ISSN 0022–1163

Volume 16 Number 2 April 1981

Journal of Food Technology

Published for the Institute of Food Science and Technology (U.K.) by Blackwell Scientific Publications
Oxford London Edinburgh Boston Melbourne

JOURNAL OF FOOD TECHNOLOGY

Institute of Food Science and Technology (U.K.)

Objects of the Journal. The Journal covers a wide field ranging from pure research in various sciences associated with food to practical experiments designed to improve technical processes. While the main object is to provide a forum for papers describing the results of original research, review articles are also included. The Editor welcomes manuscripts of original research or comprehensive reviews of specialized sections of food science or technology.

Contributions and editorial correspondence should be sent to Dr H. Liebmann, c/o Institute of Food Science and Technology (U.K.), 105–111 Euston Street, London NW1 2ED.

General correspondence should be sent to Dr P. Wix, Polytechnic of the South Bank, Borough Road, London SE1 0AA.

Business matters, including correspondence and remmittances relating to subscriptions, back numbers, advertising and offprints, should be sent to the publishers: Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL.

The Journal of Food Technology is published bimonthly, six issues form one volume. The annual subscription is £64.00 (U.K.), £77.50 (Overseas), $195.00 (N. America, including cost of airfreight). Back volumes are still available.

U.S. Mailing Agent, Expeditors of the Printed Word Ltd, 527 Madison Avenue, Suite 1217, New York, NY 10022. Second class postage paid at New York, NY.

J. Fd Technol. (1981), 16, 101–113

Physical characteristics of an air classified potato starch

F. HOLM

Summary

The physical properties of potato starch concentrate (96% starch, dry basis) produced by air classification of dried potatoes have been examined. The properties of the starch product are in many respects different from those of commercial potato starch. At a storage temperature of 20°C the product will be stable over long periods at water activities between 0.4 and 0.5. The concentrate possesses high water binding capacity which is a desirable property within food processing. Viscosity studies show that the concentrate is stable towards heat, mechanical shear, moderately acidic conditions, and that the paste viscosities are higher than for potato starch except for the gelatinization peak which indicates restricted swelling of the granules. The acid stability, rate of retrogradation and freeze-thaw stability are very similar to commercial potato starch.

Introduction

Most commercial starch products are very pure. This is especially true for potato starch, where purity is often taken as being synonymous with quality. In order to obtain high purity, it is necessary to use 10–20 tons wash water per ton potato starch. This accounts for a considerable proportion of the production cost not least through the cost of effluent treatment. Whether such a degree of refinement is required must be governed by the intended use of the starch. A considerable proportion (in Denmark more than 75%) is used in the fermentation industry, the food industry and for household and other purposes, where the residues of non-starch components may be tolerated and in some applications their presence may even prove advantageous through their property of complex formation.

Author's address: Jutland Technological Institute, Food Technology Section, 135 Marselis Boulevard, 8000 Aarhus C, Denmark.

0022–1163/81/0400–0101 $02.00

The present study was undertaken in order to determine the change in the properties of starch products by the inclusion of low levels of non-starch components, so that the utility of less pure but cheaper than conventional starch products may be evaluated. The properties examined included water absorption, viscosity, storage stability, and retrogradation. The product studied was the starch-rich concentrate produced by the process of air-classification of dried potatoes – a process that avoids the use of water (Holm, 1980). This process has the added advantage that a concentrate rich in protein and a fibre residue rich in pectin is produced at the same time. The functional properties of the potato protein concentrate are described elsewhere (Holm & Eriksen, 1980).

Materials and methods

Materials

The potato starch concentrate was produced by direct air classifiation of spray dried raw potato mash (Holm, 1980). The fine fraction constitutes the potato protein concentrate (PPC), whereas the coarse fraction is the starch concentrate (PSC). Prior to air classification, a potato fibre concentrate (PFC) is removed by sieving on a 250 μm screen. Bintje-tubers with 12.0% crude protein in DM were used as raw material. The chemical composition of the starch concentrate is shown in Table 1.

Table 1. Chemical composition and enzyme activity of PSC (Holm, 1980)

Component	Composition, PSC (%)	Activity*
Starch	91.3	—
Crude protein (N × 6.25)	3.8	—
Ash	1.7	—
Fat	0.8	—
Sugars	0.3	—
Pectin	0.2	—
Moisture	4.9	—
Lipoxygenase	—	34.0
Peroxidase	—	4.0

*Activity determined as change in absorbance per minute per gram during incubation with substrate (Lulai & Baker, 1976; Freimuth, 1972).

Some of the PSC was further treated by pinmilling, followed by a 'second' air classification step, which reduced the protein content to 3.2%, or by washing

with 2 × 400 ml tap water per 100 g PSC which reduced the protein content to 1.2% and the ash content to 0.5%.

The following products were used for comparison with PSC: potato starch (Kartoffelmelscentralen), maize starch (Sigma Chemical Company), waxy maize starch (CPC Industrial Products), acetylated distarch adipate (Col-Flo 67, Laing National), coagulated potato protein (CPC Industrial Products), soy concentrate (Aarhus Oliefabrik) and dried potato juice produced by milling potatoes, centrifuging and spray drying of the liquid phase.

Methods

For determination of the sorption isotherms and evaluation of storage stability, the products were first dried in a vacuum oven (50°C) to less than 5% moisture and then samples of 20 g were placed in desiccators adjusted to different relative humidities using various saturated salt solutions (Weast, 1970). The air humidity was monitored using a Sinascope with crystal detector (Sina AG). After equilibration, moisture content was determined by drying at 100°C until constant weight. Anisidine value was determined according to the IUPAC method (D II, 26).

The colour change of the samples during storage at the respective water activities was determined by extraction with distilled water. A 2-g sample in 20 ml of water was stirred for 5 min. The absorption spectrum of the filtrate was recorded using a Beckmann model 25 spectrophotometer. In the case of PPC and spray dried potato juice 0.2 g of sample was extracted with 20 ml water, as these products contained considerably more extractable colour.

The hydration characteristics of the products were determined according to the pipette method (Thorgersen & Toledo, 1977). The method monitors the rate of water absorption through a 0.2 μm glass filter membrane which is in direct contact with distilled water, the water absorption being read as a function of time. The method is very quick (*ca* 1 min for most starch products) and reproducible.

The water absorption was also determined by centrifugation of a 1% starch slurry at room temperature before and after heating to 90°C for 10 min. Samples were allowed to hydrate for 30 min before centrifugation in a Heraeus Christ centrifuge (model Piccolo) at 5000 rev/min.

The viscosity studies were carried out with a Brabender Viscoamylograph fitted with a 700 cmg measuring head (75 rev/min, 1.5°C/min with 60 min hold at 95°C followed by 60 min hold at 25°C). Starch suspensions (6% dry basis) in either distilled or tap water were used.

The acidities of the samples were in some experiments adjusted using citric acid monohydrate (50 g/l) and 2N NaOH. In certain cases, the viscosity was also determined using a Brookfield Viscometer (RVT) – spindle 1 and 50 rev/min.

Retrogradation of starch was measured by starch determinations of the supernatant liquid from centrifuged starch solutions as a function of time at 0°C

(Watson, 1964). The freeze-thaw properties were examined after repeated freeze-thaw cycles of a pre-gelatinized 2% starch solution. The quantity of liquid that could be drained off from the samples placed on a filter paper during 1 h was used as a measure for the freeze-thaw stability.

The chemical methods were used as previously described (Holm, 1980). Lipoxygenase and peroxidase activity in PSC was determined as change in absorbence per min per gram during incubation with substrate (Lulai & Baker, 1976; Freimuth, 1972).

Results and discussion

Storage stability

The sorption isotherms (absorption mode) of the starch concentrate (PSC), protein concentrate (PPC) and fibre product (PFC) are shown in Fig. 1. Isotherms for commercial potato starch, heat-coagulated potato protein and spray dried potato juice are included for comparison. Assuming a water activity of 0.6 as the lower limit for microbial activity, the isotherms show that for safe storage at 20°C, PSC must contain no more than 16% water whereas potato starch may be stored containing 18% water. The corresponding values for spray dried potato juice, PPC, PFC and coagulated potato protein are 28, 17, 15, and 11% water, respectively.

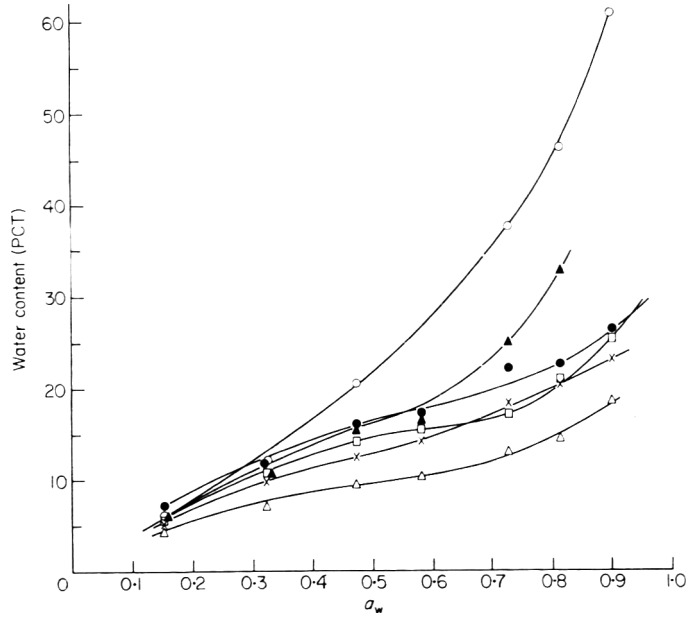

Figure 1. Sorption isotherms (20°C, absorption mode) for: ●, potato starch; □, PSC; ▲, PPC; ×, PFC; ○, spray dried fruit water; △, coagulated potato protein.

Relative to potato starch, the water activity (a_w) in PSC and PFC are both high, but low in PPC at a given water content. The sorption isotherm for PSC crosses the isotherm for potato starch at a a_w above 0.9. The effect of non-starch compounds in PSC in relation to pure potato starch is thus to increase a_w at low to medium moisture contents, but to decrease it at high moisture content.

Ions and small hydrophilic molecules such as sugars and polyalcohols normally lower a_w (Bone, Shannon & Ross, 1975). This effect is utilized in intermediate moisture food (IMF), such as salted meat and sugar-preserved food. Figure 1 does not seem to be in accordance with this, but as demonstrated by Duckworth (1978), the effect is a_w-dependent. In a model system prepared from potato starch and sorbitol he found an increase in activity at activities below 0.6 and a depression above 0.6 leading to an intersection between the isotherms of starch and mixture of starch and sorbitol. It appears from Fig. 1 that the intersection of the PSC and starch isotherms is considerably higher ($a_w = 0.93$).

Ross (1975) demonstrated that the water activity of a complex product can be estimated as the product of the activities of the single components, such that second order interactions can be neglected.

It appears from Fig. 1 that throughout the range coagulated potato protein induces higher a_w than starch of the same moisture content. This component in PSC will thus contribute to an increase in a_w and therefore displace the isotherm for starch towards higher a_w. The effect will be further intensified by the fact that at a given moisture content undenatured protein has a higher a_w in the range below 0.8 relative to denatured protein. This was shown by Hermansson (1977) to be valid for soy protein isolate and whey protein concentrate. It can hence be assumed that this effect is due to the relatively low water binding capacity of undenatured protein.

The effect of the non-starch components in PSC on the sorption isotherm thus does seem to be explained qualitatively on the basis of published studies.

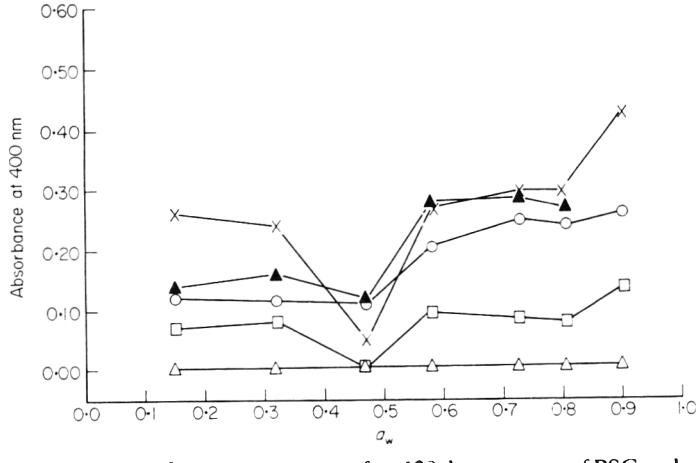

Figure 2. Absorbance of aqueous extracts after 120 days storage of PSC and reference products as function of water activity during storage. ×, PFC; ▲, PPC; ○, spray dried fruit-water; □, PSC; △, potato starch and coagulated potato protein.

A subjective evaluation of the products after 120 days storage at the respective, relative humidities indicated unchanged colour and flavour below 50% r.h. Above 50–60% r.h. PSC, PPC and PFC showed increasing grey-colouring and off-flavour, whereas the other products showed no changes below about 90% r.h. The increase in off-flavour was accompanied by an increase in anisidine value from 55–60 below 60% r.h. to approximately 150 above 60% r.h.

Figure 2 shows how the colour evolves as function of r.h. The colour is here defined as the absorbance of a water extract at 400 nm. Commercial starch and coagulated potato protein both showed low and constant absorbance after storage, irrespective of r.h., whereas the other products seem to bleach at a water activity of 0.47.

In foods, which have not been subjected to heat-treatment, e.g. PSC, the presence of some enzymes might produce off-flavour and colour changes even at low a_w values (Brockmann, 1978; Labuza *et al.*, 1972; Burnette, 1977; Brockmann & Acker, 1977).

The reactions catalysed by lipoxygenase, polyphenol oxidase, glucose oxidase and peroxidase are among the best known examples. According to Labuza *et al.* (1972) a detectable enzymic activity is already found at a_w 0.3 whereas non-enzymic discolouration starts at a_w 0.2. Brockmann (1978) mentions that lipoxygenase has a measurable activity around a_w 0.05 whereas polyphenol oxidase and glucose oxidase first become active above 0.30–0.35.

Lipoxygenase is thus active at a considerably lower water activity than most other enzymes and can cause bleaching of PSC at low a_w due to reaction between the hydroperoxides formed and carotenoides present (Blain & Todd, 1955; McDonald, 1979). At higher water activities, polyphenol oxidase becomes active, resulting in an increased grey-colouring due to the formation of dark-coloured phenol condensation products. The curves in Fig. 2 could thus be interpreted on the basis of the enzymic reactions. Storage of PSC at relative humidities between 0.4 and 0.5, i.e. 13–15% moisture content, results in an almost colourless product.

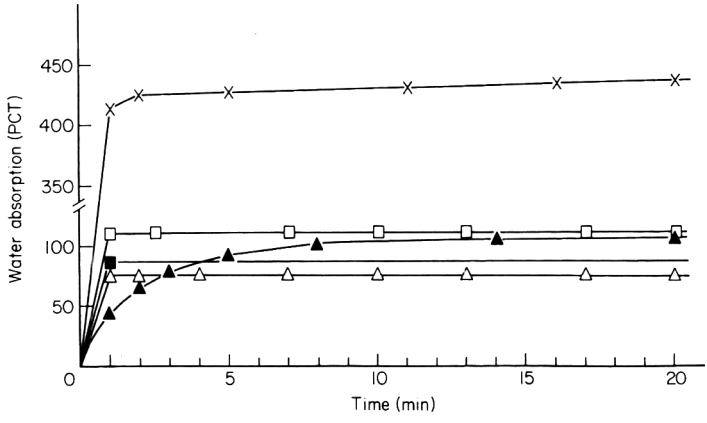

Figure 3. The rate of water absorption (pct. total water in products) of PSC and reference products. ×, PFC; □, PSC; ▲, PPC; ■, maize; △, potato.

Water absorption

Figure 3 shows the hydrating abilities of PSC, PPC, PFC and maize starch and potato starch as measured by water absorption according to the pipette method. The starch products are completely hydrated within 1 min, whereas the protein required more than 10 min. The water absorption capacity of PSC is 47% higher than for potato starch and 26% higher than for maize starch, probably due to the presence of non-starch components such as protein, fibres and minerals in PSC.

Table 2 shows the water absorption capacities of the products determined according to the pipette method and the centrifugation method.

Table 2. Water absorption capacities of PSC and reference products

	Water absorption g/g starch		
Product	Pipette method	Centrifugation method	Centrifugation method after heating to 90°C
PSC	1.13	1.5	9.6
PPC	1.26	0.8	3.4
PFC	4.30	5.3	—
Maize starch	0.86	1.2	10.2
Potato starch	0.75	1.2	5.0

It appears from the table that the water absorption capacity after heating to 90°C is almost double that of potato starch and similar to that of maize starch. This makes PSC suitable as a water binder and firming agent in many foods, such as sausages and canned foods.

Viscosity

Amylograph viscosities of potato starch and PSC, measured in distilled as well as in tap water are shown in Table 3.

Ions in tap water caused a decline in the peak viscosity for PSC and potato starch, whereas the cold paste viscosities increased significantly. PSC in tap water gave the highest hot-paste viscosity (after 1 h at 95°C) and the highest cold paste viscosity (final). The amylogram parameters of potato and maize starch and products from the air classification process are shown in Table 4.

Figure 4 shows the viscosity curves of PSC, potato starch, maize starch, PPC and PFC. None of the products – except potato starch – have a characteristic peak. Refining of the cyclone fraction by air classification and washing caused increased viscosity. This increase was higher than expectations based on the

Table 3. Amylogram parameters of PSC and potato starch in distilled and tap water

Product	Water type	Temp. (°C) Start	Temp. (°C) Peak	Viscosity, BU Peak	95°C start	95°C end	Gel peak	Final
PSC	Distilled	64	> 95	490	480	310	720	720
PSC	Tap	66	> 95	430	430	430	1200	960
Potato starch	Distilled	59	71	1800	550	320	630	530
Potato starch	Tap	60	84	870	650	320	1010	720

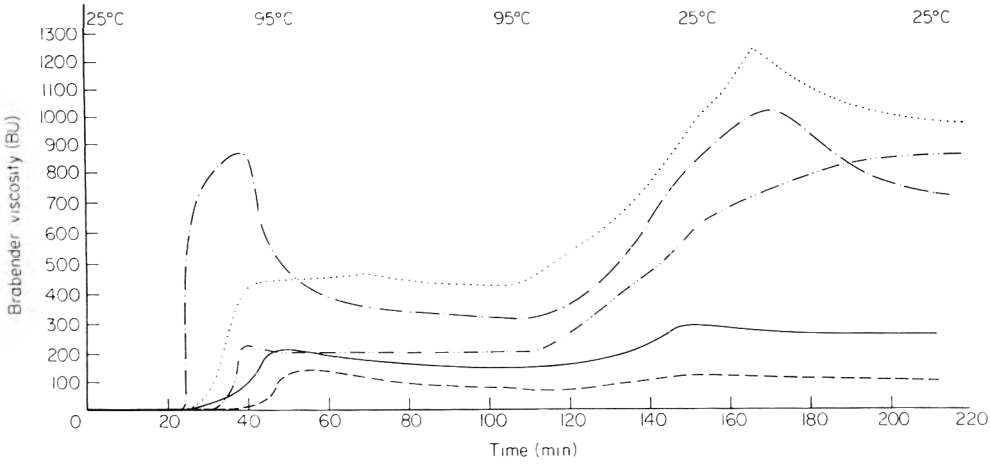

Figure 4. Amylograms (6 pct. DM suspensions) of: —·—, potato starch; . . . . . , PSC; —··—, maize starch; ———, PFC; — — —, PPC.

increased starch content (Table 4). Pinmilling followed by an additional air classification step – PSC (× 2) – however, gave decreased viscosities, which are probably due not to the air classification, but to damage of starch granules as a result of the milling. A similar effect is reported for wheat and maize starch, whereas for pea starch the viscosity increases up to 40% starch damage (Vose, 1977).

PSC has a higher peak viscosity than maize starch but lower than for potato starch. The cold-gel viscosity of PSC is higher than for maize and potato starch.

The excellent stability of PSC pastes to heat and mechanical shear is of special interest, a property that is also seen with crossbonded starch. The viscosities of the PSC solutions after 1 h at 95°C are considerably higher than for the equivalent maize and potato starch solutions. In order to examine the influence of the processing technique on the viscosity of PSC a number of samples varying in particle size were produced by changing the cut points on the air classifier.

Table 4. Amylogram parameters for PSC and reference products

Product	Temp. (°C)		Viscosity, BU				
	Start	Peak	Peak	95°C start	95°C end	Gel peak	Final
Cyclone*	64	>95	340	345	240	480	440
PSC (× 1)†	66	>95	435	435	430	1200	960
PSC (× 2)‡	64	>95	400	400	405	1090	940
PSC (purified)§	66	>95	590	550	570	1480	1130
PPC	81	>95	135	70	75	120	105
PFC	67	>95	205	200	145	290	260
Maize starch	73	89	220	210	200	—	860
Potato starch	60	84	870	650	320	1010	720

*Spray dried potato solids.
†Air classified.
‡PSC (×1) pinmilled and reclassified.
§PSC (× 2) extracted twice with water.

Table 5 shows the influence of cut point on the amylograms. The viscosity remains virtually constant on decreasing the particle size from 30 μm to 15 μm, but show significant change when smaller particles, 12 μm, are included.

The acid stability of starch solutions is important for acidic foods. The results

Table 5. Amylogram parameters for different air classifier cut points. Fractions with particles larger than the mentioned cut point have been used

Cut point (μm)	Temp. (°C)		Viscosity, BU				
	Start	Peak	Peak	95°C start	95°C start	Gel peak	Final
30	64	88	595	565	560	1670	1180
18	64	88	595	565	590	1660	1150
15	64	89	590	560	560	1640	1190
12	64	91	550	530	460	1850	1550

of acid stability examinations at the intrinsic pH of the respective products, and at pH 4.2, 3.2 and 2.0 are shown in Table 6, which for comparison includes the amylogram parameters for acetylated distarch adipate – a commercial acid-stable derivative.

The gelatinizing temperature and peak temperature initially increase on decreasing the pH to pH 4.2 and then fall on further decrease in pH.

PSC is still stable to heat and mechanical shear in weakly acidic conditions (pH 4.2). The hot-paste and cold-paste viscosities are only influenced slightly by

such a reduction in pH. However, at pH 3.2 and 2.0, respectively, PSC and potato starch are unstable to heat and mechanical shear and the paste viscosity decreases rapidly.

It is known that many chemical compounds influence the viscosity characteristics of starch solutions. Inorganic ions especially influence the gelatinization process and also the cold gel viscosity, probably as a result of bonding to phosphate ester groups with subsequent restriction of swelling

Table 6. Influence of pH on the amylogram parameters

		Temp. (°C)		Viscosity, BU				
Product	pH	Start	Peak	Peak	95°C start	95°C end	Gel peak	Final
Potato starch	—	60	81	930	630	330	1030	710
PSC	—	65	>95	400	400	410	1090	940
Potato starch	4.2	63	85	860	750	320	1080	870
PSC	4.2	67	95	380	350	390	890	880
Potato starch	3.2	61	81	725	580	70	—	310
PSC	3.2	66	93	320	320	110	—	300
Potato starch	2.0	59	69	700	70	0	0	0*
PSC	2.0	61	79	290	110	0	0	0*
Acetylated distarch adipate	3.2	67	81	760	700	505	—	1110

*Measured with a Brookfield viscometer, the viscosities are 16 and 14 cP for PSC and potato starch, respectively.

(Radley, 1976b). As potato starch is particularly rich in covalently bound phosphate, when comparing functional properties of different starch products the presence of ions must therefore be accounted for.

The viscosity curves are influenced by lipids, especially phospholipids (Madeleine, 1979; Goering, Jackson & Detlaas, 1975), but the extent of influence remains uncertain. Addition of phospholipids to potato starch (Madeleine, 1979) reduces peak viscosities; monoglycerides present also have considerable influence on viscosity curves as a result of complex formation with amylose.

Cell wall compounds have also been shown to influence the viscosity properties of starch solutions. Pentosans reduce the rate of setback, but not the course of the gelatinization (Kim & D'Appolonia, 1977) and the presence of sugar and non-denatured proteins, as in PSC, changes the amylograms (Radley, 1976b).

The non-starch compounds in PSC influence the rheological properties of the pastes by restricting the swelling at gelatinization, stabilizing the hot paste viscosity against heat and mechanical breakdown, and by increasing the paste viscosities after the gelatinization peak. The impurities are not able to influence the acid stability to any great extent. Reduction of water extractable components (Table 4) increases the viscosities indicating, by comparison with

potato starch, that the stability and the high viscosities (except for peak viscosity) are attributable to the presence of tightly bound or insoluble compounds, whereas the decreased peak viscosity of PSC is mainly attributable to water-soluble compounds.

The results in Table 3, however, indicate that this is not the complete explanation since the presence of ions in tap water – mainly calcium – decreases the peak viscosity, but increases the subsequent viscosities.

Retrogradation

Retrogradation rates of PSC, potato starch, maize starch, and waxy starch are compared in Fig. 5.

Waxy maize starch does not show any sign of retrogradation, whereas maize starch show slow and almost identical retrogradation rates. Thus, the non-starch compounds in PSC apparently have no influence on the ageing of a starch paste. This is surprising since cell wall substances are known to retard starch retrogradation (Kim & D'Appolonia, 1977). Furthermore, a number of organic and inorganic salts are claimed to retard or increase rates of retrogradation (Radley, 1976a).

Retrogradation rates of starch pastes may also be estimated from syneresis measurements after a number of freeze-thaw cycles. In this way PSC was compared to other starch products. Hydroxypropyl-distarch phosphate, a freeze-thaw stable commercial derivative, showed no sign of phase separation (Table 7) whereas the other starch products separated clearly after three cycles. The waxy maize starch was found to have intermediate retrogradation in agreement with the fact that amylopectin shows a less pronounced tendency to

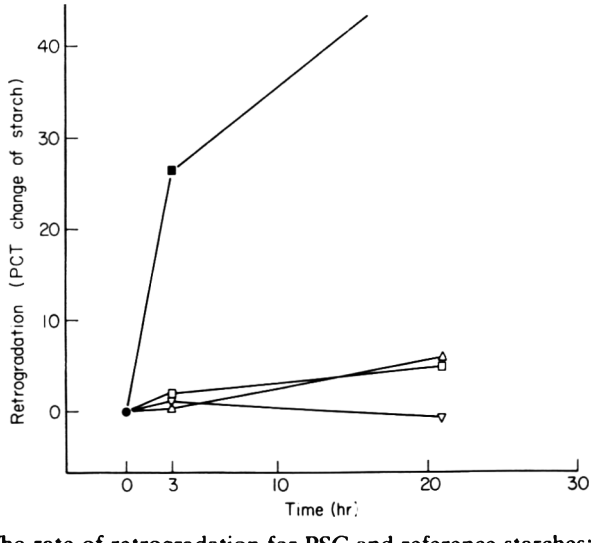

Figure 5. The rate of retrogradation for PSC and reference starches: ■, maize; △, potatoes; □, PSC; ▽, waxy maize.

retrograde. This property is useful in frozen foods, where waxy starches or crossbound esters are used.

Comparing the findings shown in Table 7 to those in Fig. 5 shows that waxy maize starch is the most stable of the natural starches during cooling and freeze-thaw cycles, irrespective which of the two methods of evaluation is used. However, the retrogradation rate of maize starch turned out to be high (Fig. 5) whereas the phase separation of maize starch solutions after three freeze-thaw cycles was not much different from those of PSC and potato starch solutions (Table 7). We are not sure whether this difference is due to different

Table 7. Freeze thaw stability of PSC and reference starches

Product	Separated water after 3 freeze thaw cycles (%)
PSC	69
Potato starch	60
Maize starch	63
Waxy maize starch	43
Hydroxypropyl distarch phosphate	No phase separation

experimental conditions or the different measuring principles. The freeze-thaw process involved a freezing of the water resulting in increasing starch concentration. This subsequently increases the intermolecular forces between the starch molecules.

Maize starch and PSC formed opaque and similar pastes unlike the translucent pastes of waxy maize and potato starch. The texture of PSC was furthermore shorter than the texture of potato starch, a result that, so far, remains unexplained.

Conclusions

From the above study it can be concluded that potato starch concentrate (PSC) is a storable product at moisture contents below 14%. The physical characteristics of PSC are in many respects better than those of conventional, high-refined starches, especially for application in the food industry.

The influence of the residue of non-starch compounds in PSC (4%, day basis) on the characteristics of the product can in many cases be interpreted on the basis of literature findings.

Acknowledgments

The author wishes to acknowledge the financial support of the Danish

Technology Board and the technical assistance of Mr Soeren Haar Soerensen and Miss Inga Boedker.

References

Blain, J.A. & Todd, J.P. (1955) *J. Sci. Fd Agric.* **6**, 471.
Bone, D., Shannon, E.L. & Ross, K.D. (1975) *Water Relations of Food*, Ed R.B. Duckworth. Academic Press, London.
Brockman, R. & Acker, L. (1977) *Lebensm.-Wiss. u. Technol.* **10**, 24.
Brockman, R. (1978) *Enzymatic Changes in Foods of Low and Intermediate Water Activity* Symposium, Paris.
Burnette, F.S. (1977) *J.Fd Sci.* **42**, 1.
Duckworth, R.B. (1978) *Water Relations of IMF*. Symposium on IMF, Paris.
Freimuth, U. (1972) *Der Nahrung* **16**, 149.
Goering, K.J., Jackson, L.L. & Detlaas, B.W. (1975) *Cereal Chem.* **52**, 493.
Hermansson, A.H. (1977) *J. Fd Technol.* **12**, 177.
Holm, F. (1980) *Starch* **8**, 258.
Holm, F. & Eriksen, S. (1980) *J. Fd Technol.* **15**, 71.
Kim, S.K. & D'Appolonia, B.L. (1977) *Cereal Chem.* **54**, 150.
Labuza, L., McNally, L., Gallagher, D., Hawkes, K. & Hurtado, F. (1972) *J. Fd Sci.* **37**, 154.
Lulai, E.E. & Baker, C.W. (1976) *Cereal Chem.* **53**, 777.
Madeleine, A.M. (1979) *J. Sci. Fd Agric.* **30**, 731.
McDonald, C.E. (1979) *Cereal Chem.* **56**, 84.
Radley, J.A. (1976a) *Examinaton and Analysis of Starch and Starch Products*, p. 116. Applied Science Publishers, London.
Radley, J.A. (1976b) *Industrial Uses of Starch and its Derivatives*, Chapter 2, Applied Science Publishers, London.
Ross, K.D. (1975) *Fd Technol.* **29**, 26.
Thorgersen, H. & Toledo, V.R.T. (1977) *J. Fd Sci.* **42**, 1615.
Vose, J.R. (1977) *Cereal Chem.* **54**, 1141.
Watson, S.A. (1964) *Methods in Carbohydrate Chemistry (IV)*, Ed R.L. Whistler, p. 150. Academic Press, New York.
Weast, R.C. (1970) *Handbook of Chemistry and Physics*. The Chemical Rubber Co., Cleveland.

(Received 21 April 1980)

J. Fd Technol. (1981). **16**, 115–125

A study of the production of clarified banana juice using pectinolytic enzymes

FLORIBETH VÍQUEZ, CELSA LASTRETO AND R. D. COOKE*

Summary

Pectinolytic enzyme treatments to increase the yield, reduce the viscosity and clarify the juice obtained from ripe banana pulp were studied. Six commercial enzyme preparations were tested on pulps of different ripenesses. Optimal conditions with respect to enzyme concentration, incubation temperature, time and pH levels are reported for the two most effective enzyme preparations. Clear juice yields of between 55 and 60% (based on pulp weight used) are obtained from pulp incubated at 45°C for 1 hr with 0.01% w/w of enzyme by subsequent centrifugation at 2900 maximal relative centrifugal force for 20 min. This corresponds to a yield of total and reducing sugars present in the pulp of over 75%. Untreated control pulps yield less than 5% of juice under these conditions. Hydraulic pressing of the pulps at 16 kg/cm^2 gives similar juice yields to those obtained by centrifugation. The juice has an excellent flavour and aroma and provides a possible use for the large quantities of reject bananas available in producer countries.

Introduction

Banana production is a key activity in several Latin-American and Caribbean countries. There is a considerable waste in these countries of the 'reject

Authors' address: Centro de Investigaciones en Tecnología de Alimentos (C.I.T.A.), Universidad de Costa Rica, San José, Costa Rica, Central America.

*Permanent address: Tropical Products Institute, 56/63 Gray's Inn Road, London WC1X 8LU. This work was undertaken while on secondment from TPI to CITA on an Overseas Development Administration Technical Co-operation Project.

0022–1163/81/0400–0115 $02.00

bananas', i.e. those which do not meet the quality norms for export. These norms are principally related to fruit size, shape and maturity. The quantity of reject bananas in Costa Rica during 1978 was about 160 000 tons, a small fraction of which was used locally, the rest being wasted.

The quantity of bananas that is processed is very small because of the competition with fresh fruit in most markets throughout the year (Wilson, 1975). A wide range of banana products has been described, such as banana powder, flour, figs, chips, canned slices, jams, beverages, etc. (Crowther, 1979). The production of most of these has been on an experimental basis or on a small scale in producer countries for domestic use.

