighed into cups of the SPS II and 5 ml of dioxan added with a pipette. A glass rod was then used to break up the minced meat and wet individual pieces. This procedure was necessary to prevent samples from lodging either in the cup or in the entry port of the SPS II unit. During the aspiration stage of the SPS II cycle the entire contents of the homogenizer vessel were drawn off into a clean dry flask (Fig. 1a). The samples were then filtered through filter paper (Whatman no. 4) and their water contents determined by Karl Fischer titration, and by the permittivity method.

Before the procedures were applied to meat samples, the system was calibrated by weighing quantities of water into the SPS II cups and determining the water content of the solutions produced. In this way a precise figure was obtained for the weight of solvent
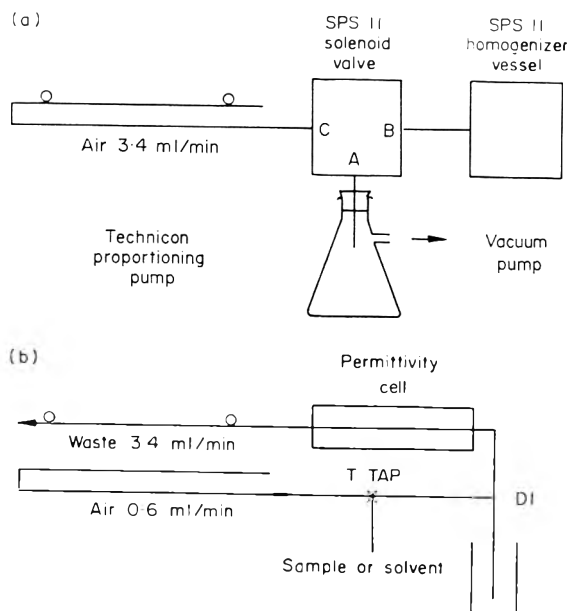
FIG. 1. (a) Air was bled into the homogenizer vessel throughout the cycle, except during aspiration when the entire contents of the homogenizer vessel was transferred to a clean dry flask.

(b) Air segmented sample or solvent was passed through the permittivity cell for three min before stopping the addition of air, and measuring the capacitance of the cell containing liquid alone.

used by the homogenizer, including the drainage in the vessel from the preceeding rinse cycles.

The handling and use of the Karl Fischer reagent is extensively discussed by Mitchell & Smith (1948). In order to obtain sharp end points with the Karl Fischer titration in dioxan 2 ml of pyridine was added to 5 ml of dioxan containing water, prior to titration, as suggested by Mitchell & Smith. However, the blank still gave a poor end point, so water was added to the pyridine (6 g/l). This has the effect of increasing the blank, but a sharp end point is then obtained.

For the permittivity method a cell was constructed from thin walled silica tubing (5 mm o.d., 3·5 mm i.d.). The external surface of this was coated with silver to form two electrodes of C cross-section (Fig. 2). The filtered sample was then passed through the tube for measurement, using the arrangement shown in Fig. 1b. The capacitance of the cell was measured with a Wayne Kerr B 642 Autobalance Universal Bridge. The cell required careful washing between measurements in order to prevent memory effects. Washing with dry dioxan segmented with air for 3 min has been found adequate. Calibration was effected using standard water/dioxan mixtures. It should also be recorded that it was necessary to run a standard in the permittivity cell each day as the detection sensitivity varied. The cell responded almost linearly to water contents up to 3·5% by weight. Above this figure the sensitivity rose sharply, however, the reproducibility became unacceptably poor. Hence no solutions above this concentration were examined.
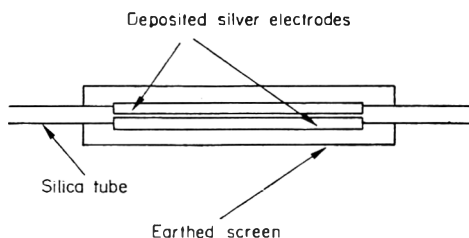

FIG. 2. Permittivity cell.

