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Micro-organisms in Food, Volume 2

Sampling for Microbiological Analysis: Principles and Specific Applications

Compiled by the International Commission on Microbiological Specifications for Foods

The first edition of this book was the first comprehensive publication dealing with statistically based sampling plans, broadly applicable to the field of food microbiology. This thoroughly revised, updated and much expanded edition of *Micro-organisms in Foods, Volume 2* retains the basic format and purpose of the first. It preserves the statistical treatment, but Part 1 (Principles) now includes a discussion of the Hazard Analysis Critical Control Point concept, and Part 2 (Specific Proposals for Sampling and Sampling Plans) has been reorganized into sixteen commodity categories. This new edition will be of even greater value than the first to food microbiologists and those responsible for food quality control and public health.

The *International Commission on Microbiological Specifications for Foods,* a standing commission of the International Union of Microbiological Societies, was formed in 1962 in response to the need for international microbiological standards for foods. Its members are internationally known food microbiologists, drawn from government laboratories in public health, agriculture and food technology, and from universities and the food industry.

Contents *Principles:* Meaningful microbiological criteria for foods; Concepts of probability and sampling; Principles of drawing samples; Appropriate sampling plans; Choice of sampling plan according to purpose; Sampling plans for situations involving direct hazard from pathogens; Control at source — the hazard analysis critical control points approach (concept); The application of variables plans; Collecting and handling sample and analytical units. *Specific proposals for sampling and sampling plans:* Introduction: the application and use of criteria; Sampling plans for raw meats; Sampling plans for processed meats; Sampling plans for poultry and poultry products; Sampling plans for feeds of animal origin and pet foods; Sampling plans for milk and milk products; Sampling plans for eggs and egg products; Sampling plans for fish and shellfish; Sampling plans for vegetables, fruits and nuts; Sampling plans for soft drinks, fruit juices, concentrates and fruit preserves; Sampling plans for cereals and cereal products; Sampling plans for spices, condiments and gums; Sampling plans for fats and oils; Sampling plans for sugar, cocoa, chocolate and confectionery; Sampling plans for formulated foods; Sampling plans for natural mineral waters, other bottled waters, process waters and ice; Shelf-stable canned foods.

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Review: Availability of iron from foods

G. O. LATUNDE-DADA AND R. J. NEALE

Introduction

The availability of iron from foods has always been of interest to nutritionists, food scientists and clinicians. Considerable research has conclusively shown that iron deficiency within population groups is not caused only by inadequacy of iron intake but by a host of interacting factors affecting iron bioavailability (Patwardhan, 1961). Iron bioavailability is a measure of the amount of iron from food that is transferred from the lumen of the gut into the blood. In recent years, the measurement of available iron in foods has undergone changes with advances in measurement technology and has led to a much greater understanding. However, despite this increasing accumulation of information, the varied dietary practices, changing eating habits, varying economic situations in different parts of the world, changing agricultural practices and different processing conditions all pose new and several yet unanswered questions on the role of food iron in the problem of iron deficiency.

Factors affecting iron availability

The availability of iron is influenced by the nutritional needs of the individual, the adequacy of intestinal secretions and the various components in foods or meals interacting in the release of iron (Table 1). Though the exact effects and mechanisms of action of some of these factors are still very controversial, the overall iron available depends on the relative proportions of promoters and inhibitors which, perhaps through synergism, influence the release of iron from foods. Physiological requirements for growth and pregnancy often lead to increased absorption of iron (Loh & Kaldor, 1971; Apte & Iyenghar, 1970). Diseases that cause increased or decreased gastric and intestinal secretions, increased motility in the gastro-intestinal tract, chronic bleeding, malabsorption etc, affect iron absorption to varying degrees (Cook, Brown & Valberg, 1964; Bezwoda *et al.*, 1978).

Enhancers

The two major enhancers of iron availability are ascorbic acid and meat. The enhancing effect of ascorbic acid on non-haem iron absorption has been well demonstrated (Hallberg, 1974, 1981; Cook & Monsen, 1977). Ascorbic acid as a reducing agent maintains the food iron in a more soluble ferrous state. It also forms a soluble iron-ascorbate chelate which remains soluble as the pH increases in the proximal small intestine (Conrad & Schade, 1968). Iron from diets containing fruits and vegetables that are rich in ascorbic acid is normally highly available (Layrisse, Martinez-Torres & Gonzalez, 1974). For instance iron absorption increased from 3.7 to 10.4% in a meal of

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Table 1. Factors affecting iron availability

Corporeal	Age	
	Pregnancy	
	Iron status	
	Diseases	
Intestinal secretions	Gastric juice	
	Bile	
	Pancreatic secretions	
Dietary constituents	Fe content	
	Chemical form of Fe (Fe^{2+}, Fe^{3+}, haem, non-haem)	
	Enhancers	*Inhibitors*
	Ascorbic acid	Phytate
	Proteins	Dietary fibre
	Available carbohydrate	Tannins
	Alcohol	Oxalates
		Calcium
		Phosphorus

bread, egg and tea after the addition of 40–50 mg ascorbic acid (Callender, Marney & Warner, 1970). Also pawpaw, rich in ascorbic acid significantly increased iron absorption from maize porridge meal (Layrisse *et al.*, 1974). Other organic acids found to promote iron absorption include citric, malic, lactic, succinic and tartaric acids (Derman *et al.*, 1980; Gillooly *et al.*, 1983).

Inhibitors

Some food components have been shown to inhibit iron absorption (Table 1). These compounds form insoluble iron chelates. The reported effects of some of these substances have sometimes been contradictory. Oxalates were earlier reported to be potent inhibitors of iron absorption (Forth & Rummel, 1973), but recent studies have shown neutral (Hallberg, 1981) and positive effect (Gordon & Chao, 1984) on iron absorption in humans and rats respectively. Fibre and its components have been shown to have varying effect on iron availability. Reinhold, Garcia & Garzon (1981) showed that fibre components of wheat and maize bind iron *in vitro*. However, the addition of wheat bran fibre to breads of similar phytate content had no effect on iron absorption in the rat (Fairweather-Tait, 1982). While lignin and hemicellulose inhibited iron absorption in humans, pectin and cellulose showed the opposite effect (Gillooly *et al.*, 1984). The inhibitory effect of phytate on iron absorption has always been questionable on the basis of a wide variety of contradictory results. Monoferric phytate, which is over 60% of the iron in wheat, was found to be soluble and readily available (Morris & Ellis, 1976). The use of different components, the pH of the interacting medium and the presence or absence of chelators of iron, are but a few of the factors that could be responsible for inconsistency in these results.

Tannin is an undisputed inhibitor of iron absorption. Its effect came to light when studies revealed the inhibitory effect of tea on iron absorbed from different foods (Disler *et al.*, 1975a; Rossander, Hallberg & Bjorn-Rasmussen, 1979; Rao & Prabhavathi, 1982). Tannate in tea was shown to form insoluble complexes (Disler *et al.*, 1975b). Coffee which also contains polyphenolic compounds also inhibits iron absorption (Derman *et al.*, 1978). Similarly, a variety of plant polyphenols especially

those that are present as non-hydrolysable tannins have recently been demonstrated to be potent inhibitors of iron absorption at the level present in various vegetables (Gillooly *et al.*, 1983; 1984).

Other substances that could depress iron availability include food additives and synthetic chelators e.g. guar gum, sodium alginate in ice-cream, marmalade, mayonnaise etc. (Bernier & Hood, 1983), tetracycline (Djaldetti *et al.*, 1981), EDTA and desferrioxamine (Hwang & Brown, 1965; Cook & Monsen, 1976).

Chemical forms of iron in foods

Iron in foods is broadly classified into two groups based primarily on the mechanism of absorption. Haem iron is organically bound to the porphyrin moiety of haemoproteins and non-haem iron could be bound to food components such as protein, phytate etc. While non-haem iron exists in both plant and animal foods, haem iron is predominantly found in animal foods.

Iron in animal foods exists in the form of myoglobin, haemoglobin, ferritin, haemosiderin and low-molecular weight iron complexes. The proportions of these iron compounds vary between different meats and within tissues of the same animal. In most red meat, the predominant compounds are the haematin compounds of myoglobin and haemoglobin. Haemoproteins account for 70 and 80% of the total iron in rabbit and veal muscles respectively (Layrisse & Martinez-Torres, 1971). Haemoproteins in beef, lamb, pork and chicken were found to be 73.3, 58.8, 47.8 and 28.5% respectively (Hazell, 1982). Pigeon meat was also found to contain a high proportion of haematin compounds (Latunde-Dada, 1984). Other non-haem iron compounds in animal foods include ferrilactin in cow's milk, phosphitin in egg white (Greengard, Sentenac & Mendelson, 1964) and the organically bound iron in fish is a nucleoprotein iron/copper complex with the iron in the ferric state (Saha & Guha, 1940).

For plant foods, there is very little information about the precise chemical form of iron. Monoferric phytate was found to be the major form of iron in wheat (Morris & Ellis, 1976). A form of phytoferritin was also found in peas and lentil seeds (Ponce-Ortiz & Crichton, 1977).

Recently, Lee & Clydesdale (1979) introduced the concept of quantifying iron in foods into forms of elemental Fe^{2+}, Fe^{3+}, soluble, ionic and complexed iron. The relative proportions of these forms in foods especially plant products could be of importance in the bioavailability of the iron present.

Estimation of available iron in foods

The introduction of radioisotopes has led to considerable advancement in iron absorption determinations. Intrinsic or biosynthetic labelling was first introduced by Moore & Dubach (1951) and successfully exploited by Martinez-Torres & Layrisse (1974). The disadvantages of intrinsic labelling include the cost and tedious work of labelling foods. It is also of limited application to studies of iron availability from composite meals. This is of particular importance since food components have been found to interact with each other to produce an effect on the overall availability of iron from a meal (Layrisse, Martinez-Torres & Roche, 1968).

The greatest breakthrough occurred when the extrinsic labelling was introduced and validated by comparison with the intrinsic technique (Layrisse & Martinez-Torres, 1972; Bjorn-Rasmussen, Hallberg and Walker, 1972). This 'extrinsic tagging' involves

Table 2. *In vivo* (animals and humans) iron availability techniques

Technique	Duration of study	Comments	Reference
Chemical balance	Days, weeks	Incomplete faecal collection, contamination problems	Heinrich (1970)
Serum iron curves	Hours	High unphysiological doses of iron	Bothwell & Finch (1962)
Plasma tolerance curves	Hours	Physiological dose of ^{59}Fe, ^{55}Fe	Bothwell *et al.* (1955)
Red cell radioiron	Days	Variations in total blood volume estimations	Pitcher *et al.* (1965)
Whole Body retention of radioiron	Days, weeks	Expensive instrument	Schiffer *et al.* (1964)
Haemoglobin regeneration	Days, weeks	Often animal studies	Pla & Fritz (1970)
Unabsorbed radioiron (intragastric dosing)	Hours	Simple, fast and cheap	Wheby & Crosby (1963) Bogunjoko *et al.* (1983)
Intestinal segments tubes and all fractions	Minutes to hours	Animal studies only	Manis & Schacter (1962)

mixing a trace amount of radiolabelled iron with the test when there is complete isotopic exchange between the extrinsic tag and the endogenous iron in the diet (Hallberg & Bjorn-Rasmussen, 1972). It is particularly useful for estimating the iron available from foods eaten in different combinations. This led to the concept of 'the common pool' of iron absorption which assumes that the non-haem iron compounds in various foods in a meal could be uniformly labelled by an extrinsic tag and that absorption of non-haem iron takes place from this common pool (Hallberg, 1974). Biosynthetically labelled haemoglobin could also be used to label the haem compounds in a meal forming another separate pool of haem compounds (Layrisse & Martinez-Torres, 1972). Problems with this technique are the possibility of incomplete exchange of the tracer in some food systems and problems associated with the mixing of food to obtain a homogeneous sample. For instance the iron storage compounds in meat ferritin and haemosiderin were not thought to undergo complete exchange with the extrinsic label (Martinez-Torres, Renzi & Layrisse, 1976). Unmilled, unpolished rice also did not undergo exchange, no doubt due to impaired diffusion of iron across the dense outer layer (Bjorn-Rasmussen, Hallberg & Walker, 1973).

Intersubject variability is another problem associated with iron availability studies. This is, however, corrected for by the use of a reference dose of 3 mg ferrous ascorbate to measure the independent absorptive capacity of the individual (Layrisse *et al.*, 1969). Thus, the relationship between the two absorption measurements (meals/reference dose) is an index of bioavailability of the non-haem iron in a meal (Bjorn-Rasmussen *et al.*, 1976). Magnusson *et al.* (1981) proposed that the bioavailability of the non-haem iron be expressed as the absorption value that corresponds to a reference dose absorption of 40%, i.e., the absorption value of subjects with borderline iron deficiency. Ethical problems associated with the use of radioisotopes in humans are now being overcome by the use of stable isotopes especially in vulnerable infants and pregnant women (Janghorbani & Young, 1980; Fairweather-Tait, Minski & Richardson, 1983).

Several *in vivo* techniques have been developed (Table 2). As these different techniques do not necessarily provide the same information, results are often related to a reference iron salt. Iron bioavailability from soya products and $FeSO_4$ determined by employing different techniques are shown in Fig. 1 (Latunde-Dada, 1984). The contro-

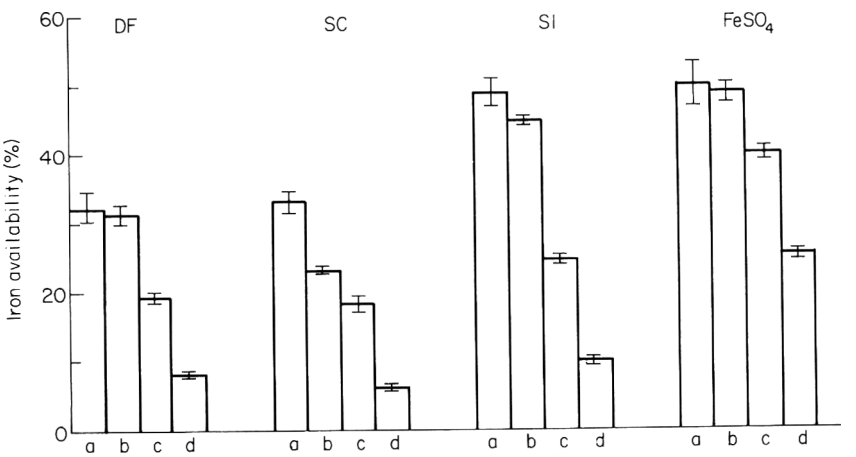

Figure 1. Iron availability from defatted soya flour (DF), soya concentrate (SC), soya isolate (SI) and $FeSO_4$ by different techniques. (a) Hb regeneration, (b) ^{59}Fe balance method, (c) 2 hr intragastric dosing technique (iron depleted rats), (d) 2 hr intragastric dosing technique (iron replete rats).

versial issue in iron availability studies is the use of laboratory animals as models for predicting iron bioavailability for humans. However, a recent survey of the literature showed a highly significant correlation ($r = 0.94$) between rats and human iron availability values for some food items (Mahoney & Hendricks, 1984).

