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Scope. Food technology is the application of scientific and technological knowledge to the production, storage, distribution, marketing and use of food and beverages from raw materials. The Journal covers a wide range of topics relevant to food, from raw material composition to consumer acceptance, from physical properties to food engineering practices, and from quality assurance and safety to storage, distribution, marketing and use. It covers basic food science and all relevant technological applications leading to the product on the table. The Journal provides a broadly based international forum for publication in English of original research and new applications in the form of research papers, concise but critical topical reviews, technical notes, and letters on material published in previous issues. All but letters are subject to multiple peer review. The Journal also publishes reviews of relevant books.

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Editorial

In the last issue of the Journal I indicated that a number of changes in the policy and objectives were in hand, and I outlined the main changes. In this issue the 'Objects of the Journal' have been replaced by a revised statement entitled 'SCOPE' which provides a brief but continuous reminder of the broader objectives of the Journal, for it will appear in each issue.

I also indicated that a revised 'Notice to Contributors' would be published in this issue. It is printed on the inside of the back cover and will continue to appear as 'Instructions to Authors'. The change of heading indicates a change of emphasis which should be noted by potential contributors, who should pay attention to the broad instructions as well as to the relatively minor details.

There are two main reasons for the changes. The first is spelt out in the new instructions—it is to improve the efficiency of communication, which is the primary purpose of publication of research. There are many secondary, and perhaps more important, purposes, such as improving one's reputation in order to increase available resources or to gain promotion, or even the old-fashioned satisfaction of sharing new data and understanding with one's peers, but all depend in the first place upon efficient communication. All skills incur time and effort to develop, and that of efficient reporting of research is no exception. Clear, concise exposition of a problem, identifying the critical questions and how to answer them, obtaining the data, testing hypotheses and drawing conclusions often represent many man-months of effort. The use of 20% of this time to distil the understanding you have gained into a concise and logical account, supported only by the necessary data, should not be resented, for what you communicate is your product, on which you will be judged. Furthermore, the greater the number of people who read your work, the greater will be its impact. It is important to realize that clear, concise papers are much more widely read and quoted than extensive, obscure and woolly ones.

The second reason for improving efficiency is simply that it takes less space. The cost of publishing is rising, whereas the resources of our main subscribers, libraries, are being held constant or declining in real terms worldwide. We also have increasing demands for space in the Journal. As a policy we do not want to use page charges, for that would produce unfair penalization on those with the least resources. If we increase the number of pages, the subscription must rise and we shall lose subscribers. Therefore, we have no choice but to make more efficient use of the space we have, or reject many more papers.

The new instructions are intended to encourage positively the submission of clear, concise contributions. The instructions will be increasingly implemented over the next two years, due allowance being made as appropriate. The effect will be to speed the processing of such papers at the expense of those that are not clear, concise and well prepared. The co-operation of contributors will be of benefit, both to themselves and to their readers.

Derek G. Land
Editor

Journal of Food Technology (1986) **21**, 535–546

Review: The direct epifluorescent filter technique

G. L. PETTIPHER

Summary

The direct epifluorescent filter technique (DEFT) for the rapid enumeration of microorganisms is described with particular reference to milk and foods. Recent development with regard to applications and automation, and the likely future of the method are discussed.

Introduction

The DEFT was originally developed for counting bacteria in raw milk at the National Institute for Research in Dairying, Reading (Pettipher *et al.*, 1980; Pettipher, 1983). It was developed at the request of the Dairy Industry which, through the Scientific Consultative Panel of the Joint Committee of the Dairy Trade Federation, expressed a need for a rapid method of estimating directly the number of bacteria in milks containing $< 1 \times 10^4 - > 10^6$ bacteria per millilitre. Such a test could be used for grading farm milks on the basis of hygienic quality and would permit better management of supplies at the creamery. The requirements of the 'ideal' new method were that it should be:

(1) Rapid. A result should be available in less than 30 min for determining the suitability of milk for processing.

(2) Sensitive. The test should be more sensitive than the 10 min Resazurin Test.

(3) Precise. The test results should correlate significantly with those obtained by other standard methods.

(4) Convenient. The method should be suitable for use by the laboratory staff of the processor or manufacturer.

(5) Economical. The test should be economical in terms of cost per sample. For a manual test at the creamery this means low labour and equipment costs because of the small number of samples per day (10–30). For centralized testing the high sample throughput permits a high equipment cost provided there is a low requirement for labour.

From the beginning a method using microscopy was envisaged as direct methods are generally more accurate than indirect methods and microscopy is one of the few rapid direct methods.

General principles

DEFT, which uses membrane filtration and epifluorescence microscopy, takes less than 30 min to complete and does not suffer from many of the disadvantages of other microscopic methods. Pretreatment of the milk with a proteolytic enzyme and surfactant at 50°C lyses somatic cells and makes fat globules sufficiently fluid to enable 2 ml of

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milk to be filtered routinely through a 25 mm diameter membrane filter. Filtration concentrates and distributes the bacteria in a manner that makes counting easier and the technique *circa* 100 times more sensitive than the Breed smear. The use of a fluorescent stain produces well stained micro-organisms that are easily distinguishable from the small amounts of fluorescent debris. A polycarbonate membrane filter is used, the flat surface of which is better suited to microscopy than the uneven surface of cellulose acetate membranes. Micro-organisms on DEFT slides can be counted automatically, thereby considerably reducing operator fatigue. DEFT can be used rapidly to enumerate micro-organisms in urines, beverages and, with additional sample pretreatment, a variety of foods in addition to milk.

Materials and methods

Apparatus and reagents

The main items of apparatus required for the DEFT include an epifluorescence microscope, filter manifold, filter towers, filters, vacuum pump, water bath, pipettes, containers for reagents, microscope slides and coverslips. The reagents needed for the DEFT include the enzyme trypsin, the surfactant Triton X-100, the fluorochrome acridine orange, citric acid — NaOH buffers, isopropanol and non-fluorescent immersion oil. Before use, all reagents, except immersion oil, are prefiltered through 0.22 μm pore size cellulose acetate membrane filters to remove bacteria and particulate matter, and are dispensed into sterile containers. A kit of concentrated DEFT reagents developed at the NIRD is available commercially (Difco Laboratories, East Molesey and Foss U.K. Ltd, York). The use of reagent kits and a reagent filtration and dispensing system developed by Foss U.K. Ltd makes the method considerably easier for the operator.

Method

Whole milk, enzyme and surfactant are incubated at 50°C for 10 min in a sterile capped test tube. A polycarbonate membrane filter (Nuclepore, 0.6 μm pore size) is mounted, shiny side uppermost, in a membrane filtration unit of a filter manifold connected to a vacuum line. The filter is warmed by filtration of surfactant at 50°C before the treated milk is filtered. Surfactant at 50°C is used to rinse the test tube and filter tower after filtration of the treated milk. After filtration of the treated milk and rinse, the membrane is overlaid with stain. After 2 min the vacuum is reapplied, and the membrane is rinsed with buffer followed by isopropanol. The stained membrane is air dried and then mounted in non-fluorescent immersion oil on a slide beneath a cover slip.

The mounted membrane filter is then examined by means of a epifluorescence microscope suitable for use with acridine orange. In the DEFT, because of the higher RNA content, metabolically active bacteria fluoresce orange whereas inactive bacteria fluoresce green (Fig. 1). Clumps of orange fluorescing bacteria are counted in microscope fields of view taken at random. Depending on the number of clumps per field, bacteria in two to fifteen fields are counted (Pettipher *et al.*, 1980). The DEFT count per millilitre is estimated from the mean clump count per field and the microscope factor (MF). The MF depends upon the amount of sample filtered through the given area of the membrane and the area of the microscope field. The MF is calculated as follows:

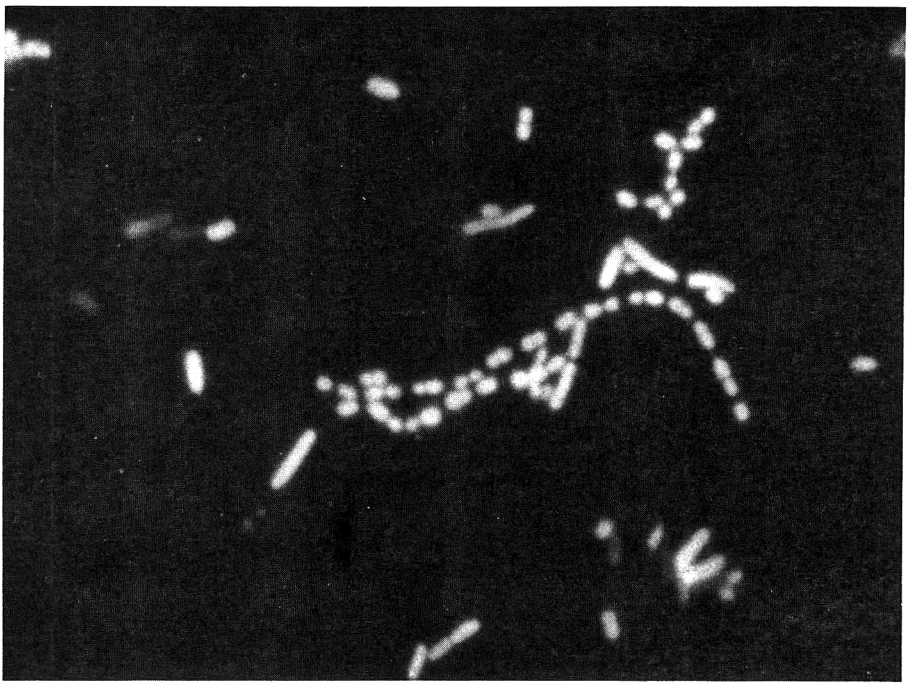

Figure 1. Bacterial cells as seen in the DEFT.

$$\text{MF} = \frac{\text{Area of membrane through which sample is filtered (mm}^2\text{)}}{\text{Microscope field area (mm}^2\text{)} \times \text{sample volume (ml)}}.$$

The MF can vary widely as it is influenced by sample volume, filter area, magnification of objective and eyepieces and type of objective used, e.g., standard or wide field. For a sample volume of 2 ml the MF will usually range from 2000 to 11000.

In addition to the count per millilitre of sample, a presumptive identification of the types of micro-organisms present on DEFT slides can be made by the operator. Generally the following types of bacteria are discernible by morphology: streptococci, micrococci, staphylococci and some bacilli and coryneforms. Fungi, yeasts and spores can also be distinguished. A tentative identification of the types of micro-organisms present in the sample may be useful in locating their source, e.g., a high bacterial count in refrigerated farm milk where the majority are streptococci is usually indicative of mastitis in the herd. A full description of the method and staining reactions can be found in a monograph of the DEFT (Pettipher, 1983).

Automation

The DEFT was developed as a manual technique designed to give rapid results on a small number of samples. However, it soon became apparent that, due to its rapidity, the usefulness of the method would be considerably increased if the sample throughput could be improved.

The DEFT could be fully automated relatively easily as the method uses low technology. It has been the cost of such a development, not practical problems, which

has prevented full automation of the technique. A three unit system involving sample pretreatment, filtration and staining, and counting has been envisaged (Pettipher, 1983). To date, the major developments have been to assist the operator in the use of the manual technique. The major progress towards automation has been made in the area of automated counting.

The manual DEFT count is only suitable for thirty to forty samples per day per operator as prolonged use of the microscope results in operator fatigue. Micro-organisms on DEFT slides can be counted automatically using the 40–10 image analyser (Analytical Measuring Systems Ltd, Shirehill, Saffron Walden, Essex, U.K.). A closed circuit TV camera (scanner) attached to the microscope camera port detects the microscopic image and the resulting video signal is analysed to obtain quantitative data about the image (Fig. 2). Using the image analyser, an operator can count *circa* fifty slides per hour. The operator has to load the slide, focus the microscope and move the microscope stage manually. The image analyser can be controlled by a micro-computer (not shown) which processes the generated data. The relevant information, e.g., sample code, count per millilitre and grade, is printed out and can be recorded simultaneously onto magnetic disc for storage purposes.

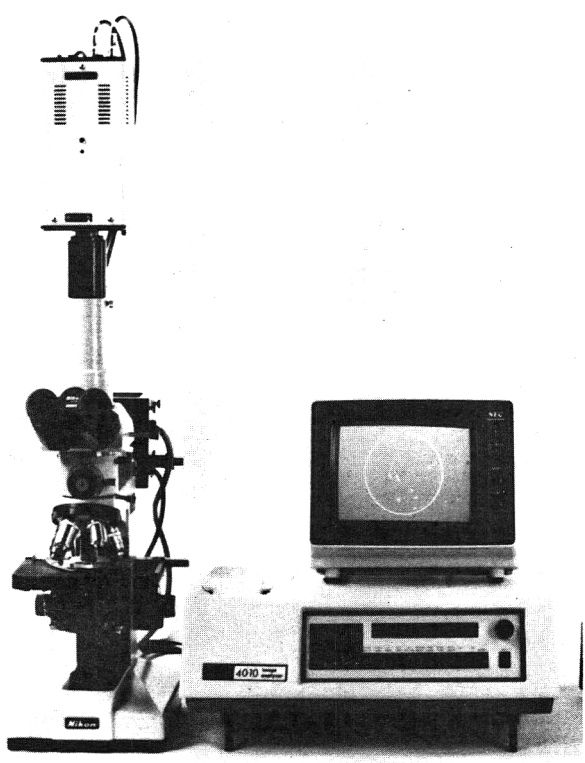

Figure 2. Apparatus for the semi-automated counting of micro-organisms on DEFT slides, microcomputer not shown.

The semi-automated count of bacteria on DEFT slides agrees well with the corresponding visual DEFT count with a correlation of 0.94 (Pettipher & Rodrigues, 1982a). For low count milks, the semi-automated count exceeds the visual count but this difference becomes proportionally less as the count increases; there is close agreement over the range 1×10^5–5×10^6/ml. The higher semi-automated count is probably due to the inclusion of particles such as sediment and the few somatic cell nuclei which remain undigested by the pretreatment. These particles are easily distinguishable from bacteria by eye and therefore would not be included in the visual DEFT count. Agreement between the semi-automated and visual DEFT counts has subsequently been improved by the inclusion of a red filter in the microscope which prevents the image analyser counting green fluorescent particles, which are usually debris or somatic cell nuclei.

One problem encountered during laboratory tests of the semi-automated counting system was the tendency for operators to 'search' for a microscopic field containing bacteria on DEFT slides prepared from low count milks. In practice this may lead to elevated counts. The use of a single directional stepping microscope stage (Foss U.K. Ltd) should ensure that random microscope fields are counted.

The counting system could be fully automated by including a cassette loading system for slides and focusing under microcomputer control. The technology for these modifications is already available (Ploem *et al.*, 1979; Thomason, Herbert & Cherry, 1975). Automation of DEFT preparations (which would involve sampling, pretreatment, filtration, staining and rinsing stages) combined with automated counting would provide a system capable of analysing hundreds of samples per day.

The use of a microcomputer for data logging also permits data manipulation. To date, the DEFT count has been based on the mean count of bacteria in the microscopic fields. Unrepresentative fields obtained due to operator error (e.g., poor focusing prior to counting), or the presence of debris in some fields of view would be included. Currently, statistical methods are being evaluated for analysing the field counts with the aim of removing unrepresentative counts (outliers) prior to calculation of the final DEFT count. This should lead to a further reduction in the counting errors.

Cost of equipment and disposable items

The cost of equipping a laboratory for the DEFT is approximately £5000 or $6000. The major capital cost is the epifluorescence microscope which retails for *circa* £4000. It is possible that a dedicated epifluorescence microscope may be produced in the future which should cost only one-third of this amount. The other expensive items of equipment are the filter manifold and waterbath. The additional cost of the instrumentation for semi-automated counting is *circa* £7800. This includes the TV image analyser system and the microcomputer which, although not essential, greatly facilitates data logging, record keeping and report writing.

The current cost of disposable items used in the DEFT is *circa* 55 pence or $0.80 per test, of which the Nuclepore membrane filter is the most expensive single item costing *circa* 35p ($0.50).

Application of the DEFT

Raw milk

The DEFT count correlates well with the plate colony count for farm, tanker and silo milks, with an overall correlation coefficient of 0.91 (Table 1). Agreement between the two methods is close at the level of 10^4–10^5 bacteria/ml, but where counts exceed

Table 1. Relationship between $\log_{10}$ DEFT count per ml (y) and $\log_{10}$ plate colony count per ml (x) for milk, meat and fish, rinses and urine

Material	a	b	r
Raw milk	0.89	0.80	0.91
Stored pasteurized milk	1.37	0.71	0.93
Fresh meat and fish	−0.41	1.00	0.91
Frozen meat and fish	1.31	0.74	0.87
Rinses of milking equipment	1.38	0.66	0.83
Urine	0.02	0.88	0.85

$y = a + bx$; r, correlation coefficient.

10^6/ml the plate colony count is higher than the DEFT count. This is possibly due to the increased break up of clumps during the preparation of dilutions in plate colony counts for milks containing high numbers of bacteria.

Comparison of DEFT counts and plate colony counts obtained by different operators on fresh and stored milks shows that there is agreement between methods and between operators over a wide range of counts (Pettipher *et al.*, 1980). For both methods the coefficient of variation (CV) between triplicate log counts within samples was ±1.6%. The CV between mean log counts obtained by different operators was ±2.2% for the DEFT count and ±2.6% for the plate colony count. Repeatability of the DEFT count seems to be similar to that of the plate colony count.

The DEFT count, which takes less than 30 min to complete and is suitable for milks containing $5 \times 10^3 - 10^8$ bacteria/ml, is both sufficiently rapid for monitoring tanker and silo milk and sensitive enough for grading farm milk on the basis of bacteriological quality. Its use should permit better control and management of supplies, reducing the risk of poor quality products resulting from a high bacterial count in the raw material.

The hygienic quality of milk is related to the number of individual bacterial cells not the number of clumps. In early work both the number of cells and clumps were counted in the DEFT (Cousins *et al.*, 1979). In order to get the method accepted the DEFT was based on a clump count as this gives closer agreement with the plate colony count. Recent work by O'Connor, Ewings & Hollywood (1983) has shown that both syringing and blending reduce the average clump size in the DEFT to approximately two bacterial cells and leads to an improved correlation with the plate colony count. Similar results on the effect of syringing and blending were reported by Rodrigues & Kroll (1985).

Milk products

For all milk products examined by the DEFT, treatment with trypsin and Triton X-100 was essential for efficient filtration and clear preparations for the microscope (Pettipher & Rodrigues, 1981). The DEFT count is not sufficiently sensitive to accurately determine bacterial numbers in pasteurized products with counts below 1×10^3/ml, but it would detect grossly contaminated products immediately after production. Any increase in bacterial numbers in pasteurized milk and pasteurized cream during storage can be immediately detected by the DEFT, the plate colony count gives a similar result 2–3 days later. Agreement between the two counting methods was good over a wide range of counts the regression line having a correlation coefficient of 0.93

(Table 1). For the DEFT to be of practical use for quality control of pasteurized products the bacterial numbers must first be increased by pre-incubation.

The microflora of pasteurized milk consists of thermoduric organisms that survive the heat treatment and post-pasteurization contaminants. In the U.K. post-pasteurization contamination by psychrotrophic Gram negative bacteria is the most significant factor in reducing the keeping quality of refrigerated pasteurized milk (Schroder, Cousins & McKinnon, 1982). In poorly refrigerated pasteurized milk, however, the thermoduric flora may also cause spoilage (Cox, 1975).

The Dairy Industry needs a rapid microbiological method for pasteurized products, preferably one that predicts the likely keeping quality. Pre-incubation of milks containing inhibitors and subsequent estimation of post-pasteurization contamination levels by measurements of bacterial ATP has been used to predict the keeping quality of pasteurized milk within 24 hr (Waes & Bossuyt, 1982). The keeping quality (KQ) of pasteurized milk stored at 5°C (satisfactory refrigeration) and 11°C (unsatisfactory refrigeration) was predicted within 24 hr by pre-incubating samples and counting bacteria by the DEFT (Rodrigues & Pettipher, 1984). For samples from 5°C storage, 0.03% (w/v) benzalkonium chloride and 0.002% (w/v) crystal violet (final concentrations) were added to inhibit the growth of Gram positive bacteria during pre-incubation. The samples from milk stored at 11°C were pre-incubated without the addition of inhibitors. After pre-incubation at 30°C there was a satisfactory relationship between the DEFT count and the KQ of milks at both 5° and 11°C. The DEFT count following pre-incubation correctly classified $> 80\%$ of pasteurized milks on the basis of KQ. Griffiths, Phillips & Muir (1984) showed that pre-incubation in the presence of inhibitors followed by use of either the DEFT or bioluminescence method could indicate the extent of post-pasteurization contamination of cream within 26 hr of production. These methods could predict whether or not the product would keep for more or less than 7 days at 6°C.

Both the DEFT count and the plate colony count failed to detect any bacteria in most samples of UHT milk which is supposed to be a sterile product. In one carton of UHT milk where after pre-incubation the growth of a *Micrococcus* sp. had occurred, the DEFT count and plate colony count were 4.3×10^4 and 1.8×10^4/ml respectively (Pettipher & Rodrigues, 1981). The DEFT could replace the plate colony count in the statutory test method, thereby giving results 2–3 days earlier.

The shortcomings of the DEFT for certain heat treated products were first revealed by an atypical sample of cream. One carton of commercially pasteurized cream had a DEFT count of $> 10^7$/ml and a plate colony count of *circa* 100/ml, whereas the two methods had been in close agreement for previous samples (Pettipher & Rodrigues, 1981). The bacteria responsible for the high DEFT count were cocci. In a follow up investigation both the raw and pasteurized cream were monitored during the 12 hr production run. An organism, which later was identified as *Strep. bovis*, gradually increased in numbers in the standardization tank during the run, finally reaching $> 10^6$/ml. This organism, although killed by the pasteurization treatment, still fluoresced orange–red in the DEFT and was therefore included in the DEFT count. The streptococcus had the same staining reaction in the DEFT even after the pasteurized cream had been stored at 7°C for 1 week. A similar phenomenon was observed for some other heat treated products.

The effect of heat treatment on the acridine orange staining characteristics of different bacterial species was examined. The persistence of acridine orange stainable

orange–red-fluorescing material in non-viable cells of *Strep. bovis* and *Micrococcus* spp. is a function of the heat treatment. Non-viable cells of these species recovered from the cultures in the decline phase of growth fluoresce green in the DEFT, as do similar cells of other bacterial species. The problem of non-viable bacteria fluorescing orange–red in the DEFT is complex and not related to Gram type e.g., the Gram positive streptococci, micrococci and bacilli behave differently. For products containing high numbers of streptococci or micrococci before heat treatment e.g., whey and milk powders, the DEFT count is probably closer to the plate colony count immediately before, rather than after, heat treatment.

Where agreement between the DEFT count and the plate colony count is good e.g., pasteurized milk, skim milk, cream, whey and sweet cream butter, the DEFT could be used for quality control and in some cases to predict the keeping quality of these products. Where the DEFT count and plate colony count are not in close agreement e.g., milk powders, pasteurized whey and ripened cream butter, the DEFT may still prove useful, because of its rapidity, in detecting bacterial contamination by monitoring the bacteriological condition of the product at various stages during its manufacture.

Foods

For stomachered suspensions of most foods tested, enumeration of micro-organisms in the DEFT proved difficult because of the presence of food debris. Pre-filtration of the suspension through nylon filters (mesh size 5 μm) removed most of the food debris and only slightly reduced the recovery of micro-organisms, as determined by the plate colony count (Pettipher & Rodrigues, 1982b). The recovery rates fell as the microbial content of the food increased e.g., approximately 100% at 10^4/g, 85% at 10^6/g, 70% at 10^8/g and 60% at 10^{10}/g. The loss of micro-organisms is unlikely to substantially affect the count of micro-organisms in the food.

For milk, 0.6 μm pore size polycarbonate membrane filters are used in the DEFT but for some 'cleaner' liquids, such as certain pre-filtered food suspensions, beverages and water, a proportion of the bacteria (up to 25%) may enter the pores of these membranes. If a sufficient volume of sample can be filtered to achieve the required sensitivity, it is recommended that a smaller pore size (0.4 or 0.2 μm) membrane filter is used for these products.

If Tween 80 is used as the surfactant for the pretreatment of samples in the DEFT, the micro-organisms appear larger than if Triton X-100 is used. These larger organisms fluoresce more brightly and are more easily counted. As Tween 80 is relatively ineffective at lysing somatic cells, even in the presence of trypsin, it should not be used as the surfactant for products containing large numbers of somatic cells. However, for other products where somatic cells are few or absent, e.g. butter and pre-filtered meat suspensions, the use of Tween 80 as the surfactant in the DEFT in place of Triton X-100 may result in better quality microscope preparations.

Depending upon the food, 4–15 ml of stomachered food suspensions (10 g food plus 90 ml diluent) could be filtered through a single nylon pre-filter and 3–10 ml of these pre-filtered suspensions could be filtered in the DEFT. A sample size of 2 ml was routinely used in the DEFT, together with a microscope factor of 57500.

For fresh meat and fish, the pre-filtered DEFT count agreed well with the plate colony count over the range 10^4–10^{10}/g, with a correlation coefficient of 0.91 (Table 1). For counts on frozen meat and frozen fish the prefiltered DEFT count also agreed well with the plate colony count over the range 5×10^4–5×10^7/g (Table 1). Of the other

foods tested, there was good agreement between the pre-filtered DEFT count and plate colony count for cooked meats, cream doughnut and spices (Pettipher & Rodrigues, 1982b). The coefficient of variation between triplicate log DEFT counts on food suspension was $< \pm 1\%$, lower than that obtained for milk.

Other workers have extended the application of DEFT to other foods. Recent results from a laboratory in Denmark show good agreement between the DEFT count and plate colony count for minced pork, beef and bacon, various types of fish, mayonnaise salads and milk (Boisen, 1983). For minced beef, the manual DEFT count and automated DEFT counts had coefficients of variation of 10.2 and 7.9% respectively. The repeatability of automated counts on a single DEFT slide was $\pm 6.1\%$. Qvist & Jakobsen (1985) reported good agreement between DEFT counts and plate colony counts for ground beef.

Beverages

Methods using the same principles as the DEFT are used to estimate viable and non-viable yeast and bacteria in wines and on winery equipment surfaces (Cootes & Johnson, 1980). The method for wine takes only 15 min to complete and has a sensitivity of less than 1 micro-organism per ml.

Water and rinses of food contact surfaces

Counts of bacteria in natural waters obtained by membrane filtration–epifluorescence microscopy techniques generally agree with counts obtained using the scanning electron microscope (Bowden, 1977). The repeatability of the microscopic method is good with a coefficient of variation between operators and between replicates of 7.5 and 3.1%, respectively.

The rinse plate method often takes 6 days or longer to locate the source of high numbers of bacteria on milking equipment. The DEFT could be used to count bacteria in rinses of milking equipment in *circa* 5 min and there was reasonable agreement between the DEFT count and plate colony count (Table 1). This enables the source of high numbers of bacteria to be traced within one working day permitting speedy corrective action. Pre-filtration through a 5.0 μm pore size nylon filter increased the sensitivity of the rinse DEFT by five times giving a sensitivity of 200 bacteria per ml of rinse.

Hunter & McCorquodale (1983) reported good agreement between the DEFT count and plate colony count of bacteria in rinses of a milking machine cleaned with chlorine based chemicals ($r = 0.82$) but poorer agreement in rinses of equipment cleaned with acidified boiling water ($r = 0.46$). The poor agreement between the methods for rinses of equipment cleaned solely by heat was probably due to the inclusion of heat killed bacteria in the DEFT count. These authors examined the staining characteristics of heat killed pure cultures in the DEFT and essentially confirmed the findings of Pettipher & Rodrigues (1981). Hunter & McCorquodale (1983) also suggested that the DEFT is a useful and rapid means of counting bacteria in rinses of equipment where disinfection is primarily achieved by chlorination but, in the absence of a stain which can differentiate more accurately between dead and living organisms, its use is limited where disinfection is carried out solely by heat. In England and Wales approximately 85% of farms use chemical disinfection and only 15% acidified boiling water.

Medical

The DEFT can be used for applications in the medical field for example detecting bacteria in intravenous fluids (Denyer & Ward, 1983) and to enumerate rapidly bacteria and somatic cells in urine (Pettipher, 1983).

Present state of acceptance and likely future of DEFT

For any new technique to gain acceptance it is advantageous if the results are similar to or correlate with those of a standard method. The DEFT count based on the number of orange fluorescing clumps of bacteria meets this requirement for raw milk, raw foods and urines, as it is in close agreement with the plate colony count. In the case of milk, these clumps may contain from 1 to 50 bacteria depending on the age of the milk. The bacteriological quality of milk and other foods is more closely related to the total number of viable bacteria rather than the number of viable clumps, as determined by the plate colony count. Once the technique has gained acceptance it is possible that a DEFT count based on the number of individual orange fluorescing cells may be preferred. This should be a more realistic measure of the bacteriological status of the product.

It is very important that any new technique should perform well in each laboratory that attempts it, otherwise its credibility may be adversely affected. The DEFT has now been introduced successfully into numerous laboratories. Where requested, assistance was given and training courses were held for prospective users. Only minor problems were encountered with the introduction of the technique, and these were quickly resolved. Operators in laboratories other than the NIRD have also obtained good correlations between the DEFT count and plate colony count. In a collaborative trial, laboratories generally achieved a correlation coefficient of > 0.9 for raw milk (Pettipher, Fulford & Mabbit, 1983). Independently, operators in Australia using minor modifications to the pre-treatment stages obtained a correlation coefficient of 0.95 between the DEFT count and plate colony count (Beck & Hehir, 1982). The reagent kits and dispensers now available commercially have made the introduction of the DEFT into a laboratory considerably easier than previously.

The DEFT is now operating satisfactorily in more than forty laboratories which are situated in the U.K., Eire, Germany, France, The Netherlands, Finland, Denmark, Sweden and Australia. Of these laboratories, more than twenty-five use semi-automated counting. More than four laboratories situated in the U.K., Ireland and Denmark have successfully used the DEFT for meat and other foods.

It has been estimated that approximately 50000 DEFT counts are made p.a. The Jersey Milk Marketing Board was quick to see the advantages of the DEFT and has been using the semi-automated DEFT count to assess the bacteriological quality of farm milk for payment purposes since April 1982. Since its introduction, a considerable improvement in the bacteriological quality of the incoming milk has been achieved. In addition, the increased speed of testing has benefitted the advisory service, and the use of microcomputers has greatly improved both the data storage and reporting. The DEFT has recently been included in British Standard 4285 'Methods of Microbiological Analysis for Dairy Products' which gives the method of 'seal of approval' in the U.K. It is hoped that other countries will include it in their standard methods. Currently the DEFT is being used mainly for raw milk but its use for other foods, most notably, meat, is increasing. No other method can give an accurate, sensitive count of bacteria in raw foods in as short a time as the DEFT.

The immediate prospects for the DEFT seem to lie in new applications, improved sample preparation, improved sensitivity and possibly improved rapidity. Now that the method is being widely used, DEFT users are likely to apply the method to different foods and therefore find new applications. DEFT will not be suitable for all products as there are some inherent problems associated with heat treated products, but it is still possible to get useful information using the method e.g., pasteurized milk. Improvements in sample preparation are possible e.g., blending to break up clumps to give a more realistic assessment of hygienic quality and different enzyme treatments for difficult to filter products. Improved sensitivity can be achieved if greater amounts of sample are filtered without increasing the background/food debris. Recent work at the Food Research Institute, Reading (Rodrigues & Kroll, 1985) has shown that the sensitivity of the DEFT for raw milk can be increased five times to 1200 bacteria per ml. Improved rapidity was also achieved enabling the result to be obtained in less than 10 min.

An area which is likely to receive increasing attention is the combination of DEFT with other staining methods. The differentiation of Gram type is possible in the DEFT using a modified Gram staining procedure with acridine orange as the counterstain (Rodrigues & Kroll, 1985). This is particularly useful for dairy applications. If the DEFT could be combined with immuno-fluorescence techniques, then it would be possible to get a total and specific count of bacteria in a single preparation.

The acceptability and use of the DEFT by microbiologists is increasing and the method is becoming widely used in the dairy, food and beverage industries. Automation of the technique is likely to speed its introduction but it should not be forgotten that no automation is required if only a small number (30–40) of samples are analysed daily. The DEFT has the advantage that as a manual technique it can be used for the rapid assessment of small numbers of samples of different foods in less than 30 min, whereas a fully automated DEFT instrument, because of its sensitivity, has the potential to become the future method of choice for the bacteriological analysis of milk in centralized testing schemes.

References

Beck, C.G. & Hehir, A.F. (1982). The rapid fluorescence method for counting bacteria in milk. *The Australian Journal of Dairy Technology*, **37**, 66–67.

Boisen, F. (1983). DEFT – analyse of levnedsmidler. *Saertryk of Dansk Veterinaertidsskrift*, **66**, 1030–1037.

Bowden, W.B. (1977). Comparison of two direct-count techniques for enumerating aquatic bacteria. *Applied and Environmental Microbiology*, **33**, 1229–1232.

Cootes, R.L. & Johnson, R. (1980). A fluorescent staining technique for determination of viable and non-viable yeasts and bacteria in wineries. *Food Technology in Australia*, **32**, 522–523.

Cousins, C.M., Pettipher, G.L., McKinnon, C.H. & Mansell, R. (1979). A rapid method for counting bacteria in milk. *Dairy Industries International*, **44**, 27–39.

Cox, W.A. (1975). Problems associated with bacterial spores in heat treated milk and dairy products. *Journal of the Society of Dairy Technology*, **28**, 59–68.

Denyer, S.P. & Ward, K.H. (1983). Rapid method for the detection of bacterial contaminants in intravenous fluids using membrane filtration and epifluorescence microscopy. *Journal of Parenteral Science and Technology*, **37**, 156–158.

Griffiths, M.W., Phillips, J.D. & Muir, D.D. (1984). Methods for rapid detection of post pasteurisation contamination in cream. *Journal of the Society of Dairy Technology*, **37**, 22–26.

Hunter, A.C. & McCorquodale, R.M. (1983). Evaluation of the direct epifluorescent filter technique for assessing the hygienic condition of milking equipment. *Journal of Dairy Research*, **50**, 9–16.

O'Connor, R.E., Ewings, K.N. & Hollywood, N.W.

(1983). Effect of agitation on bacterial aggregates in pure cultures and raw milk. *Journal of Food Protection*, **46**, 681–685.

Pettipher, G.L. (1983). The direct epifluorescent filter technique for the rapid enumeration of microorganisms. Letchworth: Research Studies Press (Innovation in microbiology series, 1).

Pettipher, G.L., Mansell, R., McKinnon, C.H. & Cousins, C.M. (1980). Rapid membrane filtration-epifluorescent microscopy technique for direct enumeration of bacteria in raw milk. *Applied and Environmental Microbiology*, **39**, 423–429.

Pettipher, G.L. & Rodrigues, U.M. (1981). Rapid enumeration of bacteria in heat-treated milk and milk products using a membrane filtration-epifluorescence microscopy technique. *Journal of Applied Bacterilogy*, **50**, 157–166.

Pettipher, G.L. & Rodrigues, U.M. (1982a). Semi-automated counting of bacteria and somatic cells in milk using epifluorescence microscopy and television image analysis. *Journal of Applied Bacteriology*, **53**, 323–329.

Pettipher, G.L. & Rodrigues, U.M. (1982b). Rapid enumeration of micro-organisms in foods by the Direct Epifluorescent Filter Technique. *Applied and Environmental Microbiology*, **44**, 809–813.

Pettipher, G.L., Fulford, R.J. & Mabbit, L.A. (1983). Collaborative trial of the direct epifluorescent filter technique (DEFT), a rapid method for counting bacteria in milk. *Journal of Applied Bacteriology*, **54**, 177–182.

Ploem, J.S., Verwoerd, N., Bonnet, J. & Koper, G. (1979). An automated microscope for quantitative cytology combining television image analysis and stage scanning microphotometry. *Journal of Histochemistry and Cytochemistry*, **27**, 136–143.

Qvist, S.H. & Jakobsen, M. (1985). Application of the direct epifluorescent filter technique as a rapid method in microbiological quality assurance in the meat industry. *International Journal of Food Microbiology*, **2**, 139–144.

Rodrigues, U.M. & Pettipher, G.L. (1984). Use of the Direct Epifluorescent Filter Technique for predicting the keeping quality of pasteurised milk within 24 hours. *Journal of Applied Bacteriology*, **57**, 125–130.

Rodrigues, U.M. & Kroll, R.G. (1985). Increased selectivity, sensitivity and rapidity in the Direct Epifluorescent Filter Technique (DEFT). *Journal of Applied Bacteriology*, **59**, 493–499.

Schroder, M., Cousins, C.M. & McKinnon, C.H. (1982). Effect of psychotrophic post-pasteurisation contamination on the keeping quality at 11 and 5°C of HTST-pasteurised milk in the UK. *Journal of Dairy Research*, **49**, 619–630.

Thomason, B.M., Herbert, A. & Cherry, W.B. (1975). Evaluation of a semiautomated system for the direct fluorescent antibody detection of salmonellae. *Applied Microbiology*, **30**, 557–564.

Waes, G. & Bossuyt (1982). Usefulness of the Benzalkon crystal violet ATP method for predicting the keeping quality of pasteurised milk. *Journal of Food Protection*, **10**, 928–931.

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Journal of Food Technology (1986) **21**, 547–558

Effect of frozen storage on protein denaturation in bovine muscle. II. Influence on solubility, viscosity and electrophoretic behaviour of myofibrillar proteins

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Summary

The effect of frozen storage on the solubility, viscosity, rheological and electrophoretic behaviour of myofibrils was studied. The solubility, viscosity, swelling and thixotropic behaviour of myofibrils showed a period in which it was constant or changed slowly, and then began to decrease. The results obtained by polyacrylamide gel electrophoresis indicated that during storage the formation of aggregates occurred in addition to alteration in the actin–myosin interaction. These results were correlated with those obtained from measurements of viscosity, rheological behaviour and swelling of myofibrils in solution.

Introduction

During freezing and frozen storage, changes in the quality of meat and fish are evident. The consequence of these changes may be loss of water-holding capacity, undesirable changes in texture and development of rancidity (Fennema, Powrie & Marth, 1973).

Most of the studies indicate that denaturation of muscle proteins, especially myofibrillar ones, plays an important role in the deterioration of meat quality during frozen storage. Changes in the structure of myofibrillar proteins, decrease of solubility and alterations in the rheological behaviour of the myofibrillar proteins have been reported (Matsumoto, 1980a).

Most of the studies have been carried out with fish muscles (King, 1966; Connell, 1968; Sikorski, Olley & Kostuch, 1976; Matsumoto, 1980b) although a little work has been performed on chicken (Khan, 1966; Yamamoto, Samejima & Yasui, 1977), rabbit (Ito, Sung & Fukazawa, 1978; Kang, Ito & Fukazawa, 1983) and bovine muscles (Awad, Powrie & Fennema, 1968; Rahelić, Pribis & Skenderovic, 1974; Carroll, Cavanaugh & Rorer, 1981). In the present work we report the results obtained on the effects of frozen storage at different temperatures on solubility, water absorption capacity and rheological and electrophoretic behaviour of myofibrillar proteins of bovine muscle frozen at two different freezing rates.

Materials and methods

Meat sample preparation

Experiments were performed within 48 hr post-mortem on semitendinosus muscle from steers, fresh and frozen at high and low rate. Meat slices were frozen as described in previous studies (Bevilacqua, Zaritzky & Calvelo, 1979; Wagner & Añón, 1985). The

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freezing process was ended when samples reached −25°C. A characteristic freezing time (t_c) was assigned to each slice (Bevilacqua *et al.*, 1979). High freezing rates correspond to $t_c < 5$ min and low freezing rates to $t_c > 60$ min. The meat slices were stored in polyethylene bags at −5±1, −10±1 and −20±1°C. For experimental purposes, the frozen samples were thawed overnight at 4°C. Measurements were carried out on the inner part of the meat slices, avoiding the surface tissue.

Preparation of myofibrils

Myofibrils were prepared according to the procedure described by Goll & Robson (1967) from 2 g of ground muscle. The purified myofibrils were suspended in 0.15 M KCl – 0.03 M Tris-HCl, pH 7.6 (solution 1). Protein concentration was determined by the modified biuret method (Robson, Goll & Temple, 1968).

Solubility of myofibrillar proteins

Myofibrillar proteins were extracted from isolated myofibrils with 0.6 M KCl – 0.03 M Tris-HCl at pH 7.6 (solution 2). Preparations were gently stirred overnight at 4°C, centrifuged at 17000×**g** for 15 min and the amount of extracted proteins in the resulting supernatant measured.

Myofibrillar ATPase activity measurements

The ATPase activity in the presence of Ca^{2+} and Mg^{2+} was assayed according to the procedure described by Hay, Currie & Wolf (1973).

Viscosity

The viscosity and the flow behaviour of purified myofibrils suspended in solution 2 was determined at 10°C with a Haake Rotavisco RV2 Viscometer using a sensor system NV and a rotor speed of 0 to 64 rpm. A PG 142 Haake programmer was used. The resulting flow curves were recorded with a Hewlett Packard y-x-t Haake recorder.

Apparent viscosity (η_{app}) was calculated as follows:

$$\eta_{app} = G \cdot \frac{S}{n} \text{(mPa.sec)}$$

where: G = instrument factor (mPa.sec/scale grad. min); S = scale value (scale grad.) and n = rotor speed (per min).

The reduced viscosity (η_{red}) of the preparation was calculated as the apparent viscosity (η_{app}) divided by the concentration of myofibrils (mg/ml).

Water absorption capacity

Myofibrils suspended in solution 1 were centrifuged at 3000×**g** for 10 min. The pellet (m_1) was resuspended with 8 volumes of distilled water and gently stirred at 4°C overnight. The viscous suspension obtained was centrifuged at 3000×**g** for 10 min. The pellet was weighed (m_2). The water absorption capacity was calculated as follows:

$$W_{ac\,1} = \frac{m_1 - m}{m} \text{ or } W_{ac\,2} = \frac{m_2 - m}{m}$$

where m = weight of myofibrils (mg); m_1 = pellet weight of myofibrils suspended in solution 1 (mg); m_2 = pellet weight of myofibrils suspended in water (mg).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Slab SDS polyacrylamide gel electrophoresis was carried out using the Laemmli discontinuous buffer system (Laemmli, 1970; Laemmli & Favre, 1973) at a gel concentration of 10%. A Pharmacia gel electrophoresis apparatus GE-2/4 was used. Myofibrils were suspended in 10% glycerol, 0.001% bromophenol blue, 2% SDS, 0.125 M Tris-HCl buffer solution (pH 6.8) with and without 5% 2-mercaptoethanol (ME) for 5 min at 100°C. The gels were fixed in isopropanol/acetic acid/water (25:10:65, by vol) and were stained with 0.02% Coomassie Brilliant Blue R-250 in 7.5% acetic acid. Gels were scanned in a Shimadzu dual wavelength TLC Scanner CS-910 (sample wavelength 570 nm and reference wavelength 395 nm) attached to a C-R IA Chromatopac Shimadzu integrator. The gels were reproducible and all peaks measured were within full scale. Molecular weights of the protein bands were estimated by use of the Pharmacia protein molecular weight calibration kit.