The procedure as described in BS 4401, part 3, 1970, concerned with the determination of moisture content of meat was used as the reference method. In this procedure meat samples are minced to 4 mm, mixed with a weighed amount of clean dry sand, ethanol is added, and the mixture is dried to constant weight at 105°C (*c.* 2 hr). The results thus obtained are referred to as moisture, in compliance with the nomenclature of the BS. Those obtained via the dioxan solutions are called water contents.

Results and discussion

Table 1 shows the results obtained for a wide range of meat samples. The moisture content by drying figures are the average of two measurements, the weight percent

water the average of three. The sausage meats were not further minced, but taken directly from the sausage. The columns of errors give the difference between the percentage water and the percentage moisture expressed as a percentage of the moisture figure, and consequently reflect all the errors in the method, including the possibility of non-representative sampling, sample sticking to the cup, and incomplete extraction of the water from the meat. The last column is the difference between the Karl Fischer and permittivity methods expressed as a percentage of the dielectric measurement.

TABLE 1. Water contents found by different methods

Sample	Moisture by BS 4401	Karl Fischer method	Water found (%)			
			% Error w.r.t. col. 2	Dielectric constant method	% Error w.r.t. col. 2	% Difference between cols 3 and 5
Kidney	80·4	79·7	−0·9	79·9	−0·6	+0·3
Chicken	75·6	73·8	−2·4	74·2	−1·9	+0·5
Veal	75·3	73·6	−2·3	72·5	−3·7	−1·5
Liver	71·8	69·3	−3·5	68·2	−5·0	−1·6
Lamb	71·6	70·6	−1·4	70·9	−1·0	+0·4
Chuck steak	69·0	68·1	−1·3	67·3	−2·5	−1·2
Ham	66·5	64·4	−3·2	64·6	−2·9	+0·3
Beef sausage	43·9	41·9	−4·6	44·1	+0·5	+5·0
Pork sausage	41·1*	38·1	−7·3	39·5	−3·9	+3·5
Streaky bacon	35·2	35·0	−0·6	36·1	+2·6	+3·0
Pork fat	11·2	11·1	−0·9	14·8	+32·2	+25·0

* Average of four not two determinations due to substantial difference between first two.

No operating problems were encountered when following the instructions above, except that the coarser samples, bacon and sausage, occasionally blocked the exit port of the homogenizer on aspiration. This was the only point at which blockage ever occurred, and it would be prudent, therefore, if working completely automatically to enlarge this aperture to prevent loss of sample.

The results obtained with the Karl Fischer method are all lower than those obtained by direct drying, the most serious discrepancies being with the sausage meats, where we might claim the possibility of a non-representative sample as the mincing stage was omitted. However, the lower liver and ham results are both supported by the permittivity measurements. It is not possible to say from these results whether this is due to any loss of volatile material from the meat on drying which is not water, or whether the homogenizer used with dioxan does not extract all the water from the meat. It is possible

that contact with hot dioxan under reflux, as suggested by Sandell (1960), might be required to effect quantitative recovery of water.

It can be seen that for the higher water content samples (greater than 50%) the permittivity and Karl Fischer methods agree well. There is, however, a positive interference with the dielectric method in the lower water content samples, i.e. those with higher fat content. The sample of pork fat allows us to estimate that this is due to the dissolution of fat of dielectric constant 3·2. This figure is in good agreement with the dielectric constant of fats reported by Mohler & Slevogt (1954). The interference could readily be removed by working with a solvent or solvent mixture of this permittivity. A mixture of methanol and dioxan was tried, but it was found that the methanol evapourated preferentially on homogenization. The mixture of solvents used by Mohler & Slevogt was not used because of an even wider discrepancy in boiling points. Water appears to be ideal as the boiling point is so close to that of dioxan (boiling range 100–102°C). A 1·2% by volume solution of water in dioxan was tried and was found to give an error of 10 7% with pork fat, a substantial reduction on the error of 32·2% with pure dioxan. A water content around 1·8% by volume solution would therefore be expected to eliminate this error altogether. Unfortunately, the use of a solution of this water content as the solvent in the method was precluded by the small range of water contents which the permittivity cell currently in use is capable of measuring reproducibly.