In vitro *estimation of chemically available iron*

In vitro estimation of iron availability involves the simulation of physiological conditions of digestion. Usually peptic and pancreatic digestions are carried out and the iron estimated as ionizable (Narasinga-Rao & Prabhavathi, 1978), soluble (Jacobs & Greenman, 1969) or dialysable iron, (Miller *et al.*, 1981). Though the '*in vitro*' methods will not duplicate '*in vivo*' conditions and cannot obviously predict 'absorption' it is useful for screening large numbers of samples, and has the advantages of low cost, speed and reduced variability.

The regulation and mechanism of iron absorption

After its release from foods, and the involvement of various intralumina! factors discussed earlier, iron is then available to the absorptive surface of the gastro-intestinal tract. The absorption of iron is a physiologically-regulated process that serves as a primary mechanism for controlling body iron balance. Despite many years of intensive studies, the mechanisms by which the intestinal mucosa regulates iron absorption remain unknown. One constant observation however, is that absorption increases during periods of iron deficiency and decreases when body iron stores are in excess (Conrad & Crosby, 1963).

Several theories have been put forward in an attempt to explain the regulation of iron absorption. The mucosal ferritin concentration was thought in earlier studies to control the amount of iron absorbed (Granick, 1946; Conrad & Crosby, 1963) but this only functions as an intracellular storage compartment for dealing with excess iron in the cell. Other proposed regulatory substances include the presence of endogenous or exogenous ligands capable of binding iron to form absorbable low-molecular weight complexes (Saltman, 1965) and the presence of gastroferrin in gastric juice which would inhibit excess absorption of iron in normal subjects. Thus reduced levels of gastroferrin would permit enhanced absorption in iron deficiency (Davis, Luke & Deller, 1966).

A recent modification of Saltman's concept was proposed by Huebers *et al.* (1983) who postulated a model of iron uptake and regulation which involves mucosal transferrin as a shuttle protein for iron absorption. Apotransferrin present in the secretions of the duodenal and jejunal mucosa reacts with food iron to form transferrin. The transferrin bound iron is taken up into the mucosal cell without appreciable degradation probably by an endocytotic process. In the cell, the iron released from transferrin can either enter the blood or be deposited in the mucosal ferritin. Small amounts of tranferrin iron could escape into the circulation in a physiologically intact form. In iron deficiency, the release of iron to the blood is predominant, whilst in iron overload, deposition as ferritin is prevalent. The iron-depleted transferrin is then returned to the intestinal lumen to continue the shuttling process. This proposal has been supported by Nunez & Glass (1983) and Ciechanover *et al.* (1983). However, the source of the mucosal transferrin is still under investigation.

Haem iron is absorbed by a different mechanism from inorganic iron. Conrad *et al.* (1967) and Weintraub *et al.* (1968) proposed a theory that haem is taken up by the mucosal cells after it has been released from its globin combination by proteolytic

enzymes i.e., without being released from the porphyrin ring. In the mucosal cell, the iron is liberated by a haem-spitting enzyme, xanthine oxidase (Dawson, Rafal & Weintraub, 1970). Iron is released from the absorbed haem faster in iron deficiency and absorption is limited by the transfer capacity of the cell rather than the uptake of haem into the cell (Wheby, Suttle & Ford, 1970). In the cell, the iron released from the porphyrin ring enters the same pool as absorbed inorganic iron and it is transferred by the same carrier (Turnbull, 1974). However, studies of Hazell, Ledward & Neale (1978) and Hazell *et al.* (1980) showed that the *in vitro* digestion of meat iron does not lead to the formation of low molecular weight non-haematin compounds. It has been suggested that the absorption of pure isolated haemoglobin is perhaps different from the haemoproteins in a 'meat environment'.

Iron in the intestinal lumen was reported to adsorb to specific receptors in the brush border of the mucosal surface (Greenberger, Balcerzak & Ackerman, 1969; Kimber, Mukherjee & Weller, 1973). The number of the receptors increases in iron deficiency especially in the distal section (Linder *et al.*, 1975). Endocytosis has also been demonstrated in neonatal rats and piglets but not in adults (Gallagher, Mason & Foley, 1973; Furugouri, 1977). Although inconclusive, many studies have provided information to support the concept that iron in the mucosal cytosol is bound to one or more specific carriers which regulate its passage across the cell. Such carriers include transferrin (Halliday, Powell & Mach, 1976), transferrin-like protein (Pollock & Lasky, 1975), and low-molecular weight compounds chelated to amino acids (Linder & Munro, 1977). Iron bound to these carriers is delivered to plasma transferrin at the serosal side by a process which probably involves a receptor in the cell membrane and could be independent of energy. The exact mechanism of this transfer process is still not resolved. Iron that is not transferred into the blood is combined with apoferritin and stored as ferritin (Linder *et al.*, 1975).

Availability of iron from common foods

In general, food iron of animal origin with a few exceptions has been shown to be better absorbed than that of vegetable origin (Layrisse & Martinez-Torres, 1971; Martinez-Torres & Layrisse, 1973). This is primarily due to the presence of haem compounds in most meat and fish products. The iron available from soya protein (a vegetable) is high and compares well with that from meat products (Layrisse *et al.*, 1969; Sayers, Lynch & Jacobs, 1973). However, this has been contradicted in some studies (Ashworth & March, 1973; Bjorn-Rasmussen *et al.*, 1973). The role of soya protein in iron nutrition has become of particular interest recently when a series of studies demonstrated inhibitory effects on iron absorption of soya products in semisynthetic meals. (Cook, Morch & Lynch, 1981), cereal-soya blended food (Morch *et al.*, 1981) and in meat products (Hallberg & Rossander, 1982; Latunde-Dada & Neale, 1984). This is of importance in iron nutrition because of the increasing use of soya protein as extenders and substitutes in various food products.

The availability of iron to humans and rats from some food items is shown in Table 3. The variability of results could be due to differing dose levels, processing conditions, methodologies or response parameters. The importance of interaction between food components has been stressed and bioavailability measurements are now being made on composite meals (Hallberg, 1981).

The major promoters of non-haem iron absorption are ascorbic acid and meat, and it is now possible to predict with some certainty the amount of non-haem iron that will

Food	Methodology*	Iron intake (mg)	Bioavailability (%) Mean (Range)±s.d.	Reference
Wheat	H–I	3.2	7.9 (0.4–22.5)	Layrisse *et al.* (1969)
Wheat rolls	H–E	1.2	24.4±4.3	Hallberg *et al.* (1977)
Brown bread	H–I	—	1.3–18.6	Callender & Warner (1968)
Wheat bread	H–I	0.3	29.3±12.4	Bjorn-Rasmussen *et al.* (1972)
	H–E	0.3	27.2±11.1	Bjorn-Rasmussen *et al.* (1972)
Rye bread	R-increase in body iron	6.28/25 d	69.7±3.98	Bing (1972)
Oat flour	R-Hb repletion	—	RBV 21	Fritz *et al.* (1970)
Wheatgerm	R-Hb repletion	—	RBV 53	Fritz *et al.* (1970)
Maize	H–I	4	5.9(0.2–14.8)	Layrisse *et al.* (1969)
Maize	H–I	4	4.1(1.9–8.6)	Martinez-Torres & Layrisse (1971)
Maize	H–I	0.5	3.9(3.3–4.6)	Cook *et al.* (1972)
	H–E	0.5	3.8(3.2–4.5)	Cook *et al.* (1972)
Rice	H–I	2	0.9	Layrisse & Martinez-Torres (1971)
Rice (polished)	H–I	—	4.5±1.1	Hallberg (1974)
	H–E	—	5.2±1.3	Hallberg (1974)
Lettuce	H–I	1.2	5.8(1.1–18)	Layrisse *et al.* (1969)
Spinach	H–I	2.0	1.7(0.3–4.8)	Layrisse *et al.* (1969)
Pulses	R-normal	3.1	4.5–9.1	Ortaliza *et al.* (1974)
Eggs	H–I	1	4.2(1–15)	Callender (1971)
Eggs+Orange juice	H–I	1	10.8(3–25)	Callender (1971)
Egg-omelet	H–I	4	1.5±1.23	Bjorn-Rasmussen *et al.* (1972)
	H–E	4	1.46±1.23	Bjorn-Rasmussen *et al.* (1972)
Egg yolk	R-Hb repletion	—	RBV 33	Fritz *et al.* (1970)
Whole egg	R-Hb repletion	—	RBV 30	Morris & Greene (1972)
Fish muscle	H–I	1–2	12	Layrisse (1970)
Breast milk	H–I		20.8(2.2–50.2)	MacMillan *et al.* (1976)
Cow's milk	H–I		13.6(1.7–34.2)	MacMillan *et al.* (1976)

Potatoes	R–I	1.6	15.2±2.7	Fairweather Tait (1983)
	R–E	1.6	49.6±1.1	
Spinach	R–I	—	73.8±1.52	Van Campen & Welch (1980)
Spinach	H–E	—	1.4	
Carrot	Red cell	—	9.6	
Potato	Incorporation	—	11.5	Gillooly *et al.* (1983)
Tomato	Incorporation	—	22.4	
Cauliflower	Incorporation	—	26.3	
Cabbage	Incorporation	—	32.7	
Soya beans	H–I	3–4	11	Layrisse (1970)
	H–I		2.6±0.7	Hallberg (1974)
	H–E		2.7±0.7	Hallberg (1974)
Boiled beans	H–I	0.5	19.0±3.3	Ashworth *et al.* (1973)
Baked beans	H–I		12.0±1.8	Ashworth *et al.* (1973)
Mature beans	R–I	46.9	34.4±6.1	Welch & Van Campen (1975)
Immature beans		32.1	48.4±4.4	Welch & Van Campen (1975)
Isolate	R-Hb repletion	61.0	6.10±1.53	Steinke & Hopkins (1978)
Soya concentrate	R-Hb repletion		29.1±4.5	Neale (1984)
Soya flour	R–E	—	42.1±1.25	Neale (1984)
Soya concentrate	R-E		49.0±2.17	Neale (1984)
Soya isolate	R–E	—	49.0±3.61	Neale (1984)
Chicken meat, raw	R–I	33	18.3±1.5	Bogunjoko *et al.* (1983)
Chicken liver raw	R–I	3	15.3±1.8	Bogunjoko *et al.* (1983)
Veal muscle	H–I	2–4	21.5	Martinez-Torres & Layrisse (1973)
Veal liver	H–I	4	11	Martinez-Torres & Layrisse (1974)

*H, humans; R, rats; I, intrinsic; E, extrinsic label.
RBV, Relative Biological Value.
s.d., Standard Deviation.

be absorbed from different diets depending on the relative amounts of these two major enhancing agents. Thus when the daily ascorbic acid level is less than 25 mg or the meat, poultry or fish is less than 30 g, the availability is low (5%). When more than 75 mg ascorbic acid or more than 90 g meat, poultry or fish are present, availability is high (20%) by subjects with no iron stores (Monsen *et al.*, 1978). Hallberg (1981) also related the amount of iron absorbed to the energy content of the meal, i.e. bioavailable nutrient density defined as the amount of iron absorbed (in mg) from a meal per unit energy (1000 kcal) by subjects who are borderline in iron deficiency.

An important consideration is the effect of processing which can affect the chemical forms of iron in foods thereby influencing its bioavailability (Lee & Clydesdale, 1981). This could involve alterations in the proportions of the soluble, insoluble, complexed, ionic, ferrous or ferric iron in food. Thus iron profiling by measurement of iron valence, solubility and complexation, especially with changes during processing, was compared and correlated with bioavailability values (Lee, 1982). For instance brewing of corn-sorghum beer increased the solubility and bioavailability of iron (Derman *et al.*, 1980) possibly due to the lactic acid produced. The baking of soda biscuits however, decreased bioavailability as high pH rendered the iron insoluble (Lee & Clydesdale, 1980).

Precipitation and insolubilization of iron in meat during cooking have been shown to decrease availability (Bogunjoko, Neale & Ledward, 1983). Iron solubility and availability from pigeon meat were also reduced by about 50% after cooking (Table 4) (Latunde-Dada & Neale, 1985). Cooking denatures the haemoproteins in meat which are then precipitated. Even a mild form of processing such as freeze drying was shown to decrease the availability of iron from beef in rats (Mahoney *et al.*, 1979). It is therefore very important that availability is measured in foods as consumed i.e., after undergoing the various processing procedures to take account of possible changes.

Table 4. Soluble and available iron from raw and cooked pigeon meat

	Percentage soluble ^{59}Fe from:			
	Raw meat		Cooked meat	
Digestion conditions	Mean	s.e.	Mean	s.e.
1. Pepsin (EC 3.4.23.1) hydrochloric acid, pH 1.5, 90 min digestion at 37°	88.8	0.5	56.1	2.3
2. Pepsin/HCl(as 1) followed by neutralization with sodium bicarbonate	37.4	2.0	21.1	1.6
3. Pepsin/HCl(as 2) + pancreatin-bile extract, 2 hr digestion at 37°	60.4	1.1	26.6	1.2
In vivo ^{59}Fe absorption in rats (% dose)	21.9	2.98	10.9	2.0

In summary therefore, the availability of iron from food is determined by a wide range of factors which include the physiological state of the individual, the presence of inhibitors, enhancers and the processing conditions. Most techniques for estimating iron from foods have limitations and attempts are being made at solving some of these shortcomings. However, the increasing use of extrinsic tagging in iron availability studies will obviously provide more relevant and useful information on this aspect of iron nutrition. The unresolved mechanism of iron absorption still requires further

investigation. On the practical side however, the diagnosis and prevention of iron deficiency are of utmost necessity, the increased consumption of enhancers of iron availability and a programme of appropriate fortification are of immense importance in this consideration.

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Derivation of physical properties of muscle tissue from adiabatic pressure-induced temperature measurements

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Summary

A novel approach to investigating the physical properties of meat is described, involving measurement of the temperature change induced by adiabatic compression. After satisfactory tests on water, the method was used to obtain data for the thermal expansivity, density and ratio of specific heats of muscle tissue between about −24 and +16°C.

Introduction

The temperature change produced when a material is compressed adiabatically is related to its thermal expansivity (α), specific heat at constant pressure (C_p) and density (ρ) by the thermodynamic relationship (e.g., Zemansky, 1957):

$$\left(\frac{\partial T}{\partial P}\right)_s = \frac{T\alpha}{C_p\rho} \tag{1}$$

where T is temperature (°K) and P is pressure. By measuring this temperature change, thermal expansivity can be derived, provided the other physical properties are known.

The thermophysical properties of muscle tissue are largely determined by the properties of its major component, water. However evidence exists that expansivity is an exception to this. For example, Jarvis (1971), in measuring the variation of density with temperature for beef muscle, obtained a mean thermal expansivity between 5 and 30°C which was more than double that for water. In order to investigate this further, thermal expansivity was determined more accurately from measurements of $(\partial T/\partial P)_s$, using equation (1). Thermal expansivity is of particular relevance to both the theory of propagation and attenuation of ultrasound and to the development of pressure in processes such as freezing, in which thermal expansion is restricted. Internal pressures as high as 60 bars can be developed in meat during freezing as a consequence of the expansion of the inside region, as it freezes, being restricted by the already frozen outer region (Miles & Morley, 1977).

The ratio C_p/C_v of the specific heats at constant pressure and constant volume can also be derived from $(\partial T/\partial P)_s$ measurements since thermodynamics (e.g., Zemansky, 1957) gives:

$$\gamma - 1 = \frac{T\alpha^2}{C_p\rho K_s},$$

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where $\gamma = C_p/C_v$ and K_s = adiabatic compressibility. Substituting α from equation (1) gives:

$$\gamma - 1 = \frac{C_p \rho}{K_s T} \left(\frac{\partial T}{\partial P}\right)^2 \tag{2}$$

For liquids $K_s = 1/U^2\rho$, where U is the velocity of sound, this relationship being also approximately valid for semi-solids such as meat (Miles, 1974). Hence the specific heat at constant volume can be obtained from the more readily measured specific heat at constant pressure.

