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**Contributions and editorial correspondence** should be sent to Dr H. Liebmann, c/o Research and Development Department, Metal Box Ltd, Twyford Abbey Road, London NW10 7XQ.

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# New techniques for the digestion of biological materials – application to the determination of tin, iron and lead in canned foods

E. V. WILLIAMS*

## Summary

A new technique for the digestion of biological materials has been developed and this has been applied to the determination of trace metals in canned foods. It is based on sample digestion in concentrated acid under pressure in a disposable polystyrene container. The digest can be analysed directly by atomic absorption or plasma emission and has the advantages of simplicity, high sensitivity (low blank value), rapid batch sample throughout rate, economy and suitability for automation. Various foods have been classified empirically into three groups on the basis of the ease with which they can be decomposed chemically to a form suitable for analysis. After classification, preparation follows one of two different procedures depending on the elements to be determined.

The system has been verified for tin, iron and lead by comparison with other more conventional procedures.

## Introduction

There are two important reasons why it is necessary to measure the trace metal content of foodstuffs. First, during recent years there has been increasing concern and associated publicity with the possible toxic effects of metals such as lead, cadmium and mercury, as a result of which there have been moves in various countries to monitor concentrations of these metals in foods and to re-examine existing legislation, sometimes proposing new and more stringent standards – MAFF (1975), FAO/WHO (1972), S.I. No. 1843 (1972). Secondly, with canned foods in particular, shelf life assessment is often made by monitoring the build up of metals, e.g. tin and iron, during storage as corrosion processes proceed. Both of these areas of activity place heavy demands on the

*Present address: Analytical Services, Tinplate Research Department, British Steel Corporation – Tinplate, Carmarthen Road, Swansea, West Glamorgan SA5 8NH.

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analyst as the need for statistically valid sampling exercises requires large numbers of measurements.

For many analytical laboratories involved in this field, batch throughput rates of at least 100 samples per day are normally required to satisfy demand, multiple sampling requirements (for statistical validity) and economy.

It is unfortunate that the methods of analysis currently used to determine metals in canned foods are extremely expensive to operate in terms of consumable ultra-purity materials and applied man hours – S.A.C. Analytical Methods Committee (1960, 1967, 1969, 1976a, b) Simpson & Blay (1966), Cameron & Brittain (1977). A comprehensive review of the methods of analysis for metals in food is given by Crosby (1977).

In the packaging context, there is a need to develop methods of analysis for tin, iron and lead in foods with high batch throughput rates. As early as 1964 it was realized that unless a radical change in the conventional methods of sample preparation, viz. dry ashing and wet oxidation, was made then this objective could not be conveniently attained.

In order to gain wide acceptance, an innovative analytical system for trace metal analysis in canned foods should satisfy the following criteria:

Versatility (e.g. applicable to a wide variety of food products); high sensitivity and accuracy; simplicity and ease of operation; low operating cost; safe and environmentally acceptable (very low effluent and emissions); suitability for automation; low contamination (low trace metal pick-up from containers and reagents – low blank value).

With these essential criteria in mind, development work on new techniques for food analysis were initiated at B.S.C. Tinplate Research Laboratories (formally the Steel Company of Wales Limited), in 1968. Two important innovative ideas were introduced at this time, viz.

(a) the use of positive pressure to facilitate acid digestion. The pressure is generated by chemical reaction of the food product with a concentrated acid within a sealed container, and

(b) the use of disposable (and sealable) rigid polystyrene containers in place of glassware.

These subsequently proved successful and became the basis of the new system of food analysis that is now established at these laboratories.

Polystyrene vessels have not been used previously as pressure containers, particularly in the sense that they are used once only to limit cross contamination possibilities from previous use. The use of pressure to decompose organic material in plastic polythene containers was used by Adrian (1971 and 1973) and Adrian & Stevens (1977) and this work in many ways proceeded along similar lines. The chemistry and mechanism of pressure control, is however, different.

High pressure and high temperature 'bomb type' decomposition devices have been used for many years and commercially constructed devices are available – NASA (1968), Bernas (1968), Cybura (1976), Holah, Krinitz & Williams

(1972). These devices, however, are unsuitable for the task described, mainly because of the cost of each container and the limited throughput rate.

The system has been developed and verified for tin, iron and lead, which are the metals currently of greatest interest in the development of tinplate containers. Samples of the foods under examination are digested under pressure either by nitric acid if they are to be analysed for lead and iron, or since tin is only partially soluble in nitric acid, digestion of samples to be analysed for tin is carried out in a mixture of hydrochloric acid and hydrogen peroxide.

Details of the digestion procedure have to be varied according to the nature and composition of the foods. Three types of food have been recognized and appropriate digestion procedures have been evolved:

Non-fatty non-alcoholic foods, e.g. vegetables, fruit and fruit juices;
Foods containing fats and oils, e.g. fish, meat, soup and cheese;
Foods and beverages containing alcohol and/or dissolved gases.

The full details of the procedures are set out in Tables 1 and 2.

The trace metal determinations in the final digests are carried out by atomic absorption spectrometry in its different modes, flame excitation for tin and iron and electrothermal atomization in a graphite furnace for lead (CRA or HGA). In these studies plasma emission spectrometry has been explored also.

## Materials and methods

### *Water baths*

Two Grant Instruments (Cambridge) Ltd stainless steel temperature controlled water baths fitted with stainless steel racks to accept the polystyrene containers are used to process samples in batches of 200–240. All immersed parts are of either stainless steel or plastic. The base of the water bath is covered with calcium carbonate cubes (British Drug Houses Ltd). Water bath temperature is controlled to 70 ± 2°C. One bath is used for Process One and the other for Process Two.

### *Sample and reagent dispensing devices*

For reagent dispensing 'Scorex' or 'Quickfit and Quartz' 10 ml dispensers are used to add 10 ml volumes of concentrated acids and acid mixtures to containers. After use they are stored with the barrel containing distilled water.

For sample dispensing Scientific Glass Engineering Syringes type 5-A-SV with p.t.f.e. tips are used for carbon rod atomization (CRA) analyses. Tips are cleaned in 40% by volume nitric acid before use and rinsed with the sample twice before final sample injection into the furnace cavity. Sample volumes of 2.5 μl are used throughout.

**Table 1.** Process 1. Determination of lead and iron

The food product is homogenized and a sample for analysis is weighed into a 'Sterilin' Universal container Type 128*

Classify the food product into its appropriate food group

| GROUP 1 | GROUP 2 | GROUP 3 |
|---|---|---|
| Non-fatty, non-alcoholic, non-effervescent, e.g. fruit juices, vegetables, fruit preserves, etc. | Foods that contain fat or oil, e.g. fish, milk, some types of soup, cheese, etc. | Foods or beverages that contain alcohol or dissolved gases, e.g. beers, wines, bitter lemon, all carbonated beverages and effervescent drinks |
| | PROCESSING STAGES | |
| STAGE 1: Add 10 ml of conc. Aristar nitric acid, seal the container and hand tighten the screw cap. Mix and place in a water bath for 24 hr at 20°C | Treat as for Group 1 Stage 1 | Heat the sample at 70°C in an unsealed container for 4 hr. Cool to 20°C |
| STAGE 2: Increase temperature to 70 ± 2°C for 4 hr | Treat as for Group 1 Stage 2 | Continue as for Group 1 Stage 1 |
| STAGE 3: Use protective gloves to release residual pressure and loosen cap. Continue to heat for 2 to 3 hr to remove dissolved gases. Cool, dilute to 25 ml, seal and mix. Centrifuge at 3000 to 4000 rev/min for 5 min or allow to stand for 24 hr to clear | As in Group 1 Stage 3 use gloves to release pressure and loosen cap. Continue to heat for 2 to 3 hr to remove dissolved gases. Dilute with hot water (70 to 80°C) to 25 ml and mix thoroughly. Observe the fat or oil ring partitioning with a central fat free area at the top of the container. Use the centre of the ring to extract an aliquot (7 ml) for analysis and transfer this to a clean container – 'Sterilin' Type 129d. Oil and fatty products have an affinity for polystyrene under the test conditions | Continue as for Group 1 Stage 2 |
| | | Continue as for Group 1 Stage 3 |

ANALYSIS: The solutions so prepared are suitable for the determination of lead and iron, by atomic absorption flame and graphite furnace methods of analysis. It is particularly suited to plasma emission techniques of trace metal measurement. The choice of analytical measurement method depends on the concentration of the element

*The sample weight for Group 1 and 2 is 5.0 g and for Group 3 is 10.0 g.

Table 2. Process 2. Determination of tin and iron

The food product is homogenized and a sample for analysis is weighed into a 'Sterilin' Universal container Type 128*

Classify the food product into its appropriate food group

| GROUP 1 | GROUP 2 | GROUP 3 |
|---|---|---|
| Non-fatty, non-alcoholic, non-effervescent, e.g. fruit juices, vegetables, fruit preserves, etc. | Foods that contain fat or oil, e.g. fish, milk, some types of soup, cheese, etc. | Foods or beverages that contain alcohol or dissolved gases, e.g. beers, wines, bitter lemon, all carbonated beverages and effervescent drinks |
| | PROCESSING STAGES | |
| STAGE 1: 10 ml conc. Aristar hydrochloric acid–hydrogen peroxide reagent, seal the container and hand tighten the screw cap. Mix and place in a water bath | Treat as for Group 1 Stage 1 | Heat the sample at 70°C in an unsealed container for 4 hr. Cool to 20°C |
| STAGE 2: Heat to 70 ± 2°C and hold at this temperature until the contents change in colour to orange-brown; nominal time 30 min depending on the food product | As in Group 1 Stage 3 use protective gloves to loosen cap. Dilute the sample with hot water (70 to 80°C) to 25 ml and then either withdraw an aliquot as in Process 1 or insert a Whatman Filter Thimble 19 mm x 90 mm to separate the phases. Transfer an aliquot to a clean container – 'Sterilin' Type 129d, before the solution cools | Continue as in Group 1 Stage 1 |
| STAGE 3: Use protective gloves to loosen cap. Dilute to 25 ml, seal and mix. Centrifuge at 3000–4000 rev/min for 5 min or allow to stand for 24 hr to clear | | Continue as in Group 1 Stage 2 |
| | | Continue as in Group 1 Stage 3 |

ANALYSIS: Atomic absorption spectroscopy, using a nitrous oxide–acetylene flame for tin and air-acetylene for iron. Plasma emission techniques offer better sensitivity and wider dynamic ranges

*The sample weight for Group 1 and 2 is 5.0 g and Group 3 is 10 g.

*Containers*

Polystyrene Universal Containers Type 128A supplied by Sterilin Ltd, Teddington, Middlesex are used for pressure decomposition. In an extensive exercise undertaken at an early stage in the development, no other containers were found to be suitable. The body of the container is manufactured from virgin polystyrene and the cap is manufactured from polythene. The containers form an integral part of the system. One important feature of the container is the ability of the screw cap to function as a pressure control valve and it is this mechanism that is exploited in the pressure decomposition process. At 20°C, the container can withstand an internal pressure of 2 bar (g), depending on the torque applied to the screw cap. Polystyrene contracts on being heated and because of this distortion the maximum allowable temperature is 70 ± 2°C. At 85°C severe distortion occurs. Polystyrene has an affinity for fats or oils and this is the second feature which is exploited in the analysis procedure.

Under the test conditions, blank values for tin, iron, chromium, copper, cadmium lead and arsenic are not detectable by flame A.A.S. methods of analysis and are less than 0.01 $\mu g\,ml^{-1}$ using CRA, and HGA methods of measurement. The lower tip of the thread mark on the body of the container is conveniently used to dilute to a volume of 25 ml following sample processing.

*Reagents*

Concentrated Aristar grade nitric acid is used for digestion in Process 1. Concentrated Aristar hydrochloric acid and Aristar grade hydrogen peroxide are used for digestion in Process 2 in the following proportions: to 500 ml of concentrated hydrochloric acid add 10 ml of 30% w/v hydrogen peroxide. Mix gently and store in an open container. The solution is unstable and only an amount sufficient to complete the task is prepared.

Standards are prepared from spectrographically pure materials or commercial atomic absorption standards in 20% nitric or hydrochloric acid as appropriate for the selected process.

*Atomic absorption spectrometry*

A Varian-Techtron A.A. 5 atomic absorption spectrometer is used for flame analysis. Tin is determined in a nitrous oxide – acetylene flame at 286.3 or 235.4 nm at an oxidant pressure of 1.6 bar (g) and a fuel pressure of 0.7 bar (g). Iron is determined at 248.3 nm in an air-acetylene flame at the instrument manufacturer's settings.

A model 63 electrothermal graphite furnace attachment (CRA) is used for the analysis of lead at 217.0 nm. Graphite furnace tubes are specially treated to improve performance and extend useful life (patent pending).

*Plasma emission spectrometry*

A limited evaluation of this technique has been undertaken with the co-operation of Techmation Ltd, Edgware, Middlesex, using a Spectrospan 3 plasma emission spectrometer operating in its single element mode. This D.C. plasma emission instrument is fitted with an Echelle grating and was used for tin, lead and iron, using Process 1 and Process 2 prepared solutions. This instrument has the capability of simultaneous analysis of up to eighteen elements on twenty channels and in combination with the procedures described would have a substantial output at high sensitivity. The other two remaining channels are used for blank and background correction. It has a wider dynamic range than atomic absorption.

*Samples*

The samples used were from cans involved in on-going research programmes at B.S.C. Tinplate Research as follows: cans packed experimentally and deliberately incorporating corrosion accelerators (e.g. nitrates) and cans originally obtained from the market place but subsequently stored under abnormal conditions (time and temperature) in the laboratory.

Cans were selected to illustrate the working range of the procedure and are not necessarily representative of the marketed product.

*Analytical methods*

*Preparation of standards.* Standard additions of fixed volume and increasing concentration of trace metals are made to foods of low trace metal content (e.g. frozen foods). When dual metal standards are prepared inverse concentration ratios of the two metals are used to establish that interferences do not arise. The fixed volume addition is mandatory to prevent dilution effects on the mechanism of pressure decomposition. The concentrations of acids following dilution for both processes is $< 40\%$, because it is consumed in the chemical reactions which take place during processing.

*Digestion procedure and chemistry*

Process 1 is designed for those elements in food products which are usually present at levels of less than $1.0\,mg\,kg^{-1}$ such as lead. Concentrated Aristar grade nitric acid is used as the scavenging digestion acid and electro thermal graphite furnace (CRA, HGA) or plasma emission spectroscopy (PES) as the measurement technique. Flame atomic absorption spectroscopy may be used for the same elements subject to sufficient sensitivity.

The chemistry of the reactions taking place under pressure is not fully understood. Invariably, the colour of the reactants is a dark green which would indicate that the active constituent is nitrous acid.

Process 2 uses a mixture of hydrochloric acid and hydrogen peroxide for the determination of tin but it may also be used for iron and possibly a number of other elements which form soluble chlorides. The chemistry of the sample/reagent mixture is complex and is only significantly reactive above 60°C. At 70°C an initial increase in container pressure occurs and this is followed shortly by a progressive decrease in pressure. Progress of the reaction is indicated by a change in colour to orange-brown. Chlorine and chlorine dioxide are the active constituents and these react with organic material to form organic chlorine complexes accompanied by a drop in container pressure. The acidity of the solution decreases significantly.

The pressure profiles within a Sterilin P.128 container for Process 1 and Process 2 are shown in Figs 1 and 2 using typical canned food samples. The colour changes that occur are also shown to indicate the progress of the reaction. In Process 1, emission of the gaseous reaction products increase the internal pressure and trigger the valve acting cap at the point shown in Fig. 1. This is because the flexibility of the plastic valve increases at the higher temperature and lowers the triggering pressure, venting occurs and the pressure

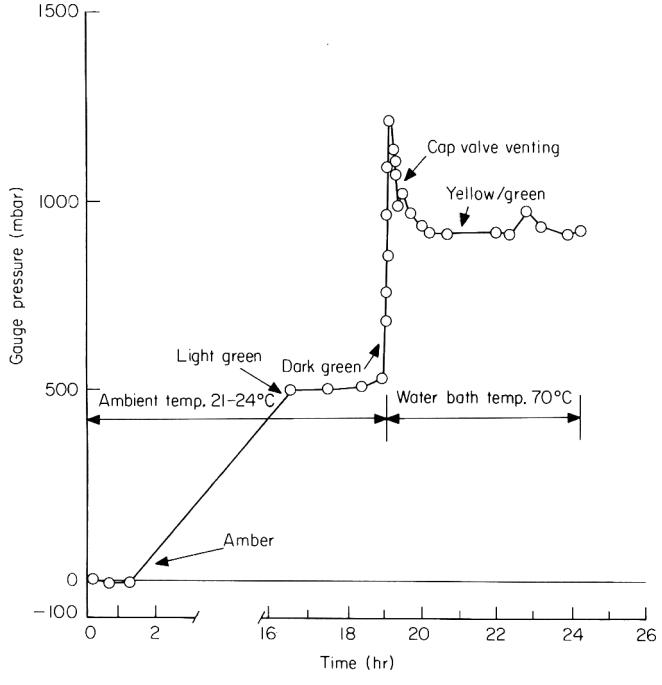


**Figure 1.** Pressures measured within a 'Sterilin' container using Process 1 for 5.0 g of canned grape fruit juice. The colour of the solution at various stages is indicated.

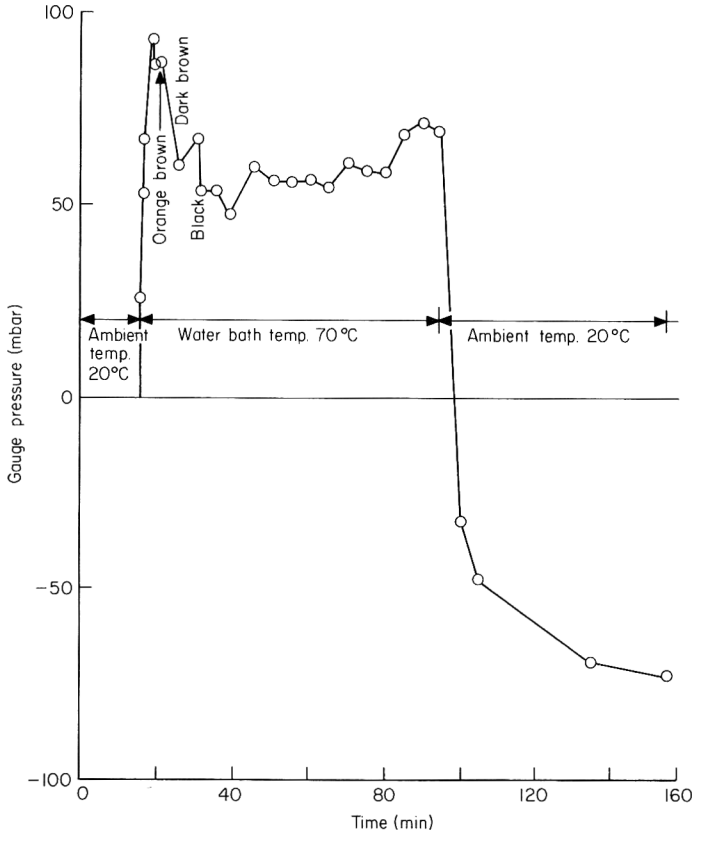


**Figure 2.** Pressures measured within a 'Sterilin' container using Process 2 for 5.0 g canned plums. The colour of the solution at various stages is indicated.

decreases. There is a gradual decrease in venting pressure as the cap temperature increases with time until a stable temperature is reached. Venting is a repetitive process, the valve opening and closing according to internal pressures being continuously regenerated. In Process 2, the mechanism is entirely different, an increase in temperature initially increases pressure which subsequently decreases because absorption of the gaseous reaction products takes place and if the reaction is allowed to continue, pressures will decrease to negative values. Possible loss of volatile metallic species is therefore prevented because venting does not arise and the reaction is only significant above 60°C and is very slow at 20°C. In both processes the maximum pressures attained at 20°C will depend on the type of food product.

*Precautions*

The gases generated in Process 1 are toxic oxides of nitrogen which are released from the sealed container when the pressure inside is sufficient to

operate the valve-acting mechanism of the screw cap. This is normally ≙ 2 bar (g) at 20°C and somewhat lower at 70°C. The gases generated in Process 2 are also toxic consisting mainly of chlorine and chlorine dioxide. The pressure due to these gases subsequently decreases to below ambient air pressure as the chlorine reacts with organic material. Consequently, the entire sample processing procedure for both Process 1 and Process 2 must be conducted inside a well ventilated (forced air) fume chamber provided with a glass fronted shutter which is partially closed during all manipulative tasks. Because of the reactivity of the processing acids at 70°C, it is essential to wear the appropriate protective clothing, gloves and eyewear.

It is very important to discard samples that contain yeast cells or have fermented on standing, e.g. fruit juices. It is dangerous for these samples to be allocated to Group 1 for processing. This is because the active yeast cells present together with alcohol or acetic acid react violently with the processing acids used. Samples may be safely stored in a freezer–refrigerator if processing cannot commence on the day of sampling, can opening or food homogenization. Similarly dry or powdered beverages or food products should not be processed without preliminary tests to establish reaction rate. This can be controlled by adding water. A layer of calcium carbonate cubes or chips placed in the bottom of the water bath is effective in preventing corrosion damage to water bath components by accidental spillage of acids from containers placed in the water bath. A thermal cut-out operating at 73–74°C is an additional device which is desirable in the interests of safety. The operating temperature of the bath must be rigidly controlled to 70 ± 2°C.

Finally, it is strongly recommended that the procedure is not undertaken in a polystyrene or other container which does not conform to the specifications and safety requirements which are outlined in this paper.

This procedure has now undergone a container test programme of over 20 000 Sterlin P.128 containers without a test failure due to a malfunction of the screw cap. However, a brief visual examination of the container walls in good light is advisable to establish the absence of fractures. Affected containers, if used, will develop a slow leak and will produce high iron values. These are easily recognized and do not present a major safety hazard. Fortunately, this is not a common occurrence. The hazards highlighted in this paper are considered to be substantially less than those that may arise during wet oxidation using perchloric acid or 50% hydrogen peroxide in conventional procedures.

## Verification and results

### *Determination of lead*

The results for the determination of lead using the new procedure are given in Table 3 (Laboratory 8). Other participating laboratories (1–7) used traditional techniques including those recommended by the S.A.C. A citric acid was

Table 3. Interlaboratory determination of lead in 15% citric acid (lead results in $mg\,kg^{-1}$)

| Laboratory | Method of analysis | Mean of at least 3 determinations | | | |
|---|---|---|---|---|---|
| | | Sample | A | B | C |
| 1 | Direct flame* | | 1.98 | 0.55 | < 0.02 |
| 2 | Direct flame | | 1.87 | 0.45 | 0.02 |
| 3 | Direct flame | | 1.82 | 0.46 | – |
| 4 | Direct flame | | 1.80 | 0.40 | – |
| 5 | Solvent extraction† | | 1.95 | 0.50 | – |
| 6 | Solvent extraction | | 1.80 | 0.48 | < 0.02 |
| 7 | Direct CRA‡ | | 1.92 | 0.43 | 0.005 |
| 8 | Process 1 and CRA§ | | 2.02 | 0.53 | < 0.015 |

A, 2.0 $mg\,kg^{-1}$ added Pb; B, 0.5 $mg\,kg^{-1}$ added Pb; C, no addition of Pb.

* Sample aspirated directly into air acetylene flame without preparation.

† Sample wet oxidized, lead solvent extracted as the APDC complex and aspirated into flame.

‡ Direct injection of diluted sample into carbon rod (CRA) analyser tube.

§ Process 1 procedure using 5 ml sample in 25 ml 40% $HNO_3$ followed by CRA analysis using 2.5 $\mu$l sample injection into a specially treated tube.

used as a matrix to ensure that lead remained in solution in the time that elapsed between the dispatch to and analysis by each laboratory. It was necessary to dilute the sample with water before analysis by the new procedure to bring the lead level within the operating range (0.01–0.1 $\mu g\,ml^{-1}$ Pb) for the carbon rod atomizer (CRA). Despite the wide range of techniques used, the overall agreement is exceptionally good.

Table 4 shows good agreement between atomic absorption (CRA) and plasmas emission results for the determination of lead in orange juice. The same samples were used for both analyses. Standards were prepared in an orange juice matrix low in lead.

*Determination of tin and iron*

Verification of tin and iron results for the new procedure is given in Tables 5–10.

The results given in Table 5 for tin show the performance of the new procedure on a range of food products which encompasses the three food groups described in the text. A similar exercise for iron is given in Table 6 and it is additionally shown that iron may be determined by either Process 1 or Process 2 (nitric acid or hydrochloric acid hydrogen peroxide mixture). However, Process 2 is a much faster technique and is therefore preferred. The results obtained agree closely with related or traditional methods of analysis. Table 7 shows the repeatability obtained on ten replicate orange juice samples for tin

Table 4. Results for the determination of lead in orange juice (lead results in mg $kg^{-1}$)
Treatment procedure: Process 1, Food classification: Group 1. Comparative results between electrothermal atomization CRA 63 and D.C. plasma emission – Spectrospan 3

| Sample no. | By CRA* | By PES† |
|---|---|---|
| 22 | 0.11 | 0.11 |
| 27 | 0.71 | 0.71 |
| 29 | 0.28 | 0.29 |
| 40 | 0.10 | 0.11 |
| 44 | 0.08 | 0.09 |
| 53 | 0.04 | 0.04 |
| 58 | 0.24 | 0.26 |
| 67 | 0.20 | 0.20 |
| 80 | 0.35 | 0.35 |
| 87 | 0.27 | 0.28 |
| 90 | 0.44 | 0.46 |
| 102 | 0.09 | 0.10 |
| 103 | 0.05 | 0.05 |
| 114 | 0.24 | 0.26 |
| 128 | 0.17 | 0.17 |
| 136 | 0.52 | 0.49 |
| 143 | 0.43 | 0.41 |
| 512 | 0.14 | 0.14 |
| 515 | 0.20 | 0.20 |

*Carbon rod analysis – tube treated to improve performance, measurements made at 217.0 nm using sample volume of 2.5 $\mu$l.

† D.C. plasma emission using Spectrospan 3 at 405.7 nm.

Table 5. Determination of tin in various food groups (results in mg $kg^{-1}$)

| Canned product | Food group | Referee result* | Process result† |
|---|---|---|---|
| Orange juice | 1 | 519 | 521 |
| Grapefruit juice | 1 | 43 | 43 |
| Brisling in oil | 2 | 60 | 63 |
| Corned beef | 2 | 70 | 72 |
| Ginger beer | 3 | 50 | 50 |
| Wine | 3 | 106 | 107 |
| Plums | 1 | 405 | 402 |
| Rhubarb | 1 | 617 | 615 |
| Milk | 2 | 52 | 53 |
| Vegetable soup | 2 | 144 | 146 |

*The referee procedure involves the wet oxidation of 50 g of food product followed by reduction and iodometric titration of $Sn^{++}$. Sample weights appropriate to the food group, i.e. 5.0 g or 10 g were used for the Process 2 procedure. Results are the mean of three determinations.

† Measured at 286.3 nm in a nitrous oxide–acetylene flame by atomic absorption. Results are the mean of at least three determinations.

**Table 6.** Determination of iron (results in $mg\,kg^{-1}$). A comparison of the results obtained between Process 1 and 2 using atomic absorption in an air–acetylene flame at 248.3 nm

| Canned product | Food group* | Process 1 | Process 2 |
|---|---|---|---|
| Orange juice | 1 | 15.8 | 16.1 |
| Orange juice | 1 | 12.6 | 12.2 |
| Grapefruit juice | 1 | 25.0 | 26.3 |
| Rhubarb | 1 | 6.6 | 6.5 |
| Soup | 2 | 4.5 | 4.6 |
| Soup | 2 | 16.0 | 14.0 |
| Ginger beer | 3 | 10.0 | 9.8 |
| Tomato soup | 2 | 38.0 | 37.0 |
| Tomato puree | 1 | 5.0 | 4.9 |

*Sample weight 5 or 10 g appropriate to food group.

**Table 7.** Process 2; repeatability and stability tests (tin and iron results on orange juice given in $mg\,kg^{-1}$)*

| Serial no. | Sn | Fe | Fe† |
|---|---|---|---|
| 1 | 16.1 | 2.42 | 2.40 |
| 2 | 17.6 | 2.30 | 2.43 |
| 3 | 17.6 | 2.57 | 2.37 |
| 4 | 17.6 | 2.54 | 2.53 |
| 5 | 17.6 | 2.42 | 2.46 |
| 6 | 17.6 | 2.51 | 2.40 |
| 7 | 17.6 | 2.39 | 2.53 |
| 8 | 17.6 | 2.63 | 2.40 |
| 9 | 17.6 | 2.63 | 2.59 |
| 10 | 17.6 | 2.57 | 2.53 |
| Mean | 17.6 | 2.49 | 2.46 |
| Spread | 16.1–17.6 | 2.30–2.63 | 2.37–2.59 |

*Tin is measured in a nitrous-oxide–acetylene flame at 286.3 nm and iron in an air–acetylene flame at 248.3 nm.

† These results were obtained after the same sample in sealed containers had been allowed to stand for a period of 2 months.

and iron using Process 2. In practice, it was not possible to obtain this precision using traditional techniques for comparative purposes. The stability of the solutions following processing is good. There is no significant difference in iron results repeated 2 months later. Tin solutions are also stable. These are useful characteristics which enable prepared samples to be stored before analysis. Plasma emission is a relatively new technique which is gaining wide acceptance. Table 8 shows the comparative results obtained for tin and iron using raspberries (frozen) as a matrix. Recoveries are excellent and compare favourably with atomic absorption (flame) values. When matrix additions are not made to

Table 8. Comparative results for the determination of added tin and iron to raspberries (results in $\mu g\ ml^{-1}$ of aspirated solution)

| Sample no. | Concentration: added tin ($\mu g\ ml^{-1}$) | Concentration: added iron ($\mu g\ ml^{-1}$) | Flame A.A.S* Found: Tin | Flame A.A.S* Found: Iron | P.E.S.† Tin | P.E.S.† Iron |
|---|---|---|---|---|---|---|
| 1 | 2 | 0.16 | 2.4 | – | 2.1 | 0.160 |
| 2 | 4 | 0.08 | 4.8 | – | 4.3 | 0.081 |
| 3 | 8 | 0.04 | 7.6 | – | 8.3 | 0.042 |
| 4 | 16 | 0.02 | 15.2 | – | 16.0 | 0.021 |
| 5 | 32 | – | 29 | – | 32.4 | – |
| 6 | 64 | – | 64 | – | 66.1 | – |
| 7 | 320 | – | off scale | | 320 | – |

* Flame A.A.S. measurements using nitrous oxide–acetylene at 286.3 nm.

† D.C. plasma emission – Spectrospan at 317.0 nm for tin and 371.9 nm for iron.

The raspberries used for this exercise were picked and immediately frozen. No tin and very low iron values were apparent in matrix blank solutions.

Plasma emission has at least 4 x linear dynamic operating range of A.A.S. for tin and iron.

