ISSN 0022-1163

Volume 18 Number 1 February 1983

Journal of Food Technology

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

JOURNAL OF FOOD TECHNOLOGY

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

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

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

General and IFST correspondence should be sent to the Executive Secretary, IFST (U.K.), 105–111 Euston Street, London NW1 2ED

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 annual subscription is £72.00 (U.K.), £86.00 (Overseas), $195.00 (N. America, including cost of airfreight). Copies of the journal are dispatched by airfreight to New York for forwarding by surface mail throughout the Western Hemisphere, and by airfreight to Singapore for forwarding by surface mail to subscribers in India, Australasia and the Far East. Back volumes are still available. This journal is covered by *Current Contents, ASCA* and *Science Citation Index.*

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

J. Fd Technol. (1983) **18**, 1–9

Comparative study of three legume starches

A. H. EL TINAY, S. B. EL HARDALOU AND A. M. NOUR

Summary

Starch content of chickpea *(Cicer arietinum),* pigeon pea *(Cajanus cajan)* and bonavist bean *(Dolichos lablab)* was 58, 52 and 50% respectively with recovery of 57.9, 48.1 and 49.0%. The amylose content was 33.5, 27.0 and 31.0%; amylose chain length was 1420, 550 and 830 glucose units and amylopectin chain length was 29, 27 and 28 glucose units for chickpea, pigeon pea and bonavist bean respectively. Chickpea starch granules ranged from large oval shaped (21 × 30 μm) to small spherical (13 μm in diameter); pigeon pea from (35 × 42 μm) to 15.2 μm and bonavist bean (35 × 42 μm) to 15.2 μm. The gelatinization temperature range was 67–76°C for chickpea, 71–78°C for pigeon pea and 78–80°C for bonavist bean starch. The swelling power for chickpea, pigeon pea and bonavist bean at 95°C were 17, 18.5 and 22.5% respectively. The legumes showed a single-stage and somewhat restricted swelling. Solubility curves for legume starches showed a similar pattern and indicated that they have higher solubility at elevated temperatures than wheat starch. The liquefaction characteristics showed that chickpea has the highest resistance to cooking and was the most sensitive to α-amylase. The three legume starches gave stabilized Brabender hot-paste viscosity; chickpea had a lower overall viscosity due to its exceptionally long amylose chains.

Introduction

Kawamura, Tuboi & Huzii (1955) developed a method to isolate starch from various legumes by treatment with a 0.2% NaOH solution, washing with water and dehydration with ethanol and ether. Schoch & Maywald (1968) found that the separation of pure starch was difficult with certain legumes because of the presence of a highly hydrated fine fibre fraction (presumably from the cell walls enclosing the starch granules) and also the high content of insoluble protein.

Authors' address: Department of Biochemistry, Faculty of Agriculture, Shambat, Sudan.

0022–1163/83/0200–0001 $02.00

Some physio-chemical properties of legume seed starches have been reported (Srivastava *et al.*, 1970; Rosenthal *et al.*, 1971; Tolmasquim, Correa & Tolmasquim, 1971; Lineback & Ke, 1975; Modi & Kulkarni, 1976). The objective of this study was to isolate starch from chickpea, pigeon pea and bonavist bean and to examine the physio-chemical properties of their starches. Comparison of information on legume starch and wheat starch could provide useful knowledge about the use of legume–cereal flour combinations in baked products.

Materials and methods

Mature and healthy seeds of chickpea, pigeon pea and bonavist bean were obtained from the local market. These were thoroughly cleaned and freed from foreign material. Wheat starch flour was supplied by BDH, U.K.

Starch isolation

The method of Schoch & Maywald (1968) as modified by Lineback & Ke (1975) was used.

Starch analysis

The isolated starches were analysed for protein, fat and ash content according to the AOAC (1970) methods.

Microscopic examination

The isolated starch granules were studied microscopically as described by McMasters (1964).

Starch determination

Starch was determined by the method of dispersal in $CaCl_2$ followed by iodine spectrophotometry (Kerr, 1950).

Starch fractionation

Fractionation of starch into amylose and amylopectin was carried out according to Lansky, Kooi & Schoch (1949) as modified by Montgomery & Senti (1958).

Determination of amylose content of starch

Amylose content were determined colorimetrically as described by Williams, Kuzina & Hlynka (1970). The amylose obtained by starch fractionation was

used to construct a calibration curve. The amylopectin values were obtained by subtractions of amylose content from 100% starch.

End group determination of amylose and amylopectin

The method of Brown *et al.* (1945) as modified by Shasha & Whistler (1964) was used. Amylose and amylopectin chain lengths were calculated according to the formulae of Potter & Hassid (1948).

Gelatinization temperature range

Measurements were made using Kofler hot stage on a polarizing microscope, according to Schoch & Maywald (1968). Temperatures were recorded that correspond to initiation and completion of the loss of birefringence.

Swelling and solubility characteristics

Swelling and solubility patterns were determined using 4 g starch by the method of Schoch (1964).

Liquefaction characteristics

The falling number method of Hagberg-Perten (1968) was used. This method measures starch resistance to α-amylase and heat, i.e. rate of liquefication of gelatinized starch. It is based upon rapid gelatinization of a flour suspension and subsequent measurement of the degradation of the starch paste by α-amylase. Barley malt with an α-amylase activity of 333 SKB units supplied by 'Intra-Malt', France, was used.

Brabender viscosity curves

Brabender curves were determined using a Brabender Viscoamylograph and analysed by the procedure described by Kerr (1950).

Results and discussion

Starch content of chickpea, pigeon pea and bonavist bean was found to be 58, 52 and 50% respectively (Table 1). This range of values is considerably lower than that reported for wheat flour (70–80%; Pomeranz, 1971). Starch recovery from chickpea was higher than that from the other two legumes. Bonavist bean has relatively higher protein and crude fibre content (Table 3) which make starch separation difficult. Schoch & Maywald (1968) reported that the separation of pure starch from certain legumes is difficult because of the presence of highly hydrated fine fibre fraction and also the high content of insoluble protein.

Table 1. Analysis and properties of legume starches

Type of starch	Starch content (%)	Starch recovery (%)	Nitrogen (%)	Fat (%)	Ash (%)	Amylose (%)	Amylopectin (%)	Amylose chain length (glucose units)	Amylopectin chain length (glucose units)
Chickpea	58	57.9	0.05	0.21	0.05	33.5	66.5	1490	29
Pigeon pea	52	48.1	0.05	0.11	0.04	27.0	73.0	550	27
Bonavist bean	50	49.0	0.06	0.10	0.07	31.0	69.0	830	28
Wheat	–	–	0.05	0.50	0.02	26.5	73.5	525	23

In Table 1 is shown the chemical analysis of the isolated legume starches together with wheat starch. The nitrogen content of the starches was low (0.05–0.06%) with values similar to those reported for wheat starch (Pomeranz, 1971). The fat content of bonavist bean was the lowest (0.1%) and that of wheat was the highest (0.5%). Wheat and bonavist bean starches had the lowest and highest ash contents respectively (0.02 and 0.07%). Schoch & Maywald (1968) reported an ash content of 0.04–0.05% for chickpea starch. In wheat starch only a trace amount of ash has been reported (Pomeranz, 1971).

The range of amylose content for the three legume starches was 27–33.5% (Table 1) which is within the range given by Whistler & Smart (1953). Wheat starch and amylose content of 26.5% (Table 1). The average amylose chain length for chickpea, pigeon pea, bonavist bean and wheat starches was 1490, 550, 830 and 525 glucose units respectively. Rao (1976) reported an amylose chain length of 1667 and 540 glucose units for chickpea and pigeon pea starches respectively. Potter & Hassid (1948) found wheat amylose chain length as 540, and that of potato 980 glucose units. The average amylopectin chain lengths of chickpea, pigeon pea, bonavist bean and wheat were 29, 27, 28 and 23 glucose units respectively (Table 1). Cori & Larner (1951) reported the same value for wheat as is found in this work.

Chickpea starch granules ranged from large oval shaped (21 × 30 μm) to small spherical granules (17 μm; Table 2). Lineback & Ke (1975) found that chickpea starch granules ranged from large oval (17–29 μm) to small spherical (13 μm). Modi & Kulkarni (1976) working on pigeon pea starch found the

Table 2. Starch granule size and gelatinization temperature

Type of starch	Granule size (μm)			Gelatinization temperature range (°C) (Kofler hot stage)
	Oval shaped			
	Average width	Average length	Spherical radius	
Chickpea	21	30	13	67–76
Pigeon pea	31	41	13	71–78
Bonavist bean	35	42	15.2	73–80
Wheat	–	–	18	59–66

Table 3. Gross composition of the three legumes

Sample	Moisture (%)	Protein (%)	Fat (%)	Crude fibre (%)	Ash (%)	Carbohydrate (%)	mg/100 g		
							Ca	Fe	P
Chickpea	6.10	19.40	5.40	3.90	3.10	62.10	125	14.00	451
Pigeon pea	6.10	19.30	2.00	6.40	3.60	62.70	132	9.80	376
Bonavist bean	6.20	22.10	1.20	7.70	3.60	59.30	116	13.70	472

granules oval in shape and large in size (20.8–33.9 μm). Bonavist bean showed the largest starch granules which ranged from large oval shaped (35–42 μm) to small spherical (15.2 μm; Table 2). This agrees with the work of Rosenthal *et al.* (1971). The average wheat starch granule size was 18 μm. Legume starch granules differ from wheat starch which has a mixture of large, intermediate and small spherical granules (Kulp, 1973).

The gelatinization temperature ranges, determined using a microscope equipped with Kofler hot stage, were 67–76°C for chickpea, 71–78°C for pigeon pea, 73–80°C for bonavist bean and 59–66°C for wheat starch. Srivastava *et al.* (1970) reported the range 71–74°C for chickpea starch while Schoch & Maywald (1968) reported 66–72°C which agrees fairly well with values reported in this work. Modi & Kulkarni (1976) found a gelatinization temperature range of 66–72°C for pigeon pea. The gelatinization temperature for bonavist bean is relatively high. Rosenthal *et al.* (1971) obtained 65–70–76°C gelatinization temperature range for bonavist bean starch.

The swelling patterns for chickpea, pigeon pea, bonavist bean and wheat starches are shown in Fig. 1. All three legume starches showed single-stage swelling while wheat starch showed a two-stage swelling pattern. The single-stage swelling pattern obtained for chickpea starch is similar to that obtained by Schoch & Maywald (1968) and Lineback & Ke (1975). The swelling pattern of pigeon pea and bonavist bean starches is similar to that of chickpea starch. The swelling power of chickpea, pigeon pea, bonavist bean and wheat starches at

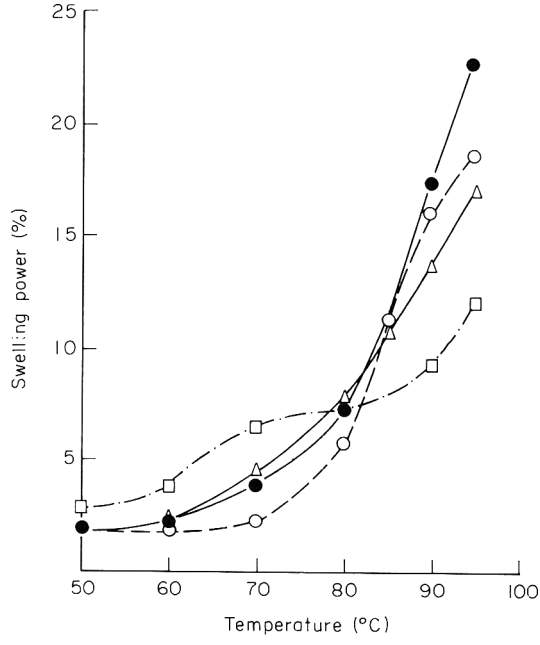


Figure 1. Swelling power (% d.b. corrected for solubles) of chickpea (—△—), pigeon pea (—●—), bonavist bean (– – ○ – –) and wheat (– · – □ – · –) starches.

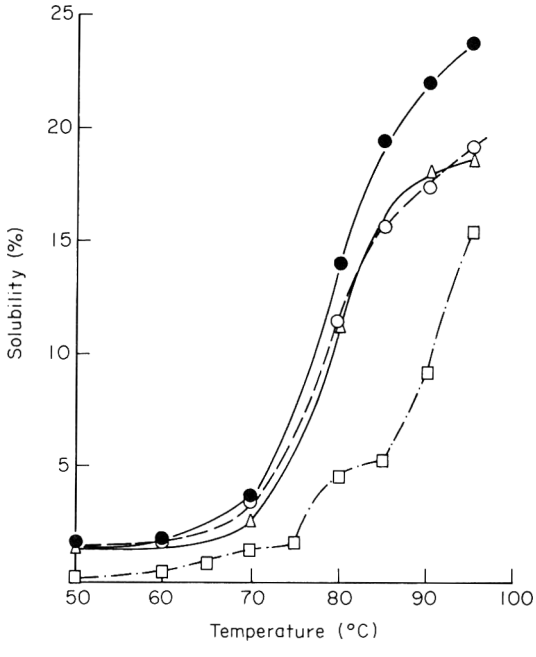


Figure 2. Solubility (% d.b.) of chickpea (—△—), pigeon pea (—●—), bonavist bean (– – ○ – –) and wheat (–·–□–·–) starches.

95°C were 17.0, 18.5, 22.5 and 12.0 respectively (Fig. 1). The swelling power for the examined legume starches is somewhat restricted. This restricted swelling plus the single-stage swelling pattern indicate starch granules of strong binding forces that relax over a narrow temperature range and not at different temperature ranges as in maize and milo (Correa, Rosenthal & Talmasquim, 1965). Rasper (1969) reported rapid and unrestricted swelling for potato starch at relatively low temperatures indicating weak and uniform bonding within the granules.

Solubility curves (Fig. 2) generally followed the same pattern as the swelling power curves (Fig. 1) indicating the direct inter-relationship of these two functions. The solubilities of chickpea, pigeon pea, bonavist bean and wheat at 95°C were 18.5, 24, 19.5 and 16% respectively. The solubility percentages and swelling powers of chickpea starch agree with the results of Lineback & Ke (1975). There seems to be a relationship between amylopectin content and solubility. Pigeon pea starch which has the highest amylopectin content (Table 1) also has the highest solubility.

Figure 3 shows that chickpea starch has the highest resistance to cooking compared to pigeon pea, bonavist bean and wheat starches. The falling number shows a negative linear relationship with malt quantity. Chickpea starch is more sensitive to malt α-amylase than pigeon pea and bonavist bean. The reference wheat starch sample has the lowest resistance to cooking in the absence of malt enzyme but is relatively more resistant against malt enzyme. The slope of the

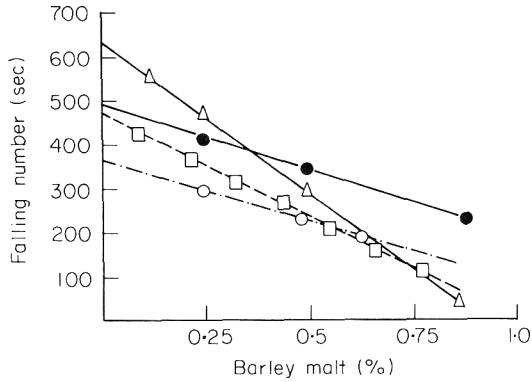


Figure 3. Liquefaction characteristic (falling number) of 4 g (d.b.) chick pea (—△—), pigeon pea (—●—), bonavist bean (-·-○-·-) and wheat (– –□– –) starches.

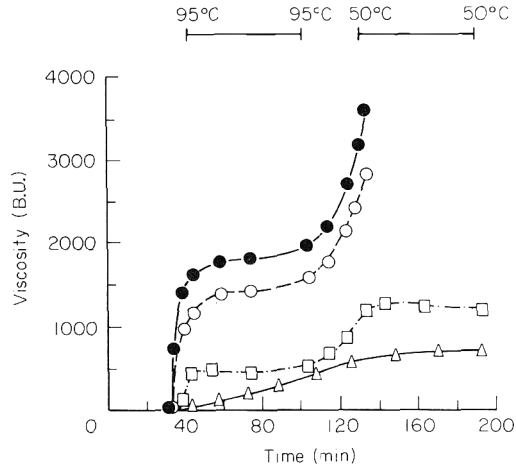


Figure 4. Graphical representation of Brabender curves (paste viscosity) for chickpea (—△—), pigeon pea (—●—), bonavist bean (– –○– –) and wheat (-·-□-·-) starches (7.5% level).

curves for chickpea, pigeon pea, bonavist bean and wheat starches were 0.7, 0.3, 0.5 and 0.28% respectively.

Paste viscosity curves for the three legumes and wheat starch are shown in Fig. 4. The starch concentration for all samples was 7.5%. The three legume starches gave a single-stage swelling with no pasting peak and an increasing viscosity during cooking as observed in the Brabender curves. This type of viscosity pattern is consistent with restricted swelling starches (Schoch & Maywall, 1968).

The initial pasting temperatures were approximately the same for the three legume starches; chickpea 76.5, pigeon pea 78 and bonavist bean 79.5°C. Lineback & Ke (1975) reported a lower initial pasting temperature of 68.5°C for chickpea while a higher temperature (81°C) for pigeon pea was reported by Modi & Kulkarni (1976).

Examination of the Brabender curves reveals that pigeon pea starch consis-

tently showed the highest viscosity throughout the cooking cycle. Bonavist bean showed higher viscosity than wheat starch while chickpea showed the lowest viscosity. Holding the pastes temperature at 95°C produced increased viscosities and cooling after 1 hr at 50°C produced an increase in viscosity for all starches (set-back) reflecting the tendency for the starch to retrograde.

Brabender curves for bonavist bean and chickpea starches varied even though their amylose : amylopectin ratios were similar. Goering, Eslick & Hass (1970) made a similar observation for barley starches for different varieties. This could be attributed to differences in granule structure or to differences in molecular structure of amylose and/or amylopectin. Chickpea starch consistently has the lowest viscosity and showed little set-back on cooling from 95 to 50°C (Fig. 4). This is probably due to the failure of amylose to form an exudate phase outside the granules although it retrogrades within the granules.

References

AOAC (1970) *Official Methods of Analysis,* 11th ed. Association of Official Agricultural Chemists, Washington D.C.

Brown, F., Dunston, S., Halsal, T.G., Hirst, E.L. & Jones, J.K.N. (1945) *Nature,* **156,** 785.

Cori, G.T. & Larner, J. (1951) *J. Biol. Chem.* **188,** 17.

Correa, A.M.N., Rosenthal, F.R.T. & Tomasquim, E.A. (1965) *Anas. Acad. Brasil Cienc.* **37,** 241.

Goering, K.J., Eslick, R. & De Hass, B. (1970) *Cereal Chem.* **47,** 592.

Hagberg-Perten (1968) *International Association of Cereal Chemists.* No. **107,** Japan.

Kawamura, S., Toboi, R. & Huzii, T. (1955) *Tech. Bull. Kagawa Agric. Coll.* **7,** 87.

Kerr, R.W. (1950) *Chemistry and Industry of Starches,* Chap. 4. Academic Press, New York.

Kulp, K. (1973) *Cereal Chem.* **50,** 496.

Lansky, S., Kooi, M. & Schoch, T.J. (1949) *J. Am. Chem. Soc.* **71,** 4066.

Lineback, D.R. & Ke, C.H. (1975) *Cereal Chem.* **52,** 334.

McMasters, M.M. (1964) In: *Methods in Carbohydrate Chemistry* (Ed. R. L. Whistler). Academic Press, New York.

Modi, J.D. & Kulkarni, P.R. (1976) *J. Fd. Sci. and Technol.* **13,** 18.

Montgomery, E.M. & Senti, F.R. (1958) *J. Polymer Sci.* **28,** 1.

Pomeranz, Y. (1971) *Wheat Chemistry and Technology,* Chap. 8. Academic Press, New York.

Potter, A.L. & Hassid, W.Z. (1948) *J. Am. Chem Soc.* **70,** 3488.

Rao, P.S. (1976) *J. Agric. Fd. Chem.* **24,** 958.

Rasper, V. (1969) *J. Sci. Fd. Agric.* **20,** 642.

Rosenthal, F.R.T., Spindola, L., Scraplao, M.I.L. & Silva, S.M.O. (1971) *Staerke,* **23,** 18.

Schoch, T.J. (1964) In: *Carbohydrate Chemistry* (Ed. R. L. Whistler) Chap. 3. Academic Press, New York.

Schoch, T.J. & Maywald, E.C. (1968) *Cereal Chem.* **45,** 564.

Shasha, B. & Whistler, R.L. (1964) In: *Carbohydrate Chemistry* (Ed. R. L. Whistler) Chap. 2. Academic Press, New York.

Srivastava, H.C., Harshe, S.N., Gharia, M.M. & Mudia, G.P. (1970) *Staerke,* **22,** 162.

Tolmasquim, E., Correa, A.M.N. & Tolmasquim, S.T. (1971) *Cereal Chem.* **48,** 132.

Whistler, R.L. & Smart, C.L. (1953) In: *Polysaccharide Chemistry,* Chap. 6. Academic Press, New York.

Williams, P.C., Kuzina, F.D. & Hlynka, I. (1970) *Cereal Chem.* **24,** 411.

(Received 14 October 1980)

J. Fd Technol. (1983) **18**, 11–19

Protection of ovalbumin against irreversible heat denaturation by a cationic amphiphile at high concentration

B. ERICSSON*†, P.-O. HEGG* AND K. MÅRTENSSON‡

Summary

The interaction of the cationic amphiphile cetylpyridinium chloride (CPC) with the egg white protein ovalbumin, was studied at various temperatures and pH values. The influence of different concentrations of CPC on the thermal behaviour of the protein was followed by specific optical rotation measurements in the pH interval 3–9. No increase in optical rotation was found at any pH when CPC was added to ovalbumin at room temperature. When heated, a gradual increase in optical rotation was registered, which became smaller the higher the pH used. At molar ratios of CPC : ovalbumin of above 70 this increase was reversed on cooling. The reversibility of the thermal unfolding on cooling was not affected by a variation in protein concentration between 0.5 and 10%. Circular dichroism measurements before and after a heating–cooling cycle confirmed the results obtained on optical rotation. When CPC was exchanged against the anionic amphiphile, sodium dodecylsulphate (SDS), a denaturing effect was achieved at neutral and alkaline pH values already at room temperature. At the acidic side of the isoelectric point, however, there was no major effect of SDS. No thermal aggregation of ovalbumin occurred near the isoelectric point when CPC was present and complete resolubility was found after thermal treatment and drying.

Introduction

The understanding of how to influence functional properties of proteins in food is still poor. However, precipitation and gelling properties of proteins under a thermal treatment have been investigated in some detail (Hegg, 1978). It was found that thermal aggregation could be influenced by rather simple methods.

Authors' addresses: * Department of Food Technology, University of Lund, Box 740, S-220 07, Lund, Sweden, and ‡Swedish Sugar Co. Ltd, Research Laboratories, Box 6, S-232 00, Arlöv, Sweden.

† To whom correspondence should be addressed.

0022–1163/83/0200–0011 $02.00

An increased salt concentration strengthened aggregation, while a high or low pH usually had the opposite effect. Even an inhibition of thermal aggregation of proteins could be achieved by adding small amounts of the anionic amphiphile sodium dodecylsulphate to the protein solution. Unfortunately, however, this leads to a simultaneous denaturation of the protein structure, which possibly changes the functional properties of the protein in a following food production process. Modification of protein properties without obtaining significant losses in protein structure is thus especially important in food technology. As thermal treatments are so frequent in food processing, also a protection against protein denaturation in general would be of technological importance. This is particularly true in processes where a protein structure with native qualities is desired after a unit operation where denaturation may occur, e.g. a pasteurization process (Lineweaver *et al.*, 1967). Protection is also particularly important if enzymatic activity is desired during or after such a process.

In model studies of the interaction between proteins and amphiphiles mostly bovine serum albumin (BSA) and sodium dodecylsulphate (SDS) have been used. Bovine serum albumin (BSA) has been reported to bind anionic amphiphiles with high affinity (Steinhardt & Reynolds, 1969) and at the same time become stabilized against structural stress as, for instance, heat (Gumpen, Hegg & Martens, 1979). The only protein reported with amphiphile binding properties similar to that of BSA is β-lactoglobulin (Steinhardt & Reynolds, 1969; Hegg, 1980). With the exception of a few proteins, e.g. glucose oxidase, papain and pepsin, which show a high resistance against unfolding by SDS (Nelson, 1971), all other proteins examined so far seem to be structurally transformed by this amphiphile (Lapanje, 1978).

If a minimal effect on protein structure is desired as a result of binding of an amphiphile, SDS seems to be an inappropriate choice. The twelve carbon skeleton, which appears to induce a maximal unfolding of proteins (Tanford,

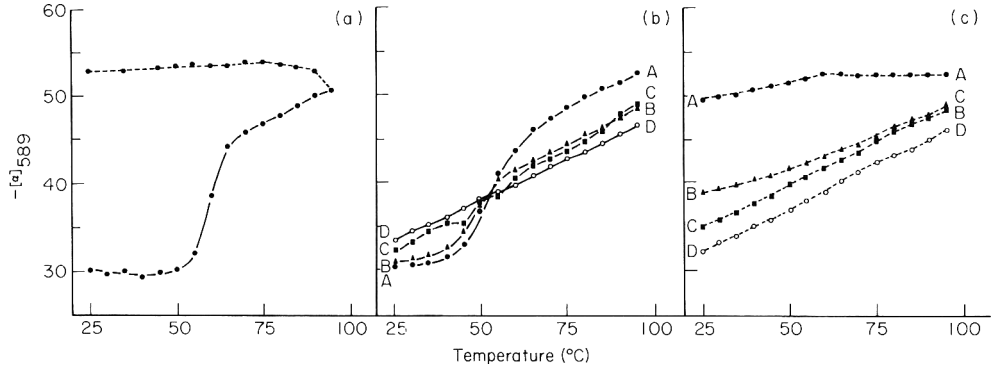


Figure 1. Specific optical rotation of ovalbumin and ovalbumin dissolved in cetylpyridinium chloride (CPC) at pH 3 as a function of temperature during heating and cooling. Protein concentration: 25 mg/ml. (a) Ovalbumin without CPC added. ——, Heating (1°C/min); ----, cooling (1°C/min). (b) Heating of ovalbumin (1°/min) in CPC of different concentrations. A, 8 mM CPC; B, 20 mM CPC; C, 40 mM CPC; D, 200 mM CPC. (c) Cooling of ovalbumin (1°C/min) in CPC. Concentrations as in (b).

1973; P.-O. Hegg, unpubl. data) combined with the sulphate head group, which intensifies the affinity to the protein more than most other head groups (Steinhardt & Reynolds, 1969), makes this amphiphile a strong denaturing agent. Thus the denaturating effect might be avoided if an amphiphile with a more apposite hydrophobic–hydrophilic balance is chosen. The interaction with such an amphiphile would probably result in a protein molecule with changed functional properties, but in addition there would also be a possibility to attain a protein structure less susceptible to external stress. The influence of amphiphiles other than SDS on protein structure is, however, poorly examined. This is particularly the case with the cationic ones.

The intention of the present work was to examine the effect of a cationic amphiphile on protein structure at different temperatures. As a model system cetylpyridinium chloride (CPC) and the egg white protein ovalbumin was used. The results obtained with CPC were compared with those obtained with SDS.

Materials and methods

Materials

Ovalbumin was prepared from hen's egg white according to Hegg (1979). No other egg white proteins could be detected in the protein preparation by SDS gel electrophoresis. Sodium dodecylsulphate (Lot No. L-5750) and cetylpyridinium chloride (Lot No. C-9002) were purchased from Sigma Chemical Co., U.S.A.

Methods

Optical rotation. The optical rotation measurements were carried out in a Perkin-Elmer 141 polarimeter at the wavelength of sodium-D-line (589 nm). The polarimeter cell (1.0 dm, 5 ml) was thermostated to temperatures within the interval 25 to 95°C at a heating and cooling rate of 1°C/min. This was obtained by coupling the polarimeter cell to a circulating water bath equipped with an electronic heat gradient control (Heto, Denmark, type 02 PH 623). Transition temperature is defined as the temperature at the midpoint of transition. Data are expressed as specific optical rotation with an accuracy of $\pm$ 0.5°.

Circular dichroism. Circular dichroism measurements were performed using a JASCO J-40 dichrograph in the wavelength range of 270–210 nm. Results are expressed as mean residue ellipticities (degree cm^2/dmol), using the value 112 for the mean residue weight of ovalbumin. A protein concentration of 0.5 mg/ml and a cell width of 0.1 cm was used in these experiments. All measurements were made at 25°C. As ovalbumin does not give a significant dichroism signal in the near u.v. region, only the far u.v. region, yielding information about the backbone conformation of the protein, was examined.

Preparation of solutions. Protein solutions of twice the final concentration were prepared using distilled water. Equal volumes of amphiphile solutions,

also of twice the desired concentrations, were then added slowly and under stirring. In most experiments a final protein concentration of 25 mg/ml was used. After addition of amphiphile the solutions were adjusted to appropriate pH using 1.0 M HCl or NaOH. A precipitate formed above the isoelectric point of ovalbumin during pH adjustment of the ovalbumin–CPC solution. This precipitate dissolved upon stirring. The time required for resolution was dependent on the pH of the solution, but was typically 1 hr.

All solutions used for analysis were optically clear and freshly prepared.

Results and discussion

Effect of cetyl pyridinium chloride on the thermal denaturation of ovalbumin at pH 3

At pH 3 ovalbumin carries a positive net charge of about 25 (Hegg, 1978; Nakamura, Hirai & Takimori, 1980), and due to net charge repulsion it does not aggregate when heated to temperatures above the denaturation temperature so far from the isoelectric point (4.6–4.9) (Rhodes, Azari & Feeney, 1958; Cannan, Kibrick & Palmer, 1941). Precipitation below the isoelectric point with anionic amphiphiles and above the isoelectric point with cationic has earlier been reported (Hegg, 1979) at low concentrations of added amphiphile. This phenomenon is thus not to be expected when the cationic amphiphile CPC is added at pH 3. These two facts made pH 3 suitable for examining the effect of various amounts of CPC on the thermal denaturation of ovalbumin.

The specific optical rotation (OR) of ovalbumin during heating and cooling is shown in Fig. 1a. In the absence of amphiphile the heat denaturation process is fast with a transition point at 59°C. On cooling the OR value does not return to the initial level, but remains above that obtained at 95°C. This is indicative of an irreversible process of denaturation, and is in accordance with earlier reports on ovalbumin (Smith, 1964).

From Fig. 1b it is seen that addition of various concentrations of CPC does not significantly alter the specific optical rotation at 25°C. This indicates that no unfolding of the protein structure occurs at this temperature. When heated at low amphiphile : protein ratios the thermal stability of the protein becomes lowered. Thus, the transition point decreases from 59 to 55°C at 8 mM CPC (molar ratio CPC : ovalbumin of 14) and to 50°C at 20 mM (molar ratio of 36). At higher amphiphile concentrations the sharp transition is replaced by a gradual increase in specific rotation and no defined denaturation temperature can be detected. The final optical rotation value obtained at 95°C is also lowered by 8°C compared to when CPC is absent. An increase in amphiphile concentration above 40 mM (molar ratio of 72) did not alter the optical rotation curve further.

The behaviour of ovalbumin on cooling at the different concentrations of CPC is given in Fig. 1c. At low CPC concentrations there is a slight decrease in the OR value as the temperature is decreased, but the major transition is not

reversed. However, for the two highest concentrations of amphiphile, the cooling process is the reverse of the heating process. Above 80% of the $\Delta[\alpha]_d$ is regained upon cooling suggesting that the heat-induced unfolding has become reversible.

Effect of pH

To examine the contribution of electrostatic forces for the formation of the protein–amphiphile complex, optical rotation measurements were carried out at increasing pH values.

The precipitate formed at and above the isoelectric point at low concentrations of added cationic amphiphile will redissolve at a sufficiently high concentration of amphiphile (Hegg, 1979). To be able to measure optical rotation of ovalbumin in CPC above the isoelectric point a concentration of 40 mM (corresponding to a molar ratio of amphiphile : protein of 72) was required. Notably, this concentration was also the requisite to obtain protection against irreversible heat denaturation at low pH (Fig. 1c).

Only a marginal increase in specific rotation was found at 25°C with increasing pH (Fig. 2), and the reversibility of the thermal denaturation process was completely maintained at all pH values examined. However, a gradual decrease in the slope of the curves was found with increasing pH when optical rotation was plotted as a function of temperature. This suggests that there is an electrostatic contribution to the protein–amphiphile interaction, and that complexes, which are progressively less heat susceptible, are formed when the negative charge on the protein increases. But since the interaction between ovalbumin and CPC is manifest already at pH 3, when both kinds of molecules are positively charged, hydrophobic interactions must also be of importance for the formation of the complex (Fig. 1b).

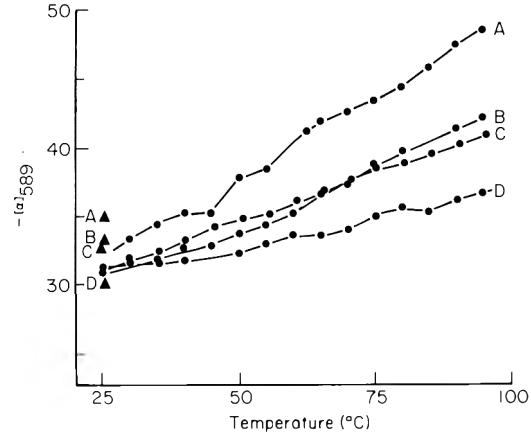


Figure 2. Specific optical rotation of ovalbumin (25 mg/ml) in 40 mM cetylpyridinium chloride (CPC) as a function of temperature at different pH values. A, pH 3; B, pH 5; C, pH 7; D, pH 9; ▲, Values after cooling.

Circular dichroism

It can be seen from the optical rotation curves (Figs 1 and 2) that ovalbumin in presence of CPC attains the same, or a very similar value of $-[\alpha]$ after heating and cooling as before heating. In order to compare the conformations corresponding to these values circular dichroism measurements of the two solutions were performed at pH 3 and the results are given in Fig. 3. At the wavelengths examined ovalbumin gave rise to a negative signal (Curve A), which diminished considerably after heating to 95°C (Curve B). The protein lost its ordered structure when denatured by heat and did not regain it on cooling. With CPC present the CD signal increased about 10% (Curve C). This small change might indicate that a slightly more dense complex is formed. The signal of the heat treated ovalbumin–CPC solution was identical to that obtained before heating (Curve D). These results show that the protein conformation is retained after the heating–cooling cycle, i.e. that CPC hampers the irreversible thermal unfolding of ovalbumin.

Effect of protein concentration

Ovalbumin normally becomes irreversibly denatured when heated. The amphiphile CPC has the ability not mainly to protect against heat denaturation but more to make the denaturation reversible. It is reasonable to assume that the reversibility of a denaturation process should be facilitated when the protein

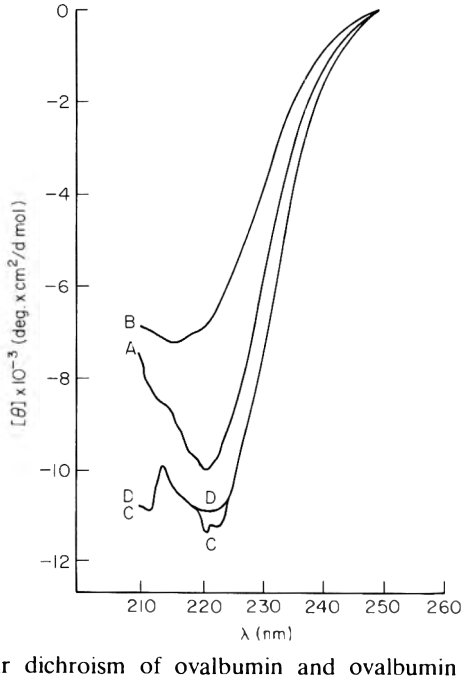


Figure 3. Circular dichroism of ovalbumin and ovalbumin dissolved in cetylpyridinium chloride (CPC) at pH 3. A, ovalbumin prior to heating; B, ovalbumin after heating to 95°C; C, ovalbumin in CPC (72 mol CPC/mol ovalbumin) prior to heating; D, the same as curve C after heating to 95°C.

concentration is decreased due to intermolecular interactions. To examine whether the reversibility of the heat denaturation process in the presence of CPC was influenced by protein concentration this was varied keeping the molar ratio of CPC to protein in (72 : 1) constant.

A protein concentration of 25 mg/ml has been used throughout this investigation. No influence on the reversibility was found when protein concentrations of 5 and 100 mg/ml were examined. However, when the protein concentration was increased a parallel displacement towards lower specific optical rotation values occurred.

Effect of SDS

Figure 4 shows the effect of 40 mM SDS on ovalbumin at pH 5, 7 and 9. At this concentration of SDS optical rotation measurements at pH 3 were not possible due to aggregation on heating. In contrast to CPC, the effect of SDS on ovalbumin seems to be extremely dependent on pH.

At pH values where ovalbumin carries a negative net charge, SDS has a strong influence on the optical rotation behaviour. Thus, the initial OR value at 25°C is increased by about 10 at pH 7 and 9, i.e. the protein conformation is altered already by the addition of SDS. Only a marginal further increase in optical rotation was registered when the temperature was raised to 95°C. Thus the protein conformation once formed at low temperature seems to be resistant to heat.

The effect of SDS on ovalbumin at and below the isoelectric point was completely different from that registered at pH 7 and 9. At pH 5 the OR curve

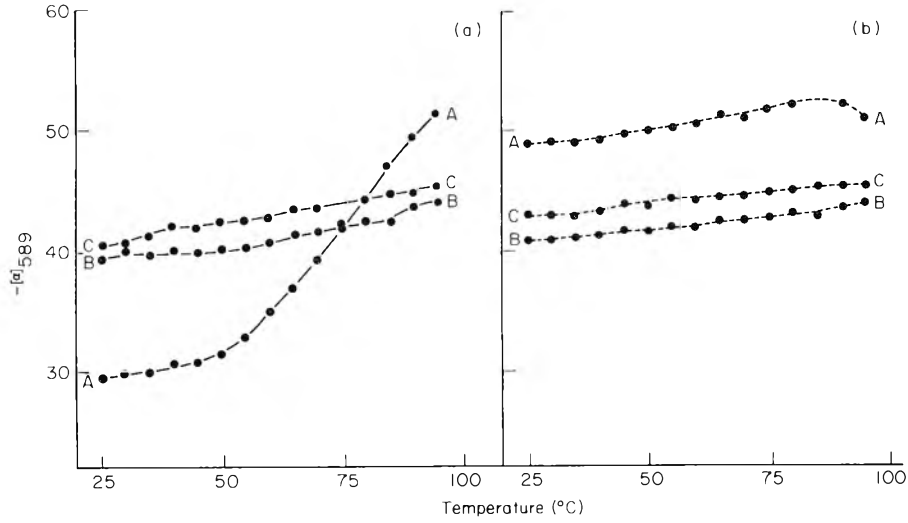


Figure 4. Specific optical rotation of ovalbumin in 40 mM sodium dodecylsulphate (SDS) as a function of temperature at different pH values. (a) Heating; (b) cooling. A, pH 5; B, pH 7; C, pH 9.

resembles the one of the protein solution without amphiphile (cf. Fig. 1a) both on heating (Fig. 4a) and cooling (Fig. 4b). The transition is, however, less sharp and the midpoint is at a somewhat higher temperature. Consequently, the effect of SDS on the conformation of ovalbumin at acidic pH values is moderate in comparison with neutral and alkaline pH values.

