Volume 14 Number 4 August 1979

Journal of Food Technology

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

JOURNAL OF FOOD TECHNOLOGY

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

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The Journal of Food Technology is published bimonthly, six issues form one volume. The annual subscription is £45.00 (U.K.), £54.00 (Overseas), $120.00 (N. America, including cost of airfreight). Back volumes are still available.

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Low methoxyl pectins from lime peel

RAJNI A. PADIVAL, S. RANGANNA AND S. P. MANJREKAR

Summary

To prepare low methoxyl pectin (LMP) from lime peel, acid deesterification at pH 0.5 for 9 hr at 60°C and for 5 hr at 70°C; sodium hydroxide deesterification at 5°C and at pH 10.5 with continuous addition of alkali for 1–2 hr; and ammonia deesterification at 25°C and at pH 10.5 for 2–3 hr or at pH 11.7 for 1–2 hr were suitable. Such LMP had ~ 4.5% methoxyl groups and a molecular weight of 40 000–70 000. Precipitation at pH 0.5 to 1.5 after saponification resulted in LMP having good gelling characteristics; precipitation at pH 4.5, as recommended hitherto, impaired the gelling property.

Saponification titre values of the pectin being deesterified and a rapid gel test, based on the sensitivity of the pectin to the calcium, are described to follow the degree of deesterification. The gel test is also indicative of the gelling property of the pectin.

Introduction

Low methoxyl pectin is prepared by deesterification of pectin using acid, sodium hydroxide, ammonia or pectinesterase (Kertesz, 1951; Doesburg, 1965; Towle & Christensen, 1973). Lime (*Citrus aurantifolia*) peel is an important source of pectin containing 15–30% pectin on dry weight basis. Procedures developed to prepare LMP having ~4.5% methoxyl, by acid, sodium hydroxide and ammonia deesterification are presented in this paper.

Materials and methods

After extraction of the juice from lime, the peel is washed with cold water, blanched in boiling water, dried in a hot air drier and coarsely ground in a hammer mill fitted with 1 × 1 cm sieve. The pectin extract was prepared

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from the peel by the procedure of Owens, McCready & Maclay (1949). Dry pectin was prepared from the extract by precipitation with alcohol.

Acid deesterification

Simultaneous extraction–deesterification using HCl was carried out according to the procedure of Woodmansee & Baker (1949) under conditions of pH and temperature given in Table 1 using a peel:water ratio of 1:20. These experiments showed that pH 0.5 was optimum for deesterification. Hence, deesterification of the pectin extract and pectin solutions (1 and 4%) was carried out at pH 0.5 and at 60, 70, 80 or 90°C in a constant temperature waterbath. The pH did not change during deesterification. The extract became turbid as the methoxyl content decreased to 6% or lower. The deesterified extract was cooled and the LMP was precipitated with alcohol at pH 0.5 or after raising the pH to 4.5.

Sodium hydroxide and ammonia deesterification

The pectin extract or pectin solution and the alkali were precooled to the desired temperature, and mixed to adjust the pH to 10.5 or 11.7. Particulars of deesterification are given in Tables 2 and 3 for sodium hydroxide and ammonia deesterification respectively.

At the end of the deesterification, the pH was adjusted to 0.5 with conc. HCl and the pectin precipitated with alcohol.

Analytical methods

Two methods were developed to measure the degree of deesterification: (i) 25 ml of the sample being deesterified were pipetted out at intervals, cooled and the saponification titre value determined as in the procedure for determining methoxyl content. In a similar aliquot, the pectin was precipitated using alcohol, redissolved in water and the saponification titre value determined. (ii) Five to 10 ml of the sample containing 50–100 mg of pectin were pipetted into a test tube, cooled and the pectin precipitated with alcohol. The precipitate was washed 3–4 times with 70% alcohol to remove excess acid and redissolved in hot distilled water, with boiling if necessary. To this hot pectin solution in the test tube, 1–2 ml of calcium chloride solution containing 2–4 mg of calcium was added, the mixture allowed to cool and the gel characteristics were observed.

Moisture, ash and methoxyl were determined by the procedures described by Owens *et al.* (1952). Molecular weight was determined by the viscosity procedure of Smit & Bryant (1967). For grading of LMP, gels were prepared at pH

Table 1. Acid deesterified LMP prepared from lime peel

Particulars of deesterification							Pectin characteristics		Gel characteristics	
Material	Temp (°C)	pH	Time (hr)	Reaction rate (k × 10^3 per min)	Activation energy (Cal/mole)	pH of pptn.	Mol. wt. AMFB* (%)	MeO AMFB*	Gel strength† (ml H_2O)	Appearance‡
Peel	60	0.3	4.0	2.63		0.3	NE	4.4	50	++++
slurry	60	0.5	12.0	1.01		0.5		4.3	56	++++
	60	1.0	24.0	0.49		1.0		4.9	38	++++
	90	0.5	0.75			0.5		5.4	10	+
Pectin	60	0.5	9.0	1.19		0.5	58 860	4.4	38	++++
extract	70	0.5	5.0	2.36	18 490	0.5	56 500	4.6	38	++++
	80	0.5	2.0	4.90		0.5	37 740	4.4	20	++
	90	0.5	1.0	11.90		0.5	NE	4.4	10	+
1% pectin	60	0.5	6.0	2.29		0.5	36 670	4.5	10	+
solution	70	0.5	3.0	5.71	21 800	0.5	36 400	4.5	10	+
	80	0.5	1.0	13.28		0.5	34 500	4.3	10	+
	90	0.5	0.4	32.70		0.5	NE	4.1	10	+
4% pectin	60	0.5	8.5	1.29		0.5	46 670	4.6	35	+++
solution	70	0.5	2.75	5.40	20 810	0.5	36 390	4.4	18	++
	80	0.5	1.75	9.08		0.5	35 060	4.4	10	+
	90	0.5	0.6	18.10		0.5	NE	4.6	10	+

* AMFB – Ash and moisture free basis

† Gels were prepared using 1% LMP solution at pH 3.0 and 40 mg of calcium per gram of LMP without sugar

‡ ++++, very good gel; +++, good gel; ++, soft gel; + coagulated gel

NE, not estimated

Table 2. Sodium hydroxide deesterified LMP prepared from lime peel

Particulars of deesterification					Pectin characteristics		Gel characteristics		
Material	Temp (°C)	pH (±)**	Time (hr)	Reaction rate†† (k × 10^3 per min)	Mol. wt AMFB*	MeO AMFB* (%)	Optimum Ca (mg/g of LMP)	Gel strength† (ml H_2O)	Appearance‡
Pectin	5	10.5–	1.0	9.107§	69 260	5.47	60	45	++++
extract	10	10.5–	1.0	9.385§	62 590	5.52	60	40	++++
	15	10.5–	1.0	9.414§	60 050	5.51	60	40	++++
	25	10.5–	0.5	30.231	47 650	4.52	40	20	++
Pectin	5	10.5+	2.0	8.736	69 630	4.60	60	46	+++
extract	5	11.7–	0.25	31.643	69 870	4.32	60	50	+++
2% Pectin	5	10.5–	0.25	28.680	47 610	3.82	40	10	+
solution	5	11.7–	0.25	47.480	40 770	1.85	20	10	+

* AMFB – Ash and moisture free basis

† Gels were prepared using 1% LMP solution at pH 3.0 without sugar

‡ ++++, very good gel; +++, good gel; ++, soft gel; +, coagulated gel

§ Reaction rate (k × 10^3) after 1 hr decreased to 0.48 at 5°C, 0.56 at 10°C and 0.65 at 15°C

** pH with (+) or without (–) continuous addition of alkali to maintain the same

†† The activation energy calculated from reaction rates at 5°C and 25°C at pH 10.5 without continuous addition of alkali was 9945 cals/mole

Table 3. Ammonia deesterified LMP prepared from lime peel

Particulars of deesterification						Pectin characteristics		Gel characteristics		
Material	Temp (°C)	pH (±)*	Time (hr)	Reaction rate (k × 10^3 per min)	Activation energy (Cal/mole)	Mol. wt. AMFB†	MeO AMFB† (%)	Optimum Ca (mg/g of LMP)	Gel strength‡ (ml H_2O)	Appearance§
Pectin extract	5	10.5–	15.0	0.996	16 590	46 580	4.48	60	53	++++
	10	10.5–	7.0	1.610			4.50	60	52	++++
	15	10.5–	6.0	2.274		42 470	4.57	60	50	++++
	20	10.5–	4.0	4.740			4.53	60	50	++++
	25	10.5–	2.5	5.431		47 670	4.37	60	50	++++
	30	10.5–	1.5	9.646			4.68	60	10	+
Pectin extract	5	11.7–	6.0	2.773	14 420	55 730	4.56	60	60	++++
	10	11.7–	5.0	3.989			4.50	60	56	++++
	15	11.7–	3.0	4.414		58 300	4.27	60	54	++++
	20	11.7–	2.0	7.535			4.25	60	53	++++
	25	11.7	1.0	15.660		48 660	4.33	60	52	++++
Pectin extract	5	11.7+	3.0	4.260	–		4.20	60	65	++++
2% prepared pectin solution	25	10.5–	2.5	3.991		38 780	4.23	60	50	++++
	25	11.7–	1.0	7.084		37 410	4.37	60	50	++++

*pH with (+) or without (–) continuous addition of alkali to maintain the same
† AMFB – Ash and moisture free basis
‡Gels were prepared using 1% LMP solution at pH 3.0 without sugar
§++++, Very good gel; +++, good gel; ++, soft gel; +, coagulated gel.

3.0 with or without sugar using a 1% LMP solution and 20–60 mg of calcium per g of LMP. The gel strength was determined using a B.F.M.I.R.A. Jelly Tester at a water flow of 75 ml per min and at 30° torque. The gel strength given in digits in the text is in terms of ml of water.

In ammonia deesterified pectins, the amide groups were determined by the method given in Food Chemicals Codex (1972). The ammonium salts in LMP were determined by the method of Ronchése (1907).

Results and discussion

Acid deesterification

The rate of deesterification increased with decreasing pH. The peel slurry required more time to deesterify to ~ 4.5% methoxyl content than the extract or pectin solution (Table 1).

Time required to deesterify the peel slurry, pectin extract or 4% pectin solution at 60°C and pH 0.5 was shorter (8–12 hr) than reported hitherto (Kertesz, 1951; Doesburg, 1965; Black & Smit, 1972; Towle & Christensen, 1973). The reaction rates were very low (Table 1).

The 4% pectin solution set to a gel as the deesterification proceeded to 6% methoxyl or lower as observed by Hills, White & Baker (1942). With 1% pectin solution or the extract, a distinct turbidity was observed on cooling to room temperature but not gel formation.

The saponification value of the pectin extract or the gel test could be used as an index of the degree of deesterification. The pectin extract being deesterified contained ~ 1% pectin. The saponification titre value of an aliquot of the extract was almost the same as that of the pectin recovered from a similar aliquot (Fig. 1). A value of 6.0 ± 0.2 ml of 0.1 N NaOH for 25 ml of the extract containing 1% pectin would be indicative of ~ 4.5% methoxyl content in the pectin.

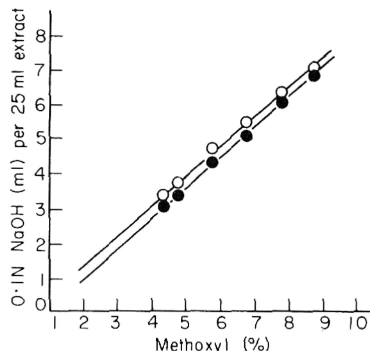

Figure 1. Interrelationship of saponification titre values of deesterified pectin extract and recovered pectin. ○, Pectin recovered from deesterified extract; ●, deesterified extract.

During deesterification of the pectin extract, it was observed that on adding calcium directly to 5–10 ml of the extract, precipitation or gelation did not occur even when the methoxyl content decreased to 6% or lower. However, when calcium was added at the rate of 2–4 mg to 50–100 mg of pectin (i.e., 40–80 mg of calcium per g of pectin) isolated from the extract as described earlier, gels or precipitates formed depending upon the methoxyl content of the pectin. High methoxyl pectins did not form gels. Between 5 and 7% methoxyl content, gelation occurred on allowing to stand for 12–24 hr, and between 2.5 and 5%, within a few minutes. Depolymerized pectins and pectic acids did not gel but formed flocculent precipitates. The test provides an objective indication of the extent of deesterification, besides indicating the functional value of LMP (i.e., gelation).

Sodium hydroxide deesterification

Without continuous addition of sodium hydroxide to maintain the pH at 10.5, the methoxyl content of the pectin extract reduced from 10.3% to ~ 5.5% in 1 hr at 5, 10 and 15°C (Table 2). The pH of the deesterifying medium lowered to ~ 9.6. Thereafter, little saponification occurred. The low reaction rates after 1 hr of saponification confirm this (Table 2). At 25°C, the methoxyl value reduced to 4.52% in 30 min.

When sodium hydroxide was continuously added at 5°C to maintain the pH at 10.5, the methoxyl content decreased to 5.2 and 3.2% after 1.5 and 2.5 hr respectively and the LMP formed good gels with gel strengths of 40 to 50. The reaction rate was uniform during deesterification.

Adjusting the initial pH to 11.7 instead of 10.5, increased the rate of deesterification and reduced the methoxyl content to ~ 4.5% in 15 to 20 min at 5°C. The pH of the deesterifying extract decreased from 11.7 to 11.0. The reaction rate was 3.6 times higher than at pH 10.5 maintained constant by continuous addition of alkali. The reaction rates found in this study are in good agreement with those reported by McCready, Owens & Maclay (1944). The activation energy of ~ 10 000 cals per mole is similar to the value reported by Merril & Weeks (1946).

Sodium hydroxide deesterification at pH 11.7 is as rapid as the enzyme deesterification and requires rigorous control of temperature and pH to prepare useful LMP samples.

Ammonia deesterification

The time required to deesterify the pectin extract to 4.5% methoxyl at pH 10.5 decreased from 15 to 1.5 hr as the temperature increased from 5 to 30°C. The pH of the extract decreased from 10.5 to 10.0 with the progress of deesterification. The reaction rates increased with increasing temperature (Table 3).

At pH 11.7, the time required decreased further (Table 3). The pH lowered from 11.7 to 11.3 during deesterification but it was not maintained constant as in sodium hydroxide deesterification. The reaction rates were higher, but the activation energy was lower than that at pH 10.5.

At 5°C, if ammonia is added to maintain the pH constant at 11.7, the methoxyl content reduced to 4.2% in 3 hr as compared to 6 hr when the pH was not maintained constant (Table 3). The reaction rate was high and the methoxyl contents at the end of 2.5, 3.0 and 3.5 hr were 4.5, 4.2 and 3.4%, and the gel strengths were 59, 65 and 78 respectively.

At pH 10.5 and 11.7 and 25°C, the time required to deesterify a 2% pectin solution to ~ 4.5% methoxyl content was 1–3 hr. The reaction rates were lower than those observed for pectin extract.

Ammonia deesterification of the pectin extract or the pectin solution at 25°C and pH 10.5 or 11.7 eliminates the cost of using refrigerated temperatures required for sodium hydroxide deesterification or the high temperature and corrosion resistant equipment required for acid deesterification.

During sodium hydroxide and ammonia deesterification, the pectin medium set to a gel as the methoxyl content reduced to 6% or lower. The appearance of the gel, the precipitation characteristics of the pectin on acidification to pH 0.5 using HCl, and the nature of the pectin precipitated could serve as a guide in determining the adequacy of deesterification. Between 5 and 7% methoxyl, the deesterified medium set to a soft gel and on acidification, the pectin did not precipitate distinctly; between 3 and 5% methoxyl, the medium set to a firm gel and on acidification, the LMP precipitated leaving a clear aqueous phase; below 3% methoxyl content, the medium set to a hard gel, and the LMP precipitated on acidification was brittle and was difficult to wash free of acid.

Gelation characteristics

Acid deesterified LMP

Low methoxyl pectins prepared from peel slurry at 60°C formed stable gels whereas those prepared at 90°C under similar conditions formed poor gels (Table 1).

The LMP prepared from the pectin extract at 60 and 70°C (pH 0.5) when precipitated at pH 4.5 as recommended in the literature (Kertesz, 1951; Doesburg, 1965) failed to form stable gels at 20–60 mg of calcium per g of LMP. Addition of citric acid to adjust the pH of LMP solution to 3.0 did not improve gelling characteristics. On washing such LMP with acidified (HCl) alcohol to liberate the carboxylic groups, the gelling ability was restored. Hence, adjusting the pH of the deesterified extract to 4.5 impaired rather than improved the gelling characteristics of LMP. The LMP precipitated without adjusting the pH after deesterification, i.e., at pH 0.5 formed stable gels (Table 1).

Deesterification of the pectin extract at 80 and 90°C, or of 1% pectin solution at 60, 70 and 80°C yielded LMP of poor gelling characteristics. A 4%

pectin solution deesterified at 60°C yielded LMP which formed stable gels; at 70°C, soft gels; and at 80 and 90°C, coagulated gels (Table 1).

Sodium hydroxide deesterified LMP

The LMP precipitated as such after deesterification had a pH of ~ 6.4 in a 1% solution and did not form satisfactory gels at 20–60 mg calcium per g of LMP. Addition of citric acid to adjust the pH to 3.0 did not aid gelation. The LMP precipitated at pH 0.5 (acidification with HCl) on the other hand, formed good gels at pH 3.0 with or without sugar at 20–40 mg of calcium per g of LMP depending upon the methoxyl content (Table 2).

Ammonia deesterified LMP

The LMP samples precipitated as such after deesterification had a pH of ~ 6.4 in a 1% solution, and formed poor gels as in sodium hydroxide deesterified samples. The saponified carboxylic groups were present as ammonium salts with a small proportion (4.2%) as amides. Adding citric acid to adjust the pH of LMP sol to 3.0 did not improve the gelling characteristics. The ammonium salts could be partially or completely eliminated by controlling the pH of precipitation but not the amides. The gels prepared from LMP samples precipitated at pH 4.5 and 3.0 coagulated. The gels from LMP precipitated at pH 1.5 or 0.5 were good. The gel characteristics shown in Table 3 are of LMP precipitated after adjusting the pH to 0.5.

Molecular weight and gelling characteristics

Among the three types, ammonia deesterified LMP had low molecular weight (39 900–50 000) as compared to sodium hydroxide deesterified samples (69 000–70 000) or acid deesterified samples (56 000–60 000). Nevertheless, ammonia deesterified LMP formed gels of higher gel strength than the other two. The molecular weights and gelling power of the LMP samples showed that apparently, the lower limit at which gelling was affected varied with the type of LMP. In acid and sodium hydroxide deesterified samples, this limit is apparently around 50 000. In ammonia deesterified LMP, it is much lower (around 30 000). This is obvious from the stable gels formed by the LMP prepared by deesterifying the pectin solution which had low molecular weight (30 000–38 000).

Acknowledgment

The authors are grateful to Drs B.L. Amla and M.V. Patwardhan for their keen interest in this investigation.

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(Received 12 October 1978)

J. Fd Technol. (1979) **14**, 343–349

Fractionation and characterization of guava pectic substances

A. H. EL TINAY, A. R. SAEED AND M. F. BEDRI

Summary

Guava pectic substances were fractionated into water soluble, oxalate soluble and sodium hydroxide soluble fractions in two canned guava cultivars as well as in fresh samples treated with calcium chloride. The results indicated that there is a gradual conversion of protopectin into soluble pectin which diffuses into the syrup.

Guava pectic substances were characterized for equivalent weight, methoxyl, free carboxyl, esterified carboxyl, degree of esterification, anhydrogalacturonic acid, weight average molecular weight and ash content in the fresh fruit and in the fresh fruit treated with calcium ions for both cultivars. Calcium ions appear to lower free carboxyl acid content and increase the weight average molecular weight of pectin.

Introduction

Since the nature of protopectin is unknown, the changes in pectin leading to greater solubility are not fully understood. According to Kertesz (1951) pectic substances contribute to rigidity of plant tissues. They are present in the middle lamella of a cell wall and act as cementing material between cells. Changes in pectic substances are responsible for the softening of fruits and other plant materials during ripening, storage or cooking (Kertesz, 1951). Postlmayr, Luh & Leonard (1956) reported that firmness of clingstone peaches is related to retention of protopectin. Hoose, Leonard & Luh (1956) showed that conversion of protopectin to water soluble pectin is responsible for softening of apricot during maturation, and that the quality of canned apricots was related to the maturity of the fresh fruit at the time of canning. They also reported that the concentration of water soluble pectin in canned apricots increased as the pressure test of the fresh fruit decreased. Dastur (1963) reported that harvest maturity, application of growth regulators and processing time were not important factors contributing to excessive softening in canned apricots.

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He observed, however, the presence of calcium oxalate crystals in the cells of severely softened canned Tilton apricots. A correlation between changes in solubility of pectin and changes in solubility of calcium led Doesburg (1957) to suggest that solubilization of pectin during ripening of apples may be caused by movement of calcium in the cell walls in response to changes in pH and organic acids. Complete characterization of pectin substances was considered necessary by McComb & McCready (1952) for controlling the texture of fruits and vegetables during processing and storage.

In the present study, guava pectic substances were fractionated into water soluble fraction, oxalate soluble fraction and sodium hydroxide soluble fraction in canned guava slices from two guava cultivars during storage; also fractionation was done on the fresh fruit from both cultivars after dipping in 1% calcium chloride solution. Guava pectic substances were characterized for molecular weight, free, esterified and total carboxyl group content, methoxyl content, equivalent weight and anhydrouronic acid content. The purpose was to determine the variations in these characteristics in relation to textural properties of the two guava cultivars.

Materials and methods

White and pink cultivars grown on Elsawhili Orchard near Khartoum were harvested at canning ripeness. They were used to study the effect of calcium ions on guava pectic substances. Calcium chloride was added to the syrups equivalent to 0, 100 and 200 ppm as calcium during the canning process.

The canning was done in the pilot plant of the Food Research Centre, Shambat, Khartoum North. Green and overripe fruits were removed before canning. The guava fruits were washed with cold tap water and then peeled, sliced and the seeds removed by hand using a stainless steel knife. The guava slices were processed in one pound cans made with differential electrolytic tin plate. The fill weight was 175 ± 0.5 g slices and 200 ± 0.5 g 25° Brix syrup made with sucrose and distilled water as added to maintain a headspace of 6/16 to 7/16in. The cans were sealed in a double seamer machine under a vacuum of 16 in Hg and heat processed at 180°F for 10 min. The cans were cooled under tap water, and stored at 68°F. For analysis a representative sample was obtained by taking the contents of four cans which were drained on 8 mesh screen and the syrup thus obtained was mixed and the slices blended in a blender.

Pectic substances were extracted from fresh slices without the addition of calcium chloride, from fresh slices dipped in 1% calcium chloride solution for 1 hr, from canned stored guava slices and syrups and were fractionated into water soluble, sodium hydroxide soluble and ammonium oxalate soluble pectin. Syrup (16 g) or slices (2 g) were weighed into a 50 ml graduated centrifuge tube and hot 95% ethanol was added to a volume of 40 ml and the mixture was heated for 10 min in a water bath at 85°C with occasional stirring

with a glass rod. The stirring rod was then rinsed off with 95% ethanol, the volume of the mixture made up to 50 ml in the tube centrifuged at 3000 rev/min for 15 min and after decanting the supernatant solution was discarded. Five ml of distilled water was added to the tube and the precipitate was dispersed with a rubber coated glass rod. The contents were made up to 35 ml with distilled water and then stirred vigorously for 10 min. This was accomplished by bubbling air through the mixture in the tube. The volume was increased to 40 ml with distilled water, centrifuged at 2300 rev/min for 15 min and the liquid decanted into a 100 ml volumetric flask. The water extraction was repeated and after centrifugation decanted into the same volumetric flask. Five ml of 1 N NaOH were added to the water extract and diluted to volume, then mixed and left for 15 min before colorimetric determination of water soluble pectin.

To the residue in the centrifuge tube 5 ml of 0.75% ammonium oxalate solution was added and the precipitate was dispersed with a rubber coated glass rod. The contents were made up to a volume of 35 ml and stirred vigorously and continuously for 10 min as described above. The volume was made up to 40 ml with oxalate solution, centrifuged as before and decanted into a 100 ml volumetric flask. The oxalate extraction was repeated, centrifuged and decanted into the same 100 ml volumetric flask. Five ml of 1 N NaOH was added to the oxalate extract and diluted to volume, then mixed and left to stand for 15 min before colorimetric determination of oxalate soluble pectin.

The residue remaining in the centrifuge tube was washed into a 100 ml volumetric flask with 5 ml of 0.05 N NaOH; 5 ml of 1 N NaOH was added and the contents were made to volume with distilled water, then mixed and left to stand for 15 min with occasional stirring and filtered before colorimetric determination of sodium hydroxide soluble pectin.

Pectin content in the extracts was determined colorimetrically by the carbazole method described by McComb & McCready (1952) and McCready & McComb (1952).

The method of Gee, McComb & McCready (1958) was used for the preparation of the marc from each cultivar which was obtained from the pulp of the fresh fruit by extraction first with 95% ethanol, acidified ethanol (750 ml of 95% ethanol plus 200 ml of hydrochloric acid), 70% ethanol and finally washed with acetone. The marc was dried at room temperature, ground and passed through 40-mesh.

Free carboxyl content was determined by taking a 0.5 g sample and 5 ml 95% ethanol were added to ensure complete wetting of the exposed surface. The product was mixed with 1 g of reagent grade NaCl and 100 ml of distilled water stirred vigorously, and the free acidity determined by direct titration with 0.1 N NaOH to pH 8.0. A blank containing the same quantities of the reagents was used.

For esterified carboxyl content determination, to the neutralized suspension obtained above, 10 ml of 0.5 N NaOH were added. The mixture was stoppered and held for 30 min at room temperature to saponify the methyl ester groups

in the pectin. After saponification 10 ml of 0.5 N HCl were added and the mixture was titrated to pH 8.0 with 0.1 N NaOH. The titre was corrected for reagent blank.

The results presented in Table 4 are on a moisture and ash free basis.

Moisture and ash were determined according to the AOAC (1960). The method of Owens *et al.* (1952) was used for viscosity determination. Intrinsic viscosity was calculated according to the formula from Doesburg (1965). Weight-average mol. wt was determined from intrinsic viscosity data according to the equation $N = 1.4 \times 10^{-6} M^{1.34}$, where N is the intrinsic viscosity and M is the molecular weight. Results presented were the average of at least three separate determinations that agreed well with each other.

Results and discussion

Tables 1, 2 and 3 show the water, oxalate and sodium hydroxide soluble pectins in canned guava slices, syrup, fresh slices and fresh slices treated with calcium chloride for the two guava cultivars.

From Tables 1 and 2 it is clear that the water soluble pectin of the canned slices from both cultivars decreased during storage while in the canned syrup it increased. The rate of decrease in slices and rate of increase in syrup was much slower in samples receiving calcium salt than in the controls. The sodium hydroxide soluble pectin in canned syrup from both cultivars was low and remained constant while it decreased in slices during storage. The rate of decrease of sodium hydroxide soluble pectin was much slower in samples receiving calcium ions than those receiving no calcium. The oxalate soluble fraction of canned slices and syrup showed slight increase during storage.

