ISSN 0022-1163

Volume 21 Number 2 April 1986

IFST

Journal of Food Technology

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

JOURNAL OF FOOD TECHNOLOGY
Institute of Food Science and Technology (U.K.)

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

Contributions and editorial correspondence should be sent to the Editor, c/o Institute of Food Science and Technology (U.K.), 20 Queensberry Place, London SW7 2DR.

General and IFST correspondence should be sent to the Executive Secretary, IFST (U.K.), 20 Queensberry Place, London SW7 2DR.

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

The Journal of Food Technology is published bimonthly, six issues form one volume. The subscription price for 1986 is £83.00 (U.K.), £99.50 (Overseas), $195.00 (N. America, including cost of airfreight). Current issues for the Indian Sub-Continent, Australasia and the Far East are sent by air to regional distribution points from where they are forwarded to subscribers by surface mail. Any back numbers are normally despatched by surface to all regions, except North America, where they are sent by a.s.p., and India, where they are sent by air freight. Back volumes are still available. This journal is covered by *Current Contents, ASCA* and *Science Citation Index*.

0022-1163/86 $02.00.

Journal of Food Technology (1986) **21**, 117–122

Assessment of the previous heat treatment given to meat products in the temperature range 40°–90°C. Part 1: Soluble nitrogen analysis

SUSAN E. PARSONS* AND R. L. S. PATTERSON

Summary

The aim of this work was to identify a technique which would yield information retrospectively about the heat treatment applied to a meat product in the temperature range 40–90°C. Saline extracts of beef samples (*M. longissimus dorsi* and *M. semimembranosus*) of known heat treatment, were analysed for soluble nitrogen by Kjeldahl analysis. The amount of residual soluble nitrogen in an extract was found to decrease as the cooking temperature increased, with the major reduction occurring between 40 and 70°C. A good correlation between nitrogen values was obtained for aliquots removed from a continuously heated raw meat extract, 5 mm slices and the centre sections of 60 mm cubes. It was also possible to detect differences in the extent of heating achieved at points between the surface and the centre of the solid piece of meat. These results indicate that it may be possible to estimate in retrospect the approximate maximum temperature reached in an unknown cooked meat sample by reference to a standard curve of soluble nitrogen *versus* known heat treatment.

Introduction

The ability to determine the previous heat treatment of a meat product would be advantageous to many areas of the meat industry. Heat treatment is given to meat to produce a palatable product, improve the shelf life and minimize the risk of food-borne illness. For these purposes time/temperature heat treatments are calculated to ensure the destruction of enzymes, pathogenic bacteria and viruses. Farm animals can also be at risk from infectious viral diseases: the swill feeding of inadequately cooked waste human food sometimes includes meat material which contains viable virus particles.

Most vegetative bacteria associated with food-borne illness and pathogenic viruses are destroyed in aqueous systems by temperatures below 90°C: *Salmonella* 70°C, African Swine Fever virus 60°C, Newcastle Disease virus 65°C, Swine Vesicular Disease virus 65°C, Foot and Mouth Disease virus 70°C and Rinderpest virus 75°C (Steele & Lambe, 1982). Confirmation that a heating process has reached the intended temperature for microbiological safety is clearly important from the point of view of both human and animal health, and also to give the desired quality of shelf-life.

Meat is a complex system and many changes occur during heating (Hamm & Deatherage, 1960; Hamm, 1966). Over the temperature range 40–90°C the major change is the denaturation of proteins which results in loss of pigment, loss of water

Authors' address: AFRC Food Research Institute—Bristol, Langford, Bristol, BS18 7DY, U.K.
*To whom correspondence should be addressed.

holding capacity, increase in pH, loss of enzyme activity, shrinkage and coagulation. By a study of the detectable changes which occur it may be possible to determine the degree of heat treatment previously applied to a meat sample.

Measurement of the undenatured protein in a heated product has been used as the basis of a method for determining the maximum temperature achieved in the product. The test, known as the Coagulation Test (USDA, Chemistry Laboratory Guidebook, 1981) is based on the principle that when proteins are heated, denaturation and coagulation occur progressively until all the proteins become insoluble and are precipitated from solution. The coagulation method claims that previous maximum cooking temperature can be estimated by observation of the temperature at which the first sign of cloudiness (protein precipitation) appears during reheating of a filtered extract of a cooked (meat) product. Weaknesses in the method include the difficulty of obtaining a clear filtered solution without removal of most of the protein, and the subjective assessment of the coagulation point (slight cloudiness) as the temperature is increased. To avoid these subjective assessments we have adapted the method to allow measurement of the residual soluble nitrogen in the extracts by Kjeldahl analysis and related these values to the previous heat treatment temperatures.

Materials and methods

Muscle samples were extracted from the carcase 24 hr post-slaughter, trimmed of all visible fat and frozen at −20°C. Whilst still frozen the samples were cut to size using a band saw. Thin (5 mm) slices of beef *M. longissimus dorsi* were used in one series of experiments to minimize the effect of temperature gradients and so produce samples of uniform heat treatment. Sixty mm cubes of beef *M. semimembranosus* were used in other experiments to provide distinct temperature gradients, samples being taken from the centre, intermediate and surface sections. Thermocouples (Chromel/Alumel), connected to a chart recorder, were positioned at the geometric centres and on the surfaces of all meat samples before being placed in thin nylon bags. Bags were closed and vacua generated by water pump before sealing. Temperatures recorded by the thermocouples were monitored before, during and after the heat treatments.

All meat samples were equilibrated initially in a 4°C stirred waterbath before being heat treated. The meat slices were immersed singly in a stirred waterbath preheated to one of the selected temperatures and removed when the temperature of the meat indicated by both thermocouples reached that of the waterbath. This was repeated at 5 or 10°C temperature intervals between 40 and 90°C. The cubes were immersed in the waterbath at 88°C, each being removed as its centre temperature reached one of the following values: 30, 50, 60, 70 and 88°C. All samples were cooled and equilibrated in a 4°C waterbath after treatment. Continuing conduction in the cubes during cooling caused the final temperatures at the centres to reach 46.5, 60, 66, 71 and 88°C respectively.

To determine the effect of heat treatment on soluble nitrogen an initial experiment was carried out using a raw meat extract. Twenty-five g of raw minced beef was soaked in 50 ml of isotonic saline (0.9% w/v sodium chloride) for 20 min, followed by centrifugation (5500 **g** for 15 min) and filtration through Whatman No. 1 paper. The extract was then heated in a waterbath from 20 to 95°C, 5 ml aliquots being removed at 5° intervals, centrifuged at 1600 **g** for 5 min then analysed for nitrogen by Büchi Kjeldahl.

Nitrogen analysis of the heated slices and cubes was carried out directly on the saline

extract produced from the first centrifugation (5500 g) and filtration stage. A minimum of 20 g of minced sample was required to produce enough extract for duplicate Kjeldahl analysis. Losses produced during cooking were not included with the sample as it is unlikely they would be available from many samples of unknown thermal history.

A combination of these two techniques was used to determine if the soluble nitrogen content of a partially cooked meat could be reduced by further heating. Extracts from two slices of meat heated to 63 or 80°C were prepared as described for the raw beef. Five ml aliquots removed at 5 and 10° intervals from the heated extract were analysed for soluble nitrogen.

Results

Figure 1 shows the relationship between soluble nitrogen content of an extract and the previous treatment temperature. Four or five trial runs were carried out over the complete or partial temperature ranges and all showed the same trend. For clarity the means of two comparable runs are shown in Fig. 1a and b. Nitrogen values for aliquots withdrawn at 5° intervals from a raw meat extract heated continuously from 20 to 95°C are shown at (a). Values for individual meat slices heated previously to specific temperatures in the range 40–90°C are shown at (b). Both graphs show a common pattern of rapid decrease in soluble nitrogen content as the temperature increased from 40–70°C which indicates the values obtained from the continuously heated extract are representative of cooked meat samples. There was little change in the soluble nitrogen content of the extracts of the two samples previously heated to 63 or 80°C, until the temperature reached that to which the meat had previously been cooked. This was particularly noticeable with the 63° sample, in which a loss of soluble nitrogen could be seen after 60°C.

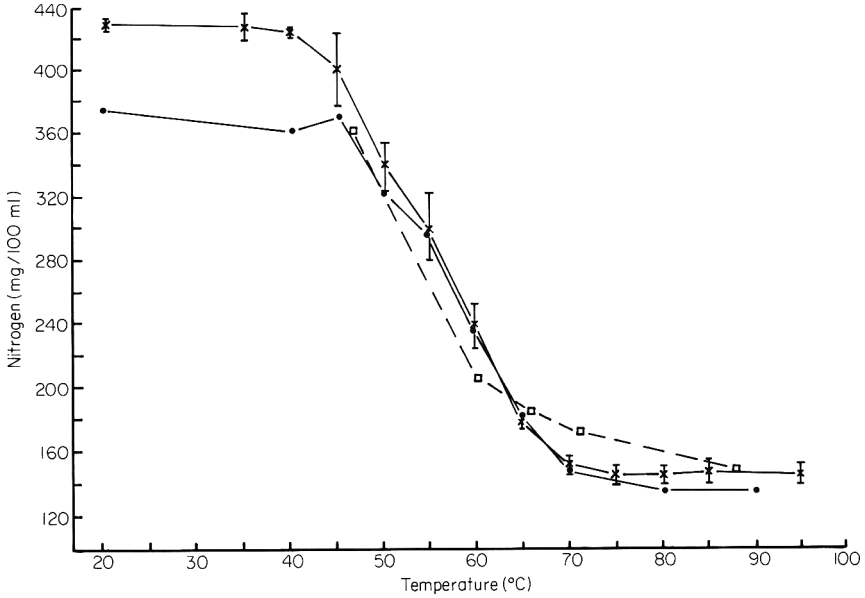

Figure 1. Soluble nitrogen analysis; (a) —x—x— nitrogen values for aliquots withdrawn at 5° intervals from a raw meat extract (mean two values, ± standard deviation); (b) —•—•— values for individual meat slices heated previously to specific temperatures; (c) —□—□— nitrogen values for the centre sections of beef cubes.

The histograms in Fig. 2 show the nitrogen values for samples taken from the three positions (centre, intermediate and surface) of each of the five meat cubes. Samples taken at each position had to be relatively thick to provide sufficient material for analysis and so contained tissue which had been exposed to temperatures either higher of lower (or both) than expected for the position. Hence the nitrogen values for the surface material of the two cubes which were heated to 46.5 and 60°C were high (Fig. 2) because although the surface was heated to 88°C the 5–7 mm thick samples mainly included material which had not even reached 50°C. When compared to curve (a), Fig. 1, the nitrogen values for these surface samples equate to a heat treatment temperature of approximately 66°C in each case, not 88°C. However, the values for the centre sections correspond well to curve (a) (see Fig. 1c).

Under certain circumstances, for example when a limited supply of material is available or if the material contains a mixture of components, it may be necessary to alter the proportions of meat to saline. Two experiments were carried out to show the effect on the soluble nitrogen content of altering the concentrations of the extract. Figure 3 shows the effect of (a) different weights of meat extracted by 100 ml 0.9% saline solution on the available soluble nitrogen and (b) the concentration of the salt solution used as the extractant on the analysable nitrogen. The amount of saline added to the different quantities of meat in (a) were adjusted to allow for the water contributed by the meat to the total volume. For example the standard preparations (assuming meat to be 76% water) contained:

50 g meat + 100 ml 0.9% saline
= 38 g H_2O + 100 g H_2O + 0.9 g NaCl
= 138 g H_2O + 0.9 g NaCl

Therefore according to the amount of meat used proportionately more or less water was added to maintain the ratio of 138 g water to 0.9 g sodium chloride.

As expected the amount of nitrogen detectable increased proportionately as the

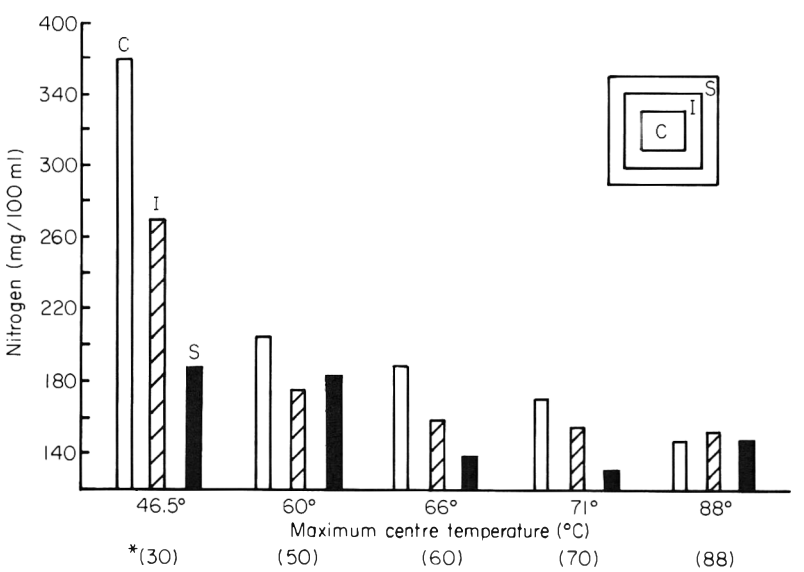

Figure 2. Nitrogen values for sections of meat cubes heated to different internal temperatures in a constant temperature waterbath (88°C); C = centre, I = intermediate, S = surface sections, * = centre temperature when removed from the waterbath.

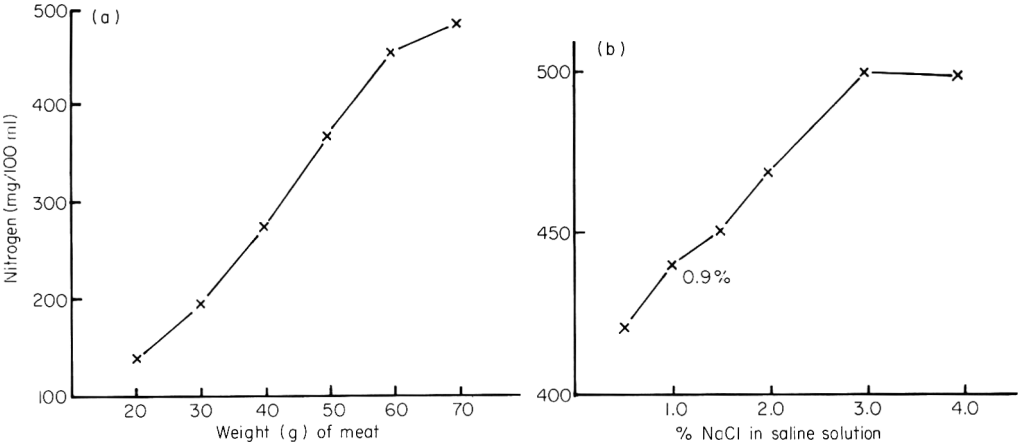

Figure 3. Effect of (a) different weights of meat extracted by 0.9% saline, on the analysable nitrogen, and (b) the concentration of the salt solution (% NaCl) used as the extractant, on the final level of nitrogen found in the extract.

amount of meat per 100 ml saline was increased over the range 20–70 g. An increase in the soluble nitrogen was also observed as the strength of 100 ml salt solution used to extract a 50 g sample was increased from 0.5 to 3.0% sodium chloride: 4% salt appeared to give no further extraction of protein, (Fig. 3b). Within these limits it would therefore be possible to standardize the procedure.

Discussion

The results showed that the amount of residual soluble nitrogen in an extract decreased as the cooking temperature increased, with the major reduction occurring between 40 and 70°C. A good correlation was obtained over this region between nitrogen values for the aliquots removed at regular temperature intervals from the continuously heated raw meat extract, values obtained from individual 5 mm slices and the centre sections of 60 mm cubes (curves (a), (b) and (c) Fig. 1), although the temperature history of these samples was quite different.

Analysis of tissue from the meat cubes after sectioning showed that it is possible to detect differences in the extent of heating achieved from the surface to the centre of a solid piece of meat. As noted in the Methods section, the requirement of the present method for a minimum of 20 g samples for each analysis resulted in less accurate values for each sampling point because of the inclusion of adjacent tissue of significantly different time/temperature history. The use of micro-Kjeldahl techniques may improve the sensitivity.

Thus it may be possible to estimate in retrospect the approximate maximum temperature reached in an unknown situation by reference to a standard curve (graph (b) Fig. 1). However it should be noted that these experiments have been carried out using only two beef muscles. Changes in the 'standard curve' could well be observed with meat of different compositions (Saffle & Galbreath, 1964), species and muscle type. For example a muscle with a high collagen content may yield lower levels of salt soluble proteins. Such a sample would also yield large quantities of soluble gelatine on heating, which would confound the final nitrogen analysis. Unusual pH, additives and salts may increase or decrease the soluble nitrogen available. It may be necessary to produce a set

of standard curves using model systems; different species, muscles and compositions, against which an unknown could be compared and classed.

The effect of duration of heating on the nitrogen curve has not yet been studied. Further experiments may show that the slope of the curve is changed but the final end point i.e., that point at which no further denaturation takes place, remains the same. This would enable one to predict under any conditions that a product had not reached 70°C (a critical temperature, see Introduction). As it stands at present soluble nitrogen analysis is a simple test which gives a good indication of previous heat treatment temperature in the range 40–70°C, where homogeneous lean beef samples of 20–25 g weight are available.

Acknowledgment

We thank the Ministry of Agriculture, Fisheries and Food (MAFF) for permission to publish this work, which was part of a research project they sponsered.

References

Hamm, R. (1966). Heating in muscle systems. In: *International Symposium on the Physiology and Biochemistry of Muscle as a Food.* (edited by E. Briskey, R. Cassens & J. Trautmann), pp. 363–385. University of Wisconsin Press, Madison, Milwaukee, London.

Hamm, R. & Deatherage, F.E. (1960). Changes in hydration, solubility and changes of muscle proteins during heating of meat. *Food Research,* **25,** 587–610.

Saffle, R.L. & Galbreath, J.W.G. (1964). Quantitative determination of salt-soluble protein in various types of meat. *Food Technology,* **18,** 1943–1944.

Steele, P. & Lambe, W.J. (1982). SDS-Gradient gel electrophoretic separation of muscle polypeptides for estimation of maximum cooking temperatures in meat. *Journal of Food Protection,* **45,** 59–62.

USDA (1981). *Chemistry Laboratory Guidebook,* Part 1, Food Safety and Quality Division, Coagulation Method. 3. 012. M.P.I. Reg. Manual 18.46.

(Received 7 June 1985)

Journal of Food Technology (1986) **21**, 123–131

Assessment of the previous heat treatment given to meat products in the temperature range 40°–90°C. Part 2: Differential scanning calorimetry, a preliminary study

SUSAN E. PARSONS* AND R. L. S. PATTERSON

Summary

Differential scanning calorimetry (DSC) was examined as a potential method for determination of the previous heat treatment of a meat product. Samples of beef (*M. longissimus dorsi and M. semimembranosus*) were given a known heat treatment using a thermostatically controlled waterbath or the DSC instrument itself. Analysis in the DSC was then carried out over the temperature range 22–97°C at a heating rate of 10°/min. Raw beef was found to produce a three peak thermogram within the temperature range 45–90°C with peak maximum at 55, 66 and 79°C. As heat treatment is applied the peaks gradually disappear and variations in the cooked sample pattern can be related to the previously applied treatment. It appears that both the temperature and duration of heat treatment are important to the degree of denaturation and therefore the thermogram pattern. A promising correlation exists between maximum heat treatment temperature and the onset of denaturation obtained by DSC analysis, whilst the effect of duration of heat treatment is reflected in the area of the thermogram. It appears from the resultant thermograms that it may be possible to obtain an indication of the temperature and possibly duration of the previous heat treatment.

Introduction

Heat treatment of a meat product is important not only to produce a palatable product but also to safeguard health by destroying pathogenic and food spoilage organisms. In part 1, analysis of soluble nitrogen in heat treated samples was reported as a potential method of eliciting information in retrospect about the previously applied heat treatments; in this paper, the possible advantages of a second, more sophisticated technique, differential scanning calorimetry (DSC) are described. DSC is based on the principle that whenever a material undergoes a physical or chemical change heat is either liberated or absorbed. Muscle has a complex protein structure and several changes occur during heating which can be monitored by DSC.

This technique measures the differential heat flow required to maintain a sample of the material and an inert reference at the same temperature while the temperature of both is gradually increased, at a pre-programmed linear rate. Any thermally induced changes occurring in the sample are then sensed as a differential power requirement, either positive or negative, and displayed on a chart recorder. From the thermograms

Authors' address: AFRC Food Research Institute—Bristol, Langford, Bristol, BS18 7DY, UK.
*To whom correspondence should be addressed.

produced, it is possible in principle to determine such characteristics as the enthalpy and temperature of transitions, and changes in heat capacity. A major advantage of the technique is that the size of sample required is very small, usually about 15 mg; thus surveying a specimen at a number of closely spaced sampling points is possible.

The DSC has been used by other workers to investigate the denaturation of meat proteins (Kvale & Martens 1977–78; Ledward, 1978). Wright, Leach & Wilding (1977) found that rabbit muscle produced three peaks and concluded that they were due to the denaturation of the myosin, sarcoplasmic proteins and actin respectively. It is also believed that connective tissue plays some part in the denaturation pattern (Martens & Vold, 1976; Stabursvik & Martens, 1980). The endothermic changes revealed in the thermograms of meat are caused by the denaturation of the proteins, which occur over a small temperature range. The aim of this preliminary study was to examine the thermograms obtained from raw and various cooked samples, to determine if there were any thermogram characteristics which related to the previous heat treatment.

Materials and methods

Thin slices of beef *M. longissimus dorsi,* sealed in nylon bags were heated in a temperature controlled water bath, as described in Part 1 (soluble nitrogen analysis), until the centre and surface temperatures reached that of the waterbath. The samples were held for 0 or 5 min at the preset temperature before cooling. Sixty mm cubes of beef *M. semimembranosus* were similarly heated in a bath at 88°C until the centre temperatures reached 30, 50, 60, 70 and 88°C, then removed and cooled.

Samples were also given precisely controlled heat treatments in the DSC (Perkin-Elmer DSC-2C) itself. For example samples, sealed in pans, were heated at 40°/min to a preset temperature held for a set time, then cooled at 20°/min. A rerun of the sample yielded the thermogram for analysis.

Raw and heat treated samples were stored frozen at −20°C until required. Ten to 20 mg samples of raw or cooked meat were excised with a scalpel to avoid visible fat and connective tissue. The accurately weighed samples were hermetically sealed into 'volatile' aluminium pans, after gentle maceration with a scalpel, which was found to improve thermal contact with the pan surfaces. All thermograms were obtained by running samples in the DSC at a heating rate of 10°/min over the temperature range 22–97°C. An empty pan was used as the reference. The instrument was calibrated using indium m.p. 156.60°C (6.80 cal/g.) and *n*-triacontane 65.8°C.

DSC measurements were carried out on raw and various heat treated samples and a method for data analysis was developed in order to make numerical comparisons of the thermograms. A typical beef thermogram is shown in Fig. 1 showing the three peaks and corresponding areas A, B and C. The baseline was drawn (estimated) and the onset temperature (T_0) (i.e., that at which the denaturation reaction begins), identified by eye from the first slope of the thermogram and its intersection with the baseline, drawing a perpendicular from the point of intersection to the temperature axis. The area under the thermogram (peaks to the baseline) is related to the enthalpy (ΔH), since the change in enthalpy is equal to the heat transfered during an isobaric process (Zemansky 1957). The conversion of area (measured by planimeter) to enthalpy was carried out according to the method given by Perkin-Elmer, using indium as the standard. The enthalpy of denaturation of meat is quoted as total ΔH/g tissue although it must be understood that the thermogram is produced by many constituents.

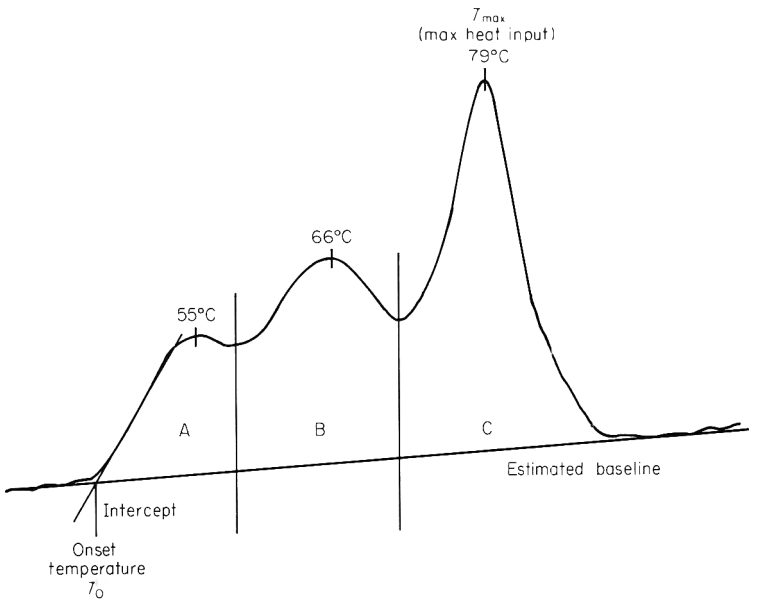

Figure 1. Analysis of the thermogram (raw beef).

The three peaks have been tentatively identified by previous workers, but disagreement exists particularly over the second peak. For the purpose of this work the peaks were taken as a partial area of the whole and the individual ΔH values per unit mass of sample. It was felt necessary to divide the thermogram to enable a numerical description of each thermogram to be made. In future studies it may be possible to show that a peak results from the denaturation of a particular protein or parts of protein and thus to quote the enthalpies as denaturation of individual proteins.

For comparative purposes it was necessary to check the effect of sample mass on the DSC thermogram. Samples of different mass might be expected to cause changes in the thermal contact, temperature gradients and conductivity of a sample, which may in turn cause variations in ΔH and temperatures T_0 and T_{max}. Samples of varying mass were therefore run in the DSC under identical conditions.

Results

Raw beef was found to give a characteristic three peak thermogram within the temperature ranges 45–90°C with peak maxima at 55, 66 and 79°C (Fig. 2). Heating the sample to a given temperature (T_H) at 40°/min followed by immediate rapid cooling at 20° min caused practically complete denaturation up to T_H. Rerunning in the DSC therefore produced thermograms that were practically featureless up to T_H and, as T_H was raised, the low temperature features of the thermogram were progressively lost (Fig. 2). By T_H = 80°C the thermogram was featureless and the meat had been totally and irreversibly denatured. Increasing the time of exposure to elevated temperature also increased denaturation, thereby reducing the area of the thermogram (Fig. 3).

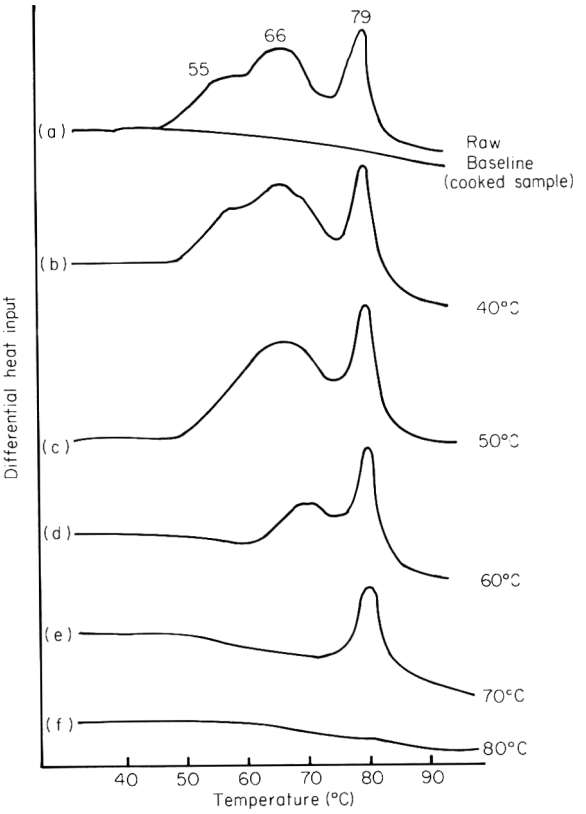

Figure 2. Thermograms of beef LD previously heated in the DSC at 40°/min to a set temperature and immediately cooled at 20°/min (a) raw beef, (b) beef heated to 40°C, (c) 50°C, (d) 60°C, (e) 70°C, (f) 80°C.

Thermograms obtained from samples heat treated in a waterbath (Fig. 4, Table 1) gave very similar results to those produced by DSC heat treated samples. Over the temperature range $45 < T_H < 70$°C there was a high positive correlation between T_H and T_0. A progressive reduction in the ΔH (enthalpy of denaturation) occurred as the temperature of heat treatment increased. The three peaks A, B and C (Fig. 1) of the

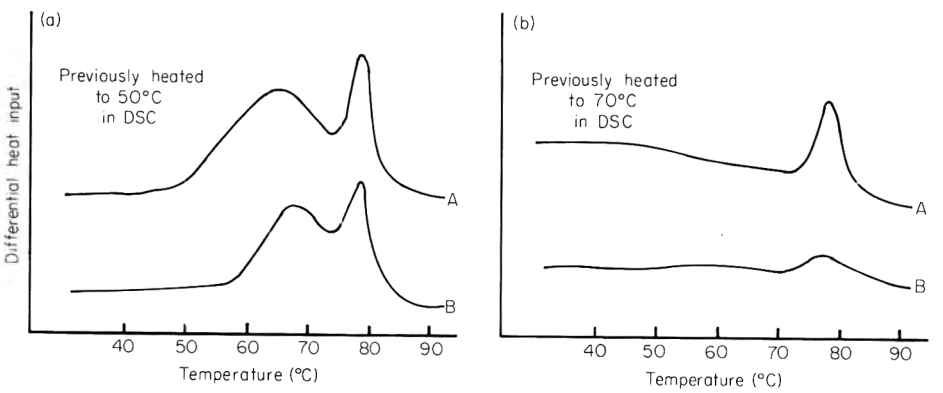

Figure 3. Effect of different heating rates and holding times. 'A' thermograms: heated at 40°/min, then immediately cooled at 20°/min. 'B' thermograms: heated at 20°/min, held for 1 min, then cooled at 20°/min.

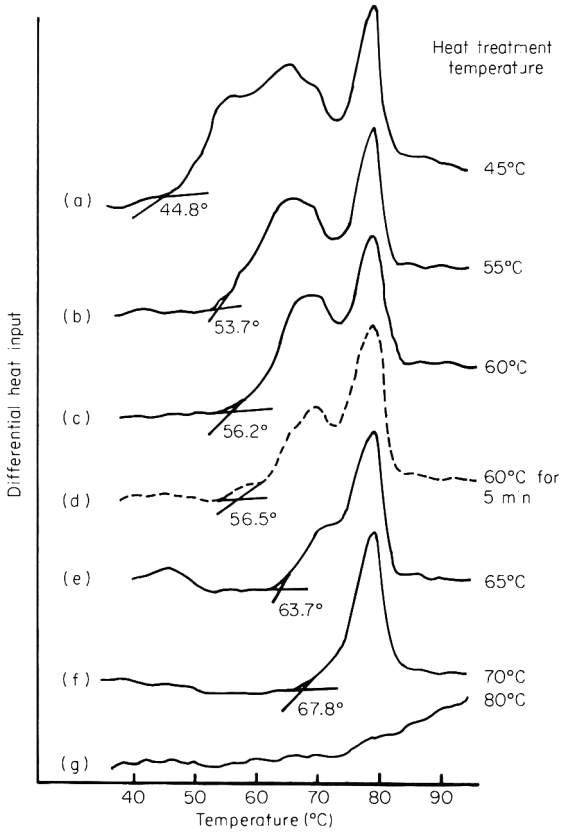

Figure 4. Samples from slices of beef LD previously heated in waterbath. Heat treatment temperature and T_o values shown for each sample.

Table 1. Analysis of DSC thermograms of beef samples previously heated in a water bath (mean values for three subsamples)

Previously applied heat treatment (°C) T_H	Onset temperature T_o (°C)	Total enthalpy ΔH (mcal/mg sample)
Raw	42.8±0.2*	0.82±0.04*
40	43.3±0.6	0.81±0.06
40 held 5 min	43.3±0.3	0.90±0.05
45	44.8±0.3	0.86±0.03
50	46.0±0.0	0.77±0.06
50 held 5 min	48.7±1.2	0.67±0.08
55	53.7±0.6	0.55±0.04
60	56.2±0.3	0.53±0.03
60 held 5 min	56.5±0.5	0.51±0.07
65	63.7±0.3	0.29±0.03
70	67.8±0.3	0.26±0.02
70 held 5 min	68.7±0.6	0.21±01
80	No peaks	No peaks
90	No peaks	No peaks

*±Standard deviation.

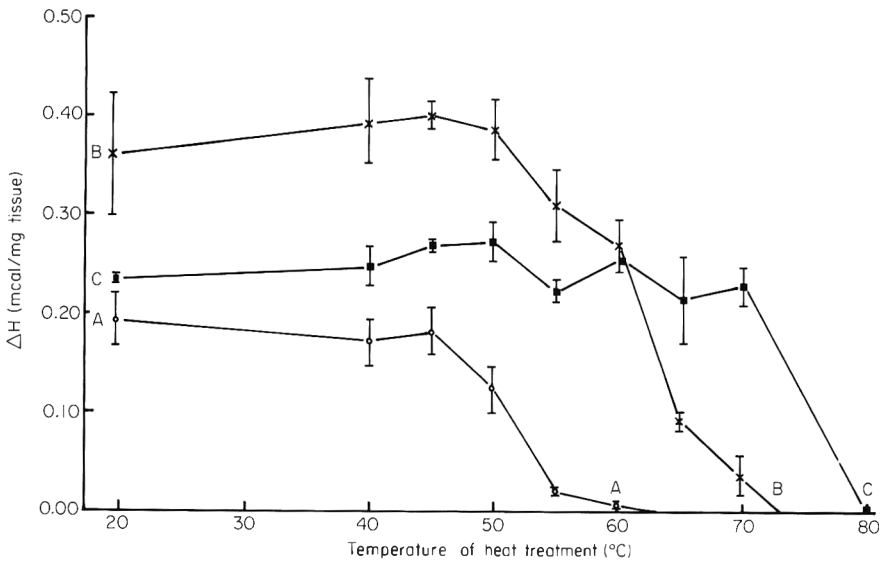

Figure 5. Effect of heat treatment temperature on the enthalpy (area) of the regions A, B and C of the thermogram. Error bars indicate standard deviation from the mean of triplicate samples.

thermogram appeared to reduce in area independently of one another, with area A reducing to zero between 45° and 60°C, area B between 50° and 70°C and area C between 70° and 80°C (Fig. 5).

Thick samples in the form of cubes, which experienced appreciable temperature gradients during the heat treatment, showed considerably more denaturation at the surface than at the intermediate and central points (Fig. 6). All samples showed complete denaturation at the surface. T_0 values of samples taken from the centre of the cubes were correlated with the maximum temperature achieved (Table 2). Profiles of T_0 through the cubes (Table 2) showed progressively more denaturation over the whole range of heat treatments. It should be noted that the maximum temperatures at the centres of the cubes were higher than at the removal from the bath due to the slow transfer of heat through the meat (Fig. 7). The maximum centre temperatures for cubes removed from the bath when the centre temperature first reached 30, 50, 60, 70 and 88°, were 46.5 60, 66°, 71° and 88° respectively.

There was a linear relationship between the energy absorbed i.e. peak area and sample mass (Fig. 8). Regression analysis yielded the following equation:

$$\text{Peak area (mcal)} = -0.67+0.839\times\text{mass (mg)},$$

with a correlation coefficient 0.992, standard error 0.023 mcal. It is important that the relationship is linear to allow comparisons between samples of different mass. The effect of varying sample mass (5–20 mg) on T_0 and T_{max} values fell within normal sample variation. It should be noted that temperatures quoted are not precise; for example variations between the thermal properties of the calibrants and meat have not been taken into account, nor the temperature lag within the sample itself or the kinetics of the denaturation processes. The analysis presented here served merely to compare the thermogram characteristics of cooked samples with those of raw beef.

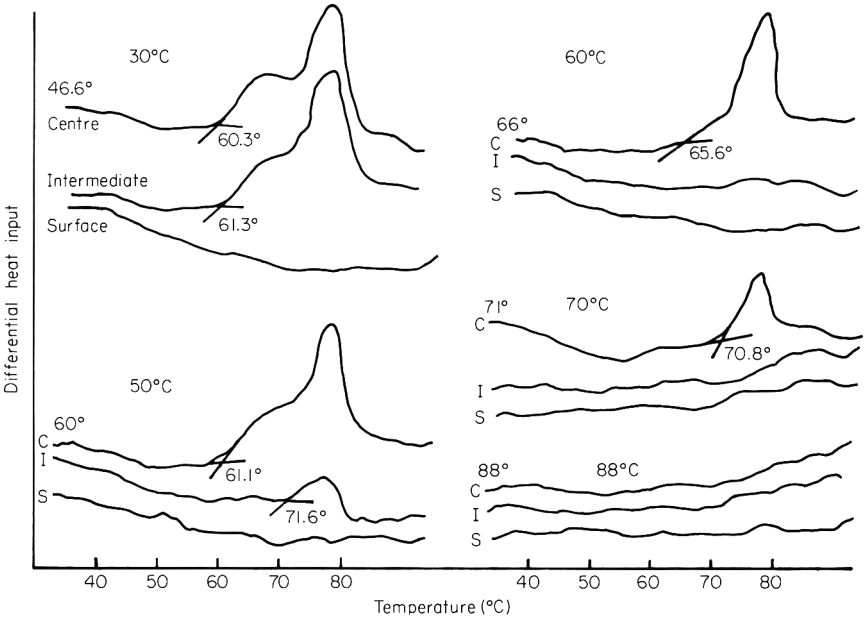

Figure 6. Thermograms taken from the centre, intermediate and surface sections of the meat cubes held in a water bath at 88°C until the centre temperature was 30, 50, 60, 70 or 80°C.

Table 2. Retrospective estimation of temperature attained in beef cubes by analysis (T_0 values) of triplicate DSC thermograms

	Temperature (°C) at cube centre (by thermocouple) when withdrawn from 88°C bath				
Mean T_0 value (°C)	30 (46.5)*	50 (60)	60 (66)	70 (71)	88 (88)
Centre	61.3±0.6†	71.6±0.0	65.6±0.0	70.8±0.3	> 75
Intermediate	60.3±0.6	61.1±0.5	> 75	> 75	> 75
Surface	> 75	> 75	> 75	> 75	> 75

*Numbers in parenthesis = ultimate centre temperature by thermocouple.
†±Standard deviation.

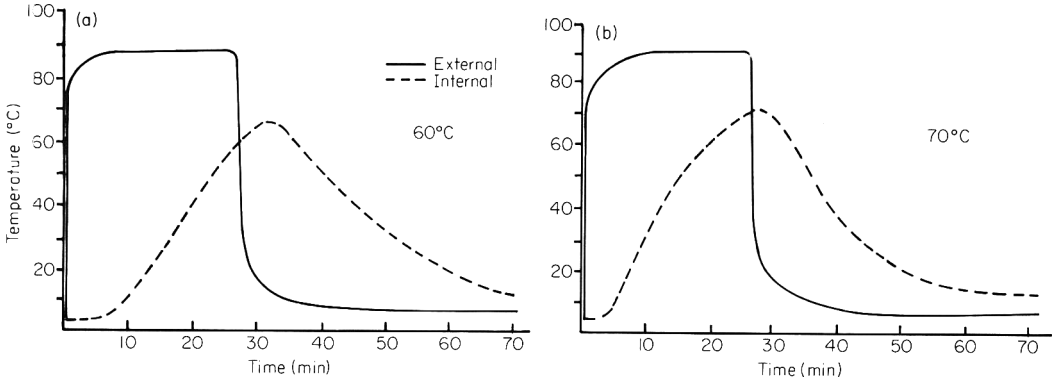

Figure 7. Heating profiles of meat cubes (60 mm sides) immersed in a waterbath at 88°C.

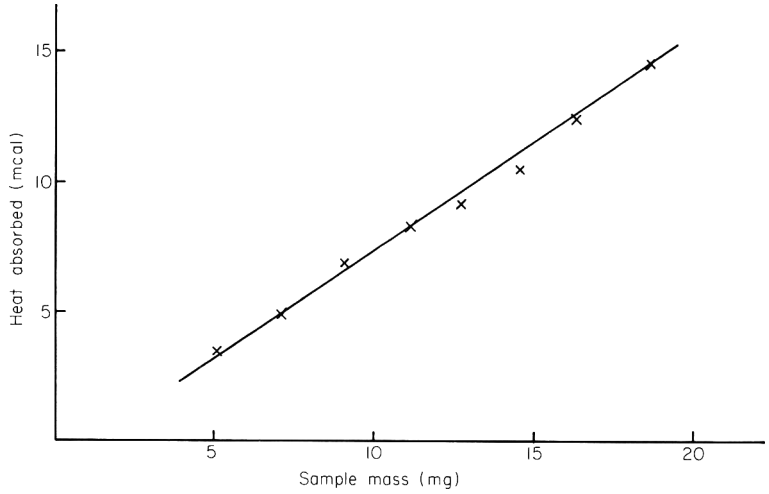

Figure 8. Effect of sample mass on enthalpy of denaturation (ΔH).

Discussion

From this preliminary study it appears that raw beef LD produces a three peak thermogram. As heat treatment is applied the peaks gradually disappear and variations in the cooked sample pattern can be related to the previously applied treatment. The peaks are caused by the energy changes associated with the denaturation of the protein. The process is not reversible; re-analysis of the cooked sample yields no peaks.

It appears that both the temperature and the duration of heat treatment are important to the degree of denaturation and therefore the thermogram pattern (Fig. 4c and d). A promising correlation exists between maximum heat treatment temperature T_H and the T_0 value obtained by DSC analysis. Measurement of the individual areas A, B and C showed that the proteins represented by these sections denature over different temperature ranges. Studies also indicated that the total area of these peaks reduced independently, as the duration of the heat treatment was extended. The transition temperature T_0, however did not appear to be grossly affected by the duration of the treatment.

The correlation between the DSC analysis and the recorded temperature of the meat cubes was not as good. The heat treatments of the cubes were of longer duration to allow the heat to penetrate to the centre. The time/temperature regime may have caused a higher degree of denaturation at lower temperatures than was previously found in the experiments using smaller samples and faster heating rates. This may be an indication of the problems to be encountered when dealing with temperature gradients within a sample. The relationship between temperature and time, on the denaturation of meat samples requires further study, but it is hoped that T_0 may be used to give an indication of the maximum temperature and the area ΔH an indication of the duration of treatment. Factors such as pH and ionic strength may also affect the denaturation of meat (Stabursvik & Martens, 1979). A ecently published study by Wright & Wilding (1984) revealed that the thermogram pattern of myosin and its sub-fragments, is dependent on the conditions of pH and salt.

Differential scanning calorimetry appears to have the potential to yield information

about previous heat treatment applied to meat materials. It also provides a means of studying the processes involved, the causes, and factors which affect the thermal denaturation of the proteins in meat.

Acknowledgments

The authors with to thank Dr R. Lyster at the Food Research Institute, Reading (formally National Institute of Research in Dairying), and Dr D. Ledward at Nottingham University, for allowing access to their differential scanning calorimeters; and the Ministry of Agriculture, Fisheries and food (MAFF) for permission to publish this work, which was part of a research project they sponsored.

References

Kvale, O. & Martens, H, (1977–78). *Food Quality and Nutrition—research priorities for thermal processing* (by Downey, W.K.) Pp. 537–542. Applied Science publishers.

Ledward, D.A. (1978). Scanning calorimetric studies of some protein-protein interactions involving myoglobin. *Meat Science,* **2,** 241–249.

Martens, H. & Vold, E. (1976). DSC Studies of muscle protein denaturation. *22nd European Meeting of the Meat Research Workers,* paper J9, J9:3–J9:6.

Stabursvik, E., Frøystein, R. & Fretheim, K. (1984). Myosin denaturation in pale soft and exudative (PSE) porcine muscle tissue as studied by differential scanning calorimetry. *Journal of the Science of Food and Agriculture,* **35,** 240–244.

Stabursvik, E. & Martens, H. (1979) Proteins in white and red muscle compared by differential scanning calorimetry (DSC). *25th European Meeting of Meat Research Workers,* **5**(21), 383–386.

Stabursvik, E. & Martens, H. (1980). Thermal denaturation of proteins in post rigor muscle tissue as studied by differential scanning calorimetry. *Journal of the Science of Food and Agriculture.* **33,** 1034–1042.

Wright, D.J., Leach, I.B. & Wilding, P. (1977). Differential scanning calorimetry study of muscle and its constituent proteins. *Journal of the Science of Food and Agriculture.* **28,** 557–564.

Wright, D.J. & Wilding, P. (1984). Differential Scanning Calorimetric study of muscle and its constituent proteins: myosin and its subfragments. *Journal of the Science of Food and Agriculture,* **35,** 357–372.

Zemansky, M.W. (1957). *Heat and Thermodynamics.* 4th edn. McGraw-Hill Book Co. Inc. New York, Toronto, London.