In order to avoid thermal aggregation at pH 3 the concentration of SDS was increased to 47 mM. This concentration corresponds to the same ratio of amphiphile : protein (w/w) as 40 mM CPC. The optical rotation *versus* temperature was almost identical to the one obtained at pH 5 and 40 mM SDS (Fig. 4a). Thus, with an addition of SDS no reversibility of the heat-induced unfolding is obtained at pH 3.

The observed difference between ovalbumin and the two amphiphiles used in this investigation might partly be explained by differences in amphiphile monomer concentration. Cetylpyridium chloride (CPC) has a critical micelle concentration (cmc) of 0.9 mM at 25°C, while SDS at the same conditions has a cmc of 8 mM (Mukerjee & Mysels, 1971). The lower monomeric concentration of CPC might be one important restriction for a co-operative binding of the monomeric amphiphile which seems to be the cause of the denaturation seen with SDS (cf. Makino, Reynolds & Tanford, 1973). The importance of micelle interaction, which has been reported for nonionic amphiphiles (Clarke, 1975; Bernath & Vieth, 1972), could therefore not be excluded in the case of CPC.

Effect on resolubility

A high solubility of dried powders is often important in food technology. Thus, in order to illustrate to what extent functional properties of a protein could be affected by addition of an amphiphile, the effect of CPC on the solubility of ovalbumin at pH 4.7 after a heating–cooling cycle was examined. To a solution of ovalbumin as added CPC to a molar ratio of 72. As a reference, an ovalbumin solution was used, to which no CPC was added. Both solutions were heated to 95°C, cooled and freeze dried. The dried samples were redissolved in distilled water and after 3 hr centrifuged at 8000 ***g*** for 30 min. The solubility was measured as the absorbance of the supernatants at 280 nm. Ovalbumin without CPC was soluble only to a marginal extent (0.6%), while ovalbumin in presence of CPC was completely soluble (100%), i.e. the addition of CPC brings about a complete resolubility of the protein after a very strong thermal treatment.

Conclusion

Addition of CPC in high concentrations, i.e. well above the cmc of the amphiphile, to ovalbumin at room temperature induces no increase in specific optical rotation. This amphiphile seems therefore not to cause a less ordered protein structure. This conclusion is supported by the circular dichroism measurements.

Conformational changes are registered when heated, however, but the thermal transition occurring without added amphiphile was to a large extent reduced in the presence of CPC. Furthermore, the transition induced by the thermal treatment was reversed on cooling. No evidences could be found from OR or CD measurements for differences in conformation between the unheated ovalbumin–CPC complex and the one which has been exposed to the heating–cooling cycle.

Work is in progress to examine the effect of different amphiphiles on various proteins in order to elucidate if other amphiphiles could be found which have similar effects as those registered for the amphiphile used as a model in this investigation, and if this effect also is applicable to proteins in general. In these studies, methods to study conformational changes complementary to those included here are used.

Acknowledgments

The authors are grateful to Gunnel Lundh for technical assistance. This investigation was supported by the Swedish Sugar Co. Ltd and the Swedish Board of Technical Development.

References

Bernath, F.R. & Vieth, W.R. (1972) *Biotech. Bioeng.* **14,** 737.
Cannan, R.K., Kibrick, A. & Palmer, A.H. (1941) *Ann. N.Y. Acad. Sci.* **41,** 243.
Clarke, S. (1975) *J. Biol. Chem.* **250,** 5459.
Gumpen, S., Hegg, P.-O. & Martens, H. (1979) *Biochem. Biophys. Acta,* **574,** 189.
Hegg, P.-O. (1978) *Thermal Aggregation and Denaturation of Egg White Proteins,* Ph.D. Thesis, University of Lund, Sweden.
Hegg, P.-O. (1979) *Biochem. Biophys. Acta,* **579,** 73.
Hegg, P.-O. (1980) *Acta Agr. Scand.* **30,** 401.
Lapanje, S. (1978) *Physicochemical Aspects of Protein Denaturation,* p. 156, J. Wiley & Sons, New York.
Lineweaver, H., Cunningham, F.E., Garibaldi, J.A. & Ijichi, K. (1967) *Agricultural Research Service* 74–39.
Makino, S., Reynolds, J.H. & Tanford, C. (1973) *J. Biol. Chem.* **248,** 4926.
Mukerjee, P. & Mysels, K.J. (1971) *Critical Micelle Concentrations of Aqueous Surfactant Systems* **140,** 51, NSRDS-NBS 36. Government Printing Office, Washington, D.C. U.S.
Nakamura, R., Hirai, M. & Takimori, Y. (1980) *Agric. Biol. Chem.* **44,** 149.
Nelson, C.A. (1971) *J. Biol. Chem.* **246,** 3895.
Rhodes, M.B., Azari, P.R. & Feeney, R.E. (1958) *J. Biol. Chem.* **230,** 399.
Smith, M.B. (1964) *Aust. J. Biol. Sci.* **17,** 261.
Steinhardt, J. & Reynolds, J.A. (1969) *Multiple Equilibria in Proteins.* Academic Press, New York.
Tanford, C. (1973) *The Hydrophobic Effect: Formation of Micelles and Biological Membranes.* J. Wiley & Sons, New York.

(Received 29 January 1982)

J. Fd Technol. (1983) **18**, 21–34

The preparation of protein hydrolysate from defatted coconut and soybean meals

I. Effect of process variables on the amino nitrogen released and flavour development

CHAY B. PHAM AND R. R. DEL ROSARIO

Summary

This study was conducted to determine the effects of temperature, time and concentration of acids on the hydrolysis of coconut and soybean meals. Using 6 N hydrochloric acid (HCl), the complete hydrolysis of soybean meal was reached after 36 hr at 95°C, while it took only 24 hr to complete the process when 18 N sulphuric acid (H_2SO_4) was used at the same temperature. Coconut protein exhibited some degree of resistance to hydrolysis. Using 10 N HCl and 18 N H_2SO_4 in two separate tests, it took 48 hr to complete hydrolysis at 95°C. Sulphuric acid caused a considerably greater decomposition of amino acids than HCl during longer periods of reaction with high acid concentration and temperature. Flavour development is a function of the free amino acids released which in turn is a function of acid concentration, reaction time and temperature.

Introduction

Protein hydrolysate in various forms is widely utilized in the Philippines as well as in other South-east Asian countries. These products have served not only as condiment but also a source of amino acids in the diet.

Coconut, a major industrial crop in the Philippines is a potential raw material for the production of protein hydrolysates. The annual production of the dried coconut meat of 'copra' in 1975 amounted to 2 million metric tons. It is a source of oil and protein. However, oil is the only component used for food in major quantities. The protein in the residue estimated at about 420 000 tons is used primarily as feed supplement just like soybean meal. There is a need to develop other uses for this large amount of protein source.

The production of protein hydrolysate from plant proteins has been done using microbial fermentation. This process however, is slow and takes from 4 to

Authors' address: Food Chemistry Laboratory, Department of Food Science and Technology, University of the Philippines at Los Baños, College, Laguna, 3720, Philippines.

0022–1163/83/0200–0021 $02.00

6 months to accomplish. The use of mineral acids reduces the time required to accomplish hydrolysis. These products made by acid hydrolysis are available in European, American and in Japanese markets (Yong & Wood, 1974; Minor, 1945).

Several studies have indicated that for analytical purposes acid hydrolysis can best be achieved by treatment of protein or peptide with 6 N HCl at 110°C for 24 hr under conditions that rigidly exclude oxygen, non-protein substances and metals (Moore & Stein, 1951; Harfenist, 1953; Smith & Stockell, 1954). The hydrolysis of protein samples as a function of time was studied by Hirs, Stein & Moore (1954) who suggested corrections necessary for incomplete hydrolysis or for destruction of amino acids. Peptide hydrolysis as shown by Vickery (1922) is nearly completed by boiling gliadin with 20% HCl for 20 hr or 4 N HCl for 50 hr. The hydrolysis rate of gelatin at three temperatures and with different concentrations of HCl and H_2SO_4 to determine the order of the reaction was also studied by Greenberg & Burk (1927). Wilcox, Cohen & Tan (1957) treated soybean protein with 5 N HCl at 110°C in an evacuated tube between 24 to 72 hr and observed the amino acids released from peptide bonds. Studies on wool, edestin and gelatin hydrolysis at 37°C with excess 10 N HCl showed that the general kinetics of acid hydrolysis is described by the splitting of bonds involving cystine, serine, threonine, proline, hydroxyproline and ammonia residues (Gordon, Martin & Synge, 1941). A mixture of soybean and wheat, hydrolysed by refluxing with constant boiling HCl (20% solution) until a maximum concentration of amino nitrogen was obtained was also studied (Minor, 1945).

Information on the conditions required in the hydrolysis process involving defatted coconut and soybean proteins is still limited. There is not enough information also on the flavour development of the proteins in the products. Therefore, this study was conducted to determine the process variables and the correlation between flavour development and amino acids released for optimizing product quality.

Materials and methods

Raw materials

The proteinaceous raw materials used were coconut and soybeans. Coconuts (*Cocos nucifera*, Linn.), Laguna variety and soybeans (*Glycine max* [L.] Merr). The UPLB, SY_2 variety were supplied by the Horticulture and Agronomy Department, respectively, of the University of the Philippines at Los Baños, College, Laguna, Philippines.

Preparation of raw materials

Mature nuts were dehusked, cracked, the meat removed from the shell and shredded through a coconut grater, then dehydrated using an electrical dryer at 68°C for 8 hr.

Soybean flour was prepared by drying the beans at 68°C for 8 hr and then dehulling them. The dehulled beans were ground to pass through a 60 mesh sieve.

Oil was extracted with petroleum ether. The sample meals obtained after milling were passed through a 100 mesh sieve, packed in plastic bags, and stored at 10°C.

Experimental design

The selection of variables was based on the fact that temperature, time and concentration of acids affect the hydrolysis rate, giving product characteristics of the different raw materials. Each treatment was replicated twice. A central composite response experimental design (Davies, 1963) was used to evaluate the influence of processing conditions in the development of flavour. Variables were transformed and coded as shown in Table 1. Results were analysed by means of a multiple regression to determine the process variables significant in flavour development and the method of least squares was used to obtain the best estimate of the independent variables. All terms not significant at the 0.05 probability level were combined with the residual.

Response surface plots were made from the regression equations which have been adjusted.

Table 1. Transformation and levels of independent variables

Variable symbols	Transformation	Levels									
		−2	−1.2	−1	−0.95	0.25	0	+0.5	+0.64	+0.93	+1.32	+2
X_1 temperature (°C)	$(x - 81)/22$	37			60				95		110	125
X_2 time (hr)	$(x - 40)/16$	8		24		36		48				72
X_3 HCl (N)	$(x - 6)/2$	2		4			6					10
X_3 H_2SO_4 (N)	$(x - 10.5)/3.75$	3	6							14		18

Experiment and variables

Two grams of the sample protein were hydrolysed with the acid solution with concentration varying from 2, 4, 6 and 10 N for hydrochloric acid and 3, 6, 14 and 18 N for sulphuric acid in a round bottom flask glass set up with condenser. Heat was applied under reflux varying from 37, 60, 95, 110 and 125°C. The hydrolysis time was set for 8, 24, 36, 48 and 72 hr. When the hydrolysis process was over, the hydrolysed product was neutralized by sodium hydroxide solution and filtered through a No. 431 Whatman paper. The filtrate was used to determine amino nitrogen and flavour.

Amino nitrogen determination

Total nitrogen in the raw materials was determined by the micro-Kjeldahl method (AOAC, 1975).

The rate of hydrolysis was determined by measuring the amount of free amino acid groups released using the different values of the formol nitrogen and ammonium nitrogen (T. Sakasai & T. Yokotsuka, pers. comm.).

Five millilitres of the hydrolysed product was diluted to 250 ml with water. Twenty-five millilitres of the sample was adjusted to pH 7 with 10 N NaOH solution. Twenty millilitres of the neutralized formalin solution was added and shaken very well. The sample was allowed to stand for 10 min and then titrated with N/10 NaOH solution until a pH of 8.5 was reached. This value is T in the following formula:

$$\text{Formol nitrogen } (\%) = 0.0014 \times F \times T \times 200,$$

where F = factor of N/10 NaOH solution.

Ammonium nitrogen of the hydrolysed product was determined by the Conway diffusion method (T. Sakasai & T. Yokotsuka, pers. comm.).

Five millilitres of the hydrolysed product and one drop of silicone or amyl-alcohol were placed in the B-bottle and then added with water to 5 cm of the height of the hydrolysed sample layer. Ten millilitres of saturated K_2CO_3 was added along the sidewall and the B-bottle was covered right away. Suction connected from the water pump to the A-bottle was applied slowly at first, and then strongly for 15 min. After suction, 20 ml of N/10 H_2SO_4 solution in the C-bottle containing two to three drops of Gluck indicator was titrated with N/10 NaOH solution (T_1):

$$\text{Ammonium nitrogen } (\%) = (T_2 - T_1) \times 0.0014 \times F \times 20$$

Table 2. Effect of HCl concentration and temperature on the amino nitrogen* released from soybean (SB) and coconut (COCO) proteins

HCl concentration (N)	Temperature (°C)									
	37		60		95		110		125	
	SB	COCO	SB	COCO	SB	COCO	SB	COCO	SB	COCO
2	20.95	22.17	54.71	42.25	85.04	72.24	98.39	94.75	98.81	98.06
4	29.87	35.14	70.92	58.64	100 (72)†	87.31	100 (36)	100 (40)	100 (24)	100 (24)
6	39.02	41.29	78.63	66.08	100 (36)	100 (48)	100 (24)	100 (24)	100 (20)	100 (20)
10	97.12	54.56	91.90	82.62	100 (24)	100 (40)	100 (18)	100 (20)	100 (12)	100 (14)

* Per cent hydrolysis.

† All figures in parentheses indicate time (hr).

where T_2 = titration value of N/10 NaOH against 20 ml N/10 H_2SO_4 and F = factor of N/10 NaOH solution.

Percentage of hydrolysis is equivalent to amino nitrogen of hydrolysed product and amino nitrogen in meal.

Sensory evaluation

The hydrolysed product was submitted to the taste panels for sensory evaluation to monitor the flavour development using the 9-point hedonic scale with: 1—very poor, 3—poor, 5—fair, 7—good, 9—very good.

Results and discussion

Effect of HCl concentration

The rate of amino nitrogen release (Table 2) increased with increasing HCl concentration, however, the extent of the reaction was determined by the temperature. At 37 and 60°C, the hydrolysis was not complete even at highest HCl concentration. At 125°C nearly all the amino nitrogen were released from soybean protein (Table 2). The minimum condition used to obtain complete hydrolysis was 4 N HCl at 95°C and required 72 hr of reaction. Beyond this condition a much shorter reaction time is needed to complete the reaction.

The two substrates used showed some variations in their reaction (Table 2), with soybean protein appearing to be more susceptible to acid hydrolysis than coconut protein. This is true for almost all temperatures used, except at 37°C where coconut protein showed a greater degree of hydrolysis. It could be possible that the free amino acid content in coconut meal was higher than in soybean meal. The higher the concentration, the faster is the rate of hydrolysis. These findings confirm those of Lawrence & Moore (1951). They found that the

Table 3. Effect of H_2SO_4 concentration and temperature on the amino nitrogen released from soybean (SB) and coconut (COCO) proteins (expressed as per cent)

H_2SO_4 concentration (N)	Temperature (°C)									
	37		60		95		110		125	
	SB	COCO	SB	COCO	SB	COCO	SB	COCO	SB	COCO
3	15.02	19.61	58.32	34.07	91.51	64.16	97.65	91.37	98.08	96.32
6	24.18	28.51	72.14	44.67	87.75	78.36	100	98.86	100	100
14	36.63	42.42	87.48	64.21	100	95.14	100	100	100	100
18	49.25	49.56	97.85	71.36	100	100	100	100	100	100
					(24)*	(48)	(14)	(20)	(10)	(12)

* All figures in parentheses indicate time (hr).

rate of hydrolysis at any acid concentration depends on the concentration of protein. On the other hand, the impurities consisting of fat (coconut 3.96%, soybean 1.96%) and carbohydrate (coconut 54.12%; soybean 34.76%) could have inhibited the reaction of the hydronium ion with the peptide of the protein. This is probably the reason why coconut protein showed an apparent resistance to acid hydrolysis.

Effect of H_2SO_4 concentration

Sulphuric acid and HCl gave the same general reaction pattern. The rate of hydrolysis (Table 3) increasd with increasing acid concentration. H_2SO_4 gave a higher reaction rate than HCl at all temperatures, except at 37°C. At 95°C, using 14 N and 18 N concentrations, hydrolysis of soybean protein was complete after about 36 and 48 hr respectively. However, the hydrolysis reaction was accompanied by extensive degradation at high concentration and temperature. Compared to soybean protein, coconut protein also showed greater susceptibility to HCl than to H_2SO_4. At 95°C, the 18 N H_2SO_4 was sufficient only to get complete conversion of coconut protein to free amino nitrogen after 48 hr. At 110°C, a much higher conversion was obtained and was accompanied by amino acid destruction.

Effect of temperature

The rate of hydrolysis of protein was greatly affected by temperature (Tables 4 and 5). As the reaction temperature was increased, the rate of hydrolysis also increased. The effect of reaction temperature was more on the amino nitrogen released from soybean protein than from coconut protein. But the reaction rate was slower for soybean protein compared to coconut protein at 37°C. At a higher temperature, however, soybean exhibited a faster rate of hydrolysis as indicated by the rapid release of amino nitrogen. There was a higher correlation between temperature and H_2SO_4 concentration than between temperature and HCl concentration. This means that the catalytic effect of acids on protein hydrolysis is proportional to the thermodynamic activity of the hydronium ion.

Table 4. Effect of temperature and time on the hydrolysis of soybean (SB) and coconut (COCO) protein using 2 N HCl (expressed as per cent)

Temperature (°C)	8 hr		24 hr		36 hr		48 hr		72 hr	
	SB	COCO	SB	COCO	SB	COCO	SB	COCO	SB	COCO
37	8.05	9.00	11.92	13.54	14.23	15.33	16.80	17.87	20.95	22.17
60	25.23	17.96	33.15	31.17	42.55	34.67	49.97	37.69	59.32	42.25
95	39.67	28.01	59.85	52.28	63.41	62.35	73.94	65.95	85.04	72.24
110	69.24	66.57	92.66	83.67	94.30	87.80	96.41	91.48	98.39	94.75
125	76.00	74.59	94.44	84.88	95.99	90.30	98.39	94.44	98.81	98.96

Table 5. Effect of temperature and time on the hydrolysis of soybean (SB) and coconut (COCO) proteins using 6N H_2SO_4 (expressed as per cent)

Temperature (°C)	8 hr		24 hr		36 hr		48 hr		72 hr	
	SB	COCO	SB	COCO	SB	COCO	SB	COCO	SB	COCO
37	13.90	15.31	17.38	19.60	19.16	22.07	21.19	25.14	24.18	28.51
60	32.51	25.36	47.53	31.14	56.00	35.05	59.97	38.49	72.14	44.67
95	65.48	43.66	78.83	57.35	80.96	64.86	85.30	71.17	87.75	78.35
110	80.41	81.96	94.95	93.12	97.27	96.96	98.65	97.49	100.0	98.86
125	86.27	85.26	96.78	92.15	98.79	97.35	100.08	99.68	100.0	99.94

Effect of variables on the decomposition of amino acids

At high temperatures (110°C, 125°C), using high acid concentrations (10 N HCl or 14 N, 18 N H_2SO_4) and a long period of reaction (48 or 72 hr), the amino acids were decomposed, so that total amino nitrogen obtainable from a protein was slightly decreased (Tables 6 and 7). This has been attributed to the secondary decomposition of amino acids. Also, the additional amount of ammonia detected probably originated from the decomposition of tryptophan

Table 6. Decomposition of amino acids from soybean (SB) and coconut (COCO) protein hydrolysates by HCl (expressed as per cent)

	110°C				125°C			
	SB		COCO		SB		COCO	
Time (hr)	6N	10N	6N	10N	6N	10N	6N	10N
24	0.00	0.00	0.00	0.00	0.00	1.07	0.00	0.00
36	0.00	1.19	0.00	0.31	1.19	4.01	1.42	2.92
48	0.06	1.88	0.87	1.81	1.69	6.55	2.80	3.86
72	1.03	4.29	1.87	2.37	4.15	9.23	5.00	6.50

Table 7. Decomposition of amino acids from soybean (SB) and coconut (COCO) proteins by H_2SO_4 (expressed as per cent)

	110°C				125°C			
	SB		COCO		SB		COCO	
Time (hr)	14N	18N	14N	18N	14N	18N	14N	18N
24	0.00	0.06	0.00	0.00	0.60	2.35	0.00	0.57
36	0.00	1.06	0.00	0.82	6.38	10.45	3.03	4.68
48	1.49	3.50	1.07	2.90	8.10	13.31	5.24	6.96
72	4.08	8.50	2.71	3.85	12.41	15.42	6.25	8.01

and cysteine. At a given temperature, the rate of decomposition increased with increasing acid concentration and time of reaction. Between the acids used, H_2SO_4 caused greater decomposition than HCl. Compared to soybean protein, coconut protein showed more resistance to acid hydrolysis as indicated by more

Table 8. Regression coefficients for flavour developments

	HCl		H_2SO_4	
Coefficient†	Soybean	Coconut	Soybean	Coconut
*0	7.33	5.45	7.15	4.78
*1	1.48	1.41	1.28	1.39
*2	0.29	0.31	0.26	0.33
*3	0.52	0.43	0.30	0.27
*11	−0.28	0.10*	−0.41	0.03**
*12	−0.16	−0.15	−0.14	−0.06*
*13	−0.25	−0.25	−0.21	−0.14
*22	−0.08	−0.09	−0.11	−0.07*
*23	−0.08	−0.06**	−0.14	−0.09
*33	−0.18	−0.15	−0.12	−0.17**
*123	−0.08	−0.10	−0.12	−0.08

*Significant at $P = 0.05$.
**Significant at $P < 0.05$.
† 1 = Temperature; 2 = time; 3 = concentration.

Table 9. Analysis of variance of flavour development regression

		HCl		H_2SO_4	
Source of variation†	d.f.	Soybean	Coconut	Soybean	Coconut
Model	10	134.3	115.7	104.8	98.6
1	1	910.5	821.8	705.1	826.9
2	1	30.1	33.9	25.8	40.3
3	1	113.9	77.0	44.6	36.7
11	1	35.0	5.0*	75.8	0.3**
12	1	19.0	16.5	17.3	29.0*
13	1	56.4	59.1	51.0	22.1
22	1	3.9	5.0*	8.0	3.9*
23	1	5.6	3.2*	20.2	8.2
33	1	20.2	14.8	5.9	2.2**
123	1	10.9	17.4	30.8	16.2
Error	189	0.5	1.0	0.5	0.8
Corrected total	199	7.2	6.7	5.8	5.7
R^2		0.93	0.86	0.91	0.87

*Significant at $P = 0.05$.
**Significant at $P < 0.05$.
† 1 = Temperature; 2 = time; 3 = concentration.

drastic conditions to effect complete hydrolysis. The rate of amino acid decomposition increased with the availability of amino acids and the presence of oxygen in the atmosphere using a catalyst like H_2SO_4.

Effect of variables on flavour development

The hydrolysed soybean protein had a higher flavour score than the hydrolysed coconut protein, either in HCl or in H_2SO_4 (Table 8). Statistical analysis showed that all variables had a strong influence on flavour development. The second order of temperature and concentration of H_2SO_4 were significant at $P = 0.05$ (Table 9). Response surface of the effect of temperature and time to soybean protein is shown by the maximum flavour score which was 9 for HCl hydrolysed (Fig. 1) and 8.2 for H_2SO_4 hydrolysed (Fig. 2). Prolonged hydrolysis time at high temperature (125°C) decreased the flavour score. For coconut protein, the maximum flavour score was 8.6 for HCl hydrolysed (Fig. 3) and 7.3 for H_2SO_4 hydrolysed (Fig. 4), considering temperature and concentration effects.

Relationship of flavour development and amino nitrogen

The relation of flavour development and free amino nitrogen formed during hydrolysis is shown in Figs 5 and 6. For treatments that did not reach complete

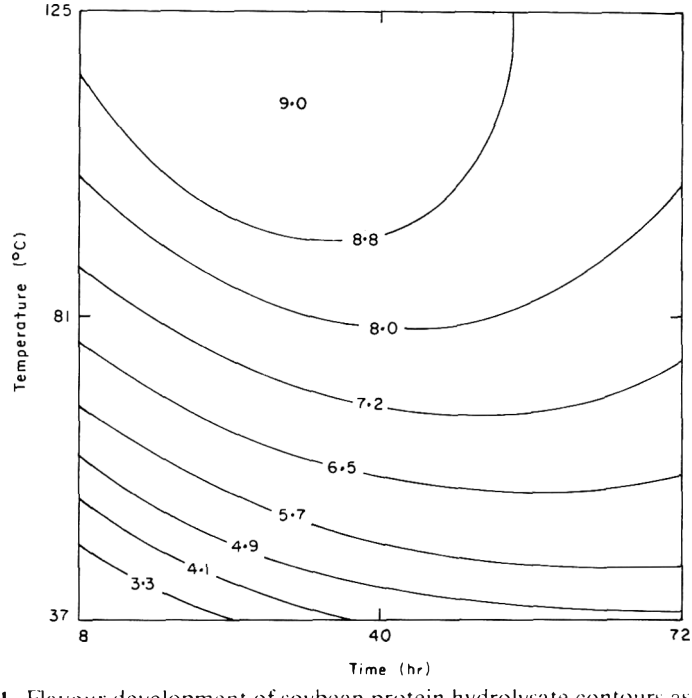


Figure 1. Flavour development of soybean protein hydrolysate contours as function of temperature and time (at 6N HCl).

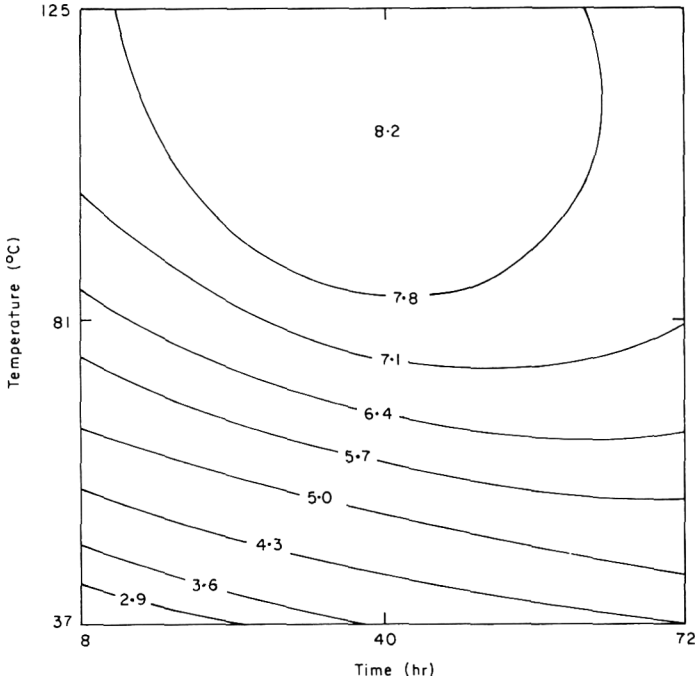


Figure 2. Flavour development of soybean protein hydrolysate contours as function of temperature and time (at 10.5N H_2SO_4).

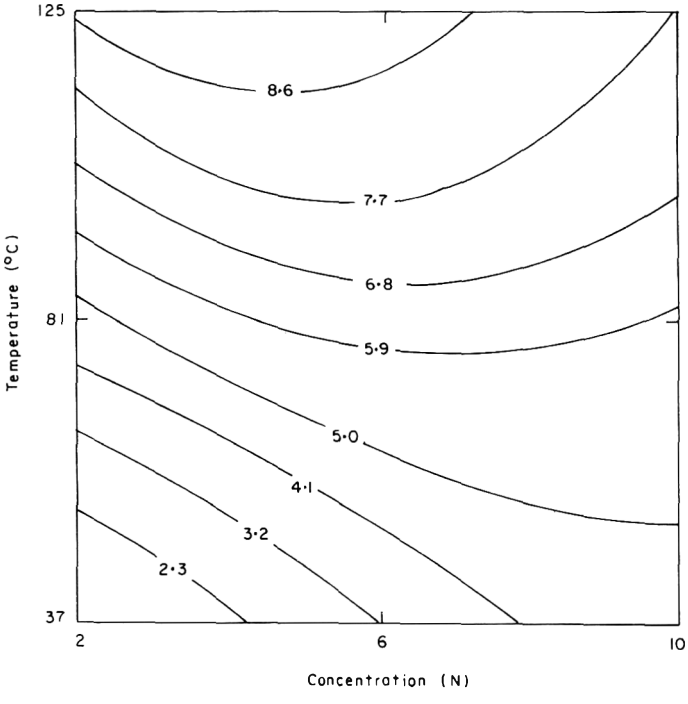


Figure 3. Flavour development of coconut protein hydrolysate contours as function of temperature and concentration of HCl (at 40 hr).

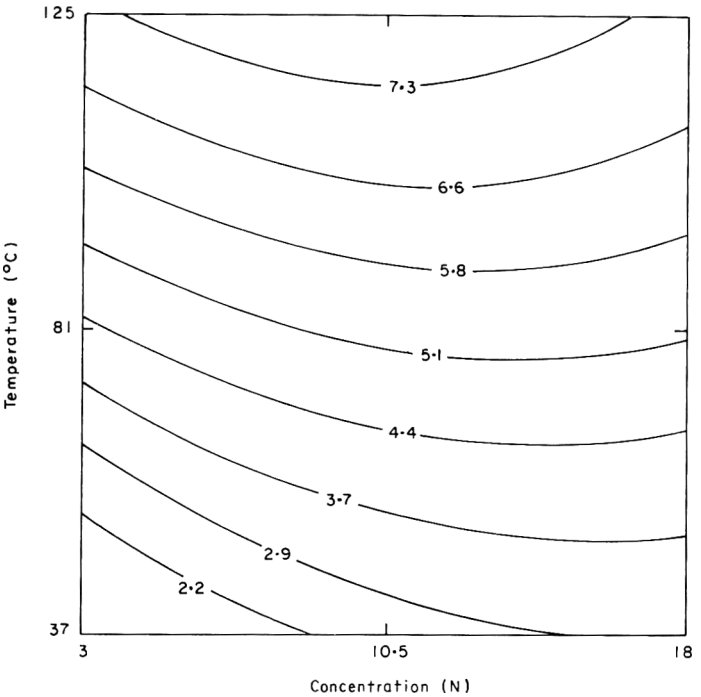


Figure 4. Flavour development of coconut protein hydrolysate contours as function of temperature and concentration of H_2SO_4 (at 40 hr).

hydrolysis, the regression line was close to the 45° line. This indicates a positive correlation between flavour development and formation of free amino nitrogen.

For treatments that have attained complete hydrolysis there was also a positive correlation between flavour score and the reduction of free amino nitrogen. The flavour score decreased rapidly in relation to the changes of the free amino nitrogen as indicated by the steep slope of the line. Apparently the relationship is governed by the formation of decomposition products like ammonia which imparts unfavourable flavour.

Comparison of the regression lines for soybean indicates faster flavour deterioration in the HCl treated sample as compared to those treated with H_2SO_4. The reverse was true with coconut samples. The reason for the difference in behaviour is not obvious but a close look at the rate of hydrolysis indicates that soybean is more susceptible to HCl than to H_2SO_4 while it is the other way around for the coconut meal.

The flavour development of hydrolysed coconut was very poor compared to the hydrolysed soybean protein. This was expected considering that the flavour being measured is associated with soybean protein hydrolysate. The inherent differences in the composition of coconut and soybean meals are not only based on the protein and amino acid content but on their fats and carbohydrates as well which could affect flavour development. The flavour of hydrolysed protein in samples which used HCl were more favourable than those which used H_2SO_4

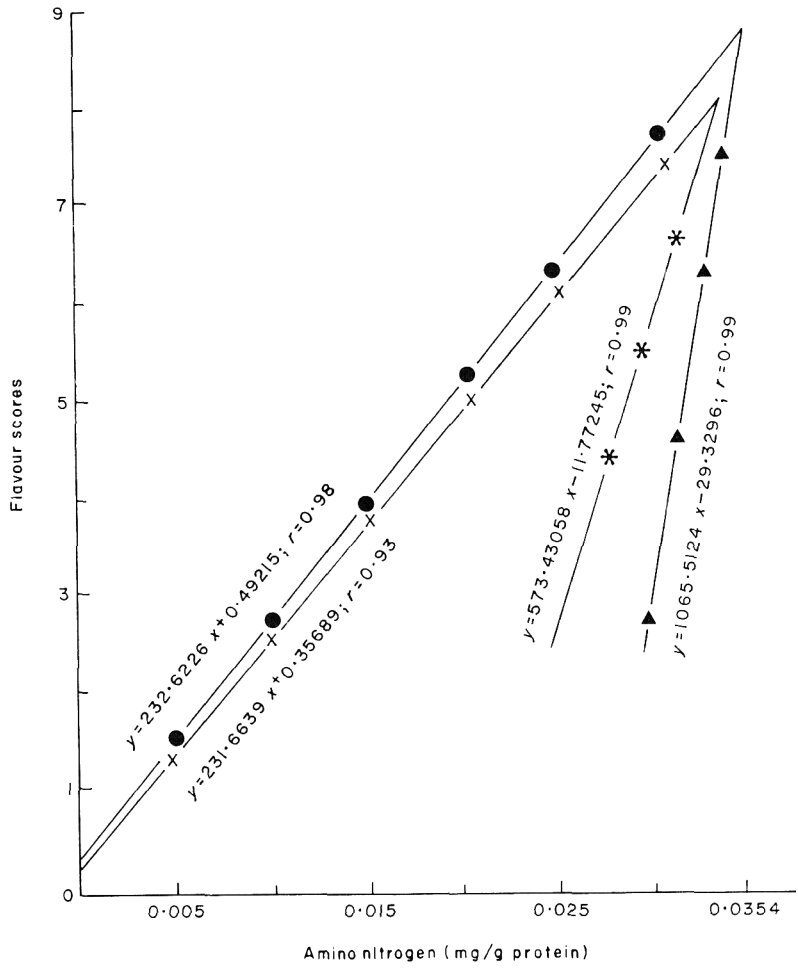


Figure 5. Relationship between flavour development and amino nitrogen on the rate of hydrolysis of soybean protein by HCl and H_2SO_4. Before complete hydrolysis: ●, HCl; ×, H_2SO_4. After complete hydrolysis: ▲, HCl; ∗, H_2SO_4.

especially at a high concentration of acid and prolonged reaction time. Evaluation of flavour development in hydrolysed soy product was complicated by a large number of compounds formed during the reaction. Although, in general, it followed the degree of hydrolysis of protein, it must be accepted also that as the reaction proceeds, other side reactions are likely to occur.

Conclusion

Three variables of temperature, time and concentration of acid and its effect on the rate of hydrolysis of soybean and coconut proteins are important and interrelated.

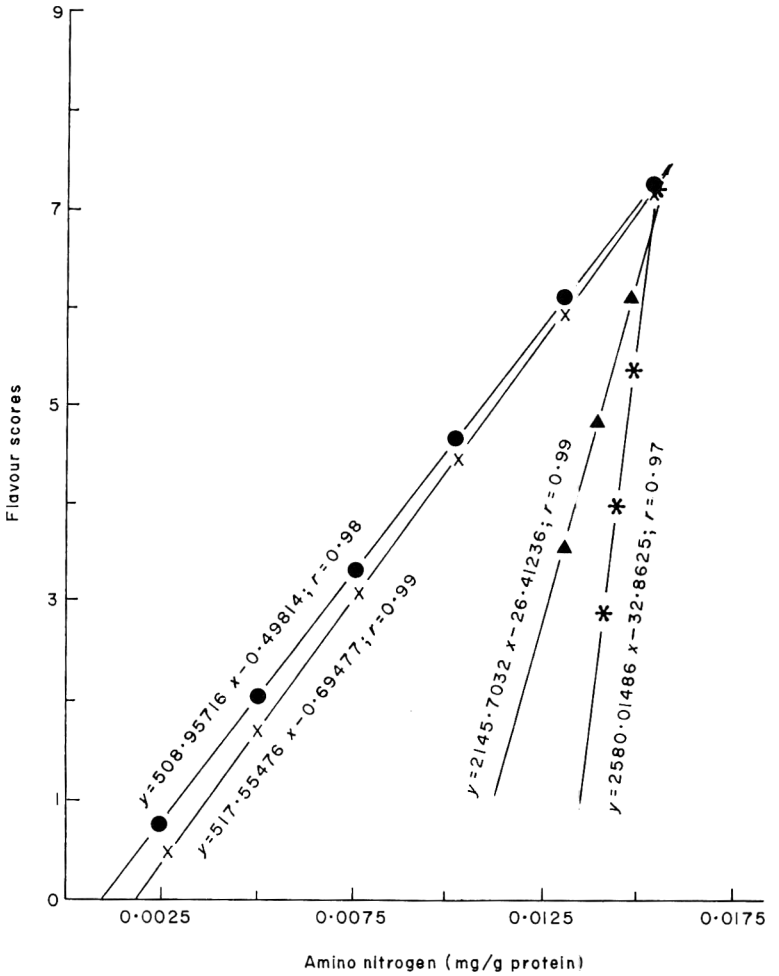


Figure 6. Relationship between flavour development and amino nitrogen on the rate of hydrolysis of coconut protein by HCl and H_2SO_4. Before complete hydrolysis: ●, HCl; ×, H_2SO_4. After complete hydrolysis: ▲, HCl; *, H_2SO_4.

The effect of HCl concentration on the hydrolysis of protein was high at low temperature (37°C, 60°C) compared to the effect of H_2SO_4 but lower at high temperature (110°C, 125°C). There was a higher correlation between temperature and H_2SO_4 concentration than between temperature and HCl concentration. This means that the catalytic effect of acids on protein hydrolysis was proportional to the thermodynamic activity of the hydronium ion. The rate of hydrolysis of soybean protein was greater than that of coconut protein probably because the structure of coconut is more complicated than that of soybean.

The percentage of amino nitrogen is at its maximum when acid hydrolysis of the protein is complete. Using 6 N HCl, the complete hydrolysis of soybean protein was reached after 36 hr at 95°C. On the other hand, it took only 24 hr to complete the process when 18 N H_2SO_4 was used under the same temperature.

Coconut protein exhibited some degree of resistance to hydrolysis. Using 10 N HCl and 18 N H_2SO_4 in two separate tests, it took 48 hr to complete hydrolysis at 95°C.

H_2SO_4 caused considerably greater decomposition of amino acids than HCl during longer periods of reaction (48, 72 hr) with higher acid concentrations (14 N, 18 N H_2SO_4). The maximum values of decomposition of amino acids from soybean protein using 10 N HCl were 4.29% at 110°C and 9.23% at 125°C. With 18 N H_2SO_4, the maximum values were 8.5% at 110°C and 15.42% at 125°C. These values were obtained after a period of 72 hr. This explained more clearly the greater effects on the relationship between temperature and H_2SO_4 than between temperature and HCl.

It was also observed in this study that the flavour of the hydrolysed products was enhanced when the amino acid content was increased. A correlation existed between the development of flavour and the release of amino acids.