The sodium hydroxide soluble pectin represents the protopectin fraction which is gradually converted to water soluble pectin in the guava tissue during

Table 1. Water, oxalate and sodium hydroxide soluble pectins of canned guava slices (mg/100 g)

	Control			100 ppm $CaCl_2$			200 ppm $CaCl_2$		
Storage time (months)	H_2O soluble	Oxalate soluble	NaOh soluble	H_2O soluble	Oxalate soluble	NaOH soluble	H_2O soluble	Oxalate soluble	NaOH soluble
(a) White cultivar									
Zero time	6.25	1.15	5.00	2.75	2.25	4.50	5.00	1.15	6.25
4	3.00	1.45	3.00	2.50	2.30	4.25	4.00	1.40	5.00
7	3.25	1.50	3.75	2.25	4.00	4.00	4.25	1.50	4.50
13	2.50	1.75	3.25	2.00	3.25	3.75	3.50	2.25	4.00
(b) Pink cultivar									
Zero time	3.25	0.93	6.25	5.50	1.04	4.50	4.25	1.13	6.25
4	3.00	1.40	3.50	4.00	1.45	4.00	4.00	1.45	5.00
7	2.75	1.50	3.00	3.50	1.50	3.75	3.75	1.50	4.75
13	2.25	2.00	2.00	3.00	2.25	3.50	3.50	2.25	4.00

Table 2. Water, oxalate and sodium hydroxide soluble pectins of canned guava syrups (mg/100 g)

	Control			100 ppm $CaCl_2$			.200 ppm $CaCl_2$		
Storage time (months)	H_2O soluble	Oxalate soluble	NaOH soluble	H_2O soluble	Oxalate soluble	NaOH soluble	H_2O soluble	Oxalate soluble	NaOH soluble
(a) White cultivar									
Zero time	1.10	0.51	0.98	1,09	0.61	0.70	0.90	0.70	0.70
4	6.50	0.65	0.90	3.50	0.90	0.70	8.00	1.05	0.75
7	7.50	0.70	0.89	4.00	0.99	0.75	8.25	1.10	0.77
13	27.50	0.77	0.99	20.50	1.04	0.77	15.00	1.50	0.70
(b) Pink cultivar									
Zero time	5.00	0.30	0.82	4.00	0.50	1.25	4.25	0.50	2.25
4	21.50	0.60	0.80	17.00	0.80	1.20	16.00	1.00	2.20
7	22.50	0.61	0.70	17.55	0.83	1.25	16.25	1.04	2.00
13	27.50	0.71	0.77	17.90	0.93	1.04	17.50	1.14	2.25

storage. Then, the water soluble pectin diffuses from the tissue into the surrounding syrup causing an increase in the water soluble fraction in the syrup and a decrease in the slices. Addition of calcium appears to inhibit the diffusion of the pectin from the tissue into the syrup. The lower rate in the decrease of sodium hydroxide soluble pectin in canned guava slices receiving calcium ions may be due to the linking of the carboxyl groups in the polygalacturonic acid units with calcium into more complex types of structures. Henglein (1947) postulated that protopectin is formed by the association of polygalacturonic acid chains among themselves, and perhaps even with cellulose,

Table 3. Water, oxalate and NaOH soluble pectins of fresh guava slices and fresh guava slices treated with calcium salt (mg/100 g)

	H_2O soluble	Oxalate soluble	NaOH soluble
Fresh white slices control	8.75	2.25	5.00
Fresh white slices treated with calcium salt	4.75	4.65	6.25
Fresh pink slices control	4.00	1.25	5.00
Fresh pink slices treated with calcium salt	2.50	2.50	5.75

Table 4. Chemical analysis of marc prepared from guava cultivars

	A	B	C	D
Moisture (%)	10.30	5.1	16.5	5.80
Ash (%)	0.30	0.75	1.0	0.25
Equivalent weight	2181	3039	3173	3132
Free carboyxl group (meq/g)	0.46	0.33	0.32	0.32
Esterified carboxyl group (meq/g)	1.04	0.98	1.33	1.09
Total carboxyl group (meq/g)	1.50	1.31	1.65	1.41
Degree of esterification (%)	69.3	74.8	80.6	73.3
Methoxyl content (%)	3.23	3.03	4.13	2.71
AUA (%)	26.4	23.1	29.0	24.8

A = marc from fresh white slices
B = marc from fresh pink slices
C = marc from fresh pink slices after treated with $CaCl_2$
D = marc from fresh white slices after treated with $CaCl_2$

exclusively through calcium linkage. Postlmayr *et al.* (1956) reported firmness in clingstone peaches is related to the retention of protopectin; the relationship between calcium, pectin quality and texture appears to be applicable to both peaches and guavas. The increase in oxalate soluble pectin in samples receiving calcium ions may be explained by the formation of calcium oxalate resulting in the removal of calcium ions, which otherwise would be involved in complexing with polygalacturonic acid units (Khayat, 1964).

Similar trends were observed for fresh guava slices and fresh guava slices treated with calcium salt (Table 4). There is an increase in sodium hydroxide and oxalate soluble pectins and a decrease in water soluble pectin as a result of addition of calcium chloride.

In Table 4 the equivalent weight for the four pectin marcs varied from 2181 to 3173 which is considerably higher than the range of values reported

Table 5. Relative, specific, intrinsic viscosity and molecular weight of pectin extracted from guava marc

Cultivar	Concentration (g/100 g)	Relative viscosity	Specific viscosity	Intrinsic viscosity dl/g	Mol. wt
Marc from fresh white slices	0.1	1.02	0.02	0.20	7.03×10^3
Marc from fresh pink slices	0.1	1.04	0.04	0.40	11.80×10^3
Marc from fresh pink slices treated with $CaCl_2$	0.1	1.05	0.05	0.50	13.90×10^3
Marc from fresh white slices treated with $CaCl_2$	0.1	1.01	0.01	0.10	4.20×10^3

by Saeed, El Tinay & Khattab (1975) for marcs from three varieties of mango fruit. Addition of calcium resulted in increase in equivalent weight. The methoxyl content is an important factor in evaluating the setting time of pectin, sensitivity to polyvalent cations and their usefulness in low solids gel, films and fibres. The methoxyl content in the fresh untreated slices was 3.23% and 3.03% for white and pink cultivars respectively. Sinclair & Grandall (1949) reported a methoxyl content of 5.72% for grapefruit peel pectin. The free carboxyl group content was 0.46 meq/g and 0.33 meq/g for fresh untreated slices from white and pink cultivars respectively. Addition of calcium ions caused a decrease in the free carboxyl group content in both cultivars. Total carboxyl content was higher in the white cultivar compared to pink cultivar. This is attributable to the higher degree of esterification of pectin in the pink cultivar (74.8%) compared to that in the white cultivar (69.3%). The anhydrouronic acid (AUA) content was 26.4% and 23.1% in the white and pink cultivar respectively.

Table 5 shows the relative, specific and intrinsic viscosity and weight average molecular weight of guava pectin. Intrinsic viscosity was 0.20, 0.40, 0.50 and 0.10 dl/g for fresh white slices and fresh pink slices, pink slices treated with calcium and white slices treated with calcium respectively. The weight average mol. wt of pectin obtained from fresh white slices, fresh pink slices and white and pink slices treated with calcium was 7.03×10^3, 11.8×10^3, 4.20×10^3 and 13.90×10^3. The lower mol. wt obtained for the white slices treated with calcium could be due to the lower ash content in this sample (Table 4).

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(Received 19 October 1978)

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Retention of volatiles during freeze drying of tomato juice

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Summary

The present work studies some factors affecting volatile retention during freeze drying of tomato juice. ^{14}C-labelled butyl acetate was used as a model volatile. The variables studied were initial solids content, freezing rate, initial volatile concentration and sample thickness.

The observed effects of initial solids content and freezing rate on volatile retention conformed to expectations. Thickness of samples was also a factor in volatile retention, but the results here obtained did not appear to be easily explained by current retention theories. The retention was found to be approximately constant for most of the range of initial volatile concentration studied.

Adsorption – desorption experiments demonstrated that adsorption does not constitute a mechanism of butyl acetate retention in freeze dried tomato juice.

Introduction

Much attention has been paid in the last decade to the behaviour of organic volatiles during freeze drying of liquid foods. This area has been comprehensively reviewed by King (1971), Bomben *et al.* (1973), and, more recently, by Karel & Flink (1974), King & Massaldi (1974), Flink (1975a, b), and Thijssen (1975). From the results obtained, either on model or real food systems, it becomes clear that the retention of the volatiles depends largely on the properties of the solute which forms the amorphous matrix of the freeze dried solid. Although the exact retention mechanism is not yet known, two mechanisms, based on selective diffusion (Thijseen & Rulkens, 1968; King & Chandrasekaran, 1973) and microregions (Flink & Karel, 1970, 1972) respectively have been proposed with considerable success. A third mechanism, namely adsorption (Rey & Bastien, 1962), has been shown (Chirife & Karel, 1973a,

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1974; Bartholomai, Brennan & Jowitt, 1974) to make only a minor contribution to total volatile retention in various systems.

Solubility of the organic volatile may also play a role in its retention behaviour. Upon freezing, highly soluble volatiles, such as methanol or acetone, remain homogeneously dissolved in the concentrated liquid, but volatiles of limited solubility may become insoluble and are retained in the form of droplets (Flink & Gejl-Hansen, 1972; Flink, Gejl-Hansen & Karel, 1973). Massaldi & King (1974a, b) developed a quantitative model to account for the retention behaviour of volatile organic substances at levels of concentration both below and above their limit of solubility.

The proposed retention mechanisms are based on data collected from freeze dried solutions containing both simple solutes (mostly carbohydrates) and a variety of volatile compounds. Real systems turned out to give complex results, which in some cases were conflicting, as reported by Flink (1975a) in a study on volatile retention in freeze dried tomato juice.

In the present study, the retention of butyl acetate in freeze dried tomato juice is characterized. The results are compared with those obtained by several workers from a wide variety of systems in order to elucidate the mechanism responsible for the retention of volatiles in freeze dried liquid foods.

Materials and methods

The system studied consisted of freeze dried tomato juice, ^{14}C-labelled butyl acetate and water.

Preparation of tomato juice

Tomatoes were washed in cold water and peeled. They were cut by hand into 2–3 mm thick slices and pressed in a home juice maker. The freshly squeezed juice was frozen in a cold chamber at −20°C during 24 hr, thawed, and filtered through filter paper (Whatman, qualitative 4) under suction. The freezing-thawing cycle allowed collection of a clarified colourless juice upon filtration. The juice so obtained was frozen at −40°C and freeze dried with unheated plates.

The systems were prepared by dissolving the desired amount of freeze dried tomato juice in distilled water and adding ^{14}C-labelled butyl acetate. The following initial concentrations were usual: tomato solids 7%, ^{14}C-butyl acetate 0.01%, water 93% (w/w).

Freeze drying

Aliquots of the above solution were pipetted into 50 ml Erlenmeyer flasks, frozen as specified below and then freeze dried for 48 h with unheated plates

and at a chamber pressure of less than 0.1 Torr in a Stokes freeze drier (Model 902-1-8). The volume of solution and resultant sample thickness were varied in some experiments as noted under 'Results and Discussion.' Samples were frozen as slabs in the flasks by one of two methods: 'fast' freezing was accomplished either by immersion of flasks in liquid air or in acetone-dry ice; 'slow' freezing refers to placing the stoppered flasks on a tray freezer at –40°C.

Butyl acetate analysis

Reagent grade butyl acetate was mixed with ^{14}C-labelled butyl acetate to give the desired specific radioactivity. The radioactive butyl acetate was obtained from the Comisión Nacional de Energía Atómica. The butyl acetate content was determined by measuring the radioactivity of the samples with a liquid scintillation counter (H. Packard, Model 574) and subtracting the background noise. For the determination the freeze dried samples were dissolved in water to its initial solids concentration and 1 ml of this solution was added to 10 ml of water miscible scintillator (DMPPO 0.15 g, PPO 3.5 g, naphthalene 50 g and dioxan 500 ml) in the counting vial. The volatile retention is expressed as a percentage of the initial volatile content.

Adsorption in layered system

Samples were prepared by freezing alternate layers of a solution containing volatile and layers of a solution without volatile (Chirife & Karel, 1973a). After freeze drying, the layers were separated for individual volatile analysis.

Adsorption–desorption experiments

Adsorption was carried out at 27°C by placing 50 ml Erlenmeyer flasks with freeze dried tomato juice in vacuum desiccators over a dilute solution of ^{14}C-butyl acetate in a non-volatile solvent (oleic acid). For the desorption experiments the volatile-containing solution was replaced by activated charcoal. Volatile retention in this experiment is expressed as a percentage of the initially adsorbed volatile after a given evacuation period.

Results and discussion

Current theories on volatile retention predict that increasing the solids concentration increases volatile retention (Chirife, Karel & Flink, 1973; King & Massaldi, 1974). Figure 1 shows the effect of initial solids concentration on retention of butyl acetate. The fractional retention increased with solids

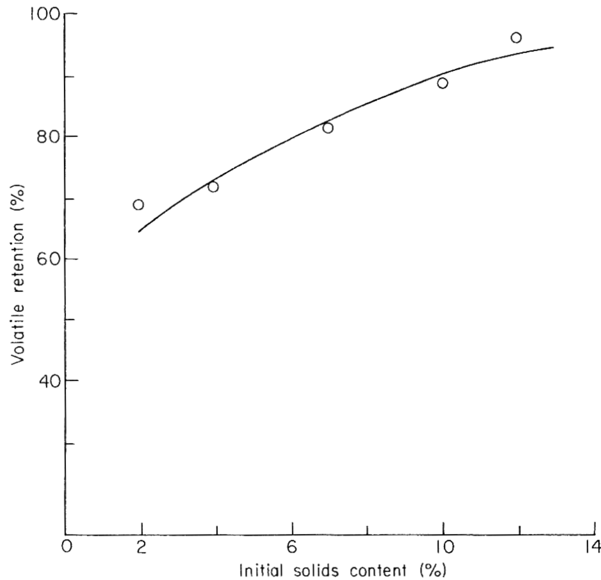

Figure 1. Effect of concentration of solids on volatile retention during freeze drying of 'fast' frozen tomato juice. Initial butyl acetate content = 0.01%.

concentration within the range of concentrations studied, in agreement with theory. Higher concentrations (above 12%) of tomato solids were not used to avoid the risk of collapse during freeze drying.

Various studies on the effect of initial volatile concentration tended to show that the fractional retention decreases with increasing concentration (Flink & Karel, 1970; Chirife *et al.*, 1973; Chirife & Karel, 1973b; Chirife & Karel, 1974; Bartholomai *et al.*, 1975). According to the microregions theory (Flink & Karel, 1970) it is expected that as the volatile concentration decreases the fractional retention would increase, approaching a maximum. King & Massaldi (1974) attributed the above behaviour to either or both of two phenomena: (a) at higher concentrations of volatile the solubility limit may be surpassed during freezing, giving droplets with lower fractional retentions and (b), dissolved volatiles can exhibit a plasticizing effect in the concentrated solutes matrix, which would increase the diffusion coefficient leading to a decrease in retention as the initial volatile concentration increases.

Figure 2 shows the effect of initial butyl acetate concentration on volatile retention in slowly frozen and rapidly frozen tomato juice. It can be seen that for both systems the fractional retention may be considered approximately constant for most of the range of volatile concentration studied, but appears to decrease at very low volatile concentration. However, additional data at very low initial volatile concentration would be needed to assess whether the decrease in volatile retention at low volatile concentration is, in fact, real. Table 1 shows some of the results of a literature review of the effect of increasing volatile concentration on fractional volatile retention. It can be seen that in various

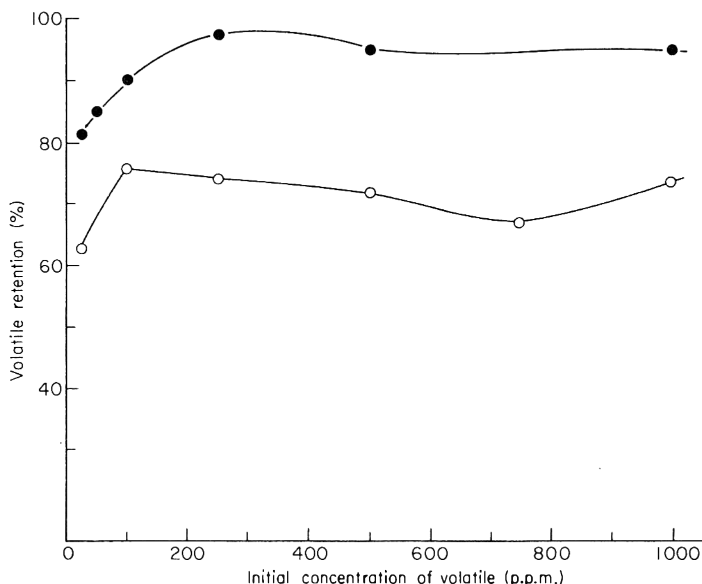

Figure 2. Effect of initial butyl acetate content on retention during freeze drying of 'slow' frozen and 'fast' frozen tomato juice (7% solids). ●, Slow freezing; ○, fast freezing (acetone-dry ice).

systems volatile retention increases with increasing volatile concentration, this behaviour being opposed to that predicted by theory. Volatiles showing this behaviour included highly soluble compounds, such as acetone, and compounds of limited solubility, which are known may become insoluble upon freezing, such as butanol (Flink *et al.*, 1973).

Comparison of both curves in Fig. 2 (slowly frozen and rapidly frozen) shows that, at all volatile concentrations, slow freezing resulted in higher retention than rapid freezing. This behaviour conforms to expectations (Flink & Karel, 1970; Chirife & Karel, 1974).

Figure 3 shows the effect of thickness of the sample on retention of butyl acetate in freeze dried tomato juice, as compared with the results reported by Flink (1975a) for alcohol retention in freeze dried canned tomato juice. Both systems show that retention increases with increasing thickness of the sample. This striking result is not in agreement with most of the results reported to date and does not conform to the predictions of either the microregion or the selective diffusion theory. If freeze drying is conducted in such a way that external resistances control it, retention should be independent of piece size (King, 1973). However, no explanation can be given for increased retention with increasing sample thickness. Flink (1975a) suggested that his results on freeze dried canned tomato juice may be explained by the fact that the thicker layers are subjected to a lower rate of freezing, thus improving retention. This may be a reasonable explanation but it is strange that this effect resulting from a balance between a lower rate of freezing and a lower rate of drying,

Table 1. Some 'anomalous' results on the effect of increasing initial volatile concentration on fractional volatile retention

Substrate or system	Volatile	Initial concentration range (ppm)	General trend in retention	Reference
Dextran (20%)	2-Propanol, FF	100–200	Increase	Flink & Labuza (1972)
Maltose (20%)	2-Propanol, SF	100–200	Increase	Flink & Labuza (1972)
Glucose	Acetone, FF	0.2–20 – 20	Increase	Voilley, Simatos & Loncin (1973)
Model solution	Ethanol, FF	100–1000	Increase	Voilley, Simatos & Loncin (1973)
Model solution	Butanol, FF	100–1000	Increase	Voilley, Simatos & Loncin (1973)
Model solution	Pentanol, FF	100–1000	Increase	Voilley, Simatos & Loncin (1973)
Sucrose (5%)	Acetone, SF	10–50	Increase	Kayaert (1974)
Sucrose (5%)	Acetaldehyde, SF	50–500	Increase	Kayeart (1974)
Sucrose (10%)	Acetaldehyde, SF	50–500	Increase	Kayeart (1974)
Gum (2%) sucrose (5%)	Acetone, SF	10–500	Increase	Kayeart (1974)
Orange juice	(+)-limonene	110–460	Increase	Berry & Froscher (1969)
Tomato juice	Ethanol	100–1000	Increase	Flink (1975a)
Tomato juice	Propanol	100–1000	Increase	Flink (1975a)
Tomato juice	Butanol	100–1000	Increase	Flink (1975a)
Clarified tomato juice	Butyl acetate, SF	25–250	Increase	Present study

FF, Fast frozen; SF, slow frozen.

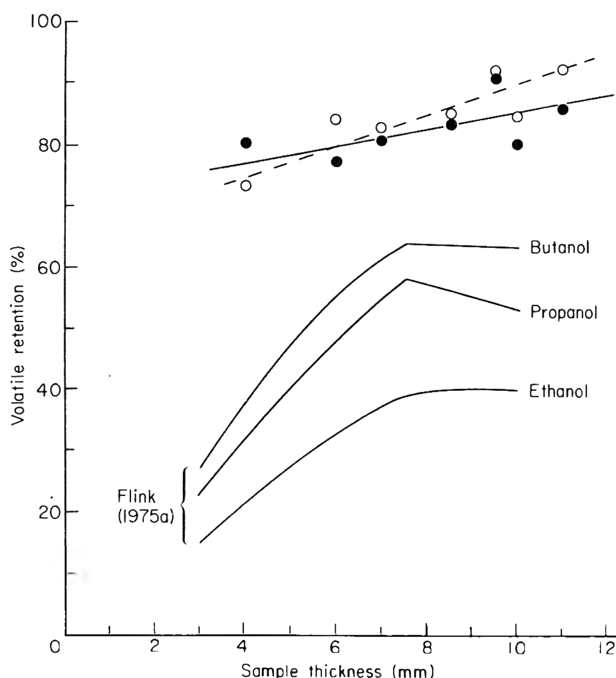

Figure 3. Effect of sample thickness on butyl acetate retention during freeze drying of tomato juice (7% solids). Initial butyl acetate content = 0.01%, ○, Frozen in acetone-dry ice (present study); ●, frozen in liquid air (present study).

should only be apparent in tomato juice and not in the wide variety of model and real food systems shown in Table 2. It can be seen that with the exception of the tomato systems, the behaviour of which is not clearly understood, all others showed a decreased retention with increasing sample thickness.

Readsorption of butyl acetate in dry layers of tomato juice was investigated in an experiment with layered systems conducted as described in 'Material and methods'. The composition of the systems for these experiments was fixed: solids 7% (w/w) and butyl acetate 0.01%. After the standard cycle of freeze drying the layers were separated for butyl acetate analysis. No butyl acetate was found in layers originally not containing any. This indicates that no re-adsorption of volatile occurred during freeze drying.

Adsorption–desorption of butyl acetate was studied as follows. Samples of freeze dried tomato juice were placed in vacuum desiccators over butyl acetate-oleic acid solutions of different volatile concentration. Although the partial pressure of the volatile in the head space was unknown, it may be considered relatively small in view of the low molar fraction of butyl acetate in the liquid phase. Table 3 shows the amount adsorbed by the tomato solids and the retained volatile (expressed as a percentage of the amount originally adsorbed) after subsequent evacuation. It can be seen that the adsorption of butyl acetate is completely reversible. This lack of any strong adsorption further indicates

Table 2. Generalized summary of results on the effect of increasing thickness of sample on fractional volatile retention

Substrate or system	Volatile and initial concentration (p.p.m.)	Thickness range (mm)	General trend in retention	Reference
Raspberry juice	Various natural volatiles	5–10	Slight decrease	Sauvageot *et al.* (1969)
Dextran-10 (20%)	2-Propanol (1000)	1.6–5	Decrease	Flink & Karel (1970)
Glucose (10%)	Acetone (100 000)	1.6–12.8	Decrease	Flink & Karel (1970)
Dextran (20%)	2-Propanol (100) SF	3–10	Decrease	Flink & Labuza (1972)
	2-Propanol (100) FF	3–10	Decrease	Flink & Labuza (1972)
Maltose (20%)	2-Propanol (100) SF	3–10	Decrease	Flink & Labuza (1972)
	2-Propanol (100) FF	3–10	Decrease	Flink & Labuza (1972)
PVP (20%)	n-Propanol (10 000) SF	2–10	Decrease	Chirife *et al.* (1973)
	n-Propanol (10 000) FF	2–10	No change	Chirife *et al.* (1973)
Pepsin (20%)	2-Propanol (500)	2–10	Slight decrease	Chirife *et al.* (1973)
Bovine serum albumin (20%)	2-Propanol (500) SF	2–10	Slight decrease	Chirife & Karel (1974)
	2-Propanol (500) FF	2–10	Decrease	Chirife & Karel (1974)
Pectin (2.5%) sucrose (12.5%)	Acetone (12.5)	3–21	Decrease	Lerici (1976)
	Ethyl acetate (12.5)	3–21	Decrease	Lerici (1976)
	Methanol (60)	3–21	Decrease	Lerici (1976)
	Ethanol (60)	3–21	Decrease	Lerici (1976)
	Propanol (60)	3–21	Decrease	Lerici (1976)
Carbohydrate gums (1.5%)	Ethyl acetate (1000)	3–8	Decrease	Kayaert (1974)
	Acetone (100)	3–8	Decrease and no change	Kayaert (1974)
	n-Propanol (2500)	3–8	Slight decrease	Kayaert (1974)
	Acetaldehyde (50)	3–8	Decrease and no change	Kayaert (1974)
	Acetaldehyde (100) SF	3–10	S. decrease and no change	Kayaert (1974)
	Acetaldehyde (100) FF	3–9	S. decrease and no change	Kayaert (1974)
Mushroom extract (5%)	Benzaldehyde (100)	2–10	Decrease	Bartholomai *et al.* (1975)
	Oct-1-en-3-ol (100)	2–10	Decrease	Bartholomai *et al.* (1975)
	Natural volatile components	2–10	Decrease	Bartholomai *et al.* (1975)
Tomato juice canned (7%)	Methanol (1000)	3–10	Increase	Flink (1975a)
	Ethanol (1000)	3–10	Increase	Flink (1975a)
	n-Propanol (1000)	3–10	Increase	Flink (1975a)
Tomato juice clarified (7%)	Buthyl acetate (100)	4–11	Increase	Present study

S, slight; FF, fast frozen; SF, slow frozen.

Table 3. Adsorption–desorption of butyl acetate in freeze dried tomato juice

Butyl acetate conc. in oleic acid solution (molar fraction)	Volatile adsorbed* ppm (dry basis)	Volatile retention† (%)
0.024	298	0
0.069	528	0
0.112	1595	3.8‡

*after 48 hr at 27°C.

† after 48 hr evacuation at 27°C; retention expressed as a percentage of the amount originally adsorbed.

‡additional evacuation decreased the retention to 0%.

that adsorption is not a mechanism of butyl acetate retention in the system studied here.

Conclusions

The results of the effect of solids concentration and freezing rate on butyl acetate retention in freeze dried tomato juice conform to the predictions of the microregion and the selective diffusion theory. However, the results of the effect of thickness of the sample on volatile retention is contrary to the predictions of the above theories. Although the data on the effect of initial volatile concentration need further investigation, this apparently 'anomalous' behaviour has also been found in a number of literature results.

A theoretical explanation of this behaviour remains to be done.

It is demonstrated that adsorption does not contribute to butyl acetate retention in freeze dried tomato juice.

Acknowledgments

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

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(Received 6 November 1978)

J. Fd Technol. (1979) **14**, 361–367

Thermal diffusivities of some unfrozen and frozen food models

F. V. ALBIN, K. BADARI NARAYANA, S. SRINIVASA MURTHY AND M. V. KRISHNA MURTHY

Summary

Using an experimental plate freezer, the time-temperature histories of some food models and food products are measured during cooling in the unfrozen state and heating in the frozen state. These time-temperature curves are matched with theoretical predictions using a nonlinear estimation technique to yield the thermal diffusivity data. Thermal diffusivities of food models of different compositions, mashed potatoes of different moisture contents and of shrimps are tabulated.

Introduction

A broad range of thermophysical properties are covered by food products because of the wide variation in their composition, structure and texture. A knowledge of thermophysical property data is useful in the prediction of thermal processing characteristics, in the design of processing equipment and in controlling the quality of the products during a thermal operation. The thermophysical properties of many food products are available in the literature (Qashou, Vachon & Touloukian, 1972; Tressler, Van Arsdel & Copley, 1968; ASHRAE, 1972; Hill, Leitman & Sunderland, 1967).

In this paper, the thermal diffusivities of some food models and foodstuffs such as mashed potatoes and shrimps in unfrozen and frozen states are given. Even though food models are extremely useful in making studies on specific thermal processes and also in ascertaining the accuracy of mathematical predictions, there is a lack of data on the thermal properties of food models. Hence, food models of various compositions are chosen as test specimens and the effects of product composition on the thermal diffusivity of foodstuffs are studied. Experimentally determined cooling curves for the unfrozen products and heating curves for the frozen products are matched with the authors' theoretical model (Albin, Srinivasa Murthy & Krishna Murthy, 1976)

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0022-1163/79/0800-0361 $02.00 © 1979 Blackwell Scientific Publications

using a nonlinear estimation technique (Badari Narayana, 1976) to obtain the thermal diffusivity data.