(Received 7 June 1985)

Journal of Food Technology (1986) **21**, 133–149

Flow behaviour and scanning electron microscopy of myosin B, soya protein components and mixtures at varying temperatures

L. C. LIN* AND T. ITO†

Summary

The relationship of shear stress *versus* shear rate of myosin B, soya protein components and mixtures of them in solution was investigated at varying temperatures. Scanning electron microscopic observations were also made at varying temperatures. Myosin B solution showed thixotropic flow behaviour at room temperature, while the solution of soya protein components and the mixture of myosin B and soya protein components were rheopectic. Upon heating the rheopectic property of soya protein components and the mixture of myosin B and soya protein components became thixotropic. Scanning electron microscopic observations revealed that a three dimensional network was formed prior to gel formation on heating for myosin B and also for soya protein components and the mixture of myosin B and soya protein components. The results suggest that in these systems thixotropic flow behaviour precedes the formation of a protein gel on heating.

Introduction

It is generally considered that a knowledge of the rheological properties of proteins in liquid state is of practical significance not only for elucidating the nature of protein–protein interactions in liquid and solid state but also for controlling the texture of meat products, fabricated foods and other proteinaceous foods. Rheological properties of protein solutions are influenced by intrinsic factors such as shape, size, surface charge and concentration of protein as well as extrinsic factors such as temperature, pH and shear rate (Kirkwood, Buff & Green, 1949; Blake *et al.*, 1965; Pradipasena & Rha, 1977; Catsimpoolas & Meyer, 1970; Ehninger & Pratt, 1974). Kinsella (1976), Schmidt, Mawson & Siegal (1981) and Acton, Ziegler & Burge (1983) have discussed the relationship in functional properties between proteinaceous foods and their constituent proteins before and after heat treatment for many kinds of fabricated foods such as beef patties and comminuted meat products. In comminuted meat products, myosin B (natural actomyosin) as well as myosin alone contributes to the development of binding quality of meat products (Fukazawa, Hasimoto & Yasui, 1961; Macfarlane, Schmidt & Turner, 1977; Siegel & Schmidt, 1979). It is also known that myosin, actin and their complex actomyosin show thixotropic flow behaviour (Nakayama & Sato, 1971; Maruyama, Kaibara & Fukada, 1974; Nakayama *et al.*, 1979). They can easily form strong gels upon heating at about 50–60°C (Samejima *et al.*, 1969; Deng, Toledo & Lillard, 1976; Haga & Ohashi, 1978). On the other hand, soya protein 7s and 11s

Authors' address: Laboratory of Chemistry and Technology of Animal Products, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan.

*Present address: Department of Animal Science, Chung Hsing University, Taichung, Taiwan.

†To whom correspondence should be addressed.

components, which make up 70–75% of total soya proteins, have a different molecular structure from myosin B and exhibit different rheological properties (Haga & Ohashi, 1984). The soya protein components are also able to form gels upon heating, but the critical temperature for inducing gelation is much higher than that of myofibrillar proteins (Saio, Sato & Watanabe, 1974). Mixtures of myofibrillar protein and soya protein have gelling properties intermediate to the constituent proteins (Hermansson, 1975; Saio *et al.*, 1974; Haga & Ohashi, 1978). In a previous paper, we have found from experiments on the extensibility of protein fibre that myosin B and soya proteins interact even at low temperature and the interaction is strengthened with increasing temperature (Lin & Ito. 1985).

In the present study, we have investigated shear rate dependent shear stress and microstructure of myosin B, soya protein components and mixtures of them at varying temperatures in order to elucidate the relationship between flow behaviour of protein solution and structure of the protein upon heating. The present results suggest that thixotropic flow behaviour precedes the gelation of the protein solution.

Materials and methods

Preparation of myosin B, soya protein 7s and 11s components

Myosin B, soya 7s and 11s components were prepared as described in a previous paper (Lin & Ito, 1985). Myosin B was extracted with Weber-Edsall solution (0.6 M KCl, 0.04 M $NaHCO_3$, 0.01 M Na_2CO_3, 1 mM ethylene diaminetetraacetate (EDTA), pH 9.2), followed by purification by repeating the usual precipitation-dissolution cycle thrice (Watanabe, 1975). Soya 7s component was extracted from defatted soya flakes as described by Roberts & Briggs (1965) and King (1977) and then purified by gel filtration with Sephadex G-100 according to the procedure of Koshiyama (1972). Soya 11s component was obtained from the cold insoluble fraction (CIF) essentially by the method of Wolf, Babcock & Smith (1962) and then purified by ion exchange chromatography with DEAE-Sephadex A-50 (Catsimpoolas *et al.*, 1967). Soya protein samples were concentrated with Aquacide II-A (Calbiochem-Behring Corp.).

Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed on 7.5–17.5% acrylamide gradient slab gels according to the procedure of Leammli (1970).

Viscometry

Viscosity of myosin B, individual soya protein components (7s and 11s), a mixture of 7s and 11s components (1 : 1), a mixture of myosin B and 7s component (1 : 1), a mixture of mysoin B and 11s (1 : 1) and a mixture of myosin B and soya protein mixture (ratio of myosin B to 7s and 11s was 2 : 1 : 1) were examined. Samples of myosin B and soya protein components were dialysed against 100 vol of a buffer (1.3 mM potassium primary phosphate, 16.25 mM potassium secondary phosphate, 200 mM sodium chloride, 300 mM potassium chloride, 5 mM β-mercaptoethanol, pH 7.6) at 0°C overnight prior to examining the viscosity. A cone and plate viscometer (Tokyo Keiki Co.) was used to evaluate flow properties of protein samples using two types of cones (4.8 cm in diameter, 1°34′ in angle; 2.8 cm in diameter, 3° in angle) at 25±0.5°C unless otherwise mentioned. The amount of protein sample was 1.2 and 0.6 ml for low angle and high angle cones respectively. Hysteresis loop effect was examined both by stepwise increase

Table 1. Time schedule for the measurements of viscosity of protein solution

Rotatory speed (rpm)	Time (sec)	
	up	down
0.5	120	1100
1.0	240	990
2.5	360	870
5.0	420	750
10.0	480	690
20.0	510	630
50.0	540	600
100.0	570	

of shear rate to the maximal (upward curve), and then from there, by stepwise decrease to the minimal value (downward curve). The time schedules for the measurements at every shear rates (rpm) are represented in Table 1. One cycle of the measurements was completed within 18.5 min. In investigating the effect of temperature on the flow behaviour of samples, a fresh sample was used at each temperature: viscosity measurements were carried out immediately after the temperature of the sample reached the desired temperature by pre-incubation. Time dependent change of shear stress was also measured at a constant shear rate (3.83/sec and 191.5/sec for soya protein; 2/sec and 100/sec for myosin B and myosin B–soya protein mixture). Protein concentration for viscosity measurements was 22 mg/ml.

Scanning electron microscopy

Scanning electron micrographs of samples incubated at varying temperatures before and after the determination of viscosity were taken essentially according to the procedure of Sjöstrand (1967) as described in a previous paper (Lin & Ito, 1985). Protein samples thinly spread on aluminium film coated-slide glasses were frozen with dry ice-isopentane mixture, followed by drying by evacuation. Then the samples were coated with gold using a vacuum evaporator (Model HUS-4GB, Hitachi Ltd). Electron microscopic observations were made with Hitachi SSM-2A scanning electron microscope using an accelerating voltage of 10 kV.

Protein concentration

Protein concentration was determined by the biuret method of Gornall, Bardawill & David (1949), which had been standardized by bovine serum albumin.

Results

Flow behaviour of myosin B, soya protein components and mixtures of them at 25°C

Figures 1, 2 and 3 show hysteresis loops of myosin B, soya protein components (7s, 11s and mixture of them) and mixtures of myosin B and soya protein components respectively, obtained by plotting measured values of shear stress *versus* shear rate. In every case the plots of shear stress *versus* shear rate exhibited non-Newtonian flow behaviour. As shown in Fig. 1, myosin B has a thixotropic property at 25°C even after

storage for 1 week at 0°C. However, the values of shear stress of stored myosin B were considerably lower than those of fresh myosin B at every shear rate examined. The time-dependent nature of shear stress at two selected shear rates (2.0/sec and 100/sec) is shown in the inset of Fig. 1. Shear stress descreased with increasing the duration of shearing during the first 10 min, and then there was almost no further change during 60 min of shearing. In addition, shear stress decreased more rapidly at higher shear rate (100/sec) than at lower shear rate (2.0/sec) during the first 10 min.

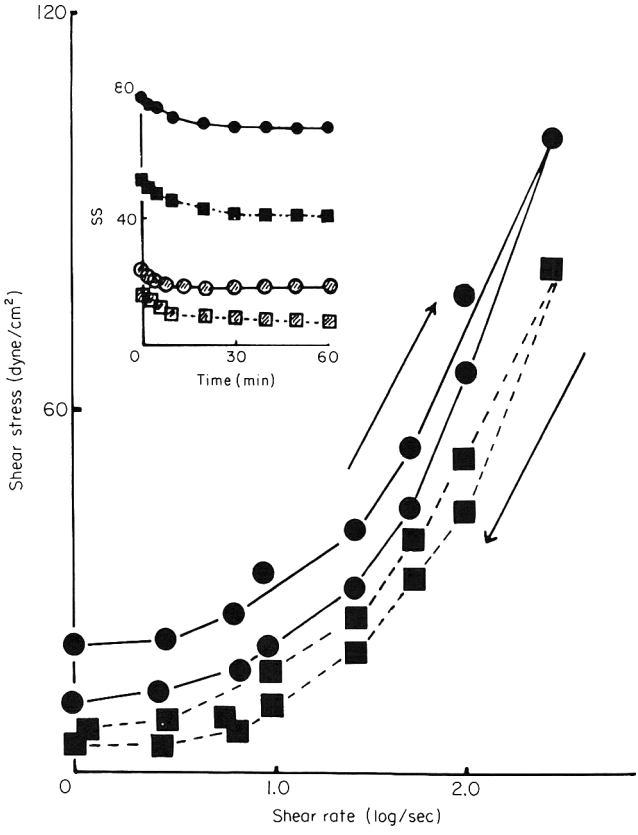

Figure 1. Hysteresis relation between shear stress and shear rate of myosin B solution. ●, Fresh myosin B; ■, myosin B stored for 1 week at 0°C. Rising arrow indicates upwards curve; declining arrow indicates downward curve. Inset: Time-course of shear stress of myosin B solution at two selected shear rates (100/sec and 2/sec). ●, Fresh myosin B (10/sec); ■, myosin B stored for 1 week at 0°C (100/sec); ▨, fresh myosin B (2/sec); ▨, myosin B stored for 1 week at 0°C (2/sec).

On the other hand, hysteresis loops of soya protein components (7s, 11s and mixture of 7s and 11s) at 25°C showed a characteristic flow behaviour of rheopectic bodies. The intensity of shear stress of these components were in order of 11s component, mixture of 7s and 11s component and 7s component (Fig. 2). Effect of shearing time on shear stress is shown in the inset of Fig. 2. Shear stress increased with increasing duration of shearing and the effect was less for lower shear rate (3.83/sec) than for higher shear rate (191.5/sec). Such flow behaviour of soya protein components contrasts with that of myosin B.

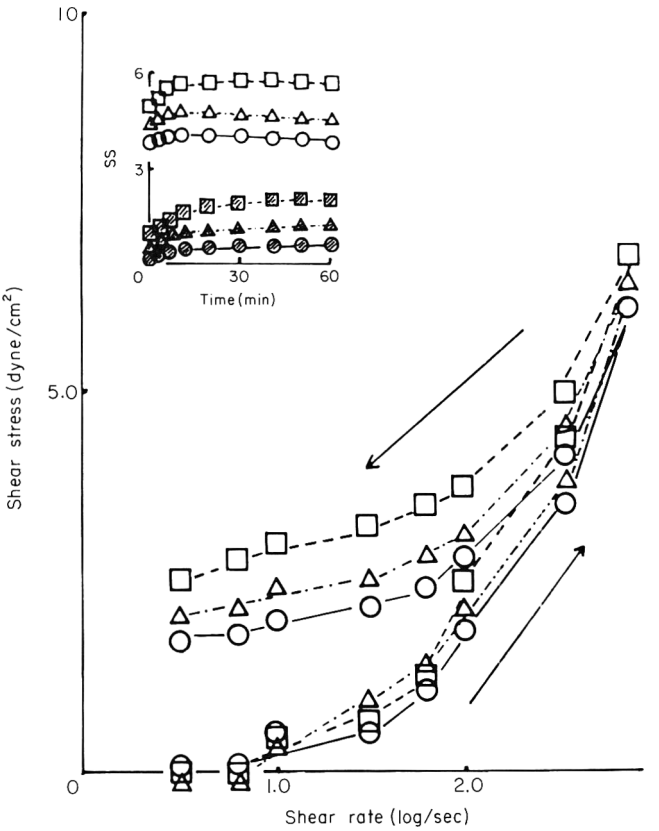

Figure 2. Hysteresis relations between shear stress and shear rate of soya protein 7s component, 11s component and mixture of them. ○, 7s Component; □, 11s component; △, mixture of 7s and 11s component (weight ratio = 1:1). Other indications are the same as in Fig. 1. Inset: Time-course of shear stress of 7s, 11s and mixture of 7s and 11s component at two selected shear rates (191.5/sec and 3.83/sec). ○, 7s component (191.5/sec); □, 11s component (191.5/sec); △, mixture of 7s and 11s component (191.5/sec); ⊘, 7s component (3.83/sec); ▨, 11s component (3.83/sec); ◬, mixture of 7s and 11s component (3.83).

As shown in Figs 3a, b and c, every combination of myosin B and soya protein components showed a rheopectic property immediately after mixing, but the flow behaviour of the mixtures became thixotropic after 1 week storage at 0°C. However, the mixtures prepared by mixing myosin B and soya protein components after storage separately for 1 week at 0°C showed a rheopectic property as in the case of freshly prepared mixture, although the intensity of shear stress decreased during storage. The intensity of shear stress was in order of freshly prepared mixture, mixture stored for 1 week and mixture prepared by mixing myosin B and soya protein components after storage separately for 1 week. Of the three types of mixtures, the mixture comprising myosin B, 7s and 11s components exhibited the strongest shear stress (Fig. 3c), while myosin B with 7s component exhibited the lowest (Fig. 3a). Insets of Figs 3a, b and c show the effect of shearing time on shear stress of the mixtures. Shear stress of samples showing thixotropic behaviour (the mixture stored for 1 week after mixing) decreased with increasing time during the first 10 min both at higher (100/sec) and lower shear rates (2.0/sec), while that of samples showing rheopectic flow behaviour (fresh mixture

and mixture prepared by mixing the constituents after storage separately for 1 week) increased.

Figure 4 shows SDS polyacrylamide gel electrophoretograms of myosin B, soya protein components and mixtures freshly prepared or stored for 1 week at 0°C after the

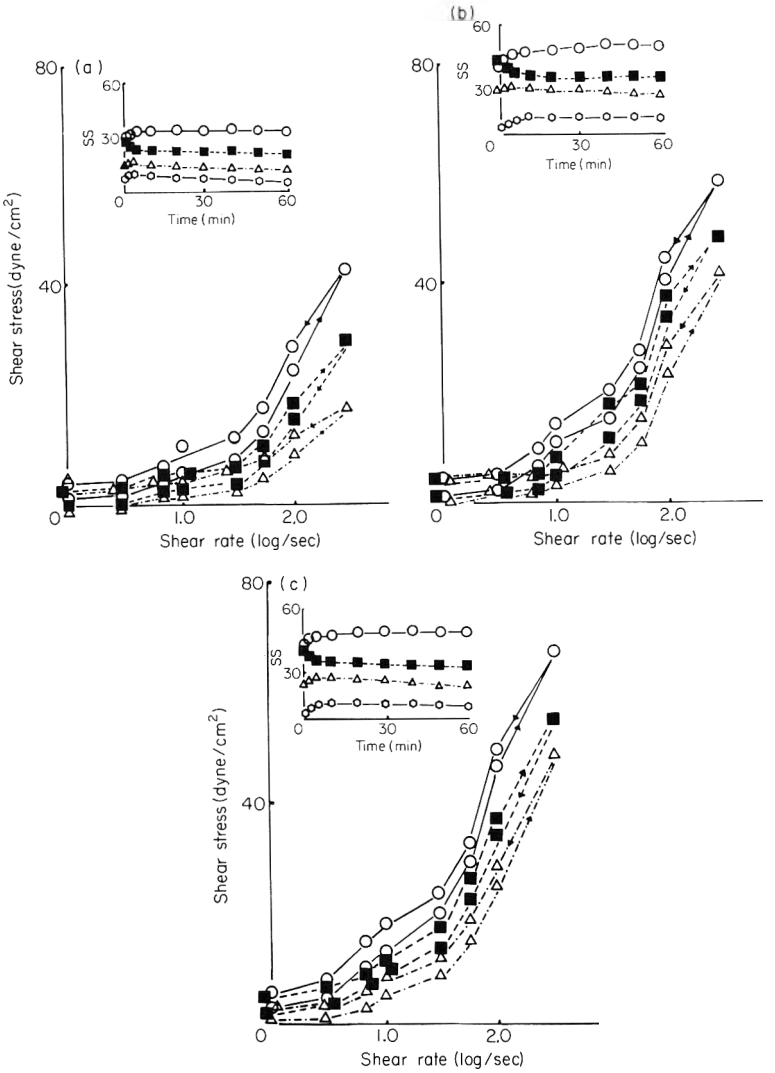

Figure 3. Hysteresis curves for the mixture of myosin B and soya protein components at 25°C. Solid symbols indicate that protein sample shows thixotropic flow behaviour, while open symbols indicate rheopexy. Other indications are the same as in Fig. 1. (a), Mixture of myosin B and 7s component (mixing ratio in weight = 1 : 1). O, Freshly prepared mixture; ■, the mixture stored for 1 week at 0°C after mixing; △, the mixture prepared after storage of the constituents separately for 1 week at 0°C. Inset; Time-course of shear stress at two selected shear rates (100/sec and 2.0/sec). O, Freshly prepared mixture (100/sec); ■, the mixture stored for one week at 0°C after mixing (100/sec); △, the mixture prepared after storage of the constituents separately for 1 week at 0°C (100/sec); ◯, freshly prepared mixture (2/sec). (b), Mixture of myosin B and 11s component (mixing ratio in wieght = 1 : 1). Symbols illustrated in Fig. 3b (including the inset) are the same as in Fig. 3a. (c), Mixture of myosin B and 7s and 11s components (mixing ratio in weight = 2 : 1 : 1). Symbols illustrated in Fig. 3c (including the inset) are the same as in Fig. 3a.

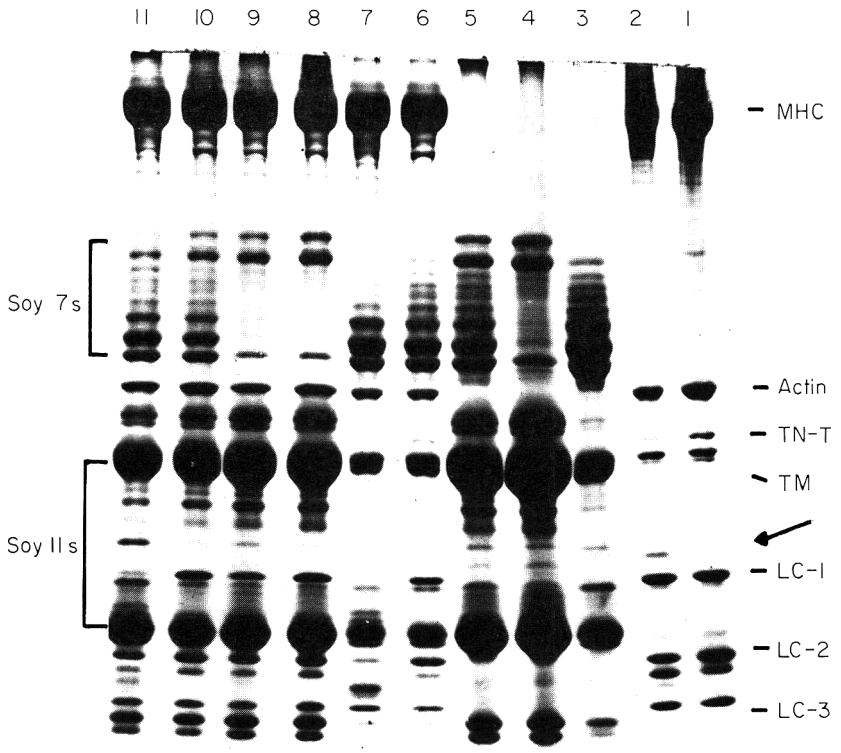

Figure 4. SDS polyacrylamide gel electrophoretograms (PAGE) of myosin B, soya protein components and mixture of them. The numbers indicated on the top of the gels were sample numbers. Thirty μg of samples were loaded on the gels. MHC, myosin heavy chains; TN-T, troponon-T; TM, tropomyosin; LC, myosin light chains. An arrow indicates the degradation product of troponin-T. Number 1, fresh myosin B; no. 2, myosin B stored at 0° for 1 week; no. 3, fresh 7s component; no. 4, fresh 11s component; no. 5, fresh mixture of 7s and 11s component; no. 6, fresh mixture of myosin B and 7s component; no. 7, the mixture of myosin B and 11s component stored for 1 week at 0°C; no. 8, fresh mixture of myosin B and 11s component; no. 9, the mixture of myosin B and 11s component stored for 1 week at 0°C; no. 10, fresh mixture of myosin B and 7s and 11s component; no. 11, the mixture of myosin B and 7s and 11s components stored for 1 week at 0°C.

mixing. In the present study, more bands are found on those electrophoretograms than on disc gel (Lin & Ito, 1985), due to the increased resolution power of gradient slab gel (7.5–17.5%). It is evident that troponin-T of myosin B preparation was degraded into its subfragment during 1 week storage. A slight degradation of troponin-T fraction was also observed in the mixture of myosin B and 7s components, while in the case of the mixture of myosin B and 11s component and the mixture of myosin B and 7s and 11s components it was difficult to find the degradation products of troponin-T on the gels.

Flow behaviour of myosin B and soya protein components at varying temperatures

Table 2 represents the effect of shear rate on shear stress of myosin B at 25–40°C. It was found that shear stress slightly decreased with increasing temperature of circulating water of viscometer from 25 to 30°C, and then increased to a higher level on increasing

Table 2. Flow behaviour and microstructure of myosin B at varying temperatures

Temperature (°C)	Flow behaviour	Shear stress at the shear rate of 100/s (dyne/cm²)		Structure before shearing	Structure after shearing
		Upward curve	Downward curve		
25	Thixotropic	76.05	69.03	Three-dimensional network composed of small granules	Porous network composed of large granules
30	Thixotropic	70.20	63.18	Almost the same as above	Almost the same as above
40	Thixotrpic	78.40	69.03	Three-dimensional network composed of sticky particles	Sponge-like structure

the temperature to 40°C. At every temperature examined, myosin B showed a thixotropic flow behaviour. The protein started to form gel upon heating above 40°C. The structure of the samples at every temperature examined are also summarized in this table (see Fig. 6).

Figures 5a, b and c show the effect of shear rate on shear stress of soya protein 7s and 11s components and mixtures at varying temperatures. As can be seen in Fig. 5a, shear stress of 7s component at every shear rate decreased with increasing temperature on the downward curve of hysteresis loops. However, such consistent temperature dependency was not observed on the upward curve of the loops. In the range 25–45°C the hysteresis loop of 7s component showed a characteristic pattern of rheopexy. However, the pattern became thixotropic on heating at 50–90°C. Partial gelation was observed above 80°C. As shown in Fig. 5b, 11s component showed rheopectic flow behaviour at 25–60°C. However, the behaviour changed from rheopectic to thixotropic at 70°C or above, as in the case of 7s component. In this case, it was observed that a small amount of white precipitate appeared at 70°C or above and a large amount at 90°C. However, no gelation was observed on heating to 90°C. As can be seen in Fig. 5b, however, thermal treatment of the 11s component at 90°C gave an unusual pattern of hysteresis loop: shear stress decreased initially and then increased greatly in the downward curve. The shear rate–shear stress relation of the mixture of 7s and 11s components showed a similar changing pattern in hysteresis loops with that of 7s component at varying temperatures examined, i.e., rheopectic flow behaviour was observed at temperatures between 25–50°C, while at 60–90°C thixotropic behaviour was noted (Fig. 5c). The change in the pattern of hysteresis loop occurred at about 60°C, the midpoint of the critical temperature for inducing the change of flow behaviour of 7s (50°C) and 11s (70°C) components from rheopexy to thixotropy.

Table 3 shows the effect of temperature on the shear rate–shear stress relation of the mixture of myosin B and soya protein components. In the case of the mixture of myosin B and 7s component, a rheopectic property of the mixture found at 25 and 30°C became thixotropic on heating at 40°C or above. In the case of the mixture of myosin B and 11s component, a rheopectic property was observed at 25°C, while at 30°C or above a thixotropic property was noted. Flow behaviour of the mixture containing myosin B, 7s

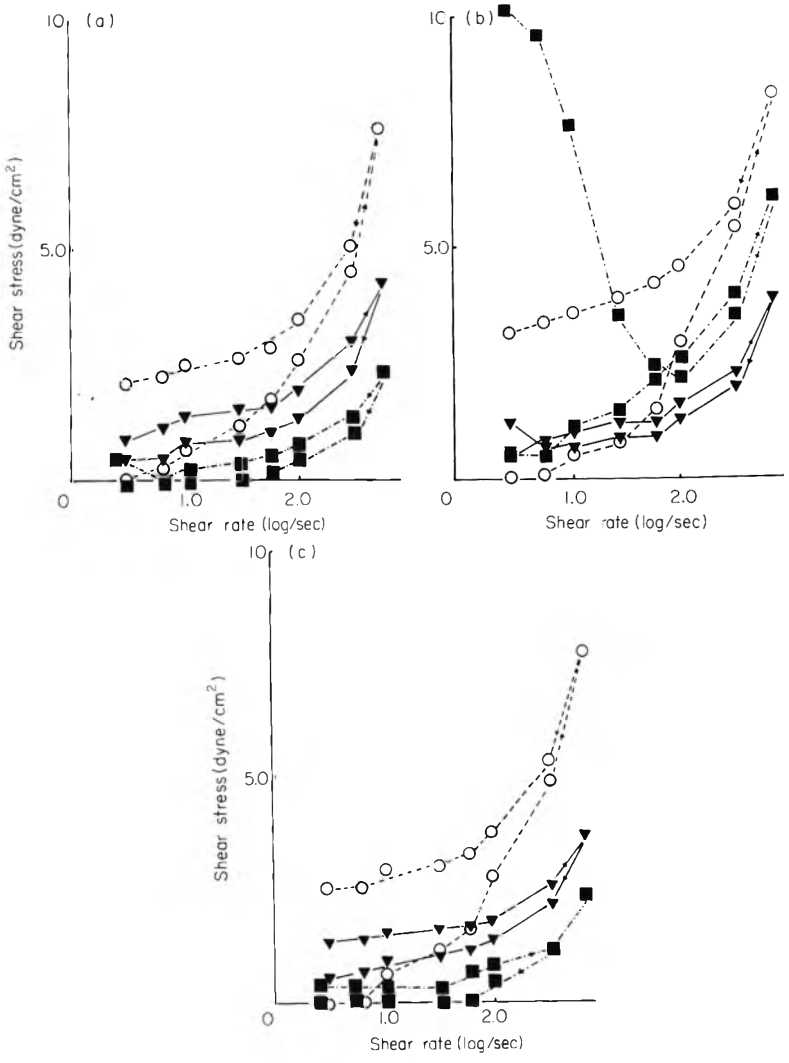

Figure 5. Hysteresis curve for soya protein components (7s, 11s and mixture of 7s and 11s) at varying temperatures: (a), 7s component: O, 25°C; ▲, 50°C; ■, 90°C (b), 11s component: O, 25°C; ▲, 70°C; ■, 90°C. (c), mixture of 7s and 11s component (mixing ratio in weight = 1 : 1): O, 25°C; ▲, 60°C; ■, 90°C. Solid symbols indicate that protein sample shows thixotropic flow behaviour, while open symbols indicate rheopectic one. Other indications are the same as in Fig. 1.

and 11s components was rheopectic at lower temperatures (25 and 30°C), but became thixotropic on heating at 40°C or above. However, all of the mixtures started to form gels on heating at 50°C for 10 min when shear rate was 20/sec.

Scanning electron microscopy of myosin B, soya protein components and the mixtures of them

The microstructure of myosin B, soya protein components and the mixtures of them was examined in order to know whether or not the change of flow behaviour of protein is dependent upon the alteration in the structure of the protein. As shown in Fig. 6a,

Table 3. Shear stress–shear rate relation for the mixtures of myosin B and soya protein components at varying temperatures

	Shear stress (dyne/cm^2)																	
	Mixture of myosin B and 7s component Temperature (°C)						Mixture of myosin B and 11s component Temperature (°C)						Mixture of myosin B and 11s component Temperature (°C)					
	25		30		40		25		30		40		25		30		40	
Shear rate (/sec)	↑	↓	↑	↓	↑	↓	↑	↓	↑	↓	↑	↓	↑	↓	↑	↓	↑	↓
1	1.2	3.5	2.3	4.7	5.9	4.7	2.3	4.7	5.3	4.7	4.7	4.7	3.5	5.0	6.4	7.6	7.6	5.3
2	1.8	4.1	2.9	7.6	10.2	5.9	3.5	6.7	7.6	6.4	10.5	5.3	4.7	6.7	8.2	11.1	11.1	7.6
5	3.5	6.4	8.8	11.2	15.2	14.0	8.8	10.0	9.4	8.8	14.0	7.6	11.1	14.0	12.3	15.2	15.8	11.7
10	5.9	10.5	13.5	16.4	15.8	15.2	11.7	13.5	15.8	12.3	15.8	12.9	13.2	17.6	15.2	17.6	19.9	17.6
20	8.8	12.9	18.1	19.3	18.7	17.5	15.2	20.5	21.6	17.5	18.1	15.2	20.2	21.9	24.0	25.7	23.4	22.2
40	14.0	17.6	23.4	24.6	21.1	19.9	25.2	26.9	28.1	26.3	24.0	21.6	29.3	29.3	30.4	30.4	28.7	26.9
100	25.7	28.1	36.3	36.3	29.3	28.1	41.0	41.0	42.1	40.9	38.6	36.8	46.8	46.8	48.6	48.6	41.0	39.2
200	42.7		51.5		39.8		58.5		60.8		53.2		66.7		67.9		55.0	
Flow behaviour	R		R		T		R		T		T		R		R		T	

Upward and downward arrows indicate the upward and downward curves of hysteresis loops respectively. R and T indicate rheopectic and thixotropic properties respectively.

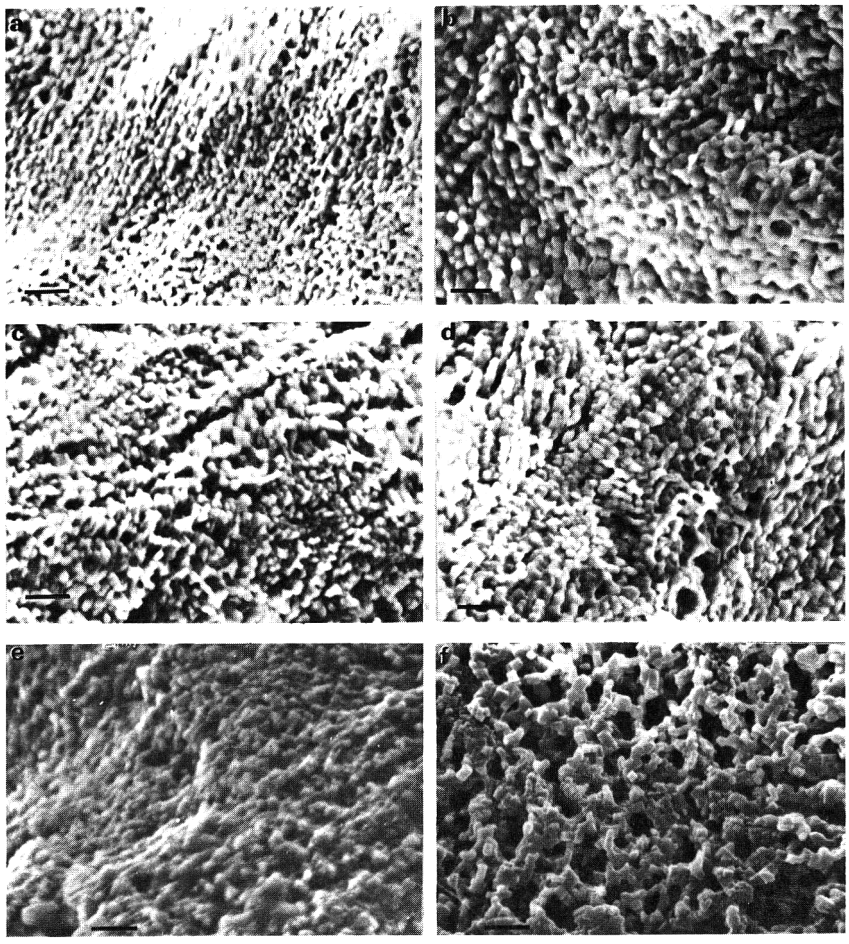

Figure 6. Scanning electron micrographs of myosin B. (a), Myosin B at 25°C before viscometric determination; (b), myosin B at 25°C after viscometric determination; (c), myosin B at 30°C before viscometric determination; (d), myosin B at 30°C after viscometric determination; (e), myosin B at 40°C before viscometric determination; (f), myosin B at 40°C after viscometric determination.

myosin B in buffer solution before the flow experiment at 25°C was tightly packed, so that it showed a fine three-dimensional network structure and the size distribution of each network was small. However, the three-dimensional network structure of myosin B in native state was changed to a porous structure with concomitant increase of the size of the particles after the examination of viscosity (Fig. 6b). Similar network structures as in the case of Figs 6a and b were observed in the samples incubated at 30°C before and after viscosity measurements respectively (Figs 6c and d). At the temperature where myosin B started to form gel (40°C or above), a drastic change was observed in the electron micrographs, i.e., three-dimensional networks composed of sticky particles Fig. 6e) and also three-dimensional sponge-like networks with slightly angular nature (Fig. 6f) were observed before and after the examination of flow behaviour of the protein respectively. Micrograph of Fig. 7 shows that 11s component in native state (at 25°C) exists as dispersed ellipsoidal particles before viscosity measurement. After the

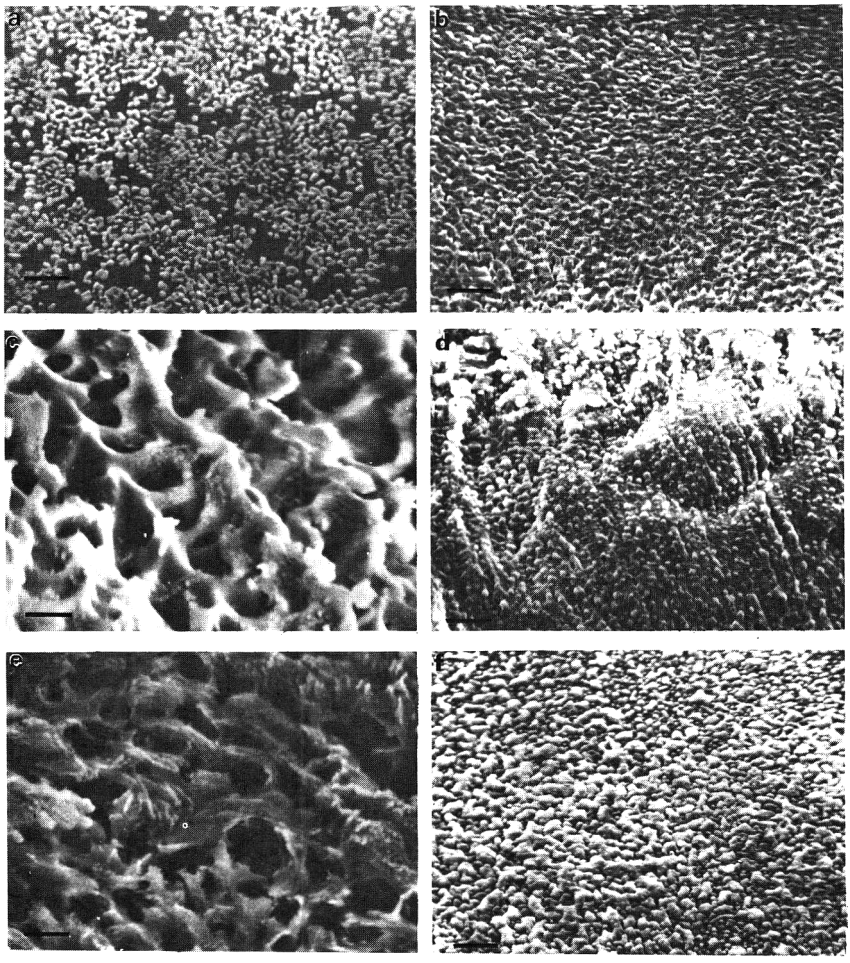

Figure 7. Scanning electron micrographs of soya 11s component. (a), 11s component at 25°C before viscometric determination; (b), 11s component at 25°C after viscometric determination; (c), 11s component at 70°C before viscometric determination; (d), 11s component at 70°C after viscometric determination; (e), 11s component at 90°C before viscometric determination; (f), 11s component at 90°C after viscometric determination.

measurement of flow behaviour with a viscometer, however, the structure of this component became more elongated with particles closely conjugated with each other. Concomitantly, the size of each particle became larger (Fig. 7b). As the temperature was raised to 70°C, a loosely structured three-dimensional network was observed (Fig. 7c). However, a fibred structure of particles was visible after the examination of viscosity (Fig. 7d). Likewise, at higher temperatures where the protein starts to precipitate (90°C), a labile three-dimensional network with crispy appearance was observed before viscosity measurement (Fig. 7e). After the measurement, however, such a characteristic network was broken to form large particles closely conjugated with each other (Fig. 7f). This indicates that the three-dimensional network of 11s component formed on heating at 70°C or above was labile under physical stress.

Figures 8a–c show micrographs of the mixture of myosin B and soya 11s component. The mixture had no characteristic structures at 25°C before viscosity measurement (Fig.

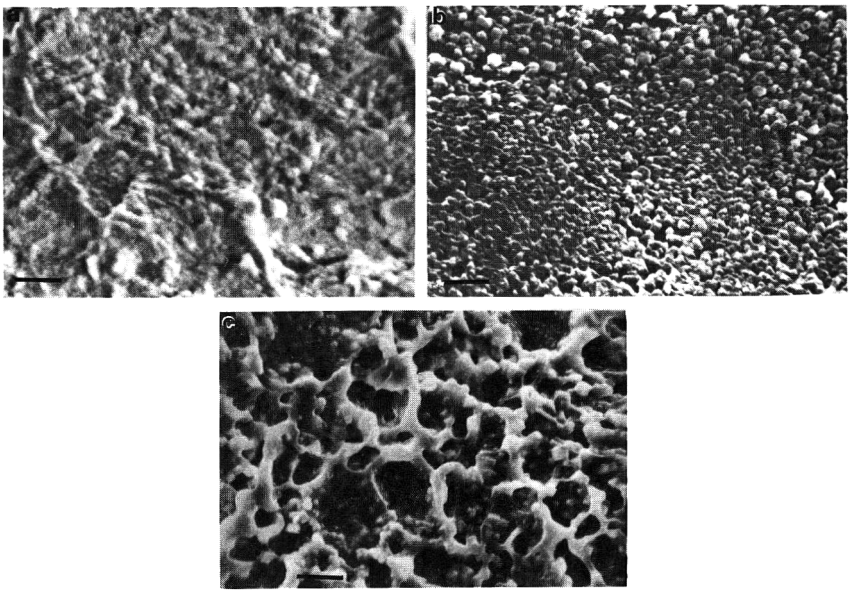

Figure 8. Scanning electron micrographs of the mixture of myosin B and 11s component. (a), The mixture at 25°C before viscometric determination; (b), the mixture at 25°C after viscometric determination; (c), the mixture at 40°C after viscometric determination.

8a). After viscosity measurement at 25°C, the structure of the mixture (Fig. 8b) was quite similar to that of 11s component shown in Fig. 7b. However, the mixture of myosin B and 11s component after the flow experiment at 40°C showed an irregular network structure with dense solid particles (Fig. 7c). This observation, together with the finding on shear stress of the mixture (Table 3), suggests that the intermolecular interaction of myosin B molecules is affected by the presence of soya protein. In the cases of the mixture of myosin B and 7s component and the mixture of myosin B, 7s and 11s components, similar structures were observed at 25°C and even after heating at 40°C (data not shown) as in the case of Fig. 8.

Discussion

Flow behaviour of myosin B, soya protein components and the mixture of them at 25°C

Food particles in both thixotropic and rheopectic liquids tend to aggregate and form a definite structural linkage. Shearing of these liquids partially destroys the structure and standing permits it to form again (Peterson & Johnson, 1978). It is considered that thixotropy is the manifestation of a temporary decrease in viscosity due to distortion of particles in liquid during shearing, while rheopexy is a phenomenon in which molecules or articles in liquid are brought into contact by a low level of shearing (Onogi, 1982). Flow behaviour of protein is characterized both by the nature of intermolecular interaction of protein under a particular shear rate of flow and by the shape and size of protein itself.

As shown in Fig. 1, the pattern of the hysteresis loop clearly indicates that myosin B has a thixotropic or a time thinning property. Such behaviour of myosin B is dependent upon the structure of a network formed by protein–protein interaction (Fig. 6). On the other hand, flow behaviour of soya protein components (7s, 11s and the mixture of 7s and

11s components) are rheopectic or time thickening (Fig. 2). This is probably due to the formation of aggregates visualised by scanning electron microscopy as closely conjugated particles having varying sizes and shapes, when the proteins are subjected to shear force at 25°C (Figs 7a and b). According to Rha (1978) commercial soya isolate dispersion also showed a shear-thickening (rheopectic) property. However, Umeya, Yamauchi & Shibasaki (1980) have found using a coaxial cylinder type viscometer that in a suspension system (12%) both crude 7s component (86% in purity) and crude 11s component (78% in purity) show a thixotropic property. In their experiment, crude 7s and 11s components were simultaneously isolated by a simple method based on differential solubility of those components (Thann & Shibasaki, 1975). This is one of the crucial differences in experimental procedures between the present study and that of Umeya, Yamauchi & Shibasaki (1980). The difference in experimental systems (dispersion and solution systems) is another explanation for the difference between the present and their results. Since the protein in dispersion systems exists as aggregated particles, while in solution systems the protein exists as molecules, the extent of protein-protein interaction in the two systems might differ.

As shown in Figs 3a, b and c, the mixture of myosin B and soya protein components (7s, 11s and mixture of 7s and 11s) showed a pattern of rheopexy. However, the pattern became thixotropic when the mixtures were stored at 0°C for 1 week. Such changes in flow behaviour of the proteins may be due to the formation of a complex formed by intermolecular linking between myosin B and soya protein components during storage at 0°C (Lin & Ito, 1985).

Shear stress of myosin B decreased greatly during storage (Fig. 1). Electrophoretograms indicate that the troponin-T fraction was degraded into its subfragment during storage of myosin B for 1 week (Fig. 4). Such degradation may be responsible for the decrease in shear stress of myosin B during storage for 1 week (Fig. 1). In the case of soya protein components, there was no change in electrophoretogram during storage (Fig. 4). This fact, together with the results of Figures 3a, b and c, suggest the inhibitory effect of soya protein components on the degradation of myosin B during storage. This result is consistent with the finding reported in a previous paper (Lin & Ito, 1985).

Flow behaviour of myosin B, soya protein components and the mixture of them at varying temperatures

In the range 25–40°C, myosin B showed a shear thinning effect of thixotropic property. The change of the pattern of hysteresis loop of myosin B solution induced by thermal treatment was small (Table 2). However, shear stress was decreased initially and then increased with increasing temperature from 25 to 40°C. The change in shear stress of myosin B seems to be correlated with its change in microstructure, i.e., the difference in microstructure was obvious between 30°C and 40°C, and small between 25°C and 30°C (Fig. 6). In the case of soya protein components (7s, 11s and mixture of 7s and 11s component), significant temperature dependency was found in shear rate–shear stress relation (Figs 5a, b and c): a shear thickening effect or rheopectic property was noted at lower temperatures, but a shear thinning effect or a thixotropic property was evident at higher temperatures. Transition temperatures from rheopexy to thixotropy were about 50, 70 and 60°C for 7s, 11s and the mixture of 7s and 11s components respectively. Likewise, freshly prepared mixtures of myosin B and soya protein components showed similar temperature-dependent change of rheological property: transition temperatures from rheopexy to thixotropy for the mixture of myosin B and 7s component, the mixture of myosin B and 11s component and the

mixture of myosin B, 7s and 11s components were about 30, 40 and about ~ 40°C respectively (Table 3). As shown in Fig. 1, myosin B originally has a thixotropic property, so that this result indicates that the transition temperatures of soya protein components were lowered to some extent by the mixing of myosin B with those components. Since the temperatures which induce gelation of proteins were approximately 50–60, 68 and 86°C for myosin B, 7s and 11s component respectively (Samejima *et al.*, 1969; Hermansson, 1978), the present results (Fig. 5 and Table 3) suggest that, in the case of soya protein components and the mixtures containing myosin B and soya protein components, the transition of flow behaviour from rheopexy to thixotropy precedes the gelation of the proteins. Ehninger & Pratt (1974) have reported that the viscosity of soya protein dispersion varied with temperature. Lee & Rha (1977) observed a slight increase in intrinsic viscosity of soya isolate dispersion upon heating to 80°C and a great increase from 80 to 90°C when the concentration of protein was 10%. Hermansson (1978) has also observed a continuous and gradual increase in viscosity of soya isolate dispersion upon heating up to 85°C, but they observed a great decrease in viscosity during further heat treatment above 90°C at 10% of protein concentration. As already noted, they used protein dispersion systems to bring the experimental systems close to the practical application, while in the present study we examined the viscosity of solution systems to focus on the nature of the interaction of myosin B and soya protein components. Therefore, the inconsistency between the present results and their results may be due to the difference in protein systems studied.