The variation of density with temperature can also be derived from $(\partial T/\partial P)_s$ measurements. Thermal expansivity is the fractional change in density with temperature i.e.,

$$\alpha = -\frac{1}{\rho}\frac{\partial \rho}{\partial T}.$$

Substituting this in equation (1) and re-arranging gives:

$$\frac{C_p}{T}\left(\frac{\partial T}{\partial P}\right)_s = -\frac{1}{\rho^2}\frac{\partial \rho}{\partial T}.$$

Integrating with respect to T between T_0 and T gives:

$$\frac{1}{\rho} - \frac{1}{\rho_0} = \int_{T_0}^{T} \frac{C_p}{T}\left(\frac{\partial T}{\partial P}\right)_s dT. \tag{3}$$

This equation enables the variation of the density of muscle tissue with temperatures in the freezing range to be obtained, for which there is a scarcity of published data.

Measurement of $(\partial T/\partial P)_s$

Pressure changes were produced by a dead-weight pressure tester (Bundenberg), accurate to within 0.05%. This was connected via hydraulic tubing to a stainless steel sample chamber of approximately 130 ml capacity, submerged in a constant temperature bath (Fig. 1). The sample temperature was measured with a shielded copper-constantan thermocouple, the emf of which was amplified using a dc chopper amplifier (Ancom, type 15C-3a). The output was measured on a digital voltmeter and recorded on a chart recorder. The thermocouple was calibrated against a platinum resistance thermometer (Tinsley), which itself was calibrated at the National Physical Laboratory, England to ± 0.01°C. The calibration was checked at intervals during the course of the experiments and found to change by $< 1\%$.

Precautions were taken to prevent possible contamination of the sample with hydraulic oil. Muscle samples were either sealed in a cling film wrapping or vacuum packaged. Measurements were also made on water, principally to check the method of measurement. Here oil contamination was prevented by positioning a thin neoprene rubber membrane beneath the lid of the sample chamber, and the hydraulic pipe connecting the pressure tester to the sample chamber was partially filled with water. On comparing measurements with and without the membrane in place, it was found that the membrane produced no measurable reduction in the pressure-induced temperature.

When the sample had reached thermal equilibrium with the constant temperature bath, it was subjected to a sudden increase in pressure (ΔP), which was maintained for

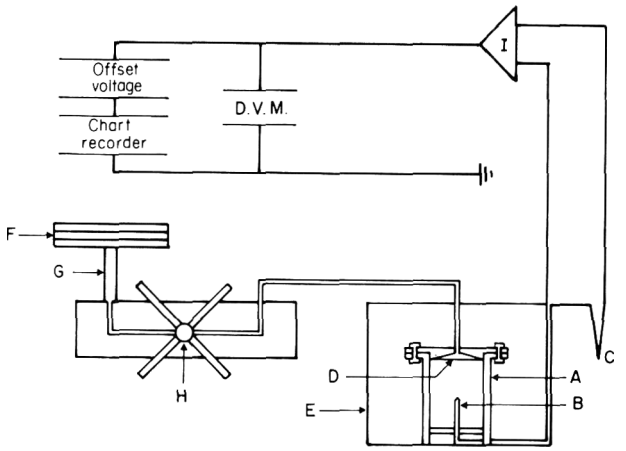

Figure 1. Measurement of $(\partial T/\partial P)_S$. A, pressure chamber, containing sample; B, thermocouple; C, ice junction; D, rubber membrane; E, constant temperature bath; F, pressure weights; G, piston unit; H, screw press; I, amplifier.

the duration of the adiabatic period, and then released. Typical temperature change responses are shown in Fig. 2. For water, the initial sharp peak obtained immediately after a pressure change, was attributed to a pressure-induced temperature change in the thin layer of epoxy-resin coating the thermocouple junction. Departure from adiabatic conditions was indicated by a decrease or increase in the sample's temperature from its steady pressure-induced value, due to heat loss or gain between the sample and its surroundings. Adiabatic conditions prevailed for generally more than a minute, and for several minutes in the freezing range. For frozen tissue below about −10°C, its high

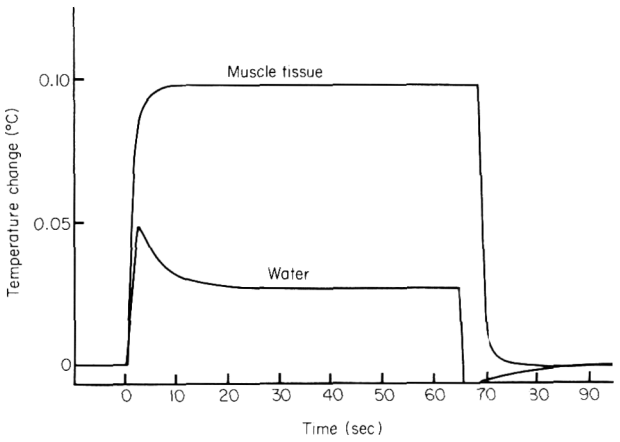

Figure 2. Typical pressure induced temperature changes, for a pressure change of 60 bars.

thermal diffusivity resulted in an adiabatic period of less than a minute, and at −20°C of only half a minute.

Samples

(a) *Water*. Purified by distillation and degassed under vacuum.

(b) *Muscle tissue.* Different samples of *M. semitendinosus* (pork, beef, lamb), trimmed of surface fat and with end pieces removed so as to fit into the sample chamber (depth 4.5 cm, diameter 6 cm). Table 1 shows the compositions of the samples, determined by standard methods.

Table 1. Compositions of the *M. semitendinosus* samples

Sample number	Type	% Water	% Fat
1	Pork	73.4	4.3
2	Pork	73.7	4.6
3	Pork	74.2	4.1
4	Pork	75.1	4.4
5	Pork	75.4	3.2
6	Beef (Calf)	74.7	2.8
7	Lamb	75.0	3.8
8	Pork	77.1	2.9
9	Pork	76.0	1.1

Results and discussion

(a) Water

The method was checked by comparing experimental $(\partial T/\partial P)_S$ values with those predicted from equation (1), using known properties of water (α and ρ from Kell, 1967). For an adiabatic temperature change ΔT, induced by a pressure change ΔP, $(\Delta T/\Delta P)_S$ was found to increase linearly with ΔP over the pressure range considered (ΔP from 20 to 100 bars). Hence $(\Delta T/\Delta P)_S$ for $\Delta P = 0$, i.e. $(\partial T/\partial P)_S$ at atmospheric pressure, was readily obtained from the regression equation of $(\Delta T/\Delta P)_S$ on ΔP (Table 2).

It can be seen from Table 2 that there is close agreement between experimental and predicted values of $(\partial T/\partial P)_S$ at atmospheric pressure.

Table 2. $(\partial T/\partial P)_S$ of water, 10^{-4}°C/bar. Variation with ΔP, $(\Delta T/\Delta P)_S = A - B\Delta P$ and comparison with predicted values for $\Delta P = 0$

Temp. (°C)	Regression Coefficients		Predicted $\frac{\partial T}{\partial P}$ $\Delta P = 0$	Difference %
	$B \times 10^2$ (Standard error)	A		
2.6	0.2043 (s.e. 0.0071)	−0.1499 (s.e. 0.0048)	−0.1483	−1.1
7.5	0.1861 (s.e. 0.0052)	0.3511 (s.e. 0.0035)	0.3561	−1.4
12.1	0.1046 (s.e. 0.0104)	0.8041 (s.e. 0.0062)	0.7852	+2.4
17.1	0.1283 (s.e. 0.0324)	1.2093 (s.e. 0.0140)	1.2151	−0.5

(b) Unfrozen muscle tissue

For pressure changes (ΔP) between 20 and 100 bars, $(\Delta T/\Delta P)_S$ increased slightly with ΔP, by on average 1% per 25 bars. Values of $(\Delta T/\Delta P)_S$ for $\Delta P = 0$ were obtained

with standard errors averaging 2%, from linear regression of $(\Delta T/\Delta P)_s$ on ΔP (Table 3). Thermal expansivities were then calculated from equation 1, taking values for C_p from Riedel (1978) and ρ from Jarvis (1971).

Table 3. $(\partial T/\partial P)_s$ of unfrozen muscle tissue, 10^{-4}°C/bar

Temp. (°C)	Sample number	$\left(\frac{\partial T}{\partial P}\right)_s$	Temp. (°C)	Sample number	$\left(\frac{\partial T}{\partial T}\right)_s$
−0.1	2	1.026	7.3	2	1.620
0.0	6	0.957	7.9	4	1.587
0.0	1	1.025	8.0	5	1.597
0.0	5	1.048	8.1	1	1.650
0.1	7	0.938	8.7	3	1.787
0.1	3	1.152	9.5	6	1.614
0.4	4	1.014	10.0	7	1.751
1.6	4	1.087	10.3	4	1.727
1.8	3	1.284	10.6	2	1.814
3.2	4	1.234	11.7	3	2.000
3.8	2	1.323	11.9	5	1.883
3.8	5	1.301	12.9	4	1.899
4.0	1	1.352	14.0	1	2.034
4.3	4	1.302	14.4	6	1.932
5.0	6	1.267	14.4	2	2.157
5.0	3	1.515	14.9	3	2.198
5.1	7	1.364	14.9	4	2.044
5.8	4	1.407	15.3	7	2.125
6.9	4	1.487	16.2	5	2.157

On comparing the thermal expansivities of muscle tissue and water (Fig. 3), it can be seen that muscle tissue has a higher expansivity, which does not become zero around 4°C. However both muscle tissue and water have large and similar variations of expansivity with temperature. Mendez & Keys (1960), in measuring the densities of mammalian muscle, obtained mean expansivities between 22 and 36°C of 3.44 (s.d. 0.85)$\times 10^{-4}$ for rabbit and 3.90 (s.d. 0.57)$\times 10^{-4}$/°C for dog. Extrapolation of the regression curves of Fig. 3 gives a mean expansivity between 22 and 36°C of 3.73 (s.d. 0.24)$\times 10^{-4}$/°C, which is similar to the values of Mendez & Keys. The density measurements of Jarvis (1971) for beef muscle gave mean expansivities of 2.23 (s.d. 0.37)$\times 10^{-4}$ between 5 and 10°C and 3.66 (s.d. 0.66)$\times 10^{-4}$/°C between 10 and 15°C. The corresponding mean expansivities obtained from $(\partial T/\partial P)_s$ measurements are 2.13 (s.d. 0.10)$\times 10^{-4}$ between 5 and 10°C and 2.56 (s.d. 0.10)$\times 10^{-4}$/°C between 10 and 15°C. The data are in close agreement between 5 and 10°C, but between 10 and 15°C, Jarvis's values are higher.

The ratio of specific heats (γ) was calculated from equation 2, taking values for the velocity of sound in muscle tissue from Miles & Fursey (1977). The γ values obtained are shown in Fig. 4. No data for muscle tissue could be found in the literature for comparison. However Sehgal & Greenleaf (1982), in fitting their theoretical model for the absorption of ultrasound to experimental values, obtained indirectly, values of $\gamma - 1$ for mammalian kidney, heart and liver at 37°C of 7×10^{-4}, 7×10^{-4} and 9×10^{-4} respectively. Extrapolation of the regression curve of Fig. 4 to 37°C gives $\gamma - 1 = 4.6\times 10^{-2}$, which is ~ 60 times greater than the values given by Sehgal & Greenleaf's hypothetical model for ultrasonic attentuation. Clearly in this respect, this data is in

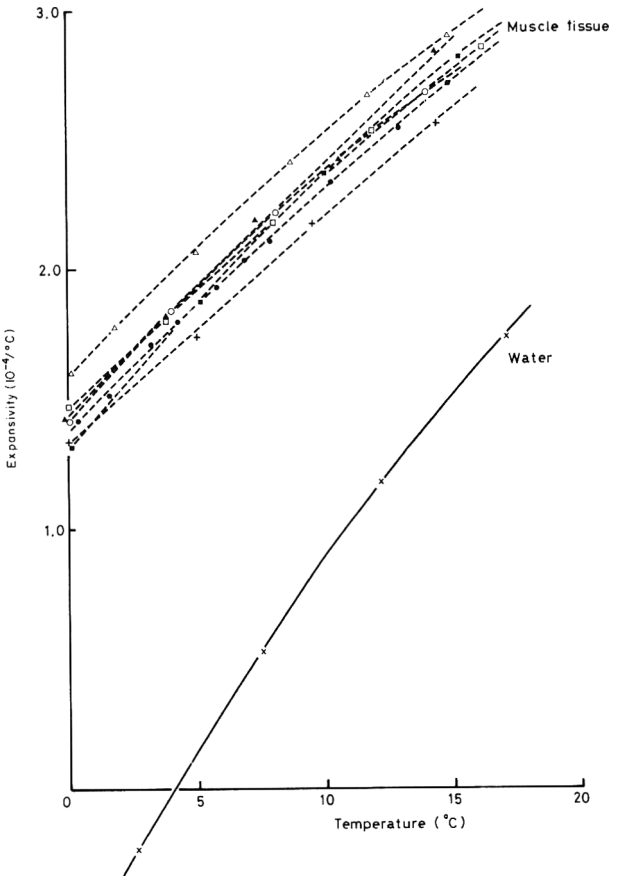

Figure 3. Thermal expansivity of unfrozen muscle tissue and water.
Symbol: O ▲ △ ● □ + ■
Sample no: 1 2 3 4 5 6 7.

conflict with their model and the assumptions they made concerning the mechanism by which sonic energy is dissipated in tissue.

(c) Frozen muscle tissue

For applied pressure changes (ΔP) in the range 10–40 bars, $(\Delta T/\Delta P)_s$ decreases linearly with increasing ΔP, mean changes per 10 bars being 0.5% above −4°C. 3% below −15°C and much greater ($\sim 2\times10^{-4}$°C/bar^{-1}) in the intermediate temperature range. Regression of $(\Delta T/\Delta P)_s$ on ΔP gave values for $\Delta P = 0$ with a mean standard error of 0.005×10^{-2}°C/bar^{-1} (Fig. 5).