Materials and methods

Test specimens

Food models were prepared from agar, cane sugar, soluble starch and water. The required proportion of ingredients are poured into hot water at 70°C and allowed to boil for 5 min with continuous stirring to obtain a consistent paste. The food model is then poured into a rectangular mould (200 × 150 mm) of 15 mm thickness in which calibrated copper-constantan thermocouples (0.1 mm diam.) are held in predetermined locations by taut threads and allowed to set. These specimens are transferred to a desiccator containing water and kept in a thermostatic bath to attain temperature equilibration prior to testing.

Fresh potatoes from the same lot are used for the investigations. These are cooked, cooled, peeled and mashed into a purée. The required quantity of water is added and the mixture is beaten to achieve a good consistency. The paste is poured into rectangular moulds in which copper-constantan thermocouples are held in position with the help of threads. A small sample of the paste is weighed and dried in a vacuum oven to determine the moisture content. Commercial frozen shrimps of the peeled and deveined variety are thawed in water at 0°C and are arranged in the rectangular mould with thermocouples in position. After the product occupies the required thickness (15 mm), water is added to the mould to completely fill the voids in between the shrimps. This method is similar to that practised in commercial freezing of shrimps.

Experimental investigations

A schematic diagram of the experimental plate freezer including the instrumentation is shown in Fig. 1. The temperature of the secondary refrigerant, i.e., ethylene glycol solution, in the bath can be thermostatically controlled to an accuracy of ± 0.1°C. The freezer plate has specially designed pathways for circulating the secondary refrigerant to ensure uniformity of temperature over the surface. The test specimen which has been equilibrated to a known initial temperature is transferred quickly on to the freezer plate. The free surface of the product is covered with 50 mm Thermocole insulation. The secondary refrigerant is circulated through the freezer plate. The temperatures at various locations across the product, and the plate and refrigerant temperatures are recorded on a potentiometric recorder. After the product is completely frozen and a uniform low temperature is attained, the refrigeration system is switched off. The secondary refrigerant is gradually heated and the heating curve of the frozen product is recorded. The reproducibility of the

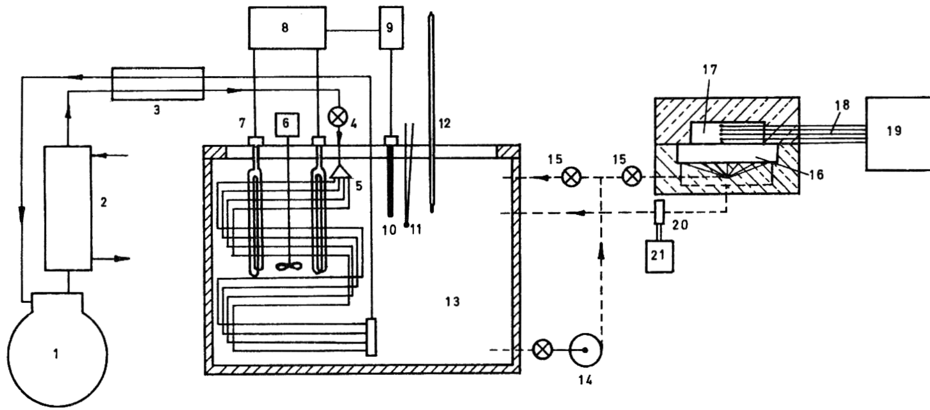

Figure 1. Schematic diagram of the freezing set-up. 1, Refrigeration compressor; 2, condenser; 3, heat exchanger; 4, expansion valve; 5, distributor and evaporator coil; 6, stirrer; 7, immersion heater; 8, dimmerstat and relay; 9, temperature controller; 10, temperature sensor; 11, glycol temperature thermocouple; 12, precision thermometer; 13, insulated tank; 14, circulation pump; 15, regulator valve; 16, freezer plate; 17, food product; 18, thermocouple connections; 19, temperature recorder; 20, orifice plate; 21, inclined tube manometer.

experimental results are found to be within ± 1% of the measured temperature values.

Evaluation of thermal diffusivity

Out of the complete time-temperature history obtained experimentally, the precooling of the unfrozen product and the heating curve of the frozen product are utilized to evaluate the thermal diffusivities in unfrozen and frozen states of the product respectively. These temperature profiles are matched with the appropriate theoretical profiles obtained from the analytical model proposed by the authors (Albin *et al.*, 1976). Though this model has the limitation that freezing occurs at a fixed temperature, this will not affect the present evaluation because the portions of time-temperature curves required for matching does not involve the phase change period.

The method used here is based on the theory of least squares for iterating upon an initial guess value of Fourier number τ. For each iteration, the correction for the assumed value of τ is computed from the condition that the sum of the squares of the deviation between the experimental and calculated temperatures at different locations inside the product given by the following objective function should be a minimum:

$$F(\tau_m) = \sum_{n=1}^{p} \int_0^{\tau_m} [\theta_{c,n}(\tau) - \theta_{e,n}(\tau)]^2 \, d\tau \qquad (1)$$

where the Fourier number τ is defined as

$$\tau = \alpha t/a^2 \tag{2}$$

and the dimensionless temperature θ is defined as

$$\theta = (T - T_o)/(T_i - T_o) \tag{3}$$

In the above equations, the various quantities are

p = no. of thermocouple locations inside the product
t = time, hr
T = temperature, °C
a = product half-thickness, m
α = thermal diffusivity, $m^2\ hr^{-1}$

and the various subscripts are

m = maximum value of time
n = thermocouple location
c = calculated
e = experimental
o = reference
i = initial

The minimization of the objective function is achieved when its partial derivative with respect to the unknown parameter is equal to zero. This yields the relation for the correction $\Delta\tau$ to be applied to the guess value of τ. The theoretical profile is evaluated at the new value of τ and the above procedure is repeated till a preset accuracy criterion is satisfied. Using the converged value of τ, the diffusivity is evaluated from eqn (2). The detailed theory, computational procedure and error analysis are recorded elsewhere (Albin, 1978).

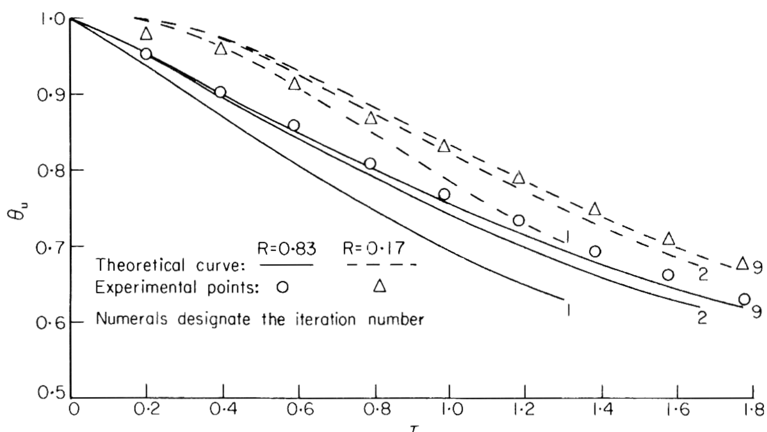

Figure 2. Thermal property evaluation by matching theoretical and experimental curves: Unfrozen product, 3 agar, 100 water; shape: slab; half-slab thickness 15 mm.

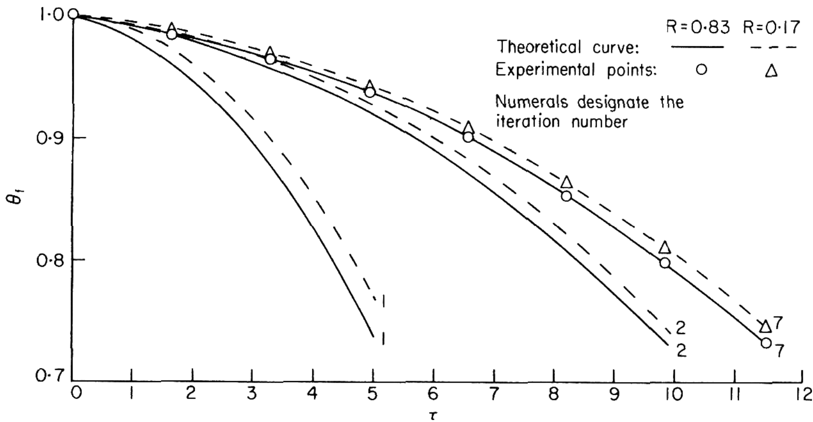

Figure 3. Thermal property evaluation by matching theoretical and experimental curves: Frozen product. 3 agar, 100 water; shape: slab; half-slab thick ness 15 mm.

The process of sequential matching of the analytical and experimental data is illustrated in Figs 2 and 3 for the unfrozen and frozen products respectively for two different locations inside the product. In the above figures, the dimensionless position R is defined as

$$R = r/a \tag{4}$$

where, r = position vector, m.

In all the cases investigated, the desired accuracy of 0.0001% deviation between the assumed and the calculated diffusivity could be achieved in five to ten iterations.

Table 1. Thermal diffusivities of some food models

Composition (by weight)	Unfrozen product		Frozen product	
	Mean temp. (°C)	$\alpha \times 10^3$ ($m^2\,hr^{-1}$)	Mean temp. (°C)	$\alpha \times 10^3$ ($m^2\,hr^{-1}$)
Agar : starch : water				
3 : 0 : 100	15	0.53	−10	2.22
3 : 5 : 100	15	0.50	−10	2.13
3 : 10 : 100	15	0.48	−10	1.98
3 : 15 : 100	15	0.46	−10	1.45
Agar : sugar : water				
3 : 0 : 100	15	0.53	−10	2.22
3 : 15 : 100	14	0.49	−13	1.35
3 : 40 : 100	12	0.47	−15	0.61
3 : 65 : 100	12	0.45	−15	0.59
Agar : sugar : water				
3 : 15 : 100	14	0.49	−13	1.35
4.5 : 15 : 100	14	0.46	−13	0.75
6 : 15 : 100	14	0.45	−13	0.72

Table 2. Thermal diffusivities of some frozen food products

Product	Unfrozen product		Frozen product	
	Mean temp. (°C)	$\alpha \times 10^3$ ($m^2\ hr^{-1}$)	Mean temp. (°C)	$\alpha \times 10^3$ ($m^2\ hr^{-1}$)
Mashed potatoes of moisture content (wet basis)				
= 0.70	15	0.30	−10	1.07
= 0.75	15	0.36	−10	1.31
= 0.80	15	0.37	−10	1.85
= 0.85	15	0.41	−10	1.96
Shrimps, peeled and deveined of grade				
= 200/300	10	0.50	−10	3.19
= 300/500	10	0.44	−10	2.16

A precise method of estimating uncertainties in experimental results as presented by Kline & McLintock (1953) is used here for making an error analysis. It is estimated that the overall uncertainty due to experimental measurements in the values of thermal diffusivity presented here is of the order of ± 5%.

Results

The thermal diffusivity values for unfrozen and frozen products are listed in Table 1 for food models of different compositions and in Table 2 for mashed potatoes of different moisture contents and shrimps.

Discussion and conclusions

The results indicate the usual trend that the diffusivity of frozen products is much higher than that for the unfrozen product. This is obviously due to the fact that water in frozen form has a higher diffusivity than water in liquid form. This is also the reason for the difference in diffusivity values between the frozen and unfrozen product being more pronounced as the water content is increased. It is observed that the effect of increasing starch and sugar contents is to decrease the diffusivity values of both unfrozen and frozen products while the converse trend is experienced when the agar content is increased.

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(Received 14 November 1978)

J. Fd Technol. (1979) **14**, 369–380

A simplified model for predicting the temperatures of foods during air dehydration

S. M. ALZAMORA, J. CHIRIFE AND P. VOILLAZ

Summary

The validity of a simplified heat transfer model to predict the temperature of fruit and vegetables during dehydration, has been examined and satisfactory results were found.

The main advantage of the model consists in its simplicity because it has an analytical solution. The temperature prediction only requires the knowledge of the food drying slope and a rough estimation of some physical properties.

Introduction

It is well known that reactions between food components are often accelerated during dehydration leading to reduction in organoleptic quality and nutritional value. The rates of the reactions are strongly affected by the temperatures and moisture contents of the food during dehydration (Labuza, 1972; Aguilera *et al.*, 1975; Wanninger, 1972; Dennison & Kirk, 1978; Riemer & Karel, 1977). For this reason a knowledge of the food's moisture-temperature distribution as a function of drying time is needed in order to predict losses of nutrients and quality during drying (Labuza, 1972; Aguilera *et al.*, 1975; Saguy *et al.*, 1978).

As drying is a simultaneous heat and mass transfer process, correlation of moisture and temperature changes of the specimen involves solution of coupled differential equations. Luikov & Mikhailov (1965) solved those equations for the case that kinetic parameters may be considered constant. However, if the kinetic parameters involved in drying, such as moisture diffusivity and thermal diffusivity, are a function of moisture content and/or temperature, the equations are no longer linear and must be solved through numerical methods. Harmathy (1969) and Husain, Chen & Clayton (1973) presented and solved numerically a set of differential equations which, when solved with the appropriate initial and boundary conditions, yields the complete moisture content

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0022-1163/79/0800-0369 $02.00 © 1979 Blackwell Scientific Publications

and temperature history of the system. Vaccarezza, Lombardi & Chirife (1974) presented a semi-empirical approach which correlated temperature and moisture changes of sugar beet during the first falling rate period of drying. While the coupled heat and mass transfer equations were not solved rigorously, the approach gave a fairly good representation of the experimental results.

The present investigation was undertaken with the aim of testing the validity of a simplified heat transfer model recently used by Alzamora *et al.* (1978) to predict temperature evolution during drying of avocado. The main advantage of this model consists in its simplicity because it has an analytical solution making computational methods unnecessary.

Materials and methods

Laboratory dryer

The drying equipment has been described in detail in a previous paper (Vaccarezza *et al.*, 1974). It consists of a centrifugal fan which blows the air through a heating section and then upwards through a vertical duct at the end of which the sample is suspended from a precision balance. A constant air velocity of 13 m/sec was used in most of the experiments. For the measurement of product temperatures during drying fine wire thermocouples were inserted in the sample at different depths.

Materials

Vegetables studied in this work included avocado, apple, potato and sugar beet root. The experimental data corresponding to sugar beet were previously obtained by Vaccarezza (1975). Foods were sliced in a food cutter and cut into square pieces with a thickness varying from 4.0 to 9.3 mm. Various measurements of the thickness were made at different points with a dial micrometer; only slices that fell within a 6% range of the average thickness were used. In all experiments reported, the thickness was approximately equal or smaller than one-tenth of the size; in this condition drying took place only from the two major faces of the slab thus simplifying the mathematical interpretation of the experimental results.

Results and discussion

Simplified heat transfer model

It is known that during air dehydration the temperature of the food sample increases rapidly at the beginning of drying towards the air dry bulb tempera-

ture (Vaccarezza *et al.*, 1974). However, the difference between the material and air dry bulb temperature becomes negligible only when most of the initial water of the food has been evaporated. Recently, Alzamora *et al.* (1978) applied a simplifed heat transfer model to predict temperatures during air drying of avocado. The energy balance for an infinite slab with moisture evaporation at the surface may be written

$$\frac{\partial T(x,t)}{\partial t} = a\frac{\partial^2 T(x,t)}{\partial x^2} \qquad (1)$$

The initial and boundary conditions for drying in a medium with constant air temperature, T_{db}, are

$$T(x,0) = T_0$$

$$-k\frac{\partial T(R,t)}{\partial x} + h\,[T_{db} - T(R,t)] - \lambda u = 0$$

$$\frac{\partial T(0,t)}{\partial x} = 0$$

where u is the evaporation rate. Experimentally it may be expressed as

$$u = u_0 \cdot \exp(-mt)$$

where u_0 and m are determined from the moisture content-time relationship,

$$(\overline{W} - W_e)/(W_0 - W_e) = \sigma \cdot \exp(-mt)$$

The values of the equilibrium moisture contents, W_e, are relatively small as compared to $\overline{W}$ or W_0 (Wolf, Spiess & Jung, 1972) and the equation may be simplified to

$$\overline{W}/W_0 = \sigma \cdot \exp(-mt)$$

Taking into account the above empirical equation, solution of eqn (1) becomes (Luikov, 1968; Alzamora *et al.*, 1978)

$$\frac{T(x,t) - T_0}{T_{db} - T_0} = 1 - \frac{\theta_{wb}\cos\{(P_d^{1/2})\,x/r\}}{\cos(P_d^{1/2}) - (P_d^{1/2})/Bi\ \mathrm{sen}\,(P_d^{1/2})}\exp(-P_d F_0)$$

$$-\sum_{n=1}^{\infty}\left[1 - \frac{\theta_{wb}}{(1 - P_d/\mu_n^2)}\right]A_n\cos(\mu_n\,x/R)\exp(-\mu_n^2 F_0) \qquad (2)$$

where

$\theta_{wb} = \lambda u_0/h(T_{db} - T_0)$

$P_d = mR^2/a$, Predvoditelev criterion

$F_0 = at/R^2$, Fourier number

$Bi = Rh/k$, Biot criterion

$a = k/\rho C_p$, thermal diffusivity

and, A_n and μ_n are defined as functions of Biot criterion

$$\cot \mu_n = (1/Bi)\,\mu_n$$

$$A_n = \frac{(-1)^{n+1}\, 2\,Bi\,(Bi^2 + \mu_n^2)^{1/2}}{\mu_n\,(Bi^2 + Bi + \mu_n^2)}$$

Equation (2) may be directly used to predict food temperature during drying.

Application of the model

Internal temperature gradients. The internal food temperature during drying may be considered uniform with little error when the Biot number for heat transfer, $Bi = hR/k$ is less than 0.1 (Welty, Wicks & Wilson, 1968). The Biot number for the various vegetables studied here calculated from literature data (Dickerson, 1968; Rha 1975) using heat transfer coefficients obtained from usual heat transfer correlations, was found to be in the range 0.2–0.5. This low value is consistent with the small internal temperature gradients reported in the literature for various foods undergoing air drying (Jason, 1958; Chirife, 1971; Vaccarezza *et al.*, 1974).

Figure 1 shows the temperature evolution at the 'surface' and centre of a thick ($\approx$ 9.3 mm) slab of avocado during air drying at 57°C. It can be seen

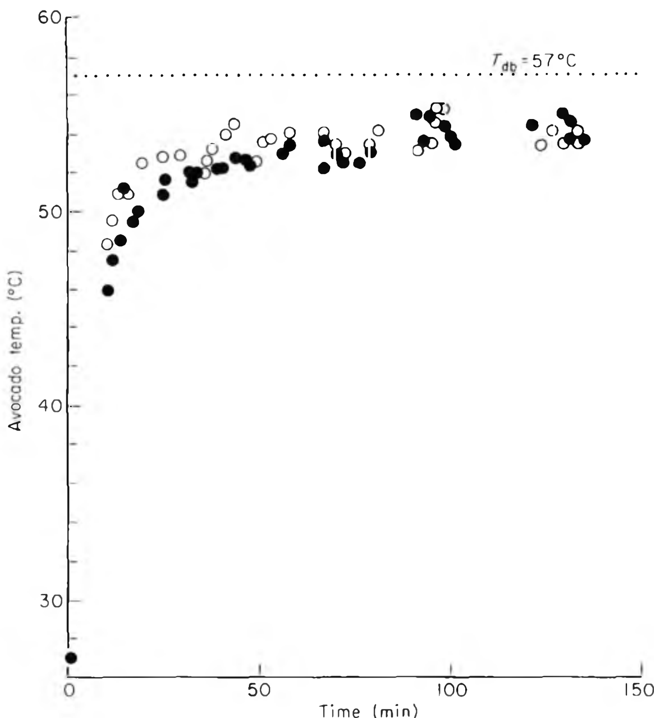

Figure 1. Surface and centre temperature during avocado slab dehydration at 57°C. ●, centre; ○, 'surface'.

that, in agreement with the previous considerations, the internal temperature gradients are very small, the maximum difference between 'surface' and centre being of about 2°C. This difference however, diminishes rapidly after the beginning of drying. It may be concluded that the sample temperature can be characterized by a single value at each instant of time.

Prediction of temperature evolution. Figure 2 shows the calculated (eqn 2) avocado temperature compared with experimental temperature-time measurements previously reported by Alzamora *et al.* (1978). The full line was obtained utilizing the physical and thermal properties (ρ, k, C_p) of the fresh fruit, while the dotted one was obtained using the properties of the 'dried' product. The values of those properties were obtained from the literature (Dickerson, 1968; Vaccarezza, 1975; Rha, 1975). It can be seen that there is very little difference between the curves predicted using the properties of the 'fresh' or 'dried' product and that a good agreement exists between experimental and predicted temperature changes. On the same Fig. 2 the end of the first falling rate period is indicated, for which the linear relationship ln $\overline{W}/W_0$ versus time applies, as reported by Alzamora *et al.* (1978). After this period eqn (2) is no longer valid and it should not be used to predict the temperature changes. However, at the end of this period the sample temperature is already so close to the air dry bulb temperature, that eqn (2) still gives a reasonable prediction as is shown in Fig. 2.

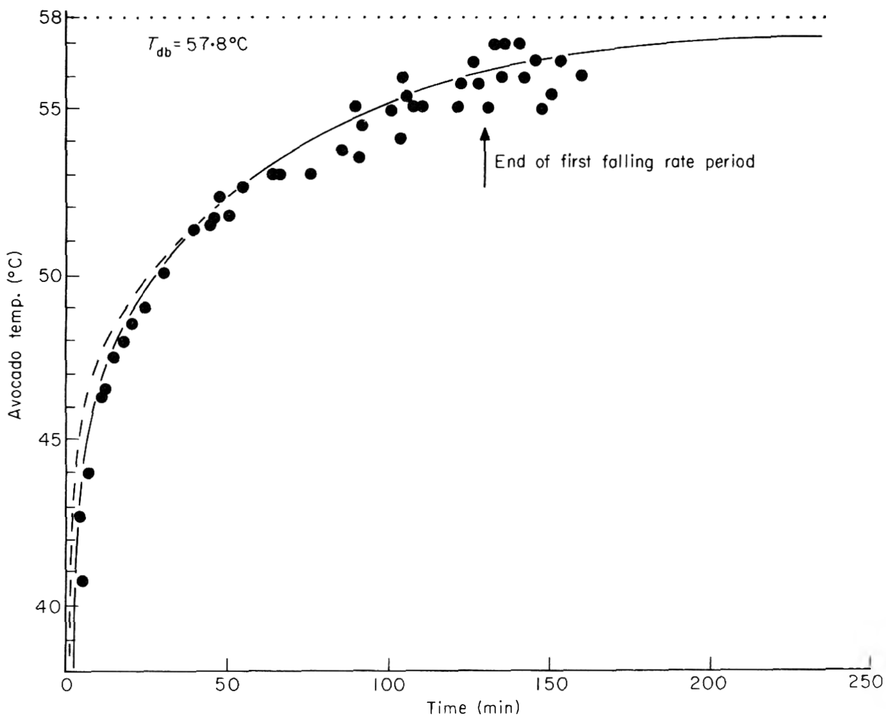

Figure 2. Comparison of predicted temperature changes of avocado with experimental results. Predicted: ——, ρ, C_p, k of fresh avocado; ----, ρ, C_p, k of dried avocado.

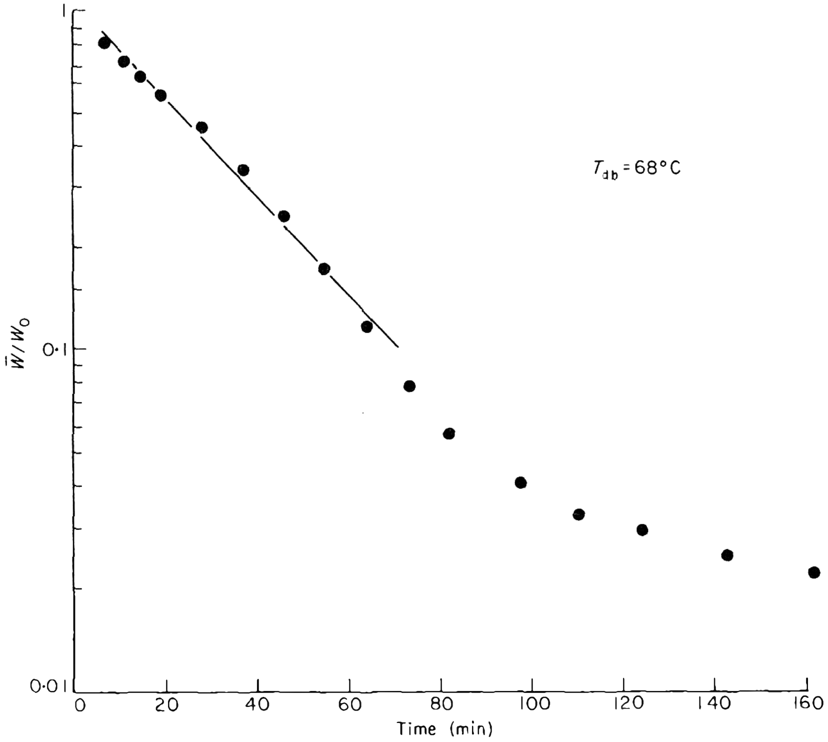

Figure 3. Drying behaviour of potato slab at 68°C dry bulb temperature.

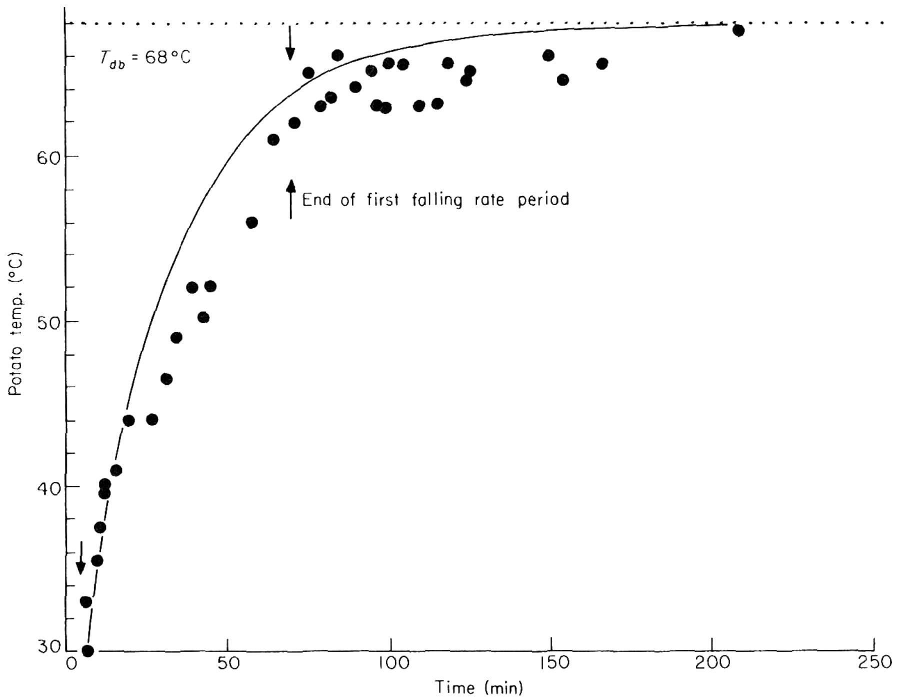

Figure 4. Comparison of predicted temperature changes of potato with experimental results. ●, Experimental; ○, predicted.

Figure 3 shows values of log $\bar{W}/W_0$ versus time for a potato slab 5.3 mm thick dried at 68°C. A straight line portion is observed and then the data deviate from the straight line relationship. The slope of the straight line was subsequently utilized together with the physical and thermal properties of fresh potato to predict the temperature changes using eqn (2). The properties of potato were obtained from the literature (Dickerson, 1968; Rha, 1975). The results are shown in Fig. 4. It can be seen that a reasonably good agreement exists between predicted and measured sample temperatures. As with avocado the temperature prediction is satisfactorily continued after the end of the straight line period, which for this case is about 60 min.

Figure 5 shows the predicted and measured temperature changes for a slab of sugar beet root 10.8 mm thick undergoing drying at 81°C. The experimental data are those previously reported by Vaccarezza (1975). As in the previous cases the prediction is satisfactory even after the finalization of the first falling rate period.