Statistical methods

Variance analysis (ANOVA) at a 95% confidence level was used for statistical evaluation of data. The mean values were of three to six replicates carried out on preparations from different muscles. No significant differences were detected among muscles from different animals. The least significant difference (LSD) was calculated.

Results and discussion

Solubility of myofibrils in 0.6 M KCl solution

Figure 1 shows the variation of the solubility of myofibrils in 0.6 M KCl solution with the time during storage at −5, −10 and −20°C following both fast and slow freezing. It

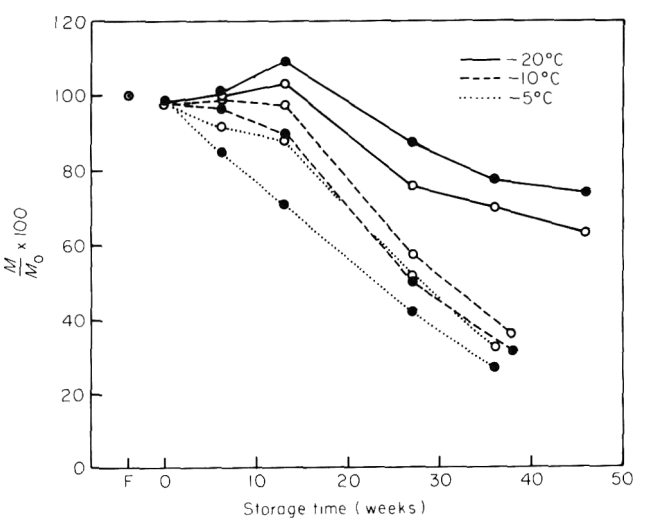

Figure 1. The time-dependent change of solubility of myofibrils during frozen storage of muscle at −20, −10 and −5°C; for fast freezing (●) and slow freezing (○). M_0 and M are respectively the solubility of myofibrils isolated from fresh and frozen stored muscle. M_0 = 81.0±5.8. Each value is an average of a minimum of four assays (maximum standard deviation: 10.5). (l.s.d.$_{0.05}$ = 9.2).

can be seen that the loss of myofibrillar solubility was greater at the higher storage temperatures. At −5°C the solubility of myofibrils decreased from the first weeks; at −10°C the solubility began to decrease markedly after a period of about 6 weeks whereas at −20°C there appeared to be an initial period of slightly increased solubility which lasted for about 13 weeks, then solubility decreased slowly. Statistical analysis showed significant differences for both time and temperature of storage (l.s.d.$_{0.05}$ =9.2). In Fig. 1 it can be seen that at −5 and −10°C the loss of solubility appeared higher in the case of fast freezing (l.s.d.$_{0.05}$ = 2.9), while at −20°C the reverse appeared true (l.s.d.$_{0.05}$ = 4.2). The yield of myofibrils did not change during storage.

In order to estimate the kinetics of insolubilization of myofibrils during frozen storage, the natural logarithm of the percentage of soluble myofibrilar protein (in relation to fresh muscle) was plotted against the time of storage, both for fast and slow freezing (Fig. 2). Two straight lines could be fitted to the points obtained for each temperature, suggesting a process of two first-order stages; an initial stage lasting approximately 13 weeks, in which the solubility remains constant (−20°C) or decreases slowly (−5 and −10°C), and a second faster stage. The values of the rate constants in this second stage were. for fast freezing, k = 0.043; 0.042 and 0.012/week at −5, −10 and −20°C, respectively; and for slow freezing k = 0.042; 0.040 and 0.014 at −5, −10 and −20°C, respectively. From these values the energy of activation (Ea) was calculated by means of the Arrhenius equation. Values for Ea of 12.6 and 10.5 kcal/mol were obtained for fast and slow freezing, respectively. In the first stage the rate of insolubilization at −5°C was more than three times that at −10°C. At these two temperatures the rates were comparable in the second stage, in which they were about three times as high as at −20°C.

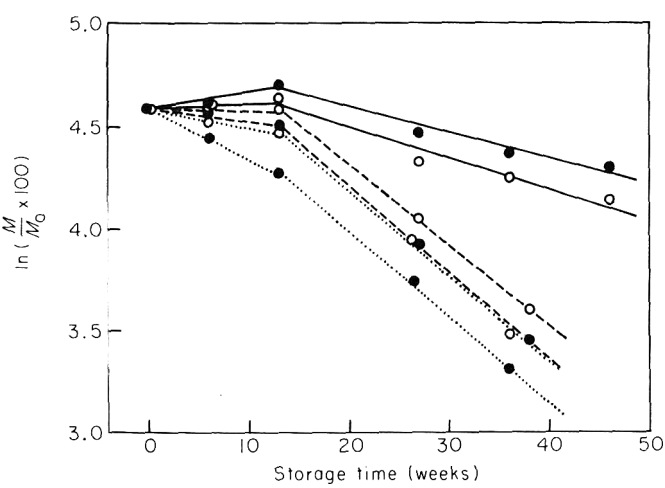

Figure 2. Estimation of the kinetics of insolubilization of myofibrils with the time of storage at −5°C (···), −10°C (---) and −20°C (——); for fast freezing (●) and slow freezing (○). M_0 and M are respectively the solubility of myofibils isolated from fresh and frozen stored muscle. Each value is an average of a minimum of four assays (Maximum standard deviation: 0.1).

Comparison of these results to those obtained by measuring the ATPase activity and the enthalpy of denaturation (ΔH) (Wagner & Añón, 1986), indicate that during the slow step of insolubilization the greatest decrease in ATPase activity and ΔH take place,

implying that rapid denaturation of the myofibrillar proteins (mainly myosin) takes place. Conversely, during the second stage the decrease in ATPase activity and ΔH is slow yet a rapid decrease in the solubility of myofibrillar proteins takes place. Thus, denaturation of myofibrillar proteins appears to occur prior to the formation of insoluble aggregates. As an illustration of this phenomenon, Fig. 3 shows the exponential correlation between the values of solubility and myofibrillar ATPase activity; it can be seen that the solubility decreased rapidly only after the ATPase activity, in the presence of Ca^{2+} or Mg^{2+}, had decreased to about 80 and 60% of the initial values respectively.

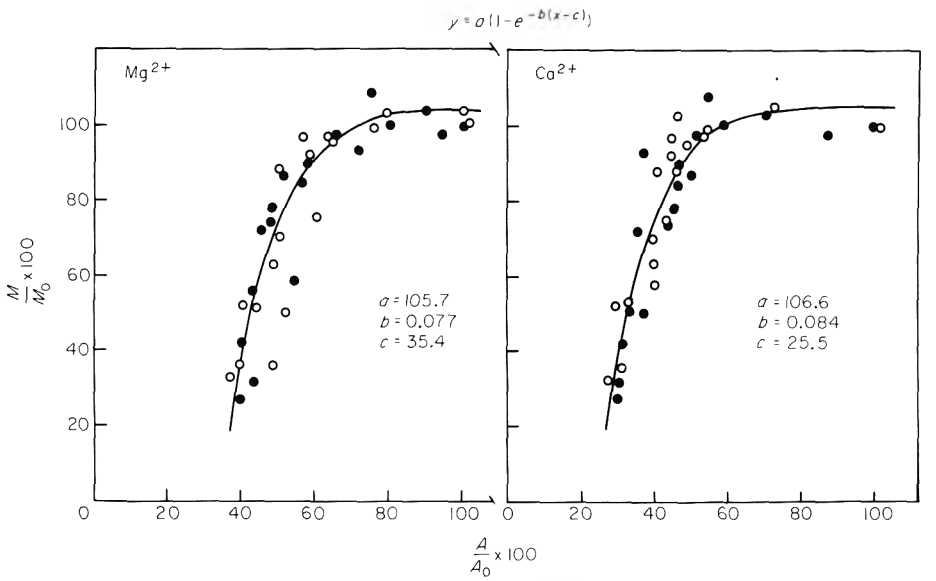

Figure 3. Relationship between myofibrillar solubility (M/M_0) and myofibrillar ATPase activity (A/A_0) in the presence of Mg^{2+} or Ca^{2+}; for fast freezing (●) and slow freezing (○). Each value is a mean of at least four assays.

Viscosity and rheological behaviour of the myofibrils in 0.6 M KCl solution

Figure 4 depicts the effect of time and temperature of storage of frozen muscle upon the reduced viscosity (η_{red}) of the myofibrils in 0.6 M KCl solution. The reduced viscosity did not change significantly upon freezing (l.s.d.$_{0.05}$ = 0.32) but was modified by protracted storage. During the first 13 weeks, η_{red} tended to remain constant at all three temperatures. After this period, η_{red} decreased rapidly at −5 and −10°C and more slowly at −20°C (l.s.d.$_{0.05}$ = 0.26). At 47 weeks of storage the decrease in viscosity at −20°C was only 35% of the initial value but had decreased by 70 and 76% at −5 and −10°C, respectively.

The rheological behaviour of myofibrils in 0.6 M KCl solution was not modified by freezing itself, though it changed with the time of storage. In the case of slowly frozen muscle with no storage, the myofibrillar solution behaved as a pseudoplastic, thixotropic liquid (Fig. 5). The myofibrils of both fresh muscle and non-stored fast frozen muscle behaved similarly (results not shown). The rheological behaviour changed gradually with the storage time from a pseudoplastic, thixotropic liquid to a newtonian, non-thixotropic one; the time needed to complete this process varied according to

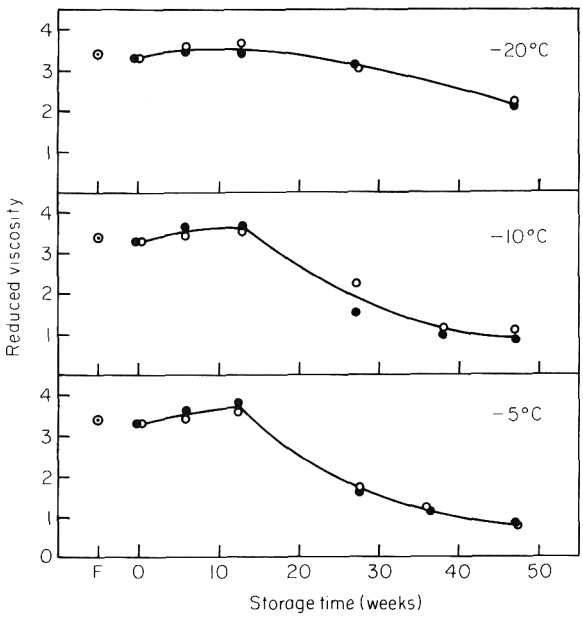

Figure 4. Effect of frozen storage on the reduced viscosity (η_{red}) of myofibrils isolated from fresh (⊙), fast frozen (●) and slow frozen (○) muscle, suspended in solution 2. Reduced viscosity was calculated as follows:

$$\eta_{red} = \frac{\eta_{app} \text{ at } 64/\text{min (mPa.sec)}}{\text{myofibrils concentration (mg/ml)}}$$

η_{app} was calculated using the maximum S value reached. Myofibrils concentration was 10–12 mg/ml. Each value is an average of at least three assays (maximum standard deviation: 0.31). (l.s.d.$_{0.05}$ = 0.26).

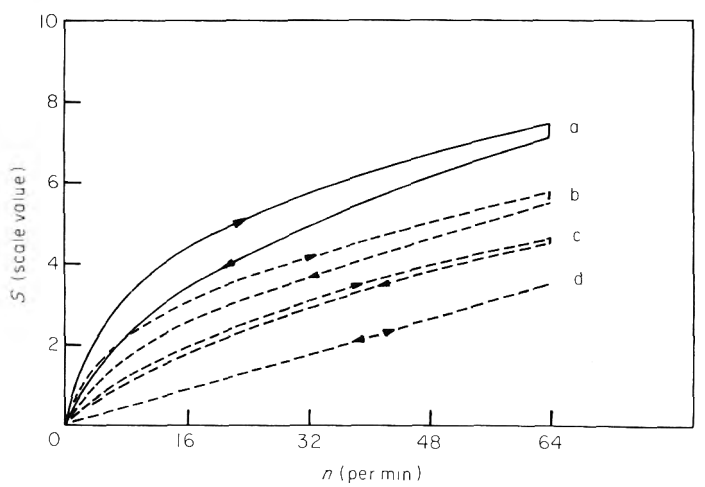

Figure 5. Flow curves obtained with myofibrils suspended in solution 2. Myofibrils concentration: 10–12 mg/ml. (a) Slow frozen muscle without storage. (b), (c) and (d) Slow frozen muscle stored 26 weeks at −20, −10 and −5°C, respectively. The programme used was:

0 → 64/min 2 rpm
64/min 1 rpm
64 → 0/min 2 rpm

the temperature. Thus, it is seen in Fig. 5 that at 26 weeks of storage the behaviour of the myofibrils was already newtonian in muscle stored at −5°C, while at −10°C the pseudoplastic behaviour and thixotropy, though diminished, were still noticeable. However, at −20°C, though the viscosity decreases (Fig. 4), the rheological behaviour was not greatly altered.

The water absorbing capacity or swelling of the myofibrils was also determined. With fast and with slow freezing, the swelling ability decreased by 9 and 14%, respectively. During storage, swelling of myofibrils (in water or in solution 1) exhibited similar behaviour to that observed with the reduced viscosity, and a linear correlation was found between these two properties (Fig. 6).

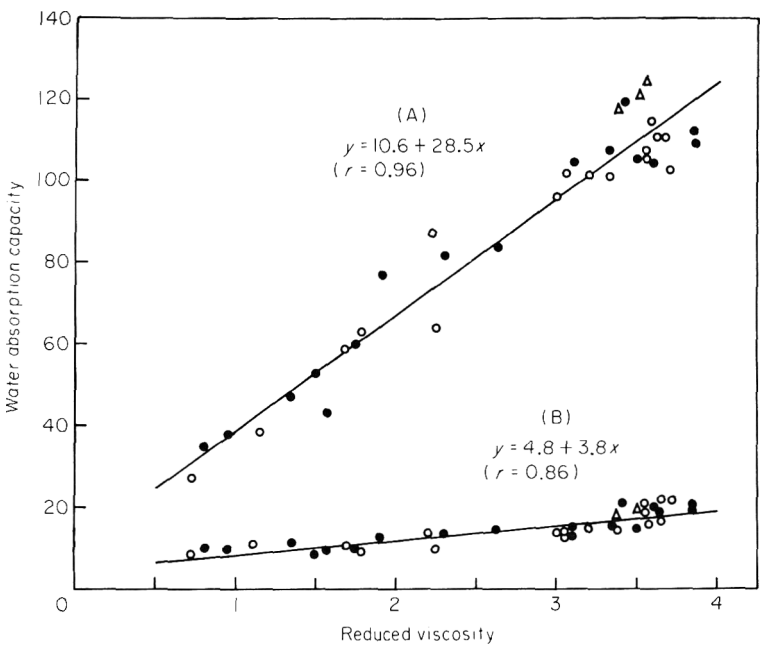

Figure 6. Relationship between water absorption capacity and reduced viscosity of myofibrils. The water absorption capacity was measured on myofibrils suspended in water (A) and solution 1 (B). The reduced viscosity was measured on myofibrils suspended in solution 2. ●: Myofibrils from fast frozen muscle. O: myofibrils from slow frozen muscle. △: myofibrils from fresh muscle. Each value is an average of three assays as a minimum (maximum standard deviation: 8.0 (A) and 1.5 (B)). *r*: Correlation coefficient.

Electrophoresis in polyacrylamide gels

Myofibrils isolated respectively from fresh, frozen and stored muscle were studied by means of electrophoresis in 10% polyacrylamide gels after being treated with SDS or SDS+ME. Figure 7 shows the electrophoretic patterns of the myofibrils of fresh muscle (a and b), fast frozen muscle (c and d) and slow frozen muscle (e and f); the main myofibrillar proteins are shown. It is seen that in the samples treated with SDS+ME (b, d and f) the area under the myosin peak (MHC) is greater than in those treated with SDS alone (a, c and e) (similar differences are seen in the areas under the peak AM). A possible explanation is that, when the myofibrils are in 0.6 M KCl solution, the molecules of myosin and actin are induced to form actomyosin filaments (AM)

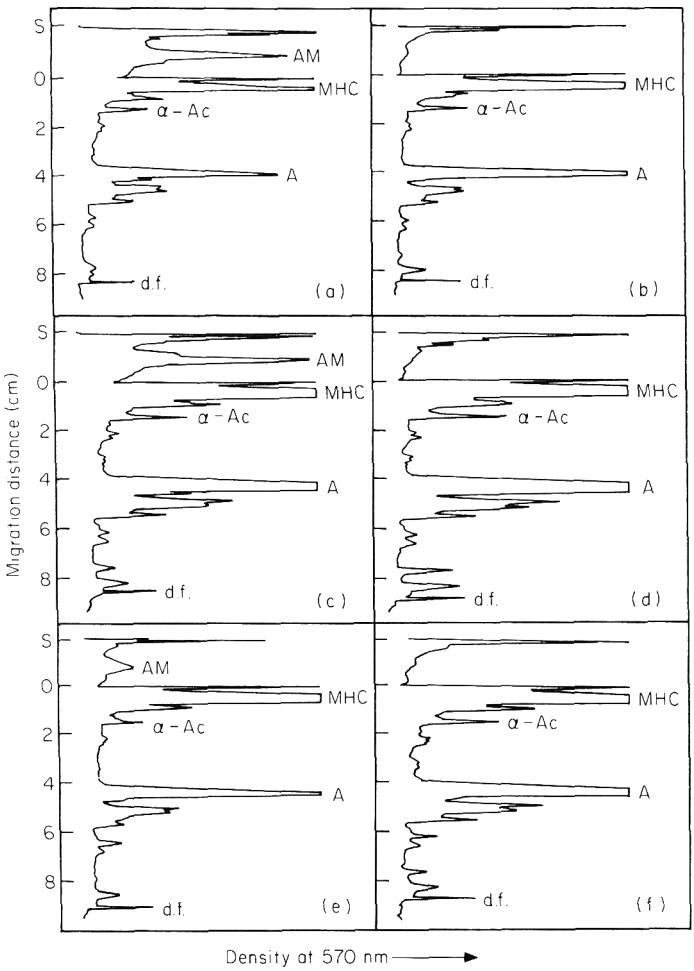

Figure 7. Densitograms of the SDS polyacrylamide gel electrophoretograms from myofibrils suspended in solution 2. Samples a, c and e were treated with SDS and samples, b, d and f with SDS+ME, respectively. s: Loading sample point; d.f.: dye front; s → 0: stacking gel AM, filaments of actomyosin; MHC, myosin heavy chain; α-Ac, α-actinin and A, actin. (a), (b): Fresh muscle; (c), (d): fast frozen muscle without storage; (e), (f): slow frozen muscle without storage.

(Matsumoto, 1980a). Disulphide bridges would be involved in the aggregate's formation since the peak AM disappeared when the samples were treated with ME. The disappearance correlated with increased amounts of myosin heavy chain (MHC) and actin (A) entering the gel. The ratio between the areas under peak MHC, with and without ME for the same sample, yields information about the actin-myosin interaction. The values of these ratios were 1.37, 1.15 and 0.93 for myofibrils from fresh, fast frozen and slow frozen muscle. Thus there appears to be a greater decrease in the actin–myosin interaction in slow frozen muscle than in the fast frozen samples (notice that the area under the peak AM is smaller for the slow frozen samples than for the fast frozen ones). Similar differences were found in the decrease of myofibrillar Mg^{2+} ATPase activity following fast and slow freezing (Wagner & Añón, 1985).

The ratio of the areas under peak MHC and peak A (actin), both with and without ME is, for myofibrils of fresh muscle, approximately 4, which is the ratio of the weights of the heavy chain of myosin and actin; i.e., in fresh muscle the heavy chain of myosin and actin interact in a 1:1 molar ratio to form the actomyosin complex. After fast or slow freezing the ratios are 3.3 and 2.3 respectively, presumably due to the modification of the actin–myosin interaction. Figures 8a,b and c show the percent variation of peak MHC, with respect to the total area of the gel, during storage at −20, −10 and −5°C, respectively, for fast and slow frozen muscle. As a result of freezing, increased MHC is seen, supporting the contention that partial loss of the actin–myosin interaction occurs.

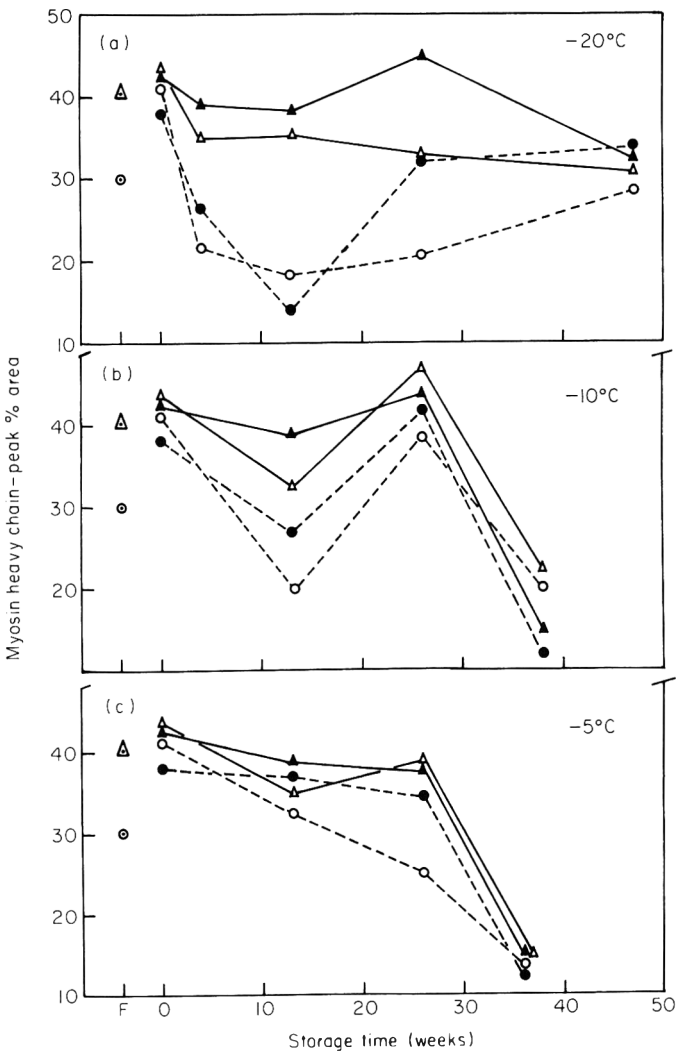

Figure 8. Effect of the frozen storage on the area of myosin heavy chain from the electrophoretic densitograms of myofibrils. ●, ▲: Myofibrils from fast frozen muscle treated with SDS and SDS+ME respectively. ○, △: myofibrils from slow frozen muscle treated with SDS and SDS+ME respectively. ⊙, ◬: myofibrils from fresh muscle treated with SDS and SDS+ME, respectively.

During storage at −20°C (Fig. 8a) there was an increase, between weeks 4 and 13, of the difference between the values of MHC with and without ME, both for fast and slow freezing; this may be attributed to an increased interaction between actin and myosin (which might indicate an alteration of the myosin head and/or actin). At 47 weeks at −20°C there is little evidence for any actin–myosin interaction. However, effects of storage at −10 and −5°C are quite different. At −10°C, there is already at 26 weeks little evidence of actin–myosin interaction, but at 38 weeks the difference between the values of MHC with and without ME can again be observed suggesting the formation of disulphide bridges in the insoluble aggregates. Furthermore, there is a decrease in MHC area in the presence of ME which would indicate a breakdown of the myosin heavy chain (in accordance with an increase of bands corresponding to polypeptides of low molecular weight in the gels). Since there is no evidence for the formation of disulphide bridges at 26 weeks but decreased myofibrillar solubility is observed (Fig. 1), the insoluble aggregates would appear to be formed by non-covalent bonds only.

On storage at −5°C, the time for total loss of actin–myosin interaction is reduced to 13 weeks and there is formation of disulphide bridges at 26 weeks. At 36 weeks, the myosin breakdowns and so the value of MHC with ME decreases and an increase in the concentration of polypeptides of low molecular weight is seen (results not shown).

At all three temperatures, prior to the formation of disulphide bridges, there must be a formation of insoluble aggregates which involve non-covalent bonds, leading to the observed decrease in myofibrillar solubility. An analysis of the actin area (A), with and without ME (Figs 9a,b and c), shows that at −20°C there are no significant changes due to the presence of ME with either freezing or storage. However, at −10°C there are, at 26 weeks, decreased A values for samples without ME. This suggests that disulphide bridges are present in the actin aggregates; these effects are more marked at 38 weeks. At −5°C the loss of the actin–myosin interaction is observed at 13 weeks. At 26 and 36 weeks there is a decrease of A with and without ME, thus indicating the formation of disulphide bridges and actin breakdown.

Conclusions

An objective of this work was to relate the present results to the changes of ATPase activity and of enthalpy of denaturation (ΔH) observed during freezing and frozen storage of beef muscle (Wagner & Añón, 1985; 1986).

From Figs 1 and 2 it can be seen that during the first 13 weeks of storage the solubility of the myofibrils remained constant (at −20°C) or fell slowly (at −5 or −10°C), thereafter the solubility rapidly decreased. The results obtained suggest a process of two first-order stages. However, the values of ATPase activity and ΔH both showed an initial stage of rapid denaturation (4–13 weeks) and a subsequent slower phase (Wagner & Añón, 1986). Thus, denaturation of myosin occurs prior to the formation of insoluble aggregates.

During the first 13 weeks the viscosity and the swelling of myofibrils tended to remain constant at all three storage temperatures; although at −5°C the myofibrillar solubility decreases from the first weeks on (Figs 1, 4 and 6). This indicates the formation of aggregates with few intermolecular bonds, allowing a high degree of hydration. After 13 weeks of storage, the viscosity, swelling and thixotropic effects rapidly decrease (Figs 4, 5 and 6) due to the formation of more compact, less hydrated aggregates which include in their structure a higher number of bonds. Breakdown of

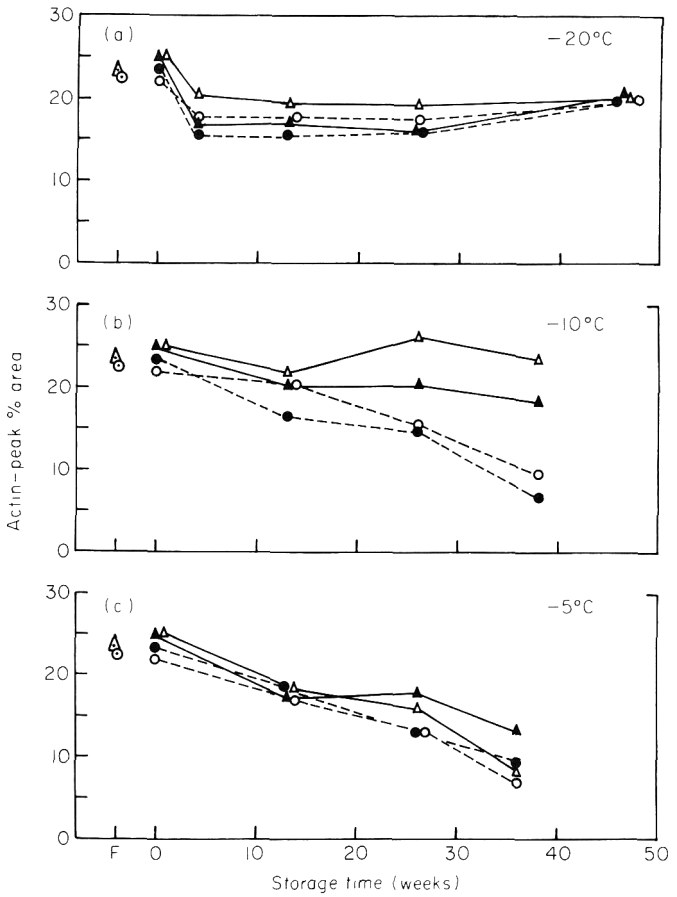

Figure 9. Effect of the frozen storage on the area of actin from the electrophoretic densitograms of myofibrils. ●, ▲: Myofibrils from fast frozen muscle treated with SDS and SDS+ME respectively. ○, △: myofibrils from slow frozen muscle treated with SDS and SDS+ME, respectively. ⊙, ◬: myofibrils from fresh muscle treated with SDS and SDS+ME, respectively.

myosin and actin, indicated by the increase of polypeptides of low molecular weights in the polyacrylamide gels, contributes to the decrease of these parameters towards the end of the storage period.

The electrophoretograms (Figs 7, 8 and 9) indicate that the first change observed after freezing is an alteration in the actin–myosin interaction. These results agree with previous studies carried out with rabbit muscle (Ito *et al.*, 1978; Kang *et al.*, 1983). During storage the formation of aggregates, probably formed by actomyosin, myosin and/or actin, occurs in addition to this phenomenon, first through non-covalent bonds, and thereafter by means of disulphide bridges (only at −5 and −10°C). King (1966), Connell (1968) and Matsumoto (1980a,b) have reported the formation of aggregates during storage of frozen fish muscle. According to Matsumoto (1980a) the aggregation is caused by the progressive formation of hydrogen bonds, ionic bonds, hydrophobic interactions and disulphide bridges.

Changes in ATPase activity, ΔH (Wagner & Anón, 1986) and water absorption capacity due to the freezing rate were observed, while during frozen storage, differences

between muscles frozen at fast and slow freezing rate were only observed in the solubility of myofibrillar proteins. The origin of this effect is being studied in our laboratory.

References

Awad, A., Powrie, W.D. & Fennema, O. (1968). Chemical deterioration of frozen bovine muscle at −4°C. *Journal of Food Science*, **33**, 227–235.

Bevilacqua, A.E., Zaritzky, N.E. & Calvelo, A. (1979). Histological measurements of ice in frozen beef. *Journal of Food Technology*, **14**, 237–251.

Carroll, R.J., Cavanaugh, J.R. & Rorer, F.P. (1981). Effects of frozen storage on the ultrastructure of bovine muscle. *Journal of Food Science*, **46**, 1091–1102.

Connell, J.J. (1968). The effects of freezing and frozen storage on the proteins of fish muscle. In: *Low Temperature Biology of Foodstuffs* (edited by J. Hawthorne and E.J. Rolfe). Pp. 333–358. Oxford: Pergamon Press.

Fennema, O.R., Powrie, W.D. & Marth, E.L. (1973). Characteristics of food myosystems and their behaviour during freeze-preservation. In: *Low Temperature Preservation of Foods and Living Matter*. Pp. 282–351. New York: Marcel Dekker, Inc.

Goll, D.E. & Robson, R.M. (1967). Molecular properties of post mortem muscle. I Myofibrillar nucleoside triphosphatase activity of bovine muscle. *Journal of Food Science*, **32**, 323–328.

Hay, J.D., Currie, R.W. & Wolfe, F.H. (1973). Effect of post mortem aging on chicken muscle fibrils. *Journal of Food Science*, **38**, 981–986.

Ito, T., Sung, S.K. & Fukazawa, T. (1978). Change of acto-heavy meromyosin ATPase of rabbit-skeletal muscle during postmortem storage. *Journal of Agricultural and Food Chemistry*, **26**, 324–326.

Kang, J.O., Ito, T. & Fukuzawa, T. (1983). Effect of frozen storage on the structure and enzymatic activities of myofibrillar proteins of rabbit skeletal muscle. *Meat Science*, **9**, 131–144.

Khan, A.W. (1966). Cryochemistry of animal tissue. Biochemical changes in poultry muscle during freezing and storage. *Cryobiology*, **3**, 224–229.

King, F.J. (1966). Ultrastructural analysis of changes in the composition of myofibrillar protein extracts obtained from fresh and frozen cod muscle. *Journal of Food Science*, **31**, 649–663.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*, **227**, 680–685.

Laemmli, U.K. & Favre, M. (1973). Maturation of the head of bacteriophage T_4. *Journal of Molecular Biology*, **80**, 575–599.

Matsumoto, J.J. (1980a). Chemical deterioration of muscle proteins during frozen storage. *ACS Symposium Series*, **123**, 95–124.

Matsumoto, J.J. (1980b). Denaturation of fish muscle proteins during frozen storage. In: *Advances in Chemistrys Series*, No 180. Pp. 205–224.

Rahelić, A., Pribis, V. & Skenderović, B. (1974). The influence of the time of freezing and period of frozen storage on the quality and characteristics of beef and port. I. Influence on water capacity. *Fleischwirtschaft*, **54**, 491–494, 497.

Robson, R.M., Goll, D.E. & Temple, M.J. (1968). Determination of protein in "tris" buffer by the biuret reaction. *Analytical Biochemistry*, **24**, 339–341.

Sikorski, Z.E., Olley, J. & Kostuch, S. (1976). Proteins changes in frozen fish. *Critical Reviews in Food Science and Nutrition*, **8**(1), 97–129.

Wagner, J.R. & Añón, M.C. (1985). Effect of freezing rate on the denaturation of myofibrillar proteins. *Journal of Food Technology*, **20**, 735–744.

Wagner, J.R. & Añón, M.C. (1986). Effect of frozen storage on Protein denaturation in bovine muscle. I Myofibrillar ATPase activity and Differential Scanning Colorimetric studies. *Journal of Food Technology*, **21**, 9–18.

Yamamoto, K., Samejima, K. & Yasui, T. (1977). A comparative study of the changes in hen pectoral muscle during storage at 4°C and −20°C. *Journal of Food Science*, **42**, 1642–1645.

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Determination of chloride, nitrate and calcium ions in sugar with ion selective-electrodes

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Summary

Chloride, nitrate, and calcium ion-selective electrodes were tested and found satisfactory for the determination of the corresponding ions in highly refined white sugar, molasses and other impure sugar samples at different stages of sugar manufacturing or refining. The interferences by Br^- and I^- ions in Cl^- determination were eliminated by oxidation with 3 M HNO_3 and passing air to remove the free Br_2 and I_2 formed. The Cl^- concentration was then determined with a chloride-selective electrode against a calibration curve. Chloride should not be determined by ashing sugar, since this causes high losses of Cl^-. It was found that NO_3^- and free Ca^{2+} ions could be determined directly in the sugar solution against corresponding calibration curves. Total calcium was determined after ashing of sugar samples. The ash was dissolved in 0.1 M HCl and passed through an anion exchange resin to remove PO_4^{3-} and SiO_3^{2-} ions, and Ca^{2+} was determined as before. Bound calcium was obtained by subtracting values of free from total calcium. Determination of bound calcium was useful to monitor the process of liming. For comparison, the three ions were also determined with acceptable precision without removing interfering ions using the method of standard addition and Gran's plot. The errors of determination for both direct and standard addition techniques were 3% for Cl^- and NO_3^- and 1–5% for Ca^{2+}.

Introduction

Ion-selective electrodes have proved to be ideal sensors for use in the analysis of industrial samples or in the control of a particular constituent in a process stream. The advantages they offer including fast response to changes in analyte concentration, and insensitivity to sample colour, viscosity or suspended solids (Thomas, 1980–1984), were the important reasons for their adoption in many applications.

The present work considers analysis of Cl^-, NO_3^- and Ca^{2+} in sugar samples and procedures with minimum pretreatment and reasonable accuracy are suggested.

The standard method for Cl^- determination in sugar was potentiometric titration with $AgNO_3$ using a Ag wire electrode. Deitz & Carpenter (1963), and Stachenko (1970) reported that Br^- and I^- were not present in sugar to such levels as to interfere. This is true in pure white sugar, but in the presence of these ions, in less pure samples of sugar or molasses, the method failed to give accurate results.

Pommez & Stachenko (1970), Brown & Pommez (1974), and later Juvier, Fernandez & Armas (1982) used a chloride ion selective electrode for monitoring the affination process in the sugar industry. These workers did not recommend procedures for accurate determination of Cl^- ion in sugar samples, especially when significant

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quantities of Br^- and I^- were present. Such objectives have been undertaken in the present work.

The second ion considered was NO_3^-. It is important to monitor its level as it can cause a reduction of sugar yield, if present in large quantities (McGaslin, Franklin & Dillon, 1970). Previous spectrophotometric methods for the determination of NO_3^- (Wood, Armstrong & Richard, 1967) had several drawbacks. They were lengthy and suffered from low accuracy. Nitrate ion-selective electrodes, however, have been found useful for analysis of beet root (McGaslin, *et al.*, 1970) and plant extracts (Jaroslav & Jiri, 1979). No application of this electrode for sugar samples could be traced in the literature up to 1983.

The third sugar analyte studied was calcium. This ion has, usually, been determined by EDTA (Gee, Domingues & Deitz, 1974) in the range 5–10 ppm. The method failed when applied for higher levels of Ca^{2+} due to the precipitation of Ca-sacchrate ($C_{12}H_{22}O_{11}$. 3CaO) especially at pH > 11 (Mark, McKetta & Othmer, 1969 and Bobrovnik, Voloshanenko & Khorunzhaya, 1981).

Other methods for Ca^{2+} determination in sugar include the indirect polarographic method (Iwasa, 1970) and atomic absorption (Lew, 1974). These methods, although useful for white refined sugar (Schiweck & Kilcioglu, 1969, ICUMSA, 1974), are not appropriate for high levels of Ca^{2+} in sugar syrups.

The calcium ion-selective electrode has only been used in sugar analysis by Juvier, Fernandez & Armas (1981), in cane juice. The present work shows the importance of a calcium electrode for control of the liming process. It presents procedures for the determination of both free and bound calcium in sugar samples.

Materials and methods

Reagents. All chemicals were analytical grade, doubly distilled water was used throughout and all readings were taken at room temperature.

Ionic strength adjusters (ISA). 5 M $NaNO_3$, for Cl^-, 2 M $(NH_4)_2SO_4$ for NO_3^- and 4 M KCl solution for Ca^{2+} determination. Two ml of the appropriate solution were added to each 100 ml portion of a standard or a sample to adjust the ionic strength. Nitrate solutions were preserved with 1 M boric acid (1 ml/100 ml of standard or sample).

Extraction reagent for NO_3^-. (Orion Methods Manual.) A solution of 16.66 g $Al_2(SO_4)_3 \cdot 18H_2O$, 1.24 g H_3BO_3, 4.67 g Ag_2SO_4, 2.43 g NH_2SO_3H (sulphamic acid) in 1 litre of water, adjusted to pH 3 with H_2SO_4.

Standard solutions

One mg/ml each of Cl^- and NO_3^- solutions were prepared by dissolving 1.74 g NaCl and 6.070 g $NaNO_3$ (dried at 110°C) respectively in 1 litre water. One thousand mg/ml Ca^{2+} solution was prepared by diluting a solution of Ca^{2+} (0.1 M) provided by Orion. Other concentrations of these solutions were prepared by serial dilutions.

Apparatus

A digital pH meter model 701A, and the following ion-selective electrodes: chloride model 94-17, iodide model 94-53, nitrate model 93-07 and calcium model 93-20 and reference electrodes single and double junction models 90-01 and 90-02, respectively were all supplied by Orion Research Inc. Mass. U.S.A.; the bromide electrode model F 1022 Br from Radiometer, Copenhagen.

Sampling

Samples were taken from Sulaimaniyah Factory which was producing sugar from two sources: (a) from local beet and (b) from imported raw sugar. A systematic procedure was used according to Tomlinson (1959) for taking the following samples directly from the factory: (a) white sugar (W.S.), (b) syrup-B: the juice produced after recycling the residual liquid from the resulting pure white sugar, (c) sugar-C: was the impure sugar produced from syrup-B, (d) molasses: the final impure residue.

Procedures

(1) Determination of Cl^- ion in sugar in the presence of Br^- and I^-

Method A: Direct Potentiometry. Oxidation of Br^- and I^- with HNO_3. The sugar sample (around 5 g) was dissolved in minimum quantities (about 4 ml) of 3 M HNO_3 in a suitable test tube. Air was bubbled (at a rate of 100–120 bubbles per minute) for 20 min to expel Br_2 and I_2 formed. The pH was then adjusted to 4 with 10 M NaOH and ionic strength to 0.5 M with $NaNO_3$ taking into consideration the quantities of HNO_3 and NaOH added for this purpose. The volume was made up to 100 ml in a volumetric flask. Chloride electrode readings were taken at the same time as those of the standard Cl^- solutions for preparing the calibration curve. The concentration of Cl^- ion in the sugar solution in (mg/l) was read from the calibration curve and the amount of Cl^- in the solid sugar samples was calculated.

Method B: Standard Addition. A 25 ml portion (or V_0) of 5% w/v sugar sample was adjusted to pH 4 and ionic strength to 0.5 M with $NaNO_3$ solution. The potential (E) of this solution was measured with the chloride electrode. One ml (or V_s) of a standard solution of mg/ml Cl^- (C_s) with the same pH and ionic strength was added and the potential was read again. This addition was repeated at least five times. Gran's function, F, where $F = (V_0+V_s)\ 10^{-E/s}$, (s = slope of the electrode) was plotted against the volume of the standard added. The straight line produced was drawn, ignoring the first two points if they were not on this line, and extrapolated back to cut the volume axis at V_e (zero value of F). The concentration of Cl^- in the original sugar solution (C_0 mg/l) was found as follows:

$$C_0 = (V_e \times C_s)/V_0.$$

The ration of Cl^- in solid sugar sample was calculated.

(2) Determination of NO_3^- in sugar

Procedure A: Direct determination of NO_3^-. The sugar sample (5 g) was dissolved in 30–40 ml water, ionic strength was adjusted to 0.04 M with $(NH_4)_2SO_4$, pH to 4 and the volume to 100 ml. Nitrate and reference electrodes were immersed into the solution and stirring was started. The potential was measured accurately and NO_3^- level was read against a calibration curve prepared under the same conditions and on the same day.

Procedure B: Standard addition method. The potential (E) of 5% w/v sugar solution 25 ml was read as in procedure A. One ml (V_s) of 100 ppm NO_3^- (C_s) standard was added, well stirred, and the potential was again recorded. The addition was repeated at least five times. Gran's function, F, where $F = (V_0+V_s)$. $10^{-E/S}$ was then plotted against V_s. The straight line obtained was extrapolated back to cut the volume axis at V_e

(zero value of F). The concentration of NO_3^- in the sugar solution (C_x) was calculated as follows:

$$C_x = (V_e \times C_s)/V_0.$$

The ratio of NO_3^- in sugar for both procedures was calculated.

3-Determination of free, total and bound Ca in sugar

Procedure A: Determination of free Ca. The sugar sample (5 g) was weighed accurately and dissolved in water in a beaker. Two ml of the ionic strength adjuster and water were added until the volume was about 80 ml. The pH of the solution was adjusted to between 5.5–6.5 with 0.1 M HCl or NaOH solution. The solution was diluted to 100 ml with water. The potential of the solution was read with the calcium electrode and Ca^{2+} ion concentration (C_x mg/l) in the solution was obtained from the calibration curve. The level of Ca in solid sugar samples was calculated.

Procedure B: Determination of total Ca. The sugar sample (5 g) was ignited slowly in a silica crucible, first on a small flame to avoid any loss, then was completed at 900°C in a muffle furnace for 2 hr. The residue, dissolved in a minimum quantity of 0.1 M HCl, was passed through a strong anion exchange resin (Dowex 2-X8 in the Cl^- form, 15–20 cm long column, 1 cm diam.) at a rate of 1 ml/min. The column was washed thoroughly with distilled water until a test for Cl^- with Ag^+ ion was negative. Ionic strength and pH were then adjusted and the concentration of Ca^{2+} was obtained as in procedure A.

