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The determination of protein in biological materials and foodstuffs

T. T. GORSUCH* AND R. L. NORTON†

Summary

Three methods for determining protein, the Folin, dye-binding and nitration procedures, have been investigated, modified when necessary, and applied to mycelial systems, flour and dried egg. All three methods give different results with different protein standards, indicating that the values obtained from different samples can only be relative rather than absolute.

Introduction

The determination of protein in materials containing variable amounts of non-protein nitrogen presents many difficulties. In an attempt to rationalize anomalous results previously obtained, a study was made of three methods of determination, the Folin, dye-binding and nitration procedures. The Folin method is probably the most widely used of the three, although dye-binding procedures are becoming increasingly popular: the nitration method was included because it appeared to be both rapid and simple. Originally, the Biuret procedure was also included but this was quickly eliminated due to its low sensitivity and the consequent interference arising from coloured samples.

Methods

Folin procedure

This technique has been extensively studied by Lowry *et al.* (1951). Protein is heated, in the presence of copper, with Folin phenol reagent in buffered alkaline solution and a blue colour, resulting from reduction of phosphomolybdate and phosphotungstate is produced. It is suggested that the tyrosine and tryptophan residues in the protein make the main contribution to the colour produced by the reaction.

The first step was a study of the conditions required to solublize the protein in mycelial systems, and samples were digested with 0·5 N, 1 N- or 2 N-sodium hydroxide solution in a boiling water bath for $\frac{1}{2}$, 1 and 2 hr before the colorimetric determination. It was found that, with the exception of the treatment with 0·5 N-alkali, digestion for

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1 hr was sufficient for complete solublization. However, it was apparent that colour development was influenced to a high degree by the pH of the solution and this was confirmed by neutralizing the above extracts to varying extents with 0·5 N-, 1 N- and 2 N-acid. Ignoring the somewhat scattered results obtained with 0·5 N-alkali, it was found that the absorbance reached a maximum when certain critical volumes of acid were added, and that at identical residual alkali concentrations, the absorbances were the same regardless of the strength of the alkali used for the preliminary digestion. As it was desirable to obtain the maximum available sensitivity partial neutralization of the alkali was carried out prior to colour development. It was shown experimentally that the simplest way to achieve this was to adopt a standard digestion procedure with 1 N-sodium hydroxide, and to dilute the Folin reagent with 1·4 N-hydrochloric acid instead of with water as described by Lowry *et al.* (1951). Using this procedure, the pH during colour development is 10·8. Lowry *et al.* (1951) suggested that a pH of about 10 is required for highest sensitivity.

Further experiments showed that full colour development occurred in 10 min and that this colour was stable for a further 20 min.

Method. Reagents:

A = 2% sodium carbonate.

B = 0·5% copper sulphate in 1% sodium citrate.

C = 1 ml B + 50 ml A, prepared fresh daily.

D = 1·4 N-hydrochloric acid (*ca.* 121 ml concentrated acid, specific gravity 1·18 diluted to 1 litre).

E = Commercial Folin–Ciocalteu reagent diluted with an equal volume of D.

F = 1 N- and 2 N-sodium hydroxide (40 and 80 g/l).

G = Crystalline bovine serum albumin or β-lactoglobulin.

Weigh the sample, containing not more than 5 mg protein, into a boiling tube, add 10 ml 1 N-sodium hydroxide, stopper the tubes loosely, and heat for 1 hr in a boiling water bath. Cool and centrifuge. To a 0·6-ml aliquot of the supernatant solution add 3 ml of reagent C and mix. Allow to stand for at least 10 min, add 0·3 ml reagent E and mix rapidly. After 10 min, measure the absorbance in 10 mm cells at 750 mμ using water as reference.

Prepare a series of standards by adding 1–5 ml of bovine serum albumin or β-lactoglobulin, solution (0·1%) to 5 ml 2 N-sodium hydroxide solution, making the solutions up to 10 ml with distilled water, and heating in a boiling water bath for 1 hr. Complete the estimation as above.

It must be emphasized that it is necessary to heat the standard protein with alkali in the same way as the samples. Proteins showed only about 80% recovery after this treatment, affirming the findings of Lowry *et al.* (1951).

Dye-binding procedure

Diacid Light Green GS (Colour Index No. Acid Green 25)* was chosen for this work, since Tsugo, Iwaida & Kawaguchi (1966) had shown that of over twenty acid dyes examined this was outstanding in its protein binding characteristics. This dye is marketed under the name Solway Green G150 by the I.C.I. Ltd.

Method. Weigh the sample containing not more than 12 mg protein into a 250 ml beaker and add 100 ml Solway Green solution (0·0075% w/v in 0·1 M-citrate buffer, pH 1·9). Homogenize for 5 min with a Silverson homogenizer† set at its slowest speed. Centrifuge the suspension and measure the absorbance of the supernatant solution at 645 mμ, using 10 mm cells and a water reference. Subtract the absorbance from that of the original dye-solution.

Prepare a series of standards by treating 3–12-mg portions of bovine serum albumin or β-lactoglobulin as described above, and plot a calibration curve.

Nitration procedure

This method, which presumably depends upon the nitration of tyrosine and phenylalanine, was applied by Dobroserdova (1966) to the estimation of milk protein. It involves heating the sample in a boiling water bath with 50% nitric acid, but this procedure gave scattered results when applied to mycelial protein. Investigations showed that colour formation decreased rapidly as the time of the acid treatment increased, suggesting that serious errors could be introduced by quite small variations in the heating conditions. From a study of the effects of variations in the acid concentration and the reaction temperature, it was found that heating in a boiling water bath with 25% nitric acid was more satisfactory, with maximum colour being developed in 8 min and remaining stable during a further 6 min heating.

Certain materials gave interferences from their inherent colours, but a correction for this was made by carrying out a parallel procedure using hydrochloric (33%) in place of nitric acid.

Method. Weigh into separate tubes, two portions of sample of similar weight, containing not more than 15 mg protein. To one tube add 2·0 ml nitric acid (specific gravity 1·42, diluted with 3 volumes water) and to the other 2·0 ml hydrochloric acid (specific gravity 1·18, diluted with 2 volumes water) as a blank. Heat both tubes in a boiling water bath for 10 min, cool and add 3·3 ml 10% sodium hydroxide to each. Dilute the solutions to 25 ml and filter through Whatman No. 1 papers. Measure the absorbance at 432 mμ in 10 mm cells using water as reference. Subtract the absorbance due to the blank from the absorbance of the sample.

* American Association of Textile Chemists and Colorists and the Society of Dyers and Colorists of the United Kingdom, 1956. *Colour Index*, 2nd edn.

† Silverson Machines Ltd, 55/57 Tower Bridge Road, London, S.E.1.

Prepare a series of standards by pipetting 0·2–1·0-ml aliquots of bovine serum albumin or β-lactoglobulin solution (1·5%) into tubes. Add 1·0 ml 50% HNO_3 and sufficient water to make the total volume to 2 ml. Treat the standards as described in the procedure above and prepare a calibration graph. No correction is normally required for inherent colour in the case of standards.

Results

The collected results for protein in mycelial systems, dried egg and flour are given in Table 1.

TABLE 1. Result on materials

Sample	Nitrogen (%)	Protein % wet basis					
		Lowry		Dye-binding		Nitration	
		A	B	A	B	A	B
	(Micro-Kjeldahl)						
Mycelial system							
I	3·45	13·1	9·7	7·7	5·5	12·6	10·3
II	4·20	24·7	18·7	12·7	9·2	25·0	19·5
III	5·11	42·0	32·6	24·7	19·0	38·5	29·9
	(Macro-Kjeldahl)						
Egg (dried)							
I	6·89	58·5	45·0	48·4	35·7	71·0	56·8
II	6·36	48·3	36·7	43·3	32·1	65·1	52·1
Flour							
I	1·78	12·4	9·2	8·2	6·0	7·5	6·0
II	1·99	14·3	10·4	9·7	7·2	7·3	5·7

A, Based on β-lactoglobulin standard; B, based on bovine serum albumin standard.

Discussion and conclusions

A study of the results presented in Table 1 enables a number of conclusions to be drawn.

(1) The calibration curves for all three methods vary considerably according to the protein used as the standard: in every case, β-lactoglobulin gives lower absorbances than bovine serum albumin for a given weight of protein. Consequently, results calculated from the β-lactoglobulin standard curves are invariably higher than those based on bovine serum albumin. Presumably, this is due – at least in part – to variations in the amino acid composition of the different proteins, since the colour developed in the Folin and nitration methods is largely dependent upon the concentrations of tryptophan, tyrosine, and phenylalanine. The mechanism involved in dye-binding is still open to conjecture.

(2) The three methods can give results differing very considerably when applied to the same material, even when the same protein is used as the standard.

(3) The ratios of the Folin results to either the nitration or the dye-binding values remain approximately constant for a single type of sample even if the protein standard is altered. The ratios obtained with different types of sample, however, vary widely (Table 2).

TABLE 2. Ratios of Folin results to nitration and dye-binding values

	Dye-binding		Nitration	
	A	B	A	B
Mycelial system				
I	1·70	1·76	1·04	0·94
II	1·95	2·03	0·99	0·96
III	1·70	1·72	1·09	1·09
Egg (dried)				
I	1·21	1·26	0·82	0·79
II	1·12	1·14	0·74	0·70
Flour				
I	1·51	1·53	1·65	1·53
II	1·47	1·44	1·96	1·82

A and B have same significance as in Table I.

The standard deviations between replicates were calculated for a series of determinations of mycelial protein, and were found to be 1·23 for the Lowry Method and 1·27 for the nitration method. However, standard curves can be reproduced with a high degree of accuracy and, for this reason, it is thought that the magnitude of the standard deviations, which must be considered high for an analytical procedure, stems from the variable extraction of protein from within the cells.

For routine use, involving batches of samples, the Folin method would seem to be the most satisfactory for the types of material investigated here. When only a few determinations are to be carried out or the samples arise singly, rather than in batches, the dye-binding procedure may be preferred.

However, it must be emphasized that the results obtained by all three methods employed in the manner described above, probably have no absolute validity. The results will vary markedly with the protein used as a standard and can, in general, serve merely as a basis for comparison. When absolute values are required, it will be necessary to prepare a standard curve using a sample of the pure protein under exam-

ination, and with microbial systems this can be very difficult, particularly when growth conditions are being altered, and the nature of the protein constituents is altering with them.

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The effect of sample dimensions on the cleaving of meat in the objective assessment of tenderness

C. L. DAVEY AND K. V. GILBERT

Summary. An examination has been made of the effect of sample dimensions on the objective assessment of meat tenderness. By adjusting shear-force values—measured at right angles to the muscle-fibre axis—to a sample cross-section of 1 cm^2 at the proposed line of cleavage, variations in tenderness values within a sample are reduced to not more than ± 5 shear-force units. Such variations represent approximately $\pm \frac{1}{2}$ unit in the mid-region of a nine-point sensory scale of tenderness. Through making such corrections to shear-force values, it is anticipated that clearer indications of the effects of animal characteristics and carcass processing on tenderness within a single muscle will be possible.

Conclusions concerning the mechanism of cleaving meat are drawn from the relationships that have been shown between sample dimensions and tenderness values.

Introduction

There have been many attempts to evaluate the tenderness of meat using mechanical devices that measure either the force or the work required to rupture cooked samples. These devices have been so designed to rupture the meat by penetration with blunt prongs (Tressler, Birdseye & Murray, 1932), by mincing (Miyada & Tappel, 1956), or by shearing with either a knife or a wedge (Warner, 1928; Satorius & Child, 1938; Volodkevich, 1938; Winkler, 1939; Sperring, Platt & Hiner, 1962; Macfarlane & Marer, 1966). Of these, that of shearing samples across the meat grain is most amenable to further physical examination. The tenderometer described by Macfarlane & Marer (1966) uses this principle and gives high coefficients of correlation (0·68–0·94) between the sensory and objective evaluations of tenderness (Marsh, Woodhams & Leet, 1966). In this tenderometer a brass wedge is forced through the sample at right angles to the muscle-fibre axis, by a load increasing linearly with time. The load-deformation curve obtained gives a clear description of the traverse of the wedge through the section, whilst the time to cleave in seconds, which is proportional to

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load, provides a convenient measure of toughness in terms of the shear-force value of the sample.

The present study using this tenderometer is aimed at reducing the dependence on statistical procedures in examining the effect of sample dimensions on shear-force values, and in so doing opening the way to a more definitive examination of the effects of animal characteristics and carcass processing on meat tenderness. In addition it has been possible to attempt an interpretation of the reaction of the meat sample to the tenderometer wedge, leading to a clearer view of meat texture.

Experimental

Bovine *sternomandibularis* (SM) muscles obtained from a variety of animals at the local meat works were prepared for experimental treatment under conditions of extreme asepsis within 90 min of slaughter. To obtain a range of toughness values, muscle samples supported horizontally on thin plastic film were allowed to cold shorten to differing extents (Locker & Hagyard, 1963; Marsh & Leet, 1966). The initial length (L_0) was measured as the distance between two small nails inserted at either end of the samples which were then stored at 15°C for periods from 0 to 24 hr before being transferred to 2°C to shorten. With the onset of rigor mortis the samples became set at the shortened length (L), thus acquiring a degree of shortening (S) given by the term $1 - L/L_0$. In all cases storage at 2°C was continued until 48 hr post mortem by which time ultimate pH values (Davey & Gilbert, 1968) had been reached. In the ageing studies, at the commencement of the ageing period – 48 hr post mortem – meat samples were sprayed with a mixture of aureomycin (100 ppm) and chloramphenicol (100 ppm) (Davey & Gilbert, 1966) and then held at 15°C and 60–70% relative humidity in a controlled-climate chamber. Strict adherence to the above procedures ensured that bacterial numbers, measured according to Davey & Gilbert (1966) were maintained at the low levels of $< 10^2$ organisms/g meat aged 100 hr at 15°C.

Cooking procedure

Oblong portions of the processed muscle samples at least 7 cm along the fibre direction × 3·5 cm × 2 cm were packed in pairs into flat, top-opening aluminium tins (internal dimensions, 9 cm × 7·5 cm × 2 cm) and vacuum-sealed in small plastic bags. Cooking was carried out in a water bath at 80°C ± 0·1°C for 40 min (Marsh *et al.*, 1966), after which time the temperature of the samples was rapidly lowered by immersing the tins in cold water for 20 min.

Objective assessment of tenderness

From the central region of the cooked samples of SM muscles, sections (1·50 ± 0·05 cm wide) parallel to the fibre were cut with a double-bladed scalpel (Love, 1958). These

sections, at least 6 cm long, were sliced longitudinally into samples ranging in thickness from 0·1 cm to 1·0 cm and in some experiments into samples ranging over the same thickness but reduced in width to 0·75 ± 0·05 cm. Despite care being taken, it was difficult in practice to cut uniform sections of the required dimensions. In this respect positive errors of 0·1 cm in the dimensions of a sample 1·5 cm wide and 0·7 cm thick would produce an error of 22% in cross-sectional area. It is important therefore that dimensions of the sample be accurately known. Width and thickness dimensions at the line of the proposed cleavage were determined with a Baty dial gauge adapted for the purpose (Fig. 1).

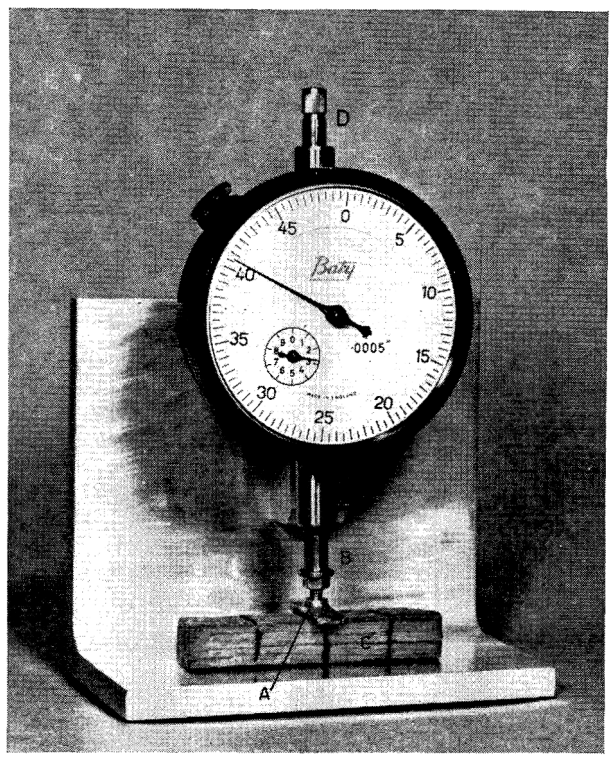

FIG. 1. The Baty dial gauge mounted on angle aluminium for measuring the width and height dimensions of cooked meat samples. A, rectangular brass foot 0·5 cm × 1·8 cm; B, spindle of gauge; C, cooked-meat sample marked for measuring with the gauge at the proposed lines of application of the tenderometer wedge; D, spindle head.

The gauge was mounted as shown on a block of angle aluminium with the rectangular brass foot (A) parallel to the base plate, replacing the point of the measuring spindle. A return spring controlling the action of the spindle (B) was removed from the gauge since spring loading would otherwise cause substantial compression of the meat

(C) held between the base plate and the brass foot. In practice the spindle head (D) was given a gentle tap before each reading to compress the points of high relief on the surface of the meat with the brass foot. With experience in both cutting rectangular sections and in using the modified dial gauge, width and thickness dimensions were determined to an estimated error of ±0·02 cm. This represented ±5% in cross-sectional area for a typical sample (1·5 cm × 0·7 cm).

Shear-force values of the precisely-cut samples were measured using the tenderometer described by Macfarlane *et al.* (1966). The tenderometer wedge was forced through the sample at right angles to the fibre axis across the line where the width and thickness had previously been determined. Lateral spreading of the meat during movement of the wedge to the point of cleavage was prevented by holding the sections firmly in position between parallel brass plates spaced to allow movement of the tenderometer wedge with little tolerance. This necessitated the replacement of the normal tenderometer wedge (width 1·50 cm) with another (width 0·80 cm) of identical cross-sectional profile, when assessing the shear-force values of the narrower samples.

Results

The effect of the cross-sectional area of the cooked-meat sample on shear-force value is shown in Fig. 2. The relationship obtained was distinctly curvilinear especially

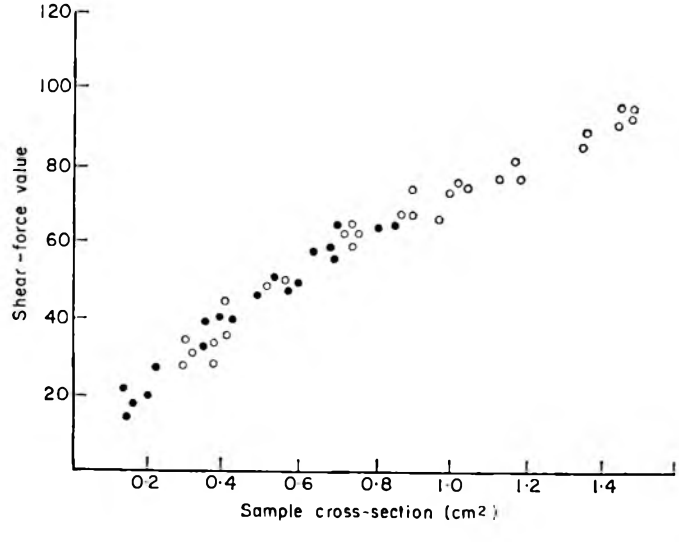

FIG. 2. The relationship between the shear-force value and the cross-sectional area of unaged samples of SM muscle (ultimate pH, 5·72) from an Aberdeen Angus bull (3 years old). ○, sample width cleaved 1·50 ± 0·05 cm; ●, sample width cleaved 0·75 ± 0·05 cm.

obvious at values of cross section below 0·4 cm². Fig. 2 also shows that there was no departure from the relationship at two-sample widths of 1·50 ± 0·05 cm and 0·75 ± 0·05 cm. Thus shape of the cross-section in rectangles having sides of different propor-

tions does not affect the shear-force value obtained. This has been verified in numerous experiments covering a wide range of both width and thickness dimensions.

The relationship between shear-force value and sample cross-section (shown in Fig. 2), is markedly dependent upon the degree of toughness induced in the meat during processing. This is clearly illustrated in Fig. 3. With an increase in cross-sectional area from 0·60 cm^2 to 0·80 cm^2 (equivalent to an increase in thickness of 0·13 cm for samples 1·50 cm in width) the shear-force value of a tough sample (curve I, S = 0·37) measured with the normal tenderometer wedge, rose by 20 units approximately. On the other hand the corresponding rise in shear-force value for a substantially more tender sample (curve III, S = 0·02) was less than half this value. Fig. 3 also shows that with the method of objective testing used, a maximum variation of ±5 units at a given cross-sectional area can be expected. This represents approximately $\pm\frac{1}{2}$ unit at the mid-point on a 9-point sensory scale of tenderness (Marsh *et al.*, 1966).

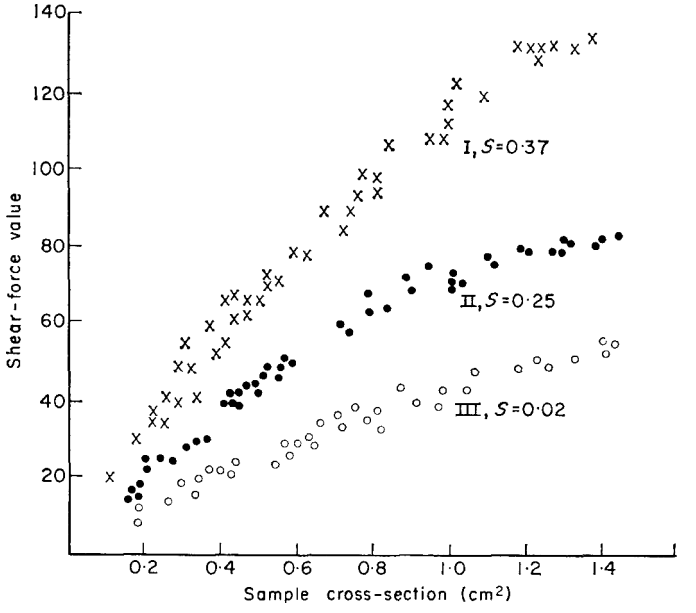

FIG. 3. The relationship between the shear-force value and the cross-sectional area of unaged samples of SM muscles from Aberdeen Angus bulls (2–3 years old) processed to give differing degrees of toughness. Curve I, muscle S value 0·37, ultimate pH value 5·73; curve II, muscle S value 0·25, ultimate pH value 5·64; curve III, muscle S value 0·02, ultimate pH value 5·68; Sample dimensions, width 1·50 ± 0·05 cm, thickness 0·1 to 1·0 cm.

Fig. 4 shows that for a variety of meat samples of differing degrees of toughness the shear-force value increases linearly with the square root of the sample cross-section.

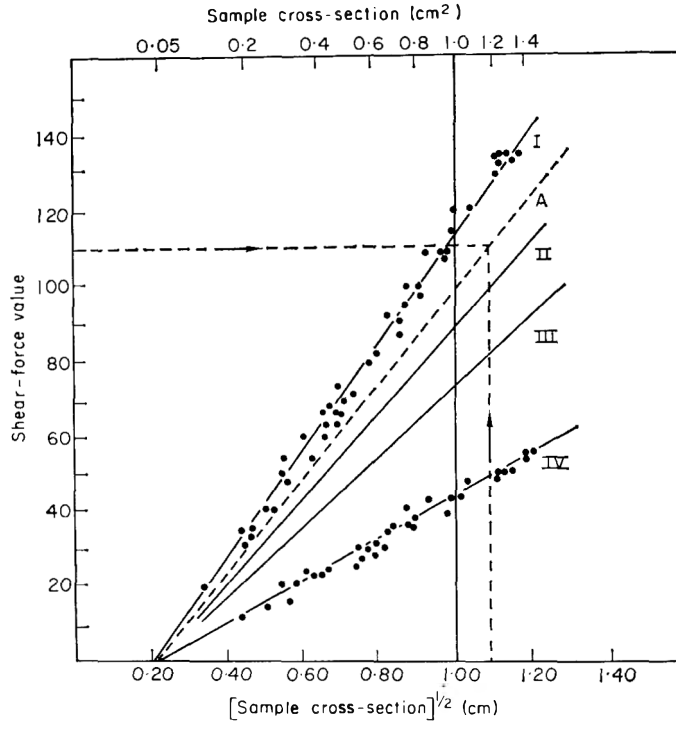

FIG. 4. The relationship between the shear-force value and the square root of the sample cross section of SM muscles from a variety of beef animals, processed to give differing degrees of toughness. Line I, unaged sample, *S* value 0·37, ultimate pH value 5·73; line II, unaged sample, *S* value 0·25, ultimate pH value 5·64; line III, unaged sample; *S* value 0·02, ultimate pH value 5·68; line IV, sample aged 3 days, 15°C, *S* value 0·04, ultimate pH value, 5·82.

These results suggest that the rather wide variations in the shear-force values, usually found within a single sample, may be due largely to variations in the cross-sectional area of the sample at the region of application of the tenderometer wedge. Indeed such variations can be reduced substantially using Fig. 4 as a nomogram to correct to a standard shear-force value at a sample cross-section of 1·00 cm^2. Thus for a test sample cleaving at 110 shear-force units and having a cross-sectional area of 1·19 cm^2, the standard shear-force value (99 units) is given by the intercept of the broken line (A) on the 1·00 cm^2 vertical co-ordinate.