Figure 6 shows the logarithmic drying curves for an apple slab 5.5 mm thick being dried at 67°C. It can be seen that the drying behaviour may be described by two straight lines of different slopes. Figure 7 shows the calculated apple temperature compared with the experimental temperature-time measurements. The full line was calculated using the drying slope corresponding to the first straight line while the dotted line was obtained using the drying slope of the second straight line. It can be seen that the prediction in the latter stages of drying is improved when the change of the slope is taken into account when using eqn (2).

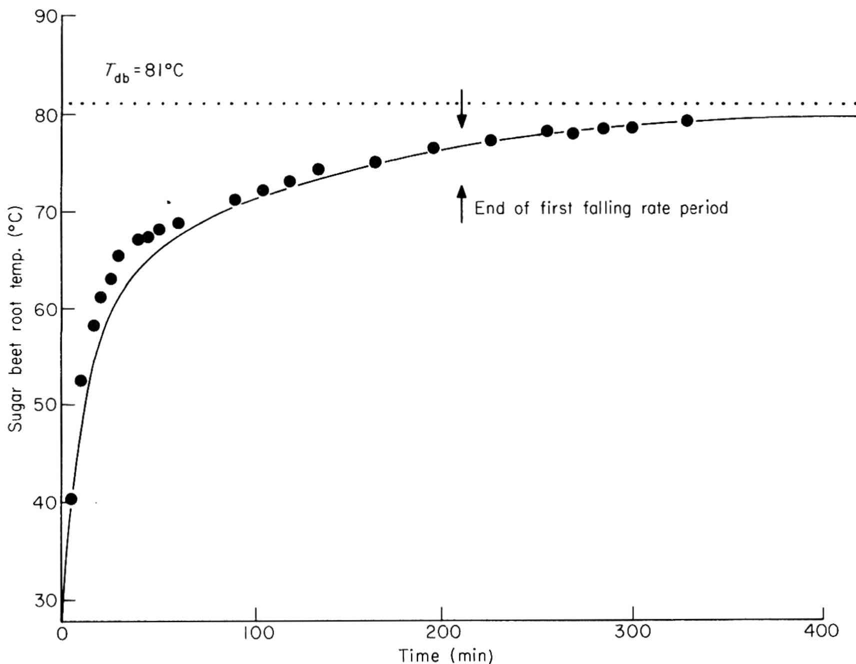

Figure 5. Comparison of predicted temperature changes of sugar beet root with experimental results. ●, Experimental; ○, predicted.

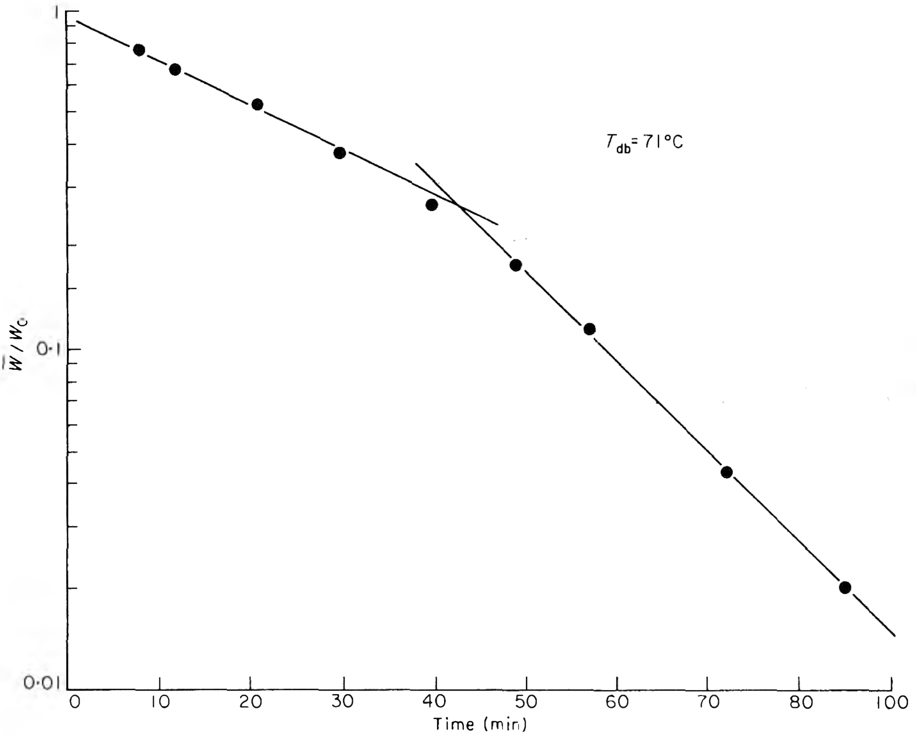

Figure 6. Drying behaviour of apple at 71°C dry bulb temperature.

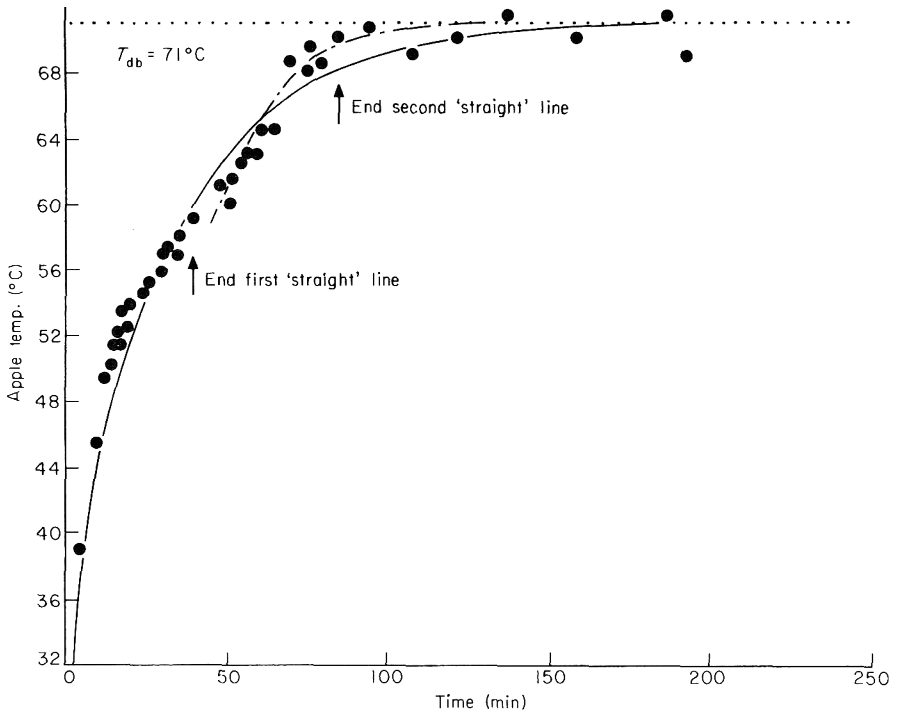

Figure 7. Comparison of predicted temperature changes of apple with experimental results. —·—·—, Predicted; ●, experimental.

All of the above predictions were made using the characteristic properties (ρ, k, C_p) of the fresh foods. It is well known, however, that these properties are a function of moisture content and consequently will not remain constant during drying. For this reason the utilization of constant values for ρ, k and C_p, as it was done here, may be questioned. However, the results shown in Fig. 2 suggested that the predictive equation (eqn (2)) is not very sensitive to variations in those properties. This may be explained through the analysis of the different terms of eqn (2). Considering the numerical values of all parameters usually encountered during air drying of vegetables, the sample temperature – as predicted by eqn (2) – is fundamentally determined by the ratio of moisture evaporation. In fact, the coefficient of the first exponential in eqn (2) is given by the slope of the drying curve. The other terms, for which the changes in food properties will be more important, are only significant at the beginning of drying for low F_0 numbers. In addition, for small Bi criterion all the terms of the series, even for low F_0 numbers, are negligible in comparison with the first one, since $\mu_n \to (n-1)\pi$ and in consequence, $A_n \to 0$ except A_1, whose value approaches one.

Table 1. Approximate range of values of density (ρ), specific heat (C_p) and thermal conductivity (k) for fruits and vegetables

	Maximum	Average	Minimum
ρ(g/cm^3	1.10	0.94	0.88
C_p (cal/g °C)	0.95	0.89	0.72
k(cal/°C sec cm)	1.91×10^{-3}	1.12×10^{-3}	0.58×10^{-3}

This observation is further tested in the following way. Rha (1975) reported values of ρ, C_p and k for a large number of fruits and vegetables. The average value of each property was calculated and shown in Table 1 together with the maximum and minimum values observed. An arbitrary drying slope was then used to simulate the temperature changes using different combinations of the extreme (low and high) values of ρ, k, and C_p. The combinations were made in a way which would generate the highest and lowest possible values of Bi and P_d. The results are shown in Fig. 8 which illustrates the expected difference in the temperature prediction when a drastic change in the values of the food properties are considered. It can be seen that the difference between the predicted curves (case 1 or case 2) rapidly diminishes with increasing drying time; i.e. the difference in the temperature prediction at 50 min drying time (about 20% of total drying time) is only 2°C.

Conclusions

The validity of a simplified heat transfer model to predict temperature changes during fruit and vegetable dehydration has been examined. Satisfactory results

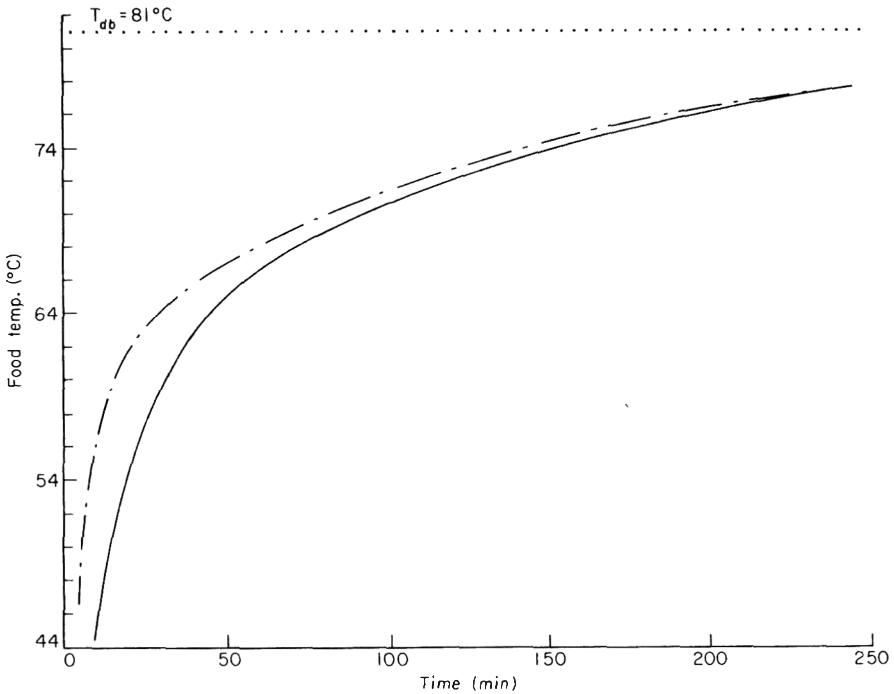

Figure 8. Effect of changes in ρ, k and C_p in the temperature prediction during food dehydration. ——, Predicted (case 1); — · —, predicted (case 2).

were found for predicting temperature during air drying of avocado, potato, apple and sugar beet root. The main advantage of the equation tested consists in its simplicity because it has an analytical solution, making computational methods unnecessary. The prediction of temperature changes during drying only requires the knowledge of the food drying slope and a rough estimation of the value of its thermal diffusivity, $k/\rho C_p$.

Nomenclature

A = drying area, cm^2
C_p = specific heat, cal/g °C
h = heat transfer coefficient, cal/sec cm^2 °C
k = thermal conductivity, cal/cm sec °C
m = slope of the experimental straight lines, ln $\overline{W}/W_0$ versus time, sec^{-1}
m_s = dry mass, g
R = half thickness of the slab, cm
t = time, sec
T = sample temperature, °C
T_{db} = air dry bulb temperature, °C
u = evaporation rate, g water/cm^2 sec

u_0 = evaporation rate at $t = 0$, g water/cm^2 sec
$\bar{W}$ = average moisture content, dry basis
$\bar{W}_e$ = equilibrium moisture content, dry basis

Subscripts

0 = initial

Greek letters

λ = latent heat of vaporization, cal/g
ρ = density, g/cm^3
σ = intercept of the experimental straight lines, ln $\bar{W}/W_0$ versus time, dimensionless

Acknowledgments

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

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(Received 30 November 1978)

J. Fd Technol. (1979) **14**, 381–388

A simple, enzymic test for monitoring the efficient thermal pasteurization of chicken egg-white

J. B. MONSEY* AND J. B. JONES

Summary

A simple, rapid test has been developed to monitor the efficient thermal pasteurization of a single sample of egg-white. The starch-degrading enzyme activity of seventy-two individual raw egg-whites and eighteen samples of commercial bulked raw egg-white has been determined. The enzyme was found in all samples and the variation between bulked samples was small. The enzyme was virtually completely destroyed when egg-white was pasteurized at 57.2°C (135°F) for 2.5 min which are the conditions currently in use commercially in the United Kingdom. A sample of egg-white, pasteurized for 2.5 min failed the test if (a) the temperature fell below 56°C or (b) the sample contained more than 0.15% w/w yolk, or (c) the sample was contaminated after pasteurization with more than 2% w/w of raw egg-white. Storage of raw or pasteurized samples up to 9 months at –20°C or of eggs-in-shell up to 20 days at 20°C did not alter the enzyme activity.

The test can be completed in 75 min and several tests can be run simultaneously.

Introduction

Because of the possible presence of salmonellae in commercially prepared egg-white (Ayres & Slosberg, 1949; Kline *et al.*, 1965) it is generally the practice now to pasteurize it. In the U.K., where no additives are used prior to heating, the conditions used are 57.2°C for 2.5 min. Confirmation that the pasteurization has been achieved is more conveniently done by a chemical test than trying by bacteriological procedures to establish the absence of salmonellae.

Such a test for the successful pasteurization of whole egg is based on the incubation of α-amylase present in egg yolk (Shrimpton *et al.*, 1962). However, the literature contains little on the presence of starch-degrading enzymes in egg-white. The 'diastase' activity of egg-yolk is claimed to be 32 times that of

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egg-white (Koga, 1923) and Henderson & Robinson (1969) thought the α-amylase activity in egg-white to be too low to be of possible use in monitoring pasteurization.

An automated test based on the destruction of β-N-acetyl glucosaminidase present in egg-white (Donovan & Hansen 1971) has the disadvantage that the enzyme activity fell sharply with the age of the egg-white (see also Lush & Conchie, 1966; Winn & Ball, 1975). Also, this method required two samples of egg-white, both before and after pasteurization.

The development of a test to confirm the effective heat-processing of egg-white, based on the destruction of a starch-degrading enzyme, is described.

Materials and methods

Materials

A flock of hens (Shaver 585) reared on a normal layer's diet at the Food Research Institute provided the eggs used in this work, unless otherwise stated. All chemicals were purchased from British Drug Houses, Poole, U.K.

Reagents

Distilled water was used throughout.

1. Starch solution: 1.40 g 'Lintner' soluble starch was made to a thick cream with water and transferred quantitatively to approx. 50 ml boiling water ensuring that the solution did not stop boiling. It was further boiled for 1 min, cooled and made to a volume of 100 ml with water. The solution was stable for 1 week.
2. Trichloroacetic acid: A.R., 15% w/v, aqueous solution.
3. Iodine solution: 12.7 g A.R. iodine was dissolved in a solution of potassium iodide (25 g in 30 ml water) and made up to 1 litre with water. This solution was stable for 6 months. One ml of this stock solution together with 0.25 g of potassium iodide were made up to 100 ml with water. The dilute solution was stable for 1 week.
4. Phosphate buffer. 0.5 M solutions of A.R. potassium dihydrogen orthophosphate and A.R. dipotassium hydrogen orthophosphate were made. The two solutions were mixed to give a buffer of pH 7.30 using a pH meter.

Method

Egg-white (30 g) was gently mixed with 7.5 ml phosphate buffer in a stoppered flask and placed in a water-bath at 44°C ± 0.1°C. After exactly 5 min, 3.0 ml of starch solution were added and mixed gently. After 60 min incubation, a 5.0 ml aliquot was withdrawn and added to 5.0 ml TCA contained

in a stoppered tube, and gently shaken. After addition of 10.0 ml water, the contents of the tube were again mixed and filtered through two thicknesses of Whatman No. 1 filter paper (125 mm diameter). To 5.0 ml of filtrate, 1.0 ml of dilute iodine solution was added. The absorbance of the coloured solution was read at 585 nm in 10 mm cells, with water in the blank cell.

Determination of optimum conditions for enzyme activity

Yolk-free egg-white from 100 eggs was combined and homogenized (M.S.E. Atomix, ½ speed for 1 min). The enzyme activity (k value) of portions of the raw egg-white was determined at (a) incubation temperatures within the range 30°–53.6°C ± 0.1°C using a constant temperature water-bath, and (b) different pH values by substituting in the test mixture the same volume of either 0.5 M Tris-maleate buffer (adjusted with varying amounts of 0.5 M NaOH) or dilute HCl in place of the phosphate buffer described.

Determination of enzyme activity of raw egg-white

The activity of raw samples was estimated using the equation for a first order reaction:

$$k = \frac{1}{t} \times 2.3 \times \log_{10} \frac{x^0}{x^t} \ \text{sec}^{-1}$$

where k = velocity constant, t = incubation time (sec), x^0 = initial starch concentration and x^t = residual starch concentration after time t. Values for x were expressed as absorbance measurements. The activity of raw egg-white was found to obey the first-order reaction (i.e. k is constant for different values of t) if incubation times of less than 10 min were used. Samples (5 ml) of incubation mixture were assayed after 2.5, 5.0, 7.5 and 10 min, and the initial starch concentration found graphically by extrapolation to zero t.

Yolk-free egg-whites were obtained from seventy-two eggs (twenty-four from each of three local shops). Each egg-white was homogenized separately in an overhead homogenizer (M.S.E.) fitted with a 100 ml vortex beaker, ensuring that frothing was kept to a minimum, and the enzyme activity (k value) of each determined.

Eighteen samples, (six from each of three leading U.K. egg-breaking plants) of raw bulked egg-white and the same material pasteurized commercially were obtained. The test was applied to a portion (30 g) of each after homogenization and k values for the raw samples were calculated.

Pasteurization and storage

Homogenized egg-white was sucked by water-pump into a stainless-steel tube with an internal diameter of 3 mm and a wall thickness of 0.5 mm (volume

35 ml) as described by Henderson & Robinson (1969). The tube was immersed in a water-bath maintained to within ± 0.05°C of the required temperature (which was measured with a narrow-range (40–70°C) thermometer supplied with a BSI certificate). After 2.5 min the tube was quickly cooled by immersion in a cold-bath and the contents ejected by pressure from an air cylinder.

One hundred yolk-free egg-whites were combined, homogenized, and samples (1) pasteurized in the stainless-steel tube at five temperatures within the range 54.5°–57.2°C; (2) mixed with raw, homogenized yolk to give yolk concentrations between 0 and 1.0% w/w and each mixture pasteurized at 57.2°C; and (3) pasteurized at 57.2°C and mixed with raw egg-white to give concentrations between 0 and 5% w/w. Samples of each of the three lots were put in closed jars and stored at –20°C. One sample of each lot (30 g) was taken for assay after 0, 2, 5 and 9 months. They were first thawed at room temperature.

Effect of age of eggs on enzyme activity

Eggs were stored at 20°C. Samples (twenty-five eggs) were broken after 0, 10 and 20 days. The yolk-free egg-whites were combined, homogenized and the enzyme activity (k value) determined.

Results

Optimum test conditions

The optimum incubation temperature for the enzyme activity at pH 7.7 was 44°C (Fig. 1). There was little change in activity between 40°C and 46°C.

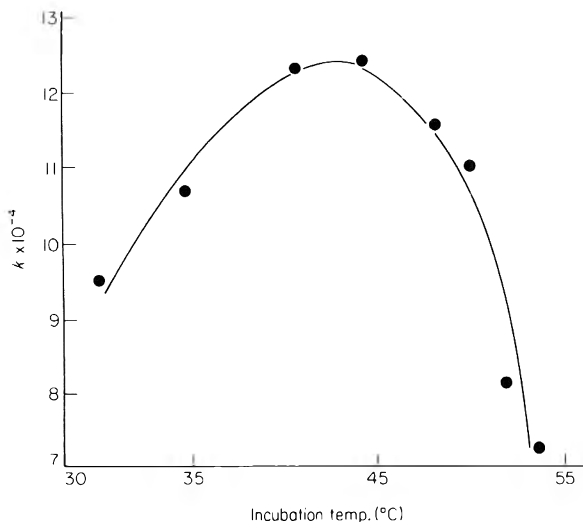

Figure 1. Effect of incubation temperature on enzyme activity. pH of incubation mixture was 7.7.

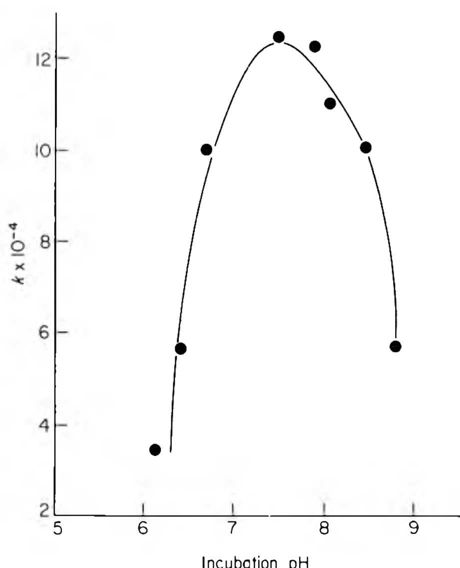

Figure 2. Effect of pH of incubation mixture on enzyme activity. Incubation temperature was 44°C.

The optimum pH at 44°C using Tris-maleate buffer was between 7.3 and 7.8 (Fig. 2). In additional tests adjustment of the pH with dilute HCl solutions (to give final test pH of 6.75 and 8.45) or with phosphate buffer (final pH 7.51) showed that these modes of adjustment did not change the slopes of the pH curve. The quantity of phosphate buffer described in the test was designed to ensure that the final pH of the test mixture, using any particular egg-white sample, fell within this range.

Enzyme activity of raw egg-white

It was found that the mean activities of egg-whites from three different sources did not differ significantly. The overall mean activity of the seventy-two egg-whites was $k = 12.2$, s.d. 3.7×10^{-4} sec^{-1} with a maximum value of 23.1 and a minimum of 5.8.

The overall mean activity of eighteen samples of raw (the mean pH value was 9.07, s.d. 0.19), commercial bulked egg-white was $k = 13.2$, s.d. 1.4×10^{-4} sec^{-1} with a range between 16.3 and 10.5. Again, the source of the samples was not significantly important. In separate experiments it was found that this variation in activity did not affect the sensitivity of the test because when samples of the raw commercial egg-white having the highest and lowest k values were pasteurized in the stainless-steel tube at 57.2°C both samples passed the test and each failed when pasteurized at 56.0°C.

It was found that the activity of raw egg-white was 60.4 times less than that of egg-yolk.

Commercial pasteurization

Each of the eighteen commercially pasteurized bulked egg-white samples passed the test (i.e. absorbance > 0.55). The mean absorbances of the six samples from each factory were 0.642, s.d. 0.010; 0.734, s.d. 0.012; 0.625, s.d. 0.033, respectively.

Contamination with yolk and raw egg-white

When egg-white contained more than 0.15% w/w yolk (Fig. 4) prior to pasteurization at 57.2°C, it failed the test. Similarly, adulteration of egg-white pasteurized at 57.2°C with 2% w/w or more of raw egg-white caused failure (Fig. 5).

Effect of pasteurization temperature

When yolk-free egg-white was pasteurized in the stainless-steel tube at 57.2°C for 2.5 min very little enzyme activity remained (Fig. 3). The activity curve for raw egg-white is included for comparison.

After the effect of yolk and raw egg-white on the sensitivity of the test has been taken into account, it was decided to fix arbitrarily the pass/failure

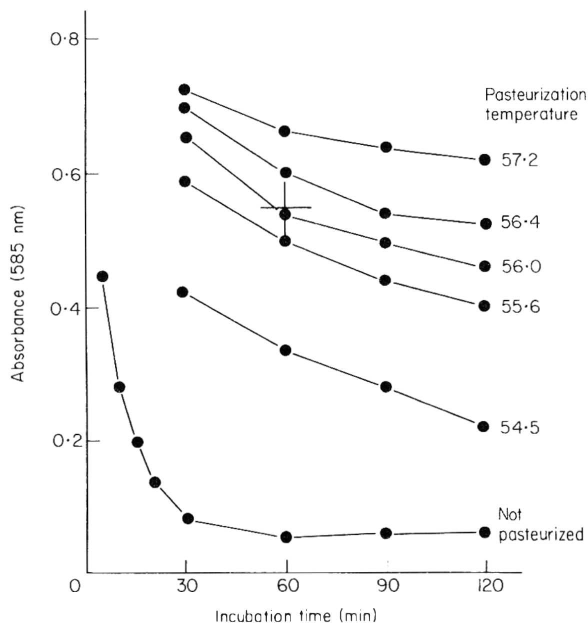

Figure 3. Effect of pasteurization temperature on enzyme activity. The cross indicates the proposed pass/fail point at 60 min incubation.

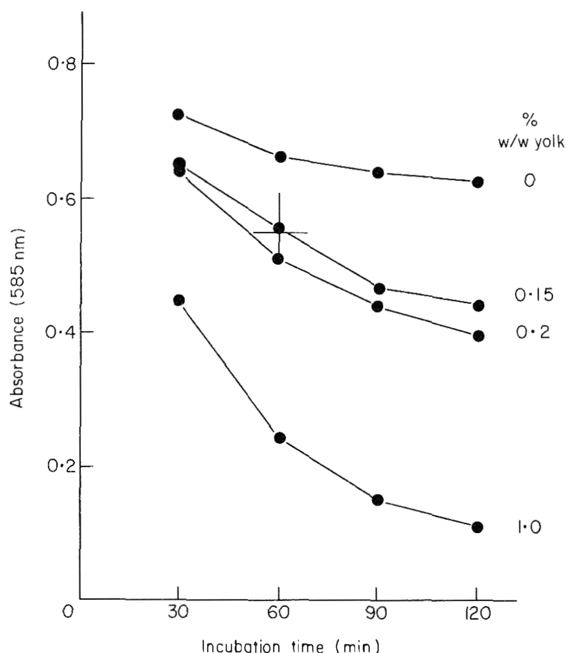

Figure 4. Effect of yolk contamination of egg-white prior to pasteurization at 57.2°C. The cross indicates the proposed pass/fail point at 60 min incubation.

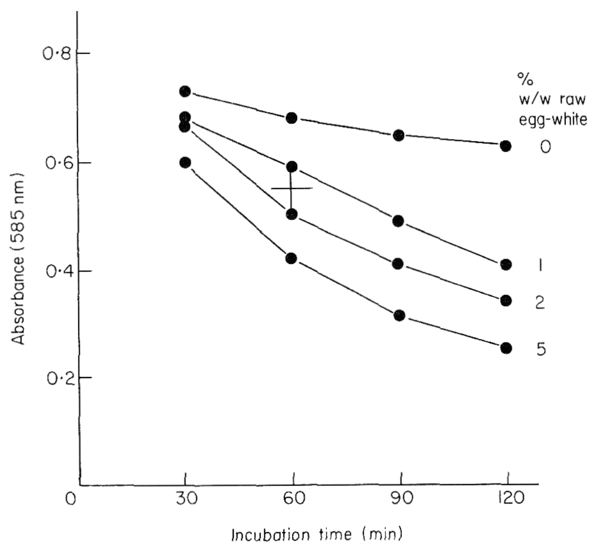

Figure 5. The effect of contamination of properly pasteurized egg-white with raw egg-white. The cross indicates the proposed pass/fail point at 60 min incubation.

absorbance as 0.55 after 60 min incubation. Egg-white pasteurized below 56.0°C will thus fail the test.