Acknowledgment

The authors thank Professor S. M. Alviar of the Computer Services Unit, University of the Philippines at Los Baños, for their statistical analysis. Gratitude is expressed to the South-east Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA) for the fellowship granted the senior author to finish his MSc programme.

References

AOAC (1975) *Official Methods of Analysis* 12th ed. Association of Official Agricultural Chemists, Washington D.C.

Davies, O.L. (1963) In: *The Design and Analysis of Industrial Experiments*, 2nd ed., p. 495. Oliver & Boyd, London.

Gordon, A.H., Martin, A.J.P. & Synge, R.L.M. (1941) *Biochem. J.* **35**, 1369.

Greenberg, D.M. & Burk, N.F. (1927) *J. Am. Chem. Soc.* **49**, 275.

Harfenist, E.J. (1953) *J. Am. Chem. Soc.* **75**, 5528.

Hirs, C.H.W., Stein, W.H. & Moore, S. (1954) *J. Biol. Chem.* **211**, 941.

Lawrence, L. & Moore, W.J. (1951) *J. Am. Chem. Soc.* **73**, 3973.

Minor, L.J. (1945) *Fd Ind.* **47**, 758.

Moore, S. & Stein, W.H. (1951) *J. Biol. Chem.* **192**, 663.

Smith, E.L. & Stockell, A. (1954) *J. Biol. Chem.* **207**, 501.

Vickery, H.B. (1922) *J. Biol. Chem.* **53**, 495.

Wilcox, P.E., Cohen, E. & Tan, W. (1957) *J. Biol. Chem.* **228**, 999.

Yong, F.M. & Wood, B.J.B. (1974) In: *Advances in Applied Microbiology*, vol. 17, 1st ed., p. 157 (Ed. by D. Perlman). Academic Press, New York.

(Received 18 March 1982)

J. Fd Technol. (1983) **18**, 35–41

Production of a food grade flour from defatted corn germ meal

R. BARBIERI AND E. M. CASIRAGHI

Summary

A defatted corn germ flour of good organoleptic and nutritional quality was obtained by applying a milling and screening process to a commercial defatted corn germ meal. The flour contains about 20% protein of good nutritional quality, above 60% starch and minor quantities of fat (1.7%), fibre (3.3%) and minerals (8.42%). The last are interesting for their content in potassium, magnesium, iron and zinc. Heat effects on protein were evaluated in the intermediate and final products of a commercial oil extraction installation. It was found that the conditioning step sharply decreased protein solubility, while protein digestibility remained substantially constant throughout the process.

Introduction

Defatted corn germ flour, the extraction residue of the corn oil industry, has a protein content of 17–18% and a total carbohydrates content of about 65%. Lipid content is 1–2% and crude fibre about 8–10% (Peri & Barbieri, 1980). The corn germ proteins are of good nutritional quality with a protein efficiency ratio (PER) Value of 2.1, comparable to that of soya protein (Satterlee, Marshall & Tennyson, 1979); EUD (enzymatic ultrafiltrate digestibility) is 67, which is similar to, or higher than the values obtained from proteins of other vegetable sources (Fidanza, 1978). Furthermore, defatted corn germ meal does not contain major anti-nutritional factors.

In spite of these interesting qualities, defatted corn germ flour is entirely destined to animal feeding, and very little has been done to promote its use in food formulations (Restani *et al.*, 1981; Blessin, *et al.* 1973).

Present availability and uses of corn in Europe

Figure 1 shows data of corn availability (usable production plus imports minus exports) in the major EEC countries in 1978–79 (FAO, 1979; Eurostat, 1980;

Authors' address: Istituto di Tecnologie Alimentari, via Celoria 2, 20133 Milano, Italy.

0022–1163/83/0200–0035 $02.00

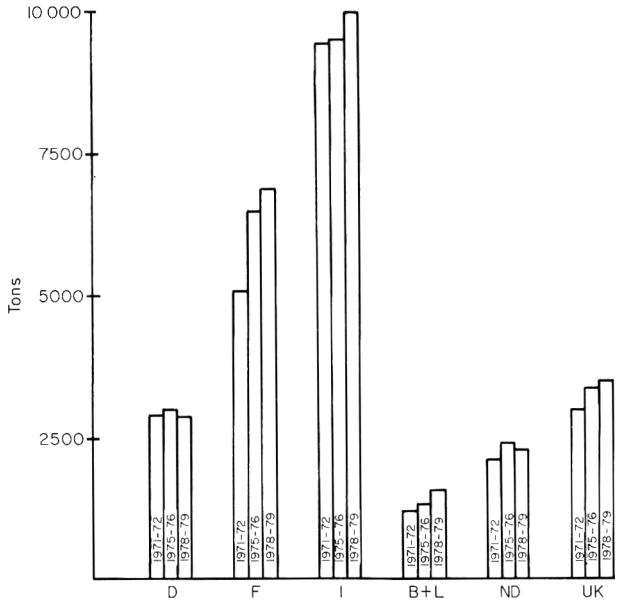
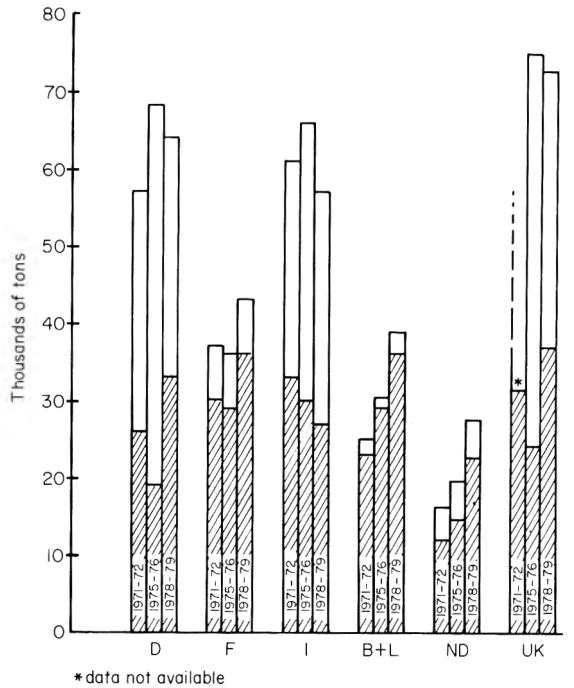


Figure 1. Corn availability in European Community Countries (1000 t).

INEA, 1979; Assitol, pers. comm.). Figure 1 represents the sum of all the corn used for animal feed stuffs and other uses such as brewing, the production of flour grits and snacks and for use as seeds in agriculture. More detailed analysis reported elsewhere (Riva, 1981) shows that corn availability in the same countries remained substantially stable during the last decade or showed a trend to a limited growth. Concerning the quantities indicated as for 'other uses' they are, for the most part, subjected to the separation of germ, which is destinated to the corn oil extraction.

Figure 2 reports the corn germ availability (FAO, 1979; Eurostat, 1980; INEA, 1979; Assitol, pers. comm.). Data are further differentiated according to the technology of germ separation from the corn kernels: the wet process is applied in the starch industry, while the dry milling process is applied for the production of corn flour for human consumption. This differentiation is of practical interest because only the corn germ deriving from the dry milling process can be used for human consumption after the oil extraction. In fact, the corn germ obtained by the wet milling process is unsuitable for food use due to the degradation of organoleptic and nutritional quality following the steeping treatment.

A few conclusions may be drawn from the observation of Figs 1 and 2. First, only a very minor proportion of corn is actually degerminated. If all or most of the corn available were degerminated it would lead to a substantial contribution in terms of oil and protein availability. At present degermination of all available corn is not practicable for economical reasons: high degermination costs, higher value of whole kernels when used for animal feeding, low commercial value of defatted meal. Changes in the oil exchange market and policies, and valuation of defatted flour as a food ingredient might change the economic terms of this



*data not available

Figure 2. Corn germ present availability in European Community Countries (1000 t). ▨. Corn germ by wet milling process; □. corn germ by dry milling process.

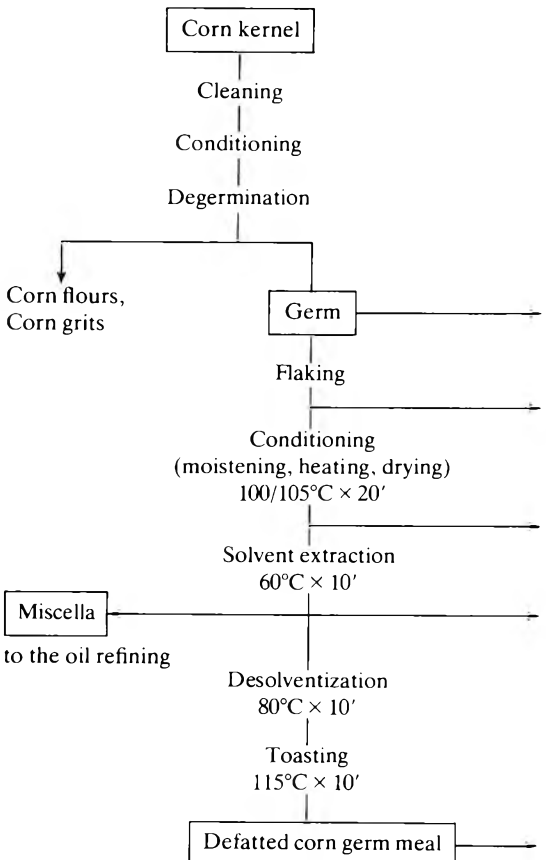
problem and make the degermination process profitable. Figures also show that Italy may be particularly interested in the prospect considering the very high consumption of corn in our country. For this reason corn germ flour has been included in a list of possible sources of protein for food use in a research project supported by the National Research Council (CNR) of Italy.

In this paper we report the results of studies carried out to evaluate the effect of the corn oil extraction process on the nutritional and functional quality of proteins in the defatted meal. A milling and screening procedure, which allows elimination of the coarser fibrous fraction from meal, obtaining a human grade flour of good nutritional and organoleptic quality, is presented.

Materials and methods

All data reported in this paper were obtained from germ separated according to the dry milling process. Proximate analysis of germ products was performed in duplicate. Moisture content was determined by drying to constant weight at 105°C, protein by macroKjeldahl technique using the nitrogen-to-protein conversion factor 6.25 (AOAC, 1970). Ash was determined by heating at 550°C overnight; an atomic adsorption spectrophotometer was employed for the determination of minerals. Lipids were determined after extraction by diethyl

Table 1. Corn germ oil extraction process; composition and protein quality of the intermediate and final products



	Moisture (%)	Total N × 6.25 (% d.w.)	Fat (% d.w.)	Crude fibre (% d.w.)	Ash (% d.w.)	Total carbohydrates (% d.w.)	Protein digestibility (%)	Nitrogen solubility index (% total N)
Germ	12.3	13.7	19.9	8.0	5.19	53.2	77.1	28.9
Flaking	15.5	14.1	20.3	7.7	5.16	52.7	75.3	28.7
Conditioning (moistening, heating, drying) 100/105°C × 20′	6.6	13.8	19.6	7.0	5.03	54.6	75.1	15.4
Solvent extraction 60°C × 10′								
Miscella to the oil refining	10.8	17.9	1.4	5.7	6.05	68.9	75.1	15.7
Desolventization 80°C × 10′								
Toasting 115°C × 10′								
Defatted corn germ meal	7.7	17.8	1.5	6.1	5.44	69.1	75.1	15.9

ether in a Soxhlet apparatus (Giuliano & Stein; 1970). Wendee's (1972) method, modified by Cirilli (1972), was used for the determination of crude fibre. Total carbohydrates were calculated as difference.

The determination of digestibility and the calculated protein efficiency ratio (C-PER) calculation were carried out following the method of Satterlee modified by Fidanza (Fidanza, 1978).

Results and discussion

Corn germ oil extraction process: composition and protein quality of the intermediate and final products

An industrial oil extraction process was studied to evaluate the extent of the degradation effects of the various process steps on protein fractions.

Table 1 gives a flow sheet of the extraction process and the analytical characteristics of the products at different stages of the process. The process does not cause a significant degradation of the protein fraction: conditioning of the flaked product, carried out at 100–105°C at high humidity determines a sharp reduction of nitrogen solubility (NSI), but does not affect protein digestibility. The following stages, including desolventization and toasting operations, though carried out at relatively high temperatures, do not produce further significant variations of protein solubility and digestibility.

Table 2 presents the mineral content of defatted germ meal. The mineral composition is interesting from a nutritional point of view, particularly with regard to potassium, magnesium, iron and zinc content.

Table 2. Defatted germ meal: mineral composition

Minerals		
P_2O_5	(mg/100g)	124.0
Na	(mg/100g)	5.4
K	(mg/100g)	945.0
Ca	(mg/100g)	18.5
Mg	(mg/100g)	425.0
Fe	(mg/100g)	16.2
Mn	(mg/100g)	2.1
Zn	(mg/100g)	11.4
Cu	(mg/100g)	0.8
Co	(mg/kg)	0.2
Ni	(mg/kg)	0.5
Pb	(mg/kg)	1.0
Cd	(mg/kg)	0.1
Cr	(mg/kg)	0.5

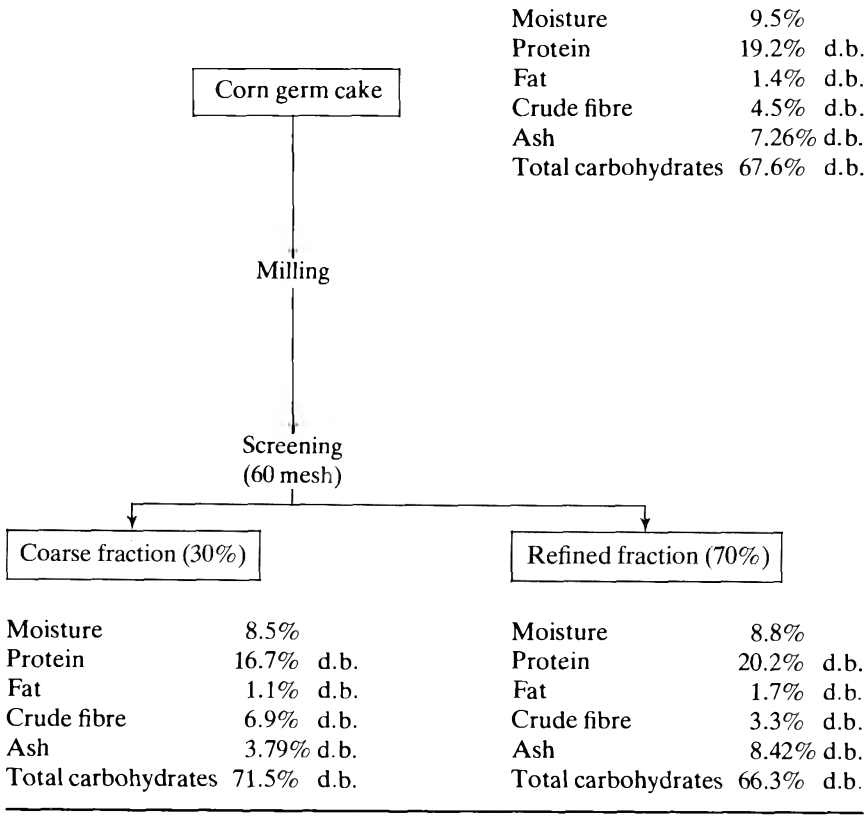
It may be concluded that the corn germ meal obtained as a residue of the extraction process can be considered a valuable source of nutrients, particularly of proteins of good nutritional quality.

Fractionation of defatted germ meal

The main obstacle to the direct use of the defatted meal in food formulations is due to the presence of fibrous fragments of the kernel tegument. They are difficult to grind to sufficiently small dimensions and are perceived with an unpleasant (sandy) sensation in the mouth. In order to eliminate the fibre fragments and to increase the protein concentration, defatted meal was subjected to a milling–screening procedure according to the scheme in Table 3. The refined fraction, representing 70% of the defatted meal, has a lower crude fibre content and a slightly higher protein and lipid content. This is due essentially to the removal of the coarse fibre fragments. The flour obtained has good organoleptic characteristics, is creamy white in colour, mild in taste and may be considered perfectly suitable as a food.

A further paper reports the results of extrusion and cooking experiments carried out on this material to produce expanded snacks (Peri, Barbieri &

Table 3. Fractionation of defatted germ meal



Casiraghi, 1982). Several other uses in bread, baked products and nutrient snacks have been succesfully experimented by food industries in the same research project.

Acknowledgments

We are grateful to Chiari e Forti S.p.A. for useful information on the oil extraction process and for the samples provided. We are grateful to Dr S. Mannino for the analysis of minerals.

References

AOAC (1970) *Official Methods of Analysis,* 11th ed., Association of Official Agricultural Chemists, Washington, D.C.

Blessin, C.W., Garcia, W. J., Deatherage, W.L., Cavins, J.F. & Inglett, G.E. (1973) *J. Fd Sci.* **38,** 602.

Cirilli, G. (1972) In: *Chimica analitica applicata al mangimi,* p. 39. Coop. Libr Univ., Bologna.

Eurostat (1980) *Yearbook of Agricultural Statistics, Crop Production,* EEC, Brussels.

FAO (1979) *Production Yearbook,* vol. 33.

Fidanza, F. (1978) Report to 'The Italian National Research Council (CNR)'.

Giuliano, R. & Stein, M., (1970) In: *Quaderni di chimica degli alimenti,* (Ed. by M. Bulzoni) vol. 1, p. 93. Roma.

INEA (1979) *Annuario Agricoltura Italiana,* vol. 33.

Peri, C. & Barbieri, R. (1980) *Riva It. Sostanze Grasse,* **57,** 185.

Peri, C., Barbieri, R. & Casiraghi, E.M. (1982).

Restani, P., Ricardi, A., Duranti, M. & Cerletti, P. *Proceedings of the Symposium 'Progress in Food Engineering'.* Foster Verlag, Zurich.

Satterlee, L.D., Marshall, H.F. & Tennyson, J.M. (1979) *J. Am. Oil Chem. Soc.* **56,** 103.

(Received 2 April 1982)

J. Fd Technol. (1983) **18**, 43–52

Physical, chemical and nutritional quality of extruded corn germ flour and milk protein blends

C. PERI, R. BARBIERI AND E. M. CASIRAGHI

Summary

An expanded nutrient snack was prepared by extrusion cooking of defatted corn germ flour added with increasing amounts of milk proteins. Two extrusion temperatures (150 and 170°C) were considered. Physical, chemical and nutritional characteristics of the final product were evaluated. The products extruded at 150°C showed better expansion and crispness than the ones obtained at 170°C. A small addition of milk protein (5%) improves the organoleptic characteristics of the extrudates produced at 150°C. On the contrary the addition of milk protein has a negative influence on the product expansion and consistency of the samples extruded at 170°C.

Introduction

Defatted corn germ flour, the extraction residue of the corn oil industry, is at present entirely used for animal feed. However its use as a food ingredient has been often proposed, particularly considering its content in protein (15–30%) of good nutritional quality (Blessin *et al.*, 1972; Blessin *et al.*, 1973). Direct use of this by-product in food formulations is unlikely because of its high fibre content and generally poor hygienic conditions.

In a previous study (Barbieri & Casiraghi, 1983) a product mild in taste and flavour, of well balanced composition and of good nutritional quality was obtained by eliminating the coarser fibrous constituents by a milling–screening process. It was demonstrated that extrusion cooking could substantially improve the organoleptic and hygienic quality of defatted corn germ flour, making it suitable for the use in food formulations (Peri & Barbieri, 1980).

This work has been carried out to optimize the extrusion cooking operation, i.e. maximize expansion and crispness, minimize heat damage of nutrients. To

Authors' address: Istituto di Tecnologie Alimentari, via Celoria 2, 20133 Milano, Italy.

0022–1163/83/0200–0043 $02.00

improve the physical and nutritional quality of the extruded product, the corn germ flour was supplemented with corn starch and milk proteins.

Corn meal has been supplemented with soy flour and dry milk (Anderson *et al.*, 1969; Anderson *et al.*, 1971) and with whey product and soy flour and isolates (Aguilera & Kosikowski, 1978) and then extruded. Sensory and functional properties of extruded corn soyblends have been studied by Maga & Lorenz (1978).

Materials and methods

For the extrusion cooking experiments the blends shown in Table 1 were used.

Table 1. Composition of blends

	Blends			
Ingredients	A	B	C	D
Defatted corn germ flour (%)	85	85	85	85
Milk protein (%)	–	5	10	15
Native corn starch (%)	15	10	5	–

Native corn starch (type: Globe 03401) obtained from an industrial wet milling process (Fragd S.p.A., Italy) was used in the mixtures. Milk proteins, purified by an ultrafiltration process were provided by the firm 'La Prosperité Fermiere' (LPF, France; Type: Prospe L801). Defatted corn germ flour (85% in each blend) was obtained with a milling–screening procedure (Barbieri & Casiraghi, 1983). As expected the composition of the blends mainly varied for the protein : starch ratio, the concentration of all other components (fat, fibre, ash and moisture) being essentially the same (Table 2).

Table 2. Analytical composition of ingredients and blends

	Moisture (g/100 g)	Protein (g/100 g d.b.)	Fat (g/100 g d.b.)	Fibre (g/100 g d.b.)	Ash (g/100 g d.b.)	Carbo-hydrates (g/100 g d.b.)
Ingredients						
Germ	8.8	20.2	1.7	3.3	8.42	66.3
Milk protein	7.8	85.2	0.9	–	1.42	12.5
Starch	9.1	–	–	–	0.13	99.9
Blends						
A	7.4	16.8	1.6	2.8	7.05	71.7
B	8.1	20.5	1.7	2.8	7.32	67.7
C	8.2	24.8	1.8	2.8	7.44	63.2
D	7.2	28.5	1.8	2.8	7.68	59.2

Table 3. Chemical characteristics and biological quality of ingredients and blends proteins

	Total protein (g/100 g d.b.)	Milk protein/ total protein (%)	Chemical score	Protein digesti- bility (%)	C-PER	Available lysine (g/100 g d.b.)	NSI (%)
Ingredients							
Germ	20.2	–	70	77.8	2.0	0.53	20.8
Milk protein	85.2	–	95	90.1	2.7	3.94	77.1
Blends							
A	16.8	–	70	77.8	2.0	0.53	20.8
B	20.5	23	80	78.7	2.5	0.65	32.2
C	24.8	36	86	81.0	2.4	0.81	39.6
D	28.5	45	91	84.1	2.5	0.89	45.0

The chemical characteristics and the biological quality of proteins are shown in Table 3. The biological quality of proteins as well as available lysine and nitrogen solubility obviously increase by increasing the amount of milk proteins.

Extrusion cooking was carried out using a double-screw Creusot-Loire BC 45 extruder (die diameter 5 mm, barrel L/D 600/55 mm). Screw rotation speed was constant at 150 rev/min; process temperatures in the barrel were 150 and 170°C and feed material moisture was 18%. Feed rate of material was ranging from 35 to 48 kg/hr.

Analytical methods

Moisture. Determined by drying to constant weight at 105°C.

Ash. Determined by dry ashing at 550°C; minerals were determined by atomic absorption spectrophotometry.

Protein nitrogen. The nitrogen to protein conversion factor used was 6.25 (AOAC, 1970).

Lipids. After extraction by diethyl ether in a Soxhlet apparatus (Giuliano & Stein; 1970).

Crude fibre. Wendee's method (Cirilli, 1972).

Total carbohydrates. Calculated as difference.

Nitrogen solubility index (NSI). AOAC method (1970).

In vitro *digestibility.* The determination of digestibility was carried out by *in vitro* proteolysis (Satterlee, Marshall & Tennison, 1979).

Available lysine. Carpenter's method (1960).

Expansion ratio. The degree of expansion of the extruded product was calculated by the ratio, extruded product diameter : die orifice diameter; expansion was evaluated as an average of ten measurements.

Textural proprieties. Texture of the experimental material was evaluated using an Instron universal testing machine (Model TM, Instron Engineering

Corp., U.S.A.). A Werner Bratzler shear was used to evaluate shear force. The sample was inserted into a triangular opening in the blade between two rectangular bars. Ten samples from each experiment were tested. Shear force was measured by height of peaks; crosshead speed was 20 cm/min and full scale deflection 10 kg.

Gelatinized starch. The degree of starch gelatinization is defined as the ratio of gelatinized and total starch in the product. This was calculated from spectrophotometric measurements at 600 nm of the starch–iodine complex formed in an aqueous suspension of the sample before and after complete solubilization of the starch by alkali (Watton, Weeden & Munk, 1971).

Water absorption index (WAI). The water absorption index is the weight of gel obtained per gram of dry sample. The method we have applied is a modification of the method of Anderson *et al.* (1969) to measure the swelling power of starch.

Viscosity. Viscosity measurements were carried out on a 40% aqueous suspension of the extruded materials, at 20°C, using a MLW-VEB Prugerate-Werk viscometer.

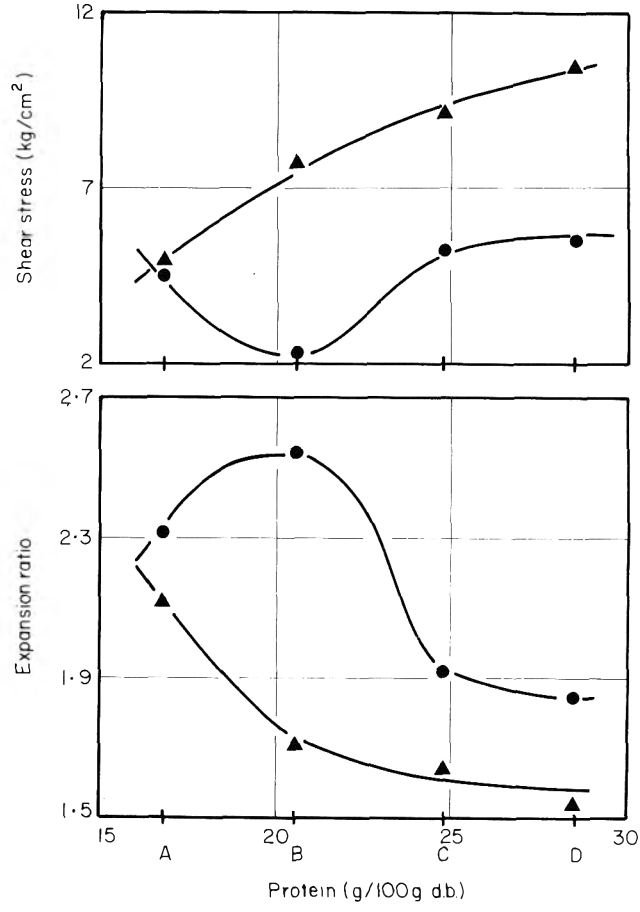


Figure 1. Effect of protein content on expansion ratio and shear stress (kg/cm²). ●, Blends extruded at 150°C; ▲, blends extruded at 170°C.

Results and discussion

Physical and functional proprieties

Figure 1 reports the expansion ratio and a measure of hardness (shear stress necessary to cut the sample in the Instron apparatus) of the extruded products as a function of total protein content. As it is evident from the osbervation of the curves, the two parameters show an almost symmetrical and opposite variation. This simply means that the hardness of the samples is greater the less expanded they are.

In general, a strong positive correlation exists between acceptability of the extruded product and its crispiness, which is in turn positively correlated to the degree of expansion. The following conclusions can be therefore drawn from data reported in Fig. 1:

(1) Samples obtained at 150°C are consistently better than samples obtained at 170°C.
(2) At the higher temperature, expansion decreases (and hardness increases) with increasing protein content, while at 150°C a maximum value of expansion was obtained at an intermediate protein concentration.

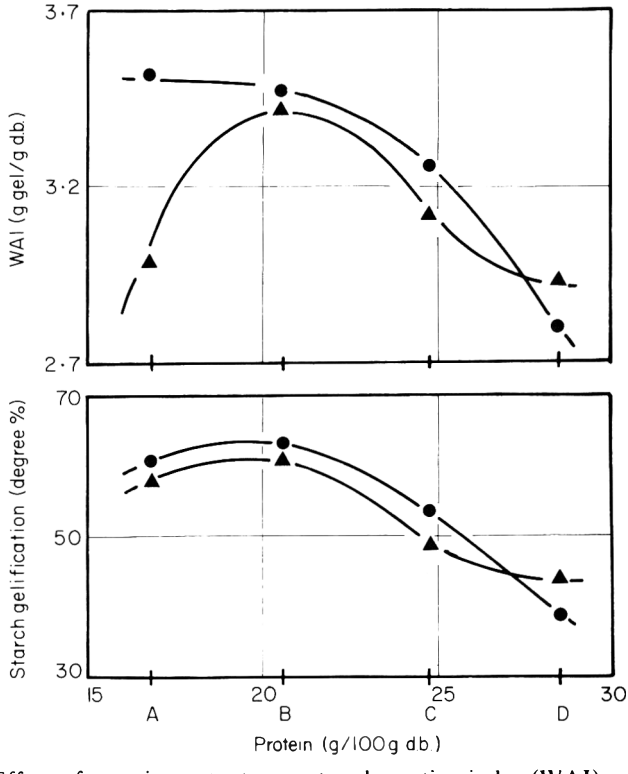


Figure 2. Effect of protein content on water absorption index (WAI) and on starch gelatinization degree. ●, Blends extruded at 150°C; ▲, blends extruded at 170°C.

Figure 2 shows the variability of the water absorption index (WAI) and of the degree of starch gelatinization. In all cases, at both 150 and 170°C these values show a maximum, corresponding to the lowest addition of milk proteins.

Figure 3 shows the rheological behaviour of the suspensions in water of the extruded samples. For purpose of comparison Fig. 3 also reports the data obtained from the non-extruded blends. As it clearly appears from the osbervation of the graphs, extrusion determines a substantial increase of viscosity at all shear rate values and a change from a newtonian to a non-newtonian, i.e. pseudoplastic, behaviour. This result is the consequence of the heating effects

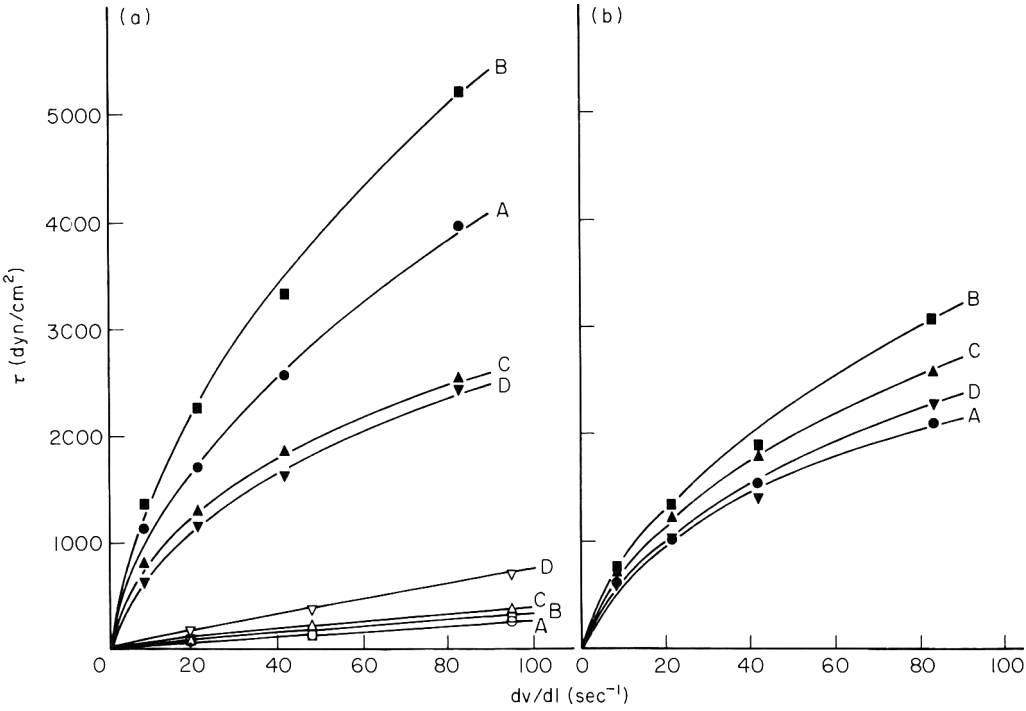


Figure 3. Shear stress (τ) *versus* shear rate (dv/dl) of different blends before and after extrusion. (a) ○, □, △, ▽, Blends A, B, C and D before extrusion; ●, ■, ▲, ▼, blends A, B, C and D extruded at 150°C. (b) ●, ■, ▲, ▼, Blends A, B, C and D extruded at 170°C.

on the structure and properties of the various constituents; particularly protein and starch. The starch gelatinization and a mild protein denaturation increase starch and protein affinity for the water phase and promote inter-molecular bonds. This results in macromolecular aggregates which interfere in the flow behaviour of the suspension. On the other hand excessive heating, causing a greater denaturation of the constituents, particularly proteins, depresses the above mentioned phenomena as it is evident comparing the rheological behaviour of the samples obtained at 170°C with those obtained at 150°C.

The consistency of data presented in Figs 1, 2 and 3 allows to draw clear indications on the extrusion cooking effects on physical parameters, which can be so summarized:

(1) Considering the effects of extrusion temperature, data show significant differences. In samples obtained at 150°C all parameters related to the water-product interactions (the starch gelatinization degree, the WAI, the viscosity of water suspension) show greater values. All these parameters are directly related to expansion and crispiness of the product, and hence to its palatability. Whatever the explanation, these findings are of significant practical interest.
(2) Concerning the milk protein addition the comparison of data is consistent. At 170°C the addition of milk proteins has negative effects on starch gelatinization, water absorption (also resulting in lower viscosity of suspensions) and expansion. The higher the milk protein concentration, the harder the extruded products are. In contrast, at 150°C a small addition of milk proteins (Sample B) clearly improves the organoleptic characteristics of the extruded products. Starch gelatinization, viscosity of the water suspension, expansion and crispiness are all increased. Higher milk protein concentrations cause a decrease of all parameters as well as of organoleptic acceptability.

Biological quality evaluation

Lysine availability, NSI and *in vitro* digestibility of protein, were evaluated to study the influence of the extrusion cooking process on the biological quality of protein; data are reported in Figs 4, 5 and 6. Available lysine (Fig. 4) and

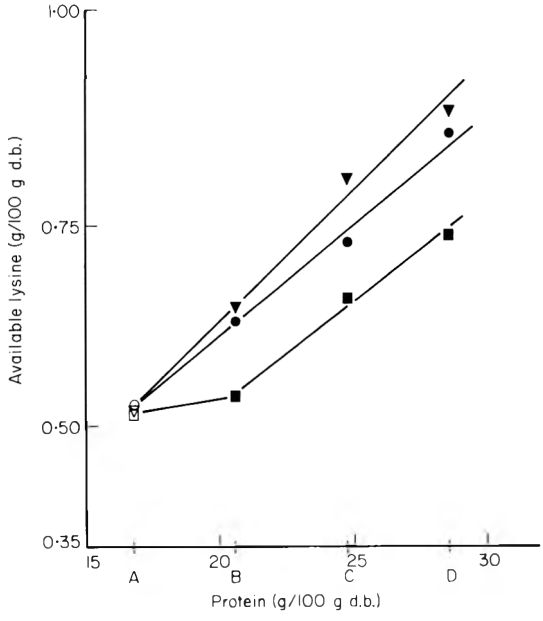


Figure 4. Available lysine of the different blends before and after extrusion. ▼, Not extruded blends; ●, blends extruded at 150°C; ■, blends extruded at 170°C.

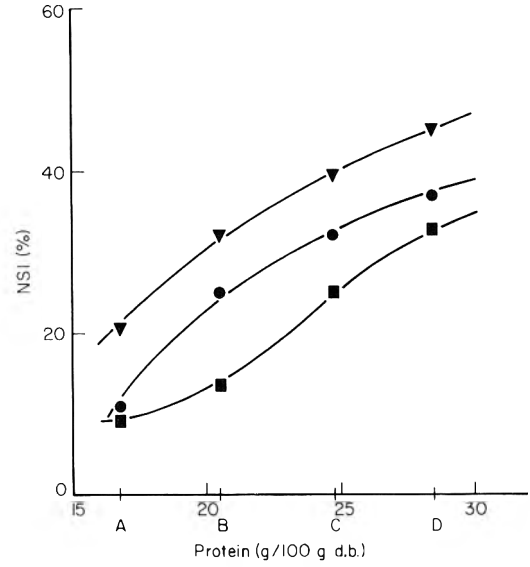


Figure 5. Nitrogen solubility index (NSI) of the different blends before and after extrusion. ▼, Not extruded blends; ●, blends extruded at 150°C; ■, blends extruded at 170°C.

nitrogen solubility index (Fig. 5) show a similar pattern of variation. Both parameters increase by increasing protein concentration as the obvious consequence of the increase of milk protein content. Extrusion determines a decrease of both parameters, this effect being substantially greater at higher tempera-

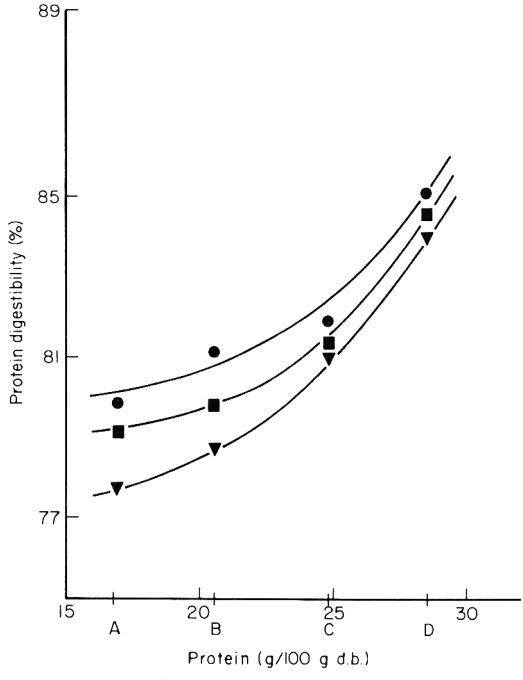


Figure 6. Protein digestibility of the different blends before and after extrusion. ▼, Not extruded blends; ●, blends extruded at 150°C; ■, blends extruded at 170°C.

ture. It is interesting to observe that protein of Sample A (no milk protein added) are more stable to heating effects. The addition of milk protein powder which also brings small amounts of lactose in the blends certainly results in an increase of heat damage due to the incidence of Maillard reactions.

These results, however related to the specific extrusion plant used in our experiments, are representative of more general trends. De Muelenaere & Buzzard (1969) studied the availability of lysine in full fat soy and in mixtures containing soy, skim milk powder and cereal. No destruction of available lysine occurred when full fat soybeans were extruded; conversely available lysine was reduced by 28% during extrusion of products containing skimmed milk.

In an extensive study of the problem (Riva, 1981) we have found similar behaviours using a single-screw laboratory extruder. The only significant difference with the findings reported in this paper was that expansion and organoleptic quality reached a maximum at a higher milk protein content, i.e. about 36% of total protein.

Contrary to the variation of available lysine and NSI, the expansion process determines an increase of protein digestibility. The overall effect of extrusion on this parameter results from the consequences of heating on protein denaturation and on browning reactions. At 150°C lower denaturation and browning results in higher digestibility than at 170°C. The benefit diminishes in the presence of increasing amounts of milk protein due to the increasing incidence of the browning reactions.

Conclusions

An expanded product mild in flavour, crisp in texture, suitable for the production of nutrient snacks may be obtained by extrusion cooking of defatted corn germ flour.

Both organoleptic and nutritional qualities are improved by the addition of a small amount of undenatured milk protein. In our case milk proteins account for about 23% of the total protein content.

The extruded products obtained at 150°C have better physical organoleptic and nutritional characteristics than those obtained at 170°C.