The lines relating the two parameters shown in Fig. 4 intercept the co-ordinate for the square root of the cross-sectional area, not at zero but at approximately 0·2 cm, equivalent to a very thin sample (width 1·50 cm, thickness $< 0{\cdot}30$ cm). The shear-force gradients of the lines (Δ shear-force value/Δ (sample cross-section)$^{\frac{1}{2}}$) are defined solely by this common intercept and the standard shear-force values, and must therefore

increase in proportion to the toughness of the meat. Fig. 5 clearly demonstrates, for a large number of samples, the linearity of the relationship between shear-force gradient and standard shear-force value inferred from Fig. 4. Although there is a considerable scatter in the values relating these parameters, meat that was shortened from $S = 0{\cdot}00$ to 0·60 through the toughness peak at $S = 0{\cdot}40$, was aged for varying periods, or meat from both young and old animals gave values which did not depart from the relationship shown in Fig. 5. Thus shear-force gradients are independent of the means of inducing changes in meat toughness but are dependent solely upon the value of the toughness itself.

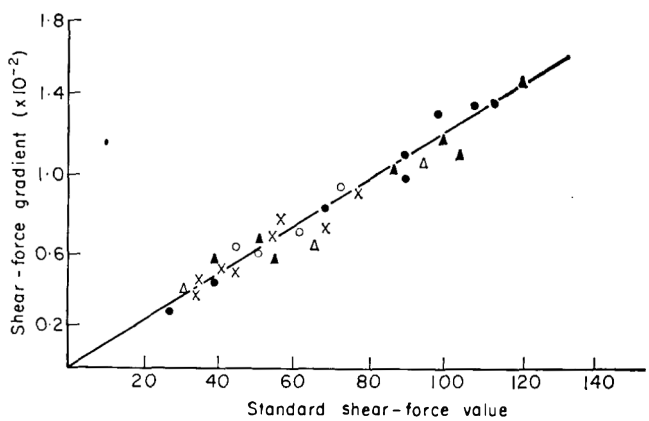

FIG. 5. The relationship between the shear-force gradient and the standard shear-force value of bovine SM muscles processed to give differing degrees of toughness. Jersey bull (4–5 years' old): ● nine shortenings, S values 0·06 to 0·39, unaged samples; ○ four shortenings, S values 0·45 to 0·58, unaged samples. Aberdeen Angus steer (1–2 years' old): ■ seven shortenings, S values 0·02 to 0·35, unaged samples; □ three shortenings, S values 0·43 to 0·52, unaged samples. Aberdeen Angus bull (2 years old), muscle S value 0·05, ×, shear-force values declining from 78 to 34 through an ageing period of 3 days at 15°C.

The procedure for reducing wide variations in shear-force values by using Fig. 4 as a nomogram finds more useful application in studies determining the effect of processing and cooking factors on tenderness within a single muscle. One such experiment is illustrated in Fig. 6 which shows the time-course of ageing in the SM muscles from two beef animals. With no correction of shear-force values for variation in sample cross-section it was not possible to separate the ageing curves for the two animals (Fig. 6(a)). On the other hand precise and different curves were readily obtained (Fig. 6(b)) when such corrections were made.

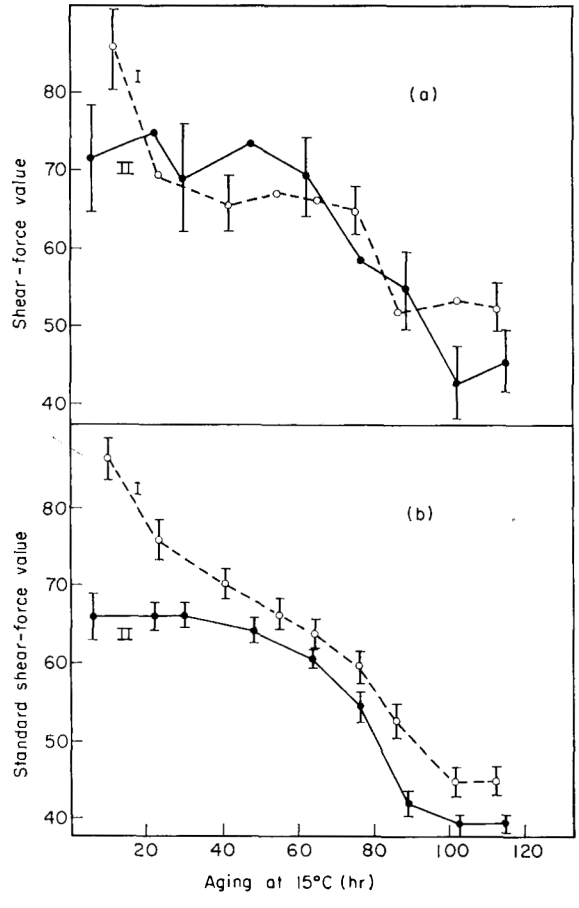

FIG. 6. The relationship between the ageing time at 15°C and both uncorrected shear-force values (a) and standard shear-force values (b) for bovine SM muscles from two animals. Curve I, Aberdeen Angus bull (5 years old), muscle *S* value 0·02, ultimate pH value 5·81, ○ mean of eight determinations; curve II, Aberdeen Angus steer (1–2 years old), muscle *S* value 0·04, ultimate pH value 5·62, ● mean of eight determinations. Vertical lines standard deviations.

Discussion

Most sudies in meat quality rely on substantial statistical analyses of results encompassing a large number of animals to obtain useful data. By this approach, average values of widely-variable tenderness measurements within a meat sample can be given significant meaning.

However the results of the present study have shown that if shear-force values are corrected to a sample cross-section of 1 cm^2 at the line of proposed cleavage, statistical

judgment of the significance of mean values is not then normally required. In this respect clearly defined and markedly different curves relating ageing time and corrected, mean shear-force values, have been described for the SM muscles from two different animals. Since the complete results are obtained on the chosen muscle of a single animal, inter-animal and intra-muscular variations in tenderness can be accurately studied, adding substantially to the meaning of the results. It is anticipated that this method of correcting shear-force values should lead to a clearer description of the relationships between both animal and carcass characteristics and meat tenderness.

In addition to these practical aspects of the study, significant theoretical conclusions can be drawn concerning the mechanism of cleaving meat, and therefore concerning meat tenderness.

Although the pressure on a meat sample for a given applied load at the tenderometer wedge is presumably inversely proportional to section width, the results have shown that shear-force values are actually not dictated by this dimension. On the other hand shear-force values increase with the cross-sectional area of the sample; the tougher the meat, the more pronounced is this effect. To explain this strict dependence of shear-force value on cross section alone, we conclude that the stress from the applied force is readily and rather evenly dispersed throughout the parallel meat fibres within the sample. Since the tensile strengths of the individual meat fibres will in large part determine the cleavage load, shear-force values will increase with the fibre numbers and hence with the cross-sectional area of the sections.

Acknowledgment

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Determination of degree of heating of fish muscle

J. J. DOESBURG AND DEIRDRE PAPENDORF

Summary

Experiments in order to control the degree of heating of lean fish (hake) and oily fish (mackerel and pilchard) are described. In the temperature range of 60–100°C the maximum temperature (T_m) of a heat treatment on a hake homogenate could be calculated from the coagulation temperature (T_c) obtained by a modified coagulation test by use of the equation $T_m = 1{\cdot}02.\ T_c - 0{\cdot}2 \pm 2{\cdot}0$. In the case of oily fish the equation $T_m = T_c + 0{\cdot}1 \pm 2{\cdot}6$ could be used to calculate the maximum temperature in the range of 60–80°C.

Introduction

In order to estimate the degree of heating of fish in cookers used for fish meal manufacture it was aimed to develop a test for the control of maximum temperatures in the range of 60–100°C (Doesburg & Papendorf, 1966, 1967).

For meat products the usual tests on residual enzyme activity (Lind, 1965; Gantner & Körmendy, 1968) or on extractability and coagulation properties of proteinaceous materials (Coretti, 1957; U.S.D.A.-A.I.Q., 1961) give indication of heat treatments within a small temperature range only. This limited temperature range is a result of the very high temperature coefficient of the conformational changes in proteinaceous materials which are often followed by heat aggregation reactions (Colvin, 1953, 1964). According to Stauff *et al.* (1961) the term coagulation as used in the tests in question refers to the fact that often—especially when high protein concentrations are present—heat aggregation was compared with the coagulation which may occur in other thermodynamically unstable colloidal systems or with the coagulation resulting from the removal of hydration layers or electric charges from the colloids in such systems.

The coagulation tests for meat products are based on the decreased extractability of muscle proteins in water or salt solutions as a result of their coagulation during heating (Coretti, 1957). According to Bendall (1964, p. 248) it is safe to say that most of the soluble proteins of the sarcoplasm and the actomyosin system of the muscle fibrils will have denatured when the temperature reaches 62°C. This statement is in agree-

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ment with the results of the coagulation tests. In these tests (Coretti, 1957; U.S.D.A.–A.I.Q., 1961) the meat is minced or homogenized and mixed with distilled water or a 0·9% salt solution, subsequently the suspension is occasionally stirred for 1 hr and finally filtered. When the clear filtrates are slowly heated to 65°C a visible coagulation will be obtained only when the meat was heated below 63°C or no longer than 5 min at 63°C (Coretti, 1957). The fact that no coagulation takes place in extracts from meat which was subjected to more intensive heat treatments could have two causes:

(a) The proteinaceous materials, made insoluble by the heat treatment, are not solubilized during the extraction procedure.

(b) The heat coagulation properties and the concentration of the extracted proteins are insufficient to produce a visible coagulum when the filtrates are heated under the conditions of the extract.

It might be, however, that after extraction by a suitable method the heat denatured proteins could show the influence of different heating conditions by their heat coagulation properties, when these proteins are subjected to proper conditions for coagulation. This hypothesis was confirmed in the next experiments, which were based on the following considerations.

It was found that the iso-electric point of some meat proteins is increased by denaturation (Bendall, 1964, p. 249), but it seems that heat coagulation of fish myofibrils (cod) produces less alteration in the titration curve than is evident from the results on pork and beef (Connell, 1964). The skeletal muscles of different animals are basically similar, but it is known that fish albumens differ considerably from mammalian albumens (Hamoir, 1951, 1955). Fish sarcoplasmic proteins contain a fraction of acidic proteins of low molecular weight which are absent in mammalian tissue (Hamoir, 1961). This fraction may cause a different coagulation pattern of proteins from fish as compared with proteins from mammalian muscle.

It is well known that the solubility of a heat denatured protein and the nature of the coagulum formed are dependent on the pH and the ionic strength (Scheraga, 1963; Bendall, 1964, p. 248). At low ionic strength denatured proteins may remain in solution when the pH is about one or more units below their iso-electric points, but at these pH values the swelling is reduced by addition of salt (Bendall, 1964, p. 248; Hamm, 1963, p. 225). Above the iso-electric point the swelling and presumably the solubilization of meat proteins is promoted by salt addition and by the presence of adenosine triphosphate (ATP) or some inorganic polyphosphates. Of several inorganic polyphosphates tested only pyrophosphates and tripolyphosphate strongly promoted the swelling and water binding capacity in cooked meat and showed a similar effect as ATP (Hellendoorn, 1962). In contrast to earlier theories on this effect (Hamm, 1963, p. 227) the increase in swelling caused by these polyphosphates should not be attributed to their Ca and Mg complexing capacity (Hellendoorn, 1962; Inklaar, 1967).

Some suitable extracting agents can inhibit coagulation of proteins during heating of the extract. According to Mackie (1967) breakdown products or fragments of heat denatured fish proteins are extracted by urea, but in some cases the presence of urea was shown to inhibit heat aggregation completely (Stauff *et al.*, 1961).

The heat aggregation properties of proteins can be influenced also by the formation or breakdown of primary valence bonds, especially disulphide cross-linkages. Under proper conditions reagents such as KCN, glutathione and cysteine can increase the speed of heat aggregation (Stauff *et al.*, 1961).

Experimental and results

Fish used

The experiments were carried out with lean fish, Cape hake (*Merluccius capensis*), and oily fish, mackerel (*Scomber japonicus*) and pilchard (*Sardinops ocellata*) from commercial catches. After catch the fish was cooled to 0–2°C as soon as possible. After cooling periods varying from 1 to 4 days a fish was filleted and representative pieces from the fillets were homogenized with an equal weight of water for 1 min in a Waring blendor.

Heating treatment of homogenates

Thirty grams of the homogenates were weighed into test tubes and placed in a waterbath at 60°C or higher temperatures. When the thermometer in the centre of the test tube showed that the temperature of the waterbath (T_m) was reached the tube was quickly cooled in running tap water after different holding times (0, 5 or 30 min) at the waterbath temperature.

Extraction methods

In the preliminary experiments 25 g of the cooled homogenates were mixed with 100 ml 0·9% NaCl, 2% NaCl or 5% NaCl solution, resulting in 0·72, 1·60 or 4·0% of added salt in these mixtures. The mixtures were stirred from time to time for 1 hr, then filtered through a Whatman paper No. 42. In the other experiments at the beginning of the extraction period the pH of the homogenates was adjusted to different values by addition of some drops of 4 N-NaOH or 4 N-HCl. When the pH was adjusted to low values (3–5) the addition of salt was omitted and the weight of the salt replaced by the same weight of water. As mentioned below in a number of cases varying amounts (0·1–0·7%) of ATP, sodium pyrophosphate, KCN, glutathione or cysteine were added to the extraction media which were adjusted to higher pH-values.

Coagulation tests

Aliquots of the clear filtrates obtained after extraction were slowly heated in a waterbath to 100°C. By use of a thermometer in the centre of the test tube the temperature at which a coagulum was formed (T_c) was noted.

TABLE 1. Coagulation temperatures (T_c values) of proteins, extracted under different conditions from fish flesh homogenates which were heated to different temperatures (T_m values)

Fish species	Characteristics of extractant		Temperature of heat treatment of homogenate					
	Added salt (%)	pH	60°C	65°C	70°C	80°C	90°C	100°C
Hake	0·72	6·5	(52), 60	(55), 65·5	(70), no floc.	No floc.	No floc.	No floc.
	1·60	6·5	—	—	(65), 81	(65), 86	No floc.	No floc.
	4·00	6·5	—	—	(67), 72	86	No floc.	No floc.
	1·60	7·2	—	—	(58), 79	(62), no floc.	No floc.	—
	4·00	7·2	—	—	(61), 83	(63), no floc.	No floc.	—
Mackerel	0·72	5·9	(48), 59·5	—	(60), 70	(73), no floc.	No floc.	No floc.
	1·60	5·9	(49), 59	—	(60), 70·5	(73), no floc.	No floc.	No floc.
	4·00	5·9	(46), 56	—	(60), 70·5	—	—	—
Pilchard	0·72	5·9	(50), 60	—	(63), 77	(62), 84	No floc.	No floc.
	1·60	5·9	(47), 60	—	(53), 73	(63), 86	No floc.	No floc.

Preliminary experiments

The results of coagulation tests on heated homogenates from hake, mackerel and pilchard, using different salt levels during the extraction, are shown in Table 1. In some cases the pH of the hake homogenate–salt mixtures was adjusted to pH 7·2; the pH values measured in the other samples were without adjustment. The hake samples were found to give a higher pH value than the oily fish samples. In most cases the filtrates became milky before a coagulum was formed during further heating; the temperatures of development of milkiness are given in parentheses. In the case of hake with 0·72% salt the heating temperatures of 60° and 65°C corresponded rather well with the coagulation temperatures, but no coagulation was obtained after heating of the homogenate to 70°C. With higher salt concentrations a coagulum was formed even after heating to 70° or 80°C, but generally there was no close relationship between the heating temperature (T_m) and the coagulation temperature (T_c). In the case of mackerel (0·72–4·0% salt) T_m and T_c corresponded rather well in the range of 60–70°C. With pilchards a coagulum was also produced after heating to 80°C, but T_m and T_c were only the same after heating to 60°C. The temperatures at which milkiness developed showed no correlation with heating temperatures. This lack of correlation was confirmed in the following experiments and for this reason the formation of milkiness is not recorded in the other tables.

Extraction and coagulation at low pH values

In the following experiments with hake at the beginning of the 1 hr extraction period the pH was decreased to values ranging from 3 to 5, presumably under the iso-electric point of most proteins in the fish flesh. As mentioned before, in these cases the addition of salt was omitted. The results of these experiments, carried out with different holding times of the homogenates at different T_m values are shown in Table 2. The extraction at pH 4·5 and 5 was repeated one or two times with single fish from different catches, indicated by the letters a–f.

Acidification to pH 3 prevented coagulation in all cases, whereas at pH 4 no suitable relationship between T_m and T_c was found. After extraction at pH 4·5 the mean T_c values were slightly lower than the T_m values and at pH 5 the mean T_c values were higher, except after heating to 100°C. Moreover, after extraction at pH 5 the mean T_c values were increased with increase of holding time, especially from 5 to 30 min. In some cases after heating to 90° or 100°C no coagulation was obtained when the holding time was 30 min. As compared with extraction at pH 5, the use of pH 4·5 (with one exception) showed a better correlation of T_m and T_c values, especially after the longest holding time. For extraction at pH 4·5 the relation between maximum, minimum and mean T_c values with the corresponding T_m values, including the different holding times, is shown in Fig. 1 (a).

Acidification during extraction was also tried for oily fish (pilchard and mackerel). However, as shown in Table 2, the results were very disappointing.

TABLE 2. Coagulation temperatures (T_c values) from heated homogenates of hake (H), mackerel (M) or pilchard (P) after extraction at different pH values

Sample	pH	Holding time (min)	Minimum and maximum coagulation temperatures after heat treatment at:				
			60°C	70°C	80°C	90°C	100°C
H, a	3·0	0	No floc.	No floc.	No floc.	No floc.	No floc.
H, a	4·0	0	90	66	77	88·5	88
H, a and e	4·5	0	58–62 (60)	67·5–71 (69·3)	74·5–79 (76·8)	87·5–90 (88·8)	98–99·5 (98·8)
H, b, d and f	4·5	5	56–60 (58)	66–69 (67·5)	77–79 (77·7)	87·5–88·5 (88·2)	94–99 (96)
H, d and e	4·5	30	59–60·5 (59·8)	68·5–68·5 (68·5)	79–82 (80·5)	90·5–91 (90·8)	99, no floc.* (> 99?)
H, b and e	5·0	0	61·5–62 (61·8)	71–72 (71·5)	81–82·5 (81·8)	88·5–91 (89·8)	98–100 (99)
H, b, c and e	5·0	5	61–63 (62·2)	71–73·5 (72·5)	81–83 (82·2)	91–95·5 (93·8)	98·5–99 (98·7)
H, b, d and e	5·0	30	64–70 (66·4)	72–81 (77·8)	81–92·5 (87·5)	98·5, no floc.† (> 99·2?)	100, no floc.† (> 100?)
M, a	4·0	0	98	No floc.	—	—	No floc.
M, a	4·5	0	No floc.	No floc.	—	—	No floc.
M, a	5·0	0	No floc.	87	—	—	No floc.
P, a	4·5	0	No floc.	No floc.	No floc.	—	—

In cases of determination on different fish per treatment minimum and maximum temperatures and averages (in parentheses) are listed.

* Coagulation developed during cooling of the filtrate after being heated to 100°C.

† See 'Discussion'.

Extraction and coagulation at higher pH values

Further experiments with oily fish by extraction at pH 7–8 with addition of salt did not give coagulation after heating to temperatures higher than 70°C. Extraction at pH 5 with 0·72% salt and 0·4% sodium pyrophosphate gave no coagulation with the filtrate adjusted to pH 5 and when the filtrate was adjusted to pH 6 flocculation already occurred at room temperature. The use of 0·3% ATP and 0·72% NaCl in the extraction medium at pH 5·4 or 6·0 gave satisfactory coagulation only after heating up to 70°C.

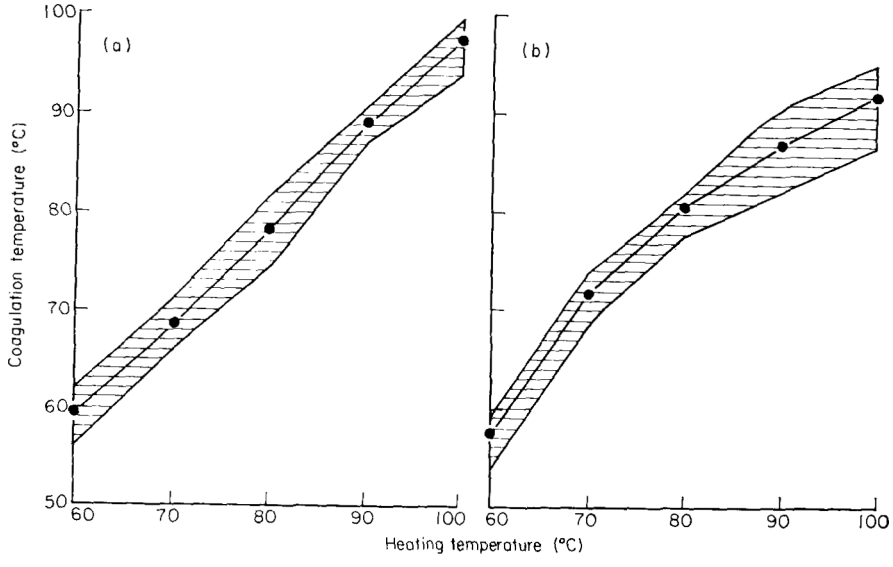

FIG. 1. Heating temperature (T_m value) of homogenates from hake (a) and oily fish (b) *versus* maximum, minimum and mean coagulation temperatures (T_c values) of their extracts.

TABLE 3. Coagulation temperature (T_c values) in filtrates adjusted to pH 6 obtained from heated homogenates of mackerel (M), pilchard (P) or hake (H) after extraction with 0·72% NaCl and 0·4% Na-pyrophosphate at pH 7

Sample	Holding time (min)	Coagulation temperature after heat treatment at: 60°C	70°C	80°C	90°C	100°C
M, a	0	—	74	—	85·5	—
M, b*	0	56	72·5	81·5	90	92
M, d	0	—	71·5	78	86·5	—
M, a	5	58·5	71	79·5	86·5	95
M, b*	5	57	72	80	91	87
M, c	5	53·5	69	81·5	91	93·5
P, a	5	59	71	82	88	90·5
M, a	30	—	74	—	83	—
M, e	30	—	—	—	86	90
P, a	30	59	72	82	87	92
Oily fish: minimum to maximum	0–30	53·5–59	69–74	78–82	83–91	87–95
average		(57·2)	(71·9)	(80·6)	(87)	(91·6)
H	5	65	82	77†	80†	80†

* Filtrates became milky after adjustment to pH 6.

† Filtrates became very cloudy but no real coagulation took place.

Coagulation after heating to higher temperatures was obtained, however, when sodium pyrophosphate was added during extraction in the pH range 7–8. When the pH of the filtrate was left at pH 7 coagulation took place at too low temperatures and a similar unsatisfactory relation between T_m and T_c values was found after extraction at pH 8, followed fly adjustment of the filtrate to pH 6 or 7. Finally the best results were found when using an extraction with 0·72% NaCl and 0·5% sodium pyrophosphate at pH 7, followed by adjustment of the pH of the filtrate to pH 6. The results are shown in Table 3. Since the difference between T_c values obtained after heating to 90° or 100°C was rather small and in these cases the variation of individual T_c readings was rather great, it was tried to improve these results by addition of varying amounts (0·1–0·7%) KCN, cysteine or glutathione, but the T_c values were not influenced by these agents. It was noted, however, that with the addition of KCN the coagulum tended to be heavier. The relation between maximum, minimum and mean T_c values with their corresponding T_m values is shown in Fig. 1(b).

Finally, the extraction and coagulation method, found to be most useful for mackerel and pilchard, was tried for hake. As shown in Table 3 the correlation between T_m values and corresponding T_c values was very poor.

Discussion

Heating temperatures of fish proteins which still gave coagulation after extraction with low salt contents (0·72%) such as used in conventional coagulation tests (Coretti, 1957; U.S.D.A.–A.I.Q., 1961) were 65°C for hake, 70°C for mackerel and 80°C for pilchard. With a salt content of 1·6–4·0% coagulation of extracted hake proteins could be obtained even after heating to 80°C. In contrast with this, application of 1·6% salt gave no coagulation of mackerel proteins after heating to 80°C (Table 1). Though both oily fish species showed a different coagulation pattern after extraction with different salt concentrations, the same modified coagulation test could be used for mackerel and pilchard in order to obtain positive coagulation results after the use of T_m values ranging from 60° to 100°C. For this purpose, however, a completely different modification had to be used for hake (Tables 2 and 3). It is not known whether these different results were caused by differences in protein composition, differences in pH during the initial heat treatment (Table 1), or by other factors such as differences in oil content or free fatty acids in lean and oily fish flesh.

At conventional low salt levels (0·72%) the results with hake (Table 1) and pork as reported by Coretti (1957) are rather close, since the maximum heating temperatures still giving a positive coagulation test were 65° and 63°C, respectively. It may be worthwhile to apply similar modifications of coagulation tests as described above to heated pork and beef products. Nevertheless, it should be remembered that the fraction of acidic low molecular weight proteins found in fish muscles, which is believed to be absent in mammalian tissue (Hamoir, 1961), may be of special importance

for the successful application of modified coagulation tests to heated fish products. As a matter of fact different protein fractions correspond in a different manner to coagulation tests, which is shown by the development of milkiness before a heavier coagulum was formed. Moreover, after application of the same extraction method the temperature of development of milkiness and the coagulation temperatures were influenced in a different manner by the variation of heating temperature (Table 1).