Storage

The activity of the samples of pasteurized egg-white described in Figs 3, 4 and 5 were unchanged after being stored at −20°C for up to 9 months. In addition, raw yolk-free samples stored and assayed at the same intervals also gave unchanged results.

When eggs-in-shell were stored at 20°C for 0, 10 and 20 days the enzyme activities (k values) of the egg-white were 11.2, 12.2 and 12.3 respectively.

Discussion

A test based on the virtual destruction of a starch-degrading enzyme in egg-white at 57.2°C has been developed. However, α-amylase in whole egg is not destroyed until a temperature of 64.4°C is reached (Henderson & Robinson, 1969). If, as seems likely, the enzyme in egg-white is α-amylase, the reason for this difference in heat-lability is not clear. Protective effect of yolk-lipids, difference of pH between yolk and white or difference in enzyme concentration are possible reasons. Alternatively, the two enzymes may have differences in molecular structure. Storage of eggs-in-shell at 20°C for 20 days showed there was no transfer of starch-degrading activity between yolk and white. The fact that presence of more than 0.15% w/w yolk in properly pasteurized egg-white will cause the sample to fail the test, might have been a problem if it had not been established that every sample of commercially-pasteurized egg-white examined passed the test. In practice, it may be important that samples should not contain more yolk because baking properties may be adversely affected (Cotterill, Cunningham & Funk, 1963).

The proposed test has been used by one of the United Kingdom processors and found satisfactory. In addition, the method was successfully used to uphold the suspicion that samples of imported egg-white had been pasteurized before entry into this country.

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(Received 8 December 1978)

J. Fd Technol. (1979) **14**, 389–404

Evaluating acceptability of fish minces and fish fingers from sensory variables

G. M. LASLETT* AND H. ALLAN BREMNER

Summary

The taste panel results from a number of similar, but separate experiments conducted on storage trials of minced flesh from Australian fish species were investigated using statistical methods to determine whether a relationship existed between the sensory variables scored by the panel and the acceptability of the samples. The important predictors of acceptability were flavour, a combination off-variable (off-aroma plus off-flavour) and texture in the case of minces, and off-variables and, to a lesser extent, flavour in the case of fish fingers.

Introduction

Evaluation of the acceptability of food products in terms of sensory variables is an important step in determining research priorities.

Acceptability of cooked fish is governed by numerous related factors. Some examples of these factors might be the species of fish, method of handling, the length of time in store, the temperature of storage, the method of cooking, the water holding capacity, the saltiness, and other flavours both natural and those produced during storage. An equation predicting acceptability could be determined in terms of such factors. The number of predictor variables may be large, and important variables may be omitted from the analysis. The scheme does not simulate the way people evaluate acceptability through the senses and thus would be difficult to interpret. This suggests that acceptability should first be related to sensory variables, and then the physical components of the important sensory variables could be determined afterwards. This two stage process has the advantage that many possibly unimportant variables are eliminated early in the evaluation process: if a sensory variable does not contribute substantially to acceptability, there is little point in investigating its physical

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or chemical components. The development of a relationship between acceptability and the sensory variables requires the determination of the form (e.g. linear, quadratic) of the relationship and the selection of the more important variables.

Kelly (1969) reported that while most of the work which had been done on frozen cod was concerned with texture deterioration, flavour was more important when the fish had a high pH (> 6.7) and recommended that more attention be given to the problem of the development of cold storage flavours. Following this Connell & Howgate (1971) assessed the relative importance of texture and flavour in determining the acceptability of frozen cod and haddock. They concluded that flavour was more important in determining the acceptability.

Horsfield & Taylor (1976) have explored the relationship between sensory data and acceptability of meat. Using the results obtained on a small sample of thirteen meat products they attempted to reduce the dimensionality of the parameter space by principal components followed by varimax rotation on the results, although the use of this technique is the subject of controversy (Francis, 1974).

Schutz & Damrell (1974) used multiple regression to assess the acceptability of twenty samples of rice (as judged by a consumer panel) in terms of fifteen sensory variables judged on the same rice samples by a trained taste panel. They attempted to eliminate the need for acceptability being non-monotonically (or non-linearly) related to some variables by artificially introducing complementary and overlapping variables, resulting in a somewhat clumsy regression. They then reduced the dimensionality by selecting some of the more important variables for inclusion. Multiple regression was also used by Connell & Howgate (1968, 1969) to analyse their results relating the acceptability of cod and haddock to flavour and texture, as judged on objective sensory scales by a trained taste panel.

The study reported here evaluates the relationship between six sensory variables and acceptability as scored by the same taste panel for sixty-nine fish minces – derived from sixteen species of fish – stored for varying periods. Similarly the same study was applied to thirty-five samples of fish fingers made from the minces. These data arose from a series of separate but similar exploratory investigations into the cold storage properties of fish minces made from a variety of Australian fish species (Bremner 1977a, 1978).

Methods

Materials and taste panel methods

Most of the sixteen species investigated were caught during exploratory midwater trawling off the New South Wales coast and were not otherwise available: some were, however, species that were of possible commercial potential.

The methods of processing, storage and sampling and the taste panel techniques have been reported previously (Bremner, 1977b, c; Bremner, Laslett &

Olley, 1978); however, some explanation, particularly of the taste panel method is necessary here. The fish were frozen whole, on board ship, in boxes which were later taken by refrigerated transport to the laboratory (in Hobart, Tasmania) where they were held at −18°C. The delay between catching and processing varied from about 4 to 6 weeks. After thawing the fish were beheaded, gutted, cleaned, scaled if necessary and then passed through a Bibun Model SDX13 meat separator. The minced flesh so produced was thoroughly mixed, then packed as 650 g blocks in aluminium trays. These blocks were frozen and held at −18°C for periods of up to 1 year. Blocks were withdrawn from store at intervals, thawed, subdivided and mixed, and two portions used for taste panel evaluation. Fish fingers were made from similar blocks.

The minces were heated for 1 hr in covered stainless steel beakers in a 60°C water bath. Individual samples were then served to the panel in pre-heated glass jars identified with abstract symbols. The fish fingers were presented to the panel on individual wire grids on which they had been heated in the oven for 45 min at 180°C.

The taste panel consisted of ten members of staff selected on the basis that they were willing to cooperate and were available for regular taste panel work over a long period of time. Eight members of this panel had previous taste panel experience and were familiar with sample evaluation and expression of the results on score sheets. The panel was asked to evaluate the samples for the attributes of fish aroma (AR), off-aroma (OAR), fish flavour (F), off-flavour (OF), toughness (T) and moisture (M), and to mark the appropriate sample symbol on the nine point objective scale (Table 1), structured at five points with descriptive terms.

In verbal instruction given to the panel the term ‘preferred texture’ was explained as ‘neither soft nor tough’. The interpretation of the term moisture has been discussed previously (Bremner, Laslett & Olley, 1978). No definite instruction on the difference between aroma and flavour and their respective counterparts off-aroma and off-flavour was given. The descriptions of off-aroma and off-flavour found in the food technology literature were presented to the panel and the choice left to them. Acceptability was measured on a nine point hedonic scale structured with five descriptive preference terms. The panellists were not asked, at the time of tasting, which variables they considered most affected acceptability.

The sample symbols were later converted from the sheets into numerical scores which were recorded on data cards. The same marking and scoring system was used for both the minces and the fish fingers. Two taste sessions, morning and afternoon with four samples per session, were held for each set of samples.

Statistical methods

All calculations were done using the GENSTAT computer package (Nelder *et al.*, 1977). The panel scores were examined for differences between panellists

Table 1. Numerical score corresponding to descriptive terms for each variable

Taste panel score	Fish aroma	Off-aroma	Fish flavour	Off-flavour	Toughness	Moisture	Acceptability
9	Very strong	Very strong	Very strong	Very strong	Tough	Very wet	Very good
7	Strong	Strong	Strong	Strong	Slightly tough	Wet	Good
5	Moderate	Moderate	Moderate	Moderate	Preferred texture	Normal moisture	Moderate
3	Weak	Weak	Weak	Weak	Slightly soft	Dry	Poor
1	Very weak	None	Very weak	None	Soft	Very dry	Very poor

by generalized Procrustes analysis (Gower, 1975) applied simultaneously to the seven scores. In this technique, the seven scores on m samples for a particular judge are regarded as a configuration of m points in seven dimensions, and the resulting configurations of all the judges are then compared (Banfield & Harries, 1975). Differences between panellists, samples and sessions were examined by analysis of variance, calculated separately for each variable.

The above techniques were applied to the original scores. For those below, taste panel mean scores adjusted for missing values estimated in the analysis of variance were used. This is a necessary consideration where differences between judges are significant, since the absence of a panellist who consistently scores toward an extreme of the scale would otherwise bias the results. These procedures were then repeated for each individual panellist's results.

Plots of means of acceptability scores against the sensory variable scores (Bremner, 1978) suggested a feasible initial model for predicting acceptability. Stepwise multiple regressions maximizing 'percentage variance accounted for' were calculated to determine the simplest predictive equation. 'Percentage variance accounted for' is equivalent to $100\ (1 - s_e^2/s_y^2)$ where s_e^2 is the residual mean square after fitting a regression model and s_y^2 is the variance of the acceptability scores. Robustness of the regression was checked by data-splitting (Snee, 1977).

An attempt was made to measure the strength of the predictor variables by doing all possible regressions, and recording the maximum and minimum change in 'percentage variance accounted for' by dropping each predictor in turn. Certain highly correlated variables were combined in this exercise, because it was impossible to distinguish their roles in determining acceptability.

The method of arriving at the predictive equation is described for the minces only; a similar procedure was used for the fish fingers.

For purposes of comparison, the largest subset of pairs of minces and fish fingers was selected on the basis of being made from the same material (both fresh and stored). For each variable, the correlation between minces and fish fingers was calculated. The best regression of acceptability of fish fingers on mince variables was found.

Results and discussion

(a) Fish minces

The Procrustes analysis revealed that the largest contribution (70%) to its analysis of variance came from the translation term (i.e. from panellists having consistently used different parts of the scale). Scaling (i.e. the range of the scale used) and rotation accounted for 18%, with the remaining 12% due to more complex differences and error. Procrustes rotation is, at present, a qualitative technique only, since no tests of significance are available. The results also suggested that there was little or no confusion between judges as to the

Table 2. Taste panel mean scores, maximum sample means, minimum sample means and residual mean square for fish minces

	Fish aroma	Off-aroma	Fish flavour	Off-flavour	Toughness	Moisture	Acceptability
Mean	4.47	3.33	4.53	2.92	5.29	4.67	4.68
Maximum sample mean*	6.11	5.41	5.94	4.85	7.83	6.42	6.76
Minimum sample mean*	3.18	1.88	2.38	1.53	1.05	3.09	2.12
Residual mean square	1.69	2.19	1.52	2.31	1.34	0.87	2.18

* These figures show the range of the scale (1–9) being used by the panel (as a whole)

Table 3. Correlation matrix between sensory variables for cooked fish minces (d.f. = 67)

	Acceptability	Fish aroma	Off-aroma	Fish flavour	Off-flavour	Toughness	Moisture
Acceptability	1.00						
Fish aroma	0.18	1.00					
Off-aroma	−0.52***	−0.15	1.00				
Fish flavour	0.49***	0.16	−0.25*	1.00			
Off-flavour	−0.57***	0.05	0.55***	0.02	1.00		
Toughness	0.11†	−0.01	0.07	0.44***	−0.01	1.00	
Moisture	−0.03†	−0.13	−0.13	−0.47***	−0.18	−0.76***	1.00

*, **, ***; significant at the 5% level (> 0.24), 1% level (> 0.31) and 0.1% level (> 0.39) respectively
† Strictly not applicable (see text), included for interest

meaning of the variables, or more intricate effects (cf. Banfield & Harries, 1975). Consequently, to a first approximation, the configuration of the panel mean scores was the same, apart from translation, as the configuration of each judge, and there was little to be lost in using the mean scores to determine the relationship between acceptability and the sensory variables. Analysis of variance on each of the seven variables showed significant differences ($P < 0.001$) between judges on all variables, supporting the conclusion that they were using different sections of the scale. Snell (1964) has discussed the application of analysis of variance to taste panel data.

Table 2 lists the mean of all samples, the maximum sample mean, the minimum sample mean and residual mean square for each variable. Quite wide variation occurred in intensity between minimum sample means and maximum sample means for all variables. The residual mean squares were quite high and were possibly due to high 'within sample variation', 'panellist × sample interaction' and scoring of variables on an integer, rather than a continuous, scale. A trained panel would probably have produced results with a lower residual mean square, but there were significant differences ($P < 0.001$) between the samples for all the variables. The panel means too, were sufficiently precise to give significance ($P < 0.05$) to a difference of approximately one unit on the nine point scale. The criterion that a change of one unit represents a 'just noticeable difference' has been suggested by Dahloff & Jul (1965).

Examination of plots of acceptability against the sensory variables revealed that acceptability increased linearly with flavour, and decreased linearly with off-flavour and off-aroma (Bremner, 1978). The plots of acceptability with moisture and toughness indicated that acceptability scores were higher in the middle of the range, which was to be expected since this accorded with preferred toughness and normal moisture being scored as five. No relationship with aroma could be discerned. Consequently a multiple regression model linear in aroma, off-aroma, flavour and off-flavour and quadratic in toughness and taste panel moisture was sought by the stepwise regression procedure.

Correlation matrix

The correlation matrix of acceptability and the sensory variables is given in Table 3.

Two variables may have a high correlation because of sampling covariation, and/or because they measure, in part at least, the same or opposite entity. Some of the latter cases may have occurred here: however, the low correlations between flavour and off-flavour, aroma and off-aroma indicated that the off-variables were not redundant. The significant correlation between off-aroma and off-flavour was not surprising since apart from the basic tastes of salt, sweet, sour and bitter (McBurney, 1974) it is the volatiles of the food stuff that are registered by the taster. Nevertheless there was only a low correlation between fish aroma and fish flavour and this may be due to loss of volatiles

before the samples were tasted. A different technique in which the samples are either served hotter or are being heated during evaluation may increase the importance of aroma (Shewan, personal communication).

Some correlations may be deceptively low because of non-linearity between variables. Examples of this were acceptability vs. taste panel moisture and acceptability vs. toughness. Graphs of all pairs of variables were drawn and this showed no other cases of non-linearity.

The high correlation between taste panel moisture and toughness has been discussed previously (Bremner *et al.*, 1978; Bremner & Snell, 1978).

Regression model

The maximum percentage variance that could be accounted for by the regression model was estimated from the analysis of variance to be 80%, the remaining 20% being due to uncontrolled variations in the data. The simplest model compatible with this error is summarized below:

$$A = -0.28 + 0.81\,F - 0.56\,OF + 1.46\,T - 0.15\,T^2 \quad (1)$$

$$= 3.00 + 0.81\,F - 0.56\,OF - 0.16\,(T - 4.53)^2 \quad (2)$$

where A is acceptability, F is flavour, OF is off-flavour and T is toughness. This model was consistent with the specified limit, since the percentage variance accounted for was 78%. Inclusion of other terms slightly increased the percentage variance accounted for, but resulted in an over-specified model. The regression coefficient of each predictor in (1) was significant at the 0.1% level, except for the constant.

Strength of effect of the variables

Although equations (1) and (2) were shown to be the simplest model, this does not imply that the other sensory variables were unimportant or irrelevant. Moreover it was difficult to determine the separate effects on acceptability of the sensory variables, because of correlations between them. An excellent account of the hazards of doing so is given in Snedecor & Cochran (1967) and by Kendall (1975) who suggests calculating all possible regressions, and choosing from the complete array. In this context moisture was found to be virtually indistinguishable from toughness, and off-flavour from off-aroma. Consequently, 'texture' was declared to be the single term encompassing the five variables (T, T^2, M, M^2, $T'M$) and 'off-variables' to be the term encompassing the pair of variables (OF, OAR) and these collective terms were included or dropped from the regressions as a single entity. This reduced the number of regressions to fifteen. The correlations (Table 3) between flavour and toughness, and flavour and moisture were high enough to cause some

problem in interpretation with this approach: however, even though deteriorative changes in flavour, toughness and moisture may be linked through the common variable of time in storage, there seemed no advantage in combining them here.

The minimum changes in percentage variance of acceptability accounted for by the addition of aroma, flavour, the off-variables and texture calculated from the regression analysis were −1, 12, 26 and 17% respectively. The corresponding maxima were 3, 32, 39 and 29%. It can be concluded that aroma had little or no influence on acceptability, but that all other variables did. They were not, however, particularly good predictors of acceptability on their own, or even, as further analysis showed, in pairs. The predictive equation seemed to require all three predictors, but the order of importance of these predictors was difficult to establish, because of the correlations between them.

The stepwise regression equation (1) includes flavour, off-flavour and toughness. Presumably toughness represents texture, and off-flavour the off-variables. The inclusion of both flavour and off-flavour again vindicated the scoring of both these variables. Flavour and toughness were both required despite the correlation between them; this was not surprising, since acceptability varies linearly with the former, and quadratically with the latter.

Robustness of the relationship

The robustness of the regression was checked by the technique of splitting the data into two parts (e.g. Snee, 1977). This was done in two separate ways according to two unrelated criteria.

For the first technique the data were split into two groups according to the chronological order in which the data were gathered. The two groups both contained data from freshly prepared material and from material which had been stored for several months at −18°C. Furthermore while the two groups contained some species in common, several species were present in the first group which were not present in the second group. The first 35 points were used to estimate a regression and the resulting equation was so similar to (1) and (2) that it is not recorded here. The final 34 points were used to validate this regression. The square of the correlation coefficient between the acceptability of the 34 final points, and their predicted acceptability from the regression of the 35 initial points was 0.80, which compares favourably with 0.79, the square of the multiple correlation coefficient (Snedecor & Cochran, 1967). These results suggest that the panel did not change its basis of judgment of the relationship of the sensory scores to acceptability over the near 2-year period of the investigations.

In the second approach the data were split according to the degree of judge absenteeism: 31 points in a 'deficient' set consisted of those session means which involved two or more absent panellists. The complementary 'complete' set with 38 points was used to calculate a regression, which again was very

similar to (1) and (2). The square of the correlation coefficient between the predicted acceptability of the inaccurate set and the recorded acceptability was 0.78, compared, as above, with 0.79 the square of the multiple correlation coefficient.

It was concluded that the predictive equation derived from the results of these ten panellists was reasonably robust, at least for the Australian species tested.

(b) Fish fingers

Table 4 lists the taste panel mean scores, maximum sample mean, minimum sample mean and residual mean square obtained on fish fingers.

Plots of panel means again suggested a model linear in aroma, off-aroma, flavour and off-flavour, and quadratic in toughness and taste panel moisture although the quadratic effect for toughness and moisture was not so evident from the results as for the minces.

The correlation matrix for the fish fingers is presented in Table 5. The correlation between off-aroma and off-flavour was even higher than for the minces. Furthermore aroma and flavour were now highly correlated. It is possible that the volatile aroma components were retained because of the batter and as a result were detected when the sample was chewed.

The stepwise regression produced a very simple equation for predicting acceptability

$$A = 5.28 + 0.47\,F - 0.75\,OF \qquad (3)$$

The equation accounted for 89% of the variance, against a specification limit of 90%. The coefficients of both variables were significant at the 0.1% level.

To determine the relative importance of the predictors, texture was again put as the five variables (T, T^2, M, M^2, $T \cdot M$) and the off-variables the pair (OF, OAR). The off-variables alone accounted for 85% of the variance of acceptability. The minimum change in percentage variance of acceptability accounted for by the addition of the off-variables was 53%, indicating that they were the dominant predictors of acceptability. The maxima due to aroma, texture and flavour were 10, 20 and 32% respectively, and this only when the off-variables were omitted. When the off-variables were included, these maxima became 2, 4 and 5% respectively. Consequently, the role of these variables as predictors of acceptability was minor when compared with that of the off-variables. Indeed, the omission of known important predictors like the off-variables will induce biases in the predictive equation (Snedecor & Cochran, 1967). In general, the influence of the included predictor variables will be increased through their correlations with the omitted variables. Hence, the maxima with the off-variables omitted were probably overestimates of the influence of aroma, flavour and texture and by the same token the maxima with the off-variables included were underestimates.

Table 4. Taste panel mean scores, maximum sample means, minimum sample means and residual mean square for fish fingers

	Fish aroma	Off-aroma	Fish flavour	Off-flavour	Toughness	Moisture	Acceptability
Mean	4.03	2.78	4.81	2.85	5.38	4.59	5.40
Maximum sample mean*	5.33	6.75	6.22	7.45	6.49	5.50	6.75
Minimum sample mean*	3.02	1.49	2.98	1.40	3.90	3.46	1.25
Residual mean square	1.54	1.59	1.71	1.91	0.94	0.62	2.09

*These figures show the range of the scale (1–9) being used by the panel (as a whole)

Table 5. Correlation matrix between sensory variables for cooked fish fingers (d.f. = 33)

	Acceptability	Fish aroma	Off-aroma	Fish flavour	Off-flavour	Toughness	Moisture
Acceptability	1.00						
Fish aroma	0.06	1.00					
Off-aroma	−0.87***	−0.05	1.00				
Fish flavour	0.47**	0.66***	−0.40*	1.00			
Off-flavour	−0.90***	0.17	0.81***	−0.18	1.00		
Toughness	−0.26†	0.23	0.35*	0.30	0.42*	1.00	
Moisture	0.20†	−0.43**	−0.32	−0.35*	−0.33	−0.73***	1.00

*, **, ***; significant at the 5% level (> 0.34), 1% level (> 0.43), and 0.1% level (> 0.53) respectively

† Strictly not applicable (see text), included for interest

Data splitting, by using the same criteria as for the minces confirmed equation (3). But when the data were split by chronological order into 18 estimation points and 17 validation points the agreement between the square of the correlation coefficient (predicted vs. observed acceptability) from the data splitting (0.62) and that from the multiple regression (0.92) was not as good as in the case of the minces. This was because the validation set (by chance) contained no points near the extremes of acceptability. The actual difference between predicted and observed acceptability however, was below 1 scale unit for all the 17 points, a satisfactory situation.

The stepwise regression included off-flavour, and one of the minor variables, flavour, whose correlation with off-flavour was low. Off-flavour presumably represented the off-variables, and flavour may have incorporated some of the influence of aroma, since their correlation was 0.66.

Analysis of each taster's results

The results for each taster were analysed for their similarities and differences. The correlation matrices for each taster varied and hence no single matrix, which could represent the whole panel, could be constructed from them. The median values of the correlation coefficients between the variables for the ten individual panellists were close to the results in Table 3 for the panel means, except in three instances all involving the texture variables. The correlations between moisture and flavour ranged from −0.29 to 0.24 (cf. −0.47 Table 3), and the correlations between toughness and flavour ranged from −0.15 to 0.43 (cf. 0.44 Table 3). While the median correlation between moisture and toughness was not as high as for the panel means (−0.76 Table 3), eight individuals showed substantial negative correlations between the two variables, ranging from −0.91 to −0.34.

All ten tasters showed negative correlations between acceptability and off-flavour (−0.78 to −0.17), between acceptability and off-aroma (−0.68 to −0.11) and positive correlations between acceptability and flavour (0.06 to 0.87) and off-aroma and off-flavour (0.27 to 0.76). The only other substantial correlation, was that between toughness and moisture.

Performing all possible regressions for each taster revealed that strengths of effect of the sensory variables were different for different tasters. A sensory variable was defined as 'of major importance' if its maximum percentage variance accounted for was greater than 10%, 'of minor importance' otherwise (no maxima occurred in the range 10−17%). A sensory variable was defined as dominant for a taster if its minimum percentage variance accounted for was greater than the maximum of any of the other variables. On these bases flavour was major for eight tasters (dominant for two tasters), the off-variables were major for nine tasters (one dominant), texture was major for seven tasters and aroma was major for two tasters.

Despite quantitative differences between tasters, qualitative conclusions from the individual results were virtually the same as from the panel means.

For the fish fingers the medians of the correlation coefficients between the variables for the ten individual panellists were similar but slightly lower than the results for the means listed in Table 4, apart from those involving toughness and moisture. All tasters had negative correlations between acceptability and off-aroma (−0.76 to −0.08) and acceptability and off-flavour (−0.90 to −0.41), and positive correlations between flavour and aroma (0.28 to 0.72), and off-flavour and off-aroma (0.48 to 0.89). The association between flavour and off-aroma was negative and weak (−0.62 to 0.03), as in Table 5. Of the correlations involving texture, that between toughness and moisture was the only one which held convincingly for most tasters (−0.71 to 0.03). All other correlations were weak, and varied in sign from taster to taster.

In doing all possible regressions for each taster, aroma and flavour were combined because of their positive correlation. All tasters had the off-variables as of major importance (four dominant), flavour and aroma were of major importance for nine tasters (one dominant) and texture was of major importance for five tasters.

The off-variables were confirmed as the most important predictors of acceptability of the fish fingers. The panel means did seem to underestimate the importance of flavour and aroma.

(c) Comparison between fish minces and fish fingers

The predictive equation (2) found for minces was different from that found for fish fingers (3) and there were some differences in their respective correlation matrices (Tables 3 and 5).

The equations and correlations were derived from different data sets, 69 scores in the case of minces and 35 in the case of fish fingers. Furthermore the fish minces and fish fingers were scored on the same scale system but the scores themselves were not necessarily comparable since the panel was asked to mark the acceptability of the samples as representatives of the form in which they were presented (i.e. as minces or fish fingers). Fish fingers are universally more acceptable than fish minces so that an acceptability score of five for both did not mean that they were equally acceptable as foods. Moreover, no session was conducted scoring the two forms together. Strictly speaking it was thus not admissible to examine the reasons for the differences in the equations. Nevertheless, it is of great practical importance to ascertain whether taste results obtained on minces can be used to predict the acceptability of products made from the minces, in this case fish fingers. Therefore, the largest possible subset of comparable fish minces and fish fingers was selected from the total data set on the basis of being made from the same material, whether fresh or stored. This subset comprised 22 data points derived from fourteen species, eight of which had repeat samplings.

Stepwise regressions on these subsets produced two equations:

Minces $$A = 2.53 + 0.90\,F - 0.45\,OF - 0.23\,(T - 4.48)^2 \quad (4)$$

Fish fingers $$A = 4.82 + 0.53\,F - 0.72\,OF \quad (5)$$

These were similar to the previous equations (2) and (3) respectively.

Examination of the interquartile ranges (Table 6) for the minces and fish fingers indicated that the scores for the minces covered a wider portion of the scale than for fish fingers. This was particularly the case with off-aroma, flavour, off-flavour and toughness scores. Toughness may not have entered the equation for fish fingers since its range (3.9 to 6.5) is less than with the minces (3.2 to 7.3).

Correlations between minces and fish fingers for each variable are presented in Table 6. Those for acceptability and off-flavour were not high. The latter is of particular concern, since off-flavour was the best single predictor of acceptability of the fish fingers. Further, a stepwise multiple regression analysis (on

Table 6. Inter-quartile range and correlations for seven sensory variables from twenty-two comparable pairs of minces and fish fingers

	Inter-quartile range		Correlations
	Mince	Fish fingers	(d.f. = 20)
Fish aroma	0.75	0.67	0.60**
Off-aroma	1.41	0.66	0.61**
Fish flavour	1.37	0.91	0.55**
Off-flavour	1.52	0.83	0.45*
Toughness	1.52	0.79	0.65**
Moisture	0.82	0.70	0.71***
Acceptability	0.78	0.89	0.41

*, **, ***; significance at 5% level (> 0.42), 1% level (> 0.54) and 0.1% level (> 0.65) respectively

the panel means), using the sensory variables obtained on the subset of minces to predict the acceptability of the fish fingers made from them, resulted in a non-significant regression in which the variance accounted for was only 18%. This was primarily because the lowest correlations in variables between minces and fish fingers were to be found in those particular variables which most influenced the prediction of acceptability of fish fingers. For some individuals there were insufficient data points (due to absenteeism) to determine a reliable correlation between fish finger acceptability and mince variables.