Procedure C: Standard addition method. This was carried out as described under procedure B (no. 1 for Cl^- determination) except that $F = (V_0+V_s)\,10^{E/S}$. The method was used for both free and total Ca determination.

Results and discussion

Chloride

Measurement of Cl^- directly in sugar solution was unsatisfactory as readings continuously increased. At first, it was thought that the viscosity of sugar solution was the main reason. A similar observation had been reported by Pommez & Stachenko (1970) and Clarke (1970). They concluded it was due to decrease in the volume of water of sugar solution due to hydration of sucrose. This was not a problem in the present work, since the sugar solution was always completed to a fixed volume before Cl^- measurement. Ashing the sugar did not stabilize the readings, indicating that sucrose was not the cause of the problem.

The determination of possible interfering ions using corresponding ion-selective electrodes showed that only Br^- and I^- ions exceeded their interfering levels, especially in solutions of 5% impure sugar or molasses, where the concentration of Br^- reached 1.6 μg/ml. The concentration of I^- was around the calibration curvature and a rough estimate (obtained by simply extrapolating the straight line) in impure samples of sugar was around 6.3×10^{-4} μg/ml. Thus, concentrations of both ions were above the interfering levels (0.4 μg/ml for Br^- and 10^{-4} μg/ml for I^- relative to minimum Cl^- concentration of 60 μg/ml). These ions effectively poison the electrode's surface (Baiulescu & Cosofret, 1977).

Oxidation of Br^- and I^- to Br_2 and I_2 respectively, is the easiest way to remove these

ions effectively, due to the high volatility of Br_2 and I_2 even at room temperature. Two methods of oxidation were tested; first was the method of Havas, Papp & Pungor (1968) who used $KMnO_4$ as an oxidizing agent. The second method, however, was by using concentrated HNO_3 followed by bubbling air through the solution to remove Br_2 and I_2 (Martin, 1951). Different concentrations of HNO_3 (0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 M) and different periods of passing air (5, 10, 15 and 20 min) for each concentration were tried. The optimum condition was found to be 3 M HNO_3 and passing air for 20 min (Fig. 1). In this figure three calibration curves were made with the electrode; one for pure standards, the others for the same standards containing added Br^- and I^- as interferences; one of which was treated with 3 M HNO_3. After treatment the calibration was restored to that of the pure standards.

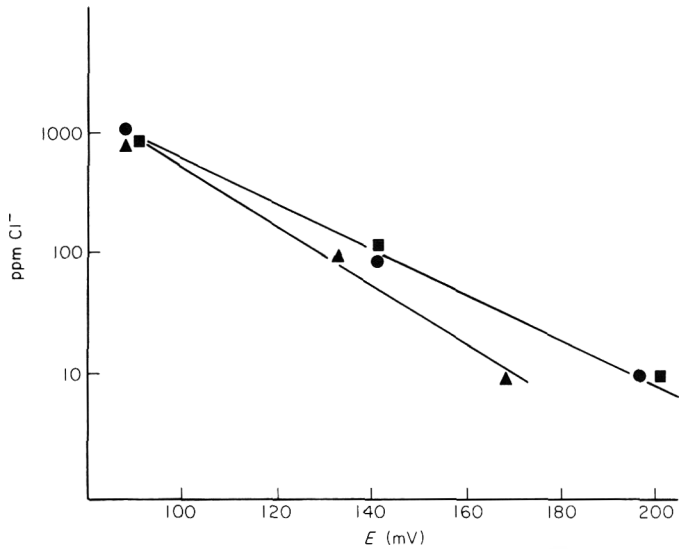

Figure 1. Calibration curve for chloride (Cl^-). (▲) with I^- and Br^- interferences. (●) after selective oxidation with HNO_3. (■) for pure Cl^- calibration.

Another convenient way to overcome the problem of interferences was by applying the method of standard addition (Moody & Thomas, 1973; Baiulescu & Cosofret, 1977). The principle of this technique depends on the fact that the effect of interfering ions is a function of concentration ratio, X^-/Cl^-, (where $X^- = Br^-$ or I^-). The successive addition of concentrated Cl^- made this ratio non-significant. This is shown in Fig. 2 which is a Gran's plot of a standard addition to a synthetic Cl^- solution containing Br^- and I^- at their interfering levels. The interfering ions lowered the first two points below the straight line drawn through the rest of the points, back extrapolation of which produced a calibration similar to that of samples containing no interfering ions.

Table 1 shows the results of Cl^- determination in sugar samples by the three methods, compared to the actual values of Cl^- present, obtained by the standard Ag^+-electrode method. The total amount of Br^- plus I^- was between 0–1.6 ppm and the maximum positive systematic error was 2.6%.

The table shows: (i) that the use of $KMnO_4$ as an oxidizing agent was not successful because of the high error; (ii) that the use of 3 M NHO_3, passing air for 20 min, gave only

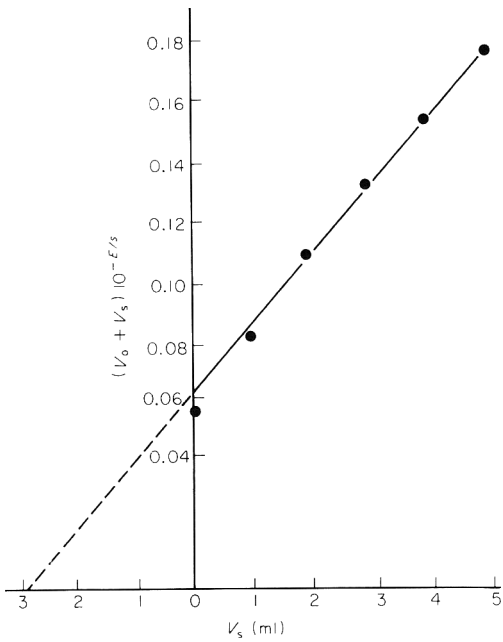

Figure 2. Typical standard addition curve (Gran's plot) for Cl^-.

a small error, not exceeding 3%; and (iii) the method of standard addition was a little less accurate, the error being around 5%.

Using the *t*-test (LeClarg, Leonard & Clark, 1962) results from both HNO_3 and the

Table 1. Methods of removing interferences of Br^- and I^- in the determination of Cl^- by the chloride ion-selective electrode

	Cl^- in mg/g sample						
		Found	% Error	Found	% Error	Found	% Error
Samples	Present (1)	(2)		(3)		(4)	
Sugar-C (Beet)	1.2	0.4	−66.6	1.16	−3.2	1.13	−5.5
Sugar-C (Raw)	2.2	0.84	−62.0	2.14	−2.7	2.30	4.8
Syrup-B	6.4	—	—	6.3	−1.5	6.24	−2.5
Molasses (Beet)	18.0	—	—	17.8	−1.1	17.9	−0.55
Molasses (Raw)	9.4	—	—	9.2	−2.1	5.32	−0.8

(1) Ag electrode reference method.
(2) Chloride determination after $KMnO_4$ treatment.
(3) After treatment with 3 M HNO_3 and passing air for 20 min.
(4) Standard addition method.
(—) Not determined.

Table 2. Effect of different ashing procedures on the loss of Cl^- from sugar samples

	Cl^- in mg/g sample				
		Found after ashing at:		Fixation	
Samples	Present before ashing	600°C for 2 hr	200°C for 12 hr	With H_2SO_4 then ashing at 600°C for 2 hr	With NaOH then ashing at 600°C for 2 hr
Sugar-C (Beet)	1.2	< 0.02	0.1	0.06	0.2
Sugar-C (Raw)	2.2	< 0.02	0.26	0.4	0.9
Syrup-B	6.4	0.6	2.64	1.0	2.4
Molasses (Beet)	18.0	2.4	9.0	7.6	7.4
Molasses	9.4	1.04	5.4	4.0	5.2

standard addition methods are different from those of the standard Ag-electrode method (95% confidence limit, d.f. = 4), possibly because the latter measures total halides instead of Cl^- ion alone. Both methods were faster than the standard method, six samples per hour compared with three by the standard procedure. Dry ashing of the samples at 600°C for 2 hr, 200°C for 12 hr, and fixation of Cl^- with H_2SO_4 or with NaOH prior to the ashing process at 600°C all gave large negative errors in Cl^- and were unsatisfactory (Table 2). Such errors can only be due to the formation and loss of volatile chlorides, and suggest that the recommended methods of ashing sugar samples for Cl^- determination (ICUMSA, 1954) should be retested. To this end NaCl and $CaCl_2$ were added to a highly refined white sugar, the samples were ashed at 600°C for 2 hr and the Cl^- was determined by both standard procedure (Ag-wire) and the recommended procedure using the chloride electrode. The samples were also analysed without ashing. Results, presented in Table 3, indicate that ashing of sugar caused a large loss of chloride, that from $CaCl_2$ being about three times more than that from NaCl. The high Cl^- loss in sugar C (Table 2) suggests that Cl^- in sugar is present mostly in $CaCl_2$ form.

Table 3. The affect of ashing at 600°C for 2 hr on Cl^- determination in white sugar using both standard and Cl^- electrode methods

	Cl^- found (mg/g)				
	On solution		After ashing		
Cl^- added (mg Cl^-/g white sugar)	Cl^- electrode	Ag-wire	Cl^- electrode	Ag-wire	Mean loss (%)
None	0.02	n.d.	n.d.	n.d.	n.d.
6.06 as NaCl	6.06	6.06	4.51	4.57	−25.5
6.39 as $CaCl_2$	6.39	6.39	1.53	1.56	−76.0

Table 4. NO_3^- levels before and after removal of interferences with extracting solution and using the Cu/Cd method

Sample	NO_3 in mg/g sample			
	Before removal of interferences	±s.e.	After removal of interferences	Cu/Cd
Sugar-C (Beet)	0.38	0.01	0.38	0.40
Sugar-C (Raw)	0.53	0.01	0.54	0.55
B-Syrup	0.60	0.01	0.60	0.63
Molasses (Beet)	4.61	0.05	4.60	4.44
Molasses (Raw)	0.81	0.02	0.82	0.75

Nitrate

Table 4 presents results of NO_3^- determination in sugar samples, using the NO_3^- selective electrode, with and without treatment to remove interfering ions. This has no effect on the NO_3^- determination, which could therefore be determined directly. Comparison with the Cu/Cd reduction method (Wood *et al.*, 1967) showed that the results were not significantly different (*t*-test, 95% confidence limit, d.f. = 4). Triplicate analysis by the direct method gave a mean s.e. of 0.02 and an average coefficient of variation of about 3%. The method is much faster than the colorimetric method using phenol disulphonic acid (PDA), ten samples per hour compared to only four samples per hour by the PDA method.

Calcium

Possible interfering ions, measured by atomic absorption and flame photometry, did not reach such levels as to interfere and free Ca^{2+} was determined without any pretreatment and compared with the method of standard addition (Table 5). As Ca^{2+} cannot be determined directly in sugar samples by the EDTA method, results using the calcium electrode could not be compared. Results are in good agreement and free Ca^{2+} can be determined directly in sugar solution with reasonable precision (coefficient of variation 1–5%).

For total Ca determination, the sugar sample was ashed at 900°C, the ash dissolved in HCl and the pH was adjusted to between 5.5–6.5. A turbid solution (white suspension) was observed, which suggested the possible presence of PO_4^{3-} and SiO_3^{2-}. Two qualitative tests were carried out. Ammonium molybdate reagent (Vogel, 1954) gave a

Table 5. Determination of free Ca^{2+} in sugar with the calcium ion-selective electrode by both direct calibration and standard addition methods and total Ca by ashing and EDTA titration

Samples	Ca in mg/g sample					
	Direct Free calibration	±s.e.	Standard addition	Ca electrode	Total EDTA	Bound (Total-Free) Ca^{2+}-electrode
Sugar-C (Beet)	1.14	0.04	1.09	1.28	1.32	0.14
Sugar-C (Raw)	2.12	0.04	2.18	2.20	2.26	0.08
Syrup-B	5.40	0.03	5.48	8.00	7.92	2.60
Molasses (Beet)	9.10	0.06	8.84	18.00	17.84	8.90
Molasses (Raw)	5.30	0.06	5.34	9.00	9.08	3.70

positive test, and u.v. absorption of ammonium molybdate treated sugar solutions and standards of PO_4^{3-} and SiO_3^{2-} solutions, at the same pH, gave peak maxima for all at 225 nm. Turbidity appearing only after ashing was probably soluble Ca phosphate, present in the sugar solution before ashing, that was converted after ignition at 900°C, to insoluble pyrophosphate (Liptay, 1975). Formation of hydrated silica on ignition and HCl treatment may also have caused some turbidity. Passing the sugar ash, dissolved in HCl, through a strong anion exchanger (Dowex 2-X8) in Cl^- form prevented formation of turbidity after pH adjustment (between 5.5–6.5). Total Ca^{2+} determined after this treatment was compared with that by EDTA titration (Table 5) and the *t*-test showed no significant difference between the two methods at 90% confidence limit (d.f. = 4).

The calcium electrode has advantages over the EDTA method in that both free and total calcium could be measured. This may be important in sugar analysis since Ca^{2+} can bind with sugar. Bound Ca^{2+} was calculated by subtracting free Ca^{2+} from total (Table 5). It shows that much bound calcium was present in molasses (5–9 mg/g); possibly as Ca-saccharate formed during the liming process and only partly decomposed by carbonation. Thus the calcium electrode maybe a useful tool to monitor the important process of liming and deliming.

References

Baiulescu, G.E. & Cosofret, V.V. (1977). *Application of Ion-Selective Membrane Electrodes in Organic Analysis*, 1st edn. (English translation edited by R.A. Chalmers and M.R. Masson). Pp. 53–86. England: Ellis Horwood.

Bobrovnik, L.D., Voloshanenko, G.P. & Khorunzhaya, L.V. (1981). Effect of sucrose on the peptization of sediments of calcium salts during liming of diffusion juice. *Sakhanaya Promyshlenno st.* **10**, 30–31. *Chemical Abstracts*, (1982), **96**, 21576.

Brown, C.R. & Pommez, P. (1974). Chloride control in the refinery. *Cane Sugar Refining Research Project, (CSRRP)*. Pp. 76–92.

Clarke, H.A. (1970). The effect of solution structure on electrode processes in sugar solution. *Cane Sugar Refining Research Project (CSRRP)*. Pp. 179–188.

Deitz, V.R. & Carpenter, F.G. (1963). Special techniques for use in sugar analysis. In: *Cane Sugar Handbook*, 9th edn. (edited by G.P. Meade). Pp. 605–615. USA: John Wiley and Sons.

Gee, A., Domingues, L.P. & Deitz, V.R. (1954). Determination of inorganic constituents in sucrose solutions. *Analytical Chemistry*, **26**, 1487–1491.

Havas, J., Papp, E. & Pungor, E. (1968). Membranes of heterogeneous structure for the determination of the activity of anions. II. Analytical application of bromide and chloride-selective membrane electrodes. *Acta Chimica* (Hungary), **58**, 9–21. *Chemical Abstracts* (1969), **70**, 83910.

ICUMSA. *International Commission for Uniform Methods of Sugar Analysis* (1954). Determination of ash content of sugar products. Reports on the Proceedings of the 11th Session, 9–14 June, 1954, Paris. Pp. 10–17.

ICUMSA. *International Commission for Uniform Methods of Sugar Analysis* (1974). Inorganic non-sugars. Reports on the Proceedings of the 16th Session, 2–7 June, 1974, Ankara. Pp. 224–234.

Iwasa, N. (1970). Polarographic determination of inorganic non-sugars. ICUMSA. *International Commission for Uniform Methods of Sugar Analysis*. Reports on the Proceedings of the 15th Session, 10–15 May, 1970, London, Appendix 1. Pp. 176–182.

Jaroslav, S. and Jiri, P. (1979). Nitrate ion-selective electrode *Chemicke Listy*, **73**, 1097–1105. *Chemical Abstracts* (1980), **92**, 51269.

Juvier, L.H.M., Fernandez, L.R.C. & Armas, T.A.R. (1981). Study of a calcium electrode in cane juices by the sample addition method. Comparison with the titration method using EDTA. *CubaAzucar*, October–December, p. 11.

Juvier, L.H.M., Fernandez, L.R.C. & Armas, T.A.R. (1982). Affination and a chloride ion-selective electrode *CubaAzucar*, July–September, p. 7.

LeClarg, E.L., Leonard, W.H. & Clark, A.G. (1962). *Field Plot Technique* 2nd edition. Minnesota: Burgess Publishing Co.

Lew, R.B. (1974). Inorganic non-sugars. ICUMSA. *International Commission for Uniform Methods of Sugar Analysis*. Reports on the Proceedings of the 16th Session, 2–7 June, 1974, Ankara. Pp. 224–234.

Liptay, G. (ed.) (1973). *Atlas of Thermoanalytical Curves*. (no. 217). Budapest: Akademiai Kiado.

Mark, H.F., McKetta, J.J. & Othmer, D.F. (1969). *Encyclopedia of Chemical Technology*, Volume 19. Pp. 151–242. USA: John Wiley and Sons.

Martin, F. (1951). *Mikrochemie*. 36137, 653. Cited by: Macdonald, A.M.G. Determination of Halogens and Sulphur in Organic Compounds. In: *Comprehensive Analytical Chemistry*, (edited by C.L. Wilson and D.W. Wilson). Vol. IB. Pp. 510–546. The Netherlands: Elsevier.

McGaslin, B.D., Franklin, W.T. & Dillon, M.A. (1970). Rapid determination of nitrate nitrogen in sugar-beets with the specific ion electrodes. *Journal of the American Society of Sugar Beet Technologists*, **16**, 64–70.

Moody, G.J. & Thomas, J.D.R. (1973). Selective Ion-Sensitive Electrodes. *Selected Annual Reviews of the Analytical Sciences*, **3**, 59–138.

Orion Research (1976). 93 series electrodes. *Methods Manual*. Cambridge, Massachusetts: Orion Research Inc.

Orion Ionalyzer (1977). Halide electrodes *Instruction Manual*. Cambridge, Massachusetts: Orion Research Inc.

Orion Research (1977). Nitrate ion electrode. *Instruction Manual*. Cambridge, Massachusetts: Orion Research Inc.

Orion Research (1977). Calcium electrode. *Instruction Manual*. Cambridge, Massachusetts: Orion Research Inc.

Pommez, P. & Stachenko, S. (1970). Use of chloride electrodes in refinery control. *Proceedings of the 1970 Technical Session on Cane Sugar Refining Research*. Pp. 82–102. Boston, Massachusetts, U.S.A.

Schiweck, H. & Kilcioglu, E. (1969). Determination of heavy metals in consumer sugars, molasses, cosettes and Pulp. *Zucker*, **22**, 321–325. *Chemical Abstracts* (1969), **71**, 69393.

Stachenko, S. (1970). Inorganic non-sugars. ICUMSA. *International Commission for Uniform Methods of Sugar Analysis*. Reports of the Proceedings of the 15th Session, 10–15 May, 1970, London. Pp. 172–182.

Thomas, J.D.R. (ed.) (1980–1984). *Ion-Selective Electrode reviews*. Volumes (1–6).

Tomlinson, R.C. (1959). Sampling. In: *Comprehensive Analytical Chemistry* (edited by C.L. Wilson and D.W. Wilson), Volume IA. Pp. 36–75. The Netherlands: Elsevier.

Vogel, A.I. (1954). *A Text Book of Macro and Semimicro Qualitative Inorganic Analysis*, 4th edn. P. 386. London: Longmans.

Wood, E.D., Armstrong, F.A.J. & Richard, F.A. (1967). Determination of NO_3^- in sea Water by Cd-Cu reduction to NO_2^-. *Journal of Marine Biological Association*, **47**, 23–31.

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Studies on the development of texturized vegetable products by the extrusion process. III. Effects of processing variables of thiamin retention*

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Summary

Thiamin retention in extruded cowpea, mungbean, defatted soybean and air classified mungbean products was studied by a least squares regression equation using process temperature, screw speed, buffer pH and moisture content as the major independent variables. The increase in process temperature, pH and screw speed variables effected the decrease of thiamin retention values, while the increase in moisture content increased retention. The heat generation due to increase of stress force caused by increased screw speed presumably resulted in the thiamin retention values which were lower than expected. The carbohydrate and protein of the feed material flours protected the thiamin during the thermoplastic extrusion process.

Introduction

Previous investigations have shown that it is possible to texturize cowpea, mungbean and air classified mungbean flours by extrusion processes. These products could be utilized in production of snack food, instant baby food, texturized vegetable protein and other food formulations (Pham & Del Rosario, 1984). The functional properties of these products have been compared with those of the texturized defatted soybean. Nitrogen solubility index and water absorption capacity values are better in texturized air classified mungbean than in texturized defatted soybean. However, there is limited information on the retention of thiamin in extruded legume products.

The first recorded property of thiamin was its relative ease of destruction by heat (Williams, 1938). According to the study, heating of thiamin in neutral solutions resulted in the cleavage of the molecule at the methylene bridge to yield pyrimidine and thiozole fragments. McIntere & Frost (1944) showed that α- and β-amino acids and some of their derivatives have a marked stabilizing effect upon thiamin at pH 6.

The observation that thiamin in natural foods is more heat resistant than thiamin in aqueous and buffered solutions indicates the existence of factors that can stabilize the reaction. Thiamin retention (88.6%) in cookies baked from commercially enriched four is predicted by a least squares regression equation as the major independent variable. At a constant soda level, the colour of the cookie may be used as a quality control guide to thiamin retention (Keagy, Connor & Schatzi, 1979).

Beetner *et al.* (1974) studied the retention of thiamin during extrusion processing. They used two temperatures, 139 and 193.4°C, feed moisture content of 13 and 16%,

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and screw speed of 75 and 125 rpm in the extrusion of corn grits. Increased retention of thiamin from lower temperature and slower screw speed were found to be very significant.

The present study of thiamin retention on the extrusion process is necessary to confirm nutrient quality and the variables of extrusion. This information can help to optimize the development of quality and nutrient content of products made from cowpea (CP), mungbean (MB), defatted soybean (DSB) and air classified mungbean (ACMB) flours.

Materials and methods

Raw Materials

Legume seeds used in this study were cowpea [*Vigna sinensis* (L.), Wilczek], mungbean [*Vigna radiata* (L.) Wilczek] and soybean [*Glycine max* (L.) Merr.]. Seed samples were obtained from the open market at Divisoria in Manila. These dried samples were contained in rice bags and stored in a cold room (10°C) prior to use.

Extrusion cooking

The preparation of samples, experimental design and extrusion processing have been described previously (Pham & Del Rosario, 1984) except for the procedure given below. Feed rates were 15 kg (d.b.)/hr for CP and MB at 100–200 rpm, and 12.5 kg (d.b.)/hr for DSB and ACMB at 10–30 rpm.

Thiamin extraction and assay

Thiamin in the raw materials and after extrusion processing was determined by the thiochrome method (Miller, Guadagni & Kon, 1973). The thiochrome method depends upon the oxidation of thiamin to thiochrome which fluoresces under u.v. light. Fluorescence was measured with a 12B Coleman Electronic Photofluorimeter model using quinine sulphate as reference standard.

Thiamin retention is that remaining in the extruded sample expressed as a percentage of the thiamin in the raw material, both on a dry weight basis.

Statistical analysis

Four process variables in the experimental design of the extrusion process used for cowpea, mungbean, defatted soybean and air classified mungbean flours were applied for coded transformation (Pham & Del Rosario, 1984). The lowest process variable value was coded -1, and the highest value $+1$.

The data were statistically analysed by means of stepwise multiple regression to determine the process variables significant in the thiamin retention. The method of least squares was used to obtain the best estimate of the dependent variables. All terms not significant at the 0.05 probability level were combined with the residual. Random error was estimated from replicate extrusion. Lack of fit was calculated as the difference between the residual sum of squares and the pure error sum of squares. The regression equation fitted a response surface type equation. First order effects, squared terms and first order interaction terms were allowed to enter the equation which corresponded to the F-test value with a confidence level of approximately 95%.

Table 1. Thiamin retention in extruded cowpea (ECP), mungbean (EMB), defatted soybean (EDSB), and air classified mungbean (EACMB) flours as affected by processing variables (%)

Source of variation	Coefficient				Mean square				Degree of freedom			
	ECP	EMB	EDSB	EACMB	ECP	EMB	EDSB	EACMB	ECP	EMB	EDSB	EACMB
Model					546.28*	583.72*	271.95*	538.77*	13	11	12	11
X_0	32.50*	27.30*	21.19*	27.15*								
X_1	2.46*	2.69*	2.01*	2.02*	400.17	468.78	228.37	251.98	1	1	1	1
X_2	−3.33*	−2.97*	−3*	−3.98*	931.42	796.81	638.96	1181.71	1	1	1	1
X_3	−2.94*	−2.74*	−1.49*	−2.37*	891.14	701.64	154.11	397.92	1	1	1	1
X_4	−9.12*	−8.91*	−6.11*	−8.32*	4371.75	4280.06	2102.86	3796.05	1	1	1	1
X_1X_1	1.24**	0.58**	0.67**	0.92**	19.30	11.82	15.60	11.65	1	1	1	1
X_2X_2	−1.24**		−0.78**		61.26		12.63		1		1	
X_3X_3	−3.11*		−1.11**	−2.29*	153.06		21.44	94.57	1		1	1
X_4X_4	−1.01**	−1.12**			17.06	22.17			1	1		
X_1X_2		0.52**	0.46**	0.54**		11.73	12.75	14.99		1	1	1
X_1X_3	−0.85*	−0.67*	−0.66*		37.82	28.58	18.15		1	1	1	
X_1X_4	−0.59**	−0.69**		−1.08*	23.88	25.50		49.50	1	1		1
X_2X_3	1.31*	1.06*	0.63*	1.00*	105.57	59.78	18.13	56.30	1	1	1	1
X_2X_4	1.25*	0.60**	0.72*	0.97*	76.59	14.95	24.30	43.84	1	1	1	1
X_3X_4	0.58**		0.56**	0.60**	12.64		13.55	16.56	1		1	1
Residual					4.76	4.48	2.98	3.54	76	78	77	78
Lack of fit					9.25	7.95	5.88	5.99	31	33	32	33
Error					1.74	2.28	1.13	1.73	45	45	45	45
Corrected total					83.86	76.08	39.22	69.56	89	89	89	89

X_0 = constant, X_1 = moisture content (%), X_2 = pH buffer, X_3 = screw speed (rpm), X_4 = process temperature (°C).
*Significant at $P = 0.01$.
**Significant at $P = 0.05$.
$R^2_{ECP} = 0.95$; $R^2_{EMB} = 0.95$; $R^2_{EDSB} = 0.93$; $R^2_{EACMB} = 0.96$.

Results and discussion

Analysis of variance for regression

The retention of thiamin in texturized vegetable products has been studied as a function of moisture content, buffer pH, screw speed and process temperature. The analysis of variance for regression of the data is summarized in Table 1.

The average thiamin retention in the extruded cowpea (ECP, 32.5%) was higher than that of extruded mungbean (EMB, 21.2%). The presence of high carbohydrate in the feed materials such as CP (66.74%) and MB (63.25%) could account for the differences in the adsorption, and perhaps provide protection from the effects of the extrusion process by the hydrophilic polysaccharides. Similar observations were made in relation to the stabilization of thiamin in conventional food process (Mulley, Stumbo & Hunting, 1975). On the other hand, thiamin retention of extruded air classified mungbean (EACMB, 27.2%) is higher than that of extruded defatted soybean (EDSB. 21.2%) used as a reference for a texturized vegetable protein, possibly due to the higher protein content (58.1%) of EACMB than EDSB (50.6%). These results confirm the findings of McIntire & Frost (1944) that protein could protect the thiamin in severe thermal processes.

Statistical analysis of the data indicated a strong dependence of thiamin retention on the process temperature and pH (Table 1). Several interactions (pH×process temperature, screw speed×process temperature, etc.) were also significant at $P = 0.05$ or better.

The regression coefficient of determination (R^2) is for example, 0.95 for ECP product, indicating that 95% of the variation in extrusion process is accounted for in the model. It contributed 88–92% of the first order terms of the effects on thiamin retention in four types of texturized products. The different R^2 also suggested that the influence of the operating variables on thiamin retention depends on the type of feed material used in the extrusion process.

Effects of moisture content and process temperature

The retention of thiamin decreased as the feed moisture content decreased from 45 to 30% (Table 1), possibly due to the viscosity of thermoplastic materials in the die zone. At a given process temperature, as moisture content of feed materials increased, this viscosity would decrease and the less viscous material would flow more rapidly out of the system, decreasing thiamin loss.

The retention of thiamin decreased as the process temperature increased, confirming earlier observations of Beetner *et al.* (1974). To illustrate the effects of both process temperature and moisture content on thiamin retention, the other variables such as pH buffer and screw speed were held constant (pH 6.8; screw speed 150 rpm for CP and MB; and 20 rpm for DSB and ACMB). The contour maps generated represent thiamin retention (Fig. 1). The maximum thiamin retention values occur at high moisture content and low process temperature for the four extruded products.

Effects of moisture content and screw speed

At a given process temperature and pH, the thiamin retention values decreased as the screw speed increased (Fig. 2), increasing the stress force and consequent induced heat generation in the extruder. However, the effect of screw speed is also dependent on moisture content. At high levels the effect of screw speed on the thiamin retention values was less than the effect at low moisture level, probably because viscosity would increase greatly as feed moisture decreased and screw speed increased.

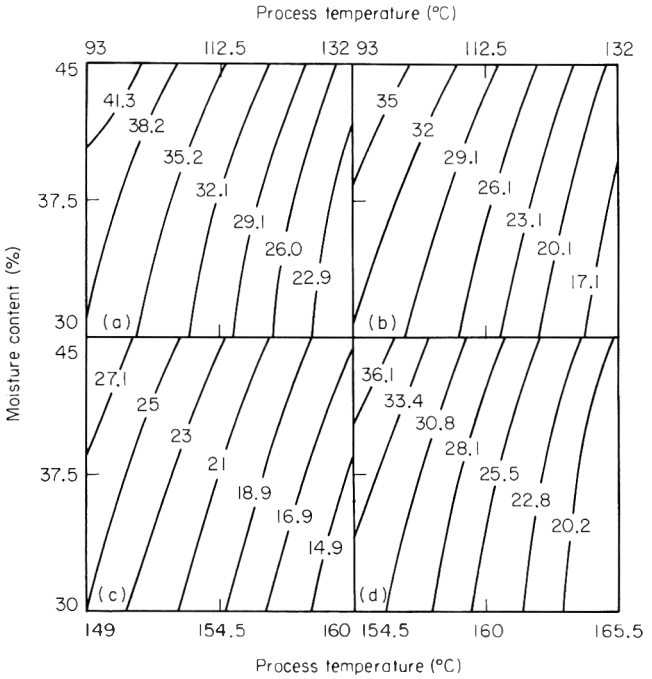

Figure 1. Thiamin retention contour maps as function of moisture content and process temperature in extruded: (a) cowpea (150 rpm, pH 6.8); (b) mungbean (150 rpm, pH 6.8); (c) defatted soybean (20 rpm, pH 6.8); (d) air classified mungbean (20 rpm, pH 6.8).

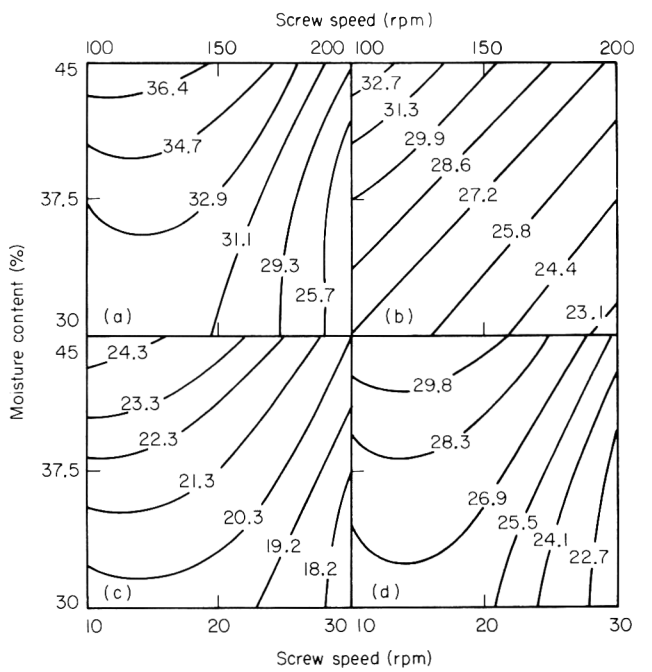

Figure 2. Thiamin retention contour maps as function of moisture content and screw speed in extruded: (a) cowpea (ph 6.8, 112.5°C); (b) mungbean (pH 6.8, 112.5°C); (c) defatted soybean (pH 6.8, 154.5°C); (d) air classified mungbean (pH 6.8, 160°C).

Effects of pH and process temperature

Thiamin retention was found to decrease with increased pH buffer of feed materials (Fig. 3). At pH 6.2, thiamin retention was higher in ECP products than in EMB products, with the same process variables in the extrusion process. With increased pH and process temperature, thiamin retention decreased more rapidly in ECP products (20.1%) than in EMB products (18.5%). Similar decreases were observed in relation to destruction of thiamin in EDSB (12.4%) and EACMB (17.4%) products in the presence of high pH buffer.

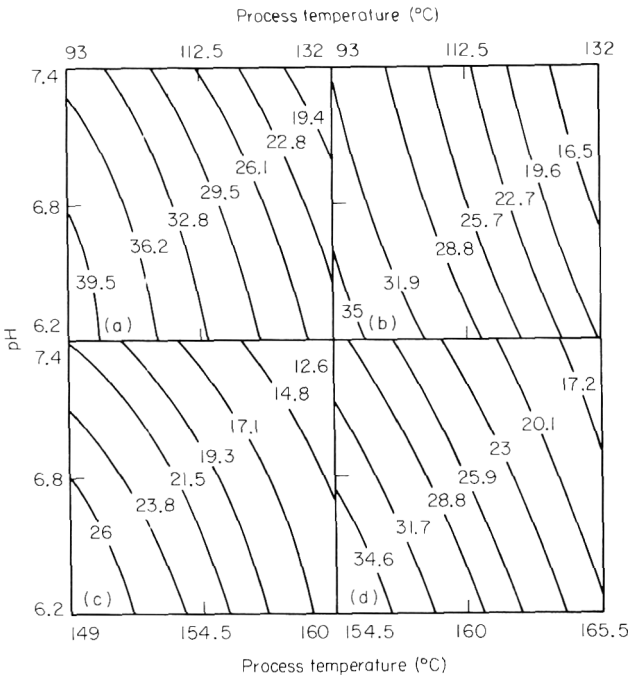

Figure 3. Thiamin retention contour maps as function of pH and process temperature in extruded: (a) cowpea (MC 37.5%, 150 rpm); (b) mungbean (MC 37.5%, 150 rpm); (c) defatted soybean (MC 37.5%, 20 rpm); (d) air classified mungbean (MC 37.5%, 20 rpm).

The rapidity of the decrease in thiamin in the alkaline medium could be due to the difference in the chemical environment of the raw materials. Compared to SB thiamin, the CP, MB and ACMB thiamins showed greater resistance to alkaline medium as indicated by more drastic conditions used to effect thiamin retention. This was possibly caused by the rapid cleavage of the sulphite from the methylene bridge in a nucleophilic displacement reaction in SB-thiamin. Similar effects were observed on the thiamin stability in food products and model systems to different pH media (Dwivedi & Arnold, 1973).

Effects of pH and screw speed

The previous results showed that pH or screw speed are significant variables in the extrusion process. Since these two variables were confounded, an experiment was conducted in which they were varied independently. It can be seen that at certain pHs and screw speeds significant improvements in thiamin retention were possible (Fig. 4).

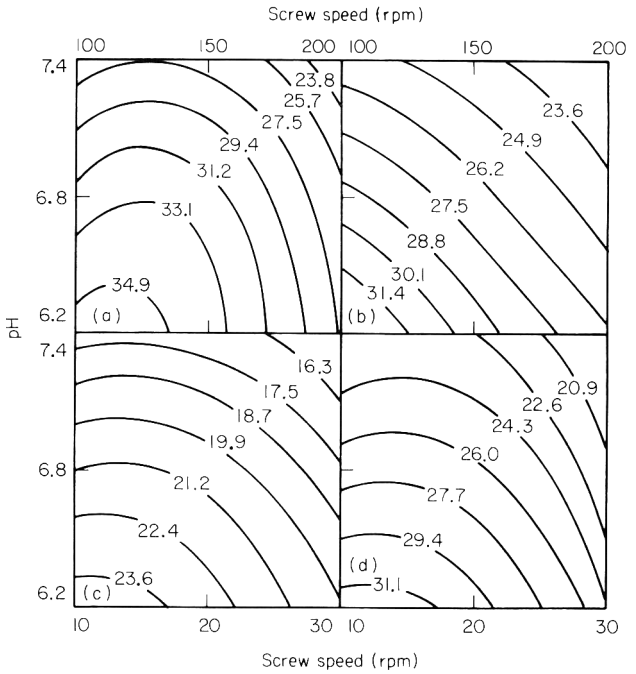

Figure 4. Thiamin retention contour maps as function of pH and screw speed in extruded: (a) cowpea (MC 37.5%, 112.5°C); (b) mungbean (MC 37.5%, 112.5°C); (c) defatted soybean (MC 37.5%, 154.5°C); (d) air classified mungbean (MC 37.5%, 160°C).

The pH was varied from 6.2 to 7.4, and screw speed from 100 to 200 rpm for ECP and EMB products, and 10 to 30 rpm for high protein content of EDSB and EACMB products. In all cases of extruded products, the lowest pH and screw speed resulted in high retention of thiamin. The figure also indicates an interaction between pH buffer and screw speed. This influence confirms previous results that starch and/or protein in feed materials play an important role in protecting thiamin retention.

Conclusion

Thiamin retention in CP, MB, DSB and ACMB products texturized by extrusion processes is a function of moisture content, buffer pH, screw speed and process temperature. The average thiamin retention value is 32.5% in ECP, 27.3% in EMB, 21.2% in DSB and 27.2% in EACMB products. At high process temperature and low moisture content, the increase of screw speed decreased thiamin retention values as buffer pH increased. The effect of process temperature is a quadratic function in ECP and EMB products, while in EDSB and EACMB products it is predominantly a linear function. The increasing screw speed at low moisture content produced heat generation which possibly resulted in a lower thiamin retention than the true values obtained.

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References

Beetner, G., Tsao, T., Frey, A. & Harper, J. (1974). Degradation of thiamin and riboflavin during extrusion processing. *Journal of Food Science*, **39**, 207–208.

Dwivedi, B.K. & Arnold, R.G. (1973). Chemistry of thiamin degradation in food products and model systems: A review. *Journal of Agricultural and Food Chemistry*, **21**, 54–60.

Keagy, P.M., Connor, M.A. & Schatzi, T.F. (1979). Regression analysis of thiamin and color and changes in enriched cookies using factorial design. *Cereal Chemistry*, **56**, 567–573.

McIntire, F.C. & Frost, D.V. (1944) Thiamin stability. Effects of amino acids and related compounds and of thiamin concentration. *Journal of the American Oil Chemists' Society*, **66**, 1317–1318.

Miller, C.F., Guadagni, D.G. & Kon, S. (1973). Vitamin retention in bean products. Cooked, canned and instant bean powders. *Journal of Food Science*, **38**, 493–495.

Mulley, E.A., Stumbo, C.R. & Hunting, W.M. (1975). Kinetics of thiamin degradation by heat. Effect of pH and form of the vitamin on its rate of destruction. *Journal of Food Science*, **40**, 989–992.

Pham, B.C. & Del Rosario, R.R. (1984) Studies on the development of texturized vegetable products by the extrusion process. I. Effect of processing variables on proteins properties. *Journal of Food Technology*, **19**, 535–547.

Williams, R.R. (1938). The Chemistry of thiamin. *Journal of American Medical Association*, **110**, 727–734.

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Thermal properties of potatoes and a computer simulation model of a blanching process

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Summary

Thermal conductivity and specific heat were experimentally determined in Bintje potatoes. The methods used were a transient hot-strip method and differential scanning calorimetry respectively. The results from the experiments were used in a theoretical calculation model using the finite element technique. Temperature profiles and heat transfer coefficients were simulated and the theoretical calculations were compared with experimental data for the blanching process. The correlation between experimental data and theoretical calculations was good if the heat transfer coefficient was assumed to be 750 $W/m^2{}^\circ C$.

Introduction

A useful tool for the food engineer when studying the behaviour of a process is a computer program based on mathematical models. When constructing this model it is necessary to study the heat and mass transport in different food products during processing, together with the knowledge about kinetic processes. Different processes like blanching, drying, frying, and combinations of processes also have to be studied.

In the future, knowledge concerning all aspects of the process will be very important for overall optimization. At present it is possible to obtain a quick answer on how to regulate and operate different stages of a process in order to obtain a product of the highest quality (Bruin, Hallström & Jowitt, 1984).

In order to construct a simulation model for the time–temperature profile for a potato blanching process, knowledge about thermal properties is necessary. The thermal properties of potatoes have been studied by Yamada (1970), who determined the specific heat at different moisture contents and also the thermal conductivity. Rao, Barnad & Kenny (1975) investigated squash and potatoes, and presented values for thermal conductivity and thermal diffusivity. Matthews & Hall (1968) and Rao *et al.* (1975) conducted studies on thermal diffusivity. Wadsworth & Spadaro (1969, 1970) determined the thermal diffusivity of sweet potatoes and also devised computer programs which were used to simulate the transient temperature distribution.

In the present work, the thermal conductivity and specific heat of potatoes were estimated for further use in a finite-element simulation program. The heat transfer coefficient and the temperature profile in potatoes during blanching were also simulated using the same computer program. The results are compared with experimental data.

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Materials and methods

The substrate was Bintje potatoes, density 1085 kg/m^3, and moisture content, 79.8%.

The method used for measuring the thermal conductivity, λ, was a transient hot strip method, by Gustafsson, Karawacki & Khan (1979) in which the strip is used as both a constant plane heat source and as a sensor of temperature.

To determine thermal conductivity the potato was cut into two slices, the metal strip held firmly between the two plane potato slices and the whole immersed in a water bath to reach constant temperature, then the voltage recording was started. The metal strip was 77.2 mm long and 5.72 mm wide. The total experimental time was 30 sec, during which about 90 voltage recordings were made.

The specific heat was measured by Differential Scanning Calorimetry (DSC, Perking Elmer DSC-2C) in lyophilized potatoes with different water contents. Water was added to the samples and equilibrated before analysis. The samples (5–10 mg) were dried after analysis (105°C, 24 hr). The moisture contents used were 85.4, 68.2, 63.5, 39.2 and 8.1% total weight. The samples were enclosed in aluminium cans. As a reference an empty can was used, and to obtain the baseline two empty cans were scanned (10°C/min from 5–95°C) at least twice and evaluated at 5°C intervals. The difference between the baseline and sample curve is proportional to the specific heat. The proportionality constant was obtained by scanning doubly distilled water, with a well known specific heat value.