With hake homogenates the best correspondence between T_c and T_m values (including varying holding times) in the temperature range of 60–100°C was obtained with filtrates produced by extraction at pH 4·5. The nearly straight-line relationship found (Fig. 1a) can be expressed by the equation $T_m = 1{\cdot}02.\ T_c - 0{\cdot}2$. The standard deviation of T_m values calculated from T_c values, and the actual applied T_m values is 2·0°C. As can be seen from Fig. 1(a) the maximum deviation of single observations from their corresponding mean T_c values was not strongly influenced by the variation of T_m values; the smaller maximum deviations after heating to 90°C might be purely incidental.

When the hake homogenates were extracted at pH 5 the T_c values tended to increase rather strongly with holding time. The fact that at this pH in some cases after heating to 90° or 100°C and 30 min holding time no flocculation was obtained (Table 2) might be caused by an increase of the coagulation temperatures over 100°C, which could not be recorded by heating of the filtrates in open test tubes in a waterbath. However, these negative results may be explained also by the assumption that after these strong heat treatments the extraction at pH 5 in some cases failed to produce coagulating proteinaceous material.

When using the technique for oily fish as described above (extraction at pH 7 in the presence of 0·72% added salt and 0·4% sodium pyrophosphate, followed by adjustment of filtrates to pH 6) the relation between T_c and T_m values in the temperature range of 60–100°C could not be expressed by a straight line (Fig. 1(b)). In the temperature range of 60–80°C the relation can be described by the equation $T_m = T_c + 0{\cdot}1$, the standard deviation between calculated and applied T_m values being 2·6°C. For the temperature range of 80–100° C the equation $T_m = 1{\cdot}47.\ T_c - 37{\cdot}0$ and a standard deviation of 3·9°C were found. From Fig. 1(b) it can be seen that maximum deviations of single T_c values from their corresponding mean values increased with increasing T_m values. For this reason and the fact that the difference between T_c values after heating to 90° and 100°C was rather small it is difficult to make an accurate calculation of T_m values from T_c values in this high temperature range.

It was found that the coagulation properties of filtrates were not altered by storage during some days at about 2°C. However, neither the influence of time delay between heating of homogenates and extraction of proteins nor the influence of homogenization of fish after heat treatment were studied. Moreover, no attention was paid to the influence of seasonal factors.

According to other experiments (Doesburg & Papendorf, 1967) the relation between heating temperature and coagulation temperature can be lost when the cooked product is dehydrated and stored in dehydrated form before application of the modified coagulation test is carried out. Samples of presscake of cooked anchovy (*Engraulis japonicus*) gave T_c readings up to 96°C when subjected to the modified coagulation test for oily fish, but the corresponding dehydrated fish meal gave no coagulation when the filtrates were tested at pH 6; in filtrates kept at pH 7·0 T_c values of 60–65°C were obtained.

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The rate of phospholipid hydrolysis in frozen fish

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Summary

Phospholipid hydrolysis was studied in lemon sole and haddock between −7°C and −29°C. The rate of reaction was much faster in the Gadoid. The haddock data showed evidence for a rapid first order reaction in which lecithin and phosphatidylethanolamine containing $C_{16:0}$, $C_{18:1}$ and $C_{20:5}$ acids were preferentially hydrolysed. The reaction proceeded to an asymptote which decreased with lowering temperature. The amount of free water available in the frozen state seemed important in these hydrolytic reactions.

The relation of these findings to protein denaturation and taste panel assessment of texture are discussed.

Introduction

Love (1962) noted that insolubilization of actomyosin in salt solution, the measurement of toughness of fish as measured by his cell fragility method and the production of free fatty acids (FFA) from phospholipids in cold stored cod (*Gadus callarias*) all had remarkably similar activation energies. The first two reactions appeared to be going to completion at all temperatures but the phospholipid hydrolysis did not appear to be approaching the same asymptote at all temperatures (Olley & Lovern, 1960; Lovern & Olley, 1962). The reaction appeared to go almost to completion at temperatures just below the freezing point while at lower temperatures the asymptote became less. The activation energy of phospholipase activity for cold stored cod was calculated by Olley & Jason (1962) based on the data provided by Baines (unpublished results 1960) who had found that the data could be reasonably well fitted to a first order reaction. He used the maximum likelihood method of Stevens (1951) to obtain the reaction coefficient and asymptote. In the earlier work only a few points were obtained at the lower temperatures but the work of Bengtsson & Bosund (1966) on unblanched frozen peas confirmed the observation. They found that at lower temperatures the ratio of polyunsaturated to saturated FFA liberated was markedly reduced in frozen peas and postulated that unsaturated fat has a low crystallization point and stays longer than saturated fat as a liquid phase when the temperature is lowered. This would facilitate enzymatic reactions by allowing the substrate to diffuse

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to the enzyme. They assumed that the reaction was very much slowed down when the liquid fat at each temperature had been hydrolysed.

When Olley, Pirie & Watson (1962) published work on the FFA production at −14°C in the cod, lemon sole (*Microstomus kitt*) and halibut (*Hippoglossus hippoglossus*) they pointed out that all three species eventually produced much the same amount of FFA although the amount of protein denaturation was very different. They later did analyses on samples of these species which had been for even longer periods in cold store at −14°C. When Baines (unpublished results 1963) later applied the maximum likelihood method of Stevens (1951) to first order reaction curves for these fish it was evident that the Gadoid was producing FFA at a much faster rate than the flat fish although eventually all species produced over 300 mg FFA/100 g flesh. Baines' (unpublished results 1963) interpretation of the data was that there seemed to be a rapid first order reaction followed by a much slower one. The data on frozen peas might also suggest this.

The present work was undertaken before the work on peas appeared. It was done to confirm the more rapid rate of production of FFA in Gadoids, to check the limited amount of data on change in asymptote with temperature and to try and assess the reason for this change. It was realized that if more than one reaction was occurring an activation energy for total phospholipase activity would no longer be valid. Had the activation energy of many reactions in the frozen state turned out to be the same then the logical conclusion in quality control would have been to use the simplest analytical criterion. FFA determination would have appeared to offer a very simple technique.

Experimental

Fish

Haddock (*Gadus aeglefinus*) and lemon sole were selected as examples of a Gadoid and a flat fish, respectively. The fish were caught in June 1965 and filleted after 3 days on ice. Fillets were stacked so that three haddock fillets and six lemon sole fillets lay on top of one another. The fillets were air blast frozen at −29°C and each pile of fillets wrapped in aluminium foil and stored at either −7°, −14°, −20° or −29°C. A pile was withdrawn from store at intervals and a frozen core taken with a special cutter which penetrated through the middle of the pile of fillets. The core was used for FFA and phospholipid determination, the outer ring was returned to the −29°C cold store wrapped in aluminium foil for further study.

Loss of FFA during chloroform–methanol–H_2O extraction of fish

Olley & Lovern (1960) noted that when iced cod had been stored for a considerable period, appreciable quantities of the FFA could be lost in the methanol–water phase of the lipid extraction. Keay (unpublished results 1967) found that the acids lost in the methanol–water phase were fairly representative of the total FFA released; for example

31% of $C_{16:0}$, 12·5% of $C_{18:1}$, 17% of $C_{20:5}$ and 24% of $C_{22:6}$ as against 21, 10, 15 and 35% of these acids in the methyl esters from the phospholipids of fresh cod (Olley & Duncan, 1965). The pH of stale fish on ice rises above 7 and significantly enhances the loss of FFA into the methanol–H_2O phase of a lipid extraction; with frozen fish the pH stays well below 7 for most samples and losses appear to be negligible. No increase in FFA was obtained by acidification of the chloroform–methanol–H_2O. Keay's (unpublished results 1967) work, however, shows that any small losses that might occur would not be preferential. It is interesting to note that Keay (unpublished results 1967) found that an acetone extraction to take the water out of a sample of stale cod preferentially dissolved polyunsaturated FFA; $C_{20:5}$ and $C_{22:6}$ accounting for 80% of the FFA extracted into acetone. In view of the small quantity of FFA lost into the methanol–water phase in frozen fish and the similarity of that which passes into methanol–water to the total, the Hanson & Olley (1963a) procedure for the extraction of lipid was used unmodified.

Analytical methods

The lipid was extracted from 10 g of the central core by the method of Hanson & Olley (1963a). Phosphorus was determined on a 1–2 ml aliquot of the chloroform phase by the method of King (1932). Another 2–5 ml aliquot was separated from phospholipid on a 2 g silicic acid column (Hanson & Olley, 1963b). The neutral lipid passed through in the chloroform phase and the FFA was determined by the method of Duncombe (1963). The outer ring was later examined to see if changes in lipid composition could explain the different asymptotes for FFA production reached at different temperatures. Samples were chosen for analysis which had produced roughly similar amounts of FFA at different temperatures. The lipids were extracted as described above and the neutral lipids and phospholipids separated on a 3 g silicic acid column. The FFA were separated from the neutral lipids by the method of McCarthy & Duthie (1962). Phosphorus was determined in the total phospholipid eluate from the silicic acid column by the method of King (1932). The phospholipid was dissolved in a small quantity of chloroform and applied as a series of close spots to a silica gel G thin-layer plate which had been activated at 100°C and then washed with chloroform. The plate was developed in a Shandon TLC Chromatank lined with filter paper soaked in the developing solvent (65 : 25 : 4, vvv, chloroform–methanol–water, Wagner, 1960) to ensure saturation of the atmosphere in the jar with solvent vapour. After development of the plate and removal of solvent it was transferred to another tank containing a few crystals of iodine. The lipids were stained as lightly as possible and the phosphatidylethanolamine and lecithin bands were removed from the plate using the filter device of Goldrick & Hirsch (1963). The solvent used to remove the two phospholipids from the plate was adapted from the recommendations of Skipski, Petersen & Barclay (1964). The lecithin was eluted with: (1) 20 ml of 25 : 15 : 4 : 2, vvvv chloroform–methanol–acetic acid–water, (2) a further 10 ml of the same solvent,

(3) 10 ml of methanol and (4) 10 ml of 94 : 1 : 5 methanol–acetic acid–water. The phosphatidylethanolamine was eluted with 10 ml of (1), 5 ml of (2), 5 ml of (3) and 5 ml of (4). An aliquot of the phospholipid extracts was used for phosphorus determination.

The FFA and phospholipids were methylated by the method of Keay (unpublished results 1967). 10-mg samples were refluxed in 2 ml of anhydrous methanolic 2 N-HCl for 1 hr. Benzene (0·25 ml) was added to maintain solution of the esters. The reaction mixture was diluted with distilled water (8 ml) followed by two extractions with ethyl ether (10 ml). The ether extract was dried with anhydrous magnesium sulphate.

In all the manipulations mentioned above including the original extraction of lipid from the fish, a small crystal of BHT was added to the solvents to prevent oxidation. The methylated fatty acids were stored at −12°C in 1 : 1 methanol–acetone with BHT and injected onto a GLC column in this solvent.

The Pye-argon gas chromatograph was fitted with a 4-ft column of 5% Apiezon L on chromasorb G (acid washed 80–100 mesh). The instrument was run at 220°C with an argon flow rate of 20 ml/min.

Results

The breakdown of phospholipid and production of FFA in haddock and lemon sole are shown in Fig. 1. The reaction was very much more rapid in the haddock and the experiment was continued for a sufficient time for a first order reaction plot to be fitted to the data. The asymptote and rate constant for FFA production is shown in Table 1. Reliable predictions of rate constant and asymptote for phospholipid breakdown

TABLE 1. Rate constant of FFA production in haddock and asymptote of first order reaction as predicted by the maximum likelihood method

Temperature	Rate constant			Aysmptote of FFA production	
(°C)	k^{-1} days	95% confidence limits		mg/100 g flesh	Standard error
−7	−0·0675	−0·085	−0·05	300·0	±12·3
−14	−0·044	−0·063	−0·025	200·0	±12·0
−20	−0·012	−0·022	−0·003	227·0	±18·2
−29	−0·0143	−0·044	+0·009	102·6	±16·4

could not be obtained as the phosphorus results were much more scattered than the results for FFA production.* The production of FFA in the sole was so much slower that in the time chosen for the experiment the final data at each temperature were too far from the asymptote to predict its value accurately. The phospholipid results at −14°C (●) were too scattered to even put a curve through them. However, it

* Olley (1965) has shown that phospholipid may be depleted relatively more rapidly than neutral lipid in starved fish. Two haddock caught in April 1968 had only 370 and 340 mg phospholipid/100 g flesh. The season would indicate that these fish were spent.

is quite apparent that phospholipid breakdown and FFA production is much slower at all temperatures in the flat fish as compared to the Gadoid.

Because of the clearer picture in the haddock this species was selected for more detailed study of the lipid breakdown. At −29°C the FFA production was proceeding to a much lower asymptote and the storage of the samples for a further year produced little extra FFA at −29°C and the GLC pattern of the FFA and proportions of lecithin and phosphatidylethanolamine hydrolysed could be taken as representative of the data at the higher storage temperature at which the haddock was first kept. There

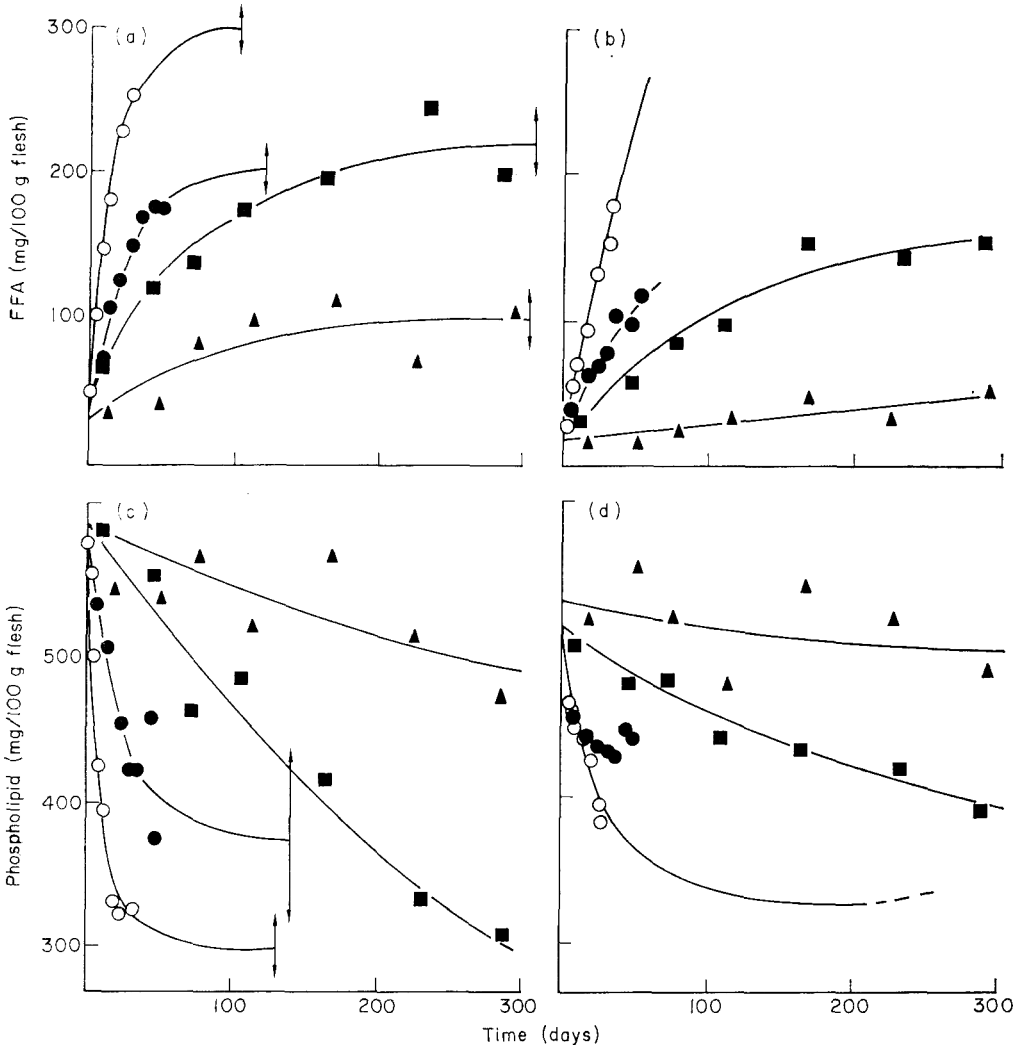

FIG. 1. Phospholipid breakdown and FFA production in cold stored haddock and lemon sole. (a) Haddock FFA, (b) lemon sole FFA, (c) haddock phospholipid, and (d) lemon sole phospholipid. ○, −7°C; ●, −14°C; ■, − 20°C; ▲, −29°C.

TABLE 2. Composition of FFA released from haddock phospholipid at four different cold storage temperatures

Temperature of storage	Time of storage (days)	FFA* produced (mg/100 g flesh)	GLC									Acids unsaturated/ saturated
			14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 4	20 : 1	20 : 5 †	22 : 6	
— 7°C	8	149	3·7	25·6	4·8	3·9	14·9	3·2	3·4	18·4	13·6	2·0
— 14°C	21	125	3·5	25·9	4·3	3·9	14·0	2·5	2·2	17·2	16·3	2·0
— 20°C	42	120	4·2	27·8	4·7	3·4	13·0	3·2	3·7	18·0	15·7	1·83
— 29°C	831	126	4·0	25·3	4·9	3·3	15·0	3·1	3·2	18·6	12·2	2·06

* Approximately 30 mg of extra FFA were produced during subsequent storage of the samples — 7°, — 14° and — 20°C at — 29°C Thus ~ 80% of the FFA was produced at the temperature stated and 20% at — 29°C. However, as there was no trend in the results the dilution by 20% of FFA produced at — 29°C would have had no influence on the composition.

† The column was not temperature programmed and therefore the $C_{22\cdot5}$ peak was very broad and flat. It never amounted to more than 4% of the total methyl esters either in the FFA or the remaining phospholipids.

was no evidence that production of large quantities of FFA at a higher temperature enabled the reaction at −29°C to proceed any faster or further (see footnote to Table 2). Table 2 shows that the GLC pattern of the FFA produced at all four temperatures was similar and Table 3 shows that up to the production of ~ 180 mg FFA/100 g flesh the proportions of lecithin and phosphatidylethanolamine hydrolysed were the same. The ratio of unsaturated to saturated acids remained at a value of ~ 2 for all storage temperatures (Table 2). As there was no trend in the composition of FFA

TABLE 3. Effect of temperature on proportions of lecithin and phosphatidylethanolamine hydrolysed on cold storage of haddock flesh

Storage		FFA produced during cold storage	Ratio phosphatidylethanolamine–lecithin
Temperature (°C)	Time (days)	(mg/100 g flesh)	
Fresh	0	0	20 : 80
−7	8	149	21 : 79
−7	12	179	19 : 81
−14	21	125	23 : 77
−14	34	170	22 : 78
−14	48	174	28 : 72
−20	42	120	27 : 73
−20	105	176	22 : 78
−29	790	138	22 : 78
−29	831	126	26 : 74

TABLE 4. Composition of the FFA produced and remaining unhydrolysed phospholipid in cold-stored haddock at different stages in the hydrolysis

Fatty acid	Less than 150 mg FFA			150–200 mg FFA		
	FFA	Lecithin	Phosphatidylethanolamine	FFA	Lecithin	Phosphatidylethanolamine
$C_{14:0}$	4·0	3·2	3·9	3·8	3·3	3·5
$C_{16:0}$	26·3	26·9	15·0	32·5	20·5	14·7
$C_{16:1}$	4·6	3·4	3·7	3·1	3·7	3·5
$C_{18:0}$	4·1	5·1	7·0	3·3	5·6	6·0
$C_{18:1}$	15·0	12·3	10·6	15·1	12·4	10·9
$C_{18:4}$	2·6	1·6	2·0	2·6	1·9	2·5
$C_{20:1}$	3·2	1·6	2·1	2·9	1·9	2·3
$C_{20\ 5}$	16·7	15·1	11·0	19·6	13·6	10·9
$C_{22:6}$	12·9	21·2	25·2	12·5	24·0	30·0

with temperature the results for Table 4 were grouped in a different way. Data for fish which had produced under 150 mg of FFA were pooled and those with a production between 150 and 200 mg. GLC of the fatty acid methyl esters of the FFA and of

the lecithin and phosphatidylethanolamine remaining unhydrolysed in these samples showed that the FFA released was not representative of the phospholipid from which it derived. The $C_{16:0}$, $C_{18:1}$ and $C_{20:5}$ acids became a greater proportion of the FFA and the $C_{18:0}$ and $C_{22:6}$ acids were concentrated in the remaining phospholipid. The effect was most noticeable after at least 150 mg of FFA had been produced, enough phospholipid having by then been degraded to markedly change the composition of the remainder. The effect applied to both lecithin and phosphatidylethanolamine.

Discussion

Change of FFA asymptote with temperature

Riedel (1956) has determined the amount of free water as a percentage of the total water in fish at temperatures below the freezing point. He quotes values of 8, 9, 11 and 16% free water in haddock at −29°, −20°, −14° and −7°C, respectively. Thus phospholipase activity in frozen fish is taking place in different water concentrations at different temperatures. This change in water content in the system in this case caused by temperature is reminiscent of the work of Acker (1965) on the effect of different relative humidities on phospholipase activity but at constant temperature. In both cases a reduction of available water results in the initial reaction not going to completion and an apparent asymptote being obtained, the less water the lower the asymptote. Acker (1965) explained these results on the basis of the necessity for capillary water. Jason (1965) considered that Acker's graph obtained by plotting rate of hydrolysis against relative humidity fitted a type II BET isotherm indicating that a monolayer of water was first laid down followed by multilayers. The monolayer water would presumably have to be subtracted from Riedel's calculation of free water. Whatever the mechanism it is apparent from Acker's work on cereals, Bengtsson & Bosund's (1966) work on frozen peas and the present work on haddock that the extent of the initial rapid reaction would appear to be limited by water. The explanation offered for cereals and peas given by Acker (1965) and the Swedish workers does not apply to fish. The latter authors postulated that the substrate must be liquid for the reaction to proceed, and as the temperature was lowered more and more polyunsaturated acids relative to the total acids were released from peas. In the present work the general effect of a lowered asymptote with a lower free moisture content was still noted although the FFA released at all temperatures had the same composition. Ganrot (unpublished results 1966) also found that the FFA released from herring at temperatures ranging from +18° to −15°C had the same composition. The fatty acids comprising the phospholipids of fish are very much more unsaturated than those of peas and cereals and it is possible that the polyunsaturated chain in the α position on almost all phospholipid molecules was sufficient to keep all the phospholipids liquid even at the lower temperatures. The changing pattern of FFA release found in peas was therefore not noted although the general effect of reduction in asymptote was.

It should be emphasized that in Acker's work the limiting amount of water was far in excess of that stoichiometrically required for lipid hydrolysis and it is presumably also far in excess in the frozen state, although the authors have made no attempt in the present paper to assess the amount of monolayer water at each temperature.

Preferential hydrolysis of $C_{16:0}$, $C_{18:1}$, $C_{20:5}$ phospholipids

The similar rates of hydrolysis of lecithin and phosphatidylethanolamine were in agreement with the previous work of Lovern, Olley & Watson (1959) on cod stored on ice and of Bengtsson & Bosund (1966) on peas stored at −20°C but were at variance with the work of Bligh & Scott (1966) with cod stored at −12°C where lecithin hydrolysed faster. The preferential hydrolysis of lipids containing $C_{16:0}$ acids was in agreement with the work of Moore & Williams (1964). Calculating $C_{16:0}/C_{18:0}$ ratios from Tables 6 and 7 of their paper shows that there is a more or less steady increase in this ratio in the lysolecithin produced by the action of phospholipase A on egg yolk lecithin while there is a decrease in the ratio on the unhydrolysed lecithin remaining in the reaction. This would appear to indicate that molecules with $C_{16:0}$ in the α position are more readily attacked by phospholipases than those with $C_{18:0}$ in the α position. Because most phospholipids have an unsaturated fatty acid in the β position and because in fish muscle both α and β fatty acids are removed from the phospholipid, one would have expected to find an unsaturated acid also preferentially removed and this in fact turned out to be the case; the $C_{20:5}$ acid being markedly enhanced in the FFA fraction while the $C_{22:6}$ acid was concentrated in the unhydrolysed phospholipid. A marked change in the palmitic : stearic ratio in the unhydrolysed phospholipid of cod stored at −14°C had previously been noted by Olley & Duncan (1965). The effect was also noted by Keay (unpublished results 1967) in plaice (*Pleuronectes platessa*) stored for 12 months at −14°C. The plaice FFA contained 19% $C_{16:0}$, the remaining phospholipid only 7%; the FFA contained 27% $C_{20:5}$ and 17%$C_{22:6}$. The position in the phospholipids was reversed, the phospholipid remaining contained 15% $C_{20:5}$ and 29% $C_{22:6}$. The effect was not noted in a Clupeoid.* Bosund & Ganrot (1968) found no difference in the composition of the lecithin and phosphatidylethanolamine from fresh herring (*Clupea harengus*) and that from herring which had been stored at −15°C for 85 days nor did the FFA released show the pattern described above.

* Ganrot (personal communication) has pointed out that about 50% of the fatty acids in the phosphilipids of the herring used in his experiments were saturated and therefore the usual hydrolysis pattern for phospholipids would have obtained, that is liberation of the unsaturated acid on the β position by phospholipidase A followed by the liberation of the saturated acid on the α position by phospholipase B. In cod, haddock and plaice examined at Torry Research Station only 30–35% of the total phospholipid FFA were saturated and there must therefore have been phospholipids with unsaturated acids on the α as well as the β position. There was, therefore, more likelihood of different rates of hydrolysis for different phospholipids in the latter three species.