References

Aguilera, J.M. & Kosikowski, F.V. (1978) *J. Fd Sci.* **43,** 225.

Anderson, R.A., Conway, H.F., Pfeifer, V.F. & Griffin, E.L. (1969) *Cereal Sci. Today,* **14,** 4.

Anderson, R.A., Pfeifer, V.F., Bookwalter, G.N. & Griffin, E.L. (1971) *Cereal Sci. Today,* **16,** 6.

AOAC (1970) *Official Methods of Analysis,* 11th ed. Association of Official Agricultural Chemists, Washington D.C.

Barbieri, R. & Casiraghi, E.M. (1983) *J. Fd Technol.* (in press).

Blessin, C.W., Inglett, G.E., Garcia, W.J. & Deatherage, W.L. (1972) *Fd Prod. Develop.* **5,** 34.

Blessin, C.W., Garcia, W.J., Deatherage, W.L. & Cavins, J.F. (1973) *J. Fd Sci.* **38,** 602.

Carpenter, K.J. (1960) *Biochem. J.* **77,** 604.
Cirilli, G. (1972) In: *Chimica Analitica Applicata ai Mangimi,* p. 39, Coop. Libr. Univ., Bologna.
De Muelenaere, H.J.H. & Buzzard, J.L. (1969) *Fd Technol.* **23,** 345.
Giuliano, R. & Stein, M. (1970) In: *Quaderni di Chimica Degli Alimenti,* vol. 1, p. 93, Roma.
Maga, J.A. & Lorenz, K. (1978) *Lebensm.-Wiss. u. Technol.* **11,** 185.
Peri. C. & Barbieri, R. (1980) *Riv. It. Sostanze Grasse,* **57,** 185.
Riva, V. (1981) MSc Thesis in Food Science, University of Milan.
Satterlee, L.D., Marshall, H.F. & Tennison, J.M. (1971) *Fd Technol. Aust.*, 612.
Watton, N., Weeden, D. & Munk, W. (1971), *Fd Technol. Aust.*, 612.

(Received 22 April 1982)

J. Fd Technol. (1983) **18**, 53–60

Influence of storage on the quality of maize meal for tortilla making

O. PAREDES-LÓPEZ* AND R. MORA-ESCOBEDO

Summary

No studies have been previously reported on the effects of storage on the quality of limed maize meal. The aim of this work was to study changes in the quality of limed maize meal for tortilla making during accelerated storage conditions. The limed meal was stored at four relative humidities (55, 62, 75 and 83%) for 10 to 90 days, using a storage temperature of 35°C. The parameters fat acidity, insoluble residue protein, available lysine, *in vitro* digestibility and protein efficiency ratio were investigated. Major alterations of these parameters suggested that storage had profound effects on the meal quality. Sensory attributes of tortillas prepared with the stored meal further confirmed that storage was responsible for quality deterioration.

Introduction

In terms of volume, maize is the most important grain produced in Mexico. Maize also represents the most important food source. About 56% of the total calories (2575 cal/day per person), and 47% of the total protein (76.5 g/day per person) in the average diet are provided by cereals and especially by maize. In the rural areas the consumption of maize provides about 70% of the calories and about one half of the proteins (Paredes-López & Gallardo, 1981; Ramírez-Hernández & Chávez, 1981). It is noteworthy that in some southern regions of the U.S.A., the tortilla making process is also quite popular (Martínez-Herrera & Lachance, 1979).

Research studies have shown that during the liming of maize, losses of some of the nutrients take place, but what is most interesting is the increase of amino acid availability, and in general the marked improvement of the overall quality

Authors' address: Depto. de Alimentos, ENCB, Instituto Politécnico Nal., Apdo. P. 42–186, México 4, D. F., México.

*Present address: CIEA del Instituto Politécnico Nal., Unidad Irapuato, Apdo. P. 629, 36500 Irapuato, Gto., México.

0022–1163/83/0200–0053 $02.00

of maize (Bressani, Paz y Paz & Scrimshaw, 1958; Cravioto *et al.*, 1945). It has also been observed by scanning electron microscopy that in the tortilla making process, liming produces important structural alterations of the maize kernel (Paredes-López & Saharópulos, 1982). The use of industrially produced limed meal for tortilla preparation, and similar products, is gaining widespread acceptance. At present, about 8% of the total amount of maize consumed for tortilla making goes to twelve industrial plants for preparation of limed maize meal.

The purpose of this work was to study changes in the quality of limed meal during short-term accelerated storage. It seems that no studies have been published so far on this specific topic.

Materials and methods

Sample

A commercial white dent maize hybrid (*Zea mays* L.) was used throughout this work. The treatment of maize kernels for the production of limed meal was carried out at one of the industrial plants of CONASUPO — the government owned food corporation.

Storage conditions

Portions of limed maize meal were placed into storage desiccators containing saturated salt solutions, to produce environments with relative humidities (r.h.) ranging from 55–83%. The four salts used, and their corresponding relative humidities, were: NH_4NO_3, 55%; $NaNO_2$, 62%; NaCl, 75%; and KCl, 83% (Rockland, 1960). The desiccators were kept in a constant temperature cabinet at 35°C for 10 to 90 days. The conditions used for storage represent some of the climatological conditions of the country. The presence of an arrow in some of the figures indicates that, at that particular storage condition, the maize meal sample showed definitive evidence of mould growth.

Analytical methods

Protein content was determined by a microKjeldahl method and fat acidity by titration with KOH (AOAC, 1975).

Protein solubility

The fractionation of maize proteins was carried out as described by Landry & Moreaux (1980), with some modifications. For this purpose, 10 g meal and 100 ml extractant were used. The 1 hr extraction was performed in a reciprocating shaker. In each case the extraction was repeated using 30 min of shaking. The solid material was isolated from extractants by centrifuging at 20 000 *g* for

25 min. The extraction was as follows: Step 1, 0.05 M NaCl; Step 2, H_2O; Step 3, 55% isopropyl alcohol (w/w) with 0.6% of 2-mercaptoethanol (v/v). After extracting albumins + globulins (Steps 1 + 2), and zeins + glutelins (Step 3), variations in the amount of remaining (residue) protein were followed very closely.

Protein quality

Available lysine was determined by the fluorodinitrobenzene (FDNB) method (Booth, 1971). The overall coefficient of variation for available lysine determinations was 11.1%. The *in vitro* digestibility of protein was measured by the multi-enzyme system of Hsu *et al.* (1977). The multi-enzyme system consists of trypsin, chymotrypsin and peptidase. The standard deviation was ± 0.82.

Six male weanling rats, 22–23 days old weighing 27–28 g, were used in each determination of protein efficiency ratio (PER). Protein levels in all maize meal diets were adjusted to 9%. The casein diet was a standard diet. PER values reported were adjusted with reference to the standard PER of casein of 2.5. The overall standard deviation was ± 0.30.

Sensory tests

Organoleptic quality of tortillas made with the stored meal was evaluated by a five member taste panel. Flavour, colour, aroma, texture and consistency were rated on a hedonic scale of 1–5 where 5 was rated 'excellent'. Data for each parameter are reported as the mean of five observations. An overall rating of tortillas was made as to whether they are acceptable or unacceptable.

Results and discussion

Hydrolytic changes in lipids during storage were followed by determining fat acidity values. The fat acidity showed a general tendency to increase at the four relative humidities and 35°C used for the storage study (Fig. 1). The most dramatic change occurred at the r.h. of 83%; the fat acidity of the sample taken after 2 months of storage at 83% r.h. increased from 19 to about 155 mg KOH/100 g meal. Determination of free fatty acids during storage of cereal grains gives a good indication of quality changes (Baker, Neustadt & Zeleny, 1959). Lai & Varriano-Marston (1980) also found that during short-term storage of millet meal noticeable amounts of fat acidity were produced.

The protein solubility experiments were carried out only with the meal stored at the r.h. of 55 and 75% (Fig. 2). The percentage of residue protein increased notably during the first 60 days of storage. The unextracted protein remained constant after this time. Production of oxidizing agents during storage may act on proteins, especially on sulphur amino acids, and give as a result a lower protein solubility (Shimada & Matsushita, 1978).

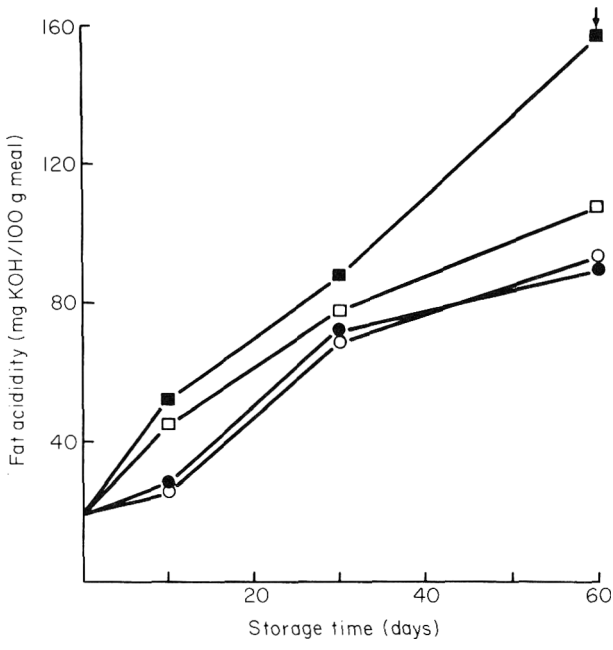


Figure 1. The effect of storage of limed maize meal on fat acidity (the arrow indicates visual evidence of mould growth). ○, 55% r.h. at 35°C; ●, 62% r.h. at 35°C; □, 75% r.h. at 35°C; ■, 83% r.h. at 35°C.

The availability of lysine is one of the critical factors determining the protein nutritional value of tortillas. Figure 3 presents the influence of the storage conditions on availability of lysine in limed maize meal for tortilla making. The initial content of available lysine of the unstored sample (2.7 g/100 g protein)

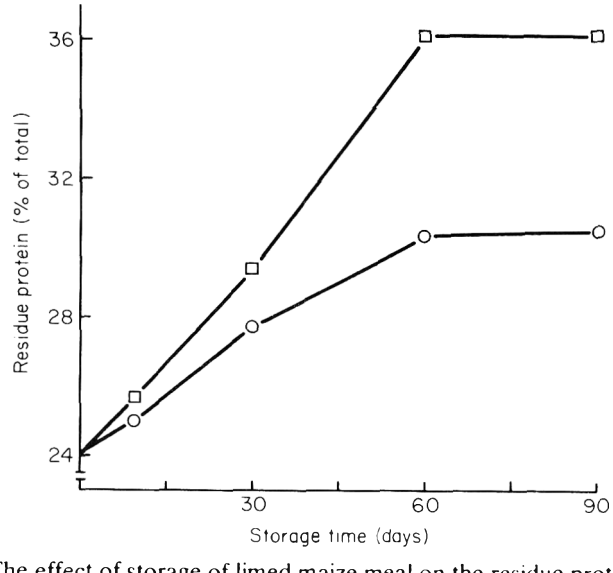


Figure 2. The effect of storage of limed maize meal on the residue protein. ○, 55% r.h. at 35°C; □, 75% r.h. at 35°C.

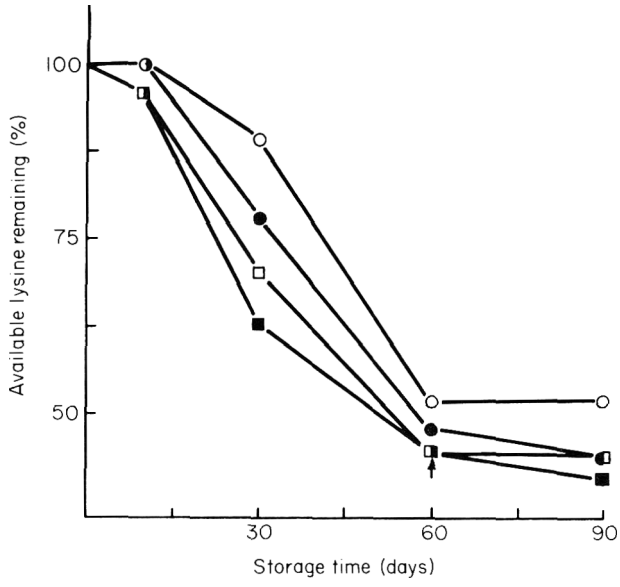


Figure 3. The effect of storage of limed maize meal on the availability of lysine (the arrow indicates visual evidence of mould growth). Key as for Fig. 1.

decreased more than 47% after 60 days of storage at all relative humidities tested. The reaction of the *epsilon* amino group with various substances, such as sugars or amide groups of other amino acids, notably decreased the available lysine. And as suggested by J. Whitaker (pers. comm.), there could also be losses due to addition to dehydroalanine produced by the alkaline conditions.

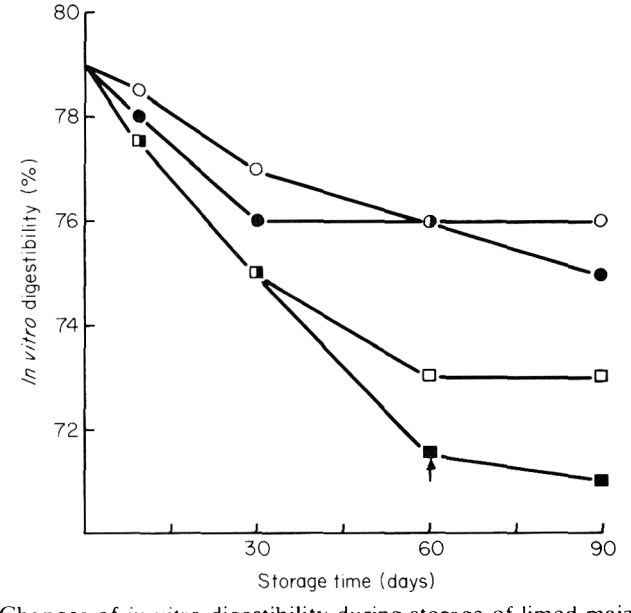


Figure 4. Changes of *in vitro* digestibility during storage of limed maize meal (the arrow indicates visual evidence of mould growth). Key as for Fig. 1.

The digestibility of a food protein is the primary determinant of the availability of its amino acids. Thus, *in vitro* digestibility studies of the stored meal were carried out as reported in Fig. 4. Increases of relative humidities produced further decreases of digestibility; at 55% r.h. and 90 days of storage the digestibility decreased from 79 to 76%, while at 83% r.h. and same time of incubation this parameter was reduced to 71%. It is also worth noting that most of the deteriorative effect, as measured by the protein digestibility, was produced during the first 60 days of meal storage.

The PER changes during maize meal storage are presented in Fig. 5. The most marked decrease occurred at the r.h. of 75% and 60 days of storage; PER decreased from 1.40 to 0.87. These data suggest that important detrimental reactions of the protein quality are taking place under the storage conditions used in this study, in agreement with the results presented in previous figures.

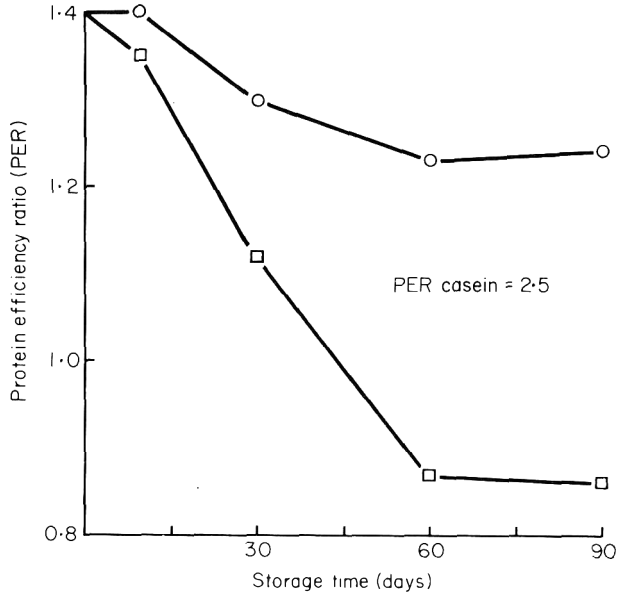


Figure 5. Changes of protein efficiency ratio during storage of limed maize meal. Key as for Fig. 2.

The deteriorative effects of the storage were reflected in the organoleptic properties of the tortillas prepared with the meal stored at the r.h. of 55, 62 and 75% (Table 1). The sample stored at the r.h. of 83% was not used for these sensory tests because of its potential content of mycotoxins. As indicated in Table 1, there were no significant differences in the aroma of tortillas for all samples tested. Tortillas made from the sample stored at 75% r.h. were rated as unacceptable.

The fatty acids produced during the meal storage might contribute to the reduction of protein solubility noted in this study, as suggested by Shimada & Matsushita (1978). The deterioration of lipids may cause changes in the form by

Table 1. Sensory properties of tortillas prepared with maize meal stored at 35°C for 60 days (All data are the mean of five replicates)

r.h. (%)	Flavour*	Colour*	Aroma*	Texture*	Consistency*	Overall rating
Control	4.2^{c}	4.0^{b}	2.8^{a}	3.2^{ab}	3.6^{ab}	Acceptable
55	3.8^{bc}	4.0^{b}	2.4^{a}	3.6^{ab}	3.6^{ab}	Acceptable
62	3.8^{bc}	3.0^{a}	2.6^{a}	3.0^{ab}	2.8^{ab}	Acceptable
75	2.4^{a}	2.8^{a}	2.4^{a}	2.2^{a}	2.4^{a}	Unacceptable

* Organoleptic properties were rated on a hedonic scale of 1–5, where 5 = excellent quality and 1 = unacceptable quality.

$^{a-c}$ Means with the same letter(s) are not significantly different ($P < 0.05$).

which lipids are bound to proteins, and it may also accelerate the aggregation of proteins. Formation of hydrophobic interaction and formation of aggregates caused by cross-linking produced through the Maillard reaction are also probably taking place, and that might also result in the decrease of protein solubility. These assumptions require further work.

Interestingly, no drastic changes of available lysine took place between 60 and 90 days of storage for all relative humidities tested. The loss of nutritional properties of the stored maize meal was reflected in the decrease of *in vitro* digestibility and PER values. Moreover, the final and most interesting evidence of maize meal deterioration in storage was observed in the sensory tests of tortillas. Important changes in the organoleptic quality of tortillas took place at 60 days and 75% r.h., which did not correspond at that storage condition to equivalent variations of some of the parameters investigated in this work, such as fat acidity and available lysine content. This study shows the difficulty in explaining the causes of changes in food quality unless one studies all the degradation reactions which are taking place.

It needs to be pointed out that most of the unwanted reactions took place during the first 60 days of storage; reactions that were accelerated by high relative humidity and temperature. It is estimated that quite frequently no less than 2 months are required for the limed maize meal to reach the final consumer; and r.h. of 75 or 83% (or even higher) with high ambient temperatures are common in some regions of the country. Thus the alterations of nutritional and functional properties found in this storage work require further considerations by those involved in food distribution programmes. It seems that there are no previous storage studies of maize meal (or maize kernel) in relation to effects on nutritional and functional properties of tortillas made from this meal. It is believed that research studies along this topic deserve further attention.

References

AOAC (1975) *Official Methods of Analysis*, 12th ed. Association of Official Agricultural Chemists, Washington, D.C.

Baker, D., Neustadt, M.H. & Zeleny, L. (1959) *Cereal Chem.* **36,** 308.
Booth, V.H. (1971) *J. Sci. Fd. Agric.* **22,** 658.
Bressani, R., Paz y Paz, R. & Scrimshaw, N.S. (1958) *J. Agric. Food Chem.* **6,** 770.
Cravioto, R.O., Anderson, R.K., Lockhart, R.E., Miranda, F. de P. & Harris, R.S. (1945) *Science* **102,** 91.
Hsu, H.W., Vavak, D.L., Satterle, L.D. & Miller, G.A. (1977) *J. Fd Sci.* **42,** 1269.
Lai, C.C. & Varriano-Marston, E. (1980) *Cereal Chem.* **57,** 275.
Landry, J. & Moreaux, T. (1980) *J. Agric. Fd Chem.* **28,** 1186.
Martínez-Herrera, M.L. & Lachance, P.A. (1979) *J. Fd Sci.* **44,** 377.
Paredes-López, O. & Gallardo, Y. (1981) *Comercio Exterior (Méx.)* **31,** 247.
Paredes-López, O. & Saharópulos, M.E. (1982) *J. Fd Technol.* **17,** 691.
Ramírez-Hernández, J. & Chávez, A. (1981) *Comercio Exterior (Méx.)* **31,** 385.
Rockland, L.B. (1960) *Anal. Chem.* **32,** 1375.
Shimada, K. & Matsushita, Y. (1978) *Agric. Biol. Chem.* **42,** 781.

(Received 7 May 1982)

J. Fd Technol. (1983) **18**, 61–73

Prediction of thiamine content in convective heated meat products

CHRISTINA SKJÖLDEBRAND,* A. ÅNÄS,* R. ÖSTE† AND P. SJÖDIN†

Summary

It is reported that thiamine is one of the most heat labile water soluble vitamins. It is therefore important to control the destruction of this vitamin during heating, reheating and keeping warm of different food items.

In this study the kinetics of thiamine degradation in ground meat were determined. The losses during roasting of a minced meat loaf under different air conditions in a forced convection oven were calculated by computer simulations. The actual losses were analysed by a chemical method. The kinetics were found to be approximately of first order. The time–temperature curves and the thiamine degradation in the loaves roasted under fairly mild conditions were easier to predict than the ones roasted at higher air temperature. This is partly due to the formation of the crust which has different thermal properties and gives different chemical changes from the inner parts of the loaf. The humidity and the velocity of the air does not effect the crust formation as much as the air temperature. This fact limits the prediction of thiamine degradation to lower air temperatures.

Introduction

To be able to optimize food processes, mathematical models for predicting what happens in the product during processing have to be made. In such models the dependence of the products on the environment have to be considered. When the process involves heating or cooling the equations of the model controlling heat and mass transfer include thermal properties of the product. Thus, these properties must be determined. Furthermore chemical, microbiological and

Authors' addresses: *Division of Food Engineering, P.O.B. 50, S-230 53 Alnarp, Sweden, and †Division of Applied Nutrition, P.O.B. 740, S-220 07 Lund, Lund University, Lund, Sweden.

0022–1163/83/0200–0061 $02.00

enzymic reactions will take place during the process and the model should describe some of these reactions. This means that the kinetics have to be known.

Thiamine is one of the most heat labile water soluble vitamins. The losses during reheating and keeping warm of foodstuffs may be considerable (Ang *et al.*, 1975, Kahn & Livingstone, 1970). Up to 75% losses have been found in restaurant handling of vegetables (Lang, 1970). Texeira and co-workers (1969, 1975a) and Manson, Zahradnik & Stumbo (1970) estimated the thiamine losses in sterilized cans by means of computer simulations. In these studies time–temperature relationships of the heating process were determined and the thiamine losses were calculated assuming a first order reaction of thiamine breakdown.

In these studies it was assumed that the surface temperature of the cans immediately reached the surrounding temperature. Texeira, Zinsmeister & Zahradnik (1975b) improved the computer program by varying the surface temperature with time.

The investigation here reported was carried out as part of a larger project where convection roasting and reheating of ground meat products were to be optimized. Parameters monitored were process time, colour of the crust, weight losses, sensory properties and thiamine content after processing at different velocities, temperatures and humidities of the air (Skjöldebrand, 1979). In this study, the kinetics of thiamine degradation in minced meat were determined. The losses during roasting of a ground meat loaf were then calculated by computer simulation. The actual losses were determined by a chemical method. The calculated and analysed values showed good agreement when the air conditions were fairly mild.

Materials and methods

Basic considerations (Jen, Manson & Stumbo, 1971)

The degradation of thiamine in ground meat products has been found to follow a first order reaction. This means that the reaction rate and thus thiamine content can be calculated from the following equation (Farrer, 1955)

$$\frac{dy}{d\tau} = k(b_0 - y) \tag{1}$$

where b_0 is the concentration at the start and k is reaction rate constant. Integration of Equation (1) gives

$$\log \frac{b_0}{b} = \frac{k \times \tau}{2.303} \tag{2}$$

where b is the concentration of thiamine at time τ.

The reaction rate constant, k, can be calculated from the following Arrhenius

equation

$$\log k = -\frac{E_a}{2.303 \times R} \times \frac{1}{T} + B \tag{3}$$

where T is the temperature, E_a is the activitation energy, B is an integration constant and R is the gas constant.

Thus the reaction rate constant depends on temperature. Plotting $\log b_0/b$ in a diagram against time at constant temperature the inclination gives the reaction rate constant at actual temperature.

When describing the time–temperature influence of the reaction, it is often convenient to translate it into terms of equivalent time at a reference temperature. This is very common when describing microbiological destruction in heat sterilization where this time is called the F value. The F value is referred to 121°C (250°F). For chemical and nutritional reactions the reference temperature is often 100°C and the time is called the cook value (C value). In this way Equation (2) becomes

$$\log \frac{b_0}{b} = \frac{C}{D} \tag{4}$$

where D (= 2.303/k) is the time for a 90% reduction of the vitamin content at 100°C (the decimal reduction time). The temperature dependence of D is calculated from Equation (3).

If log D is plotted in a diagram against temperature the Z_c value is defined as the temperature raise needed to decrease the decimal reduction time ten times for a known reduction. The Z_c value can often be assumed as constant for a specific reaction in a specific product. In this case the temperature dependence may be expressed by

$$\log \frac{D_2}{D_1} = -\frac{T_2 - T_1}{Z_c} \tag{5}$$

If the meat loaf is divided into a numbers of sections the remaining vitamin content in the entire meat loaf after processing must be equal to the sum of the content in each of these parts. The time–temprature relationship in each section can be simulated by means of the Fourier equation:

$$\frac{\partial t}{\partial \tau} = a \times \frac{\partial^2 t}{\partial x^2} \tag{6}$$

calculated for the actual geometry of the product and the appropriate boundary conditions.

Using Equation (4) for each section the following relationship is given for unit volume

$$1 \times 10^{-C/D} = \int_0^1 \frac{b}{b_0}\, dv \tag{7}$$

In this way the integrated cook value over the whole loaf may be determined.

Heating procedure

A mixed ground meat product was used consisting of meat, potato starch and bread crumbs. The recipe was similar to one used in the food industry (Skjöldebrand & Olsson, 1980). The mixture was formed into a loaf with the dimensions 200 × 50 × 50 mm. The loaf was placed in a teflon coated net cage and roasted in a specially designed convection oven made for purposes of investigation (Skjöldebrand & Öste, 1977). The temperature in the product was measured by means of chromel-alumel thermocouples. One thermocouple was placed in the

Table 1. Chosen air conditions when heating meat loaves

Air velocity (m/sec)	Air temperature (°C)	Air humidity*	Chosen air condition for thiamine analysis†
3	150	d	1
		h	
	225	d	6
		h	
	300	d	
		h	
5	125	d	2
		h	
	150	d	
		h	
	225	d	
		h	
	300	d	
		h	
7	150	d	
		h	
	225	d	5
		h	
	300	d	
		h	
9	150	d	3
		h	
	225	d	
		h	
	300	d	4
		h	

*d, 'Dry' air (0 kg water/kg dry air)' h, humid air (1.3 kg water/kg dry air).

†The figures in this column are the procedures referred to in the text.

centre of the product and the others at different distances from the surface. The temperature on the surface was measured by means of an IR-pyrometer (Raytek Thermalert LC-814) (Skjöldebrand, 1979).

The meat loaf was placed in its net cage in the oven with the short side of the loaf towards the air stream. In this way, due to the size of the product, the temperature in the centre when reaching 70°C, was only influenced by the heat transfer from the long sides (Skjöldebrand, 1979). The product was tempered to + 5°C and weighed, before heating. When the temperature in the centre reached 70°C the heating was stopped and the loaf was taken out of the oven, weighed and frozen in plastic pouches in liquid nitrogen 3 min later. It was stored for 4 weeks in the freezer before it was analysed for thiamine content. In a separate program the air conditions were varied according to Table 1. From these, six different combinations were chosen in a way that a variation air temperature, air velocity and air humidity could be covered (Table 1).

Thiamine analysis

A 1 cm thick slice from the middle of the frozen meat loaf (Fig. 1) was taken out for thiamine analysis. The piece was homogenized with distilled water in an 'Omnimixer'. From the homogenate three individual samples were analysed. Three samples from homogenized raw meat were analysed at the same time. The thiamine was analysed by the thiochrome method according to the Association of Official Analytical Chemists (1970).

Kinetics of thiamine degradation

Frozen raw minced meat was homogenized together with 40% distilled water. The obtained meat purée was transferred to small glass tubes (7 × 120 mm) which were sealed with rubber plugs. The tubes were then placed in a water bath held at constant temperature (± 0.1°C) for various lengths of time (70.5°C: 120, 240, 420 min; 85.0°C: 180, 270, 390 min; 98.0°C: 75, 180, 300 min). Using a thermocouple in the centre of the glass tube, it was found that temperature equilibrium at all temperatures was reached within 90 sec. After holding time, the tubes were immediately cooled in an ice bath and analysed for thiamine. At each time and temperature, three individual analyses were performed.

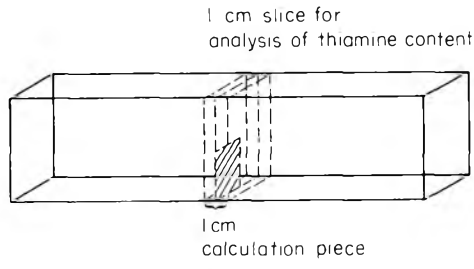


Figure 1. The meat loaf. A 1 cm slice in the centre was cut out for thiamine analysis. The calculated values were integrated over this slice.

Computer simulation

A computer program was made to simulate the temperature in a 1 cm thick slice in the middle of the meat loaf at different times during the roasting period (Fig. 1). Equation (2), (3) and (6) were used for predicting the thiamine content and the integrated cook values. The vitamin content was calculated at different places in the piece at different times. The cook value was calculated simultaneously.

The Z_c value was calculated separately using Equation (5) and assuming that the Z_c value is constant. Knowing the surface temperature as a function of time from each heating operation the heat resistance 1/Bi and the Biot number ($Bi = (\alpha \times d)/\lambda$) were calculated. The Fourier equation for an intersection between two infinite slabs was used to simulate the time–temperature curves in the centre slice and at different distances from the surface (Carslaw & Jaeger, 1959). It was assumed that heat was transferred only from the four long sides.

Programme inputs were: maximum heating time; start temperature in the product; oven temperature; time intervals (number of calculations); number of sections in the product (Fig. 1); time intervals between printing of results; heat resistance ($1/Bi = \lambda/(\alpha \times d)$; half the thickness of the loaf (d); thiamine content in raw product.

The thermal diffusivity and the thermal conductivity used in the Fourier equation were taken from another investigation where thermal properties of ground meat products were estimated (Sörenfors & Dagerskog, 1978). Knowing the fat and water content (Skjöldebrand & Hallström, 1980) the following equation for calculating the Fourier number (Fo) was used:

$$Fo = \frac{0.142 \times 10^{-6} + 0.65 \times 10^{-9}[(t + t_0)]/2 - 50 \times \tau}{d} \quad (8)$$

The simulations and calculations were made for each of the six products roasted in the convection oven.

Results

Kinetics of thiamine degradation

A plot of ln (thiamine retention) *versus* time gave straight lines as shown in Fig. 2 at all three temperatures, indicating a first order reaction under the

Table 2. Rate constants of thiamine breakdown in minced meat, assuming a first order reaction

Temperature (°C)	Rate constant (min^{-1})
70.5	1.39×10^{-4}
85.5	12.44×10^{-4}
98.0	25.11×10^{-4}

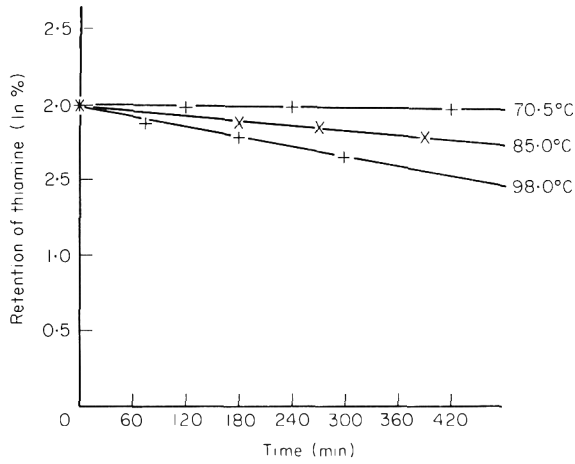


Figure 2. The thiamine retention in ground meat as a function of time for three different temperatures (70.5, 85.0 and 98.0°C). ×, Experimental value (the mean value of three samples).

conditions studied. Table 2 shows the calculated rate constant at the different temperatures. From these and Equation (3) the activation energy was calculated to 113.3 kJ/mol, which corresponds fairly well to investigations made by Feliciotti (1957) (115.4 kJ/mol) and Mullery (1975) (114.5 kJ/mol).

Estimation of the Z_c value

The Z_c value is defined as the necessary temperature rise in °C needed for a ten fold increase in reaction rate. Figure 3 shows the degradation time as a

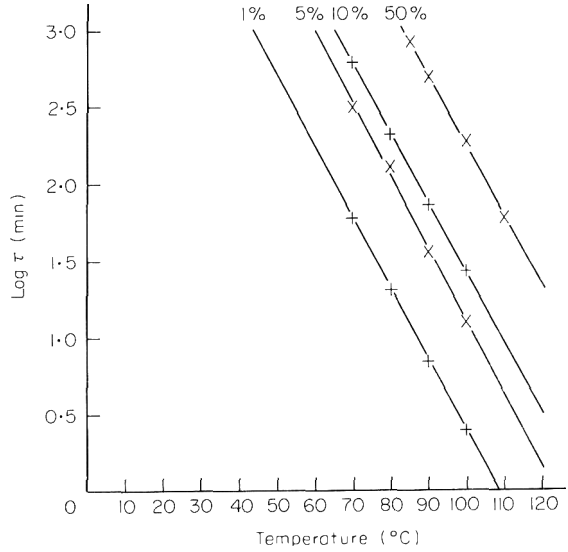


Figure 3. The degradation time for thiamine as a function of temperature. The curves correspond to different percentage of degradation. ×, Corresponds to experimentally determined data.

function of temperature in a log line diagram. The Z_c value is the slope of each curve. This value for thiamine degradation in ground meat was found to be 22°C which corresponds well to values published earlier for thiamine (Lund, 1977).

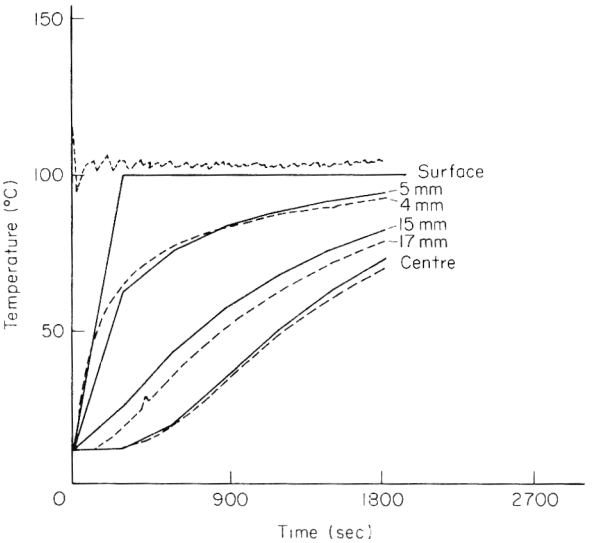


Figure 4. Simulated and measured temperature on the surface and at different distances from the surface. Air temperature, 125°C; air velocity, 5 m/sec; air humidity, 'humid' air. Bi = ∝, ----, Measured; ——, simulated.

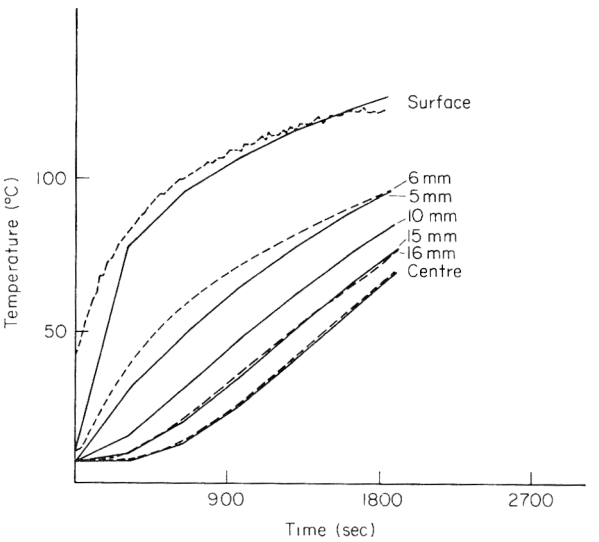


Figure 5. Simulated and measured temperature on the surface and at different distances from the surface. Air temperature, 150°C; air velocity, 9 m/sec; air humidity, 'dry' air. Bi = 1.7. ----, Measured; ——, simulated.

Temperature measurements and simulations

Figures 4 and 5 show two examples of time–temperature curves calculated and measured in the product heated. In the computer program, the heat transfer coefficient was set constant, which gives errors in the calculated surface temperature curve (Skjöldebrand, 1980). The Biot number used in the program is indicated in each diagram. The calculated curves that are shown correspond well to the measured ones. It was not possible to fix the thermocouples in beforehand defined places except for the one at the centre and in the computer program the product was divided into a number of sections of equal size. This is the reason why the measured temperature curve is not at exactly the same distance from the surface as the calculated ones.

Simulation and analysis of thiamine content

Figure 6 shows the calculated thiamine content as a function of time at different distance from the surface. This example corresponds to the same sample as in Fig. 5. The product was roasted at 150°C, 9 m/sec and dry air. In the diagram is also shown the calculated mean value over the whole slice as a function of time.

The analysed and the simulated average thiamine retention over the slice after processing of the six meat loaves are given in Table 3. The coefficient of correlation was 0.97. Figure 7 shows the simulated mean thiamine content during the process. The analysed values are indicated in the diagram.

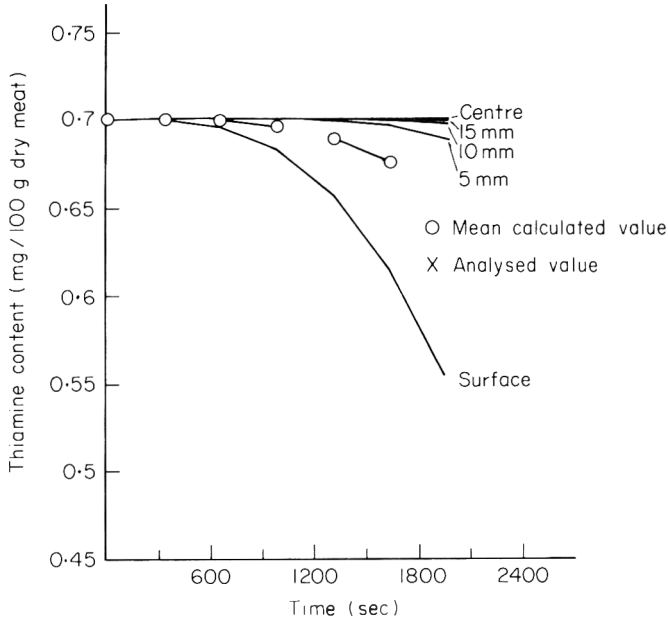


Figure 6. Calculated thiamine concentration as a function of heating time and at different distances from the surface. Air temperature, 150°C; air velocity, 9 m/sec; air humidity, 'dry' air. ○, Mean calculated thiamine content; ×, analysed thiamine content.