World demand for fruit juices, including exotic or tropical juices is increasing (Tressler & Joslyn, 1971) and the banana with its widely appreciated flavour and aroma may be able to compete in this market, either as banana juice or as mixtures with other juices. Banana juice would be a product type of sufficiently different identity to minimize direct competition with the fresh fruit. Citrus and many temperate fruit juices are obtained by simple pressing or centrifugation (Tressler & Joslyn, 1971). Tropical fruits, including banana (Dupaigne & Dalnic, 1965), are usually too pulpy and pectinaceous to yield juices by these methods without the expenditure of excessive amounts of energy. Two methods have been reported to produce banana juices. Firstly, the use of calcium oxide to precipitate the pectin, followed by neutralization with sulphuric acid (Munyanganizi & Coppens, 1974). This treatment, which has been suggested for the production of the starting juice for banana beer production, impaired flavour and aroma quality. Secondly, the use of commercial pectinolytic enzymes as processing aids; these have been used with other fruit juices such as apple and grape to disintegrate the fruit pulps, reduce viscosity and clarify the juices (Rombouts & Pilnik, 1978). The earlier reports of this technique applied to banana pulps (Dupaigne & Dalnic, 1965; Pizarro & Coronel, 1971; Munyanganizi & Coppens, 1976; Tocchini & Lara, 1977; Jaleel, Basappa & Sreekantiah, 1978) are superficial, report variable juice yields and make little comment about juice clarification; these reports are discussed later.

Fruit juices are usually cloudy, colloidal suspensions and in cases such as orange and tomato this cloud is desirable (Baker & Bruemmer, 1972). In others, such as lime and guava (*Psidium guajava* L.) a clear juice is usually more acceptable. Juices that have an unstable cloud or whose turbidity is considered 'muddy' or undesirable tend to be marketed as clear juices. The brownish colour of banana pulp and the clarifying tendency of pectinolytic enzymes favoured the selection of a clear juice. This also gives greater flexibility regarding blends with other fruit juices.

The present study examines the parameters involved in the pectinolytic enzyme treatment of banana pulps using six commercially available enzymes. The juice yields are determined both by centrifugation and hydraulic pressing.

Materials and methods

Bananas

Reject bananas (Musa AAAcv. Giant Cavendish) from the Guapiles zone of Costa Rica were used. The mature, fully green bananas were ripened under ambient conditions (21°C, 85% r.h.) Three different grades of 'ripe' fruit were studied: grade 1, completely yellow fruit without flecks; grade 2, completely yellow with small numbers of small brown flecks; grade 3, completely yellow extensively speckled with brown.

These grades correspond to the colour index grades 6, 7, 8 of the transnational banana companies (Von Leosecke, 1950). The pulp contents expressed as percentage of the weight of the whole banana for grades 1 to 3 are 66, 67 and 68% respectively. The corresponding pulp moisture contents and pH levels are 74.0, 74.4 and 75.6 and 4.85, 4.90 and 5.10. The Brix was almost constant in the range 21.0 to 21.5.

Juice extraction by centrifugation

Six commercial enzyme preparations were tested: Pectinol and Pectinol D (both of Röhm Ltd., 61-Darmstadt, W. Germany); Pectinase PV8 (Miles MKC Ltd., Hannover, W. Germany); Ultrazym 100 and Ultrazym 100 Special (Ciba-Geigy Ltd., Basel, Switzerland) and claryfine Super (Sturge Chemicals, Birmingham). An initial selection of these preparations was conducted under

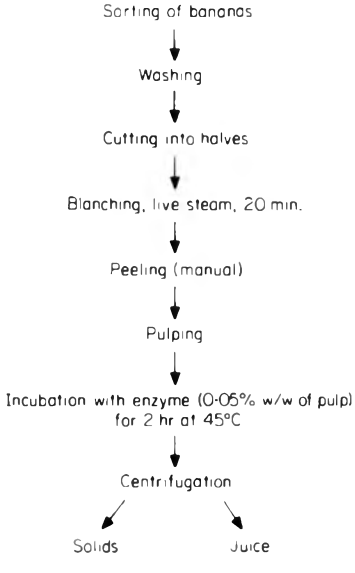

Figure. 1. Process for the extraction of clear banana juice. The incubation conditions shown are those used for the initial screening of the six enzyme preparations.

the processing conditions depicted in Fig. 1. The fruits were washed in cold tap water (chlorinated) and cut transversally in half. The halved bananas were blanched in live steam for 20 min: the pulp can then be readily squeezed out of the skins by hand. The pulp was milled in a Fitz-Mill model J.T. (Fitz-Patrick Co. Illinois, U.S.A.) using a 065 sieve, and cooled to 45°C and maintained at this temperature in a water bath. Enzyme (0.05% w/w of pulp) was added and incubated for 2 hr at the natural pulp pH level. Samples of the pulp were centrifuged in the M.S.E. Super Minor centrifuge (M.S.E., Crawley, W. Sussex U.K.) at 4000 rev/min i.e. a maximal relative centrifugal force of 2900 *g*. for 20 min. The percentage juice yield is calculated as juice weight divided by pulp weight. The three different grades of ripeness described above were tested in triplicate (three different fruit batches) with the different enzymes preparations. Preliminary experiments also tested the amalolytic preparations Termumyl (Novo Industri A/S Bugsvaerd Denmark) at 60°C and amyloglucosidase 150L (Ciba-Geigy) at 35°C.

The two most effective preparations were tested under different conditions of pulp temperature, incubation time and enzyme concentration. Two different pH levels were examined: the normal pH level between 4.8 and 5.1 and 3.8 obtained by adjusting with citric acid: the latter provides a high acid food requiring a lesser pasteurization treatment and less prone to microbiological problems.

Juice extraction by pressing

A Sapec Universal hydraulic press, model 200 HVL (St Louis, Missouri, U.S.A.) was used to compare press extraction with centrifugation. Batches of pulps of between 6 and 8 kg per batch were placed in a cotton press cloth in a stainless steel cylindrical bucket of dimensions 19.9 cm diam. × 30 cm length: the walls being perforated with eleven holes (0.5 cm diam.) per 100 cm^2 area. This cylinder was positioned in an outer steel cylindrical bucket (24 cm diam. × 34 cm) for collection of the expressed juice. A steel piston was used to apply the pressure to the pulp, the load reproducibility is ±20 kg on the scale used (0–20 000 kg) and the designated loads were maintained for 5 min intervals in each case. Comparative juice extractions by centrifugation were done on the same pulps after the same enzyme incubation periods (±15 min).

Juice evaluation and analyses

Pulp moisture contents were determined by drying to constant weight at 65°C under vacuum. Total and reducing sugars were determined spectrophotometrically using potassium ferricyanide, as described by Gaines (1973). Brix was measured refractometrically and pH using a Pye-Unicam PW 9418 pH meter. Viscosity was measured by means of juice flow time in a Cannon-Fenske

ASTM-D445 flow tube maintained at 26°C. Juice clarification was measured by the absorbance of the juice at 580 nm in a Pye-Unicam SPG-500 spectrophotometer. An empirical depectinization test consisted of mixing 1 part of juice with two parts of ethanol acidified with hydrochloric acid (5% conc. HCl v/v with 95% ethanol). The presence of pectin was indicated by the formation of a coagulum or precipitate within 5 min.

Results

Preliminary screening of enzyme preparations

Commercial enzyme preparations contain differing proportions of three classes of pectinolytic enzymes (Rombouts & Pilnik, 1978) pectin esterases EC 3.1.1.11, pectin lyases EC 4.2.2.3 and pectin hydrolases EC 3.2.1.41; these classes include enzymes with differing specificities regarding the degree of methylation of the polygalacturonic acid substrate and the endo- or exo-pattern of action. Knowledge about the pectinolytic enzyme activities present in these preparations is superficial (Cooke, Ferber & Kanagasapabathy, 1976) and few generalizations can be made about the relative effects of these preparations with the different pectin substrates of different raw materials. Consequently empirical tests are necessary.

The six enzyme preparations were tested in triplicate on each of the three grades of banana ripeness, as described in 'Materials and methods'. Preliminary experiments with two amylolytic preparations, Termamyl and amyloglucosidase (0.1% w/w) showed little effect on juice yield and none on juice clarification. Subsequent experiments included only the six primarily pectinolytic preparations referred to above. The juice yield increases with all six

Table 1. Average juice yields obtained with bananas of different grades of ripeness (1–3) using different enzymes. The figures are averages of three repetitions. The control data refer to juice yield following incubation in the absence of added enzyme.

	Average juice yield (% w/w) at different grades of ripeness		
Enzyme	Grade 1	Grade 2	Grade 3
Pectinol	49.6	53.3	57.9
Pectinol D	50.2	54.1	58.4
Pectinase PV8	46.9	46.9	51.4
Ultrazym 100	50.0	52.5	58.0
Ultrazym 100 special	66.5	68.4	68.8
Claryfine Super	66.4	67.4	68.8
Control	4.8	20.7	27.5

enzymes as the fruit ripeness increased (Table 1). The highest yields and degrees of clarification are obtained with Ultrazym 100 Special and Clarifine Super, irrespective of grade of ripeness. The subsequent analysis of treatment conditions with these two enzymes was done with both fruit of grade 1 and grade 3 ripeness. In all cases the same results are obtained with regard to optimal treatment parameter irrespective of ripeness grade and it was noted that the pulp rejected due to damaged fruit using grade 3 ripeness outweighs the extra juice yield from grade 3 pulp. Control pulps from grade 3 fruit, incubated without enzyme addition, show greater juice yields (Table 1) than that from grade 1 fruit. This is discussed in a later section; the data from grade 3 pulp were also more variable than those from grade 1.

Optimal enzyme incubation conditions

The dependence of juice yield on enzyme concentration used is shown in Fig. 2. At higher concentrations i.e. 0.025% w/w and 0.05% w/w, there is little difference between the two enzymes with regard to juice yield, viscosity reduction (Fig. 2) or clarification (Fig. 3). At lower concentrations Ultrazym produces higher juice yields and juice clarification than Clarifine. The effects on viscosity reduction (Fig. 2) are very similar.

The data in Figs. 2 and 3 emphasize that enzyme concentration has a slightly different effect on the four key parameters: juice yield, viscosity reduction, clarification and decomposition of precipitable pectin. The empirical criterion of precipitable pectin, which is a parameter recommended in much of the trade literature, is met at low (0.001% w/w) levels; while minimal viscosity does not occur until 0.01% w/w of enzyme. The enzyme dependence of juice yield and

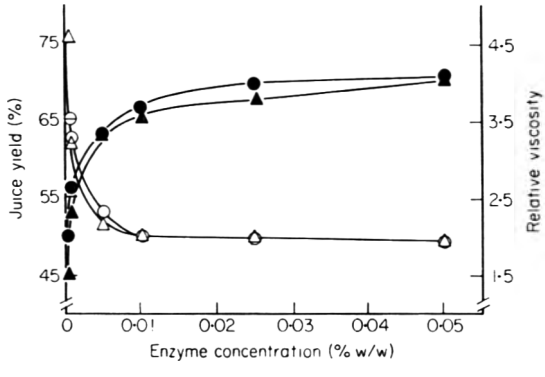

Figure 2. The enzyme concentration dependence of the viscosity and yield of juice obtained from banana pulp. The different enzyme concentrations were incubated with pulp for 2 hr at 45°C prior to juice extraction. The data refer to grade 3 bananas and are averages of triplicate determinations: the average yield from control pulps was 24 ± 5%. Juice yield: ▲, Clarifine; ●, Ultrazym. Viscosity: △, Clarifine; ○, Ultrazyme.

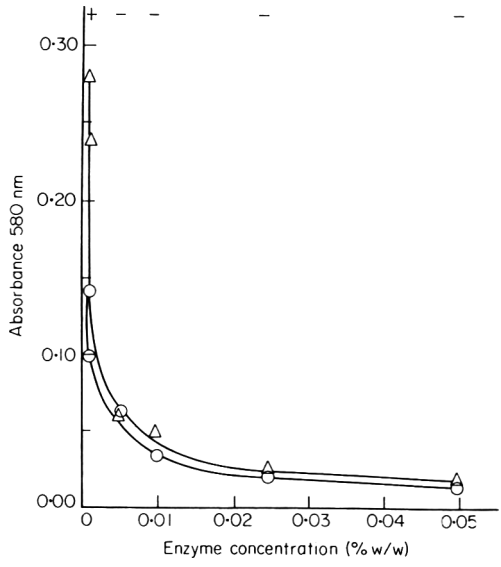

Figure 3. The enzyme concentration dependence of banana juice clarification and the removal of juice precipitable pectin. The experimental conditions and symbols are as described in Fig. 2. The symbols (+) and (−) signify the presence and absence of alcohol precipitable pectin in the juice.

clarification are similar in banana, the maximum effect occurring between 0.025% and 0.05% w/w with both enzymes. The relative kinetics of these four processes probably varies with different types of fruit depending on the pectin substrate and therefore the relative rates of the various pectin depolymerizing and pectin esterase reactions.

The time dependence of Ultrazym action on juice yield from grade 1 fruit is shown in Fig. 4; the increase in yield after 2 h is small. The differential effect of incubating at 35, 45 and 55°C with 0.01% Ultrazym for 30 min is quite small. The average juice yields from grade 1 fruit (duplicate batches) are 46.4,

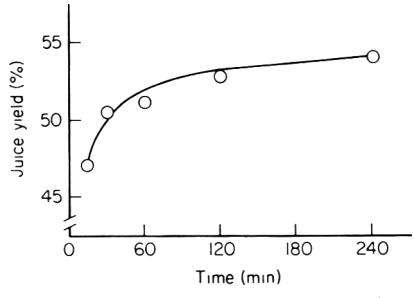

Figure 4. The dependence of the juice yield on enzyme incubation time. The pulp from grade 1 fruit was incubated with 0.01% Ultrazym at 45°C for the intervals shown, prior to juice extraction. The data are averages from duplicate batches, the control yield is 2%.

48.9 and 52%, respectively; control pulps yielding 0, 2 and 6%. The clarifications achieved are very similar but a slight browning reaction is apparent at the higher temperature (measured as an absorbance increase of 20% at 380 nm, relative to the 35°C product).

Pulp incubation at pH 3.8 with 0.01% Ultrazym for 30 min at 45°C gives the same viscosity reduction and clarification as the normal pH (4.9) incubation. The juice yield from grade 1 bananas is also very similar, 43.1% for pH 3.8 and 43.8% for pH 4.9 (the corresponding control yields being less than 2%). Preliminary taste-panel studies investigated the acceptability of juices in the pH range 3.8–5.0, the pH level being adjusted with malic or combinations of citric and malic acid. The preferred pH range is 4.4–4.6, and the juices have excellent flavour and aroma. Additional taste panel results are planned to further define optimal acidity and juice dilution factors.

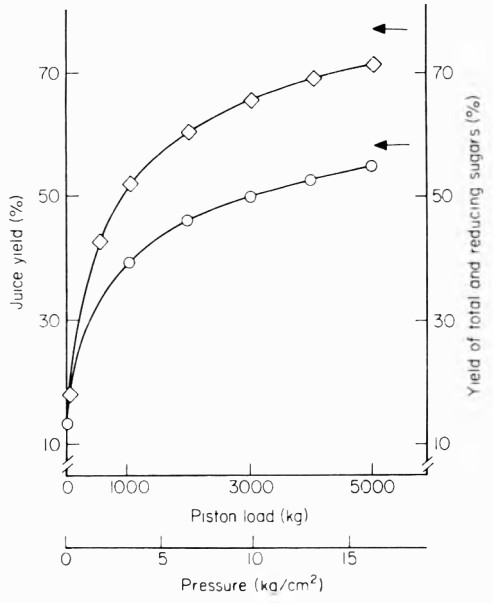

Figure 5. Comparison between juice yield by centrifugation and by hydraulic pressing. Pulp from grade 1 fruit as incubated with 0.01% Ultrazym for 1 hr at 45°C prior to juice extraction by centrifugation or pressing. The arrows refer to the corresponding yields by centrifugation, control juice yields were 3 ± 2%. Juice yield by pressing at 5000 kg for 5 min is very similar to the total yield obtained by pressing at 1000 kg intervals for 5 min up to a final load of 5000 kg (25 min total pressing). The yields of reducing and total sugars in the juices are the same (± 3) ○, juice yield; ◇, sugar yields.

Comparison of juice extraction by centrifugation and by pressing

This comparison was made because pressing often has cost advantages over centrifugation, depending on the process scale and complexity. The data in

Fig. 5 show that juice yield by pressing at 16 kg/cm^2 is very similar to that obtained by the centrifugation described in 'Materials and Methods'. The concentrates of reducing and total sugars and the appearance of the juices obtained by centrifugation or pressing are very similar. Grade 1 pulp contains about 16% total sugars and 8.5% reducing sugars (fresh weight basis) and the juice derived from this pulp contains 21% total sugars and 12% reducing sugars. The protein content of grade 1 pulp is between 1.2 and 1.4% and the juice content is between 0.6 and 0.7%. The juice obtained by pressing at 1–2000 kg has the same composition as that obtained by pressing at 5000 kg.

Discussion and conclusions

Grade of banana ripeness

Dupaigne & Dalnic (1965) recommended the use of fully ripe bananas for juice extraction to ensure complete flavour development and maximum sugar content. In the present study, three grades of ripeness were studied, the grade 3 corresponding to slightly over-ripe. The juice yields increase with fruit ripeness for all the enzyme treatments tested. This is consistent with the progressive breakdown of banana pectin due to endogenous enzyme activity and therefore to a lesser requirement for exogenous enzyme. The increase in juice yield from control pulps (5, 21 and 28% for grades 1, 2 and 3 respectively) is in agreement with this view. Adams (1978) identified galacturonic acid in over-ripe fruit which could not be detected in ripe fruit. Pectin esterase (Hultin, Sun & Bulger, 1966; Brady, 1976) and polygalacturonase (Markovic, Heinrichova & Lenkey, 1976) activities have been identified in bananas and the possible role of pectinases elaborated by contaminating microorganisms (Fogarty & Ward, 1972) in over-ripe fruit may be a contributory factor. Indeed over-ripe fruit usually displayed sufficient incidence of bruised or partially rotted fruit that had to be rejected to overrule the slight advantage in juice yield derived from use of grade 3 fruit. Consequently fruit of about grade 2 ripeness is considered optimum.

Optimal enzyme treatment

The data in Figs 2 and 3 define the enzyme concentration dependence of viscosity reduction, pectin degradation, clarification and yield of banana juice following incubation with the two most effective enzyme preparations. An earlier report (Tocchini & Lara, 1977) of banana juice production following pectinolytic treatment used viscosity reduction as the sole criterion evaluating the treatment. The different responses of juice yield, clarification and viscosity reduction (Figs 2 and 3) indicate that the latter alone is insufficient to quantify the adequacy of processing.

The optimal Ultrazym concentration for a 2 hr incubation at 45°C is between

0.01% and 0.025% w/w of pulp. The dependence of juice yield on incubation time under these conditions (Fig. 4) shows that there is only a small increase in yield between 1 and 2 hr. Juice yield does not vary much in the temperature range 35–55°C or at a reduced pulp pH 3.8 (normal pulp pH is 4.9). The use of the high acid condition reduces possible microbiological problems during the enzyme incubation. The choice of temperature, pH and incubation time within these ranges depends on cost analysis of the marginal yield differences involved and further sensory evaluation in the case of product pH. Hydraulic pressing of treated pulps produces similar juice yields of similar juice composition at low pressures (16 kg/cm^2) which are feasible with existing juice pressing equipment.

Previous reports of banana juice processing are insufficiently detailed to permit much comparison. Sreekantiah, Jaleel & Ramachandra Rao (1971) and Jaleel *et al.* (1978) refer to the use of a pectinolytic concentrate for 16–18 hr or 4 hr, respectively, at the 0.5–0.6% w/w level at 25–30°C. No details were given about extraction methods or product clarity. Dupaigne & Dalnic (1965) used an unspecified pectinolytic enzyme at 0.05 or 0.1% concentration for 1–2 hr at 50°C or overnight at 20°C, but did not describe juice yields or quality. Pizarro & Coronel (1971) extracted banana juice by centrifugation following treatment with 0.2% w/w Pectinol R-10 for 30 min at 55–60°C, no yield or clarification data were presented.

Tocchini & Lara (1977) investigated the use of four commercially available enzymes under a reduced range of conditions to that studied here, Ultrazym 100 Special was recommended and a similar yield (59%) was obtained to that reported here. Munyanganizi & Coppens (1974) obtained banana juice by treatment with quick-lime (CaO), followed by pressing or centrifugation and pH adjustment. They indicated that the organoleptic properties of the juice were considerably impaired. Under similar centrifugal conditions, i.e. 4000 rev/min for 20 min (no relative centrifugal force was stated), a 49% juice yield was obtained i.e. 10% less than reported here. Yields of up to 75% were reported by use of 17 000 rpm or 300 kg/cm^2 but these are conditions requiring high energy inputs. Munyanganizi & Coppens (1976) later recommended the use of 0.05% w/w Rapidase C-10 (no other enzymes were tested) for 1.5 hr at 50°C. A smaller range of conditions was tested than that reported here but they also indicate that pH between 3.8 and 4.9 has negligible effect on juice yield. Yields of up to 88% were reported but again using extreme conditions (300 kg/cm^2 or 35 000 rev/min for 10 min).

The present study provides extensive data about the processing parameters of the pectinolytic enzyme treatment of banana pulp and indicates that good yields of clear juice can be obtained under practical conditions

References

Adams, M.R. (1978) *Top. Sci.* **20**, 11.

A.O.A.C. (1975) *Official Methods of Analysis*, 12th ed. (Ed of W. Horwitz), p. 1094. Ass. Off. Agric. Chem., Washington, U.S.A.

Baker, R.A. & Bruemmer, J.H. (1972) *J. Agric. Fd Chem.* **20,** 1169.
Brady, C.J. (1976) *Aust. J. Plant Physiology.* **3,** 163.
Cooke, R.D., Ferber, C.E.M. & Kanagasapabathy, L. (1976) *Biochimica et Biophysica Acta.* **452,** 440.
Crowther, P.C. (1979) *Rep. Trop. Prod. Inst.*, G 122.
Dupaigne, P. & Dalnic, R. (1965) *Fruits.* **20,** 571.
Fogarty, W.M. & Ward, O.P. (1972) *Process Biochem.* **7** (8) 13.
Gaines, T.P. (1973) *J. A.O.A.C.* **56,** 1419.
Hultin, H.O., Sun, B. & Bulger, J. (1966) *J. Fd Sci.* **31,** 320.
Jaleel, S.A., Basappa, S.C. & Sreekantiah (1978) *Indian Fd Pckr.* **32** (2), 17.
Markovic, O., Heinrichova, K. & Lenkey, B. (1975). *Coll. Czech. Chem. Commun.* **40,** 769.
Munyanganizi, B. & Coppens, R. (1976) *Industries Alimentaires et Agricules.* **93,** 707.
Pizarro, S. & Coronal, M. (1971) *Politecnico.* **2,** 165
Rombouts, F.M. & Pilnik, W. (1978). Process Biochem. **13** (8), 9.
Sreenkantiah, K.R., Jaleel, S.A. & Ramachandra Rao, T.N. (1971) *J. Fd Sci. and Technol.* **8,** 201.
Tocchini, R.P. & Lara, J.C.C. (1977) *Boletim do Inst. de Tec. de Alimentos.* **51,** 93
Tresler, D.K. & Joslyn, M.A. (1971). *Fruit and Vegetable Juice Processing Technology* (2nd edn). Avi, Westport Conn., U.S.A.
Von Loesecke, H.W. (1950). *Bananas* (2nd edn). Interscience, New York, U.S.A.
Wilson, R.J. (1975) *Rep. Trop. Prod. Inst.*, G 103.

(Received 29 June 1980)

J. Fd Technol. (1981) **16**, 127–152

Alpha-amylase and bread properties

N. CHAMBERLAIN, T. H. COLLINS AND E. E. McDERMOTT*

Summary

Commercial baking trials, designed to investigate the effects of α-amylase activity during baking are described. Increasing α-amylase activity changes both the physical and chemical properties of bread, the physical changes resulting in a loss of crumb mechanical strength, and the chemical changes resulting in an increase in the amount of starch degradation products in the bread, and thus an increase in crumb stickiness. Both types of change can cause problems at the bread slicing stage. A reduction in water added at doughmaking is a successful ameliorative measure, as also is special lubrication of the slicer blades.

Results from the commercial trials have been corroborated and extended in the pilot scale bakery and the laboratory, the more controllable conditions furnishing results that implicate the high molecular weight degradation products of starch as the predominant cause of crumb stickiness. These pilot scale trials also provide evidence for the commercial usefulness of additions of acid calcium phosphate and extra fat as ameliorative measures.

Introduction

The use of home-grown wheat for bread flour is often limited by its excessive α-amylase activity, especially when sprouting occurs in wet harvest conditions. The presence of this enzyme results in an attack on the starch, which decreases the amount of water that can be held by starch both in the dough and in the bread as it is baked. Other deleterious effects from excessive α-amylase activity are the production of high crust colour, an open crumb texture and the presence of large amounts of starch degradation products in the loaf. This last effect may result in a loaf of considerable stickiness that can literally 'gum up' commercial slicing machines.

*To whom correspondence should be addressed.

Authors' address: Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Herts, WD3 5SH.

0022–1163/81/0400–0127 $02.00

The economic desirability of using more home-grown wheats can be frustrated in part by bad weather conditions at the time of harvesting, and occasionally by the growing of high yielding wheats which have a naturally high α-amylase activity even in dry harvest conditions.

Of the total bread sold in the U.K., about 75% is made by the Chorleywood Bread Process (CBP), and over half is in the form of the white, sliced and wrapped loaf, a form in which the stickiness problem is most serious. By the time such a problem occurs at the slicing machines the plant can be loaded with about 5 hr of bread production. What is worse, bread which is only marginally unacceptable will take a long time to show its true nature. The build up of gummy substances on the slicing blades will be slow and the breakdown point may not be reached for an hour or more. Thus the problem was not capable of solution in the laboratory alone but had also to be studied under commercial baking conditions.

Earlier studies of the effect of α-amylase on bread properties (McDermott, 1974) were concerned with the measurement of crumb stickiness, compressibility and recovery, and the changes in these properties that occurred during the cooling of the bread. The bread was baked under the standard conditions of test baking in our own bakery. These present studies are on bread from commercial plant bakeries as well as our own bakery and comprise industrial surveys and baking trials undertaken in an attempt to correlate commercial loaf properties and slicing characteristics with levels of flour α-amylase activity, together with laboratory pilot scale test bakes based on the results and observations made during the commercial baking trials.

Materials and methods

Commercial trials

The first commercial trial was carried out using flours of increasing α-amylase activity obtained by blending two flours, one or low and the other of a higher α-amylase activity. The α-amylase activities of the blended flours were 8, 9, 10 and 11 Farrand units (FU) respectively (Farrand, 1964).

In the second commercial trial a single consignment of wheat malt flour was diluted 1:3 with flour to give a premix with an α-amylase activity of 1400 FU. The addition of 16 oz of malt premix to one sack (280 lb) of flour, or about 0.36%, raised the activity of the flour by 5 FU. The stock bread flour in these trials had an activity of 6 FU. The commercial plant bakeries that took part in these trials were asked to standardize on common ingredients, recipes and mixing procedures as far as practicable. It was requested the recipe should be simplified on the following lines:

Flour	280 lb (1 sack)
Yeast	as normally used

Salt	as normally used
Fat	nil
Commercial compound improver*	3 lb
Water	to normal dough consistency
Mould inhibitor	nil
Malt premix	at levels required by the test.

*An improver manufacturer was asked to supply, from a single production mix, sufficient improver for each bakery. The improver contained fat, oxidants and soya flour.

Each bakery was requested to:

(1) Mix doughs to 5 Wh/lb and record mixing time and dough temperature.

(2) Make either single-piece or four-piece bread for the tests in un-lidded pans, in the shape normally used for standard 1¾ lb loaves.

(3) Prove, bake and cool the bread in the normal way, maintaining conditions as constant as possible.

(4) Slice bread to 0.3125 in (5/16 in) thickness using new blades 0.012 in thick from a single supplier.

(5) Make hour-long runs for each test variation including a control without malt premix followed, over the course of several days, by doughs with increasing levels of malt premix.

The objective of these trials was simply to carry on adding malt premix by 8 oz or 16 oz/sack increments until some problem occurred caused by the addition of too much malt premix. A duration of one hour was needed for each test run in order to give enough time for the measurement of the properties of at least six loaves during the course of the test run, and to give enough time for the proper assessment of bread slicing behaviour. A team of trained observers monitored the plant and collected samples and data.

Ameliorative measures, used by the industry, to counteract the deleterious effects of α-amylase action during baking have over the years been many and varied. A few have proved to be reasonably effective, and when, during a test run, a problem was created by the addition of malt premix it was planned to repeat the test with the application of one of these ameliorative measures. The ameliorative measures chosen were:

(1) Addition of 2 lb fat/sack to the recipe.

(2) Addition of 1.5 lb acid calcium phosphate (ACP)/sack to the recipe.

(3) Reduction in water addition by 5 lb/sack.

In the event, only one planned ameliorative measure, the reduction in water addition, was tried during the commercial trials. The other ameliorative measures were tested later in the pilot scale trials.

Pilot scale trials

Bread was made by the CBP, the recipe and procedure being as follows:

Recipe	*Per mix*	*Equivalent/lb/sack*
Flour	21.0 lb (9526 g)	280
Yeast	7.25 oz (206 g)	6
Salt	7.25 oz (206 g)	6
CBP – type commercial compound improver	3.5 oz (99 g)	3
Water	According to requirements	
Malt flour	At level required by the test.	

Procedure

Doughs were mixed to 5 Wh/lb in a Tweedy '35' using 15 in of mercury vacuum. Dough temperatures were 31 ± 1°C. Scaling was by hand at 32 oz (907 g) per piece. Dough pieces were rounded by a conical moulder, given 6 min first proof and moulded, using a Sorenson Mark II commercial moulder set for single-piece shapes. Final proof was to a height of 12 cm before baking in a gas fired reel oven without steam.

Loaves were cooled within the bakery for 2 hr before wrapping in polyethylene bags. Wrapped loaves were stored overnight at 21°C.

No attempt was made in these pilot scale trials to reproduce any one of the types of control bread made at the commercial bakeries. Previous work had indicated that bread produced using the pilot scale equipment and procedure would not be as sensitive to small increases in flour α-amylase activity as commercially produced bread. Test bread was made, therefore with levels of 0, 8, 16 and 24 oz malt flour/sack. The malt flour used had an α-amylase activity of 11 160 FU, which was higher than that used in the commercial trials. It was used without dilution into premix form giving flour α-amylase activities for the four tests of 4 FU (without malt), and 24, 44 and 64 FU when mixed with a commercial CBP-type bread flour having α-amylase activity of 4 FU.

Measurement of physical properties of bread

Commercial trials

All measurements were made on at least six loaves taken at the same time intervals after cooling, and therefore at the same time after leaving the oven. Compressibility, recovery and stickiness measurements were made at the time of slicing. In the first commercial trial, crumb density measurements were made on centre crumb at the time of slicing. In the second commercial trial, crumb

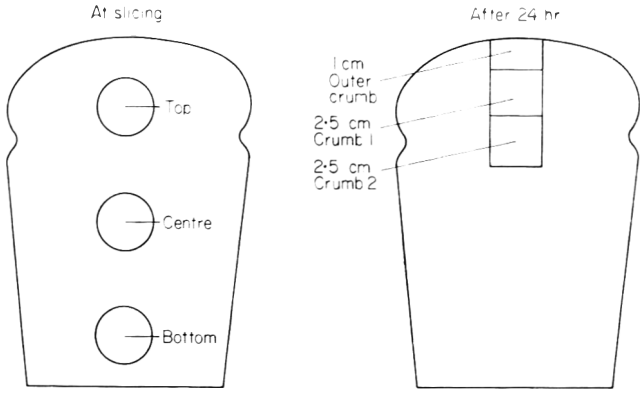

Figure 1. Sampling positions for crumb density measurements in the second commercial trials.

density measurements were made both at the time of slicing and after 24 hr on crumb at the three positions shown in Fig. 1. Loaf volumes were measured on 24 hr old bread.

Pilot scale trials

Compressibility, recovery and stickiness measurements were made on six loaves 2.5 to 3.5 hr after baking. Crumb density and moisture measurements were made on three loaves 3.5 to 4.5 hr after baking. Fig. 2 shows the positions in the loaf from which the crumb samples were taken.

Loaf volume measurements were made on two loaves 24 hr after baking. Results from four control (no malt) test bakes were used to assess the reproducibility of the physical property measurements.

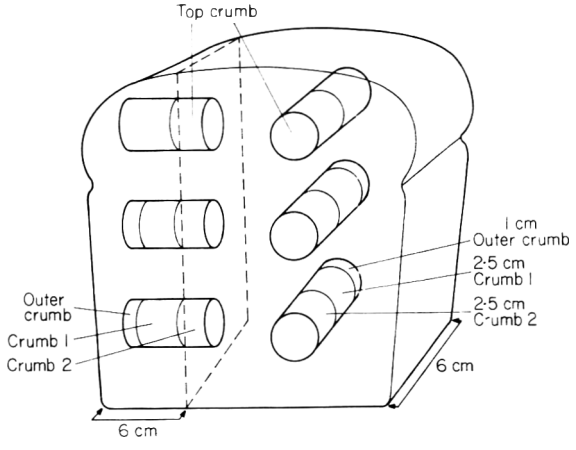

Figure 2. Sampling positions for crumb density measurements in the pilot scale trials.