The blanching procedure took place in a thermostatically controlled water bath, volume 10 litre and temperature accuracy ±0.1°C.

The potatoes, cut into pieces and placed in a wire net cage, were blanched at 65 and 75°C for 10 min. Three Cr/Al thermocouples with a thickness of 0.3 mm were placed at different distances relative to the surface (Fig. 1).

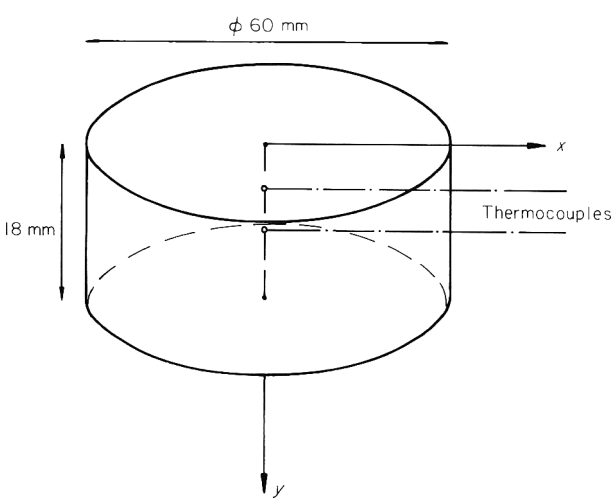

Figure 1. The experimental potato model. Diameter 60 mm, thickness 18 mm.

Computer simulation and finite element modelling

Stationary and non-stationary heat conduction may be analysed by means of the finite-element technique, a numerical procedure for solving differential equations. A model (Segerlind, 1976) is constructed as follows:

(1) A finite number of points in the domain is identified as nodal points or nodes.

(2) The value of the continuous quantity of each nodal point is denoted as a variable which is to be determined.

(3) The domain is divided into a finite number of subdomains called elements, and connected at common nodal points collectively approximating the shape of the domain.

(4) The continuous quantity is approximated over each element by a polynomial that is defined using the nodal values of the continuous quantity.

Advantages of the finite element method are: (i) irregularly shaped boundaries can be approximated; (ii) the material properties in adjacent elements do not have to be the same; (iii) the size of the elements can be varied and (iv) mixed boundary conditions can be handled. In this investigation the THAFEM program (Andersson, Fröier & Loyd, 1983) was used. This uses finite elements to solve the heat conduction differential equation for solid two-dimensional bodies and for solid three-dimensional bodies possessing cylindrical symmetry. The bodies are defined by arbitrarily set polygonic boundaries and the program allows for anisotropic heat conductivities. System size and phase transitions, as well as any potential temperature dependence of the conductivity, may be taken into account. The finite element grid is constructed by the program, and is denser in areas of large curvature and in areas where large temperature gradients are expected.

In this investigation rough estimates of initial values of the heat flux over the boundary and the node temperatures were used. The heat flux over the boundary was assumed to be convective. Any part of the boundary lacking explicitly defined boundary conditions, was treated as completely isolated. It was assumed that the surrounding volume 10 litre and temperature accuracy ±0.1°C water was well mixed and had no temperature gradient.

Nomenclature

α = Heat transfer coefficient (W/m^2°C),
λ = thermal conductivity (W/m °C),
T = temperature (°C).

Results and discussion

Thermal conductivity

The results of the measurements on thermal conductivity are presented in Fig. 2. Mathematically, the thermal conductivity may be approximated by a linear function of the temperature.

At 79.8% moisture content net weight, linear regression of 2 on T gave a best fit of

$$\lambda\,(T) = 0.00119 \cdot T + 0.624$$

with a correlation coefficient of 0.90.

These measurements were carried out on four different potatoes in a water bath. The results then represent the variation in raw material during the blanching process.

Rao *et al.* (1975) determined thermal conductivity and thermal diffusivity with a line-source method. They reported values of thermal conductivity between 0.533 and 0.571 W/m °C with a moisture content of about 82% wet weight. They also related the thermal conductivity data to moisture content and density at room temperature. The

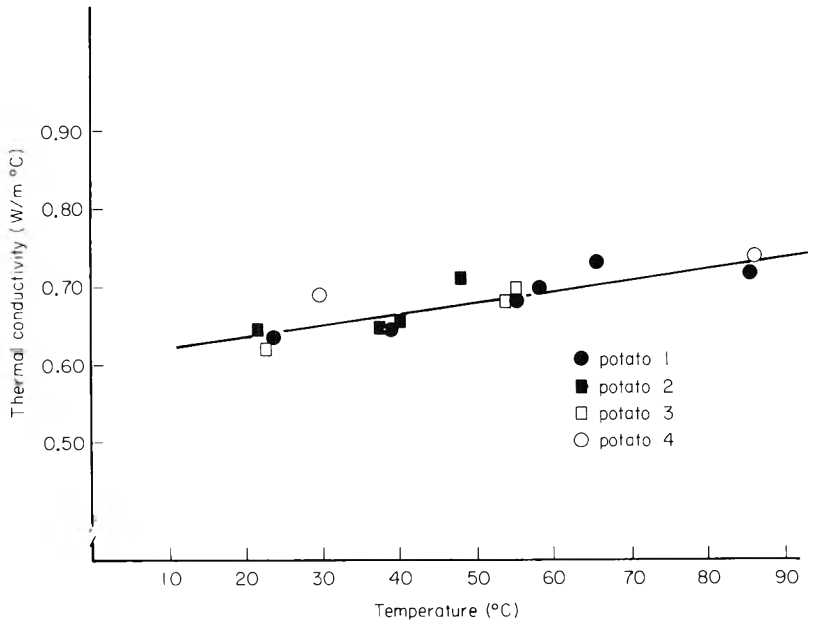

Figure 2. The thermal conductivity *versus* the temperature.

moisture content, density and heat capacity affect the thermal conductivity and it is therefore of great importance to characterize all these parameters.

Yamada (1970) measured the thermal conductivity using a method involving the non-steady-state heat conduction of a sphere. He found the thermal conductivity at 76% moisture content to range between 0.485 and 0.556 W/m °C at 10–75°C. Tschubik & Maslow (1973) reported values of 0.61–0.66 W/m °C for different potato varieties. The last-mentioned data agree well with the results of the present investigation.

Specific heat

Figure 3 shows the heat capacity as a function of temperature and water content. The heat capacity was constant over the temperature intervals 5–55 and 75–95°C at moisture contents of 85.4, 68.2 and 63.5%. The heat capacity had a maximum value in the temperature interval 55–75°C, which correspond to gelatinization. At 85.4% moisture content, the heat capacity of potatoes was found to be 3.610 kWs/kg °C. This value agrees well with the result obtained by Yamada (1970), of 3.640 kWs/kg °C at 83% water content.

Gelatinization starts at 55°C and ceases at 75°C. Maximum gelatinization is reached at 66°C. Eliasson, Larsson & Miezis (1981) investigated the gelatinization of freeze dried potato starch and original potato starch, and found that freeze drying lowered the gelatinization temperature from 66 to 60°C. In our investigation no difference in gelatinization temperature was observed; freeze dried potatoes showed the same gelatinization temperature as original potato starch, i.e., 66°C.

At moisture contents of 39.8 and 8.1% there was no peak at the actual gelatinization interval. The heat capacity increased slowly, which may be due to the gelatinization effect. The influence of water content on gelatinization, is also shown in Fig. 3.

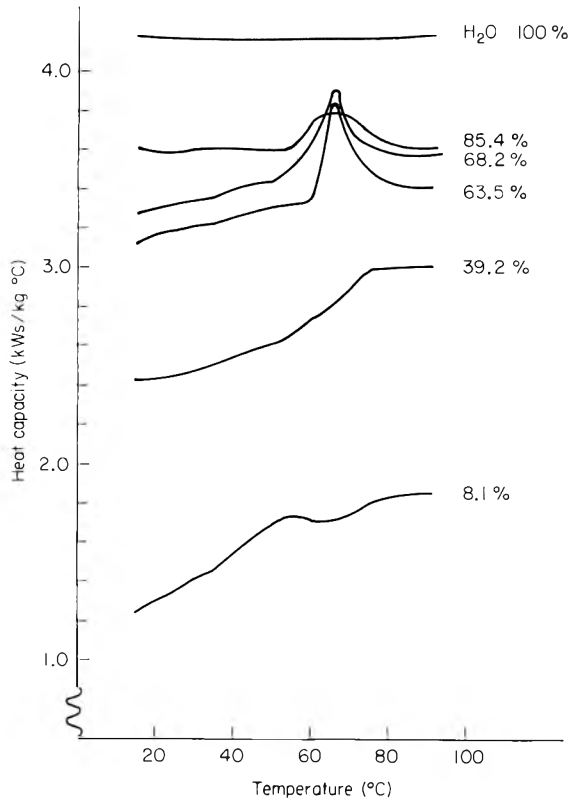

Figure 3. The heat capacity *versus* the temperature. Lyophilized potatoes with different water contents, 85.4, 68.2, 63.5, 39.2 and 8.1%, as a reference water.

In the interval 63.5–85.4% moisture content, the degree of gelatinization decreased with increase in moisture content, an effect also reported by Eliasson *et al.* (1981). With a water to starch ratio of 1:1, the gelatinization enthalpy was 9600 Ws/kg starch and increased to 21700 Ws/kg with a 2:1 water to starch ratio. The slope of the heat capacity curve increases as the water content is reduced.

Calculation model

When using the THAFEM program (Andersson, Fröier & Loyd, 1983), it is necessary to follow a program input schedule, containing material and geometry specifications as follows: thermal properties, geometry, initial temperatures, convection boundary and time steps for the calculations intervals. In this investigation heat conductivity, density and specific heat are experimental data. The geometry was chosen to simulate the heated potato in the blanching process. To satisfy the schedule, some program inputs were postulated.

* Thermal properties

Heat conductivity	0.648 and 0.719 W/m °C, (T = 20°C and 80°C, respectively)
Density	1085 kg/m^3
Specific heat	3600 Ws/kg °C

Phase transition	4300 Ws/kg
Phase transition temperature	66°C

* Geometry
See Fig. 4
* Initial tempratures

Surface	50°C
Inner part	20°C

* Convection boundary

Heat transfer coefficients	750 and 126 W/m^2 °C
Temperature of boundary fluid	75°C

* Time steps
1, 5, 10, 20 sec.

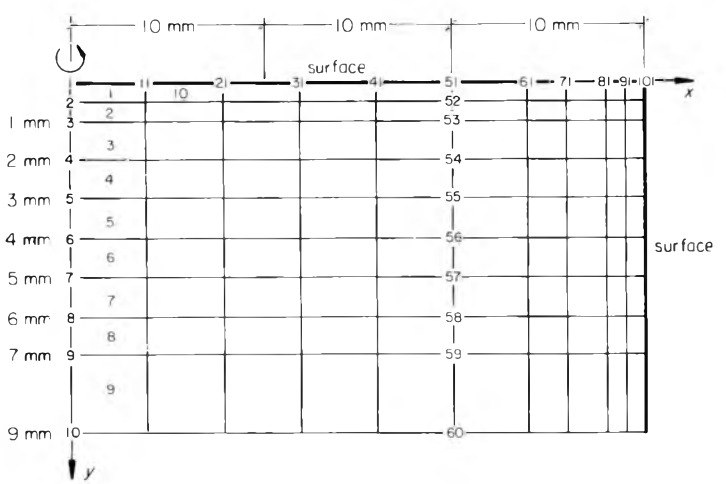

Figure 4. The potato geometry of the simulation model.

Temperature measurements and computer simulation

The measured and simulated time–temperature distribution curves, are presented in Figs 5 and 6. Figure 5 shows the variation in the time–temperature dependence at different water temperatures. In Fig. 6 the simulated time–temperature distribution is compared with the experimental. Figure 6 also shows the difference in time–temperature dependence for different heat transfer coefficients α = 750W/m^2 °C, (Mörstedt, 1976) and α = 126 W/m^2 °C (Perry, 1963).

Wadsworth *et al.* (1970) performed a computer simulation based on the finite-difference method. They compared variations in thermal diffusivity, heat transfer coefficient and size of different potatoes. The finite-element method is a modern and more general technique used in many applications and it is easy to construct a model

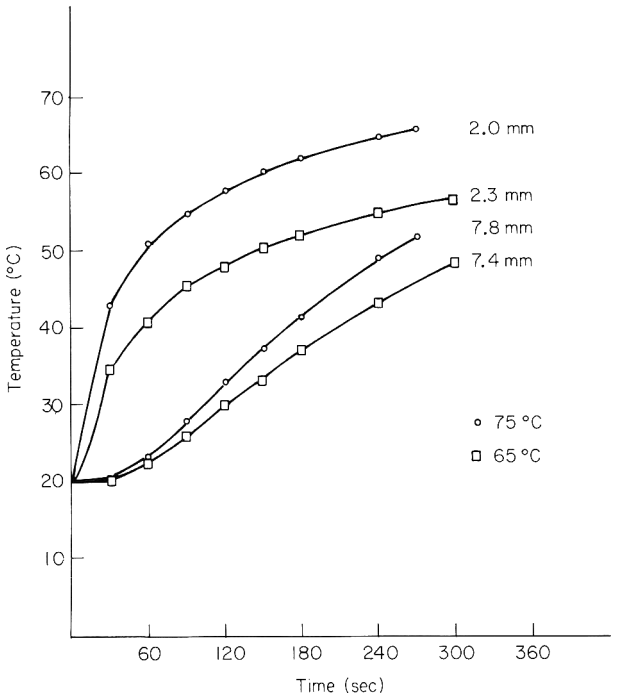

Figure 5. The variation in time–temperature dependence at different water temperatures.

even if the geometry is complex. When performing a simulation, it is important to be able to predict the heat transfer coefficient accurately, as a limited amount of experimental data are available.

Mörstedt (1976) and Perry (1963) gave the greatest difference in heat transfer values with varying moisture content. In the present investigation the largest heat transfer coefficient gave the most likely time–temperature profile compared with experimental measurements. The heat transfer is intensive in this heating procedure and the heat transfer coefficient $\alpha = 750$ W/m^2 °C therefore gives the best fit. Another aspect which must be considered in a computer simulation is the temperature dependence of the thermal conductivity. Determined experimentally, the thermal conductivities at 20 and 80°C were 0.648 and 0.719 W/m°C respectively. Figure 7 shows the variation in the simulated time–temperature curve for these values. The time–temperature curves were duplicated 3 mm from the surface. From 3 mm towards the centre there was a small deviation, less the 6% (9 mm, 180 sec) which can be neglected. In this investigation the simulation model is rectangular with rotation symmetry; this is not strictly realistic, and the model can be improved, but for the purpose of this study it was not deemed necessary.

Conclusion

Measuring thermal properties of foodstuffs is normally a delicate problem. Rapid denaturation of the materials, especially at an increased temperature, changes the

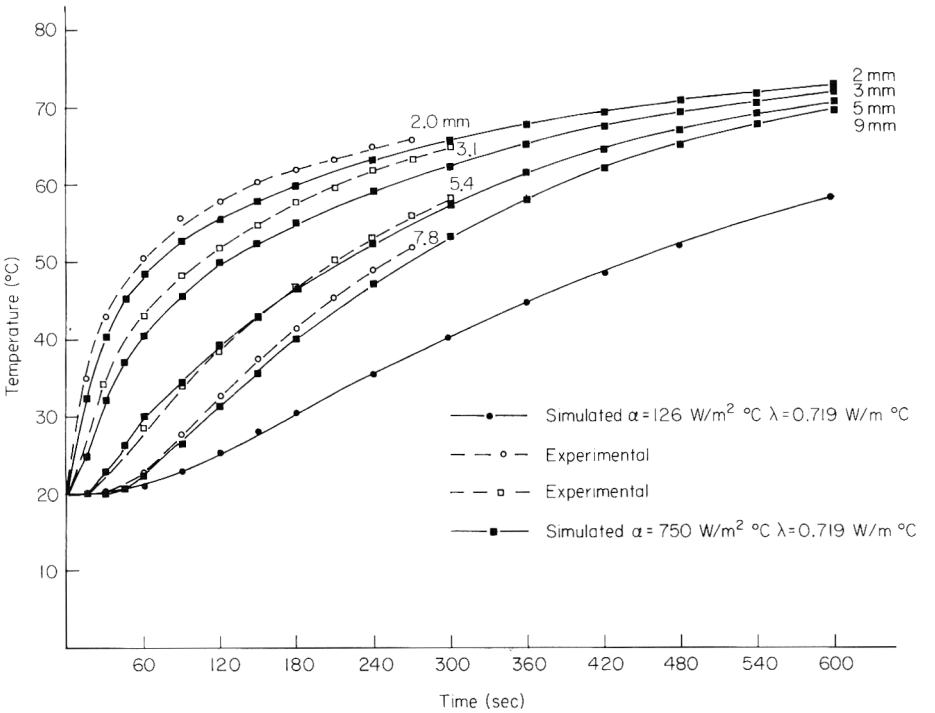

Figure 6. Simulated time–temperature distribution compared with the experimental. The difference in time–temperature dependence for different heat transfer coefficients, α.

properties and makes the results less reliable. Even a small temperature gradient applied to give measurable data may be enough to affect the result. The transient hot-strip method used here, overcomes to a great extent these difficulties, and may be used at elevated temperatures. It is the first time this method has been used for food materials, and we conclude that it is appropriate to such problems.

This work also indicates the potential applicability of finite-element simulation of temperatures in food applications. Further use is foreseen for example in the optimization of industrial processes, although this would need data on the kinetics of the chemical reactions involved; for instance, destruction of nutritional properties.

Acknowledgments

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References

Andersson, G., Fröier, M. & Loyd, D. (1983). *THAFEM Computer program.* Institute of Technology, Department of Mechanical Engineering S-581 83 Linköping, Sweden.

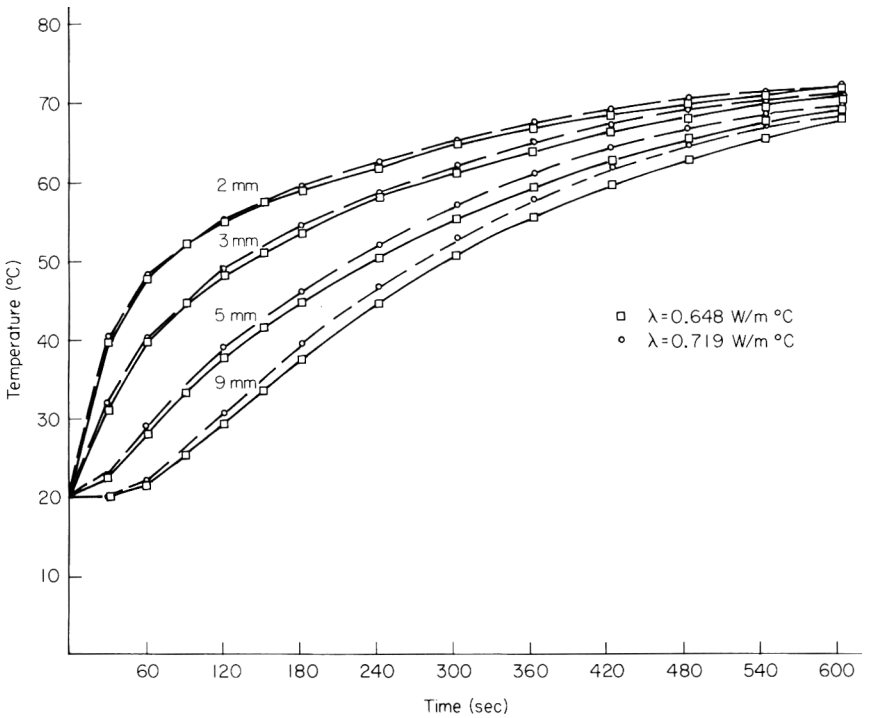

Figure 7. The variation in the simulated time–temperature curve with different thermal conductivity.

Bruin, S., Hallström, B. & Jowitt, R. (1984). Food process engineering—a model syllabus. *Journal of Food engineering*, **3**, 205–223.

Eliasson, A-C., Larsson, K. & Miezis, Y. (1981). The possibility of modifying the gelatinization properties of starch by lipid surface coating. *Stärke*, **33**, 231–235.

Froier, M. & Loyd, D. (1982). *Beräkning av värmeledningsproblem med finit elementmetod–en introduktion*, 5th edn. IKP, Linköping Institute of Technology, Sweden.

Gustafsson, S.E., Karawacki, E. & Khan, M.N. (1979). Transient hot-strip method for simultaneously measuring thermal conductivity and thermal diffusivity of solids and fluids. *Journal of Physics D: Applied Physics*, **12**, 1411–1421.

Matthews, F.V. & Hall, C.W. (1968). Method of finite differences used to relate changes in thermal and physical properties of potatoes. *Transactions of the American Society of Agricultural Engineers*, **11**, 558–562.

Mörstedt, S-E. & Hellsten, G. (1976). *Data och diagram*, 3rd edn., p 11. Stockholm, Sweden.

Perry, J.H. (1963). *Chemical Engineers' Handbook*, 4th edn. Tokyo, Japan.

Rao, M.A., Barnad, J. & Kenny, J.F. (1975). Thermal conductivity and thermal diffusivity of process variety squash and white potatoes. *Transactions of the American Society of Agricultural Engineers*, **18**, 1188–1192.

Segerlind, L.J. (1976). *Applied Finite Element Analysis*. John Wiley & Sons, Inc. New York.

Tschubik, I.A. & Maslow, A.M. (1973). *Wärmephysikalische Konstanten von Lebensmitteln und Halbfabrikaten*, 1st edn., p. 141. veb Fachbuchverlag, Leipzig, Germany.

Wadsworth, J.I. & Spadaro, J.J. (1969). Transient temperature distribution in whole sweet potato roots during immersion heating. 1. Thermal diffusivity of sweet potatoes. *Food Technology*, **23**, 85–89.

Wadsworth, J.I. & Spadaro, J.J. (1970). Transient temperature distribution in whole sweet potato roots during immersion heating. 2. Computer simulation. *Food Technology*, **24**, 77–84.

Yamada, T. (1970). The thermal properties of potato. *Journal of the Agriculaturl Chemical Society of Japan*, **44**, 587–590.

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Journal of Food Technology (1986) 21, 587–603

Effects of polysaccharide stabilizers on the nucleation of ice

A. H. MUHR*, J. M. V. BLANSHARD AND S. J. SHEARD

Summary

Sodium alginate, methyl cellulose and sodium carboxymethyl cellulose at concentrations of ~ 1% do not change the homogeneous nucleation temperature (T_H) of water or of sucrose solutions by more than the experimental uncertainty of ±2°C. Stabilizers at a concentration of 0.0067 g/ml showed a variety of effects on heterogeneous nucleation in 0.75 g/ml sucrose solution. Xanthan gum showed a slight inhibitory effect, methyl cellulose showed little effect, sodium pectate, sodium carboxymethyl cellulose and microcrystalline cellulose caused slight increases in the incidence of heterogeneous nucleation, and sodium alginate caused a substantial increase. The effect of sodium alginate can probably be attributed to inclusions rather than to the polysaccharide molecules themselves. It is concluded that none of the stabilizers affects nucleation of ice to an extent which would be of significance in frozen confectionery products.

Introduction

It is widely believed that hydrophilic gums ('stabilizers') influence ice formation in frozen confectionery products. The qualitative observation is that the use of stabilizers results in a product with a finer ice crystal texture. Several mechanisms have been proposed to explain the effect, one being that stabilizers influence the nucleation of ice (Blanshard, 1970).

Nucleation may either be homogeneous (i.e., spontaneous) or heterogeneous (i.e., initiated by nucleation agents or motes). The influence of stabilizers (which may give 'solutions' of a somewhat heterogeneous character) on both types of nucleation is of interest.

(1) *Homogeneous nucleation*

The general theory of homogeneous nucleation has been, and still is, a subject of lively debate (for a review, see Pruppacher & Klett, 1978). For the case of water the difficulties are even greater, since, unlike other undercooled liquids, water exhibits a rapid rise in specific heat and rapid approach of density to that of the crystalline phase as the temperature is reduced (Angell, 1983).

However, the classical theory of homogeneous nucleation is the only predictive model available, and provides a useful basis for discussion even though it involves a number of dubious assumptions. According to this theory, the free energy of formation

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(ΔF) of an ice-like cluster of water molecules (assumed here to be a sphere of radius r) is given by

$$\Delta F(r) = \tfrac{4}{3}\pi r^3 A + 4\pi r^2 \sigma^{iw} \quad (1)$$

where A is the bulk free energy of formation of unit volume of ice from the solution (a negative quantity) and σ^{iw} is the macroscopic surface energy of the ice-solution interface (a positive quantity). Differentiation shows that a critical radius exists

$$r^* = -2\sigma^{iw}/A \quad (2)$$

such that clusters with $r < r^*$ are unstable, but any cluster that reaches r^* by a chance fluctuation will act as a centre of growth for ice. As a first approximation we may neglect the temperature dependence of σ^{iw}, and use the simple expression

$$A \simeq -(LT/T_0^2)\Delta T \quad (3)$$

where L is the latent heat per unit volume of ice, T_0 is the melting point of ice and ΔT is the undercooling of the solution (equal to $T_E - T$ where T_E is the equilibrium freezing point of the solution). Plots of r^* *versus* ΔT obtained in this way are given in Fig. 1. As there is no ambiguity, σ is written in place of σ^{iw}.

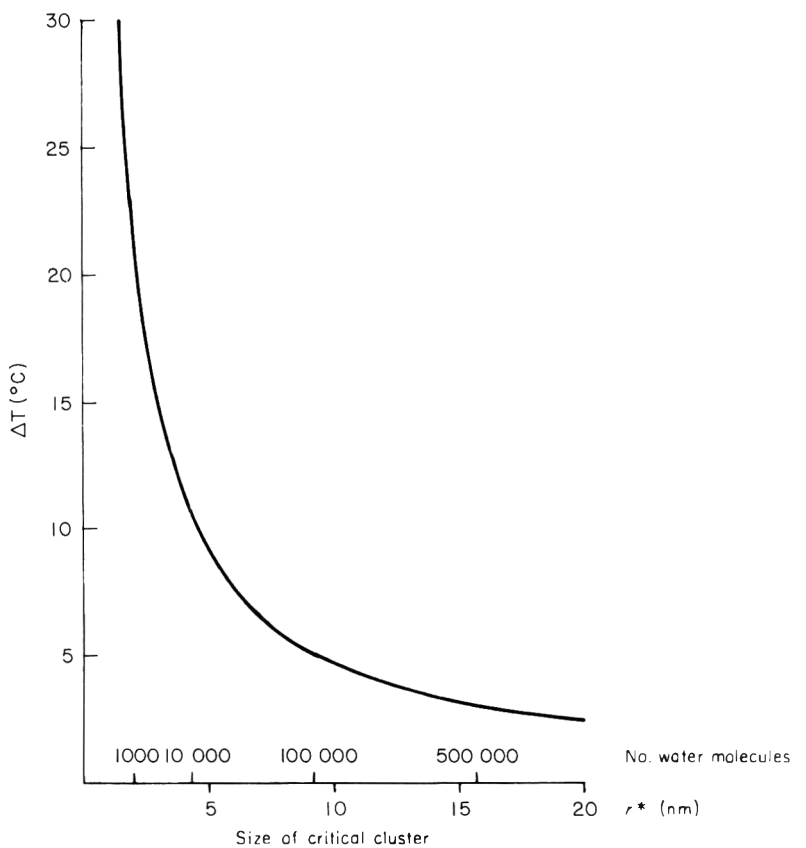

Figure 1. Relationship of undercooling (ΔT) and the radius of the critical cluster (r^*) for aqueous solutions ($\sigma = 2.5\times10^{-2}$ J/m^2, L = 3.07×10^8 J/m^3).

The rate of nucleation is thus limited by the kinetic barrier to chance growth of sub-critical clusters to the critical size. Making further approximations, the rate of formation of nuclei per unit volume, J (/m^3/sec), is a function of temperature (Muhr, 1983; Franks, Mathias & Trafford, 1984):

$$J \simeq K_1 (\sigma T)^{1/2} \phi^2 \exp(-\Delta f/kT) \exp(-K_2 \sigma^3/\Delta T^2 T^3) \quad (4)$$

where ϕ is the volume fraction of water in the solution, Δf is the free energy of activation of diffusion of the slowest moving component of the solution, and K_1 and K_2 are obtained from fundamental constants and constants relating to pure water and have the values (Muhr, 1983):

$K_1 \simeq 2.40 \times 10^{40}\ J^{-1/2} m^2 s^{-1}$,
$K_2 \simeq 6.06 \times 10^{16}\ m^6 K^5 J^{-3}$.

Plots of (4) with σ, Δf and T_E as parameters are given in Fig. 2. As Δf probably increases rapidly as ΔT increases (Stillinger, 1980; Angell, 1983), Fig. 2 (in which the parameter Δf is independent of temperature) is only a schematic representation.

From equation 4 it can be seen that solutes can affect J for a given value of ΔT by:
(i) *altering* σ; Fig. 2 (curves 1, 2 and 6) shows that small changes in σ greatly affect J.

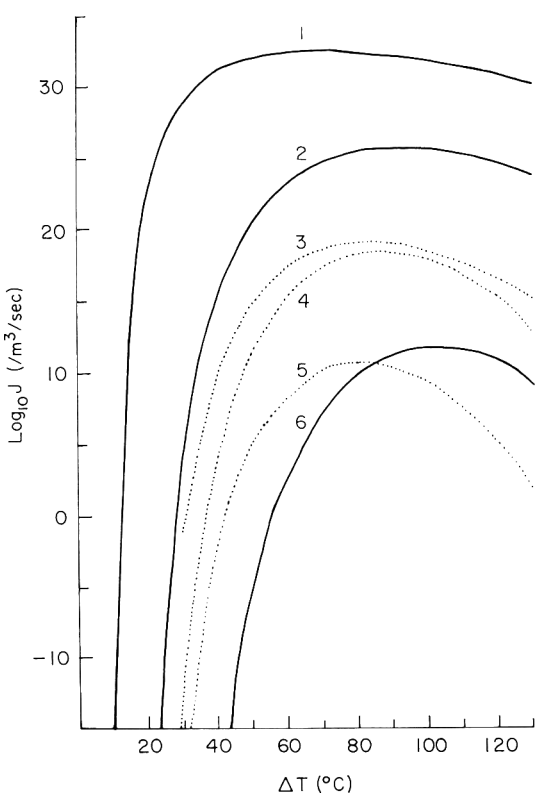

Figure 2. Rate of homogeneous nucleation (J) as a function of undercooling (ΔT) with σ, T_E and Δf as parameters.

(ii) *altering* Δf; solutes increase Δf at temperatures greater than T_E, but by reducing the 'structuring' of water they may tend to offset the rise in Δf observed in undercooled water. Rasmussen & MacKenzie (1972) have tried to interpolate Δf in some undercooled solutions (assuming a viscosity of 10^{13} poise at the glass transition temperature) but their values are by no means certain.

(iii) *reducing* T_E; this will reduce J at a given value of ΔT chiefly because $\exp(-K_2\sigma^3/\Delta T^2 T^3)$ gets smaller as T falls (for constant ΔT and σ), but the lower value of T should also lead to a smaller value of $\exp(-\Delta f/kT)$. In comparison, the pre-exponential factor of $T^{1/2}$ can be regarded as virtually constant.

(iv) *altering* ϕ; since ϕ^2 occurs only as a pre-exponential factor this effect will not be significant.

Experimental investigations of homogeneous nucleation are usually conducted on an emulsion of the solution in an inert carrier fluid. For a sufficiently small droplet size this confines motes, otherwise capable of inducing heterogeneous nucleation (and subsequent widespread crystallization), to a negligible proportion of the population.

The remaining large number of droplets thus freed from heterogeneous crystallization provides a statistically adequate number of independent nucleation events (one per droplet) to give useful information about J in a single temperature scan. For aqueous solutions either the volume (Rasmussen & MacKenzie, 1973) or the enthalpy (Rasmussen & MacKenzie, 1972) of the emulsion is monitored as a function of T. From Fig. 2 it can be seen that for a given droplet size, the number of droplets freezing per second passes from an extremely low value (i.e., negligible probability) to an extremely large value (i.e., all droplets freeze after a short time), over a short temperature interval. The temperature at which J becomes one per droplet per second is a representative point within this short temperature interval, and is spoken of as 'the temperature of homogeneous nucleation', T_H. For pure water (choosing the reasonable estimates $\sigma = 25\times10^{-3}$ J/m^2 and $\Delta f = 23.57$ kJ/mol), for droplets of volume 10^{-16}m^3 (i.e., radius ~ 3 μm and $J = 10^{16}$/m^3/sec), use of Fig. 2 curve 2 gives $T_H \sim -41$°C, which is near to the observed value ($T_H = -38.5$°C, Rasmussen & MacKenzie, 1972). The extreme sensitivity of T_H to σ makes measurement of T_H a sensitive method of determining σ. However, the values so obtained are the fruits of a theory based on dubious assumptions.

It is expected from the theory (in particular, as a consequence of equation 3) that all solutes (at a moderate concentration so that T_E is not too low) will depress T_H by only a little more than the colligative depression of the equilibrium freezing point ($\Delta T_E = T_0 - T_E$). In practice, however, the depression of T_H is around $2\Delta T_H$ for micromolecular solutes, and up to 6 ΔT_E for macromolecular solutes (Franks, 1983; Rasmussen & MacKenzie, 1972). Rationalization of such large depressions of T_H in terms of altered values of σ and/or Δf seems difficult to reconcile with the linear dependence of the depression of T_H on ΔT_E, and a full explanation of the phenomenon perhaps requires a fresh theoretical approach (Rasmussen, 1982).

(2) *Heterogeneous nucleation*

Just as solutes can alter the free energy of formation of an ice-like cluster by altering σ^{iw}, so may the free energy of a cluster be reduced if part of the solution–cluster interface is replaced by a substrate-cluster interface for which the interfacial energy (σ^{si}) satisfies $\sigma^{si} < \sigma^{iw} + \sigma^{ws}$. The appearance of σ^{ws} (the solution–substrate interfacial energy) on the right hand side of the inequality is a result of the traditional assumption

that it is the total free energy change which is involved, and this includes a contribution from the replacement of substrate–solution interface by substrate-ice interface.

This classical capillary approach is useful for examining the effect of size and shape of nucleating particles, and has been applied to a wide range of substrate geometries (Fletcher, 1969). It is generally accepted that no substance has surface characteristics more favourable for the nucleation of ice than ice itself. For our purposes it is sufficient to note that an 'ideal' nucleating agent ($\sigma^{si} = \sigma^{iw} - \sigma^{ws}$) can only bring about nucleation with certainty if the undercooling attains the value (characteristic of the size of the mote) at which the radius of the critical cluster equals that of the mote. For smaller undercoolings, the presence of the mote does enhance the probability of nucleation in a given time, but as with homogeneous nucleation, the probability becomes negligible for undercoolings which are only a little less than the characteristic value. Figure 1 may now be interpreted as giving the minimum possible radius of a mote as a function of the undercooling at which it induces nucleation.

Small ice-nucleating particles will generally be engulfed by a single nucleation event, so that a given mote will give rise to a single nucleation event at a fairly sharply defined temperature (characteristic of its size and surface properties). For a population of motes, the number of nucleation events per unit volume on cooling the suspension to a temperature T is referred to as the 'cumulative nucleus concentration', and on differentiation yields the spectrum of activity of the motes (Vali, 1971).

Similarly to that for homogeneous nucleation, the model for heterogeneous nucleation predicts that the presence of solutes which do not affect σ^{iw} or Δf, will not appreciably affect the undercooling at which nucleation occurs at concentrations such that T_E is not too greatly depressed. Few studies have tested this prediction but it does not appear to be greatly at variance with experiment (e.g., Pruppacher & Neiburger, 1963).

The size criterion generally prevents substances in true solution from acting as nucleants. However, polysaccharides can exist in solution as long, stiff molecules which may aggregate. On the size criterion alone, therefore, it is plausible that in some circumstances polysaccharides (in solution or in gel form) could act as nucleants. This ability would also require σ^{si} to be small (and σ^{sw} to be large). The most important surface characteristics of ice nucleating substrates appear to be the existence of exposed hydrogen bonding groups of strength comparable to the hydrogen bonds in ice, but more sparsely distributed (Garten & Head, 1965), and a low lattice mismatch with ice (Evans, 1965). Some polysaccharides might have an appropriate surface chemistry. Even if the polysaccharides have no nucleating activity themselves, they may affect the activity of motes from other sources by altering their surface properties (by adsorption)—such an effect has been postulated for other types of polymer (Caple *et al.*, 1983).

Experimental methods

(1) *Materials and preparation of solutions*

Details of the stabilizers used are given in Table 1. They were stored as the air dry powders at room temperature. In this work no allowance was made for the moisture content of the powders, so that weights quoted for the stabilizer actually include a small percentage of moisture.

Solutions of stabilizers in distilled and deionized water were prepared by slowly

Table 1. Suppliers and specifications of stabilizers

Stabilizer	Supplier	Viscosity grade	Batch number	Reported viscosity cps	Approx. mean DP
'Celacol' methyl cellulose (purified)	British Celanese Limited	M20	T7562	20*	—
		M450	T6250	450*	—
		M2500	T4698	2500*	—
'Courlose' sodium carboxy-methyl cellulose (purified)	British Celanese Limited	F20	780	20†	—
		F75	715	75†	—
'Manucol' sodium alginate high mannuronate content (high purity grade)	Alginate Industries Limited	DF	359522	25‡	—
		DH	362451	60‡	400
		DL	368031	175‡	—
		DM	363152	300‡	680
Guar gum	Sigma Chemicals	—	56C–0234	—	—
Locust bean gum	Sigma Chemicals	—	18C–0397	—	—
Xanthan gum	Sigma Chemicals	Grade II	88B–0200	—	—

*2% aqueous solutions, 20°C.
†1% aqueous solutions, 25°C.
‡1% aqueous solutions, 20°C.

adding the desired quantity of stabilizers (in powder form) to water (100 ml) in a conical flask. The liquid was stirred continuously by means of a magnetic flea during addition of the powder and until a clear solution was obtained.

Solutions of stabilizers in sucrose solutions were, however, prepared by a different method. A stock solution of sucrose (analytical grade) in distilled and deionized water was first prepared, and stored in a refrigerator. For each sample solution, a preweighed quantity of stabilizer (in powdered form) was added, with rapid mixing using a high speed Silverson vortex mixer, to 150 ml of the stock sucrose solution. This method was faster than that used previously and had the advantage (for experiments on heterogeneous nucleation) that the possibility of variable mote concentration in the different batches of deionized water (which might otherwise have been used) was eliminated.

The sample solutions were stored in sealed containers at 4°C for a maximum of 6 weeks to reduce the possibility of microbial activity.

(2) *Homogeneous nucleation*

(a) *Method: differential scanning calorimetry (DSC).* A preliminary study was made of homogeneous nucleation in stabilizer solutions using the emulsion technique as described by Rasmussen & MacKenzie (1972). Two methods were used for emulsification. In the first method, a 50:50 mixture of sample solution and 5% Span 65 (sorbitan tristearate) in *n*-heptane was shaken in a vial and then passed five times

through a fine (no. 26) hypodermic syringe needle under considerable pressure. In the second method, an ultrasonic disintegrator (MSE model 150W) was used to emulsify a mixture of sample solution and excess 5% span 65 in *n*-heptane contained in a small beaker. The approximate droplet size distribution of the emulsions was assessed microscopically. The second method produced a finer dispersion (most droplets in the range of 1–3 μm) than the first (most droplets in the range 5–15 μm). The extra step of passing the emulsion through packed 100 mesh gauze, used by Rasmussen & MacKenzie (1972) did not result in any appreciable refinement of the emulsions.

The instrument was a Perkin-Elmer DSC II. To enable operation at low temperatures, the heat sink reservoir was filled with liquid nitrogen. Helium was supplied to the head at a pressure of 25 psi and a positive pressure of nitrogen was maintained in the glove box surrounding the head. The sample size was approximately 5 mg and the runs were performed by cooling at 10°C/min from 300 to 200 K (or lower if necessary) using a sensitivity of 10 mcal/sec.

(3) *Heterogeneous nucleation*

Attempts were made to investigate heterogeneous nucleation by studying coarse emulsions using DSC. However, no technique for producing sufficiently uniform emulsions of large droplets to give reproducible heterogeneous nucleation peaks could be found. For this reason, a new technique was developed to study heterogeneous nucleation in stabilizer solutions by direct observation.

It was discovered that the growth of ice in 0.75 g/ml sucrose solutions (58.6 wt%) was so slow that when a bulk sample is cooled by immersion, a great number of different centres of ice formation are initiated before sufficient growth occurs to cause mutual interference. This is in sharp contrast to dilute solutions in which dendritic invasion of the whole of the undercooled solution follows rapidly after the first ice nucleus forms. In 0.75 g/ml sucrose solution the ice crystals (henceforth referred to as 'nuclei') appear as opaque white spherulites or hexagonal 'pill boxes' suspended in the transparent solutions (Figs 3–5). They are thus easily counted, as a function of undercooling, allowing an easy assay of the presence of motes in the solution. The slow growth of ice in concentrated sucrose solutions has also been exploited in studies of ice nucleation in fogs (Mason, 1961).

To determine the number of nuclei formed at a chosen temperature, the stabilizer solutions were transferred to graduated U tubes which were then immersed in a well-stirred bath of industrial methylated spirit (IMS) set at the chosen temperature (Fig. 6). After a standard incubation time the number of visible nuclei was counted and divided by the known sample volume to give the cumulative nucleus concentration at the selected temperature..

The U tubes were made from lengths of perspex tubing (internal diameter 5.15 mm, wall thickness 1.57 mm), bent using the steam from a kettle to the shape of the window of the tank. The horizontal length of each tube was marked off in four sections, 5 cm long each, using a pipe cutter to produce fine graduation marks. This enabled four determinations of the cumulative nucleus concentration to be made in each experiment. The stabilizer solutions were introduced into the U tubes with the aid of a syringe. This method had the advantage that very viscous solutions could be handled easily, and also that, by attaching a rigid tube to the syringe, a sample of the stabilizer solution could be extracted from the air free bulk of the solution below any foam formed after agitation with the Silverson mixer (Celacol solutions were particularly prone to foaming). The filled U tubes were immersed in the freezing tank. It was shown, by means of a

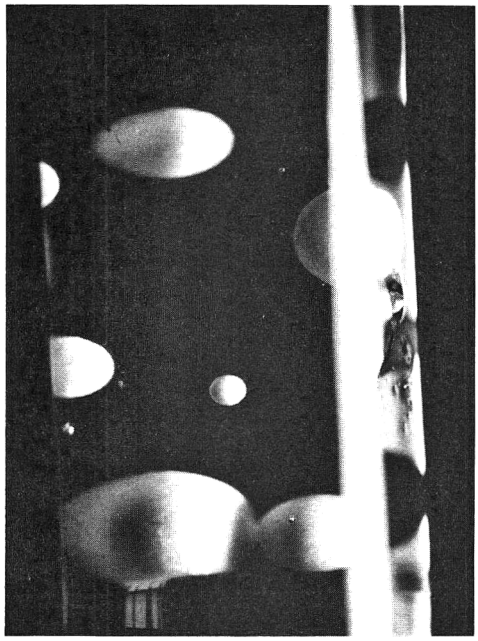

Figure 3. 'Nuclei' formed in a 0.75 g/ml sucrose solution at −30°C (test tube diameter 1 cm).

thermocouple junction inserted into a U tube and connected to a chart recorder, that within 5 min the sample solution always reached equilibrium with the temperature of the stirred IMS. For this reason, the nuclei were counted exactly 7 min after immersion, thus allowing 2 min for the growing nuclei to reach a visible size. In practice, however, the number of nuclei/ml was not very sensitive to the 'incubation' time.