Table 3. Thiamine retention in different roasted minced meat loafs calculated from chemical analysis and from computer simulations

Sample No.	Chemical analysis	Computer simulation
1	96.6	98.9
2	98.3	98.5
3	91.4	93.3
4	70.6	65.6
5	80.0	74.7
6	84.8	74.7

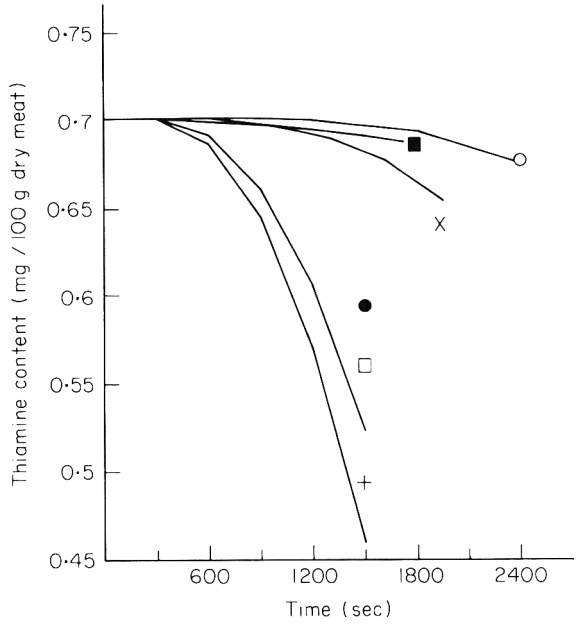


Figure 7. Calculated and analysed thiamine content in the six different meat loaves processed in the investigation. The numbers corresponds to the air conditions indicated in Table 1. (Experiment 5 and 6 gave the same calculated values). ○, 1; ■, 2; ×, 3; +, 4; □, 5; ●, 6.

Integrated cook values

Table 4 shows the integrated cook value of thiamine over the whole slice in the six loaves after the roasting process.

Discussion

The kinetics of thiamine degradation were found to be approximately of first order. This is in accordance with the findings of others (Jen, 1971; Texeira *et*

Table 4. Integrated cook value in the slice when 70°C in the centre of the meat loaf

Air combinations	Cook value (min)
1	22.4
2	26.6
3	40
4	266
5	175
6	175

al. 1975b). In a thorough examination of the thiamine breakdown in sterilized milk, (Horak, 1980) showed that the kinetics are better described by a third order reaction. However, he also showed that for smaller losses (10–15%), the first order reaction is still a good approximation. As shown in Table 3 there is a good agreement between analysed and calculated thiamine retention for losses less than 10%. However, at lower retentions the calculations seem to overestimate the actual losses somewhat. There might be a deviation from the assumed kinetics regarding both reaction order and activation energy, more pronounced with higher losses. With higher extent of heat treatment a correspondingly larger part of the loaf can be characterized as crust. The thiamine breakdown in this region, where the temperature exceeds 100°C, has been calculated from the reaction constants obtained under 100°C.

The time–temperature simulation curves fit very well to the measured ones for the loaves shown in Figs 4 and 5. These loaves have been roasted under fairly mild conditions and thus with low heat transfer coefficients. This also means that the crust is thin and the temperatures on the surface and in the crust are fairly close to 100°C. When the heat transfer coefficients increased, the simulations gave curves that did not fit as well. In the program the heat resistance was held constant during the process. This is not the case in reality (Skjöldebrand, 1980). The heat transfer coefficient changes may be one reason. The thermal conductivity and the thermal diffusivity in the crust differ comparably from the inner parts of the loaf (Skjöldebrand, 1979). When the crust is thicker and thus forms a greater part of the product, these properties will exert an influence. This fact will also give limitations for the kinetics used for thiamine reduction as mentioned above. Furthermore, many chemical and physical changes occur in the crust and they are very difficult to control. The crust formation is influenced by humidity, velocity and temperature of the air. At low air temperatures and any humidity or velocity the roasting conditions are mild and the model will be able to predict the thiamine content. At higher temperatures (> 200°C) however the relative humidity is very low and does not influence the crust formation. Furthermore, at any air velocity the air temperature influences the formation more and the model is of limited use.

Knowing the limitations, the heat transfer coefficient, the thermal properties

of the product and the thiamine content at the start of the process this model may be used for predicting the thiamine content.

The cook value is varying depending on the air conditions. Table 4 shows that the variation for a ready made product is very wide.

This model needs to be combined with models for other properties, which have optimal values for a specific cook value. The model for temperature simulations here reported can be used for these other properties if they are time–temperature dependent and their kinetics are known. Combining the different models will give the optimal conditions for the heating process. It is known that the mean thiamine content reduces with an exponential curve, which is illustrated in Figs 4 and 5. If the centre temperature is incrased from 70 to 80°C, the thiamine loss will increase 40%. This shows how important it is not to overcook the product.

Appendix

a = thermal diffusivity (m^2/sec)
B = integration constant
b = thiamine concentration at time = τ (sec)
b_0 = thiamine concentration at time = 0 (sec)
C = Cook value (min)
D = decimal reduction time (min)
d = half the thickness of the meat loaf (m)
E_a = activation energy (kJ/mol)
k = reaction rate constant (min)
R = gas constant (J/k mol °K)
T = absolute temperature (°K)
t = temperature (°C)
t_0 = temperature at time = 0 (°C)
v = volume (m^3)
X = distance from the surface (m)
y = thiamine concentration (M)
Z_c = temperature raise needed to decrease the decimal reduction time ten times (°C)
α = heat transfer coefficient (J/m^2 °K, sec)
λ = thermal conductivity (J/m °K, sec)
τ = time (sec)
Bi = $\alpha \times d/\lambda$

References

Ang, C.Y.W., Chang, C.M., Frey, A.E. & Livingstone, G.E. (1975) *J. Fd Sci.* **40**, 997.

AOAC (1970) *Official Methods of Analysis*, 11 ed., pp. 771–774. Association of Analytical Chemists, Washington D.C.

Carslaw, H.S. & Jaeger, J.C. (1959) Conduction heat in food solids, 2nd edn. Oxford.
Farrer, K.T.H. (1955) *Adv. Fd Res.* **6,** 257.
Feliciotti, E. & Esselen, W.B. (1957) *Fd Tech.* **11,** 77.
Horak, F.P. (1980) Uber die Reaktionskinetik der Sporenabtötung und chemischen Veränderungen bei der chemischen Veränderungen bei der thermischen Haltbarmachung von Milch Zur Optimierung von Erhitzungsverfahren. Ph.D. Thesis, Technischen Universität, München.
Jen, Y., Manson, J.E., Stumbo, C.R. & Zahradnik, J.W. (1971) *J. Fd Sci.* **36,** 692.
Kahn, L.N. & Livingstone, G.E. (1970) *J. Fd Sci.* **35,** 349.
Lang, K. (1970) In: *World Review of Nutrition and Dietetics*, vol. 12, pp. 266–317. Karger, Basel.
Lund, D.B. (1977) *Fd Tech.* **31,** 71.
Manson, J.E., Zahradnik, J.W. & Stumbo, C.R. (1970) *Fd Tech.* **24,** 109–113.
Mullery, E.A., Stumbo, C.R. & Hunting, W.M. (1975) *J. Fd Sci.* **40,** 989.
Skjöldebrand, C. (1979) Frying and reheating in a forced convection oven. Ph.D. Thesis, University of Lund, Sweden.
Skjöldebrand, C. (1980) *J. Fd Sci.* **45,** 1354.
Skjöldebrand, C. & Hallström, B. (1980) *J. Fd Sci.* **45,** 1347.
Skjöldebrand, C. & Olsson, H. (1980) *Lebensmittel-Wissenschaft und Technologie* **13,** 148.
Skjöldebrand, C. Öste, R (1977) In: *How Ready are Ready-to-serve Foods* (Ed. by K. Paulus, Karlsruhe), p. 236. Karger, Basel.
Sörenfors, P. & Dagerskog, M. (1978) *Bestämning av värmetekniska data för köttfärs som funktion av temperatur och receptur. SIK rapport n 434 SIK.* Swedish Food Institute, Gothenburg, Sweden.
Teixeira, A.A., Dixon, J.R., Zahradnik, J.W. & Zinsmeister, G.E. (1969) *Fd Tech.* **23,** 137.
Teixeira, A.A., Stumbo, C.R. & Zahradnik, J.W. (1975a) *J. Fd Sci.* **40,** 653.
Teixeira, A.A., Zinsmeister, G.E. & Zahradnik, J.W. (1975b) *J. Fd Sci.* **40,** 656.

(Received 29 October 1981)

J. Fd Technol. (1983) **18**, 75–84

An investigation into consumer preferences for nine fresh white fish species and the sensory attributes which determine acceptability

MORAG HAMILTON AND R. BENNETT*

Summary

Nine fresh white fish species (cod, haddock, whiting, blue whiting, ling, saithe, lemon sole, plaice, dab) were investigated by sensory panels. Assessors could distinguish between species and although they would express relative preferences, they did not feel very strongly about them. Flavour was the most significant positive determinant of acceptability, texture was neutral and appearance was either neutral or negative. It is probable that most fresh white fish could be satisfactorily interchanged with one another without causing adverse consumer reaction.

Introduction

A variety of studies have investigated consumer reaction to the sensory characteristics of a number of fish species. Kelly (1969) reported that the acceptability of frozen cod was affected more by the development of cold storage flavours than by textural changes. Rasekh & Kramer (1970) using samples of canned tuna found that consumer preference was dependent approximately equally (40%) on appearance and flavour and to a much lesser extent (20%) on texture. Connell & Howgate (1971) investigated the acceptability of cod and haddock over a wide range of freshness and concluded that, after frozen storage, flavour was a more important criterion of quality than texture. Laslett & Bremner (1979) conducted a series of storage trials with minced flesh from Australian fish species and concluded that off-flavour, off-aroma and flavour were just as important as texture variables in determining the acceptability of frozen fish products.

Such studies are drawing conclusions about acceptability as influenced by a

Authors' address: School of Home Economics, Robert Gordon's Institute of Technology, Queen's Road, Aberdeen AB9 2PG.

*To whom correspondence should be addressed.

0022–1163/83/0200–0075 $02.00

spectrum of flavours, beginning with the initial intrinsic and well-liked flavour of fresh fish through to the unpleasant off-flavours produced by spoilage.

The investigation described here was conducted with fresh white fish of good quality to establish if consumers could distinguish between nine commonly encountered species (cod, haddock, whiting, blue whiting, ling, saithe, lemon sole, plaice, dab); whether they had preferences for some species over others; and determine which of the sensory attributes contributed most significantly to any preferences established. Preliminary work using triangle and preference tests indicated that the nine species were distinguishable from one another and some of them were preferred to others (Hamilton, 1980). An investigation carried out with two of the species (whiting and blue whiting) before and after incorporation into five products had also established that preferences which existed between the species in plain steamed form were eliminated once the species were incorporated into products (Hamilton & Bennett 1981).

Materials and methods

Preparation and presentation of samples

All the samples of fish (other than the blue whiting) were selected at the Aberdeen Fish Market and had a score of between 8 and 9 on the raw odour scale devised by Shewan *et al.* (1953). Variations in the size of fillets were controlled by specifying the length of fillet for each type of fish based on the length of an average sized fillet for each fish species. The fish was bought in large batches, filleted, and then stored in shatter packs at $-30°C$ (frozen storage deterioration does not occur to any significant extent at this temperature) (Howgate, 1977). The blue whiting was caught and handled under experimental rather than commercial conditions. It was frozen at sea within hours of catching in 50 kg blocks and stored at $-30°C$ until required. The investigations were carried out on fish caught at two different times of the year. The first batch was caught, and investigated using twenty-one judges in May 1979 (summer series) and the second batch in November 1979 (winter series) was judged by thirty-four assessors.

A sufficient number of fillets of each fish species was removed from the freezer 24 hr before each test and allowed to thaw at ambient temperature. The thawed blue whiting were hand filleted immediately before being used in the tests. Five-centimetre squares from the middle part of each fish fillet were used for tasting. The samples were steamed on covered pyrex plates over boiling water. Specific cooking times were used for each species to ensure that all the samples were cooked to the same stage. Condiments were excluded during cooking to provide the optimum circumstances for assessors to discriminate between different species.

Cooked samples were served immediately in identical, coded disposable white polystyrene containers. Taste panels were carried out either mid-morning

or mid-afternoon in purpose built tasting booths in a room separate from, but adjacent to the kitchen where samples were prepared. The assessors were mainly female home economics students who had no training in sensory assessment of fish, but had basic taste training and were familiar with sensory evaluation techniques.

Test methods

Matching test. The matching test was selected as a means of determining whether assessors could match unidentified coded samples of plain cooked fish with named fish samples. The method is a variation of one described by Thompson *et al.* (1980).

The test was divided into three sets where assessors were first presented with three named and different samples of cooked white fish (A, B, C). They then received two coded samples of fish which had to be matched wih the named samples. The coded samples could be two different species, or two samples of one species (AB, AC, BC, AA, BB, CC). The three named types of fish in each set were chosen on the basis of similarities in the size and type of fillet, and in the structure of the muscle flakes. (Set 1 — cod, ling and saithe; Set 2 — haddock, whiting and blue whiting; Set 3 — lemon sole, plaice and dab). All the nine possible permutations of order for the coded samples within each set, as far as possible, were presented an equal number of times to the panel. The design allows each species to be judged on two out of three occasions on average, but the variations in sequence affect the exact number of presentations around the average.

Paired preference test. The paired preference test is designed to determine which, if any, of two products is liked more. Two samples were presented simultaneously to assessors who were asked to state which, if any, sample was preferred. Assessors were allowed to make a 'no preference' response when they found they liked both samples equally. The two possible permutations of order of samples (AB and BA) were presented equally in each test, the order of presenting the two permutations being randomized.

Since it was not feasible for all combinations of pairs of fish species to be compared, a series of pairs of fish with similar characteristics was selected, based on the sets of similar species used in the previous matching test.

Hedonic rating test. The hedonic rating test is suitable for use by untrained assessors to measure their liking for specified sensory characteristics of different foods.

The assessors rated the fish samples successively using a 5-point hedonic scale (1 = like very much; 2 = like; 3 = neither like nor dislike; 4 = dislike; 5 = dislike very much) for the three organoleptic properties, appearance, texture and flavour (in that order), they then awarded a final rating for overall acceptability. Assessors were only allowed to use unit marks. A maximum of six samples were rated at each session and the order of presentation of samples was determined randomly.

Statistical analysis of results

The results of the matching and paired preference tests were expressed as levels of significance, calculated from the appropriate tables of Roessler *et al.* (1978). When considering the results from the paired preference test, the statistical significance of the data was determined by excluding the no preference responses and calculating significance on the total number of assessors who expressed positive preference. This follows recent recommendations for evaluation of paired preference data (BSI 1980).

The hedonic rating test data for each fish for each sensory characteristic were averaged to give mean scores. The data were thus treated as scoring data by using the numerical values of the ratings. (Since hedonic rating scales cannot be considered as true interval scales, there are reservations about treating the data as such for statistical analysis. This practice is, however, generally accepted, providing that caution is exercised in the interpretation of results). The mean scores were ranked in order of magnitude and Duncan's (1955) multiple range test was applied to determine the significance of any differences between samples.

The correlations between acceptability, flavour, texture and appearance were determined by carrying out a multiple regression analysis on the data using a computer program which allowed various regression models to be tested for the purpose of describing acceptability in terms of the other sensory characteristics. This analysis is examining intra-species ratings rather than inter-species ratings and therefore allowed the hedonic rating data from all judgments on all species to be considered.

Texturometer

Cooked samples of each of the nine species in the winter series were tested for hardness on a purpose-built fish texturometer (Main, Ross & Sutton, 1972). Sutton & Main (1967) established that machine readings equated well with assessments made by sensory panels on the 'hardness' of cod. The principle of the apparatus is that cooked fish which has been mashed with a fork is packed into a small stainless steel cup and a plunger which has a very loose fit is driven into the sample a pre-determined distance and the maximum resistance recorded. The movement of the plunger has a two fold action, it compresses the fish and also extrudes some of it and the final reading therefore depends on both the resistance to compression and the ease of extrusion. Packing of the fish samples could not be absolutely standardized, therefore each species was tested seven times and the middle five readings were averaged to give the results quoted.

Results and discussion

Table 1 shows the results obtained by means of matching tests carried out by thirty-four assesors and it can be seen clearly that assessors were able to match

Table 1. The results of a matching test, where nine fish species, arranged into three sets were evaluated by thirty-four assessors

Named fish species	Coded samples (*n*)	Correct matches (*n*)	Correct matches (%)	Significance
Set 1				
Cod	24	18	75	***
Saithe	22	22	100	***
Ling	22	18	82	***
Set 2				
Whiting	20	17	85	***
Haddock	28	25	89	***
Blue whiting	20	17	85	***
Set 3				
Lemon sole	26	17	65	**
Plaice	19	12	63	*
Dab	23	13	56	*

* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.

coded samples of fish with the appropriate named samples with a high degree of accuracy. This result was by no means a foregone conclusion because of the apparent similarity and blandness of fish flesh of various species. The result gives no indication of the direction of differences between species, and this aspect was therefore examined by means of a series of paired preference tests.

Table 2 shows the results of a paired preference test where nine fish species were evaluated by twenty-one assessors. The results show that for most pairs there was a preference, although not enough for statistical significance at the 5% level. In eight out of the nine pairs of samples, the more frequently eaten and expensive fish of the pair had the greater number of preference responses. However, there is evidence from these results that the less preferred species were acceptable to assessors, since in most cases the less preferred responses plus the no preference response was very similar to the score for the preferred species. Informal discussions were held with some assessors who usually indicated that their choices were not often based on very strong feelings about the samples. The only exceptions to this were the definite reactions of dislike for the grey coloured appearance of saithe and, to a lesser extent, blue whiting.

The nature of the differences between species was investigated in detail in a series of hedonic rating tests. Table 3 shows the mean ratings of twenty-one assessors obtained in hedonic rating tests conducted in the summer with eight fish species. Table 4 shows the mean ratings from winter tests involving nine species and thirty-four to thirty-six assessors. The species have been arranged in the Tables in rank order for each sensory characteristic and it can be seen firstly

Table 2. The results of a paired preference test, where nine fish species were evaluated by twenty-one assessors

Fish pairs	Preferring each sample (*n*)	Preferring neither sample (*n*)	Positive responses (%)	Significance
Haddock Whiting	12 6	3	67 33	NS
Cod Haddock	9 9	3	50 50	NS
Cod Whiting	10 6	5	63 38	NS
Cod Ling	11 5	5	69 31	NS
Cod Saithe	12 5	4	71 29	NS
Whiting Blue whiting	11 7	3	61 39	NS
Lemon sole Plaice	13 1	7	93 7	**
Lemon sole Dab	12 5	4	71 29	NS
Plaice Dab	14 3	3	82 18	*

NS, no significant difference.
* Significant at the 5% level.
** Significant at the 1% level.

that the order of acceptability and flavour is identical, the order for acceptability and texture is very similar, and that that for acceptability and appearance is more varied. Secondly, for most fish species the scores for all characteristics are on the 'like' side of neutrality. Lemon sole which rated highly for acceptability in the summer results, is rated very badly in the winter set. We believe that this extreme shift of position and perhaps the other more minor ones on the Tables are caused by seasonal quality variations. Tables 3 and 4 also show the species 'groups' which result from the application of Duncan's multiple range test to the acceptability data. The species which are grouped by the same bracket are not significantly different from one another at the level of significance indicated, and it follows from this that any species would be an acceptable substitute for any other species which either lies below it in the table of ranked mean scores or which lies above it, but is grouped by the same bracket.

The smallest range of scores in both summer and winter tests was for texture. The two lowest scores (ling, 3.0, and lemon sole, 2.9) were probably awarded for quite different reasons, the ling having a relatively coarse structure and the

Table 3. The mean ratings arranged in rank order for each sensory characteristic from a summer rating test with twenty-one assessors using a 5-point hedonic scale (1 = like very much, 5 = dislike very much). The fish which are *not* grouped by the same bracket are significantly different for acceptability at the 1% level

Rank order	Appearance	Texture	Flavour	Acceptability	Duncan's multiple range test on acceptability data 1% significant range
1	Lemon sole 1.7	Whiting 1.7	Lemon sole 1.4	Lemon sole 1.6	
2	Dab 2.0	Haddock 1.8	Haddock 1.8	Haddock 1.9	
3	Whiting 2.0	Lemon sole 1.9	Dab 2.0	Dab 2.0	
4	Haddock 2.2	Dab 2.0	Whiting 2.3	Whiting 2.1	
5	Cod 2.4	Saithe 2.6	Cod 2.4	Cod 2.5	
6	Plaice 2.4	Cod 2.7	Plaice 2.6	Plaice 2.5	
7	Ling 2.7	Plaice 2.8	Ling 2.7	Ling 2.9	
8	Saithe 4.5	Ling 3.0	Saithe 2.7	Saithe 3.2	

lemon sole in the winter tests being rather loose and sloppy. Cooked samples of each of the nine species in the winter series were tested for hardness on a purpose-built fish Texturometer (Main *et al.*, 1972).

The mean readings (kg force) obtained from the machine tests are shown plotted against the mean panel scores for texture in Fig. 1. It can be seen that the machine scores vary considerably from hard (9.7 kg force) for blue whiting to very soft (0.9 kg force) for lemon sole, while the panel scores varied only by one point from like (1.9) for whiting to neither like nor dislike (2.9) for lemon sole. These results therefore establish that quite a wide range of normal fish textures are acceptable to consumers and that texture alone is unlikely to be a major factor in determining overall acceptability. This result with fish is in contrast to that found with most other types of flesh product where texture, particularly tenderness, is usually asserted as being the major factor in determining preference (Tarrant, 1975). It should be borne in mind, of course, that fresh fish is intrinsically tender in comparison with most meat products. The ratings awarded for the appearance of fish samples show a spread of 2.8 which considerably exceeds the spread for the other sensory characteristics. The spread is

Table 4. The mean scores arranged in rank order for each sensory characteristic from a winter rating test with thirty-four to thirty-six assessors using a 5-point hedonic scale. The fish which are *not* grouped by the same bracket are significantly different for acceptability at the 1% level

Rank order	Appearance	Texture	Flavour	Acceptability	Duncan's multiple range test on acceptability data 1% significant range
1	Plaice 1.4	Whiting 1.9	Whiting 1.8	Whiting 1.8	
2	Cod 1.5	Plaice 2.1	Cod 2.2	Cod 2.1	
3	Dab 1.6	Cod 2.2	Plaice 2.3	Plaice 2.2	
4	Haddock 1.8	Dab 2.3	Haddock 2.4	Haddock 2.2	
5	Whiting 1.9	Haddock 2.4	Ling 2.5	Ling 2.5	
6	Lemon sole 2.0	Ling 2.6	Blue whiting 2.5	Dab 2.6	
7	Ling 2.1	Blue whiting 2.7	Dab 2.8	Blue whiting 2.7	
8	Blue whiting 2.8	Saithe 2.7	Saithe 3.0	Saithe 3.2	
9	Saithe 3.8	Lemon sole 2.9	Lemon sole 3.4	Lemon sole 3.3	

largely due to the very low rating given to saithe and to a lesser extent, blue whiting, both of which have greyish coloured fillets. If the ratings for these species are excluded, the spread is only 1.0 and tends to indicate that appearance

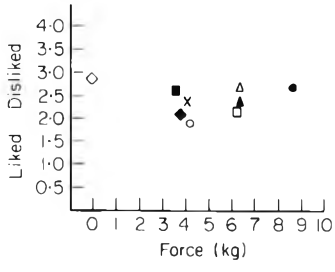


Figure 1. The relationship between the mean hedonic ratings for texture and the readings from the Aberdeen Fish Texturometer, for the same samples of nine species. Panel – 1 = like very much, 5 = dislike very much. kg Force – 1 = very soft, 11 = very hard. □, Cod; ■, ling; △, saithe; ▲, haddock; ○, whiting; ●, blue whiting; ◇, lemon sole; ◆, plaice; ×, dab.

Table 5. Matrices of correlation between the four sensory characteristics, based on the combined mean ratings for all species.

Correlation matrices	Appearance	Texture	Flavour	Acceptability
A*				
Appearance	1.00			
Texture	0.47	1.00		
Flavour	0.42	0.78	1.00	
Acceptability	0.67	0.83	0.93	1.00
B*				
Appearance	1.00			
Texture	0.50	1.00		
Flavour	0.25	0.76	1.00	
Acceptability	0.38	0.86	0.96	1.00

*Matrix A – for seventeen samples (i.e. combining the eight summer and nine winter results); Matrix B – for fourteen samples (i.e. combining seven summer and seven winter results, but omitting all ratings for saithe and blue whiting).

is neutral in positively determining acceptability, but is significant in eliciting negative reactions. Matrix A in Table 5 shows the results of the multiple regression analysis for the four sensory characteristics based on the score awarded by fifty-eight judges. It can be seen that flavour ($r = 0.93$) correlates best with acceptability. Texture has a high correlation with acceptability ($r = 0.83$) but is also highly correlated with flavour ($r = 0.78$). A reasonable model for describing the acceptability of white fish could be identified which did not require texture scores to be included in the equation. The model showed that

Acceptability = 0.8 Flavour + 0.2 Appearance (approximately).

The partial scores for each species were considered and it was evident that saithe and blue whiting showed some divergence from the model. The analysis was therefore repeated after the omission of the data from these two fish. Matrix B in Table 5 shows the correlation data obtained with the fourteen scores from fish of 'normal' whiteness. This second analysis showed that appearance now made no significant contribution to overall acceptability which was now best described purely in terms of flavour. These findings reinforce the previous conclusions that in terms of their contribution to the overall acceptability of fresh fish, texture is usually neutral, and appearance is either neutral or negative; the major factor in determining preference being flavour.

The preference tests and matching tests involved twenty-one and thirty-four judges respectively, which is possibly too few to make a definitive conclusion, however, there is a strong indication from the results of these experiments that consumers are likely to be satisfied with fresh samples of most white fish species. People are certainly able to distinguish between them, mainly on the basis of

flavour, and will express preferences, but the strength of the preference is not great and many species could be satisfactorily interchanged with one another without causing adverse consumer reaction.

It is of interest to draw attention to our previous work in which whiting and blue whiting were compared by fifty-eight judges before and after incorporation into five products (Hamilton & Bennett, 1981). This work established that such preferences as there were between the two species when tested in plain steamed form were eliminated once the two species were incorporated into products. Since this investigation was between fish species which were well separated in the ranked means shown in Table 4, it is reasonable to conclude that products made with any of the fish investigated in this study, with the possible exception of saithe, would be highly acceptable to consumers and may even be indistinguishable from one another in most forms of product.

Acknowledgments

We are grateful to the Torry Research Station, Aberdeen for providing fish samples and to Mr P. Howgate and Mr G. Smith of that establishment and Mr. A. Wilson of the School of Mathematics, Robert Gordon's Institute of Technology, for valuable discussions.

References

BSI (1980) B.S. 5929.

Connell, J.J. & Howgate, P.F. (1971) In: *Fish Inspection and Quality Control* (Ed. by R. Kreuzer), p. 155. Fishing News (Books) Ltd, London.

Duncan, D.B. (1955) *Biometrics* **11,** 1.

Hamilton, M. (1980) *J. Consum. Stud. & Home Econ.* **4,** 151.

Hamilton, M. & Bennett, R. (1981) *J. Fd Technol.* **16,** 655.

Howgate, P. (1977) In: *Sensory Properties of Foods,* p. 249. Applied Science Publishers Ltd, Essex, England.

Kelly, T.R. (1969) *J. Fd Technol.* **4,** 95.

Laslett, G.M. & Bremner, H.A. (1979) *J. Fd Technol.* **14,** 389.

Main, G., Ross, R.I. & Sutton, A.H. (1972) *Lab. Pract.* **21,** 185.

Rasekh, J. & Kramer, A. (1970) *J. Fd Sci.* **35,** 417.

Roessler, E.B., Pangborn, R.M., Sidel, J.L. & Stone, H. (1978) *J. Fd Sci.* **43,** 940.

Shewan, J.M., MacIntosh, R.G., Tucker, C.G. & Ehrenberg, A.S.C. (1953) *J. Sci. Fd Agric.* **4,** 283.

Sutton, A.H. & Main, G. (1967) In: *Freezing and Irradiation of Fish* (Ed. by R. Kreuzer), p. 371. Fishing News (Books) Ltd, London.

Tarrant, P.V. (1975) *Nutr. & Fd Sci.* **41,** 11.

Thompson, A.B., McGill, A.S., Murray, J., Hardy, R. & Howgate, P.F. (1980) In: *Advances in Fish Science and Technology* (Ed. by R. Kreuzer), p. 484. Fishing News (Books) Ltd, Farnham, Surrey.

(Received 2 November 1981)

J. Fd Technol. (1983) **18**, 85–95

Texture analysis of fish fillets and minced fish by both sensory and instrumental methods

A. J. BORDERIAS, M. LAMUA AND M. TEJADA

Summary

In an attempt to establish objective criteria for texture analysis as an effective substitute for organoleptic procedures, correlation between the results obtained by a panel of tasters and physical and chemical analyses of texture was determined. In addition, regression equations relating instrumental and sensory methods were applied to predict sensory data from those obtained during instrumental analysis.

Introduction

In view of the difficulties involved in obtaining comparative data through sensory analysis, it was felt that the use of standard instruments to measure texture would be of interest, as this would constitute a source of objective information. However, it is often difficult to relate the physical measurement obtained by means of an instrument to any one or more of the factors influencing the general sensory impression of texture, because other properties which cannot be evaluated by instruments, such as smell and taste, can affect a person's judgment at the time of tasting thus leading to results different from those expected. Nevertheless, this relationship must be correctly understood in order to be able to properly assess the significance of physical measurements.

The purpose of the present study was, therefore, to determine which instrumental (objective) methods best correlated with sensory (subjective) impressions, with a view to subsequent investigations to establish the mathematical relationship between the instrumental parameters and sensory criteria. Both fish fillets and minced fish were used in the tests.

Authors' address: Instituto del Frio, Cuidad Universitaria, Madrid-3, Spain.

0022–1163/83/0200–0085 $02.00

Materials and methods

The experiments were performed using five different fresh fish species held for a maximum of 4 days in ice chosen at random from available market supplies: rainbow trout (*Salmo irideus* Gibb), sardine (*Sardina pilchardus* Walbaum), conger eel (*Conger conger* L.), horse mackerel (*Trachurus trachurus* L.) and blue whiting (*Micromesistius poutassou* Risso). Skinned fillets and minced fish were tested, both raw and cooked.

The fish were headed, gutted and skinned. Those intended for mincing were filleted and then minced using a meat mincer with plate orifices 5 mm in diameter.

All the samples were divided into portions of 500 g, packed in aluminium foil, frozen in a plate freezer at − 40°C, and stored at − 25°C for the duration of the experiment (15 days).

Sensory methods

The texture profile method was used to determine the different sensory properties of the fish to be correlated with the instrumental data.

Six panellists were selected from among the laboratory staff and trained in the basic concepts of descriptive analysis and terminology. Several practice sessions were held until the individual ratings for the different properties showed general agreement.

A simple 7-point scale was used. The trout muscle was assigned Point 4 and was taken as a reference point for comparing the flesh of the other species tested. Every effort was made to ensure homogeneity in the trout samples used.

The cooked samples were prepared by thawing overnight and then by steaming in air-tight jars for 20 min, no more condiments added. Five tastings were made for the fillets but only three were held for the minced fish.

Fewer sessions were required for the minced samples, because, due to their higher level of homogeneity, the results exhibited less variation. All five different fish species were tasted at each session of the panel.

The following characteristics were tested as defined by Jowitt (1974) and Whittle *et al.* (1980):

(1) Initial characteristics. The response to the properties of the material on the first bite.

Wateriness: the release of water on compression; this is the initial response and is to be distinguished from juiciness. Scale points: 1, much less water released than in the trout; 4, the same amount of water released as in the trout; 7, much more water released than in the trout.

Firmness: the force required to compress the material between the molars or between the tongue and palate. Scale points: 1, much softer and less consistent than the trout; 4, similar to the trout. 7, much firmer and more consistent than the trout.

Elasticity: the ability of the material to return to its original shape after deforma-

tion; this is judged by compressing the substance slightly between the molars or between the tongue and palate and noting to what extent the material returns to its original shape. Scale points: 1, much more plastic than the trout; 4, similar to the trout; 7, much more elastic than the trout.

Cohesiveness: the extent to which a material can be deformed before it ruptures. Scale points: 1, much less cohesive than the trout; 4, similar to the trout; 7, much more cohesive than the trout.

(2) Secondary characteristics. The response to the properties of the material after chewing a few times:

Hardness: resistance to breakdown on chewing to a state, suitable for swallowing. Scale points: 1, much more tender than the trout; 4, similar to the trout; 7, much tougher than the trout.

Juiciness: the sensation of a progressive increase of free fluids in the oral cavity during mastication. Scale points: 1, much drier than the trout at the end of chewing: 4, similar to the trout; 7, much juicier than the trout at the end of chewing.

Instrumental methods

The instrumental analysis of texture was performed using an Instron model 1140 Texturometer at room temperature ($20 \pm 2°C$). For each species two different sets of measurements were taken, each set consisting of five replicates of each test.

The following parameters were tested:

Shearing: The measurements were taken using a Kramer shear cell (Ksc) (Kramer, 1961) and a Warner–Bratzler cell (WBc) (Bratzler, 1932) which combine extrusion, compression, and shear strength. Peak height was used as the measure of this parameter.

Compression: The measurements were taken with a compression assembly. Compression analysis was applied in order to obtain values for firmness, cohesiveness, and elasticity (Friedman, Whitney & Szczesniak, 1963; Henry *et al.*, 1971), relaxation (Stanley, Pearson & Coxworth, 1971) and the modulus of elasticity (Moskowitz & Kapsalis, 1976). Juiciness was also evaluated by compression by placing a sufficient amount of filter paper on both sides of the sample to collect the juice pressed out on application of a constant force of 25 kg for 1 min. The amount of juice was measured by weighing the filter paper before and after pressing and expressed as the percentage by weight.

Puncture test: This parameter was defined as the maximum force exerted when driving a flat-ended punch to a constant depth within the sample (Bourne, 1979).

Instrumental tests were applied to both raw and cooked samples which were more suitable for texture analysis. The cooked samples were steamed for 20 min in air-tight jars, cooled to room temperature, and the drip poured off. Samples of raw fillets and minced fish were cut out tempered in order to obtain more uniform pieces. The cooked minces had adequate consistencies for handling,

but this was not the case for cooked fillets, which had to be chilled after cooking to about − 3°C in order to ensure good cutting of the samples.

In the shear tests performed using the Ksc and the WBc, shearing was perpendicular to the direction of alignment of the myotomes. In the puncture tests the samples were attached as described by Kulwich, Decker & Alsmeyer (1963).

Table 1. Instrumental analysis : geometry of samples

Instrumental analysis	Fillets	Minced fish
Shear strength (Ksc)	Two parallelepipeds measuring 0.84 × 6.5 cm	Two parallelepipeds measuring 1 × 1 × 6.5 cm
Shear strength (WBc)	Two parallelepipeds measuring 0.84 × 1 × 6.5 cm	Two parallelepipeds measuring 1 × 1 × 6.5 cm
Puncture test	Cylinder measuring 5 cm in diameter × 0.84 cm in height	Cylinder measuring 5 cm in diameter × 1.77 cm in height
Firmness	Cylinder measuring 1.2 cm in diameter × 0.84 cm in height	Cylinder measuring 2.5 cm in diameter × 1.77 cm in height
Cohesiveness	,,	,,
Elasticity	,,	,,
Modulus of elasticity	,,	,,
Relaxation	,,	Cylinder measuring 2.5 cm in diameter × 0.84 cm in height
Juiciness	,,	,,

Table 2. Instrumental testing conditions

Instrumental analysis	Attachment	Sample deformation	Cross-head speed (mm/min)	Chart speed (mm/min)	No. of cycles of cross head
Shear strength	Ksc	Full shear	80	80	1
Shear strength	WBc	,,	100	100	1
Puncture test	Flat-ended punch 0.8 cm in diameter	Full penetration	80	80	1
Firmness	Compression assembly 5.7 cm in diameter	Fillets: 0.25 cm (30%) Minced: 0.5 cm (30%)	50	400	1
Cohesiveness	,,	,,	50	400	2
Elasticity	,,	,,	50	400	2
Modulus of elasticity	,,	,,	50	400	2
Relaxation	,,	Constant force of 25 kg for 1 min	50	400	1
Juiciness	,,	,,	50	400	1

Table 1 shows the geometry of the sample suggested for each individual analysis and used by the authors on the basis of the literature consulted and previous tests made at our laboratory. Table 2 gives details of the test conditions.

Hydroxiproline assay

Hydroxiproline levels in the muscle tissue were determined according to the method of Leach (1960) as an indicator of the relationship between the hardness and the amount of connective tissue.

Statistical methods

Let x_{ijk} be any of the values, 1, obtained using a given instrumental test, i, applied to a species of fish, k, treated by a given processing technique, j.

The variable $\bar{x}_{ijk}$ can then be defined as the mean of the values l thus obtained:

$$x_{ijk} = \frac{\sum_{l=1}^{q} x_{ijkl}}{q}$$

Similarly, a number of values, l', were obtained for the same batch of the same fish species, k treated in the same manner, j, from sensory ratings by a panel of tasters of the organoleptic properties, i', previously discussed, yielding the variable $y_{i'jkl'}$.

Therefore, for every value of $\bar{x}_{ijk}$ there is a number (q') of corresponding values of $y_{i'jk}$; and the number of elements comprising each statistical sample is thus $N = p \times q'$.

It was assumed that for each $\bar{x}_{ijk}$ the distribution of the corresponding values of $y_{i'jkl'}$ follows the pattern described by

$$N[\mu_{\bar{x}_{ijk}},\ \sigma^2 = \text{constant}]$$

The correlation coefficients were calculated for the samples defined as above and contrasted with the null hypothesis $P = 0$ using Student's t-test; the pertinent values and their degree of significance are presented in Table 4. Definitions of the notation employed are given at the foot of this table.

The linear regressions of $y_{i'jkl}$, on x_{ijk} were calculated using the least squares method, and the goodness of fit was calculated using Snedecor's F-test.