Crumb density and moisture

Cylindrical cores of crumb were cut from selected positions from within the loaves using a very sharp, well polished, 1 in diameter cork-borer. The cork-borer was held vertically on the crumb surface and, with as little downward pressure as possible, rotated backwards and forwards in order to cut the crumb without compression or distortion. The core was cut into measured lengths. The core pieces were weighed, dried in an oven (4 hr at 110°C) and reweighed, thus obtaining the moisture content of the crumb sample. With the volume of the core sections known, the moist and dry densities could be calculated.

Crumb compressibility, recovery and stickiness

These measurements were made as previously described (McDermott, 1974). The compressibility and recovery values were obtained using a 200 g load and 30 sec for compression and 30 sec for recovery. The load was applied to the control position on the mid-loaf surface of a 4 cm-thick slice cut from the middle of the loaf. The stickiness value was measured at this same position.

Measurement of chemical properties of bread

Commercial trials

Centre slices were cut from six loaves taken at the same intervals after cooling. The choice of the crumb sampling positions within the loaf was influenced by the distribution of soluble carbohydrates found in the first pilot scale trial. The crumb positions were at the top, 'break' and centre of the loaf as shown in Fig. 3. Crumb samples from the same positions in the six loaves were mixed, air dried and lightly powdered in a mortar. The inclusion of crust in the top and 'break' samples was carefully avoided.

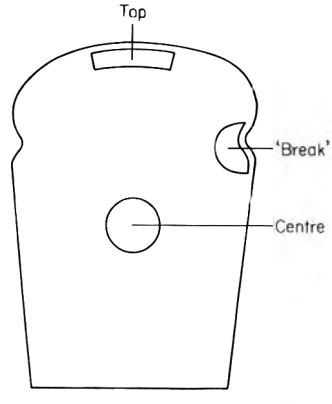

Figure 3. Sampling positions for chemical analyses.

Pilot scale trials

Crumb samples were taken from six loaves, 3 hr after baking from the top, 'break' and centre positions, and mixed and air dried as before. Crumb samples were also taken at the three positions from three loaves, kept separate, and extracted immediately as described below for the measurements of 'amylose' and higher molecular weight (mol.wt) dextrins. The results from these three control (no malt) test bakes, using this method, were used to assess the reproducibility of the 'amylose' and high mol.wt dextrin measurements.

'Amylose' and high molecular weight dextrins (blue value method)*

Reagents. Iodine solution: 0.20 g iodine, 2.0 g potassium iodide in 100 ml water. Tricholoroacetic acid (TCA): 5 g in 300 ml water.

Method

Weigh approximately 0.5 g crumb or dough into a 6 × 1 in boiling tube. Add 20 ml TCA solution preheated to 50°C and place in a water bath held at 50°C. Extract for 30 min with manual shaking very 5 min. Filter the suspension through a No. 1 Whatman filter paper into a test-tube, rejecting the first 1 ml of filtrate which is sometimes cloudy. Within 5 min of filtering pipette 2 ml of filtrate into a standard 25 ml flask already containing about 15 ml water, and add 1.0 ml of iodine solution. If the resulting colour is too weak, further aliquots of the filtrate can be added. The flask is made up to volume with water, mixed and left for 10 min. Transfer the coloured solution to an optical tube and measure the colour density against a reagent blank (1.0 ml iodine and 2 ml TCA solution made up to 25 ml with water) using an E.E.L. colorimeter. Obtain two colour density values using colour filters 607 and 622 (nominal wavelengths 600 and 470 μm respectively). 'Amylose' and dextrin values are calculated for 2 ml filtrate using equations derived from calibration curves.

$$A = \text{'Amylose'}\,(607) \times 1.2x - 0.35y$$

$$D = \text{Dextrins}\,(622) = 3.4(x - A)$$

where x = colour density with 607 filter, and
y = colour density with 622 filter.

'Amylose' values can be converted to mg 'amylose' by multiplying by the factor 0.006. Dextrin values remain as arbitrary units because of the uncharacterized mixture of high molecular weight dextrins used for the calibration. However, when calculated per gram of dry sample the dextrin results give rather high numerical values and have therefore been divided by 10 for ease of tabulation.

*Throughout this study 'Amylose' will mean polymers of glucose long enough to give a blue colour with iodine, but not necessarily native amylose as it exists within the starch granule.

Reducing and non-reducing sugars

Association of Official Analytical Chemists, Washington, U.S.A. *Official Methods of Analysis*, 11th edn, 1970, page 214.

Total soluble carbohydrate (orcinol)

Reagent. Orcinol (0.10 g) dissolved in 50 ml 66% sulphuric acid (17 ml water + 34 ml acid, sp.gr. 1.84).

Method. One ml of TCA extract (from 'amylose' and dextrin determinations) is diluted to 25 ml. To 1 ml of the diluted extract add 10 ml of orcinol reagent. Heat in boiling water bath for 12 min, then cool in ice water. Colour density is measured with E.E.L. colorimeter using 622 filter. Calibrate against glucose standards.

Low mol.wt dextrins

Calculated for dextrin chain of 5 glucose units. Association of Official Analytical Chemists, Washington, U.S.A. *Official Methods of Analysis*, 9th edn, 1960, p. 214.

Results and discussion

Industrial survey

Table 1 gives the crumb properties of standard four-piece open top bread taken from five bakeries during their normal production runs.

Table 1. Crumb properties at time of slicing of bread from different bakeries

Bakery	Compression (mm)	Recovery (%)	Stickiness (g)	Moisture (%)	α-amylase (FU)
A	17.4	54	22.4	47.0	12
B	17.7	70	10.5	44.7	9
C	15.1	84	5.8	46.0	8
D	18.3	62	14.3	45.7	12
E	22.9	50	21.7	48.4	7

All this bread was routinely sliced apparently without difficulty, and yet the variation in crumb properties, in particular the values for crumb stickiness, is

very large indeed. By past, and subsequent, experience it would certainly have been expected that bread with crumb stickiness values of over 20 g would give trouble during slicing. It was concluded that these crumb properties were not the only ones that influenced the behaviour of the bread during slicing.

Table 2 shows the variation in crumb properties of loaves taken in sequence – every 5 min – from the bread cooler. Thus, a loaf would frequently come along, for instance loaves Nos 2 and 7, with somewhat different properties. Often these departures from the norm could be associated with observations of crust colour differences.

Table 2. Variation in bread crumb properties during a single baking run

Loaf No. (in sequence)	Compression (mm)	Recovery (%)	Stickiness (g)	Dry density (g/ml)	Moisture (%)
1	20.0	50	20.0	0.093	45.6
2	17.6	49	23.5	0.104	45.4
3	18.9	55	19.0	0.086	45.5
4	20.0	53	17.6	0.086	45.5
5	19.6	52	20.8	0.100	45.4
6	19.0	54	20.8	0.091	45.5
7	17.4	69	12.0	0.099	45.5
8	19.8	54	19.5	0.086	45.7
9	20.0	51	21.3	0.091	45.4
10	19.3	55	18.0	0.093	45.5
11	18.8	59	17.0	0.092	45.4

This kind of result indicates clearly the necessity for making as many replicate measurements as possible within a test bank, but without ignoring the possibilities that the occasional abnormal loaf might initiate trouble at the slicer, perhaps by blunting the blades or by leaving a large deposit on them.

First commercial trial

Observations of the behaviour of the loaves during slicing indicated a gradual worsening of slice quality and stickiness for loaves from blend 1 flour through to loaves from blend 4 flour.

As the level of α-amylase activity increased the observed deterioration of the slice quality of the loaves was as follows: (a) Smooth, non-sticky and even slices; (b) roughness and stickiness appeared at the top and bottom of the slices, together with pills of crumb at the cut edges; (c) the loaves began to drag and deform as they passed through the slicer and the blades were under unequal pressure resulting in non-uniform slice thickness; the slices stuck together at top and bottom; (d) the loaves deformed very badly, until one broke up in the slicer (Fig. 4).

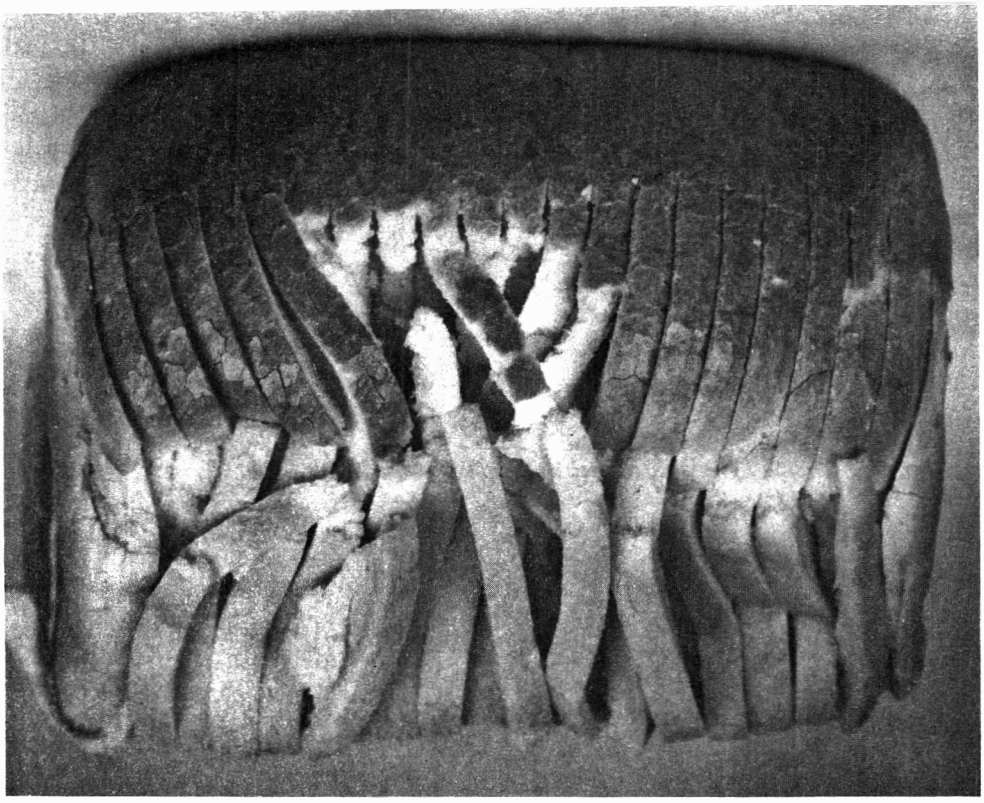

Figure 4. A loaf broken up during slicing.

No such gradual and consistent change was reflected in the results obtained for the crumb properties of bread from these four flour blends, the stickiness values being particularly inconsistent (Table 3).

Table 3. Crumb properties at time of slicing

Flour blend	Compression (mm)	Recovery (%)	Stickiness (g)	Moisture (%)	Density (g/ml)
1	17.2	82	5.2	45.4	0.080
2	15.8	85	4.8	45.4	0.089
3	15.3	82	9.8	45.2	0.089
4	19.0	73	5.5	46.1	0.077

However, the figures do show that loaves from blend 4 flour are softer, less resilient and less dense than the loaves baked from the other three flour blends.

Table 4, below, gives the weights of deposits accumulating on the slicer blades during these four test bakes. Loaves from blend 4 could only be sliced for 8 min before the machine was jammed.

Table 4. Deposit on slicer blades

Flour blend	Duration of slicing	Deposit wt (mg)	
		Per blade	Per blade/hour
1	1 hr	2	2
2	2 hr	8	4
3	1 hr	12	12
4	8 min	14	105

Analysis showed that the gum adhering to the slicer blades was rich in soluble 'amylose' abnd high mol.wt dextrins (12.9 mg 'amylose' and 147 units dextrins per g of deposit). No chemical analysis of the bread crumb was made in this first commercial trial. Observations during bread slicing indicated that most deposit collected at the top and bottom 3 in of the blade, that is, where the reciprocating blade would be in contact wih the top and bottom of the loaf. Therefore a pilot scale test bake was made in order to provide loaves for a detailed examination of the distribution of soluble 'amylose' and dextrins within the loaf.

First pilot scale test bake

Control and 16 oz malt/sack loaves were baked and examined 24 hr after baking for the distribution of soluble 'amylose' and high mol.wt dextrins within the loaf. The added malt was not premixed and raised the α-amylase activity of the flour by approximately 20 FU. Figure 5 shows the positions from which the crumb samples were taken and gives the average results, from two test bakes, for the analyses at those positions.

There is an overall increase in the amounts of 'amylose' and dextrins in the 16 oz malt/sack loaf by approximately 53 and 146% respectively over the control loaf. The concentration of 'amylose' is much higher just under the crust of the loaf than it is in the centre crumb. It is very probable that starch granule disruption and release of amylose is more extensive in regions near the crust that reach a high temperature quickly and stay at that temperature for a long time. These same conditions cause the rapid thermal inactivation of α-amylase, leading to less reduction in molecular size of the starch components.

The distribution of high mol.wt dextrins is different. The highest concentrations are found at the 'break' position in the loaf, and the lowest just under the top crust. It seems reasonable to suppose that the higher concentrations of dextrins arise where starch granule disruption is sufficient and heat penetration slow enough to allow some degradation of starch components and the accumulation of degradation products. Crumb at the 'break' position has swelled up from the interior of the loaf and has been kept relatively moist

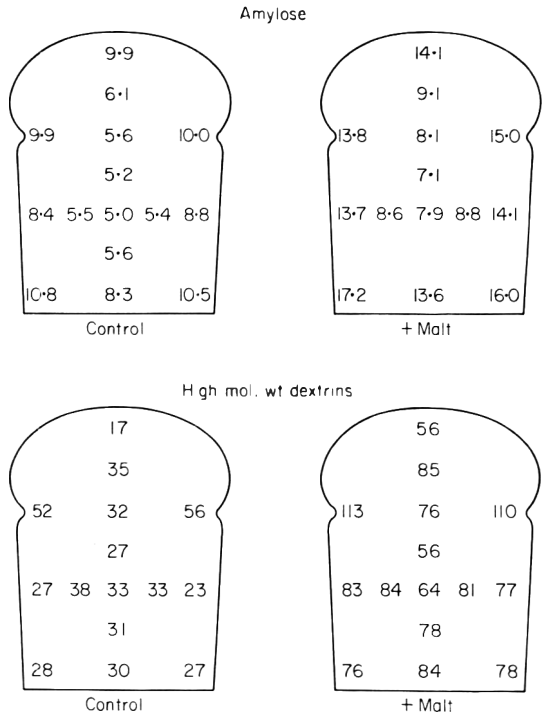

Figure 5. The distribution of 'amylose' and high mol. wt dextrins within a loaf.

and cool by the passage of steam to the outside of the loaf.

Taken together, the high concentrations of 'amylose' and high mol.wt dextrins near the outer zones of the loaf fit in well with the observed build up of gummy materials on the slicer blades.

Second commercial trial

The three bakeries which took part in these trials will be referred to as bakeries A, B and C. The bakeries differed greatly in plant type and processing conditions and, even with the recipe and procedure standardization requested, the control bread (no added malt premix) differed considerably in physical properties, as can be seen in Table 5.

Bakery B produced a soft loaf with a high compressibility and a low recovery. The crumb density was fairly uniform from top to bottom of the loaf. Bakery A produced a somewhat firmer loaf than that from bakery B, with a lower compressibility and a higher recovery. It had a lower crumb density at the top of the loaf than at the centre or bottom. Bakery C produced a much firmer loaf than those from bakeries A and B, with an even lower compressibility and higher recovery. The crumb density was correspondingly high and increased from top to bottom of the loaf.

Table 5. Physical properties of bread without added malt premix

measurement	Position	Bakery A	Bakery B	Bakery C
Crumb compressibility (mm)	Centre	17.9	19.8	12.9
Crumb recovery (%)	Centre	66	59	79
Crumb stickiness (g)	Centre	6.8	14.0	6.5
3 hr-crumb density (g/ml)	Top	0.093	0.090	0.103
	Centre	0.103	0.093	0.108
	Bottom	0.103	0.092	0.118
24 hr-crumb density (g/ml)	Outer	NM	0.190	0.243
	Crumb 1	NM	0.114	0.099
	Crumb 2	NM	0.106	0.106
Loaf volume (ml)		2857	2756	2623

NM = not measured.

Crumb density measurements on 24 hr-old bread were not made on bread from bakery A. However, it can be seen that there was a marked difference between the densities of the 1-cm outer crumb layers of loaves from bakeries B and C. The large change in crumb density through the first few centimetres from the outside of bread from bakery C must produce a loaf with slicing characteristics quite different from those of a loaf with a more gradual density change over the same region.

A further difference between the bread without added malt premix from the three bakeries was the degree of variation in properties among the six loaves taken at regular intervals after cooling. Table 6 gives the range of values found for some of the crumb properties.

Table 6. Variation in loaf properties of bread without added malt premix

	Bakery A	Bakery B	Bakery C
Compressibility (mm)	17.0–18.8	18.3–20.9	11.3–13.7
Recovery (%)	60.0–69.0	48.0–82.0	77.0–81.0
Stickiness (g)	6.2– 8.0	5.8–21.0	5.5– 7.0

Bread from bakery B was extremely variable in recovery and stickiness, the resultant average stickiness of the control bread (14.0 g) being high and not explicable in terms of the chemical analysis of the bulked crumb from the six loaves sampled. The results for the chemical analysis of the crumb from bread without added malt premix are given in Table 7.

Table 7. Chemical properties of crumb without added malt premix

Measurement per g dry crumb	Sampling position	Bakery A	Bakery B	Bakery C
'Amylose' (mg)	Top	12.0	15.0	28.8
	'Break'	14.1	15.7	15.5
	Centre	8.5	6.5	7.6
High Mol. wt. dextrins (units)	Top	60	72	48
	'Break'	98	77	76
	Centre	54	65	65
Reducing sugars (as maltose) (mg)	Centre	43	41	43
Non-reducing sugars (as sucrose) (mg)	Centre	4	4	1

Variations in the chemical properties of the control bread crumb between bakeries are not so great as the variations in the physical properties. Bread from bakery C has the highest concentration of 'amylose' and the lowest concentration of high mol. wt dextrins under the top crust, suggesting that at bakery C there is a more rapid heat penetration into the surface levels of the dough during baking. The type of oven used in bakery C differed from those in the other bakeries, though oven temperature, loaf centre crumb temperature after baking and crust colour did not show any significant variation from bakery to bakery. In general the higher concentrations were at the 'break' position and the lowest concentration of both 'amylose' and dextrins at the centre crumb position, a part confirmation of the results obtained in the pilot scale test bake.

At each of the bakeries the control (no added malt premix) test bake was followed by further test bakes with increasing amounts of malt premix included in the recipes. Only at bakery A was this plan followed without procedural or technical problems arising.

At bakery B the initial method of feeding the test flour into the mixer was manual. This method was too slow to maintain the normal throughput and created gaps in the prover and oven which affected the overall control of the plant and bread. After the 16 oz malt premix/sack addition it was found possible to change to automatic feeding and thus increase the rate of mixing sufficiently to fill the plant and improve control. A coincidental result of the change was a reduction in the amount of water required to maintain dough consistency (180 to 175 lb water/sack), thus influencing proving, baking and cooling conditions. This made interpretation of the differences in properties between breads made with additions of 16 oz and 24 oz malt premix/sack very difficult.

At bakery C, the addition of 16 oz malt premix/sack produced a volume increase in the bread such that management requested immediate steps to reduce it again. The reduction was achieved by decreasing dough temperature as a result of using a lower work input during dough mixing. The effect can be seen in Table 8, as the difference between the volume of bread from the first

addition of 16 oz malt premix/sack (2716 ml) and that from the second addition (2566 ml). It was also decided to make only a modest increase in malt premix additions at this bakery because of managerial concern about possible interruptions in throughput.

The physical and chemical properties of the bread from the three bakeries are given in Tables 8 and 9. Discussion of the results and observations made at each bakery on bread sliceability are given separately.

Bakery A

At bakery A the addition of malt premix had little effect on loaf volume, but additions beyond 16 oz/sack were accompanied by reasonably consistent changes in crumb physical properties. Thus there was an increase in crumb compressibility and stickiness and a decrease in crumb recovery. No crumb density measurements were made on 24 hr-bread at this bakery and insufficient measurements were made at 3 hr to point to any significant trend in this crumb property.

The chemical properties of bread from bakery A were again reasonably consistent with the level of added malt premix. Apart from a few anomalous results from the 16 oz/sack addition, there were increases in amounts of 'amylose', high mol. wt. dextrins and reducing sugars at all the crumb positions.

At bakery A there was a small slicing problem with all the bread. The bottom crust was always lightly smeared with deposit from the crumb, and there was some damage to the crumb structure in the area immediately above the bottom crust. This problem was thought to be caused by the practice of allowing the bottom combs in the slicer to ride free on their locating bolts, instead of bolting them firmly to the slicer base. Additions of 16 oz malt pre/sack introduced occasional smearing of the top crust with deposit from the crumb. Addition of 24 oz malt premix/sack gave slightly more sticking at the bottom crust. Addition of 32 oz malt premix/sack increased slicing problems and intensified sticking of the slices at the base; there was also some distortion of the slicer blades which resulted in slices of unequal thickness. Addition of 48 oz malt premix/sack intensified the slice distortion, caused the slices to stick together at the base and produce a roughened or 'pilled' surface of the crumb. The bread was judged to be just saleable.

Compared with bread from test bakes with lower malt premix additions, bread from the 48 oz malt premix/sack test bake had disproportionately high levels of 'amylose' and dextrins in the crumb. This was reflected in the high crumb stickiness value.

Bakery A, in a separate exercise, found that high levels of α-amylase activity which caused problems could be coped with at the bakery by spraying the slicer blades with a vegetable oil (usage rate about 3 oz/sack). The problem was, however, thus transferred to the consumer, because the slices reunited or stuck

Table 8. Commercial test bakes, physical properties of bread

		Bakery A. Added malt premix (oz/sack)					Bakery B. Added malt premix (oz/sack)						Bakery C. Added malt premix (oz/sack)				
Measurement	Position	0	16	24	32	48	0	8	16	24†	32†	48†	0	8	16	16*	24*
Compressibility (mm)	Centre	17.9	17.2	17.0	17.7	18.4	19.8	17.5	20.2	20.1	21.3	18.6	12.9	13.5	13.0	10.2	11.6
Recovery (%)	Centre	66	72	67	63	60	59	62	53	60	58	62	79	82	80	80	77
Stickiness (g)	Centre	6.8	7.8	7.8	9.9	12.8	14.0	11.2	18.9	9.6	11.5	11.0	6.5	6.3	8.4	7.6	9.6
	Top	0.093	NM	NM	0.098	0.097	0.090	0.089	0.090	0.086	0.082	0.079	0.103	0.091	0.096	0.109	0.105
3 hr-crumb density (g/ml)	Centre	0.103	NM	NM	0.109	0.100	0.093	0.096	0.095	0.090	0.087	0.091	0.108	0.097	0.102	0.115	0.110
	Bottom	0.103	NM	NM	0.109	0.091	0.090	0.097	0.096	0.087	0.083	0.086	0.118	0.106	0.110	0.118	0.107
	Outer	NM	NM	NM	NM	NM	0.192	0.202	0.202	0.206	0.200	0.200	0.243	0.232	0.258	0.278	0.285
24 hr-crumb density (g/ml)	Crumb 1	NM	NM	NM	NM	NM	0.114	0.112	0.112	0.106	0.103	Holes	0.099	0.095	0.089	0.116	0.109
	Crumb 2	NM	NM	NM	NM	NM	0.106	0.106	0.110	0.099	0.100	0.109	0.106	0.102	0.104	0.121	0.121
Loaf volume (ml)		2857	2883	2851	2837	2837	2756	2807	2800	2860	2915	2813	2623	2769	2716	2566	2582

NM, not measured; *reduced dough temperature; †automatic feeding, reduced dough water.

Table 9. Commercial test bakes, chemical properties of bread

Measurement (per g dry crumb)	Position	Bakery A. Added malt premix (oz/sack) 0	16	24	32	48	Bakery B. Added malt premix (oz/sack) 0	8	16	24†	32†	48†	Bakery C. Added malt premix (oz/sack) 0	8	16	16*	24*
'Amylose' (mg)	Top	12.0	14.3	13.2	13.8	17.8	15.0	15.1	15.5	15.8	14.6	16.7	18.8	20.9	21.0	19.5	22.1
	'Break'	14.1	14.7	14.3	17.5	17.3	15.7	16.1	16.6	18.4	20.6	22.1	15.5	16.1	21.2	20.2	22.3
	Centre	8.5	8.3	8.5	9.2	10.6	6.5	7.0	7.3	8.2	8.8	9.8	7.6	8.0	9.0	8.7	8.7
High mol. wt dextrins (units)	Top	60	81	84	87	102	72	70	93	89	115	122	48	49	54	65	52
	'Break'	98	104	97	103	136	77	77	79	101	108	120	76	74	71	70	84
	Centre	54	57	63	70	97	65	74	75	78	95	106	65	79	82	75	74
Reducing sugars (as maltose) (mg)	Centre	43	48	51	52	55	41	42	45	45	47	52	43	44	46	46	48
Non-reducing sugars (as sucrose) (mg)	Centre	4	4	4	3	5	4	5	6	5	3	3	1	3	3	3	3

*Reduced dough temperature; †automatic feeding, reduced dough water.

together so well, after the sliced loaf had been stored on end overnight, that they disintegrated when any attempt was made to separate them. This situation was reached at α-amylase levels in the region of 24 FU (56 oz malt premix/sack).

Bakery B

At bakery B the general effect of malt premix addition was to increase loaf volume up to the 32 oz addition. At the 48 oz addition the loaf collapsed slightly, resulting in a lower crumb compressibility, higher recovery and centre crumb density and a smaller loaf volume than found for the 32 oz malt premix loaf.

The change in the method of feeding the flour, with the consequent reduction in water requirement, between the 16 oz and 24 oz malt premix additions was coincident with a large reduction in crumb stickiness not explicable in terms of the chemical composition of the centre crumb. On the whole, the changes in crumb physical properties were not so consistent with the malt premix level as were those of bread from bakery A.

The changes in chemical properties were, however, more consistent with the level of added malt premix. The rather large increase in 'amylose' and dextrins at the 'break' position on changing the method of feeding the flour is possibly caused by a change in the flow pattern, during baking, of the dough containing less water.

Addition of up to 16 oz malt premix/sack had no effect on bread slicing. The addition of 24 oz malt premix/sack gave a marginal amount of slice stickiness with some pilling of the crumb. Addition of 32 oz malt premix/sack gave bread which disintegrated during slicing. Within a few minutes of the start of slicing, bread crumb was being torn by the blades; after 13 min slicing was abandoned. The slicer blades were without gummy deposit suggesting that the predominant cause of poor slicing was the low mechanical strength of the crumb as reflected in its very low density (0.082–0.087 g/ml).

The addition of 48 oz malt premix/sack was disastrous, after slicing a few dozen loaves a number of the slicing blades had collected a heavy deposit, and within 5 min all the blades were gummed up and slicing was abandoned. The main characteristic of the bread, apart from the increase in crumb dextrins, was the relatively low density of the top crumb and the, presumably, very low density of the crumb at position 1 (24 hr). Crumb density measurements could not be made at this position because of the difficulty of taking crumb core samples. This area of these loaves contained large holes and collapsed as the cork borer, used to take the crumb samples, cut into it.

Bakery C

The drop in loaf volume between the two 16 oz malt premix additions is evidence of the success of the steps taken to reduce loaf volume at the request of

the management. Concomitant changes in crumb properties were a reduction in compressibility and an overall increase in density. Consistent changes in crumb properties were more difficult to establish at this bakery because of the limited number of malt premix additions used. The addition of 8 oz malt premix/sack had no effect on slicing in comparison with that of bread without malt premix. Addition of 16 oz malt premix/sack created some slicing problems, and sticking together of slices at the top and bottom crust surfaces was clearly apparent. When steps were taken to reduce loaf volume, bread made with an addition of 16 oz. malt premix/sack sliced without difficulty. The crumb from this bread showed only a slight overall reduction in 'amylose' and dextrins, suggesting that it was the increase in crumb density which improved slicing behaviour. The addition of 24 oz malt premix/sack, again with cooler doughs, caused few slicing problems.

The effect on bread properties of reducing dough water

The ameliorative measure of decreasing dough water by 5 lb/sack improved the slicing quality of the bread containing 48 oz malt premix/sack at bakery A, the bread containing 24 oz malt premix/sack at bakery B, and the bread containing 24 oz malt premix/sack at bakery C. Examples of the changes in measured crumb properties resulting from using less dough water are given in Table 10.

Table 10. Changes in crumb properties resulting from the use of less water

		Bakery					
		A		B		C	
Malt premix level (oz/sack)		48		32		24	
Water level used (lb/sack)		181	176	175	170	174	169
Physical properties							
Compressibility (mm)		18.4	18.3	21.3	19.0	11.6	10.0
Recovery(%)		60	59	58	61	77	74
Stickiness (g)		12.8	11.8	11.5	10.8	9.6	9.1
	Top	0.097	0.091	0.082	0.090	0.105	0.118
3 hr-crumb density	Centre	0.100	0.099	0.087	0.093	0.110	0.125
(g/ml)	Bottom	0.091	0.100	0.083	0.091	0.107	0.115
Chemical properties							
	Top	17.8	16.2	14.6	15.3	22.1	21.2
'Amylose' (mg/g)	'Break'	17.3	14.8	20.6	19.7	22.3	19.8
	Centre	10.6	10.3	8.8	8.5	8.7	8.6
	Top	102	105	115	112	52	49
High mol. wt dextrins	'Break'	136	124	108	107	84	77
(units/g)	Centre	97	78	95	87	74	75

In general the reduction in dough water was ameliorative in that it reversed the changes in crumb properties associated with increases in α-amylase activity. The degree of efficacy of this ameliorative measure varied between bakeries. In bakery A the major changes in crumb properties were in the amounts of starch degradation products formed, i.e. the reductions in 'amylose' and dextrins in the 'break' and centre crumb positions respectively. The largest reduction in centre crumb stickiness was also found at this bakery. In bakeries B and C the major changes resulting from reducing the dough water were in the physical properties of the crumb, particularly in bakery C where the effect was to increase considerably the crumb density. Both the reduction in amounts of soluble starch degradation products and the increase in mechanical strength resulting from an increase in crumb density could have caused the observed improvement in bread slicing quality.

Second pilot scale test bakes

The results of the commercial trials show that trends which can be related to increasing α-amylase activity develop in both the physical and chemical properties of the bread produced. These trends are increases in crumb compressibility, stickiness and content of soluble carbohydrates, and decreases in crumb recovery and density. These trends can be reversed and the slicing properties of the bread improved by reducing dough water content.

It is reasonable to suppose that if the same trends can be reproduced on the pilot scale, then ameliorative measures which reverse these trends on the pilot scale might be successful in easing problems created by high α-amylase activity on the commercial scale.

The effects of increasing levels of α-amylase, and of the additions of ACP and fat, on the physical and chemical properties of pilot scale bread can be seen in Tables 11 and 12 respectively. The levels of addition of ACP and fat to the recipe were: ACP, 1.8 oz per mix (51 g) equivalent to 1.5 lb/sack; Fat (COVO), 2.4 oz per mix (68 g) equivalent to 2 lb/sack. Table 13 gives the 'amylose' and high mol. wt dextrin results for crumb which was not air-dried, but which was extracted immediately after sampling, and includes standard deviation (s.d.) values obtained from nine measurements (three test bakes, three loaves per bake).

There was a consistent change in the physical properties of the crumb with malt additions of 0 to 16 oz/sack, changes that followed the same pattern as those found in the commercial trials but which were larger because of the size of the α-amylase increments used. At 24 oz malt/sack the crumb compressibility and density changes were reversed, resulting in loaves with a firmer and denser crumb than that of the 16 oz malt/sack loaves. This change was not reflected in an equivalent drop in loaf volume, presumably because it was also accompanied by a reduction in the density of the 1-cm outer crumb. Assuming that a 1-cm thick outer crumb layer accounts for one-fifth of the total volume of the loaf, then the mean densities of both the 16 oz and 24 oz malt/sack loaves are identical at 0.128 g/ml, hence the similarity in their volumes.

Table 11. Pilot scale test bakes, physical properties of bread

Measurement	Position	Control	Added malt (oz/sack) 8‡	16§	24‡	16‡ + ACP 24 oz/ sack	16‡ + Fat 32 oz/ sack
Compressibility	Centre	13.1* (0.63)	14.8	16.6	15.5	15.0	14.7
Recovery (%)	Centre	83* (1.4)	68	65	64	65	67
Stickiness (g)	Centre	3.5* (0.38)	7.4	10.2	11.2	8.4	7.3
3 hr-crumb density**	Outer 1 cm	0.256† (0.008)	0.251	0.260	0.241	0.231	0.246
	1	0.110† (0.005)	0.106	0.095	0.100	0.103	0.107
(g/ml)	2	0.109† (0.002)	0.101	0.097	0.100	0.104	0.099
	Top	0.103† (0.003)	0.099	0.087	0.096	0.097	0.094
24 hr-loaf volume (ml)		2962	3190	3334	3320	3111	3206

*Mean of 24 measurements, 4 test bakes, 6 loaves per bake; s.d. in parentheses.
†Mean of 12 measurements, 4 test bakes, 3 loaves per bake; s.d. in parentheses.
‡Mean of 6 measurements, 1 test bake, 6 loaves per bake.
§Mean of 12 measurements, 2 test bakes, 6 loaves per bake.
**In all crumb density measurements, 4 × 1 cm outer, 1 and 2 crumb values and 2 × top crumb values were obtained per loaf. The means of these values were taken as one measurement per loaf.