Activation energy of phospholipid hydrolysis

Determination of the activation energy of FFA production in haddock and lemon sole was rendered impossible for a number of reasons. As has been stated above, different molecular species of lecithins and phosphatidylethanolamines hydrolyse at different rates and these reactions go to different asymptotes at different storage temperatures. An attempt was made by the maximum likelihood method of Stevens to fit a first order reaction to the fast initial breakdown of phospholipid. However, the haddock results in Table 1 show that the determination of rate constant can be deceptive. The fish at $-20°C$ were clearly producing FFA at a faster rate than those at $-29°C$ (Fig. 1) but because the ultimate asymptote of the first order reaction was higher at $-20°C$ the relative time taken to reach this was the same as the relative time taken to reach the lower asymptote at the lower temperature. It is probably not reasonable to attempt an Arrhenius plot for enzymic lipid hydrolysis in the frozen state. Tappel (1966) has reviewed the evidence for changing activation energies with decreasing temperature and points out that a multiplicity of factors are involved.

Relationship of FFA production to protein denaturation and taste panel assessment

The previous supposition that protein denaturation could not be caused by FFA in the cod and lemon sole (Olley *et al.*, 1962) because when both species had produced $\sim$ 300 mg FFA the cod was completely denatured and the lemon sole only some 30% has had to be reconsidered in the light of the possible protective effect of small quantities of neutral lipid (Olley *et al.*, 1967). A further difference in the two genus is shown in the present work. In the flat fish the initial hydrolysis occurs at a much slower rate; thus other reactions which might inhibit subsequent denaturation of muscle protein by FFA would have more time to occur. For example in the lemon sole Olley *et al.* (1967) noted that at $-7°C$ proteolysis and fragmentation of myofibrils sometimes occurred rather than aggregation. Rate of FFA production could thus be more important than absolute amounts.

Aggregation of myofibrils as measured by cell fragility and insolubilization of actomyosin in salt solution go to completion at all temperatures in the cod (Love, 1962)* and presumably in the haddock. These changes in cod could be fitted to first order reaction curves. It would appear that if FFA production does not go to completion at all temperatures or if it does so, it is at two distinct rates (an initial rapid reaction followed by a much slower one) then FFA production and actomyosin insolubilisation cannot both equate to a taste panel for texture at all temperatures. Peters *et al.* (1968) have recently correlated FFA production with overall taste panel scores for frozen cod, but these fish were all stored at one temperature, $-18°C$ and only processed in different ways. Had they attempted the correlation at different storage temperatures the significance might not have been so high.

* It should be noted that this statement is inferred from statistics rather than from experimental observations which would have taken years to make at the lower temperatures.

The insolubilization of the actin-myosin complex and the aggregation of myofibrils are reactions which presumably do not need water or enzymes. Reactions in which water is either involved or needed for transport of enzyme or substrate may not have the same time–temperature tolerance as reactions in which water is not involved.

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The connective tissues of fish. II. Gaping in commercial species of frozen fish in relation to rigor mortis

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Summary

Fish of different species frozen whole, thawed and filleted gape according to the time after death at which they were frozen. Those frozen immediately after death gaped least, a marked increase accompanying the onset of rigor mortis. Thereafter, there was a steady increase in gaping as the fish were held for longer periods in melting ice prior to freezing. The cause of this second type of gaping is unknown.

There was a marked difference between species, haddock showing the most gaping and catfish and skate none at all. The three species of flat fish gaped less than the four round fish (apart from catfish) species, which fact may relate to the shape of the fish, especially as the roundest-bodied of the flat fish, halibut, gaped the most of the group.

The site of gaping was identified histologically, and different degrees of gaping are illustrated by means of photographs.

Introduction

The first paper in this series (Love & Robertson, 1968) described gaping, a phenomenon in which the sheets of connective tissue in fillets of cod (myocommata) failed to hold the blocks of muscle (myotomes) together. It was shown that if the fish were frozen as round fish before rigor mortis, thawed and filleted, there was little or no gaping, but that if they were frozen in rigor mortis, 1 day after catching, the amount of gaping depended on the biological condition of the fish, healthy fish showing much gaping while starving or spent fish showed little or none. It was postulated that the rigor mortis contraction of the muscles was strong in healthy fish, so that when the connective tissue was weakened by ice forming within it the muscular contraction could sometimes rupture it. Conversely, the contraction of fish in poor biological condition was too weak to break the connective tissue.

In the present work, a number of species of commercial importance was examined to see whether the phenomenon was a general one, with reference to freezing before,

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in and after rigor mortis. The results of histological work identify the connective tissue strands which actually hold the fish together.

Experimental

Fish were caught by trawl net on the Station's research ship 'Sir William Hardy' between 26 April and 4 May 1967 from grounds ranging from Faroe Bank to South East Iceland. The number of fish used at each of the three times after death depended on the greatest number it had been possible to catch in a single haul, and ranged from two fish (skate and halibut) to nine (haddock).

The species, with ranges of body lengths, were as follows:

Catfish (*Anarhichas lupus* L.), 53·5–91 cm;
Haddock (*Gadus aeglefinus* L.), 38·5–70·5 cm;
Cod (*Gadus morhua* L.), 74–108 cm;
Saithe (*Gadus virens* L.), 71·5–96 cm;
Halibut (*Hippoglossus hippoglossus* L.), 43–58 cm;
Lemon sole (*Pleuronectes microcephalus* Day), 26–42 cm;
Plaice (*Pleuronectes platessa* L.), 30–58·5 cm;
Skate (*Raja batis* L.), 38–57 cm;
Redfish (*Sebastes marinus* L.), 26·5–40·6 cm.

In the case of haddock there were sufficient fish (from Faroe Bank and North Faroe) to do two complete experiments. After each haul the fish were counted, and if there was a sufficient number from any species it was gutted and divided into three groups. The first was frozen immediately and the other two were packed in melting ice, one for 18 hr and the other for 120 hr. At the end of these times the fish were frozen, like the first batch, in the cold room of the ship at − 30°C, and kept near this temperature on the ship and at the Research Station afterwards until required, up to 4 months later.

Thawing was achieved by leaving for 1 day at 4°C after which the fish were filleted and examined. The following procedures were used: gaping was scored subjectively on the scale illustrated in Plate 1, ranging from 0 (no gaping or longitudinal splitting) to 5 units (dropping to pieces) not 0 to 6 as in the previous work. The system suffers from the usual disadvantage of subjective scoring in that the numbers do not bear mathematical relationships to one another and so strictly speaking should not be averaged. However, until a suitable objective scale is found it is probably the most useful way of obtaining information.

Changes in the mechanical properties of the muscle tissue were measured by the cell fragility method (Love & Mackay, 1962).

Differential staining of connective tissue by conventional methods was found to be unreliable with the cod muscle under investigation, and a modified technique was evolved (J. Lavéty, unpublished). In this, blocks of tissue about 5 mm^3 were fixed in formaldehyde for 24 hr, washed, dehydrated in dioxan, embedded in a suitable medium

and sectioned. After removing the wax with xylol, the sections were placed in van Gieson's picro-fuchsin (microscopical stain, code No. 3127, British Drug Houses, Poole, Dorset) for 3 min. They were then rinsed in tap water, dehydrated and mounted, and the finished slides were exposed to bright sunlight for up to 5 hr. The colour was checked with the microscope from time to time, because excessive bleaching reduced the colour intensity in the connective tissue. This procedure gave muscle cells of a pale brownish colour, among which the connective tissue stood out as a slash of brilliant scarlet, and slides prepared in this way have shown no signs of deterioration after 18 months.

Results and discussion

In the first place it was desired to identify the tissue which was in fact holding the fish together. Previous sections stained with Haematoxylin and Eosin for other purposes had merely shown the ends of the muscle cells abutting the mycommata, without indicating what was retaining them there. Using the present strongly differentiated colour it was clear that fine processes of connective tissue were leaving the mycommata and reaching along between the cells (Plate 2). Since no break or termination was seen, it seems likely that they eventually joined up with the next myocomma. One can, therefore, envisage two adjacent myocommata, approximately parallel with each other, joined by a scaffolding of connective tissue strands, the spaces in between being filled with contractile muscle protein. Cross sections showed that what appeared to be 'strands' in Plate 2 are in fact tubular and surround each cell, so the distinction between them and the cell walls vanishes soon after leaving the main sheet of connective tissue. It is clear that if the tubes break at the point where they join the myocomma, gaping will occur. This locality is therefore the important one in the present study.

When the different species were studied, it was found that neither catfish nor skate gaped under any treatment, so these results are not illustrated. The remaining species showed a remarkably consistent behaviour (Fig. 1), fish frozen whole before rigor mortis gaping scarcely at all, apart from haddock.

Fish which had been frozen *in* rigor mortis all showed a sharp increase in gaping compared with pre-rigor. After 5 days in ice they showed a further increase, generally rather slight. Sufficient plaice could be obtained only for in-rigor and post-rigor freezing from one haul. Three plaice from a different haul frozen before rigor (not illustrated) did not gape. On applying Student's *t*-test at a 5% significance level, the increase in gaping from the pre-rigor to the in-rigor state was significant in all cases except halibut. The weighted mean increase for all the fish was 0·67 units. Similar testing from in-rigor to post-rigor usually gave a significant increase, the exceptions being one batch of haddock and the cod and lemon sole. The weighted mean increase for all the fish was 0·42.

In order to locate more exactly the point at which gaping markedly increased, a further trip was carried out in November 1967, cod and haddock from the East coast

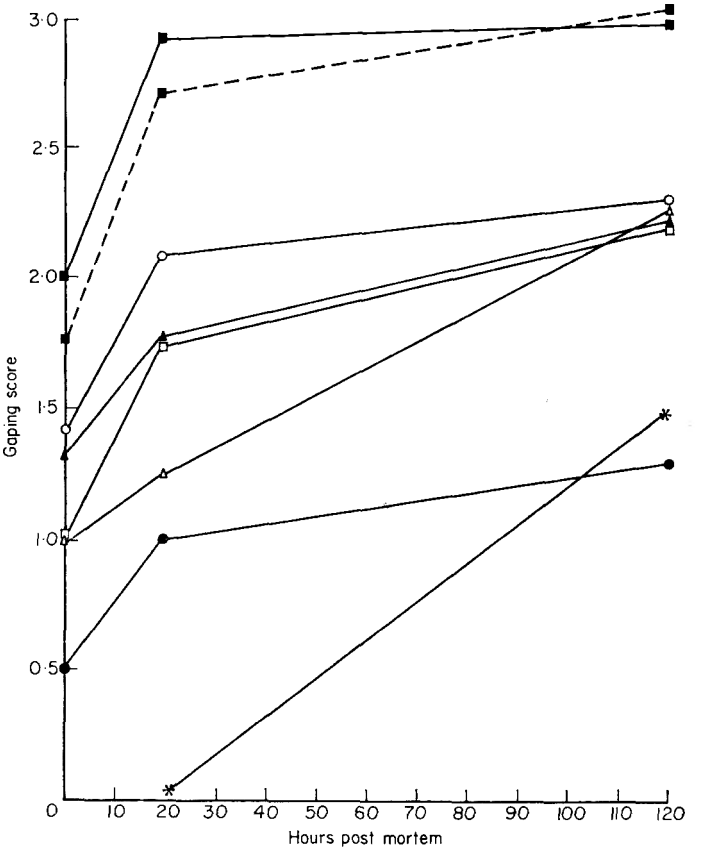

FIG. 1. Gaping in various species of fish kept in melting ice for increasing time after death before freezing (whole, gutted) at −30°C, thawing and filleting for examination. Units on the gaping scale as in Plate 1. ■, Haddock (two batches); ○, Cod; ▲, Saithe; □, Redfish; △, Halibut; ●, Lemon sole; ✱, Plaice.

of Scotland being frozen at more frequent intervals by Mr John Smith. In this case, each point is the average value of twelve fish, all twenty-four fillets being mixed and then assessed by three people. The averaged results for cod are shown in Fig. 2, which confirms that the increase in gaping is sudden, and coincides with the onset of rigor mortis, which in these fish occurred about 4 hr after death. The fish did not all enter rigor simultaneously, hence the scatter at the steep part of the curve. The slow increase in gaping between the 1st and 5th days is confirmed, and it is noteworthy that no change in the slope marked the end of rigor mortis. The results for haddock (not illustrated) were very similar, the sharp increase in gaping occurring about 4 hr after death, but in this case the minimum gaping score was 2, not 1 as in Fig. 2, and prolonged storage before freezing caused the values to rise to about 3.

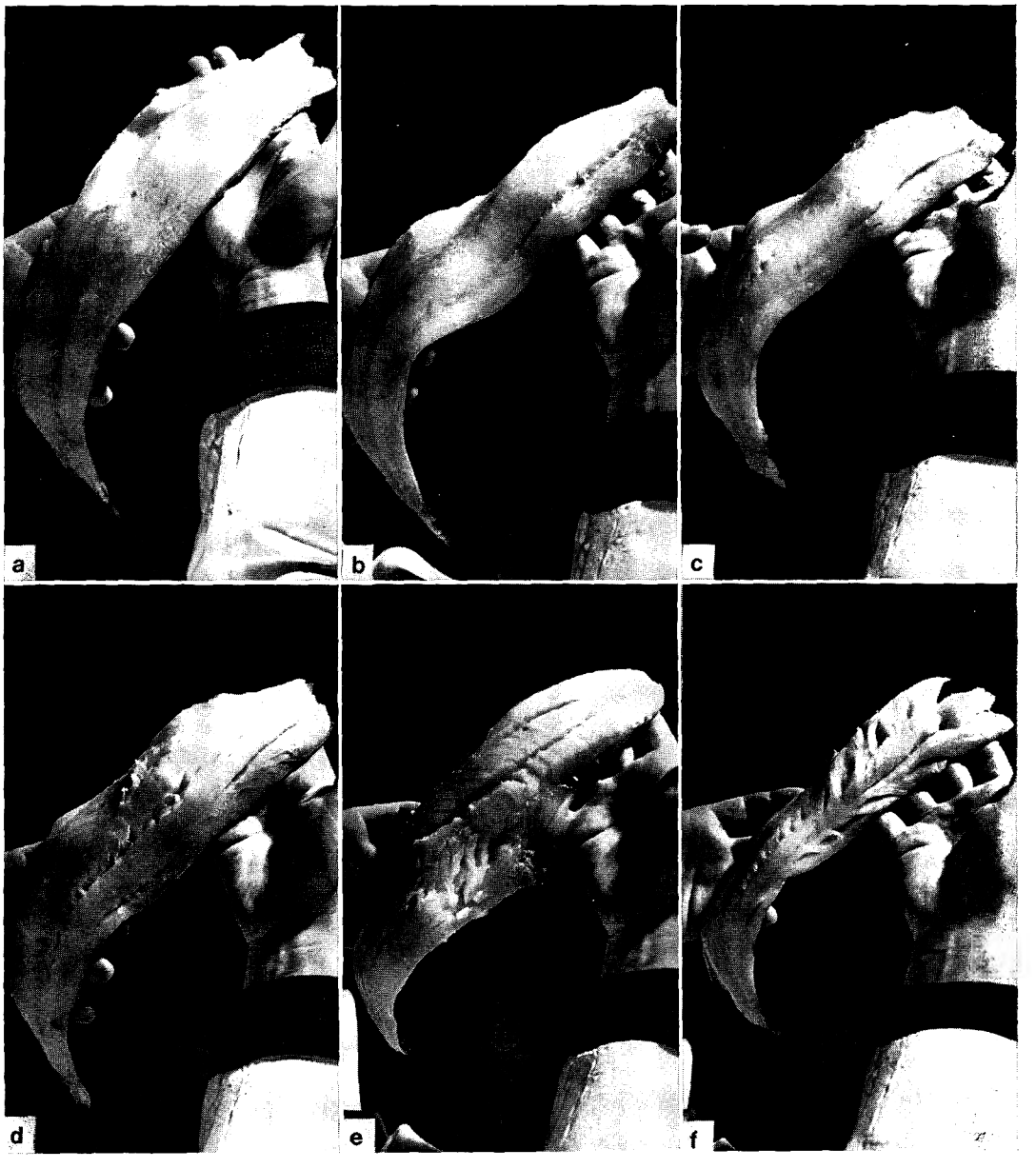

PLATE 1. Degrees of gaping in fillets made from cod frozen whole (gutted), thawed and filleted. The subjective scale of 0 to 5 is based on these pictures, each of which represents a point on the scale.

(*Facing p.* 42)

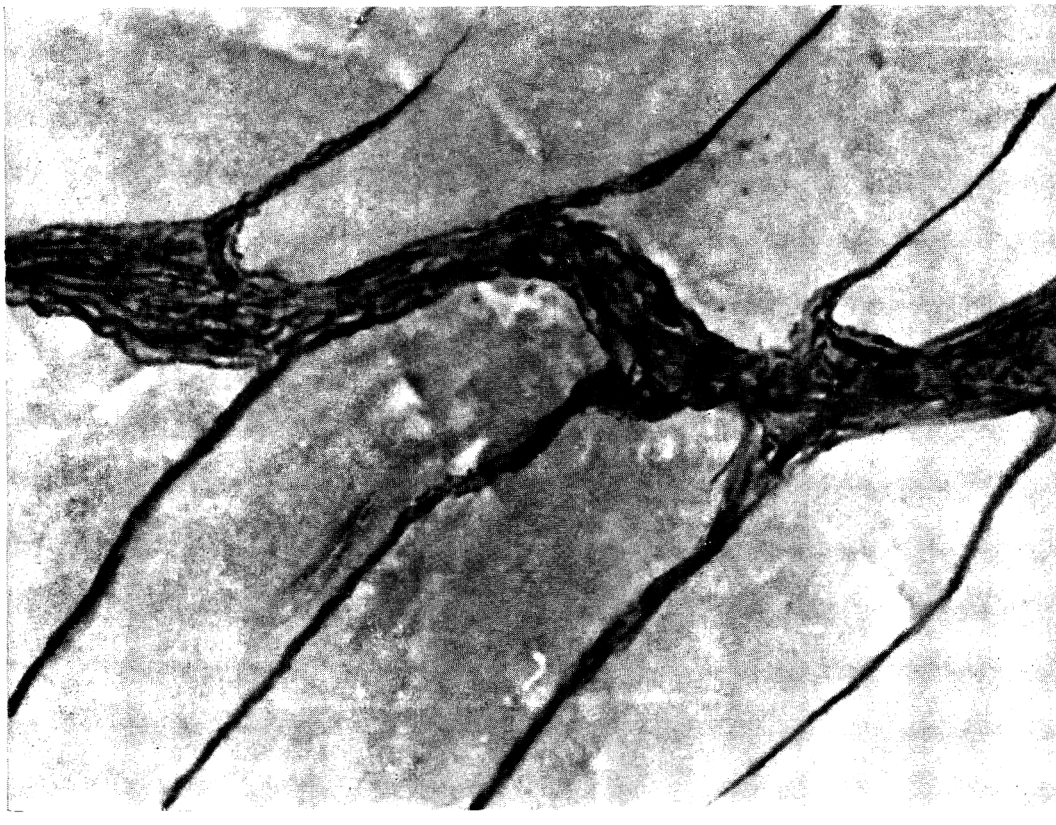

PLATE 2. Part of one myocomma (dark band running across picture), showing connective tissue strands growing between the muscle cells (light areas). Differential staining according to the text. Width of one muscle cell = *ca.* 250μ.

Since a weakening of the muscular power of the fish reduces the gaping in rigor mortis (Love & Robertson, 1968) and, from Fig. 2 of the present paper, a sharp increase in gaping coincides with the onset of rigor mortis, it now seems certain that this kind of gaping results from the rigor contraction coupled with a weakening of the connective tissue by the ice forming within it. Rigor mortis alone does not cause gaping; the fish must be frozen as well.

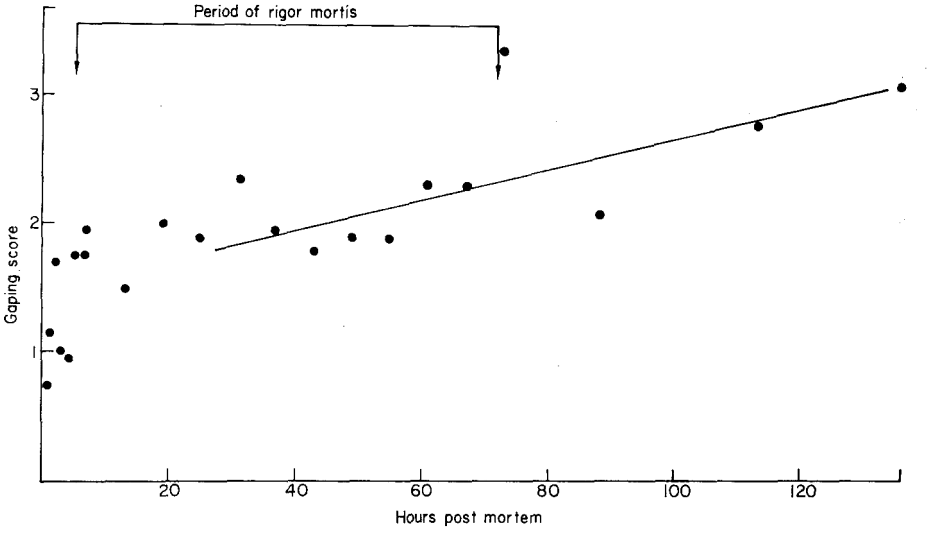

FIG. 2. Gaping in cod frozen after different periods in ice. Each point is the mean assessment of 24 fillets. A regression line has been fitted from the beginning of the 2nd day to the end of the experiment, avoiding the changes associated with rigor mortis.

Cell fragility values which increase if well-nourished fish show a decrease in toughness, showed that the texture of all species became less tough between the pre-rigor and in-rigor points, but thereafter remained steady. For example, the values for saithe rose from 0·78 to 1·05, halibut from 1·28 to 1·7, haddock from 0·94 to 1·23 and similarly for the other species. This behaviour agrees with that found in a more detailed study of cod (Love, 1962), where determinations were done at more frequent intervals than in the present work, and it was found that cell fragility values continued to change up to about 40 hr after death in the unfrozen fish, thereafter remaining steady until about 15 days had elapsed and bacterial spoilage had become significant (Love *et al.*, 1965).

Toughness as tasted in the cooked material revealed the disappearance of the 'rubbery' texture found in pre-rigor fish, but thereafter no further change, so after the resolution of rigor mortis, neither the texture as eaten nor the cell fragility values reverted to the pre-rigor state. Although such muscle has ceased to be rigid, it is not like pre-rigor muscle, as it does not stretch in the same way, and has lost its elastic resilient quality. As it is no longer actively contracting, we might have expected a

reduction in gaping, but Figs 1 and 2 show that there is a slow increase after the 1st day post-mortem, which is not influenced by the resolution of rigor, and at present we can only conclude that this represents a different kind of gaping, unconnected with the strength of contraction.

Fig. 1 showed that the three flat fish species gaped less than any of the round fishes, and also that the roundest flat fish, halibut, gaped more than the others, while the flattest (deepest-bodied) round fish gaped the least of its group. There may, therefore, be a shape factor governing the gaping of fish frozen whole *in* rigor mortis, or a strength factor which arises because flat fish are comparatively feeble swimmers.

The range of sizes of halibut and plaice were roughly comparable, so that the disparity in gaping between them is unlikely to have resulted from differences in the freezing rates. On the other hand, the haddock were considerably smaller than the cod and so would freeze more quickly. It seems unlikely, though, that an increase in freezing rate would cause the haddock to gape more than the cod. The whole question of size, and of freezing rate at constant size, in relation to gaping is now under investigation.

Acknowledgments

The work described in this paper was carried out as part of the programme of the Ministry of Technology. Mr Gordon Pedersen carried out the histological work. Crown Copyright Reserved.

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J. Fd Technol. (1969) **4**, 45–49.

An objective colour method for the determination of tomato maturity

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Summary

Tristimulus colour measurements have been made on whole tomatoes with a Colorcord colorimeter and correlated with a subjective scale of maturity numbers. The dominant wavelength and the luminance both changed characteristically with maturity number. The correlation indicated that the maturity number could be assessed objectively from the luminance alone to within $\pm\frac{1}{2}$ unit. This method would improve the routine grading of tomatoes and eliminate the subjective sorting variables of operator memory, lighting and viewing conditions. It would also allow greater certainty to be placed in inter-laboratory comparisons.

Introduction

The rapidly increasing commercial importance of tomatoes has stimulated interest in the biochemistry of maturation. A study of the biochemistry of tomato ripening processes requires, as a reference point, a reliable and preferably objective index of maturity. This is also of importance in defining the quality of tomatoes to be used in various food processes and for packaging for retail outlets. A property of tomatoes which changes radically with maturity is the colour, which can be assessed by non-destructive methods. The objective measurement of colour is achieved in many industries with a tristimulus colorimeter. This type of colorimeter gives a measure of the quality of the light reflected from the sample under standard illumination conditions and the output can be interpreted according to the standard C.I.E. system (Wright, 1964). Modern tristimulus colorimeters are suitable for use in the food industry and we have attempted to correlate measured tristimulus values with a previously used maturity scale. The successful subjective grading of tomatoes depends upon several factors, the most important of which are operator memory, lighting and viewing conditions. The variability of these factors renders inter-laboratory comparison impossible with a high degree of precision.

Previous tristimulus measurements made on a range of tomato products have been summarized by Mackinney & Little (1962). Tristimulus methods have been used for grading ripe whole tomatoes, e.g. Mackinney (1954), Desrosier (1954) and Garrett

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et al. (1960). We considered that tristimulus measurements might be used to define maturity over the entire scale; a preliminary investigation was, therefore, undertaken and the results are presented in this report.