Results and discussion

Table 3 shows the means of the results obtained by the instrumental analyses together with the coefficients of variation. With regard to the latter, it can be

Table 3. Means of the results obtained by the instrumental analyses and corresponding coefficients of variation

	Shear strength Ksc (kg)		Shear strength WBc (g)		Puncture (g)		Firmness (g)		Index of cohesion		Index of elasticity (mm)		Juiciness (%)		Modulus of elasticity (kg/cm²)		Relaxation (mm)	
	$\bar{x}$*	CV†	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV
Fillets																		
Trout	15	10	283	24	144	7	300	17	0.40	11	9.0	13	28.6	3	0.88	18	88.7	1
Sardine	17	10	375	22	128	16	184	26	0.45	11	9.6	26	36.3	15	0.54	26	84.6	3
Conger	40	30	4180	14	455	17	410	37	0.34	10	6.4	12	28.9	4	1.22	36	97.1	14
Horse mackerel	16	8	318	19	148	14	258	28	0.46	9	9.4	9	29.8	3	0.77	28	91.1	11
Blue whiting	19	31	278	35	90	16	381	31	0.34	23	7.0	21	19.2	8	1.13	32	86.2	2
Minced																		
Trout	10	5	152	12	48	20	209	10	0.37	4	19.2	6	25.8	6	0.15	10	91.2	1
Sardine	9	12	170	13	41	6	193	8	0.48	6	20.5	4	18.7	5	0.14	9	87.3	1
Conger	50	5	740	8	141	9	254	13	0.47	5	22.0	3	29.1	2	0.18	13	97.1	0
Horse mackerel	12	5	183	25	43	10	196	6	0.50	6	22.7	3	30.5	3	0.14	6	94.7	0
Blue whiting	21	10	308	24	99	19	333	11	0.56	7	26.4	3	23.8	5	0.23	9	87.7	0
Fillets																		
Trout	27	10	975	22	166	6	420	9	0.73	15	11.0	14	44.3	6	1.40	19	86.7	1
Sardine	34	16	626	18	110	5	346	8	0.45	6	11.2	3	36.6	6	1.00	8	88.6	1
Conger	58	4	1168	16	538	7	426	16	0.52	12	10.3	9	35.5	10	1.31	16	95.2	1
Horse mackerel	43	7	1600	18	191	20	308	31	0.44	16	12.2	8	32.1	6	0.90	21	92.3	2
Blue whiting	22	8	424	22	219	27	335	44	0.45	27	11.3	12	27.0	14	0.82	48	88.5	1
Minced																		
Trout	15	22	271	19	151	20	600	10	0.59	5	27.0	3	35.0	5	0.43	10	92.3	1
Sardine	19	11	547	33	128	13	491	10	0.40	8	18.8	6	26.0	5	0.35	11	90.5	1
Conger	34	14	258	31	191	21	1091	24	0.61	8	31.2	2	27.0	10	0.72	20	86.5	2
Horse mackerel	25	5	1073	18	230	14	671	11	0.52	7	29.1	2	31.0	6	0.48	11	91.3	1
Blue whiting	16	13	319	27	95	15	504	18	0.55	8	26.3	10	30.0	5	0.34	15	91.3	0

* $\bar{x}$ = Average

† CV = coefficient of variation

Table 4. Correlation between the sensory and instrumental methods. Instrumental analyses

Sensory test		N	Juiciness		Shear strength Ksc		Shear strength WBc		Puncture		Firmness		Index of cohesion		Index of elasticity		Modulus of elasticity		Relaxation		Hydroxiproline
			Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw	
Wateriness	F†	80	0.7	0.24**																	
	M†	40	0.13	0.51****																	
Juiciness	F	80	0.13	0.24**																	
	M	36	0.52****	0.008																	
Firmness	F	110			0.17*	0.01	0.15	0.17*	0.02	0.10											
	M	56			0.71****	0.37****	0.17	0.40****	0.69****	0.10	0.63****	0.58****									
Hardness	F	110			0.22***	0.05	0.08	0.27****	0.02	0.14											0.23***
	M	56			0.64****	0.25*	0.28**	0.28**	0.70****	0.008	0.53****	0.66****									0.057
Cohesiveness	F	110							0.12	0.24***											
	M	56							0.56****	0.27*	0.68****	0.43****	0.24*	0.74***							
Elasticity	F	90																	0.10	0.04	
	M	48													0.03	0.53****	0.19	0.59****	0.14	0.29*	

**** $P = 0.005$; *** $P = 0.01$; ** $P = 0.025$; * $P = 0.05$.

† F = fillets; M = minces.

The differences between the values of N are due to the fact that the number of panellists changed in the different sessions.

observed that these are significantly higher in the shear tests performed using the WBc than in the Ksc tests, which indicates less replication of results.

None of the species investigated showed variations attributable to species differences *per se*; this suggests that the texture analysis method can be applied with equal reliability to any type of fish.

It was found that the coefficients of variation in the raw or cooked fillets, were higher than in the minced fish, as can be seen in the case of the instrumental values for firmness, cohesivity, elasticity and modulus of elasticity, so that these values were not contrasted with the corresponding sensory evaluations.

The reason for this may be that this method has usually been applied only to semi-solid foods (Friedman *et al.*, 1963, Henry *et al.*, 1971), and minced fish could be considered to belong to this category. However, when applying this compression method to an item with a layered structure like fish fillets, the myotomes layers tend to slide away from the force of compression, thus making it impossible to reproduce the analysis results in different tests.

Correlation analyses

The degree of significance of the correlation between the sensory and instrumental methods is represented in Table 4 as follows: **** = 0.5%, *** = 1%, ** = 2.5%, * = 5%.

The relevant correlation coefficients were calculated only for those tests performed on the most similar physical properties.

Firmness and hardness (sensory tests) versus *instrumental analysis*

In the analyses performed using the Ksc and the puncture test, cooked batches of fish mince showed the highest correlation to the sensory data. The correlation of the values from instrumental measurements of firmness was similar for both cooked and raw minces.

The correlations between the instrumental and sensory tests were lower for fillets than for minced fish, and only those obtained for cooked fish using the Ksc and for raw fish using the WBc were found to be significant.

The low correlation for fillets and mince between the shear strength measured by a WBc and the sensory data had previously been found by Deatherage & Garnatz (1952) and Szczesniak & Torgenson (1965) in experiments on meat. Later, Pool & Klose (1969) reported that this might be caused by distortion of the muscle fibres during cutting.

The better correlation results obtained using the Ksc as compared with those obtained using the WBc, was observed in meat by Pearson (1963), who suggested that this might be due to the fact that the Ksc comprised several blades compared to the single blade in the WBc. Tests run in the Ksc take measurements for several different cuts in different parts of the sample, making such measurements more reliable.

Bosund & Beckeman (1972) found a high level of correlation between sensory tests and instrumental analysis performed using the standard shear cell of an Allo-Kramer shear press in the case of fish. However, the instrumental analysis carried out by these authors used fish dismembered in a shaker, a material more like fish mince than whole fish. As would be expected, their results were therefore closer to those obtained by the authors for the fish mince. Patasnik (1980) also found no correlation between shear strength analysis and sensory tests conducted on fish fillets.

On the other hand, Gill, Keith & Lall (1979) did find some correlation between hardness measured by sensory tests and using a modified Ksc, although the correlation was not particularly pronounced.

Hinnergardt & Tuomy (1970) stated that puncture showed good correlation as a measure of hardness and that this method could afford certain advantage over the shear test because it is less destructive.

With regard to firmness as measured by compression, the data found in the literature are contradictory. Thus, while Hiner (1960) working with meat, found close correlation with the data furnished by the taste panel, Bratzler & Smith (1963) found no correlation.

The reason that there was generally closer correlation between the results obtained using sensory and instrumental methods in the cooked products may lie in the fact that firmness and hardness are altered disproportionally by cooking as can be seen from Table 3.

Cohesiveness (sensory test) versus *instrumental analysis*

In fillets the degree of correlation between cohesiveness (sensory tests) and puncture (instrumental tests) was only significant in the case of the cooked product, and the significance was very slight.

In cooked minced fish higher correlation values were obtained from instrumental puncture and firmness tests; on the other hand, a higher correlation was obtained for cohesiveness in the case of uncooked mince.

The closer correlation found for the puncture and firmness tests in the cooked minces may result from change in the cohesive forces between the particles of the mince during cooking. The authors have not been able to give any ready explanation for the fact that the cohesiveness index was more closely correlated with sensory cohesiveness in the raw product.

Elasticity (sensory tests) versus *instrumental analysis*

In minced fish only the raw product showed significant correlations between the sensory evaluation of elasticity and the elasticity index and modulus of elasticity.

The relaxation index showed no significant correlation against elasticity, probably due to the fact that the pressure of 25 kg exerted in this analysis was perhaps excessive, thus depriving the product of its ability to recover.

Wateriness and juiciness (sensory tests) versus *instrumental analysis*

There was closer correlation between sensory ratings of wateriness and instrumental measurements of juiciness in the raw mince. However, the correlation between the sensory perception of juiciness and instrumental analysis of juiciness was closer after the mince had been cooked.

Table 5. Hydroxiproline analysis

	Hydroxiproline (mg of sample)	
Species	$\bar{x}$*	CV†
Trout	0.407	1.2%
Sardine	0.167	4.5%
Conger	0.560	3%
Horse mackerel	0.266	5%
Blue whiting	0.441	5.9%

*Mean of three replicates.
†CV = coefficient of variation.

Hardness (sensory tests) versus *connective tissue (chemical analysis)*

The percentage of connective tissue for each species is indicated in Table 5.

The hardness (sensory test) did not seem to be clearly related to the amount of connective tissue, as can be inferred from the correlation index. This had already been observed in fish by Tsuchiya & Takahashi (1950) and in meat by Pfeiffer *et al.* (1972) and Shimokomaki, Elsden & Bailey (1972). These authors considered that the type of cross-links in the connective tissue was more important than the total amount of this tissue.

Moreover, it is natural that the correlation should be closer in the fillets than in the minces, since in the former the connective tissue remains unchanged, a fact clearly perceived by the tasters, while in the latter the connective tissue structure has been completely broken down, so it has very little effect on the texture.

Regression analyses

The regression analyses carried out all showed that linear regression produced more accurate predictions than exponential regression. It follows that, according to the experiments conducted by Stanley (1976) on meat, when dealing with myosystems it would probably be necessary to resort to multiple regressions of data obtained using several different instrumental methods in order to be able to make reliable predictions of the textural properties of the product being studied.

Conclusions

(1) In fish fillets, no significant correlation was found between any of the indices obtained from the instrumental analyses and the results of sensory taste tests.
(2) In the fish minces, significant correlations between the results of instrumental analysis and taste evaluations were found for the following aspects: between hardness as determined using the Ksc and the sensory perception of firmness in cooked mince, and between the puncture test and firmness in both raw and cooked minces; between the sensory evaluation of cohesiveness and puncture and firmness tests in cooked mince, and between sensory evaluation and the index of cohesiveness in uncooked mince; between the sensory perception of elasticity and the elasticity index and modulus of elasticity in raw mince; and between wateriness and the instrumental analysis of juiciness in raw mince, and between the evaluation of juiciness by tasters and the instrumental juiciness index in cooked mince.

References

Bourne, M.C. (1979) In: *Food Texture and Rheology*. (Ed.) P. Sherman, p. 95. Academic Press, London.
Bosund, I. & Beckeman, M. (1972) *Bull. I.I.F., Annexe 1972–2*, **29**.
Bratzler, L.J. (1932) *Texture Measurements of Foods* (Eds A. Kramer & A. Szczesniak). Boston (1973).
Bratzler, L.J. & Smith, H.A. (1963) *J. Fd Sci.* **28,** 99.
Deatherage, F.E. & Garnatz, G. (1952) *Fd Technol.* **6,** 260.
Friedman, H.H., Whitney, J.E. & Szczesniak, A.S. (1963) *J. Fd Sci.* **28,** 390.
Gill, T.A., Keith, R.A. & Lall, B.S. (1979) *J. Fd Sci.* **44,** 66.
Henry, W.F., Katz, M.H., Pilgrim, F.J. & May, A.T. (1971) *J. Fd Sci.*, **36,** 155.
Hiner, R.L. (1960) *Nat'l Provisioner,* **143,** 75.
Hinnergardt, L.C. & Tuomy, J.M. (1970) *J. Fd Sci.* **35,** 312.
Jowitt, R. (1974) *J. Texture Studies,* **5,** 351.
Kramer, A. (1961) *The Food Scientist,* **5,** 7.
Kulwich, R., Decker, R. & Alsmeyer, R. (1963) *Food Tech.*, **17** (2), 83.
Leach, A.A. (1960) *Biochem. J.* **74,** 70.
Moskowitz, H.R. & Kapsalis, J.G. (1976) In: *Rheology and Texture in Food Quality* (Eds J. M. de Man. P. W. Voiseiv, V. F. Rasper & D. W. Stanley), Westport, U.S.A.
Pearson, A.M. (1963) *Proc. Meat Tenderness Symp.*, Camden, U.S.A.
Pfeiffer, N., Field, R., Varnell, T., Kruggel, W. & Kaiser, I. (1972) *J. Fd Sci.*, **37,** 897.
Pool, M. & Klose, A. (1969) *J. Fd Sci.* **34,** 524.
Shimokomaki, M., Elsden, D. & Bailey, A. (1972) *J. Fd Sci.* **37,** 892.
Stanley, D.W., Pearson, G.P. & Coxworth, V. (1971) *J. Fd Sci.* **36,** 256.
Stanley, D.W. (1976) In: *Rheology and Texture in Food Quality* (Eds J. M. de Man, P. W. Voisey, V. F. Raster & D. W. Stanley) AVI, Westport, U.S.A.
Szczesniak, A. & Torgenson, (1965) *Adv. Gd Res.* **14,** 33.
Tsuchiya, Y. & Takahashi, I. (1950) *Tôhoku J., Agr. Res.* **1,** 209.
Whittle, K.J., Borderías, A.J., Howgate, P., Mills, A. & Young, K.W. (1980) *Third National Technical Seminar on Mechanically Recovery and Utilization of Fish Flesh, Raleigh, North Carolina, December 1980.*

(Received 8 March 1982)

J. Fd Technol. (1983) **18**, 97–112

Alternative sweetening of yoghurt

LEA HYVÖNEN AND MARIA SLOTTE

Summary

The influence of different sweeteners on the quality of yoghurt was studied using a sucrose sweetened yoghurt as the reference. The alternative sweeteners used were xylitol, sorbitol, fructose, cyclamate and saccharin. The sweeteners were added to the yoghurt either prior to or after incubation.

The influence of xylitol and sorbitol were studied more closely by investigating their growth retarding effects on the yoghurt culture. The quality of the yoghurt varieties was evaluated by pH, titratable acidity, viscosity, microbiological quality, sensory properties and storage stability.

All the sweeteners used were suitable for sweetening of yoghurt after incubation. However, saccharin could be used only when mixed with xylitol to cover its disturbing bitter aftertaste when used alone.

Sweetening with sorbitol prior to incubation was problematic. To obtain a suitable sweetness of the yoghurt 15% of sorbitol had to be added. This amount retarded the growth of the yoghurt culture so greatly that no acid, aroma or coagulation was formed in the product. Even a concentration 7% made the yoghurt significantly different from the normal yoghurt. The sweetness of yoghurt prepared with less than 7% of sorbitol is very slight. Consequently, sorbitol is not suitable for use as the only sweetener in pre-sweetened yoghurt. However, its use was possible in combination with sucrose. Xylitol proved to be a good yoghurt sweetener for pre-incubation sweetening, in spite of a slight retarding effect on the growth of the bacteria. At 8% concentration, which was the most preferred, the retarding effect of xylitol was negligible.

Introduction

A sweetened yoghurt with a flavour, fruit or berry addition is a popular snack and dessert. Sucrose is the commonly used sweetener in yoghurt. It may be

Authors' address: Department of Food Chemistry and Technology, University of Helsinki, SF-00710 Helsinki 71, Finland.

0022–1163/83/0200–0097 $02.00

added either prior to pasteurization of the yoghurt milk or simultaneously with the addition of fruit or flavouring (Humphreys & Plunkett, 1969).

In this investigation the effects of alternative sweeteners on the quality of yoghurt were studied. The effects of different sweeteners, added both before and after incubation, were studied by physical and microbiological measurements and sensory evaluation. The alternative sweeteners selected for the study were xylitol, sorbitol, fructose, cyclamate and saccharin. The suitability of the sweeteners for a diabetic diet was an argument for selection. However, it should be noted that the use of cyclamate in foods is prohibited in the U.K. and that xylitol is not included in the list of permitted food additives. Xylitol and sorbitol were especially interesting because of their known retarding effect on the growth of some micro-organisms (Knuuttila & Mäkinen, 1975; Kracher, 1975; Mäkinen, 1978; Salminen & Branen, 1978). Therefore xylitol and sorbitol were studied more closely than the other alternatives.

Materials

Manufactured plain yoghurt (Kotisaari Oy) was used in the post-sweetened yoghurt study. Pasteurized milk and the same starter culture as in the manufactured yoghurt were used in the small scale preparation of the pre-sweetened yoghurt.

The following sweeteners were used. Sucrose: purity, 99.9%; ash, 0.001%; moisture, 0.003%. Fructose: purity, 99.5%; ash, 0.0014%, moisture, 0.04%. Finnish Sugar Co., Helsinki, SF.—Xylitol: purity, 99.8%; ash, 0.001%; moisture, 0.05%. Sorbitol: purity, 96.0%; ash, 0.004%; moisture, 0.28%. Sokerikemia Oy, Kotka, SF.—Na-cyclamate: DAK-63; and Na-saccharin: Ph.Nord., Apodan, Kastrup, DK. Vanilla Natural (Givaudan Duebendorf Ltd) was used as flavour.

The following media were used for microbiological quality control of yoghurt by plate count: Violet red bile agar (Difco), Malt agar (Difco), Plate count agar (Oxoid) and Soya peptone (Oxoid).

Preparation of pre-sweetened yoghurt

Pre-sweetened yoghurt varieties were prepared in the laboratory in a way resembling the factory production as much as possible. Samples were prepared in sterilized 1 litre Erlenmeyer flasks covered with aluminium foil. Sweetner, pasteurized and evaporated milk (dry matter 13%) and vanilla flavour were measured into the flask and the starter culture (0.8%) was then added. After 1 min mixing with a sterile pipette the flasks were placed into a warm bath (41°C), where they were kept for about 3.5 hr, until the titratable acidity of the unsweetened reference was at least 38 Soxhlet–Henkel degrees (°S-H). Then the flasks were cooled in an ice bath for 1 hr. After cooling the yoghurt samples were stored in the cold room (5°C) overnight. The yoghurt varieties could be analysed

on the following day. The analysis of the pre- and post-sweetened yoghurt types took 2 days. Consequently the fresh yoghurt samples were 1–2 days and the stored ones 15–16 days old.

Methods

Acidity measurements

The measurements of pH and titratable acidity were made immediately after each other. The pH was measured with a laboratory pH meter, Radiometer PHM 61. The titratable acidity of a 50 ml sample of yoghurt was determined by using 0.25 N NaOH as alkali and 2 ml 2% phenolphthalein in alcohol as the indicator. The results are expressed as °S-H (Soxhlet–Henkel degrees) which are the number of ml of 0.25 N NaOH required to neutralize 100 ml yoghurt, and are convertible to percent lactic acid by dividing 44.4.

Viscosity measurement

Apparent viscosity was measured in a Rotovisco RV 3 (Haake) rotation viscometer. The flag type rotor FL 100 was used for measurements. About 120 ml of yoghurt in a Duralex glass was tempered to 25°C for measurements. The values measured at the speed of 200 rev/min were the most reproducible and therefore the measurements in this study were made only at this speed. Three replicates of every yoghurt variety were analysed when fresh (1–2 days old) and after 2 weeks; storage at 5°C.

Sensory evaluation

The most preferred sweetness level of every sweetener was determined by a rank order test. The judges were asked to arrange four vanilla flavoured yoghurt samples sweetened with different amounts of the same sweetener in preference order. The alternative concentrations were as follows: 6, 8, 10 and 12% by weight for sucrose; 6, 8, 10 and 12% for xylitol; 5, 7, 9 and 11% for fructose; 10, 15, 20 and 25% for sorbitol; 0.03, 0.07, 0.013 and 0.019% for cyclamate, and 0.010, 0.015, 0.020 and 0.025% for saccharin.

The appearance, texture and aroma of the different yoghurt types before and after storing were evaluated by a scoring test, using the following scores: 5, excellent, 4, good, 3, satisfactory, 2, fair, 1, bad and 0, unfit to eat. When the score was 3 or lower the judges were asked to state the nature of the fault. The taste panel of eight members was familiarized in advance with the score card and the list of possible faults.

Microbiological analysis

Plate count agar (Oxoid) was used for the total bacterial count. Incubation for 3 days was at first performed at 30°C. Growth of colonies was poor at this

temperature and therefore a higher temperature, 35°C, was used during the rest of the study.

Coliforms were counted on violet red bile agar (Difco) after incubation at 35°C for 24 hr.

Yeasts and moulds were grown on malt agar (Difco) at 25°C for 5 days. The pH of the substrates was lowered to 3.5 by means of lactic acid (lactic acid : water, 1 : 10). All the plates, with less than 300 colonies, were counted. The weighted mean was used when calculating the number of bacteria per millilitre.

Storage stability

All the yoghurt types were stored at 5°C for 15–16 days, the xylitol–sucrose and sorbitol–sucrose varieties for as long as 22–23 days. The stored yoghurt varieties were examined by the same methods as the fresh yoghurt.

Results and discussion

Selection of concentration of sweeteners

The data of the rank order tests for the selection of suitable sweetener concentrations for vanilla flavoured yoghurt are given in Table 1. Since some judges found samples sweetened by saccharin totally unacceptable, in further studies a mixture of xylitol (4%) and saccharin (0.007%) was used instead of saccharin alone. The post- and pre-sweetened yoghurt types were prepared with selected, most preferred sweetness level of each sweetener.

Effects of different sweeteners on the quality of yoghurt

Acidity. The pH values of the post-sweetened and the corresponding pre-sweetened yoghurt types before and after storage and the respective values for titratable acidity are shown in Table 2.

Table 1. The most preferred concentrations of sweeteners in yoghurt by rank order test of eight judges

Sweetener	The most preferred concentration (%)
Sucrose	8
Xylitol	8*
Fructose	7*
Sorbitol	15*
Cyclamate	0.07*
Saccharin	0.010 and 0.015

* Significantly superior ($P < 0.05$).

The sweeteners had hardly any effect on the sourness of the post-sweetened varieties. The fresh pre-sweetened varieties differed from each other in acidity. The most exceptional was the sorbitol yoghurt, the pH of which was 6.8. It was not soured at all. During storage for 2 weeks at 5°C the pH of the yoghurt varieties was lowered by 0.05–0.25 pH units.

Viscosity. Viscosities of both the pre- and post-sweetened yoghurt types before and after storage are given in Table 2.

Before storage the viscosity of the post-sweetened yoghurt varieties was the same in all except sorbitol yoghurt, which was lower. After storage the viscosities of the different yoghurt varieties varied slightly, but the viscosity of sorbitol yoghurt was still the lowest.

The viscosity of the pre-sweetened sorbitol yoghurt was significantly lower than that of the other varieties. During storage the viscosities of all the yoghurt varieties with the exception of sorbitol sweetened were lowered. A possible explanation for the phenomenon might be breakdown of the precipitated proteins in the sour yoghurt samples. At pH 4.6 the precipitation is complete (Humpreys & Plunkett, 1969), hence the pH 6.6 of the stored sorbitol yoghurt was still too high for the formation of coagulum.

Sensory properties. Data on the sensory analysis of appearance, texture and flavour of the post-sweetened yoghurt type are shown in Fig. 1 and the corresponding data on the pre-sweetened yoghurt are shown in Fig. 2. The post-sweetened varieties did not differ in appearance or in texture. They were regarded as good in both characteristics before storage. After 2 weeks' storage

Table 2. The pH, titratable acidity and viscosity of the post- and pre-sweetened yoghurt varieties measured both before and after storage

Yoghurt type and variety	pH	Titratable acidity (°S-H)	Viscosity*† (cP)
Post-sweetened 2/16 days			
8% Sucrose	4.1/3.95	43.8/54.5	459/459
8% Xylitol	4.1/3.95	44.2/52.5	459/426
7% Fructose	4.0/3.90	42.6/51.0	459/459
15% Sorbitol	4.1/3.90	40.6/49.6	393/360
0.07% Cyclamate	4.1/3.90	43.8/55.0	459/459
4% Xylitol + 0.007% Saccharin	4.1/4.0	45.8/53.0	459/491
Pre-sweetened 2/15 days			
8% Sucrose	4.0/3.95	52.5/61.0	$710^{a}/546^{a}$
8% Xylitol	4.4/4.25	43.2/47.0	$666^{a}/557^{a}$
7% Fructose	4.2/4.10	42.5/50.0	$688^{a}/633^{a}$
15% Sorbitol	6.8/6.60	5.8/7.0	$109^{b}/382^{b}$
0.07% Cyclamate	4.1/3.85	54.0/59.0	$710^{a}/622^{c}$
4% Xylitol + 0.007% Saccharin	4.2/4.0	45.7/49.4	$710^{a}/655^{c}$

*Mean values with common letters are not significantly different ($P < 0.05$).

†Only one measurement of the post-sweetened yoghurt varieties.

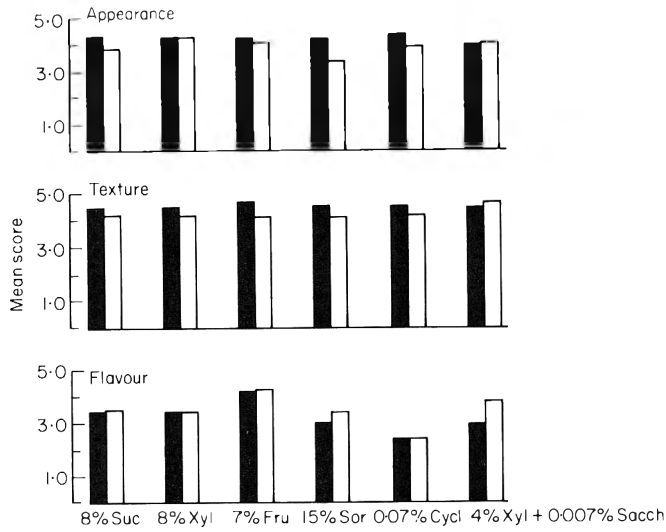


Figure 1. Mean scores for appearance, texture and flavour of the post-sweetened yoghurt before and after storage. Suc = sucrose, Xyl = xylitol, Fru = fructose, Sor = sorbitol, Cycl = cyclamate, Sacch = saccharin. ■, Before storage (2 days); □, after storage (16 days).

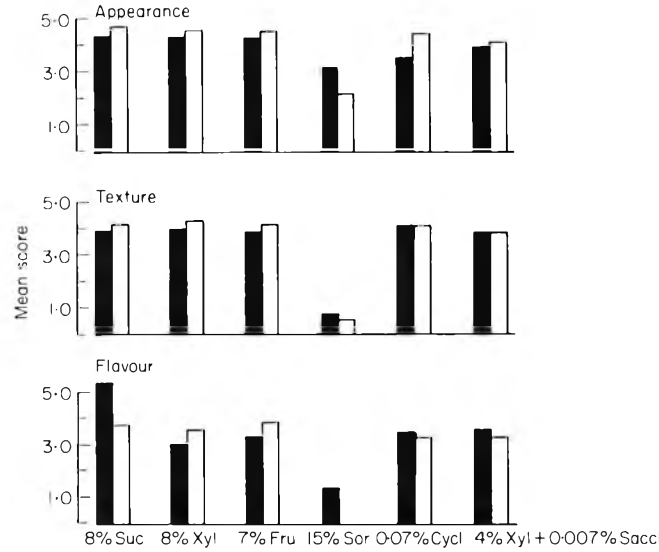


Figure 2. Mean scores for appearance, texture and flavour of the pre-sweetened yoghurt before and after storage. Suc = sucrose, Xyl = xylitol, Fru = fructose, Sor = sorbitol, Cycl = cyclamate, Sacch = saccharin. Key as Fig. 1.

the scores were lower in most cases. The sorbitol sweetened yoghurt was the only one whose appearance scores were lower than good after storage.

The mean flavour scores for the different varieties differed more from each other. The highest score was given to the flavour of the fructose yoghurt and the

lowest to the cyclamate yoghurt, which was considered to have an oxidized taste. Some judges noted a slight aftertaste in the sorbitol yoghurt and some regarded the tastes of the xylitol and the xylitol–saccharin varieties as abnormal.

Storage had either an enhancing effect or no effect on the flavour scores of the post-sweetened yoghurt varieties. The most striking change was the rise in the xylitol–saccharin yoghurt flavour score, possibly due to the taste balancing effect of the storage time on this mixture-sweetened yoghurt type.

The appearance scores given to the different pre-sweetened yoghurt varieties were very similar (Fig. 2) with the exception of the sorbitol and the cyclamate yoghurt scores. The appearance of the sorbitol yoghurt resembled milk more than yoghurt. Some whey had separated from the cyclamate yoghurt, lowering the appearance scores.

After storage the appearance of all the yoghurt varieties except the sorbitol yoghurt was regarded as good. In fact, the mean appearance scores were higher after than before storage with the exception of the sorbitol yoghurt.

The texture of all the varieties with the exception of the sorbitol-sweetened was regarded as good both before and after storage. The texture of the sorbitol yoghurt was judged to be bad. It was too thin, like milk, because of no souring.

Before storage the flavour of the sucrose yoghurt was superior and that of sorbitol yoghurt inferior to the others, which were regarded as at least satisfactory. The taste of the sorbitol yoghurt was non-typical, cooked and very sweet because no acid was formed in the product.

After two weeks' storage (at 5°C) there were so many coliform bacteria in the sorbitol yoghurt that it was regarded as unfit to be eaten or for sensory evaluation. The flavours of the sucrose, xylitol and fructose yoghurt varieties were regarded as good and the cyclamate and xylitol–saccharin varieties were still satisfactory after 2 weeks' storage.

Microbiological quality. The total bacterial counts including the yoghurt bacteria of the post-sweetened yoghurt varieties before and after storage and the number of coliforms, yeasts and moulds are given in Table 3.

Table 3. The total bacterial counts and number of coliforms, yeasts and moulds per millilitre in the post-sweetened yoghurt varieties before and after storage for 16 days at 5°C

	Total bacteria ($\times 10^8$)		Coliforms		Yeasts		Moulds	
Yoghurt variety	Before	After	Before	After	Before	After	Before	After
8% Sucrose	1.5	5.7	0	2	0	0	0	0
8% Xylitol	1.8	5.5	0	0.3	0	0	0	0
7% Fructose	3.3	7.9	0	0.5	0	0	0	0.3
15% Sorbitol	2.5	3.5	0	0	0	0	0	0
0.07% Cyclamate	2.6	3.7	0	0	0	0	0	0
4% Xylitol + 0.007% Saccharin	3.0	4.0	0	0	0	0	0	0

The total bacteria in the fresh post-sweetened yoghurt varieties varied from 1.5×10^8 to 3.3×10^8 organisms/ml. During storage the number of bacteria increased in all the yoghurt varieties, without significant differences according to the sweetener used. The number of undesirable organisms, coliforms, yeasts and moulds was very low in the post-sweetened yoghurt type.

The total bacterial counts of the fresh pre-sweetened yoghurt type were equal to the post-sweetened yoghurt, with the exception of sorbitol yoghurt which contained only 2.0×10^5 organisms/ml (Table 4).

During 2 weeks' storage at 5°C the total number of bacteria increased slightly in most of the pre-sweetened varieties. The increase was greatest in the sorbitol yoghurt. However, even after storage the total number of bacteria in the sorbitol yoghurt remained lower than in the other varieties. In the pre-sweetened xylitol yoghurt the total bacteria number did not increase during storage. These two effects probably reflect the unsuitability of these sugar alcohols for the nourishment of the yoghurt bacteria.

Table 4. The total bacterial counts and number of coliforms, yeasts and moulds per millilitre in the post-sweetened yoghurt varieties before and after storage for 15 days at 5°C

	Total bacteria		Coliforms		Yeasts		Moulds	
Yoghurt variety	Before	After	Before	After	Before	After	Before	After
8% Sucrose	1.5×10^8	4.2×10^8	0	0	0	0	0	0
8% Xylitol	2.2×10^8	1.9×10^8	0	1.3	0	0	0.3	0
7% Fructose	2.3×10^8	2.5×10^8	0.5	0	0.3	0	0.8	0.5
15% Sorbitol	2.0×10^5	6.0×10^7	32	60	25	0	0	0.3
0.07% Cyclamate	4.3×10^8	4.8×10^8	0	0	0	0	0	0
4% Xylitol + 0.007% Saccharin	2.3×10^8	3.1×10^8	0	0	0	0	0	0

A slight post-souring (higher acidity and total bacterial count) noted in both the post- and pre-sweetened yoghurt type after 2 weeks' storage means either that the temperature of the yoghurt samples did not fall to 5°C during the first day when they were stored before the first analysis, or that other lactic acid bacteria in addition to *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were present in the yoghurt. According to Buchanan & Gibbons (1974), *L. bulgaricus* will not grow at temperatures below 15°C and *Str. thermophilus* at below 20°C. There are certain lactic acid bacteria, however, that can grow slowly at the cold room temperature.

The number of coliform bacteria and yeasts was exceptionally high in the pre-sweetened, non-soured sorbitol yoghurt. In the other pre-sweetened varieties the number of these undesirable organisms was low.

Table 5. The pH, titratable acidity and viscosity of the yoghurt post- and pre-sweetened with different amounts of xylitol. Measurements were made before and after storage for 16 days at 5°C

Yoghurt (M Xylitol)	pH		Titratable acidity (°S-H)		Viscosity (cP)*	
	Before	After	Before	After	Before	After
Post-sweetened						
0.165	3.85	3.85	51.0	53.8	513[a]	393[a]
0.330	3.90	3.85	53.0	54.8	524[a]	371[a]
0.495	3.85	3.85	51.5	52.8	513[a]	360[a]
0.659	3.85	3.90	50.0	52.6	524[a]	393[a]
0.824	3.85	3.90	50.5	52.0	524[a]	338[a]
Pre-sweetened						
0.165	4.05	3.85	45.0	49.9	873[c]	753[c]
0.330	4.10	4.00	42.0	46.0	950[c]	862[c]
0.495	4.25	4.15	39.0	41.6	797[d]	830[c]
0.659	4.30	4.25	35.0	39.0	742[d]	830[c]
0.824	4.55	4.40	30.0	34.2	677[d]	710[c]

* Mean values with common letters are not significantly different ($P < 0.05$).

Effects of different concentrations of xylitol and sorbitol on the quality of yoghurt

The effects of 0.165, 0.330, 0.495, 0.659 and 0.824 M xylitol and sorbitol were studied.

Acidity. The pH values of the post- and pre-sweetened yoghurt types which were sweetened with different amounts of xylitol and the corresponding titratable acidity are given in Table 5.

Xylitol in the concentrations of 0.165–0.824 M had no effect on the acidity of the post-sweetened yoghurt. In the pre-sweetened yoghurt a slight but clear decrease in acidity could be noted as the xylitol concentration was increased. During storage a slight post-souring had occurred.

The pH values of the post- and pre-sweetened yoghurt types sweetened with different amounts of sorbitol and the corresponding titratable acidity are given in Table 7.

Increasing sorbitol concentrations seemed to have a very slight if any effect on the acidity of the post-sweetened samples. On the other hand, the effect of increasing concentrations of sorbitol on the pre-sweetened yoghurt type was drastic. The pH values of the yoghurt samples containing 0.165 and 0.330 M sorbitol were still quite normal (3.95 and 4.00), but the pH of the yoghurt with 0.495 M or higher concentrations of sorbitol was that of milk or even higher. The titratable acidity decreased as drastically as the pH values increased. Storing increased the titratable acidity slightly in most samples.

Table 6. The total bacterial counts and number of coliforms, yeasts and moulds per millilitre of the yoghurt post- and pre-sweetened with different amounts of xylitol before and after storage for 15 days at 5°C

Yoghurt type (M xylitol)	Total bacteria		Coliforms		Yeasts		Moulds	
	Before	After	Before	After	Before	After	Before	After
Post-sweetened								
0.165	2.5×10^8	5.1×10^8	0	0.3	0	0	0	0.3
0.330	1.8×10^8	4.8×10^8	0.5	0	0	0	0	0.8
0.495	1.7×10^8	5.4×10^8	0	0.3	0	0.3	0	0.5
0.659	1.5×10^8	5.7×10^8	0	0	0	0	0	1.5
0.824	8.8×10^7	4.7×10^8	0	0.3	0	0	0	0.5
Pre-sweetened								
0.165	5.3×10^8	2.7×10^8	0	0	0	0	0.5	0.3
0.330	5.5×10^8	1.9×10^8	0	0	0	0	0.8	0
0.495	4.8×10^8	1.6×10^8	0	0	0	0	2.0	0
0.659	4.6×10^8	1.0×10^8	1.0	0.8	0	0	1.0	0.5
0.824	3.8×10^8	7.7×10^7	0.3	0.3	0	0	0.8	0

Viscosity. The data on the viscosity of the xylitol sweetened yoghurt are given in Table 5.

The viscosities of the post-sweetened yoghurt varieties, although containing different amounts of xylitol, were almost similar. After 2 weeks' storage the viscosity was decreased and the values were more diverging, but the differences were insignificant ($P < 0.05$). The viscosity of the pre-sweetened yoghurt steadily decreased as the concentration of xylitol increased.

The viscosities of the sorbitol sweetened yoghurt varieties are given in Table 7. The viscosities of the freshly prepared post-sweetened samples did not vary significantly ($P < 0.05$). After 2 weeks' storage at 5°C the viscosities were lower. The viscosity of the pre-sweetened yoghurt the sorbitol concentration of which was 0.495 M or higher was that of sorbitol sweetened milk.

Microbiological quality. The numbers of total bacteria, coliforms, yeasts and moulds per millilitre of post- and pre-sweetened yoghurt with different amounts of xylitol as freshly prepared and after 15 days of storage at 5°C are given in Table 6.

The highest number of total bacteria was found in the post-sweetened yoghurt with the lowest concentration of xylitol. The difference may be due to the dilution effect of the growing proportion of the added xylitol. After storage the total bacteria count was increased and independent of the xylitol concentration used. The number of undesirable organisms was low.

The difference in the total bacterial counts between the pre-sweetened xylitol yoghurt varieties were of the same magnitude as in the post-sweetened varieties. The total bacteria counts of the pre-sweetened yoghurt decreased during 2 weeks' storage at 5°C, which was opposite to the effect in the post-sweetened

Table 7. The pH, titratable acidity and viscosity of the yoghurt post- and pre-sweetened with different amounts of sorbitol. Measurements were made before and after storage for 16 days at 5°C

Yoghurt type (M Sorbitol)	pH		Titratable acidity (°S-H)		Viscosity (cP)*	
	Before	After	Before	After	Before	After
Post-sweetened						
0.165	3.80	3.80	54.2	58.0	633[a]	437[b]
0.330	3.90	3.80	50.2	57.6	775[a]	524[b]
0.495	3.90	3.80	50.0	54.8	677[a]	611[a]
0.659	3.90	3.80	50.8	54.8	666[a]	480[b]
0.824	3.90	3.80	48.4	53.4	688[a]	600[a]
Pre-sweetened						
0.165	3.95	3.95	47.0	55.4	1059[c]	972[c]
0.330	4.00	4.05	43.6	53.0	1168[c]	1081[c]
0.495	6.45	6.70	8.7	6.0	120[d]	87[d]
0.659	6.85	6.85	4.6	4.8	131[d]	120[d]
0.824	6.90	6.90	4.6	4.8	131[d]	98[d]

* Mean values with common letters are not significantly different ($P < 0.05$).

yoghurt. Numbers of coliforms, yeasts and moulds were also low in the pre-sweetened yoghurt.

The data on the microbiological analyses of the yoghurt samples post- and pre-sweetened with different amounts of sorbitol are given in Table 8.