Table 12. Pilot scale test bakes, chemical properties of bread (air-dried crumb)

Measurement (per g dry crumb)	Position	Added malt (oz/sack) 0	8	16	24	16 + ACP 24 oz/sack	16 × Fat 32 oz/sack
	Top	20.2	25.1	31.1	36.6	28.7	29.8
'Amylose' (mg)	'Break'	11.4	19.3	26.3	27.2	19.7	22.7
	Centre	8.2	11.9	16.2	18.0	12.0	13.3
	Top	42	90	112	145	68	108
High mol. wt dextrins	'Break'	76	189	273	288	197	231
(units)	Centre	78	156	213	269	158	187
	Top	97	143	164	192	140	147
Total soluble	'Break'	103	171	217	240	167	182
carbohydrate (mg)	Centre	100	150	174	212	157	162
	Top	33.5	46.2	46.2	51.7	41.3	51.0
Reducing sugars—as	'Break'	34.8	54.3	59.3	43.7	58.2	62.2
maltose (mg)	Centre	35.7	51.8	56.9	60.2	55.7	56.7
	Top	3.2	4.4	7.1	7.6	7.0	5.7
Non-reducing sugars	'Break'	3.4	4.8	6.8	7.8	6.2	5.8
—as sucrose (mg)	Centre	3.4	3.8	5.1	6.2	5.2	5.7
	Top	30.0	41.1	59.5	62.7	43.6	53.6
Low mol. wt dextrins	'Break'	31.0	48.9	66.1	78.6	55.6	63.7
—as 5 G (mg)	Centre	30.0	45.8	54.8	68.8	47.2	52.5

Table 13. Pilot scale test bakes, chemical properties of bread (fresh undried crumb)

Measurement (per g dry crumb)	Position	Control*	Added malt† (oz/sack) 8	16	24	16 + ACP 24 oz/sack	16 + Fat 32 oz/sack
	Top	14.4 (0.45)	24.9	25.0	36.0	26.2	27.9
'Amylose' (mg)	'Break'	8.9 (0.24)	18.1	18.4	25.6	13.3	18.0
	Centre	7.8 (0.36)	14.7	14.7	18.9	12.8	13.7
High mol. wt	Top	39 (4.4)	66	90	139	61	82
dextrins	'Break'	68 (4.2)	169	218	320	154	220
(units)	Centre	69 (3.5)	128	182	244	150	151

*Means of 9 measurements, 3 test bakes, 3 loaves per bake, s.d. in parentheses.
†Means of 3 measurements, 1 test bake, 3 loaves per bake.

The 1-cm outer, crumb 1 and crumb 2 samples were cut from four crumb cores taken from different positions within the loaf (see Fig. 2). When their densities were averaged, as in Table 11, an overall picture of the crumb density gradient within the loaf was obtained. However, if the crumb density values are kept separate and arranged according to core position, then more detailed information on crumb structure can be obtained. In Table 14, the results of such an analysis are given for control and 16 oz malt/sack loaves; the top crumb values, from two core positions, are also included.

Table 14. Crumb densities, a three dimensional analysis

Core position		1-cm outer	Crumb 1	Crumb 2
Control loaves*				
	Centre	0.228 (0.013)	0.105 (0.004)	0.106 (0.002)
Side of loaf	Bottom	0.242 (0.009)	0.101 (0.003)	0.109 (0.005)
	Top			0.102 (0.002)
	Centre	0.264 (0.013)	0.111 (0.008)	0.105 (0.004)
End of loaf	Bottom	0.290 (0.017)	0.124 (0.012)	0.115 (0.004)
	Top			0.103 (0.004)
16 oz malt/sack loaves†				
	Centre	0.211 (0.008)	0.095 (0.006)	0.098 (0.003)
Side of loaf	Bottom	0.242 (0.014)	0.091 (0.005)	0.098 (0.004)
	Top			0.087 (0.004)
	Centre	0.276 (0.016)	0.096 (0.004)	0.096 (0.002)
End of loaf	Bottom	0.310 (0.024)	0.098 (0.002)	0.097 (0.004)
	Top			0.087 (0.04)

*Means of 12 measurements, 4 test bakes, 3 loaves per bake; s.d. in parentheses.
†Means of 6 measurements, 2 test bakes, 3 loaves per bake; s.d. in parentheses.

These results show that, as a result of dough expansion during baking of this type of bread, the crumb is compressed more towards the ends of the baking tin than towards the sides. Although density measurement variability increases as crumb density increases, a comparison of the control and 16 oz malt/sack results reveals that the crumb density gradient towards the ends of the loaf (crumb 2 → 1-cm outer crumb), apparent in the control loaves has disappeared in the 16 oz malt/sack loaves. The resulting loaf has a uniform low density crumb surrounded by a dense layer of highly compressed crumb, a 'hollow' loaf, similar in some respects to the control bread from bakery C, and likely to be easily deformed under pressure in the slicing machine.

The ameliorative effect of the addition of ACP or fat was reflected in the reversal of the changes in physical properties brought about by the addition of malt. The use of either ACP or fat resulted in bread with firmer, denser and less sticky crumb than that of the equivalent (16 oz malt/sack) bread without these additives, and judging from experience gained during the commercial trials, would also result in bread with better slicing characteristics.

All the values obtained for the amounts of starch degradation products present in the crumb (Table 12) increased as the level of malt addition was increased. All increases in the values for centre crumb correlated quite well with the increase in crumb stickiness. If the percentage increase (on control values) of degradation products is compared with the percentage increase in crumb stickiness, and if there is a linear relation between product weight and stickiness, then it can be seen that the high mol. wt dextrins play a predominant part in determining centre crumb stickiness (Fig. 6).

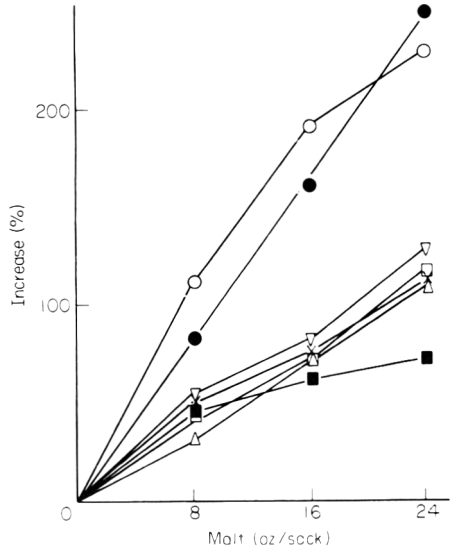

Figure 6. Percentage increases (on control values) of starch degradation products and centre stickiness. ●, high mol. wt. dextrins; ○, stickiness; ▽, 'amylose'; □, dextrins (5 glucose); ×, total soluble carbohydrate; △, non-reducing sugars; ■, reducing sugars.

The ameliorative effect of ACP and fat is reflected in the overall reduction of the amounts of starch degradation products found in the crumb, the addition of ACP being, on this basis, more effective than the addition of fat. However, by reference to the physical properties of the crumb from these test bakes, it can be seen that a greater reduction of stickiness was achieved by the addition of fat than by the addition of ACP. This suggests that the effectiveness of fat is due in part to a possible lubricating effect of extra fat within the crumb structure.

Processes such as retrogradation are most likely to effect the high mol. wt carbohydrates during the drying of the crumb. Therefore, the amounts of 'amylose' and high mol. wt dextrins found in extracts of fresh undried crumb (Table 13), should reflect more accurately the amounts of these soluble carbohydrates in the crumb at the time of slicing. However, in general, the two sets of results are reasonably close, the fresh crumb results showing the same effects with α-amylase and additives as the air dried crumb. The degree of reproducibility obtained with the 'blue-value' method is sufficient to justify the conclusions drawn from the changes in the amounts of 'amylose' and high mol. wt dextrins in both the commercial and pilot scale trials.

Based on the results from the commercial trials, the stickiness values found for the pilot scale bread were lower than would be expected from the amounts of high mol. wt carbohydrates found in the centre crumb. Crumb moisture results were not included in the tables because they were not influenced by α-amylase activity but, in fact, they were different for the commercial and pilot scale bread. This difference might be a partial explanation for the crumb stickiness results. The three commercial bakeries produced bread with the following crumb moisture contents. Bakery A. 45.9% (45.3–46.7), bakery B. 47.4% (46.6–48.1) and bakery C, 46.8% (46.2–47.2); whereas the pilot scale bread has a centre crumb moisture content of 45.0% (44.9–45.1). The figures in parenthesis are the ranges of values found.

Conclusions

The physical and chemical properties of bread change progressively as the level of flour α-amylase activity increases. Crumb compressibility, stickiness and content of soluble carbohydrate increase, while crumb recovery and density decrease. Of the soluble carbohydrates measured, the high mol. wt dextrins showed the biggest percentage increase and paralleled most closely the percentage increase in crumb stickiness.

The tolerance of bread from individual bakeries to flour α-amylase varies considerably. This appears to be the result of not only differences between the types of bread produced at the bakeries, but also the degree of control exercised at individual bakeries over the many stages in the production of wrapped, sliced bread. For example, optimum performance of the slicing machine will enable flour of higher α-amylase activity to be used. The most significant difference, in terms of sliceability between the types of bread produced at those bakeries could

be in crumb density distribution within the loaves. Thus the possibility exists for individual bakeries to increase the tolerance of their bread to α-amylase by changing the specification of the bread. For example a loaf that has a low crumb density gradient with a minimum of crumb compression against the crust, would be less likely to suffer deformation in the slicer, and would have inherently good slicing characteristics.

Bread sliceability is also affected by crumb stickiness or the soluble carbohydrate content of the crumb. The amounts and distribution of these starch degradation products in the bread also varied from bakery to bakery, possibly as a result of the different heat penetration characteristics of the doughs during baking.

It is fairly certain that differences in plant type and processing conditions other than those identified in this study, will influence the physical and chemical properties of bread and thus its tolerance to α-amylase. One result of this variability was that no maximum tolerable level of α-amylase activity common to all bakeries could be established.

The reduction of dough water and the additions of ACP or fat were generally effective ameliorative measures. The effects on the physical and chemical properties of the crumb were the opposite of those produced by the addition of malt, and in the case of water reduction slicing was shown to be improved. In some bakeries, steps taken to prevent the increase in loaf volume associated with high α-amylase levels and thus to prevent the loss of crumb mechanical strength could prove ameliorative. The expedient used at bakery C was to lower dough temperature by reducing work input at the mixing stage, but any method that resulted in reducing dough development or gas holding capacity would possibly suffice.

The earlier work (McDermott, 1974) referred to in the introduction, showed that the physical properties of bread crumb change drastically during the first 2 hr cooling, and continue to change at a lower rate afterwards. Thus the slow but steady decrease in crumb stickiness and compressibility, and increase in crumb recovery occurring through and beyond the 3 hr cooling period must, in the light of the present studies, lead to an improvement in slicing behaviour.

The remedial step of lubricating the slicer blades can be very effective. However, a situation could arise where the problem no longer occurs in the bakery but is being experienced instead by the consumer, in that the bread can be sliced and wrapped successfully, but the slices stick together in the wrapper or crumble in use.

Work is now in hand to establish, if possible, the modes of action of a number of measures, claimed to be ameliorative, including those used in this study.

Acknowledgments

The authors wish to thank the management and staff of the commercial bakeries for their cooperation. This work forms part of a research project sponsored by

the U.K. Ministry of Agriculture, Fisheries and Food to whom the authors' thanks are due. The results of the research are the property of the Ministry of Agriculture, Fisheries and Food and are Crown Copyright.

References

Farrand, E.A. (1964) *Cereal Chem.* **41**, 98.
McDermott, E.E. (1974) *J. Fd Technol.* **9**, 185.

(Received 2 August 1980)

J. Fd Technol. (1981) **16**, 153–166

Rheological studies of high ratio cake batters to investigate the mechanism of improvement of flours by chlorination or heat treatment

R. C. E. GUY* AND H. R. PITHAWALA

Summary

The rheological changes taking place in cake batters between 70 and 90°C have been studied using chlorinated, heat-treated (120°C for 7 min) and untreated flours. It was shown that, in an aqueous sucrose solution of equivalent concentration to that found in cake batters, the untreated flour forms a weaker gel system than the treated flours. This difference was probably due to the slower and less extensive swelling of the starch granules. Egg proteins were shown to contribute significantly to the overall gel strength of the cake matrix. The effect of the egg proteins was significantly greater with the treated flours than with the untreated flours.

Introduction

Early technological processes developed to improve the colour of cake flours using chlorine gas, were also found to improve the baking performance of the flour (Montzheimer, 1931). Treated flours were found to be more tolerant to high sugar levels in the formulation. This led to the development of commercial high-ratio cakes where the ratio of sugar to flour was between 1.0 and 1.4. These high ratio products with their good eating and keeping qualities proved very successful in the packaged cake market. Many studies have been made to elucidate the mechanism by which the chlorine changes the flour and brings about the improving effect. It has been shown that some of the unsaturated lipids are chlorinated (Daniels *et al.*, 1963) and that certain proteins are modified (Ewart, 1968).

Evidence for the chemical attack of chlorine on starch at the relatively low concentrations (1.5 g Cl_2 per kg of the flour) used for the chlorination of flour is limited (Gough, Whitehouse & Greenwood, 1978). Early investigators such as

*Present address: Flour Milling & Baking Research Association, Chorleywood, Rickmansworth, Herts, WD3 5SH.

Author's address: J. Lyons Central Laboratories, 149 Hammersmith Road, London W14 8QT.

0022–1163/81/0400–0153 $02.00

Uchino & Whistler (1962), Ingle & Whistler (1964) and Whistler, Mittag & Ingle (1966) used excessively large doses of chlorine at a molar ratio of 3 : 1 to the starch. They found evidence of depolymerization and increases in the levels of aldehydes, carbonyl and carboxyl groups. Kulp, Tsen & Daly (1972), working at more realistic levels of chlorination in the range 0.5–8.93 g Cl_2 per kg of the flour, found no evidence of depolymerization until the 2.23 g Cl_2 per kg level was reached. More recently, however, Johnson, Hoseney & Ghaisi (1980) have used enzymic studies to indicate that there is depolymerization and oxidation of starch polymers during the commercial chlorination process.

There is much more substantial evidence to show that the physical properties of the starch granules are changed by the chlorination process. Sollars (1958) demonstrated that the change in the starch fraction is the major factor in the mechanism of flour improvement. Howard, Hughes & Strobel (1968) showed that the granular integrity of the starch was important for the formation of stable cake structures. The improving effect of the chlorine appears to be related to the post gelatinization swelling processes since the onset of gelatinization was shown to be unaffected by the treatment (Jacobsberg & Daniels, 1974). Studies of gels formed on heating slurries (1 : 2 by weight of flour and water) by Frazier, Brimblecombe & Daniels (1974) suggested that the swelling or solubilization of the starch was increased by chlorination and by heat treatment.

The heat treatment process for flour developed as an alternative to chlorination by Russo & Doe (1968) produced a similar change in the physical performance of the starch. It did not change the iodine number of the lipids (Merryfield & Russo, 1972, personal communication) but modified the solubilization properties of the proteins (Russo & Doe, 1970). The examination of batters made with the treated flours and their untreated equivalents showed that the batters behaved in a similar manner until the final stages of baking. Photomicroscopy (Bell *et al.*, 1975) showed that the air cell structures were similar during the development of the batters up to 85°C. At this point the surrounding matrix in batters made with treated flours solidifies and the air cells change shape and burst. This change was not observed for the batters of untreated flours and the resulting effect on the cake was a collapse of the crumb structure. Cakes made from untreated flour achieved the same maximum volume in the oven as those made from treated flour but collapsed during the last 10–15 min of baking and during the cooling period after leaving the oven.

It is evident that the batter made from an untreated flour forms a weaker structure on gelling around the air cells. This gel structure is too weak to withstand the gravitational forces on the cake once internal pressures are reduced late in the baking process. The research studies herein were undertaken to examine the phenomena responsible for the differences observed in the formation of the gel structure in the batter. It was planned to study the formation of structures within the hot batter between 70 and 90°C by measuring the gel strength of the batter by compressimetry. In addition it was planned to examine model systems to distinguish the effects of flour proteins, starch and egg proteins in the formation of a gel structure in the batter.

Materials and methods

Source and preparation of the flours

An untreated cake flour (protein 6.98%, moisture 10.8%) was collected from the milling process and divided into three batches (20 kg). Batch A was used as the untreated control flour, Batch B was chlorinated using 1.2 g Cl_2 per kg of flour and Batch C was heat treated at 120°C for 7 min in a fluidized bed system. Craeta Preparata (2.35 g per kg of flour) was added to all the flours.

Five days after milling when the flour treatments had been completed all the flours were sealed in tins and stored at −18°C until required for experimental work.

Isolation of the starch fraction

A starch fraction was isolated from the flours by ultracentrifuging a flour slurry in 50% w/w sucrose solution. Flour (300 g) was mixed with 50% sucrose (700 g) in a Hobart Planetary mixer for 3 min and the slurry as subsequently ultracentrifuged at 120 000 g (*r* max.) in an MSE Superspeed 65 ultracentrifuge. The starch layer was removed and stored in sealed glass jars at −18°C until required for model system studies. A sample of starch was prepared from each of the three parent flours A, B and C.

Analytical methods

The flours and starch fractions were analysed for moisture, protein and chloride contents by the standard AOAC procedures listed in *Methods of Analysis* (1975). Starch and sucrose levels were determined by the enzymic test methods described in *Enzymic Methods of Food Analysis* (1971) by Boehringer Mannheim Ltd.

Rheological test methods

(a) Formulations for the model systems. The batters for the model systems I to IV were prepared to the formulations shown in Table 1 from the following ingredients; (i) flours A and B (starch 80.0%, moisture 10.8%, protein 6.98%) or C (starch 88.2%, moisture 1.5%, protein 7.7%) were stored at −18°C until required; (ii) sucrose: pure granulated sucrose; (iii) liquid whole egg; samples of a batch of pasteurized liquid whole egg (24.8% solids) were sealed in plastic containers and stored at −18°C until required; (iv) egg albumen; samples of Henningsen spray-dried albumen (protein 85%, moisture 5%) were sealed in plastic containers and stored at 4°C until required; (v) starch slurry; the

ultracentrifuged starch slurry (starch 45%, sucrose 20–21%, moisture 30–31%, protein 1.9–2.1%) was analysed, stored at −18°C, and used within 5 days of preparation.

Table 1. Compositions of the formulations used in model systems I–IV

Ingredients	Percentage composition in model systems			
	I	II	III	IV
Starch (dry solids)	26.90	26.25	26.52	25.89
Wheat flour protein (dry solids)	2.35	2.30	1.22	1.18
Sucrose	35.00	34.15	36.08	35.20
Whole egg solids	—	1.45	—	1.45
Egg albumen solids	—	0.97	—	0.98
Water	35.00	34.15	36.08	35.20

(b) Compression tests on batters. Batters were prepared by mixing flour or starch with the other ingredients of the model system, such as sugar and water, in a Hobart Planetary mixer for 3–4 min until a smooth batter was obtained. Special care was taken to avoid incorporating air into the batter and the final level of aeration was usually kept to below 10%. Batters with higher levels of aeration were rejected.

Small portions of batter (45 g) were weighed into cylindrical aluminium cups (35 mm high and 50 mm in diameter) and each was sealed with a No. 49 rubber bung. A set of four cups was placed in a water bath at 95°C and submerged to a depth of 30 mm. The temperature-time profiles of the batters in the cups were measured using thermocouples sealed in the rubber bungs. These temperature-time curves were used to time the heating process for subsequent sets of samples so that they could be heated to a particular temperature selected for study. On reaching the required temperature the samples were transferred to a constant temperature bath set at this temperature. After 10 min maturation in the constant temperature bath the samples were transferred to the hot cabinet of the Instron Universal Tester which was also set at the required temperature. The sample was compressed over a period of *ca* 1 min and the sample temperature was checked with a thermocouple probe in the cabinet. Samples with temperature varying by more than 0.5°C from the chosen value were rejected. Sixteen satisfactory samples were tested at each temperature.

The compression test was carried out with a 25 mm diameter disc probe, driven into the sample at 2 cm/min. A force/distance-of-compression curve was recorded using the Instron CB-cell at a chart speed of 20 cm/min. All the gelling or gelled samples showed a linear elastic region up to 7–10 mm compression followed by a yield zone and a rupture point. The value of force at 5 mm compression was chosen for comparative evaluation of the different systems as it represented the firmness of the structures in the linear elastic region.

Compression tests were also carried out on samples which have been taken through similar heating cycles and cooled to 20°C.

(c) Tensile tests on cooked batters. Batters prepared from flour, sugar, egg proteins and water as in the model system II were poured in a rectangular lidded tin (14 × 24 cm) to a depth of 10 mm. The tins were sealed and placed in the Instron oven at 100°C, baked for 40 min, removed and cooled at 4°C for 2 hr. Sections (100 × 25 mm) were cut from the slabs and two notches 3 mm deep were cut transversely at the centre of each section. The prepared sections were fixed in the jaws of the Instron tension system using the CCTM-cell and pulled apart at 5 mm/min until the sections broke at the notches. The cross-sectional area at the point of rupture was measured and the force/extension values recovered from the trace on the Instron recorder chart.

(d) Compression tests on cooked egg gels, (1) Egg gels with added soluble starch or vital gluten. Egg gel systems containing 2.5% whole egg solids and 0–10% soluble starch, or 0–5% spray-dried vital gluten, in 50% (w/w) sucrose solution were prepared by heating aliquots (45 g) in sealed aluminium cans (35 mm high and 50 mm in diameter) at 95°C for 10 min. After cooling for 60 min in running water at 20°C the gels were tested by the compression method previously described for the flour model system gels.

The soluble starch fraction was prepared by dispersing a 20% starch slurry in water at 95°C for 20 in, centrifuging at 2000 g for 10 min, and concentrating the supernatant liquor by rotary evaporation under reduced pressure at 50°C.

(2) Egg gels with added granular starch. Egg gel systems containing 2.5% whole egg solids at 0–25% Procea wheat starch in 50% (w/w) sucrose solution were prepared and tested as described above.

(3) Egg gels containing variable levels of egg solids. A series of egg gels were prepared containing 4.1–14.4% egg solids, and in one case 3.85% added albumen, in 50% (w/w) sucrose solution. The gels were prepared and tested as described in section I.

Differential scanning calorimetry

The samples of batter (10–12 mg) were hermetically sealed into sample cups and heated at 5°C/min in the range 20–120°C on the Dupont 900 Differential Thermal Analyser fitted with a DSC attachment. The onset of gelatinization was determined from the DSC trace.

Estimation of soluble starch released at 90°C

Starch (20 g) was mixed into 50% (w/w) sucrose solution (400 g) and heated at 90°C for 10 min. The slurry was ultracentrifuged in the MSE Superspeed 65 ultracentrifuge at 100 000 ***g*** for 20 min at 30°C. The supernatant liquor was analysed for total starch content.

Cake baking tests

The cake batter slurry (containing: flour 27.1%, sugar 29.4%, water 22.1%, egg 5.1%, fat 7.8%, glycerine 3.1%, egg albumen 1.0%, milk solids 1.7%, soya flour 0.7%, emulsifier 0.3%, salt 0.3%, baking powder 1.7%) was prepared by mixing in a Hobart Planetary mixer. This slurry was aerated by passing it through a 6M Oakes mixer at 4.22×10^4 kg m^{-2}, with rotor speed of 500 rev/min. The batter was piped into baking tins and baked at 207°C for 15 min.

Results and discussion

The flours A, B and C were tested in a sensitive sponge cake formulation to determine whether the chlorination and heat treatment processes had been effective. It was observed that the batters made from all three flours gave similar results during the batter expansion stage of the baking process. After all the cakes had reached approximately the same height, in the last 5 min of the baking period, the cakes made from flour A collapsed rapidly to give flat topped cakes of low volume. The cakes made from flours B and C did not collapse and retained their domed structure. The larger volumes of cakes made from flours B and C are indicated by the cross-sectional area measurements shown in Table 2.

Table 2. Analytical data and cake baking results for flours A, B and C

Flour	Moisture (%)	Protein (%)	Chloride (p pm)	Cake cross-sectional area (cm^2)
A	10.8	6.98	0	46.2
B	10.6	7.00	1260	58.1
C	1.5	7.70	0	54.9

Since the batter system of flour A had been satisfactory during the expansion phase of the baking process up to the point where the structure of the cake began to form it is clear that the differences observed between batters of treated and untreated flour are mainly concerned with their gel-forming properties. This conclusion, which has been reached by several groups of workers using a variety of approaches, appears to be fundamentally sound (Sollars, 1958; Chamberlain, 1962; Russo & Doe, 1970; Frazier, Brimblecombe & Daniels, 1974; Bell *et al.*, 1975). The cake batter contains two gel-forming systems which combine together to form the overall gel structure of the cell wall material of cake crumb. Starch, which represents *ca* 26% of the batter by weight, exists as small granular particles (1–40 μm diameter) until the temperature reaches 80 to 90°C. At this stage the granules genatinize and swell rapidly to 2–2.4 times their original

diameters (Bean & Yamazaki, 1978). This swelling process causes a rapid increase in batter viscosity and subsequently leads to the formation of a rigid gel-like structure by inter-granular contacts.

At the same time as the starch is swelling some of the egg proteins are denatured to form aggregates and polymeric forms of the native proteins. These aggregates consisting of up to twenty protein units for ovalbumen (Joly, 1965) coagulate to form a secondary gel system. The combined effect of the swollen starch granules and the continuous egg protein gel phase surrounding them provides the firm structure of the cell wall material.

Preliminary studies with starch and egg gels (Table 3) showed that a 2.5% egg solids gel is very weak. However, as starch is added to the system the effect of the egg protein is increased so that at the 25% starch level, which represents the levels found in cake batters, the firmness contribution of the egg proteins rivals that of the starch.

Table 3. The variation of gel strength with concentration of starch in 50% aqueous sucrose in the presence of egg proteins

Starch concentration (%)	Firmness of starch gel (gf)	Firmness of starch gel + 2.5% egg protein solids (gf)
0	0	10
5	0	125
10	185	790
15	1450	2400
20	2940	4660
25	4775	7785

The flours A, B and C were tested in the model systems I and II (Table 1), in which the starch level was maintained at *ca* 26% and the sucrose to water ratio was maintained at 1 : 1 w/w. Egg proteins were included in model system II to study their interaction with the flours. In order to assess the effect of the flour proteins two additional model systems were also studied in which the parent flours were replaced by a separated starch fraction. The starch fractions were isolated by a simple ultracentrifugation technique in 50% (w/w) sucrose so that the surfaces of the granules were maintained in the same environment as in the model system I. All the model systems were calculated to contain the same levels of starch and either high or low levels of the two protein types in a background solution of 50% sucrose. This permitted a direct comparison of the results to determine the relative effects of starch, egg proteins and wheat flour proteins.

Rheological testing of hot batter systems

The model batter systems I and II were heated in a simulated baking cycle from 20 to 95°C so that the gel firmness developed by each flour system could be assessed in the critical range, 70 to 90°C. Compression measurements were carried out on the hot batter systems while they were briefly held at intermediate temperatures within the range.

No firm structures were detected in any of the batters below 75°C. Incipient gel formation took place in the batters of flour C at 75°C and in the batters of the other two flours at 80°C. The firmness values of the batters of flours B and C, in model system I, increased rapidly between 82.5 and 90°C (Table 4). In contrast the batter of the untreated flour, A, firmed at a significantly slower rate as the temperature rose. Its final firmness value at 90°C was only *ca* one third that of the batters of the treated flours.

Statistical analysis of the results from model system I (Table 4) showed that the batters of the treated flours are significantly firmer (0.1% level) from 80 to 90°C than that of the untreated flour. The batter of flour C is significantly firmer (0.1% level) than that of flour B from 75 to 84°C but there is no significant difference from 85 to 90°C.

Differential scanning calorimetry measurements on the batters of flours A, B and C in model systems I and II showed that the onset of gelatinization was at 82–84°C in all these systems. This result, which confirms the earlier studies of Jacobsberg & Daniels (1974) on flours systems and extends them to cover the effect of egg proteins, shows that the starch granules start to swell at the same point in the baking cycle for all these systems. The sharp rise in firmness in model system I from 82.5 to 90°C must therefore be directly related to the swelling processes of the starch granules. Bean, Yamazaki & Donelson (1978) showed that the starch granules of flour swell in 50% sucrose (w/w) between 80 and 90°C to 2.0–2.2 times their normal diameters. The lower firmness values observed for the untreated flour in model system I batter must therefore indicate a difference in the swelling process of the starch in that flour compared to the starch in flours B and C. The starch of the untreated flour appears to swell more slowly and less extensively than that of the treated flours.

The addition of the egg proteins in model system II produced a number of interesting effects which serve to illuminate further the differences between the action of treated and untreated flours in cake batter (Table 4). In the batters of the treated flours a rapid increase of firmness was observed between 80 and 90°C. The firmness values for batters of model system II were significantly greater (0.1% level) than those for model system I and the difference increased in magnitude from 82.5 to 90°C. This difference represents the contribution of the egg proteins to the overall gel strength. It indicates that the egg gel is forming in this temperature range and that there is a substantial interaction with the starch similar to that found in the preliminary studies with cooked starch gels at 20°C (Table 3).

Table 4. Firmness values of gels made from flours A, B and C in model systems I and II

Temperature of batter when tested (°C)	Model system I firmness (gf)			Model system II firmness (gf)		
	Flour A	Flour B	Flour C	Flour A	Flour B	Flour C
70	—	5 (20)	—	—	—	—
75	12 (22)*	6 (15)	62 (22)	32 (29)	96 (26)	93 (24)
80	47 (44)	203 (29)	386 (10)	104 (27)	232 (17)	267 (22)
82.5	154 (45)	355 (22)	910 (29)	112 (30)	852 (23)	1702 (8)
84	430 (35)	715 (27)	1190 (25)	244 (35)	997 (50)	2090 (12)
85	470 (35)	1280 (13)	1265 (10)	489 (33)	1890 (17)	2300 (12)
90	600 (12)	1720 (8)	1810 (6)	1375 (16)	3033 (18)	3200 (11)

*Coefficient of variations are shown in parentheses.

The batter of the untreated flour did not show any significant increase in firmness for model system II formulations over those of model system I until the 90°C point was reached. Indeed at 82.5 to 84°C there was a small but significant negative effect (at the 0.5% level) due to the presence of the egg proteins. Johnson & Hoseney (1979) noted that whole egg solids had a deleterious effect on untreated flour, probably due to the lipids of the yolk lipoprotein fraction, whereas the egg albumen solids were beneficial to its performance. The positive contribution to gel firmness of the egg proteins, which begins to exert an influence at 80–82.5°C for the batters of the treated flours, must be counteracted in the case of the untreated flours by a decrease in the starch swelling or the starch–egg interaction.

The evidence from the gel firmness measurements of cooked batters of flours A, B and C in model systems I and II suggests that the improving action of chlorination or heat treatment is related to changes in the swelling process of the starch granules. This process may be affected either by changes in the internal structure of the starch granule or by changes at the surface of the granule. In a flour system it is possible for fats and proteins from other components, such as the wedge protein fraction (Hess, 1955) to adhere to the surface of the starch granules. This would present an alternative mechanism for the inhibition of the swelling process of the starch granule. An attempt was therefore made to remove a significant proportion of the wedge protein by ultracentrifugation. This technique was chosen so as not to disturb the natural granular surface components since Cauvain, Gough & Whitehouse (1977) have shown that even simple washing processes can cause significant changes in the properties of the starch.

Table 5. Firmness values of gels made with starches isolated from flours A, B and C and used in model systems III and IV

Starch type coded from parent flour	Model system	Firmness at 90°C (gf)
Starch A	III	2170 (11)*
	IV	4176 (9)
Starch B	III	3150 (7.5)
	IV	6620 (7)
Starch C	III	3170 (8)
	IV	6025 (6.5)

*Coefficients of variations are given in parentheses.

The results for the starch fractions in model batter systems III and IV (Table 5) show a highly significant effect due to the flour proteins. There is a marked increase in gel firmness compared to the values obtained for the parent flours in

model systems I and II. The flour proteins appear to exert a large negative effect for all the flour types both with and without egg proteins present. However, there is still a highly significant difference (0.1% level) between results of the starch fraction from flour A compared with the starch fractions of the treated flours, B and C.

The magnitude of the negative effect of the flour proteins was not significantly different for the treated and untreated flours indicating that the improving effect of the treatment processes was not significant with respect to this factor. It is clear that the major effect of flour improvement concerns the starch fraction although the knowledge of the role of the flour proteins in structure formation may prove beneficial in the use of starch/flour combinations to replace treated flours in cake systems.

Table 6. Firmness values of gels made from flours and starches A, B and C after heating to 90°C and cooling to 20°C

Component—flour or starch	Model system	Firmness at 20°C (gf)
Starch A	III	6985 (8)*
	IV	8980 (7.5)
Flour A	I	1425 (11)
	II	2970 (12)
Starch B	III	9290 (5.5)
	IV	12 480 (8)
Flour B	I	2960 (13)
	II	4565 (16)
Starch C	III	9240 (10)
	IV	11 190 (8)
Flour C	I	3650 (7)
	II	4440 (13)
†Cake A	—	4462 (6)
Cake B	—	5835 (7)
Cake C	—	5855 (6)

*Coefficients of variations are given in parentheses.

†Cakes were crumbled and centrifuged at 2000 *g* for 30 min before compression tests, to remove air cells.