Scanning electron micrographs of myosin B, soya protein components and the mixture of them at varying temperatures

It is considered that flow behaviour of protein particles is dependent upon the quasi structure which the protein particles formed (Onogi, 1982). The present results also indicate that the change of flow behaviour of proteins observed during thermal treatment seems to be associated with remarkable change of scanning electron micrographs of the proteins. Seemingly, fibrous protein myosin B, showed a labile three dimensional network structure at 40°C after viscometric determination (Fig. 6f). Flow behaviour of this protein (Fig. 1 and Table 2) suggests that this structure is formed by the rearrangement of protein particles following the breakdown of their original structure. In addition, this network structure is similar to that of myosin B gel, indicating that the formation of a labile three-dimensional network structure might be an intermediate step for the formation of thermally induced protein gel with strong rigidity. According to Hermansson (1978), gelation reaction involves the formation of a continuous network exhibiting a certain degree of order. As is well known, myosin B forms a stable gel having strong rheological properties upon heating at about 50–60°C (Samejima *et al.*, 1969; Haga & Ohashi, 1978). Such a superiority of myosin B gel could be due to the formation of a stable three-dimensional network on heating. In the case of the mixture of myosin B and 11s component, a labile three-dimensional network structure was also evident at 40°C (Fig. 8), but the structure was not so dense as that of myosin B (Fig. 6). This structure also refers to an intermediate step for the formation of heat-induced stable gel. In the case of soya 11s component, on the other hand, electron micrograph showed elongated particles conjugated each other (Fig. 7). This structure refers to a characteristic structure or rheopectic liquid (Onogi, Mikami, & Matsumoto, 1977; Mikami, 1982). However, when 11s component was subjected to heat treatment at 70 and 90°C, a loosely bound three-dimensional network and fibred structure were observed before and after the examination of viscosity, respectively (Figs 7c–f). These

structural changes upon heat treatment seem to be correlated with the change of flow behaviour of globular protein 11s component. In addition, the structure of the loosely bound three-dimensional network, labile under physical stress, may relate to an intermediate step for the formation of heat induced gel of a globular protein 11s component. Anglemier & Montgomery (1976) have stated that proteins which readily form gels normally posses a high degree of assymetry of structure. Since myosin B is a fibrous protein, while soya protein components are globular, the difference in the shape of protein might be one of the reasons for the difference in the formation of network structures on heating.

Acknowledgments

We are grateful to Professor T. Fukazawa in our laboratory for his encouragement during the course of this work. We thank Professor O. Koga for permitting us to use a scanning electron microscope in his laboratory. We also thank Fuji-Oil Co. Ltd for their donation of soya flakes.

References

Acton, J.C., Ziegler, G.R. & Burge, D.L. (1983). Functionality of muscle constituents in the processing of comminuted meat products. *CRC Critical Reviews in Food Science and Nutrition* **18**, 99–101.

Anglemier, A.F. & Montgomery, M.W. (1976). Amino acids, peptides and proteins. In: *Principle of Food Science Part 1: Food Chemistry* (edited by O.R. Fennema), p. 238. New York: Marcel Dekker, Inc.

Blake, C.C.F., Koenig, D.F., Mair, G.A., North, A.C.T., Phillips, D.C. & Sarma, V.R. (1965). Structure of hen egg white lysozyme-A three dimensional Fourier synthesis at 2A resolution, *Nature*, **206**, 757–761.

Catsimpoolas, N., Rogers, D.A., Cirile, S.J. & Meyer, E.W. (1967). Purification and structural studies of the 11s component of soybean proteins. *Cereal Chemistry*, **44**, 631–637.

Catsimpoolas, A. & Meyer, E.W. (1970). Gelation phenomena of soybean globulins. 1. Protein-protein interaction. *Cereal Chemistry*, **47**, 559–570.

Deng, J., Toledo, R.T. & Lillard, D.A. (1976). Effect of temperature and pH on protein-protein interaction in actomyosin solutions. *Journal of Food Science*, **41**, 273–277.

Ehninger, J.N. & Pratt, D.E. (1974). Some factors influencing gelation and stability of soya protein dispersions. *Journal of Food Science*, **39**, 892–896.

Fukazawa, T., Hashimoto, Y. & Yasui, T. (1961). Effect of some proteins on the binding quality of an experimental sausage. *Journal of Food Science*, **26**, 541–546.

Gornall, A.G., Bardawill, C.T. & David, M.M. (1949). Determination of serum protein by means of biuret reaction. *Journal of Biological Chemistry*, **177**, 751–766.

Haga, S. Ohashi, T. (1978). The optimum condition for gel formation of myosin B and/or soyabean protein CIF, *Journal of Japanese Society of Food Science and Technology*, **25**, 226–230 (in Japanese).

Haga, S. & Ohashi, T. (1984). Heat-induced gelation of a mixture of myosin B and soyabean protein. *Agricultural and Biological Chemistry*, **48**, 1001–1007.

Hermansson, A.M. (1975). Functional properties of added proteins correlated with properties of meat systems. Effects of concentration and temperature on water-binding properties of model meat systems. *Journal of Food Science*, **40**, 603–610.

Hermansson, A.M. (1978). Physico-chemical aspects of soy proteins structure formation. *Journal of Texture Studies*, **9**, 33–58.

King, N.L. (1977). Heat-induced complex formation between myosin and soybean 7s globulins. *Journal of Agricultural and Food Chemistry*, **25**, 166–170.

Kinsella, J.E. (1976). Functional properties of proteins in foods. *CRC Critical Reviews in Food Science and Nutrition*, **7**, 219–223.

Kirkwood, J.G., Buff, F.P. & Green, M.S. (1949). The statistical mechanical theory of transport processes. III The coefficients of shear and bulk viscosity of lipids. *Journal of Chemical Physics*, **17**, 988–994.

Koshiyama, I. (1972). A newer method for isolation of the 7s globulin soybean seeds. *Agricultural and Biological Chemistry*, **36**, 2255–2257.

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

Lee, C.H. & Rha, C.K. (1977). Thickening of soybean protein suspensions with calcium. *Journal of Texture Studies*, 7, 441–445.

Lin, L.C. & Ito, T. (1985). Rheological property of myosin B fibre and the fibre of the mixture of myosin B and soya protein, and the interaction between myosin and soya protein. *Journal of Food Technology*, **20,** 219–235.

Macfarlane, J.J., Schmidt, G.R. & Turner, R.H. (1977). Binding of meat pieces: a comparison of myosin, actomyosin and sarcoplasmic proteins as binding agents. *Journal of Food Science*, **42,** 1603–1605.

Maruyama, K., Kaibara, M. & Fukada, E. (1974). Rheology of actin. I. Network of F-actin in solution. *Biochimica et Biophysica Acta*, **371,** 20–29.

Mikami, Y. (1982). Interpretation of the rheopexy by a two-process kinetic model. *Journal of Rheology*, **26,** 263–276.

Nakayama, T. & Sato, Y. (1971). Relationships between binding quality of meat and myofibriller proteins. Part II. The contribution of native tropomyosin and actin to the binding quality of meat. *Agricultural and Biological Chemistry*, **35,** 208–218.

Nakayama, T., Niwa, E., Hamada, I. Shin, C. (1979). Viscosity changes in carp actomyosin solutions. *Journal of Food Science*, **44,** 1106–1109.

Onogi, S., Mikami, Y. & Matsumoto, J. (1977). The rheology of suspensions of titanate fibres in polymer solution. *Polymer Engineering and Science*, **17,** 1–8.

Onogi, S. (1982). *Kagakusha No Rheology* pp. 37–52 Tokyo: Tokyo Kagaku Dojin, (in Japanese).

Peterson, M.S. & Johnson, A.H. (1978). *Encyclopedia of Food Science* pp. 663–671. Connecticut: AVI Publishing Co. Ltd.

Pradipasena, P. & Rha, C.K. (1977). Pseudoplastic and rheopectic properties of a globulin protein (β-Lactoglobulin) solution. *Journal of Texture Studies*, **8,** 311–325.

Rha, C.K. (1978). Rheology of liquid foods. *Food Technology*, **32,** 77–82.

Roberts, R.C. & Briggs, D.R. (1965). Isolation and characterization of the 7s component of soybean globulins. *Cereal Chemistry*, **42,** 71–85.

Saio, K., Sato, I. & Watanabe, T. (1974). Food use of soybean 7s and 11s proteins. High temeperature expansion characteristics of gel. *Journal of Food Science*, **39,** 777–782.

Samejima, K., Hashimoto, Y., Yasui, T. & Fukazawa, T. (1969). Heat gelling properties of myosin, actin, actomyosin and myosin-subunits in a saline model system. *Journal of Food Science*, **34,** 242–245.

Schmidt, G.R., Mawson, R.F. & Seigel, D.G. (1981). Functionality of protein matrix in comminuted meat products. *Food Technology*, **35,** 235–237.

Siegel, D.G. & Schmidt, G.R. (1979). Crude myosin fractions as meat binders. *Journal of Food Science*, **44,** 1129–1131.

Sjöstrand, F.S. (1967). *Electron Microscopy of Cells and Tissues, Vol. 1. Instrumentation and Techniques*. New York: Academic Press.

Thann, V. H. & Shibasaki, K. (1975). Isolation and characterization of the multiple 7s globulins of soybean seeds. *Plant Physiology*, **56,** 19–22.

Umeya, J.I., Yamauchi, F. & Shibasaki, K. (1980). Hardening and softening properties of soybean protein-water suspending systems. *Agricultural and Biological Chemistry*, **44,** 1321–1326.

Watanabe, S. (1975). Kin-Niku. p. 243. Tokyo: Tokyo Kagaku Dojin (in Japanese).

Wolf, W.J., Babcock, G.E. & Smith, A.K. (1962). Purification and stability studies of the 11s component of soybean proteins. *Archives of Biochemistry and Biophysics*, **99,** 265–274.

(Received 29 January 1985)

Journal of Food Technology (1986) **21**, 151–157

Rapid dye reduction tests for the determination of microbiological quality of meat

D. N. RAO* AND V. S. MURTHY

Summary

Rapid dye reduction tests have been developed to determine the quality of meat. Three chemical indicators, resazurin and two tetrazolium compounds, were used to correlate the microbial numbers and reduction times in meat samples.Twenty-five surface samples from sheep carcasses were subjected to each reduction test. Total viable counts given were obtained at 37°C. Resazurin reduction time was 90–120 min when the bacterial counts ranged from 1.5×10^6 to $7.7\times10^6/cm^2$. Samples showing bacterial counts between 1.5×10^6 and $6.0\times10^6/cm^2$ reduced tetrazolium (NBT) in 360–390 min whereas samples containing bacterial counts of $2.1\times10^6/cm^2$ took 420–450 min to reduce iodophenyl nitrophenyl tetrazolium (INT) dye. Regression equations relating the number of organisms per cm^2 and reduction time were applied to predict the microbiological quality of meat samples from reduction time data. Among the three dyes, resazurin gave the lowest reduction time.

Introduction

The hygienic quality of meat is largely determined by the number of microorganisms present on the surface of carcasses. Microbiological quality of a food product will usually be judged by total bacterial counts as estimated by standard plate count procedures, but these are time consuming and there is a need to develop rapid methods. Many investigators have employed chemical indicators such as methylene blue, resazurin and tetrazolium compounds to determine microbiological quality of milk, fish, poultry and meat (Proctor & Greenlie, 1939; Jensen, 1954; Straka and Stokes, 1957; Mallman *et al.*, 1958; Walker, Coffin & Ayres, 1959; Wells, 1959; Rogers & McCleskey, 1961; Saffle *et al.*, 1961; Bradshaw, Dyett & Herschdoerfer, 1961). Nottingham (1971) employed a resazurin filter paper technique to determine the microbiological quality of meat. In this technique, the filter paper contained only a small fraction of the organisms present on the meat surface. The present study describes the practical utility of resazurin and tetrazolium compounds in determining the microbiological quality of sheep carcasses.

Materials and methods

The following nutrient medium was devised to carry out the reduction tests: meat infusion, 600 ml yeast extract, 1.0 g; tryptone, 2.5 g; proteose peptone, 2.5 g; glucose 1.25 g; dipotassium phosphate, 1.25 g; distilled water 400 ml. The ingredients were dissolved and the pH was adjusted to 7.2. The medium was distributed in 25 ml quantities in glass tubes and sterilized by autoclaving at 121°C. (Preparation of meat

Authors' address: Division of Meat, Fish and Poultry Technology, Central Food Technological Research Institute, Mysore-570 013, India.

*To whom correspondence should be addressed.

infusion lean ground sheep meal weighing 4.5 kg was mixed with 5000 ml of water and kept in refrigerator overnight and steamed for 1 hr. The material was filtered through cheese cloth and heated to boiling. The juice was kept in a refrigerator overnight. Fat was skimmed off and the juice was filtered. (Sealey and Vandemark, 1974).

Dye solutions

Resazurin solutions: Resazurin tablets (George T. Gurr Ltd., London) were used in the present experiments. A 0.5% of resazurin solution was prepared in hot sterile distilled water. The solution was stored for 3 days in a refrigerator.

Tetrazolium solutions. Nitroblue tetrazolium (NBT) and 2-(4-iodophenyl)-3-(4-Nitrophenyl)-5-phenyl Tetrazolium (INT) (Sigma Chemical Company, St Louis, U.S.A.) were employed in the present studies. Tetrazolium solution (0.1%) was prepared in distilled water and sterilized by autoclaving at 121°C. The solutions were stored in a refrigerator.

Skim milk. One hundred g of skim milk powder were dissolved in 1000 ml of distilled water, distribution in 10 ml quantities in tubes and sterilized at 121°C for 15 min.

Preparation of samples

Sheep carcasses processed at CFTRI laboratory and carcasses from the local slaughterhouse were used for sampling purposes to obtain high and low bacterial counts. An area (6.45 cm^2) on the surface of meat was scraped by a sterile scalpel using a sterile steel template. After scraping, the sample was removed with three cotton swabs (standard). The three swabs were broken into a tube containing 25 ml of nutrient medium and shaken well until cotton swabs were dispersed. This constituted one sample. Three such samples were collected from the leg region of each carcass. Each of the three samples from each carcass was tested by a different dye reduction method.

The sample was vigorously shaken until the cotton was dispersed. One ml of the sample was used for making serial dilutions to estimate total plate counts by the pour plate technique using nutrient agar (Thatcher Clark, 1978). Plates were incubated at 37°C for 24 hr.

Reduction tests

Two ml of the dye solution and 2 ml of skim milk were added to each tube containing the nutrient medium and the sample. The contents of the tube were mixed slowly by shaking the tube twice or thrice. The tubes were then incubated at 37°C in the dark. Observations were made every 15 min. In the case of resazurin tests, the contents of the tube were blue initially and appearance of pink colour was considered as the end point. The contents of the tube were colourless initially in the case of tetrazolium solutions. Appearance of blue colour was taken as the end point in the case of samples employed with nitroblue tetrazolium, whereas pink colour was taken as the end point in samples incorporated with iodophenyl nitrophenyl tetrazolium. Tubes containing 24 ml of nutrient medium, 2 ml of the dye solution and 2 ml of skim milk were kept in the incubator as control tubes.

Results

A total of twenty-five surface samples from carcasses (eleven from laboratory and fourteen from local slaughterhouse) were subjected to each reduction test. Reduction

tests and the microbial counts were made at the same time. Results of resazurin test are presented in Table 1. The results reveal that a significant negative correlation at 0.001% level was shown between bacterial numbers and reduction times. As the microbial populations increased, resazurin reduction time decreased in a regular manner. Samples showing the bacterial counts of 1.9×10^3–7.2×10^3 took 345–360 min to reduce resazurin to a pink colour whereas samples ranging from 1.5×10^6–7.7×10^6 required only 90–120 min. The regression equation regarding the relationship between the logarithm of the number of bacteria per cm^2 and reduction time at 37°C is presented in Fig. 1. Calculation of F values showed that the regression of numbers of organisms per cm^2 on reduction time was significant at 0.1% level.

The results of the reduction tests using nitroblue tetrazolium and iodophenyl nitrophenyl tetrazolium are presented in Tables 2 and 3 respectively. Samples showing more than 6.0 log/cm^2 reduced nitroblue tetrazolium in 360–390 min whereas samples containing less than 4 log bacteria reduced NBT in 720 min. INT was reduced to pink colour in 420–450 min when bacterial counts exceeded 6 log/cm^2. The regression equation regarding the relationship between numbers of organisms/cm^2 and reduction time at 37°C is presented in Fig. 2. Calculation of *F* values showed that the regression of numbers of organisms/cm^2 on reduction time was significant at the 0.1% level for both the tetrazolium compounds. Further the two regression lines are parallel and hence the meat samples employing NBT showed a faster rate of reduction than with INT. But the time of reduction was longer in the samples employed with the above tetrazolium compounds than with the resazurin.

Table 1. Relation between resazurin reduction times and bacterial counts

Sample no.	Reduction time (min)	Number of organisms per cm^2 37°C for 24 hr
1	360	4.5×10^3
2	345	7.2×10^3
3	360	3.8×10^3
4	360	1.9×10^3
5	360	1.9×10^4
6	270	8.5×10^4
7	300	1.8×10^4
8	285	3.1×10^4
9	270	5.5×10^4
10	275	2.5×10^4
11	255	6.8×10^4
12	210	5.5×10^5
13	240	1.8×10^5
14	240	3.1×10^5
15	240	1.5×10^5
16	210	5.7×10^5
17	195	6.8×10^5
18	240	5.0×10^5
19	120	1.5×10^6
20	90	5.0×10^6
21	105	3.0×10^6
22	90	6.0×10^6
23	120	2.8×10^6
24	90	4.5×10^6
25	90	7.7×10^6

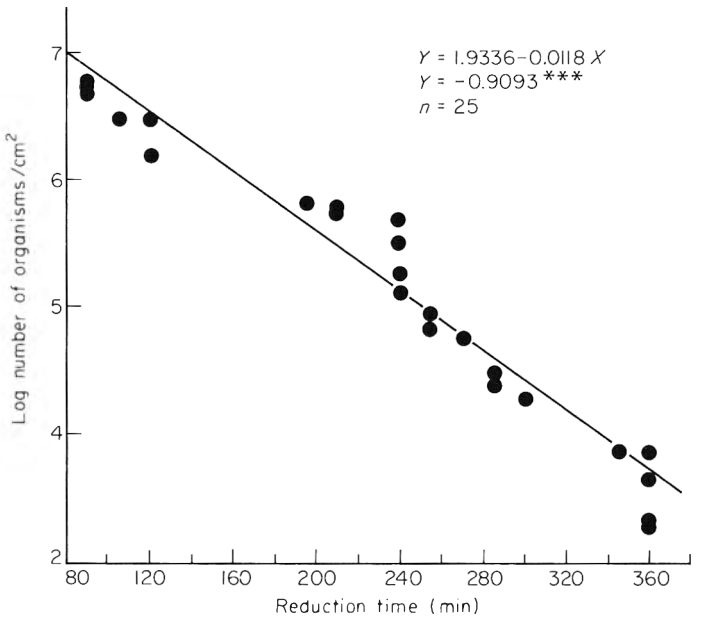

Figure 1. Resazurin reduction test. Regression of numbers of organisms/cm^2 on reduction time with total counts made at 37°C.

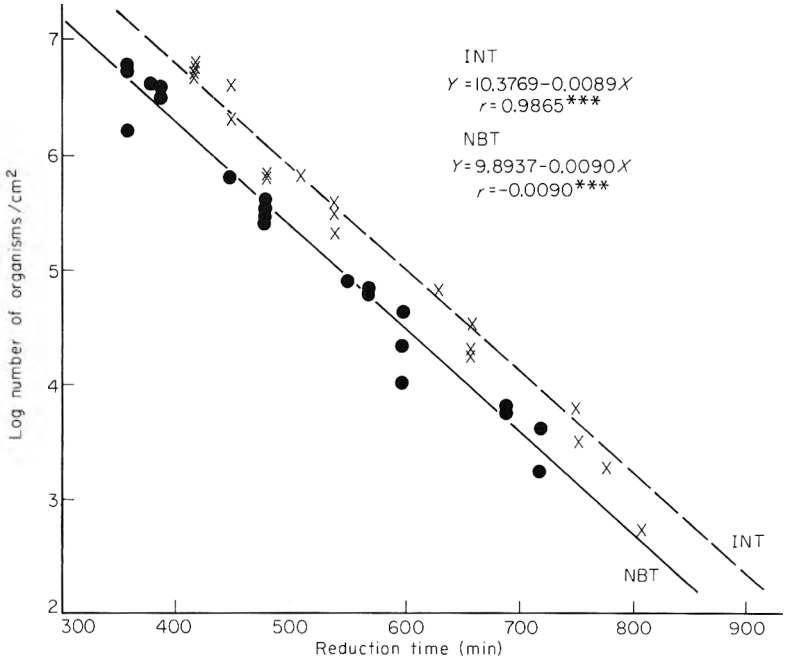

Figure 2. Reduction test with NBT and INT. Regression numbers of organisms/cm^2 on reduction time with total counts made at 37°C.

Table 2. Relation between nitroblue tetrazolium reduction times and bacterial counts

Sample no.	Reduction time (min)	Number of organisms per cm^2 37°C
1	720	3.7×10^3
2	690	6.1×10^3
3	720	4.2×10^3
4	690	5.5×10^3
5	720	1.8×10^3
6	600	2.1×10^4
7	555	7.8×10^4
8	600	1.1×10^4
9	570	5.9×10^4
10	570	6.5×10^4
11	600	4.1×10^4
12	480	3.5×10^5
13	480	3.1×10^5
14	480	4.5×10^5
15	480	2.8×10^5
16	450	7.0×10^5
17	450	6.2×10^5
18	480	4.8×10^5
19	390	3.0×10^6
20	390	3.9×10^6
21	360	5.5×10^6
22	360	1.5×10^6
23	375	4.5×10^6
24	360	5.8×10^6
25	360	6.0×10^6

Table 3. Relation between iodophenyl nitrophenyl tetrazolium reduction times and bacterial counts

Sample no.	Reduction time (min)	Number of organisms per cm^2 37°C
1	780	4.1×10^3
2	825	1.2×10^3
3	750	6.2×10^3
4	765	3.5×10^3
5	750	6.8×10^3
6	660	1.8×10^4
7	660	2.9×10^4
8	660	1.9×10^4
9	630	7.1×10^4
10	630	6.9×10^4
11	630	6.1×10^4
12	540	1.0×10^5
13	540	1.9×10^5
14	540	3.1×10^5
15	540	4.2×10^5
16	510	7.1×10^5
17	480	5.9×10^5
18	510	6.1×10^5
19	450	2.1×10^6
20	450	3.9×10^6
21	450	3.9×10^6
22	420	6.4×10^6
23	420	5.1×10^6
24	420	6.0×10^6
25	420	5.5×10^6

Discussion

Three chemical indicators were used to assess the significance of reduction tests for determining microbiological quality of meat. The results of the resazurin reduction test indicate significant correlation between bacterial numbers and reduction times. It took 345–360 min to reduce resazurin to a pink colour when the bacterial counts ranged from 1.9×10^3–7.2×10^3. Samples showing bacterial numbers from 1.5×10^6–7.7×10^6 required only 90–120 min. Proctor & Greenlie (1939) reported that hamburger steaks showing bacterial counts under one million bacteria per g took about 180 min for pink colour development. Straka & Stokes (1957) reported good correlation between resazurin reduction time and bacterial numbers in meat pies and poultry. Meat pie samples with more than 1 000 000 bacteria per g reduced in 120–150 min. The regression of numbers or organisms per cm^2 on reduction time was significant at 0.1 level in the present study. Walker *et al.* (1959) observed that the regression of reduction time on numbers of organisms per cm^2 was significant at the 0.5% level for all temperatures for poultry.

Some samples showed a variation in bacterial numbers without an effect on the reduction time. For example, total viable count was more in sample no. 16 than in sample no. 15. This difference in the counts between the two samples did not bring any change in the reduction time. This may be attributed to the differences in reducing ability of different bacterial species and strains contaminating meat from different sources (Straka & Stokes, loc. cit.). It was reported that staphylococci in milk were known to exhibit a particular aptitude in this respect (Straka & Stokes, loc. cit.).

The speed of resazurin reduction increased with increase in the temperature of incubation. Straka & Stokes (loc. cit.). reported that reduction proceeded 40–50% more slowly at 30°C than at 37°C and most of the organisms would grow at 37°C. Hence the reduction tests were conducted at 37°C in the present studies. The dye reductions may not be valid for counts made at lower temperature since the latter would reveal psychrotrophs present which would not be active reducers of such compounds.

The results of the reduction tests using nitroblue tetrazolium (NBT) and iodophenyl nitrophenyl tetrazolium (INT) indicate that meat samples employing NBT showed faster rate of reduction than with INT (Fig. 2). The time of reduction was longer with the tetrazolium compounds than with the resazurin. Straka & Stokes (loc. cit.) also reported that reduction with the tetrazolium compounds requried several hours longer than with the resazurin.

Among the three chemical indicators used in the present study, resazurin gave rapid reduction time. The test would take much less time than the plate count. It is evident from the results that resazurin reduction test could be used as a reliable means of estimating the general microbiological quality of sheep carcasses.

Acknowledgments

We thank Mr B. S. Ramesh, scientist for statistical analysis of the data in the present study.

References

Bradshaw, N.J., Dyett, E.J. & Herschdoerfer, S.M. (1961). Rapid bacteriological testing of cooked or cured meats, using a tetrazolium compound. *Journal of the Science of Food and Agriculture*, **12**, 341–344.

Jensen, L.B. (1954). *Microbiology of Meats* 3rd edn.

Champaign: U. Garrad Press.

Mallman, W.L., Dawson, L.E., Sultzer, B.M. & Wright, H.S. (1958). Studies on microbiological methods for predicting shelflife of dressed poultry. *Food Technology,*, **12,** 122–126.

Nottingham, P.M. (1971). Microbiological quality control in the meat industry. *Meat Industry Research New Zealand Bulletin* No. 217, 50.

Proctor, B.E. & Greenlie, D.G. (1939). Redox potential indicators in quality control of foods. *Food Research,* **4,** 441–446.

Rogers, R.E. & McCleskey, C.S. (1961). Objective tests for quality of ground beef. *Food Technology,* **15,** 210–212.

Saffle, R.L., May, K.N., Hamid, H.A. & Irby, J.D. (1961). Comparing three rapid methods of detecting spoilage in meat. *Food Technology,* **15,** 465–467.

Sealey, J.W.H. & Vandemark, P.J. (1974). *Microbes in Action.* 1st ed. Bombay: D.B. Taraporevalle Sons & Co. Private Ltd.

Straka, R.P. & Stokes, J.L. (1957). A rapid method for the estimation of the bacterial content of precooked frozen foods. *Food Research,* **22,** 412–419.

Thatcher, F.S. & Clark, D.S. (1978). *Microorganisms in Foods* 2nd edn. University of Toronto/Buffalo/London.

Walker, H.W., Coffin, W.J. & Ayres, J.C. (1959). A resazurin reduction test for determination of microbiological quality of processed poultry. *Food Technology,* **13,** 578–581.

Wells, F.E. (1959). Resazurin reduction tests for shelflife estimations of poultry meats. *Food Technology,* **13,** 584–586.

(Received 19 November 1984)

Journal of Food Technology (1986) **21**, 159–166

Effect of delayed icing on the storage life of rainbow trout

A. A. DAWOOD*, R. N. ROY†‡ AND C. S. WILLIAMS†

Summary

Rainbow trout (*Salmo irideus*), were divided into groups: (1) iced immediately, or (2) kept at 10°C for 6 hr, (3) 20°C for 6 hr, (4) 20°C for 18 hr, (5) 30°C for 4 hr, (6) 30°C for 6 hr. Fish in groups 2–6 were iced at the end of the stipulated temperature/time period of holding.Quality was subsequently assessed every 2 days for 14 days during iced storage: sensory assessment using criteria of appearance, texture and odour; chemical assessments by measurement of total volatile bases, hypoxanthine and thiobarbituric acid: microbiological assessments by total bacterial counts at 20°C/72 hr and 37°C/48 hr incubation. The results indicate that deamination due to bacterial action and hydrolysis of fats increases progressively with rising temperature prolonged periods of storage prior to icing, and results in final spoilage. Fish iced immediately after delivery and those iced after being kept at 10°C for 6 hr were acceptable to quality even after 14 days of iced storage. Recommendations are made for maintenance of quality and extension of shelf life.

Introduction

Freshwater trout constitutes a significant proportion of fish currently marketed in this country and marketing trends predict an increase in consumer demand for freshwater fish. Therefore there is scope for investigation in immediate post-catch handling, storage and distribution practice, to evolve a method of maintaining quality at a level that 'satisfies the customer and that is economical to the producer or seller' (Connell, 1975) and achieving predicted increased consumer demands for freshwater fish, especially trout. The ideal would be to establish a processing/chilling unit in proximity to site of harvesting of the freshwater fish and subsequent chill-chain distribution. The second option would be to establish a procedure for prompt icing and distribution of freshwater fish immediately after harvesting. Several investigators, Disney *et al.* (1971), Hansen, Olsen and Petersen (1974) and Mlay *et al.* (1982) have indicated that wet fish must be held at 0°C throughout processing and distribution in order to prevent spoilage. Numerous investigations have been carried out on microbial spoilage of marine fish, but there appears to be little information on spoilage patterns of freshwater fish. Bligh (1971) reported that most investigations on freshwater fish were directed towards identifying spoilage organisms on surface slime. The present investigation was designed to assess chemical and microbial changes and hence changes in quality of freshwater fish iced after various temperature/time periods of holding after harvesting.

Authors' addresses: *Food Technology and Dairy Department, Faculty of Agriculture, Minia University, Egypt, and †Department of Applied and Life Sciences, The Queen's College, 1, Park Drive, Glasgow G3 6LP, UK.

‡To whom correspondence should be addressed.

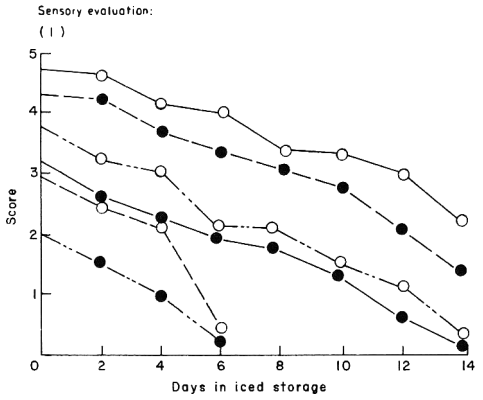

Figure 1. Whole fish: appearance. Fish iced immediately on receipt o——o. Fish kept 10°C for 6 hr prior to icing •— — — —•. Fish kept at 20°C for 6 hr prior to icing o— . . — . . — . .o. Fish kept at 20°C for 18 hr prior to icing •————•. Fish kept at 30°C prior to icing o — — — — — —o. Fish kept at 30°C prior to icing •. — . . — . .—•.

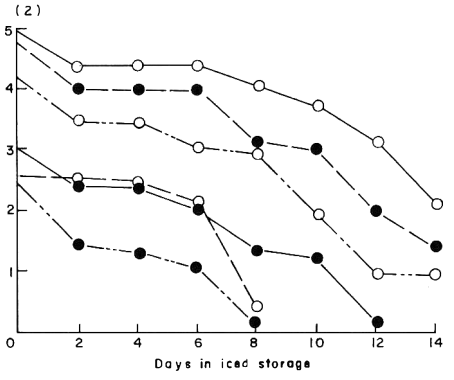

Figure 2. Fish flesh: appearance, For key, see Fig. 1.

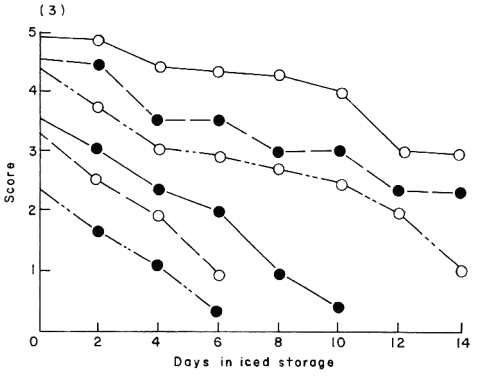

Figure 3. Fish flesh: texture. For key, see Fig. 1.

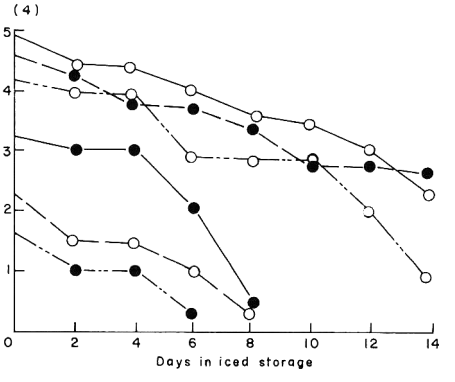

Figure 4. Fish flesh: odour. For key, see Fig. 1.

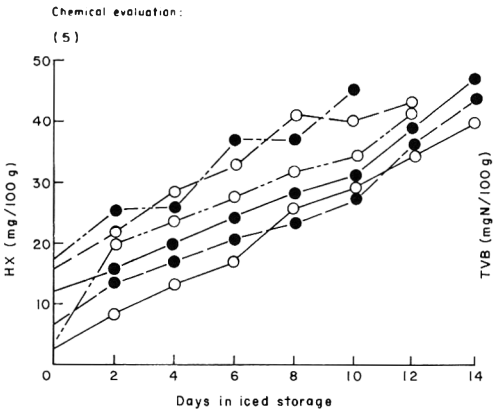

Figure 5. Changes of Hypoxanthine values. For key, see Fig. 1.

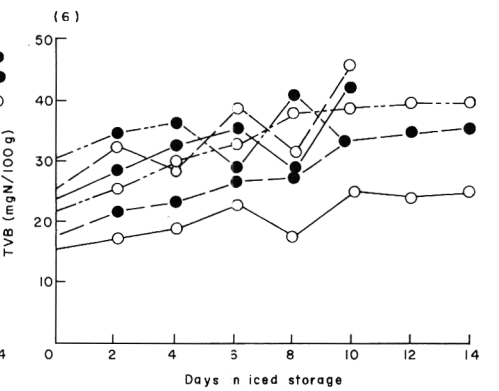

Figure 6. Changes in total volatile bases. For key, see Fig. 1.

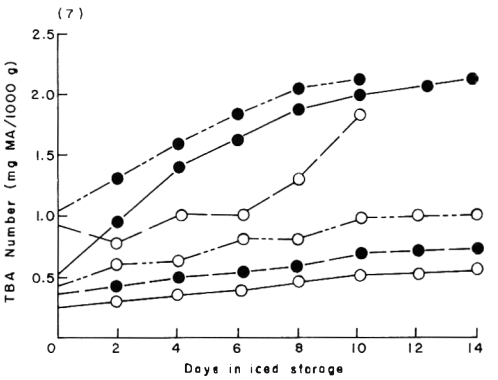

Figure 7. Changes in Thiobarbituric acid number. For key, see Fig. 1.

Materials and methods

Fish: Rainbow trout (Salmo irideus) of live weight approximately 250 g were obtained from Scot Trout Ltd, Glasgow. The fish were delivered within an hour after catching without any prior treatment of icing. The delivery was divided into six groups of thirty-six fish each and treated as follows: group 1: Iced immediately, group 2: kept at 10°C for 6 hr then iced, group 3: kept at 20°C for 6 hr and then iced, group 4: kept at 20°C for 18 hr and then iced, group 5: kept at 30°C for 4 hr and then iced and group 6: kept at 30°C for 6 hr and then iced. Samples for sensory, microbiological and chemical evaluation were taken after the stipulated periods of holding and then every 2 days during the period of iced storage. Four fish from each group were taken each time for sampling. After taking representative samples for microbiological examination, they were evaluated for sensory factors of appearance and odour against quality rating schemes (Tables 1 and 2). The fish flesh was further evaluated for appearance and texture against quality rating schemes (Tables 3 and 4). Finally the samples were evaluated for chemical quality.

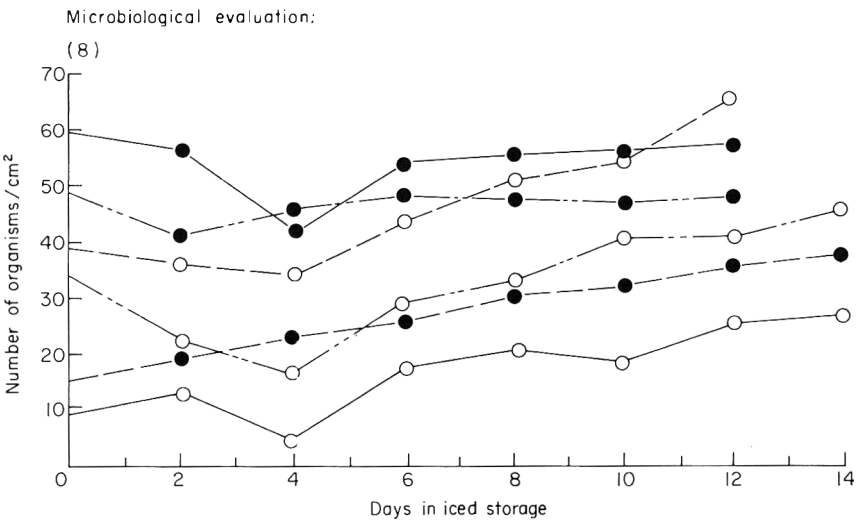

Figure 8. Total viable counts, incubation at 20°C for 72 hr. For key, see Fig. 1.

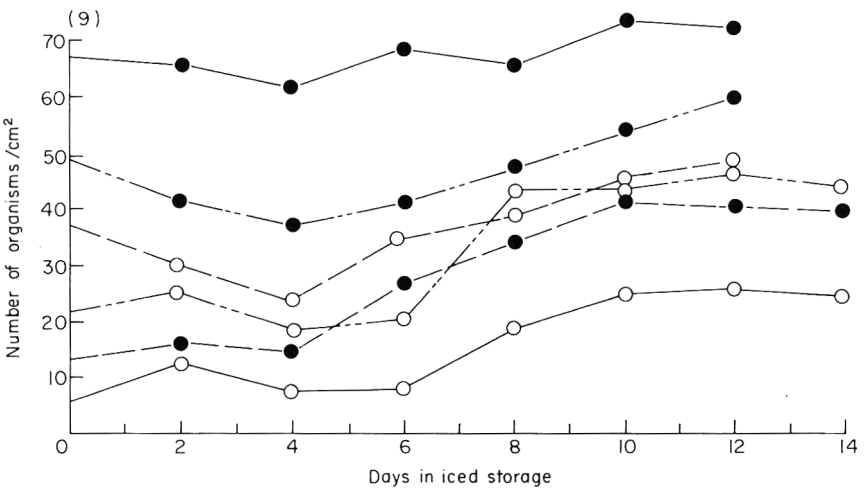

Figure 9. Total viable counts, incubation at 37°C for 48 hr. For key, see Fig. 1.

Microbiological

An area of 5 cm^2 on the surface of each fish was swabbed in duplicate and the swabs rinsed on 100 ml of sterile Ringer's solution. Serial dilutions were pour plated with Nutrient Plate Count Agar (Oxoid) and incubated at 20°C/72 hr and 37°C/48 hr. Results were reported as number of organisms per square centimetre.

Sensory evaluation

Samples were examined for appearance of skin, odour, appearance of flesh and texture using a modified quality rating scheme suggested by Howgate (1982) and obtained as personal communication from Torry Research Station, Aberdeen. (Tables 1–4).

Table 1. Quality rating scheme: appearance of skin.

Eyes fresh, convex black pupil, bright red gills, outer slime transparent.	5
Eyes flat, very slight greyness in pupil, slight loss of colour	4
Eyes slightly sunken, grey pupil, some discolouration of gills outer slime somewhat milky.	3
Eyes sunken, milky white pupil, thick outer slime with some bacterial discolouration.	2
Eyes completely sunken, gills, showing bleaching covered with bacterial mucus, outer slime thick.	0

Table 2. Quality rating scheme: evaluation of odour

Fresh trout odours.	5
Slight loss of fresh trout odours.	4
No odours, absence of fresh trout odours.	3
Slight off odours, slight rancidity present.	2
Definite off odours and rancid.	0

Table 3. Quality rating scheme: appearance of fish

No loss of fresh flesh colour, no reddening along backbone no discolouration of belly flaps, kidney blood red.	5
Slight loss of flesh colour, no reddening along backbone some discolouration of the belly flaps, loss of brilliance of kidney blood.	3
Some opacity, reddening along backbone, discolouration of flaps, brownish kidney blood.	2
Opaque flesh, brown discolouration along backbone marked discolouration of flaps, very brown kidney blood.	1

Table 4. Quality rating scheme: evaluation of texture

Firm, elastic to the finger touch.	5
Softening of the flesh.	3
Softer flesh, dried appearance.	2
Flesh soft and flabby, retains finger indentations, flesh easily torn from the backbone and skin.	1

Chemical

(a) Total Volatile Bases (TVB): Trichloroacetic acid extracts of 25 g of minced fish samples were prepared, made alkaline and distilled on Kjeltec System 1002 (Tecator); then titrated against 0.2N HCl. The titration value of acid used was a measure of the total amount of base present.

(b) Hypoxanthine (HX): This was determined according to the procedure of Jones *et al.* (1964). Protein free extracts of the fish muscle were prepared using perchloric acid, which was subsequently neutralized and filtered to remove insoluble precipitate

of potassium perchlorate. The extract was incubated with enzyme xanthine oxidase at 35°C to convert the hypoxanthine to uric acid. The uric acid present was measured at 290 nm on a Cecil 292 Digital Ultraviolet Spectrophotometer.

(c) Thiobarbituric Acid (TBA): The TBA number of 25 g samples of fish muscle were determined by the malonaldehyde distillation method of Tarladgis *et al.* (1960) and expressed as mg malonaldehyde per kg fish muscle. The number values indicated the degree of development of rancidity.

Results and Discussion

The overall quality of the skin and specific quality of flesh of all samples progressively deteriorated over the period of storage. Only fish iced immediately after receipt and those kept at 10°C for 6 hr prior to icing were of acceptable quality after 14 and 12 days of storage respectively, with scores of 2 and 2.5 for appearance of skin. Scores for appearance of fish flesh for the samples were 2 and 1.5. Fish kept at 10°C for 6 hr had a slight loss of colour of gills after the seventh day of iced storage and some loss of flesh colour with marked reddening along the backbone. In fish which were iced immediately on receipt, the eyes appeared flat and flesh retained some of the finger indentations. Fish kept at 20°C for 6 hr and 20°C for 18 hr gave scores of 3 and 2 respectively after the sixth day of iced storage. Fish kept at 30°C for 4 hr and 30°C for 6 hr prior to icing, gave scores of 2 and less than 2 as far as the appearance was concerned only 4–6 days after iced storage. The samples had sunken eyes with grey or milky white pupils, thick slime and some bacterial discolouration. The flesh was opaque with red to brown discolouration along the backbone and marked discolouration of the gill flaps with brownish kidney blood.

There were changes in texture and odour of all samples. Those kept at 20°C for 18 hr prior to icing were of soft and flabby texture, retaining finger indentation after only the fourth day of iced storage. The flesh was easily torn from the backbone and skin. There was distinctive odour development within two days of iced storage for samples kept at 30°C for 4 hr and 30°C for 6 hr prior to icing. Fish kept at 20°C for 6 hr prior to icing remained firm to touch and had no odour development up to the tenth day of iced storage. Fish iced immediately and those iced after 10°C for 6 hr remained of acceptable texture (scores of 3 and 4 respectively) after 10 days of iced storage. There appeared to be no distinctive odour development.

The results concur with the findings of Hansen (1972) who indicated that European trout stored in ice had a shelf life of 10 days. Keeping the fish at high ambient temperatures for periods up to 6 hr before icing appeared to affect their texture and flavour only marginally. There appeared to be no discernible correlation between the development of total volatile bases (mg N/100g) and the temperature/time of keeping prior to icing. There was, however, a gradual increase in TVB in samples kept at 10°C for 6 hr and 20°C for 6 hr prior to icing over the period of iced storage. There were considerable variations in TVB in samples kept at other temperature/times prior to icing. For example, fish kept at 30°C for 4 hr prior to icing had a value of 25.0 mg N/100g on the fourth day of iced storage, falling to a value of 17.7 mg N/100 g on the sixth day and finally rising to a value of 33.3 mg N/100 g on the tenth day. Herzberg, Pasteur & Rosenblatt (1977) found similar variations with *Sardinella aurita* where the initial value of TVB was high then fell over the period of storage and continued to fall even after spoilage had occurred. These variations suggest that TVB values alone cannot be accepted as an accurate indicator of the chemical quality of fish. The final readings of TVB of some samples were well within the standard of 30–40 mg N/100 g suggested by

Connell (1975) for cod (*Gadus morrhua*), yet the quality ratings of texture, odour and appearance were low. The Hypoxanthine (HX) values of fish kept at 20°C for 18 hr and 30°C for 4 hr prior to icing showed an accelerated increase over the period of iced storage. For samples kept at 30°C for 6 hr there appeared to be fluctuations in HX values. No acceptable explanation could be discerned for this variation. However, the overall increase in HX values over the period of iced storage suggests that it could be accepted as a suitable indicator of chemical quality of the fish. Burt, Murray & Stroud (1968) were also of the opinion that HX values gave good indication of the chemical quality of fish stored in ice.