The variation of the density of frozen tissue with temperature was obtained from equation (3) by integrating the regression polynomials fitting the variation of the function $(\partial T/\partial P)_s\, C_p/T$ with T. Specific heat (C_p) was obtained from Riedel (1978) and the density of unfrozen tissue just above the initial freezing point was taken to be 1078 kg/m^3. It can be seen (Table 4) that density decreased during freezing, reaching a minimum of 1020 kg/m^3 around −8.5°C (corresponding to $(\partial T/\partial P)_s = 0$) and thereafter increasing slightly with lowering temperature. The density data quoted graphically

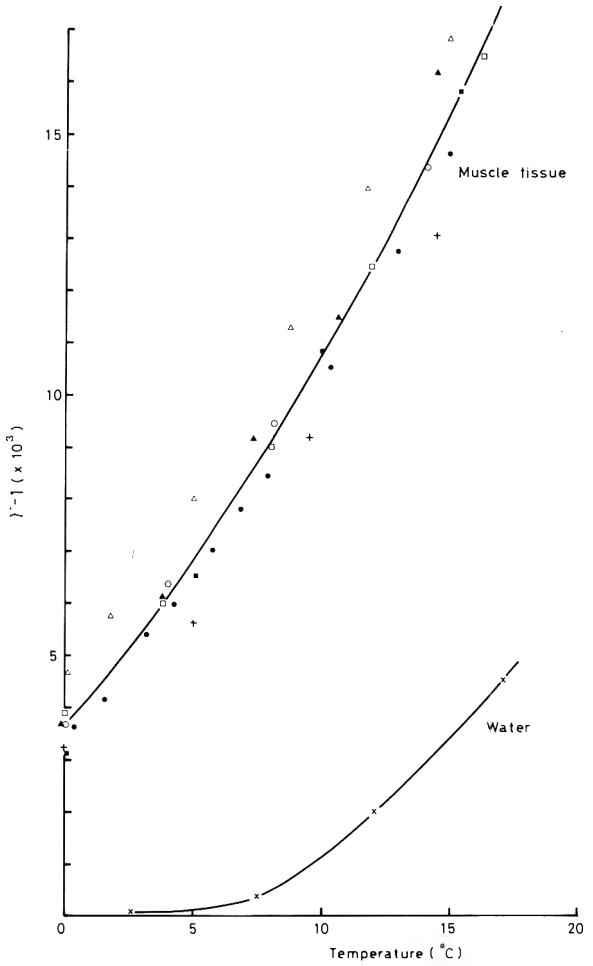

Figure 4. Ratio of specific heats (γ) of unfrozen muscle tissue and water. Symbols have the same meaning as in Fig. 3.

by Latyshev, Gritsyn & Tsirulnikova (1979) for lean beef and pork, showed minimum values of 1005–1020 kg/m^3 in the region −20– −30°C.

Conclusions

Adiabatic pressure-induced temperature measurements have enabled thermal expansivity, and related physical properties, to be determined more readily than hitherto. This approach was satisfactorily tested on water.

It was confirmed that the thermal expansivity of muscle tissue is much greater than water, and unlike water, muscle does not have a zero expansivity around 4°C. Similarly, unlike most other physical properties, the ratio of specific heats at constant pressure and constant volume of muscle tissue is much greater than water.

Muscle tissue commences to freeze at −1°C and expansivity becomes large and negative. On further reduction in temperature, expansivity decreases to zero and then becomes positive below −9°C. Correspondingly, the density of muscle tissue decreases

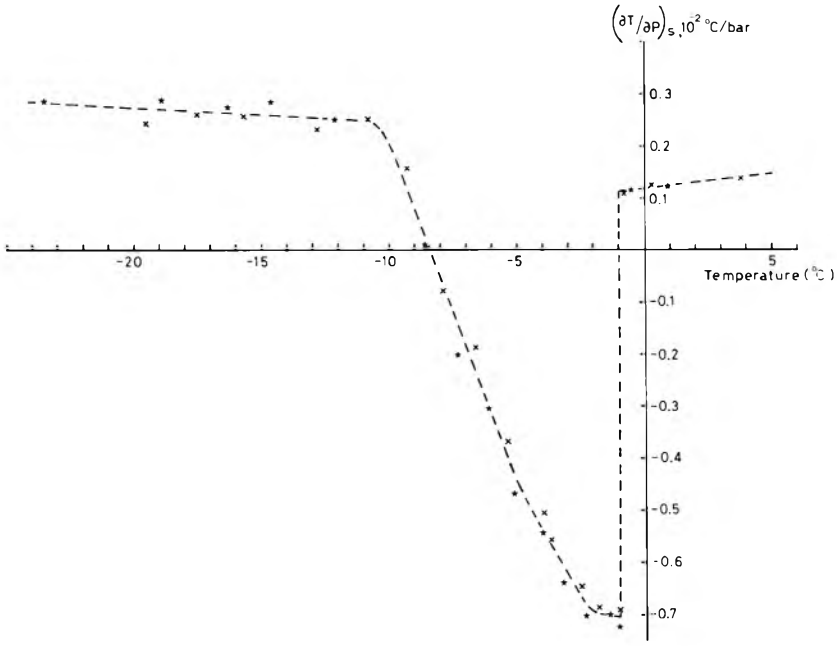

Figure 5. $(\partial T/\partial P)_S$ of frozen muscle tissue. Symbol, sample no: ★, 8; x, 9.

Table 4. Density (ρ) and expansivity (α) of frozen muscle tissue

Temp. (°C)	ρ (kg/m^3)	α (10^{-4}/°C)	Temp. (°C)	ρ (kg/m^3)	α (10^{-4}/°C)
−1	1078	−534	−13	1021	+3.3
−2	1039	−152	−14	1021	+3.2
−3	1030	−60.9	−15	1021	+3.1
−4	1025	−27.4	−16	1022	+3.0
−5	1022	−17.9	−17	1022	+2.9
−6	1021	−9.6	−18	1022	+2.8
−7	1020	−4.2	−19	1022	+2.7
−8	1020	−1.0	−20	1022	+2.7
−9	1020	+1.0	−21	1022	+2.6
−10	1020	+2.5	−22	1022	+2.5
−11	1021	+3.4	−23	1022	+2.4
−12	1021	+3.3	−24	1022	+2.3

during freezing, reaching a minimum around −8.5°C.

These measurements have provided information relevant to the behaviour of meat in a variety of thermal/pressure processing situations, and should be of particular use in the design of such processing equipment.

Acknowledgments

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Denaturation of soybean proteins related to functionality and performance in a meat system

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Summary

The effect of heat treatment on the functional properties of a soybean protein isolate was studied; heat affected the nitrogen solubility and gel properties of the isolate as well as its water absorption ability and viscosity. Significant correlations were found between protein solubility and gel viscosity. The viscosity of dispersions correlated to solubility or to water absorption depending on the protein concentration. Significant correlations were found between the functional properties of the protein and moisture loss from a model system in which the meat protein was partially replaced with soya. Protein solubility and gel properties were found to be the best predictors of moisture loss from the meat system. Viscosity of raw meat systems correlated with the solubility and water absorption of the added soya protein.

Introduction

The functional properties of a protein in a food system are affected by source, composition, prior treatment and interaction with the physical and chemical environment. Wu & Inglett (1974) reviewed how the denaturation, by heat, of plant proteins may affect their functionality and use in food applications. In addition some attempts have been made to find correlations between functional properties of proteins as affected by heat treatment (Johnson, 1970; Hermansson, 1977; Fennema, 1977; Pilosof *et al.*, 1982). Protein isolates are currently available from various sources, and one of the major applications for these proteins is in processed meats. The most important attributes of non-meat proteins in meat products are their effect on the adhesiveness and viscosity of the raw meat system, the moisture loss on cooking and the texture of the cooked product.

Data are available relating to protein functionality in simple systems and in some food systems but the relationship between functional properties in simple systems and performance in food systems has been little studied. The most detailed studies were reported by Hermansson & Akesson (1975) in which the properties of a soy isolate, caseinate and whey protein concentrate (WPC) were correlated with moisture loss in model meat systems. Without heat treatment the best correlations were obtained from a combination of the solubility, swelling and viscosity of the protein; in the heat treated systems, properties measuring gelation were highly correlated with moisture loss.

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Torgersen & Toledo (1977) studied the relationship between solubility and water absorption of WPC, peanut protein, single cell protein and chicken preen gland protein and the characteristics of a comminuted meat system containing these additives. Protein additives with high water absorption capacities yielded more viscous raw mixtures which exuded less fat and water on cooking.

The present study examines the effect of heat denaturation on some functional properties of soya protein and properties of a meat system to which this soya protein was added.

Materials

The soybean protein isolate used was a commercial sample 'Proteinmax 90 NB' from Sanbra S.A., Sao Paolo, Brazil and contained 94.1% protein. Fresh beef was trimmed of visible fat, ground through a meat grinder, fitted with a plate having 4 mm diameter holes, and mixed thoroughly. Portions weighing approximately 250 g were packed in polyethylene films, quickly frozen, and stored at −30°C. The proximate analysis of the meat was: 21.7% protein, 4.7% fat and 73.2% water. Thawing was carried out in a water-bath at 20°C.

Preparation of meat systems

10, 30 and 50% of the meat protein was substituted by soybean protein; water was added to maintain the protein/water ratio the same as in the pure meat system. For comminution, a Robot Coupe cutter was used at 1500 rpm. When meat protein was to be substituted, meat, soya protein and water were mixed for 30 sec, and the soya protein was allowed to swell for 30 min and the system was then remixed for 30 sec.

Methods

Properties of soya protein isolate

Heat treatment. The isolate was spread in a thin layer and humidified to 13.7% moisture: the moistened protein was sealed in a plastic pouch, placed in a refrigerator and allowed to equilibrate for 4 days. The moisture-equilibrated protein was transferred to cans, sealed and placed in a 90°C oven for 0.5, 1, 2 or 3 hr.

Functional properties. Solubility tests were carried out on 1% dispersions at 25°C. Soluble nitrogen was determined by the Microkjeldahl technique (AOAC, 1965). Water absorption at 24°C was determined using the device designed by Torgersen & Toledo (1977) and the water absorbing capacity was expressed as ml water absorbed/g protein.

For viscosity measurements, protein dispersions containing 5, 8 and 10% (by weight) of protein were prepared in a Virtis mixer at 11000 rpm for 2 min; the protein was allowed to swell for 30 min and the dispersion remixed for 2 min. Viscosity measurements were carried out in a Haake Rotovisco RV2 at 20°C with a MVI measuring system. The consistency index (K) of the dispersions was calculated using the power law (Bianchi, Pilosof & Bartholomai, 1985) which characterizes the overall shear stress *virus* shear rate behaviour:

$$\tau = K D^n$$

where τ = shear stress, D = shear rate, K = consistency index and n = flow behaviour index.

Gel properties of the protein were measured in 10% dispersions of protein heated at 80°C for 1 hr and cooled overnight. Gel viscosity was measured at 25°C in a Brookfield viscometer RVT using the Helipath stand with the T Spindle series at 1, 2.5 and 5 rpm. Water-binding properties of the gels were determined by the net-test developed by Hermansson & Lucisano (1982). Moisture loss was calculated as (weight of juice release/weight of sample) × 100.

Properties of meat systems

Moisture loss. The centrifugation technique developed by Hermansson & Akesson (1975) for heated meat systems was used to measure moisture loss. The meat systems were heated at 80°C for 20 min and, after cooling, were transferred to specially designed tubes and centrifuged at 510×**g** for 10 min. Moisture loss was calculated as:

$$\frac{\text{weight of water lost}}{\text{initial weight}} \times 100$$

Four replicate analyses were performed.

Viscosity of raw meat systems.

The raw meat systems were mixed with ice (1:0.8) at 10000 rpm in a chopper. Viscosity measurements of the meat homogenates were carried out at 20°C on a Haake Rotovisco RV2 with a MVI measuring system. The consistency index (K) of meat homogenates was calculated according to the power law (Bianchi *et al.*, 1985).

Statistical analysis

Correlation coefficients, and their level of significance, between the funtional properties of the proteins and the properties of the meat systems were calculated according to Davies (1965). The correlations between the functional properties of the soybean protein were also determined.

Results and discussion

Functional properties

The effect of heat treatment on the soy isolate was reflected in all the functional properties measured as shown in Fig. 1–3. The viscosity and water binding capacity of the protein gels and the nitrogen solubility of the dispersions, decreased with increased time of heating, probably due to denaturation and cross-linking of the protein.

Water absorption capacity of the protein showed a more complicated behaviour: it was enhanced by heat treatment up to 1 hr and then showed a pronounced decrease. The same behaviour has been reported by Hermansson (1977).

Viscosity behaviour of the protein dispersions, as affected by heat treatment of the isolate, depended on protein concentration. Eight and 10% dispersions exhibited an increase in the consistency index with increasing heat treatment, up to 1 hr; longer times of heating, however, resulted in a pronounced decrease in the consistency index. The consistency index of 5% dispersions decreased continuously with time of pretreatment at 90°C.

Properties of meat systems

Moisture loss. Figure 4 shows the effect of the level of meat protein substituted and the degree of heat treatment to which the soya protein has been subjected on moisture

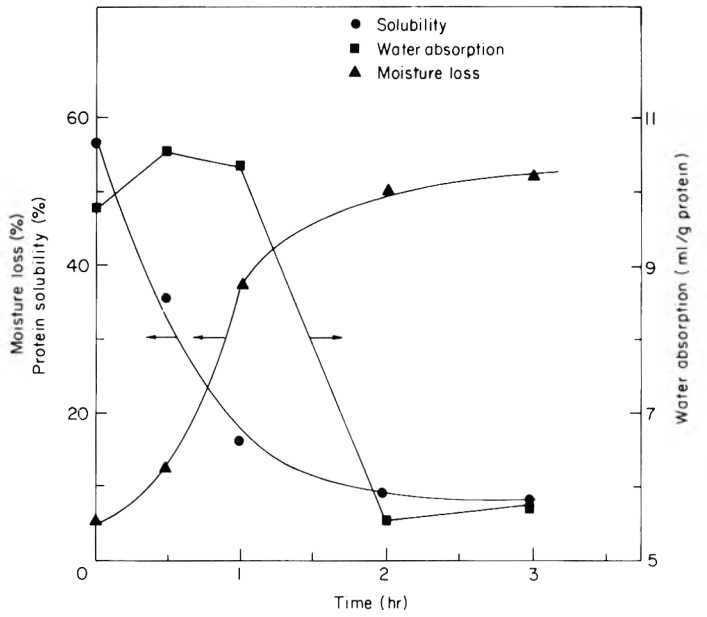

Figure 1. Effect of time at 90°C on the solubility of the isolate and the water absorption and moisture loss from gel preparations.

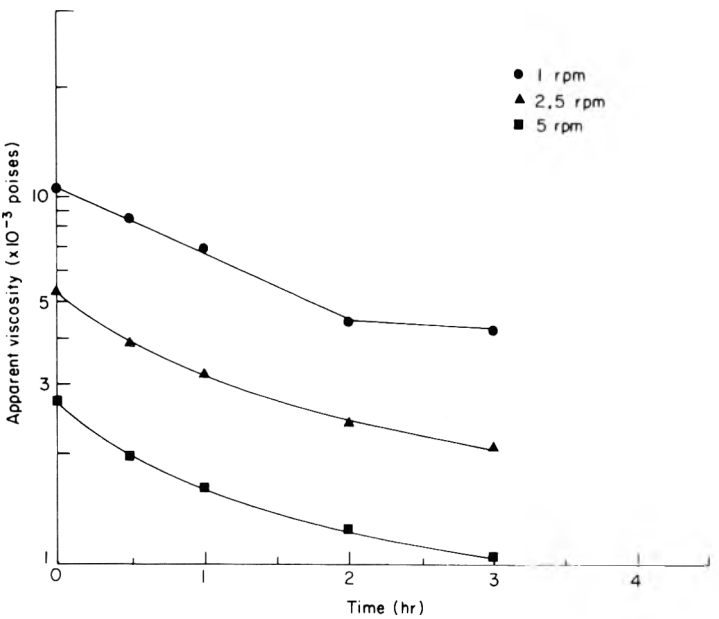

Figure 2. Effect of time at 90°C on the apparent viscosity of gels prepared from 10% dispersions of isolate.