The structures formed in the model batter systems were re-examined on cooling the cooked batters to 20°C. Gel firmness measured either by compression tests (Table 6) or tensile tests (Table 7) showed that the cooked batters of flour A were less firm and had a smaller modulus of elasticity than those of flours B and C. These results are similar to those obtained by Frazier, Brimblecombe &

Daniels (1974), using a simple flour and water slurry (1:2 by weight) but reproduce more precisely the conditions found in a real batter. Compression tests on centrifuged cake crumb prepared from the same parent flours (Table 6) showed the same pattern of results as the model systems. The cake crumb was firmer than the model system II gels but showed the same relative difference between the untreated flour and treated flour systems. It is probable that the cake crumb is firmer than the gels because of the lower moisture content (24–26%) compared with the gels (34–36%), due to moisture losses during baking. However, the results confirm that the findings of the model systems are representative of the real cake system.

Table 7. Tensile measurements of flour gels prepared in model system II

Flour	Young's modulus (dyn/cm^2)	Percentage extension at 300 gf	Tensile force at rupture (gf)	Tensile strength at rupture (gf/cm^2)
A	2.17×10^6	9.0	386 (14)*	183
B	3.23×10^6	6.1	515 (8)	272
C	3.05×10^6	6.0	502 (10)	254

*Coefficients of variations are given in parentheses.

The differences observed for the effect of egg proteins in batter systems of treated and untreated flours may help to explain the overall mechanism of improvement. There are two mechanisms by which the effect of the egg proteins can be influenced by the starch. These are a swelling process in which the egg is concentrated outside the swollen granules thus forming a much more rigid gel, or an interaction between the soluble starch polymers and the egg protein gel network. In the results shown in Table 2 it was found that the gel firmness value of 2.5% egg protein gel was increased from 10 gf to 3010 gf by the addition of 25% starch in its granular form. The simple concentration effect of the addition of starch solids raises the egg concentration to 3.2% but the results shown in Table 8 for egg gels of varying concentration indicate that this change would only increase the gel firmness to 20 gf. Concentration of the egg proteins in the inter-granular serum to five times their original level would produce a gel firmness value of *ca* 1050 gf.

Table 8. Firmness values of egg gels in 50% aqueous sucrose cooked at 90°C, as a function of concentration

Sample	Whole egg solids (%)	Egg albumen (%)	Firmness (gf)	
			At 90°C	At 20°C
1	4.1	—	10	20
2	6.7	—	45	92
3	14.4	—	421	1050
4	13.7	3.85	720	2435

Previous studies of the gelation of egg proteins in 50% sucrose (w/w) by Guy, Karmali & Willcox (1974) showed that the albumens begin to gel at 80°C. The protein aggregates would therefore begin to form before the starch granules started to swell and would be unlikely to penetrate far into the granules. Experiments with starch granules in 50% sucrose solution at 90°C showed that less than 0.2% soluble starch escapes from the granules under these conditions. Further tests with soluble starch prepared by disintegrating starch granules in excess water at 95°C, showed no significant increase in gel firmness of 2.5% egg gels in 50% sucrose occurred until the level of starch was increased to > 10%. Any starch–egg gel interactions must therefore be related to the surface of the starch granule or its neighbouring layers which can be penetrated by the egg protein aggregates.

The two major effects of flour improvement by chlorination or heat treatment therefore appear to be related to the swelling of the starch granules and the effect of the starch on the egg proteins. Flour proteins interfere with the performance of the starch and reduce the effect of the egg proteins. Experiments with up to 5% gluten in an egg gel system in 50% sucrose showed no significant difference due to the presence of the flour proteins. The mechanism for the flour proteins interference with the effect of the egg proteins in the model system II must therefore be related to its competitive interaction at the surface of the granules. This interaction could serve both to reduce the swelling of the starch and the area of contact with the egg protein network.

The major differences between the treated and untreated flours appear to concern the surface of the starch granules and its neighbouring layers. It has been shown by Hess (1955) and Barlow *et al.* (1973) that this surface is covered by proteins and lipids. The proteins are hydrophilic albumin and globulin types and could be modified by chlorination, heat treatment or aging processes at ambient temperatures to change the nature of the starch granules' surface. The lipids were shown by Daniels *et al.* (1963) and Morrison (1978) to be substituted by chlorine but are not changed chemically by heat treatment (Merryfield & Russo, 1972) or by storage at 70°C for three months (Morrison, 1978). Since the recent study by Johnson, Hoseney & Ghaisi (1980) suggests that the starch is modified by chlorination and air drying processes, further research into the changes in the starch granule surface is necessary to elucidate the fine details of the mechanism of flour improvement.

References

AOAC (1975) *Methods of Analysis*. Association of Official Agricultural Chemists, Washington.

Barlow, K.K., Butterrose, M.S., Simmonds, D.H. & Vesk, M. (1973) *Cereal Chem.*, **50**, 443.

Bean, M.M. & Yamazaki, W.T. (1978) *Cereal Chem.*, **55**, 936.

Bean, M.M., Yamazaki, W.T. & Donelson, D.H. (1978) *Cereal Chem.*, **55**, 945.

Bell, A.V., Berger, K.G., Russo, J.V., White, G.W. & Weathers, T.L. (1975) *J. Fd Technol.*, **10**, 147.

Boehringer Mannheim Ltd. (1971) *Enzymic Methods of Food Analysis*. Boehringer Mannheim.

Cauvain, S.P., Gough, B.M. & Whitehouse, M.E. (1977) *Starke*, **29**, 91.
Chamberlain, N. (1962) *Baker's Rev. (London)*, **19**, (46), 2014.
Daniels, N.W.R., Frape, D.L., Russell-Eggitt, P.W. & Coppock, J.B.M. (1963) *J. Sci. Fd Agric.*, **14**, 883.
Ewart, J.A.D. (1968) *J. Sci. Fd Agric.*, **19**, 371.
Frazier, P.J., Brimblecombe, F.A. & Daniels, N.W.R. (1974) *Chemy Inds. (London)*, 1008.
Gough, B.M., Whitehouse, M.E. & Greenwood, T. (1978) *CRC Reviews in Fd Sci. and Nutr.* (Sept), pp. 91–113.
Guy, R.C.E., Karmali, N & Willcox, C.J. (1974) Proc. IV Int. Congress Food Sci. & Technol, Vol. 1, p. 289.
Hess, K. (1955) *Kolloidzeitschrift*, **141**, 61.
Howard, W.B., Hughes, D.H. & Strobel, R.G.K. (1968) *Cereal Chem.*, **45**, 329.
Ingle, T.R. & Whistler, R.L. (1964) *Cereal Chem.*, **41**, 474.
Jacobsberg, F.R. & Daniels, N.W.R. (1974) *Chemy Inds. (London)*, 1007.
Johnson, A.C. & Hoseney, R.C. (1979) *Cereal Chem.*, **56**, 336.
Johnson, A.C., Hoseney, R.C. & Ghaisi, K. (1980) *Cereal Chem.*, **57**, 94.
Joly, M. (1965) *Physicochemical Approach to Denaturation of Proteins*, pp. 142, 143. Academic Press, London.
Kulp, K., Tsen, C.C. & Daly, C.J. (1972) *Cereal Chem.*, **49**, 194.
Montzheimer, J.W. (1931) *Cereal Chem.*, **8**, 510.
Morrison, W.R. (1978) *J. Sci. Fd Agric.*, **29**, 365.
Russo, J.V. & Doe, C.A.F. (1968) British Patent 1,110,711.
Russo, J.V. & Doe, C.A.F. (1970) *J. Fd Technol.*, **5**, 563.
Sollars, W.F. (1958) *Cereal Chem.*, **35**, 100.
Whistler, R.L., Mittag, T.W. & Ingle, T.R. (1966) *Cereal Chem.*, **43**, 362.
Uchino, N. & Whistler, R.L. (1962) *Cereal Chem.*, **39**, 477.

(Received 18 August 1980)

J. Fd Technol. (1981) **16**, 167–175

An application of the ultrasonic pulse echo technique to the measurement of crispness of biscuits

M. J. W. POVEY AND C. A. HARDEN*

Summary

The possibility of developing an objective method of measuring the crispness of biscuits using the ultrasonic pulse echo technique was investigated. An encouraging relationship between the crispness (from sensory measurement) and the velocity of longitudinal sound was found. A similar, but less convincing relationship was found between crispness and ultra sonic attenuation.

The ultrasonic velocity shows a better correlation with crispness than does the ultrasonically derived Young's modulus.

Introduction

Crispness is an important textural characteristic of some foods. The sensitivity of the consumer to texture was shown by Szczesniak (1971). This awareness of texture, coupled with the manufacturers' need to produce a consistent product has led to a need to associate sensory judgement and instrumental measurements.

An accurate study of the subject to some extent depends on the ability to define crispness. A general understanding of the word and its meaning exists (Szczesniak & Kleyn, 1963) but there seems to be little agreement on a definition. A number of attempts have been made to standardize a description in terms of easily measured quantities (see. e.g. Jowitt, 1974). Crispness tests must relate to the human senses if they are to have any bearing on consumer attitude. Thus, the problem of identification of the fundamental factors contributing to the crispness of a food makes the study of the subject an empirical one.

Attempts at an 'objective' measurement of crispness have been made by

*Now at Metal Box Limited, Metals Research Section, Wantage.

Correspondence should be sent to Dr M.J.W. Povey, Proctor Department of Food Science, The University of Leeds, Leeds LS2 9JT.

0022–1163/81/0400–0167 $02.00

many authors. Those pertinent to our experiments include Francis & Hastings (1963) who used a circular saw to cut into the biscuit, Bruns & Bourne (1975) who related Instron readings to Young's modulus, and Vickers & Bourne (1976) who measured noise emission from biscuits. These tests, as do ours, try to relate a machine measurable and hence 'objective' test to the empirical assessment of crispness by taste panel or similar technique.

Ultrasonic techniques have not, so far been used for the measurement of crispness in biscuits although diverse applications of ultrasonics exist in the characterization of foods (see, e.g., Zacharias & Parnell, 1972 on the total solids content of food; Povey & Wilkinson, 1980 on quality testing of eggs, and Steele, 1974 on water content of sucrose solutions).

It has been pointed out that there may be a relationship between Young's modulus and crispness (Brennan, Jowitt & Williams, 1974; Bruns & Bourne, 1975; and Wedzicha & Newmarch, 1980). It therefore seemed reasonable to suggest that ultrasonic velocity V_1 may correlate with crispness since for our experiments,

$$V_1^2 = \frac{E(1-\sigma)}{\rho(1-\sigma-2\sigma^2)} \qquad \text{(Pollard, 1977) (1)}$$

where E is Young's modulus, σ Poisson's ratio and ρ the density of the material. In addition, ultrasonics is a technique which has wide application in industrial, on-line, non-destructive measurement.

Materials and methods

The ultrasonic pulse echo technique

The method as described by Pellam & Galt, 1946, was used. The sample was held between an ultrasonic transducer and a metal backing plate, or between two transducers. Usually soft-tipped probes were used but hard-tipped probes were occasionally bonded to biscuits using perspex glue in order to check which acoustic modes were propagating. Figure 1 illustrates the sample holder. The jig made for the purpose could accommodate a variety of probes and a reflector plate. Since soft-tipped probes were used it was important to ensure constant pressure between the probes and the biscuit and this was done by mounting the probes on a linear bearing assembly, retained by a rod/spring arrangement. Soft-tipped probes gave better acoustic contact to the biscuit than hard-tipped probes by virtue of their flexible plastic tip which moulded itself to some extent to the irregular surface of the biscuit. Figure 2 is a simplified block diagram of the Balteau-Sonatest UFD-1, transducer and sample as used in the basic pulse echo method.

All measurements of velocity were carried out using the soft tip probes in the transmission arrangement with a narrow band pre-amplifier in series with the

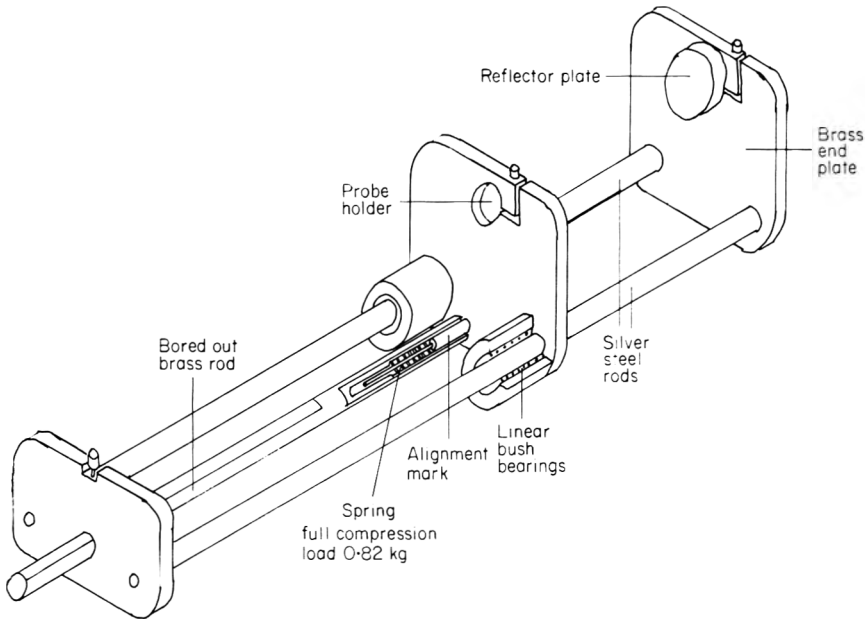

Figure 1. Transducer mounting for acoustic measurements. The sample is held between two ultrasonic probes or between opne probe and the backing plate.

detecting transducer. 1.5 MHz probes were used and the receiver was set with its centre frequency at 0.5 MHz. The echoes were broadened by multiple echoes within the soft tips of the probes and the time of arrival was taken as the arrival of the peak of the echo envelope. The probes were first pressed together at a set pressure and the electronic delay was used to bring the probe peak to the vertical line zero. The probes were then separated and the biscuit placed between them with its two faces against the probes. The probes were then pressed together at the pressure used for zeroing and the biscuit held between them. The calibrated electronic delay was then used to bring the displaced peak maximum back to the vertical line zero and the delay read off. The delay had earlier been calibrated by placing a series of spectrophotometric cuvettes, of verying path length, and full of distilled water, between the probes. Using the data of Grosso & Mader (1972), for the velocity of sound in distilled water, the arbitrary delay on the UFD-1 could be calibrated to better than 5 parts in 1000. The thickness of the biscuits was measured using Vernier calipers and the sound velocity was obtained by dividing thickness (in metres) by the delay (in seconds). The sound velocity was tabulated in the form (delay/thickness) since this is a convenient form for automatic monitoring.

That compression waves were indeed propagating was checked by glueing a hard-tipped compression probe to the biscuit and comparing the received echo's position in time with that obtained when a hard-tipped shear wave probe was glued to the biscuit. The shear wave was so highly attentuated that it could not

be detected at the far face of the biscuit and therefore there could be no doubt that the soft-tipped probes were generating and detecting compression waves.

Crispness measurements

The following eight varieties of biscuit were used: Ryebread extra-thin crispbread (Primula), Morning coffee (Fox's), Digestive biscuits (McVitie's), Cream crackers (Crawford), Vitawheat (Peak Frean), Tuc snack crackers (Crawford), Melba toasts (Buitoni), Gingernuts (McVitie's).

These brands were chosen because they were the same varieties as those used by B.L. Wedzicha & E.M. Newmarch (1980, personal communication). The biscuits were purchased from large chain stores with a rapid turnover – such biscuits could be considered fresh and acceptably crisp. The biscuits are tested straight from the pack and stored in an airtight contaioner. B.L. Wedzicha & E.M. Newmarch (1980, personal communication) had measured crispness using word association tests, obtaining a measure of crispness for the above mentioned biscuits, and we have based our assessment of crispness on their data. We are confident that these data are meeningful because a sensible correlation matrix was obtained on a twenty-word questionnaire given to nine panellists (see Wedzicha, 1980). Both positive and negative correlations were consistent. In addition our results for crispness compare well with those reported by Brennan *et al.* (1974).

Attentuation measurements

These were made simultaneously with the velocity measurements. The echo peak was attenuated using an attenuator calibrated in dB's until it was half full screen deflection. The pre-amplifier was set to give maximum echo height when the probes were attached to Morning coffee biscuits and was then left unchanged. The attenuation, given in dB, was divided by biscuit thickness to give attenuation per unit length, dB m^{-1}.

Results

In Table 1, velocity and attenuation measurements are shown as a function of percentage crispness score for the eight types of biscuit measured. The crispness data of Brennan *et al.* (1974) are presented for comparison purposes. The errors quoted in the Table are the standard deviation of the mean for five biscuits from one pack. We have included data on brittleness and crunchiness (B.L. Wedzicha & E.M. Newmarch, 1980, personal communication) in order to confirm that only crispness is measured by acoustic velocity. The data have been fitted using linear regression where r is the correlation coefficient, n is the

Table 1. Sensory and ultrasonic data for eight varities of biscuits.

Variety	1 Percentage crispness y_1*	2 Percentage crunchiness y_2*	3 Percentage brittleness y_3*	4 Percentage crispness y_4*	5 Delay/ thickness (0.3416×10^{-3}s m^{-1}) x_1	6 Gain/ thickness (10^{-3}dB m^{-1}) x_2
Digestive	23.5	82.4	11.8	6.7	15.3 (±4)	8.6 (±0.6)
Gingernut	29.4	94.1	47.1	26.7	9.1 (±2)	3.1 (± 1.0)
Morning coffee	58.8	76.5	29.4	50	15.7 (±4)	7.8 (±0.9)
Cream crackers	58.8	29.4	47.1	42.9	20.6 (±6)	11.8 (±1.5)
Primula thin (wheat)	64.7	35.3	64.7	—	14.6 (±6)	9.7 (±1.9)
Tuc	76.5	35.3	29.4	73.3	19.9 (±4)	11.1 (±4.6)
Vita Wheat (wheat)	82.4	82.4	47.1	58.3	26.5 (±6)	11.4 (±1.0)
Melba toast	88.2	64.7	47.1	41.7	13.5 (±6)	5.9 (±1.2)
	$y = 2.3x_1 + 21.4$	$-1.3x_1 + 84.3$	$-0.1x_1 + 41.8$	$2.2x_1 + 4.9$		$y_1 = 3.1x_2 + 33$
	$r = 0.52$	0.27	0.02	0.58		0.40
	$t = 1.49$	0.69	0.07	1.36		1.07
Omitting Melba toast	$y = 3.1x_1 + 3.2$	$-1.3x_1 + 85.4$	$0.1x_1 + 38.6$	$2.4x_1 + 0.5$		$y_1 = 5.2x_2 \times 8.9$
	$r = 0.76$	0.27	0.02	0.60		0.71
	$t = 2.61$	0.63	0.04	1.46		2.25

*B.L. Wedzicha & E.M. Newmarch (1980).
*Brennan *et al.* (1974).

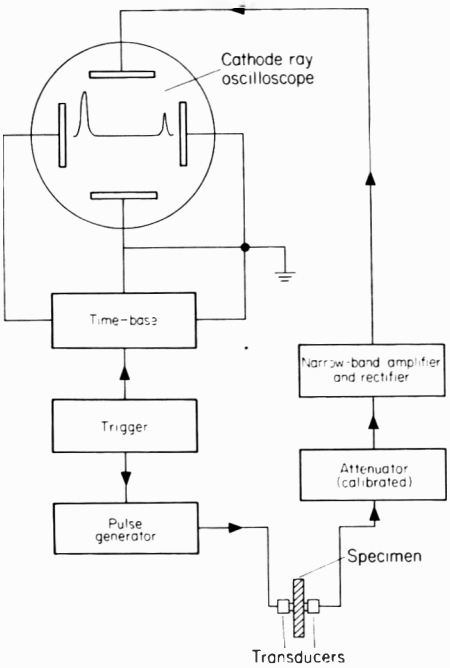

Figure 2. Simplified block diagram of the Balteau-Sonatest UFD-1, transducer and sample in the transmission configuration.

number of samples and the t statistic $r\sqrt{(n-2)/(1-r^2)}$. The t statistic may be used to determine the confidence with which it may be assumed that the slope of the fit is not zero and hence our confidence that there exists a linear relationship between the two variables uinder consideration. For instance, we conclude that there is more than 64% certainty that there is a linear relationship between attenuation (x_2) and percentage crispness (y_1) ($t_{18,6} = 1.0 < t_{calc} = 1.07$).

The calculations have also been performed with Melba toast omitted. This omission dramatically improved the fit, no other sample had such a large effect on the correlation coefficient.

In Table 2 we present Young's modulus, as computed from our velocity measurements, together with measurements of Young's modulus made by B.L. Wedzicha & E.M. Newmarch (1980, personal communication) on an Instron instrument. In our experiments the velocity of a compressional wave is given by equation 1 and we have assumed that $\sigma = 0.33$ for all our samples, a value which is typical of most solids. Biscuit density was measured by taking a complete pack of biscuits and approximating the pack to a simple geometry whose dimensions were measures. The contents of the pack were weighed and mass/volume gave density. Great accuracy was unnecessary since the standard deviation on the velocity measurements varied between 20 and 40% of the velocity.

Table 2. Velocity of longitudinal sound, Young's modulus and density of eight varieties of biscuits.

Variety	Velocity of longitudinal (compression) sound V_l ms^{-1}	Young's modulus (1.5 MHz) E (from eqn. 1) Nm^{-2}	Density ρ kg m^{-3}	Young's modulus (B.L. Wedzicha & E.M. Newmarch, 1980) Nm^{-2}
Digestive	191	0.11×10^8	464	0.15×10^8
Ginger	322	0.52×10^8	739	0.06×10^8
Morning coffee	186	0.10×10^8	427	0.19×10^8
Cream cracker	142	0.04×10^8	259	0.08×10^8
Primula thin (wheat)	210	0.10×10^8	360	2.24×10^8
Tuc	147	0.05×10^8	309	0.30×10^8
Vita Wheat (wheat)	111	0.02×10^8	272	0.25×10^8
Melba toast	217	0.08×10^8	248	0.84×10^8

Discussion

Ultrasonic velocity

Examining Table 1 we conclude that an encouraging linear relationship exists between percentage crispness, as measured by Wedzicha & Newmarch (1980) and 1.5MHz compession wave velocity. We can be more than 80% certain ($t_{calc} > t_{.1.6}$) that this relationship is not an accidental one. If we omit Melba toast from the linear regression calculation we can be more than 95% certain $t_{calc} > t_{025.5}$) that this relationship is not an accidental one. It is arguable that Melba toast is not a biscuit but a bread and, in addition, Melba toast, unlike the biscuits examined, has a very large proportion of gelatinized starch (F.O. Flint, 1980, personal communication). If it is correct to omit the Melba toast then we must admit that the mechanism producing crispness in this product is different from that in the other varieties tested.

Our general conclusions are supported by comparison with the data of Brennan *et al.* (1974) (Column 4, Table 1), although the fit to their data is not so good, nor do their results show the dramatic improvement in fit when Melba toast is removed. However, we consider that their results are not as statistically reliable as those of Wedzicha & Newmarch (1980). Supporting evidence is also provided by the very low correlation between percentage crunchiness or percentage brittleness and (delay/thickness). Thus ultrasonic velocity correlates only with percentage crispness.

Ultrasonic attenuation

Consulting Table 1, it can be concluded that we are more than 64% certain ($t_{calc} > t_{.18,6}$) that a relationship exists between percentage crispness and 1.5 MHz compression wave attenuation. However, removal of Melba toasts from the analysis improves the correlation so that for these data at least, there is a better than 90% ($t_{calc} > t_{.05,6}$) that a linear relationship exists between percentage crispness and ultrasonic attenuation.

Young's modulus

In Table 2, our data for Young's modulus, calculated from our velocity measurements and measurements of biscuit density are compared with percentage crispness, percentage crunchiness and percentage brittleness. There are two major sources of error in our measurement of Young's modulus, (1) an incorrect value for Poissons's ratio and (2) the ultrasonic method is a dynamic technique, and there is no special reason why the dynamic elastic moduli measured should be the same as the static moduli.

The Poissons's ratio merits further invesigation. This could be done by measuring the shear moduli of the biscuits using ultrasonic shear waves. An investigation of this kind may well throw more light on the physical mechanisms underlying sensory perception of crispness. We can examine Brennan *et el.*'s (1974) hypothesis that Young's modulus correlates with percentage crispness using the ultrasonically derived modulus. Using data from Tables 1 and 2, we obtain correlation coefficients for percentage crispness, crunchiness, and brittleness *vs* ultrasonic Young's modulus of $r = 0.64$, 0.53 and 0.13. These should be compared with the correlations with ultrasonic velocity in Table 1, omitting Melba toast of $r = 0.76$, 0.27 and 0.02, respectively. We can make the same comparison with the Instron measurement of Young's modulus by B.L. Wedzicha & E.M. Newmarch giving $r = 0.25, 0.44$ and 0.61. Thus the ultrasonic velocity correlates with crispness better than either the ultrasonically derived Young's modulus, or the Instron derived modulus.

We conclude that the ultrasonic technique offers promise as a method for the electromechanical measurement of the crispness of biscuits. This technique has the further advantage that it adapts readily to the industrial environment.

Acknowledgments

The authors would like to thank our colleagues in the Proctor Department of Food Science for valuable discussions, and in particular Dr B.L. Wedzicha for providing us with data, prior to publication.

The ultrasonic equipment for this project was purchased with the aid of a grant from the Agricultural Research Council.

References

Brennan, J.G., Jowitt, R. & Williams, A. (1974) *Sensory and Instrumental Measurements of 'Brittleness' and 'Crispness' in Biscuits*. Fourth International Congress of Food Science & Technology, Madrid, Spain.
Bruns, A.J. & Bourne, M.C. (1975) *J. Text. Stud.* **6**, 445.
Francis, B. & Hastings, W.R. (1963) *B.B.I.R.A. Report No.* **67**, Chorleywood.
Grosso, V.A. & Mader, C.W. (1972) *J. Acoust. Soc. Am.* **52**, 1442.
Jowitt, R. (1974) *J. Text. Stud.* **5**, 351.
Pellam, J.R. & Galt, J.K. (1946) *J. Chem. Phys.* **14**, 608.
Pollard, H.F. (1977) *Sound Waves in Solids*, 1st edn. pp. 15 and 25. Pion Ltd., London.
Povey, M.J.W. & Wilkinson, J.M. (1980). *Br. Poult. Sci.* **21**, 307.
Steele, D.J. (1974) *Br. J. Non-destr. Test.* **16**, 169.
Szczesniak, A.S. (1971) *J. Text. Stud.* **2**, 196.
Szczesniak, A.S. & Kleyn, D.H. (1963) *J. Fd Sci.* **17**, 74.
Vickers, Z. & Bourne, M.S. (1976) *J. Fd Sci.* **41**, 1158.
Wedzicha, B.L. (1979) *Nutr. Fd Sci.* **58**, 8.
Zacharias, E.M. & Parnell, R.A. (1972) *Fd Tech., Champaign.* **26**, 160.

(Received 24 May 1980)

J. Fd Technol. (1981) **16**, 177–184

The production of ethyl acetate by soy yeast *Saccharomyces rouxii* NRRL Y–1096

F. M. YONG, K. H. LEE AND H. A. WONG*

Summary

Ethyl acetate was found to be produced in large quantities in aged cultures of the soy yeast *Saccharomyces rouxii* NRRL Y-1096 grown on medium containing glucose. The synthesis of ethyl acetate was investigated. The present study shows that the process of ester synthesis is essentially an aerobic one. Glucose and ethanol were the main substrates for the synthesis of ethyl acetate although to a limited extent the yeast cells were also able to synthesize the ester from ethanol alone but not from either glucose or acetate. From the results of the present study, it is suggested that the flavour-producing moromi stage of soy-sauce fermentation is necessarily a prolonged one because ethanol has first to be produced in a semi-anaerobic environment for the synthesis of the esters responsible for the characteristic bouquet and flavour of the mature soy-sauce. It should therefore be possible to shorten this stage of soy-sauce fermentation by the addition of ethanol to the moromi.

Introduction

The ability to form traces of esters is a property of many yeasts and is partly responsible for the bouquet of wines. Ester formation by yeasts of the Hansenula species from the Carlsberg collection of yeasts has been studied by Davies and co-workers (Davies *et al.*, 1951; Peel, 1951). They found that *Hansenula anomala* formed ethyl acetate from ethanol, alone or in the presence of acetate. The process was aerobic. Their results were subsequently confirmed by Laurema & Erkama with cells that had been grown semi-aerobically (Laurema & Erkama, 1968). These workers also found that if the cells had been grown in strictly aerobic conditions, ethyl acetate was produced from ethanol only in the presence of added acetic acid.

Authors' address: Department of Biochemistry, University of Singapore, College Road, Singapore 0316, Republic of Singapore.

*To whom correspondence should be addressed.

0022–1163/81/0400–0177 $02.00

Ester formation in *Saccharomyces cerevisiae* has also been studied by Nordstrom (1961). Under anaerobic conditions, ethyl acetate formation from ethanol was found to be independent of acetic acid added to the medium.

The present study on ester formation in the osmophilic soy yeast *Saccharomyces rouxii* NRRL Y-1096 was first prompted by the fruity smell of aged cultures. It was also because little is known of its ability to form esters. In soy-sauce fermentation, the esters produced by the yeast would, no doubt, be responsible, to some extent for the bouquet of the sauce. Hence it would be important to know something of how these esters are synthesized.

Materials and methods

Purity and source of chemicals

All chemicals used were of 'Analar' grade or the purest grade available. They were obtained mainly from Merck Chemical Company, Germany and Sigma Chemical Company, U.S.A. Chemicals for yeast culture were from Oxoid, England or Difco, U.S.A.

Yeast culture, media and culture methods

These were described previously (Yong *et al.*, 1978). Cells were grown in media containing 5% glucose and 10% NaCl at pH 4.5 at room temperature (28°C) for 3 days with shaking on a rotatory shaker. They were starved overnight in glucose-free media before use.

Determination of cell weight

This was as described previously (Yong *et al.*, 1980).

Estimation of esters

The esters were separated by distillation in the presence of excess $CaCO_3$ (anhydrous) and estimated by a slight modification of the method of Peel (1951). Distillation was used as a means of separating the esters from substances in the sample, e.g. glucose, which interfered with the estimation (Table 1). Volatile substances such as alcohol were found not to interfere; and acetic acid when present in the samples was present in too small a quantity to be of any consequence (Table 2).

Table 1. Glucose interference in ester estimation

Glucose added (μmol)	Absorbance at 520 nm
1	0.14
2	0.30
3	0.41
4	0.53
5	0.71

Table 2. Acetate interference in ester estimation

Ac^- ions (μmol)	Absorbance at 520 nm
1	0.00
2	0.00
4	0.00
8	0.00
12	0.00
16	0.17
20	0.36

For distillation, each sample was diluted to 15 ml and 2 drops of liquid paraffin added to minimize frothing. Distillation was carried out in a 50 ml, long-neck, round bottom flask with the condenser cooled with circulating ice water. A micro-burner was used for the distillation. The first 9 ml of distillate was collected in a 10-ml volumetric flask (cooled in ice) and made up to volume. A more than 95% recovery of the esters was obtained.

The method for the estimation of esters consisted essentially of first converting the esters with alkaline hydroxylamine to their corresponding hydroxamic acids and reacting with acid ferric chloride to form a coloured complex. 0.5 ml of 5 N NaOH and 0.5 ml of 4 N hydroxylamine hydrochloride were added to 3-ml aliquots of the sample. After 10 min, the colour was developed by the addition of 1 ml 5.6 N HCl followed by 1 ml 15% ferric chloride solution ($FeCl_3.6H_2O$) dissolved in 0.2 N Hcl w/v. Intensities of the colour were read at 520 nm. The standard graph showed linearity up to the equivalent of 10 μmol ethyl acetate and readings were reproducible to within ±3%.

Experimental procedure and results

Identification of ethyl acetate

Distillation of the clear supernatant liquid of medium from aged cultures in the presence of excess $CaCO_3$ powder for the separation of esters was carried out according to the method of Peel (1951). Details of the procedure are recorded in 'Materials and methods'. The distillate obtained was used for the identification of esters. Since the yeast has been found to produce acetic acid and ethanol, it was very likely that if esters were present, ethyl acetate would be one of them.

Identification of ethyl acetate by gas-liquid chromatography (GLC) was inconclusive as ethyl acetate was not separated from ethanol in the system used. Therefore an indirect method had to be resorted to. The ethanol content was first determined. The distillate was then neutralized with 0.1 N NaOH and

re-distilled. The second distillate was hydrolyzed by refluxing with 0.25 N NaOH for 30 min at 100°C in a boiling water bath. The reflux condenser was cooled by circulating iced water. A suitable aliquot was used for the determination of ethanol by the Conway method (1962). The amount of ethanol obtained (after subtracting the amount before hydrolysis) was found to be equivalent to the total amount of esters present expressed in terms of ethyl acetate, as shown in Table 3.

Table 3. Identification of ethyl acetate from aged culture medium. Ester as ethyl acetate was determined by the hydroxylamine-ferric chloride method. Results are that obtained for 10 ml of culture medium and expressed as mean ± s.e.mean

Ethyl acetate	5.1±0.6 mg
Ethanol from ethyl acetate (calculated)	2.67 mg
Ethanol in medium	15.3±0.5 mg
Ethanol in medium plus ethanol from hydrolysis of ethyl acetate	17.9±0.8 mg
Ethanol due to hydrolysis of ethyl acetate	2.6 mg

An aliquot of the hydrolyzed distillate was also used for the identification and determination of acetic acid. The acetic acid was identified by thin layer chromatography of silica gel plates. There was only one band in the chromatogram and its Rf value corresponded to that of the standard sodium acetate that was chromatographed together with the hydrolysate. The acetic acid was determined by steam distillation for volatile acids according to the method of Friedmann (1938), details of which have been previously described (Yong *et el.*, 1980). Attempts at determining the amount of acetic acid more specifically and quantitatively proved unsuccessful.