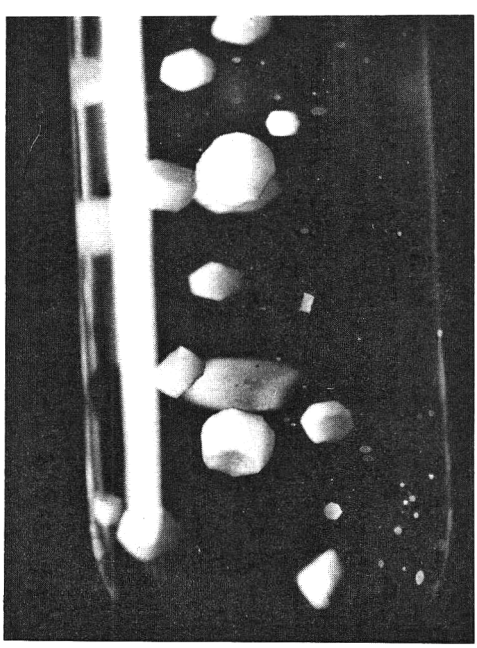

Figure 4. 'Nuclei' formed in 0.75 g/ml sucrose, 0.011 g/ml Manucol DM solution at −22°C.

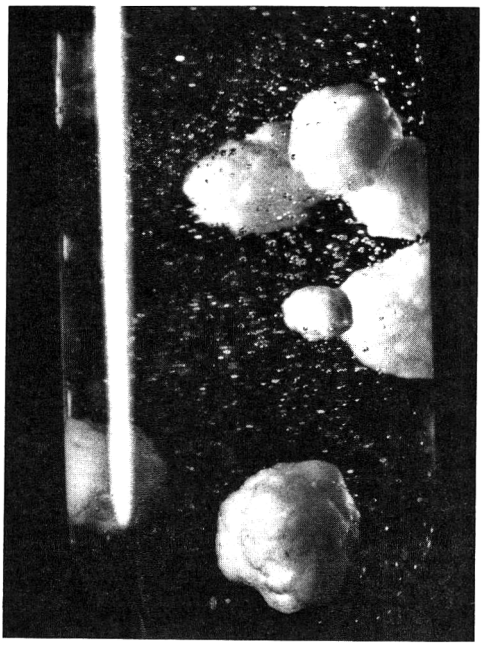

Figure 5. 'Nuclei' formed in 0.75 g/ml sucrose, 0.011 g/ml Manucol DM gel at −22°C.

Specific tests of nucleating ability

In the above experiments, the stabilizers were in true solution (except for microcrystalline cellulose). Further experiments were undertaken to check the effect of gelation and for the presence of undissolved stabilizers and other substances as promoters or inhibitors of ice nucleation.

The effect of gelation on the nucleating ability of Manucol DM was assessed on a gel prepared by adding a freshly made slurry of 1 g glucono-δ-lactone, 0.45 g Na_2HPO_4 and 0.05 g $CaHPO_4$ in 50 cm^3 of stock 0.75 g/ml sucrose solution to 1.1 g Manucol DM dissolved in a further 50 ml of stock 0.75 g/ml sucrose solution. The mean cumulative

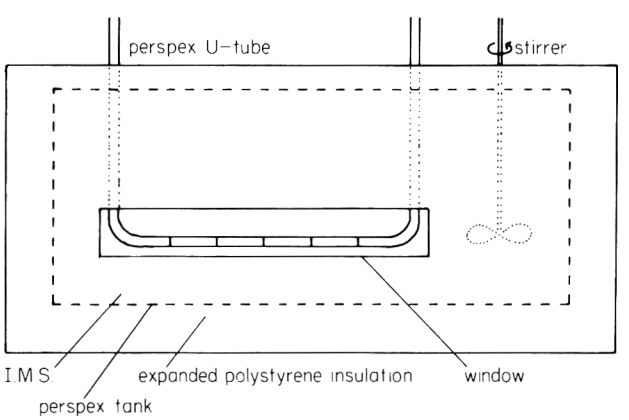

Figure 6. Diagram of freezing tank and U-tube apparatus.

nucleus concentration observed at −22°C was compared to that found in the absence of $CaHPO_4$, which, in consequence, did not gel.

Secondly, a qualitative assessment of the nucleating ability of a range of solid materials was undertaken by covering a few milligrams of each material (contained in a test tube) with enough stock 0.75 g/ml sucrose solution to half fill the test tube, which was then immersed in the tank at −23°C. The surface of the sucrose solution was sealed from the air by a layer of paraffin in order to prevent nucleation by frost particles.

Results

(1) *Homogeneous nucleation*

Typical thermograms are shown in Fig. 7. In all cases a sharp peak (width ~ 7°C, or, for the highest sucrose concentration, ~ 9°C) was present, corresponding to homogeneous nucleation. The effect of sucrose and stabilizer on the peak maximum temperature (the experimental T_H) is given in Table 2.

The results show that the effect of the stabilizers on T_H is small, and indeed that any effect is masked by experimental scatter. The effect of sucrose in depressing T_H is in agreement with the results reported by MacKenzie (1977). The significantly lower values of T_H for the emulsions prepared by the second method can be explained by the

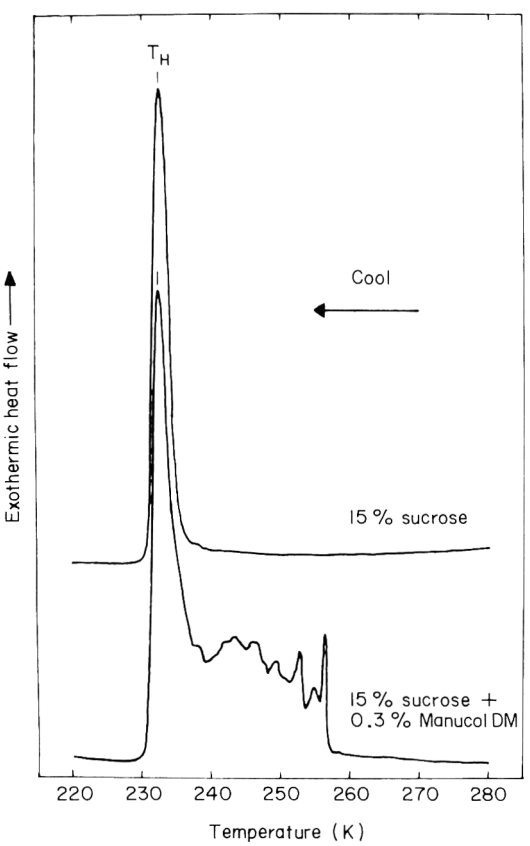

Figure 7. DSC thermograms recorded during the cooling of emulsions (15% sucrose and 15% sucrose +0.3% Manucol DM emulsified in excess 5% span 65 in *n*-heptane).

Table 2. The effect of sucrose and stabilizers on the temperature of the homogeneous nucleation peak maximum (T_H)

Sample		Emulsification method	T_H for individual experiments °C
Solutes	g/ml		
none	pure water	first	−38.2
Celacol M2500	0.02	first	−36.2
Courlose F75	0.01	first	−39.2
Manucol DM	0.01	first	−37.4
Manucol DM	0.03	first	−39.2, −39.2
sucrose	0.15	first	−40.7
sucrose Manucol DM	0.15 0.003	first	−39.2, −40.7, −40.9, −41.0, −40.8
sucrose	0.339	first	−44.2
sucrose Courlose F75	0.339 0.006	first	−45.7
sucrose Manucol DM	0.339 0.006	first	−45.2
sucrose	0.65	first	−58.7
sucrose Manucol DM	0.65 0.012	first	−60.5
sucrose	0.75	first	−70.0, −71.2
sucrose Manucol DM	0.75 0.015	first	−72.0
sucrose	0.15	second	−43.9, −43.2
sucrose Manucol DM	0.15 0.003	second	−44.2

smaller droplet size. If the mean droplet sizes are roughly 10 and 2 μm respectively, then, for the same nucleation rate per droplet at T_H, J will be approximately 100 times larger (per unit volume) for the finer emulsion at T_H. Inspection of the curve corresponding to $\sigma = 25\times10^{-3}$ J/m^2 in Fig. 2 shows that for $J \simeq$ 1/droplet/sec, a 100 fold increase in J will indeed require a lowering of the temperature by $\sim$ 3°C. It is thus apparent that the thorough characterization of the droplet size distribution is a prerequisite for the detection of differences in the rate of homogeneous nucleation caused by the addition of stabilizers (Michelmore & Franks, 1982). It is possible that the size distribution is slightly different in each sample, the difference, however, significantly affecting T_H.

The thermograms for emulsions of solutions containing Manucol DM had the unusual feature of a marked exothermic response preceding the sharp homogeneous nucleation peak (Fig. 7). The irreproducible nature of this exothermic activity indicates that it probably originates from heterogeneous nucleation in a statistically inadequate sample of the larger droplets. Since on prior examination, the emulsions of Manucol DM did not have an exceptionally high number of large droplets, either Manucol DM increases the incidence of heterogeneous nucleation and/or it destabilizes the emulsion. The former possibility is strongly supported by the observation that (at a cooling rate of

10°C/min) freezing of bulk (i.e., not emulsified) 0.75 g/ml sucrose solution takes place partly by homogeneous nucleation, whereas on the addition of Manucol DM (or other alginates) freezing is complete before T_H is reached (Fig. 8). Growth rate studies indicate that the alternative explanation of this observation—that Manucol DM increases the ice crystal growth rate—is untenable (Muhr & Blanshard, 1986).

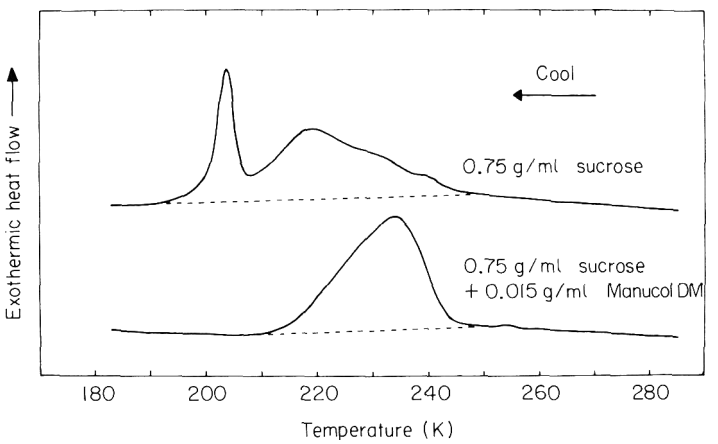

Figure 8. DSC cooling curves of non-emulsified solutions of 0.75 g/ml sucrose solution, one also containing 0.015 g/ml Manucol DM.

(2) *Heterogeneous nucleation*

Figures 9–12 give the cumulative nucleus concentrations for the stock 0.75 g/ml sucrose in deionized water solution (the control), for a range of stabilizer solutions prepared by dissolving 1 g stabilizer in 150 ml of the stock 0.75 g/ml sucrose solution, and for a 0.75 g/ml solution of commercial granulated sugar in tap water. The results show that the stabilizers investigated exhibit a range of nucleating abilities.

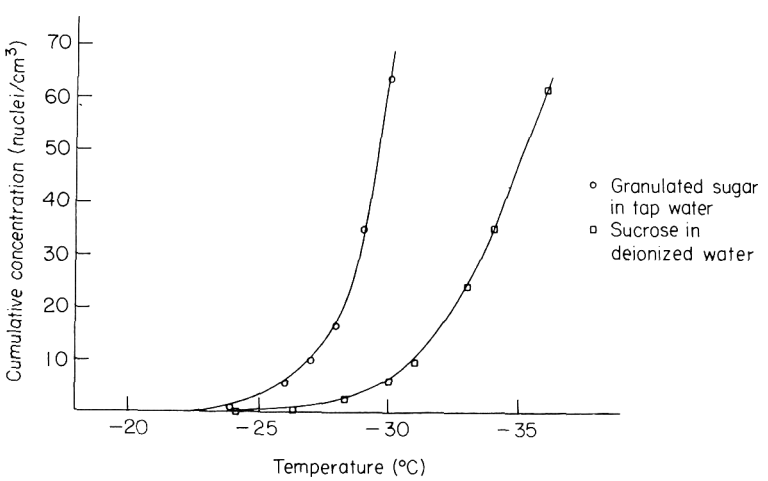

Figure 9. Numbers of ice nuclei by heterogeneous nucleation observed in 0.75 g/ml sucrose aqueous solution as a function of temperature using tap and deionized water.

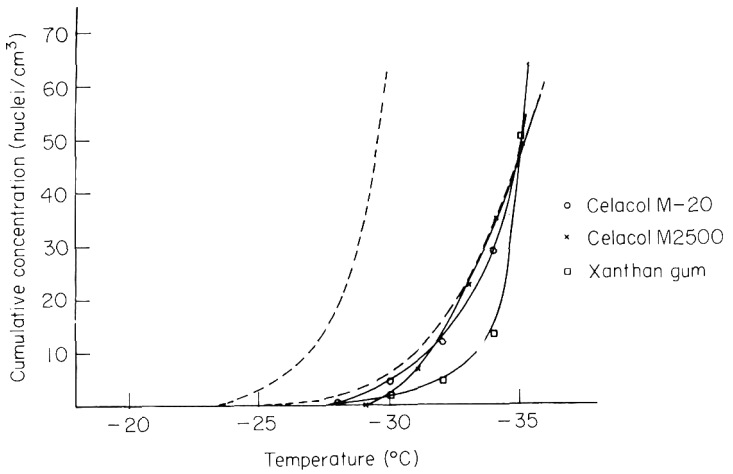

Figure 10. The effect on heterogeneous nucleation of dissolving the specified stabilizers (1 g) in the stock 0.75 g/ml sucrose solution (150 cm^{-3}).

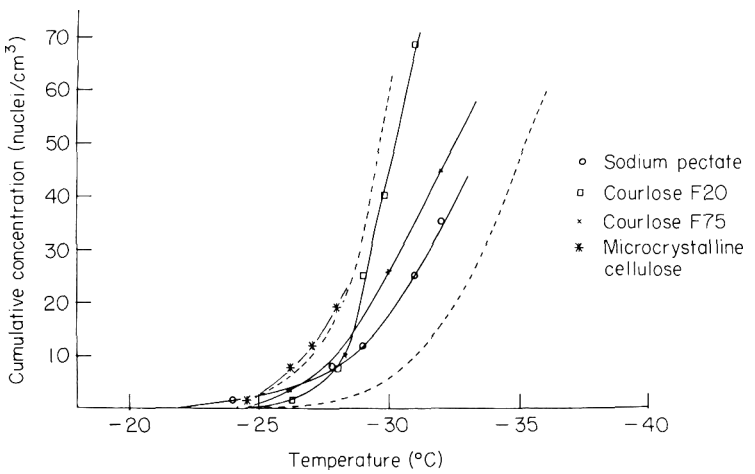

Figure 11. The effect on heterogeneous nucleation of dissolving the specified stabilizers (1 g) in the stock 0.75 g/ml sucrose solution (150 cm^{-3}).

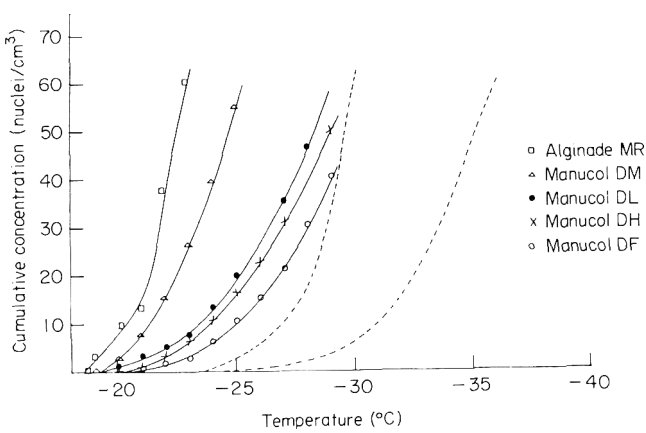

Figure 12. The effect on heterogeneous nucleation of dissolving the specified stabilizers (1 g) in the stock 0.75 g/ml sucrose solution (150 cm^{-3}).

The methyl celluloses (Celacols) and xanthan gum were found to have a slightly inhibitory effect (Fig. 10). Sodium pectate, SCMC (the Courloses) and microcrystalline cellulose slightly increased the nuclei/ml (Fig. 11). A substantial increase in the nuclei/ml was observed for the sodium alginates (Manucols), shown in Fig. 12.

The amount of nucleation attributed to the stabilizers is put into perspective by the curve representing 0.75 g/ml commercial granulated sugar in tap water (Fig. 9). It can be seen that tap water and/or granulated sugar contain a large population of ice nucleating motes, many of which are removed by purification. The results for the stock sucrose solution (the control) and for the impure sugar solution (providing the perspective) are shown as broken lines for comparison in each of the Figures. It is evident that the only stabilizers which contribute significantly to the incidence of heterogeneous nucleation are the alginates (Manucols).

Since all the stabilizer solutions were prepared using the same technique and the same stock sucrose solution, it is reasonable to assume that the difference in nucleation behaviour observed may indeed be attributed to the stabilizers. Further strong support for this interpretation comes from the linear dependence of the cumulative number of nuclei/ml on the concentration of Manucol DM (Fig. 13).

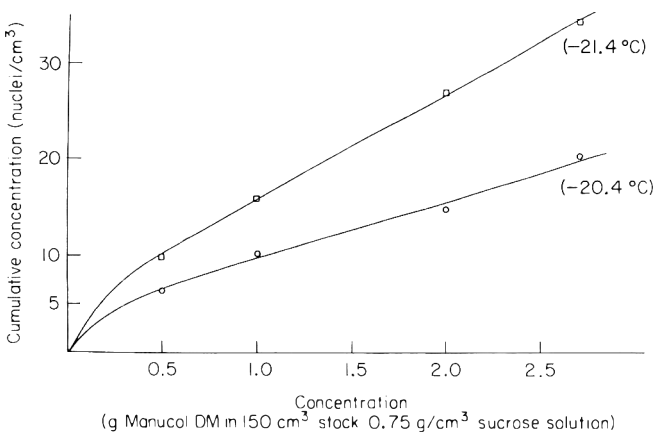

Figure 13. The effect of the concentrations of Manucol DM on heterogeneous nucleation in 0.75 g ml/sucrose stored at −21.4 and −20.4°C.

It is not clear from these experiments whether the significant increase in nucleation observed for alginate solutions results from the polysaccharide molecules themselves or from some other material present in the Manucol samples. However, since a substantial peak for homogeneous nucleation at −39.2°C was obtained for an emulsion of 3% Manucol DM (Table 2) it seems unlikely that the polysaccharide molecules are directly responsible. Further support for this conclusion comes from a preliminary study of nucleation in a film of 0.75 g/ml sucrose, 0.007 g/ml Manucol DM solution using a cold stage microscope with an eyepiece graticule to form a reference grid. It was found that out of 15 nuclei which formed in the field of view on initial cooling, the position of 10 were scarcely altered after thawing and refreezing several hours later. The other five nuclei had either moved significantly or could not be identified. It was noted that the first nuclei to form reappeared in the most reproducible manner on refreezing. This observation points to solid particles (i.e., inclusions) being the majority nucleating agents, rather than the stabilizer molecules, which should be in a state of dynamic

(albeit sluggish) motion. However, a synergistic effect of the alginate molecules and the motes present in the stock sucrose solution cannot be ruled out.

(3) *General nucleating ability*

Comparison of the mean cumulative nucleus concentration in a gel and solution of Manucol DM has shown that the mean cumulative nucleus concentration formed in the gel (at −22°C) was 12/ml (see Fig. 5), while the mean number of nuclei/ml formed in solution (at −22°C) prepared in the same way as the gel, but with 0.5 g Na_2HPO_4 and no $CaHPO_4$, was 14.5/ml (see Fig. 4). Thus gelation brings about, if anything, a very s ight reduction in the incidence of nucleation in the alginate-sucrose solution system. This provides further evidence that the polysaccharide molecules are not involved in the nucleation process, because gelation drastically affects the mobility and conformation of the polymer molecules but does not greatly alter anything else.

The experiments on the inherent nucleating ability of different materials have shown that despite nucleation in the stock sucrose solution being an unlikely event at −23°C (Fig. 9), very many nuclei formed on the surface of crystals of cholesterol immersed in the stock sucrose solutions, in agreement with the work of Head (1961). 1,3-distearin exhibited similar activity to cholesterol, although the free fatty acids (palmitic, stearic and lauric) showed no nucleating ability. Several nuclei formed on glycerol monostearate, showing it to have moderate ice nucleating ability, while the other emulsifiers Span 60 (sorbitan monosearate) and Span 65 (sorbitan tristearate) demonstrated no activity. A very few nuclei formed on the surface of Manucol DM powder, while microcrystalline cellulose, Courlose F75 (SCMC), Celacol M450P (methyl cellulose), locust bean gum and guar gum were completely inactive. The method thus rapidly demonstrated that none of the stabilizers were very active nucleating agents in solid form, although Manucol DM and glycerol monostearate (often used as an ice cream emulsifier) do have some nucleating ability.

Discussion and conclusions

(1) *Homogeneous nucleation*

According to homogeneous nucleation theory, T_H is extremely sensitive to any change in σ. T_H will also be lowered if T_E is depressed or if Δf is increased. It is believed that stabilizers have negligible effect on T_E (Muhr & Blanshard, 1983). The fact that they also have no significant effect (at low concentration) on T_H shows that neither σ nor Δf is significantly changed. Strictly speaking, this conclusion only holds at temperatures in the region of T_H. The results are in line with the work of Michelmore & Franks (1982), who found that PEG does not affect σ and changes Δf to an extent which would only be appreciable at much higher concentrations than those used in the present work for stabilizers.

(2) *Heterogeneous nucleation*

The approximately exponential increase in cumulative heterogeneous nucleus concentration as the temperature is lowered is fairly typical of heterogeneous nucleation results (Vali, 1971). The only stabilizers which significantly increased the incidence of heterogeneous nucleation (over and above that of tap water and granulated sugar) were the Manucols (sodium alginates). The relative inactivity of the other stabilizers is in accord with the work of Parungo & Wood (1968), who found that agar, gelatin, pectin, ovalbumin, BSA, RNA and DNA (as 0.01–1% solutions) have negligible effect on the

nucleation temperature of water. The slight reduction in the incidence of nucleation for xanthan gum observed here might arise from an inhibitory effect of the polysaccharide molecules on motes pre-existing in the stock sucrose solution (Caple *et al.*, 1983).

In the case of the Manucols, it seems most likely that the increased incidence of heterogeneous nucleation results from foreign motes rather than from the polysaccharide molecules themselves. Smidsrød & Haug (1968) found that their alginate samples were contaminated with approximately spherical particles (probably of plant origin) of diameter 40–120 nm; possibly these are the active motes responsible for the results in this work. Samples of organic debris from a wide variety of origins have been found to have ice nucleating ability (Schnell & Vali, 1976) and it is quite likely that such debris is present in impure ice cream ingredients.

Thus, either as a result of such impurities or because of their intrinsic properties, it is possible that other ingredients used in frozen confectionery could increase the incidence of heterogeneous nucleation, perhaps in excess of the effect observed for the Manucols. For example, according to the factors mentioned in the introduction, it is conceivable that fat globules coated with the emulsifier glycerol monostearate (thus providing a surface with exposed hydroxyls) could act as nucleating agents.

The freezing point of 0.75 g/ml sucrose solution (58.6 wt%) is −11.2°C while the onset temperature for significant heterogeneous nucleation in the alginate solutions is about −18°C. We may thus anticipate (from the discussion of the 'colligative'effect for the nucleation temperature in the introduction) that an undercooling of roughly 6°C is required at any sucrose concentration before the alginates (or the inclusions within them) initiate ice formation.

(3) *Ice nucleation in frozen confectionery products*

The ice crystal texture of frozen confectionery products is determined partly by initial formation of ice during freezing and partly by subsequent changes during storage. The significance of the nucleation results for both processes can now be examined.

Ice cream is initially frozen by continuously scraping the mix from the refrigerated wall of a metal drum, so that the initial ice crystal population is largely determined by fracture. Only a massive increase in the incidence of nucleation would significantly affect this complicated process, so the effect of stabilizers on rheological properties would be of greater consequence than their effect on nucleation. For ice lollies which are solidified in quiescent conditions, it has been observed that the initial ice crust on the wall of the mould arises from only one or two nucleation events. Moreover, the texture of the subsequent growth of the crust inwards is probably controlled by constitutional undercooling, and not by further nucleation (Muhr, 1983). The nucleating ability of even the Manucols would thus be of no significance during ice lolly production.

Further nucleation of ice may occur during storage of frozen products, and if so would be significant for the rate of coarsening. However, bearing in mind that in bulk samples the cooling rate will be small, it seems most unlikely that undercoolings as great as 6°C would occur during hardening or storage (at fluctuating temperature) of ice cream. While perhaps other ingredients may nucleate ice during cooling cycles, and thus help to keep the number of ice crystals large and their size small, none of the stabilizers studied could prevent the coarsening of ice cream by such a mechanism.

Alternatively, ice cream may coarsen during storage (even at a constant temperature) by Ostwald ripening. The driving force for coarsening by this mechanism is the total surface energy of the population of ice crystals, which is decreased when large crystals grow at the expense of small crystals. From the homogeneous nucleation

experiments it appears that stabilizers do not significantly affect the driving force for Ostwald ripening, because they do not affect σ. However, they may interfere with the kinetics of ripening by affecting the growth rate of ice (Muhr & Blanshard, 1986).

In conclusion, some stabilizers do affect the heterogeneous nucleation of ice, but not to an extent which is significant for frozen confectionery products.

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References

Angell, C.A. (1983). In: *Water, a Comprehensive Treatise*, Vol 7, (edited by F. Franks). New York: Plenum Press.

Blanshard, J.M.V. (1970). Stabilizers–their structure and properties. *Journal of the Science of Food Agriculture*, **21**, 393–399.

Caple, G., Allegretto, E., Culbertson, L.B. & Layton, R.C. (1983). Polymeric inhibition of ice nuclei activities. *Cryoletters*, **4**, 51–58.

Evans, L.F. (1965). Requirements of an ice nucleus. *nature*, **206**, 822.

Fletcher, N.H. (1969). Active sites and ice crystal nucleation. *Journal of the Atmospheric Sciences*, **26**, 1266–1271.

Franks, F. (1983). In: *Water, a Comprehensive Treatise*, Vol 7, (edited by F. Franks). New York: Plenum Press.

Franks, F., Mathias, S.F. & Trafford, K. (1984). The nucleation of ice in undercooled water and aqueous polymer solutions. *Colloids & Surfaces*, **11**, 275–285.

Garten, V.A. & Head, R.B. (1965). A theoretical basis of ice nucleation by organic crystals. *Nature*, **205**, 160–162.

Head, R.B. (1961). Steroids as ice nucleators. *Nature*, **191**, 1058–1059.

MacKenzie, A.P. (1977). Non-equilibrium freezing behaviour of aqueous systems. *Philosophical Transactions of the Royal Society of London*, Series B, **278**, 167–189.

Mason, B.J. (1961). The growth of snow crystals. *Scientific American*, **204** (1), 120–131.

Michelmore, R.W. & Franks, F. (1982). Nucleation rates of ice in undercooled water and aqueous solutions of PEG. *Cryobiology*, **19**, 163–171.

Muhr, A.H. (1983). *The influence of polysaccharides on ice formation in sucrose solutions.*, Ph.D. Thesis, Nottingham University.

Muhr, A.H. & Blanshard, J.M.V. (1983) The effect of polysaccharide stabilisers on ice crystal formation. *Prog. Food Nutr. Sci.*, Vol. 7. Pergamon Press.

Muhr, A.H. & Blanshard, J.M.V. (1986). The effect of polysaccharide stabilizers on the rate of growth of ice. Submitted.

Parungo, F. & Wood, J. (1968). Freezing of aqueous solutions of macromolecules *Journal of the Atmospheric Sciences.*, **25**, 154–155.

Pruppacher, H.R. & Klett, J.D. (1978). *Microphysics of Clouds and Precipitation*. Holland: Reidel.

Prappacher, H.R. & Neiburger, M.J. (1963). The effect of water soluble substances on the supercooling of water drops. *J. Atmos. Sci.*, **20**, 376–385.

Rasmussen, D.H. (1982). Energetics of homogeneous nucleation - approach to a physical spinodal. *Journal of Crystal Growth*, **56**, 45–55.

Rasmussen, D.H. & MacKenzie, A.P. (1972). Effect of solute on ice-solution interfacial free energy. In: *Water Structure at the Water-Polymer Interface* (edited by H.H.G. Jellinek). New York: Plenum Press.

Rasmussen, D.H. & MacKenzie, A.P. (1973). Clustering in supercooled water. *Journal of Chemical Physics*, **59**, 5003–5013.

Schnell, R.C. & Vali, G. (1976). Biogenic ice nuclei. *Journal of the Atmospheric Sciences*, **33**, 1554–1564.

Smidsrød, O. & Haug, A. (1968). A light scattering study of alginate. *Acta Chemica Scandinavica*, **22**, 797–810.

Stillinger, F.H. (1980). Water revisited. *Science*, **209**, 451–457.

Vali, G. (1971). Quantitative evaluation of experimental results on the heterogeneous freezing nucleation of supercooled liquids. *Journal of the Atmospheric Sciences*, **28**, 402–409.

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Anthocyanin colorants from Elderberry (*Sambucus nigra* L.) IV. Further studies on production of liquid extracts, concentrates and freeze dried powders

K. BRØNNUM-HANSEN* AND J. M. FLINK†

Summary

Anthocyanin extraction from elderberry pomace is improved by mechanical disintegration and use of aqueous HCl in place of acidified ethanol as extractant, with pH being important in determining extractant effectiveness. Extraction at low solvent: pomace ratios gives extracts of higher concentration and lower volume, but with reduced efficiency. Enzyme pretreatments do not improve extractability from extensively crushed pomace, and if of extended duration, can result in anthocyanin breakdown. Vacuum evaporation of extracts gave little loss of anthocyanins, and combined with freeze drying, produced materials with high anthocyanin retention.

Introduction

Interest in natural colouring agents has been increasing as the safety of artifical colours has been questioned. Among those investigated are anthocyanins, generally derived from cranberry (Fuleki & Francis, 1968a; Chiriboga & Francis, 1970; Sapers, Jones & Maher, 1983) and grape (Philip, 1974; Metivier, Francis & Clydesdale, 1980), since the solids remaining after juice/wine production can be a good, relatively inexpensive source of anthocyanins.

In Denmark, elderberry is cultivated for production of various food products, including juice and soup, from which residues contain large amounts of anthocyanins. Earlier studies on recoveries of elderberry anthocyanins by an extraction and freeze-drying process (Brønnum-Hansen, Jacobsen & Flink, 1985; Brønnum-Hansen & Flink, 1985a), and storage stability of the products obtained (Brønnum-Hansen & Flink, 1985b) are continued as a study of additional factors influencing extraction behaviour, potential for concentration of the extracts prior to freeze drying, and further evaluations of freeze drying behaviour of the extracts and concentrates.

Materials and methods

Raw material

Elderberries (Sambu variety) were obtained from the research orchards (Pometet) at the Royal Veterinary and Agricultural University's Research Farm at Tåstrup, Denmark. The elderberries were removed from their stems by hand and then frozen and stored at −25°C. Frozen material was thawed by placing at room temperature overnight.

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Pressing of elderberry

Frozen/thawed elderberries (100 or 200 g depending on the amount of pomace required for extraction) were pressed in a laboratory hydraulic press at a pressure of 100 atm for 10 sec, and then allowed to fall for 2 min (generally falling close to 0), after which it was released.

Extraction of elderberry pomace

In the general extraction procedure, the pomace sample (generally 5 g) was ground in a morter with 50 ml of extraction solvent, and the mortar was rinsed with a further 50 ml of solvent. The disintegrated pomace was then extracted at room temperature for between 2–30 min (generally 10 min) with stirring, and filtered. The residue was re-extracted by stirring with more solvent (50 ml), and filtered. This was repeated the desired number of times, each extract being analysed individually. At each step, the volume of extract recovered was measured to allow calculation of anthocyanin amounts extracted. In tests requiring larger amounts of identical material, several lots of pomace were extensively crushed individually in a mortar and pooled prior to taking material for extraction. Results were corrected for the small changes in pomace moisture and anthocyanin contents arising in crushing.

Anthocyanin concentration

Total anthocyanin concentration and Degradation Index (DI) values were measured using the spectrophotometric pH differential method (Fuleki and Francis, 1968b; Wrolstad, 1976). Anthocyanin concentration was expressed as cyanidin-3-glucoside, the major anthocyanin compound in elderberry (Brønnum-Hansen & Hansen, 1983).

Before measuring % retention of anthocyanins following evaporation, freeze concentration or freeze drying, the concentrate was re-diluted or rehydrated to the original volume. Anthocyanin concentrations before and after evaporation or drying are thus measured at the same sample solids content. Tabulated values for the anthocyanin concentrations in the concentrates are from the undiluted concentrates.

Results and discussion

In this section and in Tables the abbreviation Acy is used for anthocyanins (as in mg Acy/g berries). To compare extraction results for different experimental series, where raw materials may vary, it is necessary to express extraction efficiency as % total anthocyanin in the raw material extracted. Only when the same raw material is used, is it possible to compare anthocyanin concentration in the extracts, or amounts extracted.

Total Acy present in the raw material was generally determined by triple extraction of the elderberry pomace, as most of the elderberry anthocyanin is extracted in the first 3 extraction steps, with the incremental yield thereafter falling sharply (Brønnum-Hansen *et al.*, 1985).

Influence of various factors on extraction efficiency

Pomace disintegration. To examine the influence of pomace disintegration on extraction efficiency, samples of pomace (5 g) at four levels of disintegration (untreated, essentially whole skins), slightly crushed (skins broken into several fragments), extensively crushed (skin broken to small particles), and macerated

(essentially homogeneous mass) were extracted for 30 min with 100 ml 0.1 M HCl. Crushing was conducted in a mortar, while maceration was conducted in a Waring Blender.

Anthocyanin extractability increases (Table 1) with degree of disintegration up to maceration, which did not give increased extraction. Although high yields were obtained with maceration, in some tests the finely disintegrated macerated samples gave blockage problems during vacuum filtration. As extensive crushing prior to extraction is optimal, this was adopted as the standard method in this study.

Table 1. Effect of degree of pomace disintegration on anthocyanin extraction behaviour

Treatment	Acy concentration in first extraction (mg/l)	% Total Acy* in first extraction
Untreated pomace	712	73
Slightly crushed (in mortar)	753	80
Extensively crushed (in mortar)	860	90
Macerated (2 min in blender)	844	87

*Acy in pomace: 17.9 mg Acy/g pomace.

Extraction solution composition. Most anthocyanin extractions reported in the literature are based on acidified alcohols (MeOH or EtOH) (e.g., Fuleki & Francis, 1968b; Chiriboga & Francis, 1970). As Brønnum-Hansen *et al.* (1985) recommended 0.1M HCl as the best pomace extractant for elderberry, it was of interest to compare the two systems. Furthermore, as Brønnum-Hansen *et al.* (1985) had noted improved efficiency with increasing concentration of citric acid, the extraction efficiencies of HCl and citric acid solutions were compared at the same pH values to determine if the effect was due to pH. Since the pH differential method is used for measuring anthocyanin contents (i.e., at pH 1 and 4.5), the pH differences of the extracts do not influence the analysis technique. Results (Table 2) are given for a single raw material, so effectiveness between treatments can be compared directly using data for the total mg Acy/g dry pomace.

HCl extractions at pH 1–2 are more effective than either of the acidified EtOH solutions recommended in the literature, confirming our earlier results. Anthocyanin extraction (Total mg Acy/g dry pomace) *versus* pH gives similar behaviour for both HCl and citric acid solutions, indicating that solution pH is the determining factor for anthocyanin extractability. Solute concentrations required to obtain the same pH values are quite different (Table 2), making HCl the preferred extractant. A concentration of 0.1M HCl is preferred as it produces the highest anthocyanin concentration in the first extract.

Brønnum-Hansen & Flink (1985a,b) showed that changes in anthocyanins occurred in freeze dried extracts at pH 1 or 2 and thus freeze drying at pH 3 would be preferred. It is now clear that to obtain the most satisfactory yields, the pomace should be extracted at low pH and then the extract adjusted to pH 3 before freeze drying, instead of

Table 2. Effect of solvent composition on extraction of anthocyanins

Solution	pH	Acy conc. (mg Acy/l) Extn. 1.	Acy conc. (mg Acy/l) Extn. 2	Total Acy* extracted (mg Acy/g pomace)
99% EtOH: conc. HCl (99.9:0.1)	*circa* 0.3†	769	323	10.0
96% EtOH: 1.5 M HCl (85:15)	1.15	1113	658	16.4
Citric Acid 13.3%	1.61	1754	1111	23.3
1.8%	2.18	885	939	16.4
0.3%	2.60	515	513	9.4
HCl pH 1.0	1.03	1787	869	23.0
1.5	1.50	1407	1241	24.0
2.0	2.00	1034	1034	17.9
2.5	2.50	439	484	8.3
3.0	2.97	370	349	6.4

*Based on 5 g pomace extracted twice with 50 ml solution (pomace solids = 48.9%).
†pH in pure alcohol not necessarily a precise measure of H^+ concentration.

extracting with a HCl solution at the pH desired for freeze drying. In earlier studies (Brønnum-Hansen & Flink, 1985a,b), freeze dried samples were prepared by adjusting low pH extracts.

Solvent: Pomace ratio. The solvent:pomace (S:P) ratio is an important extraction parameter, as it influences the anthocyanin distribution between solvent and pomace (at equilibrium), and thus the concentration in the extract, as well as the amount of solvent which must be processed in subsequent process steps. For a satisfactory overall yield, the anthocyanin concentration in the extract should be as high, and the extract (solvent) volume as low as possible.

The effect of S:P ratio on various extraction parameters was investigated. Typical results (Table 3) show that while the concentration of anthocyanin in the first extraction increases strongly as the S:P ratio decreases, at ratios of 10 and below, this occurs at the expense of anthocyanin yield (decreasing % of total Acy in the first extract). The overall yield can be improved by conducting additional extractions of the pomace. In the examples given, anthocyanin yields above 93% are obtained with three and four extractions for S:P = 5 and S:P = 2.5, respectively, the anthocyanin concentration for the second extractions for S:P = 5 or 2.5 being about equal to the first extractions of S:P = 20 or 10, respectively.

The volume of solvent required to extract 100 g pomace under the respective S:P conditions, and the average anthocyanin concentrations in bulked extracts are given in Table 3. As the S:P ratio falls, smaller volumes of a higher anthocyanin concentration are obtained, though with a decreased overall yield. For given raw material and process costs, an optimum S:P ratio can be determined. A S:P ratio of 10 was generally used in this study to provide enough solution for further processing, although lower S:P ratios might have been preferred (Table 3).

Varying extractant aliquot volume, number of extractions and extraction times within totals of 400 ml and 32 min showed that multiple extractions with smaller volumes of extractant for shorter time periods gave increasing extraction yields (Table 4). With the 50 ml extractions, only three extractions of 4 min each were possible, due to

Table 3. Influence of solution : pomace ratio on extraction of anthocyanins

Solution : pomace ratio§	Total number of extractions	Acy conc. (mg Acy/l)			% of Total Acy		Solution required to extract 100 g pomace‡ (ml)
		Extn. 1	Extn. 2	Blended extracts	Extn. 1	Total extractions	
40	2	430	34	228	92	100*	8000
20	2	860	85	463	91	100*	4000
20	2	559	101	482	89	100†	4000
10	2	1125	325	955	81	99†	2000
5	3	2110	641	1206	68	94†	1500
2.5	4	4220	1225	1800	52	93†	1000

*Total Acy = 17.9 mg Acy/g pomace.
†Total Acy = 19.3 mg Acy/g pomace (pomace solids = 24.4%).
‡Includes S : P ratio and number of extractions.
§For S : P = 40 extraction volume was 200 ml; for all others 100 ml.

Table 4. Dependence of anthocyanin extractability on individual extraction volume and duration for multiple extractions at constant total solution volume (400 ml) and time (32 min)*

Total number of extractions	Solution volume for extraction (ml)	Duration per extraction (min)	Acy conc. for blended extracts ml (mg Acy/l)	Total mg Acy extracted (min/ml)			Total ml recovered
				8/100	16/200	32/400	
1	400	32	415	—	—	160	385
2	200	16	632	—	129	227	360
4	100	8	748	73	146	263	351
3†	50	4	1519	132	178‡	—	117

*Extract 10 g pomace (pomace solids = 48.9%).
†This was intended to be eight extractions. Filtration problems limited this to three extractions.
‡Value of 178 mg Acy is for 12 min/150 ml.

filtration problems, but the total anthocyanin extracted in 150 ml (12 min) is more than in 200 ml (and 16 min) for the other samples. Thus multiple-batch or continuous extractions at low S:P ratios are the most effective for recovering elderberry anthocyanins.

Enzyme treatments prior to pressing or extraction. There are many reports on juice production by dissolution of cell wall and cytoplasm macromolecule components. Enzyme technology could possibly improve anthocyanin extractability, or even eliminate the extraction step altogether (Flores & Heatherbell, 1984). A pectolytic enzyme mix (Pectolase, Grindsted Products) and a cellulolytic/pectolytic enzyme mix (Rohamat PC, Rohm GmbH) were examined in a series of tests conducted at room temperature. When added to the elderberries prior to pressing (for either 4 or 20 hr), there was little evidence for improved anthocyanin extractability. There was a tendency for a better first extraction for the enzyme treated samples, though after two extractions of the pomace, all samples were extracted to the same level (about 97–99%).

Adding enzyme to the pomace prior to extraction gave some improvement for uncrushed pomace (Table 5) for a 2 hr treatment in the first extract (about 20% higher) and a slightly higher total extraction. For crushed pomace, treatment with low enzyme levels for 20 hr gave improved extraction in one experiment (results not shown), whilst in another, 20 hr treatment with higher enzyme dosage levels resulted in greatly reduced anthocyanin concentrations, due to degradation reactions (Table 5). Enzymatic degradation of the cell wall will result in formation of low molecular weight carbohydrates which can undergo undesired browning reactions when freeze-dried as 0.1 M HCl extracts (Flink, 1983). In one test, in which an experimental (non-commercial) cell wall degrading enzyme was used on the pomace, the freeze dried material was black and had undergone structural collapse; this never happened with the regular 0.1 M HCl extracts. It appears that enzyme addition does not significantly improve anthocyanin extraction if the pomace is crushed prior to extraction.