Experimental methods

The variety of tomato used was Craigella, a greenback resistant hybrid of Ailsa Craig. Fruit initiation was defined as the date of petal drop and ripening occurred during the last 6–8 days of 42–47-day maturity span. The maturity of the fruit was classified according to the scale in Table 1.

TABLE 1. Maturity scale for whole tomatoes

Maturity No.	Description
1	8–12 days
2	24–26 days
3	34–36 days
4	44–46 days full green
5	Just colouring
6	Yellow
7	Orange
8	Just ripe
9	Ripe
10	Overripe

This scale was developed at these laboratories at the beginning of the 1967 season and is an extended version of that used by Edwards & Reuter (1967). They compared their scale with changes in the twenty pigments identified in whole tomatoes.

A Colorcord tristimulus colorimeter manufactured by Joyce Loebl and Co. Ltd, was used to measure the colour of whole tomatoes. Because the sample port of the instrument was too small the initial measurements were made only around the blossom end scar. The colours of a total of fifty-seven glasshouse summer grown tomatoes of maturities 3–10 were measured as the tomatoes became available. Illuminant C was used and chromaticity co-ordinates and the luminance values were calculated. At the end of the season we were able to modify the colorimeter so that the sample could be rotated and the colour of different parts of the tomato surface measured. The few greenhouse winter grown tomatoes of different maturities which became available were measured in this way in order to establish the range of colours occurring round the surface of the sample.

Results and discussion

The chromaticities of the initial sample of fifty-seven tomatoes are indicated in Fig. 1.

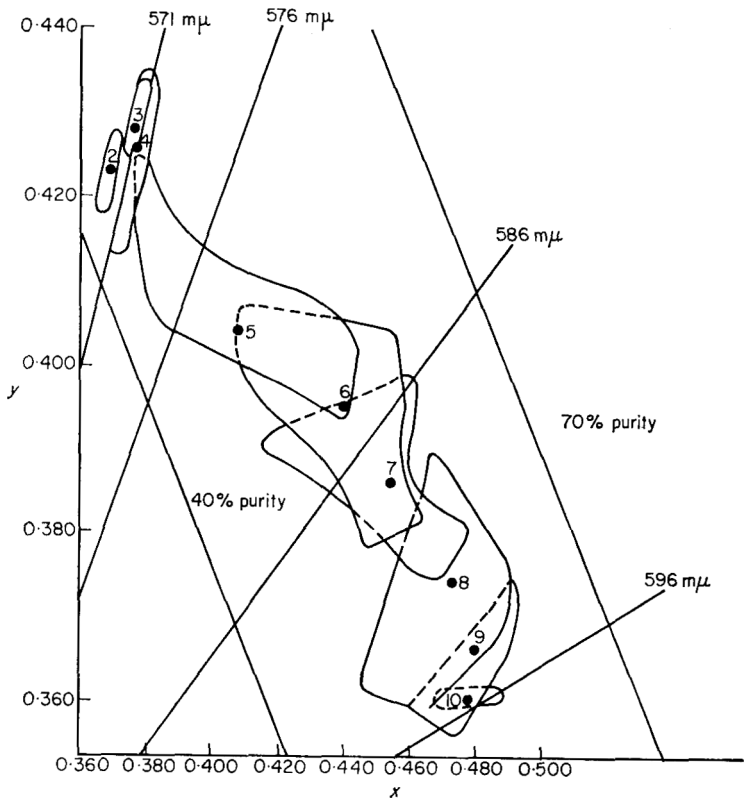

FIG. 1. The chromaticity diagram for whole tomatoes. Lines of constant dominant wavelength and purity are shown. The points indicate the mean coordinates of tomatoes in each maturity grade. The boundary around each point encloses the area inside which all the samples of that particular maturity lay.

The mean co-ordinates for each maturity number (2–10) are shown together with the boundaries of the surrounding areas inside which all the samples of a particular maturity lay. Lines of constant dominant wavelength and of purity are also shown. The dominant wavelengths of maturities 2–4 are practically constant while for maturities 5–9 they increase rapidly from approximately 571–596 mμ. The purity tends to increase between maturities 2 and 8 or 9 then falls slightly to maturity 10.

Fig. 2 shows the relation between the maturity number and luminance. The mean luminance increases to a maximum of 38% at maturity 4 and then decreases to 12% at maturity 10. The larger range indicated by the open circles to each side of the mean shows the ± standard deviations of the fifty-seven tomatoes at each maturity number and indicates that a forecast of maturity number could be made to within ± 1 unit when only one measurement is made on each tomato.

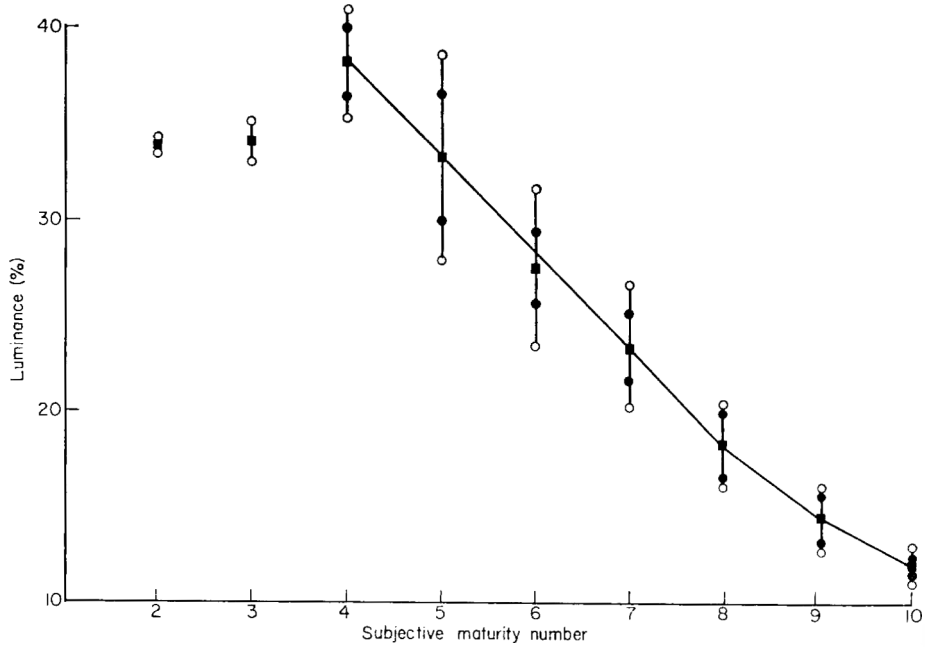

FIG. 2. The effect of maturity upon the luminance of whole tomatoes. ■, Mean luminance of each maturity; O, the ± standard deviation of tomatoes measured in one place; ●, the ± standard deviation of single tomatoes measured in four places.

It is satisfactory to note from the mean tristimulus values obtained, Figs 1 and 2, that the subjective scale is fairly evenly spaced over the most sensitive colour range, that is, between maturities 4 and 10.

There are two main reasons for the degree of overlap of the co-ordinates of consecutive maturities; subjective error in assigning maturity number and non-uniformity of colour over the whole tomato.

The same experienced operator subjectively assessed the maturity number of all samples taking account of the colour of the whole tomato. While it was comparatively simple to place tomatoes in grades 4–10 when a large number of samples were available it was much more difficult to be certain of the correct maturity when only a few were being assessed. As there are no subjectively well defined boundary colours for each grade there will always be some overlap occurring due to operator error.

The non-uniformity of colour over the whole tomato was demonstrated when the colorimeter was eventually modified so that several readings could be made on the same sample. These tests were carried out on eleven winter grown tomatoes having maturities 4–10, each tomato was measured in four places. Only those tomatoes to which the operator could definitely ascribe a particular maturity were measured. There was one tomato for each maturity number except for 7 and 9 where there were three samples. Fig. 2 includes the standard deviations of these measurements. For tomatoes of maturities 7 and 9 the largest standard deviation of the three samples are shown.

If the same scatter found in winter grown tomatoes applies to the summer grown samples then it should be possible to reduce the error of the forecast to within $\pm \frac{1}{2}$ a maturity number. This would enable us to use half maturity numbers if they were required. The error in the subjective assessment of maturity number is likely to be equal to or greater than $\pm \frac{1}{2}$ and hence the objective method would be at least as good.

It is important to note the relevance of colour blindness to tomato grading by colour. The chromaticity diagram locus (Fig. 1) of tomato colours coincides with the loci of confusion for protanopes and deuteranopes. Also protanomalous and deuteranomalous subjects have materially reduced chromaticity discrimination from red to yellow green. Approximately $8\frac{1}{2}\%$ of the population are of the above classes having these types of vision. Unfortunately people having these defects in colour vision often are not aware of them and this is an inherent danger in subjective colour assessment.

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A new method of shelling green peas for processing

R. S. MITCHELL, L. J. LYNCH AND D. J. CASIMIR

Summary

A commercial machine for shelling peas was developed after numerous tests of methods for removing peas from pods. The pods are preheated in steam and then fed to a shelling section by means of a vibratory conveyor which aligns them for end-on presentation to a pair of rubber-covered rollers. A standardized feed gap between the conveyor and the rollers separates shelled peas from unshelled pods. Pods which fail to be gripped by the roller pass to a second slightly modified shelling section.

Extensive tests showed that a greater yield of peas was obtained from the sheller operated on hand picked peas, than from conventional stationary or mobile viners operated on vines. Yield for the combination of a mechanical pea pod picker which would be necessary for commerical operations and the pea sheller was not determined. Peas from the sheller were virtually undamaged in contrast to vined peas and were consequently superior in flavour.

Storage of pods for periods of several days before shelling led to an increase in pea maturity but quality was otherwise unaffected. Ability to store pods permits greater flexibility in harvesting and processing operations and allows an extension of the growing area serving a factory. In addition use of the pea sheller eliminates the need for most of the cleaning equipment as shelled peas are clean. Shelled peas pass directly to the processing line without any delay to cause deterioration.

Introduction

Investigations into preservation of green peas have emphasized the need for care in the removal of peas from pods. Commonly pea vines are mown, taken from the field to a viner station and fed to stationary viners. The threshing action of the viners 'beats' the peas from the pods, and they are separated from the vines in the viner and subsequently cleaned by winnowing and washing. The beating action of the viner was shown to damage the peas by Moyer, Lynch & Mitchell (1954) and by Casimir *et al.* (1967). Damage predisposes peas to development of off-flavour (Lynch, Mitchell &

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Casimir, 1959; Eriksson & Sydow, 1961). Damaged peas tend to split during factory operations, and resultant losses can be appreciable.

In 1962 a pea pod picker for the mechanical removal of pea pods from vines in the field showed commerical promise in U.S.A. (Anon, 1962). Development of this machine continued, and a number of units have been produced. After harvesting with the aid of this mechanical pod picker, the harvested pods are fed to a modified viner, and the reduction in the quantity of vegetable material results in a higher rate of production of shelled peas than under normal operation.

Peas harvested in the pod offer several advantages. The weight of material is reduced by nearly half compared with vines. Flexibility in processing schedules is increased because pods detached from vines can be stored several days. Nevertheless, removal of peas from harvested pods with the aid of a viner results in damage to the peas, and other shelling procedures were investigated. A pea sheller was developed in which automatic end-wise presentation of the pods to a pair of parallel rotating rollers causes the peas to be expelled by the nipping action of the rollers while the pods are drawn between the rollers. Weakening of the pod suture by a short preheating was necessary. Pilot plant equipment was tested during several seasons, and in 1964 the first commercial unit embodying this principle was constructed by F.M.C. (Australia) Ltd. Further tests were made in 1965.

Experiments and results

Preliminary trials

Several methods for the removal of peas from pods were examined and squeezing the pods between two rotating rollers was most promising. Therefore, this method was investigated in greater detail in a further series of preliminary trials.

A short heat pretreatment of the pods was necessary for efficient recovery of peas shelled on the roller-nip principle. Untreated pods shelled in this way yielded 55% of the yield of peas obtained by hand shelling, and when pods were dipped for 15 or 30 sec in boiling water before being machine shelled the yield was increased to 94% of the yield from hand shelling. Extending the time to 45 sec or longer reduced the yield to 89% or less. In another test it was shown that pre-treatment in wet steam at atmospheric pressure was equally effective.

Several types of roller coating material were tested and best results were obtained when one roller was coated with hard rubber and the other with soft rubber. A peripheral speed of 75 ft/min for the rollers permitted a high throughput without diminishing yield. The width of the gap between the conveyor and the rollers was found to affect both the percentage of pods caught by the rollers and the rate at which pods could be fed in without overloading. After various trials a feed gap of $1\frac{1}{2}$ in. between vibratory conveyor and shelling rollers was selected. With this feed gap, 60% of the pods were shelled in a single pass through a set of rollers, and the remaining 40% fell through the feed gap without impeding the forward flow of following pods.

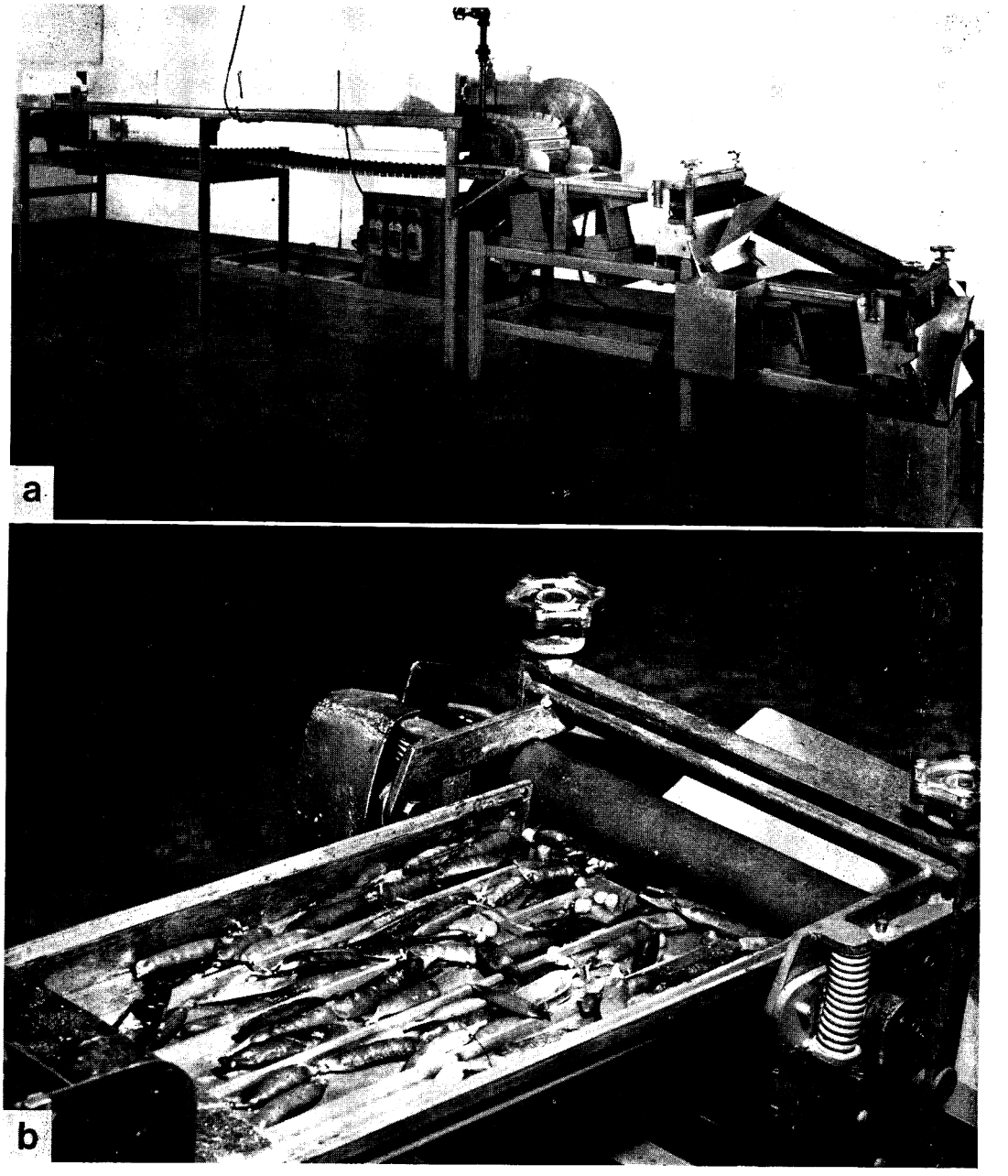

PLATE 1. C.S.I.R.O. pea sheller. (a) General view. (b) View showing vibratory conveyor, feed gap and shelling rollers.

(*Facing p.* 52)

These results led to the design and construction of a pilot-scale pea sheller incorporating two shelling sections arranged in series.

Description of C.S.I.R.O. pea sheller

The pea sheller* (Plate 1a) consists of a pre-treatment section and a shelling section. The pre-treatment section is a steam box through which the pods are conveyed on a perforated belt. Wet steam is distributed by perforated pipes placed above and below the belt. The rate of intake of pods is controlled by a brush at an adjustable distance above the belt and revolving the same direction as the belt sprockets. The speed of the belt determines the duration of the steam treatment and is adjusted according to the suture toughness of the pods which can vary with variety; 20–25 sec in the steam box is usually adequate, but some varieties require 35 sec for efficient shelling.

After leaving the steam chamber, pods pass through a cold water spray to the shelling section which has two similar units placed in series. Each unit consists of a vibratory conveyor, a pair of transversely mounted shelling rollers, a chute for shelled peas and means for removing empty pods. Vertical dividers on each vibratory conveyor provide channels which align the pods for end-on presentation to the rollers. The dividers terminate a short distance before the delivery end of the vibratory conveyor. This allows pods not immediately gripped by the rollers to turn at right angles to the direction of feed and fall through the feed gap. Plate 1(b) shows pods aligned in the first vibratory conveyor moving across the feed gap to the nip of the rollers, peas are squeezed out of their pods as the pods are drawn between the rollers. The empty pods are discharged to waste. Some pods which have been presented blunt end first or are too short for proper alignment in the first vibratory conveyor are not nipped by the rollers but fall, together with the shelled peas, through a feed gap to the second vibratory conveyor. The channels and the feed gap are narrower in the second than in the first shelling section. Thus small pods as well as the large pods are aligned and pass to the second set of rollers. Most of the blunt ended pods rejected in the first section have turned 180 degrees and travel with their sharper stem-end foremost to the second set of rollers. All the shelled peas fall through the second feed gap.

Damage to the peas during extraction

On two occasions, samples of peas were obtained from a commercially operated viner, while some vines sampled from the same source were depodded by hand and the pods fed to the pea sheller. Further comparisons, which included hand shelled samples as well as vined and machine shelled lots, were obtained from vine samples withdrawn from commercial loads at a viner station. Data were also obtained from randomized block trials each harvested on 4 successive days. Mean maturometer values are set out in Table 1. The original data from crops 3 to 10, consisted of seven-

*The machine is covered by Patent 259,115 in Australia, Patent 155,938 in New Zealand and Patent 997,274 in Great Britain.

TABLE 1. Mean maturometer index of peas from a viner, from the C.S.I.R.O. pea sheller, and by hand shelling

Crop No.	Source of samples	Maturometer index*		
		Viner	Pea sheller	Hand shelled peas
1	Viner station	226	270	
2	Viner station	253	285	
3	Viner station	212	261	294
4	Viner station	219	265	348
5	Viner station	230	274	362
6	Viner station	265	286	367
7	Viner station	311	341	398
8	Field plots†	146	200	224
9	Field plots†	166	219	265
10	Field plots†	188	241	302

* Lynch & Mitchell (1954)

† Means of four successive days harvests.

teen sets of maturometer indices and covered a maturity range of 140 to 265 maturometer index (vined). These data were analysed and highly significant linear regressions were found as follows:

$$MI_v = 1{\cdot}012\ MI_m - 47{\cdot}6,$$
$$MI_v = 0{\cdot}717\ MI_h - 17{\cdot}9,$$

where MI_v, MI_m and MI_h are the maturometer indices of vined, machine shelled and hand shelled peas, respectively. These regressions show that vined peas were lower in maturometer index than either machine shelled or hand shelled peas and had, therefore, suffered more damage. The maturometer index for hand shelled peas was greater than that for machine shelled peas.

A sample of peas from a viner operated at beater speed of 170 rev/min and a comparable sample of machine shelled peas were blanched, frozen and packaged. They were stored for 2 months and examined for damage. Approximately 3% of the machine shelled peas were found to be damaged, compared with 47% of those from the viner (Table 2).

Weight losses on blanching provided further evidence of greater damage in vined than in machine shelled peas. In one test machine shelled peas blanched for 2 min at 200°F lost 6·4% in weight compared with a 10% loss in vined peas similarly treated.

TABLE 2. Percentage by weight of vined peas and machine shelled peas in each damage class

Damage class	Viner	Pea sheller
Undamaged	53·3	96·7
Ruptured skins	36·7	2·1
Ruptured skins and damaged cotyledon	7·3	0·4
Part of skin or cotyledon missing	1·5	0·4
Skin fragments	0·4	0·4
Cotyledon fragments	0·8	0·0

Quality assessment

Comparable machine shelled and vined peas were frozen by orthodox procedures with a minimum of delay, and vined peas were likewise treated after 4 hr holding in 40-lb lug boxes on the factory floor. On another occasion, both canned and frozen samples were prepared from machine shelled and from vined peas. The machine shelled samples were obtained both from fresh pods and from pods held for 24 hr before shelling, while vined peas were processed both immediately after vining and after storage in lug boxes at ambient temperature for 2 and 4 hr. To provide a typical commercial sample of shelled peas, vined peas were stored 3 hr in a bulk 800-lb bin before feeding to a processing line. The peas passed through the blancher (60 sec at 200°F), were cooled and inspected. Sample lots removed at the end of the inspection belt were packed in polyethylene bags and frozen. The remaining peas passed to a can filler and then to a can closer. A sample lot of cans was removed from the line and processed in a retort for 28 min at 245°F.

Comparative quality of vined and machine shelled peas was assessed by taste tests using a panel of fifteen experienced tasters. Frozen peas were boiled for 6 min and served hot to the tasters, and canned peas were served unheated. All samples were rated on each of two occasions for texture and intensity of off-flavour. Texture was assessed as 0 for 'good', 1 for 'slightly hard', 2 for 'hard', with -1 and -2 for 'slightly soft' and 'soft'. Values 0–3 were allotted to represent 'absent', 'slight', 'moderate' and 'intense' off-flavour, respectively.

Three separate taste tests were carried out comparing treatments within the first experiment, within the frozen samples of the second experiment and within the canned samples of the second experiment. Mean values of panel scores for texture and off-flavour are set out in Table 3.

In frozen samples, machine shelled peas had less off-flavour than vined peas. Delay for 24 hr in pod before machine shelling did not increase off-flavour but off-flavour in vined peas increased with delay time before processing, and the commercial sample of vined peas held in bulk had a high off-flavour consistent with delay time. Texture scores for frozen peas, except those from stored pods, were approximately zero or

TABLE 3. Mean tasters' scores for vined and machine shelled peas, frozen and canned

Factor	Product	Experiment No.	Pea sheller		Viner			
			Delay Nil	Delay 24 hr	Delay Nil	Delay 2 hr	Delay 4 hr	Delay 3 hr
Off-flavour†	Frozen	1	0·4	—	0·7	—	1·5	—
	Frozen	2	0·4	0·5	0·7	1·1	1·9	1·4
	Canned	2	1·2	1·2	1·3	0·9	1·4	2·1
Texture‡	Frozen	1	−0·2	—	−0·6	—	0·4	—
	Frozen	2	0·1	0·6	−0·4	0·1	0·1	−0·7
	Canned	2	−1·3	−0·5	−0·9	−0·9	−0·7	−1·9

* Commercial sample

† 0=Absent, 1=slight, 3=severe.

‡ −2=Soft, −1=slightly soft, 0=good, +1=slightly hard, +2=hard.

negative. Peas stored in pod were harder than the other peas. All canned samples were considered to have at least a slight off-flavour. This probably reflects a preference for frozen peas by panel members. Vined peas stored for 3 hr in bulk were significantly higher in off-flavour than other treatments. Texture of canned peas was classed as too soft. Machine shelled peas from stored pods had the firmest texture.

Yield assessment

Tests of material from vines sampled at a viner station suggested that higher yields of peas were obtained from the pea sheller than from the viner. Further comparisons were made on larger quantities of material harvested at a range of maturities, and with the viner operated at a beater speed of 170 rev/min. All the peas were washed, and those smaller than $\frac{9}{32}$ in. were removed. The washed samples were spin dried to remove adhering moisture before they were weighed. Samples of 143 peas were used for determining the average weight of peas in each experimental batch. Pea yields were expressed alternatively in terms of lb/ac, as a percentage of vine weight, or as a percentage of pod weight, depending upon whether they were obtained from measured areas of crop, samples of vines, or from sample lots of pods.

An experimental area within a commercial crop was marked into five blocks each containing eight plots of 24 yd^2. Each day one plot from each block was harvested in two equal parts (sub-plots), one for vining and the other for machine shelling. Measurements were made on both size graded and ungraded peas. Statistical analysis of the yield data showed that the increase in yield with time was very highly significant. Mean yield of vined peas was 5,942 lb/ac which is significantly less than that of machine shelled peas (6,124 lb/ac). The least significant difference at the 5% level was found to be 163 lb/ac.

Additional measurements were made on peas obtained from successive daily harvests of plots within three crops by hand shelling, and machine shelling, and from four

additional crops sampled at the viner station. Yields for machine shelled peas averaged 3·5% lower than those for hand shelled.

In a semi-commercial trial, material was harvested on two separate days from areas of $1\frac{1}{4}$ ac within a commercial crop. Alternate strips were harvested by a Scott Mobile Viner and by hand. Peas were bulk handled from the viner, and hand-pulled pods were machine shelled. Pea yields from the mobile viner were 1,250 and 1,380 lb/ac and corresponding yields from the pea sheller were 1,420 and 1,470 lb/ac.