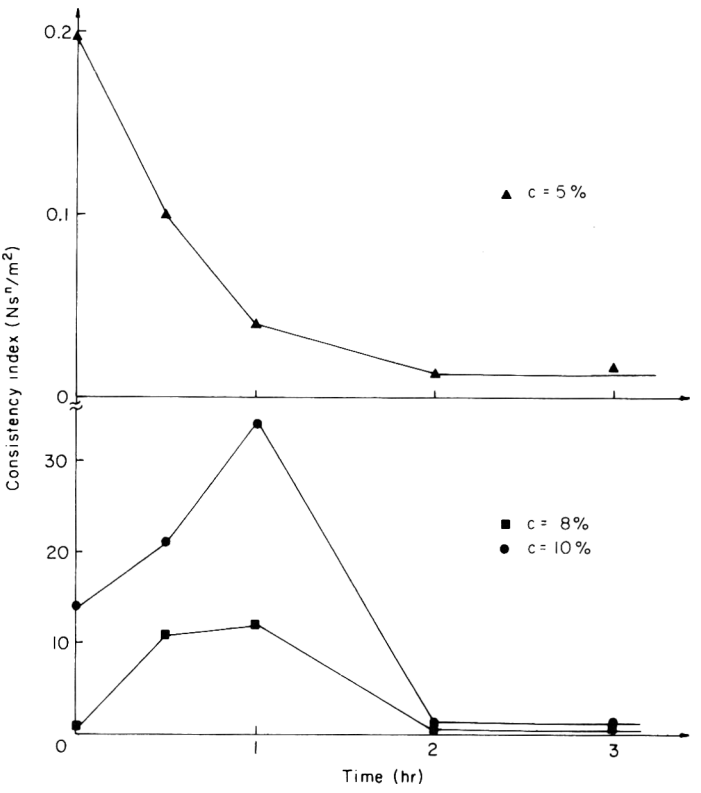

Figure 3. Effect of time at 90°C on the consistency index of dispersions of different concentrations of soya isolate.

loss. Substitution of meat protein by soya protein resulted in lower moisture losses after cooking. Increasing times of heat-moisture treatment of the protein isolate resulted in increasing moisture losses at 30 and 50% subsitution but not at 10%.

Viscosity of raw meat systems. Figure 5 shows the effect of the level of meat protein substituted and the degree of heat treatment to which the soya protein has been subjected on the consistency index of meat homogenates. Ten percent substitution resulted in a small decrease in the consistency index; 30 and 50% substitution markedly increased the consistency of the homogenates. The effect of heat treatment was only marked at 30 and 50% substitution and resulted in a pronounced decrease of the consistency index.

Correlations between functional properties of soybean protein

Correlation coefficients, and their level of significance, between the various functional properties of the heat treated soybean protein isolate are shown in Table 1. To simplify the tables, only significant correlation coefficients (at the 1 or 5% level) are shown. A significant positive correlation was found between the protein solubility and the viscosity of the gels signifying increased gel viscosity with increasing protein solubility; moisture loss from protein gels was negatively correlated with protein solubility signifying that the more soluble the protein the lower the water release from gel

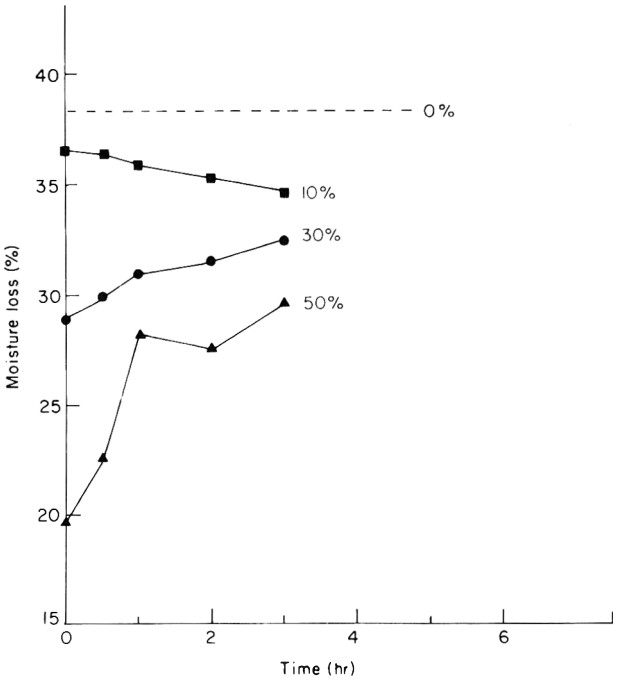

Figure 4. Effect of time at 90°C of soya protein on the moisture loss from cooked meat systems with different amounts of meat protein exchanged for soya.

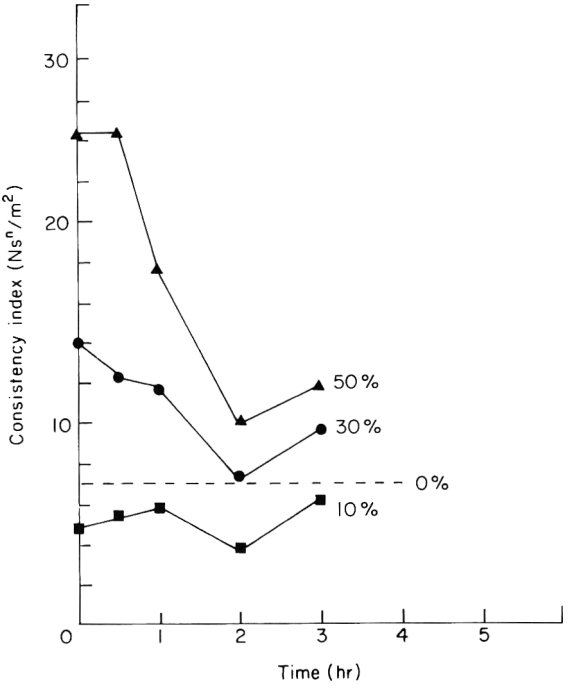

Figure 5. Effect of time at 90°C of soya protein on the consistency index of raw meat homogenates with different amounts of meat protein exchanged for soya.

Table 1. Correlation coefficients between functional properties of soybean protein*

	Gel properties		Constistency index of protein dispersions	
Function	Moisture loss	Viscosity	5%	10%
Solubility	−0.957(5)	0.981(1)	0.995(1)	—
Gel moisture loss	1.000	−0.941(5)	—	—
Water absorption	—	—	—	0.885(5)
Consistency index of 5% protein dispersions	−0.926(5)	0.974(1)	1.000	—

*Numbers in parenthesis indicate level of significance.

preparations. As expected, the two gel properties measured were negatively correlated. Solubility was significantly correlated with the viscosity of 5% dispersions but not with the viscosity of the 8 and 10% dispersions. The viscosity of the 5% was significantly correlated with both gel properties. Water absorbing capacity showed no significant correlations with either the solubility or gel properties of soybean protein. Although no significant correlation was found between water absorbing capacity and the viscosity of 5% dispersions, a significant correlation was found with 10% dispersions.

Viscometric properties are influenced both by solubility and water absorption, the relative importance being determined by the quantitative relationship between the absorbed or imbibed water and the remainder (Urbanski *et al.*, 1983).

In 10% dispersions all the water present is imbibed by the protein; the amount of absorbed water can be calculated from the water absorbing capacities; i.e., for unheated soya isolate, the water absorbing capacity is 9.8 ml water/g protein (Fig. 1), thus 10 g protein imbibe 98 ml water. In 10% dispersions the total water is 90 ml, therefore no unimbibed water exists. In these systems the viscosity with respect to heat treatment is correlated to water absorption. In 5% dispersions, extensive unimbibed or free water exists, internal friction between the solid swollen particles is drastically reduced and the viscosity is correlated to solubility.

Correlation between functional properties of protein and properties of a meat system

All the functional parameters of the soy protein were correlated with the moisture loss of the meat system after cooking except for the systems containing 10% soya protein. This is presumably due to the low level of added protein. Therefore only meat systems containing 30 and 50% soya protein were used for statitistical analysis. In Table 2, significant negative correlations (at 1 or 5% level) were found between the moisture loss of the meat systems and soya protein solubility i.e., the more soluble the protein the lower the moisture loss after cooking. Gel properties strongly influenced moisture loss; a protein which gave a more viscous gel (or a gel which released less water) allowed less moisture loss in the cooked meat systems. While the viscosity of 8 and 10% protein dispersions showed no significant correlations with moisture loss, a negative correlation coefficient was found between the later parameter and the viscosity of 5% dispersions

Table 2. Correlation coefficients between functional properties of soybean protein and moisture loss of cooked meat systems*

Function	Meat protein substituted	Solubility	Gel properties		Consistency index of 5% dispersions
			Moisture loss	Viscosity	
Moisture loss after cooking	30%	−0.941(5)	0.960(1)	−0.975(1)	−0.913(5)
	50%	−0.975(1)	0.953(5)	−0.961(1)	−0.952(5)

*Numbers in parenthesis indicate level of significance.

indicating once more the influence of protein concentration. Water absorbing capacity showed no significant correlation with the moisture loss from the meat systems.

The parameters used for correlation with the viscosity of raw meat systems were solubility, water absorption capacity and the viscosity of protein dispersions at different concentrations. None of the measured functional properties showed a high correlation with the consistency of meat homogenates (Table 3); although the results suggested that the isolates containing the higher concentrations of soluble protein and those with the better water absorbing capacities yielded the more viscous homogenates.

While the viscosity of 8 and 10% soya dispersions was not significantly correlated with the viscosity of the meat homogenates, 5% dispersions were correlated at the 10% level. This is probably because in meat homogenates the concentration of soya protein was at most 6.2% and therefore free or unimbibed water existed.

Table 3. Correlation coefficients between functional properties of soybean protein and consistency index of raw meat homogenates*

Function	Meat protein substituted	Solubility	Water absorption	Consistency index of 5% protein dispersions
Consistency index	30%	0.853(10)	0.877(10)	0.857(10)
	50%	0.828(10)	0.855(10)	0.807(10)

*Numbers in parenthesis indicate level of significance.

Conclusions

Data obtained with the soya isolate tested, showed that a high protein solubility is required to obtain strong gels on heating and to minimize moisture loss from cooked meat systems in which meat protein is replaced by soya. Thus protein solubility of the isolate and the properties of gels prepared from it were the best predictors of moisture loss from the meat systems.

Water absorption, independent of solubility, also affected the moisture loss, and it perhaps determines the behaviour when comparing two soya proteins of similar solubility. The viscosity of soya dispersions was not an adequate index of moisture loss in the meat systems since the viscosity behaviour was dependent on protein concentration. The viscosity of the raw meat systems, was determined to some extent by the solubility and water absorption of the substituted isolate. The viscosity of the soya dispersions was not a good predictor of performance unless a representative concentration was chosen.

Acknowledgments

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Cold shock reactions in iced tropical fish

C. A. CURRAN, R. G. POULTEN* A. BRUETON AND N. S. D. JONES

Summary

The development of a rigor mortis-like stiffening and the biochemical changes associated with it were investigated in tilapia (*Oreochromis aureus/niloticus* hybrid), a tropical freshwater species, and common carp (*Cyprinus carpio*), a temperate freshwater fish, during storage in ice (0°C) and at ambient temperature (22°C). Onset of stiffening in carp occurred between 16 and 17 hr after death at both temperatures but full stiffness developed much later and was a longer duration at 0°C. In tilapia, onset occurred after 7 hr at 22°C and full stiffness was established after 19 hr. However, at 0°C, tilapia experienced a cold shock reaction such that they stiffened within minutes of being placed in ice and were fully rigid within 8 hr. Resolution of stiffness in this species also occurred later at 0°C. The rate of ATP degradation was similar under both storage conditions in tilapia but more rapid at ambient temperature in carp. Although the rate of lactic acid accumulation was faster at the higher temperature in tilapia, it was not nearly so marked as for carp. Objective measurement of contractions in excised muscle fibres from trout (*Salmo gairdnerii*) and tilapia indicated that reducing the temperature delayed the occurrence of the contraction and reduced its intensity. It was concluded that cold shock stiffening and rigor mortis stiffening are different.

Introduction

Post-mortem biochemistry and the occurrence of rigor mortis have been extensively studied in mammalian and cold/temperate water fish muscle. Less work has been carried out on tropical species. There have been isolated reports in the literature that some fish, particularly those from tropical waters, may experience a cold shock reaction when placed in ice (Gianelli, 1954; Nazir & Magar, 1963; Pawar & Magar, 1965; Disney *et al.*, 1971; TDRI, unpublished data). During iced storage of *Tilapia nilotica*, Disney *et al.* (1971) noted that the fish exhibited a rigor-like hardening which usually developed within 1 hour. Uniced fish, however, did not enter rigor mortis until 2–5 hr after capture. A similar phenomenon, known as cold shortening (Locker & Hagyard, 1963) is well established in some chilled mammalian muscles but it has not been observed in coldwater fish.

Rigor mortis causes severe problems for the fishing industry (Jones, 1969) and handling of coldwater fish during rigor can result in a loss of quality and a lower yield (Bramsnaes & Hansen, 1965). The occurrence of a cold shock reaction in tropical fish could, therefore, have important implications in terms of recommended handling practices.

The objective of this study was to investigate stiffening of the tropical freshwater fish, tilapia, stored in ice and at ambient temperatures and to compare this with

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common carp, a species from temperate waters which can adapt to a wide range of temperatures. Certain concomitant biochemical changes were also investigated. The post-mortem contraction of tilapia and trout muscle at various temperatures was studied using a tensiometer to determine whether the cold shock stiffening was accompanied by shortening of the muscle fibres.

Comparative post-mortem stiffening and biochemistry of tilapia and carp.

Materials

Common carp, *Cyprinus carpio*, were aquarium-reared at the TDRI; 112 individual fish were used. Tilapia (*Orecochromis aureus/niloticus* hybrid) were aquarium-reared at the Institute of Aquaculture, University of Stirling, and 120 individual fish were used. All of the fish were killed swiftly by a blow to the head with as little struggling as possible. Each species was divided into two groups. Half of the fish were immediately placed in ice while the remaining fish were placed in polythylene bags and left at ambient temperature (22°C).

Methods

Stiffening. The onset, duration and resolution of rigor mortis, or rigor-like stiffening, were determined in ten tilapia and seven carp from each storage temperature. A simple method modified from that described by Cutting (1939) was used in which the sag of the tail was noted when the fish are held vertically by the head with the tail pointing upwards. The assessments were carried out at hourly intervals for the first 36 hr and then at 48, 53 and 56 hr following death. The stiffness of each fish was graded using the following scoring system: (i) flaccid; (ii) tail bent halfway; (iii) tail bent slightly; (iv) tail straight; and (v) whole fish very rigid.

Chemical analysis. Samples were taken for analysis at regular intervals from 0 to 36 hr following death. At each sampling time, five fish were taken from the batches at each temperature. After being weighed and their length measured, the fish were filleted. Two samples were taken from each fish for pH measurement and the remainder of muscle was frozen immediately in liquid nitrogen and stored at −30°C.

The pH of a suspension of muscle in neutralized 0.005 M iodocetic acid was measured using a glass electrode. The frozen muscle from each fish was analysed in duplicate for glycogen by the colorimetric method of Dubois *et al.* (1956) lactic acid by the enzymic method of Hohorst (1965) and adenosine triphosphate (ATP) and its breakdown products by the the layer chromatography method of Norman, Follett & Hector (1974).