Ethanol and acetate as substrates for ethyl acetate synthesis

A series of experiments was carried out to determine the ability of the yeast cells to synthesize ethyl acetate from ethanol and acetate. Experiments were carried out under anaerobic as well as aerobic conditions because it has been reported that the ester could also be synthesized anaerobically (Nordstrom, 1961; Mills & Blackwood, 1967). However, according to our earlier work on glucose fermentation by these cells (Yong *et al.*, 1980) this appears unlikely for *Sacch. rouxii*, as the ethanol and acetate together with the CO_2 produced, quantitatively accounted for the amount of glucose fermented.

The experiments were carried out in 250-ml Quick-fit Erlenmeyer flasks with a centre well and placed on a rotatory shaker. For aerobic experiments, the flasks were stoppered with non-absorbent cotton wool plugs, whilst Quick-fit

glass stoppers were used for flasks in which anaerobic reactions were carried out. The stoppers were greased to ensure that the flasks remained air-tight during the experiments, after the flasks for anaerobiosis had been set up, having carefully displaced the air inside with oxygen-free nitrogen in anaerobic jars. As some ester in the medium was found to escape into the atmosphere of the flask, alkaline hydroxylamine was placed in the centre well of the flask for complete absorption of the ester which was determined together with that in the medium. An incubation period of 4 hr was used, as a reasonable amount of ethyl acetate

Table 4. Formation of ethyl acetate from different substrates. Cells were grown in pH 4.5 medium containing 10% NaCl and 5% glucose. Reaction mixture: 39 mg cells (dry wt), various substrates in appropriate concentrations, M/10 phthalate buffer pH 4.5, total vol. 26 ml. Incubation: 28°C, 4 hr

	EtAc produced (μmol)	
Substrate	Aerobic	Anaerobic
0.25 mmol glucose	N.D.*	N.D.*
1.0 mmol ethanol	12.9	N.D.*
1.0 mmol sodium acetate	N.D.*	N.D.*
0.25 mmol glucose + 1.0 mmol ethanol	161.2	N.D.*
0.25 mmol glucose + 1.0 mmol sodium acetate	8.9	N.D.
1.0 mmol ethanol + 1.0 mmol sodium acetate	10.0	N.D.*

*Not detected.

could then be detected with the method of analysis used. The pH of 4.5 was that of the moromi stage of soy fermentation during which the bouquet of the sauce is developed. Results of these experiments are shown in Table 4.

Under strictly anaerobic conditions, there was no synthesis of ethyl acetate from any of the probable substrates tested. Preliminary experiments, however, showed that if air originally present in the flask was incompletely replaced with oxygen-free nitrogen, some ester was produced.

Under aerobic conditions, the yeast cells did not synthesize the ester from glucose alone. The yeast cells were also unable to synthesize ethyl acetate solely from acetate. On the other hand, they appeared to be able to synthesize the acetate moiety from ethanol for the synthesis of ethyl acetate though only to a very limited extent. A similar situation appears to be the case when the ester was synthesized in the presence of both ethanol and acetate. Therefore it is very

likely that the acetate cannot be utilized by the yeast cells. A small amount of ethyl acetate was, however, obtained when acetate was present together with glucose. Whether this was formed intracellularly or from ethanol resulting from glucose fermentation could not be ascertained. At the end of each experiment all reaction mixtures to which ethanol had not been added were tested for the presence of ethanol and no ethanol was detected in any of them. A large amount of the ester was produced when ethanol was metabolized in conjunction with glucose. Thus metabolism of glucose appears to be necessary for the formation of ethyl acetate from ethanol and acetate.

Effect of NaCl on ethyl acetate production

If the synthesis of ethyl acetate from glucose and ethanol is an aerobic process, then the presence of high concentrations of NaCl should have an inhibitory effect. Results of experiments shown in Table 5 shows that this was indeed the case. The experiments were carried out in Erlenmayer flasks, under aerobic conditions, on a rotatory shaker as described previously. The amount of

Table 5. Effect of NaCl on ethyl acetate production. Cells were grown in pH 4.5 medium containing 10% NaCl and 5% glucose. Reaction mixture: 41 mg cells (dry wt), 250 μmol glucose, 1 mmol ethanol, NaCl in appropriate concentrations, M/10 phthalate buffer pH 4.5, total vol. 26 ml. Incubation: 28°C, 4 hr

NaCl (%)	Amount of glucose metabolized (μmol)	EtAc produced (μmol)	EtAc/glucose (μmol/μmol)
0.0	250.0	151.3	0.61
10.0	232.7	17.8	0.08
15.0	220.0	5.0	0.02
18.0	210.0	2.6	0.01
20.00	207.0	N.D.*	0.00

*Not detected.

ethyl acetate produced, when the reaction medium was saturated with NaCl (20%), was too little to be determined by the standard method used. In soy-sauce fermentation it is a common practice to saturate the koji with brine for the commencement of the moromi stage, and 18% NaCl is usually the resulting sodium chloride concentration. At this concentration, although very little ester is produced in 4 hr under experimental conditions, during the moromi stage of soy-sauce fermentation a much greater amount of the ester would be produced

due to a greater amount of fermentable substrate and a long period of fermentation lasting for several months.

Discussion

Ethyl acetate was found to be the ester present in the growth medium of aged cultures of *Sacch. rouxii*. It was synthesized aerobically from glucose and ethanol or from ethanol alone. Its synthesis was independent of acetic acid added to the medium even though acetic acid has been identified as a metabolic product of glucose fermentation (Yong *et al.*, 1978; 1980). This is similar to that of *Sacch. cerevisiae* which has, however, also been found to be able to synthesize the ester under anaerobic conditions. On the other hand, although the synthesis of ethyl acetate in the *Hansenula* species is also a strictly aerobic process (Davies *et al.*, 1951; Peel, 1951), with *Hansenula anomala*, however, cells grown in strictly aerobic conditions produced ethyl acetate from ethanol only in the presence of added acetic acid, whilst with semi-anaerobically cultured yeast, the ester formation was distinctly enhanced by acetic acid. Thus, the synthesis of ethyl acetate by *Sacch. rouxii* does not appear to be entirely similar to either that occurring in *Sacch. cerevisiae* or yeasts of the *Hansenula* species.

The acid moiety of the ester was found to be obtained mainly from the aerobic metabolism of glucose although it could be synthesized from ethanol in a limited way. Though acetic acid is a product of glucose fermentation under certain conditions (Yong *et al.*, 1980), it does not appear to provide the acid moiety of the ester ethyl acetate synthesized by *Sacch. rouxii* NRRL Y-1096. Acetate has also been shown not to be metabolized for growth of these yeast cells (Yong *et al.*, 1978). Nordstrom (1961) in his study on the formation of ethyl acetate by *Sacch. cerevisiae* was also not able to obtain any evidence that the acid moiety of the ester could be derived from added acetate. On the other hand, it has been suggested that in *Hansenula anomala*, acetic acid supplies the acid moiety of the ester (Laurema and Erkama, 1961).

In the soy-sauce fermentation process, the flavour producing moromi stage usually requires several months. The present study suggests that this was largely due to the necessity of first establishing a semi-anaerobic environment for the fermentation of glucose to ethanol which is then utilized for the synthesis of esters. In the absence of adequate aeration and especially in the presence of the high NaCl concentration, this condition is easily attained after sufficient respiration has taken place. On the other hand, if the yeast cells were to be supplied with ethanol, the time required for this stage of fermentation could probably be shortened, thus hastening the maturation of the soy-sauce. That *Sacch. rouxii* cannot synthesize ester from glucose under aerobic conditions is consistent with our earlier findings, which showed that glucose was completely oxidized at H_2O and CO_2, giving rise to a respiratory quotient (RQ) of unity (Yong *et al.*, 1980).

Acknowledgments

The authors wish to thank Dr P.W. Hesseltine for the yeast culture. Our thanks are also due to Miss K.L. Lim and Mr W.W. Leong for technical assistance.

References

Conway, E.J. (1962) *Microdiffusion Analysis and Volumetric Error*. Chap. XXXII, 248–255. Crosby Lockwood & Son Ltd., London.
Davies, R., Falkiner, E.A. Wilkinson, J.F. & Peel, J.R. (1951) *Biochem. J.* **49**, 58.
Friedman, T.E. (1938) *J. Biol. Chem.* **123**, 161.
Laurema, S. & Erkama, J. (1968) *Acta Chem. Scand.* **22**, 1482.
Mills, C. & Blackwood, A.C. (1967). *Canadian J. Microbiol.* **13**, 1259–1270.
Nordstrom, K. (1961) *J. Inst. Brew.* **67**, 173.
Peel, J.R. (1951) *Biochem. J.* **49**, 62.
Yong, F.M. Lee, K.H & Wong, H.A. (1978) *J. Fd Technol.* **13**, 385.
Yong, F.M., Lee, K.H. & Wong, H.A. (1980) *J. Fd Technol.* **15**, 421.

(Received 21 August 1980)

J. Fd Technol. (1981) **16**, 185–197

The storage life of iced southern blue whiting (*Micromesistius australis*)

C. A. BARASSI, R. L. BOERI, M. CRUPKIN, L. A. DAVIDOVICH, D. H. GIANNINI, C. L. SOULÉ, R. E. TRUCCO AND H. M. LUPÍN

Summary

The storage life of iced southern blue whiting *(Micromesistius australis)* was studied. Pre- and post-spawning characteristics were investigated by means of organoleptic assessments of raw and cooked fish, total volatile bases, reduced viscosity of high ionic strength muscle extract and GR Torrymeter readings. Results show a great influence of the biological condition on the keeping time in ice.

The results suggest that in pre-spawning condition the whole fish can be stored up to 12 days while in post-spawning condition the keeping time cannot exceed 6 days.

The gutting of the fish could lengthen the possible storage time in the post-spawning condition for up to 2 days. Nevertheless the post-spawning fish quickly loses its quality 2–3 days after catching.

The possibility of utilizing standard white fish processing machines and the influence of parasites are also discussed.

Introduction

Southern blue whiting *(Micromesistius australis*, Norman, 1973) is a gadoid fish living off the Argentine coasts south of latitude 38–39°S (Bellisio, López & Torno, 1979). Its habitat includes the limits of the Patagonian shelf and the talus, the southern portion of the shelf, the Burdwood Bank, and the area around the islands of South Western Atlantic as far as the South Orkney Islands (60°30′S, 45°W) (Gershanovich & Lyubimova, 1971).

Specimens are mainly between 40 and 60 cm long with a mean value of 47 cm; the average weight for the specimens in the 48 to 54 cm range is 694 g (Bellisio, López & Torno, 1979). These values are notably larger than those of the North

Authors' address: Instituto Nacional de Tecnología Industrial, Centro de Investigaciones de Tecnología Pesquera, 12 de Octubre 4728, 7600 Mar del Plata, Rep. Argentina.

*Present address: FAO, Fish Utilization and Marketing Service, Rome, Italy

0022–1163/81/0400–0185 $02.00

Atlantic counterpart, the blue whiting *(Micromesistius poutassou)* for which lengths of 28 to 30 cm and weights of 120 to 200 g have been reported (Dagbjartsson, 1975; Maucorps & Fontaine, 1979).

Although there is no complete picture of the biological behaviour of southern blue whiting it seems to have the spawning period in September–October (Weiss, 1975; Otero, 1976) or October–November (Shubnikov, Permitin & Voznaj, 1969), near the islands of the Patagonian shelf and the Burdwood Bank. Apparently it migrates to the antarctic waters of the Scotia sea at the end of spring and in summer, and returns to the waters of the Patagonian shelf in March (Shubnikov *et al.*, 1969).

Southern blue whiting is also caught south of New Zealand. Although there are some differences between the populations of Patagonia and New Zealand (Inada & Nakamura, 1975) the same scientific name is kept for both in recent fisheries studies (Kerstan, 1979, 1980).

Even though there are no published data regarding the fishing potential of southern blue whiting in the South West Atlantic it can be estimated to be roughly of the same magnitude as that for Patagonian hake *(Merluccius hubbsi)*. According to Bellisio *et al.* (1979) 523 000 t/year could be captured without affecting the resource in the Patagonian shelf. In spite of the economic potential of the species, published data on either its technological characteristics or its keeping time that could provide guidelines for the handling and processing of southern blue whiting is not available.

Materials and methods

Table 1 summarizes the experiments performed, with references to the place and date of catch, vessel and types of analysis and assessments performed.

The codes PS and WH correspond to experiments performed on board the research ships PROFESSOR SIEDLECKI and WALTHER HERWIG of Poland and the Federal Republic of Germany respectively.

All the samples were placed in plastic boxes with sufficient crushed ice immediately after opening the net and held in a cold store at *ca* 0°C. For each experimental point not less than six fish were analysed except for the GR Torrymeter readings (TM) where sixteen fish were tested.

The material used for the chemical determinations resulted from pooling one fillet from each fish used in the organoleptic assessment.

The cooked organoleptic assessment (COA) was done on fillets that had been wrapped in aluminium foil and cooked by steaming in a casserole for 10 minutes.

The scores ranged from 0 for the best quality to 5 for the worst according to a score table that had originally been developed to assess the quality of cooked fillets from fresh Patagonian hake (Lupín *et al.*, 1980).

For the raw organoleptic assessment (ROA) the scores also range from 0 for the best quality to 5 for the worst on a scale based on the one developed for

Table 1. Basic information on the experiments performed

Experiment No.	Date	Experiment code*	Ship	Catch position	ROA	COA	TVB	pH	RV	TM	PR	Number of fish evaluated
1	28.12.73	UI HGI	PS	52°45′ S 63°45′ W	yes	—	—	—	—	yes†	—	73
2	10.10.78	UI	WH	48°36′ S 61°59′ W	yes	—	yes	—	yes	yes	yes	128
3	24.08.78	UI	WH	52°45′ S 63°41′ W	yes	—	yes	—	yes	yes	yes	144
4	27.10.78	UI	WH	53°48′ S 60°10′ W	—	yes	yes	yes	—	yes	yes	80
5	27.10.78	GI	WH	53°48′ S 60°10′ W	—	yes	yes	yes	—	yes	yes	80
6	27.10.78	HGI	WH	53°48′ S 60°10′ W	—	yes	yes	yes	—	yes	yes	80

*Experimental codes: UI, ungutted in ice; GI, gutted in ice; HGI, headed and gutted in ice.
†Intelectron Fish Tester V was used instead of GR Torrymeter.

Patagonian hake by the FAO–Fishery Development Project in Argentina (da Encarnaçao, 1974; Lupín *et al.*, 1980).

The existing differences between southern blue whiting and hake were taken into account. Southern blue whiting presents a bluish back with pink and violet reflections; the rest of the body is whiter than in hake and it is silver white in the belly; with deterioration these colours lose brightness and intensity. The peritoneum, that is steel grey at the beginning, tends to be easily torn away during storage.

Total volatile bases (TVB) were determined by the direct distillation method (Giannini, Davidovich & Lupín, 1979). Not less than two assays were performed on each pooled sample.

Reduced viscosity (RV) of high ionic strength muscle extract was measured as described by Crupkin *et al.* (1979). Each viscosity mesurement was done in quadruplicate.

The pH was determined directly by means of a glass electrode set in different parts of the minced, pooled sample.

The texture of the raw fish was measured by means of a penetrometer (PR) as described by Lupín *et al.* (1980).

The Intelectron Fish Tester V used in experiment No. 1 was as described by Hennings (1965).

Experiment No. 3 was performed on pre-spawning fish, experiments Nos. 1, 2, 4, 5 and 6 were conducted on post-spawning fish. The condition of the fish was assessed according to the gonad state.

Parasite infestation was determined in experiments 2–4 by candling. Hand-skinned fillets were placed on top of a translucent white plastic box (0.19 m × 1.28 m) containing two standard fluorescent lamps of 40 W, the cysts observable to the unaided eye were counted. In experiment No. 1 the determination was performed by boiling skinned fillets, slicing them carefully, and counting the cysts observable to the naked eye.

Statistical evaluations have been used wherever appropriate and possible.

Results

Pre- and post-spawning observations

The biological condition of southern blue whiting seems to affect the keeping time in ice to a great extent. Variation in ROA for ungutted fish versus days in ice is depicted in Fig. 1 with data from experiments with pre-spawning and post-spawning fish. The acceptability borderline was established at a score of 2–2.5.

When fish is pre-spawning the impairment of organoleptical quality follows a classical s-shaped curve while the quality of post-spawning fish presents an early fall.

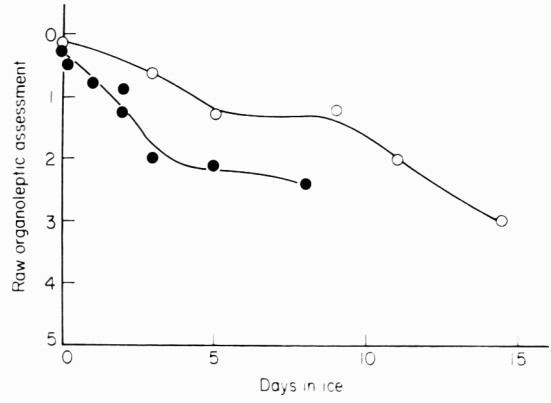

Figure 1. Raw organoleptic assessment of ungutted southern blue whiting in ice: ○, pre-spawning; ●, post-spawning.

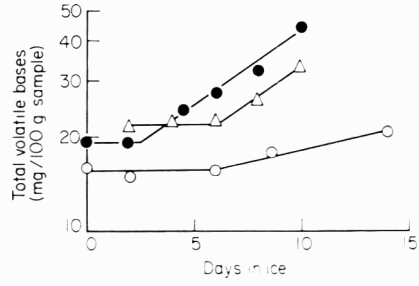

Figure 2. Average total volatile bases values for southern blue whiting stored in ice: ○, ungutted pre-spawning; ●, post-spawning; △, post-spawning headed and headed and gutted.

Variation in TVB values for ungutted fish in both pre- and post-spawning conditions can be seen in Fig. 2. The data were plotted on a semilog graph, in this way the variation can be approximated by straight lines. Again a sharp difference was found.

The RV falls dramatically for the post-spawning fish, and after 2 days in ice, or even sooner, the viscosity of the high ionic strength muscle extract becomes that of water; while the RV in pre-spawning condition remains unchanged during ice storage. The variations in RV can be seen in Fig. 3.

Penetrometer readings after *rigor mortis* and for different storage conditions, can be seen in Fig. 4.

In general there is a lowering in the resistance to penetration with time, independent of the processes to which the fish have been subjected. Whole fish lose resistance in the same proportion that gutted or headed-and-gutted fish do.

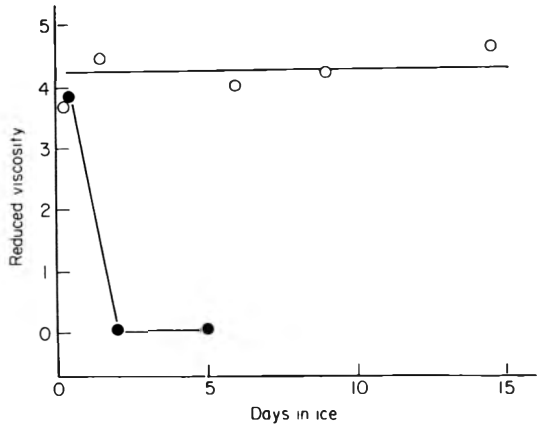

Figure 3. Reduced viscosity of high ionic strength muscle extracts of southern blue whiting stored in ice: ○, ungutted, pre-spawning; ●, post-spawning.

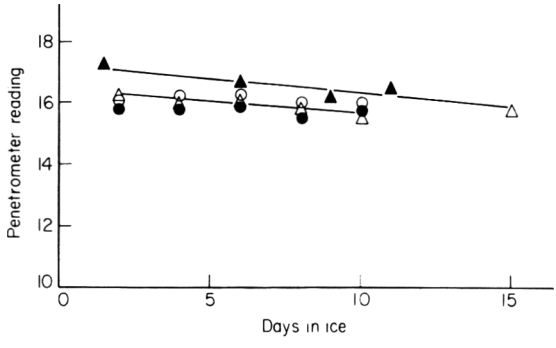

Figure 4. Penetrometer readings *vs* days in ice for southern blue whiting: ▲, ungutted, pre-spawning; ○, ungutted, post-spawning; ●, gutted, post-spawning; △, headed-and-gutted, post-spawning.

Results show a greater resistance in pre-spawning fish; however, as there may be differences from one catch to another, the data in Fig. 4 should be taken only as an objective measure of the tendency to lose texture during storage.

Numerical values of PR are similar to those obtained for hake (Lupín *et al.*, 1980).

Handling procedures

According to the COA scores, shown in Fig. 5, and as well as to TVB assays, shown in Fig. 2, gutting extended the keeping time by 1.5–2 days.

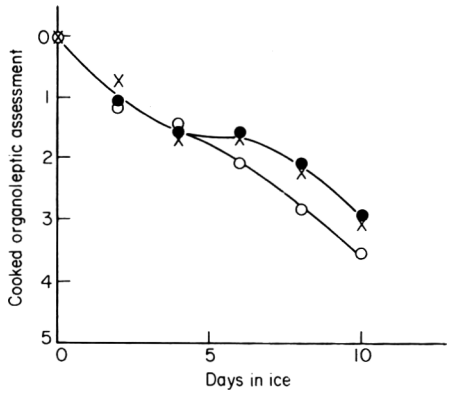

Figure 5. Cooked organoleptic assessment of southern blue whiting post-spawning stored: ○, whole in ice; ×, gutted in ice; ●. headed-and-gutted in ice.

No meaningful variations were detected in pH values during experiments 4, 5 and 6. The steady pH value reached after *rigor mortis* development, usually called the ultimate pH value, was 6.85 to 6.90, and for all the samples it increased slowly to reach 7.05 to 7.10 after 10 days in storage.

Quality assessment by electronic means

Torrymeter readings for southern blue whiting stored in ice for both pre- and post-spawning specimens and for different processing conditions are presented in Fig. 6.

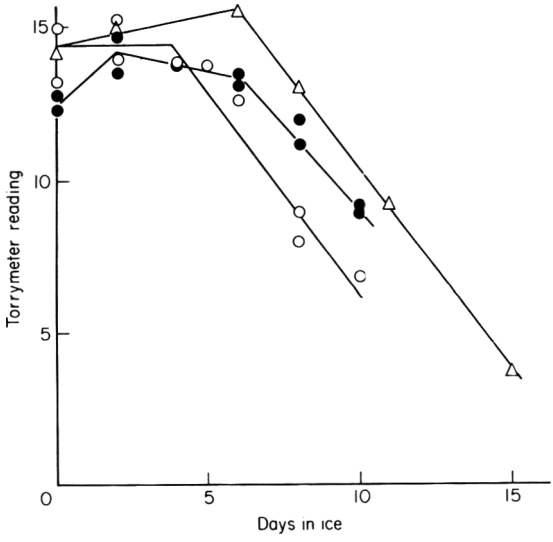

Figure 6. Torrymeter readings of southern blue whiting stored in ice: ○, whole, post-spawning, experiments Nos. 2 and 4; ●, headed-and-gutted and gutted post-spawning, experiments Nos. 5 and 6; △, whole, pre-spawning, experiment No. 3.

Although there is a strong initial influence, that includes the *rigor mortis* period, subsequent readings reflect not only differences due to processing, whole versus gutted and headed and gutted fish, but also to the biological conditions.

COA scores are plotted versus Torrymeter readings in Fig. 7. All values, except those for the *rigor mortis* period, fall on a straight line, the equation of which is:

$$COA = 5.623 - 0.301\ (TM) \quad (1)$$

with $r = -0.94$ (correlation coefficient).

ROA values and Torrymeter readings also correlate by a straight line

$$ROA = 3.956 - 0.214\ (TM) \quad (2)$$

with $r = -0.85$ (correlation coefficient).

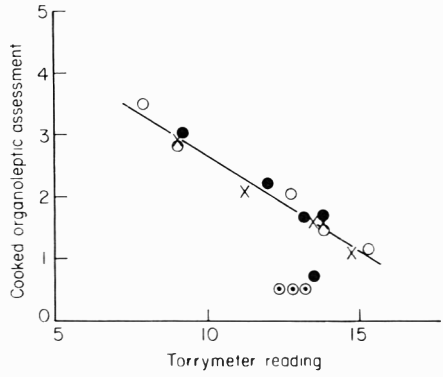

Figure 7. Torrymeter readings *vs* cooked organoleptic assessment of southern blue whiting stored in ice: ○, ungutted; ●, gutted; ×, headed-and-gutted; ⊙, in *rigor*.

Although the Fish Tester V measurements in experiment No. 1 showed the decline in quality during storage they did not show the differences between whole and headed-and-gutted fish evident from the organoleptic point of view. The readings were affected during the *rigor mortis* stage. They were greater than the pre-*rigor* values.

Discussion and conclusions

Even though southern blue whiting inhabit areas close to Patagonia and the islands of SW Atlantic only occasionally has it been caught by factory trawlers and by research ships carrying out studies in those waters.

Although it is difficult to discern from the published statistical data, it may be inferred that this species has been subjected, to some extent, to commercial exploitation. The southern blue whiting caught off the coasts of New Zealand by Soviet ships was over 40 000 t/year in 1973–1974 (Anon., 1977).

There are contradictory opinions regarding the quality of the species. It was considered a good quality fish by Gershanovich & Lyubimova (1971), while Papajewski & Schreiber (1977) expressed less favourable opinions. Nevertheless there is no research work available on this subject.

Results shown in Figs. 1, 2 and 3 indicate a very strong biological influence on the keeping time in ice and, therefore on the quality of fresh southern blue whiting. This biological influence has also been reported for blue whiting *Micromesistius poutassou)* as one of the greatest in its type found in the Gadidae family (Whittle, Robertson & McDonald, 1979; Murray & Gibson, 1979).

Post-spawning fish are softer and with a greater tendency to loose water; in general they present gaping much earlier than pre-spawning fish. The peritoneum is not resistant under these conditions and is easily torn even in 'just-caught' specimens. Yellowish discolouration occurs in flesh close to the abdomen of whole fish stored in ice after 24–48 hr. All of these changes and some additional ones considered in the organoleptic assessments took longer or did not occur at all in the keeping time assays with pre-spawning fish.

The presence of parasites does not seem to be the main cause for the observed changes. Although there is no literature on the parasites that affect southern blue whiting, there are two types of practical interest: Kudoa spp. in flesh and nematodes in the abdominal cavity, mainly in the liver. Kudoa spp. being the most usual.

Parasitism by Kudoa spp. reached 96% of the specimens used in experiment No. 1. The same determination by candling resulted in astonishing low percentages, less than 10%, in experiments Nos. 2 and 3.

The difference in the degree of parasitism between experiment No. 1 and experiments Nos. 2 and 3 can be due not only to the fish itself but also to the assessment method. In general the candling method gives lower counts than the slicing method. The candling method in itself can give different counts if measurement is made on normal or defatted fillet (fillet without the sub-cutaneous fat layer). The defatted fillet tends to show higher counts; this is obviously due to the opacity of the fat layer. In candling the very little cysts are not detected, and the operator's vision also affects the results.

Nevertheless the difference in this case is important because counts by candling on other samples of *M. australis* showed higher figures (50% more).

Even though samples lost texture during storage 'milkyness' was not observed in any of the experiments. This loss in texture was related to gaping and loss of water and not to regions with protein hydrolysis, not even in individuals having parasites of the Kudoa spp. type.

According to our observations southern blue whiting, and also Patagonian hake, show cysts with thick walls constituted by connective tissue around the parasite cluster. This wall seems to avoid noticeable protein hydrolysis of the surrounding tissue during the normal keeping time for edibility. Certainly the Kudoa spp. infestation, remains an aesthetic problem, because the cysts, if large, can be easily seen.

Although the abdominal content seems to have a great influence on the keeping time, nematodes were not detected in the flesh during the experiments with ungutted fish. Occasionally, specimens easily recognized at first sight as being heavily infested, appeared in the catches. Such fish were not used in the experiments.

Results of TVB and COA against days in ice presented in Figs 2 and 4 imply that when fish is in its most unfavourable biological condition storage can be prolonged for 2 days by gutting.

Particularly important is the information on RV (Fig. 3) which encompasses the measure of structural changes and protein extractability, and has been used to evaluate changes in frozen fish during storage.

In general RV was expected to remain almost constant during storage in ice on fresh fish. However, RV was found to diminish in Patagonian hake *(M. hubbsi)* stored in ice, specially in the post-spawning samples (Crupkin *et al.*, 1979).

A detailed analysis of experimental and seasonal conditions showed that for hake, RV could indeed be employed to identify biological quality (Barassi *et al.*, 1980). During ice storage RV remains practically constant when fish is in its best biological condition but it falls drastically when it is post-spawning, i.e. in its worst biological condition.

This same effect was observed in southern blue whiting as can be seen in Fig. 3. The fall is much more pronounced than for hake.

If RV is accepted to be a good indicator of changes during storage of frozen fish it would imply that post-spawning southern blue whiting can stand only very short times of frozen storage, even when frozen at sea.

At the same time it is possible that the most unfavourable comments on the quality of southern blue whiting are mainly based on observations made on catches in late spring and summer, which is when this species is in its worst biological condition.

After the first few days of storage in ice, the TM was capable of differentiating between whole and headed-and-gutted fish from the same batch. The TM readings for headed-and-gutted were higher than for whole fish. In the same way the TM readings for whole pre-spawning fish, were higher than the readings for whole post-spawning fish with the same storage time. These results were in accordance with the organoleptic and TVB findings.

The correlation between COA scores TM readings is better than TM readings against days in ice, except for specimens in *rigor mortis*. Thus, for practical purposes, it is advisable to use the correlation between COA scores and TM, bearing in mind that both biological quality and processing conditions influence the reading in the expected way. The capacity of GR Torreymeter to detect biological quality had been previously mentioned for hake (Lupín *et al.*, 1980).

In general, accepting the results from COA, ROA and TVB assays, southern blue whiting can be preserved in ice and still be fit for human consumption for the following periods: (a) 11 to 12 days for whole, pre-spawning fish; (b) 6 days

for whole, post-spawning fish; (c) 8 days for G or HG post-spawning fish.

However, whatever the evaluation method employed, post-spawning fish quickly lost quality in the first few days of storage and so, under these conditions, it is highly improbable that it can be used in the manufacture of frozen products.

Karsti (1971) found that whole blue whiting could be kept in ice for up to 7 days; Dagbjartsson (1975) stated that fish with full stomachs could be kept for no longer than two days, while those with empty stomachs lasted for up to a week. According to the most recent and complete paper by Smith *et al.* (1979) fish can be kept in fresh storage for periods longer than those previously mentioned, depending on the handling and the biological condition. Gutting would not affect the acceptability of blue whiting (Smith *et al,* 1979) and the nematodes present (Anisakis spp.) would not invade the muscle in the first 2 days of storage in ice (Bussmann, 1977) although they could do so in longer storage periods (Torry Research Station, White Fish Authority, 1976).

Unlike other species that show a rapid increase in pH as the fish decays, southern blue whiting presented a very slight variation. Similar results were found for blue whiting kept at ambient temperature (Bussmann, 1977).

Although TVB values can be related to organoleptic assessment of post-spawning fish, they cannot be related to pre-spawning fish and the limit amount of 30 mg N/100 g sample is not reached even when the fish is considered unsuitable for human consumption from the organoleptic point of view.

More experiments are required to evaluate the applicability of TVB as an objective index of quality, or to decide if trimethylamine (TMA) or dimethylamine (DMA) tests, as done by Smith *et al.* (1979), for blue whiting are more appropriate.

Although there are similarities between the behaviour of both *Micromesistius* species, specially regarding the seasonal variations, they differ in their keeping times, perhaps as a result of the different environments in which they live and, eventually, from differences existing between the species themselves.

The duration and development of *rigor mortis* in ore-spawning southern blue whiting are similar to those occurring in other species of white fish. No *rigor* was observed in the most exhausted individuals of post-spawning catches and in those fish largely infected by nematodes.

Finally, it is interesting to point out that, unlike blue whiting which must be handled and processed as a small fish, and for which special filleting machines had to be developed (Torry Research Station/White Fish Authority, 1976), southern blue whiting can be handled in the usual processing lines for white fish. According to Papajewski & Schreiber (1977) the Baader 38 needed only minor modifications to fillet this species. For specimens in the 45–60 cm range the yield of fillets skin on, when using a Baader 189, is 38–40% of the original weight (Lupín *et al.*, 1976). These results suggest that the usual equipment used for the processing of cod and hake can also be used for the processing of southern blue whiting.

Even though further research is needed, data presented in this work may be

useful in the development of the commercial exploitation of this species, one of the potential resources of white fish remaining in the world.

Acknowledgments

We wish to thank the authorities and crew of the research ships WALTHER HERWIG and PROFESSOR SIEDLECKI for their valuable help while on board. The experiments performed on board the RS WALTHER HERWIG were included in the Argentinean-German agreement for fishery research in 1978.

The work was partly supported by the Comision de Investigaciones Científicas of the Buenos Aires Province and the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina.