Table 9. Recovery of added tin and iron by direct comparison with aqueous standards*

| Food type and group | Added ($mg\ kg^{-1}$) Sn | Added ($mg\ kg^{-1}$) Fe | Found ($mg\ kg^{-1}$) Sn | Found ($mg\ kg^{-1}$) Fe | % recovered Sn | % recovered Fe |
|---|---|---|---|---|---|---|
| 1. Tomato puree | 50 | 5 | 55 | 5 | 110 | 100 |
| | 100 | 10 | 104 | 10 | 104 | 100 |
| | 200 | 20 | 220 | 19.5 | 110 | 97.5 |
| | 400 | 40 | 380 | 39 | 95 | 97.5 |
| 2. Ideal milk | 50 | 5 | 55 | 5 | 110 | 100 |
| | 100 | 10 | 110 | 10.5 | 110 | 105 |
| | 200 | 20 | 201 | 20.3 | 100 | 101 |
| | 400 | 40 | 420 | 38 | 105 | 95 |
| 3. Ginger beer | 25 | 2.5 | 28 | 2.4 | 112 | 98.4 |
| | 50 | 5 | 55 | 5.5 | 110 | 110 |
| | 100 | 10 | 114 | 9.6 | 114 | 96 |
| | 200 | 20 | 222 | 16.7 | 111 | 83.5 |

* Tin and iron measured by atomic absorption as in Tables 4 and 5. Standard additions were made to food samples in the range 25–400 $mg\ kg^{-1}$ for tin and 2.5–40 $mg\ kg^{-1}$ for iron, and recovery calculated using aqueous standards without matrix addition.

standards, recoveries of the order shown in Table 9 are obtained on different food types and for some products are acceptable. Finally, Table 10 provides the mean and spread values for tin and iron on samples selected as being representative of the three food groups. Tin and iron results are compared and

**Table 10.** Process 2. Determination of tin and iron (comparative results in mg $kg^{-1}$)

| *Classical reference procedure* | | | *New procedure* | |
|---|---|---|---|---|
| Wet oxidation followed by titration for tin and wet oxidation followed by colorimetric measurement for iron. | | | Measurement by A.A.S. for tin and iron following pressure decomposition. A.A.S. measurement details remain unchanged as described in Tables 5 and 6. | |
| Food classification Group 1 | | | | |
| Orange juice 50 g | | | Orange juice 5.0 g | |
| Sample | Sn | Fe | Sn | Fe |
| 1 | 142 | 6.05 | 150 | 4.2 |
| 2 | 142 | 3.02 | 143 | 5.0 |
| 3 | 157 | 4.56 | 144 | 4.0 |
| 4 | 151 | 7.56 | 146 | 4.0 |
| 5 | 145 | 3.83 | 143 | 5.2 |
| Mean | 147 | 5.00 | 145 | 4.48 |
| Spread | 142–157 | 3.02–7.58 | 143–150 | 4–5.2 |
| Food classification Group 2 | | | | |
| Ideal milk 50 g | | | Ideal milk 5.0 g | |
| Sample | Sn | Fe | Sn | Fe |
| 1 | 270 | 3.0 | 275 | 3.0 |
| 2 | 262 | 2.5 | 275 | 3.0 |
| 3 | 262 | 5.0 | 285 | 3.0 |
| 4 | 238 | 2.5 | 275 | 3.0 |
| 5 | 238 | 4.0 | 265 | 3.0 |
| Mean | 254 | 3.4 | 275 | 3.0 |
| Spread | 238–270 | 2.5–5.0 | 265–285 | Nil |
| Food classification Group 3 | | | | |
| Ginger beer 50 g | | | Ginger beer 10 g | |
| Sample | Sn | Fe | Sn | Fe |
| 1 | 2.5 | 5.8 | 5.0 | 7.2 |
| 2 | 2.5 | 5.2 | 5.0 | 7.0 |
| 3 | 2.5 | 6.9 | 5.0 | 7.1 |
| 4 | 2.5 | 4.7 | 5.0 | 7.1 |
| 5 | 2.5 | 6.8 | 5.0 | 7.1 |
| Mean | – | 5.88 | – | 7.1 |
| Spread | – | 4.7–6.9 | – | 7.0–7.2 |

show the favourable performance of the new method. It is considered that the improvements offered by the new procedure are mainly due to the preventative measures undertaken to eliminate or minimize contamination opportunities that arise to a variable extent during the course of preparation and final analysis using traditional procedures. Also because of the number of manipulative tasks that are involved in many of these procedures, variable losses are liable to take place.

### *Interferences*

Tests undertaken to establish mutual interference effects for tin, lead and iron did not show any significant deviation. Standards prepared in the same matrix as the product to be examined are a good indication of interference.

No interferences are experienced using plasma emission and it is anticipated that this technique will have much to recommend it as the preferred measurement technique in due course.

## Conclusions

The inclusion of certain criteria in the design of new methods of analysis were considered when the development of the foregoing procedure commenced. It is appropriate in conclusion to determine and discuss whether these qualities for the design of a method have been met.

(1) *Versatility* – the method is adaptable to a very wide variety of foods and also to pathological specimen analysis. It may be applied to dry or dehydrated food products providing measures are taken to control the reactivity of the product with the processing acids used.

(2) *Sensitivity and accuracy* – this is illustrated in the results given. There is an improvement in repeatability and reproducibility simply because the variable such as volumes of processing acids, etc. have been rigidly standardized. Constant blanks are therefore obtained and this leads to improved accuracy. Sensitivity is adequate with modern instrumentation and the elements of interest within the range 0.01 to 200 $\mu g\,ml^{-1}$ can be determined without difficulty.

(3) *Simplicity and ease of use* – constant supervision and manipulative skill is not required. Throughput rates now exceed the rates at which samples can be measured and it is in this capacity that the procedure described has important advantages. Samples may be stored ready for measurement without deterioration.

(4) *Operating costs* – in comparison with more conventional procedures are low, e.g. it has been calculated that the material cost saving compared with wet oxidation is at least a factor of 30. There is an even greater cost advantage in terms of applied man hours.

(5) *Effluents and emissions* – are significantly reduced and this is simply because no strong acid-high temperature fuming procedures are used. Volumes of acid for disposal are considerably less.

(6) *The suitability of the procedure for automation* – was considered early in the development stage. The entire processing procedure can be very simply automated because all tasks from sample weighing up to analysis are conducted in a single container. The geometry of the container allows it to be used readily with centrifuges or automatic presentation devices. Plasma emission instruments of the type mentioned in this paper could very readily be used for the automatic simultaneous multi-element analysis of foods prepared according to the procedure described. Similarly, automatic presentation devices for graphite furnace (CRA, HGA) atomization equipment make the tube life and performance an important consideration and this is particularly the case in hostile high acid sample environments.

(7) *Contamination from usual sources* – viz. pick-up from containers, processing reagents, airborne particles, is reduced to values that are below the detection limits for most of the measurement techniques used.

The experience gained in the analysis of many thousands of samples over several years has indicated that the procedure described is adaptable to a wide variety of products. At regular intervals, and when a new product is presented for analysis, comparative tests along the lines indicated are conducted to establish the continued validity of the technique.

In essence, the essential criteria proposed have been met and some additional advantages to the user have become apparent with experience.

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# Study of some factors affecting the growth of soy yeast (*Saccharomyces rouxii* NRRLY-1096)

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

## Summary

The effects of sodium chloride concentration, pH and temperature on the growth characteristics of *Saccharomyces rouxii* NRRLY-1096 are described. It was found that sodium chloride increased the lag phase of the growth curve and reduced the total amount of growth. Doubling time was less at 37°C during the exponential growth phase as compared to that at room temperature (28°C). Total amount of growth was however, greater at room temperature. *Saccharomyces rouxii* NRRLY-1096 grew over a wide pH range in the presence of relatively high concentrations of salt, but pH 4.0 to 6.0 was most favourable to its growth.

## Introduction

In previous papers (Yong & Wood, 1976, 1977a, b) the microbial changes in the course of laboratory soy sauce fermentation, biochemical changes in the course of the mould growth of the Koji stage, and biochemical changes in experimental soy sauce Moromi, were described. In the present paper we describe our studies on factors which are thought to affect the growth of *Saccharomyces rouxii* NRRLY-1096 which is a yeast isolated from soy mash and shown to be of importance as far as flavour development in soy sauce is concerned.

## Materials and methods

### *Purity and source of chemicals*

All chemicals used were of 'Analar' grade or the purest grade available. They were obtained mainly from Merck's Chemical Company, Germany, and Sigma

*Present address: New Zealand Milk Products Ptd Ltd, Quality Road, Singapore 22, Republic of Singapore.

Authors' address: Department of Biochemistry, University of Singapore, College Road, Singapore 3, Republic of Singapore.

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Chemical Company, U.S.A. Chemicals for yeast culture were from Oxoid, England, or Difco, U.S.A.

*Yeast culture*

The pure culture strain of *Saccharomyces rouxii* NRRLY-1096 studied was supplied by Dr C. W. Hesseltine of the Northern Regional Research Laboratory, Peoria, U.S.A.

*Media and culture methods*

The lyophilized preparation of *Sacch. rouxii* was reactivated by inoculating into broth with the composition:

| | |
|---|---|
| Malt extract | 3.0 g |
| Yeast extract | 3.0 g |
| Peptone | 5.0 g |
| Glucose | 10.0 g |

Distilled water to 1 litre.

The pH of the medium was found to be 6.8 after autoclaving at 10 lb pressure (p.s.i.) for 20 min.

The inoculated broth was incubated at room temperature (28°C) for 3 days before being used to prepare stock cultures which were then maintained on agar slopes. These stock cultures were kept at 4°C and sub-cultured at bi-monthly intervals.

Inocula were prepared by sub-culturing the yeast from the slopes into 10 ml of broth for 48 hr. The medium used for growing the inoculum had the same composition and pH as the medium used for the experiments.

*Growth studies*

Experiments were conducted with 250 ml media in 1 litre conical culture flasks which were manually shaken to suspend yeast cells, three times during the day and when samples were taken. Incubation was at room temperature (28°C).

The composition and pH of the standard media were modified according to the conditions required for each experiment.

*Estimation of dry cell weight*

Yeast cells in culture media were harvested by centrifuging at 1300*g* at 28°C, and the cells washed three times with distilled water before being dried to constant weight at 60°C.

## Experimental and results

### *Effect of* DL*-lactic acid on growth*

*Lactobacillus delbruekii* has been isolated from soy mash and thought to be of importance in soy sauce fermentation (Lockwood, 1947; Lockwood & Smith, 1950–51). Its presence in the Moromi was, however, not considered by Japanese workers to be of any consequence. Yong (1971), in a preliminary study, found that the presence of *Lactobacillus* could be omitted and a good quality soy sauce obtained if the mash was acidified to pH 4.5 with lactic acid. Results in Fig. 1 shows that the growth of yeast in medium containing 10 to 20% NaCl and acidified with lactic acid was correspondingly greater than that grown in medium acidified with HCl. For the same concentration of NaCl in the medium, there was no difference in the duration of the lag growth phase of the yeast in medium containing 10 or 15% NaCl acidified with either lactic acid or HCl. The lag growth phase was more prolonged for yeast in medium acidified with HCl at 18 and 20% NaCl concentrations than in medium acidified with lactic acid for the same NaCl concentrations. The possibility of lactic acid as a metabolisable substrate was next investigated together with acetate, pyruvate and citrate.

Figure 2 shows that lactate, pyruvate and citrate supported some growth at pH 4.5 but not at pH 7.0. The standard growth medium without glucose was able to support some growth too at pH 4.5. Growth was, however, inhibited by

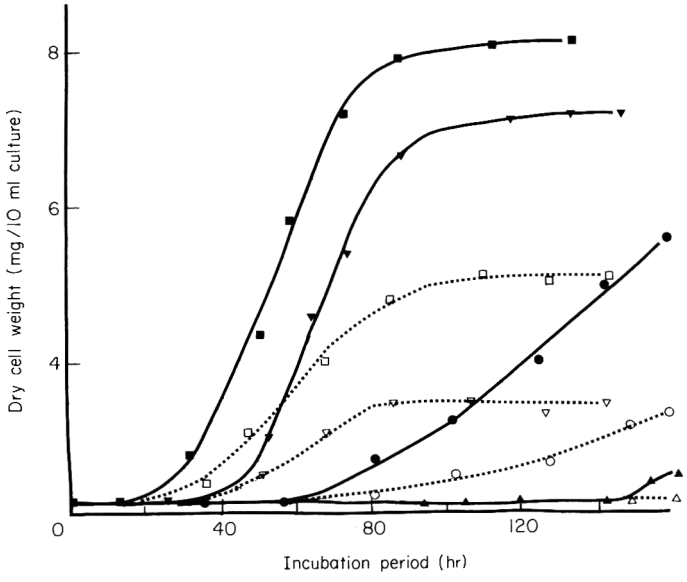


**Figure 1**. Comparison between the effects of lactic acid (solid symbols) with that of HCl (open symbols) for the acidification of growth medium to pH 4.5. Cells were grown at room temperature (28°C). Medium containing □, ■ 10% NaCl; ▼, ▽ 15% NaCl; ○, ● 18% NaCl; △, ▲ 20% NaCl.

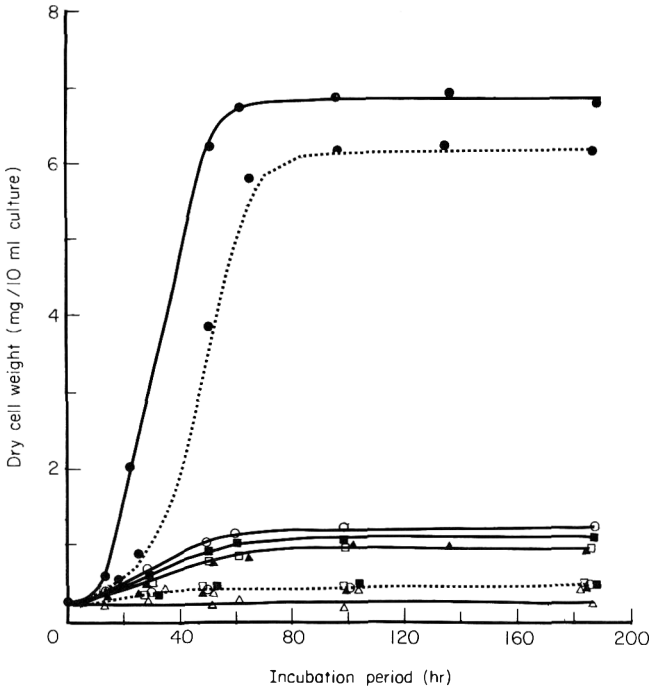


**Figure 2.** Utilization of acetate, lactate pyruvate and citrate as carbon source at pH 4.5 (continuous lines) and pH 7.0 (broken lines). Cells were grown at room temperature (28°C) in medium containing 10% sodium chloride. ●, 0.5% glucose; ○, 1.0% pyruvate; ■, 1.0% lactate; ▲, 1.0% citrate; △, 1.0% acetate; □, 1.0% no substrate.

acetate. There was no growth in the absence of glucose at pH 7.0. Phthalate buffer was used for pH 4.5 instead of the standard citrate buffer in this series of experiments.

*Optimum pH for growth*

In a study on the relationship between yeast cells and lactic acid bacterium, Yong & Wood (1976) found that when the yeast was inoculated into soy mash, the number of cells decreased until a minimum was reached after 48 hr. At this point, the pH of the Moromi had also declined from pH 6.5 to pH 6.0 and continued to do so until pH 4.5 was reached. The growth of the yeast, however, increased after its initial fall and continued to rise. The effect of pH on the growth of the yeast was therefore investigated.

Figure 3 shows that yeast cells are able to grow over a wide range of pH from pH 2 to 9 even though there were considerable differences in the growth patterns. Between pH 4 and 6, the lag growth phase was at its minimum (Fig. 4).

Changes in the pH of the growth medium were also monitored during the growth of the yeast. Table 1 shows the pH of the medium after 8 days of

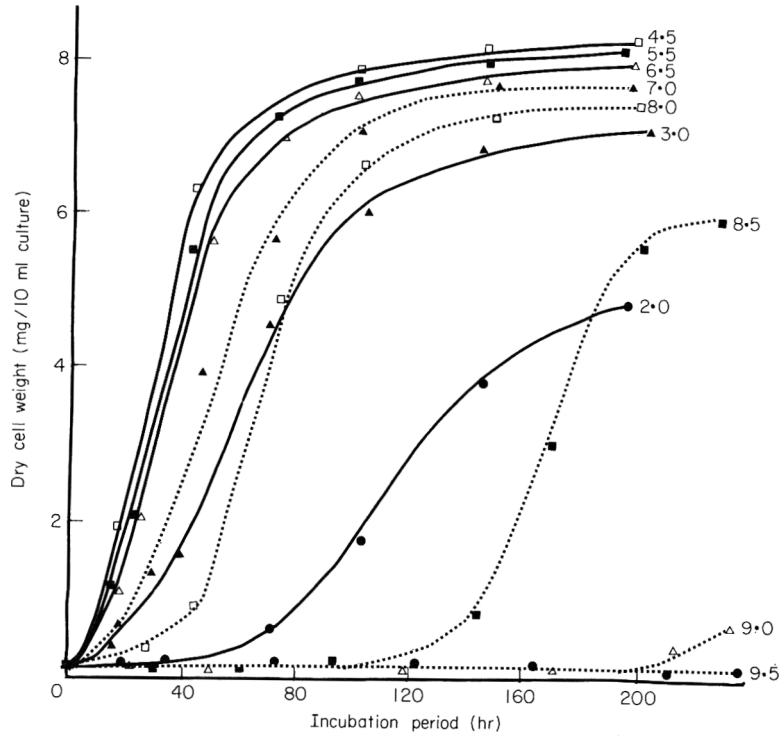


**Figure 3.** Effect of pH on growth at room temperatue (28°C) in medium containing 10% NaCl. The pH is indicated for each line.

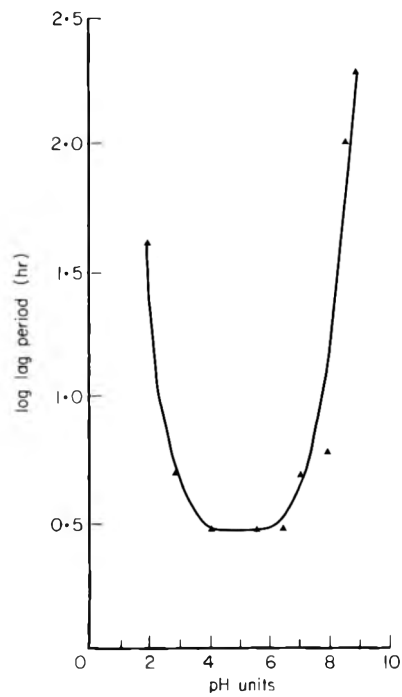


**Figure 4.** Effect of pH on the lag growth phase at room temperature (28°C) in medium containing 10% sodium chloride.

**Table 1.** Change in the pH of medium with growth

| Initial pH of medium | pH of medium after 8 days incubation with yeast |
|---|---|
| 2.0 | 2.0 |
| 2.5 | 2.5 |
| 3.0 | 3.0 |
| 3.5 | 3.5 |
| 4.0 | 4.0 |
| 4.5 | 4.3 |
| 5.0 | 4.6 |
| 5.5 | 4.8 |
| 6.0 | 5.0 |
| 6.5 | 5.0 |
| 7.0 | 5.0 |
| 7.5 | 5.0 |
| 8.0 | 5.0 |
| 8.5 | 5.5 |
| 9.0 | 8.0 |
| 9.5 | 9.5 |

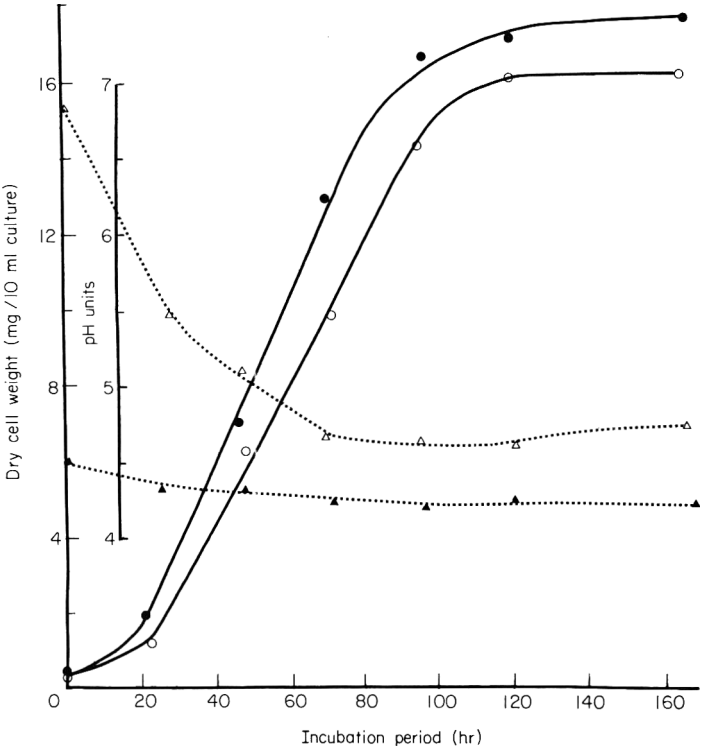


**Figure 5.** Effect of growth at room temperature (28°C) on pH of the medium containing 10% sodium chloride. ●, growth in pH 4.5 medium; ○, growth in pH 6.8 medium; ▲, pH of medium with growth from pH 4.5; △, pH of medium with growth from pH 6.8.

growth. Those with pH 4 or less remained more or less the same, whilst there was a tendency of the less acidic pH to change to a lower pH during the growth of the yeast. Figure 5 provides a comparison between changes in pH in culture medium of initial pH 4.5 and 6.8. There was a rapid fall in pH in the neutral medium inoculated with yeast and little change in pH in the acid medium during the growth of yeast. After the initial fall in pH from 6.8 to 4.6, there was a gradual rise and the pH after 8 days growth was 5.0 (Table 1). The growth characteristics of the yeast in both media, however, appeared to be similar, with a greater amount of total growth in the more acidic medium.

This was probably due to the utilization of the glucose substrate for the production of acids resulting in a lesser amount of growth. During the study of the effect of pH on the growth of the yeast, it was observed that yeast grown at pH between 2.5 and 8.5 formed pellicles. Between pH 3 and 7, the pellicles appeared after about 2 weeks of growth and the rest 4 to 5 days later. No pellicle formation was observed in media at pH 2.0, 9.0 and 9.5 even on the thirty-fifth day of incubation and after the culture grown at pH 2.0 had matured.

One of the factors which could have given rise to pellicle formation might have been the exhaustion of substrates for growth (Mills & Blackwood, 1967). Figure 6 shows that the amount of glucose (1%) present in the standard growth medium was quickly exhausted. Glucose could not be detected in the medium when maximum growth was reached. Increasing the glucose concentration

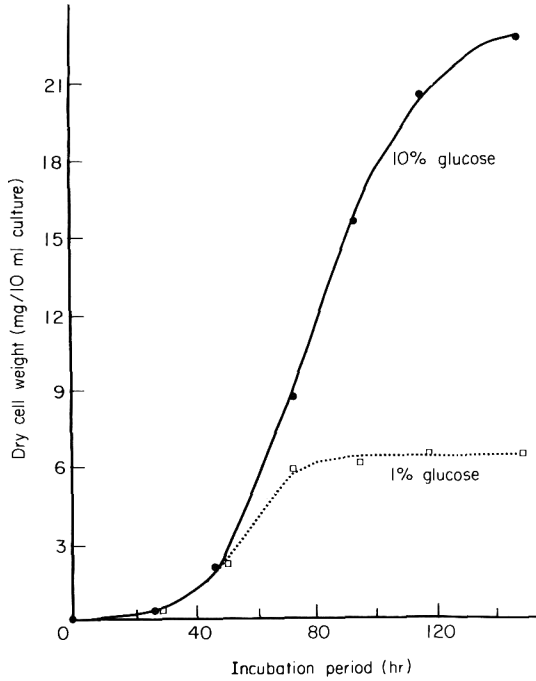


**Figure 6.** Effect of glucose concentration on growth at pH 4.5 in medium containing 10% sodium chloride. Cells were grown at room temperature (28°C).

increased maximum growth but a ten-fold increase resulted in only a three-fold increase in the growth of the yeast. Factors such as adequate aeration for the efficient utilization of the glucose are probably also important for obtaining the maximum amount of total growth.

*Effect of sodium chloride on growth*

When yeast is used in the soy mash (Moromi) stage of soy fermentation, it has first to be 'trained' for growth in 18% salt by passage through portions of liquid maintenance medium containing stepwise increase in salt concentrations, e.g. 2, 4, 8, 12, 15 and 18% (w/v). How NaCl concentration in the medium affects the growth of the yeast is, however, not known. The effect of NaCl concentration was studied at pH 4.5 in medium containing 1% glucose.

Figure 7 shows that up to 18% NaCl concentration, the lag phase of the growth curves increased exponentially with increasing NaCl concentration. A further 2% increase from 18 to 20%, however, prolonged the lag growth phase tremendously to more than 120 hr; the growth of the yeast had only just begun when the experiment was terminated at 156 hr after inoculation (Fig. 8). The yeast cells were examined microscopically after the experiments. There were no observable differences in the physical characteristics between any of the cells even though they were grown in media containing a wide range of NaCl concentrations.

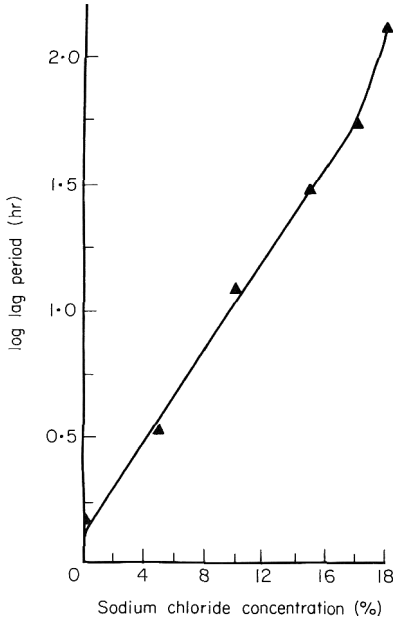


**Figure 7.** Effect of sodium chloride concentration on the lag growth phase. Cells were grown at pH 4.5 and at room temperature (28°C).

The presence of NaCl in the medium was found to affect the maximum amount of total growth of the yeast. Total amount of growth decreased with increasing NaCl concentration (Fig. 8). The rate of growth, measured at the exponential phase, also decreased, resulting in the prolongation in the doubling time as shown by Fig. 9.

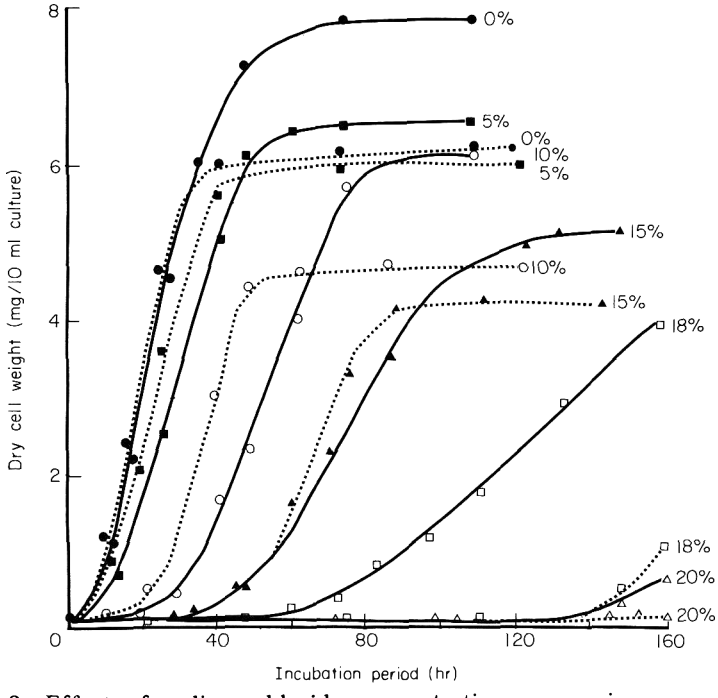


**Figure 8.** Effect of sodium chloride concentration on maximum growth at pH 4.5 medium. —— Incubation at 28°C; ···· incubation at 37°C.

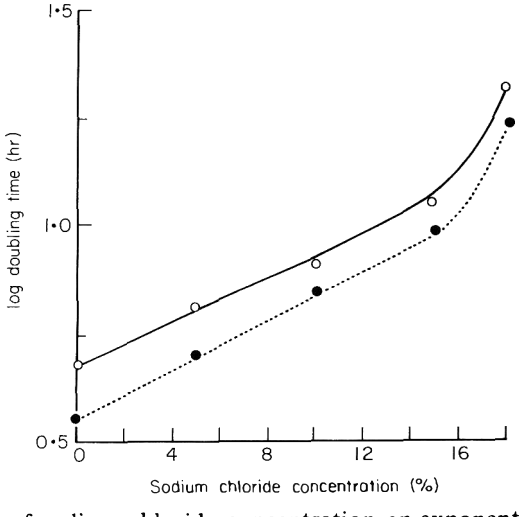


**Figure 9.** Effect of sodium chloride concentration on exponential growth phase at pH 4.5. ○ Incubated at 28°C, ● incubated at 37°C.

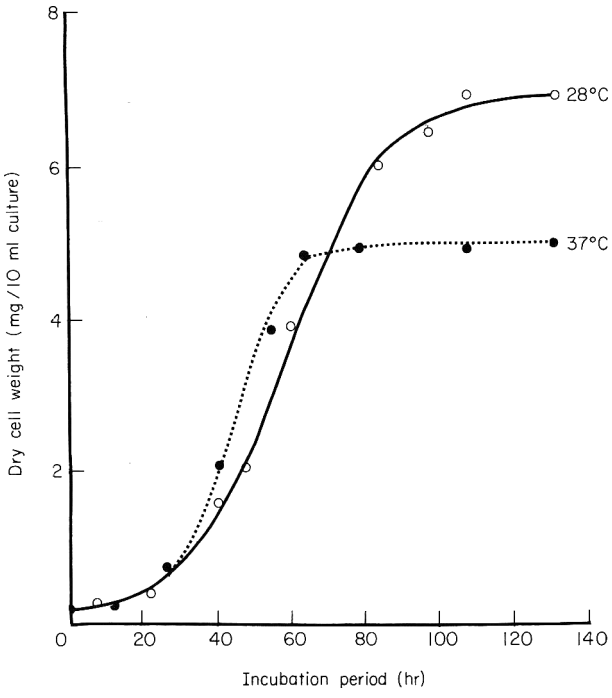


**Figure 10.** Effect of temperature on growth at pH 4.5 in medium containing 10% sodium chloride.

Figures 8 and 9 show that the growth retarding effect of NaCl on yeast cultures was independent of the temperature at which the yeast was grown, whether at 28°C (room temperature) or at 37°C. It was observed that the maximum amount of total growth obtainable at 37°C, although less than at 28°C, was attained earlier (Fig. 8). The doubling times were, however, shorter at 37°C at each specific NaCl concentration studied (Fig. 9). There was no difference in the lag growth phase. The effect of temperature on growth is shown more clearly by Fig. 10 with growth curves at both temperatures in the presence of 10% NaCl.

An attempt was made to determine whether the effects of NaCl were due to the $Na^+$ or $Cl^-$ ions or both. A comparison of the doubling time was made in

**Table 2.** Effect of $Na_2SO_4$ and KCl on doubling time as compared with the effect of NaCl

| | | Doubling time (hr) | |
|---|---|---|---|
| Salt (% w/v) | Cation conc. | Actual | Calculated (per mol cation conc.) |
| 5% $Na_2SO_4$ | 0.70 mol $Na^+$ | 5.52 | 7.89 |
| 5% NaCl | 0.85 mol $Na^+$ | 5.19 | 6.10 |
| 5% KCl | 0.66 mol $K^+$ | 4.00 | 6.06 |

the presence of NaCl and a different sodium salt as well as that of a chloride of a different metal such as potassium.

Table 2 shows that there was no difference in the effects of NaCl and KCl on the growth of the yeast. The nature of the effect of the potassium ion was probably similar to that caused by the sodium ion. The doubling time increased with $Na_2SO_4$ even though the $Na^+$ concentration in the medium was less than that in the medium containing NaCl.

## Discussion

The role of *L. delbruekii* in soya mash fermentation could not be established in the present study. Acidifying the growth medium with lactic acid, however, appeared to be beneficial to the growth of the yeast. The difference in the rate of growth of yeast grown under these conditions and those grown in medium acidified with HCl was too great to be attributed entirely to the effect of the small increase in total chloride content in the latter medium. Growth in the presence of 15% NaCl and lactic acid was very much better than growth in 10% NaCl without lactic acid and the medium acidified with HCl. The total chloride content in the latter medium was less than in the former.