Fish kept at high temperatures for varying periods (30°C for 4 and 6 hr and 20°C for 18 hr) gave high TBA values over the period of iced storage. This indicated an early development of rancidity. The TBA values for fish iced immediately and those kept at 10°C for 6 hr and 20°C for 6 hr prior to icing were low. There appeared good correlation between increase in TBA values and decrease in scores for odour and appearance. Hansen (1963) contended that for Danish rainbow trout high peroxide values correlated with poor scores of taste.

The microbiological results indicated that for all samples there was an increase in total viable counts over the period of iced storage, being highest in samples kept at 20°C for 18 hr prior to icing. The increase was not linear, as the total viable counts decreased around the fourth day of iced storage and were followed by an increase over the remaining period. This variation may be explained by a decrease in metabolic activity of the mesophiles followed by a gradual assertion of the psychrotrophic microflora. This correlates with the findings of Liston (1980) who showed that there was a shift of bacterial types during the period of storage at 3°C; *Pseudomonas* species rapidly assumed a dominant position in the microflora while other species, e.g., *Achromobacter* and *Flavobacterium* persisted but at a relatively decreasing level. Damoglou (1980) reported a similar pattern for herring during storage at 0°C in which the gram-positive species disappeared leaving the microflora comprised mainly of *Pseudomonas* species. The increase in total viable counts over the period of iced storage for all samples confirmed that icing was not a complete deterrent to microbial activity in stored fish. Nevertheless, the total viable counts for fish iced immediately and those iced after holding at 10°C for 6 hr and 20°C for 6 hr were low even after fourteen days storage. This confirms the findings of Antunnes, Britto de Castro & Novak (1971) that the total counts in fish held for six hours prior to icing were low even after ten days storage in ice. Rainbow trout (*Salmo irideus*) have an acceptable shelf life of 14 days if iced immediately on harvesting or kept for a minimum of 6 hr at not over 10°C prior to icing. Holding the catch at higher temperatures for longer periods prior to icing would negate consumer acceptibility of the fish. However, even if the catch had been held at 30°C for periods of up to 6 hr prior to icing, the quality may be acceptable up to 4 days of iced storage.

A recommendation is that the catch be kept at as low temperature as possible prior to icing. Although immediate icing is advisable, holding of the catch for periods no longer than 6 hr at temperatures no higher than 30°C prior to icing will ensure that increase in numbers of spoilage organisms is delayed.

Quality rating of fish after iced storage should include simple sensory evaluation using criteria of appearance, texture and odour of skin and flesh. These criteria, used critically, would give good indication of consumer acceptability of the catch. Laboratory quality controls of total bacterial counts and estimation of hypoxanthine concentrations would give further accurate indication of fish quality.

References

Antunnes, S.A., Britto de Castro, L.A. & Novak, A.F. (1971) Investigation on handling fish and shell-fish on board vessels in Brazil. *FAO Fisheries Report,* **115,** 1–6.

Bligh, E.G. (1971). Specific problems in the quality assessment of freshwater fish.In: *Fish Inspection and Quality Control* (edited by R. Kreuzer), pp. 81–86. Surrey, U.K: Farnham Fishing News (Books) Ltd.

Burt, J.R., Murray, J. & Stroud, G.D. (1968). An improved automated analysis of hypoxanthine *Journal of Food Technology,* **3,** 165–170.

Connell, J.J. (1975). *Control of Fish Quality* 1st edn. Surrey, U.K: Farnham Fishing News (Books) Ltd.

Disney, J.G., Cameron, J.D., Hoffman, A. & Jones, N.R. (1971). Quality assessment in Tilapia species. In: *Fish Inspection and Quality Control* (edited by R. Kreuzer), pp. 71–72. Surrey, U.K: Fishing News (Books) Ltd.

Damoglou, A.P. (1980). A comparison of different methods of freshness assessment of herring. In: *Advances in Fish Science & Technology* (edited by J.J. Connell), pp. 394–399. Surrey, U.K: Farnham Fishing News (Books) Ltd.

Hansen, P. (1963). Fat oxidation and storage life of iced trout. Part 1. Influence of gutting. *Journal of the Science of Food & Agriculture,* **14,** 781–786.

Hansen, P. (1972). Storage life of pre-packed wet fish at 0°C, Part II: Trout & Herring. *Journal of Food Technology,* **7,** 21–26.

Hansen, P., Olsen, K.B. & Petersen, T.E. (1974). Development in bulk preservation at sea of small whole fish. In: *Fishery Products* (edited by R. Kreuzer), pp. 64–69. Surrey, U.K: Farnham Fishing News (Books) Ltd.

Herzberg, A., Pasteur, R. & Rosenblatt, M. (1977) Keeping quality and freshness determination of Sardinella aurita. In: *Handling, Processing and Marketing of Tropical Fish* pp. 83–88. London: Tropical Products Insitute.

Howgate, P. (1982). Measurement of deterioration of frozen and iced fish. Personal communication. Aberdeen: Torry Research Station.

Jones, N.R., Murray, J., Livingston, E.I & Murray, C.K. (1964). Rapid estimations of hypoxanthine concentrations as indices of the freshness of chill-stored fish. *Journal of the Science of Food & Agriculture,* **15,** 763–773.

Liston, J. (1980). Microbiology in fishery science. In: *Advances in Fish Science & Technology* (edited by J.J. Connell), pp. 138–157. Surrey, U.K: Farnham Fishing News (Books) Ltd.

Mlay, M.L., Kakareh, S.M., Kirenga, J.N., Nichodemus, F.L.A., Warioba, M., Bhahinda, P., Karnicki, Z.A., Lima dos Santos, C.A.M., Nyagambi, J. & Buga, M. (1982). Effect of handling practices on the keeping quality of fish caught in Lake Victoria. *FAO Fisheries Report,* **268,** 6–14.

Tarladgis, B.G., Watts, B.M., Younathan, M.T. & Dugan, L.R. (1960). A distillation method for the quanititative determination of malonaldehyde in rancid foods. *Journal-American Oil Chemists Society,* 37, 44–48.

(Received 8 March 1985)

Journal of Food Technology (1986) **21**, 167–174

Viscosity modelling of dough in extrusion

M. BHATTACHARYA AND M. A. HANNA*

Summary

Effects of moisture level and shear rate on the rheological properties of blends of corn gluten meal (CGM) and soy protein concentrate, were studied. A simple model was used to correlate extrusion dough viscosities as a function of moisture content and shear rate. The consistency index (m) and flow behaviour index (n) of the blends were determined. It is generally accepted that viscosity is a function of product composition. Our results indicated that individual constituent differences (e.g., protein type) also significantly affected viscosity. Pseudoplastic behaviour was observed during extrusion.

Introduction

Extrusion cooking is becoming an increasingly popular food processing unit operation by which cereal, oilseed or other/carbohydrate/water mixtures are used to produce various meat analogues. The process uses high temperature short time cooking in addition to mixing and causes texturizing and shaping of food using equipment which closely resembles the screw extruders used in the processing of thermoplastics (Harper, 1981a). The state-of-the-art of protein texturization has been the subject of several reviews (Harper, 1981a; Clark, 1978; Kinsella, 1978; van Zuillichem, Witham & Stolp, 1977).

Blended foods are becoming popular in that through proper selecton of protein sources it is possible for the amino acid profile in the two protein sources to complement one another so that the resulting pattern more nearly approximates that of complete or balanced protein (Harper, 1981b). As an example, soy is relatively rich in lysine but deficient in sulphur containing amino acids (methionine and cystine) which are abundant in corn. The selection of formulations are based on a number of aspects, the important ones being: (i) cost, (ii) availability, (iii) nutritional, and (iv) consumer acceptibility of raw materials. However, the heterogeneous nature of a food system prevents one from extrapolating data for pure components and applying it to blends.

Harmann & Harper (1974) observed that equations for flow rate and torque input to an extrusion screw's metering section, derived for a plastics extruder, could be applied with sufficient accuracy to food extruders. However, these equations are applicable only if the viscosity of the dough is known. Dependence of viscosity on temperature, moisture content and shear rate has been observed by a number of researchers (Harper, Rhodes & Wanninger, 1971; Cervone & Harper, 1978; Remsen & Clark, 1978; Jao *et al.*, 1978; Chen *et al.*, 1979). Harper *et al.* (1971) (combined the effects logarithmically to get:

$$\eta = \eta^* \dot{\gamma}^{n-1} \exp(\Delta E_\eta/\mathrm{RT})\exp(\mathrm{K}M) \qquad (1)$$

where η^* = reference viscosity (Pa s), η = apparent viscosity at moisture M, temperature T and shear rate $\dot{\gamma}$ (Pa s), ΔE_η = energy of activation for flow (J/kg mole),

*Address for correspondence: Department of Agricultural Engineering, 213 L.W. Chase Hall, University of Nebraska, Lincoln, NE 68583-0726, U.S.A.

R = Universal gas constant (J/kg °K), T = absolute temperature (°K), M = moisture content, $\dot{\gamma}$ = shear rate/sec and K = constant.

Remsen & Clark (1978) applied the work of Roller (1975) to model the time–temperature effects on the viscosity of soy protein doughs. For constant moisture doughs their model can be written as:

$$\eta = \eta^* \dot{\gamma}^{n-1} \exp(\Delta E_\eta/RT) \exp \int_{t_0}^{t} K_\infty \exp(\Delta E_k/RT) dt \quad (2)$$

where K_α = apparent kinetic factor at infinite t(/sec), ΔE_k = activation energy for cooking fraction (J/kg mole), t_0 = time at start of cooking (sec), and t = time at point of interest (sec).

Cervone & Harper (1978) established a viscosity model using a combination of the power law, the logarithmic mixing rule and the Eyring kinetic theory. Morgan Suter & Sweat (1979) developed a theoretical model which described the effect of temperature–time history, temperature, shear rate and moisture content on the apparent viscosity of defatted soy flour dough. Their model used the Casson rehological equation which defines a yield stress and finate viscosities at high shear rate as:

$$\eta = \eta^* \exp(\Delta E_\eta/RT + bM)\left[\frac{\tau_0^{1/2}}{\gamma} + (\mu_0)^{1/2}\right]^2 \left[1 + B(1-M)^m (1-\exp(-K_\infty \psi)^m)\right], \quad (3)$$

where b,B,m = constants, τ_0 = yield stress (Pa), μ_0 = limiting viscosity of high shear rates (Pa-s), k_α = specific reaction velocity constant (/sec) and ψ = integral temperature-time history function, given by:

$$\psi = \int_0^t \exp(-E_K/RT) dt. \quad (4)$$

Even though a number of models exists, no single model has won universal acceptance.

During extrusion cooking two important reactions in the dough can affect viscosity. The two reactions are protein denaturation and polysaccharide gel formation. While texturizing protein based materials the shearing and stretching under the conditions of heat and pressure produces lamellar protein strands. Irreversible denaturation of a form which leads to the unfolding of the protein molecules results from the breaking and reformation of hydrogen or other bonds. This cross linking effect will depend upon two mechanisms: a temperature effect which determines the frequency of breaking and the formation of bonds and a shear effect which determines how the molecules will realign themselves.

Extrusion processing of starch gelatinizes the contents by rupturing the particles. Remsen & Clark (1978) described the gelatinization process. Initially addition of water breaks up the amylose crystallinity and as the process continues causes the starch granules to swell thereby increasing their volume by twenty-five to thirty fold. As more heat and water is added, the amylose begins to diffuse out of the granules causing the granules to collapse and the water molecules attach themselves to the exposed hydroxyl groups on the starch chain. A gel like structure with amylose supporting the collapsed granules consisting mostly of amylopectin results. Miller, Derby & Trimbo (1973) observed that the rapid rise in viscosity in starch products which is a function of temperature is associated with the presence of an extrudate outside the granule.

The objective of this experiment was to (i) develop viscosity models for three blends of corn protein (CGM) and soy protein of different proportions as a function of moisture content and shear rate (ii) determine the consistency index (m) and flow behaviour index (n) in order determine the nautre of flow of the material.

Theory

The shape of the velocity profile is dependent on the rheological properties of the fluid. For Newtonian flow, viscosity can be calculated using the Hagen-Poiseille equation which is given by:

$$\mu = \frac{\pi \Delta P r^4}{8QL} \tag{5}$$

where ΔP = pressure drop across the die (Pa), r = radius of die (m), Q = flow rate (m^3/sec) and L = length of die (m).
However, food doughs are non-Newtonian in nature, which means apparent viscosity varies with shear rate which in turn varies (non-linearly) with the radius of the capillary. For non-Newtonian doughs τ_w can be calculated the same way as for Newtonian fluid using the equation:

$$\tau_w = \frac{\Delta P r}{2L} \tag{6}$$

where τ_w = wall shear stress (Pa), but it is necessary to correct Newtonian $\dot{\gamma}_w$ or $\dot{\gamma}_a$ for non-Newtonian fluids.

It has been shown (Harper, 1981a) that wall shear rate (γ_w) is related to the apparent shear rate ($\dot{\gamma}_a$) by the formula

$$\dot{\gamma}_w = \frac{3n+1}{4n}\dot{\gamma}_a \tag{7}$$

where, n = flow behaviour index.
Also, an appreciable part of the pressure drop occurring across short capillaries is due to pressure drops which occur due to the constriction at the entrance of the capillary and expansion losses at the exit. Bagley (1957) showed that the shear stress at the wall of a short capillary can be calculated using:

$$\tau_w = \frac{\Delta P}{2(L/r + L_e/r)} \tag{8}$$

where L_e = equivalent capillary length which would increase ΔP by an amount to account for end effects (m).

Materials and methods

Materials

Defatted soy grits (Central Soya, Fort Wayne, IN) and wet corn gluten meal (ADM, Clinton, IA) were mixed in three ratios, 75:25, 50:50 and 25:75 by dry weight. Since soy and CGM have different equilibrium mositure contents, the samples were mixed just before extruding. Samples of soy and CGM (adjusted to pH 7.0) were made up to 20, 30 and 40% moisture content and allowed to equilibrate at 5°C for at least 24 hr. The samples were then brought back to room temperature, blended in the above ratios and extruded.

Extrusion

A Brabender 1.90 cm diameter laboratory food extruder was used, powered by a motor with speeds variable electrically from 0 to 200 rpm. Screw speeds of 60, 120 and 180 rpm were used. Extrusion temperature was controlled with electric heaters. Barrel temperature of the first zone was 80°C and that of the second and third zone was held at 145°C. All temperatures were within ± 1°C of the reported values. A screw compression ratio of 3:1 and die nozzle diameters of 4.25, 3.0 and 2.12 mm were used. Detailed barrel and die dimensions are shown in Figs 1 and 2 respectively. The pressure developed in the extruder at the die during the process was sensed by a pressure transducer and displayed on a Dynisco Melt Pressure Indicator. A Hewlett Packard strip-chart recorder was used to continuously monitor the pressure.

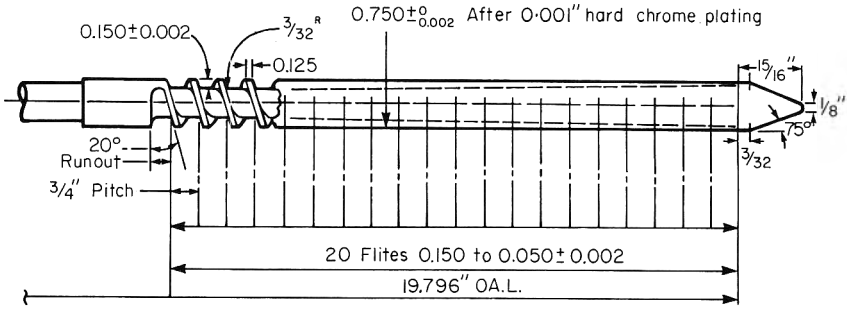

Figure 1. Configuration and dimensions of extruder screw.

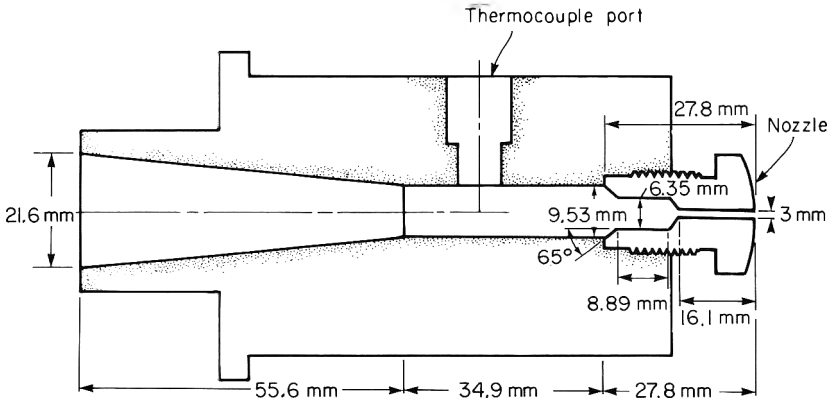

Figure 2. Configuration and dimensions of extruder die and nozzle.

Calculation of viscosity

The detailed procedure of calculating viscosity can be found in Harper (1981a). The procedure involved:

(i) measuring pressure drop and flow rate under isothermal conditions using a number of short capillary dies having varying L/r ratios; (ii) Ploting $\log \dot{\gamma}_a$ *versus* $\log \Delta P$, where $\dot{\gamma}_a = 40/\pi r^3$ with each die having a different L/r. From this figure for each single value of $\dot{\gamma}_a$ collect ΔP and L/r points; (iii) Plotting ΔP *versus* L/r for different values of $\dot{\gamma}_a$. Extrapolating this curve to $\Delta P = 0$, a value for L_e/r for each specific value of $\dot{\gamma}_a$ was

determined; (iv) Plotting L/r *versus* log $\dot{\gamma}_a$ of and determined the L_c/r for each $\dot{\gamma}_a$ of interest; (v) Calculating the corrected value of τ_w using equation (8); (vi) Calculating the flow behaviour index (n) as the slope of the plot of log τ_w *versus* log $\dot{\gamma}_a$; (vii) Calculating $\dot{\gamma}_w$ using equation (7); (viii) Calculating η using $\eta = \tau_w/\dot{\gamma}_w$.

Results

In determining the rheological properties, the method used by Bagley (1957) was applied to calculate the end correction for capillary flow. The procedure outlined by Rogers (1970) for interpreting capillary flow data was used to calculate the flow behaviour index (n) and consistency index (m). Using the values of m and n the apparent viscosity (i.e., viscosity for a particular shear rate) was calculated. The model used was of the form

$$\mu = A_1 \dot{\gamma}^{A_2} e^{A_3 M_{db}} \tag{5}$$

where μ = viscosity (Pa s), $\dot{\gamma}$ = shear rate in (/sec), M_{db} = moisture content on a dry basis, and A_1, A_2 and A_3 are associated constants.

Taking natural logarithms of both sides of equation (5) it gives

$$\ln \mu = \ln A_1 + A_2 \ln \dot{\gamma} + A_3 M_{db} \tag{6}$$

$$\text{or } \ln \mu = A_{11} + A_2 \ln \dot{\gamma} + A_3 M_{db}. \tag{7}$$

The constants A_1, A_2 and A_3 were determined using multiple regression analysis. The value of these constants for the blends of different proportions are shown in Table 1. It is seen that as the percentage of soy in the blend increases A_1 increases. Also an increase in the percentage of soy can be seen to cause a decrease in the value of A_2. The constant A_2 associated with $\dot{\gamma}$ gives the measure of the flow behaviour index ($n = A_2 + 1$) i.e., the flow behaviour index for the three blends were 0.4985, 0.5216 and 0.7344. As the percentage of soy increases in a soy/corn blend the viscosity increased for a given shear rate and moisture content. Also, the flow behaviour index of less than unity indicates that all three doughs exhibited pseudo-plastic behaviour. The negative signs associated with A_2 and A_3 indicate that any increase in shear rate or moisture content was followed by a decrease in viscosity. Water–protein interactions have been reported by Lumry (1973) and Von Hippel & Wong (1965). It is possible that water–protein complexes behave the same way as solvent–plastic complexes during thermoplastic extrusion, i.e., the higher the solvent content, the lower the flow resistance of the system. Water may be assumed to act as a plasticizer to lubricate and soften the protein making them mouldable. The viscosity will depend on the mouldability of the dough.

Table 1. Coefficient in the viscosity equation as a function of shear rate

Blend	A_1	A_2	A_3	Multiple correlation coefficient
75% soy–25% CGM*	17930	−0.502	−0.042	0.92
50% soy–50% CGM†	9558	−0.478	−0.039	0.90
25% soy–75% CGM‡	1435	−0.266	−0.041	0.64

*$275 < \dot{\gamma} < 7500$ $20 < M_{db} < 40$.
†$300 < \dot{\gamma} < 5800$ $20 < M_{db} < 40$.
‡$190 < \dot{\gamma} < 5000$ $20 < M_{db} < 40$.

Table 2. CGM and soy concentrate composition (proximate analysis, dry basis)

	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Carbohydrate (%)*
CGM	67.4	3.91	1.65	1.22	25.82
Soy	70.3	0.84	3.71	6.99	18.16

*Calculated as a difference.

It is interesting to note that the soy protein concentrate and corn protein had identical protein compositions (Table 2). CGM had a higher fat content; yet the presence of soy caused an increase in viscosity. Cervone & Harper (1978) observed that while the addition of emulsifying agent (Na stearoyl-2-lactylate) caused an increase in dough viscosity, the level of fat had no significant effect on dough viscosity. On the other hand the presence of fat did cause a significant increase in dough viscosity when added with diacetyltartaric ester of monoglycerides. The constant associated with the fat term was positive. An increase in the percentage of CGM in the blend increases fat content and if the level of fat has any effect on dough viscosity, it should lead to an increase and not a decrease as is observed in our case. There is no logical rationale to suggest that fibre or ash may affect dough viscosity in any way. Therefore, the only component that can affect the viscosity is protein. Bresnahan, Wolf & Thompson (1982) observed that it was protein type rather than quantity that altered water absorption. A similar reasoning may be applicable in our case. Very little is known, in detail, about the structure of corn and soy protein and its effect on polymerization at extrusion pressures. The flow behaviour index for pure soy flour, as reported by Chen *et al.* (1979), is 0.127. This along with other studies conducted by Bhattacharya & Hanna (1985) indicates that soy proteins require higher energy and pressure than does corn proteins.

Table 3. Consistency and flow behaviour indices of soy-CGM blends at different moisture contents

Blend	MC	m	n	Multiple correlation coefficient
75% soy–25% CGM	20	1411	0.41	1.00
	30	6837	0.47	0.92
	40	1062	0.66	0.97
50% soy–50% CGM	20	6114	0.48	0.92
	30	2317	0.55	0.86
	40	1330	0.58	0.94
25% soy–75% CGM	20	1182	0.64	0.95
	30	297.4	0.77	0.82
	40	217.4	0.78	0.85

Table 3 shows the variation of consistency and flow behaviour index for the three blends at different moisture contents. An increase in moisture content was followed by an increase in consistency index (m) but a decrease in flow behaviour index (n). Response surfaces of viscosity as a function of shear rate and moisture contents for

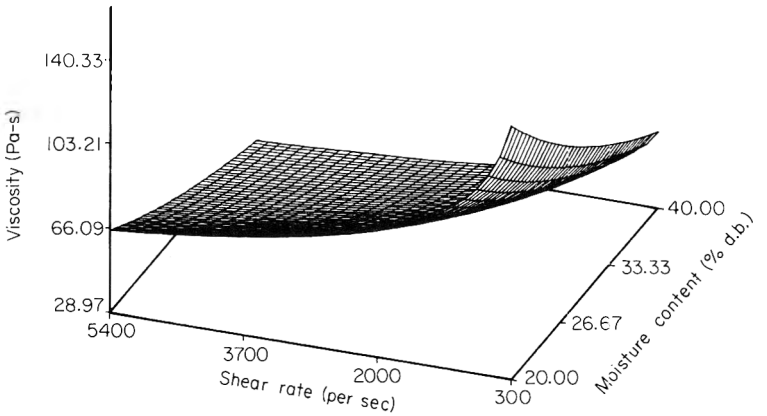

Figure 3. Response surface of viscosity as a function of shear rate and moisture content of a mixture of 25% soybean flour and 75% corn gluten meal.

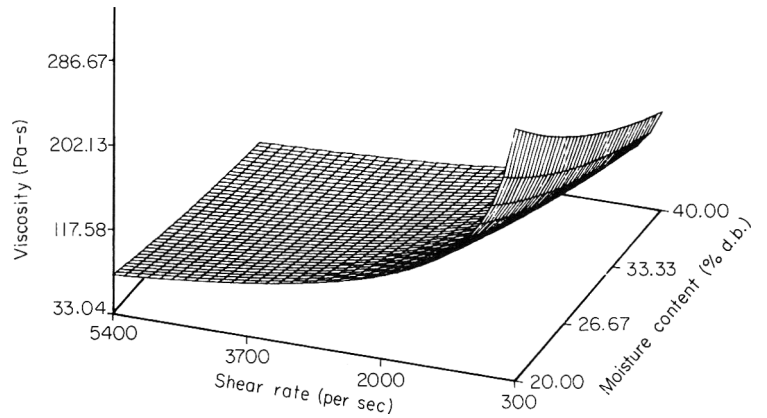

Figure 4. Response surface of viscosity as a function of shear rate and moisture content of a mixture of 50% soybean flour and 50% corn gluten meal.

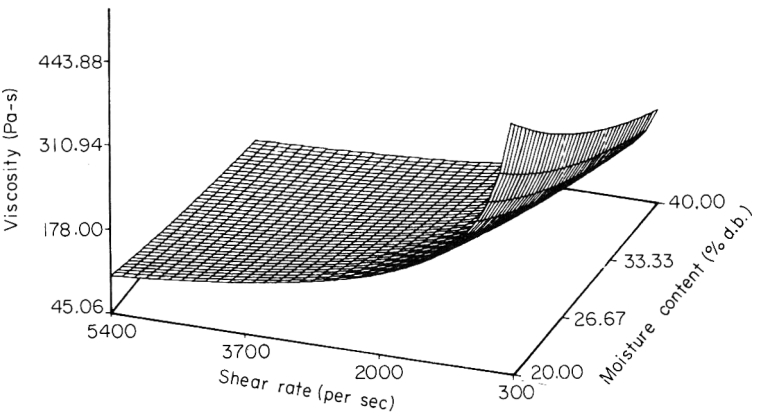

Figure 5. Response surface of viscosity as a function of shear rate and moisture content of a mixture of 75% soybean flour and 25% corn gluten meal.

the three blends are shown in Figs 3, 4 and 5. The shape of the three surfaces are identical to one another except in magnitude of viscosity. These surfaces also indicate that shear is a more important factor in viscosity than is moisture content.

Conclusions

Blends containing higher percentages of soy had higher apparent viscosities than did those containing CGM. It is also being suggested that an incresae in wall shear rate causes a higher decrease in apparent viscosity than does an increase in moisture content in the region $300 < \dot{\gamma}_w < 1500$, after which the rate of decrease of apparent viscosity is constant. Blends of higher soy percentages also indicated higher pseudoplstic behaviour as indicated by the lower values of flow behaviour index. However, the heterogeneous nature of food materials prevents one from extrapolating data from one food to another of similar chemical composition.

References

Bageley, E.G. (1957). End correction in capillary flow of polyethylene. *Journal of Applied Physics*, **28**, 624–627.

Bhattacharya, M. & Hanna, M.A. (1986). Mathematical modelling of a food extruder. *Wiss-u-Technol*, (in press).

Bresnahan, D.P., Wolf, J.C. & Thompson, D.R. (1982). Potential for utilizing 11S soy globular protein to study texture formation. *Journal of Food Process Engineering*, **5**, 113–123.

Chen, A.H., Jao, Y.C., Larkin, J.W. & Goldstein, W.E. (1979). Rheological model for soy dough in extrusion. *Journal of Food Process Engineering*, **2**, 337–342.

Clark, J.P. (1978). Texturization by extrusion. *Journal of Texture Studies*, **9**, 109–123.

Cervone, N.W. & Harper, J.M. (1978). Viscosity of an intermediate moisture dough. *Journal of Food Process Engineering*, **2**, 83–95.

Harmann, D.V. & Harper, J.M. (1974). Modelling a forming foods extruder. *Journal of Food Science*, **39**, 1099–1104.

Harper, J.M. (1981a). *Extrusion of Foods, Vol I.* Boca Raton FL USA: CRC Press.

Harper, J.M. (1981b). *Extrusion of Foods. Vol II.* Boca Raton, FL USA: CRC Press.

Harper, J.M., Rhodes, T.P. & Wanninger, L.A. (1971). Viscosity model for cooked cereal doughs. *American Institute of Chemical Engineering Symposium Series* no. 108, 40–43.

Jao, Y.C., Chen, A.H., Lewandowski, D. & Irwin, W.E. (1978). Engineering analysis of soy dough rhelogy in extrusion. *Journal of Food Process Engineering*, **2**, 97–112.

Kinsella, J.E. (1978). Texturized proteins: Fabrication, flavouring and nutrition. *Critical Review of Food Science and Nutrition*, **10**, 147–207.

Lumry, R. (1973). Some recent ideas about the nature of the interactions between proteins and liquid water. *Journal of Food Science*, **38**, 744–755.

Miller, B.S., Derby, R.I. & Trimbo, H.B. (1973). A pictorial explanation for the increase in viscosity of a heated wheat starch-water suspension. *Cereal Chemistry*, **50**, 271–280.

Morgan, R.G., Suter, D.A. & Sweat, V.E. (1979). Modelling the effects of temperature-time history, temperature, shear rate and moisture on apparent viscosity of defatted soy flour dough. Paper No. 79-6002, *American Society of Agricultural Engineers*, St. Josephs, MI.

Remsen, C.H. & Clark, J.P. (1978). A viscosity model for cooking dough. *Journal of Food Process Engineering*, **2**, 39–64.

Rogers, M.G. (1970). Rheological interpretation of Barbender plastirecorder (extrusion head) data. *Industrial Engineering Chemical Process Design and Development*, **9**, 49–52.

Roller, M.B. (1975). Characterization of the time-temperature-viscosity behavior of curing β-staged epoxy resins. *Polymer Engineering Science*, **15**, 406–414.

Von Hippel, P.H. & Wong, K.Y. (1965). Conformation stability of globular protein. *Journal of Biological Chemistry*, **240**, 3909–3923.

Van Zuilichem, D.J., Witham, I. & Stolp, W. (1977). Texturization of soy flour with a single-screw extruder, *Seminar Extrusion Cooking of Foods*. Centre de Perfectionnement des Cadres des Industries Agricoles at alimentaire, Paris.

(Received 13 May 1985)

Journal of Food Technology (1986) **21**, 175–187

Descriptive profiling *versus* direct similarity assessments of soft drinks

J. CHAUHAN* AND R. HARPER†‡

Summary

A set of seven soft drinks was profiled using a check list of descriptors and the same set of products was assessed for similarity by a panel of ten assessors. The profile data were used to generate a matrix of interstimulus distances, and this and the dissimilarity matrix obtained directly from the similarity assessment were examined using multidimensional scaling approaches (MDS/INDSCAL). The two methods yielded broadly similar results in terms of the spatial representation of the stimuli. This was further confirmed on analysis of the data using a multidimensional unfolding program which aimed to highlight any differences between the two sets of dissimilarity measures.

Introduction

Descriptive profiling, a method most commonly used to describe the sensory quality of food, has come under criticism by advocates of an alternative method where direct assessments of similarity or dissimilarity of stimuli are made (Schiffman, Reynolds & Young, 1981; Thomson, 1981; Thomson & MacFie, 1963). Descriptive profiling can consist of spontaneous characterization involving the consensus judgements of a group of specially trained assessors or the independent evaluation by a number of assessors using a pre-established product orientated vocabulary (Harper *et al.*, 1968a; 1968b; 1968c; Harper, 1977; Williams, 1983; Williams & Langron, 1984; Pangborn, 1984). Using either category scaling or graphical scaling, depending upon the particular profile method being used, each of the attributes for the product under study is scored for the amount present. A sample or perceptual space can then be derived for that product by representing each of the descriptors that is scored as a dimension in space, a unique position being given for each sample depending upon how it was scored by the assessors. For a series of samples, points are obtained in multidimensional space where each point represents a sample such that distances between samples show their similarities and differences (Moskowitz & Von Sydow, 1975; Moskowitz & Barbe, 1976). In similarity–dissimilarity scaling, the samples are presented in pairs and their degree of similarity scored using a category or interval scale. A similarity matrix is generated which can be subjected to statistical analysis to yield a sample space similar in concept to that derived from profiling.

A major criticism of descriptive profiling is that a profile which consists of the various 'sensory elements' perceived does not necessarily convey the same impression as the original, spontaneous experience, it is pre-supposed that words can adequately convey what is being perceived. This similarity approach, on the other hand, does not

Authors' address: Department of Food Science, Reading University, Whiteknights Park, Reading RG6 2AP, U.K.

Present addresses: *Department of Foods and Nutrition, University of Alberta, Edmonton, Canada T6G 2M8; and †29 Harrow Court, Bath Road, Reading RG1 6JF, U.K.

‡To whom correspondence should be addressed.

require the use of words except for interpretation of the results which is done by reference to description of the products. One of the major drawbacks of the similarity method is the large number of samples which must be assessed. The application of the two methods to the same set of food stimuli has not been reported although a number of instances of obtaining similar results from verbal ratings and global dissimilarity judgements can be found in the psychological literature (Rosenberg & Sedlak, 1972). The present study includes both methods and is intended to show their comparative advantages.

Metric multidimensional scaling (Davison, 1983; Torgerson, 1958) is justified only when the similarity-dissimilarity data have the form of ratio scaled distances. The INDSCAL model (Carroll & Chang, 1970), which is 'based on a psychological model that postulates dimensions that are unique in the sense of being modifiable (in salience, or perceptual importance) across, and perhaps even within, individuals' (Carroll & Wish, 1974), is robust to mild departures from linearity. In addition what makes INDSCAL an important and useful technique is the fact that it takes account of individual differences in perception, in terms of differential weights of a common set of unique underlying dimensions. Thus, INDSCAL was used for analysis of the dissimilarity data.

As in every study of this type, the pattern of responses is invariably determined by the particular selection of samples assessed and the list of descriptors provided. Previous studies (Chauhan, 1982) involved ginger drinks and thus formed the basis of their choice here. However, the final choice was influenced by availability of drinks and therefore in addition to five ginger drinks, two citrus flavoured drinks were also included. Lemon and lime oils are often constituents of 'ginger ales' and the chemical components of lemon and lime oils such as citral and nerolidol have been reported to be important constituents of ginger oil for eliciting 'ginger' flavour (Bednarczyk, 1973; Bednarczyk & Kramer, 1975). Thus, in this instance, inclusion of lemon and lime flavoured drinks was felt to be a minor departure from ginger flavour.

There is a formal restriction on the number of independent 'dimensions' which can be identified in any study, depending upon the number of stimuli, in this instance the number of drinks. Even if the data contained no error, something in excess of three stimuli as a minimum would be essential to give rise to more than one dimension. Subsequently, the opinions of experts as to the number of stimuli which are essential under different circumstances has been recorded. Quoting from Schiffman *et al.* (1981), ideally one should have about twelve stimuli for two-dimensional solutions and eighteen stimuli for three-dimensional solutions. Kruskal & Wish (1978) recommend nine stimuli for two dimensions, thirteen for three and seventeen for four; Young (1970) and Spence & Domoney (1974) recommend six for one dimension, eleven for two and seventeen for three. A large number of stimuli are needed in order to allow enough stimuli to identify a dimension. Thus, in the present studies,two dimensions might reasonably be identified, if that number of different sources of variation between the samples exist. In any case, it would have been impracticable to have included a larger number of different drinks, with the workload involved.

Materials and methods

Samples

Seven drinks were used: (1) Hunts Dry Ginger Ale; (2) Schweppes Dry Ginger Ale; (3) Hunts American Ginger Ale; (4) Schweppes American Ginger Ale; (5) Idris Ginger

Beer; (6) Corona Lemonade; (7) Corona Limeade. The drinks were presented as marketed, with the labels masked, for the task of profiling. However, for comparisons of (dis)similarity the drinks were poured by the experimenter into coloured glasses and covered with a lid prior to assessment. All drinks were presented at 10–15°C. For profiling, a balanced 7×7 latin square design was used for presentation of the drinks, all of them being assessed separately, twice. For similarity assessments, each assessor worked through the controlled orders of presentation of the different pairs, the order being changed for each assessor. Self-comparisons of each drink were also included and, in total, twenty-eight pairs of drinks were assessed, each being assessed twice on separate occasions. All assessors were given three pairs of drinks to assess for similarity at the beginning of the study in order to familiarize themselves with the procedure.

Assessors

Ten assessors, four females ranging in age 20–55, and six males ranging in age 28–60 participated in the study. All assessors were familiar with profiling of soft drinks but not with direct assessments of dissimilarity.

Procedures

Profiling (indirect assessments of dissimilarity). The seven drinks were profiled in terms of aroma, flavour and after-flavour, using a check-list of thirty descriptors (Table 1) which were applicable for characterization of ginger drinks in particular. The usual six-point category scale (0–5) was used to rate the intensity of the characteristics perceived. The results and discussions here, are confined to the data collected for 'flavour in the mouth' characteristics only. The data collected from the duplicate assessments by the ten assessors were treated as representative of twenty assessors, for the purpose of analysis.

Table 1. Descriptors used in profiling the flavour characteristics of the seven soft drinks

Fruity, natural	Green, leafy
Fruity, synthetic	Green, herbal
Fruity, citrus	Woody, resinous
Fruity, other	Cardboard-like
Sweet	Earthy
Sharp, acid	Spicy, ginger-like
Sour	Spicy, other
Bitter	Metallic
Pungent, cough-provoking	Soapy
Pungent, like sulphur dioxide	Stale
Caramel	Body
Burnt	Drying
Vanilla-like	Astringent
Fragrant, perfumery	Warming
Floral	Burning/hot

In order to facilitate comparison of the two methods, direct and indirect assessment of dissimilarity, each profile assessment was converted into a dissimilarity matrix between all pairs of drinks by computing the Euclidean distance between their respective ratings. For any one pair of drinks, the square root of the sum of the squares of the

differences in their ratings for each of the thirty descriptors was taken to give the Euclidean distance. This yielded twenty-one inter-drink distances for each assessment, as self-comparison of a drink will always produce a zero distance. Thus, twenty individual matrices were generated.

Direct assessment of dissimilarity. The seven drinks were also assessed in pairs for dissimilarity in terms of flavour, using a nine-point category scale (0 = identical, 8 = extremely different). On any one occasion, a maximum of three pairs of drinks was assessed, all drinks being assessed twice. Only the data concerning flavour by mouth are considered in this paper. A matrix of twenty-eight inter-drink dissimilarities was obtained from each direct assessment (i.e., including self-comparison). Thus, twenty such matrices were obtained in all. The twenty dissimilarity matrices generated by each of the two methods were then subjected to Multidimensional Scaling (MDS) using the computer program INDSCAL (Carroll & Chang, 1970).

Multidimensional unfolding of dissimilarity data on seven drinks. The two assessment methods can be compared qualitatively in terms of the group stimulus spaces output by the INDSCAL solutions. However, in order to highlight in a formal way, any differences which may exist between the methods, the data were analysed using the multidimensional unfolding program METUNF. The theory on which this FORTRAN IV program is based is given by Schonenmann (1970) while some illustrative examples of the method are provided by Constantine & Gower (1978).

To use multidimensional unfolding, the two dissimilarity matrices obtained from the two methods of assessment are treated as a single matrix, values from one method forming the lower triangle and values from the other method the upper triangle of this matrix. The program then produces a group stimulus space containing two points for each drink, one point corresponding to each method of assessment. If these two points are close together, then the two methods terpreted as having treated this stimulus comparably. If the points are far apart, however, then the two methods have treated the stimulus differently.

Averages of the twenty individual matrices for each method were initially scaled so that the sum of the squares equalled unity for each matrix. This made the two sets of measures comparable. One half of each of the dissimilarity matrices were then combined into one (7×7) matrix, representing the comparison of each of the seven drinks with each other, including the self-comparisons, by the two methods in comparable form. This is best illustrated by the composite matrix itself (Table 2). The upper triangular arrangement of elements corresponds to the dissimilarity measures obtained

Table 2. Matrix input for Metric Multidimensional Unfolding Standardized (sum of squares = 1 for each half-matrix); direct assessment of dissimilarity *below* diagonal; indirect *above* diagonal

Drinks	1	2	3	4	5	6	7
1	0.000	0.175	0.210	0.208	0.221	0.213	0.234
2	0.143	0.000	0.206	0.221	0.260	0.220	0.222
3	0.170	0.221	0.000	0.183	0.208	0.220	0.226
4	0.223	0.199	0.126	0.000	0.206	0.210	0.219
5	0.291	0.301	0.214	0.231	0.000	0.259	0.272
6	0.223	0.243	0.177	0.189	0.299	0.000	0.161
7	0.189	0.221	0.185	0.192	0.284	0.141	0.000

by the indirect method and the lower triangular arrangement to the dissimilarity measures obtained by the direct method. This matrix then constituted the input to the METUNF program.

Results and discussion

Indirect assessment of dissimilarity

As insufficient stimuli were used to sustain more than two dimensions, the results and discussions will be confined to the two-dimensional solution only. The two-dimensional INDSCAL solution accounted for 47% of the variance. The coordinate values for the stimuli in the two-dimensional solution are plotted in Fig. 1. The graphical representation of these coordinates is the group space. The assessors' weights on the respective dimensions are represented graphically in the weight space given in Fig. 2 where a and b refer to the replicate assessments by the same assessor.

Tests of significance in respect of the differences for INDSCAL are not immediately available although Weinberg, Carroll & Cohen (1984) discuss the use of bootstrap and jack-knife techniques to estimate confidence regions of the group stimulus points derived from INDSCAL. In the present instance, the mutual arrangement of the various stimuli for the different assessors has to be evaluated in broadly qualitative terms. With this restriction in mind, the following interpretation seems reasonable.

The group stimulus for the two-dimensional INDSCAL solution shows (Fig. 1) that the seven drinks are differentiated into two separate groups, the ginger drinks and the citrus flavoured drinks. Drink 5, Ginger Beer, and Drink 2, Schweppes Dry Ginger Ale, are seen to be quite distinct, the three other ginger drinks falling intermediate

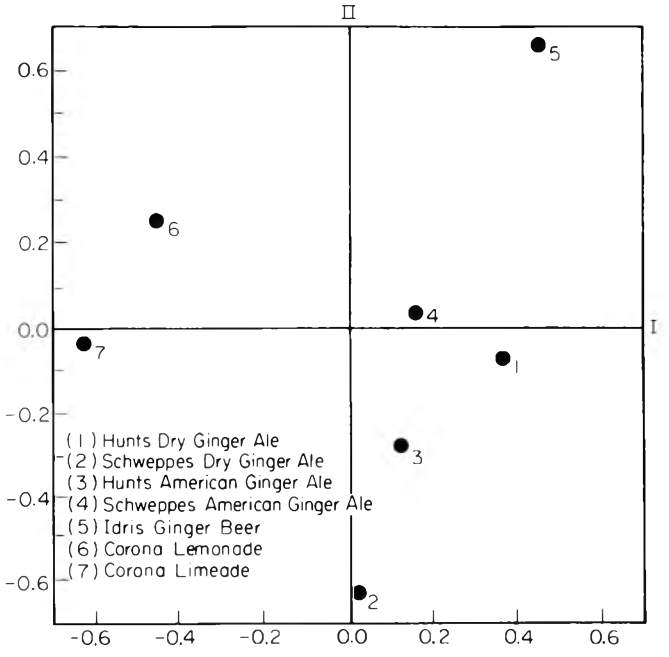

Figure 1. INDSCAL Group Stimulus Space: A two-dimensional INDSCAL analysis of twenty matrices of dissimilarities among drinks by indirect assessment.

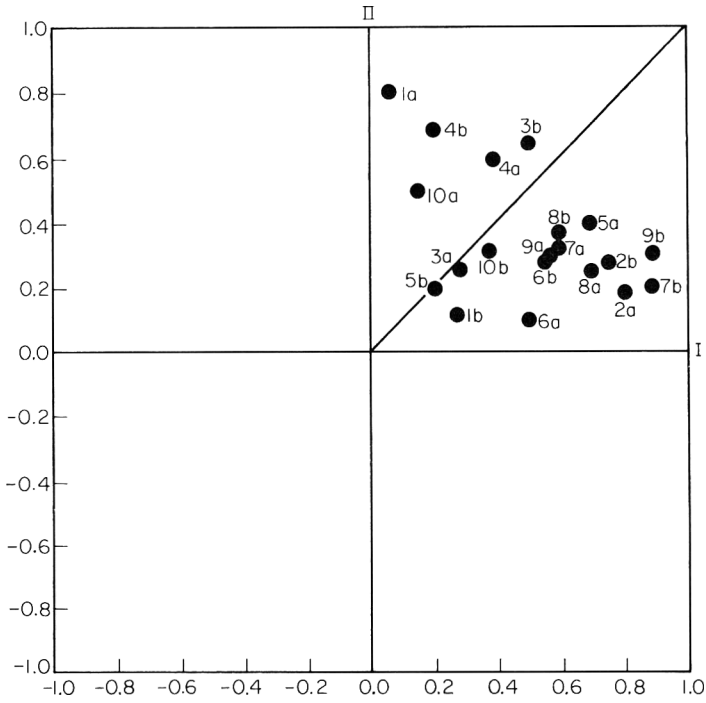

Figure 2. INDSCAL Weight Space: Dimension weights from a two-dimensional INDSCAL analysis of twenty matrices of dissimilarities among drinks by indirect assessment.

between the other two. The distance between drinks 3 and 4 is relatively small compared with that between drinks 1 and 2, indicating the greater degree of similarity between the two American Ginger Ales than between the two Dry Ginger Ales. The American Ginger Ales are closer to the citrus drinks, indicating the similarity between these two groups.