The above results suggest that taste panel work to determine acceptability must be done on the finished product, not on the raw material. A similar conclusion was reached by Ruello & McBride (1973) in work on two different species of prawns which were compared as boiled prawns or as fried prawn cutlets. A reversal of taster preference was found according to the manner of presentation of the product. This does not exclude the possibility of putting the relationships of the individual variables to predictive practical use where the correlations are high, as for example with moisture.

Conclusions

This paper outlines an approach for analysing taste panel data and discusses the problems involved in unravelling the effects of (at times) related sensory variables on the acceptability of fish minces and fish fingers.

For fish minces, it was inferred that

(a) off-flavour and off-aroma were not correlated with their complements flavour and aroma, and hence it was appropriate to score both in the taste trial,

(b) in this study aroma had little effect on acceptability although in different experimental circumstances it may prove important and

(c) texture, the off-variables and flavour were the important predictors of acceptability, particularly in combination.

For fish fingers, it was inferred that

(a) the off-variables were the most important predictors of acceptability, with off-flavour alone a very good predictor and

(b) aroma, flavour and texture were poor predictors of acceptability.

Overall, it could be concluded that taste panel work to determine acceptability of fish fingers should not be done on the minces.

The regression relationships contained in this paper will not apply to samples where the high correlations reported here, are not maintained, nor to any other situation outside the scope of this work. Nevertheless, the implication of this work is that off-aroma, flavour and off-flavour are just as important in determining acceptability of frozen fish products as texture variables. Much of the research work on fish has, however, been directed towards determining changes in textural qualities. Textural changes lend themselves to controlled measurement by objective methods whereas the detection of flavour changes is difficult and relies on the use of sophisticated analytical equipment (cf. McGill, Hardy & Butt, 1974), or taste panels. At present, the taste panel is the most useful and versatile tool for this work.

Acknowledgments

The authors wish to acknowledge the skilled technical assistance of Ms A. Wright and Ms K. Hales in conducting the taste panels and of Ms E. Longmore in data processing. The useful and stimulating discussions with Dr June Olley are also gratefully acknowledged. The work was supported by a grant from the Fishing Industry Research Trust Account.

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(Received 3 January 1979)

J. Fd Technol. (1979) **14**, 405–419

Development of an emulsion-based product from minced silver carp in Israel*

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Summary

Minced whole fish, fillets, remains after filleting, and connective tissue of silver carp were employed in various combinations to produce an emulsion-based product. A fairly firm-textured emulsion product could be obtained with 30% minced whole silver carp + 70% minced or ground fillets. The remains after filleting gave the poorest products in any combination with the other kinds of meat.

It is concluded that experiments need to be carried out with mixtures of fish species in order to increase the amount of whole fish mince that can be used in emulsion-based products and perhaps also to enable the inclusion of minced leftovers after filleting of silver carp.

Introduction

In continuation of work on the development of further processed products from fish in Israel (Angel & Baker, 1977), investigations are reported on the production of fish bologna sausages from whole and/or parts of silver carp fish. Tanikawa (1963) reported on the production of fish sausages in Japan, and frozen minced fish is used to produce Surimi from which sausages and other products are made (Fisheries Research Board of Canada, 1972). However, as Grabowska & Silorski (1976), Karmas & Turk (1976) and others have claimed, the leftover parts of fish after filleting lack water-binding properties, and sausages made from mechanically deboned (minced) fish flesh often lack the desired texture.

The aim of these investigations was to utilize silver carp carcases minced after filleting – alone or in combination with ground or minced whole fish or fillets – to produce a good-textured sausage.

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*Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. 1978 series no. 263-E.

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Materials and methods

Raw material

One to 3-day-old whole iced silver carp were bought on the local wholesale market, brought to the laboratory, cleaned, eviscerated, headed, skinned, and then kept overnight at 0°C. Some of the fish were filleted by hand, and the fillets and frames were also kept overnight at 0°C.

After overnight storage at 0°C, the following operations were performed:

(a) Some of the fillets were ground in a precooled grinder with 3 mm end plate. These were referred to as 'ground fillets'.

(b) The rest of the fillets were passed through a small meat/bone separator (Yanagiya Meat Bone Separator). These are referred to as 'minced fillets'.

(c) The tissue remaining on the external surface of the deboner screen was collected, separated from bones and tendons by hand, spread on a tray, frozen until stiff, and ground. This is referred to as 'connective tissue'. Protein level of the 'connective tissue' and ground fillet was determined by micro-Kjeldahl, and hydroxyproline according to the international standard ISO method (ISO/DIS 3496-2, 1978).

(d) Whole fish passed through the deboner is referred to as 'MDF-whole'.

(e) Remains after filleting were mechanically deboned. This is referred to as 'MDF-fr.' (MDF-frames).

The meat from the five kinds of raw material was used for the preparation of bologna, an emulsion-type product. Samples contained the following raw silver carp fish meat combinations:

100% ground fillet
100% minced fillet
70% ground fillet + 30% MDF-whole
70% ground fillet + 30% MDF-fr.
70% minced fillet + 30% MDF-whole
70% minced fillet + 30% MDF-fr.
100% MDF-whole
100% MDF-fr.
80% MDF whole + 20% 'connective tissue'
80% ground fillet + 20% 'connective tissue'
80% MDF fr. + 20% 'connective tissue'
80% minced fillet + 20% 'connective tissue'

Product preparation

The meat at 0°C was chopped in a silent cutter (Scharfen, Germany), in an air-conditioned room, for 3 min (with two 30-sec pauses for cleaning the flesh which clung to the cover). The following ingredients were added per kg: 20 g NaCl, 5 g Kena, 5 g sodium glutamate, 2 g potassium sorbate, 0.4 g sodium

erythrobate, 0.1 g sodium NO_2, 3 g garlic powder, and 100 g hydrogenated soybean oil (slip point 34%). The emulsion which was obtained was stuffed (with a manual stuffer) into cellulose casings, and stored overnight at 0°C. The next day the products were brought to room temperature and cooked in a smoker (Afos-mini Kiln), without smoke, at 82°C, to an internal temperature of 67–68°C as determined by a thermocouple. The products were cooled and kept at 0°C. Five sausages were prepared for each treatment combination.

Moisture and fat content of the five kinds of raw meat were determined with an Ultra X-70 apparatus (Groner, West Germany). The basis for this determination is evaporation of moisture using infrared radiation, and then extracting the lipids with petrol ether, and evaporating the solvent with the infrared heat source.

Shear press determinations were made using a Lee Kramer Shear-Press. Following cooking and cooling to 0°C the bologna-type products were sliced (5 mm thick) and arranged in one layer inside a Kramer Shear Cell. Ram speed was 0.11 cm/sec, and determinations were made using a 250 lb ring. Duplicates were carried out in each case. The dial on the proving ring had 20 divisions, with each division equivalent to 1.9 lb/in^2.

Light micrographs of the products were made by preparing frozen blocks in a Slee Cryostat Microtome. Sections of 10 μm were mounted on slides and stained by Milligam's Trichrome stain (Humason, 1967) for proteins, and with Oil Red O or Sudan Block B for lipids. The slides were photographed with a Nikon research microscope using an AFM camera.

Scanning electron micrographs of the samples were prepared by dehydrating blocks of 5 mm thickness through a series of alcohol concentrations and then through acetone. From these blocks small samples were dried in a critical point dryer (Polaron, England), gold coated, mounted on stubs and observed at low and medium magnifications in a Cambridge 180 scanning electron microscope. Photographs were taken from the CRT with a Nikon 35 mm camera.

Statistical analysis

Results were analysed according to the Duncan Multiple Range Test (Duncan, 1955).

Results and discussion

Table 1 shows the moisture and fat contents of the different kinds of raw materials. The results are means of four experiments performed in duplicates.

Only the connective tissue differed significantly ($P < 0.05$) from the other meats in moisture and fat content. The hydroxyproline content of the 'connective tissue' was 2.2% of the total protein N as compared with 0.63% for the ground fillet, thus verifying the fact that this was collagenous tissue.

Table 1. Moisture and fat content of the meats

Meat sample	Moisture (%)	Fat (%)
Ground fillet	75.5	6.4
Minced fillet	78.8	3.1
MDF-whole	78.2	5.0
MDF-fr.	78.3	5.4
Connective tissue	66.8	15.0

Table 2. Shear press values of the tested fish products

Treatment	Shear press units
100% MDF-fr.	0.85
80% MDF-fr. + 20% connective tissue	0.89
70% Minced fillet + 30% MDF-fr.	0.89
100% MDF-whole	0.91
80% MDF-whole + 20% connective tissue	1.80
70% Minced fillet + 30% MDF-whole	1.90
70% Ground fillet + 30% MDF-fr.	1.95
70% Ground fillet + 30% MDF-whole	2.20
100% Ground fillet	2.80
80% Ground fillet + 20% connective tissue	2.86
100% Minced fillet	3.48
80% Minced fillet + 20% connective tissue	3.53

Ground fillet contained 6.4% fat and minced fillet 3.1% fat; due to the variance between samples these differences were not significant (at $P < 0.05$).

Minced fillet had half the fat content of ground fillet, while 'connective tissue' had five times as much fat as the minced fillet. Thus, the fat of silver carp fillet which is minced in the deboning machine is lost together with the 'connective tissue' during mechanical deboning. Whole minced fish (MDF-whole) or parts left after filleting (MDF-fr.) had a somewhat higher fat content than minced fillet. This is in agreement with the findings of Angel & Baker (1977). There was no significant difference between 3.1% fat of the minced fillet and 5.0% to 5.4% fat for the MDF and MDF-fr.

Table 2 presents the list of the products in order of increasing mean shear press values (each value being the mean of five sausages, which were tested twice).

The differences between values within groups bracketed by the same vertical line are not significant, and values below that line are significantly different ($P = 0.05$).

The 80% MDF-whole + 20% connective tissue product had a value of 1.80 as compared with values of 0.85 and 0.91 for 100% MDF-fr. 80% MDF-fr. + 20% connective tissue, 70% minced fillet + 30% MDF-fr. and 100% MDF-whole, and the resistance to shear of 80% MDF-whole + 20% connective tissue was significantly higher than that of the other products. 100% ground fillet has a value of

2.80 and was siginficantly more resistant to shear than the 80% MDF-whole + 20% connective tissue (1.80).

Shear values between 2.86 and 3.53 were obtained for various ground and minced fillet products of silver carp. These were not significantly different from the value of 2.80 obtained with the 100% ground fillet. However, 100% minced fillet and 80% minced fillet + 20% 'connective tissue' were highly significantly more resistant to shear than 70% ground fillet + 30% MDF-whole or any of the MDF products (which ranged in values from 0.85 to 2.20).

Figures 1–16 are microphotographs of the products and raw material from which fish bologna was prepared. Figures 1–7 and Figs 13–16 show the emulsions of the various products. The best emulsions can be seen in Figs 1, 2, 3, 13 and 14 Figures 1, 2 and 3 are 70% minced fillet + 30% MDF-whole, 70% ground fillet + 30% MDF-whole, and 80% MDF-whole + 20% connective tissue, respectively. Figures 13 and 14 are SEM preparations of 70% minced fillet + 30% MDF-whole and 80% minced fillet + 20% conn. tissue. The photographs show a fine protein matrix and small, well dispersed fat droplets. The microstructure of the matrix of good emulsion has previously been demonstrated by Theno & Schmidt (1978), who photographed sections of commercial frankfurters and compared them with SEM preparations.

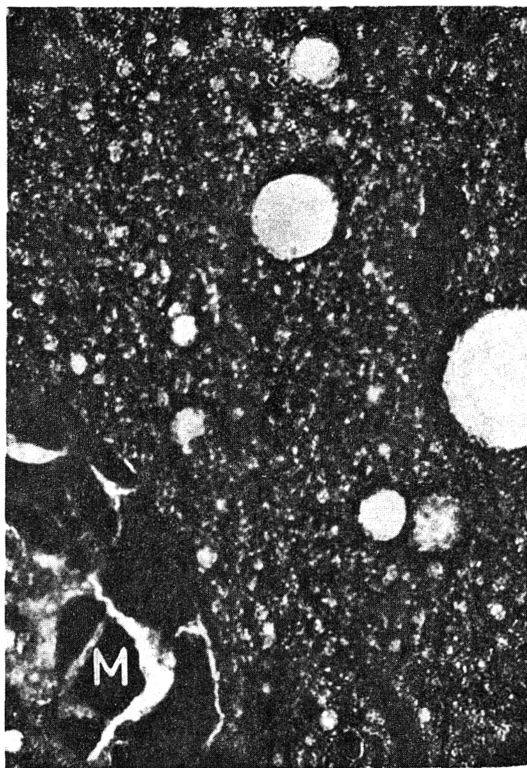

Figure 1. 70% minced fillet + 30% MDF-whole. M = unchopped muscle.

Figures 1–7 are light micrographs of 10 μm cryosections × 100, stained for proteins.

Figure 2. 70% ground fillet + 30% MDF-whole. M = unchopped muscle.

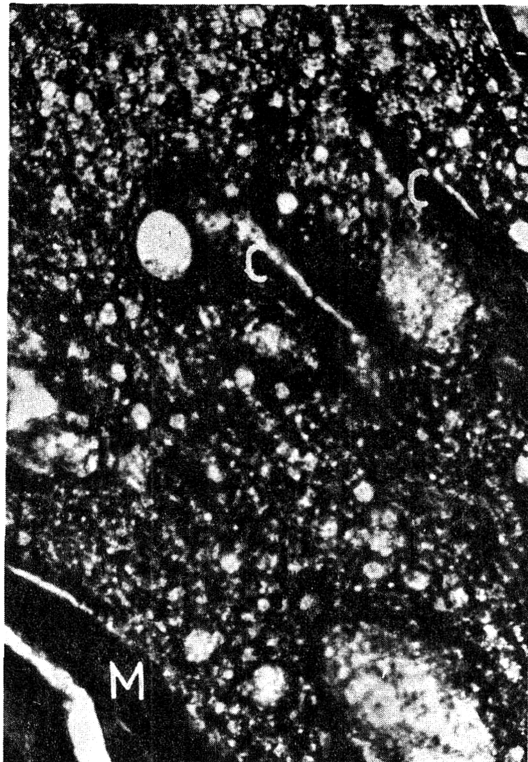

Figure 3. 80% MDF-whole + 20% connective tissue. M = unchopped muscle; C = connective tissue.

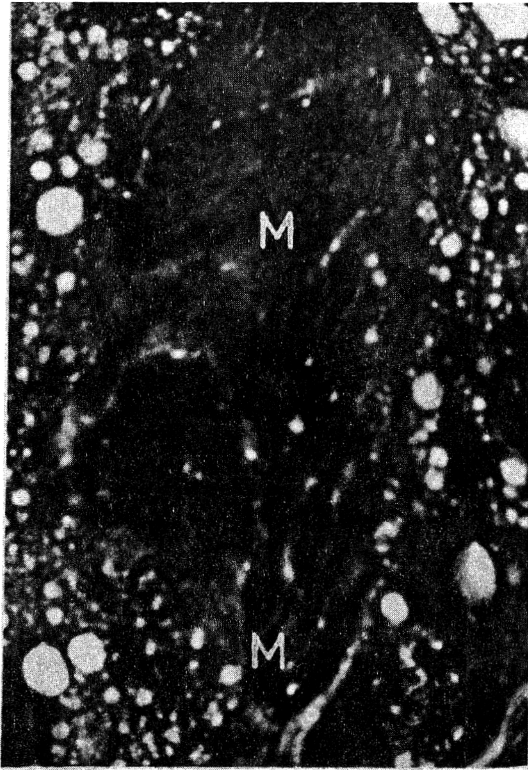

Figure 4. 100% ground fillet. M = unchopped muscle.

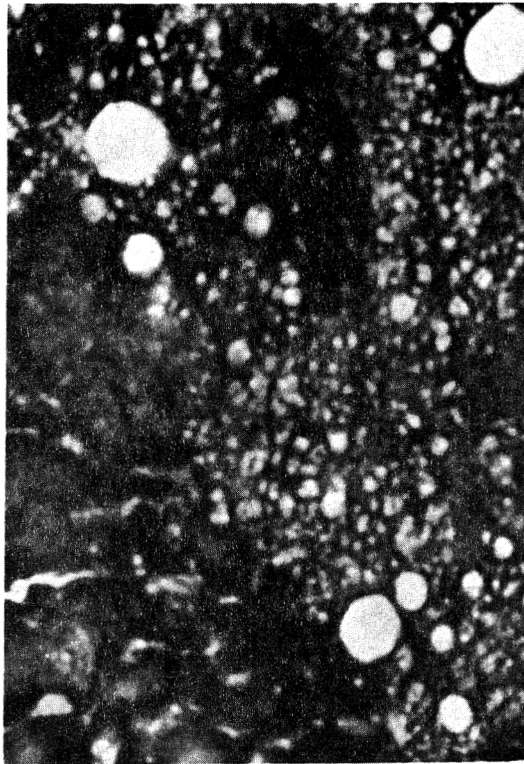
Figure 5. 70% ground fillet + 30% MDF-whole.

Figure 6. 100% MDF-fr.

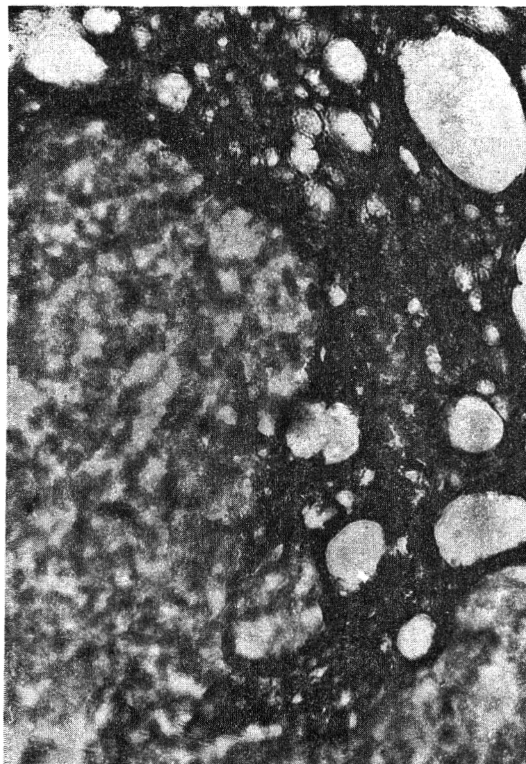

Figure 7. 80% MDF-fr. + 20% connective tissue.

Fairly good emulsions can be observed in Figs 4 and 5 (100% ground fillet, and 70% ground fillet + 30% MDF, respectively). The poorest emulsions were obtained with 100% MDF-fr. (Fig. 6) and 80% MDF-fr. + 20% connective tissue (Fig. 7). In the figures with the poor emulsions one can see large areas of un-chopped muscle and 'connective tissue'. Figure 15 is a SEM preparation of the 80% MDF-fr. + 20% 'connective tissue'. Figure 16 is an enlargement of one area of the collagen fibres.

Figures 8–12 are micrographs of the raw material used in the preparation of the products. Figure 8 (MDF-fr.) shows that the muscles were for the most part crushed and lost their identity. In Fig. 9 (MDF-whole), the muscles retained their character, but show signs of compression. Figure 10 and 11 are cross-sections of the muscle fibres of the ground and minced fillets, respectively. There was elongation of the muscle fibres in the ground fillets, and the minced fillet fibres were elongated, compacted and distorted. Figure 12 shows distorted collagen fibres from the 'connective tissue' separated from the minced fillet.

There was fairly good agreement between an increase of shear press value and emulsion quality as observed by the micrographs. Products prepared from MDF silver carp leftovers after filleting, whether wholly from MDF-fr. or mixed MDF-fr. and minced and ground fillet, had lowered shear values and gave poor

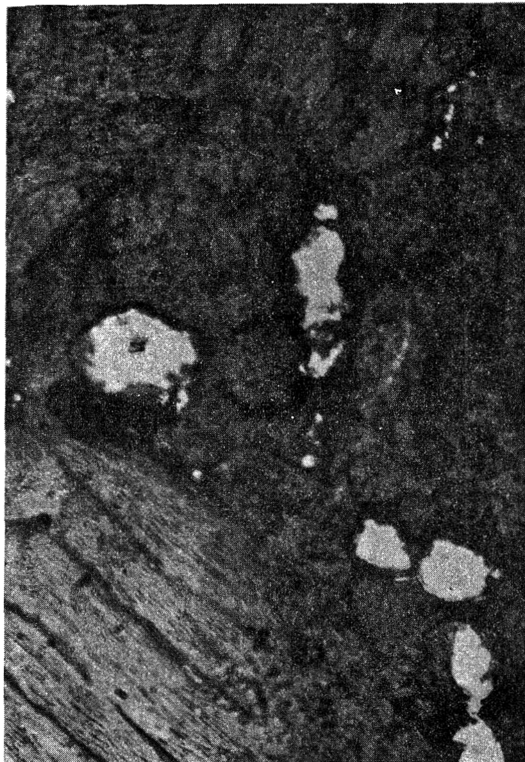

Figure 8. MDF-fr.

Figures 8–12 are light micrographs of 10 μm cryosections of raw material used in the bologna products, stained by toluidine blue × 200.

Figure 9. MDF-whole.

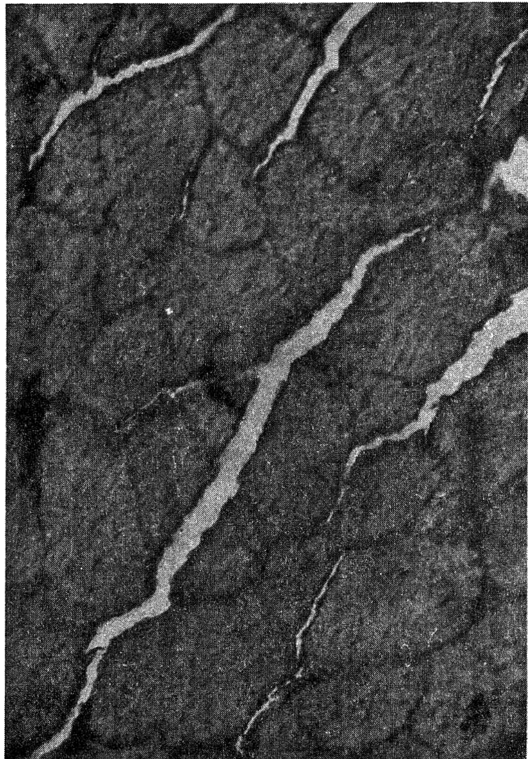

Figure 10. Ground fillet.

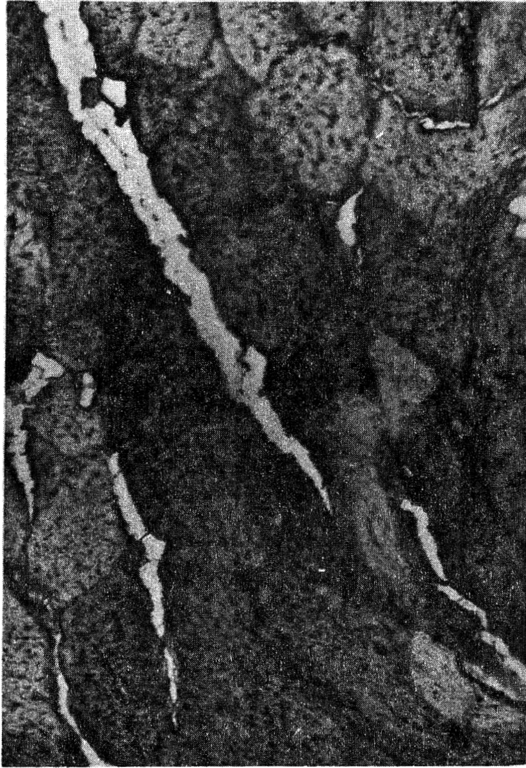

Figure 11. Minced (mechanically deboned) fillet.

Figure 12. Connective tissue. ×400.

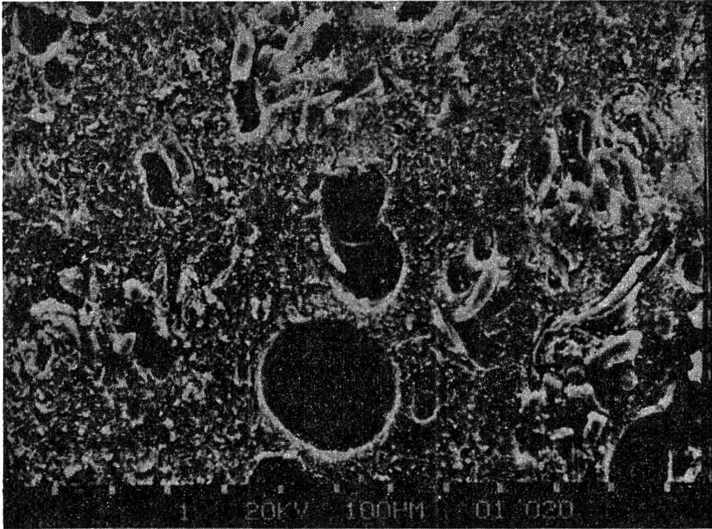

Figure 13. 70% minced fillet + 30% MDF-whole. x 100.
Figures 13–16 are SEM micrographs of fish bologna products.

emulsions, while products from MDF of whole silver carp gave higher shear press values. The best emulsion formations appeared with the minced fillets and these also gave the highest shear values. Addition of 'connective tissue' in all cases increases the shear press values of the corresponding products. Bologna prepared from 100% MDF-fr. or 100% MDF had a lower shear value than the corresponding product mixed with ground or minced fillets.

In some isolated cases there was no agreement between increase in shear press value and the quality of the emulsion as seen in the micrographs. This may have been due to randomly dispersed connective tissue, which increases

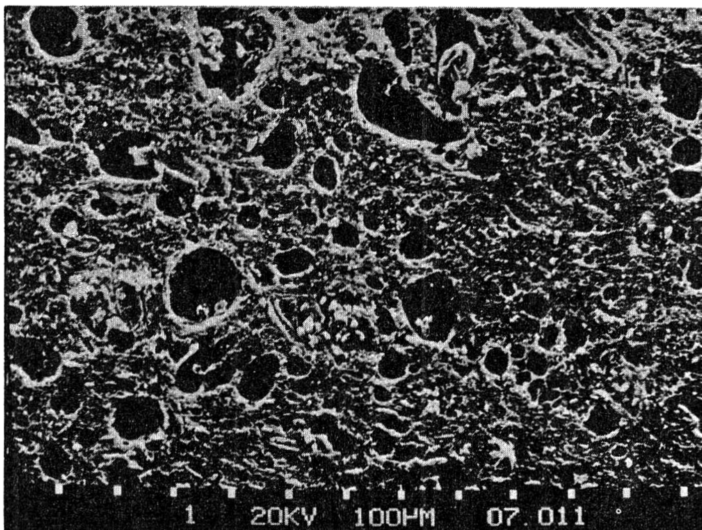

Figure 14. 80% minced fillet + 20% connective tissue. x 100.

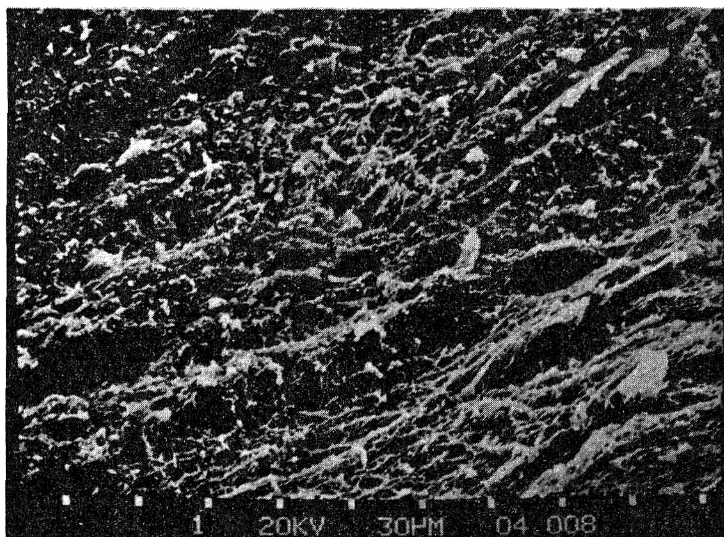

Figure 15. 80% MDF-fr. + 20% connective tissue. ×400.

the resistance to shear. In the vicinity of the connective tissue (which had the highest fat content), there were cases where emulsion formation was improved (Fig. 3).