Measurements of size graded peas

Size graded material from the replicated field trial was examined for differential effects due to vining and machine shelling. Data on maturometer readings, yield, weight per pea, and yield of peas expressed in numbers are set out in Table 4.

TABLE 4. Mean values for maturometer readings, yield, weight per pea and numbers of peas in size grades

Factor	Treatment	Size grade							
		2	3	4	5	6	7	8	Un-graded
Maturometer reading	Viner	117	132	160	198	239	284	318	
	Pea sheller	155	187	223	263	304	336	365	
Yield (lb/ac)	Viner	247	626	1021	1598	1546	755	149	5942
	Pea sheller	188	463	837	1527	1731	1108	270	6124
Weight/pea (mg)	Viner	236	304	375	448	521	595	667	427
	Pea sheller	233	291	357	430	508	586	657	446
No. of peas/ac ($\times 10^{-3}$)	Viner	475	934	1236	1619	1347	576	101	6317*
	Pea sheller	366	722	1064	1612	1547	858	186	6234*

* Values represent sums of size grades. When calculated from ungraded yield and weight per pea values are 6,290 for vined and 6,357 for peas from the pea sheller.

Yield per acre was significantly higher for machine shelled peas than for vined peas in size grades 7 and 8 but the reverse was true of size grades 2, 3 and 4. Total yield was significantly greater for machine shelled peas. The total numbers of peas were not significantly different for the two shelling methods.

The interaction of size and shelling method was significant. Numbers in each of size grades 2, 3 and 4 were greater for vined than for machine shelled peas. In size grades 6, 7 and 8 there were more peas in machine shelled than in vined samples.

Weight per pea was greater for vined than for machine shelled peas in each size grade; however weight per pea in ungraded peas was greater for machine shelled peas than for vined peas.

Examination on many occasions of shelled peas and waste pods from the pea sheller showed that peas of all sizes were efficiently shelled.

Field to factory transport

The weight of material to be transported from field to factory depends on whether the peas are recovered by viner, by sheller, or by mobile viner. Data from successive daily harvests from plots within one crop are set out in Table 5. At the optimal harvest time (Lynch & Mitchell, 1954) the weights of material transported were therefore in the ratio of 2 : 5 : 10 for the mobile viner, the C.S.I.R.O. pea sheller and the stationary viner, respectively. The proportion of peas to vines and the proportion of pods to vines were lower for earlier harvests (Table 5).

TABLE 5. Mean yields of vines, pods, and peas from daily harvests of an experimental area in a commercial crop

Days from OHT*	Vine yield (lb $\times 10^{-3}$/ac)	Pod yield (as % of vines)	Pea yield (as % of pods)	Pea yield (as % of vines)
−6	31	42·6	28·0	12·0
−5	—	—	28·3	—
−4	—	—	33·5	—
−3	36	45·5	39·5	18·0
−2	37	52·8	43·2	17·5
−1	33	50·7	42·7	21·6
0	36	47·7	42·3	21·0
+1	35	55·9	41·2	23·0

*OHT, optimal harvest time (Lynch & Mitchell, 1954).

Storage of pods

One of the advantages claimed for handling peas in pods is the ability to store and transport them with a minimum of physical and biological deterioration. A number of tests were made of the effect of pod storage on quality changes. Pods from vines harvested from field plots were stored in 30-lb lots in lug boxes on the cannery floor for 0, 1, 2 and 3 days before machine shelling. On each of these days, pods from random plots within the same experimental area of crop were machine shelled and the peas tested without delay. The maturometer indices for peas stored in pod were 278, 358, 357 and 407 on each of these successive days, and the corresponding results from pods from the growing crop were 261, 292, 336 and 343. Thus rate of change of maturity (as indicated by the maturometer index) for peas stored in pod was rapid during the initial 24-hr period, but thereafter was of the same order as that for peas from the growing crop.

Six replicate lots of pods were tested at intervals over a 7-day storage period at ambient temperatures. Initially the maturometer index was 252 and rose to 287,

313, 324, 336 and 365 after 1, 2, 3, 4 and 7 days storage, respectively. The rate of change during the first 24 hr in store was similar to that of the stored pods in the previous experiment, but thereafter the rate of change was slower.

Discussion and conclusions

A prototype pea shelling machine of the roller type was constructed after preliminary tests had defined basic requirements. The operation of this machine was compared with methods of extraction of peas from pods by hand shelling, vining and the use of a mobile viner. The prototype sheller was found to perform efficiently, and after suitable modifications a prototype commercial sheller was produced which could satisfactorily shell up to 2,400 lb pods/hr.

In vining, damage to peas is excessive and is a primary cause of off-flavour in the canned and frozen product. Visual counts showed that 47% of vined peas were injured in varying degrees compared with 3% of peas recovered by the C.S.I.R.O. pea sheller. Damage also results in tenderization, which is reflected in lowered maturometer values. Maturometer values were highest for hand shelled, intermediate for machine shelled and least for vined peas. The heat treatment necessary for efficient removal of peas from pods is the main factor in the lower maturometer value for machine shelled than for hand shelled peas but it is of no practical importance since shelling is always followed immediately by blanching.

Deleterious effects of damage were demonstrated by taste tests for frozen peas. When machine shelled and vined peas were processed immediately after removal from the pod, the machine shelled samples were preferred, though differences were small. In commercial practice, delays between vining and processing are common and result in development of off-flavour especially in frozen peas. The logical placement of the pea sheller is in the factory line after pod holding facilities and pod washers, with shelled peas passing directly to the blancher. Consequently, delays associated with machine shelling are restricted to pod storage during transport and prior to processing in the factory. It was shown that this delay does not induce off-flavour because damage is not involved. The only notable change was an increase in maturity; peas held in pod at ambient temperature matured very rapidly during the first day's storage but subsequently did not differ from field peas in their maturation rate.

The yield of peas obtained by different mechanical procedures depends on the numbers left in pods and on the losses due to damage or fragmentation. The correctly adjusted pilot model sheller gave yields of 96·5% of those from shelling by hand, while the commercial model gave the same yields as hand shelling; for comparison the yield value for a commercial stationary viner was 97·1% of that from the pilot model sheller. Losses during blanching due to damage caused by shelling were about 4% higher for vined than for machine shelled peas, while further appreciable losses of vined peas occur in the processing line because of the predisposition of damaged peas to breakage.

Damage during vining is accompanied by loss of rigidity and when size graded many vined peas pass through a finer screen than they would if undamaged (Casimir *et al.*, 1967). Thus data on yields and numbers of peas in size graded vined peas show net losses from size grades 6, 7 and 8 and net gains in size grades 2, 3 and 4 as compared with machine shelled peas. Average weight per pea in ungraded lots was higher for machine shelled than for vined peas showing that material was lost from individual peas during vining. In contrast average weight per pea, within each size grade, was greater for vined peas than for machine shelled peas. Peas which pass through a screen only because of loss of rigidity are marginal in size and it is logical to assume that their individual weights are between the average weights per pea of the adjacent size grades. Consequently the average weight per pea is increased for both size grades. The pea sheller, therefore, gives lower yields of peas in small size grades than the viner because vining affects the grading and not because of loss of small peas in the sheller. On the contrary the sheller satisfactorily extracts very young peas.

Harvesting pods in the field offers a considerable saving in transport costs compared with the vining procedure; if crops are harvested before the optimal harvest time when the ratio of pea to vine weight is lower, the saving becomes proportionately greater. Transport savings from handling pods are of course less than those associated with transport of shelled peas obtained by use of mobile harvesters.

Factors favouring adoption by industry of a pea sheller of the type described are that the peas obtained from it are practically free of damage, and are consequently superior in appearance and flavour to those obtained from machines (viners and hullers) based upon the impact principle. Additional factors of economic importance are reduction in loss due to breakage in the processing line, reduction in capital outlay and space required for cleaning equipment, and saving in transport costs compared with carriage on the vine. Yield was greater from the pea sheller than from viners but as the former operated on hand harvested pods commercial yields would depend on the relative efficiency of machine picking of pods. Also to be noted is the ability to store pods for relatively short periods, e.g. 24 hr, permits more precise control of factory operations and enables peas to be grown at greater distances from the processing factory.

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The relationship between maturity and quality of canned broad beans (Vicia faba L.)

V. D. ARTHEY AND CHRISTINE WEBB

Summary. This paper describes a co-operative investigation carried out by the Fruit and Vegetable Preservation Research Association and eight of its member firms to determine optimum maturity of broad beans for canning.

The results indicate that (a) there was no difference in flavour in canned broad beans harvested at tenderometer readings from 90 to 200, (b) there was a significant linear deterioration in texture as beans matured, (c) alcohol insoluble solids of raw and canned beans increased linearly as beans matured, (d) a linear relationship was found between tenderometer readings and sample size, (e) consumers equally preferred canned broad beans harvested at tenderometer readings of 120 and 160 and (f) optimum maturity of broad beans for canning from the data obtained was at a tenderometer reading of 136 using a 5 oz sample of raw beans.

Introduction

The number of crops which are harvested by hand for processing is gradually diminishing as new and more sophisticated mechanical harvesters are introduced on to the market. The effect of this evolution is not inconsiderable and has resulted in changes in the methods of crop production used, thus ensuring that a more economic use of the available land is made and that plants are of the desired habit and spacing for efficient operation of the machine. Another important characteristic which must be considered is the choice of the correct stage at which the fruit or vegetable should be harvested since the whole crop is now taken at one time and not in a number of successive harvests which was necessary with hand labour. It is recognized that fruits soften and vegetables toughen but both increase in yield as they mature. A compromise must, therefore, be reached between the grower and processor so that yield is satisfactory to the former and quality is acceptable to the latter.

The broad bean has recently been added to the list of those vegetables which can be harvested mechanically. The problems which exist at present in this operation will be overcome in the next few years as mechanical harvesting is developed more extensively for this crop. The time required to hand pick broad beans has meant that

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little control over maturity of the bean seed could be exercised and quality has consequently suffered. Mechanically harvested beans, however, can be obtained at a desired maturity, all other factors being favourable, and this paper describes three years' co-operative work carried out at Campden to determine the optimum stage of maturity at which broad beans should be harvested for canning. The detailed results of each year's work have been published elsewhere (Anon, 1966; Arthey & Webb, 1967, 1968).

In the case of the garden pea crop, which is of major economic importance to canners, the tenderometer was introduced in the United States in 1937 (Martin) and is now in wide commercial use in the United Kingdom for the measurement of pea maturity. Similarly in Australia, Lynch & Mitchell (1950) introduced the maturometer for measurement of pea maturity. Other instruments, such as the texturemeter and hardnessmeter have also been used for this purpose. In the case of beans, however, no similar methods are in general commercial use, but the tenderometer has been shown to be a useful instrument for the measurement of raw broad bean maturity (Anthistle, Ashdown & Dickinson, 1959), and therefore this instrument was used for the tests described in this paper.

In addition the use of the alcohol insoluble solids content of peas as an index of their maturity was first suggested by Kertesz (1934). This method, with others, was reviewed by Makower in 1950 and the commercial applications of several methods of measuring maturity was discussed in 1959 by Lynch, Mitchell & Casimir. The alcohol insoluble solids method of measuring maturity of peas was used in this research project on broad beans.

The following factors were assessed:

(1) Consumer acceptance of canned beans harvested at two different maturities.
(2) The relationship between texture (measured organoleptically on the canned product) and the maturity of the raw material measured with a tenderometer.
(3) The relationship between flavour (measured organoleptically on the canned product) and the maturity of the raw material measured with a tenderometer.
(4) The relationship between the alcohol insoluble solids content of the raw and processed beans and the maturity of the raw material measured with a tenderometer.
(5) The relationship between tenderometer readings and the sample size, using three sample sizes of beans.

Experimental procedure

The three years' work was carried out with the co-operation of eight member firms of the Research Association whose assistance is acknowledged at the end of this paper.

With the exception of two samples of beans, all were of the cultivar Triple White. The individual firms were responsible for growing the beans and recording agronomic

data. The tenderometer readings and alcohol insoluble solids contents were determined in a similar way to that described below for the work at Campden.

Each firm used its own commercial technique for the canning of the beans which were later assessed organoleptically for quality at Campden.

The experimental procedure at Campden is described below.

In each of the three years, the beans were hand sown and the growing crops managed according to the best methods of husbandry. The first harvests were taken when three 5 oz samples of raw beans gave a mean tenderometer reading of between 80 and 90. Thereafter, beans were harvested at 2–3-day intervals until a tenderometer reading of approximately 200 was attained.

At each harvest all the pods from one row of beans were removed from the plants and weighed. The pods were hand shelled and the weight of the beans recorded.

The tenderometer was checked daily in a similar manner to that described by Graham & Evans (1957). Three 5-oz, three 3-oz and three 2-oz samples of raw beans were used to obtain tenderometer readings for different sample sizes of beans of the same maturity. The three readings from each sample size were averaged and recorded.

Samples of raw beans were analysed for alcohol insoluble solids content and the remainder of each harvest was canned, using a standard method.

The method used for the alcohol insoluble solids determination of beans was similar to that described for peas by Dickinson & Holt (1954). The beans were cut into portions according to their size and a 50-g sample was macerated with 50 ml water to a smooth paste. 20 g of macerate were transferred to a 250 ml flask and boiled with 150 ml 80% alcohol under a reflux condenser for half an hour. The mixture was filtered through a Buchner funnel under suction using a weighed 9-cm No. 1 Whatman filter paper dried at 98–100°C and cooled in a desiccator for 30 min. The residue was washed with 80% alcohol until the washings were colourless. The filter paper and residue were dried at 98–100°C for two hours, cooled in a desiccator for 30 min, and weighed. The alcohol insoluble solids content of each bean sample was calculated from two determinations.

The samples of beans for canning were blanched for 3 min in 50% distilled and 50% tap water at 200°F. After cooling, 12½ oz of beans were filled into each A2 can, covered with brine consisting of 3 oz salt per gallon of 50% distilled and 50% tap water at 190–200°F and processed at 240°F for 35 min. The cans were stored for quality assessment later in the year.

The tasting panel was comprised of five persons from the quality inspection panel and the agricultural department. Not more than 15 samples were tasted each day. Each maturity sample was replicated three times and scored for flavour and texture according to the Standards of Quality Revised Memorandum Q.C.6 (1965) as follows:

Flavour (maximum number of points 20)	
Full natural flavour of the bean	20
Fine natural flavour (above the average)	18–19
Good natural flavour (normal)	16–17
Rather weak natural flavour, or very slightly harsh or bitter	14–15
Weak natural flavour or slightly harsh or bitter	12–13
Distinct foreign flavour	6–11
Strong objectionable foreign flavour	0–5

Texture (maximum number of points 30)	
Each bean whole, plump and very tender in flesh and skin. Absolutely clear liquid	30
Very slight divergence from perfection	27–29
Flesh tender but skins slightly too firm	24–26
Beans rather too firm; sides partially collapsed	21–23
Beans distinctly too firm, but not tough; sides collapsed. Not more than 5% split or broken. Liquid slightly cloudy	18–20
Beans tough, liquid cloudy	9–17
Beans very tough. Liquid very cloudy	0–8

Samples from each source were examined together on the same day or, when necessary, on consecutive days.

Consumer preference tests

Two member firms of the Research Association carried out consumer preference tests using the techniques with which they had experience.

Results

1. *Consumer preference tests*

The data obtained from the first year's work indicated that optimum maturity of broad beans for canning (based on texture only) was about tenderometer reading 130 (actually T.R. 128·7). It was felt, however, that it would be desirable to determine whether the public preferred tender beans with a weak flavour or tough beans with a good flavour; it being thought at that time that flavour of canned broad beans improved with increase in tenderometer readings. Two batches of cans were prepared, one using beans harvested at T.R. 120 and the other using beans at T.R. 160.

The consumer tests were carried out by the Research Departments of two companies represented on the Canning Panel of the Research Association. The samples for the tests were prepared by a third member firm.

Samples of the canned beans were assessed for flavour and texture by expert tasting panels at one company (A) involved in the consumer opinion tests and at Chipping Campden. The results, using the Campden Standards of Quality Revised Memorandum Q.C.6, were as follows:

Specialist panel A (8 members – mean scores)

	T.R. 120	T.R. 160
Texture	21·1	19·4
Flavour	13·4	14·5

Campden panel (5 members – mean scores)

Texture	26·7	22·0
Flavour	15·6	15·1

A considerable overall difference is apparent between the assessment by the two specialist panels but in both cases a larger difference was recorded in texture than in flavour between the two different maturities.

The texture of the immature sample was scored more highly than that of the mature sample.

The preference of the two consumer panels (A and B) are given in Table 1. The detailed preferences of panelists for the different items measured are given in Table 2. The reasons for panelists' preferences are summarized in Table 3.

TABLE 1

Preference	Consumer Panel A		B	
Broad beans T.R. 160	65	(41)	34	(38)
Broad beans T.R. 120	66	(42)	30	(34)
No preference	27	(17)	25	(28)
Total	158	(100)	89	(100)

Figures in brackets are percentages.

2. *The relationship between organoleptic assessment of texture and tenderometer reading*

One of the most important agronomic factors affecting the texture quality of the canned broad bean is the maturity at which the bean is harvested.

The relationship was measured in the present survey, using canned beans from six sources in 1966 and three sources in 1965 and 1967.

The quality of the canned product, from the point of view of texture, was found to deteriorate linearly with maturity of the beans. The relationships, which are calculated from all the data from all sources for each year and presented in Fig. 1, were highly significant.

TABLE 2. Details of preferences (%)

Consumer Panel A			Consumer Panel B		
Appearance	T.R. 120	T.R. 160	Appearance	T.R. 120	T.R. 160
Very good	28·4	34·1	Very good	26·1	27·9
Good	43·6	40·5	Good	31·8	37·2
Fair	20·8	20·8	Average	31·8	27·9
Poor	6·3	4·4	Poor	10·2	5·8
Very poor	0·6	0·0	Very poor	0·0	1·1
Flavour			Flavour		
Very good	38·6	32·9	Very good	29·5	28·4
Good	28·4	31·0	Good	26·1	36·3
Fair	22·1	28·4	Average	27·2	22·7
Poor	9·4	5·0	Poor	12·5	11·3
Very poor	1·2	2·5	Very poor	4·5	1·1
Texture			Texture		
Very tender	50·7	22·6	Much too soft	1·2	1·1
Tender	36·7	44·9	Slightly too soft	28·3	9·4
Firm	10·7	25·9	Just right	55·5	60·0
Hard	1·8	6·3	Slightly too tough	7·4	25·8
Very hard	0·0	0·0	Much too tough	7·4	3·5
Texture (By preference)					
Very good	32·9	29·1			
Good	37·9	45·5			
Fair	20·8	21·5			
Poor	8·2	2·5			
Very poor	0·0	1·2			

TABLE 3. Panelists' reasons for preferences

	T.R. 120		T.R. 160	
	Panel A	Panel B	Panel A	Panel B
Reasons associated with flavour	45	23	52	27
Reasons associated with appearance	6	4	8	5
Reasons associated with texture	29	12	19	14

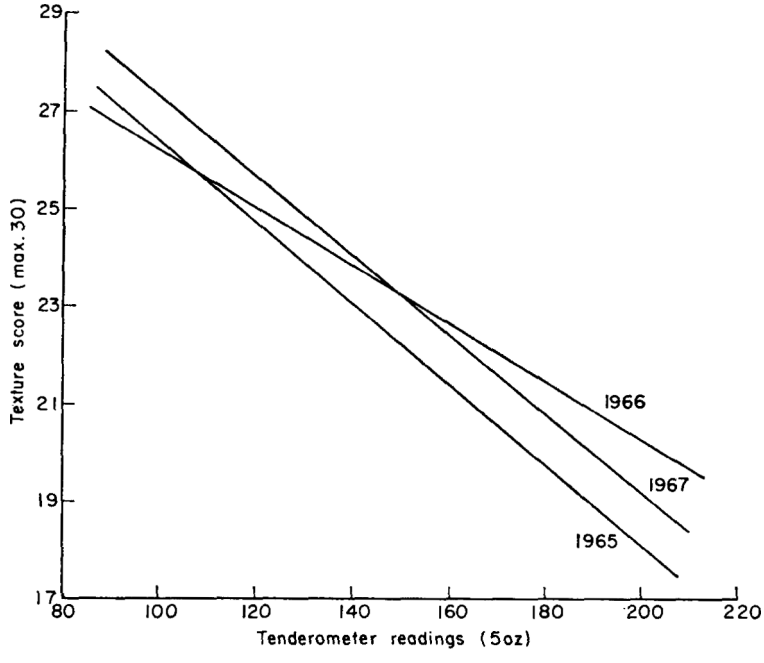

FIG. 1. The relationship between organoleptic texture scores and tenderometer readings (5-oz samples).

$$Tx_r = 34{\cdot}4856 - 0{\cdot}0815\ T5 \text{ (1965)}$$
$$Tx_r = 32{\cdot}0457 - 0{\cdot}0586\ T5 \text{ (1966)}$$
$$Tx_r = 35{\cdot}4039 - 0{\cdot}0810\ T5 \text{ (1967)}$$

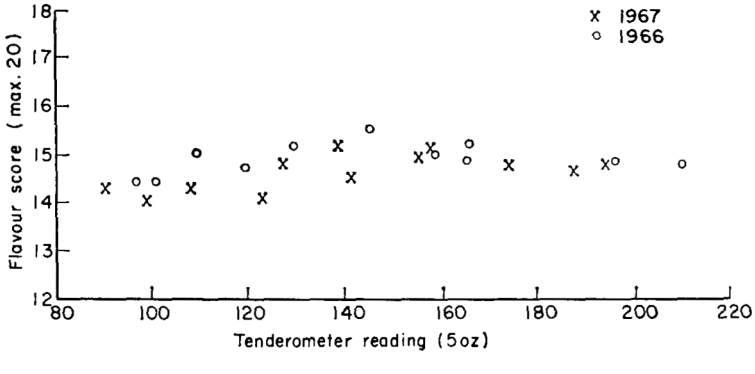

FIG. 2. The relationship between organoleptic flavour scores and tenderometer readings (5-oz samples). Campden data.

3. *The relationship between organoleptic assessment of flavour and tenderometer reading*

This relationship was included in the project for 1966 and 1967, since it was suggested that flavour of canned broad beans improved as the beans matured.

The expert panel at Campden was unable to detect any relationship between flavour of canned beans and tenderometer readings in any of the nine batches of samples. Fig. 2 presents the results obtained from the Campden trials only. Data from all other sources followed a similar pattern.

4. *The relationship between alcohol insoluble solids content and tenderometer reading* (5-*oz sample*)

The determination of alcohol insoluble solids (A.I.S.) was considered to be an important aspect of the survey, since it is one of the most accurate methods of assessing the maturity of peas and could be of similar value for broad beans.

The A.I.S. content was measured on raw and canned beans and the data are presented in Figs 3 and 4 respectively. The relationship between A.I.S. and maturity, measured in terms of tenderometer readings, was linear in both years. In 1966 two member firms provided data for each of the canned and raw beans and a further set of data for raw beans was available from the Campden trial.

In 1967 data were available from only one source for raw beans and one source for canned beans.

The relationships were highly significant for both years.

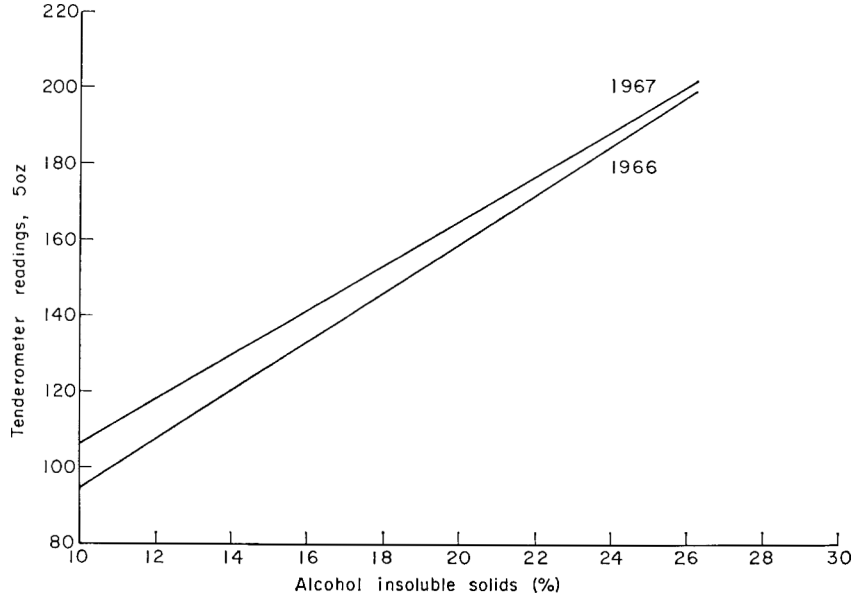

FIG. 3. The relationship between alcohol insoluble solids content of raw beans and tenderometer readings (5-oz samples).