Individual differences in assessments of dissimilarity between the drinks are clearly evident on examination of the weight space shown in Fig. 2. Hence, the use of INDSCAL as the method of analysis of the dissimilarity matrices is justified. In Fig. 2 assessors 2, 4, 6 and 8 show relatively better agreement between the two replicate assessments compared with assessors 1, 3, 5, 7, 9 and 10. All assessors have positive weights and thus there are no aberrant assessors as far as the theoretical model is concerned. The individual spaces of assessor 3a and 5b (not reproduced here) correspond to the group stimulus space, but the points representing these assessors in the weight space lie close to the origin (Fig. 2). This indicates that only a small proportion of the total variance in the original data for these particular assessors is accounted for by the INDSCAL solution. The individual space for assessor 10b is almost equivalent to the group stimulus space since both dimensions are weighted almost equally.

Assessors 1b, 2a, 2b, 5a, 5b, 6a, 6b, 7a, 7b, 8a, 8b, 9a, and 9b give a higher weight to Dimension I than II (Fig. 2), so that their individual spaces correspond to the group stimulus space stretched out along Dimension I. This is in contrast to assessors 1a, 3b, 4a, 4b, and 10a who give a higher weight to Dimension II than I, so that their individual spaces corresponds to the group stimulus space stretched out along Dimension II. The first group of assessors still differentiate the ginger from the citrus drinks and drinks 2

and 5 are perceived to be fairly distinct from the other drinks. However, the distance between the drinks is relatively small compared with that in the group stimulus space. This is in contrast to the second group of assessors (1a, 3b, 4a, 4b and 10a) in whose configurations drinks 2 and 5 are pulled further apart, indicating their dissimilarity from the other drinks.

Assessors 1b and 6a give negligible weight to Dimension II and can be contrasted with assessor 1a who weights Dimension II substantially. The individual spaces for these assessors are one-dimensional, the relative positions of the drinks being projected on to that one dimension. It becomes evident that for assessor 6a the biggest difference between the drinks perceived is that between the two major groups of drinks, whereas for assessor 1b that Dry Ginger and the Ginger Beer are the most different of all seven drinks.

Although not reproduced here, the individual pattern representing the relationship between the seven different samples by the same methods are remarkably similar, even when they involve differential stretching in the particular dimensions in accordance with the weights given for each assessor in Figs 2 and 4, dealing respectively with indirect and direct assessments of similarity.

Direct assessment of dissimilarity

The two-dimensional INDSCAL solution for the dissimilarity matrices obtained by the direct method also accounted for 47% of the variance in the original data. The coordinate values are represented graphically in Fig. 3, which is the group stimulus space. The weight space is given in Fig. 4.

The group stimulus space for the two-dimensional solution shows that the 7 drinks can be considered in pairs: (1) the two Dry Ginger Ales (drinks 1 and 2), (2) the two

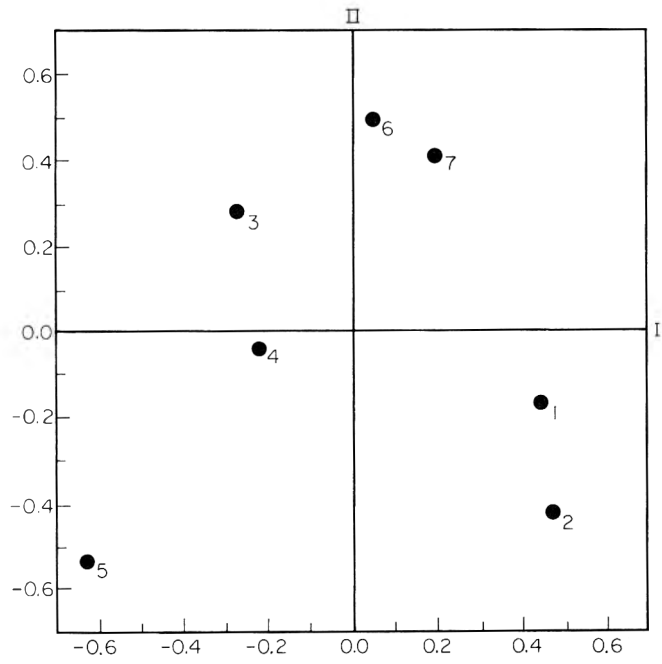

Figure 3. INDSCAL Group Stimulus Space: A two-dimensional INDSCAL analysis of twenty matrices of dissimilarities among drinks by direct assessment.

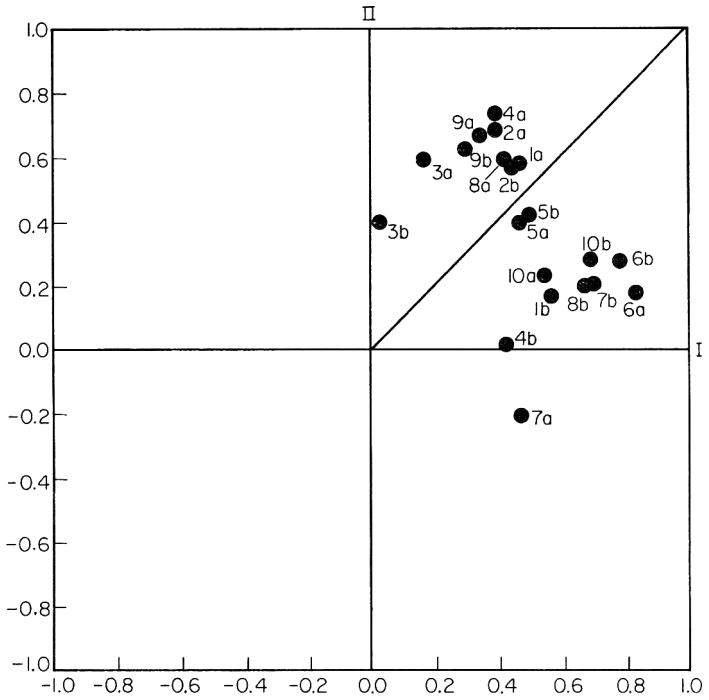

Figure 4. INDSCAL Weight Space: Dimension weights from a two-dimensional INDSCAL analysis of twenty matrices of dissimilarities among drinks by direct assessment.

American Ginger Ales (drinks 3 and 4), (3) the two citrus drinks (drinks 6 and 7) with the Ginger Beer (drink 5) being fairly distinct amongst the seven drinks. The Lemonade and Limeade are relatively closer to the American Ginger Ales than to the Dry Ginger Ales indicating their similarities.

An appreciable amount of individual variation becomes evident from the weight space (Fig. 4). Assessors 2, 3, 5, 6, 9, and 10 are fairly consistent in their replicate assessments. In contrast, assessors 1, 4, 7, and 8 show poor replication in the criteria used to base their assessments of dissimilarity.

All assessors have positive weights, with the exception of assessor 7a, who is given a negative weight for Dimension II. This fact needs further examination, since reference to the individual spaces reveals that the arrangement of the samples in the replications for 7a and 7b are almost mirror images about the line representing Dimension I. The results for 7a form an open U-shaped figure, whereas the rest of the individual spaces, including 7b, consist of an inverted U-shaped figure, similar to Fig. 3.

Assessor 5 gives an equal weight to both dimensions and the individual spaces of this assessor correspond well to the group stimulus space in Fig. 3. Assessors 1b, 4b, 6a, 6b, 7b, 8b, 10a, and 10b give substantial weight to Dimension I compared with Dimension II. Therefore, the individual spaces for these particular assessors correspond to the stimulus space stretched out along Dimension I. These assessors discriminate well between the two types of ginger ales. Also, as pairs of drinks, the two citrus drinks are perceived to be relatively less similar to one another compared with the drinks within the pairs of ginger ales.

Assessors 1a, 2a, 2b, 3a, 3b, 4a, 8a, 9a, and 9b, give greater weight to Dimension II

than I. Consequently, the individual spaces for these assessors correspond to the group stimulus space stretched out along Dimension II. These assessors do not discriminate well between the two types of ginger ales.

Assessor 3b gives negligible weight to Dimension I in contrast to assessor 4b who gives negligible weight to Dimension II. The stimulus points are projected on to one dimension in the individual spaces of these assessors. In this instance, the two types of ginger ales are well discriminated by assessor 4b in contrast to assessor 3b. Assessor 4b perceives the Dry Ginger Ales to be just as different from one another as are the American Ginger Ales from the two citrus drinks.

In most instances, cross reference to the original dissimilarity matrices of the different individuals confirms the interpretation of the configurations output by the INDSCAL analysis. Examination of the weight spaces output for the two different methods of dissimilarity (Figs 2 and 4) indicates that assessors 2, 6 and 9 are reasonably good at both direct assessment of dissimilarity and profiling of drinks; assessors 3, 5 and 10 are relatively more consistent at direct assessments of dissimilarity, whereas assessors 4, 7 and 8 are more consistent at profiling. Assessor 1 is highly variable in both methods.

A learning effect in assessing dissimilarity is evident on comparison of the distribution of dissimilarity estimates given to self-comparisons of the seven drinks for each of the two replicate assessments (Table 3). Compared with the first assessment, nearly 16% more ratings 0 or 1 (identical or very similar) were obtained on the second replication. An analysis by individuals clearly indicates assessor 1 to be the least consistent (Table 4). The samples can also be placed in order of difficulty in comparison by examining the number of O's assigned to the self-comparison of each drink (Table 4). Ginger beer (13), Limeade (13), Lemonade (11) and Schweppes Dry Ginger Ale (11) were assigned the highest numbers of O's on self-comparison (these are given in brackets after each of the four drinks). This may be attributed to the fact that these drinks were almost completely different in their flavour characteristics from the rest of the samples in this particular set of seven. Consequently, the four drinks listed above were easily recognized when identical pairs were presented for the assessment of dissimilarity. This study clearly indicates that for some individuals assessment of dissimilarity between different stimuli may be just as difficult as characterizing each stimulus in descriptive terms.

Table 3. Distribution of dissimilarity estimates for self-comparison of drinks

Dissimilarity estimates	0	1	2	3	4	5	6	7	8
Replicate 1	34	13	9	6	4	1	1	2	0
Replicate 2	38	20	7	2	2	0	1	0	0
Difference 2–1	+4	+7	−2	−4	−2	−1	0	−2	0
Total shift		+11				−11			

Multidimensional unfolding of dissimilarity data on seven drinks

Figure 5 is the configuration output by METUNF. The overall pattern is similar to that of the group stimulus space output by INDSCAL solution: three separate groups of

Table 4. Distribution of dissimilarity estimates of self-comparisons of drinks for each individual

	Dissimilarity estimates																				
	Assessors 1–10																				
Drinks	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	7a	7b	8a	8b	9a	9b	10a	10b	*
1	2	1	1	1	3	1	1	3	2	0	0	0	0	2	1	0	0	0	0	0	9
2	5	6	0	1	3	1	3	0	0	0	2	0	1	0	0	0	0	0	2	0	11
3	7	2	1	1	2	3	0	0	1	0	0	2	4	4	0	0	0	0	3	2	8
4	2	2	1	1	1	1	1	0	0	1	1	4	0	2	0	0	0	1	0	1	7
5	1	1	0	1	1	1	0	0	0	2	0	0	3	0	0	0	0	0	0	0	13
6	4	4	0	1	1	1	0	0	4	1	0	0	1	2	0	0	0	0	0	0	11
7	6	2	0	1	7	1	0	0	3	0	0	0	0	0	0	0	0	0	2	0	13

*Last column: Distribution of numbers of 'O's' (no difference) for self-comparisons of each drink.

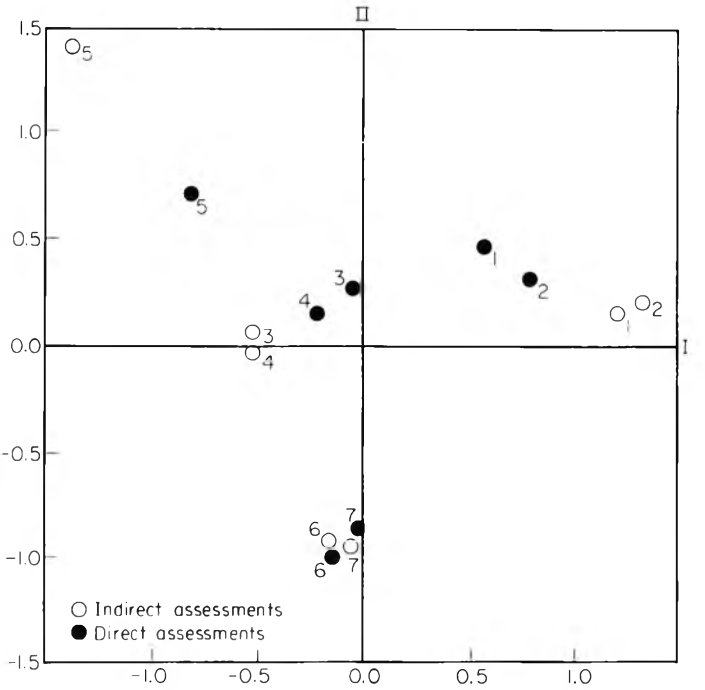

Figure 5. Metric Multidimensional Unfolding using MEˉUNF.

drinks with the Ginger Beer being unique amongst the seven drinks. With the exception of points representing Drinks 1 and 5, the two points for each of the other five drinks are fairly close together, indicating that the two dissimilarity measures for the latter five drinks are almost equivalent.

Conclusions

The two sets of dissimilarity matrices when subjected to MDS both yield two-dimensional configurations which lead to broadly similar conclusions. There is evidence in both INDSCAL solutions, that the seven drinks consist of two main groups: the ginger on one hand and the citrus flavoured drinks on the other. Within the ginger drinks there are two subgroups: the American and the Dry Ginger Ales, with the two samples of the former being more similar to each other than the latter two. In fact, the American Ginger Ales are more similar to the citrus drinks than to the Dry Ginger Ales. The only sample of Ginger Beer is placed uniquely in both configurations. The study demonstrates that direct dissimilarity scores can be substituted by dissimilarity measures derived from profile data without any real loss of information.

There is some underlying logical weakness in using Euclidean distances as dissimilarities, since there is no distinction between minimum and maximum scores for any two descriptors, i.e., the difference between a descriptor scored 0 for a particular pair of drinks in one instance and then in another pair scored 5, is the same, namely zero. In addition, Euclidean distances between replicates prove to be substantial and not zero. The implications of these statements warrant further consideration. Ideally, Euclidean distances assume that the descriptors are uncorrelated, but this is not so.

In the present study, Euclidean distance seemed to be the best approach. The multidimensional unfolding solution showed little difference between the two methods within the limits of accuracy of the data.

The use of suitable product-orientated terminology is essential in profiling. Ideally, all descriptors should be clearly understood with minimal ambiguity between assessors. It is considered that in descriptive profiling, assessors are confused by words and their responses biased by preconceived ideas of foods (Schiffman *et al.*, 1981). Schiffman advocates that for interpretation purposes similarity assessments should be followed by the use of words, but never the reverse. A number of examples are given indicating considerable individual variation in the descriptions of the products but in most examples, the words are of a non-specific nature covering a wide range. Furthermore, as far as one can tell, the assessors in these studies were not trained in the use of words. In any systematic profiling exercise, considerable attention is paid to the words using group discussion, illustration by reference standards and, as far as possible, words included to represent every identifiable characteristic relevant to the product. Free-choice word profiling where each assessor uses his/her own vocabulary to describe a particular product, the data being analysed by Procrustes analysis (Williams & Langron, 1984) is recently being applied to many products with great success. Thus descriptive language is not necessarily an inadequate medium for communication of flavour characteristics perceived by different people. For many purposes this information is indispensable.

The particular panel of assessors in this study was unfamiliar with assessing dissimilarities but it is clearly evident that an untrained panel can provide meaningful results, although the stimulus spaces and dimensions as derived by MDS analysis are not always easy to interpret. In the case of profiling, descriptors are established for each product and differences between them readily discernible from the profiles themselves.

One of the main disadvantages of the direct method is the number of comparisons involved which with a substantial number of stimuli increases rapidly to unmanageable level (number of comparisons including self-comparisons $= n(n+1)/2$, where n is the number of stimuli). In the present instance, with only seven samples (a maximum of three paired comparisons on any one occasion) involved the assessors coming in for some 20 days. Fatique and loss of interest on the part of the assessor may occur, especially in primarily experimental (scientific) investigations. This might result in unreliable assessment, but this suggestion is largely speculative. Even so, the importance of motivation and of management support in practical studies is well recognized and may be easier to achieve in applications than in methodological studies. It should be added that profiling allows data on different stimuli to be accumulated over a period of time, whilst with direct comparisons, an additional sample (new) would have to be compared with all the previously compared samples.

Acknowledgments

The authors wish to thank Beecham Products as collaborators in these studies, research students and staff of the Department of Food Science for their participation, and Ms P. Simpson for helping with the manuscript preparation.

References

Bednarczyk, A.A. (1973). *Identification and Evaluation of the Flavour Significant Components of Ginger Essential Oil.* Ph.D. Dissertation, University of Maryland, College Park.

Bednarczyk, A.A. & Kramer, A. (1975). Identification and evaluation of the flavour significant components of ginger essential oil. *Chemical Senses and Flavour,* **1,** 377–386.

Carroll, J.D. & Chang, J.J. (1970). Analysis of individual differences in multidimensional scaling via an N-way generalisation of 'Eckart-Young' decomposition. *Psychometrika,* **35,** 283–319.

Carroll, J.D. & Wish, M. (1974). Models and methods for three-way multidimensional scaling. In: *Contemporary developments in mathematical psychology,* Vol. 11 (edited by D.H. Krantz, R.C. Atkinson, R.D. Luce and P. Suppes). San Francisco: W.H. Freeman.

Chauhan, J. (1982). *Characterisation and Discrimination of Soft Drinks by Sensory Methods.* Ph.D. Thesis, University of Reading.

Constantine, A.G. & Gower, J.C. (1978). Graphical representation of asymmetric matrices. *Applied Statistics,* **27,** 297–304.

Davison, M.L. (1983). *Multidimensional Scaling.* New York: John Wiley and Sons.

Harper, R. (1977). Flavour characterization and its development. In: *Olfaction and Taste,* Vol. VII (edited by J. le Magnen and P. MacLeod), pp. 393–400. London: Information Retrieval Ltd.

Harper, R., Bate-Smith, E.C. & Land, D.G. (1968a). *Odour description and odour classification.* London: Churchill, J. & A.

Harper, R., Bate-Smith, E.C., Land, D.G. & Griffiths, N.M. (1968b). A glossary of odour stimuli and their qualities. *Perfumery & Essential Oil Record,* **59,** 22–37.

Harper, R., Land, D.G., Griffiths, N.M. & Bate-Smith, E.C. (1968c). Odour qualities: a glossary of usage. *British Journal of Psychology,* **59,** 231–252.

Kruskal, J.B. & Wish, M. (1978). *Multidimensional Scaling.* Beverly Hills, CA: Sage Publications.

Moskowitz, H.R. & Von Sydow, E. (1975). Computer derived perceptual maps of flavours. *Journal of Food Science,* **40,** 788–792.

Moskowitz, H.R. & Barbe, C.D. (1976). Psychometric analysis of food aromas by profiling and multidimensional scaling. *Journal of Food Science,* **41,** 567–571.

Pangborn, R.M. (1984). Sensory techniques of food analysis. In: *Food Analysis Principles and Techniques. Volume I. Physical Characterisation* (edited by D.W. Gruenwedel and J.R. Whitaker), pp. 37–93. New York: Marcel Dekker Inc.

Rosenberg, S. & Sedlak, A. (1972). Structural representations of perceived personality trait relationships. In: *Multidimensional Scaling* Vol. II (edited by A.K. Romney, R.N. Shepard & S.B. Nerlove), pp. 133–162. New York & London: Seminar Press.

Schiffman, S.S., Reynolds, M.L. & Young, F.W. (1981). *Introduction to Multidimensional Scaling-Theory, Methods and Applications.* New York: Academic Press.

Schonemann, P.H. (1970). On metric multidimensional scaling. *Psychometrika,* **35,** 349–365.

Spence, I. & Domoney, D.W. (1974). Single subject incomplete designs for nonmetric multidimensional scaling. *Psychometrika,* **39** (4), 469–490.

Thomson, D.M.H. (1981). *An Investigation of Non-Verbal Sensory Techniques for Evaluation Meat Flavour.* Ph.D. Thesis, University of Bristol.

Thomson, D.M.H. & MacFie, H.J.H. (1983). Is there an alternative to descriptive sensory assessment? In: *Sensory Quality in Foods and Beverages: Definition, Measurement and Control* (edited by A.A. Williams and R.K. Atkin), pp. 96–107. Chichester: Ellis Horwood.

Torgerson, W.S. (1958). *Theory and Methods of Scaling.* New York: John Wiley and Sons.

Weinberg, S.L., Carroll, J.D. & Cohen, H.S. (1984). Confidence regions for Indscal using the jack-nife and bootstrap techniques. *Psychometrika,* **49** (4), 475–491.

Williams, A.A. (1983). Defining sensory quality in foods and beverages. *Chemistry and Industry,* 740–745.

Williams, A.A. & Langron, S.P. (1984). The use of free-choice profiling for the evaluation of commercial ports. *Journal of the Science of Food and Agriculture,* **35,** 558–568.

Young, F.W. (1970) Nonmetric multidimensional scaling: recovery of metric information. *Psychometrika,* **35,** 455–474.

Journal of Food Technology (1986) **21**, 189–197

Effect of non-enzymic browning, starch and sugars on total cyanide determination in cassava by an enzymic assay

M. B. ARIHANTANA AND K. A. BUCKLE*

Summary

Maillard (non-enzymic) browning pigments and gelatinized starch interfered in the assay of total cyanide in cassava products by an alkaline picrate-enzymic method. Interference from browning pigments in cyanide determinations was overcome by adding charcoal followed by filtration, while gelatinized starch was precipitated by the addition of ethanol. Glucose, fructose and maltose but not sucrose also interfered in the assay and produced high total cyanide results. A more specific reaction than alkaline picrate is still required for free cyanide estimations in cassava products.

Introduction

Cassava (*Manihot esculenta* Crantz) roots are an important dietary staple for possibly as many as 300 million people in tropical countries (Nestel, 1973; Okezie & Kosikowski, 1982). Increasing world population coupled with the limited availability of energy in some countries has prompted a recent surge of interest in cassava, not only in its traditional forms as human food and for specialized starches, but also for animal feedstuffs and other industrial uses.

Cassava roots and tissues can contain considerable quantities of the cyanogenic glucoside linamarin which, together with smaller levels of lotaustralin, is hydrolysed by the endogenous enzyme linamarase liberating hydrogen cyanide when the plant tissue is damaged (Conn, 1969; Coursey, 1973). The utilization of cassava for human and animal food is limited by the possibility of chronic and acute cyanide toxicity when untreated or poorly processed cassava is consumed continuously.

In Indonesia cassava is processed into a variety of foods, the most popular being tape (peujeum), kripik and krupuk (Reynvaan & Vos, 1954; Stanton & Wallbridge, 1969). Tape is steamed cassava which is then fermented with ragi, an inoculum consisting of a mixture of rice flour and spices. Kripik is produced from peeled fresh tubers which are thinly sliced, dried and fried in oil. Krupuk is made from cassava flour which is steamed, dried and fried, or from grated tubers, which are flattened and formed into strips, steamed, dried and fried in oil.

Determination of the cyanide level in cassava and cassava products is important to assess their toxicity and the adequacy of detoxification procedures applied to reduce the cyanide content. Cooke and co-workers developed a method using the purified enzyme linamarase for estimating total cyanide in cassava products (Cooke, 1978, 1979; Cooke, Blake & Battershill, 1978). Ikediobi, Onyia & Eluwah (1980) modified Cooke's enzyme assay, replacing the pyridine/pyrazolone method of free cyanide quantitation (follow-

Authors' address: Department of Food Science and Technology, The University of New South Wales, P.O. Box 1, Kensington, NSW 2033, Australia.

*To whom correspondence should be addressed.

ing enzymic hydrolysis of cyanogenic glucosides) with the well known but less specific alkaline picrate method. This obviated the need for the rather more complex chemicals used in the former method, and the need for a fume cupboard. The authors claimed that a trained operator could analyse at least 700 samples/day, yet Rao & Hahn (1984), who recently automated the Cooke method using an autoanalyser, claim only 300 samples/day. The method of Ikediobi *et al.* (1980), however, has not been applied to cassava products which have undergone non-enzymic browning (NEB), which produce very viscous extracts or which contain high levels of reducing sugars (e.g., fermentation products). The present paper outlines the effect of NEB pigments and sugars on total cyanide levels determined by the enzymic assay procedure of Ikediobi *et al.* (1980).

Materials and methods

Dark-skinned cassava tubers from 13 month old plants (cultivar M.Aus 4) were obtained by overnight air freight from Australian Cassava Products Pty Ltd, Bundaberg, Queensland. The tubers (5–7 cm diameter) were washed free of adhering soil and peeled.

Preparation of gelled extracts of dried cassava

Cassava starch (20 g) was mixed with 250 ml extract of dried cassava (Ikediobi *et al.*, 1980) then heated to 60–70°C and stirred continuously until a sticky, gelled extract was formed. Gelled extracts were divided into three 75 ml portions. One portion was used to prepare an extract for total cyanide determination following the procedure of Ikediobi *et al.* (1980). The other two portions were added to the same volume of ethanol to precipitate the gelatinized starch. The supernatant was decanted, the ethanol evaporated in a rotary vacuum evaporator at 50°C and the residue centrifuged for 5 min at 3000×**g**. The clear extract obtained was used for the analysis of total cyanide content.

Preparation of extracts from browned cassava and cassava products

Fresh peeled tubers were cut into 2 mm thick slices and fried for 15 min in polyunsaturated vegetable oil in an electric frying pan at 120–140°C. The slices were washed with petroleum ether (b.p. 40–60°C) to remove the remaining oil and held over a boiling water bath for 10–14 min to evaporate residual petroleum ether. Krupuk and kripik were fried for less than 15 sec in oil.

Extracts of fried cassava products were prepared by the method of Ikediobi *et al.* (1980). Gelatinized starch in these extracts was precipitated by the addition of ethanol and the supernatant decanted. Residual ethanol was evaporated, the pH adjusted to 6.8 and the residue centrifuged for 5 min at 3000×**g**. The brown extract obtained was mixed thoroughly with charcoal (5% w/v, activated decolorizing powder, Ajax Chemicals, Sydney, Australia) and filtered with a Sartorius Clarification kit (0.45 μm, 47 mm diam.). The clear extract obtained was used for the analysis of total cyanide content.

Preparation of fermented cassava

Fresh cassava tubers were peeled, sliced lengthwise in half and steamed for 25 min until tender. The tubers were cooled at room temperature then inoculated with finely powdered ragi (0.3% w/w, NKL, Solo) obtained from Pasar Badung, Denpasar, Bali, Indonesia. The cassava was wrapped in aluminium foil, placed in a small plastic box and fermented for 2–3 days at ambient temperature (25–30°C). Extracts of fermented cassava were prepared following the method of Ikediobi *et al.* (1980).

Preparation of NEB mixtures

NEB mixtures of glucose/glycine, fructose/glycine and sucrose/glycine were prepared by the method of Baloch, Buckle & Edwards (1973). NEB mixtures free of sugars were prepared by passing aliquots (150 ml) of these solutions through a glass column (150×25 mm diam.) packed with the ion exchange resins AG501-X8 (Bio-Rad Laboratories, Richmond, CA, U.S.A.) or Dowex SBR (Sigma Chemical Co., St Louis, MO, U.S.A.) until all sugars were extracted (Baust, Lee & James, 1982). NEB mixtures with or without residual sugar were added to fresh cassava extracts and total cyanide content determined.

Analytical methods

NEB of cassava and cassava products was measured by the method of Baloch *et al.* (1973). Total cyanide content was determined by the enzymic procedure of Ikediobi *et al.* (1980). Sugars were determined by high performance liquid chromatography by the method of Wills, Balmer & Greenfield (1980). The equipment (Waters Associates, Milford, MA, U.S.A.) consisted of a model M6000A solvent delivery system, model R401 refractive index detector, model U6K universal injector and silica column (Radial Pak liquid chromatography cartridge, Waters Associates) in a model RCM100 radial compression module. A linear instrument recorder (Omniscribe, series D5000) was used to record peak areas.

Results and discussion

Effect of starch gelatinization on total cyanide determination

The clarification of extracts of cassava products requires filtration or centrifugation (Cooke, 1979; Ikediobi *et al.*, 1980). However, gelatinized starch in processed cassava products tended to encourage extensive gel formation which hindered filtration. Centrifugation alone was ineffective, especially when preparing extracts from fried and

Table 1. Mean total cyanide content (mg/kg±s.d.) of steam cooked cassava extract containing starch and prepared by three methods

	Method of preparation*		
Sample	A	B	C
Steam cooked cassava†	3.3±0.1‡[a]	3.5±0.1[a]	3.4±0.1[a]
Cassava extract containing gelatinized starch$	5.1±0.1[b]	4.4±0[c]	4.4±0[c]

*A. Ikediobi *et al.* (1980).

B. Method A, ethanol added, supernatant adjusted to pH 6.8, ethanol evaporated and residue centrifuged.

C. Method A, ethanol added, ethanol evaporated from the supernatant, residue adjusted to pH 6.8 and centrifuged.

†Total cyanide content of fresh cassava tuber before cooking was 12.3 mg/kg fresh weight.

‡Each value is the mean of six replications analysed in duplicate±s.d. Any two means not followed by the same letter are significantly different at the 5% level according to Tukey's test.

$Total cyanide content of fresh cassava tuber before drying was 14.2 mg/kg fresh weight.

steamed dried cassava products. Ethanol precipitated the gelatinized starch and the resulting supernatant was filtered or centrifuged without difficulty. Table 1 shows the total cyanide content of steam cooked cassava and cassava extracts containing gelatinized starch and prepared by three different methods. There were no significant differences in cyanide content ($P > 0.01$) for steam cooked cassava prepared by the three methods, but there were significant differences in cyanide content ($P < 0.05$) for cassava extracts containing gelatinized starch prepared by the same methods. Extracts containing gelatinized starch did not pass through the glass fibre filter and centrifugation alone did not produce clear extracts, leading to high absorbance readings because of turbidity.

Effect of NEB on total cyanide determination

Brown but clear extracts were obtained from fried cassava and cassava products since NEB pigments, which had formed during frying, remained in the prepared extract. NEB interfered in the measurement of total cyanide in these products and gave high results whether sugars were present or not (Tables 2, 3 and 4). Total cyanide contents in the presence of fructose were higher than in extracts containing glucose because fructose reacts strongly with alkaline picrate. The dark brown colour of heated cassava products as reflected by the NEB values (Table 4) resulted in calculated cyanide contents higher than actually present in the products. The brown colour was removed by the addition of charcoal followed by filtration (Tables 2 and 3). Charcoal treatment did not affect the measured level of cyanide (Table 5).

Table 2. Effect of NEB pigments on total cyanide content of cassava extracts

Fresh cassava extract (ml)	NEB mixture added (ml)	Charcoal added+filtration	Total cyanide content (mg/kg)
	Glucose/glycine		
5*	–	–	14.8±0.1†
5	2.5	–	15.1±1.1
5	2.5	+	39.2±0.6
5	5	–	81.5±1.2
5	5	+	31.7±0.5
	Fructose/glycine		
5*	–	–	15.4±0.2
5	2.5	–	57.1±0.4
5	2.5	+	18.4±0.7
5	5	–	80.9±1.3
5	5	+	55.3±1.2
5	Sucrose/glycine		
5*	–	–	14.5±0.2
5	2.5	–	13.9±0.1
5	2.5	+	12.0±0.4
5	5	–	12.9±0.2
5	5	+	9.7±0.1

*Different cassava samples.

†Total cyanide content of fresh cassava: mean values and standard deviation of four replications.

Table 3. Effect of sugar free NEB mixtures on total cyanide content of cassava extracts

Fresh cassava extract (ml)	NEB mixture added (ml)	H_2O added (ml)	Charcoal added +filtration	Total cyanide content (mg/kg)
	Free of glucose			
5	2.5	–	–	11.6±0.2*
5	2.5	–	+	11.0±0.1
5	–	2.5	–	11.0±0.1
5	5	–	–	5.8±0.2
5	5	–	+	5.0±0.0
5	–	5	–	5.0±0.0
	Free of fructose			
5	2.5	–	–	11.5±0.1
5	–	2.5	–	11.6±0.0
5	5	–	–	5.2±0.1
5	–	5	–	5.2±0.0
	Free of glucose and fructose			
5	2.5	–	–	11.0±0.2
5	2.5	–	+	10.2±0.1
5	–	2.5	–	10.1±0.0
5	5	–	–	5.5±0.1
5	5	–	+	5.0±0.0
5	–	5	–	5.0±0.0

*Mean values and standard deviation of four replications.

Table 4. NEB and total cyanide content of cassava products

Cassava product	NEB (absorbance at 420 nm)	Total cyanide content* (mg/kg dry weight basis)	
		Charcoal not added	Charcoal added
Krupuk	0.003	19.3±0.4	18.3±0.0†
Kripik	0.005	26.8±0.1	25.2±0.1
Gaplek (dried sliced tuber)	0.010	30.6±0.6	28.6±0.2
Fried krupuk	0.015	18.6±0.4	14.6±0.0
Fried kripik	0.026	25.9±0.1	20.6±0.1
Fried fresh tuber	0.042	34.1±0.2	15.4±0.2

*Mean values and standard deviation of four replications.

†Significant differences at the 5% level between charcoal added and not added.

Table 5. Recovery of cyanide from extracts of cassava and cassava products

Sample	CN^- added (mg/kg)	Linamarin added (mg/kg)	Total cyanide content (mg/kg fresh weight)		Recovery (%)
			Charcoal not added	Charcoal added and filtered	
Fresh cassava extract (initial cyanide level 13 mg/kg)†	20	—	—	32.9	99.5
	40	—	—	53.5	101.3
	60	—	—	73.8	101.3
	80	—	—	92.8	99.8
	100	—	—	112.7	99.7
	120	—	—	135.1	101.7
Extract of dried cassava (initial cyanide level 4.4 mg/kg)†	20	—	—	24.6	101.0
	40	—	—	44.9	101.3
	60	—	—	63.9	99.2
	80	—	—	83.5	98.9
	100	—	—	104.2	99.8
	120	—	—	123.4	99.2
Fresh cassava extract (initial cyanide level 15.4 mg/kg)†	—	500	16.3	16.3	100
	—	1000	17.4	17.4	100

*Mean of four replications.
†Different cassava cultivars.

Effect of sugars on total cyanide determination

Glucose, fructose and maltose were also found to interfere in the analysis of total cyanide in cassava products (Tables 2, 6 and 7). A deep orange colour resulted from reaction of these sugars and alkaline picrate giving high spectrophotometer readings and high total cyanide results. Sucrose was the only sugar present in cassava products which did not react with alkaline picrate (Table 7), but during heating it was hydrolysed into small amounts of glucose and fructose leading to interference (Table 2). These results do not agree with those of Ikediobi *et al.* (1980) who reported that glucose did not react with alkaline picrate.

Table 6. Effect of added glucose and fructose on total cyanide content of fresh cassava extract

Glucose added (mg/kg)	Fructose added (mg/kg)	Total cyanide content (mg/kg)
—	—	10.7±0.4*
100	—	16.1±0.1
200	—	20.4±0.2
300	—	26.9±0.1
—	100	18.6±0.3
—	200	24.3±0.1
—	300	29.6±0.1
100	100	27.0±0.2
200	200	35.7±0.5
300	300	39.1±0.3

*Mean values and standard deviation of four replications.

Table 7. Absorbance at 490 nm of sugar solutions* subjected to the enzymic cyanide assay of Ikediobi *et al.* (1980)

Sugar concentration (g/100 ml)	Added sugar			
	Glucose	Fructose	Sucrose	Maltose
0.3	0.2	0.2	0	0.1
0.5	0.3	0.4	0	0.3
1.0	0.7	0.8	0	0.6

*Mean of duplicate determinations.

The sugars present in cassava products were removed by filtration through a column of ion-exchange resin which retained 95–100% of mono-saccharides (glucose and fructose) and 45–85% of disaccharides (sucrose and trehalose) as reported by Baust *et al.* (1982). Linamarin was also removed since it contains hydroxyl groups, leading to undetectable cyanide levels in sample extracts. The Dowex SBR ion-exchange resin retained not only sugars but also NEB colour prepared from fructose (Table 3).

The sugar content of some cassava and cassava products is reported in Table 8. Sucrose formed the bulk of the sugars in these products except in tape (fermented cassava) which also contained fructose, glucose and maltose. The highest concentration of glucose (> 7.2%) was attained after 48 hr fermentation. The concentration of sugars

Table 8. Sugar levels in cassava and cassava products

Cassava product	Sugar content (g/100 g)			
	Glucose	Fructose	Sucrose	Maltose
Fresh	trace	trace	0.8	0
Steamed	trace	trace	0.7	0
Fried	0.1	0.2	0.9	0
Dried	0.6	0.6	2.4	0
Kripik	0	0.1	0.3	0
Krupuk	0.1	0.2	0.5	0
Tape:				
Fermented 24 hr	1.6	0.3	0.2	0.8
Fermented 48 hr	> 7.2	0.4	0.2	1.9
Fermented 72 hr	> 7.2	0.4	0.2	0.9
Fermented 96 hr	> 7.2	0.4	0	0.5

in sample extracts was also low except in the fermented cassava products and consequently interference was greater in these products.

A quantitative method using alkaline picrate for free cyanide quantitation has been employed by Wood (1965) who reported that aldehydes, acetone and hydrogen sulphide gave a red colour with alkaline picrate. Zitnak (1973) also reviewed earlier studies of the specificity of the picrate reaction. However, there are several factors (e.g., sugars, NEB, starch) which interfere in the alkaline picrate assay to determine the total cyanide content of cassava and cassava products.

Conclusions

The work presented here shows that during heat processing (drying, frying) of cassava products NEB pigments are formed and the brown colour is extracted during cyanide assays leading to erroneous results for total cyanide content using alkaline picrate for cyanide quantitation. Cassava fermented using ragi as an inoculum contained glucose, fructose, sucrose and maltose. These sugars (except sucrose) interfered in total cyanide determinations by the method of Ikediobi *et al.* (1980) which is applicable for cassava and cassava products only if NEB pigments and sugar are not present. The method used in the present study is applicable for total cyanide determinations on cassava products in the presence of both NEB pigment and low levels of reducing sugars but is not applicable for fermented products (e.g. tape) containing high levels of reducing sugars. A more specific method for measuring free cyanide levels in cassava products is still required.

Acknowledgments

M. B. Arihantana thanks the Commonwealth of Australia and the International Development Program of Australian Universities and Colleges for the award of an IDP Fellowship.

References

Baloch, A.K., Buckle, K.A. & Edwards, R.A. (1973). Measurement of non-enzymic browning of dehydrated carrot. *Journal of the Science of Food and Agriculture*, **24**, 389–398.

Baust, J.G., Lee, R.E. & James, H. (1982). Differential binding of sugars and polyhydric alcohol to ion-exchange resins: inappropriateness for quantitative HPLC. *Journal of Liquid Chromatography*, **5**, 767–779.

Conn, E.E. (1969). Cyanogenic glucosides. *Journal of Agricultural and Food Chemistry*, **17**, 519–526.

Cooke, R.D. (1978). An enzymatic assay for the total cyanide content of cassava. *Journal of the Science of Food and Agriculture*, **29**, 345–352.

Cooke, R.D. (1979). *Enzymatic Assay for Determining the Cyanide Content of Cassava and Cassava Products*, pp. 1–14. Cali, Columbia: Centro International de Agricultura Tropical (CIAT). Series 05EC.

Cooke, R.D., Blake, G.G. & Battershill, J.M. (1978). Purification of cassava linamarase. *Phytochemistry*, **17**, 381–383.

Coursey, D.G. (1973). Cassava as foods: toxicity and technology. In: *Chronic Cassava Toxicity* (edited by B.L. Nestel and R. MacIntyre), pp. 27–36. Ottawa, Canada: International Development Research Centre. Report IDRC–010e.

Ikediobi, C.O., Onyia, G.O. & Eluwah, C.E. (1980). A rapid and inexpensive enzymatic assay for total cyanide in cassava (*Manihot esculenta* Crantz) and cassava products. *Agricultural and Biological Chemistry*, **44**, 2803–2809.

Nestel, B.L. (1973). Current utilization of future potential for cassava. In: *Chronic Cassava Toxicity* (edited by B.L. Nestel and R. MacIntyre), pp. 11–26. Ottawa, Canada: International Development Research Centre. Report IDRC–010e.

Okezie, B.O. & Kosikowski, F.W. (1982). Cassava as food. *Critical Reviews in Food Science and Nutrition*, **17**, 259–275.

Rao, P.V. & Hahn, S.K. (1984). An automated enzymic assay for determining the cyanide content of cassava (*Manihot esculenta* Crantz) and cassava products. *Journal of the Science of Food and Agriculture*, **35**, 426–436.

Reynvaan, J. & Vos, L. (1954). Cassava production. *De Surinaamese Landbouw*, **2**, 202–208.

Stanton, W.R. & Wallbridge, A. (1969). Fermented food processes. *Process Biochemistry*, **4**, 45–51.

Wills, R.B.H., Balmer, N. & Greenfield, H. (1980). Composition of Australian foods. 2. Methods of analysis. *Food Technology in Australia*, **32**, 198–204.

Wood, T. (1965). The glucoside content of cassava and cassava products. *Journal of the Science of Food and Agriculture*, **16**, 300–305.

Zitnak, A. (1973). Assay methods for hydrocyanic acid in plant tissue and their application in studies of cyanogenic glucosides in *Manihot esculenta*. In: *Chronic Cassava Toxicity* (edited by B.L. Nestel and R. MacIntyre), pp. 89–96. Ottawa, Canada: International Development Research Centre. Report IDRC–010e.

(Received 21 March 1985)

Journal of Food Technology (1986) 21, 199–208

Kinetics of sun and air drying of different varieties of seedless grapes

M. RIVA AND C. PERI

Summary

The drying kinetics and the quality parameters of seedless grapes were evaluated in a screening test carried out to select the variety or varieties best suited to the production of dried grapes. Experiments were carried out on a pilot scale by sun drying and air convection drying under controlled conditions and with the grapes pretreated by ethyloleate dipping. The kinetics data, analysed according to a simplified form of Fick's law, allow the calculation of a rate constant, which we tried to correlate to the grapes' geometrical parameters: i.e., the berries' average radius and skin thickness. A systematic computing procedure showed that the following simple relationships were well correlated to the experimental values and could be used to forecast drying kinetics:

(a) Sun drying: $K = 0.05 - 12.8 \times R^2 \times \mathrm{Th}$
(b) Air drying: $K = 0.42 - 87.8 \times R^2 \times \mathrm{Th}$

where:

K = rate constant, (per hr),
R = average berries; radius (cm),
Th = average skin thickness ($\mathrm{cm} \times 10^{-3}$).

The following scale of drying speeds for the varieties was found:

Ruby > Emerald > Thompson > Delight > Rodi

with a two-fold decrease of the rate constant value from Ruby to Rodi, which are respectively the fastest and the slowest drying varieties. Composition and colour data show that experimental samples compare favourably with commercial samples of dried grapes, as a consequence of faster drying due to the dipping pretreatment and careful control of drying conditions.

Introduction

World production of dried grapes averages about 700 000 tons per year. The major producers are: U.S.A. (about 35%), Turkey (31%) and Greece (15%). About 40% of this production reaches the international market and is mainly directed to the industrialized countries of Europe, where dried grapes are used as ingredients by the confectionery industry. Italy, which was traditionally a producer of dried grapes, has become a large scale importer of this commodity with about 15 000 t per year mainly imported from Turkey (77%). This fact, and the parallel increase in wine and table grape surpluses suggest the resumption of dried grapes production in Italy through the partial reconversion of existing vineyards.

Authors' address: Istituto di Tecnologie Alimentari, Università di Milano, Via Celoria, 2-20133, Milano, Italy.