Table 8. The total bacterial counts and number of coliforms, yeasts and moulds per millilitre of the yoghurt post- and pre-sweetened with different amounts of sorbitol before and after storage for 16 days at 5°C

Yoghurt type (M sorbitol)	Total bacteria		Coliforms		Yeasts		Moulds	
	Before	After	Before	After	Before	After	Before	After
Post-sweetened								
0.165	3.8×10^8	4.8×10^8	0	0	0	0	0.5	0
0.330	3.4×10^8	4.6×10^8	0	0	0	0	0.3	0.3
0.495	3.9×10^8	3.8×10^8	0	0	0	0	0	1.8
0.659	4.2×10^8	4.0×10^8	0	0	0	0	0.3	0.8
0.824	4.1×10^8	3.3×10^8	0	0	0	0	0.5	1.5
Pre-sweetened								
0.165	2.6×10^8	2.0×10^8	0	0	0	0.3	1.3	0.5
0.330	2.3×10^8	2.3×10^8	0	0	0	0	0	0.3
0.495	2.0×10^5	4.8×10^4	0	0	0	0	0	0
0.659	$< 1 \times 10^5$	1.0×10^4	0	0	0	0.3	0.3	0
0.824	4.0×10^4	1.0×10^3	0	0	0	0	0.3	0.3

No significant differences were noted in the total bacteria counts of the post-sweetened sorbitol yoghurt varieties either before or after storage. The post-sweetened yoghurt contained some moulds but no coliforms or yeasts. The yoghurt samples were presumably contaminated during the addition of sorbitol.

Table 8 shows a normal total bacteria count in the pre-sweetened sorbitol yoghurt samples with the two lowest concentrations of sorbitol, whereas the counts in the following higher sorbitol concentrations were significantly lower ($\leq 2.0 \times 10^5$ organisms/ml). During 2 weeks' storage the total bacteria in the latter samples decreased significantly. Some yeasts and moulds were found in the pre-sweetened sorbitol yoghurt, but the number was very low.

Xylitol–sucrose and sorbitol–sucrose combinations

In order to balance the positive and negative effects of xylitol and sorbitol as bacterial growth inhibiting agents, pre-sweetened yoghurt was prepared with combinations of xylitol and sucrose and of sorbitol and sucrose. The ability of the sugar alcohols to prevent acid formation was regarded as positive if an excessively high acidity of yoghurt could be prevented, but as negative if the inhibitory effect was so great that appearance, texture or flavour was no longer typical of yoghurt.

The combinations were prepared so that the sweetness of each variety of yoghurt was at an acceptable level and that all were as near one another as possible. The dry matter added into the yoghurt as sweetener was maintained constant. The pre-sweetened yoghurt containing 8% sucrose was regarded as the reference in studying the xylitol–sucrose combinations. The combinations used were: 6% xylitol + 2% sucrose; 4% xylitol + 4% sucrose; and 2% xylitol + 6% sucrose. The sorbitol–sucrose combinations in the pre-sweetened yoghurt were: 4% sorbitol + 6% sucrose; 6% sorbitol + 4% sucrose; 7% sorbitol + 3% sucrose; and 8% sorbitol + 2% sucrose. The pre-sweetened yoghurt with 10% sucrose was regarded as the reference in this case.

Acidity of xylitol–sucrose and sorbitol–sucrose sweetened yoghurts. The pH and titratable acidity of the xylitol–sucrose sweetened yoghurt are given in Table 9.

With a decreasing proportion of xylitol in the combination there was a slight but steadily increasing acidity. In all combinations, however, the acidity was close to the acidity of the reference yoghurt and it increased somewhat during storage.

The pH and titratable acidity of the sorbitol–sucrose sweetened yoghurt (Table 9) give more detailed information than the earlier part of this study (Table 2) about the concentration of sorbitol which causes a serious inhibiting effect on the growth of yoghurt bacteria.

The yoghurt containing 6% sorbitol showed incipient growth inhibition. When the sorbitol concentration of the yoghurt was 7%, the pH of the product was significantly higher (4.75) than the normal pH of yoghurt. At the sorbitol

Table 9. The pH, titratable acidity and viscosity of the yoghurt pre-sweetened with various combinations of xylitol and sucrose, and sorbitol and sucrose. Similarly prepared yoghurt samples containing 8 and 10% of sucrose were used as respective references. Measurements were made of yoghurts freshly prepared and after 22–23 days of storage at 5°C

Pre-sweetened yoghurt variety	pH		Titratable acidity (°S-H)		Viscosity (cP)*	
	Before	After	Before	After	Before	After
2% Sucrose 6% Xylitol	4.25	4.00	40.5	44.6	611[a]	611[a]
4% Sucrose 4% Xylitol	4.20	3.95	42.0	45.2	721[b]	600[a]
6% Sucrose 2% Xylitol	4.15	3.90	43.6	46.8	721[b]	600[a]
8% Sucrose	4.10	3.90	46.8	49.8	677[b]	655[a]
6% Sucrose 4% Sorbitol	4.10	3.90	39.0	44.9	917[c]	633[d]
4% Sucrose 6% Sorbitol	4.30	4.20	34.4	35.7	873[c]	666[d]
3% Sucrose 7% Sorbitol	4.75	4.45	25.8	28.4	622[d]	459[e]
2% Sucrose 8% Sorbitol	6.00	5.20	11.4	22.8	120[e]	175[f]
10% Sucrose	4.15	3.95	38.8	45.6	884[c]	710[c]

* Mean values with common letters are not significantly different ($P < 0.05$).

concentration of 8% the acidity (pH 6.0) was no longer adequate to cause coagulation.

During three weeks' storage at 5°C all the sorbitol–sucrose varieties were soured more, but only the combination of 6% sucrose and 4% sorbitol produced the normal yoghurt acidity (Table 9).

Viscosity of xylitol–sucrose and sorbitol–sucrose yoghurts. The viscosities of the xylitol–sucrose and sorbitol–sucrose sweetened yoghurt varieties are given in Table 9.

The viscosity of the fresh yoghurt containing 6% xylitol was lower than that of the other xylitol–sucrose yoghurt varieties or the reference yoghurt. After 3 weeks' storage at 5°C there was no significant difference between the viscosities of the different varieties.

The viscosities of the sorbitol–sucrose sweetened yoghurt varieties containing 4 or 6% of sorbitol did not differ from the viscosity of the sucrose reference ($P < 0.05$). Those varieties that contained 7 or 8% of sorbitol had a significantly

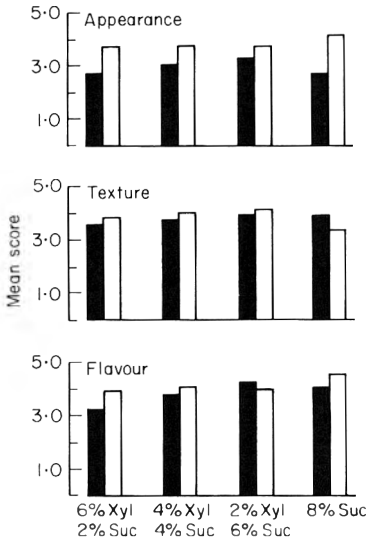


Figure 3. Mean scores for appearance, texture and flavour of the yoghurt pre-sweetened with various combinations of xylitol and sucrose. Sensory evaluation was made both before and after storage. Suc = sucrose, Xyl = xylitol. ■, Before storage (2 days); □, after storage (23 days).

lower viscosity than any of the others. The viscosities of the sour samples were lowered during storage.

Sensory evaluation. The data on the sensory evaluation of the pre-sweetened xylitol–sucrose yoghurt varieties are shown in Fig. 3.

The appearance of the yoghurt varieties containing both xylitol and sucrose and also the pre-sweetened sucrose reference was considered to be satisfactory. Formation of whey was often mentioned as a fault in the appearance of all the xylitol–sucrose varieties.

The texture of the xylitol–sucrose varieties as well as that of the sucrose reference was judged to be fairly good. No significant differences were noted between the varieties.

The flavour of the yoghurt varieties containing 2 and 4% xylitol was judged to be as good as the reference. The variety with 6% xylitol was given lower flavour scores because of poor flavour and insufficient sourness.

The appearance scores for the pre-sweetened sorbitol–sucrose variety with 4% sorbitol, was regarded as good and even better than the reference. The reason for the low appearance scores for other varieties was stated to be the separation of whey and formation of gas bubbles in the yoghurt.

After 3 weeks' storage at 5°C the mean appearance scores of all varieties except that containing 8% sorbitol were higher than before storage. The tendency to whey separation decreased during storage.

The mean texture scores for the reference yoghurt containing 10% sucrose and for the varieties containing 4 or 6% sorbitol were good. The variety with 8% sorbitol was judged to be bad. The last was not coagulated at all. Three weeks' storage at 5°C did not change the mean texture scores significantly.

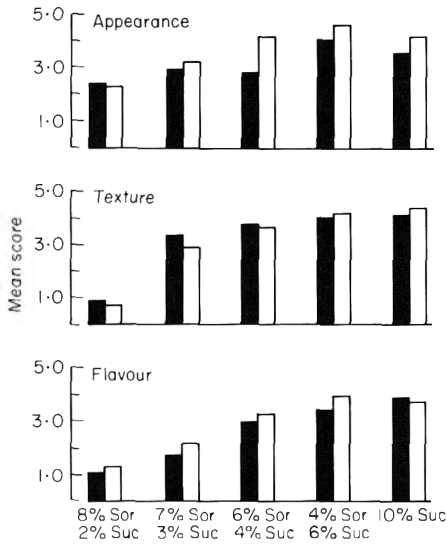


Figure 4. Mean scores for appearance, texture and flavour of the yoghurt pre-sweetened with various combinations of sorbitol and sucrose. Sensory evaluation was made both before and after storage. Suc = sucrose, Sor = sorbitol. Key as Fig. 3.

With decreasing proportions of sorbitol in the sorbitol–sucrose combinations the mean flavour scores of these yoghurt varieties increased.

The flavour of the sorbitol–sucrose varieties was considered to have improved during storage. However, the more sorbitol was used in the yoghurt the more non-typical the flavour was considered to be. The faults noted were an oxidized taste, a taste of cardboard, or excess sweetness.

Conclusions

In evaluating the data of this study the following conclusion can be drawn. The pH and titratable acidity of the yoghurt were in good correlation. The changes in acidity were accompanied by changes in the total bacteria counts and viscosity. The sensory properties correlated with the titratable acidity in that when the acidity was low the flavour scores in the sensory evaluation also were low.

The sweetening of yoghurt after incubation succeeded well with any of the sweeeteners (fructose, xylitol, sorbitol, cyclamate, xylitol–saccharin) without marked deterioration of any quality characteristic. The sweetening of yoghurt with sorbitol before incubation proved impossible. The product produced had no characteristics typical of yoghurt. The amount of sorbitol (15%) needed to obtain a suitable sweetness had too great an inhibiting effect on bacterial growth in the yoghurt culture.

It proved possible to use xylitol also for pre-sweetening yoghurt, although this sweetener had some inhibiting effect on bacterial growth. At the 8% concentration needed for sweetening, the inhibiting effect of xylitol was not appreciable.

The inhibiting effect of sorbitol on the growth of bacteria in the pre-sweetened yoghurt began to affect the properties of the product at the concentration of 7%. The yoghurt with 7% of sorbitol was not sufficiently sweet. Therefore sorbitol is not suitable for sweetening of yoghurt before incubation, but it succeeded in combination with sucrose. For example the pre-sweetened yoghurt containing 4% sorbitol and 6% sucrose did not differ from the sucrose sweetened reference.

References

Buchanan, R.E. & Gibbons, N.E. (1974) *Bergey's Manual of Determinative Bacteriology*. 8th ed., p. 490. The Williams & Wilkins Co., Baltimore.

Humpreys, C.L. & Plunkett, M. (1966) *Dairy Sci. Abstr.* **31,** 607.

Kracher, F. (1975) *Kakao Zuck.* **27,** 108.

Knuuttila, M. & Mäkinen, K. (1975) *Caries Res.* **9,** 177.

Mäkinen, K. (1978) *Biochemical Principles of the Use of Xylitol in Medicine and Nutrition with Special Consideration of Dental Aspects*, p. 23. Birkhäuser Verlag, Basel und Stuttgart.

Salminen, S.J. & Branen, A.L. (1978) Paper presented at the *38th Annual IFT Meeting, June 4–7, Dallas 1978* (unpublished).

(Received 1 February 1982)

J. Fd. Technol. (1983) **18**, 113–123

Dehydration of carrots

Effects of pre-drying treatments on moisture transport and product quality

G. MAZZA

Summary

The effect of blanching, sulphiting, starching, freezing and sucrose and sodium chloride dipping before air drying on moisture transport during carrot cube dehydration was studied. The results, interpreted in terms of existing theories combined with physical considerations, provide evidence that factors such as blanching, freezing and sucrose dipping affect moisture transport and product quality.

Introduction

Even with the development of newer drying techniques, most vegetables are still air dried because this method of dehydration is still the simplest and most economical. Major problems associated with air dehydration are the considerable shrinkage caused by cell collapse following the loss of water, the poor rehydration characteristics of the dried product, and the unfavourable changes in colour, texture, flavour and nutritive value caused by drying.

A number of authors have suggested pre-drying treatments of solid food materials as a means to both improve product quality and reduce product water load to the dryer. Karel & Flink (1978) and Flink (1980) have reported the results of studies on concentration of carrots in mixed lactose–salt and sucrose–salt systems. Hawkes & Flink (1978) have reported on the effectiveness of mixed carbohydrate systems for osmotic concentration of apple slices. Speck, Escher & Solms (1977), Curry, Burns & Heidelbaugh (1976), Neumann (1972) and others have shown that treatment of sliced vegetables with sodium chloride, sugars and glycol prior to dehydration improves rehydration, colour, texture and flavour of the finished product.

Author's address: Alberta Horticultural Research Centre, Brooks, Alberta T0J 0J0, Canada.
Present address: Agriculture Canada Research Station, P.O. Box 3001, Morden, Manitoba R0G 1J0, Canada.

0022–1163/83/0200–0113 $02.00

Saravacos & Charm (1962) found that steam blanching of potatoes had no significant effect on drying rate when compared with the control sample dried at 66°C. Vaccarezza, Lombardi & Chirife (1974) found that water blanching of sugarbeet root slabs (using wrapped samples to avoid soluble solid loss) had no significant effect on drying rate at 60°C, but at 47°C it somewhat increased the rate. Alzamora & Chirife (1980) reported that blanching and freezing had little influence on the drying rate of avocado.

The aim of this study was to determine the effect which water blanching, sulphiting, starching, freezing, and sucrose and sodium chloride dipping applied before air drying had on the rate of moisture movement during carrot cubes dehydration and on finished product quality.

Materials and methods

The dehydration equipment and experimental method have been described in detail in a previous paper (Mazza & LeMaguer, 1980). Therefore, only modifications are presented here.

The carrots, 'Spartan Fancy', were washed, mechanically peeled and diced into 0.95 cm cubes. The cubes were then treated in the following ways:

(1) One part of carrots was blanched in three parts of boiling water for 3 min and immediately cooled in ice water.
(2) Carrots which were treated as described under (1) were dipped additionally, after cooling, into 1% sodium bisulphite solution for 1 sec.
(3) Carrots which were treated as described under (2) were dipped, after draining for 3–4 min, into a 2.5% waxy maize starch (Col–Flo 67) or corn starch (Melojel) suspension at 80°C for 1 sec.
(4) Carrots which were treated as described under (1) were immersed in 5, 10 or 60% sucrose or 10% sodium chloride solution for 30 min at 20°C.
(5) Carrots that were treated as described under (1) were frozen by placing in a freezer at − 20°C for 60 hr then allowed to thaw at room temperature.

All samples were dehydrated in a Vibro Fluidizer at 50°C, 8.1 m^3/min air flow rate and at a loading ratio of 16 kg/m^2 of drying area.

Drying curves were determined by periodic weighing of 5% of the mass of carrots in the dehydrator and by graphical integration of the thermograms. The carrot subsample used to follow the change in moisture content with time, was in a pre-tared wire screen basket (11 × 14 × 9 cm) fastened to prevent loss of small pieces. The shrinkage of the carrot cubes after various times of drying was determined from specific volume measurements by the displacement of toluene.

The equilibrium moisture contents (X_e) of carrots treated as described under (4) were determined by placing subsamples in vacuum desiccators containing saturated salt solutions which give different constant relative humidities (Rockland, 1960). The desiccators were kept at constant temperature until equilibrium was reached. The time required for the samples to reach equilibrium varied

with the relative humidity and the temperature. By plotting the moisture content of the samples, expressed in kg water/kg dry solids *versus* water activity (a_w), the desorption isotherms were obtained. To obtain adsorption isotherms, the carrots treated as described under (4) were first freeze dried and then placed into atmospheres of water activity range $0.11 \leqslant a_w \leqslant 1.00$ and kept there until equilibrium was reached. The moisture content of the samples, expressed again as kg water/kg dry solids, was plotted *versus* a_w.

Rehydration of dehydrated carrots was measured in distilled water at 25°C. Five grams of dehydrated carrots were added to 150 ml of distilled water in a 250 ml beaker, mixed thoroughly and allowed to rehydrate for various lengths of time. At the end of the rehydration period the carrot cubes were filtered off using a No. 4 Whatman filter paper and slight vacuum and weighed. Soak water was retained and analysed for solid content. Rehydration ratios were calculated by dividing rehydrated weight by the initial dry matter weight (including sugar or salt in dry matter).

Results and discussion

The effect of blanching, sulphiting, starching and freeze thawing on the rate of drying of carrot cubes is shown in Fig. 1. Although both blanching and freeze thawing increased the drying rate, blanching had a more significant influence than freezing on the rate of moisture transport in carrots. Sulphiting and starching of blanched carrots had no effect on the rate of drying. Blanching is

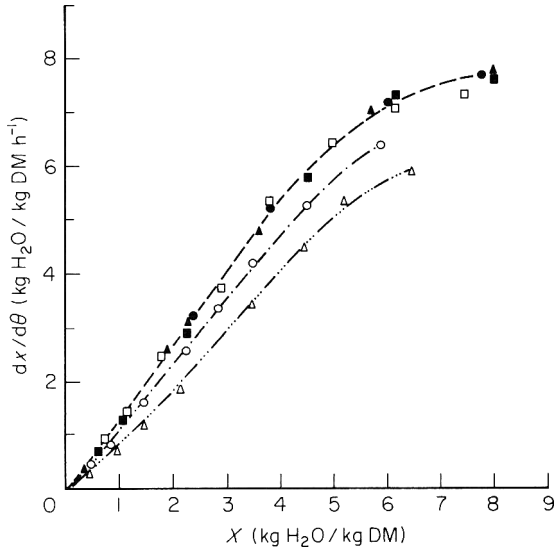


Figure 1. Rate of dehydration of raw, freeze thawed, blanched, blanched and $NaHSO_3$ and blanched, $NaHSO_3$ and starched carrot cubes at 50°C and 8.1 m³/min air flow rate, ●, Blanched, $NaHSO_3$ and melojel; ■, blanched $NaHSO_3$ and col-flo; ▲, blanched and $NaHSO_3$; □, blanched; ○, freeze thawed; △, untreated.

considered in the literature to affect the rate of vegetable drying although there is little experimental evidence. It is believed that the aspects responsible for the effect of blanching are: (a) change in physical properties of the tissue, such as destruction of the cell membranes by heat; and (b) loss of soluble solids, which as shown by Vaccarezza & Chirife (1975), affect the rate of drying. Freeze thawing was shown to have a less significant effect than blanching on the rate of drying of carrots, possibly because it altered only the physical characteristics of the tissue.

The effect of sucrose dipping on the rate of moisture removal of carrots is shown in Figs 2 and 3. From Fig. 2 it can be observed that as the concentration of sucrose, used for dipping the cubes for 0.5 hr was increased from 5 to 60%, moisture content of the carrots decreased from 9.0 to 3.6 kg H_2O/kg DM (same as from 90 to 78% H_2O). With air drying, the moisture content of carrots dropped rapidly, however, in spite of the fact that some water had been removed during the dipping in the sucrose solutions, drying times necessary to reach low residual moisture content were actually increased. Figure 3 is a plot of the drying rate of sucrose- and NaCl-dipped carrots *versus* mean moisture content. It can be observed that with NaCl and increasing concentration of sucrose treatment, the rate of moisture transport decreased substantially.

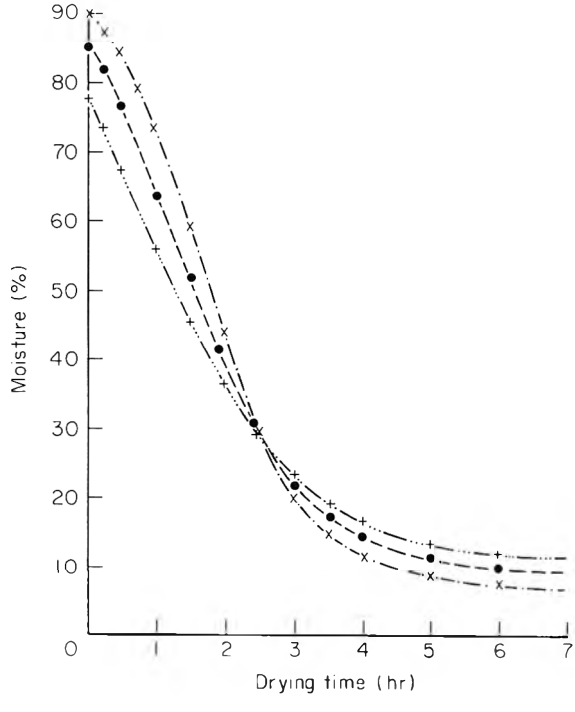


Figure 2. Effect of pre-drying sucrose dipping on the rate of moisture removal from carrot cubes dehydrated at 50°C, 16 kg/m² drying surface and 8.1 m³/min air flow rate. ×, 5% Sucrose; ●, 20% sucrose; +, 60% sucrose.

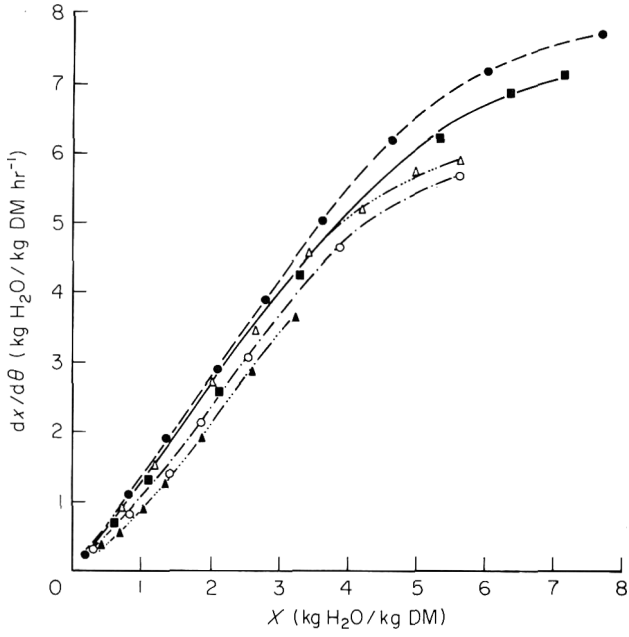


Figure 3. Rate of dehydration of blanched, blanched and NaCl, and blanched and sucrose dipped carrot cubes dehydrated at 50°C and 8.1 m³/min air flow rate. ●, Blanched; ■, 5% sucrose; △, 10% NaCl; ○, 20% sucrose; ▲, 60% sucrose.

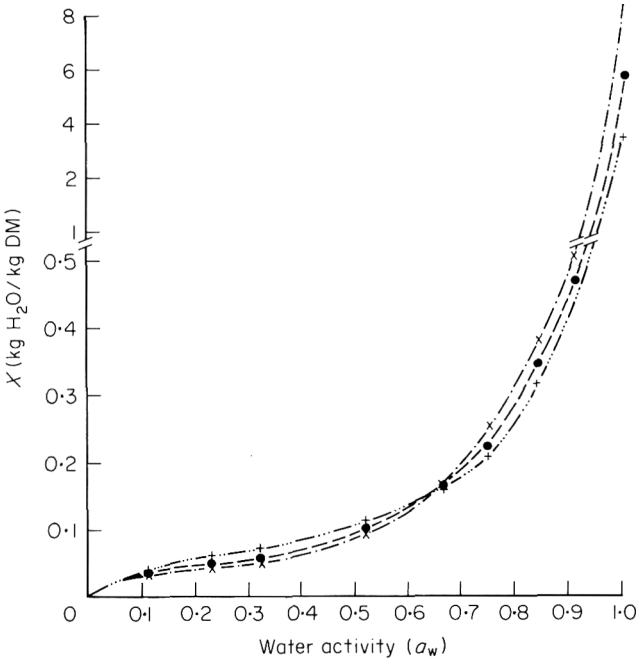


Figure 4. Desorption isotherms of blanched and sucrose treated carrot cubes at 40°C. +, 5% Sucrose; ●, 20% sucrose; ×, 60% sucrose.

It is well known (Ponting *et al.*, 1965; Hawkes & Flink, 1978) that the concentration effect of sucrose results from osmotic dehydration and from sugar uptake by the treated material on its surface. The lower rates of moisture transport observed in air drying of sucrose treated carrots can be attributed to two reasons: (1) sucrose that crystallizes during the air drying process lowers the diffusivity of water vapour and impairs heat transfer within the product; and (2) the vapour pressure of water in the product is depressed due to dissolved sugar. Thus, the vapour pressure difference between drying air and product decreases and this results in a lower drying rate.

The equilibrium moisture contents of sucrose treated carrots determined using raw and freeze dried samples were also affected by the sucrose concentration (Figs 4, 5 and 6). At low water activities, the increase in sucrose concentration increased the equilibrium moisture content when this was determined using raw samples (Fig. 4). This finding is consistent wih the observed effect of sugar concentration on air drying of carrots. Figure 4 also shows that although the sucrose treated product contains more moisture than the untreated material its a_w is lower, therefore the higher sucrose containing product is as stable as the blanched material with less moisture but same a_w.

When the equilibrium moisture content was determined using sugar dipped, freeze dried carrots, at low and intermediate water activity, the increase in sucrose concentration decreased the equilibrium moisture content (Fig. 5). This

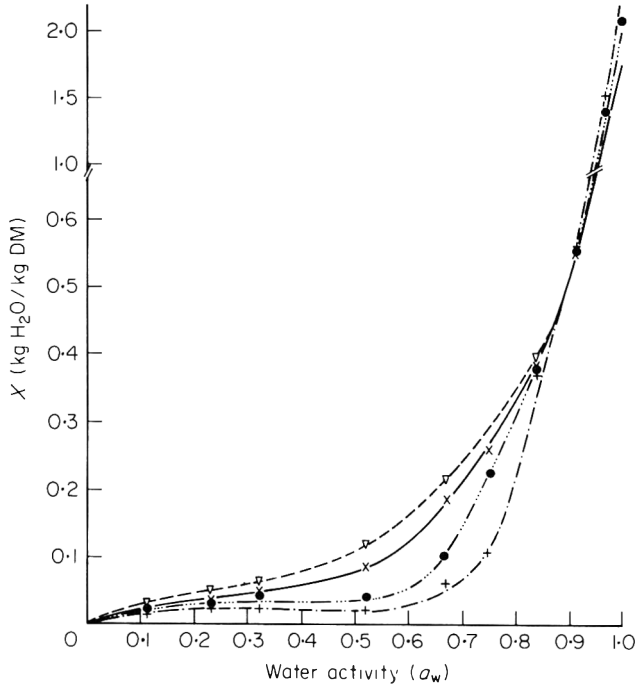


Figure 5. Adsorption isotherms of blanched, sucrose treated and freeze dried carrot cubes at 40°C. ▽, Control; ×, 5% sucrose; ●, 20% sucrose; +, 60% sucrose.

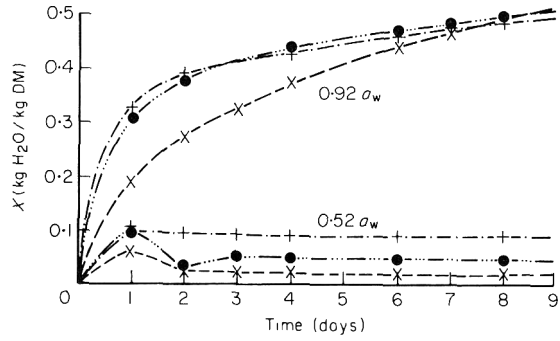


Figure 6. Water uptake by blanched, sucrose treated and freeze dried carrot cubes at 40°C. +, 5% Sucrose; ●, 20% sucrose; ×, 60% sucrose.

effect of sucrose is due to change in state of the sugar. It has been reported (Karel, 1975) that sucrose may be present in one of several states: crystalline solid, amorphous solid (bound to other food components) and aqueous solution. When carrots treated with sucrose are freeze dried, the sugar probably goes into the amorphous form. In this form, it is very hygroscopic and unstable. However, the very high viscosity of the medium and the presence of non-sugar fraction in the carrots act as a support and prevent molecular rearrangement. The adsorption of water imparts mobility to the sugar molecules and this mobility results in the transformation of sugar from the metastable amorphous state to the more stable crystalline state which sorbs very little water until water activity reaches approximately 0.8 and the sucrose begins to dissolve. Also in this process of transformation the sucrose treated material loses water (Fig. 6) (Iglesias, Chirife & Lombardi, 1975; Varshney & Ojha, 1977).

When the equilibrium moisture content was determined using carrot cubes immersed in sugar solutions but not followed by freeze drying (Fig. 4), the sequence of events was different. During the immersion step water diffused through the cellular surface structure from the carrots to the concentrated solution. After 30 min, when the carrots were removed from the concentrated sucrose solution and placed in atmospheres of various water activity (desorption), the sucrose concentration on the surface of the piece was higher than in the middle. Exposure of this material to decreasing humidities resulted in sufficiently slow water loss to produce amorphous sucrose which sorbs more water than crystalline sucrose (Karel, 1975).

Figures 7 and 8 show the adsorption and desorption isotherms of sodium chloride and sodium chloride treated carrot cubes at 40°C. It is evident from these isotherms that at low water activities, the higher the sodium chloride concentration, the lower the sorption capacity of the carrots. Sodium chloride adsorbed practically no water at water activities below 0.5. At higher water activities, however, as for the sucrose treated material, the increase in sodium chloride concentration increased the equilibrium moisture content. This reflects the change in state of the salt.

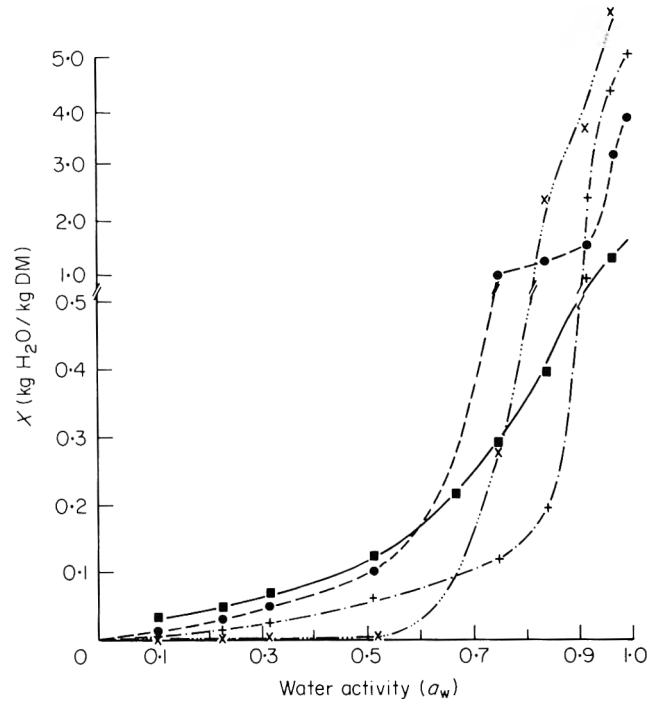


Figure 7. Adsorption isotherms of blanched and NaCl treated carrot cubes at 40°C. ■, 0% Nacl; ●, 10% Nacl; +, 30% NaCl; ×, 100% NaCl.

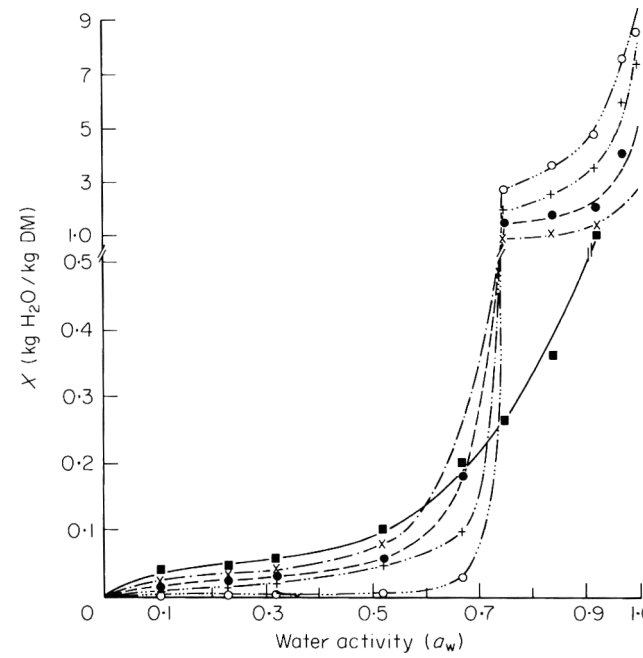


Figure 8. Desorption isotherms of blanched and NaCl treated carrot cubes at 40°C. ■, 0.0% NaCl; ×, 2.5% NaCl; ●, 10.0% NaCl; +, 30.0% NaCl; ○, 100.0% NaCl.

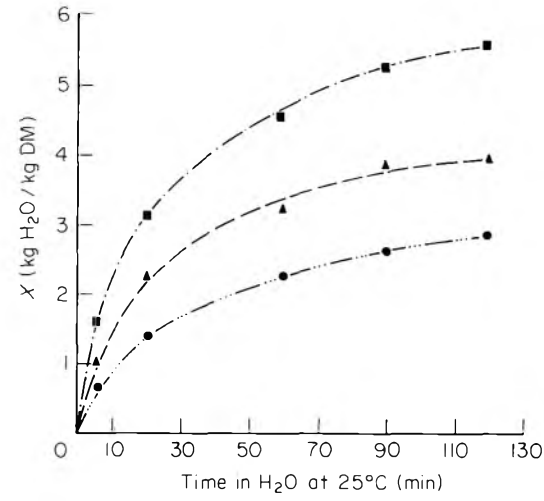


Figure 9. Rate of rehydration of carrot cubes blanched and dipped in 5, 20 and 60% sucrose solution for 30 min prior to drying at 50°C and 8.1 m³/min air flow rate. ■, 5% Sucrose; ▲, 20% sucrose; ●, 60% sucrose.

The sorption isotherms of sucrose- or NaCl-treated carrots can then be interpreted on the basis of their basic components, i.e. polimeric material (protein, starch, cellulose, pectin) and soluble solids (sugars, salts, acids). The soluble solids adsorb very little water at low water activities, and adsorption is mainly due to the polymers. As vapour pressure is increased above the vapour pressure of the saturated solution of the soluble solids, adsorption increases considerably and a solution is produced.

This demonstrates that the presence of sucrose or salt on the surface of cubed carrots impairs moisture uptake and removal. Hence, at the same dehydration temperature, carrots treated with sucrose or NaCl will always yield more moist finished product than untreated material. The finished product, however, will also absorb less moisture and the rehydration ratio will be lower for treated product than for untreated material.

Measurement of the rehydration ratios of the dry materials used in this experiment showed significant differences between the control and the treated samples. At room temperature, the rehydration ratio decreased by over 50% as the sucrose concentration was increased from 5 to 60% (Fig. 9). This is because of the sugar on the dehydrated carrots, which rehydrates less than the vegetable tissue. This has good and bad features; the sugar treated material does not take up as much water, which may be a disadvantage in hot pies, glazed and candied cakes, gelatin salad, etc. On the other hand, less rehydration means a crisper texture. This would be an advantage for eating in soups and stews. Loss of water soluble solids during the rehydration period was not negligible and was affected by the pre-drying treatment (Fig. 10). Pre-drying sucrose treatment resulted in less shrinkage of carrots during air drying. Values for shrinkage were lowest for the 60% sucrose treated samples and highest for the blanched material.

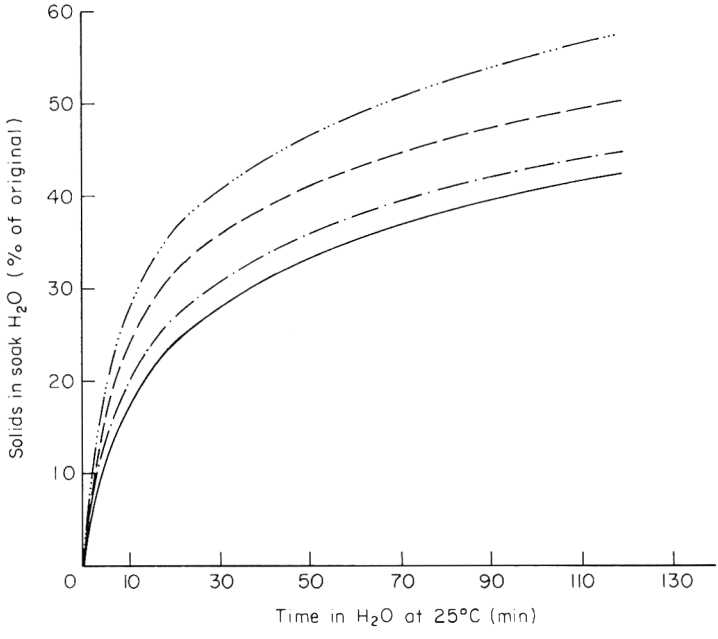


Figure 10. Loss of solids during rehydration (in distilled H_2O at 20°C) of blanched and sucrose treated carrots. – · · · –, 60% Sucrose; – – –, 20% sucrose; – · –, 5% sucrose; –, 0% sucrose.

From the above discussion it appears that carrots, to be air dried after partial osmotic drying, will require higher air temperature or more time to reach the same final moisture content as blanched, blanched and sulphited or blanched, sulphited and starched carrots. However, the stability of a dehydrated product is not associated with its minimum total moisture content, but with its water activity, therefore partial osmotic drying of carrots may still reduce air drying time and energy consumption. Also, although the texture of osmotically dried material is more or less open and rigid and the tissue does not collapse as much during drying, the presence of sugar or salt on the surface of the material has great bearing on the rehydration characteristics of the finished product and on the transport of heat and mass during air drying.

Acknowledgments

The author thanks Dr Marc LeMaguer for suggestions in preparation of this manuscript.

References

Alzamora, F.M. & Chirife, J. (1980) *J. Fd Sci.* **45**, 1649.

Flink, J.M. (1980) In: *Food Process Engineering* (Ed. by P. Linko, Y. Mälkki, J. Olkku and J. Larinkari) vol. 1, p. 412. Applied Science Publishers Ltd., London.

Curry, J.C., Burns, E.E. & Heidelbaugh, N.D. (1976) *J. Fd Sci.* **41,** 176.
Hawkes, J. & Flink, J.M. (1978) *J. Fd Proc. Preserv.* **2,** 265.
Karel, M. (1975) In: *Freeze Drying and Advanced Food Technology* (Ed. S. A. Goldblight, L. R. Rey and W. W. Rothmays) p. 643. Academic Press, New York.
Karel, M. & Flink, J.M. (1978) In: *Mechanisms of Deterioration of Nutrients,* p. 5–41–5–46. Annual Report on Phase V of NASA/JSC Control. No. 9-12485.
Iglesias, H. A., Chirife, J. & Lombardi, J. L. (1975) *J. Fd Technol.* **10,** 385.
Mazza, G. & LeMaguer, M. (1980) *J. Fd Technol.* **15,** 181.
Neumann, H.J. (1972) *J. Fd Sci.* **37,** 437.
Ponting, J.D., Watters, G.G., Forrey, R.R., Jackson, R. & Stanley, W.L. (1966) *Fd Technol.,* Champaigne, **20,** 1365.
Rockland, L.B. (1960) *Anal. Chem.* **32,** 1375.
Saravacos, G.D. & Charm, S.E. (1962) *Fd Technol.,* Champaigne, **16,** 78.
Speck, P., Escher, F. & Solms, J. (1977) *Lebensm.-Wiss. u.-Technol.* **10,** 308.
Vaccarezza, L.M. & Chirife, J. (1975) *J. Fd Sci.* **40,** 1286.
Vaccarezza, L.M., Lombardi, J.L. & Chirife, J. (1974) *J. Fd Technol.* **9,** 317.
Varshney, N.N. & Ojha, T.P. (1977) *J. Dairy Res.* **44,** 93.