Conclusion

It has been shown that the SPS II produces solutions of water in dioxan from meat which give moisture values marginally lower than those obtained by drying. This may be interpreted as either the loss of non aqueous volatiles on drying, or the incomplete extraction of the water by the homogenizer. For routine water determinations of products of a similar composition this should not prove a significant disadvantage. These results may equally well be obtained by Karl Fischer or permittivity measurements. The latter are more suited to routine day-to-day analysis with an automated system. Fat gives a positive interference when dioxan alone is used as the solvent for the permittivity measurements, but this interference is reduced by working with a water/dioxan mixture as solvent, and should be negligible with a 1·8 volume percent solution.

The manual operations in this work were the filtration and subsequent water estimation. Use of a Technicon continuous filter and a more sophisticated bridge capable of recording or printing out capacitance results would enable the process to be automated completely.

Acknowledgment

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Book Reviews

Dehydration of Fruits and Vegetables: Food Technology Review No. 13. By
M. Torrey.
New Jersey: Noyes Data Corporation, 1974. Pp. xi + 287. US$36.

Food technologists who have to concern themselves with the patent literature must be immensely grateful to the Noyes Data Corporation for the excellent reviews covering the US patent literature. In each of the Food Technology Review Volumes the patents are condensed and presented without legislative phraseology. The information is then collected together under specific headings and suitably indexed.

In this volume the patent literature concerning various aspects of the dehydration of fruits and vegetables is presented for the period 1960–73 together with a few earlier patents. Freeze drying is not included as this was dealt with in Food Technology Review No. 1.

The first chapter covers general techniques and includes hot air drying, drum drying, foam mat processes and a number of miscellaneous topics such as osmotic dehydration, solvent distillation and explosion puffing. This is followed by two chapters on fruit and vegetable juices, one concerned with drying techniques and the other with concentration, in particular freeze concentration and reverse osmosis.

The remaining chapters cover the drying of individual fruits, vegetables and potatoes. The final chapter on potato products reviews no less than fifty-seven patents reflecting the immense commercial interest in this topic. The author has taken care to cross-reference throughout so that information covered in a number of sections can easily be located.

The contents of the book indicate the immense interest in dehydration in the United States during the last fifteen years, together with the large amount of research and development work which must have been undertaken.

Previous volumes in this series have covered this subject as well and No. 15 Fruit Juice Technology 1970, No. 19 Vegetable Processing 1971 and No. 21 Fruit Processing give, in some cases, more details of processes described in this volume. Other processes are given which are omitted in the present volume. However, more recent patents not covered in the previous reviews are given.

The author must be congratulated on presenting the mass of information from 195 patents in a readable and readily understood form.

S. D. Holdsworth

Food and Man. By E. N. TODHUNTER, E. D. WILSON, J. R. SAVAGE and J. Z. LUBAWSKI. New York: John Wiley and Sons, 1974. Pp. xi + 459. £6.10. 2nd edition.

There is sometimes debate in university common rooms on whether first-year students should be taught by the youngest and least experienced lecturers while the professor reserves himself for higher things, or whether—since as the twig is bent, so is the tree inclined—the professor himself should attend to the education of the freshmen. This book by Todhunter, Wilson, Savage and Lubawski is full of many sorts of facts. The facts are fitted into a long, chatty narrative about 'man' and what he has been up to since 40 000 B.C., what food patterns were like in the Early and High Middle Ages, and how the great religions of the world (Christianity, Judaism, Islam, Hinduism and Buddhism) have affected him and his food. It is intended for American students for whom nutrition is not a major interest and who have no background of chemistry or physics. British readers, therefore, have no cause for complaint at finding more space devoted to Mrs Ellen White and the Seventh-Day Adventists, who recommended Dinner Cuts as manufactured by Lorna Linda Foods, than to Islam. Nor will they object to references to authorities in the literature on the world's eating habits of which they might otherwise have been ignorant, to Jennie Rowntree, Poppy Cannon of the *Saturday Review* and Marion Schibsby.