From the proximate composition of MDF-whole and MDF-fr. in Table 1, there is no explanation for the higher shear value of MDF-whole over MDF-fr., or for the differences between ground and minced fillet. However, during the deboning process it was observed that the MDF-fr. was very loose textured and watery, while the MDF from the whole fish (and the fillets) was more rigid and could, for instance, be rolled into a ball. An explanation for this can be found in the literature (Grabowska & Silorski, 1976). Although the moisture

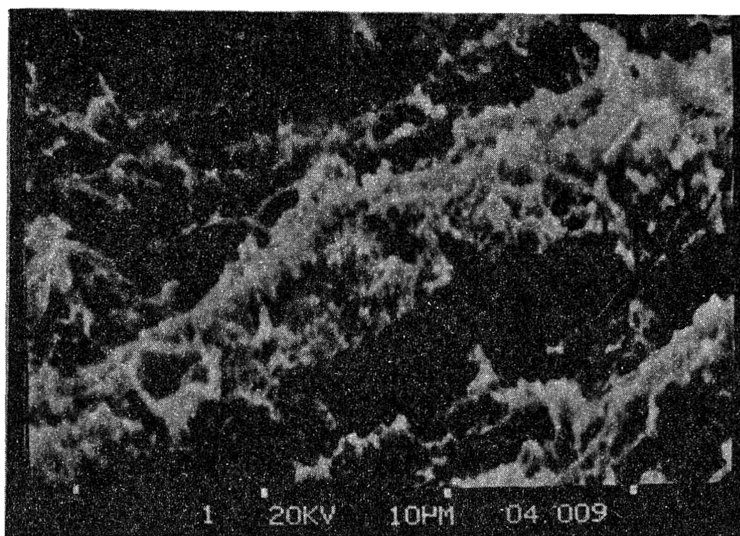

Figure 16. 80% MDF-fr. + 20% connective tissue (collagen fibre). ×3000.

content of the MDF and MDF-fr. did not differ (see Table 1), the ability of the protein in the MDF-fr. to hold water appeared inferior to that of the whole fish and fillets, and the water separated following mincing. The nature of the fish protein from the various raw materials is now the subject of investigation.

Conclusions

The above results indicate that minced silver carp could be used at a level of up to 30% to prepare the emulsion-based product with either ground or minced fillet.

There was no significant difference in shear press value for the products from the mixtures between 70% ground fillet + 30% MDF-whole and 70% minced fillet + 30% MDF-whole (see Table 2). In order to avoid possible bone fragments, minced fillets could be used for the preparation of such products.

The above proportions of MDF-whole and minced fillet produced an emulsion-type product of satisfactory texture, requiring no addition of non-fish binders.

It is possible that, minced meat from various fish species could effectively be mixed to produce strong-textured emulsion-type products. The use of such a mixture might conceivably increase the proportion of MDF-whole that could be mixed with ground or minced fillets to produce firm emulsion-based products.

The use of MDF from several species might also enable a certain amount of minced fish prepared from the leftovers after filleting where such operations would exist, to be utilized to prepare emulsion products without adversely affecting their texture.

Acknowledgments

This research was supported by a grant from the United States – Israel Bi-national Science Foundation (BSF), Jerusalem, Israel.

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(Received 5 January 1979)

J. Fd Technol. (1979) **14**, 421–428

Preliminary studies on the storage stability of intermediate moisture beef formulated with various water binding agents

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Summary

Samples of intermediate moisture beef (water activity ≈ 0.83) were prepared by 'moist-infusion' using different combinations of water binding agents (or humectants) and stored for 60 days at 27 and 34°C. Humectants utilized included glycerol, sodium chloride and sucrose. Microbiological analyses were performed on samples after storage at 34°C and the results indicated good microbiological stability.

No significant loss of available lysine – as measured by the fluordinitrobenzene method – was observed in any of the intermediate moisture beef samples after the storage period either at 27 or 34°C. The possibility of sucrose hydrolysis during storage, in sucrose-containing samples, was also investigated. The results indicated a lack of significant sucrose hydrolysis over the period of storage considered.

Introduction

In recent years with the advent of intermediate moisture pet foods there has been a resurgence of interest in intermediate moisture foods (IMF) for human consumption. However, with the prospects for the development of IMF for human nutrition there is a great need for studies on the quality and mechanisms of deterioration of such foods. A good example of such studies may be found in a series of papers by Obanu, Ledward & Lawrie (1975a, b, c) who have studied the state of the proteins of glycerol-salt infused IM meat during storage.

Nonenzymic browning is specially important in IMF because the rate of this reaction usually has a maximum in the range of water activity (a_w) corresponding to IMF; e.g. 0.65–0.85 (Labuza, 1972; Lea, 1958; Loncin, Bimbenet

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& Lenges, 1968). Nonenzymic browning can take place when reducing sugars and proteins react in the presence of water to form brown pigments. This results in the production of off-flavours and loss of solubility and protein nutritional value (Lea, 1958; Schnickels, Warmbier & Labuza, 1976). This loss of protein nutritional value is mainly due to reactions involving primarily the ϵ-amino groups of lysine. Several solutes also called humectants are usually incorporated in IMF to lower the a_w to the desired range. These water binding agents or humectants include polyols, sugars and salts (Kaplow, 1970; Bone, 1973; Heidelbaugh & Karel, 1975) and may be incorporated in the food by different techniques (Brockmann, 1970; Heidelbaugh & Karel, 1975). Because of the nonenzymic problem the use of reducing sugars as water binding agents, such as corn syrup solids, is not convenient for IMF formulations. Schnickels *et al.*, (1976) and Warmbier, Schnickels & Labuza (1976) showed that a significant loss of available lysine occurred in IM model systems containing glucose and proteins when stored up to 2 months at 35 or 45°C.

The purpose of the present work is to study the loss of available lysine in stored IM beef prepared by 'moist-infusion' using different combinations of humectants. These humectants included, glycerol, sucrose and sodium chloride. The role of sucrose in the susceptibility of protein beef to nonenzymic browning damage was particularly considered.

Experimental part

Preparation of IM beef ('moist-infusion')

Post-rigor beef trimmed of visible fat and connective tissue was cut into small pieces of about 1.5 × 1.5 × 1.5 cm. The pieces were immersed in different infusing solutions containing predetermined amounts of various solutes and water to give after equilibration a water activity of about 0.83. Table 1 gives the composition of the infusing solutions. The beef pieces were cooked in the infusing solutions for 15 min at 85°C. Heat was then removed and the beef pieces allowed to equilibrate overnight at room temperature. A small amount

Table 1. Composition of the infusing solutions

Component	Percentage weight in infusing solution							
	1	2	3	4	5	6	7	8
Sodium chloride	25.4	14.9	14.9	19.9	10.9	25.8	25.8	11.0
Glycerol	–	–	–	23.8	43.6	12.1	–	21.4
Water	74.1	34.8	34.6	55.7	44.7	61.5	61.5	35.9
Sucrose	–	49.7	49.7	–	–	–	11.9	30.9
Potassium sorbate	0.53	0.59	0.59	0.60	0.60	0.60	0.60	0.60
Sodium bisulphite	–	–	0.2	–	0.2	–	0.2	0.2
Weight ratio: $\frac{\text{solution}}{\text{beef}}$	3.0	2.0	2.0	2.0	2.0	1.72	1.82	2.0

of citric acid was added to adjust the pH to the range 5.5–5.8. Potassium sorbate was added to all solutions as antimycotic agent. Sodium bisulphite (browning inhibitor) was also added to some infusing solutions after they were cooled at room temperature. At the end of the equilibration period the pieces of beef were drained for 10 min and analysed for water activity, pH and moisture content.

Moisture-proof pouches (Trenel, Ducilo) were packed with about 400 g of IM beef without vacuum, heat sealed, and stored at 27 and 34°C in constant temperature rooms for 60 days. At the end of the storage period the samples were removed and analysed for available lysine, total and reducing sugars and microbial count. Control samples were stored in a freezer at –30°C.

Analysis

Water activity of the IM samples was determined at 27°C by an electric hygrometer indicator (Hygrodynamics, Inc.). The sensor was checked against standard salt solutions for accuracy. Various precautions such as adequate temperature control, equilibration time, etc. were taken in order to obtain accurate determinations of a_w (Labuza *et al.*, 1976).

The pH of homogenates of the IM samples in distilled water was measured with a glass electrode.

Moisture content was determined gravimetrically by drying 3–5 g aliquots in a vacuum oven at 70°C for 48 hr. For glycerol containing samples a gravimetric procedure at 40°C was utilized in order to avoid evaporation of glycerol (Favetto, Chirife & Bartholomai, 1978).

Available lysine was determined by the fluordinitrobenzene (FDNB) method of Carpenter modified by Conkerton & Frampton (1959).

The total and reducing sugars were determined according to AOAC Official Method 22.043 (1965), AOAC Official Method 29.038 (1965) and AOAC Official Method 29.026 (1965).

For microbiological tests 100 g of sample were blended with 900 ml of 0.1% peptone-distilled water. Aliquots were taken for the standard plate count (at 35°C for 48 hr), anaerobic spore formers (SPS-Agar, Merck Art. 10235 at 35°C for 48 hr), Enterobacteriaceae (Resuscitation: Medium M_9, 35°C, 3 hr; Enrichment: Mossel Broth 35°C, 18 hr; Isolation: MacConkey Agar + 1% Glucose, 35°C, 18 hr), *Staphylococcus aureus* (Baird-Parker Medium at 35°C, for 48 hr) and yeast and mould count (OGA Medium) at 27°C for 5 days.

Results and discussion

Prediction of a_w of infused IM beef samples

Ross (1975) proposed a simple equation for estimating the water activity of complex mixtures. Ross' equation is based on the Gibbs-Duhem relationship

and may be written as

$$(a_w)_f = (a_w^0)_1 (a_w^0)_2 (a_w^0)_3 \ldots \quad (1)$$

Equation (1) assumes that in a food system each a_w lowering component behaves independently. The final a_w, $(a_w)_f$, is a product of each component water activity, $(a_w^0)_1$, $(a_w^0)_2$, $(a_w^0)_3 \ldots$ based on its being dissolved in all of the water in the system.

Assuming that equilibrium was reached between beef pieces and infusing solutions (Chirife, 1978) Ross' equation can be utilized to calculate the final (or equilibrium) water activity in the beef pieces, from the following data: initial moisture content of beef, ratio of initial beef weight/solution weight and solution composition. In addition it is needed to know the a_w lowering effect of each individual solute. The a_w lowering effect of the solutes used in this work have been obtained from the following literature data:

glycerol: National Bureau of Standards (1951)
sodium chloride: Robinson & Stokes (1959)
sucrose: Robinson & Stokes (1959), Norrish (1966).

In view of the relatively high moisture content of IM beef samples, the a_w-lowering effect of non-soluble solids (meat solids) was not taken into account for predicting a_w (Chirife, 1978). Table 2 shows the results of the application of Ross' equation (Eqn 1) to the various infused IM beef samples as compared to experimental a_w measurements. It can be seen that the agreement between measured and predicted values is fairly good. Table 2 also shows the moisture content and pH of the equilibrated samples; moisture content of the various samples ranged between 35.8–51% (total basis).

Microbiological analysis

Only samples stored at 34°C were subjected to microbiological analysis. All eight IM beef products were in good microbiological condition after 60 days storage. With the exception of three samples for which the standard plate count (SPC) was between 400 and 1000, all others have SPC ranged between 30 and

Table 2. Initial water activity, moisture content and pH of the different infused IM beef samples

Infusing solution	(a_w) experimental	(a_w) predicted	Moisture content(%)	pH
1	0.84	0.83	51.0	5.7
2	0.83	0.83	33.9	5.9
3	—	0.83	—	5.8
4	0.83	0.82	42.9	5.7
5	0.83	0.82	38.1	5.7
6	0.85	0.82	44.3	5.4
7	0.83	0.83	42.9	5.5
8	0.84	0.83	35.8	5.6

100. Enterobacteriaceae, anaerobic spore formers and *Staphylococcus aureus* were not detected in any of the samples. All samples were completely resistant to yeast growth and moulds were detected only in four samples having a mould count of 10 per g of product.

Available lysine and sucrose hydrolysis

Table 3 shows the available lysine content of the controls (kept at –30°C) and stored (at 27 and 34°C) IM beef products infused in the different solutions. Total sugar (as invert sugar) and reducing sugars (as invert sugar) content of samples infused in sucrose-containing solutions, were also determined and shown in the same Table 3.

All determinations were made in duplicate and the average is shown. It can be seen that no significant loss of available lysine occurred in any of the IM samples stored for 60 days at 27 or 34°C, irrespective of the presence of

Table 3. Available lysine and sugar content of IM beef samples after 60 days storage at 27 and 34°C

Sample no.	Storage temp. (°C)	Available lysine g/16 g N	Total sugars (as invert sugar) (%)	Reducing sugars (as invert sugar) (%)
1	control (–30)	10.4	–	–
	27	10.6		
	34	10.5		
2	control (–30)	10.2	20.7	< 0.1
	27	9.9	20.7	0.2
	34	9.8	20.8	0.3
3	control (–30)	10.6	19.3	< 0.1
	27	10.1	19.2	0.2
	34	10.4	19.7	0.3
4	control (–30)	10.5		
	27	10.5	–	–
	34	10.6		
5	control (–30)	10.8		
	27	10.7	–	–
	34	10.7		
6	control (–30)	11.1		
	27	11.1	–	–
	34	11.0		
7	control (–30)	10.6	4.9	< 0.1
	27	10.6	5.2	< 0.1
	34	10.1	5.2	0.1
8	control (–30)	10.5	12.7	< 0.1
	27	10.4	12.7	< 0.1
	34	10.1	13.0	0.3

added sodium bisulphite (browning inhibitor). No significant sucrose hydrolysis was found in any of the sucrose-containing IM beef samples, as indicated by the data (total and reducing sugar content) also shown in Table 3.

Sharp & Rolfe (1958) studied the nonenzymic browning reaction in dehydrated meat and indicated that the active carbonyl groups which react with amino nitrogen in the browning reaction were provided in meat by free reducing sugars (mainly glucose) and phosphate esters. They studied the part played by the different reactants present in meat and concluded that the aqueous soluble substances (obtained by extraction with boiling water) had an important role in the browning reaction. The results obtained in the present work with stored IM beef may be explained on the basis of the above observations. The retention of available lysine during storage may be a consequence of the leaching of nonenzymic browning reactants from beef during cooking and soaking in the infusing solutions.

The presence of sucrose in heated protein foods may be considered a potential danger from the point of view of nonenzymic browning reaction. It is generally accepted, however, that the rate-limiting step in the reactions responsible for the browning damage in protein-sucrose systems is the inversion of sucrose to glucose and fructose; the Maillard reactions then proceed rapidly (Hurrell & Carpenter, 1977). Hurrell & Carpenter (1977) did not find loss of lysine in an aqueous-sucrose solution (pH 5.4) kept at either 35° C for 16 hr or at 100°C for 2 hr, nor could they detect reducing sugars in these solutions after heating. Only after heating for 4 hr at 121°C loss of available lysine was observed. The same authors also heated a 'dry' albumin-sucrose mix (pH 5); heating for 1 hr at 100°C only slightly reduced its FDNB-reactive lysine but as the heat process was increased the lysine became unreactive to FDNB. Karel & Labuza (1968) showed that freeze dried model systems containing sucrose and organic acids underwent nonenzymic browning even at low equilibrium relative humidities. The browning was due to reducing sugars produced by the acid-catalysed hydrolysis of sucrose. Significant hydrolysis of sucrose was found to occur at an $a_w = 0.75$ and lower. It should be noted, however, that these experiments were carried out at a considerably higher temperature (55°C) and much lower pH than those corresponding to the present experiments with IM beef. Schoebel, Tannenbaum & Labuza (1969) studied the kinetics of sucrose hydrolysis in freeze dried models at limited water concentrations stored at 37°C. They found that sucrose hydrolysis can occur in systems in which the water activity is low and that the same kinetics of hydrolysis occur in the small amounts of sorbed water as in saturated solutions. Their model systems, however, contained a considerable amount of citric acid which catalysed the reaction; in fact, Schoebel *et al.* (1969) assumed that the pH of the aqueous phase in the low moisture models was below 2.

It may be concluded that although hydrolysis of sucrose in intermediate moisture beef ($a_w = 0.83$) theoretically may occur, the relatively high pH (5.5–5.8) of the aqueous phase and the moderate storage temperature prevented the reaction over the period of storage studied.

Conclusions

All IM beef samples showed good microbiological stability after storage at 34°C for 60 days. No loss of available lysine was observed in IM beef samples infused with different humectants stored either at 27 or 34°C. No significant sucrose hydrolysis was observed in sucrose-containing samples after 60 days' storage period.

Although the data presented suggest a good stability of the systems studied to nonenzymic browning reactions, it may not be concluded that the nutritional stability will remain unchanged during storage. As suggested by Obanu *et al.* (1975a, b) other changes may occur in the proteins of glycerol-infused IM beef, such as crosslinking and breakdown reactions. By this reason further consideration should be given to the nutritional value of such proteins after storage.

Acknowledgments

The authors acknowledge the technical assistance of Mr Cayetano Montenegro. This study was supported in part by the Secretaría de Estado de Ciencia y Tecnología de la República Argentina (Programa Nacional de Investigaciones en Tecnología de Alimentos). The authors are grateful to Sasetru S.A. for donating the beef used in this study.

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(Received 8 January 1979)

J. Fd Technol. (1979) **14**, 429–440

Protein quality of milk-cereal based foods for infants and children in relation to processing methods and composition of the products*

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Summary

The protein quality of milk-cereal based infant foods, i.e. gruel and porridge powders prepared by different industrial processing methods, was assayed biologically. Net protein utilization (NPU) determinations were made in order to elucidate causes of previously observed wide variations in the protein quality of marketed products and to permit recommendations for improvement of processing methods and formulations.

In a pre-mix of a milk-cereal based gruel with about 58% of the total protein content derived from milk, the NPU varied between 15 and 85, depending on the method of roller-drying and on the type of sugar added to the mixture before drying.

Heat treatment of porridge powder products was found to reduce the availability of lysine. Thus when the wheat flour was roller-dried and the milk spray-dried, the NPU value was 78. When wheat flour, glucose and milk were all present on the drum, the NPU decreased to 40. The presence of reducing sugars, e.g. glucose and fructose, on the drum invariably seemed to result in protein destruction. The advantages and disadvantages of changing sucrose to fructose, glucose or lactose in baby foods are discussed.

Introduction

Processed foods for infants and children are commonly used in industrialized countries. In Sweden ready-to-serve milk-cereal based gruel powders are given at 1–2 meals per day to almost all infants after the age of 4–6 months and to young children. It has long been known that different heat processing methods may destroy the protein, and decrease its utilization to different extents

*This paper is listed as No. 2 in the series 'Protein quality of foods for infants and children' published by the Institute of Nutrition, Uppsala University, Sweden.

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0022-1163/79/0800-0429 $02.00 ©1979 Blackwell Scientific Publications

(Fairbanks & Mitchell, 1935; Bigwood, 1972). Cereal-based baby foods often contain sucrose, which increases both the acceptability and the energy density of these products but the choice of carbohydrate used for sweetening has been under debate. It is well recognized that sucrose is a highly cariogenic substance. Dental caries is a health problem starting at a young age in Sweden, as in many other industrialized countries (Holm, 1975). In order to reduce the risk of caries, it has been suggested that sucrose be replaced by glucose or fructose, as these monosaccharides are less cariogenic than sucrose (Mäkinen & Sheinin, 1971). However, it is well known that reducing sugars, e.g. glucose, fructose and lactose, very easily react with protein, i.e. the lysine in the Maillard or nonenzymic browning reaction (Bigwood, 1972). This reaction renders the lysine unavailable and hence reduces the nutritive value of the protein (Mauron & Mottu, 1958).

As no data from biological evaluations showing the effect of different compositions and processing procedures of dried baby food products appeared in the literature, and as this seemed of importance, a series of investigations were carried out to elucidate these questions. This paper reports one study in the series, in which the effect of different processing methods on the protein quality of dried milk-cereal baby foods with different formulations was evaluated in order (1) to explain the wide variation of the protein quality of these types of marketed products found in an earlier study (Abrahamsson & Hambræus, 1977) and (2), to find appropriate methods of production. Some preliminary results from this study were presented at the 2nd European Nutrition Conference (Abrahamsson, Bengtsson & Hambræus, 1977).

Material

Cereal-milk based gruel products recommended for use in infants from 4 months of age and porridge powders recommended from 5 months of age, were evaluated. Both the gruel and the porridge powders were pre-cooked and thus ready for use after mixing with hot water. Table 1 shows the composition of the gruel powder investigated. The protein content was determined by analysis, while quantities of other nutrients were calculated on the basis of the given formulation.

The corresponding compositions and nutrient contents of the two porridge powders tested are presented in Tables 2 and 3. Standardized and pasteurized cows' milk purchased in a grocery shop were freeze dried and included as standards, in addition to wheat flour (80% extraction rate) and AOAC reference casein.

The processing methods of the dried commercial products have been described elsewhere (Abrahamsson & Hambræus, 1977). The gruel powder is commercially processed as two separate portions (called 'pre-mixes' in the following) (Table 1). Pre-mix I, containing cows' milk and cereal flour, is roller-dried, while the pre-mix II consists mainly of spray-dried cows' milk

Table 1. Composition and nutrient content of the model gruel powders tested

	Pre-mix I roller dried	Pre-mix II spray-dried	Complete product III (50% of pre-mix I 50% of pre-mix II)
Components (g/100 g)			
Oat + oat flour	56		28
Dried skim milk	25	78	51.1
Carbohydrate added (glucose, fructose, lactose or sucrose)	19		9.5
Fat		20	10
Minerals and vitamins		2	1
Total	100	100	100
Nutrients (g/100 g)			
Protein (N x 6.25) total	16.8	28.1	22.5
from cow's milk	9.0	28.1	18.6
Fat	1.2	20.2	10.7
Lactose from added cow's milk	13.3	41.0	27.2
Carbohydrates added	19	–	9.5
Carbohydrates, fibre, minerals, water, etc.	49.7	10.7	30.3
Total	100	100	100

Table 2. Composition and nutrient content of the porridge powder tested

	g/100 g
Components	
Wheat flour	40
Dried skim milk	28
Glucose + fructose	13
Apple concentrate	12
Fat	6
Salt	1
	g/100 g dry material
Nutrients	
Protein (N x 6.25) total	17
from cows' milk	10.5
Fat	7
Lactose from cows' milk	17
Glucose + fructose	11
Carbohydrates + ash	48

Table 3. Composition and nutrient content of a cereal-sucrose-mix tested

	g/100 g
Components	
Barley flour	50
Wheat flour	20
Rye flour	10
Sucrose	20
	g/100 g dry material
Nutrients	
Protein (N x 6.25)	9.2
Sucrose	20
Fat	1
Carbohydrates + ash	69.8

and fat. Pre-mixes I and II are finally mixed together in dried form. In the first experimental series in this study the test mixtures were thus produced according to the same procedure (Table 4). In the second series, four different test pre-mixes I of the gruel powder were produced in a pilot plant (Table 5). These contained sucrose, glucose, fructose or lactose and each variety was processed in one of the following ways:

(i) C_0, D_0, E_0 and F_0 with the sugar component added after roller-drying and dried to a final moisture content of 4%;
(ii) C_1, D_1, E_1 and F_1 with the sugar component added before roller-drying and dried to a final moisture content of 4%;
(iii) C_2, D_2 and E_2 as in mixes C_1 to F_1 but dried to a moisture content of 1.5%. (Sample F_2 was not possible to obtain due to lack of material.)

The mixtures of the tested porridge described in Table 2, were produced in a pilot plant as follows (Table 6):

(i) G_1 with only the wheat flour suspension roller-dried, and the spray-dried milk as well as the rest of the components mixed in dried form:
(ii) G_2 with all the ingredients roller-dried;
(iii) G_3 with the wheat flour, fruit concentrate and monosaccharides roller-dried and with spray-dried milk added afterwards;
(iv) G_4 with the wheat flour and the cows' milk roller-dried and the monosaccharides added afterwards;
(v) G_5 to G_7 with lyophilized cows' milk added to the roller-dried mixes G_2 to G_4 in the same relative amount as the fresh milk recommended to be served with the prepared porridge.

In order to study the effect of supplementation with lysine, lysine and isoleucine, and cows' milk, respectively, a cereal-sucrose mix was roller-dried. One batch with 2% glucose added was roller-dried by conventional methods. Another

Table 4. Protein qualities of roller-dried gruel pre-mixes type I with sucrose or glucose and their corresponding complete products with spray-dried cows' milk

Product No.	Type of sugar added	Test mixture	NPU		
			n	$\bar{X}$	s.d.
A_1	sucrose	Pre-mix I	3	60	± 4.0
$_2$	sucrose	Complete product	3	70	± 8.5
B_1	glucose	Pre-mix I	3	34	± 3.2
$_2$	glucose	Complete product	3	52	± 2.7

Table 5. The NPU values of roller-dried gruel pre-mix I with addition of different sugars and with different processing methods

Test mixture no.	Type of sugar	Sugar added		Moisture content		NPU		
		before roller drying	after roller drying	4%	1.5%	*n*	$\bar{X}$	s.d.
C_0	sucrose		x	x		3	72	± 2.3
$_1$	sucrose	x		x		3	69	± 12.1
$_2$	sucrose	x			x	3	51	± 4.8
D_0	glucose		x	x		3	80	± 3.3
$_1$	glucose	x		x		3	35	± 5.7
$_2$	glucose	x			x	3	15	± 8.2
E_0	fructose		x	x		3	83	± 6.7
$_1$	fructose	x		x		3	48	± 1.5
$_2$	fructose	x			x	3	30	± 11.6
F_0	lactose		x	x		3	70	± 10.0
$_1$	lactose	x		x		3	38	± 4.5
Wheat flour (80% extr. rates)	–	–	–			3	38	± 1.7
Freeze dried cows' milk	–		after freeze drying			3	85	6.0
Casein	–					3	85	0.7

batch without glucose was roasted on the drum in order to achieve a special taste. To these two batches of pre-cooked and roller-dried cereal mixes, respectively, amino acids and spray-dried whole cows' milk were added as shown in Tables 7 and 8. Lysine and isoleucine were included as they were the first and the second limiting amino acids, respectively, in the milk-cereal mix.

Total nitrogen was determined by a modified Kjeldahl method described elsewhere (Hambræus *et al.*, 1976). The protein content was expressed as nitrogen × 6.25.