Statistical analysis. An analysis of variance was carried out on the data for both species to determine the variance with temperature and time, and the interaction between these two factors. Between fish (and within fish) variance was initially included but was found to be small. In addition regression analysis was conducted to investigate the changes in pH, lactic acid, ATP and inosine monophosphate (IMP) with time.

Post-mortem muscle contraction in tilapia and trout

Materials

Muscle samples were taken from the shoulder region of freshly killed rainbow trout (*Salmo gairdnerii*), reared at a commercial farm, and tilapia (*Orecochromis moss-*

ambicus/niloticus hybrid), aquarium reared by the University of Stirling. Small muscle strips, approximately 12 mm long, 3 mm wide and 2 mm thick, weighing about 0.1 g, with the myofibrils running along the length, were mounted between the platinum foil jaws of the tensiometer (see Fig. 1). For the purposes of this investigation the mounts were prepared so that only muscle fibres, and not connective tissue, were visible between the jaws; in this way, only the development of tension in the fibres was measured.

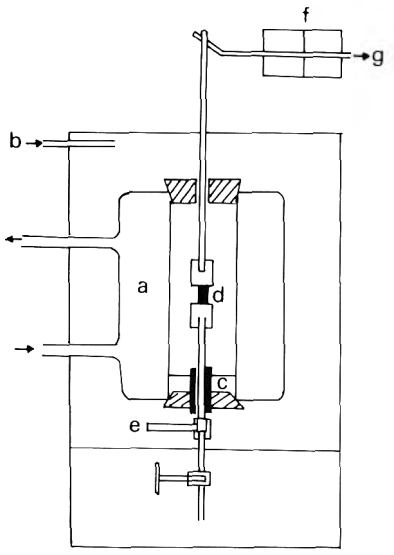

Figure 1. Schematic diagram of the tensiometer (not to scale); (a) circulating-water thermal jacket; (b) moist air; (c) water; (d) mounted muscle strip; (e) locking screw; (f) strain gauge resistors; (g) output to recorder.

Measurement of muscle contraction

A tensiometer (Fig. 1), adapted from the electo-magnetic force balance described by Burt *et al.* (1970), was constructed. It is designed to hold muscle samples in a humid, constant temperature environment. The muscle strips, held between the platinum jaws, were placed in this chamber. The bottom jaw was held in position by an adjustable rack and pinion at the base of the chamber. The upper jaw was attached to a beam on which four strain gauge resistors, connected in a full Wheatstone bridge circuit, were mounted. The tensiometer was zeroed initally for normal muscle tension when the sample was loaded into the chamber. The amplified output from the bridge supplied a signal to a chart recorder, which indicated the force applied to the beam as a deflection from zero giving a measure of the development of tension in the muscle strip as it contracted. The full scale deflection on the chart was calibrated to a force of 5 g (49 mN). The investigations with trout were carried out at 0 and 30°C and those with tilapia at these temperatures and also at 10 and 20°C.

Results and discussion

The changes in the mean values for stiffness score with time in carp and tilapia stored at 0 and 22°C are shown in Fig. 2. the results of the analysis of variance of the results is

given in Table 1. For the latter the development of stiffening was broken down into five stages corresponding to the following stiffness scores (S): Stage A: S < 1.5; Stage B: 1.5 < S < 2.5; Stage C: 2.5 < S < 3.5; Stage D: 3.5 <S < 4.5; and Stage E: S > 4.5 to start of decline. For the carp the last two categories were not easily distinguished and were merged into one.

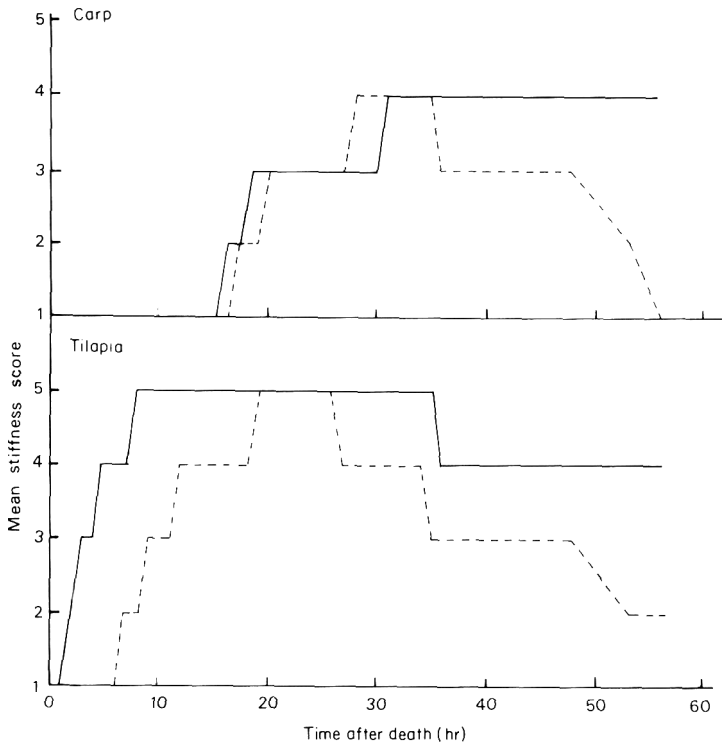

Figure 2. Post-mortem development of stiffening in carp and tilapia stored at 0 (——) and 22°C (– – – –).

Table 1. Results from the analysis of variance of the stiffening data for carp and tilapia stored at 0 and 22°C

Factor	Carp	Tilapia
Time	***	***
Temperature	***	n.s.
Interaction between time and temperature:		
Stages A–E	***	***
At Stage A	*	*
At Stage B	*	n.s.
At Stage C	n.s.	n.s.
At Stage D	*	
At Stage E	*	*

***, $P \leq 0.001$.
*, $P \leq 0.05$.
n.s., not significant.

The results for tilapia show that stiffening occurred after 2 hr in fish stored at 0°C and after 7 hr at 22°C and that this difference was statistically significant. This apparent cold shock reaction in iced tilapia was similar to observations of earlier workers on other tropical speices (Nazir & Magar, 1963; Pawar & Magar, 1965; Disney *et al.*, 1971; TDRI unpublished data) and occurred in some fish within a few minutes of being placed in ice.

For the tilapia at 0°C, full stiffening was reached after 8 hr and was maintained for 27 hr. At 22°C, however, full stiffening was not reached until 19 hr after death and was maintained for only 7 hr. Again, the difference found in the duration of full stiffening at the two temperatures was found to be statistically significant.

In contrast, the development of stiffening in carp was much later than for tilapia and the effect of temperature was less marked as regards the time of onset of the stiffening. Nonetheless, the 1 hr difference between the time the fish at 0°C started to stiffen (16 hr after death) and that for the fish at 22°C (17 hr after death) was statistically significant. The intensity of rigor in carp at both temperatures was not as great as that in tilapia and this is indicated by the lower scores which were awarded. However, as in tilapia, the duration of full stiffening was longer in fish stored at 0°C than at 22°C (7 hr at 22°C compared with over 25 hr at 0°C) and this difference was statistically significant.

The results of the analysis of ATP and its breakdown products, adenosine diphosphate (ADP), adenosine monophosphate (AMP), IMP, inosine (Ino) and hypoxanthine (Hx), for the two species at both temperatures are presented in Fig. 3. Those for glycogen, lactic acid and pH are given in Fig. 4. Regression lines have been plotted for ATP and IMP in Fig. 3 and for lactic acid and pH in Fig. 4. The correlation coefficients (r^2) are given along with the legend to Figs. 3 and 4. In all cases the

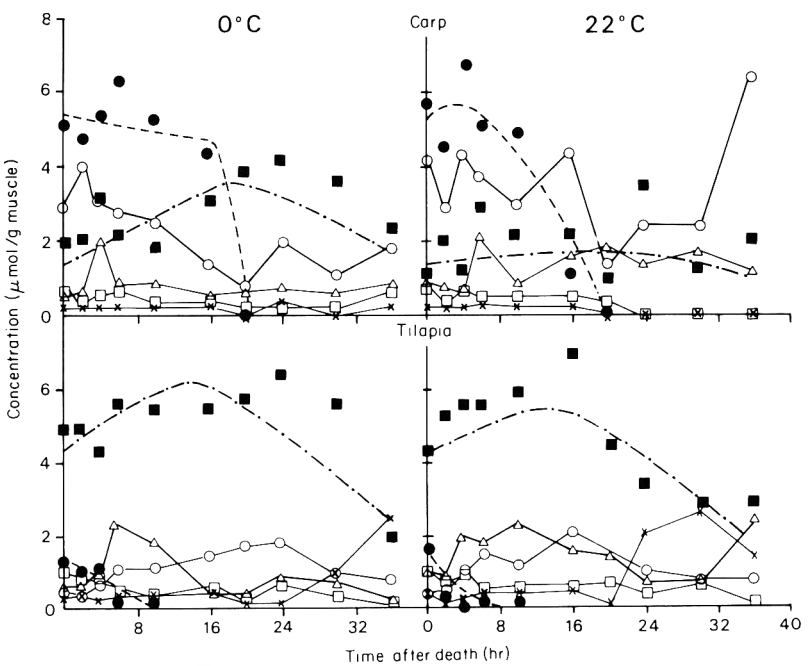

Figure 3. ATP and its degradation products in carp and tilapia stored at 0 and 22°C. ●---● ATP; □-□ ADP; ×-× AMP; ■-·-·-■ IMP; △—△ Ino; 0-0 Hx; Regression lines for only ATP (carp, r^2 = 0.567; tilapia, r^2 = 0.184) and IMP (carp, r^2 = 0.141; tilapia r^2 = 0.347) are incorporated (where r^2 is the correlation coefficient for each pair of variables).

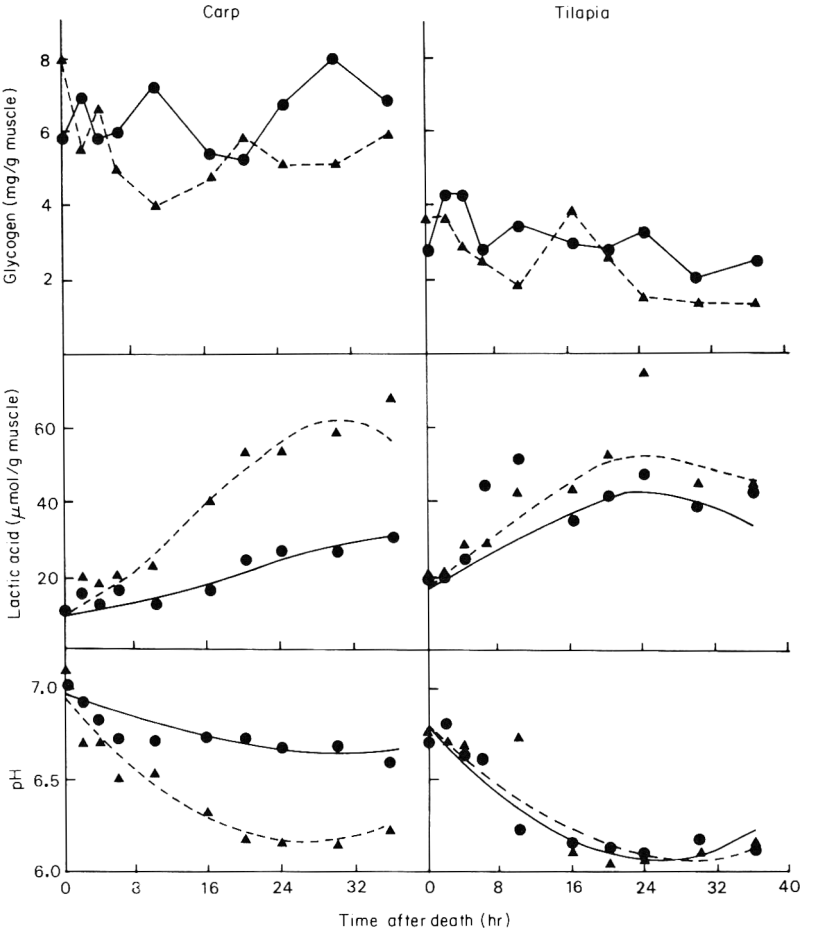

Figure 4. Glycogen, lactic acid and pH in carp and tilapia stored at 0 (●——●) and 22°C (▲- - -▲). Regression for only lactic acid (carp, $r^2 = 0.627$; tilapia, $r^2 = 0.283$) and pH (carp, $r^2 = 0.865$; tilapia, $r^2 = 0.814$) are incorporated (where r^2 is the correlation coefficient for each pair of variables).

regression lines were plotted using a log quadratic model: $\text{Log}_e(y) = a+bt+ct^2$ (for $T = 0°C$) and $\text{Log}_e(y) = a+bt = ct^2+b't+c't^2$ (for $T = 22°C$); where a, b, c, b , c′ are coefficients, t is time in hr and T is temperature in °C. The analysis of variance results for all the chemical data are given in Table 2.

The results of the biochemical analysis (Figs. 3 and 4) show that the initial post-mortem level of ATP in tilapia was considerably lower than that in carp but, together with the correspondingly lower glycogen concentration and higher IMP and lactic acid levels, it indicates that, apart from the expected species differences, the former was probably more active or struggled more prior to death. Temperature had different effects on the post-mortem metabolism of the two species used in this study and the analysis of variance (Table 2) showed that there were significant temperature effects and interactions between temperature and time for most of the variables in carp. For tilapia, however, the temperature effects alone were less often significant but, for most of the variables, there were significant interactions between temperature and time.

Table 2. Results from the analysis of variance of the biochemical data for carp and tilapia stored at 0 and 22°C

Species	Factor	Glycogen	Lactic acid	pH	ATP	ADP	AMP	IMP	Ino	Hx
Carp	Temperature	***	***	***	n.s.	n.s.	*	***	***	***
	Time	n.s.	***	***	***	***	***	*	n.s.	***
	Interaction between time and temperature	n.s.	***	***	***	***	***	*	***	**
Tilapia	Temperature	***	n.s.	n.s.	n.s.	n.s.	**	n.s.	***	n.s.
	Time	***	***	***	***	***	***	***	***	***
	Interaction between time and temperature	***	**	***	n.s.	n.s.	***	**	***	n.s.

***, $P \leq 0.001$.
**, $P \leq 0.01$.
*, $P \leq 0.05$.
n.s., not significant.

ATP was degraded more rapidly at 22°C than at 0° C in carp whereas in tilapia the rate of ATP depletion was not significantly different at the two temperatures. When the products of ATP catabolism are considered (Fig. 3 and Table 2), it can be seen that, in the same manner as ATP, ADP was depleted earlier at 22°C in carp and at the same rate in tilapia stored at the two different temperatures. In both species, at the higher temperature, the metabolism of AMP, IMP and Hx appeared to be more rapid than at 0°C but Ino depletion was slower. The general pattern for ATP catabolism in carp is in good agreement with the results of other workers, for example, Noguchi & Yamamoto (1955), Saito & Arai (1957) and Tomiyama *et al.* (1966).

Glycogen degradation (Fig. 4 and Table 2) in both species was significantly affected by temperature but it was only in the tilapia that any significant time/temperature interaction was found. There appeared to be very little overall decrease in glycogen concentration with time in carp but there was a slight fall in tilapia which was faster at the higher temperature.

The production of lactic acid and the corresponding decrease in pH (Fig. 4 and Table 2) was much more rapid in carp at 22°C compared with 0°C while, in tilapia, the rate was only slightly greater at 22°C. A discrepancy between glycogen disappearance and lactic acid formation has also been observed by other workers (Tarr, 1966).