Thanks are given to H. O. Otero and J. D. Ciechomski, marine biologists, who helped us to identify the biological condition of southern blue whiting, and to M. Stelli for helping in experiment No. 1.

References

Anon. (1977) *Fishing News Int.* **16**, (4), 22.

Barassi, C.A., Crupkin, M., Martone, C. & Trucco, R.E. (1980) (unpublished paper).

Bellisio, N.B., López, R.B. & Torno, A. (1979) *Peces Marinos Patagónicos*. Subsecretaría de Pesca Argentina.

Bussmann, B. (1977) *Infn Fischw.* **24**, 77.

Crupkin, M., Barassi, C.A., Martone, C. & Trucco, R.E. (1979) *J. Sci. Fd Agric.* **30**, 911.

da Encarnaçao, J.D. (1974) Fisheries Development Protect FAO-Argentine. Tech. Co. No. 39.

Dagbjartsson, B. (1975) *J. Fish. Res. Board Can.* **32**, 747–751.

Gershanovich, D.E. & Lyubimova, T.G. (1971) *VNIRO Proceedings (USSR)*, Vol. **LXXIX**, 21.

Giannini, D.H., Davidovich, L.A., Lupín, H.M. (1979) *Rev. Agroq. Tec. Alimentos (Spain)* **19**, 55.

Hennings, C. (1965) *The Technology of Fish Utilization*, pp. 158–61 (ed. by R. Kreuzer). Fishing News (Books) Ltd., Farneham.

Inada, T. & Nakamura, I. (1975). *Far Seas Fish. Res. Lab. Bull.* (Shimizu, Japan) **13**, 1.

Karsti, O. (1974) *Fiskeridirektorates, Teknisk-Kemiske Forsoksinstitute (Norway), Annual Report 1973.*

Kerstan, M. (1979) *Infn. Fischw.* **26**, 167.

Kerstan, M. (1980) *Infn. Fischw.* **27**, 10.

Lupín, H.M., Stelli, M. Kogan, E. & Soulé, C.L. (1976) *Boletín Cámara Marp. de Ind. del Pescado (Argentine)* **91**, 1.

Lupín, H.M., Giannini, D.H., Soulé, C.L., Davidovich, L.A. & Boeri, R.L. (1980) *J. Fd Technol.* **15**, 285.

Maucorps, A. & Fontaine, B. (1979) *Science et Pêche*, 294.

Murray, C.K. & Gibson, D.M. (1980) *Advances in Fish Science and Technology*, pp. 503–507 (ed. by J.J. Connell). Fishing News (Books) Ltd., Farneham.

Norman, J.R. (1937) Coast fishes. Part II. The Patagonian region. *Discovery Reports*, **23**, 1, i.

Otero, H. (1976) *Physis (Secc. A)* **35** (91), 155.

Papajewski, H. & Schreiber, W. (1977) *Infn Fischw.* **24**, 114.

Shubnikov, D.A., Permitin, J.E. & Voznaj, S.P. (1969) *VNIRO Proceedings (USSR)*, Vol. **LXVI**, 299.

Smith, J.M., Hardy, R., Thompson, A.B., Young, K.W. & Parsons, E. (1980) *Advances in Fish Science and Technology*, pp. 299–303 (ed. by J.J. Connell). Fishing News (Books) Ltd., Farneham.

Torry Research Station/White Fish Authority (1976) *Machine Filleting and Processing of Blue Whiting*. TRS/WFA, Stornoway, U.K.

Weiss, G. (1974) *Physis. (Secc. A)* **33** (87), 537.

Whittle, K.J., Robertson, I. & McDonald, I. (1980) *Advances in Fish Science and Technology*, pp. 378–387. Fishing News (Books) Ltd., Farneham.

(Received 22 August 1980)

J. Fd Technol. (1981) **16**, 199–212

Behaviour of the mechanically separated flesh of some common fish species of the Mexican shrimp by-catch during storage at −20°C

M. A. TABLEROS* AND R. H. YOUNG†

Summary

Deboned minces prepared from fish species predominant in the Mexican shrimp by-catch were examined with respect to their stability during storage for 10 months at −20°C. Reductions in the extractability of salt-soluble proteins occurred to varying levels in all samples, particularly in the initial storage phase. These changes were shown to influence the textural characteristics of the minces, albeit to a minimal extent. Considerable variability in the colour of minces from different species was observed and a washing procedure which effectively removes dark pigments is described. In general, the acceptability of the products was maintained throughout the storage period. It is thus concluded that deboning and the freezing of the resultant minces could offer a potential means of processing currently wasted by-catch fish into human food.

Introduction

The shrimp by-catch, or those fish and other marine organisms captured incidentally during shrimping operations in Mexico and other regions, represents a significant resource which is presently wasted. The need to utilize the shrimp by-catch to satisfy the growing demand for fishery products has often been emphasized (FAO, 1975; Krone, 1979). This has led to the application of processing techniques to the by-catch and a salt/fish product for human consumption has recently been developed (Young *et al.*, 1979).

The wide variety of extremely small demersal fish in the by-catch obviously poses problems for conventional distribution and use. However, the development of mechanical deboning techniques now permits efficient utilization of the flesh of fish which were hitherto underutilized due to their

Authors' addresses: *Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM), Apartado Postal 484, Guaymas, Sonora, Mexico and †Tropical Products Institute, 56/62 Gray's Inn Road, London WC1X 8LU.

0022–1163/81/0400–0199 $02.00

small size or unacceptable appearance. Subsequent freezing in blocks should play an important future role in the marketing of deboned fish minces from by-catch, both for home consumption or for export. For example, in the United States alone, it is estimated that the requirement for frozen fish blocks in 1985 will be about 270 000 tonnes greater than in 1974 (FAO, 1975). Since the fish species conventionally utilized are near or beyond their maximum yields, future supplies of frozen blocks can only be increased by exploiting minces prepared from alternative species. In Mexico, where there is an existing infrastructure for the distribution of frozen fishery products, the freezing of minced blocks may also have potential in the development of new fishery products for the home market.

To support the current interest in this area, there is a need for more information on factors which may affect the stability, and hence the acceptability, of different fish minces during frozen storage. The disruption of the fish flesh by mechanical separation encourages a variety of biochemical reactions to occur at rates greater than those observed in whole or filleted fish. Such reactions are caused by the increased access of oxygen to the system, the spreading of bacteria, the mixing of intracellular and extracellular components and the release of enzymes. In the frozen state, certain changes promoted by these factors have been shown to influence the eating quality of the mince. For instance, the lipids of frozen fish can be hydrolyzed by lipases and phospholipases to yield free fatty acids which are more susceptible to oxidation than the parent compounds (Bremner, 1977). Thus, the development of rancidity in frozen minced fish may be a particular problem.

In some species, the osmoregulatory compound trimethylamine oxide (TMAO) may be converted enzymatically during frozen storage to dimethylamine (DMA) and formaldehyde (FA) (Amano & Yamada, 1964; Tokunaga, 1964; Dyer & Hiltz, 1974). The formation of these compounds in the fish flesh has been shown to be related to a loss of extractability of the muscle protein (Castell, 1971; Castell, Smith & Dyer, 1973). It is thought that complexing of the FA with proteins is the major causative factor of protein aggregation in gadoid species, such as Alaska pollock and red hake (Dyer, 1973). Decreases in protein extractability in fish muscle are associated with increased toughness when cooked and a consequent decline in product acceptability. Moreover, there is a reduction in the water-holding capacity (WHC) of the muscle which may promote difficulties in secondary processing, such as the preparation of fish sticks.

In addition to the effects of FA, other compounds such as free fatty acids and malonaldehyde (a fat oxidation product) may also render fish myofibrillar proteins insoluble (Sikorski, Olley & Kostuch, 1976). These compounds encourage protein cross-linking reactions, the end result being textural changes, loss of WHC, poorer manufacturing properties and development of off-flavours and aromas.

Since the fish species predominant in the shrimp by-catch and other underutilized resources may ultimately be used to some extent in frozen minced

products, it is important to provide data on their susceptibility to the changes described above. The present article reports studies recently undertaken in Mexico on a variety of common shrimp by-catch species.

Materials and methods

Raw materials

The fish studied included*: orangemouth corvina *(Cynoscion xanthulus)*, Gulf croaker *(Micropogonias altipinnis)*. bronzestriped grunt *(Orthopristis reddingi)* and various species of flatfishes (Bothidae).

These were small fish (maximum length 20 cm) obtained from commercial shrimping vessels operating in the Gulf of California and immediately stored in ice for transfer to the laboratory. The period during which the fish were kept in ice varied between 1 and 2 days.

Preparation of fish

Fish were beheaded and eviscerated manually. Particular care was taken to remove the swim bladder, kidney tissue and excess blood from the abdominal cavity. This was aided by scrubbing under a continuous flow of water. Samples were thoroughly washed and stored in ice ready for deboning.

Deboning and freezing

Elimination of the fish bones and skin was effected using a Paoli model 19 automatic meat and bone separator. The distance between the cylinder and the pressure plate of the separator was adjusted to 0.008 cm. Deboned flesh was packed in rectangular metal freezing trays and plate frozen at −40°C. The frozen flesh was removed from the trays and cut into rectangular portions (of approximately 60 g in weight) using a band saw. Portions were wrapped individually in aluminium foil, labelled and stored in polyethylene bags at −20°C.

Washing procedure

A portion of the mince recovered from Gulf croaker was washed by stirring with distilled water in a stainless steel tank previously lined with a cheesecloth.

*In Mexico, the common fish names are as follows: corvina *(C. xanthulus)*, chano *(M. altipinnis)*, rayadillo *(O. reddingi)* and lenguados (Bothidae).

Washing was carried out in two stages on the material recovered from the mincing section of the Paoli deboner (i.e. prior to deboning). This was found to facilitate washing and, in particular, excessive losses of solids during the procedure were avoided. Throughout washing the temperature was maintained at 3±2°C by the addition of ice. In the first washing stage, the ratio of mince to water was 1:2 and in the second stage equal parts of mince and water were used (taking into account the weight of added ice). Each stage was of 10 min duration and the water/mince mixture was stirred gently but continuously. Between the two washing stages and after the final stage, excess water was expressed by squeezing manually through the cheesecloth.

Analytical determinations

Analyses were carried out in triplicate on each sample at the beginning of the trial and then at monthly intervals throughout the storage period.

Extractable protein nitrogen (EPN) was determined by applying the biuret procedure (Snow, 1950) to the centrifuged supernatant of an extract prepared at 0–3°C by blending 11.25 g of the sample with 214 ml of an iced saline solution (mixture equivalent to 0.719 M NaCl; 0.02 M $NaHCO_3$; Sorensen, 1976). Extraction of the sample was carried out in a two-speed Waring blender for 1 min at slow speed followed by 2 min at maximum speed and extracts were centrifuged at 3500 *g* for 30 min.

Water holding capacity (WHC) was measured using an adaptation of the method of Wierbicki, Kunkle & Deatherage (1957). A 250-ml plastic cup with 2-mm diameter holes drilled in the base was supported in a second similar plastic cup. The distance between the upper and lower cap bases (due to the conical shape of the cups) was approximately 2.5 cm. Samples of 50 g of fish mince were allowed to thaw at room temperature in the upper cup for 3 hr. The amount of free liquid which had drained into the lower cup during this time was weighed. The cups and samples were then centrifuged at 800 *g* for 5 min and the final amount of liquid obtained was weighed to give a value for total expressible fluid.

Dimethylamine (DMA) was determined using the Cu-dithiocarbamate method (Dyer & Mounsey, 1945). Extracts were made by blending 25 g of sample in a mixture of 25 ml distilled water and 50 ml of 7.5% (v/v) trichloracetic acid (TCA). The TCA homogenates were allowed to stand for 30 min at 2–4°C and then filtered through Whatman No. 42 filter paper.

The oxidation of fats was estimated using 2-thiobarbituric acid reagent according to the modified method of Yu & Sinnhuber (1966). Extracts were prepared by blending 20 g of deboned flesh with 40 ml of 7.5% TCA containing 0.1% propyl gallate and 0.1% disodium-EDTA (Vyncke, 1970). Results are expressed as TBA numbers (mg malonaldehyde/kg minced flesh).

Total protein (N × 6.25) contents of the fish minces and waste water from the washing procedure were determined by the microkjeldahl method (Lillevick, 1970).

Lipid, water and ash contents of the minces were determined using the procedures described by the AOAC (1975).

Organoleptic evaluation

In order to define the textural characteristics of the samples, taste panel assessment was carried out employing a texture profile (Fig. 1) modified from that of Sorensen (1976). The profile is divided into three sections. Firstly, the initial mouthfeel sensation is defined in terms of resilience and hardness on 5-point hedonic scales. The second part of the scheme is designed to evaluate the particle characteristics of the sample when chewed. In both these sections, no attempt is made to assess the acceptibility of the product. However, panellists were also asked to separately judge the colour and flavour of the samples using 5-point hedonic scales. Scores below 3 were considered acceptable for colour whilst scores above 2 were acceptable for flavour.

Ten panellists selected from ITESM staff accustomed to consuming fish carried out the tests at monthly intervals. Samples were thawed for 3 hours at ambient temperature, cooked for 30 min in plastic bags immersed in boiling water and served hot.

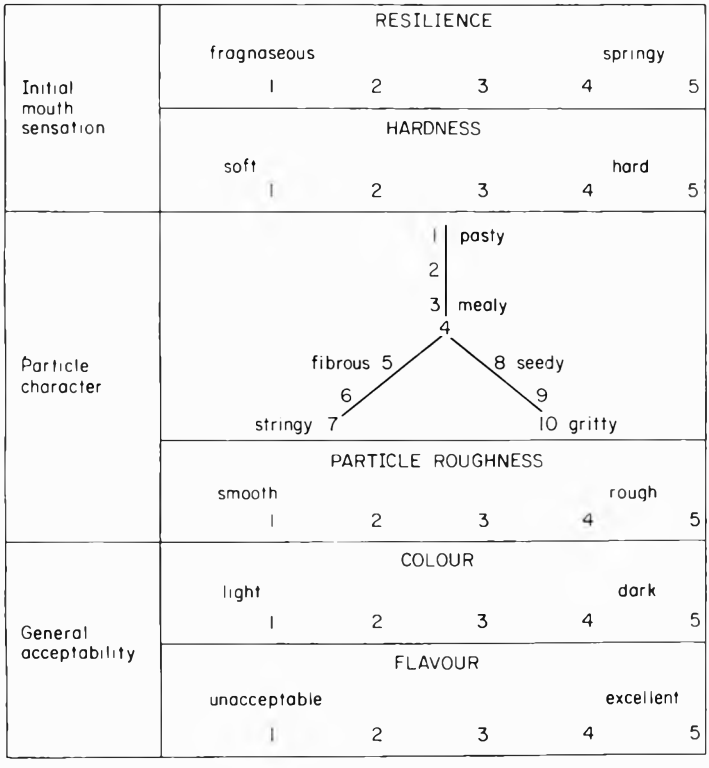

Figure 1. Texture profile and acceptability ratings used for taste panel evaluation of cooked minced fish.

Results

EPN

Figure 2 demonstrates the changes in EPN in the flesh of the fish species investigated during 10 months' storage at −20°C. The equivalent for the washed sample is also included. There was a gradual reduction in EPN for all species although this was more marked in the samples of flatfishes (Bothidae). The washed mince showed a minimal change in EPN throughout the storage period. The range of EPN values encountered and the extent of the reduction in EPN were found to vary between samples from different species.

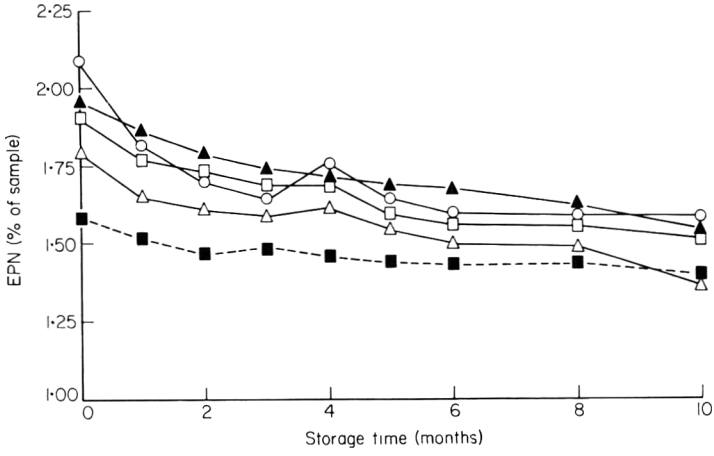

Figure 2. The effect of storage time at −20°C on the extractable protein nitrogen of the minced flesh of shrimp by-catch fish. ▲, Orangemouth corvina; ○, flatfishes; △, bronzestriped grunt; □, Gulf croaker; ■, washed Gulf croaker.

WHC

The variation in WHC of the frozen minces is indicated in Fig. 3. Gradual losses of WHC occurred throughout the storage period and these appeared to correspond with the results for EPN. In this case, the change in WHC of the mince from orangemouth corvina was especially notable.

DMA

Table 1 shows the concentrations of DMA in the minced flesh of the various species at different stages of frozen storage. The maximum level of DMA found was 19.5 μmol/100 g of sample.

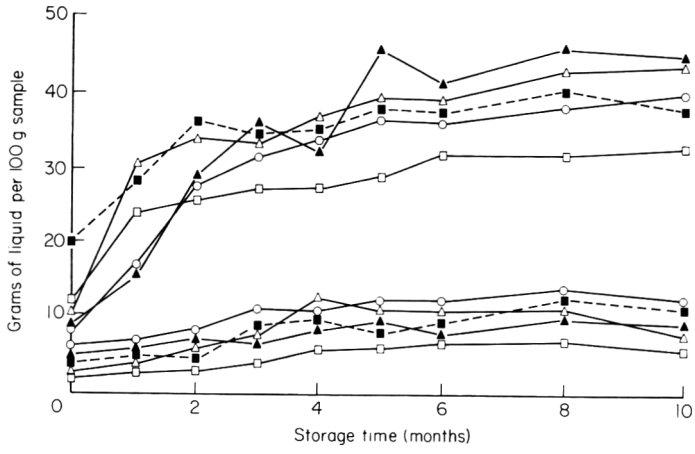

Figure 3. The effect of storage time at −20°C on the free liquid (lower plots) and expressible fluid (upper plots) released from the minced flesh of shrimp by-catch fish. ▲, orangemouth corvina; ○, flatfishes; △, bronzestriped grunt; □, Gulf croaker; ■, washed Gulf croaker.

Table 1. Variations in the concentration of dimethylamine in the minced flesh of shrimp by-catch fish during storage at −20°C

Storage time (months)	DMA (μmol/100g)				
	Corvina	Flatfishes	Grunt	Croaker	Washed croaker
0	3.5	6.4	10.8	8.4	5.5
2	4.4	7.8	19.5	17.4	7.0
4	8.5	9.2	16.8	17.6	7.2
6	11.6	14.9	19.5	18.0	7.0
8	10.9	13.0	17.5	17.6	6.5
10	11.7	14.8	19.5	17.8	8.0

Fat oxidation

Variations in TBA no. (mg malonaldehyde/kg sample) for the fish minces during 5 months' frozen storage are demonstrated in Fig. 4. Initial values tended to be relatively high (1–2 mg malonadehyde/kg) and continued to increase gradually during the initial three months of storage. With the exception of that from Gulf croaker, however, reductions in measured malonaldehyde were observed in the minces after 3–4 months of frozen storage.

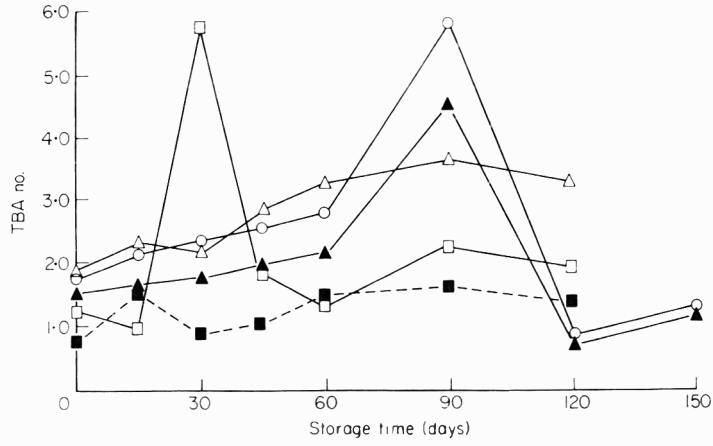

Figure 4. The effect of storage time at −20°C on lipid oxidation, as measured by TBA no., in the minced flesh of shrimp by-catch fish. ▲, Orangemouth corvina; ○, flatfishes; △, bronzestriped grunt; □, Gulf croaker; ■, washed Gulf croaker.

Texture

Changes in texture are shown in Fig. 5. In general, relatively little variation was seen during the first 3 months' storage. Subsequently, the initial mouthfeel of the minces tended towards hardness and the particle roughness increased. This effect appeared more marked in the minces prepared from orangemouth corvina and flatfishes than in the other samples. The washed mince was comparatively stable with respect to textural changes.

Colour and flavour

The variations in the colour of minces prepared from the different species may be appreciated from the photograph (Fig. 6). Minced flesh from bronze striped grunt was particularly dark whilst those of orangemouth corvina and flatfishes were acceptably light in colour. The flesh of the latter two groups, however, tended to darken during frozen storage (Fig. 5). The washing procedure described appreciably lightened the colour of the minced flesh from Gulf croaker as may be seen from Figs 5 and 7.

The flavour of all minces was considered acceptable throughout the storage period (Fig. 5), although flavour losses occurred in the flesh of orangemouth corvina after 4 months' storage. Flavour losses were also apparent in the washed mince (Fig. 5). However, in general, relatively little variation in flavour was detected.

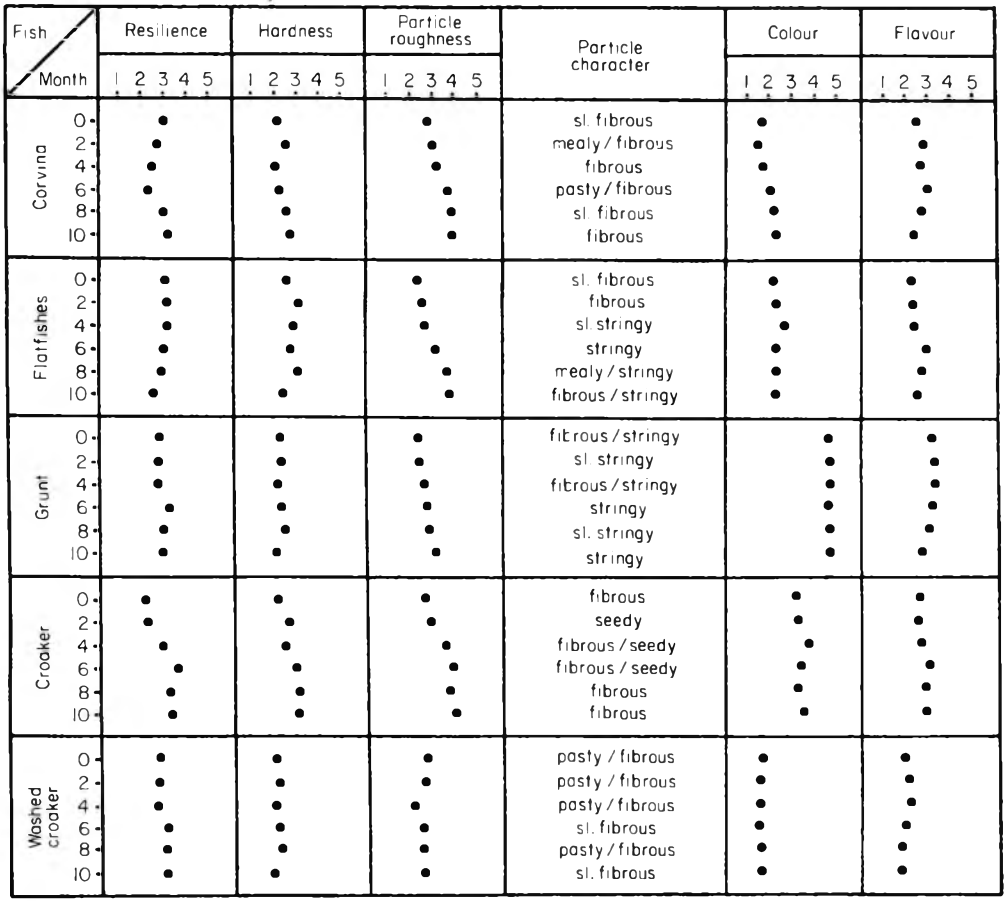

Figure 5. The effect of storage time at −20°C on the textural characteristics, colour and flavour of the minced flesh of shrimp by-catch fish (see Fig. 1 for key).

Table 2. Proximate analyses of minced fish flesh and waste water from washing procedure

Fish	Protein (g/100 g)	Fat (g/100 g)	Moisture (g/100 g)	Ash (g/100 g)
Corvina	18.8	1.9	81.0	1.6
Flatfishes	19.0	1.2	84.0	1.8
Grunt	18.1	1.5	83.0	1.9
Croaker	18.6	1.3	84.0	1.8
Washed croaker	13.2	0.8	89.0	1.5
Wash water I	3.6	0.5	—	—
Wash water II	1.6	—	—	—

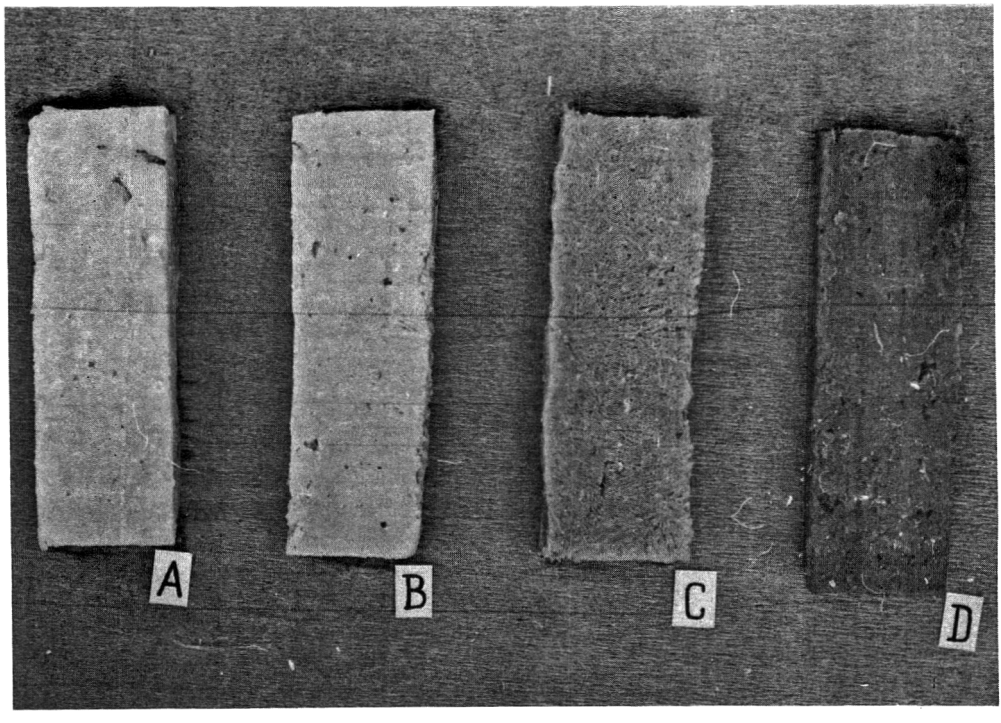

Figure 6. Minces prepared from different shrimp by-catch fish showing variations in colour. (A), flatfishes; (B) orangemouth corvina; (C) Gulf croaker; (D) bronze-striped grunt.

Proximate composition of minces

The results of proximate analyses for fish minces and effluent from the washing process are given in Table 2. Washing reduced the crude protein and fat contents of mince from Gulf croaker from 18.6 g/100 g to 13.2 g/100 g and 1.3 g/100 g to 0.8 g/100 g respectively.

Discussion

It is well known that fish muscle is susceptible to biochemical changes during frozen storage and that these modifications subsequently influence texture and sensory properties (Dyer & Dingle, 1961; Awad, Powrie & Fennema, 1969). Such changes are particularly marked in frozen minced fish due to the greater surface areas available for reaction. A variety of possible mechanisms exists for causing protein aggregations in fish muscle when stored at low temperatures. These phenomena may in turn alter the texture of fish minces and lower their acceptability for food uses.

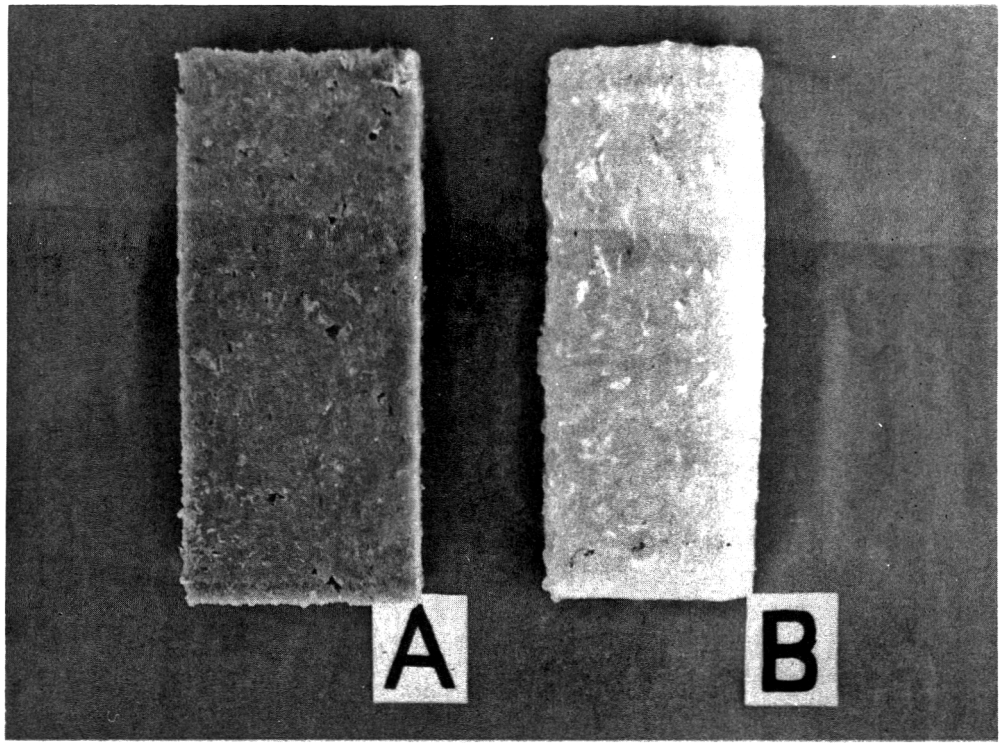

Figure 7. The effect of washing on the colour of the minced flesh of Gulf croaker. (A) normal sample; (B) washed sample.

The data of Fig. 2 indicate that the EPN for all four types of minces investigated was reduced to varying extents over the frozen storage period. The effect was most noticeable during the initial three months of storage after which the losses in EPN were comparatively small.

The WHC of the fish muscle proteins also decreased (Fig. 3). The production of DMA and FA was negligible in the minced flesh of all the species investigated, indicating that this reaction was not responsible for any decline in EPN or WHC. Indeed, if these compounds had appeared in the fish flesh at more significant levels, then it is likely that much greater losses in EPN would have been observed (Dingle & Hines, 1975; Dingle, Keith & Lall, 1977).

The Paoli deboning system was found to provide an efficient means of separating bones and scales from the flesh of the small by-catch fish. Nevertheless, the flesh is subjected to a relatively severe mechanical action during the process. It may be that this treatment, together with associated temperature rises, rendered the muscle proteins more susceptible to aggregation during the initial freezing period, with a consequent reduction in the measured EPN. Moreover, the fish flesh is exposed to the metallic surfaces of the deboner for relatively extended time periods during processing. This, in conjunction with the appreciable mincing of the flesh, may rapidly promote the oxidation of fats.

Thus, although the fat contents of the muscle of all the species studied were low (Table 2), values for malonaldehyde were higher than expected, even at zero freezing time (Fig. 4). Malonaldehyde may also contribute to increased protein aggregation in fish muscle and it is feasible that this was so for the samples currently examined. The fact that malonaldehyde values showed an apparent decrease in the minced flesh of some species at certain stages during frozen storage further suggests that linkage of the compound with the muscle protein hindered its extraction under the conditions employed.

The susceptibility of the fish flesh to the changes caused by deboning and freezing appears to differ between the species investigated since variations occurred in both the initial EPN values and the extent of reduction in EPN (Fig. 2). For instance, the muscle proteins of flatfishes and orangemouth corvina retained a higher extractability than those of bronzestriped grunt. In general, throughout the frozen storage period, the extractable protein of all minces was maintained above the 8.5–11.0 g/100 g flesh suggested by Sorensen (1976) as the minimium level required to ensure satisfactory binding properties in heat-gelled products. This is particularly important if the preparation of items such as breaded fish sticks is envisaged. Adequate formation of such products from the minces tested in this study has been confirmed in practice (M.A. Tableros, unpublished data).

Despite the modifications in the characteristics of the fish muscle proteins which occurred during frozen storage, little alteration in the texture of the minced blocks was recorded. As indicated in Fig. 5, some variation in consistency and particle roughness was noted after 3–4 months' frozen storage, particularly in the minces prepared from orangemouth corvina and flatfishes. However, it was evident that, although the minces were generally acceptable, they possessed a rather granular texture and some particle roughness at the beginning of the frozen storage period. This again suggests that, in the present case, the deboning mechanism was more influential on product texture than freezing.