Experiments are under way for the selection of suitable seedless varieties with high production yields and optimum drying characteristics. This paper reports data on the kinetics and quality aspects of sun drying and air drying of five varieties obtained by an experimental cultivation in Apulia, a southern Italian region typically suited for grape production. The choice of the experimental conditions for the drying operation was based on a state-of-the-art knowledge of industrial practice and on the results of previous experiments (Breekke & Nury, 1964; Exarchos, 1970; Daris, 1977; Riva & Peri, 1983; Peri & Riva, 1984). Energy saving and quality preservation were considered as critical parameters for the optimization of the process. Grapes were surface treated by dipping in ethyloleate, which greatly increases the drying rate by altering the waxy layer structure at the grape surface and by reducing the internal resistance to water diffusion (Pointing & McBean, 1970; Riva & Peri, 1983). As a consequence of this treatment, drying time is greatly reduced, even when operating at low temperatures, with a consequent marked reduction of browning and other degradative reactions affecting quality (Peri & Riva, 1984). In one set of experiments grapes were sun dried, while in another set a drying cabinet with air under forced convection at 50°C was used. This temperature can easily be obtained by using solar collectors or recovering heat from low-enthalpy sources which are readily available in the region.

Materials and methods

Five seedles varieties were used for the drying experiments; a red grape (Ruby) and four white varieties: Thompson, Emerald, Delight and Rodi. The grapes were hand harvested and stored in a cold room at 4°C (+/−1) for a few days before the experiments. Samples were prepared for the drying tests by hand removing the stems and subsequently by sulphiting the grapes in a confined atmosphere under sulphur dioxide vapour for 6 h: the average sulphur content of the grapes was 700 ppm on fresh weight. According to a procedure suggested by Pointing & McBean (1970), pretreatment was carried out by dipping the grapes in a solution containing 3% ethyloleate and 2.5% potassium carbonate, for 3 min at 40°C, followed by free draining and a few hours' rest. For the sake of comparison, two batches of Ruby and Emerald varieties were dried both as such and after dipping.

Drying tests

About 5 kg of grapes of each batch were air dried under forced convection conditions in a cabinet equipped with five trays in parallel: therefore 1 kg of grapes was spread ever each tray in a single-berry layer. Air was fed at 50°C and 15% relative humidity at an exceedingly high flow rate so that air conditions could be considered as constant in each part of the dryer and at all times during the experiment. At 30-min intervals trays were weighed and their position in the cabinet changed in order to obtain completely comparable experimental conditions. The experiments were stopped when the weight loss during the last hour was less than 1 per thousand by weight.

Sun drying tests were carried out by exposing a single layer of berries in wooden trays in the sun during the day and sheltering them in the night. The temperature and relative humidity of the surrounding atmosphere were monitored continuously. Their values varied during the day from about 20°C and 75% relative humidity in the morning and in the evening to about 40°C and 40% relative humidity in midday hours. During the night the temperature yas roughly constant at 22°C and relative humidity was around 70% (+/−5%).

As a consequence of these variations, the drying cycle showed a typical fluctuating pattern, as can be seen in the example reported in Figure 1. Variations are relatively small and become negligible in the last part of the experiment and the continuous dotted line can be taken to represent the entire process without appreciable error. Sun drying experiments were terminated after 10 days.

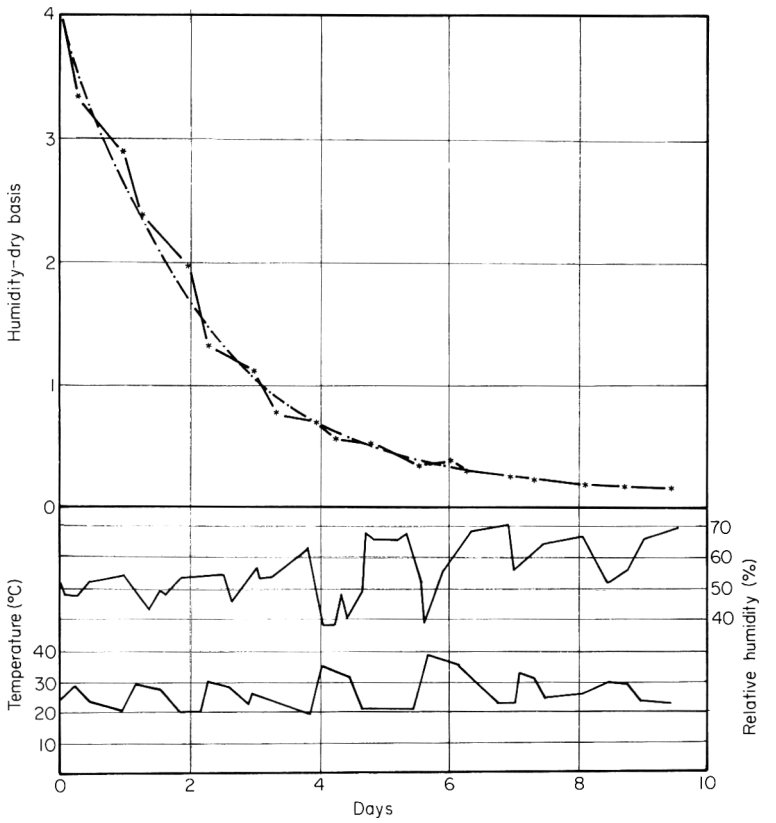

Figure 1. An example of sun drying kinetics. The upper graph shows the drying curve of Thompson variety. The lower graph reports the corresponding variations of the temperature and relative humidity of the surrounding atmosphere.

The dried grapes were washed (rinsing with water at 40°C for 10 min followed by a brief drying treatment) to remove the ethyloleate layer, which may develop an objectionable rancid flavour. The following analyses were carried out:

(i) Dry weight was determined by drying to constant weight under reduced pressure in an oven at 70°C. Data are expressed as absolute moisture, kg water per kg dry weight.

(ii) Total acidity was determined by titration according to the Italian Official Methods for musts and wines (MAF, 1964). Data are expressed as grams of tartaric acid per 100 g of product.

(iii) Reducing sugars were determined by the Fehling procedure according to the Italian Official Methods for musts and wines (MAF, 1964). Data are expressed as weight percent of glucose.

(iv) Total and free sulphur dioxide, were determined by a oxydimetric method according to the Italian Official Methods for musts and wines (MAF, 1964). Data are reported as mg sulphur dioxide/100 g products.

(v) The average volume of the berries was evaluated by a toluene displacement method. The average radius was then calculated assuming a spherical shape.

(vi) For the evaluation of skin thickness of the fresh grapes the following procedure was adopted: the skin was carefully hand removed from the frozen berries and thoroughly washed with 80° ethanol to remove flesh fragments and soluble material adhering to the internal surface. The volume of a given amount of skin material corresponding to a known number of berries was then measured by the toluene displacement method. The average thickness was calculated by dividing the volume by the known surface of the berries.

(vii) Colour: the values of lightness (L), redness (a) and yellowness (b) of fresh and dried grapes were determined using a Hunterlab colorimeter.

(viii) Relative humidity of dried samples were evaluated by direct measurement with a Rotronic Hygroscope DT.

(ix) Kinetics data are reported as absolute humidity values against the time and as drying rate against the absolute humidity. The kinetics constants were evaluated by applying a simplified form of Fick's law (Riva & Peri, 1983):

$$\frac{ns-ns_{\infty}}{ns_0-ns_{\infty}} = \frac{6}{\pi^2} \exp \frac{-\pi^2 D t}{2.3 R^2},$$

where:

ns_0 = absolute humidity at time zero;
ns = absolute humidity at time t;
ns_{∞} = absolute humidity at infinite time (equilibrium values);
t = time;
D = diffusion coefficient;
R = average radius of the berries.

The best fit of the experimental points to the above exponential equation, obtained by computer procedure, allowed the calculation of the absolute humidity at equilibrium (i.e., at infinite time, ns_{∞}) and a rate constant value (K, in hr^{-1}) including geometrical and diffusivity parameters. This constant can also be defined as the reciprocal of the time required to reduce by tenfold the total 'extractable' water (i.e., the value of $[ns-ns_{\infty}]$).

Results and discussion

Table 1 reports data of composition and geometrical parameters of fresh grapes. The five varieties differ widely in composition and ripeness, calculated as the sugars-to-acids ratio. The sulphur dioxide content of the berries is inversely related to the berry's radius. Also, there are considerable differences in the geometrical characteristics of the berries, which may play a part in diffusion phenomena. Emerald shows the maximum radius and minimum skin thickness, which may be related to a high value of the maturity index. Conversely, the low value of Thompson's maturity index may account for the lower radius and greater skin thickness.

Figures 2 and 3 show the drying curves of the grapes under forced convection and in the sun, respectively. In both charts the bottom curve represents the drying kinetics of a sample which was not subjected to the dipping pretreatment. As previously observed (Riva & Peri, 1983), drying proceeds at a rate decreasing from the beginning of the

Table 1. Composition of fresh grapes (after sulphiting and dipping)

		Ruby	Emerald	Thompson	Delight	Rodi
Dry matter	(g/100 g)	18.25	20.24	20.03	18.71	23.26
Reducing sugars	(g gluc./100 g)	16.85	16.56	16.87	14.54	21.09
Titrable acidity	(g tart.ac/100 g)	0.68	0.56	1.03	0.50	0.65
Sugars/acid ratio		24.8	36.1	16.4	29.1	32.4
Total SO_2	(mg SO_2/100 g)	64.9	48.9	77.2	72.1	64.5
Free SO_2	(mg SO_2/100 g)	18.9	14.9	19.3	19.4	16.5
Average volume of berries	(cm^3)	1.72	3.05	1.52	2.11	1.86
Average radius of berries	(cm)	0.743	0.899	0.713	0.796	0.763
Average thickness of skin	($cm \times 10^{-3}$)	3.08	3.04	5.01	4.33	5.13

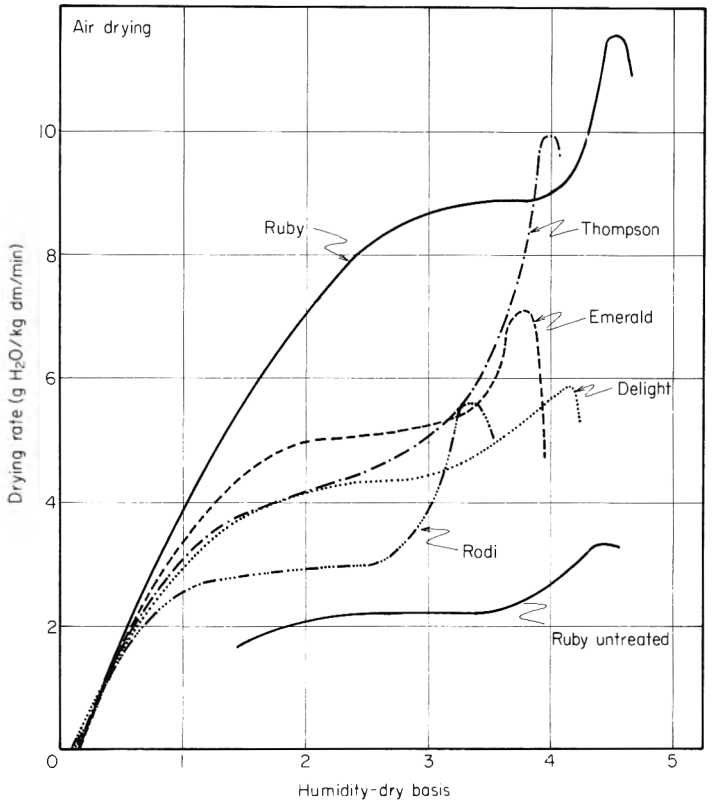

Figure 2. Drying rate *versus* absolute humidity curves in the air drying experiments.

process after an initial short period of increase due to temperature rising in the product. In all cases, after an initial rapid drop, the drying rate curves flatten out at the intermediate moisture values and decrease again at a faster pace in the final phase of the process.

Comparing the plots in the two figures it can be observed that drying curves follow a similar pattern in the air and sun drying experiments, with a kinetics which is lower in

the sun drying by almost an order of magnitude. The untreated grapes show a drying-rate three to six times lower than the sample of the same variety pretreated by the ethyloleate dipping.

The rate constants calculated from the drying experiments are reported in Tables 2 and 3 and arranged by decreasing value. Differences between varieties are very significant, with a two-fold decrease from Ruby to Rodi, which are respectively the fastest and the slowest drying varieties.

Comparing these kinetics data to the geometrical parameters reported in Table 1, it can be observed that the berry's radius and skin thickness have a kind of cumulative effect on the drying rate. In fact the Emerald variety, which has the greatest radius, shows comparatively high drying rates, which may be justified considering the limited thickness of its skin. On the other hand, the Thompson variety has a very similar rate with constant but opposite geometrical characteristics, i.e., a small radius and a large skin thickness. This observation suggest that the classical equations of diffusion limited drying of homogeneous materials cannot be applied to grape drying. A similar case has

Table 2. Kinetics data and composition of air dried grapes

		Ruby	Emerald	Thompson	Delight	Rodi
Kinetics data:						
Equilibrium moisture as absolute humidity(n_∞)	(kgH_2O/kg DM)	0.082*	0.160	0.105	0.130	0.105
Drying-rate constant (K)	(per hr)	0.265†	0.214	0.213	0.173	0.142
Composition:						
Absolute humidity (n_s)	(kg H_2O/kg DM)	0.144	0.149	0.166	0.169	0.142
Relative humidity (r.h.)	(%)	51.3	52.1	51.6	52.8	52.0
Reducing sugars	(g gluc./100 g DM)	73.5	75.1	68.3	66.9	68.7
Titrable acidity	(g tart.ac/100 g DM)	0.11	0.18	0.20	0.13	0.22
Total SO_2	(mg SO_2/100 g DM)	52.6	20.3	52.3	64.6	43.9
Free SO_2	(mg SO_2/100 g DM)	23.8	7.9	9.0	13.8	7.0

* = Ruby untreated: 0.205.
† = Ruby untreated: 0.046.

Table 3. Kinetics data and composition of sun dried grapes

		Ruby	Emerald	Thompson	Delight	Rodi
Kinetics data:						
Equilibrium moisture as absolute humidity (n_∞)	(kg H_2O)/kg DM)	0.080	0.151*	0.111	0.151	0.115
Drying-rate constant (K)	(per hr)	0.0288	0.0186†	0.0204	0.0144	0.0114
Composition						
Absolute humidity, (n_s)	(kg H_2O)/kg DM)	0.188	0.199	0.197	0.263	0.270
Relative humidity r.h.	(%)	54.1	57.1	54.7	56.2	59.1
Reducing sugars	(g gluc./100 g DM)	66.8	63.9	63.0	65.1	64.7
Titrable acidity	(g tart.ac/100 g DM)	0.13	0.11	0.20	0.16	0.11
Total SO_2	(mg SO_2/100 g DM)	10.5	4.6	10.5	5.9	9.7

* = Emerald untreated: 0.225.
† = Emerald untreated: 0.0052.

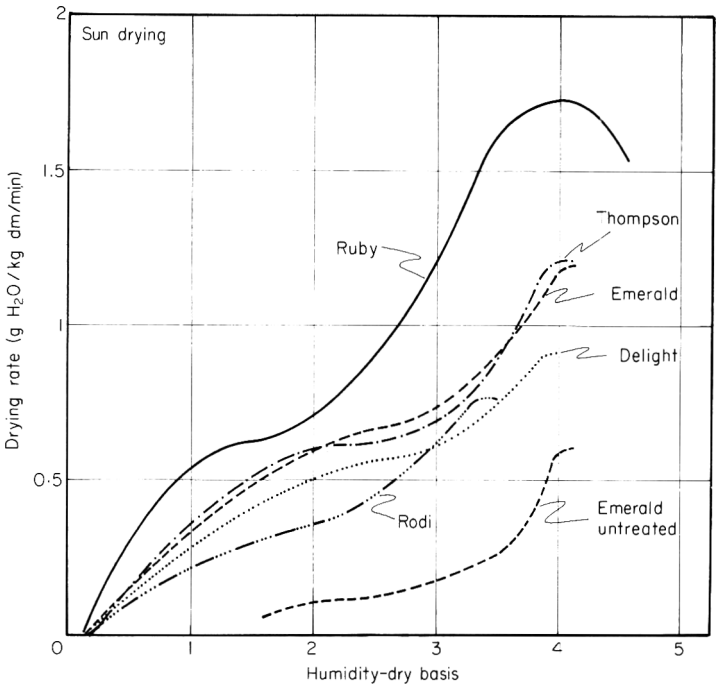

Figure 3. Drying rate *versus* absolute humidity curves in the sun drying experiments.

been thoroughly investigated by Bakshi & Chhinnan (1984), studying the kinetics of rice and corn drying which can be considered as composite spherical bodies of two different materials consisting of an inner spherical core of one component and an outer concentric shell of another component. The study carried out by these authors is based on the solution of the transport equations by a mathematical modelling technique and the optimization of transport parameters by comparing experimental and predicted values of drying kinetics and time.

In a more empirical approach we tried to correlate the rate-contrast value calculated according to the classical diffusion expression, to a value combining both geometrical parameters. In fact, a systematic computing procedure led to the observation that the constant rate value is inversely related to the product of the square of the average radius times the skin thickness. This relationship is shown in Fig. 4. The experimental data fit, with a high correlation coefficient, straight lines according to the following equations:

For sun drying: $K = 0.05 - 12.8 \times \mathrm{Th} \times R^2$ (corr.coeff. = 0.986),

For air drying: $K = 0.42 - 87.8 \times \mathrm{Th} \times R^2$ (corr.coeff. = 0.977),

where:

K= rate constant, (per hr);
R= average berries' radius (cm),
Th = average skin thickness ($\mathrm{cm} \times 10^{-3}$).

This result may be taken as an indication of the relative importance of the two parameters on the drying kinetics. Furthermore, when the K value obtained in previous

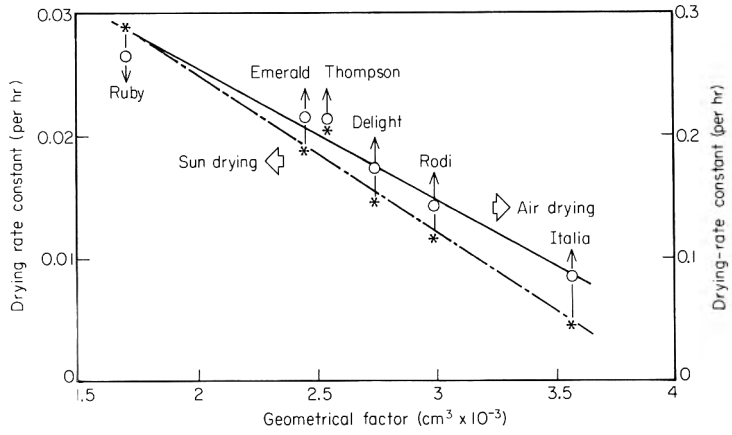

Figure 4. Graph shows the straight relationship between the drying rate constant and a geometrical factor of berries.

experiments on a different grape variety (Italia) having a much larger berry radius, was plotted (Fig. 4), a very good agreement was found. After confirmation by further experiments we expect this relationship to be useful in predicting the drying rate of grapes.

In Tables 2 and 3 the kinetics data and analytical parameters of dried grapes are also reported. The residual moisture is higher in sun dried than in air dried grapes as a result of higher equilibrium moisture values and lower drying rates. Futhermore, the equilibrium moistures calculated by extrapolating the drying data are considerably higher than the values that would be achieved if the drying led to a true equilibrium with the drying atmosphere. Considering that the relative humidity of the air in the sun drying and the air drying experiments was 40 and 15%, the equilibrium moisture calculated from the sorption isotherms (Peri & Riva, 1984) should be of about 0.09 and 0.02, respectively. This means that the particular structure of the grapes prevents the system from reaching equilibrium with the surrounding atmosphere, by promoting the formation of an almost impermeable superficial layer. This phenomenon is the more evident as skin resistance rises, as can be observed by comparing the equilibrium moisture values of untreated and pretreated grapes.

Concerning the other data in Tables 2 and 3, it is interesting to note that the residual sulphur dioxide content is much higher in air dried that in sun dried grapes. This is due to the fact that the release of sulphur dioxide is a rate limited phenomenon, due to the slow displacement of equilibria from the combined to the free form. Therefore, the sulphur dioxide is less dependent on evaporation rate and drying temperature than on drying time.

As a consequence of the longer exposure to the drying process and of the lower content of sulphur dioxide, the sun dried grapes undergo more marked browning, as seen in Fig. 5, where lightness (L) and redness (a/b) values are reported for the fresh, sun dried and air dried samples. Also, data obtained from three commercial samples are reported for comparison (Fig. 5). Among the experimental samples, Ruby has not been reported because it is a red variety and therefore shows colour parameters incomparable with the other samples. Air dried samples have higher lightness and

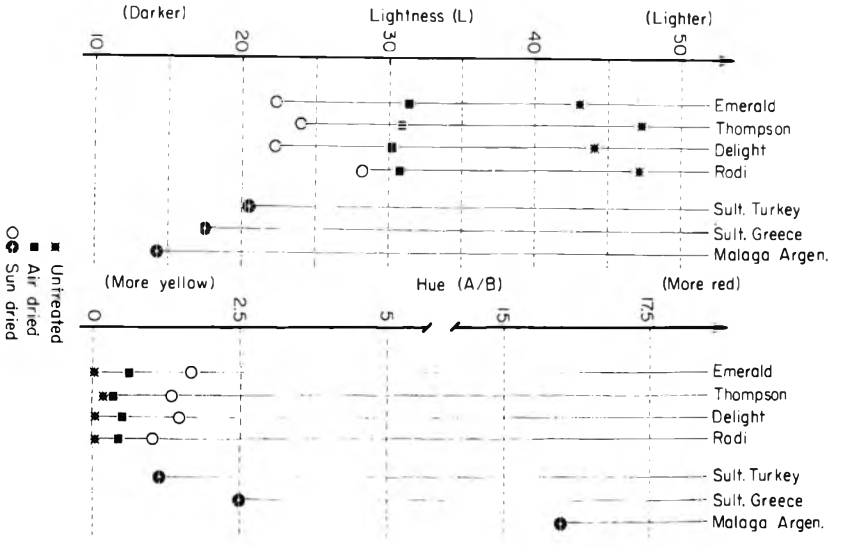

Figure 5. Colour parameters of fresh and dried grapes. The experimental samples are compared with three commercial raisins.

lower redness values compared with the sun dried samples. This difference is particularly significant if we consider that air drying has been carried out in more drastic conditions, leading to lower residual moisture. The commercial samples are considerably darker than the experimental ones, which may be due to the fact that they were dried in the sun for very long times, without prior dipping.

Conclusions

The results of this research show that the various seedless varieties have very different drying kinetics as a result of different berry radius and skin thickness. A simple inverse ratio was found between an empirical geometrical factor given by the product of the square of the radius multiplied by the skin thickness and the drying rate constant, in both the sun and air drying experiments. It was found that this relationship, which can be of practical interest in predicting the drying behaviour of grapes, holds true with a correlation coefficient of about 0.98 over a wide range of grape dimensions and characteristics. Compared with commercial raisins, the dried grapes obtained in our experiments are much better in quality, being lighter in colour and shelf stable, even in the absence of antimicrobial additives and at very low residual sulphur dioxide content. This is mainly due to the positive effects of the dipping pretreatment on drying rate and skin integrity (Bolesco, 1984). These products are therefore particularly attractive for consumption as natural snacks.

Acknowledgment

This research work was supported by CNR, Italy, special grant I.P.R.A.—sub-project 3, paper no. 583.

References

Bakshi, A.S. & Chhinnan M.S. (1984). Estimation parameters for moisture transport in foods. *Journal Food Processing Engineering*, **7**, 43–155.

Boleso, W. (1984). *Studio delle cinetiche di disidratazione osmotica dell'uva*. Thesis, University of Milan, Italy.

Breekke, J.E. & Nury, F.S. (1964). Fruits. In: *Food Dehydration* (edited by W.B. Van Ardsel and M.J. Copley) Vol. II., pp. 467–507. Westport, Connecticut: Avi Publishing Co. Inc.

Daris, B.T. (1977). La production de raisins secs à partir de varieté de table. *Bulletin de l'O.I.V.*; 557–558, 496–506.

Exarchos, C. (1970). Technologie moderne des raisins secs. *Bulletin de l'O.I.V.*, 469, 263–269.

MAF (1964). *Metodi ufficiali di analisi per vini, mosti ed aceti*. Roma, Italy, Ministero Agricoltura e Foreste.

Peri, C. & Riva, M. (1984). Etude du sechage des raisins. 2: Effet des traitements de modification de la surface sur la qualité de produit. *Sciences des Aliments*, **4**, 273–286.

Pointing, J.D. & McBean, D.M. (1970). Temperature and dipping treatment effects on drying times of grapes, prunes and other waxy fruits. *Food Technology*, **24**, 1403–1406.

Riva M. & Peri, C. (1983). Etude du sechage des raisins. 1: Effet des traitements de mc dification de la surface sur la cinetique du sechage. *Sciences des Aliments*, **3**, 527–550.

(Received 23 May 1985)

Journal of Food Technology (1986) **21**, 209–219

Simplified equation for predicting the freezing time of foodstuffs

Q. T. PHAM

Summary

A simple method is presented for predicting freezing times of predominantly aqueous foodstuffs. The method assumes that heat is released at a 'mean freezing temperature', common to all foodstuffs. Internal resistance effects are calculated for any shape from a 'mean conducting path', intermediate between the shortest and longest distances from surface to centre. The method agrees with published data better than any previously published method, except that of Pham (1984, 1985) from which it was derived. It applies equally well to different materials, geometries and final product temperatures.

Introduction

The freezing of food is an important and widespread industrial process. Therefore, a simple equation for predicting freezing times would be useful. However, exact analytical equations cannot be derived from first principles because of the complex nature of the process.

Many studies have been done on phase change phenomena in other fields of engineering, but most of them are not directly applicable to food freezing. The latter differs from other freezing phenomena in one important aspect: phase change for foodstuffs is gradual rather than sharp, and thermal properties vary continuously over a wide range of temperature. Any predictive method (other than numerical methods) has to use some kind of average or representative property values, and cannot be expected to give completely accurate solutions [an engineering accuracy of about $\pm 10\%$ is generally the best that can be expected (Cleland & Earle, 1982)].

Thus, the general approach of workers in the food freezing field has been to seek approximate or empirical relationships, rather than to try to derive exact analytical equations. Various approximate methods have been proposed in recent years, as reviewed by Cleland & Earle (1977, 1979a, 1984), Mascheroni & Calvelo (1982), Hung & Thompson (1983) and Pham (1984, 1985). The methods can be classified into two groups: (i) methods relying on analytical approximations, such as those of Plank (1941), Fleming (1967), Mascheroni & Calvelo (1982) or Pham (1984, 1985); (ii) methods relying on regression of computer results or experimental data, such as those of Cleland & Earle (1977, 1979a,b), Hung & Thompson (1983), Hayakawa, Nonino & Succar (1983) and Succar & Hayakawa (1984).

The methods vary considerably in complexity and accuracy, the number of arbitrary or empirical parameters used ranging from 0 to more than 50. Pham's (1984, 1985) equations, which involve no empirical factor for the simpler shapes, have been shown to be more accurate than other methods when checked against several hundred previously published data (Cleland & Earle, 1984). These equations can be further simplified.

*Author's address: Meat Industry Research Institute of New Zealand, P.O. Box 617, Hamilton, New Zealand.

Theory

In most freezing applications, the boundary or surface conditions are usually assumed to be of the third kind (i.e., of Newton's cooling-law type). For this situation Plank's equation (1941) is usually taken as the starting point for calculating the freezing time. This equation can be written in the general form;

$$t = \frac{LV}{hA(T_f - T_a)}(1 + Bi_s/4). \tag{1}$$

The derivation of Plank's equation assumes that: (i) freezing takes place, and hence latent heat is released, at a single temperature T_f. Thus, thermal conductivity changes sharply from k_u to k_s at that temperature. (ii) Sensible heat effects are negligible; i.e., $c_u = c_s = 0$.

Neither of these assumptions is true in practice. However, effect (i) can be accommodated by using a 'mean freezing point' somewhat lower than the temperature at which the material starts to freeze, while effect (ii) can be accounted for by separately calculating the precooling, freezing and subcooling times. These modifications result in the following equation (Pham, 1984):

$$t_i = \frac{V\Delta H_i}{hA\Delta T_{mi}}(1 + Bi_i/k_i) \tag{2}$$

where $i = 1$ for precooling, 2 for phase change, 3 for subcooling, $k_1 = 6$, $k_2 = 4$, $k_3 = 6$, $Bi_1 = (Bi_u + Bi_s)/2$, $Bi_2 = Bi_3 = Bi_s$

Equation (2) is accurate for a wide variety of freezing conditions (Pham, 1984, 1985; Cleland & Earle, 1984). However, for many purposes an expression that is easier to handle would be useful. The following approximations will be introduced:

(1) Equation (2) shows that for the precooling period, the resistance to heat transfer is about $(1 + Bi_u/12 + Bi_s/12)$, compared to $(1 + Bi_s/4)$ for the freezing period. For Tylose, meat and other predominantly aqueous materials, the two expressions differ by 30% at most. Furthermore, in ordinary applications the precooling time is never more than about 25% of the total processing time. Thus we can take $(1 + Bi_s/4)$ as the resistance to heat transfer for the precooling period as well. From similar arguments, the factor $(1 + Bi_s/4)$ can be extended also to the subcooling period.

(2) According to equation (2), the effective temperature driving force for the precooling period is the log-mean between the start and end values of the temperature difference. However, since the precooling period is short, the arithmetic mean can be used.

(3) Since subcooling times and sensible heat of subcooling are usually small, the subcooling period can be combined with the freezing period. This latent-plus-subcooling heat, ΔH_2, is assumed to be released at a mean freezing temperature T_{fm}.

The use of a *mean* freezing temperature is central to this method (Pham, 1984). The definition of T_{fm} involves replacing the varying temperature driving force $(T - T_a)$ by a mean driving force $T_{fm} - T_a$. Thus, it can be expected that T_{fm} depends on T_a, T_c, T_f and the shape of the specific heat curve. The shape of the curve and T_f do not vary greatly for most foodstuffs, so T_{fm} should depend mainly on T_a and T_c. A linear relationship is chosen for simplicity. Curve-fitting against existing freezing data (Cleland & Earle,

1977, 1979a,b; de Michelis & Calvelo, 1983; Hung & Thompson, 1983) yielded the following equation:

$$T_{fm} = 1.8+0.263T_c+0.105T_a \text{ (in °C)}. \quad (3)$$

(Note that this mean freezing temperature is different from that defined by Pham (1984), although the concept is similar.) Equation (3) applies equally to all the materials considered (Tylose, beef, carp, potato) and is probably valid for most predominantly aqueous biological materials.

Combining the foregoing approximations, one gets the following equation:

$$t = \frac{V}{hA}\left(\frac{\Delta H_1}{\Delta T_1}+\frac{\Delta H_2}{\Delta T_2}\right)(1+Bi_s/4) \quad (4)$$

where $\Delta H_1 = c_u(T_i-T_{fm})$ (5)

$$\Delta H_2 = L+c_s(T_{fm}-T_c) \quad (6)$$

$$\Delta T_1 = (T_i+T_{fm})/2-T_a \quad (7)$$

$$\Delta T_2 = T_{fm}-T_a \quad (8)$$

and T_{fm} is given by equation (3).

Equation (4) shows that the freezing time is given by Newton's law of cooling, with a correction factor for the internal resistance. Equation (4) is equivalent to that suggested by Fleming (1967), except that he used the onset-of-freezing temperature T_f instead of T_{fm}.

Calculation of Biot number for various shapes

(a) Infinite slabs, infinite cylinders and spheres. The Biot number for simple shapes is defined by:

$$Bi_s = hD/k_s \quad (9)$$

(b) Bricks. For brick-shaped blocks, Bi_s is similarly calculated using a length scale D equal to twice the 'mean' conducting path' (mcp) from the surface to the thermal centre (Pham, 1985). Pham discussed various equations that may be used to correlate the mcp to the dimensions of the brick, and concluded that a geometric mean relationship is adequate while retaining the advantage of simplicity. By regressing against available data, one obtains the following equation:

$$D = 1.46\sqrt{W_1W_2} \quad (10)$$

Equation (10) was found by a curve-fitting procedure. If extrapolated to large values of W_2/W_1 and Bi_s, it leads to the prediction that a rectangular block takes longer to freeze than an infinite slab of the same thickness. Therefore, when calculating the freezing time of rectangular blocks, the time for an infinite slab ($V/A = 2W_1$, $D = W_1$) must also be calculated and used as an upper limit.

(c) Finite cylinders. For finite cylinders, an approximate calculation method can be derived as follows: data (Cleland & Earle, 1979b) indicate that for prisms of square cross-section $W\times W$, the mean conducting path is about $2W/3$ (Pham, 1985). Hence, the freezing time for an infinite square prism of cross-section $W_e\times W_e$ is proportional to $W_e\ (1+hW_e/3k_s)$, while for an infinite cylinder of diameter D the freezing time is

proportional to $D(1+hd/4k_s)$. For the two to freeze in the same time, we must have:

$$W_e(1+hW_e/3k_s) = D(1+hD/4k_s). \quad (11)$$

This equation can be solved to give:

$$W_e/D = \frac{3}{2Bi_{sc}}(-1+\sqrt{1+4Bi_{sc}/3+Bi_{sc}^2/3}) \quad (12)$$

where $Bi_{sc} = hD/k_s$. (13)

The square cross-section $W_e \times W_e$ is thus equivalent to the round cross-section of diameter D. A reasonable assumption is that this equivalence remains valid when the rod and the cylinder are of finite lengths. Thus, we can replace a finite cylinder of diameter D and length L by a square prism with side $W_e \times W_e$ and length L, and apply the brick equation (equation 10 together with the infinite slab limit) as above.

When $Bi_{sc} = 0$ (no internal resistance), equation (12) reduces to $W_e = D$ and the method correctly reduces to Newton's cooling law ($t = V\Delta H/hA\Delta T$). When Bi_s tends to infinity (internal resistance controlling), $W_e = 3D/4$. If only a crude estimate is required, then the average of these limiting values will do:

$$W_e \simeq 0.88D. \quad (14)$$

(d) Other shapes. For other shapes the value of the length scale D or that of the mean conducting path $D/2$ must be found either by experiment or by numerical computation. For squat shaped bodies where the shortest and longest dimensions are not very different, a sufficiently accurate estimate can be made from *a priori* reasoning. For example, for an egg with shortest and longest dimensions of 40 and 60 mm, $D = 50 \pm 10$ mm. Thus if $Bi_s \simeq 1$, the maximal error due to this approximation will be only 4% (since freezing time is proportional to $1+Bi_s/4$), and the probable error will be much less. Similarly, for a cube of side W_1 the shortest dimension is W_1 and the longest is 1.732 W_1 (the long diagonal). Taking the mean between these yields, $D = 1.37\ W_1$, which is quite a good estimate. (Pham (1985) found, by working back from data, that $D/W_1 = 1.39 \pm 0.15$ for a cube. Pham also found that for rectangular blocks where the longest-to-shortest-side ratio is 2.6 or less (ratio of longest diagonal to shortest side is 3.8 or less), this simple method of calculating D leads to freezing time predictions accurate to $\pm 16\%$.) To illustrate the method a worked example is presented in the Appendix.

Results

Equation (4) was applied to four published sets of freezing data (Cleland & Earle, 1977, 1979a,b; de Michelis & Calvelo, 1983; Hung & Thompson, 1983; Hayakawa *et al.* 1983), totalling 283 freezing times. Three data points from de Michelis & Calvelo were excluded from consideration because of ambiguities ($T_c < T_a$), and the data of Hyakawa *et al.* (1983) for six infinite rectangular rods will be discussed separately. The total data cover a wide range of parameters: test materials (Tylose (23% methylcellulose in water), lean beef, ground beef, potato and carp), initial product temperatures (up to 34.5°C), final product centre temperatures (−10, −18 and −27.8°C), air temperatures (−16 to −44°C), Biot numbers (0.045–21) and shapes (slabs, infinite cylinders, spheres, bricks and finite cylinders).

Table 1 compares the method of this paper with several others over all data, using

Table 1. Comparison of various methods for predicting freezing times. (Freezing times were compared to four sets of previously published data)

Method	Mean error (%)	s.d. (%)	Error range (%)	Range enclosing 90% of data (%)
Plank (1941)*	−54.5	19.1	−81 to −16	−78 to −25
Modified Plank* (Slatter & Jones, 1972)	−8.8	13.0	−46 to +10	−35 to +4
Cleland & Earle (1982)*	−2.5	8.5	−30 to +23	−19 to +9
Hung & Thompson (1983)*	+12.8	11.8	−17 to +44	−4 to +31
Pham (1984, 1985)	+0.3	6.9	−24 to +20	−10 to +9
This paper	−0.1	7.1	−23 to +24	−11 to +10

*For these methods, Cleland & Earle's (1979b) ehtd formula was used to take into account the effect of complex geometry.

thermal property data (i.e., c_s, c_u, k_s, and L) collated by Cleland & Earle (1977) and Hung & Thompson (1983). Apart from Pham's full method (1984, 1985), from which this method is derived, the present method is the most accurate by all the criteria applied; yet it is also the simplest (apart from Plank's original equation). Cleland & Earle's (1982) and Hung & Thompson's (1983) methods, for example, each contain seven regression coefficients for the basic geometries and also require values for T_f and k_u.

In terms of mean error, the present method applies equally well to the datasets used (Table 2). Cleland & Earle's data has the least scatter, probably because most are from tests on Tylose, a synthetic substance whose properties are more reproducible than real food. When different materials, geometries and final centre temperatures are considered separately (Tables 3, 4, 5), the method applies equally well to almost all cases: the mean error remains close to 0% and the errors are always evenly distributed about the mean. The exception is for finite cylinders, for which only ten data are available.

Hayakawa *et al.* (1983) also presented data for the freezing time of six infinite rods of lean beef with rectangular section (these are the only infinite rod data available). Table 6 indicates that none of the methods tested, including finite-difference results, agrees well with this small set of data. There may be two reasons for this.

(i) There may have been experimental errors in measuring freezing conditions or in estimating material properties. For example, in Hayakawa *et al.*'s sample 6, the measured time to −20°C, 3.93 hr, is less than that calculated assuming infinite material thermal conductivity (i.e., based on Newton's cooling law), 4.07 hr. This shows that

Table 2. Accuracy of present method for individual datasets

Data from	No. of data	Mean error (%)	s.d. (%)	Error range (%)
Cleland & Earle (1977; 1979a,b)	187	0.0	5.7	−13 to +13
de Michelis & Calvelo (1983)	28	+1.1	6.5	−12 to +20
Hung & Thompson (1983)	59	−0.9	10.5	−23 to +24
Hayakawa *et al.* (1983)*	6	+1.7	10.0	−9 to +15

*Freezing time to −27.8°C

Table 3. Acuracy of present method for different materials

	No. of data	Mean error (%)	s.d. (%)	Error range (%)
Tylose	204	+0.2	6.2	−14 to +17
Lean beef	43	−0.0	8.0	−20 to +20
Ground beef	9	−2.9	15.6	−23 to +24
Potato	15	+0.8	4.8	−7 to +8
Carp	9	−0.9	12.7	−20 to +20

Table 4. Accuracy of present method for different geometries

	No. of data	Mean error (%)	s.d. (%)	Error range (%)
Slabs	119	+1.1	8.7	−23 to +24
Infinite cylinders	30	+0.1	4.9	−11 to +6
Spheres	30	+0.5	3.4	−7 to +6
Bricks	91	−2.2	5.2	−13 to +10
Finite cylinders	10	+5.0	9.9	−9 to +20

Table 5. Accuracy of present method for different final centre temperatures

T_c	No. of data	Mean error (%)	s.d. (%)	Error range (%)
−10°C	204	−0.1	5.6	−13 to +13
−18°C	70	−0.0	10.2	−23 to +24
−27.8°C	6	+1.7	10.0	−10 to +15

Table 6. Comparison of various methods for predicting freezing times of infinite rods. (Times were compared with six freezing-time data in Hayakawa *et al.*, 1983)

	Mean error (%)	s.d. (%)	Error range (%)
Plank	−14.6	6.2	−21 to −5
Modified Plank	+8.2	7.7	0 to +20
Cleland & Earle*	+21.0	8.4	+12 to +34
Hung & Thompson†	+18.4	8.5	+9 to +31
Pham (1984, 1985)	+13.1	7.9	+4 to +26
This paper	+25.0	8.6	−15 to +29
Finite differences	+8.2	8.2	−7 to +26

*Using freezing time to −15°C, which is close to Cleland & Earle's specified value of T_c (−10°C).

†Using freezing time to −20°C, which is close to Hung & Thompson's specified value of T_c (−18°C).

either the measured heat transfer coefficient was wrong or the assumed specific enthalpy change was wrong.

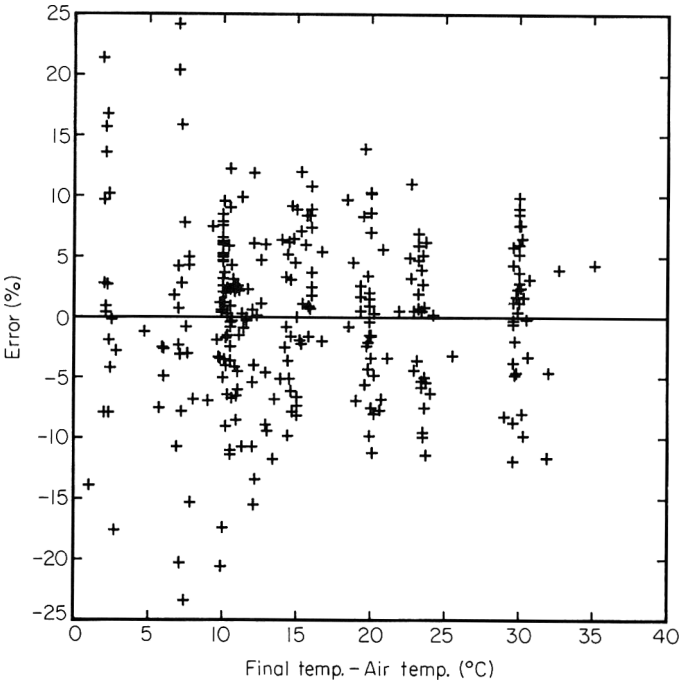

Figure 1. Influence of final centre temperature minus air temperature ($T_c - T_a$) on percentage errors.

(ii) The formulae for estimating the ehtd (number of equivalent heat transfer dimensions: Cleland & Earle, 1982) or mcp (equation 10) may be inappropriate for infinite rectangular rods. Both ehtd and mcp formulae were found by regression on data from brick freezing tests. Thus, there is no guarantee that they would hold for infinite rectangular rods.

Since the mean error for the finite-difference calculation is about one-third that of the simple formula, it is likely that both factors play a part in causing the discrepancies. Thus, more experimental data on the freezing of long prisms are needed.

Discussion

To be useful for engineering applications, a calculation method should not only be accurate, it should also be easy to apply. Therefore, it should require as few input data as possible and preferably should avoid lengthy or complex operations or reference to graphs and tables. Table 7 compares the present method with others proposed in recent years. The present method is clearly the easiest to apply (and visualize); it has no complex operations and requires knowledge of only four parameters relating to material properties (c_s, c_u, L, k_s).

The main limitation of the present method is its use of a single temperature driving force ($T_{fm} - T_a$) to describe heat transfer during the freezing and subcooling period.

Table 7. Complexity of freezing time prediction methods

	No. of empirical parameters used (simple shapes only)	No. of material parameters required	Operations used (apart from + − ×).
Cleland & Earle (1982)	7	6	—
Mascheroni & Calvelo (1982)	0	19	graphical
Hung & Thompson (1983)	14	6	—
Succar & Hayakawa (1984)	52*	12	log & exp
Pham (1984, 1985)	0	6	log
Present method	3	4	—

*Eighteen of which are found by regression.

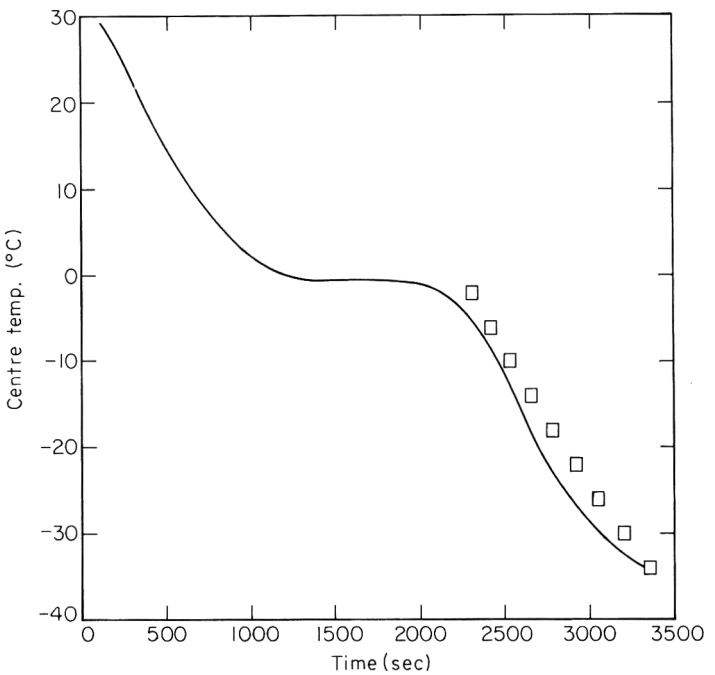

Figure 2. Comparison of centre temperature curves calculated by two methods, for Cleland & Earle's test 1 ($T_a = -40$°C). ——— finite differences, □ present method.

Thus, the method (incorrectly) predicts that the freezing time remains finite when T_c equals T_a.