The pH range between pH 4.0–6.0 appeared to be the optimum for growth of the yeast. The presence of the lactic acid bacteria in the Moromi would serve to hasten the lowering of the pH, thus promoting optimum growth in the shortest possible time.

The growth of the yeast was found to be greatly affected by the presence of NaCl. To terminate the Koji stage fermentation, the fermented soya bean mixture is saturated with NaCl to completely inhibit the growth and metabolism of the mould *Aspergillus oryzae* present in the Koji. The concentration of the NaCl is generally between 18 and 20%. Whether such a high concentration of NaCl is required for the termination of the Koji stage is not known and ought to be investigated. If the concentration of NaCl could be reduced by as little as 3% from 18 to 15%, the lag period and doubling time in the growth of the yeast would be greatly decreased and the maximum amount of total growth increased.

Thus the result of the present study seem to suggest that the Moromi stage of soy fermentation could be accelerated if the soy sauce Koji was first acidified with lactic acid to pH 4.0 to 6.0 and the yeast fermentation (Moromi stage) carried out in the presence of 15% NaCl instead of 18%, attained by saturating the Koji with NaCl.

## Acknowledgments

The authors wish to thank Dr P. W. Hesseltine for the supply of the yeast culture and Mr W. W. Leong for technical assistance.

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# The breadmaking potential of products of cassava as partial replacements for wheat flour

JOAN CRABTREE,* E. C. KRAMER* AND JANE BALDRY†

## Summary

Various fresh and dried cassava products were prepared on a pilot scale and incorporated into wheat flour bread at a 20% level of substitution. The cassava products were assessed for ease of preparation and ease of incorporation. Their effects on bread quality and sensory evaluation were investigated. Fresh minced cassava was the most easily prepared and incorporated into the bread. Blanched minced cassava gave the most sensorily acceptable loaf. Low temperature drying gave the most acceptable dried product, but sensory evaluation indicated that the bread made from dried products was inferior to that made from fresh products. An attempt was made to reduce the fibre content of the dried flours: these flours marginally improved loaf sensory acceptability, but gave rise to loose and soft crumb texture which had little strength. Soaking generally improved the baking quality of the dried flours: higher soaking temperatures had the greater effect but gave a product which was difficult to handle.

## Introduction

Numerous studies conducted over the last 40 years on the use of dried cassava products, notably flour and starch, in breadmaking have been reviewed by Dendy *et al.* (1975) and Kasasian & Dendy (1977).

The incorporation of fresh minced cassava into bread and the acceptability of the baked product has been studied by the present authors (Crabtree, Kramer & Baldry, 1978). This technique has the advantage of eliminating the need for an energy-consuming drying stage and should be of special interest to bakeries in rural areas of the developing world where fresh cassava is readily available. The longer storage life of dried cassava products may alternatively be advantageous in urban situations remote from cassava-producing districts.

Authors' address: *Tropical Products Institute, Industrial Development Department, Culham, Abingdon, Oxon OX14 3DA and † Tropical Products Institute, Plant Food Commodities Department, 56/62 Gray's Inn Road, London WC1X 8LU.

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This paper extends the work previously described to include the preparation of several fresh and dried cassava products and their incorporation into bread. Following peeling and slicing of the cassava tubers, four main processing operations were investigated: mincing, blanching, cooking and drying. Comparisons were made between the products prepared using one or more of these operations.

The literature makes little reference to the enzymes present in cassava but effective blanching would inactivate those enzymes which might adversely affect the quality of the bread containing a fresh cassava product. Blanching also removes oxygen from the tissues, reduces cell turgidity and could gelatinize some of the starch. These effects might also be important.

Cooking may be considered as a more severe form of heat treatment which produces the effects associated with blanching but also involves major textural changes associated with, for example, cellular breakdown and hydration. It is usually easier to mash root crops after cooking as with, for example, potatoes or yams (Gooding, 1972).

It has been suggested (Hudson & Ogunsua, 1976) that the presence of fibre is largely responsible for the inferior baking quality of cassava flour when incorporated into bread. Removal of the fibre from the flour should therefore effect an improvement in loaf quality. As fibre is unlikely to be reduced during milling to the same particle size as the other softer components of the flour, separation of the larger particles should reduce its fibre content. Instead of attempting to remove the fibre by classification the cassava flour could be soaked to soften the fibres prior to its use in breadmaking.

## Materials and methods

Cassava tubers were obtained through the Kenya Trading Corporation and air-freighted from Nairobi.

### *Preparation of the cassava products*

The cassava tubers were washed and partially peeled with a mechanical bench peeler, peeling being completed by hand: peeling losses were between 20 and 30%. The tubers were sliced mechanically to 1 cm thickness. Portions of the slices were processed as follows:

(i) Slices were minced mechanically by passing successively through $^3/_8$ inch and $^3/_{16}$ inch plates (sample 1). Part of the minced material was boiled for 1 hr in a closed sleeve of mutton cloth and cooled (sample 3).

(ii) Slices were blanched in water at 80°C for 5 min and plunged into cold water. These conditions were determined by testing for phosphatase inactivity using hydrogen peroxide. Some of the blanched slices were minced as in (i) (sample 2). The remainder were dried in a tray dryer with vents open for 16 hr at 50°C, allowed to cool and milled in a hammer mill fitted with a $^1/_8$ inch screen (sample 6).

(iii) Slices were boiled in water for 1 hr and minced as in (i) (sample 4) or hand mashed using a potato masher (sample 5).

(iv) Slices were dried in a tray dryer with vents open for 16 hr at 50°C. The dried slices were milled in a hammer mill fitted with $\frac{1}{8}$ inch screen (sample 7). Part of this sample was sieved: material passing a 250 $\mu$m screen was retained as 'defibred' (sample 8, Table 1). Soaking of sample 7 at temperatures of 30, 50 and 70°C was carried out at the breadmaking stage (samples 9, 10 and 11 respectively).

(v) Slices were dried similarly for 16 hr at 95°C. The milling, sieving and soaking operations were carried out as in (iv) (samples 12, 13, 14, 15 and 16 respectively).

A flow diagram of the preparation of the various cassava products is shown in Fig. 1.

The cassava products were scored for ease of preparation (Table 4) with regard to: the numbers of operations required to process the product, the capital cost and labour and energy inputs to the equipment used in the operations, handling difficulties, yield and keeping qualities of the product.

*Breadmaking procedures*

The moisture contents of the cassava products were determined by the AACC method (1969) (Table 2). The results obtained were used to determine

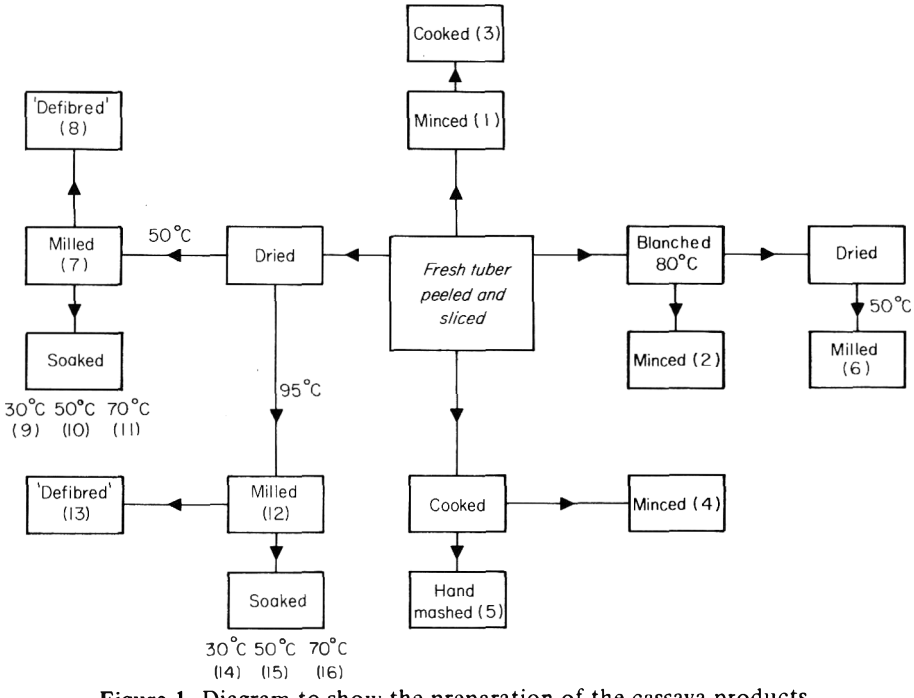


**Figure 1.** Diagram to show the preparation of the cassava products.

Table 1. Fibre contents of whole and 'defibred' flours dried at 50 and 95°C

| Sample | Fibre content (%) 50°C | 95°C |
|---|---|---|
| Whole flour | 2.54 | 2.84 |
| 'Defibred' (particle size less than 250 μm) | 1.88 | 2.32 |

Table 2. Moisture contents of cassava products used in breadmaking

| Sample no. | Treatment | Moisture content (%) |
|---|---|---|
| 1 | Minced | 62.4 |
| 2 | Blanched, minced | 67.8 |
| 3 | Minced, cooked | 78.0 |
| 4 | Cooked, minced | 69.0 |
| 5 | Cooked, hand mashed | 63.0 |
| 6 | Blanched, dried 50°C | 13.4 |
| 7 | Dried 50°C | 8.5 |
| 8 | Dried 50°C, 'defibred' | 6.9 |
| 9 | Dried 50°C, soaked 30°C | 8.5 |
| 10 | Dried 50°C, soaked 50°C | 8.5 |
| 11 | Dried 50°C, soaked 70°C | 8.5 |
| 12 | Dried 95°C | 4.5 |
| 13 | Dried 95°C, 'defibred' | 2.7 |
| 14 | Dried 95°C, soaked 30°C | 4.5 |
| 15 | Dried 95°C, soaked 50°C | 4.5 |
| 16 | Dried 95°C, soaked 70°C | 4.5 |

the amount of cassava product required to give a substitution level of 20%, calculated on a 14% moisture basis.

The soaked samples (9, 10, 11, 14, 15 and 16) were prepared immediately prior to breadmaking as follows. The weighed sample was mixed with 500 ml water at the required temperature (30, 50 or 70°C) in a stainess steel jug and maintained at that temperature in a thermostatically controlled water bath for 1 hr. For the samples soaked at 50 and 70°C, the stainless steel jugs were removed from the water bath after 55 and 50 min respectively and placed in ice for 5 and 10 min respectively, to cool the samples prior to incorporation into the bread dough.

Batches of several different samples were baked on each occasion together with a wheat flour control.

A bulk fermentation method of breadmaking was used, according to the recipe in Table 3.

The bakers flour used was of about 72% extraction and 12.4% protein content. To give a dough of suitable consistency, the volume of mixing water

Table 3. Recipe for breadmaking

| | | |
|---|---|---|
| *Dried yeast reconstitution* | | |
| Active dried yeast | 10 g | Aerobic respiration 15 min at 38°C |
| Sugar | 3 g | |
| Water at 38°C | 110 ml | |
| Baker's flour<br>Cassava product (at 14% moisture basis) | 1300 g | |
| Salt | 23.4 g | |
| Fat | 9.1 g | |
| Sugar | 10 g | |

was varied according to the moisture content of the cassava product used. The breadmaking procedure was carried out as previously described (Crabtree *et al.*, 1978). The cassava products were scored (Table 4) for ease of incorporation into the dough, with regard to ease of handling and ease of mixing.

The specific volumes of the baked loaves were determined by seed displacement and expressed as a percentage of mean control loaf volume (Table 4). The loaves were scored for shape, crust colour and structure (crust appearance) crumb texture, colour and odour (crumb appearance) and crumb feel and recovery from compression (tactile characteristics) (Table 4). Photographs were taken of slices cut from the loaves, a selection of which is illustrated in Fig. 2. The loaves were frozen in sealed polythene bags and stored at −10°C prior to sensory evaluation.

### *Sensory evaluation*

A panel was drawn from laboratory staff, all of whom had had experience of sensory assessment. Preliminary tests were conducted for panel training and selection which were identical in form and design to those used in the actual assessment.

Samples were warmed to ambient temperature by microwave thawing shortly before presentation and assessed in randomized batches of three, including where possible the appropriate control. The panel, consisting of a minimum of eighteen assessors, judged hardness or softness, dryness or moistness, doughiness or sponginess, and degrees of freshness and acceptability by marking along a 10 cm line. These assessments were converted into scores out of forty (Table 5).

## Results and discussion

The results of the physical assessment of the cassava products and the test loaves are summarized in Table 4. A sample with a score of less than half of the

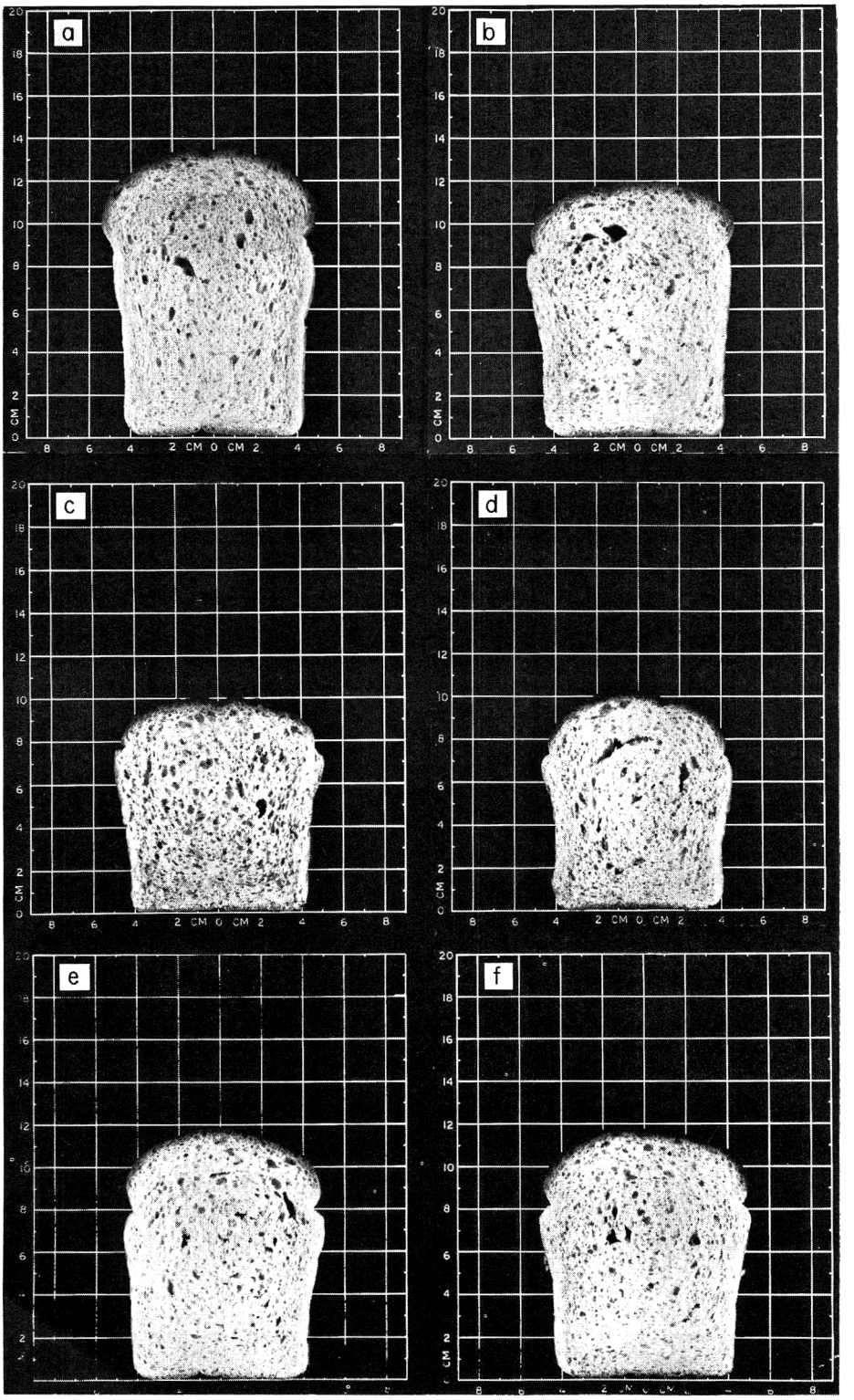


**Figure 2.** a, control; b, minced cassava (sample 1); c, minced, cooked cassava (sample 3); d, cooked, minced cassava (sample 4); e, Cassava flour, dried 50°C (sample 7); f, Cassava flour, dried 50°C, soaked 70°C (sample 11).

Table 4. Cassava product and loaf assessments

| Sample no. | Treatment | Ease of preparation (10) | Ease of incorporation (10) | Loaf shape (10) | Crust appearance (10) | Crumb appearance (10) | Tactile characteristics (10) | Overall loaf score (40) | Loaf specific volume as % of control |
|---|---|---|---|---|---|---|---|---|---|
| Control | – | – | – | 9 | 10 | 9 | 9 | 37 | 100 |
| 1 | Minced | 9 | 8 | 7 | 8 | 7 | 8 | 30 | 82.2 |
| 2 | Blanched, minced | 6 | 7 | 8 | 6 | 6 | 7 | 27 | 78.3 |
| 3 | Minced, cooked | 5 | 6 | 5 | 6 | 4 | 3 | 18 | 77.1 |
| 4 | Cooked, minced | 5 | 7 | 7 | 6 | 6 | 5 | 24 | 78.8 |
| 5 | Cooked, hand mashed | 3 | 7 | 8 | 7 | 6 | 7 | 28 | 84.6 |
| 6 | Blanched, dried 50°C | 5 | 9 | 6 | 7 | 6 | 6 | 25 | 69.3 |
| 7 | Dried 50°C | 6 | 9 | 8 | 4 | 6 | 6 | 24 | 82.2 |
| 8 | Dried 50°C, 'defibred' | 3 | 9 | 8 | 7 | 6 | 5 | 26 | 87.8 |
| 9 | Dried 50°C, soaked 30°C | 6 | 6 | 7 | 4 | 5 | 6 | 22 | 80.0 |
| 10 | Dried 50°C, soaked 50°C | 6 | 3 | 8 | 5 | 6 | 6 | 25 | 86.3 |
| 11 | Dried 50°C, soaked 70°C | 6 | 1 | 8 | 6 | 5 | 8 | 27 | 84.1 |
| 12 | Dried 95°C | 6 | 9 | 7 | 3 | 4 | 5 | 19 | 79.5 |
| 13 | Dried 95°C, 'defibred' | 3 | 9 | 6 | 3 | 4 | 4 | 17 | 73.2 |
| 14 | Dried 95°C, soaked 30°C | 6 | 6 | 8 | 5 | 4 | 5 | 22 | 86.8 |
| 15 | Dried 95°C, soaked 50°C | 6 | 3 | 7 | 5 | 4 | 6 | 22 | 83.2 |
| 16 | Dried 95°C, soaked 70°C | 6 | 1 | 7 | 6 | 4 | 7 | 24 | 84.1 |

**Table 5.** Sensory evaluation of the control and test loaves (mean scores out of forty)

| Sample no. | Treatment | No. of judgements | Softness | Moistness | Sponginess | Freshness | Acceptability |
|---|---|---|---|---|---|---|---|
| Control | | 87 | 30.2 | 23.8 | 23.1 | 28.1 | 27.7 |
| 1 | Minced | 87 | 26.7* | 21.8 | 23.9 | 25.2* | 25.2† |
| 2 | Blanched, minced | 87 | 30.3 | 27.4† | 22.4 | 29.0 | 28.5 |
| 3 | Minced, cooked | 20 | 29.9* | 30.5 | 24.4 | 30.4 | 27.0* |
| 4 | Cooked, minced | 20 | 32.1 | 30.1 | 22.9 | 31.6 | 30.0 |
| 5 | Cooked, hand-mashed | 20 | 32.4 | 29.8 | 23.2 | 32.5 | 30.0 |
| Control | | 18 | 32.2 | 24.9 | 23.5 | 29.9 | 28.9 |
| 6 | Blanched, dried 50°C | 18 | 25.4* | 22.0 | 19.4 | 24.2† | 25.8 |
| 7 | Dried 50°C | 18 | 27.3* | 18.3* | 20.9 | 24.7 | 22.9* |
| Control | | 59 | 30.1 | 27.1 | 23.0 | 28.6 | 26.5 |
| 7 | Dried 50°C | 38 | 26.4 | 22.5 | 25.5 | 26.0 | 25.8 |
| 9 | Dried 50°C, soaked 30°C | 18 | 24.6* | 22.1 | 26.6 | 25.4† | 23.9 |
| 10 | Dried 50°C, soaked 50°C | 18 | 27.3 | 22.1 | 23.6 | 27.0 | 25.2 |
| 11 | Dried 50°C, soaked 70°C | 39 | 28.1 | 21.3 | 22.8 | 27.5 | 25.5 |
| 12 | Dried 95°C | 38 | 25.7† | 18.8* | 23.5 | 24.2† | 24.6 |
| 14 | Dried 95°C, soaked 30°C | 18 | 26.9 | 20.2* | 20.9 | 26.8 | 25.1 |
| 15 | Dried 95°C, soaked 50°C | 18 | 25.7† | 21.1† | 21.3 | 28.7 | 24.4 |
| 16 | Dried 95°C, soaked 70°C | 39 | 28.2 | 23.0 | 25.3 | 27.8 | 27.1 |
| Control | | 18 | 33.6 | 26.6 | 23.8 | 32.5 | 28.7 |
| 8 | Dried 50°C, 'defibred' | 18 | 26.8* | 22.1† | 27.2 | 26.5* | 25.9 |
| 13 | Dried 95°C, 'defibred' | 18 | 26.2* | 20.1* | 23.3 | 26.2* | 22.8* |

*Significantly different from control or other best sample at 1% level or † 5% level.

maximum in any of the measured parameters may be considered unacceptable and would not be recommended for preparation.

The results obtained from the sensory evaluation of the control and test loaves are given in Table 5. Differences significant at the 5 and 1% levels are noted. The number of judgements for each sample varied from eighteen to eighty-seven, some of the assessments being repeated so that smaller differences could be assigned a level of significance.

Mincing of the fresh cassava (sample 1) gave a product which was easily prepared in a single operation and which was easily blended with the dry ingredients used in breadmaking.

Blanching and subsequent mincing produced a sticky product (sample 2) which tended to block the holes of the mincer. The material was bulky and difficult to blend with the dry breadmaking ingredients. This was also true of the cooked and minced cassava (sample 4). Hand mashing of the cooked slices (sample 5) produced a less sticky product which was easier to handle. This agrees with the results of Gooding (1972) who compared gentle and vigorous mashing of cooked yams. However, the operation was difficult and slow due to the fibre content of the cassava and would be impractical on anything larger than the kitchen scale. Generally, loaves containing blanched or cooked samples had large pieces of meal embedded in the surface. However, this did not detract from their overall acceptability by the panel (Table 5). Bread containing cassava which had been blanched and minced (sample 2), minced and cooked (sample 3), cooked and minced (sample 4), cooked and hand mashed (sample 5) had lower overall loaf scores than bread containing fresh minced cassava (sample 1). However, the panel rated samples 2, 3, 4 and 5 higher than sample 1 for softness, freshness and acceptability.

Cooking of the fresh minced cassava in a muslin bag gave a dark gelatinous product (sample 3) which was very difficult to handle. There was also a loss of material during cooking due to the leaching of solubles and fine particles passing through the mesh into the cooking water. Dough containing this sample was very soft and the resultant loaf had well defined edges where the dough had spread into the corners of the tin. The loaf score was low due mainly to the open and sticky crumb texture and dark crumb colour.

Although the various dried products were easily incorporated into the dough, their preparation was costly in terms of equipment, number of operations and the energy required. Overnight drying for 16 hr at 50°C (sample 7) was adequate and found to be convenient. In the early stages of drying at 95°C (sample 12), the slices developed a hard, dry crust, which restricted moisture loss from the centre of the slices. By the time the centre was dry, the crust had scorched and the resultant flour was darker in colour than that prepared from slices dried at 50°C. Loaves containing the dried flours had a pale crust which appeared stretched at the sides. Those containing flour dried at 95°C had a dry, hard crumb which was dark in colour. In sensory evaluation, the dried products were generally inferior to the fresh products.

The method used to defibre the dried flours (samples 8 and 13) was time

consuming. As shown in Table 1, sieving is only partially successful for the removal of fibre. However, this would be the only practical method available in rural areas of less developed countries for fibre reduction. Moreover, as the rejected material, with particle size greater than 250 $\mu$m, amounted to nearly half of the total this method of fibre reduction could be justified only if the bread made from this material was greatly superior to the bread made from unclassified material. These 'defibred' flours gave loaves with loose and soft crumb texture which had little strength, and were less acceptable than the loaves containing the whole cassava flours. It would seem unlikely that the small reduction in fibre content would cause such an effect on crumb texture: it may be due to the small particle size of the 'defibred' flour. The panel found that the partial removal of fibre from the flour dried at 50°C gave a marginal improvement in the loaves.

Soaking of the dried flours was carried out at the breadmaking stage. Although it was relatively easy to maintain the flour and water suspensions at 30 and 50°C, some difficulties were experienced in maintaining a temperature of 70°C for the necessary time. These difficulties could be overcome but equipment unlikely to be readily available in a small bakery would be required. Gelatinization of the starch was a problem at 70°C, and to a lesser extent at 50°C; this made stirring of the suspension and its incorporation into the dough difficult. Cooling of the 50 and 70°C suspensions before addition to the dough was necessary as these temperatures would have been sufficient to reduce yeast activity. Cooling was done as rapidly as possible but was hindered by the high viscosity of the suspensions.

Cooling would also be slow in situations where refrigeration was not available. The bread produced from flours soaked at 70°C (samples 11 and 16) had a higher overall loaf score than those soaked at 30°C (samples 9 and 14) or 50°C (samples 10 and 15). For the flour initially dried at 50°C the panel rated sample 11 softer, fresher and more acceptable than sample 10 which in turn was rated higher than sample 9. This last sample was also found to be significantly harder than the control. Similar panel assessments were given for the loaves made from the soaked flour which was dried at 95°C (samples 14, 15 and 16). In general, soaking at temperatures of 50°C and 70°C improved the overall loaf characteristics compared with the unsoaked flours but the difficulties experienced in handling and incorporation would tend to reduce the advantages.

Further work in cassava growing areas using different cassava cultivars would be required before definite recommendations could be made for the commercial use of the cassava products in bread. Moreover, consideration would have to be given to the local breadmaking techniques, the quality of the breadmaking flour and consumer preferences.

## Conclusions

It has been shown that it is feasible to incorporate fresh cassava into wheat

flour bread thus eliminating a drying stage. Bread containing 20% fresh minced cassava rated higher than any other product in all assessments except sensory evaluation. The panel found bread containing blanched or cooked minced cassava at the same level to be the most acceptable. However, practical difficulties were experienced during their preparation.

If cassava is to be processed to a dried flour before incorporation into bread, low temperature drying at around 50°C is recommended to ensure that the flour is light in colour. The baking potential of dried flours was improved by soaking prior to incorporation: higher soaking temperatures had a greater effect but rendered the product more difficult to handle. Unless these difficulties could be overcome, a compromise temperature of about 50°C would appear to be more appropriate.

## Acknowledgements

The study of the inclusion of fresh cassava in bread was made at the suggestion of Dr N. R. Jones and it formed part of the T.P.I. programme of work on composite flour technology.

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# Proteolytic activity and the frequency of burst bellies in capelin

ASBJØRN GILDBERG

## Summary

This paper describes the risk of burst bellies in capelin caught during the intense feeding period ('summer capelin') and during the spawning period ('winter capelin') and discusses this phenomenon in relation to the proteolytic activity in the digestive tract of the fish. The risk of belly bursting is highest in summer capelin probably because the fish caught during the intense feeding period contains more digestive enzymes than at other times of the year. It is suggested that the risk of burst bellies is highest if the fish is caught at a late stage of digestion after heavy feeding, because the enzymes are then free to diffuse into the surrounding tissue. A protein-solubilizing mechanism active at neutral pH is discussed.

## Introduction

Capelin is the predominant fish resource in Norway. More than 99% of the total catch is processed to fish meal and oil. There is a growing interest in using more of the capelin for human consumption. However, capelin is very susceptible to enzymatic tissue degradation, which often leads to burst bellies before the catch can be delivered. This renders the fish unsuitable as a raw material for food production. To avoid such spoilage, it is important to know how the degradation proceeds.

There are no published papers on tissue degradation in fresh capelin. Some knowledge, however, exists on this process in herring and sprat, which are also susceptible to burst bellies under certain biological conditions (Almy, 1926; Marvik, 1974).

The tendency of bellies to burst is apparently associated with the feeding cycle of the fish, both qualitatively and quantitatively. It is well known that

Author's address: The Institute of Fishery Technology Research (FTFI) N-9000 Tromsö, Norway.

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fish eating certain pteropoda (especially *Limacina retroversa*) are very susceptible to burst bellies (Dahl, 1972). Generally the tendency is highest in heavy feeding periods, but there is no simple relation between food content of the stomach and the extent of burst bellies (Marvik, 1974).

Burst bellies are first of all a result of connective tissue breakdown. It is shown that collagen structures in fish are strengthened during periods of starvation, and weakened in periods of heavy feeding (McBride, MacLeod & Idler, 1960; Hughes, 1963; Lavéty & Love, 1972). Low tensile strength of the tissue combined with a high level of digestive enzymes probably maximizes the risk of tissue degradation *post mortem*.

Seasonal changes of pH in the tissue may also affect tissue degradation, because of the pH dependence of activity, and because the strength of fish connective tissue decreases markedly with decreasing pH (Love, Lavéty & Garcia, 1972). High feed intake usually causes low pH in the tissue *post mortem* (Love, 1975).

This paper discusses the influence of such factors on burst bellies in capelin.

## Materials and methods

Capelin (*Mallotus villosus*) caught by trawl in the Barents Sea were used in all the experiments, except for one where they were caught in Balsfjorden near Tromsö (Norway). Capelin caught in August and September were designated '*summer capelin*', while capelin caught in March and April, prior to spawning, were designated '*winter capelin*'. The length of fish used was between 11 and 19 cm.

Freshly caught fish that had been wrapped individually in aluminium foil, packed in sealed plastic bags and stored at −20°C were used for most of the experiments although some fresh non-frozen material was also used.

*pH measurement.* The pH was measured on a suspension of mince from six fish in an equal weight of water.

*Protease activity* was determined according to Barret (1972). The incubation mixtures consisted of 0.5 ml Johnson/Lindsay-buffer (Johnson & Lindsay, 1939), 0.25 ml enzyme sample and 0.25 ml 8% haemoglobin. The concentration of *Folin positive material* in the supernatant after adding TCA (5% end concentration) was determined by Lowry's method (Lowry *et al.*, 1951) using tyrosine as a standard.

*Autolysis.* 10.0 g of minced summer capelin were mixed with 30 ml Johnson/Lindsay-buffer and placed on a water bath (25°C) for 18 hr. At the end of the incubation, the samples were centrifuged (20 min, 20 000 *g*). Total protein in the supernatant and peptides soluble in 5% TCA were estimated by the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin as a standard.

The recorded pH's are the average values of pH's measured at the start and at the end of the incubation.

*Leakage of enzymes.* Leakage of enzyme from the digestive tract was determined as a function of storage time of whole capelin. Fresh winter capelin (caught in Balsfjorden) were stored in a covered plastic tray at 4°C. After 1, 3 and 5 days of storage, two male and two female capelin were gutted. The digestive tracts were suspended inside a 60 ml glass beaker by bending the anal and oral ends of the tracts over the edge of the beaker and taping them at the outside of the beaker wall. The beaker was filled with 60 ml 0.9% NaCl solution and stirred magnetically for 1 hr at 4°C. The extract was then decanted, and the digestive tracts were homogenized in 60 ml of 0.9% NaCl solution. Protease activities were determined in the extracts and homogenates at pH 8.9 and 2.8 respectively after incubation for 1–4 hr at 25°C. The sum of the two activities was designated total activity.