(Received 18 January 1981)

J. Fd Technol. (1983) **18**, 125–128

Bound and free water determination by pulsed nuclear magnetic resonance

A method for data analysis in the presence of exchange

A. DI NOLA AND E. BROSIO

Summary

In the present paper the analysis of the transverse magnetization decay curves of water is discussed. Whenever the spin–echo decay curve of water does not show a single exponential behaviour and the apparent relaxation rates of bound and free component vary with the moisture content, an exchange process must be hypothesized. A method for obtaining the amount of bound and free water, in the presence of exchange, is reported. If the described procedure is applied, the amount of bound water as determined by pulsed nuclear magnetic resonance (NMR) is in good agreement with the amount of unfreezable water as determined by other techniques.

Introduction

The longitudinal and transverse magnetization decay curves of water have been extensively exploited to obtain information on water binding in many foods such as wheat flour, corn starch, casein, etc. (Leung *et al.*, 1976; Leung, Magnuson & Bruinsma, 1979), as well as in other substances like silica gel (Zimmerman, Holmes & Lasater, 1956; Zimmerman & Brittin, 1957; Zimmerman & Lasater, 1958; Woessner & Zimmerman, 1963). The results so far obtained show that the pulsed nuclear magnetic resonance (NMR) technique is very suitable for determining the amount and the mobility of free and bound water in the samples.

In many cases however the results obtained by pulsed NMR do not agree with other observations: for example, the amount of bound water in corn starch (Leung *et al.*, 1976) and in wheat flour doughs (Leung *et al.*, 1979) as determined by pulsed NMR does not correspond to the amount of unfreezable water as determined by wide-line NMR (Leung, 1975; Toledo, Steinberg & Nelson,

Authors' address: Istituto di Chimica-Fisica, University of Rome, p. le A. Moro 5, 00185 Rome, Italy.

0022–1163/83/0200–0125$02.00

1968), differential thermal analysis (DTA) (Davies & Webb, 1969) or differential scanning calorimetry (DSC) (Bushuk & Mehrotra, 1977). The discrepancies have been ascribed to a real difference between the bound water as determined by pulsed NMR and the unfreezable water.

We believe that, in most cases, a different elaboration of the NMR data can solve these discrepanices: in such a way the bound water contents determined by the different methods may be related to each other. In the present paper we report the method to evaluate the experimental data.

In the studies so far reported (Leung *et al.*, 1976, 1979) the spin–echo decay curve of water does not show single exponential behaviour. This feature was interpreted as the presence of two types of water molecules, with different relaxation times, that do not exchange magnetization. The curve was therefore fitted by the sum of two exponentials:

$$Q(t) = Q_0[a_b \exp(-\mu_b t) + a_f \exp(-\mu_f t)] \tag{1}$$

According to that, the extrapolated values at the time $t = 0$ of the two exponentials, a_b and a_f, were supposed to coincide with the relative amount of bound and free water, P_b and P_f respectively, and the rate constants μ_b and μ_f were identified with the relaxation rates $1/T_{2b}$ and $1/T_{2f}$ of the two types of water in the sample.

Let us suppose that only two species of water molecules are present, we want to point out that, even in the presence of exchange between bound and free water, it is possible that the echo decay curve does not show a single-exponential behaviour and it can still be fitted by the sum of two exponentials. In this case however the values a_b, a_f, μ_b and μ_f do not correspond to P_b, P_f, $1/T_{2b}$ and $1/T_{2f}$, respectively. The echo decay curve, will be described by a formula like Equation (1) (Zimmerman & Lasater, 1958; Woessner & Zimmerman, 1963) where

$$a_b = \frac{1}{\mu_f - \mu_b}\left(\mu_f - \frac{1}{T_{av}}\right); \qquad a_f = \frac{1}{\mu_f - \mu_b}\left(\mu_b - \frac{1}{T_{av}}\right);$$

$$2\mu_b = \left(C_1 + C_2 + \frac{1}{T_{2b}} + \frac{1}{T_{2f}}\right) - \left[\left(C_2 - C_1 + \frac{1}{T_{2f}} - \frac{1}{T_{2b}}\right)^2 + 4C_1C_2\right]^{1/2};$$

$$2\mu_f = \left(C_1 + C_2 + \frac{1}{T_{2b}} + \frac{1}{T_{2f}}\right) + \left[\left(C_2 - C_1 + \frac{1}{T_{2f}} - \frac{1}{T_{2b}}\right)^2 + 4C_1C_2\right]^{1/2};$$

$$1/T_{av} = P_b/T_{2b} + P_f/T_{2f}$$

and C_1 and C_2 are the rate constants of the exchange process. Only if $C_1 = C_2 = 0$ the a_i and μ_i coincide with the P_i and $1/T_{2i}$ respectively.

Moreover the real (P_i, T_{2i}) and the apparent (a_i, μ_i) quantities will be related by:

$$\frac{P_b}{T_{2b}} + \frac{P_f}{T_{2f}} = a_b\mu_b + a_f\mu_f \tag{2}$$

The problem, whenever the magnetization decay curves do not show a single exponential behaviour, is to ascertain whether an exchange process is present, that is to state whether the apparent quantities can be identified with the real ones. In many cases it is possible to solve the question: in fact if there is not exchange, the apparent relaxation rates of bound and free water must not vary with the moisture content of the sample and they must be equal to the measurable rates where only one type of water is present.

On the other hand, if an exchange process is present, the apparent relaxation rates may vary with the moisture content of the sample and their values may differ from those of bound or free water. For this reason, whenever a variation of one, or both, relaxation rates is observed an exchange process is to be hypothesized. This seems to occur on the reported studies on the transverse magnetization decay of water in corn starch (Leung *et al.*, 1976) and in flour doughs (Leung *et al.*, 1979). In these cases the reported amount of bound water (0.194 ± 0.01 g H_2O/g DM for corn starch and 0.62 ± 0.03 g H_2O/g DM for flour dough) measured without taking into account the exchange process clearly coincides with the quantity a_b.

However, in spite of the greater complexity of the process, in many cases it is possible to obtain the real quantities, P_i, provided that some conditions are met. Starting from Equation (2), with $P_b + P_f = 1$, we obtain:

$$P_b = (a_b \mu_b + a_f \mu_f - 1/T_{2f})/(1/T_{2b} - 1/T_{2f}) \tag{3}$$

In most cases the transverse relaxation time of free water, T_{2f}, even if reduced because of macromolecular interactions, is at least one order of magnitude greater than T_{2b}, $1/\mu_b$ and $1/\mu_f$, so that it can be neglected in Equation (3), which becomes

$$P_b = T_{2b}(a_b \mu_b + a_f \mu_f) \tag{4}$$

If the real relaxation time of bound water, T_{2b} is known, the amount of bound water can be derived from Equation (4).

Generally in water binding studies progressive amounts of water are added to the dried material and for the first additions no free water component is detected. The measured relaxation time of water in these samples can be then assumed as the relaxation time of bound water. As concerns the reported studies on corn starch (Leung *et al.*, 1976) the transverse relaxation time of bound water can be set as the average value of the relaxation times of the fast decaying component (1.43 msec) that practically coincides with the relaxation time of water in the dried sample (A. Di Nola and E. Brosio, unpubl. data). As concerns the studies on flour doughs (Leung *et al.*, 1979) the relaxation time of bound water can be taken as the measured value of the immobile fraction of water in the sample with the lowest moisture content (about 10 msec).

If the described procedure is applied to the reported data obtained for corn starch (Leung *et al.*, 1976) and wheat flour doughs (Leung *et al.*, 1979), the amount of bound water, P_b, calculated from Equation (4) results 0.28 g H_2O/g DM

and 0.34 g H_2O/g DM respectively. The amount of bound water as determined by pulsed NMR according to Equation (4) is now in good agreement with the amount of unfreezable water as determined by different techniques. In fact for corn starch wide-line NMR (Leung 1975) gives 0.26 g H_2O/g DM of unfreezable water; for flour doughs wide-line NMR (Toledo *et al.*, 1968), DTA (Davies & Webb, 1969) and DSC (Bushuk & Mehrotra, 1977) give 0.29, 0.33 and 0.30 g H_2O/g DM of unfreezable water respectively. That confirms the assumptions made.

References

Bushuk, W. & Mehrotra, V. K. (1977) *Cereal Chem.* **54,** 526.
Davies, R.J. & Webb, Y. (1969) *Chems. Ind.* **33,** 1138.
Leung, H.K. (1975) PhD thesis, University of Illinois, Urbans, Illinois, U.S.A.
Leung, H.K., Magnuson, J.A. & Bruinsma B.L. (1979) *J. Fd Sci.* **44,** 1408.
Leung, H.K., Steinberg, M.P., Nelson, A.I. & Wei, L.W. (1976) *J. Fd Sci.* **41,** 297.
Toledo, R., Steinberg, M.P. & Nelson, A.I. (1968) *J. Fd Sci.* **33,** 315.
Woessner, D.E. & Zimmerman, J.R. (1963) *J. Phys. Chem.* **67,** 1590.
Zimmerman, J.R. & Brittin, W.E. (1957) *J. Phys. Chem.* **61,** 1328.
Zimmerman, J.R. & Lasater, J.A. (1958) *J. Phys. Chem.* **62,** 1157.
Zimmerman, J.R., Holmes, B.G. & Lasater, J.A. (1956) *J. Phys. Chem.* **60,** 1157.

(Received 14 May 1982)

J. Fd Technol. (1983) **18**, 129–133

Technical note: Determination of organic nitrogen by Kjeldahl digestion using hydrogen peroxide

L. N. SRIKAR* AND R. CHANDRU

Introduction

The standard method for the determination of nitrogen is by Kjeldahl digestion, whereby the organic nitrogen is converted to ammonia by digestion with concentrated sulphuric acid in presence of copper sulphate, mercury or other suitable catalysts. The ammonia formed may be quantitated by removal from digestion mixture through steam distillation followed by titration, or nesslerization. In recent years automated systems have been employed for the estimation of nitrogen in the digestion mixture (Davidson, Mathieson & Boyne, 1970; Gehrke, Killingley & Wall, 1972; Florence & Milner, 1979; Wall & Gehrke, 1975).

The presence of a catalyst in the digestion process accelerates oxidation and completes digestion. A number of catalysts have been tested by many investigators, and mercury has been established as the most effective catalyst. For the analysis of organic nitrogen in fertilizers, animal feeds, fish and other marine products, either mercury or mercuric oxide is used as a catalyst in the A.O.A.C. (1975) standard method. However, mercury is extremely toxic and toxicity is cumulative. In addition to being expensive, mercury may cause environmental contamination and disposal problems. Consequently much research has been carried out to find an efficient catalyst of low cost and lower toxicity.

Copper sulphate alone or with potassium sulphate was used as a catalyst by Folin & Farmer (1912). Stirrup & Hartley (1975) and Williams (1973) used cupric sulphate with titanium dioxide. Zirconium oxide alone and in combination with copper sulphate has been tested by Glowa (1974). Further, selenium dioxide has also been employed as a catalyst in the Kjeldahl digestion procedure.

The use of hydrogen peroxide to accelerate the decomposition and clarification of a variety of nitrogenous products, was reported by Bradstreet (1965). Florence & Milner (1979) have described the digestion of nitrogenous materials

Authors' address: Department of Biochemistry, College of Fisheries, Mangalore 575002, India.
* To whom correspondence should be addressed.

0022–1163/83/0200–0129$02.00

with hydrogen peroxide determining the ammonia formed, using automated flow system.

In the present investigation the routine determination of organic nitrogen in fish and some other foods on a semi-micro scale by Kjeldahl digestion in the absence of a metallic catalyst have been examined and the hydrogen peroxide method is compared with the conventional copper sulphate digestion method.

Materials and methods

Apparatus

Digestion flask: 30 ml capacity, corning with B_{24} joint.

Digestor: Electrically heated multimantles for six flasks, with individual temperature regulators (Toshniwal make) and vapour manifold made of corning glass to hold the flasks and to remove acid fumes.

Distillation assembly: Paranas wagner apparatus.

Reagents

Hydrogen peroxide solution 30% G.R. grade; sulphuric acid analar; potassium sulphate; copper sulphate analar; sodium hydroxide solution 40% w/v, analar; boric acid solution 2% w/v, analar; mixed indicator solution 0.1%w/v in alcohol containing methylene blue and methyl red in 1 : 3 ratio; L—tryptophan analar (99–100.5% pure); DL—alanine analar (98.5–100.5% pure); L—cystine analar (98.5% pure).

Procedure

Hydrogen peroxide method. Dried samples of fish and other foods used in the experiment were ground and sieved through an I.S.S. standard test sieve having an aperture of 500 μ. Thirty to one hundred milligrams of samples were weighed on a pre-weighed glazed paper of size 70 × 50 mm. The samples along with the paper were transferred into digestion flask. In another digestion flask, glazed paper of the same size was introduced to serve as a blank. One hundred milligrams of potassium sulphate was added to each of the digestion flasks, followed by 2 ml sulphuric acid. To each flask 1 ml hydrogen peroxide was added and the reaction was allowed to take place at ambient temperature. The flasks were fixed to Kjeldahl digestion rack inclining the flasks at an angle of approximately 45°. The flasks were heated controlling the heat in such a manner that bumping was prevented and digestion was smooth and uniform throughout. The digestion was complete in about 30 min, when the digest was clear and colourless. For samples, where the digest was not colourless at the end of 30 min, hydrogen peroxide was added carefully dropwise down the wall of the flask to minimize effervescence, allowing the reaction to complete before

further addition of hydrogen peroxide, until the digest became permanently colourless.

Copper sulphate method. To the sample in digestion flask 100 mg of digestion mixture containing copper sulphate and potassium sulphate in the ratio 1 : 10 was added, followed by 2 ml sulphuric acid. The flask was then heated on the rack until the digest became clear and colourless. Normally the time required for digestion was 2–2.5 hr.

Macro procedure

In order to compare the semi-micro method described with that of macro method, commercial grade casein was employed. Samples containing about 1 g casein were digested both by the copper sulphate and hydrogen peroxide methods. The digested sample was made up to 100 ml and from these, aliquots of 10 ml were used for distillation.

Analysis

To the digested sample a small quantity of ammonia-free distilled water was added, and the resulting sample was transferred directly into the distillation chamber of Kjeldahl distillation apparatus, which was thoroughly cleaned previously by passing steam. The flask in which the digestion was carried out, was rinsed with small quantities of ammonia-free water and the washings transferred into the distillation chamber.

In the case of macro procedure 10 ml of the made-up solution was pippetted into the distillation chamber. Ten millilitres of 40% sodium hydroxide was added and the distillation allowed to take place by passing steam. The liberated ammonia was steam distilled into 25 ml of 2% boric acid containing two to three drops of mixed indicator. As ammonia was absorbed into boric acid, the colour of the solution changed from purple–violet to green. Distillation was continued for further 5 min and the ammonium borate was titrated against standard 0.05N sulphuric acid until the original purple–violet colour was restored. The amount of nitrogen in the sample was calculated.

Results and discussion

The results of nitrogen determination by hydrogen peroxide method and copper sulphate method are given in Table 1. The nitrogen content obtained for all the samples tested was higher in the peroxide method when compared to the values obtained by the copper sulphate digestion method. The results obtained by the semi-micro and macro procedures using commercial casein indicate that the nitrogen value obtained was comparatively high in the peroxide method than

Table 1. Organic nitrogen determination in fish and other foods by peroxide and copper sulphate digestion methods

Sample	Peroxide method		Copper (II) sulphate method	
	Nitrogen (% Mean ± s.e. mean)	*n*	Nitrogen (% Mean ± s.e. mean)	*n*
Mackerel* *(Rastrelliger kanagurta)*	3.25 ± 0.02	9	3.09 ± 0.03	8
Common carp* *(Cyprinus carpio)*	2.23 ± 0.01	6	2.14 ± 0.05	3
Tilapia* *Tilapia mossambica)*	2.42 ± 0.02	4	2.37 ± 0.04	4
Catfish* *(Tachysurus tenuispinis)*	2.66 ± 0.05	8	2.51 ± 0.03	9
Cuttle fish* *(Sepia aculeata)*	2.61 ± 0.03	12	2.49 ± 0.05	8
Egg albumin (commercial)	12.63 ± 0.05	8	12.32 ± 0.14	6
Casein (commercial) make A	13.81 ± 0.04	8	13.34 ± 0.05	7
Bengal gram	3.86 ± 0.13	4	3.39 ± 0.06	4
Green gam	4.55 ± 0.06	5	4.20 ± 0.11	4
Casein (commercial) make B				
Semi-micro	12.32 ± 0.09	23	ND	
Macro	12.30 ± 0.04	9	12.10 ± 0.03	10

* Values expressed on wet weight basis

the copper sulphate method while the results obtained were similar both by semi-micro and macro procedures, when peroxide was used for digestion.

For assessing the efficiency of digestion the amino acid tryptophan is considered a good reference standard (Florence & Milner, 1979). The recovery of nitrogen by peroxide and copper sulphate methods was examined using the amino acids tryptophan, alanine and cystine. The results in nitrogen recovery for the amino acids are given in Table 2. The recovery of nitrogen was higher in peroxide method than in the copper sulphate method. The results obtained

Table 2. Recovery of nitrogen from amino acids by peroxide and copper (II) sulphate methods

Sample	Method	*n*	Nitrogen (% Mean ± s.e. mean)	Recovery (%)
Tryptophan	Peroxide	16	13.64 ± 0.29	99.49
	Copper sulphate	10	12.82 ± 0.47	93.51
Alanine	Peroxide	3	15.52 ± 0.24	98.79
	Copper sulphate	3	15.45 ± 0.68	98.35
Cystine	Peroxide	4	11.54 ± 0.56	98.89
	Copper sulphate	3	10.72 ± 0.18	91.86

indicate that the hydrogen peroxide digestion method is superior to copper sulphate method both in accuracy and precision for tryptophan, while the two methods compare well in the case of amino acid alanine. The results obtained in the present investigation indicate that hydrogen peroxide digestion followed by steam distillation and titration compares well with the auto analysis results reported by earlier workers (Florence & Milner, 1979; Tingvall, 1978).

The hydrogen peroxide digestion followed by steam distillation of ammonia, described in the present paper is quite rapid, reliable and accurate. Therefore, it can be employed for the routine determination of total organic nitrogen in fish and other foods. By this method, not only the digestion time is minimized, compared to the copper sulphate digestion method, but costly and toxic catalysts like mercuric oxide, zirconium oxide, titanium oxide and selenium oxide, can be safely dispensed with.

References

AOAC (1975) *Official Methods of Analysis.* 12th ed. Association of Official Agricultural Chemists, Washington D.C.

Bradstreet, R.B. (1965) *The Kjeldahl Method for Organic Nitrogen.* Academic Press, London.

Davidson, J., Mathieson, J. & Boyne, A.W. (1970) *Analyst,* **95,** 181.

Florence, E. & Milner, D.F. (1979) *Analyst,* **104,** 378.

Folin, O. & Farmer (1912) *J. Biol. Chem.* **11,** 493.

Gehrke, C. W., Killingley, J.S. & Wall, L.L. Sen. (1972) *J. Ass. off. Analyt. Chem.* **55,** 467.

Glowa, W. (1974) *J. Ass. off. Analyt. Chem.* **57,**1228.

Oser, B.L. (1976) *Hawk's Physiological Chemistry,* 14th ed. Tata McGraw-Hill Co., New Delhi, India.

Stirrup, J.E. & Hartley, A.W. (1975) *J. Ass. Publ. Analysts,* **13,** 72.

Tingval, P. (1978) *Analyst,* **103,** 406.

Wall, L.L., Sen. & Gehrke, C.W. (1975) *J. Ass. off. Analyt. Chem.* **58,** 1221.

Williams, P.C. (1973) *J. Sci. Fd Agric.* **24,** 343.

(Received 23 October 1981)

J. Fd Technol. (1983) **18**, 135–141

Book reviews

Recent Advances in the Biochemistry of Fruit and Vegetables. (Phytochemical Society of Europe Symposia Series No. 19) Ed. by J. Friend and M. J. C. Rhodes.
London: Academic Press, 1981. Pp. xiii + 275. ISBN 0 12 268420 6. £24.00

This volume of thirteen reviews originated as the proceedings of a Symposium held by the Phytochemical Society in Norwich in April 1980. Unlike the majority of symposia proceedings which tend to have limited appeal and to become rapidly out-dated, this collection of essays should command a wide audience of readers among food and plant scientists. Moreover, it should serve as a useful reference to the subject for a number of years.

This book has appeared some 12 years after the publication of that major reference to fruit biochemistry edited by A. C. Hulme, namely *The Biochemistry of Fruits and their Products*, and in many ways it provides an excellent addendum to that work, since it reviews the progress that has been made in fruit biochemistry since 1970. Thus in the opening chapter J. B. Biale and the late R. E. Young discuss the respiration and ripening of fruits, including among other items up-to-date lists of those fruits showing the respiratory climacteric during ripening and those that do not. An important recent advance in fruit biochemistry has undoubtedly been the full elucidation of the pathways of biosynthesis and turnover of the key ripening hormone ethylene. It is therefore valuable to have here two complementary chapters by S. F. Yang and E. M. Beyer dealing with ethylene biosynthesis, its regulation, action and metabolism.

During fruit ripening, many changes occur in fruit enzymes, in texture, cell wall structure and in phenolic metabolism and these are variously considered here by G. E. Hobson, by M. Knee and I. M. Bartley and by M. J. C. Rhodes and his collaborators. Particular attention is given to the enzyme(s) responsible for fruit browning and there are two chapters dealing with changes in polyphenol oxidase levels by A. M. Mayer and E. Harel and with the molecular properties of tyrosinase by D. A. Robb. The molecular biology of ripening is also authoritatively discussed by D. Grierson and his co-workers, with special reference to the tomato fruit.

The title of the book under review includes vegetables as well as fruits and this explains the inclusion of chapters by C. Vance on cyanide-insensitive respiration and by T. Ap Rees and co-workers on low temperature sweetening, two phenomena which are more closely associated with tuber than with fruit tissue.

Anthocyanin colour can be extracted impartially from both fruits and vegetables and C. F. Timberlake in the one chapter on pigments reviews recent developments in the study of the anthocyanins of both types of tissue.

In summary, this is a book which has a particular appeal to food scientists concerned with post-harvest behaviour of fruits and vegetables. It also contains sufficient general plant biochemistry to be of value and reference to many other plant scientists. It has been thoroughly edited and is nicely produced, with many figures and tables and excellent indexes. Although relatively expensive for the individual purchaser, it deserves its place on the library shelf next to Hulme's *The Biochemistry of Fruits and their Products.*

J. B. Harborne

Food Composition and Nutrition Tables, 1981/82. 2nd ed. Ed. by S. W. Souci, W. Fachmann and H. Kraut. (In English, German and French.) Stuttgart: Wissenschaftliche Verlagsgesellschaft, 1981. Pp. xxiii + 1352. ISSN 0721 6912. DM 96.–.

This book provides a comprehensive reference on the composition of a wide range of foodstuffs. To make the Tables useful to as many international users as possible, the Introduction, Preface and Table of contents are written in English, German and French, with the names of the foods at the head of the columns in all three languages. All other headings are in English to save space, but there is a useful glossary of these terms in German and French at the end of the book. Dealing with 700 foodstuffs, this is the first edition to appear in the form of a computer print-out. It is envisaged that by using a computer data bank in this manner, the Tables can be readily up-dated in future. The aims of the editors are to meet the needs for information on food composition of those involved in dietetic and nutritional guidance and in the Food Industry. As the aims of the book are very similar to those of McCance and Widdowson's Composition of Foods (Paul & Southgate, 1978), it is of obvious interest to make some comparison between the two. There are a number of points on which they differ, but on the whole the two books are complementary. Each food commodity is given a full two-page spread for easy reference and the book has a useful index in English, German and French.

Data are included on foods such as tropical and Mediterranean fruit and vegetables which are now more generally available throughout Europe. Also included are a wide range of less commonly eaten oils and fats such as shark oil, and other foods which would not normally feature greatly in the diets of people in the U.K. Thus there are, for example, fifteen entries dealing with different types of edible fungi. For the first time much detailed data on the composition of certain major food components have been included, such as the fatty acid and sterol composition of fats, the amino acid composition of proteins and the

carboxylic acid composition of fruit and vegetables. In addition there is a breakdown of carbohydrates into monosaccharides, oligosaccharides and polysaccharides with particular emphasis on dietary fibre. Trace elements such as nickel, molybdenum, chromium, selenium and boron are included for the first time as well as phenolic constituents and amines such as serotonine.

Food constituents are expressed as averaged values for the edible portion and for the food as purchased. This is welcome information for research workers, as the latter data are excluded from Paul & Southgate (1978) for reasons of space saving. Another welcome feature is the addition of a range of values for food constituents of the edible portion of food. However, these data may be of more use to a research worker than to a dietitian, indeed, as far as dietitians are concerned, information providing a range of values may prove difficult to interpret in dealing on a day-to-day basis with patients. In contrast to Paul & Southgate (1978), very few cooked or made up dishes are included and this may limit the usefulness of these tables for dietitians.

Two points may render the book less useful for research workers. Firstly, there is no information on methods of sample collection or any references given where data were taken from the literature. The reason for this omission was lack of space and readers are invited to write to the editors for further information. Secondly, there is no reference to the methodology used. Although this would have considerably increased the work needed to compile the tables, not a great deal of extra space would have been required for this information, and the addition of such references would have been valuable as different methodologies may yield very different data. Despite these points, this book will provide a great deal of valuable information for nutritionists and food scientists involved in research or education.

Reference

Paul, A.A. & Southgate, D.A.T. (1978) *McCance and Widdowson's Composition of Foods*. MRC Special Report No. 297. H.M.S.O. London.

A. F. Walker

Malting and Brewing Science. Volume 1: Malt and Sweet Wort, 2nd ed. By D. E. Briggs, J. S. Hough, R. Stevens and T. W. Young.
London: Chapman & Hall, 1981. Pp. x + 387. ISBN 0 412 16580 5. £17.50.

The first publication of *Malting and Brewing Science* as a single volume in 1971 was a most welcome event. Its appearance filled a void caused because other well known English language texts on brewing were becoming out-dated. During the last decade, as in many other areas of food technology, malting and

brewing sciences and their related technologies have continued to develop apace. Thus the time is now right for this book to have been completely revised and for its second edition to be published. The decision to split the text into two volumes will make for convenient handling and durability of the books, though I suspect that it will have added significantly to the purchase price.

The first volume comprises eleven chapters. It begins by outlining malting and brewing in very general terms. Indeed this first chapter can be recommended as reading for anyone not directly concerned with brewing and its allied trades and who perhaps wishes to gain the flavour of this subject. There are then five chapters on barley and malting: these cover barley; barley grain physiology; the biochemistry of malting grain; malting conditions and malt types; and the technology of malting and kilning. The text then turns to consider brewing itself with a chapter on brewing water: this encompasses natural brewing water, desalination, deionization, water purity and effluent treatments. There then follows a new chapter on adjuncts, sugars, wort syrup and a disappointingly short section on industrial enzymes. Finally there are three chapters on sweet wort production: these discuss the chemistry and biochemistry of mashing, preparation of the dry grist (i.e. milling technology as applied to brewing) and mashing. The volume concludes with a useful appendix on units of measurement. The subject index is comprehensive and effective in practice: there is no author index as references, which are appended at the end of each chapter, are referred to by number rather than by authors' names.

The text is nicely printed with a clean readable typeface. The book is well illustrated with tables, graphs, line drawings and other figures of a generally high standard. It is remarkably free from minor typographical errors.

The book avoids the pitfall of launching itself into a detailed analysis and discussion on the current status of plant biochemistry and other aspects of general chemistry: these are left to be covered by other more specialized textbooks. This allows the subject matter to be concentrated upon those chemical and biochemical matters which are special to barley and malt and which have specific influences upon the production of wort and beer.

In this second edition the discussion on many topics has been brought up to date to include material published elsewhere in 1979 and early 1980. The following items are particularly worth mentioning: Clova malting plant, barley abrasion (physiological and technological aspects), the occurrence of nitrosamines in malt and methods for their reduction, dimethyl sulphide, effluent treatments, energy conservation, a short section on industrial enzymes, some newer methods of analysis applicable to barley and malt and newer mashing technologies with some reference to the economies of process.

In their preface the authors suggest that this text is primarily intended as a teaching aid particularly for the MSc course in Brewing Sciences at Birmingham University. It will, however, have a rather wider readership, for the undoubted quality of its text will widen its appeal to all those who are concerned with research, development and production within the malting, brewing and distilling industries.

The republication of this text in an up-dated and slightly expanded form will ensure that this volume remains as one of the standard text books for brewing and its associated industries.

R. W. Scott

Pearson's Chemical Analysis of Foods, 8th ed. By H. Egan, R. S. Kirk and R. Sawyer.
Edinburgh: Churchill Livingstone, 1981. Pp. vi + 591. ISBN 0 443 02149 X. £17.00.

The eighth edition of *The Chemical Analysis of Foods* was separated from the seventh edition by the untimely death of David Pearson, who is commemorated by the inclusion of his name in the title. We are extremely fortunate that the difficult task of revision has been undertaken by the Government Chemist and two of his staff. They have managed to achieve this revision with only a slight increase in the number of pages. As the preface notes, there has been an attempt to keep a balance between the needs of the different types of potential reader, both from the point of view of interests in the various fields of food analysis and also in terms of recognition that the book is used by workers in industrial and enforcement laboratories, *and* by students. Egan and colleagues have been particularly concerned to extend the range of instrumental techniques.

Inevitably this has meant that whereas some methods are included in working detail, other methods are only mentioned briefly with appropriate references to the works which detail those methods. It should be noted in this context that the reader should retain his or her copy of the seventh edition as certain methods have been omitted from the eighth edition. For example, tests for a number of specific oils (arachis, cotton seed, sesame and tea seed) have been omitted with the reader being referred to the seventh edition for details.

The overall layout has been retained with, as before, twelve chapters devoted to the analysis of specific commodity groups. The discussion on the determination of food additives and contaminants has been separated into two chapters although the total length remains the same. A new twenty-three page chapter has been added to provide an elementary outline to some of the main instrumental techniques.

There are frequent references provided for TLC, GC and HPLC methods for determinations and analyses, and working details of some of these analytical methods are given. Also a number of analytical techniques are added which reflect changes of emphasis in the topics considered to be of concern to the food analyst. For example there has been the addition of methods of determining histidine and histamine in scombroid fishes, and of determining erucic acid in fats and oils. In some cases however, the topic is covered only by a brief discussion and provision of literature references — for example in the cases of determination of antithyroid and anabolic compounds in flesh foods, or of soya protein in the presence of meat using the ELISA method.

Typographically this edition is perhaps rather less successful than the seventh edition. The choice of type faces for the hierarchy of headings and sub-headings, together with the line spacings adopted around those headings, is sometimes rather confusing and makes it difficult to discern the beginning of a new topic (see for example pp. 454–6). Also the proof-reading does not appear to have been as rigorous as in the previous edition. There are a number of minor spelling mistakes, but of more concern are a number of mistakes in the data presented. For example, the compositional table of fruits records the sugar content of raw bananas as 1.2%. Although the source of the data is not indicated, this table in fact seems to derive from McCance & Widdowson (1967) or Paul & Southgate (1978) in which case the sugar content should read 16.2%. Throughout the book there is difficulty in identifying the source of data presented in tables — when the reference is given it is often buried in the text some pages away from the table. It would be far better to attach the reference to each table, so that the reader can check the accuracy of the data if these are in doubt.

Nevertheless these minor shortcomings in presentation should not be allowed to detract too much from the welcome to be given to the latest edition of a well established and indispensable text.

W. F. Harrigan

Techniques in Visible and Ultraviolet Spectrometry. Volume 2: Standards in Fluorescence Spectrometry. Ed. by J. N. Miller for the UV Spectrometry Group.
London: Chapman & Hall, 1981. Pp. ix + 115. ISBN 0 412 22500 X. £8.50.

Standards in Fluorescence Spectrometry is the second publication that has arisen from the working parties set up by the UV Spectrometry Group in 1977. Originally this material was to be circulated to members only but it was subsequently realized that it would be of wider interest. The first volume published earlier in 1981 covered standards in absorption spectrophotometry.

This second volume adheres closely to the style adopted in the first volume. The first chapter provides a very brief introduction to fluorescence but in no way can this be considered even a basic introduction to the theory involved. Whether a somewhat fuller treatment is desirable is open to debate. The next three chapters, all of which were written by the editor, cover wavelength calibration, stray light and sensitivity. The first of these on wavelength calibration is well composed and contains much useful information on methods available for this most important check procedure. The chapter on stray light is short (four pages) and functional whereas that on sensitivity seems rather confused. In Table 4.1 the 'Raman method' of determining sensitivity is described as 'not very meaningful in practice', while in the summary the same method is described as the 'most suitable method'.

Inner filter effects form the basis of Chapter 5 and this topic is extensively

covered. Indeed as compared with the earlier chapters this topic receives a considerably more thorough treatment. Chapter 6 deals with the problems of temperature effects and photodecomposition, which are often not fully appreciated. The final two chapters are devoted to correction of spectra and determination of quantum yields, topics which should be understood by anybody using fluorescence quantitatively. The remainder of the book contains an Appendix of corrected spectra, which will prove useful as standards for quantum yield determinations and for spectral correction of other solutes. The style of placing the figure legends on a separate page, and in a different direction, is untidy and adds sixteen unnecessary pages to the book.

In conclusion the book forms a useful partner to Volume 1 although the balance between the topics covered is perhaps not ideal. The standard of production is good but the quality of paper used is very poor, being cream and insufficiently opaque.

R. Macrae

Developments in Meat Science, Volume 2. Ed. by R. Lawrie.
London: Applied Science, 1981. Pp. xii + 299. ISBN 0 85334 986 X. £32.00.

The multi-disciplinary nature of food science is nowhere more evident than in the science of meat and meat products. The literature is diverse in nature and scattered, and comprehensive reading habits are the privilege of the few. Authoritative review articles have a special place in the communication process, but even these can be difficult to locate. The present volume, therefore, is doubly welcome: for its own content and for the prospect that the meat scientist can look to this series as a source of timely and well chosen surveys. Its coverage is typical of the whole field, ranging from detailed anatomical and physiological characteristics of 'normal' and 'abnormal' animals to the problems of enforcing regulations and the nutritional status of individual humans. Eight topics of current interest are reviewed by leading workers from six countries: Muscular hypertrophy in cattle (Double muscling) by R. Boccard; Connective tissue by T. J. Sims and A. J. Bailey; Stress in meat animals by D. Lister, N. G. Gregory and P. D. Wariss; Post mortem changes in relation to comminuted meat products by R. Hamm; Recent studies on meat freezing by A. Calvelo; Intermediate moisture meats by D. A. Ledward; Methods of determining connective tissue free muscle protein in meat products by W. J. Olsman and P. Slump; and Meat and health by A. M. Pearson.

Some deal with the progress in established research fields while others draw together widely scattered findings in more general fields, but all are readable, concise and fully documented. This is a volume with something for most workers in the field and the whole series should be regarded as a basic source of reference in any library concerned with meat or the wider aspects of food.

S. R. Hannan

J. Fd Technol. (1983) **18,** 142

Erratum

J. Fd Technol. (1982) **17,** 649–652
Technical note: The significance of the presence of β-hydroxybutyric acid in hen eggs.

N. L. Thomas and S. W. Stock

The caption to Table 2 on p. 651 should read: **Table 2.** Fertilized eggs.

Corrected
A. J.
26 06 86.

CALL FOR RESEARCH REPORTS

Comtex Scientific

announces a new Advanced Scientific Research (ASR) Report Series in

Food Science and Technology

Comtex and the editors of* Food Science *and* Technology *invite you to submit your current research reports for publication in this ASR Series.

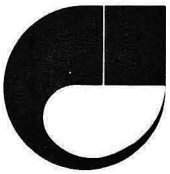
Comtex ASR reports are preliminary unedited results of your research in progress, containing all experimental details, such as instrumentation, processes and equipment, calculations and derivations, algorithms, and documentation. To ensure rapid dissemination, review consists of a yes/no decision by the Series Editor. Texts are not abridged or changed.

Early access to your technical research reports will become available as part of an ongoing subscription in microfiche when your report is accepted. ASR reports will soon be part of a complete online information retrieval system in science and technology to subscribers throughout the world.

Food Science and Technology

This series contains current research reports on all aspects of food science and engineering—production, processing, distribution, and protection. The scope of topics includes materials science of foods; quantitative analysis of food processing operations; chemistry and biology of nonconventional food production; and recent breakthroughs impacting on the industry, such as genetic engineering and enzyme technology.

For additional editorial and subscription information on ASR Report Series and other Comtex publications please write or call. You may also dial direct from your terminal to Comtex Online Information Service 212-759-8511. No online charges. (300 baud full duplex, 8 bits, 1 stop bit, no parity; press carriage return when modem on line.)



Comtex Scientific Corporation

850 Third Avenue

New York, NY 10022

212-838-7200

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

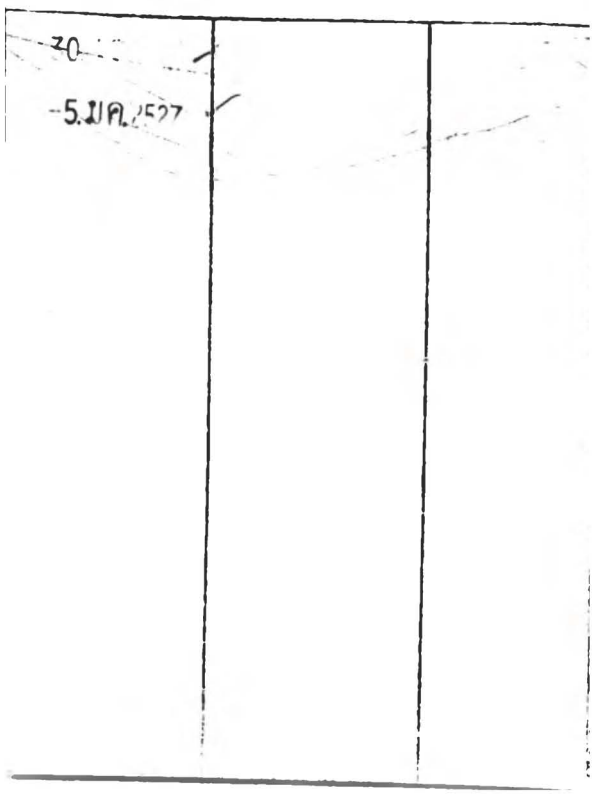
Figures. In the text these should be given Arabic numbers, e.g. Fig. 3. They should be marked on the backs with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of a figure the top should be marked with an arrow. Each figure must bear a 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



Journal of Food Technology
Volume 18 Number 1 February 1983

Contents

Printed by Adlard and Son Ltd, Bartholomew Press, Dorking