$T5_r = 28{\cdot}9539 + 6{\cdot}5371$ A.I.S. (1966)

$T5_r = 47{\cdot}5192 + 5{\cdot}8938$ A.I.S. (1967)

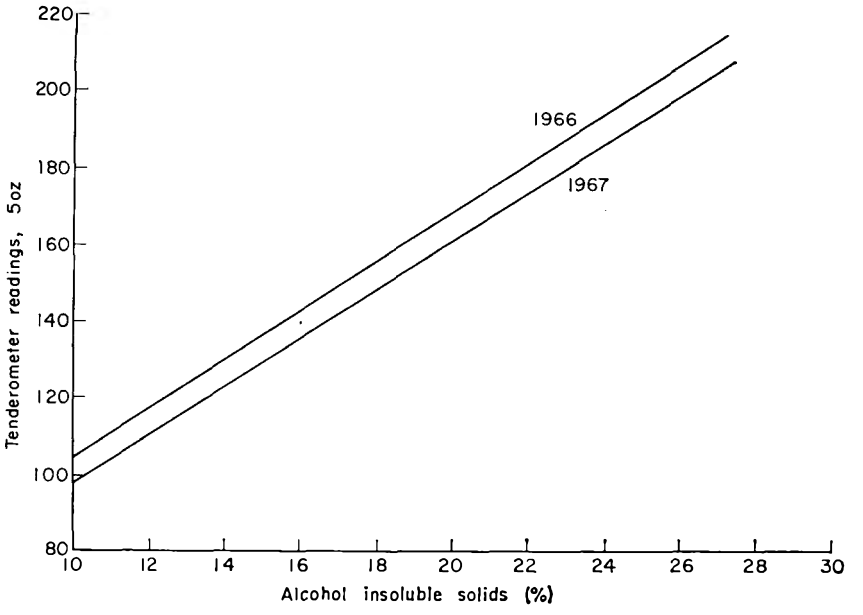

FIG. 4. The relationship between alcohol insoluble solids content of canned beans and tenderometer readings (5-oz samples).

$$T5_r = 40{\cdot}4947 + 6{\cdot}3876 \text{ A.I.S. (1966)}$$
$$T5_r = 34{\cdot}6451 + 6{\cdot}3427 \text{ A.I.S. (1967)}$$

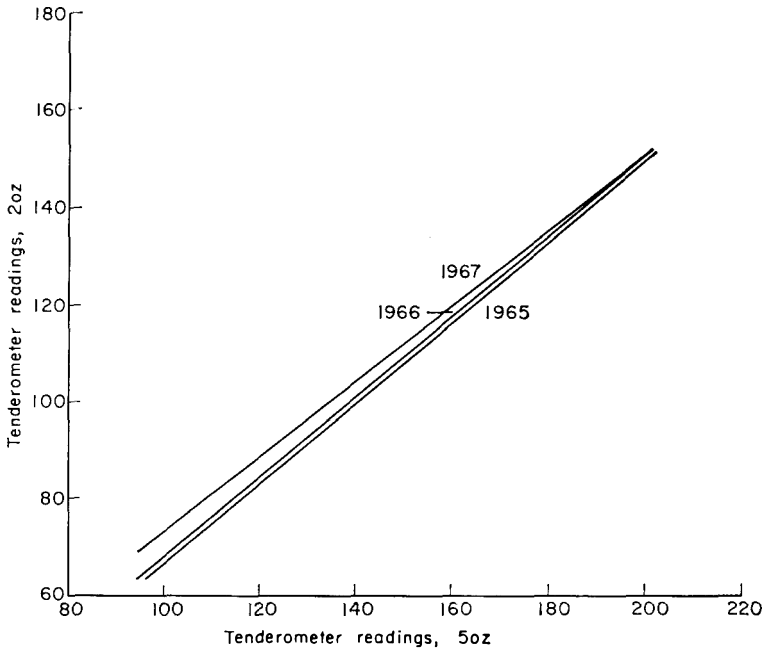

FIG. 5. The relationship between tenderometer readings using 2-oz and 5-oz samples.

$$T2_r = 0{\cdot}8375T5 - 16{\cdot}9829 \text{ (1965)}$$
$$T2_r = 0{\cdot}8373T5 - 16{\cdot}2578 \text{ (1966)}$$
$$T2_r = 0{\cdot}7815T5 - 4{\cdot}8921 \text{ (1967)}$$

5. *The relationship between tenderometer reading and sample size*

This relationship was measured because some factories, when assessing maturity, use a smaller quantity of beans than is necessary to fill the grid of the tenderometer. The three sample sizes selected for the survey were 2 oz, 3 oz and 5 oz, the last being approximately equal to a full grid of beans. All the samples were weighed carefully since it has been suggested that differences of up to 0·5 oz may occur if the grid is randomly filled with beans due to the large unit size of the seeds.

The mean data for the three years are presented in Figs 5 and 6. In each case a linear relationship was found to exist between tenderometer reading and size of sample for 5-oz vs 3-oz and 5-oz vs 2-oz samples. The regressions were highly significant.

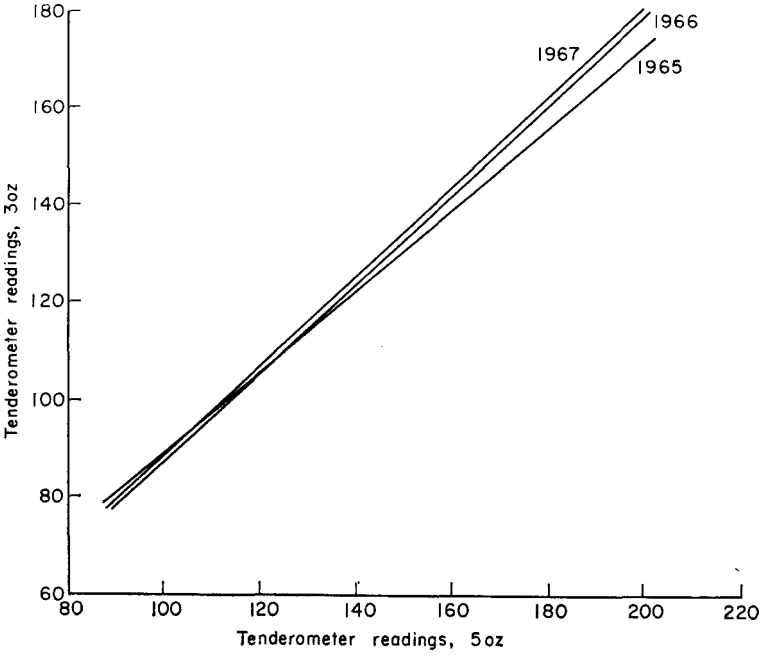

FIG. 6. The relationship between tenderometer readings using 3-oz and 5-oz samples.

$T3_r = 0{\cdot}8324T5 + 5{\cdot}8094$ (1965)

$T3_r = 0{\cdot}9166T5 - 4{\cdot}9958$ (1966)

$T3_r = 0{\cdot}9182T5 - 3{\cdot}3982$ (1967)

Discussion

The two consumer tests carried out on behalf of the Research Station both showed that preferences were evenly divided for both of the samples of canned broad beans harvested at T.R. 120 and T.R. 160.

Expert tasting panels were able to detect differences in texture but only negligible differences in flavour between the two samples of broad beans.

In both consumer tests, the majority of the tasters arrived at their decisions on the basis of flavour but the results showed that the preferences were evenly divided for both samples. In one test there was a slight preference in favour of the more mature beans and in the other test the majority of the panel thought that the immature beans were slightly better in flavour.

The texture of the beans was not the most important consideration when deciding preference although in one test the greater difference recorded was in the texture, when those who preferred the tender beans found the mature beans too tough. The converse was also true. In the other test the immature beans were considered to be more tender than the mature ones although when rated, a slightly greater number of consumers thought the mature sample to be 'very good' or 'good'. It is suggested that tenderness is not of great importance in consumer preferences.

From these results, it appears that canned beans harvested at T.R. 120 and 160 were acceptable to equal proportions of consumers and a fair proportion were indifferent. There is no indication to suggest that canned beans harvested at some point between these two maturities would not be more acceptable, neither is it possible to predict how quickly beans become less acceptable at more immature stages than T.R. 120 or more mature stages than T.R. 160.

In this paper all tenderometer readings refer to 5-oz samples of beans, except where it is stated as otherwise. In some factories samples of other sizes are used and the relationship between sample size (2 oz, 3 oz and 5 oz) and tenderometer readings was measured and found to be linear. Table 4 shows the mean equivalent 2-oz and 3-oz readings for a range of 5-oz tenderometer readings.

TABLE 4

5 oz	3 oz	2 oz
90	79	61
100	88	69
120	106	86
140	123	102
160	142	118
180	159	135
200	177	151

The alcohol insoluble solids content method of measuring maturity of peas is an important one and the maturity of some beans in this survey was assessed in this way.

The relationship between tenderometer readings and alcohol insoluble solids for raw and canned beans were linear and statistically significant. The lines for raw and

canned beans compared closely with the data given by Anthistle (1961) for raw peas. The lines for raw and canned beans are similar, but those for raw and canned peas diverge. At tenderometer readings of 120 and 160, canned beans gave alcohol insoluble solids contents of 12·4% to 13·5% and 18·7% to 19·8% respectively and raw beans gave 12·3% to 13·9% and 19·1% to 20·0% respectively.

Two aspects of quality—texture and flavour—were measured organoleptically on the canned beans. Texture scores decreased linearly as the beans matured, i.e. the beans became tougher as the tenderometer readings increased. The practical canning stage of peas occurs when increase in firmness of the skins and flesh and onset of mealiness is first readily detectable; the practical canning stage of broad beans could occur at a similar point. Organoleptically this stage occurs when six of the available 30 points have been deducted for failure of the texture to reach perfection. Although a different system is used for scoring the texture of broad beans, a similar stage to that for peas also occurs at a texture score of 24 out of 30. In the three years during which this experiment was conducted, this stage was reached when the canned beans had been harvested at tenderometer readings of 129 in 1965, 137 in 1966 and 141 in 1967. The mean value for the three years was 136.

The question of yield is of economic importance. The processor may wish to state the maturity at which he requires broad beans to be harvested so that some control over the quality of the final product can be achieved. The lower the tenderometer reading at which the beans are harvested, the lower will be the yield and the grower will expect some financial compensation. The higher the tenderometer reading at which the beans are harvested the higher the yield and the more satisfied the grower will be to have more nearly reached the maximum potential yield. Product quality may deteriorate, however, at tenderometer readings above 160. It was not possible to measure yield per acre in this project, but at the two maturities known to be acceptable to the consumer (i.e. T.R. 120 and 160) the mean seed percentages of beans in pod delivered to factories were 30·3 and 32·5 respectively in 1966 and 29·5 and 35·0 respectively in 1967. Thus at the higher maturity the seed percentage of the beans in pod had increased by a maximum of 5·5%. In all the samples examined in this experiment, there was no indication that the maximum potential yield had been reached, although at high maturities (i.e. high tenderometer readings) increases in seed percentage may have been apparent rather than real due to pod senescence. The number of days required for the beans to mature from T.R. 120 to T.R. 160 in 1966 and 1967 was seven to nine.

Conclusions

Since it is likely that canned broad bean quality will be worse, rather than better, if beans are harvested at tenderometer readings below 120 or above 160, it is reasonable to suppose that optimum maturity lies between these two maturities (used for the con-

sumer preference tests). The expert panel was able to detect no difference in flavours of beans over the range of tenderometer readings examined and the consumer panel showed an equal preference for beans harvested at T.R. 120 and T.R. 160. Texture, however, was shown to deteriorate significantly as the beans matured when measured organoleptically by the trained panel. Thus, this remains the only item of product quality, measured in this project, which can be used to assess optimum maturity.

From the results available for texture assessment, optimum maturity was reached at a tenderometer reading of 136 (5-oz samples). This is equivalent to an alcohol insoluble solids content in the raw beans of 15·7%.

To some degree the results of the consumer test Panel A support this conclusion since the data suggest that the practical canning stage of broad beans lies closer to T.R. 120 than T.R. 160.

Whilst the results show that optimum maturity of broad beans for canning may be at a tenderometer reading of 136, the consumer tests show conclusively that beans are equally acceptable to the public at tenderometer readings of 120 and 160.

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Schweppes (Foods) Limited
Smedley's Limited.

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The sorption isotherms of maize meal

P. VAN TWISK

Summary. The equilibrium moisture contents of the three main grades of South African maize meal over a range of 1·4–90·7% relative humidity (r.h.) were determined at 30°C. It was found that the fat content of maize meal influenced the moisture content at equilibrium, i.e. the higher the fat content, the lower the equilibrium moisture content for a specific relative humidity. Both desorption and adsorption isotherms for maize meal were found to be sigmoid and the effects of hysteresis were encountered in all cases. On prolonged storage mould growth occurred in maize meals held in an atmosphere of 84% r.h. and higher. From the sorption isotherms the maximum r.h. at which maize meal could be stored without the danger of mould contamination could be predicted.

Introduction

Maize is the major cereal crop of the Republic of South Africa and maize meal (or mealie meal as it is known locally) forms the staple diet of the majority of its Bantu population. Three main grades of maize meal are produced commercially and are classified as 'unsifted granulated', 'sifted granulated' and 'special sifted granulated'. The Mealie Industry Control Board controls the marketing of maize and maize products and has specified requirements for each grade. The limits for fat and fibre contents of maize meals are given in Table 1.

TABLE 1. Specifications for granulated maize meal

	Unsifted granulated	Sifted granulated	Special sifted granulated
Fat %	3·7 minimum	3·2 minimum	2·5–3·4
Fibre %	1·4–2·5	1·4 maximum	1·4 maximum

The stability of dried foods stored under specific ambient conditions may be predicted by means of their sorption isotherms. Nemitz (1962) considered that the relations between equilibrium moisture contents and the corresponding relative humidities

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are best described by sorption isotherms. In this connection, Hertzendorf (1965) has given a good review of the critical properties of air, such as relative humidity, dew point and water-vapour capacity and he has also described the use and application of psychrometric nomographs.

Labuza (1968) describes the sorption isotherm of a food material as a plot of the amount of water adsorbed as a function of the relative humidity. This amount of water is that which is held after equilibrium has been reached at a constant temperature.

Because of the fact that maize meal is such an important commodity in South Africa and because r.h. and equilibrium moisture content may play such important roles in storage stability, it was decided to investigate the relationship of these factors in relation to the storage of maize meal.

Most of the work done in this connection has dealt primarily with the properties of whole grain. Davey & Elcoate (1965) have summarized published data concerning the moisture content/relative humidity equilibrium values for maize (yellow and white), rice (paddy, husked, under-milled, milled), sorghum and wheat. Hubbard, Earle & Senti (1957) studied the 'moisture relations in maize' and found both desorption and adsorption isotherms to be sigmoid in shape. Furthermore, 'hysteresis loops' were found for the sorption isotherms. Similar results were obtained by Shelef & Mohsenin (1966) for whole yellow dent maize as well as for germ and endosperm fragments of this maize. Ballschmieter (1967) published data on the sorption characteristics of different food materials, including maize meal. This author, however, investigated only the adsorption isotherm for only one grade of maize meal, viz. special sifted granulated. In the present study all three major commercial grades of maize meal were studied and both adsorption and desorption characteristics were determined.

Materials and methods

The maize meals used in the investigations reported below were commercial samples obtained from different mills. Six samples of each grade of maize meal were examined and the average fat and fibre contents for each grade are given in Table 2.

TABLE 2. Average fat and fibre content of experimental samples

Maize meal grade	Fat (%)		Fibre (%)	
	Average	Range	Average	Range
Unsifted granulated	5·0	4·0–7·6	1·9	1·6–2·3
Sifted granulated	3·7	3·3–4·3	0·96	0·88–1·1
Special sifted granulated	2·6	2·5–2·9	0·74	0·65–0·85

Atmospheres of different relative humidities were established by using saturated solutions of different salts as well as sulphuric acid solutions of specific concentrations as described by Carr & Harris (1949), O'Brien (1948), Richardson & Malthus (1955), Rockland (1960), Wexler & Hasegawa (1954) and Wink & Sears (1950). For convenience, and as reference, the solutions applied are tabulated in Table 3.

TABLE 3. Solutions used to provide atmospheres of specified relative humidities

Relative humidity at 30°C (%)	Solution
1·4	Sulphuric acid, 75·5% (S.G. = 1·680 at 30°C)
7·1	Sulphuric acid, 67·0% (S.G. = 1·580 at 30°C)
11·8	Saturated lithium chloride solution
22·0	Saturated potassium acetate solution
32·8	Saturated magnesium chloride solution
43·5	Saturated potassium carbonate solution
54·2	Saturated sodium dichromate solution
64·6	Saturated sodium chromate solution
72·8	Saturated sodium citrate solution
75·6	Saturated sodium chloride solution
79·6	Saturated ammonium sulphate solution
84·5	Saturated potassium chloride solution
90·7	Saturated potassium nitrate solution

When sulphuric acid was used the specific gravity of the solutions was checked regularly in order to detect and correct dilution of the acid caused by moisture absorption from the samples. Care was also taken to ensure that undissolved crystals of each of the salts used were present in the salt solutions.

The apparatus used for exposing the materials to a humid atmosphere was based on that of Nemitz (1963). Nemitz's apparatus consisted of a hermetically sealed fruit-canning jar of capacity approximately 1 litre, which was approximately one quarter filled with the required solution. The air in the jar was circulated continuously by means of a mechanical stirrer attached to a small electric motor. Fig. 1 shows the general plan adopted. Only one jar is shown in the illustration, but in practice stirrers in more than one jar were operated by one motor by means of a belt and small pulleys. The tests were performed in a temperature-controlled room maintained at 30°C and each of the jars held seven weighing bottles.

The method used for determining the desorption equilibrium moisture contents was weighing 2–2·5 g material into previously weighed and dried glass-stoppered bottles (small weighing bottles) 4 × 2 cm in size. The bottles were then placed in the constant humidity apparatus after removal of the stopper. Thereafter, the bottles

with contents were weighed at weekly intervals until a constant weight had been attained. The moisture contents of the maize meal were then determined by the vacuum oven method of the A.O.A.C. (1965). For the determination of adsorption isotherms the samples were first dried in a vacuum oven for 72 hr at 50°C before continuing as with desorption investigation. Most determinations were triplicated or, at least, duplicated. Replicates were not run simultaneously. Finally, the values obtained for moisture contents were plotted against the corresponding figures for r.h.

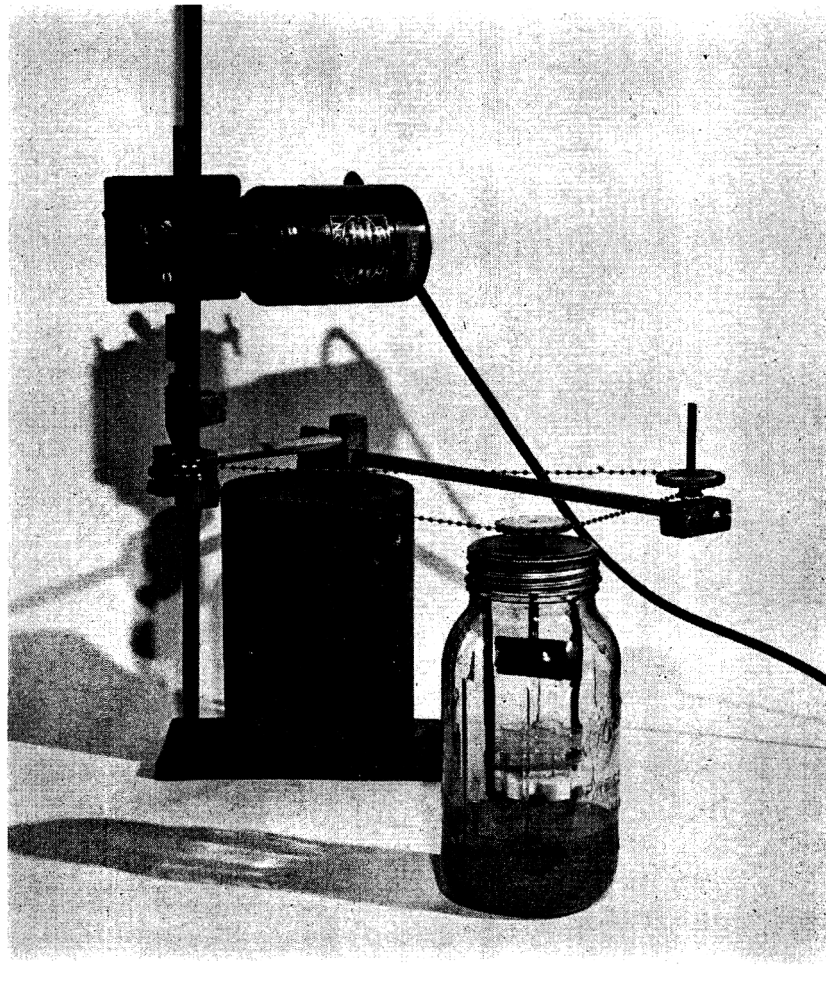

FIG. 1. Constant relative humidity apparatus.

Results

Fig. 2 depicts the sorption isotherms for unsifted granulated maize meal. The isotherms for the sifted granulated and special sifted granulated grades follow the same pattern

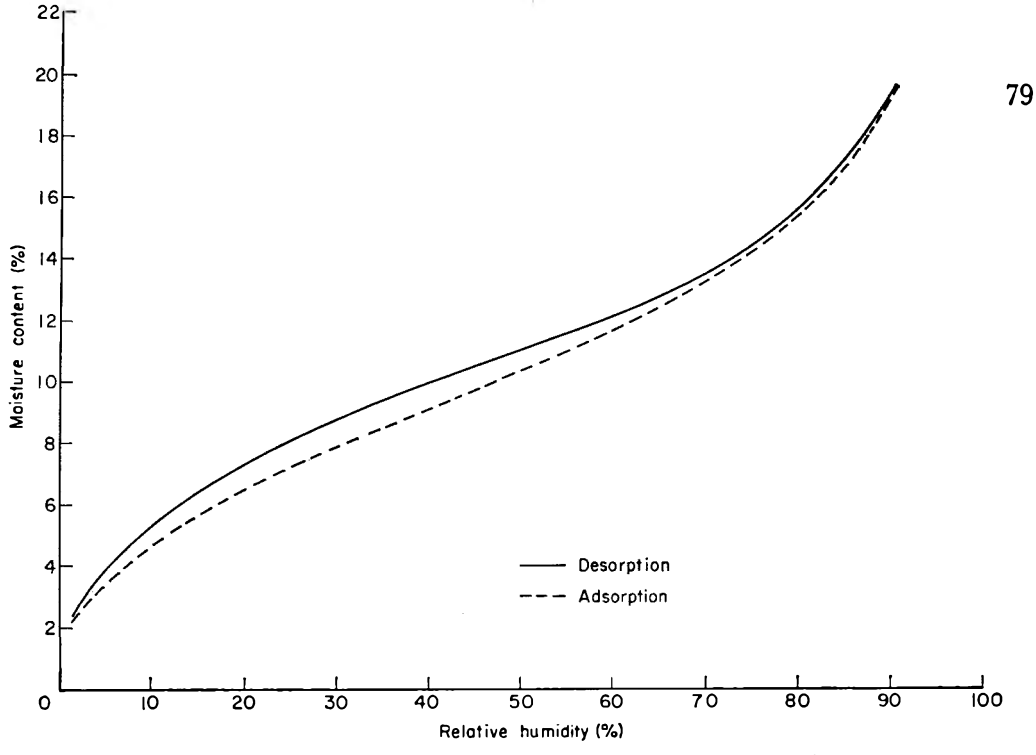

FIG. 2. Sorption isotherms of unsifted granulated maize meal.

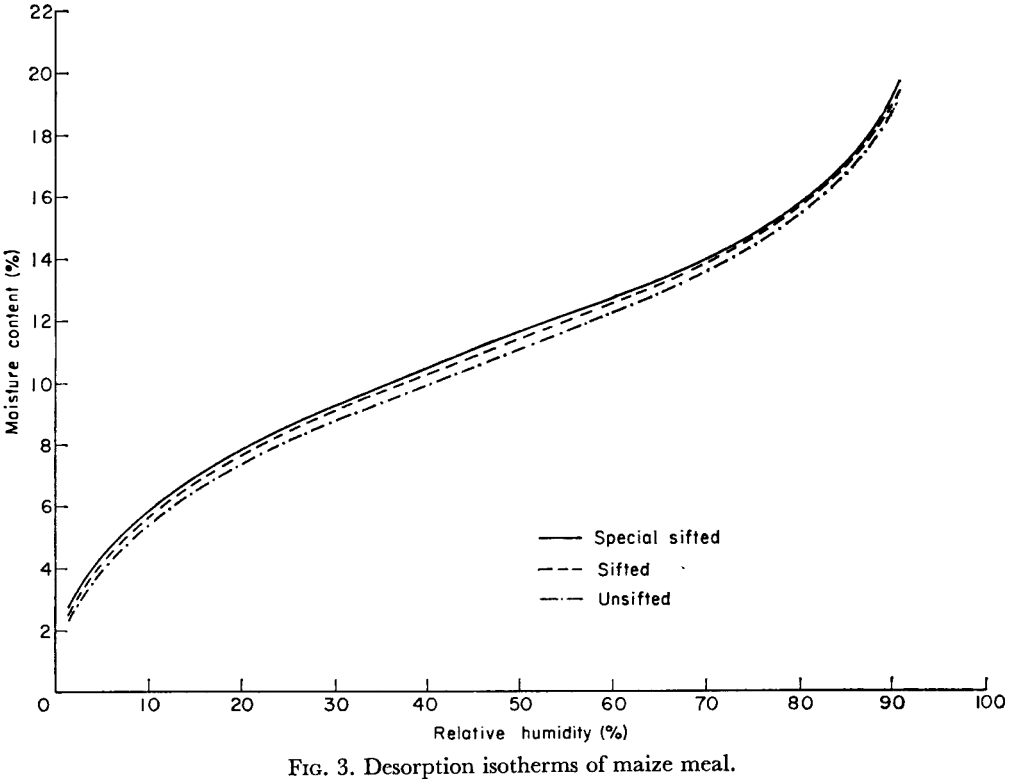

FIG. 3. Desorption isotherms of maize meal.

and are, therefore, not given. As can be seen from Fig. 2, hysteresis loops were found for the sorption isotherms and both desorption and adsorption isotherms are sigmoid in shape. The maximum hysteresis effect is between 10 and 50% r.h. disappearing at approximately 90% r.h. Maximum hysteresis corresponds to about 1% moisture.