*Frequency of burst bellies.* Immediately after capture, twenty capelin were placed at 4°C in a covered plastic tray. Three days later the number of fish with burst bellies was taken as those with holes at one or more places along the belly side between the gills and the anal opening.

*Soluble protease.* Protease activity at neutral pH was determined in extracts of homogenized digestive tracts. Twenty capelin from each catch were gutted. The digestive tracts were homogenized in nine volumes of distilled water (1 min in a Waring blender). The homogenates were centrifuged (20 min, 20 000 *g*) and the protease activities in the supernatant solutions were determined.

## Results

Figure 1 shows the changes of pH of winter and summer capelin during storage

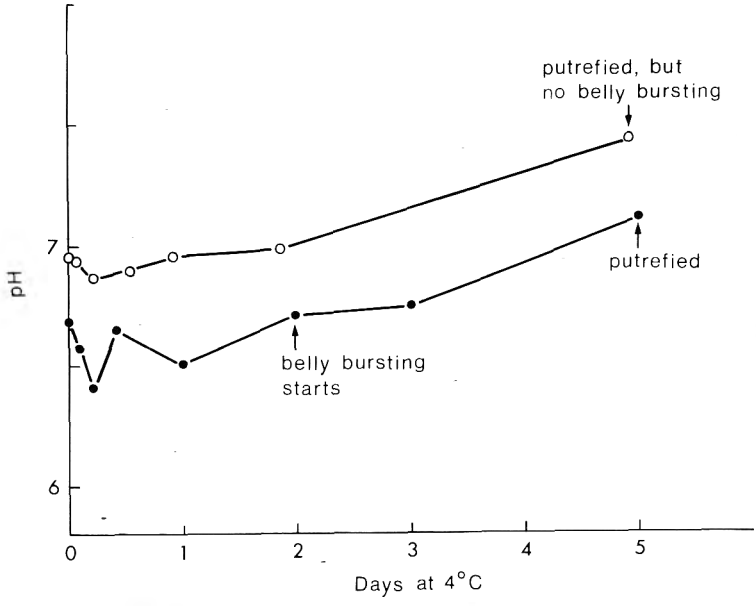


**Figure 1.** The pH of winter capelin (○) and summer capelin (●) as a function of storage time at 4°C.

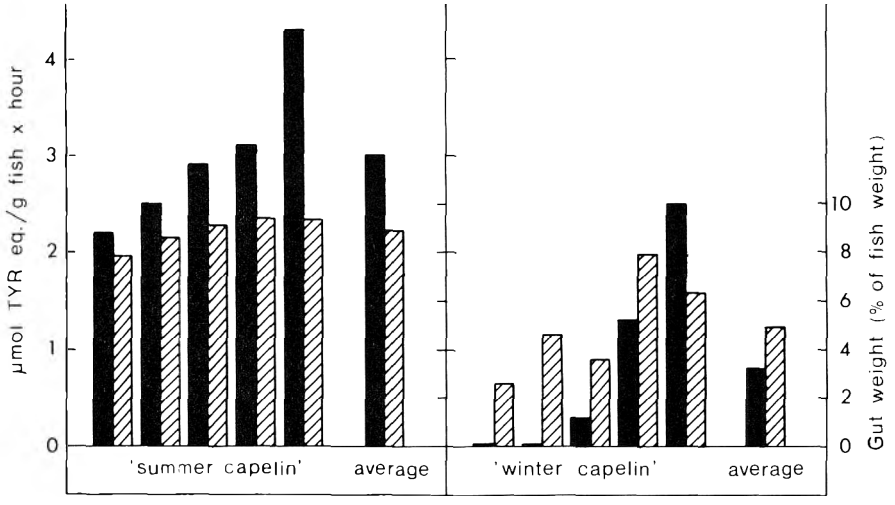


**Figure 2.** Protease activity in homogenized digestive tracts per g of whole fish (filled columns) and weight of digestive tracts expressed as percentage of whole fish (w/w) (hatched columns) in ten catches. The given results are based on measurements on batches of four fish from each catch. Protease activities were determined after incubation for 1–2 hr at 23°C at a pH of 6.6.

at 4°C. The pH is generally 0.4 units lower in the summer than in the winter capelin. In the former the belly bursting starts after 2 days whereas in the latter visible belly bursting does not develop even after the fish became putrid. In spite of this distinct difference in tendency to burst bellies, the two catches putrefied after the same time of storage.

The amount of protease activity at neutral pH is usually higher in summer capelin than in winter capelin (Fig. 2). This may reflect the heavy feeding during late summer and early autumn. Just before spawning the feed intake is usually very low, and ratio of gut weight to whole fish weight is relatively low.

The digestive tract of capelin contains proteases with optimal activity at pH 3–4 and at pH 9 when measured with haemoglobin as substrate (Fig. 3(a). The autolytic degradation of protein in whole fish mince to TCA solubles is highest at alkaline conditions (Fig. 3(b)). At slightly acid and neutral pH, release of TCA solubles is rather low. In this pH range, autolysis yields high molecular proteinous material, thus suggesting the presence of a solubilizing principle which is active at conditions where the degradation of proteins to peptides is rather slow.

Figure 4 shows that proteases present in the digestive tract can leak through the walls even though the tissues are without visible damage. The rate of this leakage increases with the storage time of the capelin prior to excision of the digestive tract.

Tissue degradation and burst bellies are probably the result of the cooperation of many different enzymes acting on the tissue. Figure 5 shows the tendency to burst bellies in different catches as a function of the amount of soluble digestive protease active at neutral pH. The risk of burst bellies seems

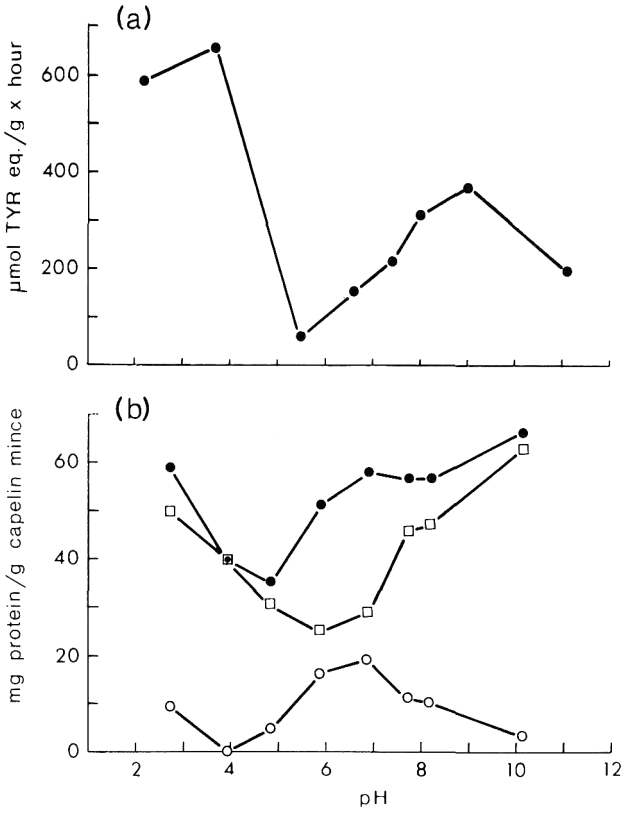


**Figure 3.** (a) Protease activity in homogenized digestive tracts of summer capelin as a function of pH. Activities were determined after incubation for 1 hr at 25°C. (b) Autolysis of minced capelin as a function of pH: water soluble protein material (●), *Folin positive material* soluble in 5% TCA (□) and the difference between water soluble and TCA soluble protein material (○).

to increase with increasing amount of soluble protease in the digestive tract. However, the small number of catches makes the conclusion uncertain.

Figure 6 indicates that the concentration of soluble protease active at neutral pH, is highest when the digestive tracts contain little feed.

## Discussion

For capelin living in the Barents Sea the access to food is usually much higher in the summer and autumn than in the winter and spring. The heavy feeding summer capelin has a high glycogen level leading to low *post mortem* pH, which subsequently reduces the strength of connective tissue. The summer capelin also has a high production of digestive enzymes, which thus increase the risk of *post mortem* autolysis. Moreover, fish which feed heavily generally have a weaker connective tissue than starving fish (Hughes, 1963; Lavéty & Love, 1972).

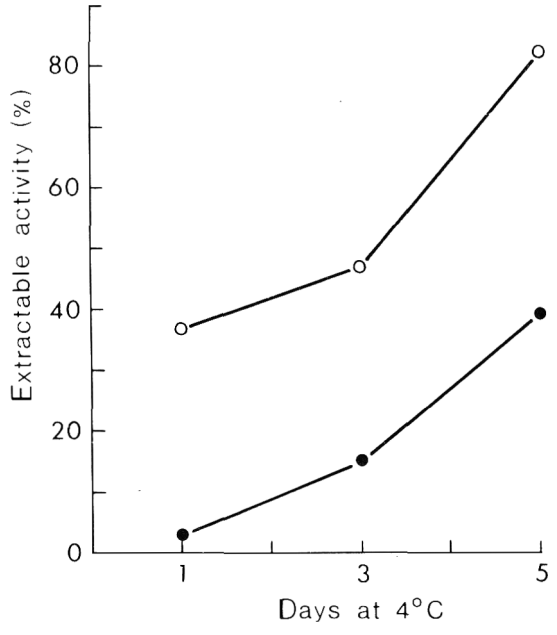


**Figure 4.** Protease activity extractable from digestive tracts as a function of storage time of whole capelin at 4°C. The activities are given as a percent of the total activities at pH 8.9 (○) and at pH 2.8 (●).

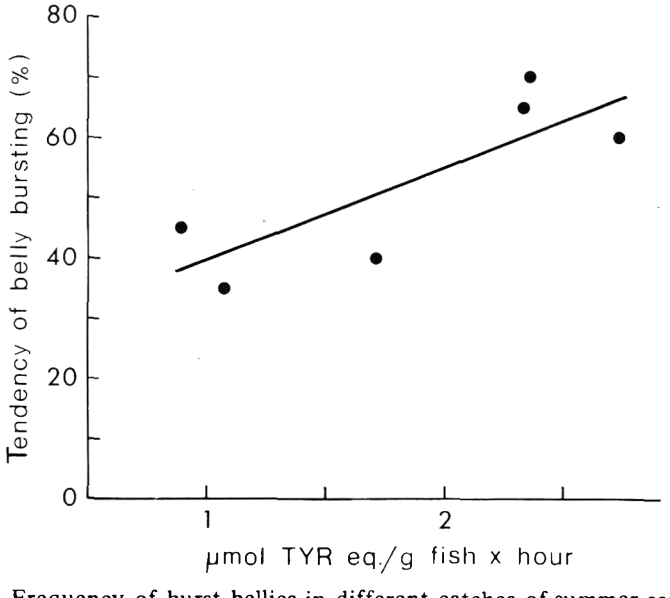


**Figure 5.** Frequency of burst bellies in different catches of summer capelin as a function of activity of soluble digestive proteases per g whole fish. Protease activities were determined after incubation for 2 hr at 23°C and pH 6.8. Each point represents one catch, and the given protease activities are calculated from measurements in batches of digestive tracts from twenty fish.

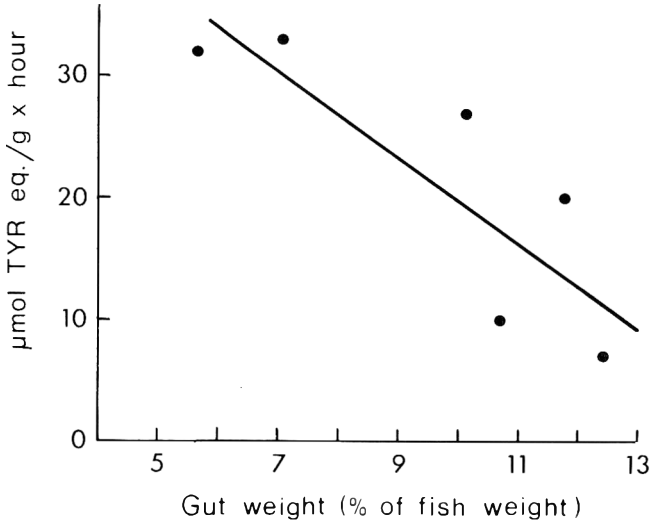


**Figure 6.** Activities of soluble proteases in homogenates of digestive tracts from summer capelin as a function of percent digestive tracts of whole fish (w/w). Determination of the activities is described in the legend of Fig. 5.

All these factors may add up to the explanation that the summer capelin is more susceptible to tissue autolysis than the winter capelin and thus be less suitable as a raw material for human food products.

The proteolytic activity of digestive enzymes is relatively low under slightly acid and neutral conditions. The autolysis of fish tissue, however, is rather high in this pH range, and there seems to be a system capable of releasing higher molecular protein. This is probably an enzyme catalysed mechanism, as the chemical extractability of fish protein is very low at slightly acid conditions (Meinke & Mattil, 1973). This activity need not necessarily be a protease, but it may very well be the result of cooperative action of enzymes which release protein components from the tissue.

It was shown that proteases can leak out from a seemingly intact digestive tract.

Proteases active at alkaline pH leak out faster than those active at acid pH. This may be due to differences in molecular structure (weight and charge). It may also be that the stomach, which contains most of the proteases active at acid pH, has a wall that is less susceptible to leaking than other sections of the alimentary canal.

It seems to be a positive relation between amount of neutral digestive protease activity and the tendency of burst bellies.

Capelin with high feed content in the stomach does not necessarily have to be susceptible to belly bursting. Secretion of digestive enzymes is induced by food in the stomach (Kapoor, Smit & Verighina, 1975). In a fish with a full stomach caught immediately after feeding, the enzyme secretion may not have been properly induced. It is also possible that the undigested food can effec-

tively absorb digestive enzymes so that the amount of available soluble enzyme remains low until most of the food is digested.

The experiment that shows a decreasing concentration of soluble protease with increasing gut content supports this assumption. If this holds, the risk of burst bellies should be highest in fish which are caught during late stages of digestion after heavy feeding.

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# Prediction of water activity in intermediate moisture foods

JORGE CHIRIFE

## Summary

Ross' equation for estimating the water activity of multicomponent solutions was tested with experimental data in solid intermediate moisture foods. Literature experimental data on water activity and food system composition in a wide variety of intermediate moisture foods (IMF) were utilized to test the validity of Ross' equation.

The results obtained were satisfactory and indicate that Ross' equation constitutes a simple and reasonably accurate method for predicting the water activity in IMF.

## Introduction

In recent years with the advent of intermediate moisture pet foods and the possibility of intermediate moisture foods (IMF) for human consumption there has been an increased interest in methods for measuring (Labuza *et al.*, 1976; Troller 1977) and predicting (Sloan & Labuza, 1976) the water activity in IMF. Several solutes, also called 'humectants', are usually incorporated in IMF to lower the water activity ($a_w$) to the desired range, e.g. 0.65–0.90. 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, like 'moist-infusion', 'dry-infusion' or blending (Brockmann, 1970; Heidelbaugh & Karel, 1975).

When a given solute or a mixture of them are incorporated into a food it is not an easy matter to predetermine the final $a_w$ of the product. However, it is necessary to do it in order to formulate the food to the desired $a_w$ with a minimum of product development time. This can be accomplished if a predictive equation for $a_w$ lowering in a complex food system is available. A number of

Author's address: Departmento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Núñez 1428 Buenos Aires, Argentina.

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equations have been proposed for calculating the $a_w$ lowering effect of simple solute in solution (Grover, 1947; Money & Born, 1951; Norrish, 1966). Most of these equations were recently examined by Sloan & Labuza (1976), Chuang & Toledo (1976) and Sloan, Schulueter & Labuza (1977). Recently, Ross (1975) proposed a simple equation for estimating the $a_w$ of complex solutions. Ross' equation is based on the Gibbs-Duhem relationship and may be written as,

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

Equation (1) assumes that in a food system each $a_w$ lowering component behaves independently. The final $a_w$ ($a_{wf}$) is a product of each component water activity, $(a_w^\circ)_1$, $(a_w^\circ)_2$, $(a_w^\circ)_3$ ... based on its being dissolved in all of the water in the system. Ross (1975) demonstrated the validity of his equation by measuring the $a_w$ of various solutions of sucrose and salts. Sloan & Labuza (1976) studied the validity of Ross' equation for predicting the $a_w$ in an intermediate moisture (IM) dog food system, and Chuang & Toledo (1976) tested eqn (1) with mixtures of water, sucrose and sodium chloride and also with solid mixtures of starch and wheat. Both authors reported that Ross' equation gives reasonably good results for estimating $a_w$ in the IM range.

In view of these promising results and the simplicity of Ross' equation its validity for predicting the $a_w$ in IMF is now further examined. Literature experimental data on water activity and food system composition in a wide variety of IMF were utilized to test Ross' equation.

## Results and discussion

### *Utilization of Ross' equation*

In order to apply eqn (1) for calculating the $a_w$ of multicomponent mixtures, we need first to know the $a_w$ lowering effect of each individual solute. Several authors have experimentally determined the $a_w$ lowering effect of potential IMF humectants, and Sloan & Labuza (1975) recently reviewed this area. The humectants utilized to depress $a_w$ in the IMF discussed in this work (shown later) were mainly glycerol and sodium chloride; propylene glycol, corn syrup DE 42 and sucrose were also used in same systems. The $a_w$ lowering effect of these solutes at various concentrations have been obtained from the experimental data reported by the following authors:– glycerol: National Bureau of Standards (1951), sodium chloride: Robinson & Stokes (1959), propylene glycol: Verlinde, Vebeeck & Thun (1975), corn syrup DE 42: Norrish (1966), sucrose: Robinson & Stokes (1959) and Norrish (1966).

The presence of non-solute materials in an IMF introduces a problem for the use of Ross' equation. As acknowledged by Ross (1975), the non-solutes bind water and help to lower water activity. Ross suggested that an, '$a_w^\circ$' factor may be also assigned to each non-solute on the basis of its sorption isotherm considering that all the water is sorbed in each non-solute separately. In this manner

each non-solute may give the appropriate '$a_w°$' which can be included in eqn (1). Preliminary results reported by Ross (1975) using corn starch, collagen and gluten were satisfactory, but he suggested that further investigation was needed in order to verify the concept of '$a_w°$' factors for non-solute food ingredients. There is a given ratio of non-solute solids/moisture ($r$) below which the $a_w$ of the solid-moisture mixture is very close to 1. In this condition the non-solute component need not be taken into account to estimate the water activity. According to Ross (1975) this critical ratio is about 1:1. This may be verified in the following manner. The critical ratio may be obtained by inspecting the sorption/desorption isotherm of the non-solute considered and finding the solid/moisture ratio, $r$, which correspond to an $a_w$ just below 1.0, let us say 0.99. Ideally, it would be necessary to know the value of $r$ at an $a_w$ as close as possible to 1.0, but this is difficult because of the well known uncertainties associated with the determination of sorption isotherms at very high water activities. As a matter of fact, most of the isotherms reported in the literature usually end at an $a_w$ of about 0.90–0.95 or below. Nevertheless, a revision of the literature was made in order to find the critical ratio, $r$, which for the purposes of this work is evaluated at an $a_w$ of 0.99. Some values at an $a_w$ of 0.98 are also reported when no data were available at higher water activity. The results are shown in Table 1; it shows the $r$ values for various non-solute food ingredients as well as for some foods containing very little soluble solids. It can be seen that the $r$ values range between about 1.7 and 3.6 depending on the type of solid considered.

Ross' equation will be applied to two classes of experimental data on IMF reported in the literature. The first one corresponds to IMF systems for which the composition was directly reported by it authors. The second one corresponds to IMF prepared by 'moist-infusion'. In this procedure the food is immersed and cooked in a solution of predetermined composition and held

**Table 1.** Ratio solid/moisture ($r$) at water activity 0.99–0.98 for various non-solute food ingredients

| Product | Specifications | $a_w$ | $r$ | Reference |
|---|---|---|---|---|
| Beef, raw | Ads., 20°C | 0.99 | 2.1 | Saravacos & Stinchfield (1965) |
| | Ads., 40°C | 0.99 | 3.1 | |
| Casein | Ads., 20°C, at isoelectric point | 0.98 | 1.7 | Rüegg & Blanc (1976) |
| Gelatin | Ads., 20°C | 0.99 | 2.7 | Saravacos & Stinchfield (1965) |
| | Ads., 40°C | 0.99 | 3.0 | |
| Potatoe, blanched | Ads., 20°C | 0.99 | 2.6 | Saravacos & Stinchfield (1965) |
| | Ads., 40°C | 0.99 | 2.9 | Stinchfield (1965) |
| Soybean | Ads., 15°C | 0.98 | 2.3 | Gane (1948) |
| Soy isolate | Ads., 25°C | 0.98 | 1.5 | Fett (1973) |
| Starch gel | Ads., 20°C | 0.99 | 3.3 | Saravacos & Stinchfield (1965) |
| | Ads., 40°C | 0.99 | 3.6 | |

until equilibration. Several literature results indicate (Brockmann, 1970) that pieces of food of moderate thickness (e.g. 1 cm thick) completely equilibrate following cooking at 95–100°C and held overnight in a refrigerator or at room temperature. Assuming that equilibrium was reached, Ross' equation can be utilized to calculate the final (or equilibrium) $a_w$ in the food if the following data are known: initial moisture content of the food, ratio of initial food weight/solution weight, and solution composition. In most of the literature results discussed here, the $a_w$ of the IMF systems were measured using electronic hygrometers. In accordance with the study of Labuza *et al.* (1976) all the $a_w$ values, either experimental or predicted, are rounded to two decimal places.

*Application to experimental data*

Table 2 shows the results of the application of Ross' equation to various IMF systems reported in the literature. It can be seen that the agreement between measured and predicted $a_w$ values is fairly good. The application of Ross' equation in the presence of non-solute components is illustrated by the

**Table 2.** Comparison between measured and predicted (Eqn 1) water activity values in various intermediate moisture foods

| No. | Product | Composition | $a_w$ measured | $a_w$ predicted | % diff. | Reference |
|---|---|---|---|---|---|---|
| 1 | IM Meat | Meat solids: 70.5%<br>Water: 15%<br>Frodex (42 DE); 14.5% | 0.83 | 0.81 | 2.4 | Hass *et al.* (1975) |
| 2 | IM Meat | Meat solids: 60.4%<br>Water: 25%<br>Glycerol: 12%<br>NaCl: 2.6% | 0.83 | 0.83 | 2.4 | Haas *et al.* (1975) |
| 3 | IM Meat | Meat solids: 3.3%<br>Water: 40%<br>Glycerol: 25%<br>NaCl: 3.7% | 0.83 | 0.83 | — | Haas *et al.* (1975) |
| 4 | IM deep-fried fish (catfish) | Fish solids: 22.7%<br>Fat: 13.2%<br>Glycerol: 20.1%<br>NaCl: 4.1%<br>Water: 39.9% | 0.85 | 0.84 | 1.2 | Collins *et al.* (1972) |
| 5 | IM deep-fried fish (cod) | Fish solids: 21%<br>Fat: 22.4%<br>Glycerol: 21.5%<br>NaCl: 3.6%<br>Water: 31.5% | 0.83 | 0.81 | 2.4 | Collins *et al* (1975) |

Table 2. (*Cont.*)

| No. | Product | Composition | $a_w$ measured | $a_w$ predicted | % diff. | Reference |
|---|---|---|---|---|---|---|
| 6 | IM Irish stew gravy | Moisture content: 25.7%<br>Cottonseed and Coconut oils: 38.4%<br>Glycerol: 12.5%<br>Corn syrup solids: 5.5%<br>Sucrose: 5%<br>NaCl: 3%<br>Fresh egg yoke: 10%<br>Seasonings, colour, acid, flavour, etc: 2.1%<br>Water: 23.5% | 0.85 | 0.83 | 2.4 | Kaplow & Halik (1972) |
| 7 | IM carrots | Carrot solids: 6.3%<br>Glycerol: 51.1%<br>NaCl: 2.1%<br>Prop. glycol: 0.9%<br>Water: 39.3% | 0.77 | 0.74 | 3.9 | Kaplow (1970) |
| 8 | IM carrots | Carrot solids: 34.2%<br>Glycerol: 35.9%<br>NaCl: 1.5%<br>Prop. glycol: 0.7%<br>Water: 27.5% | 0.76 | 0.74 | 2.6 | Kaplow (1970) |
| 9 | IM sauce (ham) | Moisture content: 19.8%<br>Cottonseed oil: 47.7%<br>Non fat dry milk: 12.8%<br>Glycerol: 9.83%<br>Whole egg yolk: 10%<br>Corn syrup solids: 2.1%<br>Prop. glycol: 1%<br>NaCl: 1%<br>Water: 14.25% | 0.86 | 0.87 | 1.2 | Kaplow & Halik (1972) |
| 10 | IM sauce (chicken) | Moisture content: 19.8%<br>Cottonseed oil: 47.22%<br>Non fat dry milk: 12.75%<br>Glycerol: 11.75%<br>Whole egg yolk: 10%<br>Corn syrup solids: 2%<br>NaCl: 1%<br>Water: 14.1%<br>Others: 1.2% | 0.86 | 0.86 | – | Kaplow & Halik (1972) |
| 11 | IM Tuna | Moisture content: 38.8%<br>Equilibration in glycerol-NaCl solution | 0.81 | 0.81 | – | Brockmann (1970) |
| 12 | IM carrots | Moisture content: 51.5%<br>Cook-equilibration in glycerol-NaCl solution | 0.81 | 0.82 | 1.2 | Brockmann (1970) |

Table 2. (*Cont.*)

| No. | Product | Composition | $a_w$ measured | $a_w$ predicted | % diff. | Reference |
|---|---|---|---|---|---|---|
| 13 | IM marconi elbow | Moisture content: 46.1% Cook-equilibration in glycerol-NaCl solution | 0.83 | 0.83 | – | Brockmann (1970) |
| 14 | IM pork loin | Moisture content: 42.5% Cook-equilibration in glycerol-NaCl solution | 0.81 | 0.84 | 3.7 | Brockmann (1970) |
| 15 | IM beef, cubed chuck | Moisture content: 31% Cook-equilibration in glycerol-NaCl-Prop. glycol solution | 0.77 | 0.80 | 3.9 | Kaplow & Halik (1972) |
| 16 | IM carrot dices | Moisture content: 35.7% Cook-equilibration in glycerol-NaCl-Prop. glycol solution | 0.71 | 0.73 | 2.8 | Kaplow & Halik (1972) |
| 17 | IM potato, diced | Moisture content: 36.8% Cook-equilibration in glycerol-NaCl-Prop. glycol solution | 0.79 | 0.77 | 2.5 | Kaplow & Halik (1972) |
| 18 | IM peas | Moisture content: 50.2% Cook-equilibration in glycerol-NaCl solution | 0.83 | 0.81 | 2.4 | Kaplow & Halik (1972) |
| 19 | IM lamb pieces | Moisture content: 40.2% Cook-equilibration in glycerol-NaCl solution | 0.86 | 0.85 | 1.2 | Kaplow & Halik (1972) |
| 20 | IM onions | Moisture content: 59.8% Cook-equilibration in glycerol-NaCl solution | 0.85 | 0.82 | 3.5 | Kaplow & Halik (1972) |
| 21 | IM smoked ham | Moisture content: 40% Cook-equilibration in glycerol-NaCl solution | 0.85 | 0.84 | 1.2 | Brockmann (1973) |

example nos 1, 2 and 3 (Table 2). In these IMF systems the $a_w$ (measured) is the same, but the non-solute (meat solids)/moisture ratio varies widely. In the example no. 3 the meat solids need not be taken into account to predict the $a_w$ in view of the low solids/moisture ratio ($r = 0.783$). However, in examples nos 1 and 2 an '$a_w^\circ$' factor has to be assigned to them in view of the much higher $r$ values (4.7 and 2.42 respectively). This is done from the above ratios and the sorption isotherm of beef reported by Saravacos & Stinchfield (1965). The results are shown in Table 3.

In the rest of IMF systems reported in Table 2 the relatively high moisture content (generally well above 30%) makes negligible the $a_w$ lowering effect of non-solute solids.

**Table 3.** See text for details

| | Composition | | $a_w^\circ$ |
|---|---|---|---|
| No. 1 (IM meat) | | | |
| | Meat solids: | 70.5% | (0.833) |
| | Frodex (DE 42): | 14.5% | (0.967) |
| | Water: | 15.0% | |
| | $(a_w)_f = (0.833)\,(0.967) = 0.81$ | | |
| No. 2 (IM meat) | | | |
| | Meat solids: | 60.4% | (0.982) |
| | Glycerol: | 12.0% | (0.903) |
| | NaCl: | 2.6% | (0.941) |
| | Water: | 25.0% | |
| | $(a_w)_f = (0.982)\,(0.903)\,(0.941) = 0.83$ | | |

It may be concluded that Ross' equation constitutes a simple and relatively accurate method to predict the water activity in intermediate moisture foods. It is believed, however, that more research is needed in order to clarify the application of Ross' equation to predetermine the $a_w$ in IMF systems containing significant amounts of mixtures of non-solute solids, in addition to the humectants.

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# The use of sodium pectate as a thickener in canned foods

J. MITCHELL, A. SOMMERVILLE AND C. SPEIRS

## Summary

The thickening properties of sodium alginate, low methoxyl pectin (degree of esterification, 22%) and sodium pectate (degree of esterification, 4%) in systems that had been heat processed were compared with those of starch. Heat penetration data were obtained for pectate and starch solutions processed at 121.1°C in both static and rotating retorts.

By controlling the level of calcium release in the solution using a sparingly soluble calcium salt in conjunction with a thermolabile sequestrant, partial gelation of 0.5% sodium pectate solutions could be induced on completion of the heat process cycle giving a high viscosity. This effect could not be obtained using either sodium alginate or the low methoxyl pectin. When low levels of sodium pectate (0.2–0.4%) are mixed with starch, a considerably higher viscosity after processing is obtained compared with starch alone.

In a rotating retort a high rate of heat transfer is obtained for the pectate solutions in comparison with solution containing levels of starch required to give comparable post process viscosities. It is suggested that sodium pectate would be of use as a thickener in canned products particularly where rapid heat penetration is desirable.

## Introduction

Sodium alginate and sodium pectate are polysaccharides with similar properties. In particular both are capable of forming thermostable gels in the presence of divalent ions. There are however some differences between the gelation behaviour of these two substances. For example alginates will not gel in the presence of magnesium ions whereas pectates will. Another difference high-

Authors' address: Food Science Laboratories, Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD.

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lighted by recent work (Mitchell & Blanshard, 1976a, b) is that sodium pectate requires the addition of lower levels of calcium ions both to initiate gelation and to give maximum gel strength, compared with sodium alginate, even if the latter contains a relatively high proportion of guluronic acid residues.

Neither of these two polysaccharides are extensively used as thickeners in low acid canned foods because both show a substantial drop in viscosity when their solutions are autoclaved due to thermal degradation of the polymer (Pilnik & McDonald, 1968). However, an interesting process has been described where the formation of a calcium alginate gel on cooling after retorting, has been used to thicken canned foods (Messina & Pape, 1966). This is possible since alginates that have been extensively degraded are still capable of gelling (Smidsrod & Haug, 1972). One advantage of this process is that because during retorting the alginate solution has a very low viscosity, heat penetration is much more rapid than with conventional starch based thickeners therefore giving a considerable reduction in the process time required. Thermo-reversible gelling agents would not be so effective as the increased viscosity obtained on cooling due to gelation would be lost when the food is reheated.

In formulating a thickening system of this type it is important to prevent gelation until cooling after retorting, since if gelation occurs prior to processing the gel will not subsequently melt but break up and synerese at autoclaving temperatures to give a non-homogeneous product. Control of the gelation reaction through the processing cycle may be accomplished for example, either by using a sparingly soluble calcium salt which increases in solubility with decreasing temperature and maintaining the food at a high temperature prior to retorting, or by employing a thermally degradable sequestrant. In either of these cases the increase in calcium concentration after retorting that can be achieved is small. It therefore seems possible that the greater calcium sensitivity of sodium pectate would enable the gelation reaction to be controlled more easily than with sodium alginate. In this paper, results comparing the behaviour of pectates, alginates and low methoxyl pectins as thickeners in near neutral pH sterilized systems are reported.