But perhaps there may be thoughtful people on both sides of the Atlantic, even among the authors' young students, who, reflecting on the history of the last generation or so, would challenge the statement that 'man's concern for his fellow men, rather than concern for science and technology as such, is a development of the twentieth century'. Nevertheless, the last chapter listing all the diverse programmes that warm-hearted people have struggled to implement to improve nutrition in different parts of the world is a moving one. So much has been attempted—by FAO, WHO and UNICEF, the Friends, the Salvation Army, the Red Cross—and yet so much remains to do. The book describes the various sorts of undernutrition and malnutrition. It particularly recounts the work done in Biafra. But perhaps the uninformed readers for whom it is intended should also have been told that to blockade food supplies has been used as a 'legitimate' instrument of war, whether in Biafra in an all-Nigerian conflict, in Paris in a Franco-Prussian campaign or in Germany after Versailles.

'The problems now are', the authors write, 'to make food available where it is needed and to teach people to eat it'. We can agree, looking out from the shadow of our own uneatable mountain of beef. There is teaching and teaching. One way, as at the end of the section on 'Food, Man and the Influence of Business', is to give the pupil as a topic for individual investigation the problem 'Think up a name for a new snack food based on the guidelines given in the chapter'. Another way would be to expose the neophyte more directly to the astringent atmosphere of thinking by which the best scientists have made such progress as has been achieved.

MAGNUS PYKE

Plastics in Contact with Food. By J. H. Briston and L. L. Katan.
London: Food Trade Press Ltd, 1974. Pp. xii + 466. £12.00.

The basic needs of the human race are food, drink, sunlight, air, shelter, sex and love.
The manner in which these interact is an ecological study of the first magnitude and
Briston and Katan's book is concerned with the assistance given by plastics in satisfying
the first two of these needs. It is an attempt to answer the question 'How can plastics
assist in delivering food from its source to the consumer, economically, safely and in
unimpaired quality?' Packaging forms an essential part in the chain of operations, and
it is proper to consider any potential hazards to health that arise from it, particularly
from plastics packaging, and to compare them either with the hazards due to the absence
of packaging, or with the hazards presented by alternative methods of packaging. As
Briston and Katan point out, comparison with a hypothetical zero hazard is neither
valid nor meaningful. Although packaging hazards are very much less serious from the
practical point of view than those of food spoilage, they present a more difficult theoreti-
cal problem.

For these reasons the problem of contact between plastics and food has not had a
great deal of attention from the theoretical point of view although much empirical
work has previously been reported. Legislation on food additives, on packaging and on
materials for the processing of food varies from country to country throughout the
world and the picture overall is both muddled and inconsistent. It shows a spectrum
of relevant legislation from statute law and codification at one extreme to the use of
common law at the other.

Plastics in Contact with Food is divided into three parts, Part I dealing with materials,
Part II with health and quality and Part III with manufacture. Undoubtedly, the
most important and interesting is Part II and the need for Parts I and III will vary
according to the requirements of the reader. Since the book has been written mainly
with the education of the technologist foremost in mind, possibly Parts I and III serve
a valid purpose in this context. However, for the more advanced reader the omission
of the first chapters would prove no great handicap since the meat of the book is
undoubtedly sandwiched in the middle portion.