In the assays presented in Tables 4, 5 and 6, Sprague–Dawley rats from Anticimex, Norrviken, Sweden, were used, and in the remaining assays Wistar rats. The studies on the net protein utilization (NPU) and the nitrogen balance

Table 6. The NPU values of a milk-cereal based porridge processed in various ways and the effect of addition of freeze dried milk

	Composition of roller-dried mix				Components added after roller-drying				NPU		
Test mix no.	Wheat flour	Skim milk	Apple conc.	Fructose + glucose	Spray-dried skim milk	Apple conc.	Fructose + glucose	Freeze dried skim milk	n	$\bar{X}$	s.d.
G1	×				×	×	×		3	78	± 3.4
G2	×	×	×	×					3	40	± 5.4
G3	×		×	×	×				3	60	± 2.7
G4	×	×				×	×		3	71	± 0.9
G5	×	×	×	×				×	3	59	± 2.3
G6	×		×	×	×			×	3	68	± 4.8
G7	×	×				×	×	×	3	75	± 2.6

Table 7. The effect of lysine or lysine + isoleucine and addition of 2% glucose on a roller-dried, roasted porridge mix (described in Table 3)

Type of mix	Amino acids added	Type of heat process	NPU n	NPU $\bar{X}$	NPU s.d.
Cereal-sucrose-glucose	–	not heated	5	55	± 3.7
Cereal-sucrose-glucose	–	glucose added after heating	5	47	± 3.7
Cereal-sucrose-glucose	–	glucose added before heating	5	25	± 2.7
Cereal-sucrose-glucose	+ 4 g lysine/$_{16N}$	glucose added before heating	5	55	± 2.0
Cereal-sucrose-glucose	+ 4 g lysine and 2 g isoleucine/$_{16N}$	glucose added before heating	5	57	± 2.2

Table 8. The effect of dried cows' milk supplementation on a roller-dried, roasted cereal-sucrose mix (described in Table 3)

Relative amount of protein derived from		NPU		
dried cows' milk (%)	cereal mix (Table 3) (%)	n	$\bar{X}$	s.d.
0	100	5	32	± 3.6
27	73	5	55	± 1.8
42	68	5	66	± 2.2
59	41	5	74	± 4.1
68	32	5	70	± 6.5

tests were performed using a 10% protein level in the diets. The methods have been described elsewhere (Abrahamsson & Hambræus, 1977). The NPU values given in Tables 4, 5 and 6 were obtained from carcass analysis. The BV values in Tables 7 and 8 are calculated values based on results from nitrogen balance studies in three replicates with four rats per trial (Eggum, 1973).

Amino acid data for the chemical score calculations in Fig. 1 were taken from the FAO amino acid table (FAO, 1970). Chemical score was calculated by the FAO/WHO method, and using the reference pattern from 1973 (WHO, 1973).

Statistical evaluations of means and standard deviations were made by conventional methods. The significances of the differences of the means were determined by Student's t-test or by one-way analysis of variance. $P < 0.01$ and $F < 0.005$ were regarded as being statistically significant.

Results

The results of the biological assays are presented in Tables 4–8. Table 4 shows that the addition of spray-dried milk can mask prior destruction of

protein. The pre-mix A_1 with sucrose had an NPU of 60, and B_1 with glucose 34, while the NPU values of the complete products A_2 and B_2 were 70 and 62. The difference between the means of A_1 and B_1 and that between B_1 and B_2 were statistically significant.

Table 5 shows the NPU of the gruel powder tested with addition of different types of sugar and with different degrees of drying. The NPU values of wheat flour, freeze dried cows' milk and reference casein are included. In the products where the sugars were added to four different parts of one batch of porridge powder after the roller-drying process, the NPU were 72, 80, 83 and 70 with sucrose, glucose, fructose and lactose, respectively. These differences were not statistically significant. Roller-drying to a final water content of 4% resulted in a decrease of the NPU values to 69, 35, 48 and 38, respectively. The difference between the NPU of the samples in which the sugar was added before and after heat treatment, respectively, was statistically significant except for $C_0 - C_1$. When the drying was continued to a final moisture content of 1.5%, the NPU values were 51, 15 and 30 in the porridges containing sucrose, glucose and fructose, respectively. These values were significantly lower than those of the control products C_0, D_0 and E_0.

Finally, the NPU of the porridge powder prepared in different ways varied between 78 and 40 (Table 6). When freeze dried milk was added to these prepared products in the same relative amount as when the prepared porridge is served with fresh milk, the NPU varied between 75 and 59. The completely roller-dried milk-cereal based porridge G_2 had a significantly lower NPU than G_1, in which the only cereal was roller-dried. The addition of lyophilized milk to the completely roller-dried porridges (G_2 and G_5) increased the NPU value significantly. However, the NPU of G_5 was still significantly lower than that of G_1.

The NPU of a cereal-sucrose mix decreased significantly from the expected reference value after roller-drying to obtain a roasted taste, both with and without the presence of glucose on the drum (Table 7). Lysine supplementation restored the NPU levels. Addition of isoleucine resulted in no further improvement. About 20% of the protein needed to be milk protein in order to compensate for the cereal protein destroyed by heat processing (Table 8). It is also seen in this table that the NPU increased significantly ($0.001 < P < 0.005$) until 50% of the protein consisted of milk protein.

Discussion

The protein quality of foods intended for infants and young children must be high in order to cover their relatively high need of essential amino acids (WHO, 1973). The products tested in this study fulfil this recommendation, as the protein is derived mainly from milk, giving them a high essential amino acid content as indicated by chemical scores (Fig. 1). However, the availability of the amino acids varies considerably depending on the drying procedure used.

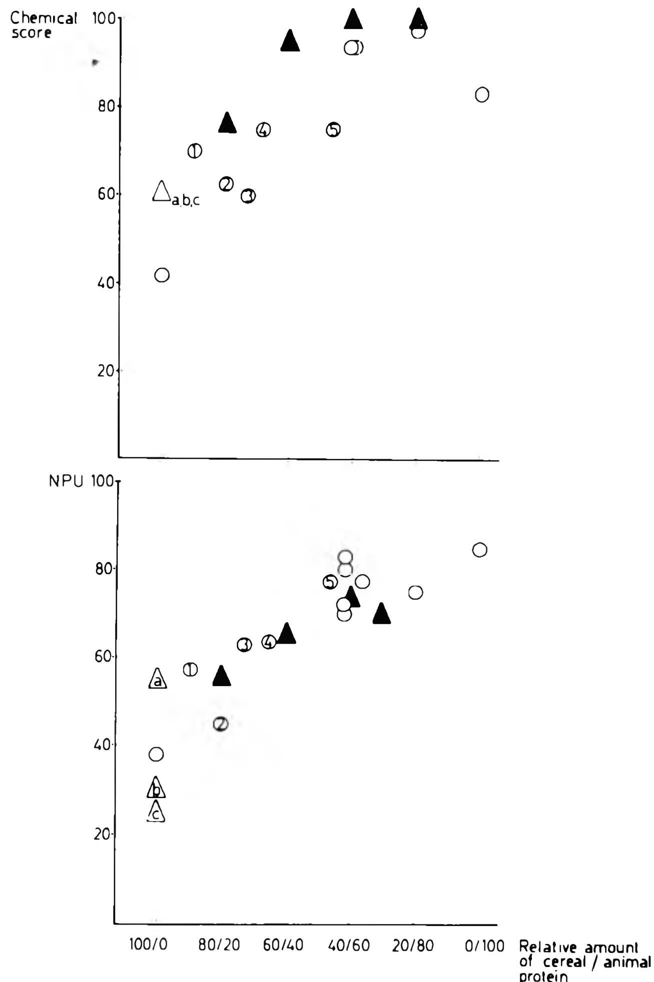

Figure 1. Chemical score and NPU of different cereal mixes in relation to increasing content of animal protein. △a, Unheated product (Table 7); △b, heated and roasted without reducing sugar (Table 8); △c, heated with reducing sugar (Table 7); ▲, △b + added milk (Table 8); ○, heated without reducing sugar; ①, 95% wheat + 5% DSM, unheated; ②, 70% wheat + 30% SEF unheated; ③, 95% wheat + 5% WPC unheated; ④, 50% wheat + 50% SEF unheated; ⑤, SEF unheated.

In an earlier report the protein qualities (NPU) of three different types of milk or cereal and milk-based baby foods (human milk substitutes, gruel and porridge powders) produced and marketed in Sweden were presented (Abrahamsson & Hambræus, 1977). The NPU of spray-dried human milk substitutes were high (75 to 78) and corresponded to the values expected from the composition of the product. Roller-dried milk and cereal-based gruels with subsequent addition of spray-dried cows' milk had NPU of between 69 and 77. The NPU values of totally roller-dried porridge powders showed an astonishingly wide variation,

between 28 and 69, which was assumed to be due to the processing method and the composition of the products. Interestingly, similarly low NPU of certain cereal and milk-based products have also been reported from Norway (Holm, 1973). This pointed to the need for further analysis of the reasons underlying these wide variations in nutritive value. In the study reported here, some of the effects of various sweetening agents in roller-dried cereal-milk based products were examined. In the complete gruel products, the milk protein covers 85% and in the pre-mix I 58% of the total protein content. The NPU of the optimally produced pre-mixes were found to vary between 70 and 83 (Table 4) when no saccharide was added before the heat processing. This range was about the same as was found in breast-milk substitutes and in freeze dried milk. The addition of more milk to the pre-mix does not seem to be necessary according to the NPU results. When pre-mixes were roller-dried to a final moisture content of 4%, the original NPU of the mix containing sucrose was unchanged, while the NPU of mixes containing reducing sugars was drastically reduced. With a more intensive drying method to a moisture content of 1.5%, the protein quality was even further reduced to as low as NPU 15 in the mix with glucose. Landes & Miller (1976) observed the same effect when substituting glucose for sucrose in baked products. Our results confirm that the addition of reducing sugars should be avoided. However, if such saccharides are used they should be added subsequent to the drying procedure. Furthermore, the advantages of glucose or fructose over sucrose in roller-dried milk-cereal mixes with respect to dental caries need to be further examined, taking into consideration the fact that these food products contain both protein and fat which decrease the cariogenic effect of sucrose, and also that they are not particularly sticky, which likewise will reduce the risks of caries. Moreover, milk and cereal-based baby foods are served as meals once or twice a day and not as snacks several times daily. The effect of storing a product containing reducing sugar is another problem. If the moisture content is raised due to humid storage the Maillard reaction may accelerate. Thus, it would seem that the role of sucrose and the question of its replacement as an ingredient in these types of products should be re-evaluated.

The NPU values of the cereal-milk mixes tested were expected to be 70–80, because of their high content of cows' milk. This is illustrated in Fig. 1, where the NPU and chemical scores of different non-heat-dried cereal-milk mixes (Abrahamsson & Hambræus, 1977) are compared with those of the mixes presented in Tables 5, 7 and 8. The NPU of wheat flour is low (38) compared with that of the unheated barley-wheat-rye mix (55). Roller-drying with glucose present on the drum reduced the NPU from 55 to 32. Addition of milk to the heat-treated product representing 20% of the total protein improved the NPU to the original value. Of the cereal mixes Nos. 1–5 included in the figure (Abrahamsson, unpublished results) one contained wheat flour and dried skim milk (DSM), one contained whey protein concentrate (WPC) and three were based on supplementary enriched food (SEF, 1977).

The figure shows that the NPU are already high when 50 to 60% of the

total protein content is derived from animal sources. Hence the complete gruel product, with 85% of the protein derived from milk, contains more milk than is needed for the amino acid supplementation. The milk protein content of the tested porridge powder represents about 60% of the total protein. The porridge is recommended to be served with fresh cows' milk, as this is the usual way of eating porridge in Sweden. When the fresh milk is taken into consideration, the total milk protein represents 80% of the protein in the mix. Thus, it should be stressed that this addition of milk is not based on nutritional needs but rather on food habits. In a home-prepared meal of cereal porridge served with milk, 40–70% of the protein is supplied by the added milk, depending on the relative amount of milk consumed.

As heat-drying, e.g. roller-drying, always involves a risk of protein destruction, only components requiring pre-cooking, should be dried on the drum. The remaining components, particularly reducing saccharides, should be mixed with the dried products. In these products where cows' milk is considered a necessary component, spray-dried cows' milk is to be preferred. It should be remembered, however, that the addition of dried milk can largely mask previous protein destruction, as is shown by the NPU results in Tables 3, 5 and 8.

It has been argued that a low NPU can be accepted in products that are usually served with cows' milk or other animal protein sources. This is true when the NPU represents the value for the raw unsupplemented material. However, a NPU as low as 28, which was found previously in a porridge product containing a considerable amount of milk, cannot be accepted, as this value is lower than the NPU of unsupplemented wheat, which lies between 40 and 56 (FAO, 1970). The expected NPU is between 70 and 80. The protein quality may also be achieved in mixtures with only a limited amount of animal protein or based solely on vegetable protein sources as, for example, in low-cost weaning foods for use in developing countries (Abrahamsson, Forsum & Hambræus, 1974; Forsum, 1973; Velarde & Abrahamsson, 1978).

The importance of proper processing of these kinds of food was realized twenty years ago in a malnutrition rehabilitation programme in Africa, when the effect of protein damage in a heat treated milk-cereal-legume product was shown both chemically and clinically (Clegg, 1960; Clegg & Dean, 1960).

Conclusions

To obtain an optimal protein quality of pre-cooked cereal-milk based baby foods, the choice of processing method and of sweetening agents must be considered. An unsweetened product is to be preferred, but if a sweetened cereal or cereal-milk based product is to be produced, any reducing sugar, e.g. glucose or fructose, must be added after the heat-treatment involved, otherwise sucrose should be chosen as the sweetening agent. Lactose may be suitable in some types of dried mixes. Furthermore, the product should not be

dried to a lower moisture content than is necessary for safe storage. Finally, as a general rule, the nutritional aspects must be taken into account when processing foods, particularly baby foods. Protein destruction through processing should always be avoided, and this refers to every step in the production.

Acknowledgments

The able technical assistance of Miss Ann-Sofie Eriksson is gratefully acknowledged. Part of this work was financially supported by the Swedish International Development Authority and the Semper Fund for Nutrition Research.

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(Received 30 January 1979)

J. Fd Technol. (1979) **14**, 441–447

The quantitative determination of trimethylamine in egg

A. HOBSON-FROHOCK

Summary

A freeze-drying technique followed by gas chromatography of the distillate is described for the analysis of trimethylamine. The method is routinely applicable at concentrations of 0.05 $\mu g\ g^{-1}$ egg using samples of 1.0 g and could be used with other foods of sufficient moisture content.

Introduction

An investigation into the nature of a 'crabby' or 'fishy' odour in brown-shelled eggs (Hobson-Frohock *et al.*, 1973) indicated that trimethylamine (TMA) was responsible for the taint and it was necessary therefore to develop a sensitive and reproducible technique for the measurement of TMA in whole egg at the ppm level. Many of the existing methods for the measurement of TMA generally refer to its presence in fish or fish products, for example as an index of quality during frozen storage (Gruger, 1972; Castell, Smith & Dyer, 1974; Ritskes, 1975). It has also been implicated in cases of fishy taint in milk from cows grazed on a wheat pasture (Mehta, Bassette & Ward, 1974; Von Gunten *et al.*, 1976). Other papers have been concerned with its involvement in atmospheric pollution (Andre & Mosier, 1973) or its presence in biological fluids (Dunn, Simenhoff & Wesson, 1976; Marks *et al.*, 1977). Keay & Hardy (1972) established a quantitative method for the analysis of TMA and dimethylamine by gas chromatography (GC) of the steam distillate from an alkaline fish muscle extract. Murray & Gibson (1972a, b) compared this method with the Hoogland modification (Hoogland, 1956) of the Dyer picrate method (Dyer, 1945), with a microdiffusion (Conway) technique (Beatty & Gibbons, 1937) and with a method based upon the Technicon Auto Analyser (Murray & Burt, 1964). The use of the microdiffusion technique was critically reviewed by Spinelli (1964) who concluded that it was neither easy nor accurate.

However, none of these methods was satisfactory because it was necessary to establish the relationship between the 'free' TMA content of the egg and an

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odour panel assessment of the intensity of the taint and this was not possible if the egg had been subjected to chemical or thermal degradation during the analysis. The presence of TMA in biological fluids has been investigated by Dunn *et al.* (1976) who devised an ultrafiltration method to prepare samples for GC analysis and by Marks *et al.* (1977) who injected urine samples directly on to the GC column. Unfortunately, neither of these techniques could be applied to the analysis of homogenized whole egg and the equilibrium vapour analysis method for TMA and dimethylamine in fish described by Miller *et al.* (1973) lacked the required sensitivity. Initial experiments had shown that the lyophilization technique for fish protein concentrate used by Wick, Underriner & Paneras (1967) could be applied to homogenized whole egg and produced a distillate containing the characteristic odour of the taint; this technique was therefore modified to handle small (1.0 g) samples of egg.

Materials and methods

(a) *Freeze-drying of egg*

The egg (40–60 g) was broken out into the 100 ml vortex beaker of an MSE Homogenizer (MSE Scientific Equipment) and the yolk and white blended at maximum speed for 5–10 sec. Aliquots (1.0 g) were weighed into the lyophilizer which was constructed from a Q & Q MF 24/3/8 test tube (Quickfit & Quartz Ltd.), fitted with a side-arm (Fig. 1). A 'cold finger' made from a Q & Q CEB 24 coned stem, tapered to a point 12 cm below the cone joint, was inserted into the tube, the joints lightly greased with silicone high vacuum grease and the whole connected to a manifold which could take twelve such tubes. The egg was frozen by immersion of the tube into liquid nitrogen for 5–10 min and liquid nitrogen added to the cold fingers. The manifold and

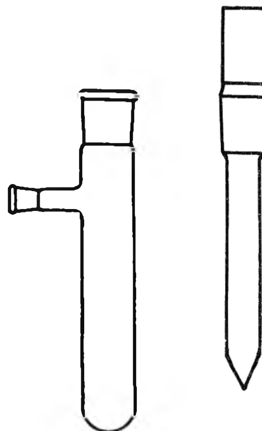

Figure 1. Lyophilizer for freeze drying homogenized whole egg.

tubes were evacuated using a two stage rotary oil pump through a liquid nitrogen cooled trap until the pressure in the system was a low as possible, at least 0.05 Torr, with the egg still frozen in liquid nitrogen. The pump was then isolated from the manifold and the coolant was removed from around the egg. The sample was freeze-dried for about 2.5 hr, care being taken to maintain the system pressure at 0.05 Torr by occasional use of the pump. At the end of the distillation the vacuum was released with nitrogen, the cold finger quickly removed, emptied of coolant, placed in a Q & Q MF 24/3 test tube containing 150 μl of N/10 HCl and the condensate allowed to melt. The cold finger was rinsed with approximately 1 ml of water, the acidic solution taken to dryness on a rotary evaporator at 40°C and the residue of hydrochlorides then dissolved in N/10 HCl (250 μl) for gas chromatographic analysis.

(b) *Gas chromatography*

Aliquots (7 μl) of the hydrochloride solution were analysed on a 2.7 m, 4 mm i.d. glass column packed with 50–60 mesh Chromosorb 103 coated with 20% (w/w) KOH. The column was installed in a Pye-Unicam Model 104 gas chromatograph coupled to the Pye S4 Autojector and a Perkin-Elmer thermionic detector in the nitrogen mode replaced the existing flame ionization detector. Hydrogen and air flows to the detector were controlled by Brooks Model 8944 flow controllers (Brooks Instruments Ltd.) and set at 5 and 100 ml min^{-1} respectively with a detector temperature of 225°C. The detector response varied with the temperature of the electrically heated rubidium silicate bead; for routine use the heating current dial was set between 400 and 500 (2.6 to 2.9 A) to give a response similar to that of the flame ionization detector, although a tenfold increase in sensitivity could be obtained (with a consequently reduced bead life) by increasing the dial to 600–650. The column was operated isothermally at 140°C with a nitrogen carrier gas flow rate of 30 ml min^{-1}. Trimethylamine was released from its salt by injection onto soda lime (prepared from calcium oxide slaked with 5M NaOH, dried, ground to 40–80 mesh and then coated with 20% KOH) which was packed into the initial 4 cm of the column and separated from the Chromosorb 103 by a quartz wool plug. Under these conditions trimethylamine had a retention time of 3.5 min. An Autojector programme was selected which gave duplicate injections of each sample followed by a syringe washing with water and this enabled fifty samples to be analysed in one run. Normally, the first five sample vials contained standard solutions of TMA.HCl ranging from 3 to 30 μg ml^{-1} followed by egg distillates with a vial containing a 30 μg ml^{-1} solution of TMA.HCl after each group of eight sample vials. The last five sample vials also contained the 3–30 μg TMA.HCl ml^{-1} standards. A calibration curve was drawn from all standards and in this way the performance of the column could be monitored during the run which, if at full capacity, could take several hours to complete. Broadening of the TMA peak and a reduction in detector

response indicated the need to replace the soda lime packing, although it was normally replaced weekly. At the lowest working amplifier sensitivity of 2×10^{-11} A fsd, the minimum detectable gas chromatographic peak of TMA at 1 cm peak height was calculated to be equivalent to 1.0 ng TMA injected, corresponding to 0.05 μg TMA g^{-1} egg and this is regarded as the lower concentration limit for routine analysis, although, as stated above, the sensitivity of the nitrogen detector could be 'tuned' to a ten-fold increase if required.

(c) *Recovery of TMA from water and egg*

Recovery experiments were conducted using a solution of TMA.HCl (Sigma) in water over the concentration range 1.4–414.0 μg ml^{-1}. Aliquots (50 μl) of solution at each concentration were added to 1.0 ml water or 1.0 g egg in the lyophilizer, two drops of 4% (w/v) KOH added, the cold finger inserted and the whole gently mixed before connection to the manifold.

Results and discussion

(a) *Gas chromatography*

Analysis of amines by gas chromatography is made difficult by the highly polar nature of these compounds. A variety of different column preparations exists in the literature, many of which require treatment of the liquid phase with alkali. Much of the development work in this paper was carried out using a flame ionization detector and the Dowfax DN9/KOH on Silocell C22 column described by O'Donnell & Mann (1964) and modified by Keay & Hardy (1972) but it proved unreliable at the low levels of trimethylamine involved in this problem. (The Dowfax DN9 phase has now been discontinued and Triton X-100 suggested as a replacement (Phase Separations Ltd.) but we have not investigated this phase.) The base-coated support in the injection area needed frequent replacement, a process which involved lengthy conditioning to reduce the column bleed to an acceptable level. The initial 4 cm column packing was therefore replaced with soda lime prepared from calcium oxide which needed only a short conditioning period *in situ* (about 1 hr at 200°C) before the column was ready for use. Chromosorb 103 (Johns Manville), a porous cross-linked polystyrene used for the analysis of short-chain aliphatic amines by Andre & Mosier (1973), was investigated and proved to be the most suitable material when coated with 20% (w/w) KOH. The ease of column preparation with Chromosorb 103 made it preferable to tetraethylenepentamine on Graphon, a partially graphitized carbon black (Di Corcia, Fritz & Bruner, 1970) and to polyethyleneimine 40M + KOH on a modified carbon support (Di Corcia, Liberti & Samperi, 1974). However, at the higher sensitivities (2×10^{-11} to 5×10^{-11} A fsd), the TMA peak was not fully resolved from

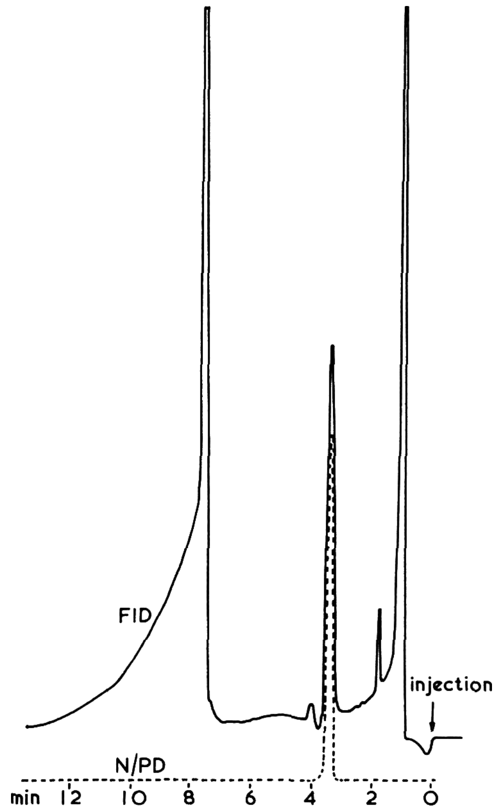

Figure 2. Gas chromatogram of distillate (concentrated to 250 μl) from egg containing 0.7 μg TMA g^{-1} ; 7 μl injected on to Chromosorb 103/KOH column at 140°C. Amplifier sensitivity 5×10^{-11} A fsd; chart speed 1 cm min^{-1} ; carrier gas (nitrogen) 30 ml min^{-1}. -----, Nitrogen detector; ——, flame ionization detector.

several early running peaks which arose from injections of water as solvent, a phenomenon noted by Di Corcia & Samperi, 1974. The use of the nitrogen/ phosphorus detector operating in the specific nitrogen mode overcame all the difficulties of 'ghost' peaks and produced a chromatogram in which only the TMA peak was visible (Fig. 2).

(b) *Recovery of TMA added to water and egg*

As shown in Table 1, the recovery of TMA from aqueous solutions was perfectly adequate with the values ranging from 81 to 96% and demonstrated the efficiency of the method. However, some difficulty was experienced when samples of homogenized whole egg were 'spiked' with TMA.HCl, taken to pH 10 with 4% (w/v) KOH and distilled. When TMA was added to a 'control', i.e. untainted egg, even at levels of 30–40 μg g^{-1}, the recoveries were low and erratic. Only when a tainted egg was used could recoveries be considered

Table 1. Recovery of trimethylamine from aqueous solution

TMA added (as hydrochloride) µg	Recovery* % ± s.d.
0.07	81 ± 7
0.28	94 ± 3
0.46	93 ± 2
1.38	95 ± 1
1.88	96 ± 3
6.90	96 ± 2
13.80	96 ± 2
20.70	96 ± 1

*Mean of 12 replicates

adequate and perhaps this is not surprising in view of the adsorptive properties of egg proteins (Maier, 1970) and the difficulty of producing tainted samples by the addition of an identified tainting compound to the untainted food at comparable concentrations. The addition of alkali to the homogenized egg, not a part of the normal analytical technique, produced a marked change in the structure of the matrix from an open 'foam-like' structure to a rather viscous liquid and this could have contributed to the lower recoveries shown in Table 2. Extension of the distillation time from 2.5 to 7 hr only increased the TMA value of a 'spiked' egg by approximately 10%. Unfortunately, few authors appear to report recovery experiments from the food material under examination; some of those that have done so reported difficulties with recovery of amines from 'spiked' samples. Patterson & Mottram (1974) could only recover 40% of methylamine added to meat samples although recovery of dimethylamine and ethylamine was somewhat higher (75–85%). Gruger (1972) added a mixture of ten amines to a sample of Coho salmon at concentrations ranging from 17 to 3700 $\mu g\ g^{-1}$ and obtained an average recovery of

Table 2. Recovery of trimethylamine added to tainted egg

TMA added (as hydrochloride) µg	Recovery* % ± s.d.
0.94	53 ± 11
1.88	63 ± 10
4.69	69 ± 12
9.38	76 ± 5
18.76	75 ± 4

* Mean of 12 replicates

only 42% (the range being 26–47%); no recoveries were given for the methylamines.

The method of TMA determination described in this paper should be applicable to any biological material or food which has a sufficient moisture content to enable freeze-drying to take place; the addition of water would be necessary for dry foods such as fish protein concentrate.

Acknowledgments

The author gratefully acknowledges the skilled technical assistance of Mrs J.A. Harrison in carrying out the analytical part of this work.

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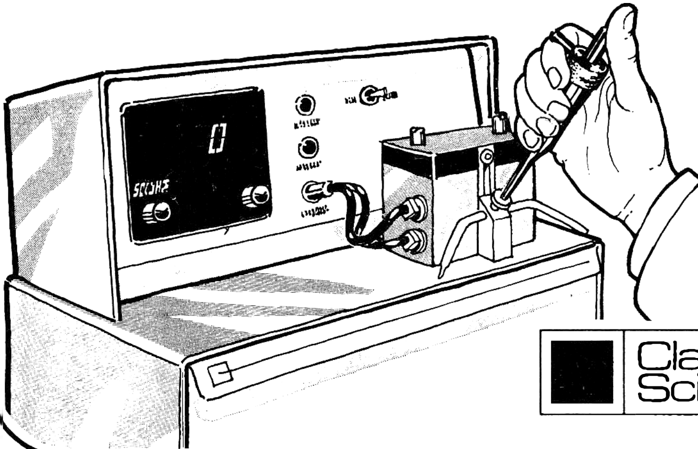

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Abbreviations. Abbreviations for some commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular. Wherever possible the metric SI units should be used unless they conflict with generally accepted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

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3} m	hour	hr
micrometre	μm = 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^3
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·5461 l
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'.

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Printed by Adlard and Son Ltd, Bartholomew Press, Dorking