## Materials and methods

### *Materials*

Sodium polypectate (pectate) was purchased from Sigma Inc. (Cat. 27879). Although the term pectate is normally reserved for pectic substances containing a negligible proportion of methoxyl groups as reported by a committee of the American Chemical Society (1944), methanol determination by gas chromatography on the saponified pectate sample shows a residual degree of esterification of 4%. Sodium alginate was kindly donated by Alginates Industries Ltd. The sample used in this investigation has a guluronic/mannuronic acid ratio of approximately 0.8. The rheological properties of gels prepared from this material has been previously reported (Mitchell & Blanshard, 1976a). Low

methoxyl pectin with a degree of esterification measured as 22% was kindly donated by H.P. Bulmer Ltd Col. Flo. 67, a crosslinked, acetylated waxy maize starch was purchased from Laing-National Ltd.

Calcium sulphate dihydrate ($CaSO_4\ 2H_2O$) and sodium tripolyphosphate (S.T.P.P.) were standard laboratory reagents.

### *Methods*

A $408^{7/8} \times 300$ can was half filled with a 0.5% S.T.P.P. solution and the required amount of pectate, alginate and/or starch was dissolved or dispersed in this solution using a 'Silverson' mixer. The can was then topped up with the 0.5% S.T.P.P. solution leaving 1 cm of headspace and heated in a water bath until the temperature reached 80°C, when any starch present had gelatinized. The $CaSO_4 2H_2O$ was mixed in and the can sealed. Thermocouples were positioned in the can centre and when the indicated temperature dropped to approximately 50°C processing was commenced.

Processing was carried out at a temperature of 121.1°C either for an operators process time of 40 min in a rotating retort or for 60 min using a static retort. In the rotating retort end over end rotation was employed at a speed of 10 rev/min. The centre of the can was approximately 15 cm from the axis of rotation. After retorting the cans were water cooled, rotation being continued for 30 min during cooling.

Cans were opened 24 hr after retorting and the viscosity was measured in the can with a 'visco-tester' (Rion Co. Ltd, Tokyo) using the most sensitive spindle. Although the viscosities were read from the instrument in poise, in view of the non-Newtonian behaviour expected for solutions of this type, the results should only be regarded as an empirical measurement of the comparative viscosities. A more detailed rheological study of these systems is planned. The temperature dependance of the solution viscosity was studied by heating the cans with continuous stirring in a water bath and taking measurements of viscosity at approximately 10°C intervals.

## Results

The effect of $CaSO_4\ 2H_2O$ level on the viscosity after processing 0.5% sodium pectate solution in the rotating retort is illustrated in Fig. 1. At levels of $CaSO_4\ 2H_2O$ higher than 0.8% the cans contained gel pieces dispersed in a low viscosity liquid. This is believed to be due to the formation of a gel prior to retorting which subsequently disintegrated and syneresed during processing. Similar gel pieces were observed in 0.5% sodium alginate solutions processed in the same manner at $CaSO_4\ 2H_2O$ levels greater than 1.0%. In contrast to pectate, alginate solutions did not show measurable viscosities ($> 0.3$ poise) at lower $CaSO_4\ 2H_2O$ levels when the cans were opened 24 hr after processing.

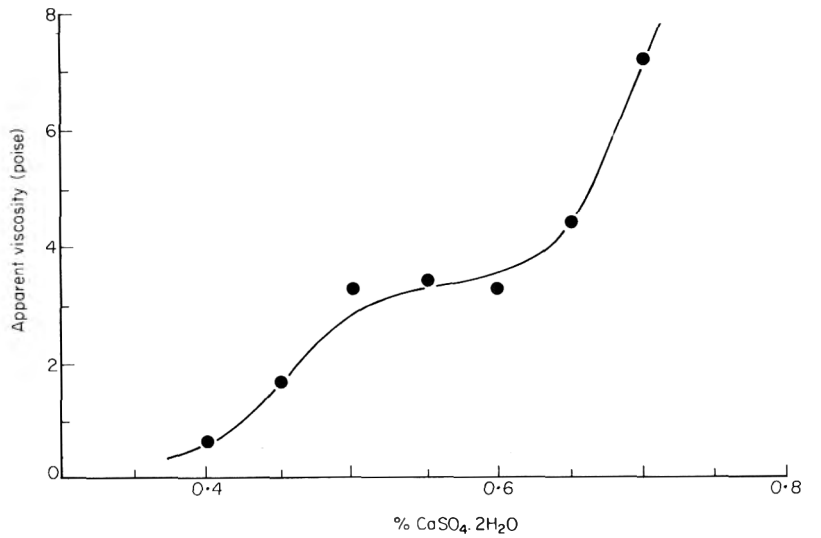


**Figure 1.** The effect of $CaSO_4 2H_2O$ levels on viscosity for processed 0.5% pectate solutions.

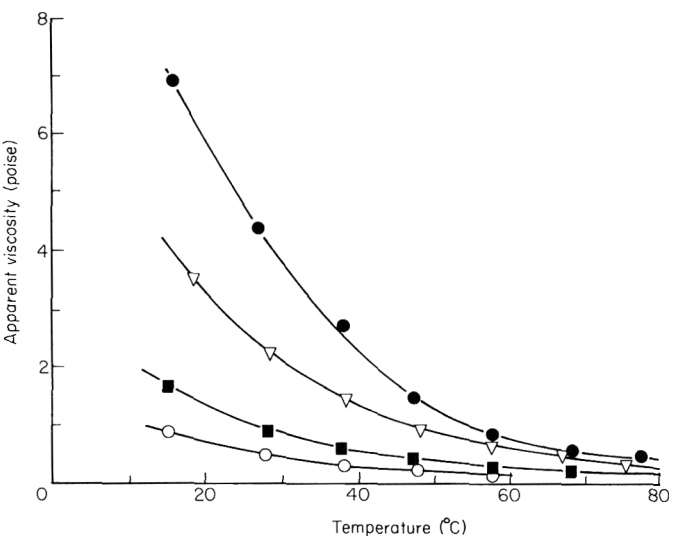


**Figure 2.** The effect of temperature on viscosity for processed solutions containing ● 0.6%, ▽ 0.5%, ■ 0.4% and ○ 0.3% pectate.

Solutions containing low methoxyl pectin at the 0.5 and 1.0% levels with 0.6% $CaSO_4 2H_2O$ also gave no measurable viscosity after processing in the rotating retort. In contrast to the pectate and alginate solutions which has pH values after processing in the range 6.2–6.9; the pH decreasing with increasing $CaSO_4 2H_2O$ level, the pH of the low methoxyl pectin solution had dropped from 7.5 to 4.2 during retorting.

Figure 2 displays the result of varying pectate concentration on the temperature dependence of the viscosity of solutions containing 0.6% $CaSO_4 2H_2O$.

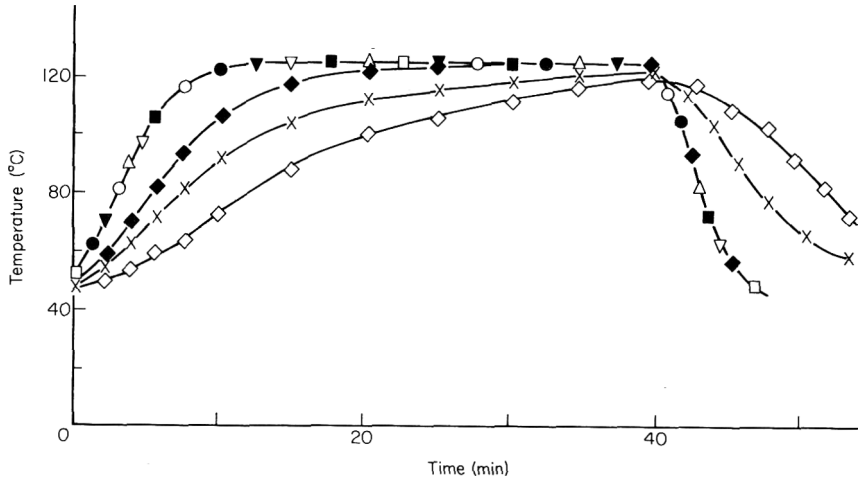


**Figure 3.** Comparison of the rate of heat penetration into solutions containing △ 0.1% , ▽ 0.2%, ▼ 0.3%, □ 0.4%, ● 0.5% and ○ 0.6% pectate with solutions containing ■ 1%, ◆ 2%, × 3% and ◇ 4% starch.

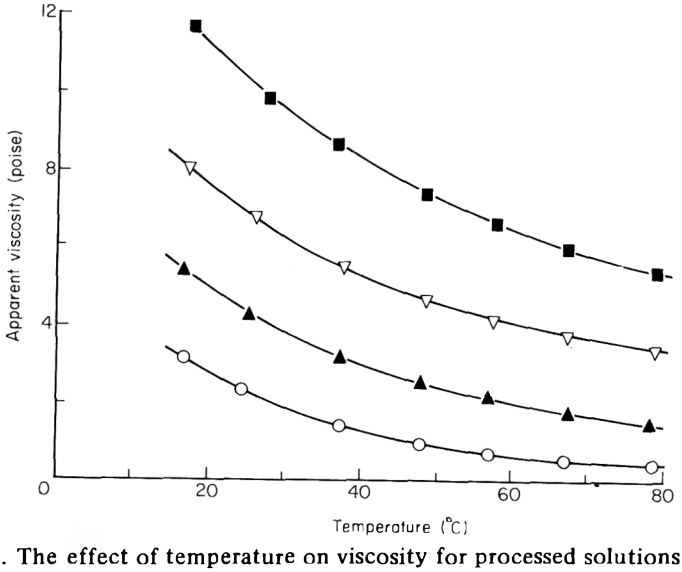


**Figure 4.** The effect of temperature on viscosity for processed solutions containing ■ 6%, ▽ 5%, ▲ 4% and ○ 3% starch.

The temperature/viscosity relationship was reversible on cooling. The rapid rate of heat penetration into these solutions during processing is shown in Fig. 3 and can be compared with the starch systems. The corresponding starch viscosities as a function of temperature after processing are shown in Fig. 4. It is apparent that the viscosity of the pectate system decreased more rapidly with increasing temperature than starch despite the thermostable characteristics of the calcium pectate gel. However, there was not a sharp discontinuity in the viscosity temperature curve that would be expected if a thermoreversible gelling agent was employed.

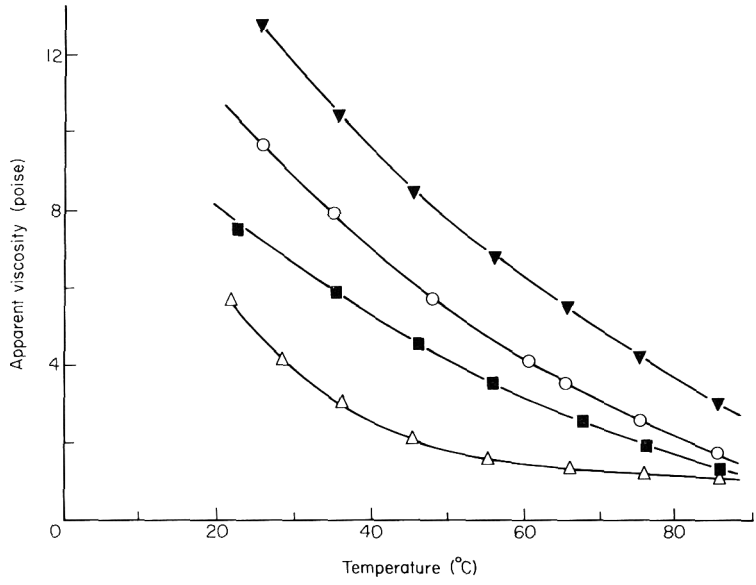


**Figure 5.** The effect of temperature on viscosity for processed 4% starch systems containing △ 0.1%, ■ 0.2%, ○ 0.3% and ▼ 0.4% pectate.

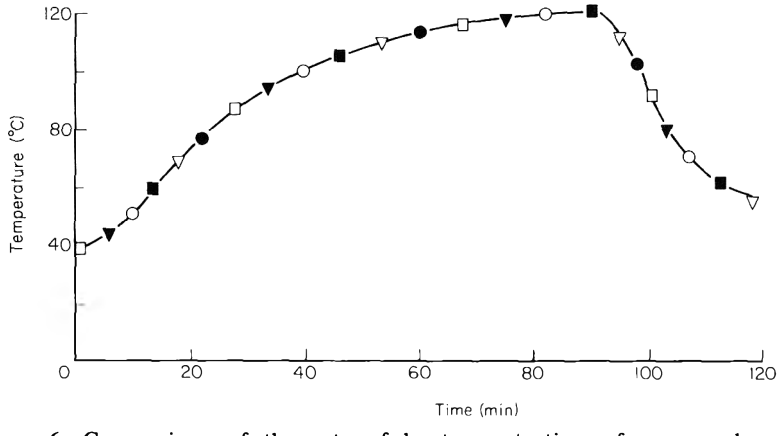


**Figure 6.** Comparison of the rate of heat penetration of processed systems containing ● 4% starch with those containing 4% starch plus □ 0.1%, ▲ 0.2%, ○ 0.3%, ■ 0.4% or ▽ 0.5% pectate.

It was not possible to obtain homogeneous viscous solutions of sodium pectate after processing in the static retort without the addition of another thickener. This is because the pectate viscosity during processing is too low to maintain a homogeneous dispersion of $CaSO_4\,2H_2O$. In the rotating retort this dispersion is maintained by agitating the cans until thickening occurs as the gelation reaction commences on cooling. Figure 5 displays the temperature dependence of the viscosity of starch/pectate mixtures processed in the static retort with 0.3% $CaSO_4\,2H_2O$. The pectate gelation system is compatible with

starch although at these relatively high starch levels heat transfer is predominantly by conduction and no difference in processing time is achieved using pectate, rather than additional starch to increase viscosity (Fig. 6). The pH of the starch and starch pectate mixture was 5.9 ± 0.1. At these pH values it appears that lower $CaSO_4\,2H_2O$ is necessary to initiate pectate gelation on cooling than at the higher pH's encountered using pectate alone.

## Discussion

An examination of the data on Figs 2, 3 and 4 would suggest that a reduction in process time might be achieved by the replacement of starch by moderately low levels of sodium pectate. For example at 40°C the viscosities after processing at 0.6% pectate and 4% starch were similar. If the two systems had to be processed at 121.1°C to give an Fo value of 10 then the operators process time for the pectate system would be 12 min compared with 60 min for starch.

The marked difference in the behaviour of pectate and low methoxyl pectin is due to the extensive degradation of the latter during retorting. When heated at neutral pH pectin degrades because of the translimination reaction (Albersheim, Neukom & Deuel, 1960). This mechanism will not operate if the methoxyl group is removed. It therefore follows, as has been shown by Pilnik & McDonald (1968) that the resistance of pectin to degradation at neutral pH increases with decreasing methoxyl content. An additional consideration is the distribution of residual methoxyl groups along the polygalacturonic acid chain. The low methoxyl pectin sample employed for this work was prepared by acid de-esterification, whereas the sodium pectate was de-esterified by enzyme action. If de-esterification is by acid the remaining methoxyl groups will be distributed randomly along the chain whereas in the case of enzyme de-esterification the methoxyl groups will be in blocks (Kohn & Furda, 1967) with large regions of unsubstituted chain in between. Hence if degradation on heating occurs mainly between methoxylated residues then enzyme de-esterified pectin will have a higher mean molecular weight after processing than the acid de-esterified materials. There will be a threshold molecular weight below which gelation will not take place. It has been shown that calcium alginate gels can just form when the primary weight average degree of polymerization is as low as 65 (Smidsrod & Haug, 1972), although corresponding information is not available for pectate, in view of the similarity between the two polymers a similar value would be expected for calcium pectate gels.

In the work described in this paper the calcium ion release through the retorting cycle has been achieved using S.T.P.P. as a sequestrant in conjunction with the sparingly soluble salt $CaSO_4\,2H_2O$. It seems probable that these salts are effective because during processing S.T.P.P. degrades to lower molecular weight phosphates that are less effective at chelating calcium ions, thus more calcium is available for gelation after retorting than before. In this way gelation can be made to occur after retorting without the undesirable possibility of a

thermostable gel forming prior to processing. An additional factor may be slight increase in $CaSO_4\,2H_2O$ solubility as the pH drops during retorting. Further studies, perhaps using a specific ion electrode to measure changes in calcium ion concentration would be necessary to elucidate the role of the salts in these systems.

Although there is scope for a more detailed investigation, the data presented in this paper shows that sodium pectate has advantages as a thickener in low acid canned foods compared with both low methoxyl pectin with a higher degree of esterification and sodium alginate. Compared with the latter material, it appears that the greater calcium sensitivity of pectate compared with alginate facilities the control of the gelation reaction through a retorting cycle, whereas compared with pectins with higher degrees of esterification sodium pectate has the important advantage that it does now show extensive degradation on heating at neutral pH.

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# The analysis of tocopherols in corn oil and bacon fat by thin-layer chromatography and spot density measurement

G. F. M. BALL AND P. W. RATCLIFF

## Summary

The two major tocopherols (α and γ forms) present in corn oil were separated, after removal of interfering lipids, by thin-layer chromatography (TLC), and detected on the TLC plate by spraying with Gibbs' reagent (2,6-dichloro-*p*-benzoquinone-4-chlorimine). Quantitation was achieved by densitometric scanning of the coloured spots, using a calibration curve prepared from reference samples of the pure α- and γ-tocopherols. α-tocopherol, added to bacon fat, was quantitatively recovered without measurable loss during the analysis. Other antioxidants commonly added to foodstuffs, with the exception of BHA and BHT, were not extracted. The method described has a detection limit of 20 ppm of α- or γ-tocopherol for a given fat sample, hence permits the quantitation of either tocopherol in vitamin E-rich materials, such as vegetable oils, but lacks the sensitivity to detect the trace amounts of tocopherols normally present in animal fatty tissues.

## Introduction

Eight chemically related forms of vitamin E occur in nature; four tocopherols, designated α-, β-, γ- and δ-tocopherol, and four analogous tocotrienols (Bauernfeind, 1977). Vitamin E occurs in the lipid fraction of a variety of plant materials, especially in vegetable oils, the different analogues varying greatly in their relative distribution (Table 1). Animal food products, such as pork and bacon, in which α-tocopherol is the only analogue which has been reported (Table 2), are poor sources of vitamin E. The vitamin is deposited throughout the fatty tissues of animals, having been introduced solely through the diet. Quantitation of the vitamin E analogues separated by TLC usually involves scraping off the area of adsorbent containing the spot, eluting the analogue from the adsorbent, and determining colorimetrically the amount of analogue

Authors' address: J. Sainsbury Ltd, Stamford House, Stamford Street, London SE1 9LL.
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**Table 1.** Vitamin E content of vegetable oils estimated by TLC and colorimetry (Müller-Mulot, 1976)

| Oil | Total vitamin E content (ppm) | Concentration (ppm) of individual tocopherols and tocotrienols: percentage of the total vitamin E content | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | α-T | | β-T | | γ-T | | δ-T | | α-T3 | | β-T3 | γ-T3 | | δ-T3 | | |
| | | ppm | (%) | ppm | (%) | ppm | (%) | ppm | (%) | ppm | (%) | † | ppm | (%) | ppm | | |
| Corn oil | 1198 | 191 | (16) | trace | | 942 | (79) | 42 | (3) | 23 | (2) | – | n.d. | | n.d. | | |
| Castor oil | 478 | 28 | (6) | 29 | (6) | 111 | (23) | 310 | (65) | n.d. | | – | n.d. | | n.d. | | |
| Edible oil C | 375 | 165 | (44) | n.d. | | 63 | (17) | n.d. | | 50 | (13) | – | 97 | (26) | n.d. | | |
| Edible oil I | 429 | 65 | (15) | n.d. | | 140 | (33) | trace | | 56 | (13) | – | 168 | (39) | n.d. | | |
| Rapeseed* | 271 | 70 | (26) | 16 | (6) | 178 | (65) | 7 | (3) | n.d. | | – | n.d. | | n.d. | | |
| Maize germ oil* | 603 | 134 | (22) | 18 | (3) | 412 | (68) | 39 | (7) | n.d. | | – | n.d. | | n.d. | | |
| Soyabean oil* | 1162 | 116 | (10) | 34 | (3) | 737 | (63) | 275 | (24) | n.d. | | – | n.d. | | n.d. | | |
| Wheat germ oil | 2188 | 1179 | (54) | 398 | (18) | 493 | (23) | 118 | (5) | trace | | – | n.d. | | n.d. | | |

T = tocopherol, T3 = tocotrienol, n.d. = not detected.

* Refined oil. † β-T3, if present, is included in the γ-T values.

Table 2. α-tocopherol content of pig products

| Product | α-tocopherol* (ppm) | Reference |
|---|---|---|
| Bacon | 3.7 | Thompson, Erdody & Maxwell (1972) |
| Bacon fat (fried) | 1.0 | Bunnell *et al.* (1965) |
| Pork back fat | 4.5 | Hvidsten & Astrup (1963) |

* Results for other tocopherols not reported.

in each sample of eluate (Glavind & Holmer, 1967; Müller-Mulot, 1976). This procedure is somewhat laborious and requires considerable technical skill. The susceptibility of the vitamin E analogues toward atmospheric oxidation, and the errors associated with each step, constitute further problems.

The technique of spot density measurement using a scanning densitometer was investigated as a means of quantifying tocopherols separated on a TLC plate. This technique offers the advantage of measuring the separated components *in situ* on the TLC plate, thus saving time and removing several sources of error.

Two applications of densitometry were studied. The first involved transmitted light measurement of the coloured spots obtained from the reaction between Gibbs' reagent, (2,6-dichloro-*p*-benzoquinone-4-chlorimine; van der Heide, 1966) and the tocopherols separated on the TLC plate. Gibbs' reagent couples to phenols forming characteristically coloured indophenols. The production of stable coloured spots against a uniform white background has been found to facilitate successful spot density measurements.

Alternatively, the uv-absorbing property of tocopherols (Martelli & Nano, 1968) was utilized in developing a fluorescence quenching method for determining the tocopherols separated on a fluorescein-impregnated TLC plate. The coating of the latter is activated by uv light at λ 254 nm, emitting green light with λ max. 526 nm. Preliminary attempts at measuring α-tocopherol by fluorescence quenching yielded non-reproducible and random results, but when densitometric scanning was delayed until the fourth day after chromatographic development, satisfactory results were obtained. This phenomenon was due to the gradual oxidation by air of α-tocopherol to the α-tocopherylquinone, which exhibits strong fluorescence quenching properties. The quenching zones remained quantitatively stable for at least another 5 days.

## Materials and methods

### *Materials*

Standard solutions containing (w/v) D,L-α-tocopherol 1% and D,L-γ-tocopherol 1%, biochemical grades (Roche Products Ltd) were prepared in absolute ethanol and stored at 0–5°C in amber-coloured glass bottles.

TLC plates 20 × 20 cm (Scientific Supplies Ltd) were coated with a 0.5 mm layer of fluorescein-impregnated silica gel $HF_{254}$, type 60 (Merck 7739) using a Gallenkamp TLC plate spreader.

Gibbs' reagent, 0.5% (w/v) 2,6-dichloro-*p*-benzoquinone-4-chlorimine in absolute ethanol was prepared immediately before use.

*Methods*

Vitamin E is readily oxidized by strong light, thus bright daylight must be excluded. The extraction and chromatography were carried out in a darkened fume cupboard; all other stages were performed in subdued light.

*Saponification and extraction of unsaponifiable matter*

A 10 g sample of corn oil or chopped bacon fat was saponified for 30 min at 80°C with a mixture of 25 ml of absolute ethanol, 10 ml of 50% (w/v) KOH and 0.5 g of ascorbic acid in an atmosphere of oxygen-free nitrogen. This procedure prohibited the oxidation of the vitamin E analogues which would otherwise occur in hot alkaline conditions.

The unsaponifiable matter was extracted with diethyl ether according to Cocks & van Rede (1966), any emulsions that formed being broken by the addition of solid sodium chloride. The final ether layer was dried with anhydrous sodium sulphate, then reduced to about 10 ml on a Büchi rotary evaporator (Orme Scientific Ltd) *in vacuo* at ambient temperature. Approximately 5 ml of absolute ethanol was added to the flask contents and the mixture re-evaporated *in vacuo* to obtain a dry residue of unsaponifiable matter. The residue was dissolved completely in 2.0 ml of absolute ethanol and the solution transferred to a light-proof sample bottle.

*TLC of unsaponifiable extract*

A multiple plate chromatography tank (Shandon Southern Products Ltd) was charged with developing solvent and the tank allowed to equilibrate for 3 hr in a darkened fume cupboard. Two solvent systems were investigated (i) petroleum ether, 40–60°C/glacial acetic acid, 4:1 (Endean & Bielby, 1975) and (ii) *n*-hexane/ethyl acetate, 185:15 (Müller-Mulot, 1976). Using Drummond microcaps, suitable volumes of the unsaponifiable sample extract were applied to a TLC plate in a series of aliquots not exceeding 5 μl, and each application was dried with cold air. To obtain calibration curves at the required concentration range, appropriate loads of α- and γ-tocopherol standard solutions were applied to the same plate. The plates were placed in the equilibrated chromatography tank, adsorbent surfaces facing each other, and developed over

a distance of 15 cm. The plates in the petroleum ether/acetic acid tank were subjected to a single development (migration time approximately 75 min). The plates in the *n*-hexane/ethyl acetate tank were developed three times successively (migration time 3 X 40 min).

*Detection of separated vitamin E analogues*

The TLC plates were blown dry with cold air and observed under uv light (254 nm). Faint fluorescence quenching zones corresponding to the tocopherol standards were visible as blue/black spots against the green fluorescent background produced by the fluorescein in the plate adsorbent. The positions of the fluorescence quenching zones were noted without marking the zones, and the plate was then sprayed with Gibbs' reagent. After heating the plate at 100°C for 10 min, the tocopherols were revealed as characteristically coloured spots against a white background. The Rf values of the coloured spots coincided with those of the fluorescence quenching zones. With the petroleum ether/acetic acid developing solvent α-tocopherol spots were yellow (Rf 0.50) and γ-tocopherol spots were dark brown (Rf 0.45). The *n*-hexane/ethyl acetate solvent system produced a better separation of the tocopherol analogues (α-tocopherol, light brown, Rf 0.45; γ-tocopherol, grey, Rf 0.30).

*Densitometric measurement of coloured spots*

The coloured spots obtained after spraying the TLC plate with Gibbs' reagent were scanned by the Vitatron TLD 100 densitometer (MSE/Fisons Ltd) using the tungsten lamp and the following settings: mode −log; diaphragm 0.5 mm; filters 523 nm and UVB; recorder speed 20 cm $min^{-1}$. The level, span, and scan speed were adjusted for each plate to give optimal peak height and width.

A calibration curve was constructed of densitometer peak area plotted against the corresponding load (μg) of α- and γ-tocopherol reference standards applied to the TLC plate. Within a load range of 5–40 μg of either tocopherol, a linear relationship obeying Beer's Law was obtained (Fig. 1). Thus, provided the same peaks lay within the range of the calibration curve, the concentration of α- or γ-tocopherol present in a sample extract could be interpolated. At plate loads above 40 μg of either tocopherol, a departure from linearity occurred. The limit of detection with Gibbs' reagent was 3 μg of α-tocopherol and 1 μg of γ-tocopherol reference standard applied to the TLC plate and developed in either of the solvent systems.

*Densitometric measurement of fluorescence quenching zones*

The fluorescence quenching zones were scanned by the densitometer on the fourth day, or up to the ninth day, after chromatographic development using

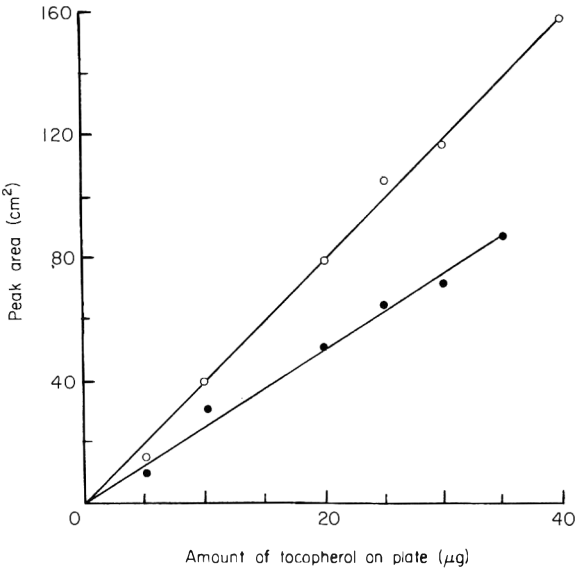


**Figure 1**. Spot density measurement of α- and γ-tocopherol reference standards by transmitted light, after developing the TLC plates in petroleum ether, 40–60°C/glacial acetic acid (4 : 1) then spraying with Gibbs' reagent. ● D,L-α-tocopherol, ○ D,L-γ-tocopherol.

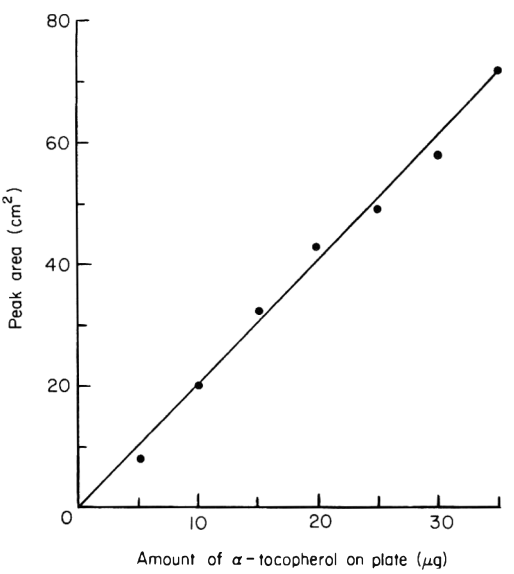


**Figure 2**. Densitometric measurement of the fluorescence quenching zones arising from the atmospheric oxidation of D,L-α-tocopherol reference standards. TLC plate scanned on the fourth day after chromatographic development in petroleum ether, 40–60°C/glacial acetic acid (4 : 1).

the mercury lamp and the settings described above for transmitted light measurements. Linear calibration curves were obtained within a plate load range of 5–40 μg of α-tocopherol, above which a departure from linearity occurred (Fig. 2). The limit of detection of the α-tocopherylquinone quenching zone was equivalent to 5 μg of α-tocopherol reference standard applied to the TLC plate and developed in the petroleum ether/acetic acid solvent system.

## Results

### *Recovery of α-tocopherol from bacon fat*

In order to determine the percentage recovery of two levels of α-tocopherol from bacon fat, calculated amounts of pure D,L-α-tocopherol were added to 10 g of bacon fat such that, assuming (a) a complete absence of indigenous α-tocopherol, and (b) no loss of α-tocopherol during the process of saponification and ether extraction, appropriate volumes of the final ethanolic extract applied to the TLC plate contained 20 μg and 40 μg of α-tocopherol.

Following chromatographic development in petroleum ether/acetic acid and densitometric scanning of the fluorescence quenching zones, the peak areas of the two 'recovery' samples were compared with the 20 and 40 μg α-tocopherol reference standards that were applied directly to the TLC plate. The results from two experiments showed recoveries ranging from 96 to 101%. No signs of fluorescence quenching appeared in extracts derived from 10 g of bacon fat with no added α-tocopherol, signifying an α-tocopherol concentration below the detectable limit in the fat sample.

It must be emphasized that protection of the test samples from bright light throughout the entire analysis is essential. Preliminary recovery experiments, in which inadequate precautions were taken against bright sunlight, yielded poor results with recovery factors as low as 30%.

### *Analysis of corn oil*

*Qualitative evaluation.* A 10 g sample of corn oil was saponified and extracted with diethyl ether. A total of 50 μl of the final ethanolic extract was applied to a TLC plate spotted with reference standards (40 μg) of α-tocopherol, and a mixture of both. The plate was developed in the petroleum ether/acetic acid solvent system, observed under uv light, then sprayed with Gibbs' reagent.