It has been suggested (Poulter, Curran and Disney, 1981) that, if a drop in temperature from 37 to 5°C stimulates mammalian muscle to contract, rapid cooling of tropical fish from the temperature of their natural environment (25–30°C) to 0°C (in ice) might

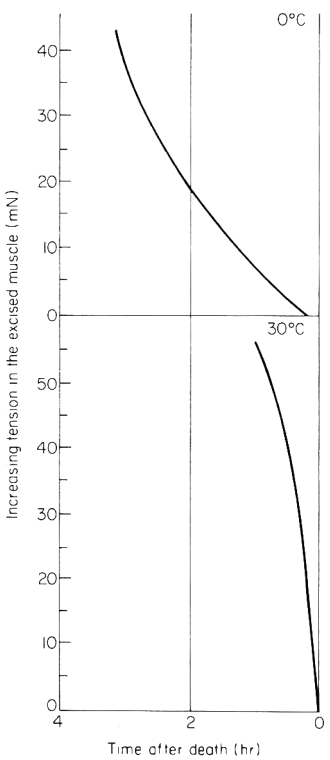

Figure 5. Recorded tensiometer traces showing the contraction of trout muscle at 0 and 30°C.

also cause stimulation of the muscle. An attempt was made to determine whether the cold shock reaction of tilapia was accompanied by shortening of the muscle fibres as occurs in cold-shortened mammalian muscle (Hamm, 1982). The recorded tensiometer traces for trout held at 0 and 30°C are given in Fig. 5 and those for tilapia at 0, 10, 20 and 30°C are presented in Fig. 6.

The results for trout show that there was a rapid strong contraction of the muscle strip at 30°C. At 0°C, the contraction was delayed for approximately 15 min and was less intense. In tilapia, as the temperature decreased, the delay before any contraction was recorded increased: 5 min at 30°C, 15 min at 20°C, 25 min at 10°C and 80 min at 0°C. The contraction at 30°C was fairly rapid but much weaker than that in trout at the same temperature. The initial rate at 20°C was fairly rapid but decreased after 30 min. The rate of contraction at 0 and 10°C was fairly slow and similar to the later rate at 20°C. Since there would have been some delay before the muscle strips actually adjust to the air temperature within the chamber while the chilling effect of ice on whole fish would be fairly rapid, one tilapia was chilled for half an hour in iced freshwater before the muscle strip was taken and placed in the instrument. This still resulted in a delay of 60 min before any contraction was recorded.

The results for both species agree with the data in the literature in that the muscle contractions, presumably rigor mortis, were delayed by lowering the temperature, and the tension developed was also reduced. Further, it appeared that a reduction in temperature had a greater effect in this respect on tilapia than on trout.

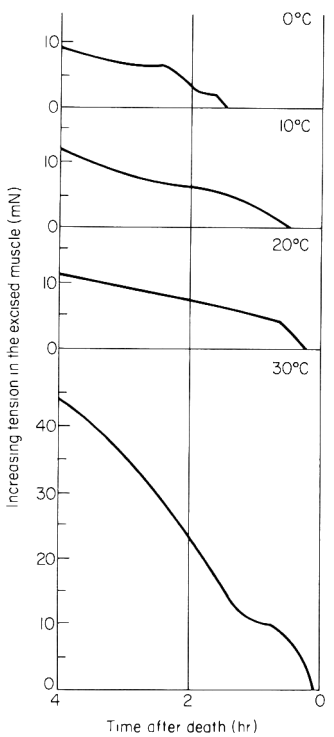

Figure 6. Recorded tensiometer traces showing the contraction of tilapia muscle at 0, 10, 20 and 30°C.

Conclusions

This study has shown that whole tilapia place in ice immediately after death undergo a form of cold shock reaction which causes the body of the fish to stiffen almost immediately. A similar stiffening which is presumably due to rigor mortis occurs in fish stored at 22°C only after some 7 hr. In carp at 0 and 22°C stiffening did not occur until 16 and 17 hr respectively after death.

Analysis of muscle metabolites which are associated with the normal rigor mortis reaction in fish were undertaken. As is often the case with complex biological and biochemical changes, no clear relationship could be identified between the biochemical indices and the physical changes occurring. However, there was some indication that storage at 0°C reduced biochemical activity (compared with that at 22°C) more in carp than in tilapia.

Direct measurement of muscle contractions in excised muscle strips showed that, in both carp and tilapia, as the temperature was reduced the delay before a contraction occurred increased and the intensity of the contraction was reduced. In tilapia, this is apparently the opposite of the stiffening results observed in whole fish.

At present there is insufficient evidence to allow speculation on the cause of the stiffening reported in iced whole tilapia. However, like cold shortening in mammalian meats and thaw rigor in frozen cold water fish, it could have important commercial implicatons. Work recently completed on the effect of the cold shock stiffening on fillet yield and quality is reported in a separate paper (Curran *et al.*, 1986).

Acknowledgments

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Effect of handling treatment on fillet yields and quality of tropical fish

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Summary

The effect on fillet yields and quality of the cold shock reaction of tropical fish when they are iced immediately following death was investigated. Tilapia (*Oreochromis mossambicus/niloticus* hybrid) were subjected to three handling treatments: filleting immediately following death; icing the whole fish immediately following death and filleting after 3 days; ageing the fish for 6 hr at ambient temperature prior to icing and then filleting after 3 days. All fillets were stored on ice until 9 days after death. Pre-rigor filleting resulted in the highest filleting yields with the least drip loss and gaping on storage but the shortest shelf life. This was accompanied by the highest post-mortem metabolic rate. The lowest yields and highest drip loss were obtained by icing the fish immediately at death followed by filleting after 3 days. A 6-hr delay before icing gave yields and drip loss which were between these two extremes and also the slowest post-mortem metabolism. The two post-rigor filleting procedures produced fillets with increased gaping but a longer shelf life compared with pre-rigor filleting. The implications of the cold shock reaction in terms of recommended codes of practice and handling operations in tropical fisheries are discussed.

Introduction

Recently, Curran *et al.* (1986) investigated the cold shock reaction which has been found in some tropical species of fish (Nazir & Magar, 1963; Pawar & Magar, 1965; Disney *et al.*, 1971; TDRI unpublished data). They reported that, in tilapia, a tropical freshwater species, onset of rigor mortis at ambient temperature (22°C) occurred 7 hr after death. Full rigor mortis was established after 19 hr. However, the fish stiffened within minutes of being placed in ice and were completely rigid within 8 hr. The duration of rigor was also longer at the lower temperature. In contrast lowering the temperature generally prolongs both the pre-rigor and the rigor periods in coldwater species (Amlacher, 1961). The metabolism of tilapia appeared to be accelerated by icing leading to the suggestion that the cold shock reaction may be similar to the cold shortening phenomenon which occurs when certain mammalian muscles are chilled (Hamm, 1982). However, it was shown that the stiffening of iced tilapia was not accompanied by the contraction of muscle fibres which occurs in cold shortening muscles (Curran *et al.*, 1986).

Rigor mortis can cause acute problems for the fishing industry (Jones, 1969) and

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handling of coldwater fish during rigor can result in a loss of quality and a lower yield (Bramsnaes & Hansen, 1965). It would, therefore, be expected that the earlier rigidity of tropical fish caused by the cold shock reaction could cause additional problems. The objective of this study was to investigate the effect of handling procedures on fillet yields and quality of tilapia.

Materials and methods

Materials

Aquarium-reared tilapia (*Oreochromis mossambicus/niloticus* hybrid) from the Institute of Aquaculture, Stirling University, were killed by a blow to the head with as little struggling as possible. Three handling treatments prior to filleting were investigated.

(i) *Pre-rigor fillets.* The fish (fifty) were filleted and skinned immediately after being killed. The fillets were then wrapped in waterproof paper (Wetpack) and placed with ice in waxed cartons. Half of the fillets were used for assessing drip loss and quality and the remaining half for chemical analysis.

(ii) Post-rigor fillets. The fish (sixty-six) were immediately stored in ice and were held for 3 days before fifty were filleted and skinned. The fillets were treated in the same way as the pre-rigor samples.

(iii) *Aged post-rigor fillets.* The fish (sixty-six) were aged at ambient temperature (22°C) for 6 hr following death; they were then iced. After 3 days, fifty were filleted, skinned and treated in the same manner as the pre-rigor and post-rigor fillets.

Methods

Filleting yields. The filleting was carried out by a commercial filleter. The yields of the skinned fillets were expressed as a percentage of the weight of the whole fish.

Fillet drip loss and quality. The pre-rigor fillets were stored in ice for 9 days and the post-rigor and aged post-rigor fillets for 6 days (9 days storage in total). Each day, the fillets were weighed and the free drip loss determined as the percentage weight loss. The odour and visual quality of the fillets were also assessed daily with particular attention being paid to the occurrence of gaping.

Stiffness measurements. The onset, duration and resolution of rigor mortis, or rigor-like contractions, were determined during the first 3 days following death in the fish used to produce post-rigor and aged post-rigor fillets. Visual assessment of the stiffness of ten fish from each treatment was carried out hourly up to 18 hr and then at 24, 48 and 72 hr, following death using the method described previously (Curran *et al.*, 1986). The stiffness of each fish was graded using the following scoring system: (i) flaccid; (ii) tail bent halfway; (iii) tail bent slightly; (iv) tail straight; (v) whole fish very rigid.

Chemical analysis. Samples were taken at intervals from 0 to 216 hr following death for analysis of pH, lactic acid, adenosine triphosphate (ATP) and inosine monophosphate (IMP) as described previously (Curran *et al.*, 1986). In addition, the solubility of the muscle protein nitrogen (% SPN) in a solution of 5% NaCl was determined by the method of Ironside & Love (1958) as modified by Cowie and Little (1967). At each sampling time, either four fillets or four fish (in the case of post-rigor and aged post-rigor samples before filleting was carried out) were taken for analysis.

Statistical analysis. An analysis of variance was carried out on the data from all three handling procedures. This was used to indicate the effects of treatment and time and the

interaction between these two factors. In addition, a regression analysis, as described previously (Curran *et al.*, 1986), was used to investigate the chemical parameters. The correlation coefficients (r^2) are given along with the legends to figures.

Results and discussion

The tilapia used in this study were approximately 400 g in weight and 27 cm long. The mean filleting yields, drip losses and the overall processing yields of the three treatments are given in Table 1 together with the statistical analysis of the data. The yields on filleting for the three handling treatments, pre-rigor, post-rigor and aged post-rigor, were 31.18, 29.79, and 30.62% respectively. These yields were all significantly different at the 5% level.

Table 1. Mean filleting yields, drip losses and processing yields for the three treatments and the results of analysis of variance †

Treatment	Mean filleting yield (%)	Mean drip loss (%)	Mean processing yield
Pre-rigor ‡	31.18^a	-0.73^a	31.40^a
Post-rigor	29.79^b	1.43^b	29.36^b
Aged post-rigor	30.62^c	1.09^b	30.29^c
L.S.D. ($P \leq 0.05$)	0.51	0.79	0.24
Significance of effect of treatment	*	*	**
Significance of effect of time	—	*	***

† Means in each column with the same letter superscript are not significantly different ($P \leq 0.05$).

‡ To allow direct comparison of the data, only the values from days 0–6 for drip loss and processing yields of the pre-rigor treatment have been included in the analysis.

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

The post-rigor filleting yield was also found to be significantly lower than those from the other two treatments at the 1% level. Only the first 6 days data for drip loss and processing yield of the pre-rigor fillets were included in the statistical analysis to allow a direct comparison of the results for all three treatments. During this time, the pre-rigor fillets showed no drip loss but, instead, gained slightly in weight due to uptake of ice melt water even though they and been loosely wrapped in waterproof paper during storage. Although there was more total free drip from the post-rigor fillets (3.8%) compared with the aged post-rigor fillets (2.6%), these were not significantly different. The overall processing yields, calculated from the filleting yields and drip loss, were all significantly different with the greatest obtained from the pre-rigor handling treatment and the least from the post-rigor treatment.

The initial quality of all the fillets was very good regardless of the handling treatment. After 8 days in ice, the pre-rigor fillets started to develop off-odours and were considered to be spoilt the following day. At that time, the fillets from the two other treatments were still acceptable. Only approximately 20% of the pre-rigor fillets suffered from gaping whereas about 90% of both the post-rigor and aged post-rigor

fillets were affected. For all three treatments, however, the gaping was a single longitudinal split along the fillet and would have only been awarded a score of 1 on the subjective scale of Love, Laverty & Steel (1969) which ranges from 1 (no gaping or longitudinal splitting) to 5 (fillet dropping to pieces).

There are several causes of gaping, one of which is rigor mortis (Burt *et al.*, 1970). When whole fish enter rigor, the muscle attempts to contract but this is prevented by the skeleton and connective tissue and, consequently, tension increases within the muscle. If the connective tissue cannot withstand the increased tension, then gaping will occur. In addition, the higher the temperature of the fish as it enters rigor mortis, the more the flesh will gape because the rigor tension becomes greater and the connective tissue weaker. If the fish is filleted pre-rigor, the restraint of the skeleton is removed but the fillet may shrink considerably, can become distorted and its surface can take on a corrugated appearance. The extent of shrinkage is dependent upon the temperature of the fish in the same way that gaping is affected. The muscle pH also affects gaping (Love & Haq, 1970): as the pH decreases, gaping increases. Thus the pre-rigor fillets in this study suffered the least gaping since they underwent rigor off the bone. Surprisingly, shrinkage was not a serious problem in these fillets possibly because they were chilled immediately after their removal and also because the pH was relatively high (6.9). The pH values of the post-rigor and aged post-rigor samples which entered rigor as intact fish were 6.3 and 6.5 respectively and at these relatively low pH's gaping was quite extensive. The severity of the gaping was relatively low, however, again possibly due to the low storage temperature.

The free drip from unfrozen fillets of coldwater species has been found to be dependent, in part, on the pH of the muscle at filleting. There is a trend for the drip to increase as the pH decreases (Tomlinson, Geiger & Dollinger, 1966; MacCallum *et al.*, 1967). The results of this work are in agreement with this since the pre-rigor fillets, which had the least drip, had the highest pH at filleting while the post-rigor fillets with the highest drip had the lowest pH.

One of the objectives of this study was to determine whether 'ageing' the fish for a few hours at ambient temperature before icing would avoid the cold shock reaction in tropical fish in the same way that it has been suggested that accelerated rigor might be used to control some of the problems of rigor mortis encountered with coldwater species (Jones, 1969). A delay of 6 hr before icing was chosen since previous work (Curran *et al.*, 1986) had shown that uniced tilapia start to stiffen at this time as rigor mortis commences.