Table 5. Effect of enzyme treatment on anthocyanin extractability

Treatment prior to extraction	Time (hr)	Anthocyanin extracted (mg/g pomace)*			
		Uncrushed sample		Crushed sample	
		1st extn.	Total	1st extn.	Total
None	—	15.1	19.9	18.1	20.4
1 ml pectolase	2	18.0	20.4	18.1	19.5
5 ml pectolase	2	19.2	21.6	16.7	18.0
5 ml pectolase	20	2.2	2.5	0.9	1.0
100 mg Rohamet PC	2	20.3	21.8	19.8	21.3
100 mg Rohamet PC	20	14.8	15.8	13.3	14.3

*Based on 5 g treated pomace extracted twice with 0.1 M HCl (100 ml).

Concentration and freeze drying of anthocyanin extracts

Freeze concentration. A simple freeze concentration method was utilized. A 500 ml glass bottle containing 450 ml anthocyanin extract was frozen at −20°C. The frozen extract was then partially thawed in a microwave oven by sequential 2 min exposures at defrost setting (power = 210 watts). After each 2 min period, the melted material was separated and the volume and anthocyanin concentration measured. In a typical test, one-half the original sample weight contained 71% of the anthocyanins, giving a concentration factor of 1.5. The residual material, which still contained 29% of the anthocyanins, could be recycled. There was no indication of anthocyanin degradation and thus this is a simple laboratory-scale method for obtaining concentrate of high quality.

Vacuum evaporation of anthocyanin extracts. Several tests were conducted using a laboratory vacuum rotary evaporator operating at 125 torr (b.p. (H_2O) = 56°C) and a bath temperature of 80°C, where it took about 10 min to concentrate 50 to 25 ml in a 250 ml round-bottom flask, or 100 to 50 in a 500 ml flask.

Results of three evaporations with 50 ml extract samples are shown in Table 6. A concentration of 17.3 is obtained by initially 'drying' the sample under vacuum, and then

dissolving the residue in a minimum amount of water. This was a simple method of obtaining highly concentrated material, in which there is no loss of anthocyanins, and the degradation index (DI) does not vary with concentration, while pH decreased with increasing concentration.

Table 6. Effect of vacuum evaporation on pH, anthocyanins, acid retention and degradation index (DI) of extracts*

Conc. factor (wb/wb)	pH of conc.	Acid† retn. %	Acy levels (mg/l) conc.	% retn.	D.I.
2.21	0.82	95	629	100	1.16
5.77	0.42	97	1763	100	1.17
17.30	ca. 0	96	4914	97	1.18

*Original sample = 50 ml; flask volume = 250 ml, pH 1.18, Acy 285 mg/l, D.I. 1.21.

†Based on titration with 0.1 M NaOH.

In a second test series, anthocyanin extracts at 3 pH values, with or without 1% maltodextrin (DE = 20), a freeze drying structure stabilizer, were evaporated about two-fold. While there was no loss of anthocyanin at pH 1 and 2, or pH 3 with maltodextrin, there was some loss at pH 3 in the absence of maltodextrin (Table 7). Thus vacuum evaporation can be used to rapidly concentrate anthocyanin extracts without loss of the anthocyanin component, though with lower pH, which can be of some importance.

Table 7. Effect of extract pH and 1% maltodextrin (MD) addition on vacuum evaporation of anthocyanin extracts

Extract pH	Conc. factor (v/v)	Sample pH original	Sample pH conc.	Acid retn.* %	Acy levels† conc. (mg/l)	Acy levels† % retn.	D.I.‡
1	1.92	1.01	0.76	103	1320	96	1.13
1 MD	1.76	1.01	0.76	103	1158	97	1.13
2	1.76	2.00	1.76	100	1175	100	1.13
2 MD	2.21	2.00	1.64	102	1456	99	1.13
3	1.91	2.91	2.66	91	1142	84	1.14
3 MD	1.92	2.91	2.79	95	1175	100	1.13

*Based on titration with 0.1 M NaOH.

†Original extract: 637–687 mg Acy/l (depending on MD addition and pH adjustment); D.I. = 1.10–1.18.

‡D.I. = Degradation Index.

Combined vacuum evaporation and freeze drying. Anthocyanin extracts prepared at three different concentrations using different solvent:pomace ratios were vacuum evaporated approximately two-fold. Maltodextrin was either added to the extract prior to evaporation, or after evaporation to give 2.5 or 5% in the final concentrate. These gave 'extracts/concentrates' with anthocyanin concentrations varying from 650 to 5000

mg Acy/litre and maltodextrin levels of 0, 2.5 and 5%. These concentrates were then freeze dried at ambient temperature conditions. All samples were analysed for anthocyanin and hydrogen-ion retention before and after evaporation, and after freeze drying; the physical structure of the freeze dried material was visually assessed.

In all cases (Table 8), the anthocyanin retention is greater than 90%. While the pH of the evaporated samples decreases, the pH of the rehydrated freeze dried material has increased, indicating a loss of hydrogen ions during freeze drying. The retention of hydrogen ions during freeze drying depends on the level of maltodextrin present (very low retention without maltodextrin) and is essentially independent of the anthocyanin concentration. The structure of the freeze dried material depends on both maltodextrin and anthocyanin concentrations, an increase in either giving a better structure. At the low anthocyanin concentration a maltodextrin concentration above 5% is necessary for good structure, whilst at the highest concentration (5000 mg Acy/litre), only 2.5% is necessary. Practically, addition of maltodextrin prior to evaporation is preferred as the lower extract viscosity will make mixing easier.

Conclusions

The extractability of anthocyanins from elderberry pomace is improved by a mechanical disintegration. Macerating the pomace in a blender did not improve extraction, and in some cases, resulted in filtration problems.

Aqueous HCl is superior to acidified ethanol as extractant and pH is important for extractant effectiveness. HCl was preferred to citric acid due to the lower solute concentration at equal pH values. For preparing freeze dried powders, it is desirable to extract at low pH to obtain the highest yield and then increase pH prior to freeze drying.

Low solvent:pomace ratios gave extracts of higher concentration and lower volume, but with reduced extraction efficiency. Multiple extractions at low S:P ratios improved extraction efficiency, whilst total volumes were still low. At a given overall S:P ratio and extraction duration, extraction efficiency will be increased by using a continuous process.

Enzyme pretreatments did not improve extractability from extensively crushed, previously frozen pomace. Enzyme pretreatment of fresh pomace was not investigated, but little difference is expected (Brønnum-Hansen *et al.*, 1985). Extended treatments can result in anthocyanin breakdown.

By a simple freeze-concentration process, 1.5-fold concentrates were prepared with no anthocyanin loss. Vacuum evaporation of extracts gave little loss of anthocyanins, even with a seventeen-fold concentration. A drop in pH is generally noted.

Freeze drying of extracts, or vacuum evaporated concentrates, produced materials with high anthocyanin retention independent of the initial anthocyanin concentration, and independent of addition of maltodextrin prior to freeze drying. The loss of hydrogen ions during freeze drying, which was reduced by increasing maltodextrin concentration, resulted in a higher pH in the rehydrated powder than in the original extract. The structure of the freeze dried material depended on the maltodextrin and anthocyanin concentrations, with no cake being observed in the absence of maltodextrin. To prepare high concentration extracts for freeze drying, it is preferable to add maltodextrin prior to evaporation, rather than add it at the desired level to the evaporated extract.

Table 8. Effect of anthocyanin concentration and maltodextrin (MD) addition on concentration and freeze drying (FD) of anthocyanin extracts

Nominal final Acy*	Conc. MD	Sample preparation			Acy retention (%)			Sample pH			H^+ ion retn. after FD§	Visual evaluation of structure**
		Process†	Extract	MD	Evap.	FD	Overall‡	Orig. Extr.	After Conc.	FD+ Rehyd.		
650	0	None	650	0	—	98	98	1.23	—	2.58	5	Total coll.
	2.5	Mix	650	2.5	—	97	97	1.22	—	1.49	54	s. cake: surf. layer
	5.0	Mix	650	5.0	—	93	93	1.28	—	1.43	71	ris. cake
1275	0	None	1275	0	—	96	96	1.24	—	2.35	8	coll.
	0	Evap	650	0	104	86	90	1.23	0.90	2.28	4	coll.; part.
	2.5	Mix	1275	2.5	—	93	93	1.28	—	1.53	56	surf. layer
	2.5	Evap	650	1.25	101	99	100	1.21	0.92	1.32	40	ris. cake; s. coll.
	5.0	Mix	1275	5.0	—	96	96	1.27	—	1.45	66	cake
	5.0	Evap	650	2.5	94	97	91	1.22	0.96	1.12	69	p. ris. cake
2400	0	None	2400	0	—	96	96	1.37	—	2.24	14	coll.
	0	Evap	1275	0	103	92	95	1.24	0.97	2.04	9	part.
	2.5	Mix	2400	2.5	—	95	95	1.37	—	1.66	51	shrunken cake
	2.5	Evap	1275	1.25	97	98	95	1.28	0.94	1.27	47	sl. ris. cake
	5.0	Mix	2400	5.0	—	100	100	1.34	—	1.56	60	dense cake
	5.0	Evap	1275	2.5	98	98	96	1.28	0.94	1.12	66	sl. ris. cake
5000	0	Evap	2400	0	101	94	95	1.37	1.03	1.83	16	por. cake; s. coll.
	2.5	Evap	2400	1.25	96	97	93	1.34	1.02	1.27	56	por. cake
	5.0	Evap	2400	2.5	119	99	118	1.37	0.89	1.09	63	dense cake

*Measured Acy conc: 650: 650, 1275: 1250–1300, 2400: 2300–2500, 5000: 4500–5500.
†Concentration factors: None = 1, Mix = 1, Evap. = 1.9–2.0.
‡Overall = (Evap.) (FD).
§Based on measured pH values.
**coll. = collapsed, ris. = risen, p. = partially, surf. = surface, por. = porous, part. = particles, s. = some, sl. = slight.

References

Brønnum-Hansen, K. & Hansen, S. (1983). High-performance liquid chromatographic separation of anthocyanins of *Sambucus nigra L. Journal of Chromatography*, **262**, 385–392.

Brønnum-Hansen, K., Jacobsen, F. & Flink, J.M. (1985). Anthocyanin colorants from Elderberry (*Sambucus nigra* L.) 1. Process considerations for production of the liquid extract. *Journal of Food Technology*, **20** (6), 703–711.

Brønnum-Hansen, K. & Flink, J.M. (1985a). Anthocyanin colorants from Elderberry (*Sambucus nigra* L.) 2. Process considerations for production of a freeze dried product. *Journal of Food Technology*, **20** (6), 713–723.

Brønnum-Hansen, K. & Flink, J.M. (1985b). Anthocyanin colorants from Elderberry (*Sambucus nigra* L.) 3. Storage stability of the freeze dried product. *Journal of Food Technology*, **20** (6), 725–733.

Chiriboga, C. & Francis, F.J. (1970). An anthocyanin recovery system from cranberry pomace. *Journal of the American Horticultural Society*, **95** (2), 233–236.

Flink, J.M. (1983). Nonenzymatic browning of freeze-dried sucrose. *Journal of Food Science*, **48**, 539–542.

Flores, J.H. & Heatherbell, D.A. (1984). Optimizing enzyme and pre-press mash treatment for juice and colour extraction from strawberries. Flussiges Obst **7**, 327–328 (complete in German 320–324).

Fuleki, T. & Francis, F.J. (1968a). Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science*, **33**, 72–77.

Fuleki, T. & Francis, F.J. (1968b). Quantitative methods for anthocyanins. 2. Determination of total anthocyanin and degradation index for cranberry juice. *Journal of Food Science*, **33**, 78–83.

Metivier, R.P., Francis, F.J. & Clydesdale, F.M. (1980). Solvent extraction of anthocyanins from wine pomace. *Journal of Food Science*, **45**, 1099–1100.

Philip, T. (1974). An anthocyanin recovery system from grape wastes. *Journal of Food Science*, **39**, 859.

Sapers, G.M., Jones, S.B. & Maher, G.T. (1983). Factors affecting the recovery of juice and anthocyanin from cranberries. *Journal of the American Horticultural Society*, **108** (2), 246–249.

Wrolstad, R.E. (1976). Color and pigment analysis in fruit products. Bull. 624. Oregon Agriculture Station, Corvallis, OR.

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Residual NO_3^-, NO_2^-, carbonyl and TBA values of Turkish soudjouk manufactured by adding different starter cultures and using different ripening temperatures

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Summary

No standard technologically based manufacturing method exists for dry, fermented, spicy Turkish style sausage (soudjouk) and current techniques yield unacceptable, mostly rancid and putrid products with high levels of residual NO_3^- and NO_2^-. A standard method of soudjouk processing was developed. Three different starter cultures (*Lactobacillus plantarum*, *L. plantarum*+*Micrococcus aurantiacus*, *L. plantarum*+*M. aurantiacus*+*Debaryomyces hansenii*) were added to the soudjouk mixture and ripened at three different temperatures (12–14, 16–18 and 20–22°C). The addition of mixed cultures significantly reduced the residual NO_3^- and NO_2^- levels, increased the lipid soluble carbonyl levels, and produced low thiobarbituric acid values in the ripened soudjouks. For better quality characteristics, addition of *D. hansenii* is advisable and a ripening temperature of 20–22°C should be used.

Introduction

Turkish soudjouk is a spicy, typically dry, fermented sausage manufactured from beef, water buffalo meat and/or mutton and usually contains 10% tail fat of fat-tailed sheep. No standard technologically based method of soudjouk manufacture has been developed (Gökalp & Ockerman, 1985), causing serious problems as characteristic soudjouk quality attributes may not develop and end products are generally rancid and putrid (Aktan, 1979; Ertaş and Gögüş, 1980; Gökalp, 1984).

A basic component of fermented sausage processing is bacteria producing lactic acid (Deibel, Niven & Wilson, 1961; Nurmi, 1966; Coretti, 1977), but in Turkey soudjouk processing depends upon chance inoculation from the individual meat plant. Development of suitable starter cultures for soudjouk processing has been very limited. The use of pure cultures of *Lactobacillus casei v. alactosus* or *Pediococcus cerevisiae* (Özer & Özalp, 1968) and *L. plantarum* and *P. cerevisiae* (Ertaş & Göğüş, 1980) was recommended, but two different commercial starter cultures, Bactoferment (Özer & Özalp, 1968) and Duploferment (Yildirim, 1977) were not satisfactory.

Lactobacillus and *Micrococcus* are two widely used genera of bacteria for fermented sausage starter cultures. Among the micrococci, *M. aurantiacus* is commonly used for nitrate reduction, and it may eliminate hydrogen peroxide (Niinivaara, 1955; Nurmi, 1966; Coretti, 1977; Favier *et al.*, 1980). *Lactobacillus plantarum* is the *Lactobacillus* predominantly used for acid production (Nurmi, 1966; Eilberg, 1978; Favier *et al.*,

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1980). Fermented sausage starter cultures may be single bacterial strains or mixed cultures, usually of lactobacilli or micrococci (Nurmi, 1966; Krylova *et al.*, 1976; Coretti, 1977; Winter, 1977; Bacus & Brown, 1981), and the addition of certain strains of mould and yeast, especially the yeast Debaryomyces hansenii, to European fermented sausages improves flavour and colour development (Rossmanith & Leistner, 1972; Coretti, 1973).

The levels of NO_2^-, NO_3^- and N-nitrosamine in soudjouk sold in Turkey are generally very high and vary greatly (Özdemir, Bati & Gökalp, 1984). One method of reducing residual NO_3^-, NO_2^- and N-nitrosamine in fermented sausage products might be to use mixed starter cultures, e.g., micrococci and lactobacilli. Appropriate mixed cultures give a reliable and rapid pH drop and accelerate the reduction of nitrate to nitrite (Deibel *et al.*, 1961; Nurmi, 1966; Krylova *et al.*, 1976; Sutik, 1978). Effective reduction of nitrate to nitrite, and possibly to nitric oxide (NO), which readily combines with myoglobin to form nitrosomyoglobin (cured colour), would lower the required levels of these added salts (Coretti, 1973; Winter, 1977; Eilberg, 1978; Sutik, 1978). The level of lipid soluble carbonyl compounds, which contribute to the unique flavour characteristics of fermented sausages (Nurmi & Niinivaara, 1964; Demeyer, Hooze & Mesdom, 1974), could also be increased by starter cultures, especially those containing catalase active *Micrococci* (Nurmi & Niinivaara, 1964; Cantoni *et al.*, 1967). Little information is available on lipid oxidation of soudjouk, other than very low 2-thiobarbituric acid (TBA) values found in soudjouk from starter cultures (Ertaş & Gögüş, 1980).

Another process parameter, ripening temperature, has not been optimized, although different manufacturers have been using temperatures varying from 12 and 25–30°C: research workers have indicated temperatures ranging from 12–14 to 18–20°C. Relative humidity (% RH) and ventilation of the ripening rooms have not been mentioned at all (Özer & Özlap, 1968; Özhan, 1975; Ertaş & Gögüş, 1980).

The objective of this investigation was to study the effect of *L. plantarum* as a single, and *L. plantarum*, *M. aurantiacus* and *D. hansenii* as mixed, cultures on the residual NO_3^-, NO_2^- levels, lipid soluble carbonyl levels and TBA values during and at the end of the ripening period of soudjouk samples ripening at three different temperatures (12–14, 16–18 and 20–22°C). The duration of the ripening period and some other chemical, physical, microbiological and organoleptic quality characteristics of the samples are discussed elsewhere (Gökalp & Ockerman, 1985; Gökalp, 1986).

Materials and methods

Soudjouk mixture

The formulation used for the soudjouk samples was: 45% beef, 45% water buffalo meat, 10% tail fat; plus, as the percentage of the total meat and fat mixture, the following additives and spices: 2% salt, 1% garlic, 0.7% red pepper (similar to chilli), 0.5% black pepper, 0.9% cumin, 0.25% pimento, 0.6% sucrose, 0.25% refined olive oil, 0.033% $NaNO_3$ and 0.005% $NaNO_2$. The olive oil was added to soften the mixture and to help in peeling the casing from the soudjouk while preparing it for eating.

Additives and spices were added during mincing of the meat using a 2.5 cm plate mincer. The mixture was held at 0–4°C for 12 hr, and then re-minced using a 3 mm plate while frozen tail fat was slowly added. The ground mixture was then mixed well and divided into four equal parts of dough for the three different starter cultures and for the control.

Lyophilized starter cultures (*L. plantarum* Lb8, *M. aurantiacus* Mc56 and *D. hansenii* HI29; Federal Meat Research Institute, Kulmbach) were activated and total viable counts determined by plating before adding to the soudjouk mixture. The broth and agar media used for activation, growth and total plate count were APT (all purpose medium with Tween) for *L. plantarum*; TGE (tryptone glucose extract) for *M. aurantiacus*; and YM (yeast medium) for *D. hansenii*. The inoculum were sprayed on to the corresponding dough in sterile water (100 ml) as the dough was thoroughly mixed. The numbers of active microorganisms added to the three portions of soudjouk dough were 3.4×10^7 *L. plantarum*/g mixture; 3.4×10^7 *L. plantarum* plus 3.6×10^7 *M. aurantiacus*/g mixture; and 3.4×10^7 *L. plantarum* plus 3.6×10^7 *M. aurantiacus* plus 3.2×10^7 *D. hansenii*/g mixture. This level of inoculum is similar to those recommended elsewhere (Nurmi, 1966; Rei *et al.*, 1973). Nothing was added to the control group.

After the addition of the cultures, dough was stuffed into air dried bovine small intestine, and soudjouk links weighing 250–275 g were made, tied on a long string, and washed with pressured water. Subgroups of each were held in ripening rooms at 12–14, 16–18 and 20–22°C, ventilated at 0.5–1.5 m/sec. The RH was 95% on the first day, 90–95% on the second and fourth days and then gradually decreased to 80–85% on the sixth day and continued at this level until the end of the ripening period.

Ripening was considered to be completed when the water content had fallen to about 35% (Aktan, 1979; Gökalp, 1984). Soudjouks made with *L. plantarum*+*M. aurantiacus*+*D. hansenii* were ripened at 20–22, 16–18 and 12–14°C in 9, 12 and 17 days respectively. The ripening periods of the soudjouk containing *L. plantarum*+*M. aurantiacus* were very similar. Ripening periods for the soudjouk with *L. plantarum* were 12, 14 and 18 days and for the control groups 15, 16 and 19 days.

Analyses

Two links of soudjouk were randomly selected from each group for chemical analyses on the first day of the ripening and at 3-day intervals. Casings were removed with a knife and the soudjouk were separately ground in a small grinding machine. Duplicate analyses were carried out on each sample.

Determination of residual NO_3^- and NO_2^- content

Residual NO_3^- and NO_2^- values were determined by the modified methods of Ockerman (1976) and Muneta (1980) using a specific ion meter (Orion Research, Model 407A with Model 93–07 nitrate, Model 90–02 reference and Model 95–46 NO_2^- electrodes) calibrated using specific standard solutions.

Isolation and determination of lipid soluble carbonyl compounds

Concentration of hexane-soluble total carbonyls was determined by a modification of the methods of Lawrence (1965), Dimick, MacNeil & Grunden (1972) and Thomas, Dimick & MacNeil (1971). The procedure was based upon the conversion of carbonyls into their 2,4-dinitro-phenylhydrazine (DNPH) complexes and then extraction of lipid soluble carbonyls with carbonyl-free hexane. Ten grams of soudjouk sample were homogenized with 40 ml distilled water in a small blender, DNPH complexes of carbonyls were developed by rinsing the homogenate with 50 ml DNPH reagent and 6 hr allowed for the completion of the reaction. The DNP hydrazones of carbonyls were then extracted using successive 60 and 40 ml portions of carbonyl-free hexane. DNP hydrazone concentration was determined by reading the absorbance at 340 nm with the

carbonyl-free hexane as a blank. A molar extinction coefficient (E) of 22500 was used (Jones, Holmes & Seligman, 1956) to calculate the concentration of DNP derivates. These results were referred to as total carbonyl concentration and expressed as μmol of total lipid soluble carbonyl per 10 g of soudjouk sample.

TBA analysis

The degree of rancidity in each soudjouk sample was objectively measured by using TBA analysis. The procedure was the modified method of Tarladgis *et al.* (1960) and Ockerman (1976). Mean values are reported as mg malonaldehyde/kg of soudjouk or TBA numbers.

Organoleptic evaluation of the fried soudjouk

On completion of ripening, one soudjouk link from each group of soudjouk was randomly chosen and evaluated by a laboratory panel consisting of eight trained panellists. The casing was removed and the soudjouk was cut into slices of 1 cm thickness, fried, and evaluated for flavour, odour, juiciness, off-flavour and odour and general acceptability using a 1 to 9 hedonic scale (for the characteristics: 9–acceptable, 1–not acceptable; for off-flavour and odour, 9–none, 1–intense).

Results

Residual NO_3^- and NO_2^- values

Changes in the residual NO_3^- and NO_2^- values of the various soudjouk samples containing different starter cultures and ripening at three different temperatures are given in Tables 1 and 2 respectively. In the Tables, the last numbers of each column indicate that at this stage the soudjouk was fully ripened (35% moisture) and ready for sale.

During the ripening period, soudjouk samples containing mixed cultures and ripening at the higher temperaures showed lower NO_3^- and NO_2^- values than the control samples. These differences became greater toward the end of ripening. In the final product, samples prepared with the mixed cultures of *L. plantarum*+*M. aurantiacus*+

Table 1. The mean residual NO_3^- values (ppm) of soudjouk ripened at different temperatures with different starter cultures

Ripening period (days)	Ripening temperatures											
	12–14°C Starter cultures				16–18°C Starter cultures				20–22°C Starter cultures			
	C	L	LM	LMD	C	L	LM	LMD	C	L	LM	LMD
1	372	336	328	306	356	315	244	253	348	285	300	288
3	355	310	303	298	348	300	215	218	332	283	245	228
6	322	273	233	245	335	245	230	223	305	235	203	180
9	290	255	223	215	285	225	203	205	285	203	145	155
12	265	265	213	212	293	213	208	195	243	153	—	—
15	260	218	195	202	273	203	—	—	235	—	—	—
18	235	185	198	180	—	—	—	—	—	—	—	—

C = control; L = *L. plantarum*; LM = *L. plantarum*+*M. aurantiacus*; LMD = *L. plantaurm*+*M. aurantiacus*+*D. hansenii*.

Table 2. The mean residual NO_3^- values (ppm) of soudjouk ripened at different temperatures with different starter cultures

Ripening period (days)	Ripening temperatures											
	12–14°C Starter cultures				16–18°C Starter cultures				20–22°C Starter cultures			
	C	L	LM	LMD	C	L	LM	LMD	C	L	LM	LMD
1	24	21	17	15	19	14	13	13	16	10	9.0	11
3	22	20	16	16	21	16	15	14	15	14	13	13
6	16	16	14	21	19	14	13	12	16	19	16	17
9	20	19	16	18	21	17	18	17	25	17	11	10
12	18	17	16	17	21	15	17	15	22	12	—	—
15	20	16	15	13	20	13	—	—	19	—	—	—
18	18	13	12	11	—	—	—	—	—	—	—	—

C, L, LM, LMD are as in Table 1.

D. hansenii (LMD) and ripening at the higher temperatures showed lower residual NO_3^- (145–155 ppm) and NO_2^- (10–11 ppm) values than their corresponding control group (235 and 19 ppm respectively).

On the residual NO_3^- and NO_2^- values of the ripened soudjouks, highly significant ($P < 0.01$) effects of starter cultures and ripening temperatures, and a significant ($P < 0.05$) effect of starter culture X ripening temperature interactions were determined. These interactions for NO_3^- and NO_2^- values are seen respectively in Figs 1 and 2. As seen from the figures, effects of the starter cultures on the residual NO_3^- and NO_2^- levels varied according to the different ripening temperatures. In the ripened soudjouk,

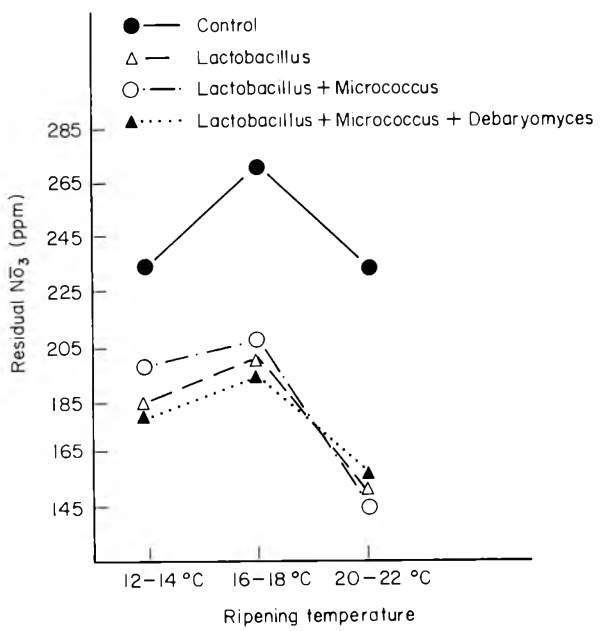

Figure 1. The effect of starter culture and ripening temperature on the residual NO_3^- values of the ripened soudjouks.

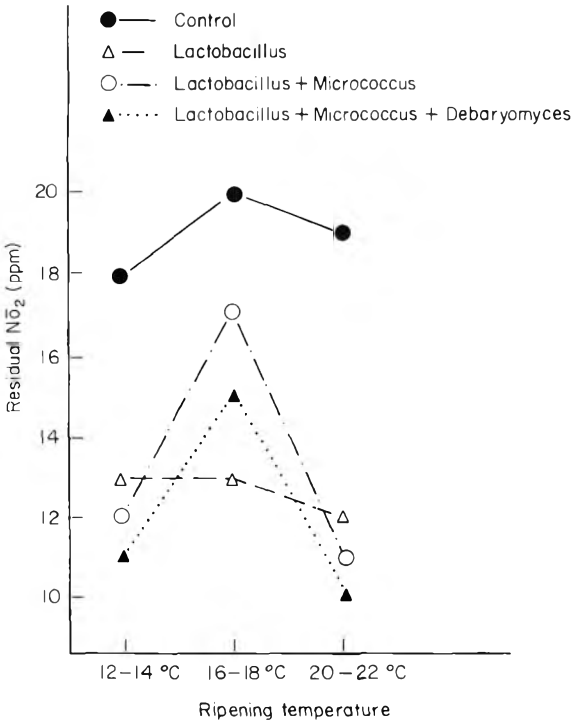

Figure 2. The effect of starter culture and ripening temperature on the residual NO_2^- values of the ripened soudjouks

the three samples made with the mixed culture LMD (NO_3^- 176 ppm, NO_2^- 12 ppm) and samples ripened at the 20–22°C (NO_3^- 172 ppm, NO_2^- 13 ppm) gave the lowest mean values of NO_3^- and NO_2^-. The control groups (NO_3^- 248 ppm, NO_2^- 19 ppm) and samples ripened at the 12–14°C (NO_3^- 200 ppm, NO_2^- 14 ppm) resulted in the higher mean values of NO_3^- and NO_2^-.

Lipid soluble total carbonyls

Total lipid-soluble carbonyl content was determined to be 6.37 μmol/10 g sample in the starting soudjouk dough mixture. This carbonyl content fluctuated but showed a general increase during the ripening period, and at the end, ripened soudjouk gave values between 12.61 and 26.86 μmol/10 g sample (Table 3). Among the ripened soudjouk, samples with the starter cultures, especially *L. plantarum*+*M. aurantiacus* (LM) and LMD mixed cultures added, and ripened at the higher temperatures (20–22 and 16–18°C) yielded higher carbonyl concentrations.

Again, the variance analysis of the total lipid soluble carbonyl data of the ripened soudjouk indicated that starter culture and ripening temperature interaction had highly significant ($P < 0.01$) effects. This significant effect of interaction can be followed in Fig. 3. Highly significant ($P < 0.01$) correlations were determined between the total lipid soluble carbonyl values of the ripened soudjouk and the panel flavour ($r = 0.82$), off-flavour and odour ($r = 0.77$) and general acceptability ($r = 0.66$) scores of these ripened and fried soudjouks.

Table 3. The mean values of the total lipid soluble carbonyl content (μmol/10 g) of soudjouk ripened at different temperatures with different starter cultures

Ripening period (days)	Ripening temperatures											
	12–14°C Starter cultures				16–18°C Starter cultures				20–22°C Starter cultures			
	C	L	LM	LMD	C	L	LM	LMD	C	L	LM	LMD
1	11.6	9.3	10.4	13.8	12.1	13.0	13.5	14.7	12.9	16.8	13.4	17.8
3	13.3	10.9	14.2	15.6	15.9	18.5	14.6	17.4	18.0	20.2	19.3	21.6
6	19.8	18.6	21.3	19.9	19.3	21.6	29.7	22.1	18.9	18.3	15.6	18.4
9	20.8	21.6	22.3	23.1	19.8	18.6	17.6	19.0	21.0	20.9	23.6	26.9
12	22.0	22.1	22.9	24.0	17.6	20.1	23.8	25.9	22.5	24.7	—	—
15	14.3	15.2	16.5	17.4	20.5	23.5	—	—	18.3	—	—	—
18	13.6	12.6	14.6	14.2	—	—	—	—	—	—	—	—

C, L, LM, LMD are as in Table 1.

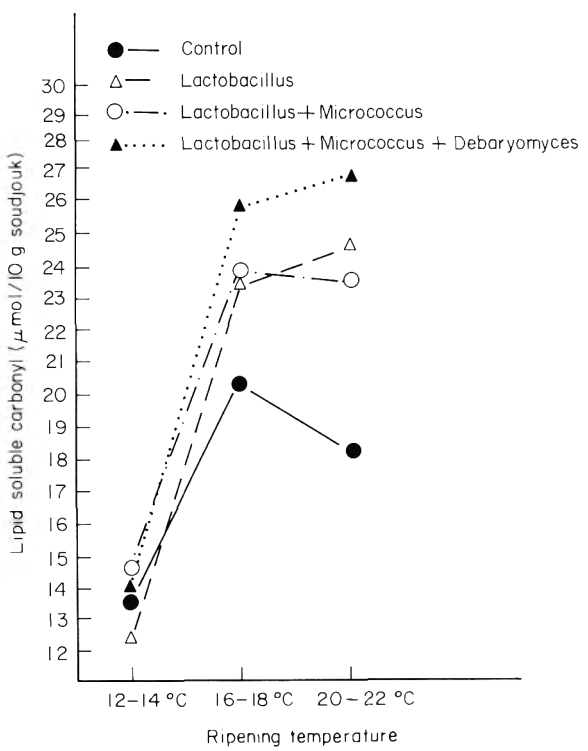

Figure 3. The effect of starter culture and ripening temperature on the lipid soluble carbonyl concentration of the ripened soudjouks.

TBA (2-thiobarbituric acid) values

TBA values of the soudjouk samples ripening at the three different temperatures generally showed increasing values from day 1 to 6 of the ripening period (Table 4). The rate of the TBA number increase was greatest in the samples ripened at 20–22°C, and the lowest in those ripened at 12–14°C. After the sixth day of the ripening, TBA values generally levelled off or started to decline very slowly.

Table 4. The mean values of TBA numbers (μmol malonaldehyde/kg soudjouk) of soudjouk ripened at different temperatures with different starter cultures

Ripening period (days)	Ripening temperatures											
	12–14°C Starter cultures				16–18°C Starter cultures				20–22°C Starter cultures			
	C	L	LM	LMD	C	L	LM	LMD	C	L	LM	LMD
1	1.53	1.62	1.28	1.45	1.31	1.47	1.24	1.31	1.16	1.33	0.93	1.42
3	1.61	1.81	1.45	1.53	1.72	1.61	1.34	1.39	1.37	1.52	1.20	1.59
6	1.73	1.83	1.59	1.53	2.08	1.80	1.61	1.58	1.57	2.15	1.86	2.13
9	1.57	1.22	1.53	1.50	1.76	1.73	1.49	1.46	1.66	2.08	1.75	1.84
12	1.07	1.19	1.46	1.41	1.55	1.54	1.35	1.24	2.12	1.90	—	—
15	1.22	1.25	1.36	1.46	1.26	1.44	—	—	2.00	—	—	—
18	1.12	1.06	1.08	1.17	—	—	—	—	—	—	—	—

C, L, LM, LMD are as in Table 1.

On the TBA numbers of the ripened soudjouks, the effect of the ripening temperatures was found to be highly significant ($P < 0.01$). While the samples ripened at 20–22°C gave the highest average (1.87), samples ripened at 12–14°C indicated the lowest (1.11) average value and samples ripened at 16–18°C (1.33) had intermediate values. However, the effects of the starter cultures and starter culture × ripening temperature interaction on the TBA values were not found to be significant ($P < 0.05$). In the ripened and market ready soudjouk TBA values ranged from 1.06 to 2.00.

Discussion

Significantly lower values of the residual NO_3^- and NO_2^- in soudjouk samples made with mixed cultures and ripening at the higher temperatures are probably produced by the starter cultures, especially the effect of *M. aurantiacus* on the nitrate and perhaps on nitrite, and the pH lowering by *L. plantarum* in these samples. Residual NO_3^- values of the ripened soudjouk samples were lower than the recommended limits of Ruusunen, Puolanne & Niinivaare (1978) and of the World Health Organization (Yıhdırım, 1977), and also lower than in soudjouk sold in Turkish markets (Özdemir *et al.*, 1984). The effects of starter cultures on the falling level of residual NO_3^- and NO_2^- have also been determined by other workers (Niinivaara, 1955; Nurmi, 1966; Coretti, 1977; Favier *et al.*, 1980). Changes in the NO_3^- values during the ripening period and NO_3^- levels in the ripened products were also similar to the values determined during Thuringer sausage fermentation (Dethmers & Rock, 1975).

Residual NO_2^- in the cured meat products accumulates from the added $NaNO_2$, especially at the lower pH (5.5–6.0), and also by the bacterial reduction (especially by *Micrococcus*) of added $NaNO_3$. While some of the reduction intermediates of $NaNO_3$

and $NaNO_2$ further reduced to NO chemically or bacteriologically and combined with myoglobin to produce the desired nitrosomyoglobin colour and anti-botulinal effects, some could be converted to N_2O_3, N_2O_5, HNO_2, HNO_3, via different reactions (Möhler & Scheerer, 1979). All these and the activity of the starter cultures could affect the final accumulation level of the NO_2^- in the soudjouk products, and may account for variations during the ripening period (Table 2). But when ripe, soudjouks made with starter cultures, especially mixed cultures, and at higher temperatures, had lower residual NO_2^- levels than the control groups demonstrating the advantage of these conditions.

Since the starter cultures increase the efficiency of nitrate and nitrite, their use could allow a reduction in the added level of $NaNO_3$ and $NaNO_2$. Similar results have been found by other workers (Winter, 1977; Ruusunen *et al.*, 1978; Eilberg, 1978).

In general, rates of decrease in NO_3^- and NO_2^- in the samples ripening at 12–14°C were very slow, because at this low temperature starter cultures did not multiply well enough to produce a noticeable effect, and on the other hand at the lower temperatures chemical reduction reactions could also proceed very slowly.

In the early stages of ripening, the carbonyls in the mixed culture soudjouks increased rapidly and ripening at the higher temperature could be due to early bacterial removal of added sugar as suggested by Demeyer *et al.* (1974) who indicated that starter cultures first attack sugar so carbonyl levels would increase in the early stages of sausage ripening. Subsequently, lipids would start to oxidize and produce lipid soluble carbonyls. The catalase activity of *M. aurantiacus*, LM and LMD mixed culture probably resulted in higher carbonyl values at all the ripening intervals and may result in the desired unique flavour and odour characteristics of the soudjouk samples, since the lipid soluble carbonyl values correlated significantly with the panel flavour and general acceptability scores. Soudjouks ripening in shorter time and with higher carbonyl values also gave more acceptable panel results, similar to the findings of Rossmanith & Leistner (1972) and Coretti (1973).

Lower TBA numbers during and at the end of the ripening could indicate less fat auto-oxidation and less rancid flavoured short chain carbon products under these processing conditions. TBA numbers using LM and LMD starter cultures ripened at all three different temperatures, were lower than those (2.28–2.40) found by Ertaş & Göğüş (1980) in soudjouks to which a pure culture of *P. cerevisia* and *L. plantarum* had been added and which had been ripened at 18–20°C.

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References

Akan, T. (1979). Determination of protein quality characteristics of soudjouks sold in Turkish market by hydroxyproline content determination. *Food Science and Technology Journal of Turkey*, **2** (1), 78–94.

Bacus, J.N. & Brown, W.L. (1981). Use of microbial cultures: Meat products. *Food Technology*, **35** (1), 74–83.

Cantoni, C., Molnar, M.R., Renon, P. & Giolitti, G. (1967). Untersuchung über die Lipide von Dauerwürsten. *Die Nahrung*, **11**, 341–344.

Coretti, K. (1973). Warum Interessiert den Praktiker

die Mikrobiologie der Rohwarstreifung? *Fleischwirtschaft*, **53**, 907–913.

Coretti, K. (1977). Starter Kulturen in der Fleischwirtschaft. *Fleischwirtschaft*, **57**, 386–393.

Deibel, R.H., Niven, C.F. Jr & Wilson, G.D. (1961). Microbiology of meat curing. III. Some microbiological and related technological aspects in the manufacture of fermented sausages. *Applied Microbiology*, **9**, 156–161.

Demeyer, D., Hoozee, J. & Mesdom, H. (1974). Specificity of lipolysis during dry sausage ripening. *Journal of Food Science*, **39**, 293–296.

Dethmers, A.E. & Rock, H. (1975). Effect of added sodium nitrite on sensory quality and nitrosamine formation in Thuringer sausage. *Journal of Food Science*, **40**, 491–495.

Dimick, P.S., MacNeil, J.H. & Grunden, L.P. (1972). Poultry product quality. Carbonyl composition and organoleptic evaluation of mechanically deboned poultry meat. *Journal of Food Science*, **37**, 544–547.

Eilberg, B. (1978). Verfahren zur Hertstellung Verbesserter Fermentierter Fleischprodukte. *German Federal Republic Patent Application*, **2**, 645–654.

Eratş, A.H. & Gögüş, A.K. (1980). Research works on the different level of tail-fat and different starter culture added soudjouks. *Journal of Scientific and Technological Council of Turkey*, D_1 **4** (3), 48–53.

Favier, M.J., Ramet, F., Favre, C. & Larpent, J.P. (1980). Use of starter for the manufacture of French dried sausage. *Zentralblatt Fur Bakteriologie*, 1B **170**, 126–132.

Gökalp, H.Y. (1984). Development of new method for Turkish style soudjouk manufacturing by adding different starter cultures at the various ripening temperatures. *Journal of Scientific and Technological Council of Turkey*, D_1 **8** (2), 116–1127.

Gökalp, H.Y. & Ockerman, H.W. (1985). Turkish-style fermented sausage (soudjouk) manufactured by adding different starter cultures and using different temperatures. I. Growth of total, psychrophilic, proteolytic and lipolytic microorganisms. *Fleischwirtschaft*, **65** (10), 1235–1240.

Gökalp, H.Y. (1986). Soudjouk manufacturing by adding different starter cultures and using different ripening temperatures. II. Ripening period, some chemical analysis, pH values, weight loss, color values and organoleptic evaluations. *Fleischwirtschaft*, **66**, 573–575.

Jones, L.A., Holmes, J.C. & Seligman, R.B. (1956). Spectrophotometric studies of some 2,4-dinitro phenylhydrazones. *Analytical Chemistry*, **28**, 1396–1339.

Krylova, V.V., Likhonosova, N.D., Mikhailova, M.M., Mirzoeva, V. Sh., Marushkina, V.I., Anisinova, I.G. & Chistyakova, T.G. (1976). Study of ripening of soft-consistency raw-smoked sausages using a bacterial preparation and gluconodeltalactone. I. *Proceedings 22nd Meeting European Meat Research Workers Conference*, G4, 1–6.

Lawrence, R.C. (1965). Use of 2,4-dinitrophenylhydrazine for the estimation of micro amounts of carbonyls. *Nature*, **205**, 1313–1314.

Möhler, K. & Scheerer, C. (1979). Bilanz der Bildung von Pökelfarbstoff im Muskelfleisch. VI. Bilanzvergleich zwischen Fleischerzeugnissen des Handles und Modellversuchen mit Muskellfleisch und Nitrit. *Zeitschrift für Lebensmittel-Unterschung und Forschung*, **168**, 381–388.

Muneta, P. (1980). Analytical errors resulting from nitrate contamination of filter paper. *Journal of Association of Analytical Chemists*, **63** (4), 937–938.

Niinivaara, F.P. (1955). Über den Einfluss von Bakterien-Reinkulturen auf die Reifung und Umrötung von Rohwurst. *Fleischwirtschaft*, **35**, 603–607.

Nurmi, E. & Niinivaara, F.P. (1964). Lipolytic changes of fats in dry sausage. *Proceedings 10th Meeting European Meat Research Workers Conference*, 115–118.

Nurmi, E. (1966). Effect of bacterial inoculations on characteristics and microbial flora of dry sausage. *Acta Agralia Fennica*, No. 108, 1–77.

Ockerman, H.W. (1976). *Quality Control of Post-Mortem Muscle Tissue*, 1st edition. The Ohio State University, Columbus, Ohio U.S.A.