Five coloured spots, all completely separated, that exhibited fluorescence quenching properties, were observed in the corn oil extract. The main component (Rf 0.45, dark-brown) was judged to be γ-tocopherol on the basis of the characteristic reaction to Gibbs' reagent, and its Rf value compared with the γ-tocopherol reference standard. Similarly, a smaller sized spot (Rf 0.50, yellow) was judged to be α-tocopherol. Further evidence of the identity of these

two components was provided by developing similar TLC plates in *n*-hexane/ethyl acetate, and spraying plates developed in both solvent systems with DPPH (1,1-diphenyl-2-picrylhydrazyl)/ethanolic sulphuric acid (Müller-Mulot, 1976) and potassium ferricyanide/ferric chloride (Endean & Bielby, 1975) reagents. The remaining three components (Rf 0.62, 0.70, 0.80, brown) were regarded as impurities because of their negative reaction to either of the above reagents.

*Quantitative estimation.* A 10g sample of corn oil was saponified and extracted with diethyl ether. A 10μl volume of the final ethanolic extract was applied in duplicate to a TLC plate spotted with 5, 10, 15, 20 and 25 μg loads of α- and γ-tocopherol reference standards. The plate was developed three times successively in the *n*-hexane/ethyl acetate (185:15) system, then sprayed with Gibbs' reagent. The plate was scanned with the densitometer level set at d2, the scan speed at 1 cm $min^{-1}$, and the span control at 9.00 (α-tocopherol spots) and 8.00 (γ-tocopherol spots).

Table 3 lists the results of five separate determinations from the same sample of corn oil. The results were calculated from individual calibration curves constructed from the tocopherol standards applied to each plate. The difference between the smallest and largest estimated values of the tocopherols expressed as a percentage of the mean is 8.1% for α-tocopherol and 6.6% for γ-tocopherol.

The α- and γ-tocopherol spots in the corn oil extracts were clearly separated from one another and, apart from two distinct non-tocopherol spots near the solvent front, were free from interfering coloured impurities. Results were also obtained from plates developed in the petroleum ether/acetic acid solvent system but these were non-reproducible, possibly because of interference by residual acetic acid with the spray reagent. Fluorescence quenching scanning of the corn oil extracts was not carried out owing to the presence of interfering uv-absorbing impurities near the tocopherol quenching zones.

**Table 3.** α- and γ-tocopherol content of corn oil estimated by TLC*, and spot density measurement of the coloured spots developed with Gibbs' reagent

| | Concentration (ppm) of α- and γ-tocopherols: percentage of the total estimated vitamin E content | | | | |
|---|---|---|---|---|---|
| | α-tocopherol | | γ-tocopherol | | Total |
| | ppm | (%) | ppm | (%) | ppm |
| | 125 | (22) | 443 | (78) | 568 |
| | 125 | (22) | 438 | (78) | 563 |
| | 120 | (20.5) | 465 | (79.5) | 585 |
| | 118 | (20) | 468 | (80) | 586 |
| | 128 | (22) | 454 | (78) | 582 |
| Mean | 123 | (21.3) | 454 | (78.7) | 577 |

* The TLC developing solvent was *n*-hexane/ethyl acetate (185:15).

*Detection of other food antioxidants*

Pure samples of the following food antioxidants were applied directly (200 μg loads) to a TLC plate and developed in the petroleum ether/acetic acid solvent system: propyl, octyl and dodecyl gallates (Rf 0.15, 0.25, 0.17), ascorbyl palmitate (0.15), BHA (butylated hydroxyanisole, 0.40) and BHT (butylated hydroxytoluene, 0.98). All of these antioxidants gave positive reactions to Gibbs' reagent and displayed fluorescence quenching properties. In order to ascertain whether or not the antioxidants were extractable by the method described, a 10 g sample of corn oil was analysed after adding the three gallates, ascorbyl palmitate, and α-tocopherol at concentrations calculated to yield, theoretically, 50 μg of each when applied in the final extract to the TLC plate. BHA and BHT were also added at twice this concentration.

The absence of quenching or coloured spots at Rf values below 0.4 on the TLC plate, as also shown by a sample of corn oil without added antioxidants, demonstrated that the three gallates and ascorbyl palmitate were not extracted from the test sample by the method described.

The α-tocopherol spot in the test extract was, logically, more pronounced than that in the control extract. Both extracts revealed the relatively high concentration of γ-tocopherol (Rf 0.45) in the corn oil sample.

The presence of a spot in the test extract, whose Rf value (0.40), fluorescence quenching activity, and reaction to Gibbs' reagent, coincided with those of the reference BHA standard, indicated that an appreciable recovery of BHA was obtained. Finally, a faint fluorescence quenching spot (Rf 0.98) in the test extract, which was undetectable by Gibbs' reagent, might have been BHT. There were no equivalent spots at Rf values of 0.40 or 0.98 in the control extract.

## Discussion

Of the two principal methods of densitometry described for the determination of tocopherols, measurement of light transmitted by the coloured spots produced with Gibbs' reagent is considered to be more suitable than the measurement of fluorescence quenching. Using the former technique, the TLC plate can be sprayed and scanned as soon as the plate is dry. This contrasts with the 4 days' delay period necessary for the complete oxidation of the tocopherols to the fluorescence-quenching tocopherylquinones. Moreover, Gibbs' reagent distinguishes between α- and γ-tocopherols on a colour basis, the visible spots are easier to scan, and the reagent is more selective for detecting tocopherols than the visualization of fluorescence-quenching spots.

Despite these advantages, it is considered worthwhile to use TLC plates containing the fluorescent indicator. This enables the plates to be visualized under uv light before spraying with the reagent, thus providing information regarding the presence and positions of impurities in the unsaponifiable sample

extract. The fluorescence indicator does not appear to interfere with the reaction between tocopherols and Gibbs' reagent.

The *n*-hexane/ethyl acetate solvent system (triple development) was found to be superior to the petroleum ether/acetic acid system in terms of spot separation, uniformity of spot shape, consistency of Rf values, and reproducibility of spot density measurements. Possibly the poor performance of plates developed in the latter solvent system was due to the incomplete removal of residual acetic acid. According to Müller-Mulot (1976) the *n*-hexane/ethyl acetate system completely separates six of the eight known vitamin E analogues (α-, β- and δ-tocopherol; α-, γ- and δ-tocotrienol). The γ-tocopherol and β-tocotrienol form a seventh, indistinguishable spot. This author described several spray reagents suitable for characterizing the separated analogues. The *n*-hexane/ethyl acetate system and a suitable spray reagent may facilitate the quantitation of the other vitamin E analogues by spot density measurement.

Other antioxidants commonly used in foods, (gallates, ascorbyl palmitate, BHA, BHT) are separable by the same TLC system, and exhibit similar fluorescence quenching properties and colour reactions with Gibbs' reagent. Of these substances, however, only BHA and BHT are extractable by the procedure described.

Vegetable oils, including corn oil, contain high proportions of autoxidizable unsaturated fats, thus the vitamin E analogues, in exerting their antioxidant action, will themselves be gradually oxidized. Thus the difference between the estimated tocopherol concentrations in corn oil (Table 3) and those reported by Müller-Mulot (1976; see Table 1) could be explained by differences in the degree of refinement and the storage histories of the product. The estimated mean percentage (78.7%) of γ-tocopherol in the corn oil was in close agreement with the quoted value of 79%. The relatively high percentage of α-tocopherol (21.3% compared with 16%) obtained in the present study was probably due, at least in part, to the lack of detection of the minor vitamin E analogues (δ-tocopherol and α-tocotrienol) which together make up 5% of the vitamin E constituents in corn oil (Müller-Mulot, 1976).

On the basis of a detection limit of 5 μg of α- or γ-tocopherol on the TLC plate, either by fluorescence quenching or reaction with Gibbs' reagent, it can be calculated that the detection limit of either tocopherol for a given fat sample by the method described (10 g fat sample; 2 ml unsaponifiable extract; 50 μl extract applied to the plate) is equivalent to 20 ppm.

There are three stages in which the sensitivity of the method might be increased. In the case of bacon fat, a 10 g fat sample was the maximum amount that could be saponified without scaling-up the saponification and extraction process. Experiments in which larger amounts of fat were refluxed with the stated volume of alcoholic KOH proved unsatisfactory, since an incomplete saponification was obtained. This resulted in an excess of fat in the 'unsaponifiable' extract which was impossible to dissolve in a small volume of solvent. TLC of such fatty extracts has been proved useless, owing to the physicochemical barrier imposed by the mass of lipid at the origin of the TLC plate.

Alternatively, several saponifications and extractions could be performed on a number of 10 g fat samples, and the extracts bulked, then re-evaporated to produce a combined, concentrated extract. This would necessitate an extra purification step, e.g. a sulphuric acid wash, to remove interfering impurities.

There is also a limit (2 ml) upon the smallest volume of solvent that can be used to quantitatively re-dissolve the unsaponifiable residue. The final way to increase the sensitivity is to apply a larger load of the final extract to the TLC plate. In practice, 100 $\mu$l, applied by a multiple loading schedule, is the maximum load that a plate can tolerate without experiencing the problem of spot 'tailing'.

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# Changes in total solids, ascorbic acid and total pigment content of capsicum cultivars during maturation and ripening

F. M. M. RAHMAN, K. A. BUCKLE AND R. A. EDWARDS

## Summary

Quantitative variations in total solids, ascorbic acid and total pigment content of fifteen capsicum cultivars grown under field conditions were assessed. A progressive increase of total solids was found in all cultivars at all stages of fruit maturation and ripening. A direct relationship was found between ascorbic acid content and capsicum maturity. Total pigment contents increased between two and seventy fold as the result of transition from the immature to the fully ripe condition.

## Introduction

Capsicums, members of the family Solanaceae, are used in a variety of ways, yet little attention has been given to their nutritional value, although they are rich in vitamin C and have a high content of the precursors of vitamin A. The influx of European migrants to Australia, who are accustomed to eating capsicums in their homeland, has resulted in the fruit becoming a product of increasing demand in the market.

Reports of quantitative determinations of ascorbic acid (Gomolyako, 1937; Beckley & Notley, 1943; Shukla & Pande, 1967) and total pigment content of capsicum cultivars (Cholnoky *et al.*, 1955; Curl, 1962, 1964; Davies, Mathews & Kirk, 1970) differ considerably from laboratory to laboratory due to variations in factors such as fruit variety, agronomic and environmental conditions and stage of maturity.

The quality parameters of the major capsicum cultivars grown in Australia have not been investigated previously. The present study involved a determination of total solids, ascorbic acid and total pigment content at four stages of maturation and ripening of fifteen capsicum cultivars grown under local conditions.

Authors' address: School of Food Technology, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia.

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## Materials and methods

### *Growing and harvesting of capsicums*

Fifteen capsicum cultivars were grown at Hawkesbury Agricultural College, Richmond, N.S.W. during the summer season. Fruits were selected at the immature, mature, half ripened and fully ripened stages. Immature and mature fruits of a particular cultivar of the same colour and similar size were differentiated by touch. Soft fruits were graded as immature and tough fruits as mature. Half-ripened and fully-ripened stages were graded by colour. Capsicums were classified as green (eight cultivars) or yellow (seven cultivars) based on the colour of the fruits at maturity (i.e. before ripening).

Five large bell-type fruits or ten small fruits of each cultivar at one maturity level were de-stemmed, de-seeded, cored and cut into pieces approximately 1 $cm^2$ and thoroughly mixed. The resulting sample is referred to as the stock capsicum sample.

A 20 ± 0.1 g stock sample was taken for immediate pigment extraction and the remaining sample was used for ascorbic acid and total solids determinations.

### *Determination of total solids and ascorbic acid*

A stock sample (4.5–5.0 g) from fruits of the fifteen cultivars at four stages of maturity were weighed into nickel crucibles and total solids determined by evaporation under vacuum following the AOAC (1970) procedure.

Stock samples (10.0 ± 0.1 g) of prepared fruit were extracted with 3% metaphosphoric acid by blending with an Ultra-Turrax comminutor. Ascorbic acid in the filtered metaphosphoric acid solution was determined by the indophenol method of Kefford (1957).

### *Estimation of total pigment content*

Stock samples of capsicums (20 ± 0.1 g) were added to a flat-bottomed glass tube (25 × 4 cm) and 25 ml of cold (−27°C) acetone was added. Pigment was then extracted by homogenizing with the Ultra-Turrax for 2 min after adding 2 g magnesium carbonate. The contents of the tube were vacuum filtered through sintered glass, the residue transferred to the glass tube and extracted with acetone, and the process repeated four times until the final acetone extract was colourless. The acetone extracts were combined in a 500 ml stoppered separatory funnel and the pigment immediately transferred to petroleum ether (b.p. 40–60°C) by adding 10% sodium chloride using the method of Jeffrey (1968). All extractions were performed as rapidly as possible under reduced light.

The extracted pigment solution in petroleum ether of all fifteen cultivars at four maturity stages was diluted (if necessary) with petroleum ether, and the spectral curve recorded in a Unicam SP 800 spectrophotometer from 340 to 700 nm for immature, mature and half-ripened fruit, and from 340 to 600 nm for fully ripened fruit. Total pigment contents were calculated as β-carotene using an $E^{1\%}_{1\,cm}$ value of 2500 at 451 nm following the method of Davies (1965).

## Results and discussion

### *Total solids*

Table 1 shows that a progressive increase of total solids occurred in all cultivars during maturation and ripening. The range of total solids was in agreement with the results of Shipton & Last (1967) who found total solids to increase from 6.4 to 8.7% during the ripening of fully matured capsicums. On the whole the yellow cultivars contained a lower percentage of total solids than the green cultivars at the immature stage, but no significant difference existed at the fully ripened stage. Long Red Cayenne and Red Chilli cultivars showed higher total solids contents (10–15%) compared to the other cultivars, a result consistent with their small size and low flesh content.

**Table 1.** Total solids content of capsicum cultivars at different stages of maturity

| Cultivar | Total solids (%) | | | |
|---|---|---|---|---|
| | Immature green | Mature green | Half ripened | Fully ripened |
| Green cultivars | | | | |
| Large Green | 6.2 | 6.3 | 6.6 | 8.3 |
| Pacific Bell | 6.4 | 6.7 | 7.6 | 8.4 |
| Long Red Cayenne | 10.0 | 13.3 | 14.4 | 15.0 |
| Tomato-Shaped Green | 6.2 | 7.3 | 7.6 | 8.9 |
| Red Chilli | 10.5 | 13.8 | 14.5 | 15.6 |
| Golden Californian Wonder | 6.5 | 7.5 | 8.5 | 9.6 |
| Long Green | 6.4 | 6.6 | 8.6 | 8.9 |
| Californian Wonder | 6.0 | 6.1 | 7.3 | 8.0 |
| Yellow cultivars | | | | |
| College Gold | 5.1 | 6.3 | 7.2 | 8.3 |
| Tomato-Shaped Yellow | 5.1 | 5.4 | 7.4 | 7.7 |
| Bogyiszlo' | 5.3 | 6.2 | 7.9 | 8.6 |
| Hungarian Yellow | 6.4 | 7.3 | 7.9 | 8.3 |
| Yellow Wax | 4.8 | 6.5 | 8.2 | 8.8 |
| Ram Horn | 5.9 | 6.5 | 8.6 | 8.9 |
| Romanian Hot Yellow | 5.6 | 6.1 | 8.4 | 8.9 |

Table 2. Ascorbic acid content of capsicum cultivars at different stages of maturity

| Cultivar | Ascorbic acid content (mg/100 g) | | | |
|---|---|---|---|---|
| | Immature green | Mature green | Half ripened | Fully ripened |
| Green cultivars | | | | |
| Large Green | 82.2 | 87.0 | 94.9 | 125.6 |
| Pacific Bell | 100.6 | 115.1 | 120.8 | 160.3 |
| Long Red Cayenne | 145.0 | 185.2 | 203.6 | 212.2 |
| Tomato-Shaped Green | 84.9 | 131.4 | 138.1 | 166.4 |
| Red Chilli | 129.0 | 113.3 | 194.0 | 217.7 |
| Golden Californian Wonder | 89.8 | 107.7 | 153.7 | 185.4 |
| Long Green | 105.0 | 115.7 | 162.6 | 184.4 |
| Californian Wonder | 103.0 | 137.6 | 153.7 | 174.8 |
| Yellow cultivars | | | | |
| College Gold | 123.8 | 147.7 | 159.0 | 162.0 |
| Tomato-Shaped Yellow | 104.3 | 117.7 | 136.5 | 161.6 |
| Bogyiszlo' | 118.4 | 130.3 | 160.7 | 164.8 |
| Hungarian Yellow | 117.9 | 137.6 | 142.3 | 149.2 |
| Yellow Wax | 83.5 | 144.4 | 168.5 | 188.4 |
| Ram Horn | 104.9 | 138.0 | 162.6 | 189.8 |
| Romanian Hot Yellow | 113.7 | 146.3 | 176.7 | 199.1 |

*Ascorbic acid*

With the advance of fruit maturation and ripening, ascorbic acid concentration increased in all cultivars and was highest in the fully ripened stage (Table 2), contrary to the results of Paul (1940). The trends of ascorbic acid increase during maturation agreed closely with those reported by Sugiura (1937) and Beckley & Notley (1943).

Considerable differences in ascorbic acid content existed within the cultivars examined. Ascorbic acid concentrations in immature fruit ranged from 82 to 145 mg/100 g fresh tissue, while in fully ripened fruit ascorbic acid concentrations ranged from 125 to 228 mg/100 g. The results are in agreement with the findings of Beckley & Notley (1943) and Shukla & Pande (1967) who found significant differences in the ascorbic acid content of chillies (162 to 344 mg/100 g) and sweet peppers (226 to 342 mg/100 g) of differing maturity. In the Tomato-Shaped Green, Golden Californian Wonder and Yellow Wax cultivars the increase of ascorbic acid during ripening was of the order of approximately 100%.

The high ascorbic acid content of the matured capsicums examined is nutritionally important since capsicums are often consumed in Australia at the matured stage. The capsicums examined contain approximately five to ten

**Table 3.** Total pigment content of capsicum cultivars at different stages of maturity

| Cultivar | Pigment content of fresh tissue calculated as β-carotene (μg/g) | | | |
|---|---|---|---|---|
| | Immature green | Mature green | Half ripened | Fully ripened |
| Green cultivars | | | | |
| Large Green | 14.6 | 26.6 | 52.4 | 158.0 |
| Pacific Bell | 13.0 | 31.1 | 69.8 | 192.6 |
| Long Red Cayenne | 38.1 | 61.0 | 148.9 | 702.4 |
| Tomato-Shaped Green | 8.9 | 13.2 | 32.3 | 112.1 |
| Red Chilli | 33.4 | 41.6 | 146.9 | 780.0 |
| Golden Californian Wonder | 20.2 | 24.8 | 55.4 | 92.5 |
| Large Green | 13.8 | 22.8 | 64.8 | 228.3 |
| Californian Wonder | 11.4 | 21.7 | 50.1 | 202.6 |
| Yellow cultivars | | | | |
| College Gold | 2.3 | 3.1 | 51.0 | 218.4 |
| Tomato-Shaped Yellow | 3.5 | 5.1 | 42.7 | 161.1 |
| Bogyiszlo' | 2.0 | 3.3 | 27.4 | 118.4 |
| Hungarian Yellow | 5.7 | 9.3 | 54.9 | 197.6 |
| Yellow Wax | 1.8 | 2.4 | 39.3 | 174.1 |
| Ram Horn | 6.8 | 9.7 | 48.0 | 181.5 |
| Romanian Hot Yellow | 4.1 | 5.7 | 29.3 | 83.9 |

times more ascorbic acid than fresh tomatoes which usually contain 12 to 19 mg ascorbic acid/100 g fruit (Rahman, 1970).

*Total pigment*

Total pigment contents (Table 3) increased in all cultivars as the fruit advanced from the immature to the fully ripened stage. However, large differences existed in total pigment content within the fifteen cultivars examined at all stages of maturation and ripening. Significantly lower pigment contents were observed in all yellow cultivars at both immature (range 1.8–6.8 μg/g) and mature (range 2.4–9.7 μg/g) stages compared to immature (range 13.0–38.1 μg/g) and mature (range 13.2 to 61.0 μg/g) stages of the green cultivars. Total pigment contents of individual cultivars increased significantly during the conversion from the mature to the half and fully ripened stages. The increase of pigment content in the yellow cultivars was twelve to seventy fold, and in the green cultivars two to eight fold. Cholnoky *et al.* (1958) found a ten fold increase in pigment content in yellow cultivars and a thirty-five fold increase in the red cultivars examined. Davies *et al.* (1970) also reported a five fold

increase of total pigment in yellow and orange fruits whereas in Chilli peppers the increase was 100 fold.

Significant differences existed in the total pigment content of the fully-ripened red cultivars. The highest pigment content was in the Red Chilli cultivar (780 $\mu$g/g) and the lowest in the Romanian Hot Yellow cultivar (83.9 $\mu$g/g). The differences among the cultivars are probably due to the low flesh content of some of the cultivars, especially the Red Chilli and Long Red Cayenne, compared to the other cultivars, and to the differences in the genetic background of these cultivars.

No strict environmental control was exercised during the growing of the fruit and accordingly there is no certainty that the total pigment levels found in the present work are invariably characteristic of the cultivars examined. It needs to be established whether the large pigment differences determined indicate varietal differences only, or whether variations in environmental, soil and nutrient parameters contribute to the differences in pigment levels found.

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# Emulsifying properties of proteins and meat from broiler breast muscles as affected by their initial pH values

J. KIJOWSKI AND A. NIEWIAROWICZ

## Summary

Emulsifying capacity (EC) and emulsion stability (ES) in myofibrillar, sarcoplasmic, and a mixture of both protein fractions isolated from normal, PSE- and DFD-type broiler breast muscles were investigated. The EC and ES in sausage mixtures formulated from the three kinds of broiler meats mentioned above, as well as the influence of frozen storage on the EC and ES in meat, were also studied. Generally, myofibrillar proteins demonstrated considerably higher EC and ES than the sarcoplasmic ones. In the mixture of both proteins intermediate values of EC and ES were found.

Myofibrillar and sarcoplasmic proteins originating from normal broiler meat ($pH_{15}$ 6.2) showed better EC and ES than proteins from PSE- and DFD-type meat, were observed. EC in sausage mixtures was not affected by the initial pH no significant differences in EC and ES, as influenced by initial pH values of meat, were observed. EC in sausage mixture was not affected by the initial pH of broiler breast muscles, but the best ES was found in the mixture containing normal broiler meat. The mixture prepared from PSE-type meat showed intermediate ES values, and that containing DFD-type meat demonstrated the lowest ES.

Proteins extracted from sausage meats using 2% of sodium chloride revealed the highest EC in the samples produced from normal meat of $pH_{15}$ 6.5.

Frozen storage over 3 months decreased the EC and particularly the ES in broiler breast muscles.

## Introduction

Fat emulsifying and emulsion stabilizing capacities are the required technological properties of meat. Muscle proteins liberated in the course of mincing

Authors' address: Institute of Animal Products Technology, Agricultural Academy of Poznań, ul. Wojska Polskiego 31 60-624 Poznań, Poland.

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and salting (curing) procedures are recognized as natural fat emulsifiers in the sausage mixture.

Both salt and water soluble proteins are involved in stabilizing, however, the latter ones to a lesser extent. Myosin, actin, and actomyosin demonstrate the highest emulsifying capacity (Neelekantan & Froning, 1971), while connective tissue (stroma proteins) is one of the factors decreasing emulsifying properties of meat (Maurer & Baker, 1966).

The kind of meat and its ageing period, degree of protein solubility, pH, ionic strength, fat composition, and the processing procedures influence fat emulsifying and emulsion stabilizing capacities of meat emulsions.

Among hen, broiler, turkey and duck meat the highest emulsifying capacity was found in broiler meat (Hudspeth & May, 1967, 1969; McCready & Cuningham, 1971). The same authors and many others proved better emulsifying capacity in poultry dark meat than in light meat, in spite of the fact that in the latter the content of total and soluble nitrogen is higher.

Wismer-Pedersen (1968) and Merkel (1971) showed reduced fat emulsifying properties in PSE pork meat. No references have been found on the emulsifying properties of DFD pork and beef, and no information is available concerning this property in PSE- and DFD-type meat from broilers.

The occurrence of PSE- and DFD-type meat in broilers was reported by Trojan & Niewiarowicz (1971, 1973), but the assignment of certain broiler breast muscles to the DFD group is rather tentative. The method of identification of PSE-type normal, and DFD-type meat in broilers is based on pH measurement in the breast muscle 15 min after slaughter of the birds. The criteria estimated by these authors were as follows: PSE meat – $pH_{15}$ 5.7 or below; normal meat – $pH_{15}$ 5.9–6.2; and DFD-type meat – $pH_{15}$ 6.4 or above. The breast muscles of $pH_{15}$ 5.8 or 6.3 showed no definite features, and can be assigned to both proximate groups of meat. The thigh muscles exhibited always higher $pH_{15}$ values, ranging mostly from 6.4 to 6.6, irrespective of the initial pH in breast muscles.

The aim of this study was to evaluate fat emulsifying properties of the isolated proteins and of the sausage mixtures prepared from broiler breast muscles of PSE-type, normal, and DFD-type of meat.

## Materials and methods

Chicken broilers 8–9 weeks of age, processed in a commercial processing plant, were used in this study. Measurement of pH was performed 15 min after slaughter by inserting glass and calomel electrodes into the middle of the breast muscle, close to but not touching the sternum. The carcases were divided into three experimental groups of low $pH_{15}$ 5.7 (PSE-type meat); intermediate $pH_{15}$ 6.2 (normal meat); and high $pH_{15}$ 6.5 (DFD-type meat). The material was transported to the laboratory, stored at +4°C for 24 hr and the ultimate $pH_{24\,hr}$ was measured in the same way. The breast muscles were excised, comminuted,

and samples were taken for analyses, separately for each carcase. The other samples of excised breast muscles were wrapped in plastic film, frozen at −25°C, and stored at −18°C. After 1, 3 and 6 months of frozen storage the emulsifying capacity of meat and its emulsion stability in model system were determined.

Sarcoplasmic proteins were extracted with 0.05 M, myofibrillar proteins with 1.2 M, and the mixture of both protein fractions with 0.67 M solution of sodium chloride, and purified according to method of Trautman (1966). The amount of proteins in the solutions was determined by the biuret procedure.

Emulsifying capacity (EC) of muscles proteins was determined as follows: 10 mg of protein sample was filled up to 20 ml of volume with sodium chloride of 0.05, 0.67 or 1.2 M concentrations, depending on the kind of protein to be examined, and 5 ml of soya oil was added. The analysis was then conducted according to the method of Webb *et al.* (1970). Results were expressed in grams of oil per 100 mg of protein.

EC in meat and in sausage mixture was examined as reported by Webb *et al.* (1970), but instead of using 20 ml muscle tissue suspension, the 3 ml of meat or mixture suspensions were made up to a volume of 20 ml with 2% sodium chloride.

EC in a model system was determined in the same manner as that of protein fractions. The pH of the protein solutions was adjusted to the required value using 0.1 M hydrochloric acid or 0.1 M sodium hydroxide, before filling up to 20 ml volume. In this experiment broiler muscles of $pH_{15}$ 6.2 were used.

Emulsion stability (ES) was determined in a protein model system consisting of 135 mg of protein, 25 ml of 2% NaCl, and 70 ml of soya oil. ES of meat after frozen storage was investigated in model meat compositions obtained from 5 g of broiler breast muscles, 35 ml of 2% NaCl, and 40 g of soya oil. After homogenization the further analysis was carried out by a procedure combining the methods reported by Townsend *et al.* (1968), Inklar & Fortuin (1969), and Haq *et al.* (1973): 30 g of emulsion in a centrifuge test-tube (28 × 102 mm) was stoppered and heated in a water-bath at 70°C for 30 min when a temperature of 68.8°C was reached in the centre of the tube. Centrifugation of the emulsion at 700*g* for 15 min followed, the volume of drip, water and oil separately, was measured, and recalculated in millilitres of drip per 100 g of emulsion.

ES in the sausage mixture was determined by the method of Townsend *et al.* (1968), but the temperature of the heated emulsion sample was checked according to the procedure of Haq *et al.* (1973). The formulation of the mixture was as follows: 54% of breast broiler meat, 23% of pork jowl, and 23% of added water.

## Results and discussion

The myofibrillar proteins showed markedly higher emulsifying capacity than sarcoplasmic ones (Table 1). This is in accord with many of the research papers

Table 1. Emulsifying capacity of proteins from broiler breast muslces (g oil/100 mg protein)

| $pH_{15}$ of meat | Number of samples | Sarco-plasmic proteins | Myo-fibrillar proteins | Mixture of both proteins (1:1) |
|---|---|---|---|---|
| 5.7 | 12 | 277.8* | 425.3* | 370.7 |
| 6.2 | 12 | 293.7 | 441.2 | 377.2 |
| 6.5 | 12 | 281.2* | 427.0* | 371.0 |

*Differences significant ($P = 0.01$) within the columns in relation to the normal meat type ($pH_{15}$ 6.2).

reviewed by Saffle (1968) and by Cuningham & Froning (1972). In the mixture of myofibrillar and sarcoplasmic proteins, intermediate EC values were found.

EC of myofibrillar and sarcoplasmic proteins originating from the normal ($pH_{15}$ 6.2) breast muscles was higher than of PSE ($pH_{15}$ 5.7), and DFD ($pH_{15}$ 6.5) type muscles. These differences were statistically significant at $P = 0.01$. No significant differences were found in EC between the myofibrillar or sarcoplasmic proteins extracted from PSE- or DFD-type breast meat of broilers. However, in the mixture of both kinds of proteins no significant differences in EC, as influenced by initial pH value of meat, were observed, but that at $pH_{15}$ 6.2 showed the inclination to a higher EC.

Wismer-Pedersen (1968) and Merkel (1971) reported on the lower EC of the PSE pork muscle. No papers were found on EC neither of the isolated proteins nor directly of the tissue of farm animals and poultry demonstrating symptoms of DFD meat.

Emulsion stability (ES) was measured by the amount of water and oil released during heating of emulsion samples at a standard temperature. As it can be seen in Table 2, myofibrillar proteins showed markedly higher ES than sarcoplasmic ones, as was demonstrated earlier in various kinds of meat.

The higher ES of myofibrillar proteins was mainly due to their better oil-binding ability. The water-binding ability seemed to be better in sarcoplasmic proteins, except for samples originating from normal ($pH_{15}$ 6.2) broiler breast muscle.

Mixed proteins showed an intermediate ES, however, the experimental results are closer to the ES of myofibrillar proteins.

Stability of emulsion containing proteins isolated from normal muscles ($pH_{15}$ 6.2) was in all cases significantly ($P = 0.01$) better than emulsions of proteins from muscles of PSE and DFD-type groups. The highest ES of myofibrillar protein originating from normal breast muscles was mainly attributed to its oil-binding ability, while the ES of sarcoplasmic proteins, although lower than in myofibrillar ones, was due to equal oil and water-binding capacity.

ES in proteins from PSE-type broiler meat was lower than that of DFD-type meat proteins but in the case of myofibrillar proteins the data were statistically not significant.

Table 2. Emulsifying stability of emulsion from broiler breast muscles (ml drip/100 g emulsion)

| $pH_{15}$ of meat | Number of samples | Sarcoplasmic proteins | | | Myofibrillar proteins | | | Mixture of both proteins (1:1) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Water | Oil | Total | Water | Oil | Total | Water | Oil | Total |
| 5.7 | 10 | 9.5** | 69.0** | 78.5** | 24.8** | 0.8** | 25.6** | 24.1 | 13.5** | 37.6* |
| 6.2 | 10 | 25.2 | 26.9 | 52.1 | 19.8 | 0.2 | 20.0 | 25.4 | 6.1 | 31.5 |
| 6.5 | 10 | 5.2** | 67.5** | 72.7** | 23.8** | 0.4** | 24.2** | 24.5 | 7.2 | 31.7 |

Differences significant within the columns in relation to the normal meat type ($pH_{15}$ 6.2), $*P = 0.05$, $**P = 0.01$.