However, this limitation is not expected to be serious in practice. Figure 1 shows that errors remain evenly distributed about zero, even with $T_c - T_a$ as low as 2°C, although the scatter tends to increase when T_c is close to T_a (probably due to the difficulty in determining end points accurately). Figure 2 shows curves of T_c versus time calculated by the present formula and by the finite-difference procedure, using the conditions of Cleland & Earle's (1977) run No. 32 for a flat slab of Tylose. The two

curves remain very close down to $T_c - T_a = 5°C$ or less. In practical industrial situations, T_a will be at least several degrees lower than T_c, so no difficulty should arise.

A more serious limitation is the scarcity of experimental data for the freezing of finite cylinders and rectangular rods of infinite length (or, more generally, data for the freezing of elongated bodies). This is an area that future experimenters may fruitfully investigate.

Conclusions

Simplification of Pham's (1984, 1985) full freezing prediction equations leads to the present method, which at first sight is quite similar to Fleming's (1967) method. However, the success of this approach depends on the following features: (i) the use of a mean freezing temperature, which varies with the final centre and air temperatures and is independent of the material; and (ii) the use of a 'mean conducting path' to extend the method to irregular geometries.

The method requires few empirical parameters (three for simple geometries) and data for only four material properties (c_u, c_s, k_s, and L). It does not require data for the freezing point or the thermal conductivity of unfrozen material. Yet this method agrees with experimental results better than more complex methods. It fits the data equally well for different geometries, materials and final centre temperatures.

The simplicity of the present method makes it suitable for hand calculation or for microprocessor control when computational power is limited. The 90% error range of −11–+10% is adequate for most practical purpose. If more accuracy is needed, Pham's (1984, 1985) full method may be used.

Notation

A	Surface area, m^2
Bi	Biot number, hD/k
c	volumetric specific heat, J/m^3K
D	length scale equal to twice the mean conduction path (slab thickness, cylinder diameter, sphere diameter, or length given in equation 10), m
h	surface heat transfer coefficient, W/m^2K
ΔH	enthalpy change, J/m^3
k	thermal conductivity of material, W/mK
L	volumetric latent heat of freezing, J/m^3
t	freezing time, sec
T_a, T_c, T_i	air, final centre and initial temperature, °C
T_f	temperature at which freezing commences, °C
T_{fm}	mean freezing temperature, °C
ΔT	temperature driving force, K
ΔT_m	log-mean temperature driving force, K
V	volume of body, m^3
W_e	side of equivalent square cross-section, m
W_1	shortest side of rectangular block, m
W_2	second shortest side of rectangular block, m

Subscripts

s frozen phase (−30°C)
u unfrozen phase
1 precooling
2 freezing, or freezing and subcooling.

References

Cleland, A.C. & Earle, R.L. (1977). A comparison of analytical and numerical methods of predicting the freezing times of foods. *Journal of Food Science*, **42**, 1390–1395.

Cleland, A.C. & Earle, R.L. (1979a). A comparison of methods for predicting the freezing times of cylindrical and spherical foodstuffs. *Journal of Food Science*, **44**, 958–963, 970.

Cleland, A.C. & Earle, R.L. (1979b). Prediction of freezing times for foods in rectangular packages. *Journal of Food Science*, **44**, 964–970.

Cleland, A.C. & Earle, R.L. (1982). Freezing time prediction for foods—a simplified procedure. *International Journal of Refrigeration*, **5**, 134–140.

Cleland, A.C. & Earle, R.L. (1984). Assessment of freezing time prediction formulae. *Journal of Food Science*, **49**, 1034–1042.

Fleming, A.K. (1967). Immersion freezing of small meat products. *Proceedings 12th International Congress of Refrigeration, Madrid*, **2**, 683–694.

Hayakawa, K.I., Nonino, C. & Succar, J. (1983) Two-dimensional heat conduction in food undergoing freezing: predicting freezing time of rectangular or finitely cylindrical food. *Journal of Food Science*, **48**, 1841–1848.

Hung, Y.C. & Thompson, D.R. (1983). Freezing time prediction for slab-shaped foodstuffs by an improved analytical method. *Journal of Food Science*, **48**, 555–560.

Mascheroni, R.H. & Calvelo, A. (1982). A simplified model for freezing time calculations in foods. *Journal of Food Science*, **47**, 1201–1207.

de Michelis, A. & Calvelo, A. (1983). Freezing time predictions for bricks and cylindrical-shaped foods. *Journal of Food Science*, **48**, 909–913.

Pham, Q.T. (1984). An extension to Plank's equation for predicting freezing times for foodstuffs of simple shapes. *International Journal of Refrigration*, **7**, 377–383.

Pham, Q.T. (1985). An approximate analytical method for predicting freezing times for rectangular blocks of foodstuffs. *International Journal of Refrigeration*, **8**, 43–47.

Plank, R. (1941). Beitrage zur Berechnung und Bewertung der Gefriergeschwindikeit von Lebensmitteln. *Zeitschrift fur die gesamte Kalte Industrie*, Beih Reihe **3**, 1–24.

Slatter, MA. & Jones, M.C. (1972). Development of an accurate method of predicting freezing rates in watery solids. Pap. D2, *International Symposium on Heat and Mass Transfer in Food Engineering, Wagerningen*.

Succar, J. & Hayakawa, K.I. (1984). Parametric analysis for predicting freezing time of infinitely slab-shaped food. *Journal of Food Science*, **49**, 468–477.

(Received 24 June 1985)

Appendix: worked example

Problems

Calculate the time for freezing lean beef from 15 to −18°C in the form of (a) a 0.1 m thick slab of infinite area; (b) a 0.1 m diameter sphere; (c) a 0.1 m side cube; (d) a $0.1\times1\times1$ m rectangular block. The coolant temperature is −30°C and the surface heat transfer coefficient is 16.0 W/m²K. For lean beef the thermophysical properties are: $k_s = 1.55$ W/mK, $L = 2.09\times10^8$ J/m³, $c_u = 3.65\times10^6$ J/m³K, $c_s = 1.90\times10^6$ J/m³K.

Solution

(a) Thermal factor (common to all shapes). Mean freezing temperature:

$$T_{fm} = 1.8+0.263(-18)+0.105(-30) = -6.1°C.$$

Precooling heat:

$\Delta H_1 = (3.65\times10^6)[15-(-6.1)] = 7.7\times10^7 \text{J/m}^3$.

Freezing and subcooling heat:

$\Delta H_2 = (2.09\times10^8)+(1.90\times10^6)[-6.1-(-18)] = 2.32\times10^8 \text{J/m}^3$.

Precooling temperature driving force:

$\Delta T_1 = [15+(-6.1)]/2-(-30) = 34.45°\text{C}$.

Freezing temperature driving force:

$\Delta T_2 = -6.1-(-30) = 23.9°\text{C}$.

Thermal factor:

$(\Delta H_1/\Delta T_1+\Delta H_2/\Delta T_2)/h = 7.46\times10^5 \text{ s/m}$.

(b) Geometric factors Volume to area ratio:

V/A = 0.05 m for slab,
= 0.0167 m for sphere and cube,
= 0.0417 m for rectangular block.

Meat dimension ($2\times$mcp) D = 0.1m for slab and sphere,
= (0.1 m+0.173 m)/2 = 0.137 m for cube (taking mean of side and diagonal),
= $1.47\sqrt{0.1\times1.0}$ = 0.465 m for rectangular block.

(Note: D for the cube can also be calculated from equation 7).

Thus, geometric factors are: $\frac{V}{A}(1+Bi_s/4) = \frac{V}{A}(1+hD/4k_s)$

= 0.063 for slab,
= 0.021 for sphere,
= 0.023 for cube,
= 0.092 for rectangular block.

(c) Freezing times. Multiplying the thermal factor (7.46×10^5s/m) from (a) by the geometric factors from (b), one obtains the following freezing times: 47000 sec or 13.1 hr for the slabs, 15666 sec or 4.4 hr for the sphere, 17158 sec or 4.8 hr for the cube, 68632 sec or 19.1 hr for the rectangular block. Since the rectangular block cannot take longer to freeze than the slab, a freezing time of 13.1 hr should be taken for the block.

Journal of Food Technology (1986) **21**, 221–228

A general method for predicting the water activity of simple and multi-component mixtures of solutes and non-solutes

M. CAURIE

Summary

Caurie's (1985) equation for predicting the water activity (a_w) of binary solutions has been revised by replacing molal concentration units and activity coefficient with equivalent expressions for weight concentration (g/kg H_2O). Results obtained using the revised equation, which now applies to both solutes and non-solutes, agree closely with measured literature values. The equation reveals that the relationship between the weight concentration of solute/non-solute binary solution or dispersion and the corresponding a_w exhibits a minimum for carbohydrates and proteins. Electrolytes exhibit no such minimum and are the most effective in depressing a_w while proteins are the least effective. The substitution of the new binary equation into the Ross (1975) equation results in an equation for a mixture identical with the recently corrected Ross one (Caurie, 1985), except that the correction factor in the new equation is expressed in g/kg H_2O making it applicable to simple and multi-component mixtures of both solutes and non-solutes. The new equation has been applied to literature data from various sources and has been found to agree very closely with measured values.

Introduction

The rate of a number of chemical and biological reactions in foods depends on the water activity or the effective concentration of water. For this reason, when formulating new foods in the laboratory it is desirable to keep the chemical activity of water at a level that will minimize the deteriorative reactions. Thus the ability to predict the water activity (a_w) of the formulation from the properties of the components, which may include both solute and non-solute, is needed. However, most of the available methods for making this prediction are limited to the aqueous phase because they are based on defined molal concentrations which non-solutes, which constitute the bulk of most dry food materials and contribute substantially to a_w depression, do not possess.

A method suggested by Chuang & Toledo (1976) for predicting the a_w contributions of insoluble solids suffers from severe limitation in that it only applies to binary mixtures. The Ross (1975) method is applicable to any number of ingredients, as is its recent correction (Caurie, 1985) but these methods are limited to the aqueous phase.

To overcome the problem posed by the lack of defined molality for non-solutes it is proposed in this paper to replace the molal concentration units used in Caurie's (1985) corrected Ross equation with weight concentration units. This will allow the equation to be applied to both solute and non-solute ingredients of a food material.

Author's address: Department of Food Science and Technology, University of Jos, Makurdi Campus, Makurdi, Benue State, Nigeria.

Methods

A binary solution containing only one solute at molality m_s has been described (Caurie, 1985) by the equation

$$a_w^0 = 1 - \frac{m_s}{55.5}(1+\ln \gamma_s^0), \tag{1}$$

where γ_s^0 is the activity coefficient of the component solute and the superscript 0 indicates that the solution contains only one solute. The application of this equation requires a knowledge of an activity coefficient at the concentration of the solute, which may not always be available (Ross, 1975). For non-solutes this activity coefficient does not even exist (Bone, Shannon & Ross, 1975) because of a lack of any defined molality for these components.

The problem posed by this dearth of information may be solved if γ_s^0 could be expressed in terms of m_s. In this respect the variation of m_s with γ_s^0 has been shown (Maron & Prutton, 1965) to follow a parabolic curve described by the equation:

$$\gamma_s^0 = 1 + A'm_s + B'm_s^2, \tag{2}$$

where A′ and B′ are constants. Substituting equation (2) into equation (1) we have:

$$a_w^0 = 1 - \frac{m_s}{55.5}[1+\ln(1+A'm_s+B'm_s^2)]. \tag{3}$$

For small values of $(A'm_s+B'm_s^2)$ relative to unity, equation (3) may be simplified as:

$$a_w^0 = 1 - \frac{m_s}{55.5}(1+A'm_s+ B'm_s^2). \tag{4}$$

Being in molal concentration units equation (4) is still not in a form that can be applied to non-solutes. To apply to non-solutes equation (4) can be restated in gram solids (W) per kg H_2O units as:

$$a_w^0 = 1 - \frac{W}{55.5M_w}\left(1+\frac{A'W}{M_w}+\frac{B'W^2}{M_w^2}\right)$$

$$= 1 - \frac{W}{K}(1+AW+BW^2) \tag{5}$$

where $K = 55.5M_w$; M_w = molecular weight of solute/non-solute; $A = A'/M_w$; $B = B'/M_w^2$.
Rearranging equation (5) yields

$$\frac{1-a_w^0}{W} = \frac{1}{K}+\frac{AW}{K}+\frac{BW^2}{K} \tag{5a}$$

a second degree polynomial equation of the general form:

$$y = a+bx+cx^2 \tag{6}$$

in which $a = 1/K$; $b = A/K$; $c = B/K$ are constants; $y = 1-a_w^0/W$ and $x = W$.
The constants a, b and c may be found by applying the least squares method of analysis (Yeomans, 1968) to the sorption data of solute or non-solute ingredients of a food. The

characteristic constants K, A, B so obtained for each ingredient (Table 1) can be substituted into equation (5) to estimate the a_w of simple binary solutions and dispersions (Table 2) without the need to know either molal concentrations or activity coefficients.

Table 1. Characteristic K, A, B Constants of selected food ingredients at 25°C*

Solute/ non-solute	K ($55.5\ M_w$)	M_w (equivalent)	A	B
NaCl	1762	32	2.1915×10^{-4}	1.1316×10^{-6}
KCl	2322	42	-8.9364×10^{-6}	9.0541×10^{-8}
Propylene glycol†	4238	76	4.0989×10^{-5}	64.9570×10^{-9}
Sucrose	20060	361	3.8297×10^{-4}	-7.4839×10^{-8}
Gluten	36630	660	-7.2146×10^{-7}	-2.4536×10^{-8}
Collagen	54339	979	1.2172×10^{-3}	-4.7822×10^{-7}
Starch	35788838	644844	9.999×10^{-1}	-1.0674×10^{-4}
Wheat§ (Manitoba)	75296	1357	3.5130×10^{-5}	5.6750×10^{-8}

Sorption data from: *Ross (1975), †Labuza (1976), §Ayerst (1965).

Table 2. Concentrations and calculated and observed a_w's of ingredients in Bone's (1975) solution D

Ingredient	Concentration		a_w^0	
	molality	g/kg H_2O	Calculated (eq. 5)	Measured* (Robinson & Stokes, 1959)
Sucrose	2.72	924.55	0.9405	0.9400
NaCl	0.75	40.68	0.9767	0.9769
KCl	0.40	23.41	0.9899	0.9871
Propylene glycol	3.68	284.09	0.9318	0.9378†
Ross product method a_w			0.8473	0.8500*
Corrected Ross product method a_w			0.8184	—
Measured mixture			—	0.8220

*From Bone *et al.* (1975).
†Established by Raoult's Law.

a_w *of mixed solutions*

The substitution of equation (5) into the Ross (1975) equation for a two component mixture gives:

$$a_w = \left[1-\frac{W_1}{K_1}(1+A_1W_1+B_1W_1^2)\right]\left[1-\frac{W_2}{K_2}(1+A_2W_2+B_2W_2^2)\right]$$

$$=\left[\left(1-\frac{W_1}{K_1}\right)-\frac{W_1}{K_1}(A_1W_1+B_1W_1^2)\right]\left[\left(1-\frac{W_2}{K_2}\right)-\frac{W_2}{K_2}(A_2W_2+B_2W_2^2)\right]$$

where the subscripts indicate components 1 and 2.

From equation (5)

$$\left(1-a_{w_1}^0-\frac{W_1}{K_1}\right)=\frac{W_1}{K_1}(A_1W_1+B_1W_1^2) \tag{i}$$

$$\left(1-a_{w_2}^0-\frac{W_2}{K_2}\right)=\frac{W_2}{K_2}(A_2W_2+B_2W_2^2) \tag{ii}$$

substituting these expressions into equation (7) yields:

$$a_w=\left[\left(1-\frac{W_1}{K_1}\right)-\left(1-a_{w_1}^0-\frac{W_1}{K_1}\right)\right]\left[\left(1-\frac{W_2}{K_2}\right)-\left(1-a_{w_2}^0-\frac{W_2}{K_2}\right)\right]$$

which upon expansion and rearrangement gives:

$$\begin{aligned}a_w=\;&a_{w_1}^0a_{w_2}^0-\frac{2W_1W_2}{K_1K_2}+\frac{2W_1W_2}{K_1K_2}\\&-2\left(1+\frac{W_1}{K_1}+\frac{W_2}{K_2}\right)+2\left(1+\frac{W_1}{K_1}+\frac{W_2}{K_2}\right)\\&-(a_{w_1}^0+a_{w_2}^0)+(a_{w_1}^0+a_{w_2}^0)\\&-\left(\frac{W_1a_{w_2}^0}{K_1}+\frac{W_2a_{w_1}^0}{K_2}\right)+\left(\frac{W_1a_{w_2}^0}{K_1}+\frac{W_2a_{w_1}^0}{K_2}\right)\end{aligned} \tag{8}$$

Equation (10) may be reduced to:

$$a_w=a_{w_1}^0a_{w_2}^0-\frac{2W_1W_2}{K_1K_2}+\frac{2W_1W_2}{K_1K_2}. \tag{8a}$$

We observe from this simplification, as before (Caurie, 1985), that the Ross (1975) equation results from a cancellation of two equal but opposite sets of solute-solute weight interaction factors. This cancellation is known to overestimate the true a_w especially at high solute or solid concentrations (Chuang & Toledo, 1976). The extent of overestimation has been attributed (Caurie, 1985) to the positive interaction factors which when eliminated from equation (8a) results in the mixture equation:

$$a_w=a_{w_1}^0a_{w_2}^0-\frac{2W_1W_2}{K_1K_2}. \tag{9}$$

This equation is identical with Caurie's (1985) corrected Ross equation for a two component mixture except that the correction factor in equation (9) is in weight concentration units (g/kg H_2O) instead of molal concentration units. From the similarity between the two equations, equation (9) may be expressed, from Caurie (1985), for a three component mixture as:

$$a_w=a_{w_1}^0a_{w_2}^0a_{w_3}^0-N\left(\frac{W_1W_2}{K_1K_2}+\frac{W_1W_3}{K_1K_3}+\frac{W_2W_3}{K_2K_3}\right)+\frac{(N+1)W_1W_2W_3}{K_1K_2K_3} \tag{10}$$

where N, $(N+1)$ in the parenthesis are respectively the first and second order solute-solute weight interaction coefficients in which N is the number of component ingredients (Caurie, 1985). Thus, provided the characteristic K, A, B constants of the component ingredients are known the a_w's of simple and mixed solutions and dispersions, as well as solid mixtures may be estimated from the weight concentrations of their component ingredients.

Results and discussion

We have applied equation (5a) to sorption data (Ross, 1975; Labuza, 1976; Ayerst, 1965) to calculate the K, A, B values characteristic of certain food ingredients (Table 1).

From the estimated values of K, equal to 55.5 M_w, the equivalent molecular weights of substances in aqueous systems are seen to be greater or smaller than those of the corresponding dry values (Table 1). For example the equivalent molecular weights of the electrolytes KCl and NaCl in solution are seen to be smaller while those of the non-electrolytes sucrose and propylene glycol are identical to their actual values.

The proteins gluten and collagen are non-solutes and show moderate molecular weights in solution while starch maintains its high polymeric form in solution with a large equivalent molecular weight of 644, 844 equivalent to 3582 glucose molecules.

The substitution into equation (5) of the calculated K, A, B constants characteristic of a given solute/non-solute gives a characteristic equation for the solute or non-solute ingredient.

A plot of the relationship between weight concentration and a_w, calculated from equation (5) for the food ingredients listed in Table 1, is shown in Fig. 1. Both carbohydrates and proteins, non-electrolytes, show minimum a_w's beyond which the a_w of their dispersions increase with increasing concentration; the carbohydrates are seen to depress the a_w more effectively than the proteins. The electrolytes NaCl and KCl exhibit no such minima and depress the a_w more effectively than either the proteins or the carbohydrates.

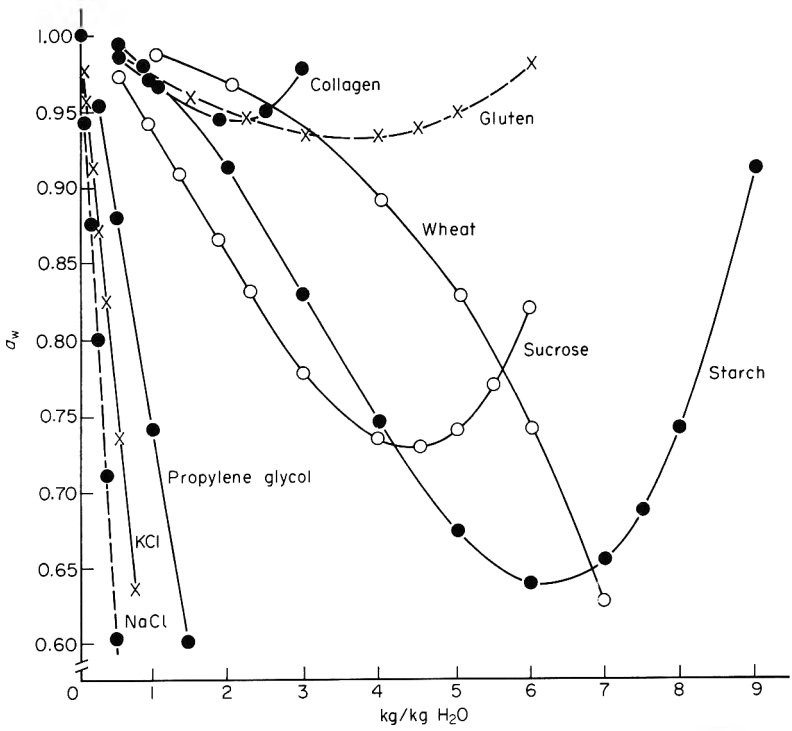

Figure 1. Plots of concentrations (g/kg H_2O) of selected food materials against a_w calculated using their characteristic K, A, B constants derived from equation (5).

To explain the parabolic nature of the curves in Fig. 1. Bone (1973) has suggested that water does not consist of a simple homogenous mixture of separate molecules but rather of molecules linked together into three dimensional structures with variable molecular memberships. When a solute or non-solute is added to water the interaction of the solute with water may either break up the three dimensional structure of water into smaller units to increase its effective concentration or the solute may encourage hydrogen bonding among the water molecules immediately adjacent to the solute (Nemathy & Scherega, 1962) thus reducing the effective water concentration and therefore the a_w of the solution. If this hypothesis is correct it is clear from Fig. 1 that this breaking and formation of the three dimensional structure of water depends on both the nature and concentration of the solute or non-solute.

To evaluate the ability of the K, A, B constants of an ingredient to define characteristic binary equations for the prediction of a_w, we have substituted into equation (5) weight concentrations for the ingredients used in Bone's (1975) model food solution D (Table 2). It is seen that calculated a_w's agree closely with the measured values (Robinson & Stokes, 1959) indicating that the calculated K, A, B constants characterize the ingredients.

The application of the calculated binary a_w's and their corresponding weight concentrations to equation (10) predicts an a_w value for Bone's solution D of 0.818 (Table 2) which compares favourably with the measured value of 0.822. In a previous paper (Caurie, 1985) in which the molal concentration form of equation (10) was used a calculated value of 0.827 was found representing an error of plus 0.61% compared with an error of minus 0.49% recorded using equation (10).

The difference between the estimated a_w based on molal concentration and that based on weight concentration could arise from differences between equivalent molecular weights (M_w) of component ingredients estimated from K (Table 1) and the actual M_w of the ingredients used by Caurie (1985). For example the calculated equivalent M_w of the two electrolytes, NaCl and KCl, are lower than their actual values (Table 1). This lower M_w can result in higher ingredient concentrations leading to correspondingly lower calculated mixture a_w's (Table 2). The equivalent molecular weights M_w recorded in Table 1 may therefore be taken as constituting the effective M_w of the respective ingredients.

Table 3. Concentrations and calculated and observed a_w's of ingredients in Bone's (1975) solution E

Ingredient	Concentration		a_w	
	molality	g/kg H_2O	Calculated (eq. 5)	Measured* (Robinson & Stokes 1959)
Sucrose	2.72	930.40	0.9401	0.9400
KCl	0.40	29.60	0.9873	0.9871
NaCl	0.75	40.80	0.9766	0.9769
Propylene glycol	3.68	286.00	0.9314	0.9068†
Collagen	—	1713.00	0.9470	0.9659†
Ross product method a_w			0.7995	0.7939
Corrected Ross product method a_w			0.7373	—
Measured mixture a_w			—	0.7940

*From Bone *et al.* (1975).

†Established by interpolation.

When collagen is added to solution D to give a mixture containing 25% moisture and 42.83% collagen (Table 3) (solution E of Bone *et al.*, 1975), equation (5) predicts, from the constants in Table 1, that the a_w contribution of the added collagen will equal 0.9470 a_w. The a_w contribution of this collagen was previously estimated by interpolation (Bone *et al.*, 1975) to equal 0.9659. With the now calculated collagen contribution of 0.9470, the water activity of the mixture according to the Ross (1975) product method is 0.7995 (Table 3) which compares well with a measured (Bone *et al.*, 1975) value of 0.7940. But from equation (10) this calculated Ross value must be corrected downwards by an amount equal to 0.0622 to give a final value of 0.7373.

The agreement between the calculated and observed a_w values for solution E is obviously less satisfactory than for solution D. One major difference between solutions D and E is that while equilibration in solution D, as a four component solution, has been shown (Caurie, 1985) to depend on the completion of only ten solute–solute interactions, solution E, being a five component system, requires twice as many such interactions to occur before equilibration is achieved. This large number of interactions means that Solution E will require more time than solution D to equilibrate. If this is not allowed for, erroneously high observed values may be expected to make predictions from equation (10) appear low.

Equations (5) and (10) have similarly been applied to the collagen-sucrose mixture of Bone *et al.* (1975) in which 12.8 g collagen was dispersed along with 474.5 g sucrose in 255.4 g distilled water with equally good results. This mixture is equivalent to 50.1 g collagen and 1857.87 g sucrose per kg of distilled water which upon substitution into equation (5) along with the appropriate K, A, B constants (Table 1) gives an equivalent a_w value of 0.9990 for collagen and 0.8654 for sucrose. The resulting Ross product value then becomes 0.86453 which may be corrected downwards according to equation (10) to give an a_w of 0.8644 in very good agreement with the observed value (Bone *et al.*, 1975) of 0.8650.

Chuang & Toledo (1976) suggested a method for predicting the a_w of multi-component solid mixtures from the water sorption isotherms of individual components. These authors tested their method with a mixture of 70 g starch (14.9% H_2O wet basis) and 30 g wheat (11.44% H_2O wet basis). On a dry weight basis the moisture content of the starch would be 17.51% and that of wheat would be 12.92%. This means that the 70 g starch used by the above authors on a dry weight basis was equivalent to 57.743 g dry starch dispersed in 12.257 g H_2O or 4711 g dry starch per kg H_2O. Substituting this weight concentration of starch into equation (5) along with corresponding K, A, B values (Table 1) results in a calculated a_w of 0.6916. Similarly the 30 g wheat would be equivalent to 26.125 g dry wheat solids dispersed in 3.875 g H_2O or 6742 g dry wheat solids per kg H_2O. Substituting estimated K, A, B values for Manitoba wheat (Table 1) into equation (5) for 6742 g dry wheat per kg H_2O results in a calculated a_w of 0.6583. Applying equation (10) to the above starch/wheat data gives an a_w of 0.455 compared with 0.460 measured by Chuang & Toledo (1976). The latter authors calculated a value of 0.491 with their method.

A major problem with the development of a rigorous theoretical model to predict the a_w contribution of non-solutes has been the assignment of a proper solids to moisture ratio (Ross, 1975). Ross (1975) suggested the assignment of all moisture in a mixture to each ingredient separately but he pointed out that in a product containing several non-solutes some water will be bound to each non-solute thereby reducing the quantity available for the others. We have preserved, unchanged, the natural solids to moisture ratio in each ingredient and only converted the ratio to g/kg H_2O.

By way of comparison, if Ross' (1975) technique were used in the above calculation the total water in the starch/wheat mixture would equal the sum of 12.257 g H_2O from starch and 3.875 g H_2O from wheat which would add up to a total of 16.132 g H_2O. Thus from the Ross technique 57.743 g of dry starch and 26.125 g of dry wheat solids would be dispersed in 16.132 g H_2O instead of in 12.257 and 3.875 g of H_2O respectively as in the present treatment. The dispersion of each ingredient in 16.132 g H_2O in the Ross technique would give an equivalent concentration of 3579 g dry starch/kg H_2O and 1619 g dry wheat/kg H_2O. If these lower weight concentration values are substituted into equation (5) along with the corresponding K, A, B values (Table 1) a_w contribution of 0.7787 for the starch and 0.9741 for the wheat are calculated. The a_w of the non-solute starch/wheat mixture according to the Ross (1975) technique would then equal (0.7787) (0.9741) = 0.7585 instead of the observed value of 0.460. The higher a_w calculated for this mixture using the Ross (1975) technique clearly arises from the assumed exposure of the individual component solids in the ingredients to more water than is available to them. This dilution effect using the Ross technique will increase with an increasing number of ingredients and therefore with increasing total solid content, as pointed out by Chuang & Toledo (1976).

We have expressed the recent Caurie (1985) equations for predicting the a_w levels of simple and mixed solutions in weight concentration (g/kg H_2O) units. These modifications have permitted the application of the equations to both solutes and non-solutes, and their mixtures in both dry and solution forms. The modified equations have been tested against independent literature data and found to agree closely with the measured values.

References

Ayerst, G. (1965). Determination of the water activity of some hygroscopic food materials by a dew point method. *Journal of the Science of Food and Agriculture*, **16**, 71–78.

Bone, D. (1973). Water activity in intermediate moisture foods. *Food Technology*, **27**(4), 71–76.

Bone, D.P., Shannon, E.L. & Ross, K.D. (1975). The lowering of water activity by order of mixing in concentrated solutions. In: *Water Relations of Foods* (edited by R.B. Duckworth), p. 613 London: Academic Press.

Caurie, M. (1985). A corrected Ross Equation. *Journal of Food Science*, **50**, 1445–1447.

Chuang, L. & Toledo, R.T. (1976). Predicting the water activity of multicomponent systems from water sorption isotherms of individual components. *Journal of Food Science*, **41**, 922–927.

Labuza, T.P. (1976). Storage stability and improvement of intermediate moisture foods. Final contract NAS 9-12560. Final report, p. 264. NASA Food and Nutrition Office Houston, Texas.

Maron, S.H. & Prutton, C.F. (1965). *Principles of Physical Chemistry*, 4th edn., p. 438. New York: Macmillan Press Co.

Nemathy, G. & Scherega, H.A. (1962). The structure of water and hydrophobic bonding in proteins 2. Model for the thermodynamic properties of aqueous solutions of hydrocarbons. *Journal of Chemical Physics*, **36**, 3401.

Robinson, R.A. & Stokes, R.H. (1959). Electrolyte solutions. London: Butterworths.

Ross, K.D. (1975). Estimation of water activity in intermediate moisture foods. *Food Technology*, **29**(3), 26–34.

Yeomans, K.A. (1968). *Statistics for the Social Scientist*. Vol. 2, p. 184. Middlesex: Penguin Books Ltd.

(Received 18 January 1984)

Journal of Food Technology (1986) **21**, 229–232

Technical note: Multiple homogenization of cream liqueurs

D. D. MUIR* AND W. BANKS

Introduction

Approximately 25 million litres of cream liqueur are produced commercially in the British Isles and the incidence of product defects is now comparatively low. Nevertheless, spasmodic problems occur in which a solid plug of material is found in the neck of bottles after prolonged storage. This material comprises largely of fat. Banks, Muir & Wilson (1982) considered the problem of fat-plug formation in cream liqueurs and found that relatively severe homogenization conditions were necessary to inhibit creaming. However, the industry has been unable to correlate cream plug formation with deficiences in homogenization (few producers evaluate indices of homogenization efficiency) and there is a general feeling that the problem may be associated with excessively severe homogenization. This reasoning is derived from the behaviour of double cream where slight homogenization results in clumping and clustering of fat globules and marked thickening (Phipps, 1985; Mulder & Walstra, 1974). In double cream, the protein to fat ratio is *circa* 0.04 and there is insufficient protein available to cover completely the new fat surface formed during homogenization (e.g., Mulder & Walstra, 1974). Cream liqueurs employ a much higher protein to fat ratio with values in the range 0.19–0.21 (Banks, Muir & Wilson, 1981a). In our work, creaming has never been observed in normal liqueurs, but more severe homogenization conditions have not been extensively tested (Banks *et al.*, 1982). This report describes the effect of severe homogenization conditions on the properties of cream liqueur.

Materials and methods

Pasteurized double cream (48% butterfat) and sucrose were purchased locally. Sodium caseinate was bought from the Scottish Milk Marketing Board (Underwood Road, Paisley) and neutral spirit was provided free of charge by various distillers. Analar grade trisodium citrate was used. A caseinate syrup was prepared by mixing water, sodium caseinate and sucrose in the ratio 1:0.18:1.17 w/w. After complete dispersion at 85°C, to this syrup was added double cream (1:0.83), alcohol to give a final proof of 30° (17.1% vol/vol) and trisodium citrate (1.9 g/l of liqueur). The mixture was then blended and homogenized at 50°C and 4500 psi using a Manton-Gaulin pilot-scale homogenizer (55 l/hr). After homogenization, the liqueur was quickly cooled to 20°C and the total solids content adjusted to 40% (w/w) using 30° neutral spirit.

Fat, crude protein, sugar and total solids contents of the liqueurs were estimated by the methods detailed in Banks, Muir & Wilson (1981b). The efficiency of homogenization was measured using a Coulter Counter (Model ZM, Coulter Electronics) and a 20 μ orifice. Before estimation of globule size, the liqueur was diluted 1:10000 with Isoton II (Coulter Electronics). The viscosity of the liqueur was estimated by rotational viscometer (Rheolog type, Brookfield Engineering Labs Inc., Staughton,

Authors' address: The Hannah Research Institute, Ayr KA6 5HL, Scotland, U.K.
*To whom correspondence should be addressed.

U.S.A.) in a temperature controlled small sample holder at a fixed shear rate (79/sec) at 25°C. The stability of the product was the elapsed time at 45°C before visible separation of serum or particulate matter was observed.

Results and discussion

Liqueur was formulated as described above, warmed to 50°C, homogenized and, when necessary, re-equilibrated to 50°C. This process was repeated sequentially seven times. The composition of the products did not change significantly (Table 1). As the number of homogenization treatments increased, there was an increase in viscosity but this was not marked and probably reflected differences in particle size distribution. It is extremely difficult to fully characterize the particle size distribution in cream liqueurs because of the discrete populations of casein micelles (0.05–0.5 μ diameter) and emulsified fat globules (0.2–2.0 μ). However, we have found that an index of homogenization efficiency (the weight percent of large fat globules) relates to the formation of cream plug during storage (Banks *et al.*, 1982). The results of the analysis of homogenization efficiency, calculated on the proportion of particles over 1 and 0.5 μ diameter, are also shown in Table 1. A single homogenization did not adequately reduce the number of large particles, i.e., those responsible for creaming (Banks *et al.*, 1982). Further treatments steadily reduced the proportion of oversize particles although there was no significant improvement once four sequential treatments had been carried out.

Table 1. Effect of multiple pass homogenization on properties of cream liqueur

No. of homogenizations	Fat (%)	Total solids (%)	pH	Viscosity (cP)	Particles greater than (wt %)	
					0.5 μ	1.0 μ
1	15.3	40.7	6.86	24.5	14.7	5.3
2	14.7	40.8	6.84	25.5	4.5	0.2
3	14.4	39.1	6.85	24.8	2.7	0.1
4	14.5	39.7	6.84	22.3	1.4	0.1
5	14.7	40.7	6.83	23.4	1.9	0.1
6	15.4	39.2	6.81	28.0	0.9	0.1
7	16.2	39.6	6.79	33.0	1.1	0.1

The properties of the liqueurs were examined after storage at 45°C for 100 days and at 20°C for 9 months. During storage at 45°C no fat separation was noted, with the single exception of the sample which had received one homogenization treatment only: a distinct fat ring developed after a week at 45°C. No serum separation or precipitate was found in any of the samples on storage for 100 days at 45°C. There were some slight, but commercially insignificant, changes in the physical properties of the liqueurs after storage for 9 months at 20°C (Table 2). The changes in the weight percent oversize particles and in viscosity were most marked in the samples which had been homogenized six or seven times. Clearly, very severe homogenization can be applied to cream liqueurs of the composition used here without significant detriment to product quality.

Table 2. Properties of homogenized liqueur after storage at 20°C for 9 months

No. homogenization	Viscosity (cP)	Particles greater than (wt %)	
		0.5μ	1.0μ
1	26.0	16.1	6.1
2	28.7	7.1	1.4
3	24.1	4.2	0.8
4	33.5	2.7	0.4
5	25.4	1.7	0.3
6	44.3	2.7	0.8
7	38.4	2.7	0.8

Whilst it is inconceivable that any complete batch of liqueurs would be as severely homogenized as above, conditions occur when a small proportion is severely homogenized. For example, at plant start-up liqueur may be continuously circulated in a by-pass loop. The effect of such treatment was therefore investigated. Liqueur (3 l) was introduced to the homogenizer at 50°C and continuously recirculated at 4500 psi. During homogenization, the temperature of the liqueur rose and after 40 min had reached 70°C. At this point, homogenization was stopped, the liqueur cooled to 50°C, then the product was further recirculated at 4500 psi for an additional 40 min. The total treatment was equivalent to 24 cycles of homogenization. As shown in Table 3, this combined treatment did result in the formation of some larger particles. Nevertheless, the impact of over-homogenization of such a small quantity (probably less than 1%) of a total production batch will be commercially insignificant because of the dilution effect.

We have previously noted small differences in stability at 45°C between liqueurs with different protein:fat ratios (Banks *et al.*, 1983). At the lower protein:fat ratios, longterm stability was poorer. Therefore, a liqueur was formulated in which the protein:fat ratio was 0.10, i.e., half the recommended level. In this case, recirculation homogenization had a marked effect (Table 3) on the number of oversized particles. A

Table 3. Effect of recirculation homogenization on the fat-globule size of cream liqueurs

	Particles > 1.0 μ (%)	
	Control	Treated
Normal protein level	0.5	5.6
Low protein level	0.9	29.0
Intermediate protein level	0.6	6.9

Mean values from three trials.

Control = homogenized twice at 4500 psi: Treated = control continuously homogenized at 4500 psi for 40 min, cooled to 50°C then repeated.

Normal protein:fat = 0.19; low protein:fat = 0.10.

A 50:50 mixture of normal and low protein liqueur.

compound sample comprising 50:50 of normal and low protein ratio liqueur was also recirculated. Its properties were intermediate, but the prevalence of large particles was not as marked as in the low protein sample (Table 3).

In conclusion, there is strong evidence from this work that cream liqueurs with a protein:fat ratio 0.2 cannot be easily over-homogenized. Therefore, when cream plug formation is evident in stored samples, under-homogenization, perhaps due to valve wear, should first be suspected. Notwithstanding this general observation, caution should be exercised to minimize the extent to which liqueur is continuously homogenized by recirculation. When low protein:fat ratios are used, severe homogenization can lead to formation of large particles with subsequent creaming and fat plug formation. This observation, together with our earlier findings on longterm emulsion stability (Banks, Muir & Wilson, 1983) suggests that low protein:fat ratios should be avoided.

Acknowledgments

Miss Anne Allan, Miss Julie Jones and Mrs Sheila Golightly are thanked for their expert technical assistance.

References

Banks, W., Muir, D.D. & Wilson, A.G. (1981a). The formulation of cream-based liqueurs. *The Milk Industry*, **83**, 16–18.

Banks, W., Muir, D.D. & Wilson, A.G. (1981b). Extension of the shelf-life of cream-based liqueurs at high ambient temperatures. *Journal of Food Technology*, **16**, 587–595.

Banks, W., Muir, D.D. & Wilson, A.G. (1982). Formulation of cream-based liqueurs: comparison of sucrose and sorbitol as the carbohydrate component. *Journal of the Society of Dairy Technology*, **35**, 41–43.

Banks, W., Muir, D.D. & Wilson, A.G. (1983). Stabilization of alcoholic beverages by sodium caseinate. In: *Proceedings of an International Dairy Federation Symposium, Helsingør, Denmark*, 331–338.

Mulder, H. & Walstra, P. (1974). In: *The Milk Fat Globule: Emulsion science as applied to milk products and comparable foods*. 1st edn, Farnham Royal, U.K.: Commonwealth Agriculture Bureaux.

Phipps, L.W. (1985). *The high pressure dairy homogenizer. Technical Bulletin No. 6, Chapter 4*. Shinfield, Reading: The National Institute for Research in Dairying.

(Received 3 May 1985)

Journal of Food Technology (1986) **21**, 233–238

Technical note: Effect of low pH preservation on the colour and consistency of tomato juice

G. S. MUDAHAR, J. S. SIDHU* AND K. S. MINHAS†

Summary

The changes in colour and consistency are reported for tomato juice produced by low pH preservation and stored under ambient conditions. The juice preserved under acid conditions showed higher consistency, probably due to the higher concentration of pectin and the presence of low methoxyl pectin. The furfural and hydroxymethylfurfural which were detected in the juice indicated that darkening of the juice colour was due to non-enzymic browning involving both ascorbic acid and sugars (hexoses). Although the retention of ascorbic acid was higher under low pH conditions, it led to a significant darkening of the juice colour due to non-enzymic browning involving mainly ascorbic acid.

Introduction

Tomato is an important vegetable which, apart from its culinary properties, is a good source of certain vitamins and minerals (Tressler & Joslyn, 1961). An economical method of preservation of tomato juice under low pH conditions has already been reported by Sidhu, Bhumbla & Joshi (1984). These authors observed that the juice preserved under acid conditions darkened in colour considerably during storage. This investigation examined the reasons for the darkening of colour and changes in consistency of tomato juice during storage under low pH conditions.

Materials and methods

Extraction of juice

Tomatoes (var. 'Punjab chhuhara') were procured from the seed farm of the Punjab Agricultural University, Ludhiana. After washing thoroughly, the tomatoes with sunburns, excessive cracks and blemishes were discarded. The juice was obtained by cold pressing (Sidhu, Jain and Bains, 1974), using a superfine pulper (Raylons, Bombay). After adding 2% sugar, 0.7% sodium chloride and 0.1% citric acid, the juice was divided into two portions. One lot (control) was bottled and preserved by the method proposed by Sidhu *et al.* (1974). The pH of the second portion was lowered to 1.40 with hydrochloric acid and preserved by the method of Sidhu *et al.* (1984). The room temperature during the storage period varied between 25–30°C.

Chemical analysis

The fresh, as well as preserved, juice samples were analysed at intervals of 2 months for a period of 6 months for total soluble solids, total solids, pH, acidity and ascorbic

Authors' address: Department of Food Science and Technology, Punjab Agric. University, Ludhiana, India.

*Present address: Central Food Technological Research Institute, Mysore.

†To whom the correspondence should be addressed.

acid contents. The total soluble solids were determined using a hand refractometer with a percentage sugar scale; the pH by a Toshniwal Digital pH meter (Model CL-46); total solids and acidity by AOAC (1975) methods. Ascorbic acid was estimated by titration against 2,6-dichlorophenol indophenol dye (Ruck, 1965). The pectin, methoxyl, furfural and hydroxymethylfurfural contents of tomato juice were determined initially (after 1 week of processing), and after 6 months storage by the methods proposed in the laboratory manual for fruit and vegetable products (Ranganna, 1979).

Measurement of colour and consistency

The colour of tomato juice was determined by reflectance method (I.C.C. system) using a B and L spectronic -20 colorimeter with reflectance attachment. The consistency of tomato juice was measured at room temperature (27±1°C) in a Brookfield synchrolectric viscometer (Model LVT) using spindle No. 1 at 30 rpm and reported in centipoise (cP). The colour and consistency of juice were measured initially as well as after 6 months storage.

Results and discussion

Changes in chemical constituents

The data presented in Table 1 show that total soluble solids, total solids, pH and acidity did not vary significantly in both the control and low pH samples over the storage period of 6 months. However, there was a significant loss of ascorbic acid during storage; the control sample showed the highest loss (33.8%) when compared with the low pH sample (12.5%). These findings are corroborated by the work of Pruthi *et al.* (1952). According to Inagaki (1950), the oxidation of ascorbic acid is retarded in natural food juices due to the presence of substances like pectin, naringin, thiamin, and β-carotene. So apart from low pH, the higher pectin content (0.31%) of this sample might have helped the retention of ascorbic acid by this method of preservation.

Colour and consistency

The data for the colour and consistency of tomato juice, one week after processing

Table 1. Effect of storage on the chemical composition of the tomato juice (average of triplicates)

Attribute	Fresh juice	Storage period (months) Initial*		2		4		6		Loss (%)	
		S_1	S_2	S_1	S_2	S_1	S_2	S_1	S_2	S_2	S_1
Total soluble solids (°Brix)	5.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	—	—
Total solids (%)	5.18	7.28	7.40	7.25	7.35	7.28	7.30	7.30	7.34	—	—
Ascorbic acid (mg/100 g)	18.5	17.00	18.00	15.80	17.00	13.30	16.00	11.25	15.75	33.8	12.5
pH	3.98	3.84	1.43	3.80	1.42	3.81	1.42	3.85	1.40	—	—
Acidity (% citric acid)	0.35	0.47	1.32	0.45	1.30	0.46	1.32	0.45	1.30	—	—

*One week after processing.
S_1 = Control sample.
S_2 = Low pH sample.

and after six months storage, are presented in Table 2. The colour of the control sample was much brighter (Y = 12.09%) than the low pH sample (Y = 9.56%), the latter being on the dull side. There was a small but significant decrease in brightness of colour of the low pH sample during storage. The dominant wavelengths of the control samples (590–591 nm) indicated the hue as orange, whereas the hue of the low pH sample had shifted towards reddish orange (596–603 nm), revealing that a certain amount of non-enzymic browning of tomato juice had taken place. This darkening of colour can also be inferred from the lower values of chroma of the low pH sample (15.15%) when compared with the chroma of the control sample (29.82%).