Özdemir, M., Bati, B. & Gökalp, H.Y. (1984). Nitrate, nitrite and N-nitrosamine contents of Turkish soudjouks. *Fleischwirtschaft*, **64** (12), 1476–1477.

Özer, I. & Ozalp, E. (1968). Research work on the determination of microbial flora and enterotoxigenic *Staphylococcus*. Bulletin No. 3, *Food Hygiene and Technology Association of Turkey*, Ankara, Turkey.

Özhan, M. (1975). *Beef Cattle and Meat Technology*, 1st edn. Erzurum, Turkey: Ataturk University Press.

Rei, M.K., Kirikall, V.V., Redel, Y.R., Luts, V.Kh. & Teemusk, A.R. (1973). Use of concentrated starter cultures in the manufacture of cold smoked sausages. Myasnaya Industry, SSSR No. **7**, 21–24.

Rossmanith, E. & Leistner, L. (1972). Hafen als Starter Kulturen für Rohwürste. *Mitteilungsblatt der Bundesanstalt für Fleischforschung*, **38**, 1705–1709.

Ruusunen, M., Puolanne, E. & Niinivaara, F.D. (1978). Use of nitrite and nitrate in Finnish Meat Products. *Suomen Elainlaakarilehti*, **84**, 271–280.

Sutik, M. (1978). The effect of additives on pure and conjoint cultures for sausages. *24th Meeting European Meat Research Workers Conference*, 1–8.

Tarladgis, B.G., Watts, B.M., Younathan, M.T. & Dugan, L.R. Jr (1960). A distillation method for the quantitative determination of malonaldehyde in

rancid foods. *Journal of the American Oil Chemists' Society*, **37**, 44–46.

Thomas, C.P., Dimick, P.S. & MacNeil, J.H. (1971). Foultry product quality. 4. Levels of carbonyl compounds in fresh, uncooked chick and turkey skin. *Journal of Food Science*, **36**, 527–532.

Winter, F.F. (1977). Reifekulturen für Rohwurst. *Die Fleischerei*, **28** (6), 46–50.

Yıldırım, Y. (1977). Effect of the different technological soudjouk processing methods on the microbial flora and the quality characteristics. *Firat University, Veterinary College Journal (Turkey)*, **4** (1–2), 52–79.

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Extrusion behaviour of different soya isolates and the effect of particle size

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Summary

Eleven different commerically produced soya isolates, and one defatted soya flour, were extruded at temperatures of 120 and 180°C at a moisture content of 35% on a dry solids basis (dsb), using a Brabender laboratory extruder. The pressure, torque and flow rate during extrusion, and product expansion and texture of the final extrudate following rehydration varied considerably between different soya isolate preparations. Analysis by multiple regression yielded about twenty significant ($P < 0.05$) correlations between the three extrusion variables (pressure, torque and flow rate), the four feed variables (bulk density, solubility in water, particle size distribution and angle of slope) and the three extrudate characteristics (resistance to shear, solubility in water and product diameter) measured. It was concluded that the best extrusion behaviour was obtained with feed containing insoluble but native protein. Fine powders containing insoluble protein were less prone to agglomeration, and, hence, were easier to feed than powders containing high levels of soluble protein. In addition, higher shear force values after retorting were obtained with extruded products prepared from feed containing insoluble, but native protein. This was interpreted in terms of the formation during extrusion of covalent disulphide linkage between initially insoluble regions of protein. The wide variation in extrusion behaviour of chemically similar materials prepared in different ways suggests that extrusion studies on single materials should be interpreted with caution.

Introduction

Almost all investigations of soya extrusion have been confined to a single sample of soya grits. Although the protein content of this material is only about 50%, the texture of the resultant product has generally been explained in terms of interactions between protein molecules alone. The carbohydrate component has largely been ignored.

Recently, using a Brabender laboratory extruder we have compared the extrusion behaviour of a soya flour and a soya isolate (a flour was used rather than a grit because it had a similar particle size to the isolate). Although the composition of the two materials was different it was found that both could produce well-textured products. The extrusion behaviour of the flour was more dependent on moisture content and temperature than the isolate. It was suggested that this could have been because the flour contained native protein, whereas all the protein in the isolate was denatured (Sheard, Mitchell & Ledward, 1985).

An obvious question raised by investigations of this type is whether the conclusions are specific to the materials used or whether they have general validity i.e., would

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different preparations of isolates and flours behave in the same way? In this paper we describe the extrusion behaviour of a number of commercially available soya isolates. One of the objectives of this study was to determine whether there were differences between isolates prepared by different procedures. A second objective was to attempt to establish a relationship between the state of the protein in the feed material and the texture of the products without the complication of the presence of substantial quantities of non-protein material.

A major complication with work of this type is the possibility that the feed rate will vary considerably from sample to sample. This is because most soya isolates are only available as fine powders. It is well known that it is difficult to feed small extruders with fine particles and that the extrusion behaviour will depend on the particle size (Anderson *et al.*, 1969; de Muelenaere & Buzzard, 1969). For this reason the flow behaviour of the powders has been studied as well as the particle size distribution, both before and after hydration, and an attempt has been made to relate these parameters to flow rate through the extruder under identical feeding conditions. By examining the correlations between flow rate, the extrusion process variables and the properties of the extruded product, we have endeavoured to separate effects due to varying feed rate from those due to more fundamental characteristics of the feed material.

Materials and methods

Materials

Defatted soya flour and isolated soya proteins (product codes Pp860, Pp830, Pp710, Pp660, Pp610, Pp500E, Pp220) were obtained from McAuley Edwards Ltd. Further isolates—Ardex D, Ardex DHV, Ardex F and Ardex R—were obtained from British Arkady Ltd. Information supplied by the manufacturers on the composition and properties of these materials is given in Tables 1 and 2.

Particle sizing of the dry powders: diffractometry and sieve analysis

All the feeds were available as spray dried powders, except Pp220 which was granular. These powders were suspended in propan-2-ol and sized using a Malvern 2200 lazer diffractometer. The size distribution of Pp220 was obtained by sieve analysis as outlined below for the hydrated feeds.

Sieve analysis of the hydrated feeds

Ten grams of material was rehydrated to 35% moisture (dry solids basis) and equilibrated overnight. A known mass of the hydrated products (or dry Pp220) was gently shaken through a series of analytical sieves (Endecotts Ltd) and the mass retained on each sieve accurately weighed.

Water solubility of the dry powders

Two grams of material was stirred overnight in 98.0 ml of distilled water. After centrifuging at 40000 **g** for 20 min, the suspension was filtered through Whatman no. 4 filter paper and the protein content determined by a micro-kjeldahl technique using 2.0 ml of filtrate. The original protein content was taken as 91% for the isolates and 52% for the flour. The protein content was determined in duplicate and expressed on a dry weight basis.

Table 1. Composition of soya flour and various soya isolates

	McAuley Edwards Ltd							British Arkady			
	200/70*	Pp860	Pp830†	Pp710	Pp660	Pp610	Pp500E	Pp220	Ardex D	Ardex DHV	Ardex F	Ardex R
Protein ($n \times 6.25$ moisture free basis)	50	91.0		92.0	92.0	92.0	91.0	91.5	91.5	90.0	90.0	91.5
Moisture	8	5.5		5.6	5.5	5.5	5.5	6.0	6.0	6.0	6.0	6.0
Fat‡ (pet. ether extract)	1.25	0.2		0.3	0.5	0.3	0.8	0.2	0.5	0.5	0.5	0.5
Crude fibre	3.5	0.2		0.1	0.1	0.1	0.1	0.1	0.5	0.4	0.5	0.5
Ash	6.5	3.8		3.8	3.8	3.8	3.8	3.8	4.5	4.5	4.5	4.5
pH	NA	6.8		6.9	6.8	6.7	7.0	7.1	7.0	7.0	7.0	4.6

*The carbohydrate content of the flour was determined by difference.

†The manufacturers specification for this product was not available.

‡ Oil may be added at source to minimize dust; lecithin may also be added at source to aid dispersibility in water.

Table 2. Functional and other properties claimed by the manufacturers

	Physical properties	Hydration characteristics	Emulsifying and gelation properties
Soya flour (200/70)	PDI 68–76 95% min through 200 mesh	—	—
Pp860	90% through 100 mesh High density	Low water solubility and moisture absorption	—
Pp830*	—	—	—
Pp710†	—	Low viscosity	—
Pp660	—	Easily dispersible Excellent suspension properties	—
Pp610	—	High moisture absorption	Good fat absorption
Pp500E	—	—	Good emulsification properties and gelation properties
Pp220	10–40 mesh	High moisture absorption	—
Ardex D	Non-dusting	Highly dispersible Medium viscosity	Good gelling properties Emulsifier and emulsion stabilizer
Ardex DHV	0.48 g/cm^3	High solubility High viscosity	Emulsifier and emulsion stabilizer Gells irreversibly
Ardex F	Non-dusting	Highly dispersible Medium viscosity	Does not gel
Ardex R	90% through 100 mesh 0.72 g/cm^3	Good water absorption and water binding	—

*The manufacturers specification for Pp830 is not available, though its properties are said to be similar to Pp660.
†Pp710 is partially hydrolysed.

Angle of slope and bulk density

Bulk density was determined by weighing the mass of material (in a 100 ml calibrated container) after gently tapping for 4 min. A sample of powder (50 g) was placed on a piece of paper (14×9 cm), stuck onto a flat board, and gently patted by hand to give a rectangular block (approximately 14×9×1 cm). The board was gradually raised at one end and the angle at which the powder slid off the paper was recorded as the angle of slope.

Differential scanning calorimetry

Samples were hydrated to a moisture content of 66% (wet solids basis) and allowed to equilibrate for 4 h before recording the thermogram on a Perkin-Elmer DSC 2 at a heating rate of 5 K/min and a sensitivity of 0.2 mcal/sec.

Extrusion

A Brabender laboratory extruder (model DN), powered by a Docorder drive, was used. The grooved barrel had a L/D ratio of 20:1. The compression ratio of the screw was 2:1, and the die diameter was 3 mm, and its length 40 mm. The screw speed was kept at 250 rpm. The material was fed from the hopper mounted vertically above the feed end of the extruder, and equipped with an auger screw which rotated at a constant 125 rpm on a vertical axis. Pressure was measured using a Dynisco pressure transducer as described by Sheard *et al.* (1985). Two different sets of operating conditions were used. The barrel and die heaters were set at 120°C for the low temperature conditions, and at 180°C for the high temperature work. The feed section was kept at 120°C for all the experiments. The required amount of distilled water was mixed with the feed using a Kenwood mixer, and equilibrated overnight at 2–3°C prior to extrusion. The mean product diameter was determined at three to twelve points, depending on uniformity, using a micrometer gauge.

Texture and protein solubility after retorting

Distilled water (175 g) was added to protein isolates (25 g) in a can (7×11 cm) and allowed to stand for 4 hr, before retorting at 120°C for 60 min. The processed cans were left for 16 hr before making texture measurements. An Ottawa Texture Measuring System (OTMS) cell, equipped with a 9 wire grid and a base area of 30 cm^2, was filled with the contents of a single can. The peak force was recorded, using an Instron Universal Tester (model 1140), at a crosshead speed of 50 mm/min and a chart speed of 100 mm/min.

Protein solubility was measured after retorting a sample of ground extrudate (2.0 g) in distilled water (98 ml) for 60 min at 120°C in a 6×7 cm can. The can contents were filtered through Whatman no. 4 filter paper, and soluble protein determined as previously described.

Correlation coefficients

Correlation coefficients were calculated by multiple regression.

Results

Characteristics of the feed materials

The angle of slope, the bulk density and the protein solubility of the isolates and flour are shown in Table 3. The angle of slope for the particulate isolate Pp220 is far less

Table 3. Angle of slope, bulk density and protein solubility of the feed materials (dry)

	Angle of slope	Bulk density (g/ml)	Protein solubility (%)
Soya flour	63	0.56	20
Pp860	57	0.72	21
Pp830	69	0.53	32
Pp710	61	0.41	54
Pp660	73	0.55	57
Pp610	76	0.54	20
Pp500E	76	0.41	52
Pp220	35	0.55	10
Ardex D	75	0.53	44
Ardex DHV	76	0.48	45
Ardex F	79	0.46	40
Ardex R	66	0.75	3

than for any of the powders and it has low water solubility. Ardex R, an isolate prepared by isoelectric precipitation, is also very insoluble.

Thermograms are shown in Fig. 1 for soya flour and three of the isolates. No peaks were observed for the other isolates indicating that the protein component had been fully denatured during manufacture. The most stable (11S) protein fraction of the soya is still predominantly in its native form in the flour, Pp860, Ardex D and Ardex R, but the 7S fraction has been fully denatured only in Pp860.

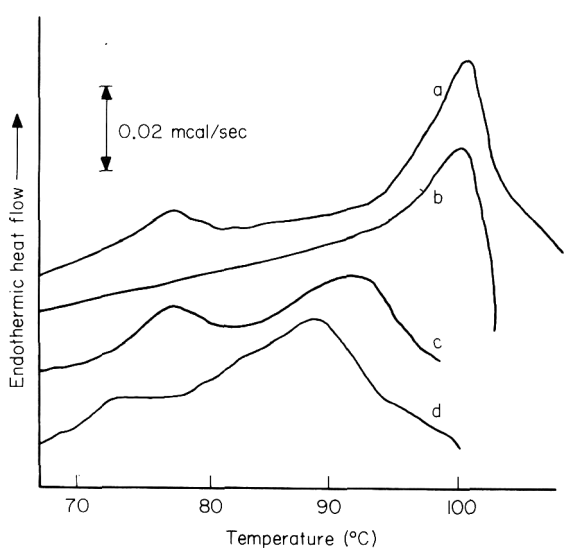

Figure 1. Typical thermograms showing denaturation of the 7S and 11S globulins in (a) soya flour (11.07 mg) and soya isolates, (b) Pp860 (11.01 mg), (c) Ardex D (8.70 mg) and (d) Ardex R (10.70 mg) at a moisture content of 66%.

The particle size distribution of the dry powder is shown in Tables 4 and 5. With the exception of Pp220 (which had a much larger size distribution), all the isolates consisted primarily of particles between 20 and 180 microns diameter (> 70%). The flour had a higher proportion of fine material than the isolates with over 50% having diameters of less than 20 microns.

Figures 2–4 show the size distribution of the hydrated feed materials. For Pp220 the size distribution is narrow and little different from the dry material, but the other feeds contained large agglomerates in addition to the finer material.

Table 4. Percentage of material by weight in the indicated size range for the dry feed materials

Size band (microns)	Soya flour	860	830	710	660	610	500	D	DHV	F	R
87–188	5.6	8.5	14.4	12.3	2.3	10.7	18.4	20.9	27.0	31.7	4.6
53–87	9.9	21.5	34.5	35.1	6.6	22.7	27.2	34.9	33.7	28.7	35.5
38–53	0.0	19.3	1.7	5.6	2.0	17.4	7.6	16.2	10.0	14.7	20.5
28–38	10.7	16.6	19.8	15.5	22.9	13.5	10.6	10.6	14.4	10.5	16.7
21–28	12.5	12.4	11.8	12.8	20.3	10.6	13.9	8.7	5.9	7.2	9.1
16–21	9.0	5.1	7.2	6.8	13.2	7.5	8.1	3.6	3.9	3.4	6.1
13–16	8.4	6.2	4.8	3.6	9.4	4.8	5.3	2.9	3.0	1.3	2.7
10–13	6.8	1.4	1.5	0.9	6.8	3.4	3.6	0.0	0.0	0.0	1.2
8–10	10.8	3.1	0.7	2.0	4.9	3.1	2.2	0.3	0.0	0.0	0.6
6–8	6.9	0.2	0.0	1.3	2.5	1.5	0.0	0.0	0.0	0.0	0.0
5–6	3.1	0.0	0.0	0.8	1.4	0.9	0.6	0.0	0.0	2.2	0.0
4–5	2.0	0.0	1.7	0.0	1.5	0.0	0.1	0.0	0.0	0.0	0.0
3–4	3.4	1.3	0.0	1.2	0.0	1.0	0.0	0.0	2.0	0.0	2.4
2–4	2.6	1.2	0.0	1.0	1.6	0.8	0.7	0.0	0.0	0.0	0.0
< 2	3.5	1.2	0.0	0.9	1.5	0.8	0.7	0.8	0.0	0.0	0.0

Table 5. Percentage of dry feed material by weight in the indicated size range for Pp220

Nominal sieve size (microns)	1680	1400	1200	1000	850	710	600	355	210	< 210
Weight %	1.5	11.9	18.8	27.0	13.5	12.3	5.9	6.5	1.8	0.8

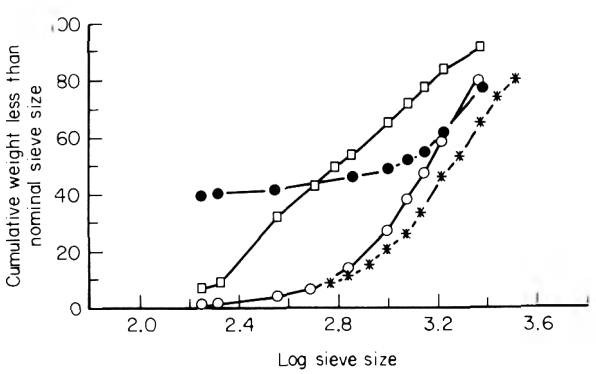

Figure 2. Size distribution of soya flour (*), Pp860 (□), Pp830 (●) and Pp710 (○) in the hydrated feed (30% dsb).

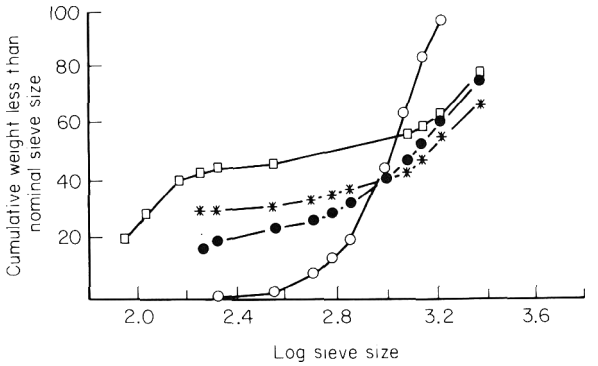

Figure 3. Size distribution of Pp660 (*), Pp610 (□), Pp500E (●) and Pp220 (○) in the hydrated feed (30% dsb).

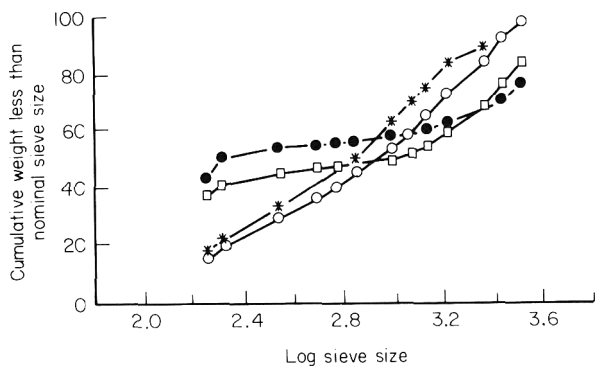

Figure 4. Size distribution of Ardex D (*), Ardex DHV (□), Ardex F (●) and Ardex R (○) in the hydrated feed (30% dsb).

Extrusion data

The extrusion data is shown in Tables 6 and 7, and the data in Table 6 agrees satisfactorily with the values predicted by the equation derived by Sheard *et al.* (1985) for soya flour and soya isolate (610), with one or two notable exceptions (Table 8). These exceptions may reflect screw wear over the intervening 12 months and the fact that different batches of flour and isolate were used. Another possibility is that the differences were due to the different screw speeds of the auger used in this work. This unfortunately occurred as the motor had to be changed in the intervening period.

The flow rates for low and high temperature extrusion were similar but, because of the decrease in viscosity, torques and pressures were lower at 180 than at 120°C.

Product characteristics

The products produced on extrusion of the different feed materials are shown in Figs 5–7 and it can be seen that they differ markedly from one another. There were also wide

Table 6. Pressure, torque and flow rate for soya proteins extruded at 120 and 180°C

	120°C			180°C		
	Press (psi)	Torque (Nm)	Flow (g/min)	Press (psi)	Torque (Nm)	Flow (g/min)
Soya flour	1700	22	57	425	16	53
Pp860	2700	27	76	1290	21	66
Pp830	1540	12	30	635	7	21
Pp710	585	9	35	115	4	30
Pp660	1655	14	33	810	9	40
Pp610	1950	18	44	625	11	36
Pp500E	1675	15	28	575	11	38
Pp220	2425	21	111	1675	20	122
Ardex D	1560	16	53	725	10	51
Ardex DHV	1820	17	42	595	9	39
Ardex F	1555	13	24	625	5	24
Ardex R	3200	36	67	1020	24	62

Table 7. Product diameter, OTMS texture* and protein solubility* for soya proteins extruded at 120 and 180°C

	120°C			180°C		
	Product diameter (mm)	Peak force (N)	Solubility (%)	Product diameter (mm)	Peak force (N)	Solubility (%)
Soya flour	4.1	116	47	5.3	233	46
Pp860	5.1	1290	21	6.6	1300	21
Pp830	5.1	225	45	7.4	166	49
Pp710	3.6	190	54	4.3	403	56
Pp660	4.6	159	50	7.4	164	50
Pp610	4.8	325	40	5.8	325	39
Pp500E	5.3	110	54	5.8	77	57
Pp220	5.3	79	63	5.1	50	62
Adrex D	5.1	42	63	7.9	52	65
Adrex DHV	6.6	26	80	8.4	22	80
Adrex F	4.8	94	58	7.6	83	62
Adrex R	4.1	4500	8	4.1	4500	7

*Average of duplicate results.

variations of OTMS texture. However, this does not depend strongly on extrusion temperature (Table 7). It is also seen from this table that extrudates having a low solubility in water appear, not unexpectedly, to possess the tougher textures.

Correlation between feed characteristics and the extrusion behaviour of soya isolates

Tables 9 and 10 show the correlations for all the measured variables. It is interesting to note that these were similar for extrusion at 120 and 180°C.

Table 8. Pressure, torque and flow rate data for soya flour and Pp610 extruded at 120 and 180°C and their values predicted by Sheard *et al.* (1985)

	120°C			180°C		
	Press (psi)	Torque (Nm)	Flow (g/min)	Press (psi)	Torque (Nm)	Flow (g/min)
Soya flour						
experimental	1700	22	57	425	16	53
predicted	1889	24	72	605	13	79
Pp610						
experimental	1950	18	44	625	11	36
predicted	2294	19	44	1540	16	61

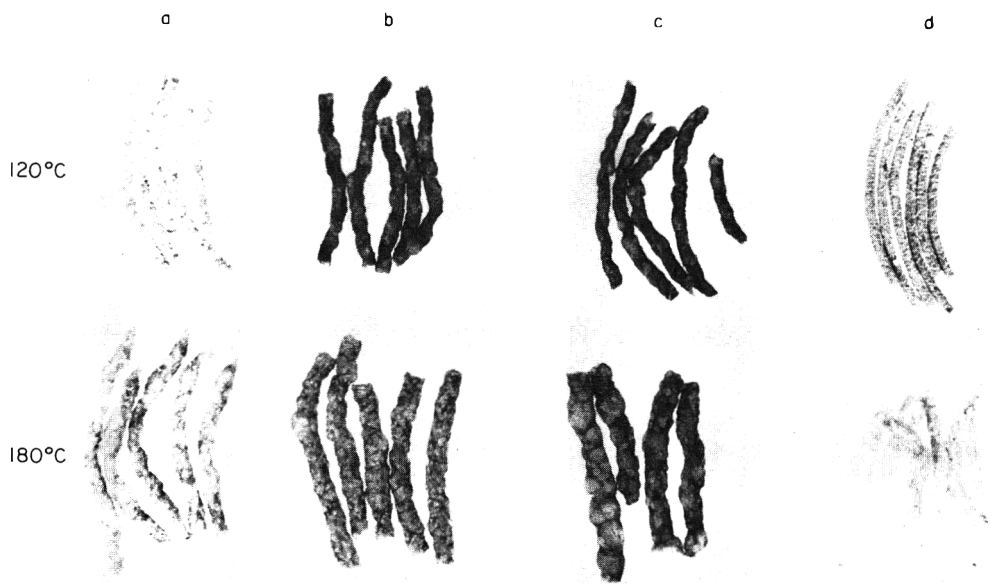

Figure 5. Soya extrudates at 120 and 180°C from (a) soya flour, (b) Pp860, (c) Pp830 and (d) Pp710.

Discussion

Flow rate

It is apparent from the data in Table 6 that there is a wide variation in extruder output between samples. This suggests that, with the possible exception of the granular material (Pp220), the extruder is operating under starve feed conditions with the screw flights incompletely filled except near the die end. Continuous extrusion was observed with all samples, although, when the flow rate was low there were considerable fluctuations in the diameter of the extrudate.

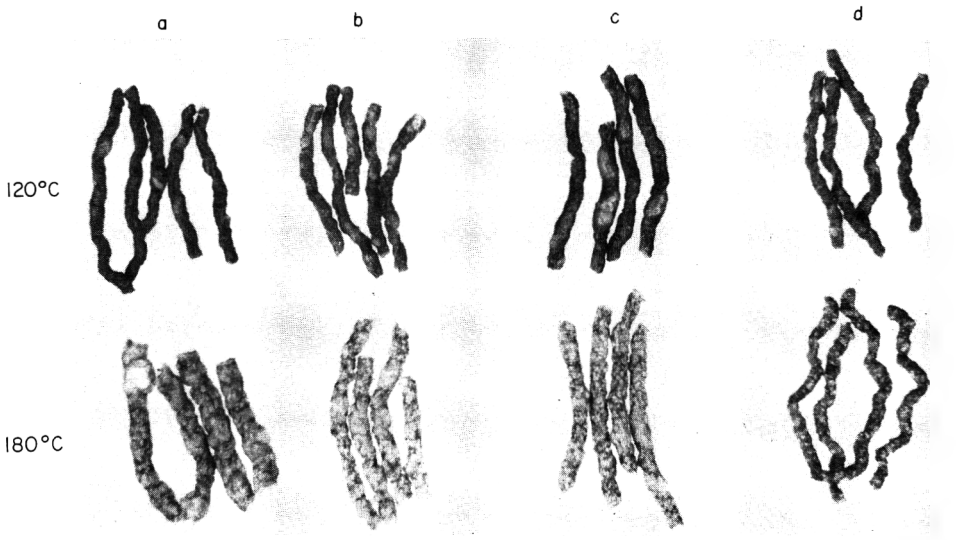

Figure 6. Soya extrudates at 120 and 180°C from (a) Pp660, (b) Pp610, (c) Pp500E and (d) Pp220.

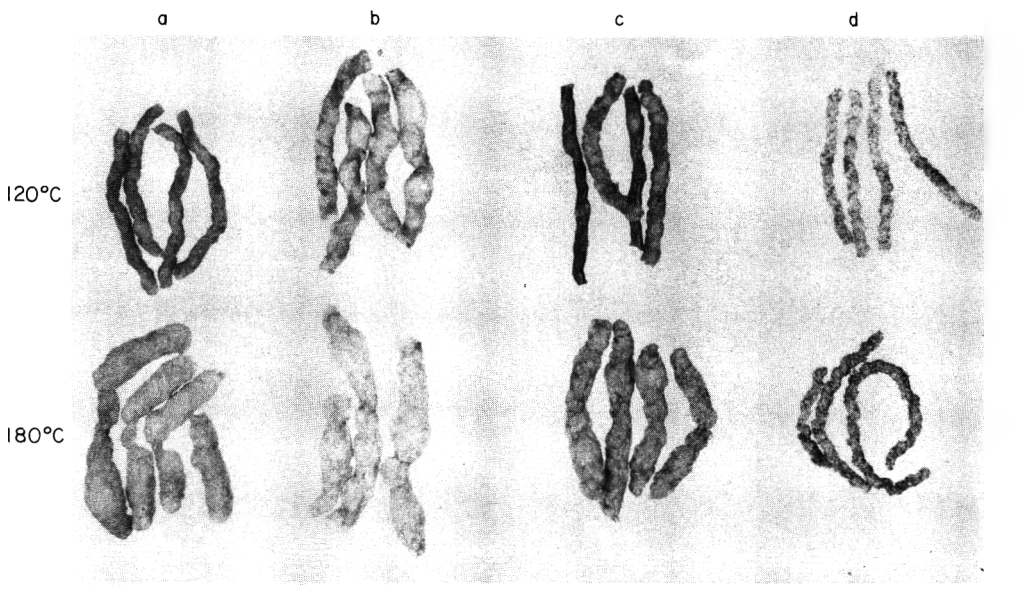

Figure 7. Soya extrudates at 120 and 180°C from (a) Ardex D, (b) Ardex DHV, (c) Ardex F and (d) Ardex R.

The flow rate gave the expected positive correlations with torque and pressure. For materials that show rheological behaviour that can be represented by a power law equation, the pressure drop across the die will be proportional to Q^n where n is the flow behaviour index and Q the volumetric flow rate (Levine, 1982). The torque will be governed primarily by the extent to which mechanical energy is dissipated as heat. This will occur, to a large extent, in the clearances between the flights and the extruder barrel

Table 9. Matrix of correlation coefficients for soya proteins extruded at 120°C

1	1.00									
2	0.92	1.00								
3	0.63	0.63	1.00							
4	−0.07	0.18	0.09	1.00						
5	0.83	0.72	0.28	−0.32	1.00					
6	−0.81	−0.80	−0.75	0.11	−0.59	1.00				
7	−0.71	−0.62	−0.22	0.53	−0.80	0.62	1.00			
8	0.88	0.84	0.55	−0.19	0.77	−0.76	−0.80	1.00		
9	−0.31	−0.31	−0.85	0.13	−0.09	0.53	0.14	−0.32	1.00	
10	0.35	0.54	0.74	0.43	0.18	−0.45	−0.03	0.37	−0.53	1.00
	1	2	3	4	5	6	7	8	9	10

1 Torque,
2 Pressure,
3 Flow rate,
4 Product diameter,
5 OTMS texture,
6 Protein solubility of feed,
7 Protein solubility of extrudate,
8 Bulk density of feed,
9 Angle of slope,
10 % of particles < 1600 microns.
Regression coefficients > 0.602 are significant at the 5% level, and > 0.735 at the 1% level.

Table 10. Matrix of correlation coefficients for extruded soya proteins at 180°C

1	1.00									
2	0.74	1.00								
3	0.75	0.84	1.00							
4	−0.42	−0.06	−0.38	1.00						
5	0.64	0.26	0.19	−0.50	1.00					
6	−0.83	−0.61	−0.61	0.43	−0.58	1.00				
7	−0.68	−0.28	−0.14	0.48	−0.84	0.65	1.00			
8	0.82	0.59	0.42	−0.23	0.77	−0.76	−0.86	1.00		
9	−0.55	−0.65	−0.87	0.52	−0.10	0.53	0.13	−0.32	1.00	
10	0.47	0.82	0.69	0.05	0.15	−0.45	−0.05	0.37	−0.53	1.00
	1	2	3	4	5	6	7	8	9	10

1 Torque,
2 Pressure,
3 Flow rate,
4 Product diameter,
5 OTMS texture,
6 Protein solubility of feed,
7 Protein solubility of extrudate,
8 Bulk density of feed,
9 Angle of slope,
10% of particle < 1600 microns.
Regression coefficients > 0.602 are significant at the 5% level, and > 0.735 at the 1% level.

where the shear rate will be very high (Harper, 1981a). Clearly the extent of this energy conversion will decrease as the screw becomes less completely filled.

The flow rate is positively correlated with the percentage of particles in the hydrated feed with a size less than 1600 microns. Although the particle size of the dry granular Pp220 material is much larger than the powders, on hydration the powders show a greater tendency to agglomerate. Thus, in the hydrated feed Pp220 has more particles less than 1600 microns, compared to any of the powders. It seems probable that for the powders, the large agglomerates collect in the bottom of the feed hopper and in the entrance to the extruder forming a partial blockage through which the smaller particles have to percolate.

There is a negative correlation between the flow rate and the protein solubility in the feed. This may well be because material containing soluble protein is more prone to agglomeration, although the negative correlation between the percentage of particles less than 1600 microns and protein solubility in the feed is not significant at the 5% level.

It seems that differences in adhesion between the hydrated particles are also present in the dry materials as there is a high negative correlation between the angle of slope and the flow rate.

Product texture

An obvious question to consider is, whether the variations in OTMS texture arise because of the different flow rates or whether they are due to differences in the nature of the protein? There are two reasons why we believe that the different textures are due to something more fundamental than differences in flow rate.

Firstly, there is not a significant correlation between flow rate and OTMS texture (Table 9 and 10). Secondly, the average residence time will increase with decreasing flow rate. Thus, one of the differences between material that feeds poorly and material that feeds well will be the severity of the heat and shear treatments applied; although in view of the similarities in texture between isolates extruded at 120 and 180°C, variations in heat treatment over a wide range do not seem to be important.

The material that gave the highest OTMS texture was the isoelectric isolate Ardex R. This suggests that electrostatic interactions play an important role in determining the final texture, either because of ionic bonding, or, alternatively, because at pHs different from the isoelectric point, electrostatic repulsion limits the extent to which other aggregation reactions can occur. Both Rhee, Kuo & Lucas (1981) and Simonsky & Stanley (1982) found that pH greatly affected interior and exterior morphology of extruded soya grits. If the results for Ardex R are omitted then the correlations shown in Tables 9 and 10 are not substantially changed.

There is a negative correlation between texture and protein solubility in the feed, and a positive correlation between protein solubility in the feed and in the extrudate after retorting. The correlation between protein solubility in the feed and in the extrudate suggests that heat stable covalent bonds, presumably disulphide (Sheard *et al.*, 1986), may form during extrusion primarily between regions of the protein that are already associated non-covalently in the feed material. The formation of intermolecular disulphide bonds in soya following initial stabilization by non-covalent bonding has also been used to explain the solubility and heat denaturation behaviour of this protein system (Shen, 1981).

There is some disagreement on whether the state of denaturation of the protein in the feed material effects extrusion behaviour e.g., compare Harper, 1981b; and Lillford, 1986. Our data suggests that, although it is possible to obtain satisfactory

textures from feed containing no native protein, the highest OTMS textures are given by materials containing some native protein, with the important proviso that the solubility must also be low. Thus the highest OTMS values are given by Ardex R and Pp860. It is interesting that, unlike other feeds of low solubility, the solubility of the retorted extrudates prepared from Pp860 and Ardex R was not substantially higher than the cold water solubility of the feed. This suggests that the insoluble material in the feed is more prone to associate covalently during extrusion if it contains at least some native protein.

The question of whether these ideas extend to flour and grits is of practical interest. In general, flour did not depart from the relationships illustrated by the correlations in Tables 9 and 10. However, despite the fact that the flour contained native protein, the protein solubility in the retorted extrudate was substantially higher than in the feed suggesting that the carbohydrate component, either directly (Sheard, Ledward & Mitchell, 1984), or indirectly by dilution of the protein component (Sheard *et al.*, 1986), interferes with the formation of some protein–protein interactions.

Conclusions

We suggest that two conclusions of particular interest may be drawn from this work. First, there is a very wide variation in the extrusion behaviour of materials with similar chemical composition. We have interpreted these differences in terms of the protein component present though we would not wish to eliminate the possibility that the addition of small quantities of oil or lecithin may also contribute to these differences. Because there are such large differences, the results of experiments on a single material should be treated with caution. By altering the procedure by which the feed is prepared it may be possible to texturize proteins which, hitherto, have not been amenable to extrusion processing.

Second, better results are obtained when the protein in the feed material has a low water solubility. If the feed is in the form of a fine powder, then insoluble protein shows less tendency to agglomerate on the addition of water and feeding improves. In addition, feed containing protein with a low water solubility gives a better texture after extrusion. This appears to be particularly the case if some native, as opposed to denatured, protein is present.

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References

Anderson, R.A., Conway, H.F., Pfeifer, V.F. & Griffin, E.L. Jr (1969). Gelatinisation of corn grits by roll and extrusion cooking. *Cereal Science Today*, **14** (ii), 372–375 & 321.

Harper, J.M. (1981a). *Extrusion of Foods*, Vol. 1. Florida: CRC Press.

Harper, J.M. (1981b). *Extrusion of Foods*, Vol. 2. Florida: CRC Press.

Levine, L. (1982). Estimating output and power of food extruders. *Journal of Food Process Engineering*, **6**, 1–13.

Lillford, P.J. (1986). Texturisation of proteins. In: *Functional Properties of Food Macromolecules* (edited by J.R. Mitchell & D.A. Ledward). Pp. 355–384. London: Elsevier Applied Science Publishers.

de Muelenaere, H.J.H. & Buzzard, J.L. (1969) Cooker extruders in the service of world feeding..

Food Technology (Chicago) **23**, 24.

-hee, K.C., Kuo, K. & Lucas, E.W. (1981). Texturisation. In: *Protein Functionality in Foods* (edited by J.P. Cherry). Pp 51–88. ACS Symposium Series, 147.

-imonsky, R.W. & Stanley, D.W. (1982). Texture structure relationships in textured soya protein 5. Influence of pH and protein acetylation on extrusion texturisation. *Canadian Institute of Food Science and Technology*, **15** (4), 294–301.

heard, P.R., Ledward, D A. & Mitchell, J.R. (1984). Role of carbohydrates in soya extrusion. *Journal of Food Technology*, **19**, 475–483.

Sheard, P.R., Mitchell, J.R. & Ledward, D.A. (1985). Comparison of the extrusion cooking of a soya isolate and a soya flour. *Journal of Food Technology*, **20**, 763–771.

Sheard, P.R., Fellows, A., Ledward, D.A. & Mitchell, J.R. (1986). Macromolecular changes associated with the heat treatment of soya isolate. *Journal of Food Technology*, **21** (1), 55–60.

Shen, J.L. (1981). Solubility and viscosity. In: *Protein Functionality in Food*, (edited by J.P. Cherry). Pp. 89–109. ACS Symposium Series, 147.

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Technical note: Textural quality of cooked Malaysian fresh water prawns (*Macrobrachium rosenbergii*) as influenced by the moulting cycle

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Introduction

The Malaysian freshwater prawn, *Macrobrachium rosenbergii* is currently being aquacultivated world-wide. These crustaceans have been reported to have only a short shelf life on ice, due to loss of texture on cooking. (Kurtzman & Snyder, 1960; Miyajima & Cobb, 1977; DPED, 1978; Waters & Hale, 1981; Rowland, Finne & Tillman, 1982; Baranowski, Nip & May, 1984 and Angel *et al*, 1986).

V.D. Sidwell (pers. comm.), felt that quality was more closely associated with the stage of moulting than any other factor, but no data was presented to support the assumption. The present study was carried out to determine whether mushiness in freshly caught, cooked prawns is related to the moulting cycle.

Materials and methods

Collection and preparation of prawns

The prawns were taken from polycultured fish ponds in Israel, on three separate occasions from three locations in the Bet She'an Valley. In the first two experiments (Exp. 1 and 2) the live prawns were lifted off the conveyors or picked out of the shallow water at the ponds edges or out of the mud, as the ponds were drained. The live prawns were taken to a field laboratory for cooking and organoleptic testing. In the third experiment (Exp. 3) 1 day iced prawns were taken from a commercial packing house. Most of them had been taken from the mud of a large pond that was being drained.

In the three experiments the prawns were divided into three moulting stages, according to the hardness or softness of the dorsal shield exoskeleton in the cardiopericardial and branchial regions (Patwardhan, 1937), and of the rostrum. External exoskeleton pigmentation, which undergoes changes during the moult cycle of this prawn (Peebles, 1977), was used as an additional indicator of the moult stage.

Intermoult prawns had a hard exoskeleton and a hard rostrum. Premoult prawns had a flexible exoskeleton and a hard rostrum. Postmoult prawns had a soft exoskeleton and a deflectable rostrum. The terminology was adopted from Peebles (1977).

Sensory evaluation

In Exps 1 and 2 the prawns in each moulting stage were cooked for 3 min in boiling water and then air cooled. The cephalothorax was separated from the tail leaving the lobes on the segment intact. The lobes refer to the two protruding sections of the first

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segment which lie in proximity to the hepatopancreas. The shell was then removed from the tail and the tails evaluated for texture.

Sensory evaluation of texture was carried out by an expert tester on the lobes of the first segment and the other segments randomly using a double blind procedure. The lobes and segments were bitten once by the incisor teeth and the texture classified as firm, soft or mushy. Firm is defined as offering maximum resistance to a single bite by the incisor teeth or having a springy texture. Soft is defined as offering little resistance to biting, and lacking springiness. Mushy is defined as offering no resistance to biting and possessing a mealy texture.

In Exp. 1 both lobes were tasted at the same time and the average texture noted. In Exp. 2, first one lobe was tasted, the prawns randomized and the second lobe tasted, using the same double blind procedure. The experiments are designated Exp. 2A and 2B respectively. For Exps 1 and 2, chi-square tests were carried out, yielding two way frequency tables of groups×degrees of textural quality.

Histological examination

In Exp. 3, following cooking, the prawns were cooled for several minutes in ice water and drained for about 10 min. Mushiness was determined histologically by measuring the degree of fibre disruption of the muscle. In preliminary experiments this method showed a marked difference in fibre disruption between the firm muscle tissue of freshly caught prawns and the tissue of prawns stored on ice for 1 week.

The lobes from the first segment of each prawn were placed in a 10 ml test tube with 1.0 ml distilled water. Each tube was covered with parafilm and inverted by hand three times. Immediately after inversion the entire 1.0 ml of water was removed and three 0.03 ml aliquots pipetted onto three precleaned, sulphochromic acid washed microscope slides. Each aliquot of water on the slide was smeared evenly over 1 cm^2 of surface. The slides were covered, allowed to dry for 24 hr, and stained with Giemsa stain (Humason, 1967).

The myofibrils were counted and their lengths measured at ×40 magnification using a moveable scale placed under the slide. The myofibrils were classified into long (0.5–1.0 mm), medium (0.1–0.5 mm) and short (0.1 mm or less), and the average number of fibres in each group was calculated for each slide. Both lobes of 105 prawns were examined separately.

The significance of the differences between the number of fibres in each group for the three stages of the moulting cycle was estimated by analysis of variance using Duncan's multiple range test with a GLM procedure in a SAS programme using an IBM 4341 computer.

Results

The differences in firmness between the pre- and postmoult prawns were not significant. Thus these two groups were combined and compared with the prawns in the intermoult stage. Only the lobes of the first segment showed a significant difference in firmness between the intermoult, and pre- and postmoult prawns.

Table 1 shows the textural quality of the lobes in the intermoult and the pre- and postmoult prawns. In Exp. 1 the percent of firm lobes in the intermoult stage was greater than in the pre+postmoult, and the percent of soft lobes in the pre+postmoult was greater than in the intermoult stage. The percent of mushy lobes was not dependent on the moulting stage.