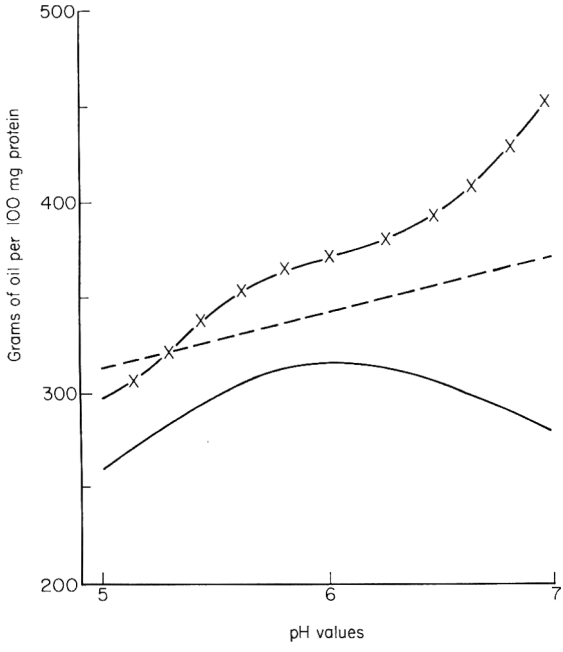


**Figure 1.** Emulsifying capacity of proteins as influenced by pH in model system. —— sarcoplasmic proteins, —x— myofibrillar proteins, – – – mixture of sarcoplasmic and myofibrillar proteins (1:1).

Emulsifying capacity of proteins as affected by pH of solution in a model system is illustrated in Fig. 1. In sarcoplasmic proteins pH 6.0 was the optimum value, and a pH below or above 6.0 decreased emulsifying capacity. In myofibrillar proteins pH 7.0 appeared to be the optimum in regard to their EC, while lower pH values decreased the EC. In the mixture of both protein fractions a linear relationship (positive correlation) between pH and EC was noticed. Generally, as in the above experiments, the myofibrillar proteins showed a higher EC than sarcoplasmic ones, at all pH values.

McCready & Cuningham (1971) reported that the amount of salt-soluble proteins extracted from poultry meat was approximately the same at normal pH (5.8–6.5) and pH 7.0, but lower at pH 5.0. Emulsifying capacity at pH 7.0 was significantly better than that at normal pH and pH 5.0, although the amount of salt-soluble protein was not always higher.

Emulsifying capacity of sausage mixture suspension (Table 3) was not affected by the initial $pH_{15}$ of breast muscles used as component; The EC of samples containing meat of a high $pH_{15}$ 6.5 was slightly lower but the difference was not significant.

Proteins extracted from sausage mixture (Table 3) with 2% of sodium chloride showed the best EC in the samples of emulsion produced from meat of $pH_{15}$ 6.2.

Tsai, Cassens & Briskey (1972) reported on investigation of potential emulsifying capacity in a model system, carried out in protein solutions of lower

**Table 3.** Emulsifying capacity of sausage mixture suspension and of the proteins extracted from the mixture

| $pH_{15}$ of meat | Number of samples | Mixture suspension (g oil/g mixture) | Proteins extracted from the mixture (g oil/ 100 mg protein) |
|---|---|---|---|
| 5.7 | 12 | 178.2 | 254.7 |
| 6.2 | 12 | 177.4 | 263.0 |
| 6.5 | 12 | 174.3 | 258.8 |

concentration than in commercial emulsions. It was found that EC in protein solutions of higher concentration was considerably lower, and thus differences between myofibrillar and sarcoplasmic proteins appeared negligible.

It may be supposed that after the curing process the sausage mixture used in our experiment, formulated on the basis of normal broiler breast muscles should reveal a better EC than the mixture prepared from PSE- and DFD-type meats.

However, emulsifying stability of sausage mixtures (Table 4) was considerably dependent on the initial $pH_{15}$ in broiler breast meat. The highest ES was proved in the samples of normal meat, intermediate ES in the PSE-type meat, and the lowest ES was found in the DFD-type meat. These differences were significant at $P = 0.01$. Unexpected and rather difficult to explain was the lowest ES of stuffing produced from DFD-type meat of high initial $pH_{15}$ 6.5.

It is generally considered (Saffle, 1968) that ES determination of commercial meat emulsion is of higher practical value than that of EC.

EC in frozen broiler breast meat (Table 5) decreased gradually during storage. This decrease was significant at $P = 0.05$ after 3 months, and at $P = 0.01$ after 6 months of storage. The normal meat ($pH_{15}$ 6.2) showed a constant tendency to have an EC higher than that of meat of $pH_{15}$ 5.7 and 6.5, but the differences were statistically not significant. This is in accord with earlier findings, that in the mixture of myofibrillar and sarcoplasmic proteins the influence of $pH_{15}$ on its EC is negligible.

**Table 4.** Emulsion stability of sausage mixture (ml drip/100 g emulsion)

| $pH_{15}$ of meat | Number of samples | Water | Fat | Total |
|---|---|---|---|---|
| 5.7 | 12 | 12.5* | 1.2* | 13.7* |
| 6.2 | 12 | 8.2 | 0.8 | 9.0 |
| 6.5 | 12 | 15.1* | 1.3* | 16.4* |

*Differences significant ($P = 0.01$) within columns in relation to the normal meat type ($pH_{15}$ 6.2).

Table 5. Emulsifying capacity of broiler breast meat after frozen storage at −18°C

| $pH_{15}$ of meat | Number of samples | Time of frozen storage (months) | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 3 | 6 |
| Proteins extracted from meat with 2% NaCl (g oil/100 mg protein) | | | | | |
| 5.7 | 18 | 256.5 | 246.2 | 243.4 | 181.8** |
| 6.2 | 18 | 259.8 | 252.3 | 249.6 | 208.8** |
| 6.5 | 18 | 258.3 | 248.8 | 242.9* | 189.6** |
| Meat suspension in 2% NaCl solution (g oil/g tissue) | | | | | |
| 5.7 | 18 | 191.6 | 185.4 | 180.9* | 171.3** |
| 6.2 | 18 | 196.3 | 186.3 | 185.9* | 176.7** |
| 6.5 | 18 | 189.0 | 185.1 | 180.0 | 170.3** |

Differences significant within the raws as related to non-frozen meat samples, $*P = 0.05$, $**P = 0.01$.

ES in model emulsions prepared from frozen meat decreased slowly, and significantly for the third month of frozen storage (Table 6). Slight, but significantly ($P = 0.05$) better ES was found in model emulsions from normal meat than in emulsions from PSE- and DFD-type meat irrespective of the duration of frozen storage. Generally, it may be concluded that frozen storage longer than 3 months affects negatively the EC, and particularly the ES of broiler breast muscles.

Dhillon & Maurer (1975) observed a decline in EC of ground beef and of manually deboned broiler meat after 6 months of frozen storage at −25°C. However, mechanically deboned broiler and turkey meat showed no significant decrease of EC under the same conditions of frozen storage.

It should be pointed out that these broiler breast muscles were taken for the experiments 24 hr after the birds were slaughtered. At this time the ultimate pH was markedly lower than the $pH_{15}$ value used for selection and was almost

Table 6. Emulsion stability of model meat composition after frozen storage at −18°C (ml drip/100 g emulsion)

| $pH_{15}$ of meat | Number of samples | Time of frozen storage in months | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 3 | 6 |
| 5.7 | 18 | 30.4 | 31.6 | 31.8* | 32.9** |
| 6.2 | 18 | 29.0 | 31.1 | 31.5* | 32.1** |
| 6.5 | 18 | 31.0 | 32.2 | 32.8** | 34.1** |

Differences significant within the raws as related to non-frozen meat samples, $*P = 0.05$, $**P = 0.01$.

the same for all samples. The corresponding $pH_{15}$ and ultimate pH values were as follows: 5.7, 5.5, 6.2, 5.6, and 6.5, 5.65.

This fact suggests that some changes in the meat properties, necessary for emulsion formation and stability, occurred shortly after the slaughter of the birds, and was influenced by the pH at that time but was not reversed by the subsequent equalization of pH values during storage.

The causes of the poor technological properties of PSE-type meat in pigs and in broilers are generally recognized, whereas those of impaired properties of DFD-type meat are not clear and need further investigations.

## Acknowledgment

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# Effect of initial pH in broiler breast muscles on gel forming capacity of meat proteins and on rheological characteristics of frankfurter-type sausage

J. KIJOWSKI AND A. NIEWIAROWICZ

## Summary

Gel forming capacity (GFC) of proteins extracted from broiler breast muscles of various initial pH values, as well as some technological and rheological characteristics of frankfurter-type sausages manufactured from normal, PSE- and DFD-type muscles of broilers were investigated.

Myofibrillar proteins showed generally a considerably higher GFC than sarcoplasmic proteins. Both proteins isolated from normal muscles ($pH_{15}$ 6.2) demonstrated significantly better GFC than proteins from PSE ($pH_{15}$ 5.7) and DFD ($pH_{15}$ 6.5) type meat.

GFC was affected by the pH of the solution in a model system and showed an optimum at pH 6.0 for all three protein fractions.

Dynamic viscosity was markedly higher for myofibrillar than for sarcoplasmic proteins. No differences in viscosity were found between myofibrillar and sarcoplasmic proteins as influenced by $pH_{15}$ in the muscles. Myofibrillar proteins produced a considerably more rigid gel than sarcoplasmic ones, as measured by its linear strain. Protein gels from normal muscles exhibited stronger consistency than those from PSE- and DFD-type muscles. Viscosity of sausage meat was higher in the samples originating from meat of $pH_{15}$ 6.2 in comparison with meat of lower and higher $pH_{15}$ values.

The lowest free water content was found in model sausages produced from normal meat, intermediate in these of PSE-type meat, and the highest one in the sausages obtained from DFD-type meat. Such rheological characteristics as linear, plastic, and elastic strains showed the best quality in sausage manufactured from normal meat. It may be concluded that the initial condition of

Authors' address: Institute of Animal Products Technology, Agricultural Academy of Poznań, ul. Wojska Polskiego 31, 60-624 Poznań, Poland.

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broiler breast muscles affects markedly some technological properties of meat and the quality of the ready-to-eat product.

## Introduction

Gel forming capacity is equal in importance to the emulsifying ability of sausage meat influencing the yield and rheological properties of ready-to-eat meat products. Gel formation of adequate rheological characteristics is affected predominantly by the kind and properties of meat proteins and by conditions of thermal processing in the presence of sodium chloride.

Fukazawa, Hashimoto & Yasui (1961a, b), Trautman (1966), Samejima *et al.* (1969) and Grabowska & Sikorski (1976) reported that fibrillar proteins played the main role in gel formation. Fukazawa *et al.* (1961 a, b) proved that adequate quantities of native myosin A in fibrils were necessary to obtain good binding in sausage meat. The water soluble protein and the actin and tropomyosin had no immediate effect on the binding quality in sausage. Actin appeared to be useful in maintaining stability in the form of actomyosin, being bound with myosin A.

Trautman (1966) performed an experiment with the salt soluble protein fractions extracted from muscles of ham. He showed the influence of meat pH on the gel forming capacity tested by the determination of the least concentration endpoint (LCE) method. The most desirable post-rigor muscle pH varied from 5.8 to 6.2 in which the heat denatured salt soluble proteins produced a more rigid gel. Grabowska & Sikorski (1976) also reported on the influence of pH on yield during extraction and on gel forming properties of proteins obtained from fish meat.

Water soluble, sarcoplasmic proteins only influence to a small extent the forming ability of sausage because these proteins coagulate and precipitate when heated above 40°C.

Connective tissue proteins seem to play a negligible role, if any, in gel formation in mixtures of frankfurter-type sausages. Ten hours heating at 70°C did not dissolve the stroma proteins (Paul, Buchter & Wierenga, 1966), however gel was formed at temperatures up to 50°C, and above this temperature no significant changes in gel properties occurred (Sikorski & Grabowska, 1973). The stroma proteins may only strengthen and improve the gel structure formed by the other meat proteins (Samejima *et al.*, 1969).

Trautman (1966) reported that gel forming capacity in porcine meat proteins was affected by the glycolytic reaction in meat but he did not observe whether it was different in PSE-type and DFD-type meat.

In the available literature no papers have been found on gel forming capacity of poultry meat proteins, especially in broilers demonstrating PSE-type and DFD-type meat.

The aim of this study was to evaluate the gel forming capacity of proteins extracted from broiler breast muscles of various initial pH values. Moreover,

some rheological characteristics of frankfurter-type sausages made from PSE, normal, and DFD-type muscles were also investigated.

## Material and methods

Carcases of commercial broilers 8–9 weeks old were taken and divided into three experimental groups by measuring the pH in the breast muscles 15 min after slaughter, according to the technique reported by Trojan & Niewiarowicz (1971). By this procedure the required material such as: PSE-type meat – $pH_{15}$ 5.7; normal meat – $pH_{15}$ 6.2, and DFD-type meat – $pH_{15}$ 6.5 was selected for experiments.

Carcases were transported to the laboratory, stored at +4°C for 24 hr, and ultimate pH of meat was measured. Then the breast muscles were excised, and samples taken for analyses, and for model sausage meat and for frankfurter-type sausage production.

Sarcoplasmic, myofibrillar, and the mixture of both protein fractions were extracted by sodium chloride solutions, and purified according to Trautman's (1966) technique. The content of proteins in the solutions was determined by the biuret procedure.

Gel forming capacity was tested by the determination of the least concentration endpoint (LCE) using a slightly modified Trautman (1966) technique. The modification consisted of changes of temperature, 70°C instead of 80°C; time of heating: 15 min instead of 10 min; the cooling time of the sample was shortened to 15 min, instead of 1 hr.

Protein samples for determination of gel forming capacity in a model system were prepared as follows. The pH of the protein solution was adjusted to the required value using 0.1 M HCl or 0.1 M NaOH. For these experiments the broiler breast muscles of $pH_{15}$ 6.2 were used.

Dynamic viscosity of protein fractions in sodium chloride solution was tested on the Rheo-Viskometer of Höppler (Prüfgeräte-Werk Medingen, Freital, GDR). The results were calculated according to formula: $\eta = P.t.K_0 (N \times \text{sec}/m^2)$; $P$ = weight of the glass bead ($N/m^2$); $t$ = time (sec) of bead movement through the cylinder 30 mm length; $K_0$ = coefficient depending on the type of measuring cylinder, in this case 0.01.

Consistency of the protein gel was characterized by measurement its linear strain (tensile strength). Glass cells (25 × 30 mm) were filled up with protein solution of 20 mg/ml protein concentration, heated at 70°C for 30 min and chilled afterwards to form a gel. Linear strain was measured on Höppler-Konsistometer (P.W.M., Freital, GDR). The depth in millimeters of mandrel immersion into gel sample was read after 30 sec, using loads in the range of $0.2–1.0 \times 10^4$ N m$^{-2}$.

The formulation of sausage meat for experimental production of frankfurter-type sausages was as follows: 54% broiler breast meat, 23% pork jowl, 23% added water, and curing salts.

The viscosity of sausage meat was determined on the Rotationviskosimeter-Rheotest 2 (P.W.M., Freital, GDR).

Free water content in sausages was determined according to the procedure reported by Uchman *et al.* (1975). Fifty grams of sausage sample were placed in a perforated metal cylinder and kept under pressure of $10^5$ N m$^{-2}$ for 1 hr. The amount of free water was calculated from the weight difference before and after pressing the samples.

The rheological properties such as linear (unit) strain, plastic strain and elastic strain of the model sausages were measured using a Höppler-Konsistometer:

Linear strain – depth of mandrel immersion in millimetres under load of $7.5 \times 10^4$ N m$^{-2}$ after 30 sec,

Plastic strain – immersion of mandrel in mm after 15 sec up to the moment when the load was taken off, in the same sausage sample,

Elastic strain – was calculated in mm from the difference between linear and plastic strain values.

## Results and discussion

Experimental data on gel forming capacity (GFC), measured by least concentration endpoint (LCE) in three protein fractions are shown in Table 1. Generally, sarcoplasmic proteins exhibited low GFC, resulting probably from the partial thermal coagulation. GFC of sarcoplasmic proteins was about three times lower than GFC of myofibrillar proteins, which is in accord with the investigation of Fukazawa *et al.* (1961), Trautman (1966), Samejima *et al.* (1969), Grabowska & Sikorski (1976), and some others. In the mixture of both protein fractions in the ratio of 1:1 an intermediate GFC was noticed being closer to GFC of sarcoplasmic proteins.

The best GFC proved to be in the protein fractions obtained from normal muscles ($pH_{15}$ 6.2), as compared with protein samples from the PSE meat ($pH_{15}$ 5.7), and DFD-type meat ($pH_{15}$ 6.5). The differences were statistically significant at $P = 0.01$. The differences in GFC between PSE- and DFD-type meats were significant for sarcoplasmic and the mixed proteins, but not for myofibrillar proteins.

Table 1. Least concentration endpoint (LCE) in per cent of protein

| $pH_{15}$ of meat | Number of samples | Sarcoplasmic proteins | Myofibrillar proteins | Mixture of both proteins (1:1) |
|---|---|---|---|---|
| 5.7 | 12 | 1.8* | 0.6* | 1.3* |
| 6.2 | 12 | 1.3 | 0.3 | 0.7 |
| 6.5 | 12 | 1.6* | 0.6* | 1.1* |

*Differences significant ($P = 0.01$) within the columns in relation to the normal meat type ($pH_{15}$ 6.2).

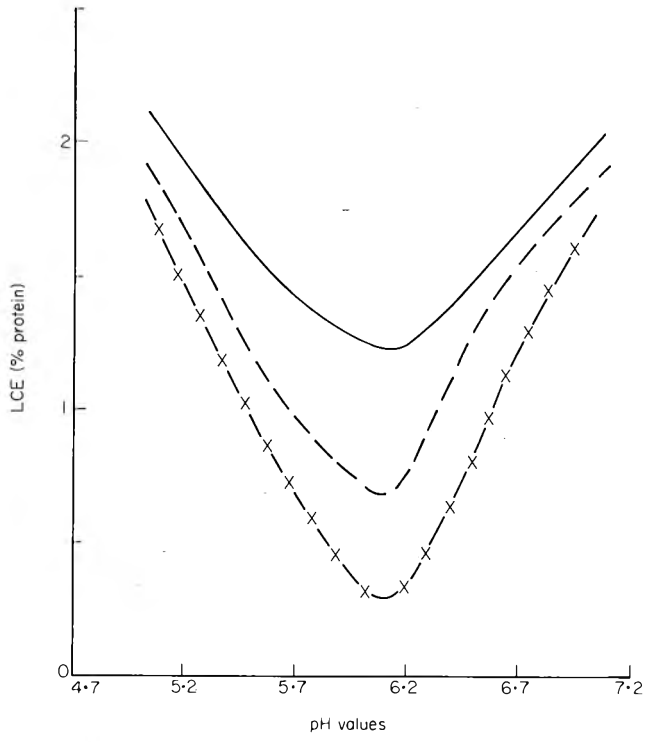


**Figure 1.** Gel forming capacity of proteins as influenced by the pH in model system. —— Sarcoplasmic proteins, ——x—— myofibrillar proteins, – – – mixture of sarcoplasmic and myofibrillar proteins (1:1).

GFC was affected by the pH of the solution in a model system (Fig. 1) and pH 6.0 was the optimal value, securing the best GFC in all three kinds of investigated protein fractions. However, this finding did not clarify the influence of $pH_{15}$ on GFC of proteins obtained from normal, PSE- and DFD-type of broiler breast muscles. Moreover, it should be remembered that the GFC determination within this study was carried out in muscles taken for analyses or/and to sausage production 24 hr after slaughter of birds. At this time the ultimate pH of muscles was 5.5, 5.6, and 5.65 for PSE-type, normal, and DFD-type meats, respectively.

Trautman (1966) reported on a definite correlation between post-rigor muscle pH and the LCE of extracted salt soluble proteins. The most desirable post-rigor muscle pH in ham was 5.8 to 6.1, but no mechanism was proposed to explain the effect of pH on LCE of the salt soluble protein. Grabowska & Sikorski (1976) found the best GFC in myofibrillar proteins isolated from Baltic cod fish in the range of pH 5.5–6.0, while at pH 5.0 GFC was considerably and at pH 7.0 slightly worse only. A shift of the pH in the minced cod flesh from the range of the isoelectric point resulted in an increase of GFC.

Dynamic viscosity (Table 2) in myofibrillar proteins was approximately four times higher than in sarcoplasmic ones. The mixture of two proteins showed

**Table 2.** Dynamic viscosity of protein in sodium chloride solution in $10^{-3}$ ($N \times sec/m^2$)

| $pH_{15}$ of meat | Number of samples | Sarcoplasmic proteins | Myofibrillar proteins | Mixture of both proteins (1:1) |
|---|---|---|---|---|
| 5.7 | 12 | 4.4 | 16.5 | 6.9 |
| 6.2 | 12 | 4.3 | 16.6 | 6.8 |
| 6.5 | 12 | 4.4 | 16.1 | 6.8 |

**Table 3.** Linear strain of protein gels (mm)

| $pH_{15}$ of meat | Mandrel load ($10^4$ N $m^{-2}$) | Sarcoplasmic proteins | Myofibrillar proteins | Mixture of both proteins (1:1) |
|---|---|---|---|---|
| 5.7 | 0.2 | 4.6 | 0.1 | 1.6 |
| | 0.4 | 7.6 | 0.3 | 4.1 |
| | 0.5 | 12.0 | 3.0 | 5.8 |
| | 0.7 | – | 5.9 | 9.2 |
| | 0.9 | – | 9.2 | – |
| | 1.0 | – | – | – |
| 6.2 | 0.2 | 4.4 | 0.03 | 1.3 |
| | 0.4 | 7.4 | 0.2 | 4.1 |
| | 0.5 | 12.0 | 2.2 | 5.1 |
| | 0.7 | – | 5.4 | 9.0 |
| | 0.9 | – | 8.2 | – |
| | 1.0 | – | – | – |
| 6.5 | 0.2 | 4.7 | 0.1 | 1.9 |
| | 0.4 | 7.8 | 0.3 | 4.1 |
| | 0.5 | 12.0 | 2.1 | 5.6 |
| | 0.7 | – | 6.3 | 9.2 |
| | 0.9 | – | 9.2 | – |
| | 1.0 | – | – | – |

**Table 4.** Viscosity of emulsion, and free water in model sausages

| $pH_{15}$ of meat | Number of samples | Viscosity ($N \times sec/m^2$) | Free water content (%) |
|---|---|---|---|
| 5.7 | 12 | 5.2* | 10.1* |
| 6.2 | 12 | 6.0 | 6.5 |
| 6.5 | 12 | 5.4* | 14.7* |

*Differences significant ($P = 0.01$) within the columns in relation to the normal meat type ($pH_{15}$ 6.2).

intermediate viscosity values. No significant differences were found in relation to $pH_{15}$ of muscles from which the proteins were extracted.

The consistency of protein gels, measured by linear strain is presented in Table 3. Myofibrillar proteins produced a considerably more rigid gel than sarcoplasmic ones, at the same mandrel load, while the mixture of both proteins showed an intermediate value of linear strain. Breakage of gel structures was observed at mandrel loads of $0.7 \times 10^4$ N m$^{-2}$ in sarcoplasmic proteins, at $0.9 \times 10^4$ N m$^{-2}$ in the mixture, and at $1 \times 10^4$ N m$^{-2}$ in myofibrillar proteins. Gels from the muscles after normal *post-mortem* glycolysis demonstrated stronger consistency than these obtained from PSE- and DFD-type muscles. Differences were significant for $P = 0.01$ when calculated for mandrel load $0.4 \times 10^4$ N m$^{-2}$. No significant differences in linear strain were found between the samples originating from PSE- and DFD-type meats.

The viscosity of sausage meat (Table 4) was higher ($P = 0.01$) in the samples prepared from normal broiler breast muscles of $pH_{15}$ 6.2 in comparison with muscles of $pH_{15}$ 5.7 and 6.5. The lowest quantity of free water (Table 4) was found in model sausage manufactured from normal meat, higher in that of PSE-type meat, and highest in sausage made from DFD-type meat. The differences were significant at $P = 0.01$. The ease with which water was released in sausage obtained from DFD-type meat of high initial $pH_{15}$ value was rather surprising. However, the sensory evaluation and to some extent the measurement of rheological properties (Table 5) confirmed the lowest quality in such model sausage.

Data concerning the rheological properties of model frankfurter-type sausages are presented in Table 5. All values of linear, plastic, and elastic strain indicated the best quality in sausage manufactured from normal meat, followed by PSE-type meat, and by DFD-type meat which showed extremely poor rheological characteristics. Differences were significant at $P = 0.01$. The observed sequence of decreasing rheological properties was confirmed by the sensory evaluation of experimental sausages.

The evident effect of various initial pH values of broiler breast muscles on the quality of frankfurter-type sausages could be attributed not only to variation in GFC in the normal, PSE- and DFD-type meat, but also to the other

**Table 5.** Rheological properties of model sausages

| $pH_{15}$ of meat | Number of samples | Linear strain (mm) | Plastic strain (mm) | Elastic strain (mm) |
|---|---|---|---|---|
| 5.7 | 12 | 7.6** | 5.9** | 1.7* |
| 6.2 | 12 | 5.7 | 3.2 | 2.5 |
| 6.5 | 12 | 10.8** | 9.5** | 1.3** |

Differences significant within the columns in relation to the normal meat type ($pH_{15}$ 6.2); *$P = 0.05$, **$P = 0.01$.

technological properties of such types of meat. Kijowski & Niewiarowicz (1978) reported on lower emulsifying stability in sausage mixture prepared from PSE- and from DFD-type meat. Trojan & Niewiarowicz (1973) showed lower water holding capacity of PSE- and DFD-type broiler breast meat.

The mechanism involving the inferior technological properties of PSE meat seems to be well recognized in regard to pork and broilers. However, the causes of decreased technological value of meat with extremely high initial pH values need further investigation.

## Acknowledgment

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# Factors influencing the adhesion-cohesion forces between butter layers

J. P. BRADY, J. BUCKLEY AND J. FOLEY

## Summary

Fracturing of bulk-packed butter was associated with poor hesion of butter layers. Hesion of butter to butter surfaces improved with increase in applied load and with rise in temperature up to about 18°C, while moisture condensation on surfaces and partial setting before bulk packing impeded it. Pumping of butter through a length of pipe increased the free oil index and lowered hesion values of the surfaces which had sheared against the pipe wall. Methods of overcoming problems of fracturing in commercial circumstances due to the inability of layers of butter to coalesce are outlined.

## Introduction

The occurrence of a defect described by butter graders as 'brittleness' or 'fracturing' coincided with the introduction into Ireland of continuous buttermaking machines. The defect was clearly associated with either the manufacture or packing of continuously made butter. It was more serious in some factories than in others and was not confined to any particular continuous buttermaking system.

When grading, fracture lines were apparent in plugs of butter withdrawn from bulk packs with a trier. A sideways thrust of a fully inserted trier, by the grader, caused fracture lines to appear along the surfaces of a defective block. In extreme cases 25 kg blocks of butter tended to fall apart when emptied from their cartons and during transport on conveyor lines to blending and printing machines. Bulk packs showing pronounced fracturing were rejected for export by graders.

This study was undertaken to establish the nature of the defect, to identify and quantify as far as possible the influence of the factors involved, and to

Authors' address: Department of Dairy and Food Technology, University College, Cork, Ireland.

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determine the means to overcome the problem in commercial butter manufacture.

## Methods and materials

### *Hesion of butter surfaces*

An Instron Universal Testing Machine, fitted with specially designed Perspex clamps was used to measure the force required to detach 9 $cm^2$ surfaces of butter from each other. The temperature used during testing was 10°C and the crosshead speed 10 cm/sec. Rectilinear samples of butter (3 × 3 × 3.5 cm) were prepared by cutting with a piano wire to obtain smooth flat surfaces. The freshly cut surfaces (3 × 3 cm) were placed in contact with each other for 1 min and a load applied for some experiments.

When testing the samples were held by the clamps. Details of the clamp are shown in Fig. 1. Each clamp wall (w) had a sliding movement on the base (b) and could be secured in position by the nut (n). The walls, which were of scored Perspex, were fitted with screw projections (s) which protruded into the butter sample when the walls were moved into the clamping position to hold the sample for testing. One clamp was secured on the crosshead of the Instron by screwing and the other was linked to the tension cell. The vertical pull required to detach the butter surfaces from each other was sensed by the tension cell, graphed on a chart and recorded as 'hesion' values in Newtons/$m^2$ ($N\,m^{-2}$). The term 'hesion' was coined by Claassens (1959) to describe the combined forces of adhesion and cohesion.

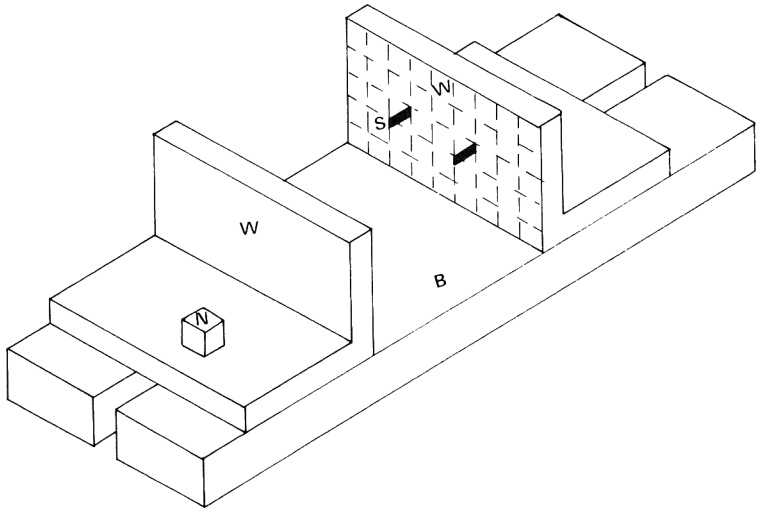


**Figure 1.** Details of the clamp used to hold portions of butter for testing in tension.

*Free oil index*

The method used was essentially that of Schultz, Knoop & Voss (1960). An aluminium foil sheet (0.003 mm in thickness), with a circular hole, was placed on each print of butter. Whatman No. 1 chromatogram paper stained with an alcoholic solution of Sudan 3 dye was placed on the aluminium foil. The paper was overlaid with a glass plate (165 g) and a gentle pressure carefully to ensure good contact between the butter and test paper. Each sample was then held at 12°C for 3 days. The discoloured area of the test paper, due to diffusion of free oil, was measured and after correcting for the area of the punched hole, recorded as 'free oil index'.

## Results

A preliminary examination of defective packs from different factories at a public cold store identified the nature of the defect as one associated with hesion of butter layers rather than one of classical brittleness. It was possible to separate and to trace the contour of folded layers in 25 kg blocks of butter (Fig. 2). The fracture lines of plugs withdrawn with a butter trier correspond with the different layer interfaces within a block. Apparently the smooth surfaces of the butter ribbon extruded from the filling head of the continuous buttermaker had not coalesced to any significant extent during packing.

**Figure 2.** Photograph showing layering of butter emptied from a 25 kg carton which was filled from a pipe of a continuous buttermaking machine.