Although, during the preparation of all the minces, care was taken to remove visceral tissues and excess blood from the fish abdominal cavity, there was a notable variation in colour between minces from different species. Indeed, samples prepared from bronzestriped grunt were especially dark in colour. The effect was probably caused by contamination of the mince by pigments from the skin of the fish or by the presence of greater concentrations of haem compounds associated with the flesh of this species. The washing procedure adopted was found to substantially remove pigment from the minces and, thus, lighten their colour. Furthermore, washed mince appeared less susceptible to deteriorative changes during frozen storage. For example, EPN losses were minimal in the washed sample (Fig. 2) and the mince texture remained stable (Fig. 5). Nevertheless, washing did promote flavour losses and, in practice, may give rise to effluent problems due to the leaching of soluble solids, mainly protein, from the mince. For these reasons, Bremner (1978) surmises that the disadvantages of washing may outweigh the advantages. In the case of shrimp by-catch,

however, washing may provide a means of conferring some uniformity on the colour, flavour and texture of fish minces which may feasibly be produced from a heterogeneous mixture of species. Moreover, bronzestriped grunt, which produced the darker flesh, is one of the more common fish species found in Mexican shrimp by-catch (Young & Romero, 1979). Preliminary studies have indicated that washing has beneficial effects in stabilizing the appearance and organoleptic characteristics of breaded fish sticks fabricated from mixtures of small by-catch fish (M.A. Tableros, unpublished data). On the other hand, Mexican consumers are not familiar with such products and it is possible that darker coloured minces may find acceptability in the local market. Appropriate research is needed to assess the consumer's requirements in this respect.

Clearly, the shrimp by-catch fish examined in this study have potential as raw materials for the manufacture of frozen fish minces. This represents a further option for converting a presently wasted fishery resource into palatable food products for human consumption.

Acknowledgments

This work forms part of a joint research project between the Tropical Products Institute and the Mexican Fisheries Department as part of the technical cooperation programme between the Overseas Development Administration and the Government of Mexico. The authors gratefully acknowledge the provision of a Tropical Products Institute Extra Mural Contract to one of us (M.A.T.). We are also indebted to the Director and staff of the School of Marine and Food Sciences of the Monterrey Institute of Technology (ITESM) for the provision of the facilities required for the study.

References

Amano, K. & Yamada, K. (1964) *Bull. Jap. Soc. Sci. Fish.* **30,** 430.
AOAC (1975) *Official Methods of Analysis*, 12th edn. Association of Official Agricultural Chemists, Washington, D.C.
Awad, A., Powrie, W.D. & Fennema, O. (1969) *J. Fd Sci.* **34,** 1.
Bremner, H.A. (1977) *Fd Technol., Aust.* **29,** 89.
Bremner, H.A. (1978) *Fd Technol., Aust.* **30,** 393.
Castell, C.H. (1971) *J. Am. Oil Chem. Soc.* **48,** 645.
Castell, C.H., Smith, B. & Dyer, W.J. (1973) *J. Fish. Res. Board Can.* **30,** 1205.
Dingle, J.R. & Hines, J.A. (1975) *J. Fish. Res. Board Can.* **32,** 775.
Dingle, J.R., Keith, R.A. & Lall, B. (1977) *Can. Inst. Fd Sci. Technol. J.* **10,** 143.
Dyer, W.J. (1973) *Refrigeration.* **48,** 38.
Dyer, W.J. & Dingle, J.R. (1961) *Fish as Food*, Vol. I (ed. by G. Borgstrom), p. 275. Academic Press, New York.
Dyer, W.J. & Hiltz., D.F. (1974) *Halifax Lab. Fish. Mar. Serv. New Ser. Circ.* **45,** 5 p.
Dyer, W.J. & Mounsey, Y.A. (1945) *J. Fish. Res. Board Can.* **6,** 359.
FAO (1975) *FAO Fish. Rep.* No. 175, 47p.
Krone, W. (1979) *Fd Policy.* **4,** 259.

Lillevick, H.A. (1970) *Methods of Food Analysis*, 2nd edn. (ed. by M.A. Joslyn), p. 605. Academic Press, New York.
Sikorski, Z.E., Olley, J. & Kostuch, S. (1976) *Crit. Rev. Fd Sci. Nutr.* **8**, 97.
Snow, J.M. (1950) *J. Fish. Res. Board Can.* **7**, 594.
Sorensen, T. (1976) *The Production and Utilization of Mechanically Recovered Fish Flesh (Minced Fish)* (ed. by J.N. Keay), p. 56. Torry Research Station, Aberdeen.
Tokunaga, T. (1964) *Bull. Hokkaido Reg. Fish. Inst.* **29**, 108.
Vyncke, W. (1970) *Fette, Seifen, Anstrichm.* **72**, 1084.
Wierbicki, E., Kunkle, L.E., & Deatherage, F.E. (1957) *Fd Technol., Champaign.* **11**, 69.
Young, R.H. & Romero, J.M. (1979) *Trop. Sci.* **21**, (in press).
Young, R.H., Coria, E., Cruz, E. & Baldry, J. (1979) *J. Fd Technol.* **14**, 509.
Yu, T.C. & Sinnhuber, R.O. (1966) *J. Am. Oil Chem. Soc.* **44**, 256.

J. Fd Technol. (1981) **16**, 213–217

Technical note: Semolina BAPA-ase activity and its possible relationship to pasta cooking quality

LUCIANA PETRUZZELLI*, C. DELLA GATTA, P. DE LEO AND G. COLAPRICO

Introduction

It is known that semolina made from different wheat varieties produces spaghetti of widely different cooking quality. On the basis of chemical and physical parameters of wheat and semolina, it has been established that among the factors regarded as possibly responsible for spaghetti cooking quality, the protein content and gluten quality are two very important factors relating to pasta cooking quality (Matsuo, Bradley & Irvine, 1972; Dexter & Matsuo, 1977a, b; Matsuo & Irvine, 1970; Walsh & Gilles, 1971).

Wheat proteolytic enzymes are generally believed to be too low to affect the quality of doughs by alteration of gluten proteins. On the other hand, it is well known that proteolytic enzymes are poorly soluble in common buffers, difficult to extract (Wang & Grant, 1969) and able to change the physical characteristics of gluten proteins if present in small amounts (Kruger, 1971). It has been attempted also to relate BAPA-ase, the proteolytic enzyme hydrolyzing α-benzoyl-L-arginine-p-nitroanilide, and protease activities changes during wheat maturation to breadmaking quality of flour (Kruger, 1973).

In view of these considerations, it cannot be excluded that proteolytic enzymes could affect significantly the ability of semolina to produce high quality spaghetti by enzymatic breakdown of only a few peptide bonds of semolina proteins. Moreover, the literature does not provide any easily measurable characteristics of durum wheat varieties by which their pasta making quality could be assessed (Scarascia-Venezian, 1973; Fabriani, Quaglia & Maffei, 1975; Dexter & Matsuo, 1977a, b).

In our laboratory, studies have been undertaken to extract and measure semolina proteolytic enzymes. In the present paper, BAPA-ase activity in semolina from five Italian durum wheat varieties from two annual crops and with different spaghetti cooking quality have been examined. In addition, in order to obtain information on changes in the level of BAPA-ase during pasta

*Author's address: Germplasm Institute, National Research Council, Bari, Via Amendola 165/A, Italy.

0022–1163/81/0400–213 $02.00

processing, the enzymic activity has been determined in the same semolina samples mixed with 33% water and after storage at 37°C for 15 hr (mixed semolina).

Materials and methods

Five Italian durum wheat cultivars (Appulo, Capeiti, Cappelli, Creso and Valgerardo), grown in the same experimental field in Southern Italy (Castelluccio) during 1976 and 1978, were used for the study.

For the crops of both years the wheat was stored for 6 months (at 20°C and 65% r.h.) before milling. Thereafter 3-kg samples of each wheat were washed, tempered overnight and milled at 16.5% moisture in a Buhler laboratory mill for durum wheat (mod. 202) in conjunction with a laboratory purifier. Kjeldahl protein contents (N × 5.7) and wet gluten were determined according to the standard AACC (1961) procedures.

BAPA-ase activity determinaton

Samples (3 g) of semolina and/or mixed semolina from all the varieties were homogenized in three steps of 30 sec in a omni-mixer with 30 ml of cold sodium phosphate buffer (pH 7) containing 0.2 M NaCl, 1 mM EDTA, 5 mM B-mercapto ethanol. The resulting suspension was centrifuged for 20 min at 33 000 *g*. The supernatant, after filtration through two layers of cheesecloth, was used to measure the hydrolysis of benzoyl-DL-arginine-p-nitroanilide (BAPA-ase activity). An aliquot of each semolina extract (0.3 mg of proteins determined by biuret assay) was mixed with 1 ml of 2 mM BAPA in phosphate buffer (pH 7) and the volume made up to 2 ml with the above phosphate buffer.

The resulting mixture was incubated for 30 min at 37°C and the reaction was stopped by adding 1 ml of 1 N acetic acid. After centrifugation, the release of p-nitroanilide was estimated by measuring spectrophotometrically the increase in absorbance (Δ A) at 410 μm with respect to the control (reaction mixture stopped at zero time). Each experiment was repeated at least three times with two replicates. The standard deviation did not exceed $\pm 3\%$.

Spaghetti making

All semolina samples were premixed with tapwater (absorption was 33% for each variety) and made into spaghetti essentially as described by Matsuo *et el.* (1972). The spaghetti were dried for 36 hr at 42°C with a controlled decrease in relative humidity. In the cooking tests, the sphagetti (100 g) were cooked in boiling distilled water (1 litre) containing NaCl (1%) for about 12 min. For determination of their cooking quality, each sample of cooked spaghetti was subjected to organoleptic evaluation by three experienced tasters who measured stickiness and tenderness or firmness (Vallega, 1977).

Table 1. Quality data for semolina from 1976 crop wheats

Variety	Crude protein (%)	Wet gluten (%)	Extract protein		Specific BAPA-ase activity		Spaghetti cooking quality
			Semolina (mg/ml)	Mixed semolina (mg/ml)	Semolina (Δ A/mg prot)	Mixed semolina (Δ A/mg prot)	
Appulo	16.74	14.82	0.671	0.663	0.636	0.322	6.0
Capeiti	15.59	14.72	0.643	0.586	0.771	0.353	6.0
Cappelli	16.75	15.54	0.699	0.669	0.652	0.296	6.0
Creso	14.97	14.17	0.843	0.678	1.032	0.711	7.2
Valgerardo	16.93	15.72	0.647	0.640	0.750	0.345	6.5

Table 2. Quantity data for semolina from 1978 crop wheats

Variety	Crude protein (%)	Wet gluten (%)	Extract protein		Specific BAPA—ase activity		Spaghetti cooking quality
			Semolina (mg/ml)	Mixed semolina (mg/ml)	Semolina (ΔA/mg prot)	Mixed semolina (ΔA/mg prot)	
Appulo	16.02	14.06	0.984	0.779	0.581	0.360	3.0
Capeiti	13.45	12.80	0.910	0.816	0.654	0.445	8.0
Cappelli	15.60	15.28	0.874	0.872	0.702	0.325	7.5
Creso	14.65	13.98	0.876	0.807	1.306	1.049	9.5
Valgerardo	16.01	15.20	1.004	0.916	0.853	0.538	8.0

The stickiness is the index to evaluate how much cooked spaghetti is sticking; the firmness or tenderness is a measure of the extent to which the sample is firm, with 'good bite'. To both the parameters the tasters assigned a numerical value, from 1 (very much) to 5 (very little) for the first and from 1 (very poor) to 5 (very good) for the second. The two numbers were added up in order to have the overall spaghetti cooking quality data expressed on a scale from 1 to 10: 1–2 = very poor; 3–4 = poor; 5–6 = sufficient; 7–8 = good; 9–10 = very good. All the determinations were verified by repetition, usually several times.

Results and discussion

As reported in Tables 1 and 2, the protein and gluten content differ among the varieties of the crops of the same year and between the crops of the two years for the same variety. Nevertheless, all the values were higher in the 1976 crop than in that of 1978. Also the amount of protein extracted changes among the varieties of the same year, but the values were always 20–30% greater in the 1978 crop than in the 1976 crop.

After mixing, the protein recovery in each semolina extract becomes slightly lower, presumably due to protein denaturation. In respect to the BAPA-ase activity, the two Tables show clear differences in relationship to the variety or to year of growing and it is also clear that the Creso variety has the highest semolina peptidase activity of both years. After mixing, there is a proportional decrease of BAPA-ase activity in all the semolina samples so that Creso shows again the greatest enzymatic activity. At the same time, Creso is the semolina which presents the best overall quality in spaghetti cooking in both the years.

Moreover, if the data of BAPA-ase activity from the two Tables are considered together, a significant correlation (P=5%) is found between enzyme activity and cooking quality. The correlation coefficients are 0.69 for unmixed and 0.65 for mixed samples. These correlation coefficients are not high but it must be pointed out that the pasta cooking quality is judged by organoleptic tests and is related to many factors. The enzymic technique is rapid, easy and requires very small amounts of substrate. Further studies are in progress to ascertain whether this biochemical parameter can be a good tool to recognize in the early phases of the breeding work durum wheats producing high quality pasta.

References

AACC (1961) *Approved Methods of the AACC*. American Association of Cereal Chemists, St Paul, MN.

Dexter, J.E. & Matsuo, R.R. (1977a) *Can. j. Plant Sci.* **57**, 717.

Dexter, J.E. & Matsuo, R.R. (1977b) *Cereal Chem.* **54**, 4.

Fabriani, G., Quaglia, G.B. & Maffei, A., (1975) *Tecn. Molitoria.* **26**, 81.

Kruger, J.E. (1971) *Cereal Chem.* **48**, 121.

Kruger, J.E. (1973) *Cereal Chem.* **50**, 122.
Matsuo, R.R. & Irvine, G.N. (1970) *Cereal Chem.* **47**, 173.
Matsuo, R.R., Bradley, J.W. & Irvine, G.N. (1972) *Cereal Chem.* **49**, 707.
Scarascia-Venezian, M.E. (1973) *Genetics and Breeding of Durum Wheat.*
Vallega, J. (1977) *Inf. Agr.* **21**, 2669.
Walsh, D.E. & Gilles, K.A. (1971) *Cereal Chem.* **48**, 544.
Wang, C.C. & Grant, D.R. (1969) *Cereal Chem.* **46**, 537.

(Received 4 July 1980)

J. Fd Technol. (1981) **16**, 219–221

Technical note: A refinement of Ross's equation for predicting the water activity of non-electrolyte mixtures

C. FERRO FONTÁN* AND J. CHIRIFE†‡

The interest in water activity (a_w) control in intermediate moisture foods (IMF) has stimulated research into the prediction of the water activity in single and mixed electrolyte and non-electrolyte solutions (Ross, 1975; Sloan & Labuza, 1976; Chuang & Toledo, 1976; Benmergui, Ferro Fontán & Chirife, 1979; Ferro Fontán, Benmergui & Chirife, 1980; Chirife, Ferro Fontán & Benmergui, 1980). Ross (1975) developed a very simple equation for estimating the water activity of complex solutions which proved to be useful for most a_w predictions in the food area (Chuang & Toledo, 1976; Chirife, 1978; Chirife *et al.*, 1980). Ross (1975) assumed that interaction effects between different solutes cancel on the average, and with this condition the Gibbs-Duhem equation reduces to the simple expression

$$(a_w)_M = \pi_s (a_{w,s}) \tag{1}$$

Equation (1) says that the water activity of a complex solution $(a_w)_M$ becomes simply the product of the water activity values of the aqueous solutions of each s component when measured at the same molality as in the complex solution.

Chirife *et al.* (1980) extensively examined the validity of Ross's equation for mixtures of non-electrolytes or single electrolyte-non-electrolyte solutions relevant to a_w control in IMF and found a good agreement between predictions and reality.

It is the purpose of the present communication to propose a refinement of equation (1) which allows even more accurate predictions of a_w of non-electrolyte mixtures. The proposed equation is

$$(a_w)_M = \pi_s \left[a_{w,s}\,(\mathrm{m})\right]^{m_s/m} \tag{2}$$

where m_s is the molality of the s component and m is the total molality of the

Authors' address: *Departamento de Física and †Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Núñez, 1428 Buenos Aires, Argentina.

‡Member of Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

0022–1163/81/0400–0219 $02.00

Table 1. Comparison of the error in a_w prediction upon application of Ross's (1975) equation and the proposed modification for various ternary non-electrolyte mixtures (m: molality)

	m_1	m_2	a_w (obs.)	Percentage deviation on $(1 - a_{w\,obs.})$ Ross's (eqn. 1)	Ross's mod. (eqn. 2)	
(a) Sucrose (1)–glucose (2)	1.7391	1.8737	0.925	7.1	1.3	Stokes & Robinson (1966)
	2.3504	1.0996	0.926	4.1	1.3	"
(b) Sucrose (1)–sorbitol (2)	1.693	1.0593	0.943	3.5	0.0	Stokes & Robinson (1966)
	1.2683	1.5709	0.943	5.3	0.0	"
(c) Sucrose (1)–glycerol (2)	2.0405	1.3615	0.930	4.5	1.4	Stokes & Robinson (1966)
	1.5022	2.065	0.930	3.0	2.9	"
(d) Sucrose (1)–mannitol (2)	1.9707	1.0494	0.936	4.7	1.6	Robinson & Stokes (1961)
	4.9503	0.7051	0.863	2.2	0.7	"
	3.0949	0.6557	0.915	3.5	1.2	"
	2.5321	0.3874	0.936	1.6	0.8	"
	2.2820	0.6829	0.936	3.1	1.1	"
	5.1174	0.4889	0.863	2.2	0.5	"
(e) Sucrose (1)–urea (2)	3.6375	0.243	0.912	2.3	1.1	Ellerton & Dunlop (1966)
	4.2556	0.2843	0.895	1.9	1.4	"

mixture. The difference in equation (2) from that of Ross (1975) is that the water activity of each non-electrolyte must be taken at the total molality of the mixture and then raised to the m_i/m power.

The idea for this modification of Ross's equation was originated in a previous theoretical paper on a_w prediction in multicomponent electrolyte mixtures (Ferro Fontán *et al.*, 1980).

Literature experimental data are now utilized to test the validity of the proposed equation as compared to Ross's (1975) equation. The data utilized correspond to accurate isopiestic vapour pressure measurements obtained by workers in the physical-chemistry area which permit the expression of a_w values to 3 or 4 decimal figures. Solutes tested include combinations of sorbitol, glucose, glycerol, sucrose, mannitol and urea. The a_w data for single non-electrolyte solutions were obtained from the excellent compilation of Teng & Lenzi (1974).

Table 1 compares the error in a_w predictions (in the range most relevant to IMF) when using Ross's equation or the proposed modification (eqn. 2). The deviation between observed and calculated values is expressed on $(1 - a_w)$ which is more significant than the error in a_w itself since it gives directly the error in a_w lowering (Sangster & Lenzi, 1974). As it was shown previously (Chirife *et al.*, 1980) the error upon application of Ross's (1975) equation is small; however, it can be seen that the proposed equation is even more accurate. Although the accuracy of Ross's (1975) equation is sufficient for most requirements of a_w predictions in foods, the proposed refinement may be used for some special cases where an even better estimate is needed.

Acknowledgments

The authors acknowledge the financial support of the Secretaría de Estado de Ciencia y Tecnología de la República Argentina (Programa Nacional de Tecnología de Alimentos).

References

Benmergui, E.A., Ferro Fontán, C. & Chirife, J. (1979) *J. Fd Technol.* **14**, 625.
Chirife, J. (1978) *J. Fd Technol.* **13**, 417.
Chirife, J., Ferro Fontán, C. & Benmergui, E.A. (1980) *J. Fd Technol.* **15**, 59.
Chuang, L. & Toledo, R.T. (1976) *J. Fd Sci.* **41**, 922.
Ferro Fontán, C., Benmergui, E.A. & Chirife, J. (1980) *J. Fd Technol.* **15**, 47.
Ellerton, H.D. & Dunlop, P. (1966) *J. of phys. Chem.* **70**, 1831.
Robinson, R.A. & Stokes, R.H. (1961) *J. phys. Chem.* **65**, 1954.
Ross, K.D. (1975) *J. Fd Technol.* **13**, 715.
Sangster, J.A. & Lenzi, F. (1974) *Can. J. of chem. Engng.* **52**, 392.
Sloan, E.A. & Labuza, T.P. (1976) *J. Fd Sci.* **41**, 532.
Stokes, R.H. & Robinson, R.A. (1966) *J. of phys. Chem.* **70**, 2126.
Teng, T.T. & Lenzi, F. (1974) *Can. J. of chem. Engng.* **52**, 387.

(Received 1 September 1980)

J. Fd Technol. (1981) **16**, 223–228

Book reviews

The Science of Food. By M. Bennion.
San Francisco: Harper and Row, 1980. Pp. x + 598. ISBN 0 06 453532 0. $14.95.

The primary readership for this book is meant to be college and university students who have a good background knowledge of chemistry and who have already taken an introductory course in food science. The aim of the book is to examine the chemical and physical characteristics of food and the way in which preparation and processing operations affect these properties. This is done with the help of fairly frequent reference to relevant research publications, with the literature sources being given at the end of each chapter. The objective is to enable the student to obtain a basic understanding of the chemical and physical effects of preparation and processing and an appreciation of the role of research in increasing the body of knowledge in this area.

Thirty-two chapters are organized into seven sections. The first section 'Relationship of structure to quality in food' contains four chapters on evaluation of food quality; on chemical composition and physical structure; on pH and acidity; and on enzymes.

The next three chapters outline preservation by canning and by freezing.

Part III contains two chapters on chemical additives and food standards.

Part IV concerns carbohydrates in food systems and contains seven chapters. Part V, of five chapters, deals with gels, emulsions and foams in food systems, and it is within this section that fats and oils are considered. Part VI concerns proteins in food systems and also contains seven chapters and the final section, of four chapters, deals with baked flour products.

It can be seen that the subject matter of the book does not provide a complete text for food science, even within the terms of reference of the declared aims and objectives. Relatively few types of food processing operation have been considered in detail; for example neither UHT sterilization nor reverse osmosis are discussed.

A considerable use of the index is needed to obtain much of the information contained in this book. For example, a reader interested in the effect of heat treatment on the vitamin content of food will find some information in this book, but it is well scattered and dealt with on a commodity basis, the bulk of the information being found in the chapters on fruits and vegetables in Part IV and obviously relating to those commodities alone. In fact sixteen of the thirty-two chapters are devoted to specific commodities. Thus, for example, in Part IV ('Carbohydrates in food systems') chapters are found on sugars and candies,

frozen desserts, fruits, vegetables, cereal grains; in Part VI ('Proteins in food systems') are to be found chapters on eggs and egg products, milk and milk products, meat, poultry and fish, etc. This orientation would seem to make it extraordinarily difficult, if not impossible, for the student to gain a basic understanding of the fundamental chemical and physical aspects of the preparation and processing of foods.

The book offers a wealth of titbits of information to any reader, whether student or practising food scientist or technologist. This reviewer has learnt much about the strange behaviour of angel cake mixtures, for example. A well presented book, with clear and easily read typeface in a larger than usual page size (23 × 18 cm) which I much enjoyed reading, but it seems to make an ideal coffee-table book or Christmas or birthday present, rather than to be readily assimilable into any normal course in food science of food technology.

W.F. Harrigan

Polysaccharides in Food. Ed. by J. M. V. Blanshard and J. R. Mitchell. London: Butterworths, 1979. Pp. ix + 368. ISBN 0 408 10618 2. £19.50.

This collection of twenty-one original papers presented at the 27th Easter School 1978, held at Nottingham University provides a comprehensive text for both postgraduate students and research workers. The text reviews research from a variety of disciplines on the topic of the growing use of polysaccharides in food, providing broad coverage ranging from the basic structure of these macromolecules through their use in the food industry both from commercial and health aspects.

The book is systematically divided into six sections, the first of which comprises four chapters which deal with the fundamentals of polysaccharides, their synthesis, structure and solvation. Northcote's chapter on the biochemistry of polysaccharide synthesis in the plant cell is particularly worthy of mention, as it succinctly describes synthesis of polysaccharides by plant cells in a manner that is easily comprehensible to those interested readers whose discipline is not biochemistry.

Section two focuses on polysaccharide enzymes, their function and properties. In the light of recent research on the effect of raw starch on the mammalian intestine, Chapter Five by D.J. Manners is of particular pertinence. It not only describes the enzymology of starch hydrolysis, but also highlights some areas of uncertainty with regard to these hydrolyses that may have some bearing on the aforementioned phenomenon. Starch in food processing is the subject of section three which emphasizes the physicochemical aspects of starch gelatinization, structural modification by extrusion cooking and the use of modified starches in the food industry. The application of the properties of

polysaccharides to the development of new food products comprises the subject of section four. The novel use of polysaccharides in foods, will of necessity require modification of the current legislation if their use is to become viable; this is adequately discussed in section five. The compilation finally concludes with two papers devoted to the social and nutritional aspects of these food ingredients.

In conclusion, it can be said that this book provides a well balanced and broad outlook on the current status of this science. Adequate literature sources accompany most chapters, thus, completing an excellent text which serves both those workers presently engaged in the field and those wishing to enter it.

S. Z. Dziedzic

Additive Migration from Plastics into Food. By T. R. Crompton. Oxford: Pergamon Press, 1979. Pp. xi + 234. ISBN 0 08 022465 2. £20.00.

For a long time there has been increasing pressure on all industries concerned with the manufacture and packaging of food, and with articles that come into contact with food, to reduce or eliminate any contamination of food with traces of extraneous materials. This pressure, which such industries must expect and accept, comes from many sources including national and European governments. When the extraneous materials are those that could produce off-flavours or present a potential safety problem, then reaction must be fast and sensible.

This book by T. R. Crompton is an attempt to cover just one aspect of this field: the potential migration of non-polymeric components from plastics into foods. Its scope is slightly wider than the word 'additive' in the title might suggest. The first chapter describes, in some detail, the types of polymers used in food packaging while the second chapter lists and discusses the types of additives, etc., that may be present in such polymers. The principles of extractability testing, i.e. using food simulants, are covered in the next chapter; subsequent chapters cover analytical methods that can be applied to both aqueous and fatty foods and their simulants. There are also a number of specific examples of analyses in which the author has presumably had some direct interest. Following a chapter on degradation products of additives there are finally eighteen pages on the legislative aspects of the use of additives in food grade plastics.

At this point the reviewer must declare his feelings and say that he found this a disappointing book. Its cover in some areas is uneven and much of the subject matter is out of date. These points are shown by examining the list of references. There are only 150, a small number for the range of topics that the author has attempted to cover, and all the references are 4 years old or more. About half are over 10 years old. The unevenness of the coverage is illustrated also by

reference to the monomers the author has chosen to include in Chapter 5. There is here no mention at all of vinyl chloride, which has deeply concerned analytical chemists, polymer manufacturers and legislators since 1974. Vinyl chloride is mentioned in earlier chapters only as the monomer of polyvinyl chloride. Styrene and acrylonitrile monomers are covered in much detail, but the analytical methods described are regrettably too insensitive for today's needs. The modern analyst needs a limit of detection for acrylonitrile, in foods, of 0.01 ppm, not the 1.0 ppm quoted. The same is true for styrene monomer. At the limit of detection quoted for one analytical method a food would be inedible due to tainting, and analysis would not be required.

The examples of analytical methods quoted in other parts of the book have frequently been superseded by newer and better methods. Has the author not heard of headspace gas chromatography or high performance liquid chromatography or mass spectroscopy? In a similar fashion, parts of the chapter on legislation are now outdated. Surely the progress, however slow, towards harmonization of legislation in the EEC deserves more than six uninformative lines, and the resultant U.K. legislation of 1978 (S.I. 1927) should have been mentioned. Similarly the author appears unaware that the FDA completely changed the numbering of all the relevant paragraphs early in 1977; indeed, his index mentions no FDA regulations later than 1967.

When a number of anomalies, out-dated methods, etc., are seen in a book that might otherwise be useful for reference, then it is hard to give credence to the remainder of the subject matter. With this in mind, it is difficult to commend this book – particularly to persons fresh to this field. The kindest comment that the reviewer can make is that he might have been more favourably inclined towards this book if he had been asked to read it 7 or 8 years earlier.

J. T. Davies

Fibrous Proteins: Scientific, Industrial and Medical Aspects. Vol. 1 and Vol. 2. Ed. by D. A. D. Perry and L. K. Creamer.
London: Academic Press, 1979–80. Vol. 1 (1979) Pp. xvii + 508. ISBN 0 12 545701 4. £19.80. Vol. 2 (1980) Pp. xvi + 257. ISBN 0 12 545702 2. £13.00.

These volumes are based on the proceedings of the International Conference on Fibrous Proteins held at Massey University, New Zealand, in February 1979. Volume 1 is a series of fifteen invited reviews of research topics of special current interest, written by eminent authorities; whereas volume 2 brings together twenty-three of the research papers presented at the Conference. The two volumes are rightly separated as they will attract somewhat different groups of readership.

Volume 1 comprises four reviews on aspects of muscle, six on collagen, three

on keratin, one on fibrinogen and one general chapter on the determination of structural information from the amino acid sequences of fibrous proteins. These topics stem from the basic industrial needs of a country still based mainly on an animal products economy with its need to sustain production of meat, wool and hides.

Scientifically, the pace of development in this field is often not realized. To quote from the review on the primary structure of collagen – 'It has been known for a long time that the thermal stability of collagen is related to its imino-acid content' with a reference to Schimmel & Flory's paper of 1968. Eleven years is certainly 'a long time' in this subject, since most of the cited references are from the 1970s.

The subject matter covered has very diverse application. For example 'Muscle structure and meat toughness' (food technology), 'Mechanical properties of connective tissues' (medicine) and 'Chemistry of keratin processing' (textiles) bring together scientists who, on the face of it have little in common. At the molecular level, as gradually emerges for the general reader there is a great deal in common, owing to their need for a familiarity with fibrous protein structures.

The value of these volumes is to research workers, technologists and teachers in the areas represented. The conjunction of several different disciplines makes them useful not only for up-to-the minute information but also for the 'browser' in search of ideas and explanations just outside his own area of study.

The food technologist concerned with meat muscle or the various forms of collagen will find much of interest in these books. A notable gap, however, is in nutrition, which, except for a rather casual treatment in a collagen chapter, receives hardly any attention.

B. J. F. Hudson

Commodity Year Book, 1980: The Public Ledger.
London: U.K. Publications Ltd., 1980. Pp. 112. £25.00.

In the preface of this first edition of 'the Public Ledger' *Commodity Year Book*, the editor states this volume will serve as a 'soft' commodity guide for both 'newcomer' and 'seasoned trader'. Unfortunately, it appears that neither goal is achieved, a surprise for regular readers of the very informative *Public Ledger* daily market report.

The book is divided into four sections: grains, feeds and proteins; oilseeds, oils and fats; produce markets, e.g. sugar, cocoa, coffee, tea, etc.; and freight markets. Commodity parts essentially contain data over the 1975–1979 period, with some more recent statistics for 1979–80, on production, imports, exports and prices; with brief notes on recent trends for the commodity and its major

producers/traders. Details of freight markets, cargo rates, ship sales and purchases are covered in the final part of the Year Book, with also a small directory of market contacts.

To achieve comprehension or predict future price movements in any commodity sector, the mere listing of production/trade/price figures, as in this text, provides only part of the market scenario. Instead what is more desirable would be provision of 'balance sheets', that is additional data concerning stocks, wastage, domestic supply, consumption, utilization, etc. For example, the decline in stocks of many 'soft' commodities during the late 1960s was considered an important contributory factor to the commodity price 'boom' of the early 1970s. This style of presentation has already been publicly endorsed, with the FAO's recent publication of commodity balance sheets, by country and product, an innovation which it appears to be developing.

A number of smaller points also raise scepticism about the Year Book. Many tables list countries' production/trade performance in alphabetical order, rather than in descending sequence of importance, although the oilseeds section is an exception in this respect. With reference to the preface that the book will allow 'interesting comparison' with other sources, such comparison will be frustrated by the fact that many other published series of commodity figures employ the calendar year, instead of the crop year as in this case. The directory of market contacts, listed under commodity headings, also has a strange layout, with over a third of the entries (which number almost 100) composed of the same three companies.

In compensation, albeit relatively modest for these limitations, the oilseed section is well covered (but excluded oilcakes, except soyameal which is contained in the protein section); the occasional future estimates are useful but might have been listed with other estimates for comparison and the pages devoted to freight markets were welcome.

At £25 one wonders who will be persuaded to purchase the *Public Ledger Community Year Book*, especially when competition is fierce from chapter comparable volumes currently available, i.e. FAO, *Production/Trade Yearbooks* and Frank Fehr, *Annual Commodity Review*, both of these under half the price.

Kevin P. Parris

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

The Journal of Food Technology publishes original contributions to knowledge of food science and technology and also review articles in the same field. Papers are accepted on the understanding that they have not been and will not be, published elsewhere in whole, or in part, without the Editor's permission. Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Typescripts (two complete copies) should be sent to the Editor, Dr H. Liebmann, c/o Institute of Food Science and Technology (U.K.), 105–111 Euston Street, London NW1 2ED. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be doubled-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

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

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

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

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

SI UNITS

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

NON SI UNITS

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

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

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

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

Journal of Food Technology
Volume 16 Number 2 April 1981

Contents

Printed by Adlard and Son Ltd, Bartholomew Press, Dorking