Table 1. Textural quality of cooked prawn tail first segment lobes from the intermoult and pre- and postmoult stages

Exp.	Moulting stage	Texture (%) Firm	Soft	Mushy	χ^2	P'
1 (n = 94)	Intermoult	63.3	6.7	30.0		
	Premoult+ postmoult	43.8	31.3	25.0	7.03	0.03
2A (n = 79)	Intermoult	57.4	31.2	11.5		
	Premoult+ postmoult	17.7	47.1	35.3	9.81	0.007
2B (n = 79)	Intermoult	55.74	31.15	13.11		
	Premoult+ postmoult	5.56	44.44	50.00	17.4	0.0002

In Exp. 2A the percent of firm lobes in the intermoult stage was much greater than in the pre+postmoult stages, while the percent of soft lobes in the latter was somewhat greater than in the former. The percent of mushy lobes was much greater in the pre+postmoult than in the intermoult prawns.

In Exp. 2B the percent of firm lobes in the intermoult stage was much greater than in the pre+postmoult stages, while the percent of soft lobes in the three stages was similar. The percent of mushy lobes was, however, much greater in the pre+postmoult prawns.

The histological studies, summarized in Table 2, of the disrupted myofibrils in the prawn lobes of Exp. 3 showed there were no significant differences in the number of long fibres among the three moulting stages. However, there were significantly more disrupted medium size fibres in the pre- and postmoult than in the intermoult prawns.

The greatest differences between moult cycle stages were in the average number of disrupted short fibres, with the fewest (17) in the intermoult and most (102) in the postmoult stage.

Table 2. Histological examination of disrupted first segment lobe myofibrils in three moult cycle stages (Exp. 3, n = 105)

Moulting stage	Average number myofibrils per prawn* Long fibres	Medium fibres	Short fibres	Fibre disruption index Ratio short to long fibres
Intermoult (n = 79)	4.3[a]	7.6[a]	17.0[a]	4.25
Premoult (n = 11)	6.3[a]	20.1[b]	102.0[b]	17.0
Postmoult (n = 15)	8.4[a]	19.5[b]	60.1[c]	7.5
S.D.	11.5	17.7	60.9	

*Means with same letter in a column are not significantly different. ($P > 0.05$).

A fibre disruption index was calculated as the ratio of short-to-long fibres in each of the three stages, the premoult having the highest ratio.

Discussion

The pre + postmoult prawns were softer or mushier and had a larger proportion of short fibres, than the intermoult stage prawns.

Prawns in the three stages differ in their muscle composition and the present results can be explained by the biological changes which take place between the moulting stages. Intermoult prawns have firm compact tissue and upon entering the premoult stage begin to absorb water. This water uptake, which can increase the body weight by 60%, aids the prawns in the process of ecdysis (the shedding of the old exuvia) (Passano, 1960). Such absorption of water may soften the tissue by disrupting the inter-fibre connections within the muscle. Muscle tissue in the lobes of the first segment, which lie on both sides of the hepatopancreas and thus in proximity to digestive and proteolytic enzymes (Baranowski *et al.*, 1984), tends to disrupt and disintegrate on cooking.

After moulting, the amount of water in the tissues gradually decreases (Passano, 1960), although sufficient may still be present to cause significant myofibrillar disintegration and mushiness.

The sensory test suggested there was no significant difference in texture between pre- and postmoult prawns, perhaps because a proportion of prawns which had been classified pre- and postmoult were either just going into or just coming out of the intermoult stage. However, there were highly significant differences in the number of short fibres in the first segment lobes for the pre-, post- and intermoult stages. Thus, the histological test may be a better indicator of the disintegration state of the tissue after cooking than the sensory test.

Conclusions

Premoult prawns are apparently most prone to tissue degradation. Such prawns would have the shortest shelf life, since degradation advances rapidly due to both endogenous proteolytic enzymes in the muscle (Mykles & Skinner, 1982) and the action of hepatopancreas digestive enzymes. Postmoult prawns would tend to be less mushy, but since they also contain large amounts of water they are also prone to tissue degradation.

Due to the vulnerability of the pre- and postmoult prawns, it would be best to consume them within hours of catching. If storage on ice is necessary, the heads should be removed. It would be advisable not to freeze prawns in these two stages of moulting.

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References

Angel, S., Juven, B.J., Weinberg, Z.G., Lindner, P. & Eisenberg, E. (1986). Effects of radurization and refrigerated storage on quality and shelf life of freshwater prawns, *Macrobrachium rosenbergii*. *Journal of Food Protection*, **49**, 142–145.

Ba-anowski, E.S., Nip, W.K. & Moy, J.M. (1984). Partial characterization of crude enzyme extract from the freshwater prawn, *Macrobrachium rosenbergii*. *Journal of Food Science*, **49**, 1494–1495, 1505.

DFED Department of Planning and Economic Development (1978). Aquaculture Planning Program. Aquaculture Development for Hawaii. Assessment and Recommendations. Hawaii, U.S.A.

Humason, G.L. (1967). *Animal Tissue Techniques*. San Francisco: Wolf Freeman and Company.

Kurtzman, C.H. & Snyder, D.G. (1960). The picric acid turbidity test. A possible practical freshness test for iced shrimp. *Food Technology*, 7, 337–342.

Miyajima, L.S. & Cobb, B.F. (1977). Preliminary observations on the frozen storage stability of freshwater prawn, *Macrobrachium rosenbergii*. Second Annual Tropical and Subtropical Fisheries Technological Conference of the Americas. Texas A & M University Publication. TAMU-SG 78–101 p. 253.

Mykles, D.L. & Skinner, D.M. (1982). Moult cycle associated changes in calcium — dependent proteinase activity that degrades actin and myosin in crustacean muscle. *Developmental Biology*, **92**, 386–397.

Passano, L.M. (1960). Moulting and its control. *Physiology of Crustaceae. Vol. I. Metabolism and Growth*, (edited by T.H. Waterman). Pp. 473–536. New York: Academic Press.

Patwardhan, S.S. (1937). The Indian Zoological Memoirs. p. 9. Lucknow, India: Lucknow Publishing House.

Peebles, J.B. (1977). A rapid technique for moult staging in live *Macrobrachium rosenbergii*. *Aquaculture*, **12**, 173–180.

Rowland, B., Finne, G. & Tillman, R. (1982). A morphological study of muscle proteolysis in the tails of *Macrobrachium rosenbergii*. *Proceedings of the 7th Annual Tropic and Subtropic Fish Technology. Conf. of the Americas*. P.p. 105–112.

Waters, M.E. & Hale, M.B. (1981). Quality changes during iced storage of whole freshwater prawns, *Macrobrachium rosenbergii*. Contribution Number 81–25c. South East Fisheries Center, National Marine Fisheries Service S.C., U.S.A. p. 1–13.

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Book Reviews

Principles of Plant Biotechnology: An Introduction to Genetic Engineering in Plants. By S.H. Mantell, J.A. Matthews & R.A. McKee. Oxford: Blackwell, 1985. Pp. iii+269. ISBN 0 632 01215 3. £10.80 (paperback).

The ancient but rapidly expanding craft of plant biotechnology has its roots in many fields of science, and depends on diverse techniques. These range from manipulation and modification of the genes, through methods applied at cell and tissue levels, to industrial-scale fermentations. This book gives the scientific background to these techniques, and describes how newly emerging methods are being used in the exploitation of plants and the development of marketable products or services from them.

After a brief introductory chapter in which the importance of plants in the global economy is established, and 'Biotechnology' is defined, there are four chapters on techniques and background science, followed by three on practical applications of these. Interestingly, the 'science' chapters occupy a larger proportion of the book, at 122 pages, than the 'applications' chapters with only 90. The diverse topics covered in the earlier chapters—plant molecular biology, cloning plant genes and transferring them to other plants, and tissue culture methods—inevitably mean that some of the treatment is rather sketchy. Each individual topic has been, after all, the subject of a number of recent full-length texts. Nevertheless, the present volume does manage to give a good introduction to these topics, and a useful feature is a list of suggested further reading at the end of each chapter. The reader who is not familiar with recombinant DNA and plasmid techniques may find the going fairly heavy in some places, for example in the description of the construction of gene vectors based on the *Agrobacterium* Ti plasmid.

The 'applications' chapters cover use of culture techniques in rapid propagation methods, the use of tissue, cell and molecular techniques in plant breeding, and some industrial uses of plants, cells and enzymes derived from them. The writing here is quite nicely balanced between existing achievements—the clonal propagation of oil palms, and the use of plants as sources of pharmaceuticals are notable examples—and the difficulties which remain before much of the technology which works in the laboratory can have a commercial manifestation. In the area of gene transfers, there is a good discussion of the problems associated with modification of storage proteins, and insertion of genes of N-fixation into non-leguminous crops. In contrast, the intriguing possibilities of controlling weeds, pests and diseases by novel techniques are scarcely explored.

The book includes no fewer than eight appendices, covering topics as diverse as some extremely basic molecular biology, and numerous recipes for tissue culture media. I am not convinced that these are useful: anyone who requires the basic molecular biology should read a book on the subject, and anyone who is embarking on, say, another culture, will need to consult the primary literature.

Apart from the reading list at the end of each chapter, some 460 primary references are cited. There has clearly been an effort to be up-to-date in this; most of the citations are to papers published in the 1980s. However, the coverage falls off seriously in 1983 and there is almost nothing for later years. This means that important recent and

not-so-recent developments, especially in the areas of gene vectors, and transformation of monocotyledons, are missing from the text.

The book is well produced, with numerous line drawings, quite reasonable photographs and much tabulated information. There is a comprehensive and useful index, and a short glossary. There are comparatively few typographical or minor factual errors; plant RNA viruses acquire a mysterious manifestation as DNAs at one stage, for example, and some of the definitions in the glossary may raise an eyebrow.

For undergraduates in plant sciences, this book should provide a good general introduction to biotechnology at a reasonable price. For those already engaged in biotechnology, whether in plant production, the food industry or industrial fermentation, it will provide a very basic overview of the emerging technologies, and some indication of where to go for more detailed treatments.

R.S.S. Fraser

Quality Control in the Food Industry, Volume 1. 2nd edn. Ed by S. M. Herschdoerfer. London: Academic Press, 1984, Pp. xiii+469. ISBN 0 12 343001 1. £45.00.

The first edition of this work appeared in 1967. This second edition has been enlarged to four volumes from the original three, with this expansion being largely in the commodity chapters in Volumes 2, 3 and 4. However, Volume 1, which is being reviewed here, has itself been extended from 385 to 469 pages.

A number of the original contributors have been replaced by new authors for this new edition. There are eight chapters. The first, 'The organization of quality control' by John Hawthorn, is essentially unaltered from the first edition, with the bulk of the chapter being comprised of the hypothetical case study of the production of 'Okebits' canned acorns.

The second chapter, 'Health problems in foods: chemical aspects' by Arnold Bender (replacing Alastair Frazer of the first edition), is substantially new—partly because it is by a different author, but also because of the significant changes which have occurred since 1967. To give just three examples of fresh topics, hyperactivity, lysinoalanine production, and the Ames test are discussed. There is then a new chapter, also by Arnold Bender, entitled 'Food processing and nutritional values', which deals with nutritional losses and nutritional gains during processing, and mentions briefly both nutritional labelling and the problems of analysis.

The chapter on microbiological aspects has been rewritten for this edition by David Mossel and colleagues, and although the principal microbial hazards and their methods of detection are briefly and severally described, the emphasis of the chapter is different from that in the first edition, reflecting as it does the current awareness of the limitations of end product examination in the attainment of a supply of microbiologically safe products, and the correspondingly great importance of risk analysis and the application of Codes of Good Manufacturing Practice. Methods of monitoring processing procedures are also discussed.

The chapter on 'Statistical methods in quality control' by E.H. Steiner mostly follows that of the first edition, but an additional topic now covered is that of Cusum control charts, and there is also an additional section on the precision of analytical methods. One surprising omission is discussion of the 3-class attributes sampling plans which were developed for the International Commission on Microbiological Specifi-

cations for Foods (ICMSF), and which now feature so frequently in draft microbiological specifications. The next contribution, 'Sensory assessment in quality control' by N.T. Gridgeman, is almost identical to that in the first edition, recent work not having been incorporated and critically reviewed, the principal change being the addition of an appended 'Bibliographic note' of 1½ pages.

As might be expected, the chapter on 'National and international standards' by R.H. Murray (in place of Francis Townshend in the first edition) is considerably modified, with discussion of EEC standards, and also of standards relating to pesticides, food additives, labelling, and nutrition. The final chapter on 'Quality standards and specifications in the food industry' by N. Goldenberg is new to this edition. It gives examples of microbiological and chemical standards which can be adopted, and of Codes of Practice relating to processing, packaging and storage.

To summarize, this first volume of the second edition contains two new chapters, and three of the remaining six chapters are newly written (in all cases by new contributors).

The index unfortunately is minimal with very many topics not listed. To give just two examples from those already mentioned, the Ames test and cusum charts cannot be found through the index. This restricts the usefulness of this volume in particular as a work of reference, so that it needs to be viewed as a book which offers a number of essay reviews on the topics of the chapter titles. Consequently, I feel that it is unlikely that readers who already possess Volume 1 in the first edition will feel compelled to spend £45.00 to purchase this book.

W.F. Harrigan

An Evaluation of the Role of Microbiological Criteria for Food and Food Ingredients. By the National Research Council (U.S.) Food Protection Committee, Subcommittee on Microbiological Criteria. Washington, D.C.: National Academy Press, 1985. Pp. xiv+436. ISBN 0309034973. £34.30.

In 1962 the International Association (now Union) of Microbiological Societies set up the International Committee (now Commission) on Microbiological Specifications for Foods (the ICMSF). From 1964 onwards, the ICMSF has been responsible for a number of notable publications on, *inter alia*, microbiological methods, sampling plans and specifications. At around the same time national and international bodies such as the US Food and Drug Administration (FDA) and the US National Research Council (NRC), the EEC, and Codex Alimentarius have also paid attention to the same topics. Amongst the previous publications of the NRC have been *An Evaluation of Public Health Hazards from Microbiological Contamination of Foods* (1964) and *An Evaluation of the Salmonella Problem* (1969).

At the begining of this period emphasis tended to be placed on end product specifications. In Britain, although many companies have operated their own end product specifications for decades, the thrust of law enforcement has traditionally been toward inspection of the food production premises, under such legislation as the *Food Hygiene (General) Regulations*. In 1974 the United States FDA formalized the monitoring of the hygienic aspects of food production through the introduction of the Hazard Analysis Critical Control Point (HACCP) concept.

This latest publication from the NRC suggests plans for implementing the HACCP

system in food production programmes and for developing meaningful specifications, guidelines and standards. The book arose from a request directed to the NRC by the FDA, USDA, the National Marine Fisheries Service and the US Army Natick Laboratory. The volume opens with a 15 page 'Executive Summary' and 25 pages of 'Recommendations', the latter providing a summary of the recommendations which derive from Chapters 1 to 11. Chapter 1, 'Introduction', examines the sources and activities of microorganisms in foods, and their control. Chapter 2 discusses the 'Definitions, Purpose and Needs for Microbiological Criteria'. Following a brief chapter giving examples of particular foods of microbiological concern, Chapter 4 ('Selection of Pathogens as Components of Microbiological Criteria') and Chapter 5 ('Selection of Indicator Organisms and Agents as Components . . .') briefly outline respectively the principal public health hazards and a range of microbiological quality criteria such as aerobic plate counts, direct microscopic counts, mould counts, coliform tests, dye reduction tests, and metabolites (e.g., TMA, indole, diacetyl, histamine, thermonuclease, aflatoxin). Chapters 6 and 7 then consider attributes and variables sampling plans, and the action to be taken when a limit is exceeded.

The next chapter provides an interesting overview of the current sources of microbiological criteria (e.g., FAO, ICMSF) and the US national and state legislation.

Chapter 9, 'Application of Microbiological Criteria to Foods and Food Ingredients', is the longest chapter in the book, at 124 pages. It discusses the microbiological aspects of the principal food commodity groups, and it can be likened to a US-oriented version of the commodity volume (Volume 2) of the ICMSF book *Microbial Ecology of Foods*. The final chapter concerns plans of action for implementation of HACCP and of microbiological criteria. There are seven appendices and a 16-page index. The text throughout is supported by an extensive bibliography. The book provides a valuable source of information for readers interested in or concerned with the subject of US food protection programmes, but it also has much to offer the student of food protection in general.

W.F. Harrigan

Sensory Evaluation of Food: Statistical Methods and Procedures. By Michael O'Mahony. New York: Marcel Dekker, 1985. Pp. xv+487. ISBN 0 8247 7337 3. US $102.00.

There are sixteen chapters of text occupying about 400 pages. The first third covers basic statistical material—probability, normal, binomial, chi-square and *t*-distributions. Another third concerns the analysis of experiments designed in a number of standard ways, the last being 'Split-plot Designs'. In this section of the book there are separate chapters on 'Multiple Comparisons' and 'Fixed- and Random-Effects Models'. In the last third of the text 'Correlation and Regression' merits only a few pages compared with a long chapter on 'Additional Nonparametric Tests' and a selection of appendices. It is very much a book about statistical methods, rather than about sensory evaluation methodology. One need only remove a dozen main entries out of well over 200 from the index to make it quite impossible to discern the field of application of the statistical techniques, except to infer that the author had learned his statistics from psychologists rather than from statisticians.

The book claims to be aimed at University students, at 'the unwitting food scientist

who has just been told that he or she is in charge of sensory evaluation, despite having had no training', and at workers in the field who want a ready reference at an introductory level.

For British students the price of $102 looks prohibitive. Food sciences students seem to be heavily burdened with lectures, practicals and assignments and many may well want a less self-indulgently long-winded text.

The author's second category of unwitting food scientists with no training will be disappointed to find little in the book about the basic techniques of sensory analysis. There could be an excellent book laying out those principles and integrating them with elementary but sensible statistical procedures. This is not that book. I looked up Magnitude Estimation via the index and found only a couple of trite sentences, which certainly would not have led me to think there might be a technical literature on this subject. References are few and far between except in the acknowledgments below the many photoreproduced statistical tables.

Workers in the field who want a ready reference would probably want some reference to the handling of profile data, where many characteristics of each sample are rated. There seems to be no effective treatment herein of this extremely common feature of sensory data. Admittedly most statistical analysis is a little advanced, by the standards O'Mahony sets, but some descriptive discussion would have been quite in order.

This reviewer is loath to reject a statistics book just because the author does not have professional qualifications in the subject; its universal application means many brilliant people have become fascinated by statistics after qualifying in other fields. There is however something very strange in a Preface which boasts, 'The book is hard-headed but it is also very simple to understand. This is because it is written by a very simple non-statistican. If I can understand it, you can too!' If I removed the prefix non-, and put this phrase into my book on 'Do-it-yourself Brain Surgery' I would still not expect to be taken seriously by any publisher, but with statistics books the profession of ignorance seems to be treated much more positively. In this instance, the worker looking for an authoritative ready reference should discount the first quoted sentence, believe the second, and underline the first word of the last.

The excessive concentration on hypothesis testing, and the uncritical use of multiple comparison methods, are examples of a very limited perception of what statistics is about. There are plenty of examples of plain error. Opening the book at random, I found Fig. 7.1 giving a population pyramid with indeterminate numbers of people at the lowest ages, and a discrete distribution drawn as continuous, one axis out of four being labelled, one having a (wrong) scale. Despite being quite well-produced, and despite laudable intentions to communicate at a realistic level of discourse, this book has to be condemned as too ill-judged and too inaccurate to find a place in scientists' libraries.

I.M. Wilson

Intestinal Microbiology. By B.S. Drasar and P.A. Barrow. (Aspects of Microbiology 10). Wokingham: van Nostrand Reinhold, 1985. Pp. vii+80. ISBN 0442305982. £5.25 (paperback).

About 90% of the cells in the human body are bacteria: the cells that comprise the body are numerically in the minority, though of course their volume is greater. Most of the

bacteria are in the gut, and for most of the time they exist in balance with the host. It is of importance to the food industry that products marketed do not contribute to upsetting this balance. In fact there have long been claims that certain gut microbes can be beneficial: by contributing to longevity, by making invasion by pathogens less likely, by contributing vitamins, and, of course, as symbionts in the rumen. This book provides an interesting introduction to structural physiology and function of the gut of man and animals in the context of microbiology. There are four sections: methods for investigating intestinal flora; nature of intestinal flora of the normal intestine; intestinal flora in diarrhoea and malabsorption; benefit and mischief, including cancer, from the intestinal flora. Each chapter is supported by a short guide to further reading, but recent literature is not widely mentioned. Type and diagrams are clear and accurate, there is a useful index, and altogether the book represents good value for money.

The authors, eminent in their field, have provided a useful broad introduction to the gut ecosystems in man and animals. For example, we are told that of 7 litres of fluid per day entering the small intestine of man only 2 enter as diet, the rest entering as intestinal secretions—a fact that emphasizes the complexity of study of fluid balance in the gut in the context of diarrhoea. The physiological basis of coprophagy by rabbits and other animals is explained, as are various other gut-related topics. We learn that over 300 species of bacteria are known to occur in the gut, and so we come to appreciate the difficulty of investigating relationships between diet and types of microbes in the gut. The problems with such investigations are also hinted at in other ways. The detection of correlations between presence of particular microbes in the gut and particular nutrition is difficult enough but even more difficult is determining whether correlations are merely incidental or represent causal relationships.

More guidance on the types of bacteria in the gut might have been given. In a book of this sort it seemed inappropriate to recommend readers to Bergey's Manual for further guidance on identification. The authors appeared to use the term 'niche' in its three dimensional, architectural, sense, rather than in its multidimensional, ecological sense. I did not like the apparent equating of Eh with oxygen tension; Hungate clearly indicated long ago that Eh values could be high enough to inhibit growth of some bacteria, even in the complete absence of oxygen. It seemed inadequate to quote a 1972 reference in support of a statement that mechanisms of pathogenesis in many types of diarrhoeal diseases had not been elucidated. While the statement is still true, the field is a fast moving one and much useful and interesting information has come to light since then. Despite these minor criticisms I recommend the book for undergraduates and others wishing to have a useful introduction to the complexities of the relationships between the gut and its microbes.

R.W.A. Park

Common Fragrance and Flavor Materials: Preparation, Properties and Uses. By Kurt Bauer and Dorothea Garbe. Weinheim: VCH, 1985. Pp. 213. ISBN 3 527 26038 2. DM 112.

This book is stated to be essentially a translation of the chapter on fragrance and flavour materials in *Ullmann's Encyclopädie der technischen Chemie*, Volume 20, 4th edn, 1981, supplemented by relevant information from other sections and by recent

developments. Flavour should not have been used in the title, since the book deals with odorous compounds only, deliberately setting sapid compounds aside.

The bulk of the book is concerned with individual odorous compounds (107 pp. subdivided into aliphatic compounds, acyclic terpenes, cyclic terpenes, other cyclo-aliphatic compounds, aromatic compounds, phenols and phenol derivatives, O-heterocycles, and N- and S-heterocycles); essential oils (60 pp.); animal secretions (3 pp.); quality control (1! p.); toxicology (1! p.); literature (11 pp.; including almost 300 references, many to documents of the Essential Oil Association of the USA and of ISO); formula index including CAS registry numbers (12 pp.); and subject index (9 pp.).

For each compound, there is given the molecular formula, the molecular weight, boiling point and/or melting point, density, and refractive index, followed by natural occurrence, odour qualities, isolation, synthesis, and uses (but not formulation), the length of the entry being roughly in proportion to the compound's importance. Essential oils are dealt with in a parallel manner, additional data, such as specific rotation and solubility, usually being included.

The book thus offers a great deal of information, but it is not clear for whom it is intended. The specialist would want to have a fuller treatment and would thus go to Guenther's *The Essential Oils* (1952) or Gildemeister and Hoffman's *Die ätherischan Oele* (1966); the lay reader is likely to become bogged down in detail, unless he were only seeking the answer to fairly specific questions, such as, 'what is coriander oil' and 'what is its principal constituent'.

There are surprises in the book; a selection follows. The omission of sweet from the primary tastes. The statement that an objective classification of odour qualities is not possible. Only rarely are thresholds given for odorants. Sulphur compounds, except for allyl isothiocyanate, are dealt with rather cavalierly, and even there the difference between English and French mustard is not pointed out, nor is horseradish oil mentioned. Damascones rate a section, damascenones don't. The odour of furfural is described as freshly baked bread and that of furaneol as relatively weak and non-specific. No details of the synthesis of N- and S-heterocycles are given. Elemicin appears under nutmeg, but not under elemi oil. Terms like fixative are not explained. Words such as almond, apple, beef, butter, coconut, cheese, chocolate, coffee, curry, honey, meat, mushroom, peach, pineapple, and strawberry are not to be found in the subect index. There is no reference to V. Formáček and K.-H. Kubeczka, *Essential Oil Analysis by Capillary Gas Chromatography and Carbon-13 NMR Spectroscopy*, Wiley Heyden, Chichester, 1982.

Overall, the book leans more towards fragrances than towards flavours, but, in spite of the above criticisms, explicit or implied, the book is recommended as occupying a niche left largely vacant. The book is very well produced with many clear formulae and very few misprints and Germanicisms.

H.E. Nursten

Digestibility and Amino Acid Availability in Cereals and Oilseeds. Edited by John W. Finley & Daniel T. Hopkins. St. Paul, Minnesota: American Association of Cereal Chemists, 1985. Pp. ix+304. ISBN 0 913250 40 6. US $58.00

This compilation results from a symposium sponsored in 1983 by the Protein Division of the American Association of Cereal Chemists. The twelve chapters are each written by well-known specialists. The objectives of the symposium were to determine the effect of digestibility on the nutritional quality of cereals and oilseed proteins, to recommend procedures for determining the availability of individual amino acids, to ascertain the chemical and physical reasons for differences in digestibility and availability, including processing factors, and to decide whether protein quality can be determined by estimates of digestibility or amino acid content.

Not surprisingly, the answers to these questions are complex, and subject to reservations, but the authors have, on the whole, presented their conclusions as clearly as could have been expected, backed by convincing evidence.

This text will be of particular value to specialists, including research workers, in the nutritional sector of the feeds, and to a lesser extent, the food industry. The food industry is increasingly concerned with nutritional issues, but the consequences of present day highly diversified diets are that, so long as deficiencies and hazardous components are avoided, the diet as a whole is what matters, not the features of minority commodities within it. Further, the protein component of the diet is less emphasized nowadays than formerly. Another trend in the food industry, towards increasing and more sophisticated food processing, is also important, and is recognized in several chapters. No doubt this book is not aimed at the non-specialist reader but, even so, it would have been useful to refer occasionally to implications for the human diet of low availabilities, absorptions and digestibilities.

The standard of presentation is high. References are up-to-date and carefully documented, tabulation is a strong feature and there is a good index. In general both authors and publishers must be congratulated.

B.J.F. Hudson

Fungi and Food Spoilage. By John I. Pitt and Ailsa D. Hocking. Sydney: Academic Press, 1985. Pp. xi+413. ISBN 0 12 557730 3. £49.00.

Introduction to Food-borne Fungi. By Robert A. Samson, Ellen S. Hoekstra, Connie A.N. van Oorschot and others. Baarn, Netherlands: Centraalbureau voor Schimmelcultures, 1984. Pp. ii+248. ISBN 90 70351 03. £7.50 (obtainable from the Commonwealth Mycological Institute, Kew).

Both of these books are intended as laboratory guides for food microbiologists who are involved in the isolation and identification of food-borne fungi. Consequently they feature identification keys (in both cases dichotomous) and a large number of black and white illustrations. In addition, both books have a number of supporting chapters discussing various aspects of the growth and physiology of food spoilage fungi, food as a fungal habitat, and the cultural and identification methods available. These chapters in *Pitt & Hocking* are: The ecology of fungal food spoilage; Naming and classifying fungi; Methods for isolation, enumeration and identification; Spoilage of fresh and perishable food; and Spoilage of stored, processed and preserved foods. The corresponding

chapters in *Samson et al.* are: The detection and quantification of fungi in food; Fungal growth on foodstuffs related to mycotoxin contamination; Mycotoxins, sampling and chemical detection; Heat resistance of yeast cells and fungal spores; Food preservatives; Fermentation foods by moulds; and a Glossary of mycological terms. It is obvious from the chapter headings listed above that *Samson et al.* pays more attention to the question of mycotoxigenesis and to the possibility that fungi spoiling food may produce mycotoxins.

The food microbiologist with little mycological background will find these books different in the accessibility of the information. The provision by *Samson et al.* of the glossary of many of the more specialized mycological terms avoids the necessity for a user to refer too frequently to a book such as Ainsworth & Bisby's *Dictionary of the Fungi*. Indeed Samson's glossary includes a useful half-page of diagrams which illustrate the subtle differences between shapes such as falcate and fusiform; ampulliform and pyriform; obovoid and clavate. In contrast, although *Pitt & Hocking* state that 'the identification keys have been designed for use by microbiologists with little or no prior knowledge of mycology', the reader is offered little or no help in dealing with the terminology used in the keys, e.g., 'intercalary chlamydoconidia and arthroconidia present; aleurioconidia on tiny pedicels or solitary'.

The keys in *Pitt & Hocking* are based on an initial culturing on a number of standard media incubated at 5, 25 and /or 37°C. Many of the differential characteristics relate to appearance, size and colour of colonies on the standard media. Without such standard culturing it will be nearly impossible to identify fungi with this book. *Samson et al.*, on the other hand, use the more conventional characteristics observed by microscopic examination in their keys. Although *Samson et al.* recommend the culture of fungi on standard media, the descriptions of colonial morphology are given within each genus or species to provide confirmatory identification; the user will thus frequently be able to achieve a good identification to generic or even to species level from microscopic observation of sporulating fungi from the primary isolation medium or from the foodstuff. Of course, a standardized identification procedure is to be preferred, but often a food microbiologist will need to make a rapid identification and this is not really possible with *Pitt & Hocking*.

The two manuals are similarly illustrated with black and white photographs of colonies on standard media, photomicrographs taken mainly using Nomarski interference contrast, and line drawings. However, the larger format of *Samson et al.*, in which a typical group of five illustrations occupies a full printed page of 24×16 cm, makes the book more comfortable to use than that of *Pitt & Hocking* in which a group of three illustrations is typically only allocated 11.5×6 cm.

By now readers will have suspected that I prefer the manual by *Samson et al.* and you will be correct. This elegantly produced manual with excellent illustrations *must* be a 'best buy' at £7.50, and I strongly recommend the purchase of a personal copy by any food scientist, practising or student, who needs to identify food microfungi. However, the book by *Pitt & Hocking* is likely to be valuable to food microbiologists needing an entrée into the literature relating to the occurrence and significance of a given fungal species in foods, since the bibliography is more extensive, but at £49 purchase is likely to be restricted to one copy in the laboratory library.

W.F. Harrigan

Short reviews

Cheesemaking Practice, 2nd edn. By R. Scott.
London: Elsevier Applied Science, 1986. Pp. xxii+529. ISBN 0 85334 392 6. £42.00.

Five years have elapsed since the publication of the first edition (reviewed in this journal, Vol 17, page 787). The author has identified changes as including a greater use of references to the literature, and a more detailed treatment of the topics of casein micelles, starters, coagulation of milk, and the ultrafiltration of cheese milk. In line with the usual practice in Elsevier Applied Science publications, the references have been placed at the end of each chapter, rather than consolidated at the end of the book, and do not include titles of papers. There has been a slight increase in the overall length of the book (529 pages, instead of the 475 pages of the first edition), although most of this increase is used for the references.

Meat Science, 4th edn. By Ralston A. Lawrie.
Oxford: Pergamon, 1985. Pp. xviii+267. Hard cover: ISBN 0 08 030790 6, £25.00; flexicover: ISBN 0 08 030789 2, £14.00.

The third edition was published in 1979, and the author observes that there has been a considerable advance in our knowledge relating to the science of meat in the intervening time. Topics which have been added to, or which have been amended substantially include: the hormonal control of reproduction and growth, preslaughter stress, methods of stunning and bleeding, use of refrigeration, eating quality, and consumer health. New sections have been included on the electrical stimulation of carcasses post-mortem, the mechanical recovery of meat, the modification of meat by high pressure, reformation after comminution, and the incorporation of proteins from abattoir waste or from non-meat sources.

Advances in Refrigeration and Heat Pump Technology Achieved by the Application of Micro-electronics and the Control of Systems by Micro-electronic Devices, 2 vols.
Paris: Institut International du Froid, 1985. Pp. 462. FF200.

These two volumes constitute the proceedings of the meeting of Commission B2 on 3–7 September, 1984. Most papers are presented in English, with a French summary; a few are in French with an English summary.

Books received

Modern Control Techniques for the Processing Industries. By T.H. Tsai, J.W. Lane and C.S. Lin.
New York: Marcel Dekker, 1986. Pp. ix+277. ISBN 0 8247 7549 X. US $71.50.

Vitamin Deficiency in the Elderly: Prevalence, Clinical Significance and Effects on Brain Function. Ed. by J.R. Kemm and R.J. Ancill.
Oxford: Blackwell Scientific Publications, 1985. Pp. xi+210. ISBN 0 632 01365 6. £15.00.

Chemical Changes in Food during Processing. Ed. by Thomas Richardson and John W. Finley. Westport, Conn.: AVI, 1985. Pp. xv+514. IBN 0 87055 504 9. £63.75 (distributed by D. Reidel, 3300 AZ Dordrecht, The Netherlands).

Concentration and Drying of Foods. Ed. by Diarmuid MacCarthy. London: Elsevier Applied Science, 1986. Pp. x+303. ISBN 0 85334 442 6. £32.00.

Food Packaging and Preservation: Theory and Practice. Ed. by M. Mathlouthi. London: Elsevier Applied Science, 1986. Pp. xiii+402. ISBN 0 85334 413 2. £48.00.

Functional Properties of Food Macromolecules. Ed. by J.R. Mitchell and D.A. Ledward. London: Elsevier Applied Science, 1985. Pp. xii+433. ISBN 0 85334 373 X. £52.00.

Economic Aspects of Biotechnology. By Andrew J. Hacking. Cambridge: Cambridge University Press, 1986. Pp. x+306. ISBN 0 521 25893 6. £35.00.

Biotechnology of Marine Polysaccharides. Ed. by Rita R. Colwell, E. R. Pariser and Anthony J. Sinskey. Hemisphere Publishing Corp.: Washington, 1985 (distributed by McGraw Hill). Pp. xi+559. ISBN 0 89116 433 2. US $79 95.

Modern Methods of Food Analysis. Ed. by Kent K. Stewart and John R. Whitaker. Westport, Conn.: AVI, 1984. Pp. xx+421. ISBN 0 87055 462 X. £58.25.

A Colour Atlas of Food Quality Control. By Jane P. Sutherland, Alan H. Varnam and M.G. Evans. London: Wolfe, 1986. Pp. 272. ISBN 0 7234 0815 7. £75.00.

Ice Cream Making. By James R. Rothwell. Reading: J. Rothwell, 1985. Pp. 102. ISBN 0 7049 933 2. £3.35 incl. p.&p. (obtainable from Dr J. Rothwell, 68 Lowfield Road, Caversham Park Village, Reading, Berks RG4 0PB).

Membrane Separations in Biotechnology. Ed. by W. Courtney McGregor. New York: Marcel Dekker, 1986. Pp. xx+386 ISBN 0 8247 7465 5. US $78.00.

Machinery Adhesives for Locking, Retaining and Sealing. By Girard S. Haviland. New York: Marcel Dekker, 1986. Pp. xi+340. ISBN 0 8247 7467 1. US $71.50.

Food Additives: Taking the Lid off What We Really Eat. By Erik Millstone. Harmondsworth: Penguin, 1986. Pp. 163. ISBN 0 14 052369. £2.95.

Rheology of Wheat Products. Edited by Hamed Faridi. St Paul, Minn.: American Association of Cereal Chemists, 1985. Pp. vi+273. ISBN 0 913250 422. US $41.00.

Principles of Cereal Science and Technology. By R. Carl Hoseney. St Paul, Minn.: American Association of Cereal Chemists, 1986. Pp. vii+327. ISBN 0 913250 43 0. US $46.95.

Interactions of Food Components. Ed. by Gordon G. Birch and M.G. Lindley. London: Elsevier Applied Science, 1986. Pp. xi+343. ISBN 0 85334 398 5. £42.00.

Modern Dairy Technology, 2 vols. Ed. by Richard K. Robinson. London: Elsevier Applied Science, 1986. Vol. 1, Advances in Milk Processing. Pp. ix+438. ISBN 0 85334 391 8. £44.00. Vol 2, Advances in Milk Products. Pp. x+440. ISBN 0 85334 394 2. £44.00.

Rice: Chemistry and Technology, 2nd edn. Ed. by Bienvenido O. Juliano. St. Paul, Minn.: American Association of Cereal Chemists, 1985. Pp. xvi+774. ISBN 0 913250 41 4. US $87.00.

Solar Dryers: Their Role in Post-Harvest Processing. By B. Brenndorfer, L. Kennedy, C. O. Oswin Bateman, D. S. Trim, G. G. Mremo and C. Wereko-Brobby. London: Commonwealth Science Council, 1985. Pp. vi+337. ISBN 0 85092 282 8. £7.50.

Foliar Feeding of Plants with Amino Acid Chelates. Ed. by H. DeWayne Ashmeed, Harvey H. Ashmeed, Gene W. Miller and Hsin-Hung Hsu. Park Ridge, N.J.: Noyes, 1986, Pp. xii+370. ISBN 0 8155 1059 4. US $54.00.

Control of Pesticide Applications and Residues in Food: A Guide and Directory, 1986. Ed. by Bengt v. Hofsten and George Ekström. Uppsala: Swedish Science Press, 1986. Pp. vi+315. ISBN 91 86992 01 5. SEK 240.00.

Moredun Research Institute, Annual Report 1984–85. Edinburgh: Moredun Research Institute, 1986. Pp. 70.

JOURNAL OF FOOD TECHNOLOGY: INSTRUCTIONS TO AUTHORS

Original contributions relevant to the science and technology of food and beverages are accepted on the strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. Topics of only narrow local interest will not be accepted unless they have wider potential or consequences. Papers accepted become the copyright of the Journal, and may not be published elsewhere in whole or in part without the Editor's permission in writing. No page charges are levied.

Contributions are: *Papers*. These are reports of substantial research (typically <6000 words equivalent, including tables, figures, references), and comprise:

(a) a concise Summary (<150 words) containing the main results and conclusions;

(b) an Introduction giving essential background but no subheadings; objectives must be clearly stated;

(c) Materials and Methods with sufficient full experimental detail (where possible by reference) to permit repetition; sources of material must be given and statistical methods must be specified by reference, unless non-standard;

(d) Results should be presented concisely, using well-designed tables and/or figures; the same data may not be used in both; appropriate statistical data should be given;

(e) Discussion should cover the implications and consequences, not merely recapitulating the results; conclusions should be concise;

(f) brief Acknowledgements;

(g) References as shown below.

Technical Notes (<2000 words). These describe smaller investigations and applications, useful techniques and apparatus. They have no summary, a brief Introduction, Materials and Methods, Results and Discussion, Acknowledgements and Reference. They can provide accelerated publication if needed.

Reviews (<8000 words). These are concise, critical but constructive and conclusive topical accounts written for non-specialists. References must be in the form shown below. A small honorarium may be given.

Letters. These are brief comments on material published in previous issues; they are published at the discretion of the Editor. They are the only items not subject to multiple peer review.

Arrangement and presentation. As the purpose of publishing work is to communicate it to others, it is necessary to design the arrangement and presentation to do this in the most efficient way. This requirement is often quite different from the sequence in which the work was done. The logical sequence is to describe a problem or need, analyse it in operational or testable terms, obtain the data, and then use them to clarify, solve or satisfy the problem or need. This is an excellent basis for both design and reporting of investigations, although in practice the path may be devious. This should not cloud the report, which should aim to clarify the situation for the reader rather than demonstrate how difficult and complex the work was. It is rarely necessary to present all data. It is much more difficult and time consuming to produce clear and concise reports than rambling and woolly ones, but the end result is of far greater value to the reader, both in the shorter time taken to read it and in greater understanding. Furthermore, it reaches more people, for many will not waste time on unnecessarily extensive reports.

Authors should very carefully consider the preparation of papers to ensure that they communicate efficiently. We are much more likely to accept those that are carefully designed and laid out, have few or no errors, are concise, and conform to the style and instructions. They will also be published with much less delay than those that require much scientific and editorial correction. Authors who are not fluent in written English should seek help from a fluent person before the final version is typed.

The Editor reserves the right to make literary corrections and to make suggestions to improve brevity.

Typescripts (three complete copies) must be single-sided, double-spaced and with wide margins. The title page should carry an informative title that reflects the content, a running title (<46 characters+spaces), the names of the authors, and the place(s) where the work was carried out. The full postal address of the indicated corresponding author *must* be given as a footnote. Up to six keywords or very brief phrases must be given to aid data retrieval and indexing.

Standard Usage, Abbreviations and Units. The Concise Oxford English Dictionary is the reference for spelling and hyphenation. Statistics and measurements should always be given in figures, e.g. 10 min, except when the number begins a sentence. When the number does not refer to a unit of measurement it is spelt out unless it is 100 or greater

Abbreviations should be used sparingly, only when long or unwieldly names occur frequently, and never in the title; they should be given at the first mention of the name.

Metric SI units should generally be used except where they conflict with current practice or are confusing. For example, 1.5 l rather than 1.5×10^{-3} m^3 or 3 mm rather than 3×10^{-3} m.

Chemical formulae and solutions must specify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units.

Common species names should be followed by the Latin binomial (underlined) at the first mention. For subsequent use the generic name should be contracted to a single letter if it is unambiguous.

Figures should be drawn boldly in black Indian ink on separate sheets of white paper. They should be drawn so that on 50% reduction they will fit within the type area, and that lines, figures and symbols will be still clearly legible and not overcrowded. Photographs must be unmounted, glossy prints that have not been retouched. Colour prints will incur charges to the author. All figures must be given Arabic numbers, e.g. Fig. 3, in the text and on the back of the figure, where the title of the paper and the authors' names must also be marked. Self-explanatory legends of all figures should be typed separately under the heading 'Legends to Figures'.

Tables should be few in number, carefully designed, uncrowded, and include only essential data. Each must have an Arabic number, e.g. Table 3, a self-explanatory caption, and be on a separate sheet. Vertical lines must not be used.

References. Only closely relevant references may be included. References in the text must give the author's name with the year in parentheses. Where there are three authors, all three must be cited on the first occasion and subsequently only the first, followed by *et al.* The *et al.* form must always be used for four or more authors. Several papers by the same author and year must be followed by a, b, c etc. after the year. References must be listed alphabetically in full at the end of the paper in the following standard form, and journal titles must be given in full.

Chen, J., Reineccius, G. A. & Labuza, T. P. (1986). Prediction and measurement of volatile retention during extrusion processing. *Journal of Food Technology*, **21**, 365–383.

Stone, H. & Sidel, J. L. (1985). *Sensory Evaluation Practices*. Pp. 56–59. Orlando: Academic.

Dubois, P. (1983). Volatile phenols in wines. In: *Flavour of Distilled Beverages* (edited by J. R. Piggott). Pp. 110–119. Chichester: Ellis Horwood.

Unpublished work must only be cited where necessary, and only in the text. Copies of references in press in other Journals must be supplied with submitted typescripts. It is essential that all citations and references are carefully checked before submission, as mistakes or omissions will cause delays.

Proofs will be sent to the corresponding author.

Offprints. Fifty will be supplied free. Further copies must be ordered when the proofs are returned.

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Contents

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