Table 2. Effect of storage on the colour and consistency of tomato juice (Average of triplicates)

	Control		Low pH	
	Initial*	6 months	Initial*	6 months
Colour				
% Y (Brightness)	12.090	11.480	9.560	8.800
x	0.403	0.391	0.391	0.350
y	0.349	0.348	0.332	0.325
λ nm (dominant wavelength)	591	590	596	603
% chroma (purity)	35.08	29.82	26.23	15.15
Consistency (cP)	52	51	74	72

*One week after processing.

The consistency of the tomato juice control (52 cP) and the low pH sample (74 cP) was significantly different. The higher consistency of the low pH sample may be due to two reasons: (a) the increased pectin contents (0.31%) or (b) the presence of increased levels of low methoxyl pectin in this sample. Higher levels of pectic substances have been demonstrated to increase the consistency of tomato juice by a number of workers (McColloch, Keller & Beavans, 1952; Hand *et al.*, 1955; Foda & McCollum, 1970). According to Padiwal, Ranganna & Manjrekar (1979), the gel formation involves the transformation of a sol state of Low Methoxyl Pectin (LMP) to the gel state caused by added calcium. Gel formation occurs only at the optimum concentration of LMP (≃ 1% LMP) and calcium content of 20–30 mg/100 ml (Padiwal, Ranganna & Manjrekar, 1980). Because the concentration of LMP in low pH tomato juice is much lower (0.31%) than this optimum, probably a weak gel is formed with the calcium ions present therein. This association of LMP with calcium ions may be responsible for the considerable increase in the consistency of low pH juice when compared with the control sample.

Non-enzymic browning

The pectin, methoxyl, furfural and hydroxymethylfurfural contents of the control and low pH tomato juice at the end of 6 months storage are shown in Table 3. The presence of hydroxymethylfurfural and furfural indicate that both the sugars (hexoses) as well as ascorbic acid are involved in the darkening of the colour of tomato juice under

Table 3. Non-enzymic browning between control and low pH samples during storage (Average ± standard deviation of three replicates)

	Control		Low pH	
	Initial*	6 months	Initial*	6 months
Pectin content (% calcium pectinate)	0.180±0.0054	0.160±0.0078	0.310±0.0051	0.270±0.0067
Methoxyl content (%)	6.72±0.080	6.18±0.144	1.78±0.118	1.14±0.050
Furfural content (mg/100 ml)	$(6.08\pm0.085)\times10^{-1}$	$(8.17\pm0.148)\times10^{-4}$	$(33.19\pm0.209)\times10^{-4}$	$(44.9\pm0.154)\times10^{-4}$
Hydroxy methyl furfural (mg/100 ml)	$(6.10\pm0.116)\times10^{-1}$	$(7.50\pm0.096)\times10^{-4}$	$(1.12\pm0.064)\times10^{-1}$	$(1.46\pm0.104)\times10^{-4}$

*One week after processing.

these storage conditions. It can be seen from the higher furfural contents that there is a significant amount of non-enzymic browning in the low pH sample, which might have taken place due to the oxidation of ascorbic acid during storage. In the case of the control sample, both the sugars and ascorbic acid seem to have contributed towards the darkening of the colour of tomato juice due to non-enzymic browning. Dulkin & Friedemann (1956) and Deuel & Stutz (1958) have demonstrated that both reductic acid (derived from pectin) and ascorbic acid, in acid or buffered solutions, undergo types of browning reactions analogous to those occurring in stored or heat processed foods, ultimately leading to the development of an intense brown colour. Joslyn (1957) has also suggested that ascorbic acid is the most reactive component in browning systems containing ascorbic acid, amino acids and sugars. Singh, Dean & Cantor (1948) have suggested that ascorbic acid alone in aqueous solutions undergoes browning on heating at 98°C or above, forming furfural and carbon dioxide. From the foregoing discussions, it can be concluded that the tomato juice preserved by the low pH method (Sidhu *et al.*, 1984) exhibited a higher consistency due to the increased amount of pectins, and the presence of low methoxyl pectins, but darkened in colour due to non-enzymic browning involving mainly ascorbic acid.

Acknowledgment

The authors are grateful to Dr L. N. Shetty, Scientist, CFTRI, Mysore, for his kind help in the measurement of colour and consistency of tomato juice.

References

A.O.A.C. (1975). Official methods of analysis, 12th edn (Association of Official Agricultural Chemists). Washington D.C.

Deuel, H. & Stutz, E. (1958). Pectic substances and Pectic Enzymes. *Advances in Enzymology*, **20**, 341–382.

Dulkin, S.I. & Friedemann, T.E. (1956). The role of dehydro-ascorbic and dehydroreductic acids in the browning reaction. *Food Research*, **21**, 519–527.

Foda, Y.H. & McCollum, J.P. (1970). Viscosity as affected by various constituents of Tomato Juice. *Journal of Food Science*, **35**, 333–338.

Hand, D.B., Moyer, J.C., Ransford, J.R., Haning, J.C. & Whittenberger, R.T. (1955). Effect of processing conditions on the viscosity of tomato juice. *Food Technology*, **9**, 228–235.

Iragaki, C. (1950). Oxidation of ascorbic acid in relation to acids and sugars. *Journal of Agricultural Chemists Society, Japan*, **26**, 486–493.

Joslyn, M.A. (1957). Role of amino acids in the browning of orange juice. *Food Research*, **22**, 1–14.

McColloch, R.J., Keller, G.J. & Beavens, E.A. (1952). Factors influencing the quality of tomato products 1. Surface localised pectic enzymes inactivated by blanching. *Food Technology*, **6**, 197–199.

Padiwal, R.A., Ranganna, S. & Manjrekar, S.P. (1979). Mechanism of gel formation by low methoxy pectin. *Journal Food Technology*, **14**, 277–287.

Padiwal, R.A., Ranganna, S. & Manjrekar, S.P. (1980). Cloud stabilisation in citrus beverages by low methoxyl pectin. *Journal Food Technology*, **15**, 25–34.

Pruthi, J.S., Tandon, G.I., Jain, N.L. & Natarajan, C.P. (1952). Ascorbic acid and colour changes in citrus fruit juices during processing. *Journal of Scientific and Industrial Research*, **11A**, 32–34.

Ranganna, S. (1979). *Manual of Analysis of Fruit and Vegetable Products*. Pp. 20, 24, 324–325. New Delhi, India: Tata McGraw Hill Publishing Company Ltd.

Ruck, J.A. (1965). *Chemical Analysis of Fruit and Vegetable Products*. Pp. 25–27. Canada Dept of Agriculture, Ottawa, Canada.

Sidhu, J.S., Jain, V.K. & Bains, G.S. (1974). Processing of Tomatoes for Juice and Viable Seeds. *Research and Industry*, **19**(4), 155–157.

Sidhu, J.S., Bhumbla, V.K. & Joshi, B.C. (1984).

Preservation of tomato juice under acid conditions. *Journal of the Science of Food and Agriculture*, **35**(3), 345–352.

Singh, B., Dean, G.R. & Cantor, S.M. (1948). Role of 5-hydroxy methyl furfural in the discoloration of sugar solutions. *Journal American Chemical Society*, **70**, 517–522.

Tressler, D.K. & Joslyn, M.A. (1961). *Fruit and Vegetable Juice Processing Technology*. Westport. Connecticut: Avi Publishing Co. Inc.

(Received 6 June 1985)

Journal of Food Technology (1986) **21**, 239–253

Book Reviews

Speiseeis.By Fritz Timm. (Vol. 19 of 'Grundlagen und Fortschritte der Lebensmitteluntersuchung und Lebensmitteltechnologie'), (in German).
Berlin: Paul Parey, 1985. Pp. 264. ISBN 3 489 61514 X. DM 96 (paperback)

The book, after an initial brief but interesting history of ice cream in Europe (6 pp.), is divided into three parts. Part A is made up of sections describing the various types of ice cream, the ingredients used, and their significance by I. Hirsing (19 pp.), ice cream as a food (8 pp.), and the law as pertaining to ice cream by P. Lipps (21 pp.). Part B is concerned with the industrial production of ice cream by H. Buchner (38 pp.), its storage and distribution (11 pp.), and hygiene (43 pp.). Part C is concerned exclusively with all aspects of quality control (34 pp.), covering the general aspects of QC, sensory analysis, microbiological testing, as well as chemical and physical analysis, this last by J. Gayer.

The author's intention is to provide an up-to-date text book for German technologists working in the area of industrial preparation of ice cream, and thus filling a very long standing need. Whilst there are several books on the subject in English, the subject has only received scant reference in varying German food science and technology books, and only one book on the subject in German in 1969. As intended, and the contents confirm, the book concentrates on the hygiene aspects of industrial production and handling of ice cream. With his co-authors, Dr Timm covers the subject very effectively, providing a very readable text book particularly for those not working in the industry, and providing a very useful student text.

The coverage of all aspects of quality control and hygiene is very thorough, providing an overview of most of the current methods of testing and control that are used, with very adequate background and rationale for these. As expected, it places great emphasis on the many German laws and regulations that apply to the composition, manufacture, and standards applicable to ice cream, with many useful comparisons between these and those of other countries. It is however regretted that there is little objective qualification to the various methods and results quoted, and bearing in mind that the book is published in 1985, it is surprising that of the many references provided very few are post 1979.

The section on production gives a very useful and professional overview of the modern method of industrial manufacture, often providing names of equipment suppliers, a section which would provide very much useful information if expanded in a future edition. Particularly interesting is the reference to ice cream confection and stick manufacture as found in Europe.

With its useful coverage of the comparative consumption and legal aspects of ice cream in different countries of the world, it is surprising that the unique situation existing in the U.K. is sparsely referred to, i.e. where standard ice cream is based on non-dairy fats, nor is any reference made to some of the modern vogues in ice cream, e.g., soft scoop etc., this is in common with the little reference made to the marketing of ice cream.

It is a very easily readable and well laid out book which certainly fills the long standing need for such a book in German, and provides a valuable contribution which

would benefit by being translated into the English language, particularly if the section on the manufacture, storage and distribution were to be expanded, and an objective evaluation of the useful information given added in the areas dealing with quality control. The publishers should be commended on the quality of the typescript and illustrations, and the very thorough index that is provided.

D. E. Blenford

Meade-Chen Cane Sugar Handbook. 11th edition. By James C. P. Chen. New York; John Wiley, 1985. Pp. xxi+1134. ISBN 0 471 86650 4. £139.35.

'Comprehensive' is the only way to describe this latest edition of one of the standard works on Cane Sugar Technology. In the forward, an authority on the Sugar Industry speaks of previous editions being used by beginners and experienced sugar technologists alike and this will be equally true of the 11th edition. For the beginner there is ample material for an introduction to any branch of the subject, and for the specialist this will be a constantly used reference work.

This edition includes 30% of new material with almost every chapter having new sections. After an introductory chapter on Sugar Cane and Sugar, Part 1 goes chapter by chapter through the natural sequence of the raw sugar manufacturing and refining processes and the properties and handling of their products and byproducts. Each chapter details the scientific basis for the process steps as well as the technology and plant employed. The major variations in the worldwide practice are described, together with the alternative technical terms used in different countries. Part 2 deals with all the major analytical procedures used in the industry and is set out in such a way as to allow the readers to make a brief survey of the methods and gain an overall understanding; or to follow the details given in order to use the methods themselves. Part 3 describes Chemical and Process Controls in equal detail and includes a useful chapter which defines terms commonly used in the industry. The chapter on Energy Conservation is new to this edition. All the descriptions in the book are supported by numerous pictures, diagrams and graphs with many worked examples and tables; and the book concludes with 100 pages of tables of data of especial value to workers in any field of sugar technology.

For the reader who wishes to go more deeply into any of the subjects covered, there are copious references covering the literature up to late 1983, drawing not only from standard sugar works and specialist journals, but also from publications over a wider field. However, in a very brief survey, although the correct subjects were referred to, I found four errors in the references and two of these made it difficult to track down the paper in question.

The index is adequate, but not comprehensive, and needs to be supplemented by the readers' own knowledge of the book and the subject. There are a few typographical errors which are either of a trivial nature or such that the correct reading is readily discerned. This is a book which can be recommended as a useful work of reference and should find its place on bookshelves throughout the cane sugar industry. I fear, however, that its use will be limited by the very high price.

J. C. Williams

Immunoassays in Food Analysis. Ed. by B. A. Morris and M. N. Clifford. Barking, Essex: Elsevier Applied Science, 1985. Pp. x+222. ISBN 0 85334 321 7. £25.00.

This book is based on the proceedings of a symposium organized by the editors and held at the University of Surrey, U.K., in September 1983. The book starts well with a 7 page glossary of terms associated with immunoassay e.g., monoclonal antibodies, Hook Effect etc. The papers are sensibly divided into three sections namely Principles of Immunoassay, Application to Macromolecules and Application to Small Molecules.

In the first section the history of food immunoassay is traced from Yallow and Berson's paper of 1959, and reasons proposed to explain why food analysts were slow to adopt the technique. Note is made of the exponential growth of current interest in food immunoassays, and an excellent bibliography of 160 references is given indexed by author and analyte. The next three papers discuss the principles of classical and enzyme linked immunosorbent assays (ELISA), production, assessment, avidity and specificity of antisera, and the use of enzyme labelled reagents. Choice of enzyme, formation of enzyme-conjugates, hapten-enzyme conjugates and factors influencing the sensitivity of assays are detailed.

The section on macromolecules consists of seven papers which discuss for example the speciation of meats, the detection of soya proteins and the determination of staphylococcal enterotoxins, typical analytical problems which face food analysts daily. The third section on small molecules contains papers which discuss the determination of mycotoxins, hormones and potato glycoalkaloids.

Excellent diagrams and graphs are used throughout the book to supplement the text which is clear and easily read. This book should be in the library of all food laboratories irrespective of whether they are involved in immunoassay, because of the large amount of basic information and the number of references it supplies. A very useful book for food science students although the price may be beyond their means.

The papers are well written and concise as one would expect from the well known contributors. Some of the techniques described are beyond the scope of small (and some large) analytical laboratories. For example one paper decribes the preparation of immunoglobulins and antibodies which are subsequently used for the speciation of meats. Hopefully as immunoassays become more common in food analysis more antisera and associated reagents will become commercially available. Experience has shown however that not all commercially available antisera have the desired specificity, and several papers in the book give information on essential purification techniques.

The papers describing the raising of antibodies to small non-immunogenic molecules by bonding them covalently to an immunogen should stimulate readers' ideas for development of future assays, especially when note is taken of the specificity achievable. The book perhaps could have included some papers discussing recent developments in, and uses of, gel diffusion immunoassay, a technique which is still extensively used by food analysts. The symposium was held in September 1983, and the book has only recently been published, so it is not describing the present state of the art considering the exponential growth of immunoassay applications to food analysis. It must almost be time for another symposium on the subject, and hopefully a compilation of papers as excellent as this book will be produced.

I. D. Lumley

Microbiology of Frozen Foods. Ed. by R. K. Robinson.
Barking, Essex: Elsevier Applied Science, 1985. Pp. x+290. ISBN 0 85334 335 7. £33.00.

At first I wondered whether I was reviewing the correct book: the first two chapters making up about a third of the volume barely mentioned a microorganism! These two chapters by Boast, and Boegh-Soerensen and Jul, respectively covered the technology of freezing and the effects of freezing, cold storage and thawing on the organoleptic quality of the food. Perhaps the title should have reflected the inclusion of such a large amount of non-microbiological content.

The remaining six chapters discussed the general response of microorganisms to freeze-thaw stress, frozen meat, fish, dairy and related products, catering and finally the laboratory microbiological examination of frozen foods. In view of the varied content of this book the target readership is not easy to define, but those presently engaged in (or contemplating) Quality Control or Development in the frozen food industry, or those setting up a food freezing operation would find it of value and interest.

The chapter by Davies and Obafemi reviews the effect of freezing particularly, and cold storage and thawing, on microbes and indicates the mechanisms associated with the freeze injury of susceptible genera. Lowry and Gill of the Meat Industry Research Institute of New Zealand present a very satisfactory review of the microbiology of frozen meat and meat products. They deal adequately with yeasts and moulds as well as bacteria. Their statement, that the activity of microbial exoenzymes including lipases is not particularly important unless near-spoilage densities of bacteria are present prior to freezing, could be misleading. Whilst the statement is probably correct when applied to carcase meat or cuts, the situation existing in comminuted meats is probably different with microbial lipases being of greater importance in reducing organoleptic quality during cold storage.

The chapter on fish and related products, by Simmonds and Lamprecht, is a good review of the microbiology and spoilage characteristics of the unfrozen cold-, warm- and fresh-water fishes. The section on freezing is relatively small but the effects on microbial content of holding thawed fish (Cape Hake) are well illustrated graphically. Overall this chapter provides a statement of Good Manufacturing Practice as applied to catching, handling, freezing and thawing of fish and the production of hygienic products.

Rothwell's chapter on dairy products briefly reviews the freezing of milk, cream, butter, starter cultures for cheese and fermented milk (and other) uses, cheese and ice cream. In most of these the essential point is made (as it is throughout the book) that the microbiology of the final frozen material reflects that of the material for freezing with some reductions due to freezing and cold storage. Ice cream is essentially a pasteurized commodity and the microbiology of the final product therefore reflects the efficiency of pasteurization and subsequent handling. Many items are nowadays added to such desserts and often added after heat treatment; therefore Quality Control is necessary to ensure the safety and microbiological quality of the fruit, nuts, biscuit and chocolate employed.

The editor's chapter on catering covers the obvious precautions required for setting up catering/cook–freeze operations particularly when considering the use of materials e.g., meat, poultry, shellfish, etc which may be contaminated with the well known food-poisoning organisms. This section deals with the hygienic requirements of the kitchen at the preparation, cooking and handling/serving stages.

Finally White and Hall's chapter on the laboratory microbiological examination of frozen foods provides sensible and well tried methods of sampling, preparation and enumeration. Freeze damage and its effects on enumeration are well covered. There is one small confusion in the section relating to enumeration of *Bacillus cereus* using Mannitol egg yolk polymyxin agar: the words used indicate that *B. cereus* does not give a positive egg yolk precipitation reaction when in fact it does.

W. E. Crabb

Microbiology of Fermented Foods, 2 volumes. Ed. by B. J. B. Wood. Barking, Essex: Elsevier Applied Science, 1985. Volume 1: Pp. xx+385. ISBN 0 85334 332 2. £54.00. Volume 2: Pp. xx+306. ISBN 0 85334 333 0. £42.00.

This two volume book aims to cover food fermentations of the developed and developing world with an emphasis on practical aspects and on their historical origins. It includes chapters on vinegar, cheese, other fermented milks, fermented sausages, fish fermentations, vegetable fermentations, silage, soy sauce and miso (fermented soybean paste), sour-dough breads, koji (moulded rice) manufacture and use, mushroom production, African fermented foods, tea, coffee and cocoa, as well as more general chapters dealing with yeast-lactic acid bacteria interactions in food fermentations, solid substrate fermentations of cereal straw and animal feedlot waste, production of industrial enzymes, vitamin enrichment in fermented foods, tropical food fermentations and technology transfer, strain selection and improvement, and a final chapter on miscellaneous food related fermentations not covered elsewhere.

All of these areas are adequately, if sometimes rather briefly surveyed and the book serves a valuable purpose in bringing them all together between four covers. However, as is inevitable with any compilation that attempts to be comprehensive in its coverage, a number of the chapters add rather little to what is already published in other recent books or reviews.

The book will find use by researchers in the field and will be of particular value to workers in developing countries since a number of the articles are slanted towards low technology processes that might be introduced relatively easily. With the possible exceptions of the chapters on sour-dough breads, fermented sausages and silage, the information provided is mainly descriptive, with only limited attempts at interpretation. In part this is, of course, a consequence of the little that is known about some of the fermentations. It is to be hoped that this book will stimulate more basic research on the nature of the microbe–microbe and microbe–substrate interactions occurring in these natural food fermentations.

The book is well presented and the relatively few photographs are of good quality. My main criticism concerns the inclusion in certain chapters of a diversity of non-S.I. units, including temperature in °F, salt concentrations in ° Salometer and volume in gal (whether U.S. or Imperial is not stated). References generally reach 1982 or 1983, which is as expected for a multi-author book with its inevitably long gestation period.

J.D. Owens

Health or Hoax? The Truth about Health Foods and Diets. By Arnold E. Bender. Goring on Thames: Elvedon Press, 1985. Pp. 184. ISBN 0 906552 25 7. £8.50.

For those consumers who believe that the ills of modern industrialized society are due to bad diet and who seek youth, vitality, sexual vigour and freedom from disease, this new book by Professor Arnold Bender will provide food for thought. Many of the myths and misconceptions about the health benefits of specific foods and nutrients, and the frequently incredible claims made by the more unscrupulous purveyours of nutrition quackery are exposed by Professor Bender for what (in most cases) they are, largely nonsense, at best promotions by misguided but sincere believers, and at worst by outright frauds and swindlers.

In this 184 page hardback book, Professor Bender collects together a wealth of information about the huge and expanding health food and diet aid industry, and takes care to point out to readers that the food laws in the U.K. ensure that very strict standards of microbiological and food quality are adhered to by the vast majority of food manufacturers. The book is a joy to read with one anecdote after another to ease the reader through a complex and informative text.

In Chapter 1, 'Science or Salesmanship', the author states there is no such thing as a health food, just a health food industry, and this latter point is illustrated by the extraordinary statistic that there is now one health food shop for every 45 000 people in the U.K. The chapter describes how the manufacturers of health foods define their products; explores why people buy them, expresses some concern regarding the potential risks such as vitamin overdosage, toxic supplements and plant extracts, malnutrition from fad diets etc., and draws attention to the awesome power of the media to sensationalize and persuade a gullible public to literally pour their money down the drain.

Chapter 2, 'Nature knows best?' asks the question how safe are unprocessed foods describes what processing aids are, identifies some of the more common natural toxins and dispels the myth that organically grown produce is more nutritious than that grown on commercial farms using inorganic fertilizers. Chapter 3, 'Commodities and Claims' includes sections on ginseng, garlic, honey, bee's royal jelly, laetrile, lecithin and a host of potions and cure-alls, many of which have extravagant claims which are completely unsupportable under the close scrutiny of the professional nutritionist.

In Chapter 4, 'Value for Money' the author explores the willingness of the many people who are keen to pay a premium price for health foods in preference to 'ordinary foods' which are frequently regarded by protagonists of health foods as mass-produced, overprocessed and overpackaged. Sections of the chapter are devoted to processing and nutritional value and a plethora of the old wives' tales—many of which could make your hair curl! This latter theme is expanded in Chapter 5 with answers to over 60 questions; ranging from 'Is fish good for the brain?' and 'Does grapefruit burn off fat?' to 'Is vitamin E good for sex?'. The magic of vitamins is the subject of Chapter 6. Professor Bender explains why we need them, how much we need, how they were discovered and 'megavitamin therapy'. Chapter 7 on 'Diets and Slimming' focuses on the health risks of the Zen macrobiotic diet, the story behind the so-called 'starch-blockers' and the irresponsibilities of using a liquid protein diet without proper supervision.

Finally, in Chapter 8, Professor Bender points the way to a healthy diet, summarizes the role of the individual nutrients and dietary fibre in good nutrition and finally gives an outline of the best available scientific research. Perhaps the only criticism of the book and of Chapter 6 and 7 in particular, is the omission of a summary of the actual nutrition

claims permitted under U.K. food regulations so that readers could at least have an understanding of how to interpret what they see on labels on packaged goods from reputable manufacturers.

The book has a good index and is an interesting collection and examination of the more spurious claims made for a range of 'wonder diets', herbal remedies and nutrients. The lay reader will find the book fascinating and entirely comprehensible and the scientist will enjoy a book full of anecdotes written by Professor Bender in his own inimitable style.

D.P. Richardson

Spray Drying Handbook. 4th edn. Ed. by K. Masters.
London: George Godwin, 1985. Pp. xiv+696. ISBN 0 7114 5805 7. £45.00.

Spray drying technology because of its association and dependence on atomization has seen a vast increase in applications and a growth in diversity. Though the text is divided into six sections containing eighteen chapters, the contents can be conveniently considered in three groups of nearly equal volume. Atomization and spray drier designs constitute one third of the book and are well supported by chapters on representation of sprays, their drying and less extensively, drying principles. Wheel atomization in particular is treated thoroughly. The next third of the text deals with the separation and recovery of the dried product, operational aspects of the drier plant, including operational measurements and a survey of the many auxiliary plant items that make up a complete spray drying plant. The final third of this book is connected with the applications of spray drying. This section includes a patent survey of processing systems involving atomization. The period covered is from 1971 to the 1980, with only a few patents after that year. A large number of spray drying applications are described, many with process diagrams, flowsheets and useful process details. Though a large section covers applications in the food industry, spray drying of many products is described from inorganic, organic, pharmaceutical, biochemical, timber and waste product industries.

The wide range of applications, shown with clarity and very good detail, require many different forms of plant and different operating parameters. This versatility of spray drying is also its weakness, because it leads to drying operations which are poorly understood and only too frequently wasteful in energy. The problem of efficient drying, though recognized as compromise of heat and mass transfer, is not treated adequately and for detailed treatment of drying principles the reader is referred to other sources, notably the excellent book by R.B.Keey (Introduction to Industrial Drying, Oxford, Pergamon Press 1978). The second ommission is total absence of any treatment on fluidization which is correctly claimed as an essential step in many efficient spray drying plants. Particle agglomeration is another aspect of spray drying omitted, that would have enriched this book.

The good presentation of the subjects is well supported by numerous figures and tables and also some excellent photographs. Though the text has been updated there are some chapters that have not been changed from the initial publication. The book is however a most useful source of reference on nearly all subjects connected with spray drying, up to 1979. Some very useful references presented in tabular from, on a number of specific items, such as Dryer Design Procedures, Atomization, Theory of Air

Dispenser Design and others, have been practically lost in the text because of their absence in the index. The text is supported by worked out examples, though in fewer numbers than in previous issues. Some equations are presented without deduction and indication of their origin. Nomenclature of symbols used, placed at the end of the book is not comprehensive enough because of the diversity of subjects discussed and causes some duplication of symbols. This leads to the use of the same symbol for different properties and some confusion.

The text covers many fields of engineering and technology and has essentially a practical bias. For this reason this book remains a valuable, but in some aspects dated, treatment on the basics of single stage spray drying. Though its main use is possibly as a reference book and a theoretical text for engineering students, operators of spray drying plant will find it useful as well.

J.S. Olejniczak

Food Analysis: Principles and Techniques. Vol. 3. Biological Techniques. Ed. by Dieter W. Gruenwedel and John R. Whitaker.
New York: Marcel Dekker, 1985. Pp. xvi+395. ISBN 0 8247 7183 4. $90.00.

This volume describes and discusses a number of biological techniques widely used in assessing the quality and safety of food components including macro- and micro-nutrients, additives and contaminants. The techniques employed range from the use of intact animals to *in vitro* procedures using tissue preparations, mammalian cells in culture, bacteria, fungi, protozoa and yeasts.

The six chapters comprising the book are: the use of whole animals; the use of bacteria, fungi, protozoa and yeast; analysis of food products for microorganisms or their products; cell and tissue culture methodology; immunological techniques and analytical uses of enzymes. The treatise is intended for use as a reference book for graduate students and for scientists involved in food analysis, and with this objective in mind the seven authors involved in writing the various chapters have in the main placed particular emphasis on the underlying principles of the various techniques described. Additionally, each of the chapters provides numerous examples of the applications of the techniques in the context of food analysis.

The chapters have been generally well written, and two in particular need to be commended; one is on cell and tissue culture methodology. The text is lucid, wide ranging and detailed. The author has made considerable effort in describing the various techniques used, the genetic endpoints measured, the precautions required to ensure standardized conditions and avoidance of contamination and the application of the techniques to a variety of food chemicals. In addition an extensive review of the literature and references are provided. The other chapter also to be specially commended deals with the analytical uses of enzymes. This chapter explains in considerable detail the underlying principles of enzyme kinetics and the effects of a variety of factors on enzyme mediated reactions. Not surprisingly these two chapters were written by the editors.

This volume is well presented, relatively free from typographical errors, with clear illustrations and an index. Any intended reader must note however, that this book, in

common with other volumes in the series, is not a handbook of analytical methods and not designed to serve as a primary book of instructions for undergraduates.

S.D. Gangoli

Basic Science for Food Studies. Ed. by V.L. Brownsell, C.J. Griffith and Eleri Jones. Harlow: Longman, 1985. Pp. 209. ISBN 0 582 41328 1. £5.95.

This useful text has eighteen chapters in 209 pages. It gives a first introduction on the fundamentals of physical, chemical and biological science written from a catering point of view. The basic science starts with SI units, density, energy, heat, electricity then moves into properties of matter, atomic theory, chemical reactions, acids, bases and salts, then some simple organic chemistry leading into the nature of living things: the microbes, plants and animals, to include physiological processes of bacterial growth, modes of nutrition, human nutrition and digestion. The final chapter describes twelve practical experiments including measurements of relative density, pH and microscopic examination of food materials. Each chapter ends with five self assessment questions to which no answers are provided.

The text is designed to meet all the requirements of students of Basic Science for Catering Level I; it meets this need very well. In addition it is suitable for all those following BTEC Food Technology, Catering and similar Food courses with a need for Basic Science at Level II or even III, including Bridging packages in Higher BTEC courses.

This is an excellent *first* text on basic science for students. It is concise and easy to read. It is simple yet comprehensive: good for the beginner and useful for those moving onto higher studies. It will be helpful as a quick reference book on the elementary principles of science which frequently crop up in food studies. The authors time and time again make reference to food, catering or the food industry to positively relate basic science to food studies.

The contents are logically developed through physics to chemistry to biology. The authors have done well to condense the biology into meaningful accounts plus the importance to food studies of the microbes, the Plant and Animal Kingdoms. The illustrations on virus structure and cultures of microorganisms could be improved. Human nutrition is linked with energy but food chains and ecology stop short of including kilojoule values. Suggested texts for further reading or references are not provided.

The twelve experiments do not include discussion about expected or other results. The fullsome descriptions of the methods for the laboratory work do not draw attention to safe working methods. Students should know about hazardous substances and have training in the handling of for example Millions reagent and concentrated sulphuric acid. The text does emphasize the potential hazards involved with electricity and the importance of basic food hygiene.

This is a paperback, size 21×14 cm with all the pages packed with relevant facts about basic science. The typeface is easy to read. The layout is generally good. The numbering of chapters, sections and subsections makes it easy to follow, especially for cross-referencing purposes. The text is pleasantly broken up by interesting tables,

diagrams and illustrations, signs that the authors have drawn upon lecturing experience to catering students. The index is adequate for the level intended. Use this as a first introduction to basic science for food studies and you will be well satisfied.

R.P. Goodchild

Sensory Evaluation Practices. By Herbert Stone and Joel L. Sidel. Orlando: Academic Press, 1985. Pp. xi+311. ISBN 0 12 672480 6. £39.50.

Sensory analysis has developed as a science over the last 150 years, but its extensive use as a practical tool in product development and quality control in industry has only occurred over the past 50 years. Over this period, relatively few textbooks or manuals have appeared. These range from benchtop 'cookbooks' to more academic treatises. This book is intended to provide guidance for the development and application of sensory analysis within an industrial environment, and, with some justification, claims to be unique in providing extensive coverage of principles, design and procedures for business rather than for academic purposes. To this end, it covers the development and definition of the field, placing considerable emphasis on organization, goals, strategy, facilities, and many other general aspects of current use of sensory analysis, mostly discussed in practical details, e.g. design of test facilities and selection, screening and monitoring of subjects. It then proceeds to cover the principles of sensory measurement and the tools used, together with statistical methods of data analysis. This is followed by a chapter on design of experiments, with extensive discussion of both psychological errors (biases) and statistical constraints on design. The next three chapters cover detailed accounts of how to conduct the various descrimination tests and descriptive and affective (hedonic) methods. These chapters include further guidance on when to select particular methods, data analysis and interpretation of results. The chapter on descriptive methods, not surprisingly, is mainly devoted to the author's own version of this family of techniques, Q.D.A. These powerful and extensively used techniques now form one of the most important areas of sensory analysis. The chapter covers a range of applications not really well covered elsewhere, e.g. relation to advertising, stability and storage, product development, optimization and Q.C. The book closes with a useful epilogue.

The book is intended for those responsible for sensory resources, technical and research managers, and for a wide range of professional workers whose areas relate to sensory quality and marketing. It is also directed towards teaching, and to more experienced workers in sensory science. It will certainly be useful to many of these people, although probably more to refer to than as a laboratory manual. It very largely succeeds in its objectives, and is particularly useful in the extent to which it discusses the strategy of using sensory resources efficiently within an industrial organization. The experience of the authors in this respect makes a valuable and unique contribution.

The book does not claim to be comprehensive; its over 200 references are well chosen, up to 1983, and with only a few minor errors. It is well laid out, easily read and the illustrations apt and clear. However, it does have a major drawback in that important, if not vital item, the index. Although it contains over 400 terms, it is difficult to use, because of the method of sub-classification. It demands either a knowledge of the main heading, or a search through much of the index. For example, although most of one chapter is devoted to different forms of bias, it is only indexed as such under

Discrimination tests (one sample bias; response bias) and under Q.D.A. (number bias). However, thirteen different frms of bias are indexed under sub-headings of 'Psychological' error without using the term 'bias'. Similarly 'fatigue' is indexed only under Discrimination test and Ordinal Scales, but a useful reference to it is not indexed (p. 251). It is a great pity that so many otherwise excellent reference books are made much less valuable because too little thought and effort is given to indexing. Nevertheless, it will prove to be a useful addition to many bookshelves.

D.G. Land

Trends in Modern Meat Technology. Ed. by B. Krol, P.S. van Roon and J.H. Houben.
(Proceedings of an International Symposium, Wageningen, 1984.)
Wageningen: Pudoc, 1985. Pp. 125. ISBN 90 220 880 0. Dutch Fl 50.00

The editors in their preface say that the Symposium was held (in October to November 1984) because there are so many current developments in meat technology that: 'it seemed useful to have a discussion on *trends* of new developments [such] as these with ... colleagues working in research institutes, industry and government bodies . . . and to publish the proceedings and the results of the discussions as soon as possible'. They have succeeded excellently with the second objective, and in my view, however useful the discussion may have been to the participants, this published version will be useful also to many others.

It is predominantly the *research* worker's view of the present trends. Among the addresses of the participants I find twelve research institutes, university departments and government bodies, three industrial research laboratories or consultancies and only two manufacturing establishments, which are the sort of proportions to be expected at international conferences of the kind. The papers presented, or chapters in the book, therefore lean heavily either to research studies of topics which are somewhat in advance of actual present day commercial technology, or to deeper scientific investigation of technologies which have long been, and are still, successfully practised without the benefits of that science. In the former category are the studies of applications of electrical stimulation and hot boning techniques, which industry, for its own good reasons, is much slower to adopt than the researchers seem to believe they should be; in the second are studies, which I personally find exciting, which propose new and usually simpler ways of making well established, very complex products, still imperfectly understood, such as dried fermented sausage or liver sausage. In most cases we are not told, and it is difficult or impossible to deduce, which of the research proposals are actually in use in factories, to what extent, under what precise practical conditions and with exactly what benefits.

With that reservation, however, I find the book a good summary of what is new in meat technology now. Discussion of injection, massaging, tumbling and comminution processes is sparse (perhaps the research technologists have finished with them!) and only one packaging application which is quite specialist is considered, but otherwise the coverage of new developments, from slaughter onwards and over all the main kinds of meat products, is fairly comprehensive.

Most of the contributors write in the English which non-English scientists use at conferences. It is incomparably better than most of our attempts at Dutch, German,

French or Danish but it does contain occasional small traps or difficulties if you are not used to it. And it may raise an innocent smile occasionally: 'Will meat research make or brake the sector?'

If you could not find time or funds to go to the last few Meetings of European Meat Research Workers, or to read their massive Proceedings, or if you have a general interest in this area, then I think that you will find this well produced little volume very good value for money.

M.D. Ranken

Functional Properties of Food Components. Ed. by Yeshajahu Pomeranz. Orlando: Academic Press, 1985. Pp. x+536. ISBN 0 12 561280 X. £69.00.

Apart from its cost this well produced book makes a useful background text for first and second year Food Science undergraduates as it is clearly written and most of the explanations of the behaviour of the different food components in a foodstuff are lucidly presented. It attempts to describe what in the chemical nature of the component makes it behave the way it does, why it performs, or not, in this way and which of such functions still require more research.

The book is essentially divided into three parts. The first consists of 324 pages, and discusses each of the major components in foods, namely water, carbohydates (including starch, structural polysaccharides, pectins, gums, corn sweeteners and wheat carbohydrates), proteins (both general and in specific foods), enzymes and lipids. The second part, of 120 pages, discusses engineered foods including both traditional foods and foods of the future and the final part, of 4 pages, discusses information and documentation.

Not surprisingly in view of the author's background, the discussion on the carbohydrate components is most extensively covered and certainly the chapters on water and specific food proteins would only serve as background reading for all but the most slothful student. However, each chapter carries its own comprehensive list of references quoted to cover the period up to 1982/3 and thus more extensive information is easily acquired. The clarity of the diagrams and the freedom from typographical errors are a feature of this book and the index, although not as extensive as one might wish, is adequate and helpful to the seeker of specific information.

My only reservation regarding the book is the rather dogmatic way in which several statements are made, statements which, if not actually incorrect, would certainly be the subject of some debate. For example as early as page 5 it is claimed that deteriorative changes induced by heat processing are less at lower water contents; this is obviously not true for non-enzymic browning and several antoxidative reactions. Similarly the statement that actomyosin toughness is on average 10 times as powerful as background (collagen/elastin) toughness in meat tenderness would not be universally accepted. This fault though is certainly true of most undergraduate texts, and occurs more frequently in most others and would not stop me recommending the book for background reading to any student. It would though be advisable to read it in conjunction with a more traditional food chemistry text to ensure a balanced approach.

D.A. Ledward

Food Allergy. Ed. by Patricia Scowen, Gisela Medhurst and Jill Leslie.
(Edsall Summaries for Health Professionals No. 2).
London: Edsall, 1985. Pp. 96. ISBN 0 90263 45 1. £3.90 (paperback)

One of the problems in the allergy field is epitomized in the foreword: 'Although members of the general public are well aware that a number of common clinical disorders can be caused by foods, a similar understanding by many health professionals has been slow to gain acceptance (despite some 40000 research papers in the last 10 years)'.

There is no unequivocal diagnostic test for allergy: none of the twenty currently used tests is reliable: as a result, estimates of the prevalence of allergic disease vary from less than 1% to as much as 20%. The picture is further clouded by the number of people who think that they are allergic to a food—about a quarter of those questioned. 'A leading expert' estimates that only two or three out of ten of them can be shown to have an intolerance, and perhaps one in ten, a food allergy. A figure from the U.S. illustrates the poor evidence; estimates of immunoglobulin-E (IgE)-mediated food allergy range from 1 in 1000 to 70 in 1000; 'so wide a range of possibilities as to be almost meaningless'.

Allergy has become so popular a topic in the media that people forget, or do not know, that the greatest potential source of harm from food is contamination by microbes, followed by nutritional imbalance. Note that additives are very low in this list. Tartrazine, for example, has been bandied around until it has become the archetypal food problem, yet it affects, perhaps, one person in 10000.

For medical and biochemical purposes it is necessary to distinguish between true allergy, which is a response to an immunological cause, and food intolerance, which covers any abnormal response to food. Since true allergens are protein or carbohydrate in nature tartrazine is not an allergen, but this is of little comfort to those who suffer. Intolerance includes inborn enzyme defects, or temporary ones following some disorder, and 'false food allergies' involving the release of histamine or prostaglandins without the immune system. The latter are thought to be about ten times as common as true allergies, and are common in children, who tend to outgrow the problem by about 8 years of age. This highly complicated subject is presented in a simple, easy-to-read fashion with an explanation of the specialized terms so often used, and misused.

We are warned not to attempt self-diagnosis and self-treatment since this has given rise to cases of severe malnutrition. We are also warned against quack treatment with the illustration that 35% of subjects with seasonal rhinitis improve after a course of placebo. The parallel is the numerous, usually expensive, nostrums that circulate in western society through word-of-mouth testimonials and are based on the placebo effect. This book will prove of value to all those engaged in teaching public health, in food research or manufacture, or in any chemical area related to food—and it is cheap.

A.E. Bender

Toxigenic Fungi - Their Toxins and Health Hazard. Ed. by H. Kurata and Y Ueno.
(Developments in Food Science, 7).
Amsterdam: Elsevier, 1984. Pp. xix+363. ISBN 0 444 99630 3. $86.50.

This volume represents the Proceedings of the Mycotoxin Symposium held during the

Third International Mycological Congress in Tokyo, 30 August to 3 September, 1983 and is Volume 7 of the series 'Developments in Food Science'.

The study of mycotoxins has been a major commitment in Japan and this is reflected in the existence of the Japanese Association for Mycotoxicology which was established in 1974. Since 1964 there has existed a Joint U.S. - Japan Cooperation on Development and Utilization of Natural Resources which provided a panel on toxic microorganisms as a forum for the interchange of information on mycotoxin research. So it was very appropriate that these two bodies should sponsor a major symposium on mycotoxins during the Third International Mycological Congress.

It must be recognised that the book presents the work of eighty contributors and has apparently been produced using camera-ready copy. This means that the editors can have very little editorial jurisdiction over presentation, but it also means that the proceedings have been produced soon after the meeting generating them and will reasonably reflect the present state of a range of mycotoxin studies. It is necessary for the reader to be very patient with the grammatical deficiencies of some of the contributions.

The book is divided into five sections being in fact, the collected proceedings of five separate symposia. The first of these deals with the ecology of mycotoxin-producing fungi and includes studies on the moulds associated with koji manufacture, the possible use of indicator mould species in the evaluation of the significance of fungal deterioration of foods in the market place, the interactions of insect damage and aflatoxin formation in cotton seed, and cereals, the influence of drought and soil temperature on aflatoxin formation in groundnuts, as well as mycotoxin production in malted barley and in maize before harvest. Two of the papers illustrate the value of a resource allowing environmental control of crop plants during the study of the preharvest formation of mycotoxins. Thus, the extensive facilities in Georgia, U.S.A., have provided an insight into just how critical soil temperature is in the development of aflatoxin in drought stressed groundnut plants.

The second section concerns the taxonomy of mycotoxin producing fungi with a particular emphasis on *Aspergillus, Penicillium, Fusarium* and *Chaetomium* but with an additional overview on toxigenic ascomycetes.

The third section deals with foods and feed mycology in relation to the presence of mycotoxins and includes papers presenting survey data and analytical methods. The fourth section is devoted to the toxicology of mycotoxins and there is a concluding brief section on the epidemiology of mycotoxins including papers on risk assessment of human cancer arising from exposure to aflatoxins. The book is completed by a fungal species index as well as a useful general index.

It is unfortunate that the book does contain so many trivial errors of grammar and spelling although these do not usually lead to any ambiguity of meaning. For the specialist in mycotoxin studies it will none-the-less provide a useful source of information and an entrée into the recent literature, for each contribution has a bibliography, but it cannot be recommended to a wide readership because it has not been subjected to a sufficiently ruthless editorial scrutiny.

M. O. Moss

Books Received

Developments in Meat Science, Volume 3. Ed. by Ralston Lawrie.
Barking: Elsevier Applied Science, 1985. Pp. x+227. ISBN 0 85334 361 6. £30.00

Analysis of Food Carbohydrates. Ed. by G.G. Birch.
Barking: Elsevier Applied Science, 1985. Pp. viii+311. ISBN 085334 354 3. £40.00.

Zucker und Zuckerwaren. Ed. by Hartmut Hoffmann, Werner Mauch and Werner Untze.
Berlin: Paul Parey, 1985. Pp. 431. ISBN 3 489 617142. DM 148. (paperback).

Microwaves in the Food Processing Industry. Ed. by Robert V. Decareau.
Orlando: Academic Press, 1985. Pp. xiii+234. ISBN 012 208430 6. £33.00.

Common Fragrance and Flavor Materials: Preparation, Properties and Uses. Ed. by Kurt Bauer and Dorothea Garbe.
Weinheim: VCH, 1985. Pp. 213. ISBN 3 527 26038 2. DM 112.

Journal of Food Technology
Volume 21 Number 2 April 1986

Contents

Printed by Adlard and Son Ltd, Bartholomew Press, Dorking

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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

Typescripts (two complete copies) should be sent to the Editor, Mr W. B. Adam, George and Dragon Cottage, Chipping Campden, Glos. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be double-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

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

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

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

Abbreviations. Abbreviations for some of the commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular unless confusion is likely to arise.

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

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

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

Page proofs will be submitted to the contributors for minor corrections and should be returned to the Editor within 3 days. Major alterations to the text cannot be accepted.

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

กำหนดส่ง