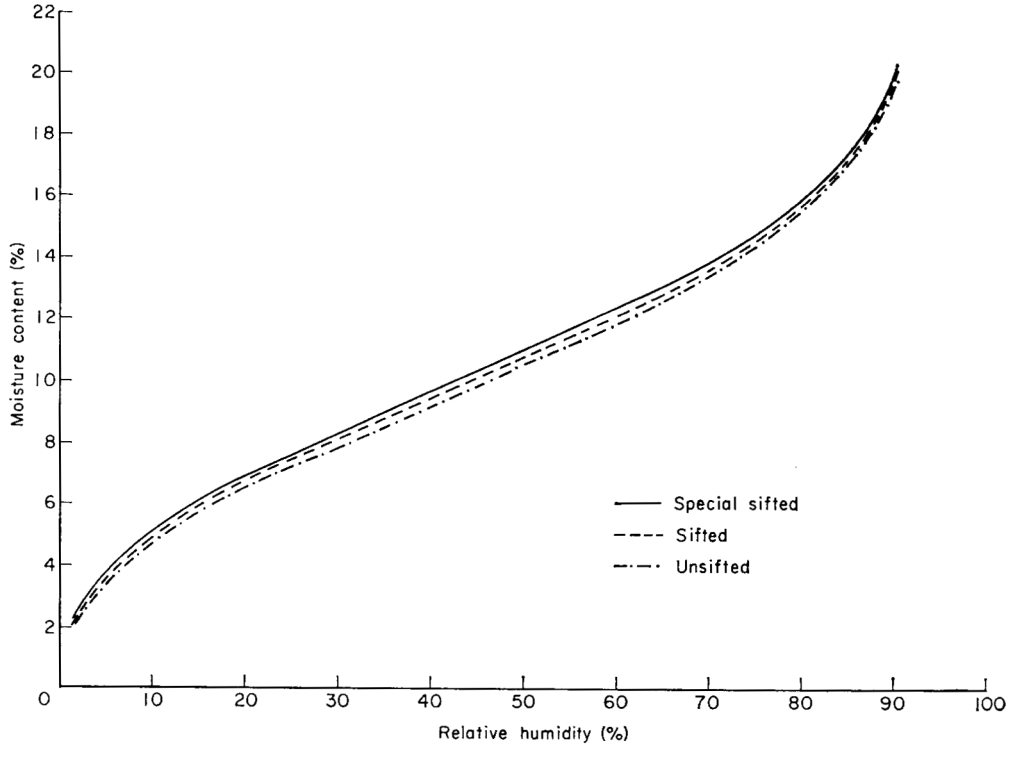

FIG. 4. Adsorption isotherms of maize meal.

Figs 3 and 4 respectively illustrate the desorption and adsorption isotherms for the three grades of maize meal. The curves follow the same pattern, but do not coincide; the graphs for unsifted granulated and sifted granulated maize meal being slightly lower than that for special sifted granulated maize meal in that order.

Mould appeared in the samples stored at relative humidities of 84% and higher. When mould appeared, equilibrium could not be accurately established and the values are only presumptive. As an important aspect of the study was to determine the r.h. levels at which mould growth first occurred, no attempt was made to suppress mycological contamination.

Apart from a few exceptions, especially in the case of mouldy samples, the standard deviation for moisture content was in the region 0·03–0·07. This seems most satisfactory especially when evaluating the differences between the different grades of maize meal.

Discussion and conclusions

Several theories have been put forward to explain hysteresis. These have all been based on the effects of water which has condensed in the capillaries of the material concerned. Labuza (1968) explained hysteresis as a change in the contact angle of this water and the capillary walls during adsorption and desorption. Rao (1941) on the other hand, used his so-called 'ink bottle theory' to explain this phenomenon. According to Gerzhoi & Samochetow (1958), sorption hysteresis may be attributed to the presence (after the removal of the capillary moisture during the drying process) of air which enters the capillaries and is adsorbed on their walls. For this reason, it is necessary to increase the partial water vapour pressure in order to overcome the resistance of air and to achieve complete wetting of the walls of the capillaries, when increasing the moisture content of cereal products.

It was noticed that the differences between equilibrium moisture contents for the three grades of maize meal were approximately inversely proportional to the fat contents of the samples for all relative humidities. It was, therefore, suggested that these differences were due to the differences in fat content. In order to obtain some information in this connection, the equilibrium moisture contents of the three grades, for several different humidities, were determined after extraction of the fat by means of petroleum ether. It was found that the equilibrium moisture contents for both desorption and adsorption, for all three grades of meal were identical within the limits of experimental error, thus indicating that the differences originally found were due to varying fat content.

Although the differences in equilibrium moisture contents between the different maize meals are not appreciable, they may be important when storing these commodities at high relative humidities.

Acknowledgments

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Routine rheological tests in the British food industry

H. G. MULLER

Introduction

As an organized branch of knowledge, rheology is fairly new. The British Rheologists Club, now the British Society of Rheology, was founded only in the 1940's. Nevertheless, today rheology is well organized and many countries have their own rheological societies. The research worker can keep abreast of modern development by studying the Rheology Abstracts and two lists are available (British Society of Rheology, 1966; Hayward, Findley & Gibbs, 1967) giving the fields of research of most rheologists working in these islands.

The food scientist is also able to find rheology books of varying mathematical complexity (Eirich, 1960; Van Wazer *et al.*, 1963; Wilkinson, 1960) instructing him in techniques and there are three collections of papers specifically on the rheology and texture of food (Scott-Blair, 1953; Society of Chemical Industry, 1960 and 1968).

In spite of this readily available information, it has not been possible to say which of the many methods are in fact used from day to day in the British food industry, how they are conducted in practice, what instruments are used and what the user's opinion of them is. For this reason a questionnaire was sent to the first 450 entries in the British Food Manufacturers Directory 1967–68. In this contribution the replies are analysed.

The questionnaire

The questionnaire was designed in two parts, only one of which was to be completed. The first part contained six questions for those not using rheological tests and the second 15 questions for those using them. Wherever possible, the questionnaire was sent with a covering letter to an individual by name. It was stated that the contents of the returns would be published but the identity of the person and firm replying would not be divulged. Only information on established routine methods was requested and not on any rheological research or development work. The questionnaires were sent out during May 1968 and the last returns considered were received at the end of September.

The questions for those *not* using rheological tests on a routine basis were as follows:

1.1 What is the product? (One only)

1.2 Do you have a laboratory?

1.3 Do you use a chemical test on the product?

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1.4 Did you once use a rheological test and then give it up?
1.5 If you gave it up, was that because the test was too difficult to perform or because it was unreliable?
1.6 If you were given a reliable test which would take a technician 20 min per day, would you use it or is the volume of your material so low that it would not be worth it?

It was the last question which had been difficult to formulate. Obviously rheological flour testing might be of importance to a flour miller whose life's work consists of making flour. If a food manufacturer uses one bag a week for, say, dusting purposes he would hardly be expected to use such tests.

The most informative question would have been 'what is your market share of the product?', but the average technologist would not have been able to answer it. The writer might have obtained the net assets (total fixed assets plus total current assets less total current liabilities), information which is publicly available. Unfortunately this information is only rarely applicable to an individual product and in any event is not relevant to a subsidiary company. Eventually question 6 was phrased as it now appears and it served its purpose reasonably well.

The second part of the questionnaire dealt with those products for which a rheological test *was* used (one product and one test only).

2.1 What is the product?
2.2 Is it a final or intermediate product or a raw material?
2.3 Do you use a chemical test for it?
2.4 Do you have a laboratory?
2.5 What is the name and
2.6 the manufacturer of the rheological instrument?
2.7 Do you use the standard method or your own?
2.8 Is the instrument in the laboratory or the plant?
2.9 Do you use it for batch tests or does the instrument work continuously, 'on line'?
2.10 If 'on line', does the instrument automatically adjust the process?
2.11 Is the temperature controlled during the test?
2.12 If yes, how? (Automatic computation, constant temperature room or cabinet, waterbath.)
2.13 Do you use statistical methods of sample analysis?
2.14 Are you satisfied with the test?
2.15 If not, why not?

The replies

(a) *The number of replies*

450 questionnaires were sent out and 125 firms responded (28%) by returning

them properly completed. Since a firm could return more than one, thus covering different products or different tests on the same product, a total of 228 usable questionnaires were returned. Several replies were received which could not be used and have not been considered. (Examples: we are only distributors; we have passed on your questionnaire to our central laboratory; it is not our policy to give information; we are tired of people sending us questionnaires.)

A reply from a central laboratory was evaluated as being equivalent to one manufacturing unit only. One executive replied on behalf of 27 bakeries belonging to his group. Since questionnaires had been sent to only three of these, only three out of 27 returns were utilized. Of the 125 firms replying, 97 (73%) had their own laboratory (or *were* a central laboratory), 28 (22%) had not. A few of the latter stated that their product was tested by their parent company, a consultant or a supplier.

(b) *Chemical and rheological testing by product*

Table 1 shows the type of product for which questionnaires were returned, the number of replies for each product, the number of rheological tests done on the product and for comparison the number of chemical tests done on it.

As compared with 54 chemical tests recorded on these products, there were 125 rheological ones. For chocolate, cream, flour, gelatin, jelly and peas more rheological than chemical tests were conducted.

The returns on flour, chocolate and meat account for almost half of the total returns. The high number of flour returns could be explained by the great importance of rheological testing in that field, but it must not be forgotten that the present writer is a cereal chemist and for that reason a more positive response might have been obtained here.

The absence of rheological testing for meat, fruit and vegetables (with the exception of peas) seems surprising in view of the considerable literature on just these topics. The Kramer shear press (Kramer & Twigg, 1966), the Wolodkewitch (1957) and the Szczesniak (1963) systems, the Instron (Bourne, 1966) and the sonic testing technique (Abbot *et al.*, 1968), all suitable for these products, were not mentioned at all.

Table 2 deals essentially with the first part of the questionnaire: with those replies indicating that no rheological testing was being conducted.

Of all 228 replies, 103 (45%) indicated that no rheological tests were being done. Of these 103, nine (9%) had tried such a test in the past and given up. 48 (47%) would use a rheological test if there was a good one, 21 (20%) were not interested because it would not be worth it. 26 (25%) did not comment. It should be noted that this was the only question in the whole questionnaire to which some workers did not reply.

(c) *The rheological instruments used*

Table 3 gives the name of the product which was tested, the number of replies stating that a test was being employed on it and the instrument used. If the instrument

TABLE 1

Product	Total replies	Chemical tests	Rheological tests	Neither
Baby food	3	3	2	0
Bacon	3	1	0	2
Bean (baked)	1	1	0	0
Breakfast cereals	1	1	0	0
Butter	1	1	1	0
Cake mixes	1	1	0	0
Caramel	2	1	1	1
Cheese	1	0	0	1
Chocolate	21	11	14	3
Cocoa	1	1	0	0
Cream (inc. synthetic)	6	4	5	1
Creme fillings	1	1	0	0
Cyder	1	1	0	0
Dextrins	2	2	2	0
Eggs	2	1	1	1
Essences	1	1	0	0
Fat	7	5	3	2
Flour	73	51	53	12
Fruit	1	0	0	1
Fruit (dried)	1	0	0	1
Gelatine	7	6	7	0
Gum	1	1	1	0
Jam	3	3	3	0
Jelly	3	1	3	0
Liqueurs	1	1	0	0
Marsh mallow	1	1	0	0
Meat	12	6	0	6
Meat extract	2	2	2	0
Milk	2	1	0	1
Milk (condensed)	1	1	1	0
Milk (evaporated)	1	0	0	1
Mince meat	1	1	0	0
Nuts	1	0	0	1
Oil	2	2	2	0
Orange peel	1	0	1	0
Pasta	2	1	0	1
Pectin	4	4	4	0
Peas	6	4	5	0
Potatoes	3	1	0	2
Potatoes (chipped)	1	1	0	0
Salad cream	1	1	1	0
Salt	1	1	0	0
Sauce (Meat)	2	2	2	0
Sausages	2	2	0	0

TABLE 1 (*continued*)

Product	Total replies	Chemical tests	Rheological tests	Neither
Semolina	2	1	0	1
Soft drinks	7	5	2	2
Soya flour	2	2	0	0
Starch	8	7	6	1
Sugar	4	1	0	3
Sugar (soln)	4	3	0	1
Sugar (confy)	3	0	0	3
Tea	2	0	1	1
Tomato paste	3	3	1	0
Wines	1	1	0	0
Yogurt	1	1	1	0

TABLE 2

Product	Replies	Not using rheological test	Used it and gave it up	Would use one	Not worth it	No comment to quest. 1.6
Baby food	3	1	0	0	0	2
Bacon	3	3	0	1	2	
Bean (baked)	1	1	0	1	0	
Breakfast cereals	1	1	0	1	0	
Butter	1	0	0	0	0	
Cake mixes	1	1	0	1	0	
Caramel	2	1	0	0	1	
Cheese	1	1	0	1	0	
Chocolate	21	7	1	3	2	1
Cocoa	1	1	0	0	0	1
Cream (inc. synthetic)	6	1	0	1	0	
Creme fillings	1	1	0	0	0	1
Cyder	1	1	0	1	0	
Dextrins	2	0	0	0	0	
Egg	2	1	0	1	0	
Essences	1	1	0	0	0	1
Fat	7	4	1	3	1	
Flour	73	20	5	11	4	
Fruit	1	1	0	0	1	
Fruit (dried)	1	1	0	1	0	
Gelatine	7	0	0	0	0	
Gum	1	0	0	0	0	
Jam	3	0	0	0	0	
Jelly	3	0	0	0	0	
Liqueurs	1	1	0	0	0	1
Marsh mallow	1	1	0	0	0	1

TABLE 2 (*continued*)

Product	Replies	Not using rheological test	Used it and gave it up	Would use one	Not worth it	No comment to quest. 1.6
Meat	12	12	0	3	6	3
Meat extract	2	0	0	0	0	
Milk	2	2	0	1	0	1
Milk (condensed)	1	0	0	0	0	
Milk (evaporated)	1	1	0	0	0	1
Mincemeat	1	1	1	1	0	
Nuts	1	1	1	1	0	
Oil	2	0	0	0	0	
Orange peel	1	0	0	0	0	
Pasta	2	2	0	2	0	
Pectin	4	0	0	0	0	
Peas	6	1	0	1	0	
Potatoes	3	3	0	1	0	2
Potatoes (chipped)	1	1	0	1	0	
Salad cream	1	0	0	0	0	
Salt	1	1	0	1	0	
Sauce (meat)	2	0	0	0	0	
Sausages	2	2	0	2	0	
Semolina	2	2	0	1	1	
Soft drinks	7	5	0	2	0	
Soya flour	2	2	0	2	0	
Starch	8	2	0	0	0	2
Sugar	4	4	0	1	2	1
Sugar (solution)	4	4	0	0	0	4
Sugar (confy)	3	3	0	2	1	
Tea	2	1	0	0	0	1
Tomato paste	3	2	0	0	0	2
Wines	1	1	0	0	0	1

TABLE 3

Product	Replies	Instrument used
Baby food	2	Bostwick Consistometer, Amylograph
Butter	1	Penetrometer
Caramel	1	Hardness Tester
Chocolate	14	Torsion Wire (6), Redwood Cup (2), Koch (3), Cone, Brookfield, Ferranti portable
Cream	5	Torsion Wire (3) Brookfield, Penetrometer
Dextrin	2	Brookfield, Corn Products Viscometer
Egg	1	Ford Cup

TABLE 3 (*continued*)

Product	Replies	Instrument used
Fat (vegetable)	3	Penetrometer, Hobart mixer, F.I.R.A./N.I.R.D. Extruder
Flour	54	Research Extensometer and water absorption meter (17), Water absorption meter only (2), Farinograph (11), Extensograph (7), Amylograph (7), Ford Cup (2), Hagberg (3), Falling Ball, Penetrometer, Wattmeter on mixer, Ammeter on mixer (2)
Gelatine	7	Brookfield, F.I.R.A., Rigelimeter, Boucher, Bloom (3)
Gums	1	Ferranti portable
Jam	3	Brookfield (2), Gravity flow apparatus
Jelly	3	F.I.R.A. (2), Brookfield
Meat extract	2	Brookfield, Techne Capillary Tube
Milk (condensed)	1	Ferranti portable
Oil	2	Ford Cup, Falling Ball
Orange peel	1	Tenderometer
Pectin	4	Rigelimeter (3), F.I.R.A.
Peas	5	Tenderometer (5)
Salad cream	1	Falling Ball
Sauce (meat)	2	Ford Cup, Falling Ball
Soft drinks	2	Falling Ball, Brookfield
Starch	6	Scott (2), Amylograph (2), Brookfield, Farinograph
Tea (instant dried)	1	Powder flow meter
Tomato paste	1	Bostwick Consistometer
Yogurt	1	Penetrometer

was named more than once, this is indicated by the figure in brackets behind the name. It is striking that with a few exceptions the methods employed are empirical. This might support the jest that theoretically sound instruments do not work in practice and those that are theoretically unsound, do.

The great majority of workers were satisfied with their tests and instruments. Only nine out of 125 (7%) were not. In some cases the reasons for the failure were obvious. For dough and chocolate testing the temperature must be controlled and a falling ball, capillary tube and emptying cup viscometer, which are designed for Newtonian liquids, do not necessarily work with non-Newtonians. Theoretically, they should of course not be used at all for such liquids, but in practice they are, and apparently often satisfactorily!

Apart from those few that were dissatisfied with their tests outright, several made critical comments. The greatest number of these dealt with the Research Extensometer, a dough testing instrument. Nevertheless, it was the most frequently used instrument of the entire survey. In contrast the Swanson Mixograph and the Chopin Alveograph received no mention.

Only 24% of the rheological instruments were used together with statistical methods

of analysis (30 out of 125). There was no continuous (on line) testing except in the baking industry, where several mixers were fitted with various electrical recorders to depict the mixing process. No rheological instruments were mentioned which automatically adjusted the manufacturing process. One chocolate manufacturer pointed out that he intended installing a Rheometer of the Contraves Epprecht type in the near future.

Most of the tests were done in the laboratory rather than the plant (102 out of 125 or 82%). Nevertheless, all six tenderometers stood on the factory floor. Three chocolate and eight dough tests were done there too.

73 (59%) used their own testing method, 62 (50%) the official one. 9%, mainly flour millers, used both, presumably to satisfy their customers who might use their own method. (Here is a field for some useful committee work!)

Conclusion

There is without doubt a very considerable amount of rheological testing in the food industry. The overwhelming majority of tests are regarded as satisfactory by those workers using them. This confidence is shared neither by those who would use tests if good ones were available, nor is it shared by the present writer.

Rheology is much like psychology: We all have the same psychological attributes but these vary quantitatively in great measure. Similarly, all materials have all rheological properties but some predominate (Reiner, 1960). Hence it is legitimate to test, say, caramel either as an elastic solid or a viscous liquid. Whichever is done depends on the purpose to which the material is to be put.

None the less, with some goodwill it is possible to classify foods into various groups.

(1) *Newtonian liquids*

Here shear stress is linearly related to the rate of shear. Examples are: water, carbonated beverage, meat extract, corn syrup, sucrose and salt solutions, milk and alcoholic beverages as long as they do not contain long chain molecules. For measurement almost any accepted viscometer can be used as long as the temperature is controlled and there is no turbulence.

(2) *Non-Newtonian liquids*

Shear stress is not linearly related to the rate of shear. Examples are almost all liquid foods other than those mentioned above. Non-Newtonian liquids fall into four groups, those that harden and those that soften with shear and in each of these two are those that are time dependent and those that are not. For the testing of these liquids the literature should be consulted (Van Wazer *et al.*, 1963; Wilkinson, 1960) but as a general rule narrow gap concentric cylinder or cone and plate viscometers should be used. This rule may be broken if the general rheology of the material is known;

chocolate is an example of a well-documented material for which one point measurements may be successful. Otherwise, orifice and capillary viscometers and rotating devices such as pins, paddles and discs, as well as wide gap concentric cylinder viscometers, should be avoided. Some of these instruments (e.g. capillary tube viscometers) may be satisfactory if the liquid is not time dependent. Of course, if the apparent viscosity difference from sample to sample is really massive (flour batters, starch pastes) almost any instrument can be used including one's fingers.

(3) *Plastic materials*

The material has a yield value. Its subsequent flow behaviour may be linear or non-linear. Examples are mayonnaise, stiff creams, fats, chocolate, marzipan, foams. Many methods, both c.g.s. (Capillary viscometer, concentric cylinder viscometer) and empirical (penetrometer, extruder), are available. Any possible time dependence should be considered before an instrument is chosen.

(4) *Visco-elastic materials*

The material has both viscous and elastic properties. Examples are gels, wheat flour dough, liquorice, egg white, ice cream, fruit, breadcrumb and very many others. Testing methods are: vibrational techniques, loading/unloading experiments, stress relaxation methods and measurement of normal stress components. There is no doubt in the writer's mind that these methods should replace most of the ones at present in use.

(5) *Solids*

There are very few of these materials in the food industry: hard boiled sweets, caramel and uncooked pasta products are examples. Conventional hardness testers are useful.

Additional difficulties in rheological testing arise where the property measured (and badly at that) is of no direct interest, but merely serves as an indication of a different phenomenon. Thus the rheology of flour dough is used to forecast baking performance, the apparent viscosity of a gelatin solution is used to predict gel strength, or the hardness of fat to indicate subsequent creaming properties. These exercises are very risky.

Nevertheless, a poor test is better than none. It is hoped that this survey has helped to indicate how foods are tested rheologically at present and how standards of testing can be raised.

Acknowledgment

I wish to thank most sincerely the representatives of the 125 companies who co-operated in this survey.

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Note on sequential tasting tests

E. H. STEINER

In his article on 'Some aspects of statistics of small numbers of triangular taste tests' Long (1968) puts forward an argument which he alleges leads to significant differentiation between samples with smaller numbers of tastes than in previously published tables (Steiner, 1966).

For the development of his theme Long makes some statements which, if they are to be accepted, would appear to require more substantiation than is given to them. In particular, in criticizing the 1966 tables, Long implies that error was introduced from the use of the normal approximation to the binomial distribution. This is not, in fact, the case since these tables were constructed according to Wald's (1947) theory of sequential analysis for the discrimination between two population values of a proportion which does not involve any use of the normal approximation.

Secondly, on page 71 Long makes a statement 'To test the hypothesis that the fraction of tests in which true discrimination occurred was f, a score of m successes out of n trials may be regarded as equivalent to a test of a null hypothesis of no detectable difference for a score of $(m-fn)$ out of $n(1-f)$'. The interpretation of a 50% discrimination is that individuals may vary in their sensitivity, but taken over a long (infinite) period of time, this ability to discriminate will be for 50% of the time, or alternatively a panel of tasters is regarded as a sample from the whole population, 50% of whom can truly discriminate. In either case, this will lead to an expected proportion of successes of $\frac{2}{3}$ in any given tasting test, and the purpose of the sequential scheme is to discriminate between the two hypotheses of $p=\frac{1}{3}$ and $p=\frac{2}{3}$. This does not mean, as required by Long's statement, that $\frac{1}{2}$ of the results obtained by the panel must be correct, and it is only necessary to consider the significance of successes in the other half.

Finally, in deriving Table 3 Long gives limits for each successive number of tests which are derived from the number required to disprove the null hypotheses in fixed panel size tests of corresponding number. It is not valid, however, to use ordinary significance tests in a sequential scheme. Thus, if the null hypothesis of $p=\frac{1}{3}$ (no difference) is true, then the chance of rejecting it is 0·05 at the smallest possible panel size of 3. If tests are continued and rejection of the null hypothesis is repeatedly allowed on the fixed panel size limits the chance of rejection will increase the more the oppor-

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tunities for rejection that are given. This would indicate that the decisive scores given in Table 3 correspond with probabilities greater than the stated risk of error of 5%.

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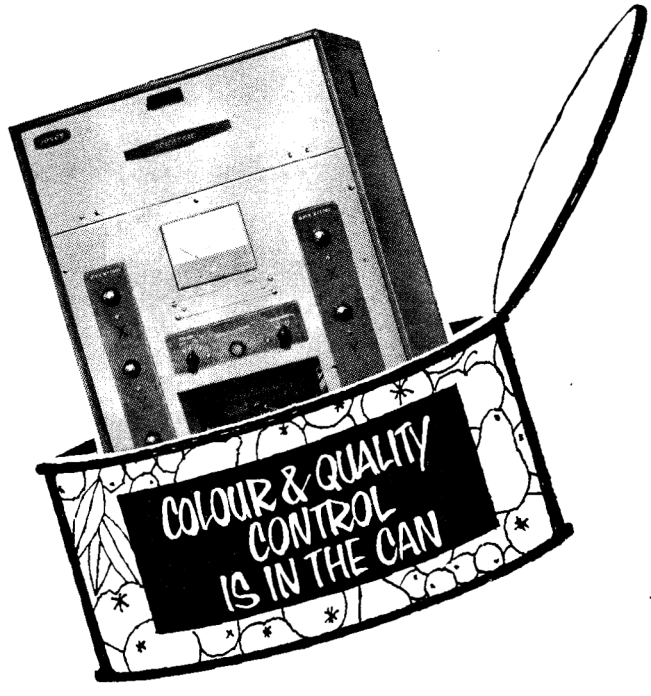

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gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	μg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

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