These six chapters dealing with health and quality are a first-time attempt to bring
together in collected form all the many aspects of the subject in a logical and interesting
manner. It attempts, first of all, to place the potential hazards of adulteration into
perspective with all the other hazards arising from food preparation and from deliberate
additives. In relation to natural food hazards it is only a matter of convenience that we
refer to substances of relatively high toxicity as poisons. Most materials, if taken in
sufficient quantity, are liable to have bad effects. In fact one man's poison may well be
another man's cure in many areas of food processing. It is, therefore, correct to recog-
nize that plastics packaging should be encouraged where any beneficial effect it has
equals or exceeds any hazard deriving from the natural state of the food. The authors

then postulate a basic model pointing out that toxicity as such never really enters into questions either of deliberate additions to food or adventitious contamination by materials which may migrate from packaging, particularly plastics packaging. Chapter VIII is probably the most important chapter of the book and deals essentially with a basic mathematical model for phenomena and points out the difficulties and the lack of knowledge existing in this area. It is, perhaps, unfortunate that there is some confusion in the symbols used, particularly the use of a lower case letter 'ell' for thickness since this appears in the type face used as the number 'one' rather than a lower case 'ell' and consequently gets mixed up when the figure one is used deliberately. Nevertheless, in spite of this, the basic principles of considering migration in a logical manner are admirably expressed. The final chapter of Part II deals with the laws and regulations in the various countries of the world and should prove a most valuable chapter for all concerned with food packaging.

The book should be studied by all concerned with food manufacture, processing and distribution. Moreover, having studied the current techniques, the realization that we have a lot to do before any systematic method of assessing the potential hazards of materials used in the processing and packaging of foodstuffs can be agreed, should result in action to achieve this. Briston and Katan have pointed the way. It is now necessary to take action to get work done which will enable us to write the correct legislation to ensure that the nations of the world receive the food they need in the right form, in good condition and without hazard from adventitious or deliberate additives.

So much, then, for the meat of the book contained in the central six chapters of Part II. The other Parts (I and III) must be judged against previously available books dealing with the subject of plastics and their manufacture, respectively. Personally, I felt that Part I was rather elementary in its approach and probably more useful for a layman in the subject than to the serious student. Part III, on the materials and their manufacture, is a useful summary of the various techniques available but provided nothing new that had not been said previously.

The book ends with five Appendices. The first is a useful glossary of terms, the second deals with the basic theory of diffusion and permeability, the third with waste disposal and litter, the fourth gives some useful data and choice criteria about the various plastics and the last outlines the symbols and units used throughout the text. Most of these appendices are of some value, but that on waste disposal and litter seems a little irrelevant to food contact problems and is rather too short to make much impact.

Altogether an interesting book which should be read and remain on the shelves of all concerned with food processing, manufacturing and distribution.

F. A. PAINE

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Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Verbs which contain the suffix ize (ise) and their derivatives should be spelt with the z. 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 greater than one hundred.

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

SI UNITS

gram	g	Joule	J
kilogram	$kg = 10^3$ g	Newton	N
milligram	$mg = 10^{-3}$ g	Watt	W
metre	m	Centigrade	°C
millimetre	$mm = 10^{-3}$ m	hour	hr
micrometre	$\mu m = 10^{-6}$ m	minute	min
nanometre	$nm = 10^{-9}$ m	second	sec
litre	$l = 10^{-3}$ m³		

NON SI UNITS

inch	in	$= 25\cdot4$ mm
foot	ft	$= 0\cdot3048$ m
square inch	in²	$= 645\cdot16$ mm²
square foot	ft²	$= 0\cdot092903$ m²
cubic inch	in³	$= 1\cdot63871 \times 10^4$ mm³
cubic foot	ft³	$= 0\cdot028317$ m³
gallon	gal	$= 4\cdot5461$ l
pound	lb	$= 0\cdot453592$ kg
pound/cubic inch	lb in⁻³	$= 2\cdot76799 \times 10^4$ kg m⁻³
dyne		$= 10^{-5}$ N
Calorie (15°C)	cal	$= 4\cdot1855$ J
British Thermal Unit	BTU	$= 1055\cdot06$ J
Horsepower	HP	$= 745\cdot700$ W
Fahrenheit	°F	$= 9/5\ T°C + 32$

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

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