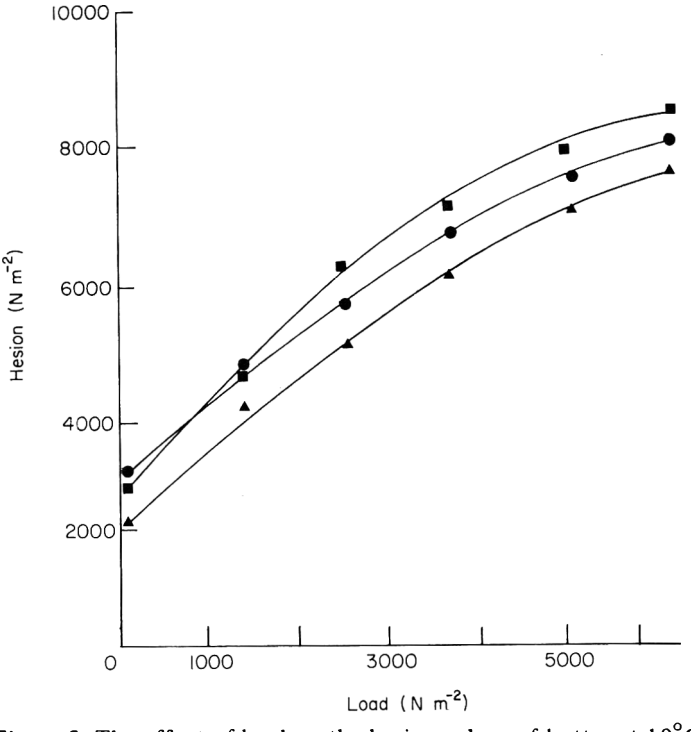


**Figure 3.** The effect of load on the hesion values of butter at 10°C.

Figure 3 shows that the hesion values at 10°C increased when the load applied during contact of the surfaces was increased from 0 to 6250 N $m^{-2}$. When the time of contact was increased by 15 sec intervals up to 90 sec the hesion values at 10°C for the different load levels used were only marginally increased. Increasing the time of contact at 15°C gave a definite increase in readings. Above 15°C the hesion values dropped and the samples tended to break instead of the test portions detaching from each other, thus making further measurements by this method impossible. The test no longer reflected the hesion values at the interfaces but rather the cohesive forces of butter itself which had begun to increase with rise in temperature. Claassens (1959) studied the hesion of butter to metal, wood and some other solid materials using a Westphal balance and regarded the weight of mercury to detach the test material from the butter as a measure of hesion. He found that hesion values increased with nominal load values at temperatures up to 12°C.

The hesion value (N $m^{-2}$) of freshly worked butters was considerably greater (1700 s.d. ± 60) than for those which had been allowed to set for 6 hr (600 s.d. ± 50) before bringing the surfaces into contact. Deposition of a moisture layer by aerosol on the freshly worked butter surfaces also reduced very considerably the hesion values (500 s.d. ± 50), i.e. the ability of the surfaces to stick together.

The influence of the shearing action against a metal surface when butter was pumped through a stainless steel pipe 2.5 m long and 100 mm in diameter on

**Table 1.** Free oil index and hesion values of butter pumped through a pipe: (a) samples from the metal sheared surface, (b) samples from the interior

| | Mean values for four trials | | | |
|---|---|---|---|---|
| | (a) | | (b) | |
| Free oil index after 3 days ($cm^2$) | 3.08 | s.d. ± 0.11 | 2.29 | s.d. ± 0.11 |
| Hesion values ($N\,m^{-2}$) | 2400 | s.d. ± 70 | 3000 | s.d. ± 70 |

**Table 2.** The influence of different packing methods on the susceptibility of butter to fracturing at 10°C

| Method of packing | Extent of fracturing after holding at 2 and −9°C | |
|---|---|---|
| | 3 days at 2°C | 10 days at −9°C |
| Packed from the buttermaking machine | None | None |
| Packed from the bulk packer | Pronounced | Pronounced |
| Bulk packed and pounded with mallet | Very slight | Slight |

the free oil index and hesion values is given in Table 1. When determining the hesion values, the butter surfaces which had sheared against the pipe wall were placed face to face and a load of 1200 $N\,m^{-2}$ applied. The values obtained were compared with sample surfaces prepared by cutting with a light wire but otherwise similarly treated. The free oil index of the surfaces which had sheared along the pipe wall increased and the hesion values decreased.

The influence of different packing procedures on the susceptibility of set butter to fracturing at 10°C is shown in Table 2. Butter packed into cartons directly from a continuous buttermaking machine did not fracture. When delivered through a 2.5 m length of 100 mm diameter stainless piping into the hopper of a machine and bulk packed into 25 kg cartons, the blocks of butter fractured when probed with a trier. The defect was barely noticeable in butter packed directly from the pipe before the bulk packer. Pounding with a butter mallet after packing considerably reduced the degree of fracturing.

## Discussion

The study clearly shows that the defect called fracturing by graders was related to the failure of adjacent layers of butter to coalesce during packing into 25 kg cartons. The defect, which was hardly detectable immediately after packing, became more noticeable when butter had set for 24 hr.

Increase in temperature and applied pressure improved hesion between butter layers (Fig. 3). Ripened cream butter, which comes from the butter-making machine at a higher temperature than sweet cream butter, did not fracture. The problem of fracturing at one factory was overcome by cutting off the chilled water supply to the working section of the machine and increasing the counter hydraulic pressure during packing. Pounding with a butter mallet after filling into cartons also reduced the tendency of butter to fracture.

A detailed study at a factory where the problem was acute, indicated that factors such as partial setting, moisture condensation on butter surfaces and pumping through a length of piping were relevant. At this factory, butter was delivered from the buttermaking machine through a length of stainless steel piping into the hopper of a bulk packer for filling into 25 kg cartons. When packed directly from the continuous buttermaker the blocks did not fracture. Fracturing was only very slight in blocks filled from the pipe feeding the packer while butter packed from the bulk packer fractured. Furthermore, it was noticed that defective blocks in this case tended to develop symmetrical longitudinal and transverse fracture lines which divided each block into four portions. The vertical fracture was caused by a vertical partition in the hopper of the packer and the horizontal fracture by a staybar at the entrance to the block-forming compartment which supported the augers.

When the residence time in the packer was increased the fracturing defect was intensified. Apparently the ability of butter surfaces to coalesce diminished when there was a time lag between churning and bulk packing. Freshly made butter sets rapidly after manufacture as fat crystals grow and interlock, forming a more rigid structure (Foley, 1969). When butter is allowed to set the ability of surfaces to coalesce is diminished. Furthermore, when butter is held in the hopper of a packer in a creamery environment where both the ambient air temperature and humidity are often high, water vapour condenses on the relatively cold butter surfaces. The presence of a layer of moisture acts as a barrier to cohesion between layers of butter. This effect is more pronounced after butter is held at sub-freezing temperatures. The expansion, associated with crystallization of water as ice below 0°C, appears to have a wedging effect between layers.

The problem of fracturing of butter from the bulk packer was overcome by modifying the design such that it extruded a freshly worked butter surface. The modification involved fitting a stainless steel partition, having a 100 mm diameter hole at its geometric centre, at the entrance to the block-forming compartment of the packer. Extrusion through the perforation in the partition created freshly worked surfaces which coalesced during packing.

The results in Table 1 show that the free oil index of butter pumped through a pipe increased and confirm the observations relating to cohesion of surfaces made by De Man & Poulsen (1970). They associated layering in Danish butter with pumping butter through pipes, and considered that a film of liquid fat on the surface prohibited interlocking of the surfaces.

## Acknowledgment

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# Technical note: Microbial load in blood meals*

G. P. PATGIRI AND A. K. ARORA†

In India the losses incurred due to improper utilization of slaughterhouse by-products are of the order of Rs30 million annually (Moorjani, 1971). Absence of facilities in slaughterhouses, scattered slaughtering, general ignorance and lack of interest among people have been responsible for the under-utilization of blood and other slaughterhouse by-products thus resulting in the loss of valuable proteins and other useful substances.

There are few microbiological studies on blood meal although several workers have determined its nutritive value and recommended blood meal as livestock and poultry feed supplement (Fratzer & Green, 1957; Ali & Momin, 1964). Recently Patgiri & Arora (1976) examined the microbial load and chemical quality of various slaughterhouse by-products including blood meal. Further, these workers have also determined the most probable number of coliforms and *Escherichia coli* in blood meal samples and reported characterization of *E. coli* organisms isolated from them (Patgiri & Arora, 1977).

Keeping in view the importance of blood meal in supplementing the proteinaceous foods, the present study was undertaken to determine the microbial load in blood meal samples prepared by two different methods.

## Materials and methods

The blood, collected from two to six goats, was pooled and weighed in the fresh state as well as after sun drying to determine percentage recovery of blood meal. The pooled blood was processed immediately after the collection as described by Patgiri & Arora (1977).

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† Correspondence should be addressed to Dr A. K. Arora at Pantnagar.

Authors' addresses: Ph.D. Scholar, Division of Bateriology & Mycology, Indian Veterinary Research Institute, Izatnagar, and College of Veterinary Sciences, G.B. Pant University of Agriculture & Technology, Pantnagar, District Nainital (UP), India.

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A total of fifty blood meal samples were prepared, of which twenty-five were prepared without adding CaO and were termed as 'Untreated' samples while the remaining twenty-five samples were prepared with the addition of CaO in the fresh blood at a ratio of 1:8 (1 part of CaO and 8 parts of blood) and were termed as 'Treated' samples.

*Microbiological studies*

The samples were examined after storing them from 7 to 15 days and providing sufficient aeration during the preparation of dilutions (Patgiri & Arora, 1977).

*Standard plate count (SPC)*

Standard plate count was done on nutrient agar using serial ten fold dilutions according to the method of Warmbrod & Linda (1966).

*Enumeration of staphylococci*

Staphylococcal count was done using a selective medium staphylococcus No. 110 (S 110) as described by Mildred, Edward & Marquitta (1966). Average number of colonies from two plates of the same dilution were recorded.

*Aerobic spore count*

Standard nutrient agar was used and the counting of spores was done according to the method of Gollakota & Halvorson (1960). A 10% suspension of the sample in sterile normal saline solution (NSS) was made. After thorough shaking the tubes were placed in a water bath at 80°C for 0.5 hr. A series of ten fold dilutions were made in sterile NSS. Pour-plate method was adapted and the plates incubated at 37°C for 48 hr and colonies counted in a colony counter.

For isolation of salmonellae tetrathionate broth was used as enrichment medium with the incorporation of brilliant green and iodine as suggested by Edwards & Ewing (1972). One gram of powdered blood meal sample was dissolved in 10 ml of tetrathionate broth and the tubes incubated at 37°C. A loopful of this material was streaked on brilliant green agar (BGA). Non lactose fermenting colonies (pink colonies) were not observed in any of the BGA plates, hence further tests were not carried out.

## Results and discussion

The amount of blood in an animal accounts for one-eleventh to one-fourteenth of its body weight (Mann, 1967). On average 1 kg of blood could be collected from each goat weighing approximately 20–25 kg. In the process of preparation of blood meal an average of 170 g (17%) dried meal was obtained from each goat slaughtered. The recovery percentage was in agreement with that of Vickery (1968). The method employed for the preparation of blood meal in the present study was simple, rapid and less expensive.

Viable microorganisms were found to be present in all the blood meal samples by SPC method. The SPC of the untreated samples ranged from $1 \times 10^5$ to $6.3 \times 10^7$ with an average of $1 \times 10^7$ organisms $g^{-1}$. Like viable count, staphylococcal and aerobic spore counts also ranged from 0 to $1.4 \times 10^3$ and 0 to $3.8 \times 10^4$ with averages of 83 and $3.4 \times 10^3$ $g^{-1}$ respectively. The corresponding values (and averages) of SPC, staphylococcal and aerobic spore counts $g^{-1}$ of the treated blood meal samples were: 100 to $2.6 \times 10^3$ ($8.1 \times 10^2$), 0 to 70 (8) and 0 to $4.2 \times 10^2$ (32) respectively (Fig. 1). Out of the total of twenty-five untreated samples examined, twelve (48%) were found to be positive for *Staphylococcus* and twenty-three (92%) for aerobic spores. Eight (32%) and fourteen (56%) of the twenty-five CaO treated samples resulted in the isolation of *Staphylococcus* and aerobic spores respectively (Table 1).

Calcium oxide treated blood meal samples showed significantly lower microbial count as compared to the untreated samples. Blood is known to contain high amount of phosphate. Addition of CaO will result in the formation of calcium phosphate which is known to be a good absorbant of

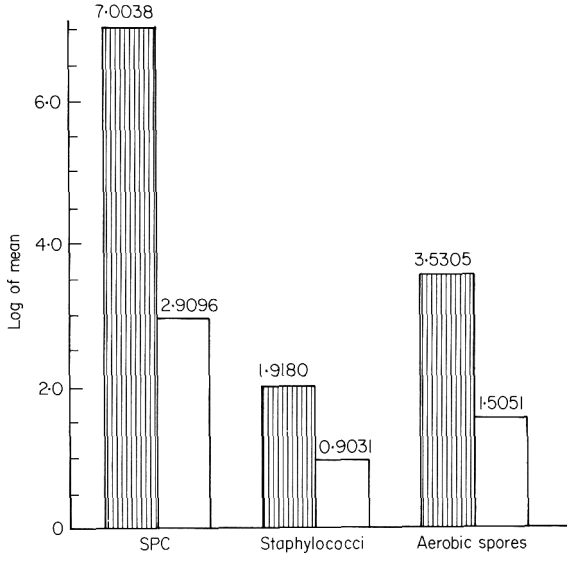


**Figure 1.** Microbial load in untreated (hatched) and CaO treated (open) blood meal samples (per gram).

**Table 1.** Recovery of microorganisms from blood meal samples (per gram)

| | SPC | | *Staphylococcus* | | Aerobic spores | |
|---|---|---|---|---|---|---|
| | CaO treated | Untreated | CaO treated | Untreated | CaO treated | Untreated |
| Maximum | $2.6 \times 10^3$ | $6.3 \times 10^7$ | 70 | $1.4 \times 10^3$ | $4.2 \times 10^2$ | $3.8 \times 10^4$ |
| Minimum | $1 \times 10^2$ | $1 \times 10^5$ | 0 | 0 | 0 | 0 |
| Mean | $8.1 \times 10^2$ | $1 \times 10^7$ | 8 | 83 | 32 | $3.4 \times 10^3$ |
| % positive | 100 | 100 | 32 | 48 | 56 | 92 |

several organic compounds. It is possible that CaO thus added removes or limits some of the nutrients which supports the growth of microorganisms in blood. Preliminary experiments have also indicated that CaO does inhibit growth of *E. coli* (Patgiri & Arora, 1977). Incorporation of CaO in fresh blood was also reported to improve net protein utilization and limit microbial growth (Mann, 1967; Abou, Abbady & Shanein, 1971).

Hobbs & Gilbert (1970) while summarizing the microbial standards of food products for human consumption suggested that a colony count of $1 \times 10^5$ organisms $g^{-1}$ is usually acceptable and $1 \times 10^6$ $g^{-1}$ or above is a sign of warning. Considering this, the load in the untreated samples can be accepted for livestock feed as the standards suggested by these workers were for human food stuffs. The low counts in the CaO treated blood meals were probably due to the inhibitory effect of CaO on the germinated, outgrowing and vegetative cells of the spore formers and other aerobes.

None of the samples examined resulted in the isolation of members of the genus *Salmonella*. This finding is significant from the human and animal health point of view.

Another contributory factor for the low microbial load could be that both CaO treated and untreated samples were prepared, handled, processed and stored under most hygienic conditions. Therefore, the possibility of post storage contamination was too little and whatever organisms recovered were probably because of contamination during the drying process.

It is concluded that CaO treated samples were of better quality and as such incorporation of CaO in the fresh blood can be safely recommended for commercial blood meal production.

## Acknowledgments

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# Book reviews

**Developments in Food Analysis Technique. 1.** Ed. by R. D. King.
London: Applied Science Publishers, 1978. Pp. 323. £25.

'The object of the book', according to the preface, 'is to examine a range of analytical techniques that have been used and are being used by analysts'. There is no indication, however, for whom this book has been written – the student or the expert? It is evident from the content that this has not been communicated to the various authors and the impression given is that the latter have merely been asked to write something on food analysis. Some authors have given concise accounts of recent developments in their fields whilst others have only given a sketchy and incomplete account which is barely adequate for students. In short, the content is like a curate's egg – good in parts only.

A lot of the content in the various chapters should have been deleted by the editor since it is often more adequately covered in other chapters. As is usual in a book of this kind, the style of the different chapters varies enormously; some authors are concise, some are very verbose and others show a lack of knowledge of simple English grammar.

Six of the chapters are concerned with instrumental techniques (including enzymic analysis) and four with the determination of groups of compounds. The chapters have been written by analysts from the Laboratory of the Government Chemist (two), Food Research Association, Leatherhead (two), teaching establishments (five), and an instrument manufacturer.

Chapter 1 ['Vitamin analysis' (42 pages, 194 references), Christie and Wiggins] is obviously written by experts in this field. It is a concise and critical account of recent developments in vitamin analysis and covers extraction, spectrophotometric, h.p.l.c. and g.l.c. methods.

Chapter 2 ['Nitrogen and protein' (32 pages, 185 references), Lakin] gives a very practical account of modern instruments, with full names and addresses of manufacturers, available for the rapid determination of nitrogen, e.g. block digestors and automated procedures, and for estimating protein, e.g. dye-binding and i.r. procedures. The author discusses the need to reconsider established nitrogen to protein conversion factors. The separation and estimation of individual proteins in foods are only briefly mentioned.

Chapter 3 ['Role played by water in food' (47 pages, 111 references), Hardman] has a section of about twelve pages wherein the food analyst interested in determining the equilibrium relative humidity of foods will find a mass of useful information. The author describes the large number of available commercial instruments (full addresses are given) and a careful reading should

help in a right purchase. Words of caution are given to those who expect too much from these instruments. The rest of the chapter deals with thermodynamic relations, dielectric dispersion and n.m.r. techniques, and is covered with a mass of complex equations more of interest to the researcher than the analyst.

Chapter 4 ['High pressure liquid chromatography' (29 pages, 59 references), Saxby] gives examples of the use of h.p.l.c. for the analysis of vitamins, flavour compounds, carbohydrates, lipids, vegetable tannins, fish amines, food additives, mycotoxins, pesticides, nitrosamines and polycyclic hydrocarbons. The examples given for carbohydrates do not strictly refer to food analysis; this is unfortunate since there are excellent examples in the literature of the use of this elegant technique for the determination of sugars in, e.g. chocolate, honey and milk products. It is also unfortunate that the only example given for the determination of benzoic acid and saccharin in beverages should be the original method of Nelson. To the reviewer's knowledge, most analysts prefer the more stable reverse phase system and examples of the use of the latter have appeared in the literature. The section on vitamins should have been omitted since this aspect is very thoroughly covered in Chapter 1. The inclusion of tea, coffee and cocoa alkaloids, and of tea theoflavins in the 'Flavour Compounds and Essential Oils' is somewhat odd.

Chapter 5 ['Gas chromatography' (23 pages, 74 references), Manning] is divided into sections with the following headings – Capillary columns, Headspace analysis, Identification techniques, Sulphur and phosphorous specific detectors, and Computer techniques. A great deal of space is taken up into giving lengthy procedures for preparing glass capillary columns. The author states, 'Preparing and coating capillary columns is still an art' and 'unfortunately, the performance obtained with the different column depends to a large extent on the experience of the person preparing the column'. The reviewer suspects that most food analysts have not the time to become experienced in this art and consider it more productive to purchase columns with guaranteed efficiencies. The only application of g.l.c. to food analysis given by the author is direct analysis of vapours above foodstuffs. His statement that 'headspace analysis is now the preferred method for obtaining quantitative information about the compounds that contribute to the flavour of foodstuffs' is, to put it mildly, rather sweeping. The section on identification techniques should have included at least two references to recent review articles which cover this subject very thoroughly. The author seems to underestimate the use of mass spectrometry as an identification tool; his reference to a 1969 review article as an indication of the state of the art of g.l.c.–m.s. interface is somewhat ludicrous. Aspects of computer techniques cover less than half a page. A reader who really wants to know the full potential of g.l.c. in food analysis is advised to refer to 'Gas Chromatography in Food Analysis' by Dickes and Nicholas (cf. review in *J. Fd Technol.* (1977) **12**, 318).

It is interesting to note that although the author does not refer to it, it *is* mentioned in another chapter.

Chapter 6 ['Enzymic methods' (16 pages, 84 references), Wiseman] gives an adequate review of the methods available for carbohydrates, organic acids and bases, lipids, amino acids, $NO_3^-$ and $PO_4^{3-}$. The author should have admitted that most food analysts make use of commercially available kits, e.g. by Boehringer, and the accompanying copious literature supplied free usually explains the reactions involved in some detail.

Chapter 7 ['Ion selective electrodes' (26 pages, 70 references), Comer] is written by a representative of a manufacturer of ion selective electrodes and this perhaps explains the omission of some references which critically assess their use compared with other analytical techniques and where they have been found to be inferior. Most of the methods are given in various suppliers' free literature.

Chapter 8 ['Automatic methods' (37 pages, 42 references), Stockwell] is divided into two parts. The first part is concerned with the 'philosophy and practice of automatic analysis' and the analyst might find some ammunition here to help him convince his superior that a certain black box is a must. The second part describes the various automatic systems (mostly purpose built) that are used at the Laboratory of the Government Chemist. They include systems for determining original gravity, alcohol and total sugar in beer, $SO_2$ and alcohol in beverages, Se in water, and for digestion of foodstuffs for trace metal analysis. It is interesting to note that nearly two pages are devoted to a system for the microbiological assay of unspecified vitamins by turbimetric measurement but only one sentence is given to it by the authors of the first chapter who are from the same establishment; an editorial cross reference would have been useful here.

Chapter 9 ['Determination of Carbohydrates' (31 pages, 123 references), Lee] has sections on clarification methods, polarimetry, different chromatographic methods, colorimetry and 'enzymatic' methods. In attempting to cover all aspects of the subject in the short space available, the author has presented a rather insipid product. He does, however, refer to recent reviews, and he does admit to the fact that 'enzyme systems ... are now readily available commercially ... and analytical procedures based on these are well established'.

Chapter 10 ['Atomic absorption spectroscopy' (22 pages, 21 references), Cowley] begins with a simple and clear description of the principle of the technique. However, the rest of the chapter which gives a few examples of the use of the technique to the determination of trace metals in foods is at a superficial level. One reference (1973) given is that of a publication by an instrument manufacturer; it is an excellent review of the subject and is, as far as the reviewer is aware, available free on request. Editorial cross references to other chapters, e.g. determination of Se in chapter 8, would again have been useful.

A Subject Index is provided at the end of the book; the reviewer noted a number of omissions, e.g. patulin, p. 147.

The '1' in the title would indicate that more volumes are to be expected but there is no indication when the next volume is to appear or of their contents.

It is difficult for the reviewer to give his recommendation as to the purchase

of this book but the information given in one or two of the chapters could be of immense benefit to an analyst entering the relevant fields. One feels that the publication of this type of book benefits only the publisher; although it will probably reach many libraries, it is unlikely that the information in it will reach the food analyst. It is unfortunate that some of the authors did not see fit to publish their contributions as review articles in analytical journals, which would have reached a wider audience.

*D. I. Rees*

**Diet of Man; Needs and Wants.** Ed. by John Yudkin.
London: Applied Science Publishers, 1978. Pp. ix + 358. £20.

This is a useful book to dip into since it provides information on a wide range of nutritional topics. It contains nineteen papers presented at a Symposium of the Rank Prize Funds together with discussion.

The titles indicate the subject areas covered; three papers deal with recommended intakes and how various diets relate to these standards; there are particularly useful sections relating diet to various diseases – useful because they review the field in a reasonable unbiased fashion, are written by authorities, and to ensure that they are balanced each is discussed and criticized by other participants. The diseases dealt with are diabetes, intestinal disease, obesity and heart disease. Possibly the heat was taken out of the argument on the last subject by the author's (Dr M. F. Oliver) opening remarks that he believes diet contributes to CHD mortality but is not the major component and that the adverse effects of fats are determined to a considerable extent by genetic factors. He reviews the recent epidemiology (CHD falling in the United States but reasons unknown) and discusses the risk factors including saturated fat, cholesterol, sucrose, fibre and hardness of water. In the discussion the design of the large scale experiments on dietary changes and their effect in reducing coronary disease were criticized.

There is a considerable section on social nutrition, including dietary changes in U.K., and the effects of culture, economics, marketing, physiology and anthropology. Finally food production is discussed in four papers.

Useful facts often come to light in discussions, so it can be of considerable value to record such discussions. For example there was once a potential vitamin termed the grass juice factor and one might well wonder what ever happened to it. In fact, it was verbally withdrawn at a meeting in answer to a question addressed to its originator – but that discussion was never recorded. In this book there is a correction by Dr N. Zöllner from Germany, that despite a recommendation that table salt be fortified in Germany this and all other fortification of food are strongly opposed by Consumer Organizations and so are not done – despite the fact that 1% of all hospital admissions of children have florid rickets. Such information does not appear to be generally available despite the harmonization that is under way in Europe.

An interesting slant on fat consumption was given by Lord Trenchard, in discussing the inter-relation between marketing and nutrition. Due to the trend over many years towards leaner meat carcases, the argument that fat consumption has gone up since the beginning of the century is not true – instead of an increase from 98 to 133 g/day between 1909 and 1974 he re-calculated this to be 128 and 133 g respectively. This is illustrated by the new Food Composition Tables which show less fat in the meat than in the older figures.

All in all a useful reference book.

*A.E. Bender*

**McCance and Widdowson's The Composition of Foods** (4th ed). By A. A. Paul and D. A. T. Southgate.
London: Her Majesty's Stationery Office, 1978. Pp. xii + 418. £12.

The long awaited fourth edition of this familiar book has now been published with an attractive jacket and clear print on quarto size pages. The presentation of the tables has been dramatically altered; the lay-out is logical and the composition, proximate analysis, inorganic constituents, major vitamins (retinol and carotene are presented separately) and minor B-vitamins/100 g for any one commodity are on adjacent pages. Cooked dishes and foods important to immigrants, e.g. chapatis with and without fat, have now been included within the category of the major ingredient. The dietary fibre content of cereals and cereal products based on direct analysis has been added in this edition; similarly, some fruit and vegetables have been analysed in the same manner but provided values identical to those obtained by the previous method for unavailable carbohydrate evaluation.

The section for amino acid composition is given separately, as before, but is considerably enlarged – even to including haggis! A new section covers fatty acid composition, and the final section retains phytic acid phosphorus levels and also incorporates cholesterol contents.

To interpret the tables to best advantage, the authors point out the need to study the general introduction (34 pages) to appreciate, for example, the conversion factors for energy calculation as the factor for fat has now been reduced from 9.3 to 9.0 kcal/g; the tabulated values for free folic acid appear authoritative but the accompanying text rightly draws attention to the difficulties involved when analysing its level in raw food material. The iodine and organic acid contents of foods depend on environmental conditions and are too variable to warrant tabulation but are discussed in the text.

The appendices provide cross-reference arrangements between the sections which are excellent because of the clear code number allocated to each commodity. The sources of data for new entries in the tables are readily detected by cross-reference of commodity number and literature reference number; bold type indicates new analyses carried out by the Laboratory of the Government Chemist or the Dunn Nutritional Laboratory. The recipes for the cooked

dishes for which analyses have been made are included in an appendix and are given in metric units.

This brief review cannot do justice to the quantity of work undertaken by the innumerable individuals and research teams throughout Britain who have assisted in the compilation of *The Composition of Foods*. This epic publication provides the cornerstone for everyone who has any kind of interest in food and will be especially appreciated by dietitians, food technologists, research and nutritional survey workers.

*K. Mary Clegg*

**Sensory Properties of Foods.** Ed by G. G. Birch, J. G. Brennan and K. J. Parker.
London: Applied Science Publishers, 1977. Pp. xi + 326. £16.00.

This book records the papers and discussion at a symposium of the above title held at the National College of Food Technology, Weybridge, U.K. from 18–20 April 1977. The purpose was to explore and discuss the whole range of sensory attributes of food, their measurement and their basis in food.

Appearance is covered by papers on the many different methods for colour measurement, the influence of optical properties, physical form and mode of presentation on consumer behaviour, and on its measurement and factors influencing it in fresh and cured meats (Francis, Hutchings & McDougal).

Taste and its modification is extensively discussed by Bartoshuk, who demonstrates the influences of shape of the psychophysical functions, methods of presentation and taste modifying substances. The effect of chemical structure on sweetness of amino acids and sugars and Kier's proposed extension of Shallenberger's AH-B theory are discussed by the latter and by Beets. Birch *et al.*, in studies of bitterness of sugar derivatives suggest that both bitter and sweet receptors may be stimulated by the same molecules by orientation to adjacent receptors in different ways. Taste attributes of amino acids, peptides and proteins are well reviewed by Higginbotham & Hough, who include interesting work on Thaumatin and speculation on its mode of action.

The importance of volatile components in flavour is reviewed by Nursten by chemical class and complexity of contributing substances. Langlois *et al.*, describe data on volatile composition and sensory differences in lemon oils and von Sydow & Akesson successfully use newer psychometric techniques to evolve predictive relationships between analytical data on certain volatile components in canned meats and those sensory attributes indicative of deterioration.

Harper puts the development of sensory analysis in the U.K. into perspective and Moskowitz describes the application of magnitude estimation to descriptive analysis, product development and preference, and psychological demand curves.

Texture measurement in a variety of foods is critically discussed by Brennan, and Jowett & Howgate describe texture profiles and factor analysis of fish

texture descriptors. The advantages and disadvantages of protein spinning for texturizing novel protein foods (Plaskett), and factors influencing texture in chocolate and other confectionary products (Beesley) are followed by an account by Sherman of his critical instrumental and sensory analysis of foods which flow.

This book is essential for those involved in sensory and quality research, and also for updating those involved in food technology and quality appraisal. It is well produced with few mistakes and a small but adequate index.

*D. G. Land*

## Books Received

**Yeasts for Food and Other Purposes**
**Food Technology Review No. 45.** By J. C. Johnson.
New Jersey: Noyes Data Corporation, 1977. Pp. xi + 344. US$39.

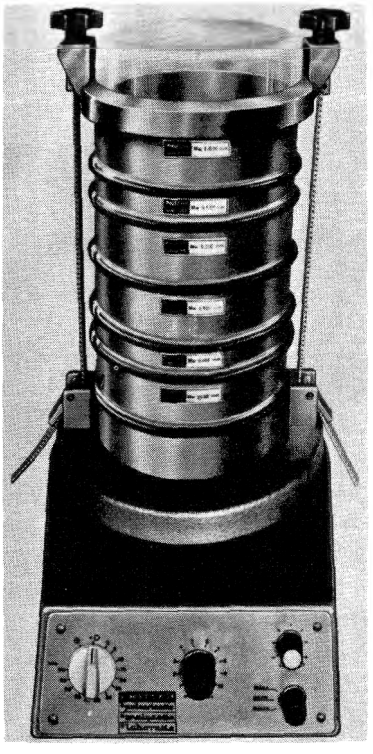
A survey of the American patent literature on the utilization of yeasts. The chapter headings relevant to the food technologist are: Growth of Yeast on Hydrocarbons and Other Carbon Sources, Treatment of Yeast Cells and Proteins, Yeast Modified Food Products, Yeasts in Bakery and Pasta Products, Yeast as Condiments and Flavour Enhancers.

**Human and Veterinary Nutrition. Biochemical Aspects of Nutrients.** Volume 30 of World Review of Nutrition and Dietetics. Ed. by G. H. Bourne.
Basle: S. Karger, 1978. Pp. x + 235. US$74.

A collection of short review articles on nutritional subjects.

**Meat, Poultry and Seafood Technology.** By R. L. Henrickson.
New Jersey: Prentice-Hall, 1978. Pp. ix + 276. £10.90

An elementary introduction intended for students of the catering trades.



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Applicants must have a Ph.D. in Food Science or a field related directly to Food Science, an established record of scholarly achievement and demonstrated administrative ability. The applicant must have an appreciation for the relationship between basic and applied Food Science and will be expected to work closely with the Food Industry.

The closing date for applications is 1 December 1978. They should include a curriculum vitae and the names and addresses of three persons who can be contacted for reference and should be mailed to:

Dr A. Gordon Ball, Chairman of Selection Committee, Dean's Office, O.A.C., University of Guelph, Ontario, Canada, N1G 2W1.

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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 | $\mu$m = $10^{-6}$ 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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