The development of stiffening in the two groups of fish used to prepare the post-rigor and aged post-rigor fillets is shown in Fig. 1. The tilapia which were chilled immediately after death exhibited the typical cold shock reaction: they started to stiffen within minutes of being placed in ice and were fully rigid after 11 hr, which was slightly later than the fish used in the earlier study (Curran *et al.*, 1986). This may partly be explained by the fact that a different hybrid species of tilapia were used but also the fish used in the current work were considerably larger than the earlier samples and the development of rigor mortis is faster in smaller sized fish (Amlacher, 1961). For the same reason, the rigor period itself was also longer in the larger fish: 37 hr compared with 27 hr in the previous study with the smaller fish. The 'aged' fish also started to stiffen soon after being iced and were rigid after 16 hr. Statistical analysis of the data indicated that the immediately iced whole fish reached each stage of the stiffening grading system significantly earlier ($P > 0.01$) than the aged tilapia. However, there was no significant difference in the time taken for the fish of either group to reach full

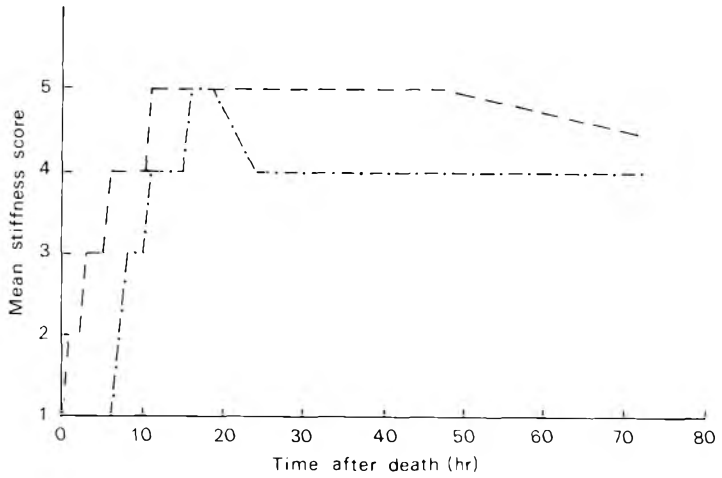

Figure 1. Post-mortem development of stiffening in tilapia which were either iced immediately after death (— — —) or held at ambient for 6 hr before being iced (—·—·-).

rigidity once they had started to stiffen (i.e., 10 or 11 hr from when they were iced). In comparison with the fish which were held at ambient temperature throughout storage in the earlier study, the aged fish became fully rigid much earlier (16 hr *versus* 19 hr). Unexpectedly, since they were larger and the lower temperature should prolong rigor mortis, the fish used in this study also had a much shorter rigor period (3 hr *versus* 7 hr). The aged post-rigor handling treatment would, therefore, not appear to avoid the cold shock reaction completely.

The results of the ATP, IMP, pH, lactic acid and % SPN analysis are given in Figs 2–4 with their regression lines. Table 2 presents the results of the analysis of variance of these data. It can be clearly seen that the handling procedures had different effects on the post-mortem metabolism of the tilapia. Treatment, time and the interaction between these two factors had significant effects ($P < 0.01$) on all of the variables measured.

Table 2. Results of the analysis of variance of the chemical data for the three handling treatments

Treatment	pH	Lactic acid	ATP	IMP	% SPN
Pre-rigor	6.800^a	24.53^a	1.37^a	4.29^a	58.41^a
Post-rigor	6.625^b	33.13^b	1.93^b	7.02^b	63.81^b
Aged post-rigor	6.675^c	27.79^c	2.23^c	6.14^b	52.90^c
LSD ($P \leq 0.05$)	0.039	1.79	0.379	1.036	4.17

Mean values in each column with the same letter superscript are not significantly different ($P \leq 0.05$).

Unlike the previous study (Curran *et al.*, 1986) with a different hybrid of tilapia where no clear relationship could be established, in the present study the post-mortem metabolism of the tilapia which were iced immediately following death was accelerated,

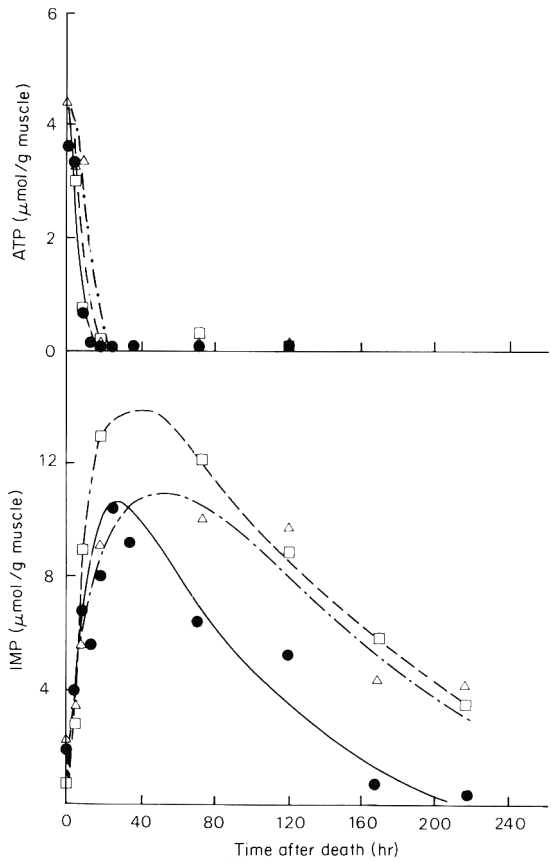

Figure 2. ATP(r^2 = 0.743) and IMP (r^2 = 0.50) in pre-rigor (•--•), post-rigor (□- - -□) and aged post-rigor (△-·-△) fillets from tilapia (where r^2 is the correlation coefficient for each pair of variables).

presumably due to the cold shock reaction. ATP degradation (Fig. 2) and lactic acid accumulation (Fig. 3) were faster in these fish compared with the tilapia which were held at ambient temperature before icing. However, the most rapid metabolism was found in the pre-rigor fillets. This may possibly be explained by the fact that the fillets would be chilled much more rapidly than the whole fish and, therefore, the cold shock reaction may be expected to be more severe. The pre-rigor fillets also had the fastest accumulation and subsequent breakdown of IMP (Fig. 2). The rates of IMP metabolism in the post-rigor and aged post-rigor samples were significantly slower than in the pre-rigor fillets but not significantly different from each other. The latter result is unexpected since Fraser, Pitts & Dyer (1968) found that IMP was completely dephosphorylated much earlier in Nova Scotia mackerel stored at 13–20°C for 7 hr before icing compared with fish which were immediately iced on capture. The maximum concentration of IMP found in some of the treatments was higher than normally expected and at present this cannot be explained. The change in muscle pH would normally be expected to reflect the changes in lactic acid concentration described above. This was true for the two post-rigor treatments. In the pre-rigor fillets, however, the pH remained fairly constant throughout storage while the lactic acid concentration varied quite considerably.

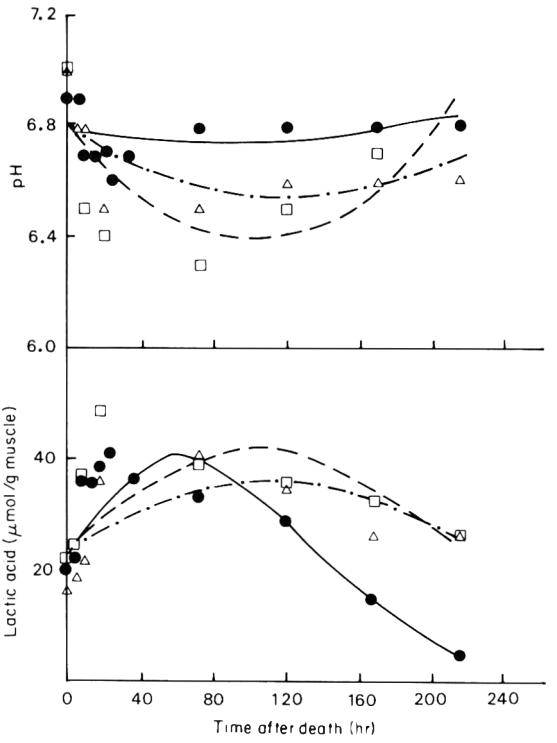

Figure 3. Lactic acid (r^2 = 0.653) and pH r^2 = 0.327) in pre-rigor (●--●), post-rigor (□- - -□) and aged post-rigor (△-·-△) fillets from tilapia (where r^2 is the correlation coefficient for each pair of variables).

There is a certain amount of scatter inherent in the SPN method (Love, 1962) such that it is more pertinent to observe the trends rather than individual values. Differing results have been reported for the change in SPN in relation to rigor mortis and prolonged iced storage (Moorjani *et al.*, 1962; Connell, 1968; Disney *et al.*, 1971; Suzuki, 1981). In addition, Partmann (1960), Love (1962) and MacCallum *et al.* (1967) all found that the proteins of iced pre-rigor fillets were less soluble than those of iced whole fish. The results of this study concur with this since the post-rigor fillets had a slight increase and subsequent decrease in SPN but remained fairly constant around 60% while the pre-rigor fillets showed a gradual decrease from about 60% to 40–50% SPN. The aged post-rigor fillets also showed a slight increase in protein solubility but this decreased to below 40% by the end of the storage period. The solubility of the muscle proteins in dilute salt solutions is related to the condition of the proteins themselves and the pH of the muscle (Suzuki, 1981). The pH of the three treatments studied here varied, with the aged post-rigor treatment having the lowest pH.

Conclusions

This study has shown that the best filleting and processing yields and the least drip loss and gaping were obtained when the tropical fish, tilapia, were filleted pre-rigor. On-board filleting, which is often highly impracticable in the tropics, would be a requirement for this operation. A further disadvantage is the shorter shelf life of the

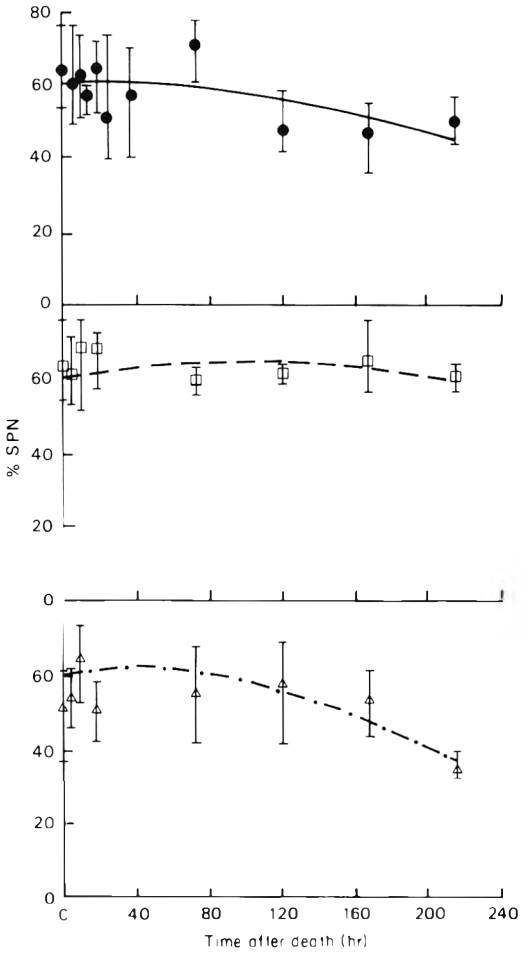

Figure 4. Soluble protein nitrogen ($r^2 = 0.302$) in pre-rigor (●--●), post-rigor (□- - -□) and aged post-rigor (△-·-△) fillets from tilapia (where r^2 is the correlation coefficient for each pair of variables).

pre-rigor product. Icing tilapia immediately on capture, i.e., the recommended handling procedure, induced the cold shock reaction; as a consequence, filleting these fish after 3 days produced the lowest filleting and processing yields, the highest drip loss and more gaping. Delaying icing for 6 hr produced a 3% overall increase in yield compared with immediate icing of whole fish and a drip loss which was midway between the extremes of the other two handling procedures. The same amount of gaping occurred as in the post-rigor fillets and both of these treatments allowed a longer overall shelf life compared with pre-rigor filleting. These results suggest that ageing fish at ambient conditions for a few hours before icing could produce higher yields in some tropical fish intended for filleting.

Thus a cold shock reaction in tropical fish has important commercial implications. FAO/WHO International Codes of Practice (FAO/WHO, 1977; FAO, 1982) recommend that fish are chilled quickly to the temperature of melting ice soon after capture and are maintained at that temperature. The Codes are widely accepted as being of

considerable value but they are based upon experience and established practices in the fisheries of temperate and cold regions. From the results of this study it is clear that some modification of these Codes to accommodate characteristics peculiar to tropical fish may be required.

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Consumer acceptability of cod and whiting after chilled storage and freezing and thawing

R. BENNETT AND M. HAMILTON

Summary

The effect of freezing and thawing, and of storage at 0°C on the acceptability of two white fish species (cod and whiting) was investigated. It was concluded that samples which had been frozen and thawed were no less acceptable than fresh samples and that storage of fresh fish at 0°C produced a linear decline in overall acceptability of both species. A method of presenting the data to allow decisions to be taken on the shelf life of chilled products is discussed.

Introduction

Now that distant water fishing grounds are no longer available, the U.K. catch of white fish is obtained from medium distance and inshore waters and is usually stored on ice after catching, rather than being frozen at sea. Depending on circumstances the consumer could typically be purchasing fish which had spent 2–12 days on ice post-harvest and much of it would be deep frozen at this stage for later consumption.

Love (1980) suggested that the 'seaweedy' flavour of fresh fish was lost after about 5 days on ice at which time it became bland until off flavours developed. Early changes are caused by autolytic enzyme action and subsequent changes by bacteria. Love also observed that some consumers might prefer spoilt fish and may never have encountered the genuine fresh product.

Hamilton & Bennett (1983) investigated the acceptability to consumers of nine fresh white fish species and established that flavour was the major determinant of preference, that species were distinguishable from one another by taste and that consumer preferences were not held very strongly. The work was carried out with very fresh fish, which had been frozen and stored at −30°C before thawing, and tasted as plain steamed samples. For all practical purposes, 'very fresh fish' can be regarded as fish which has spent 2 days on ice since it can take this time for post-mortem rigor to resolve.

In addition to the established markets for fresh-wet and frozen fish, there is now a growing market for sales of chilled fresh fillets or steaks in transparent 'controlled atmosphere' packs which have been flushed with carbon dioxide before sealing. Legislation requires these packs to be stamped with 'best by' or 'sell by' dates and at present there is limited information in a form which will enable decisions about this dating to be taken, even though practicable storage life is of great commercial significance. Howgate (1982) has recently put forward ideas for criteria on which such quality judgments might be made.

The effects of iced storage and freezing and thawing on cod (Connell & Howgate, 1968) and on haddock (Connell & Howgate, 1969) were investigated using small expert panels and showed that storage at −30°C for 6 months did not further affect quality.

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Later work on cod and haddock (Connell & Howgate, 1971) used somewhat larger non-expert panels and found wide variation in the abilities of individuals to distinguish between fish of a wide range of qualities. Despite this the mean panel scores did discriminate successfully.

We felt that it would be useful to develop aspects of this work with an additional species, using larger consumer panels with an experimental design which allowed direct comparisons to be made of the scores awarded by individual judges to each treatment. This work is therefore largely concerned with describing the reaction of consumers to the fish which they are likely to encounter and to presenting the information in ways which may be useful to the processor or supplier. For these reasons the effects of freezing and thawing and of chilled storage were explored.

Materials and methods

Iced–age tests

In order to make direct comparisons between the various ages of samples a large batch of freshly caught fish was gutted and held on ice. Samples were removed periodically, filleted, deep frozen in polythene-lined waxed cardboard boxes and held at −30°C, to produce a series of samples which had been held on ice between 3 and 15 days on the day of test.

Freeze–thaw tests

Bulk samples of good quality cod and whiting which scored 8 or 9 on the Shewan *et al.* (1953) Raw Odour scale were selected (equivalent to fish which had been 2 or 3 days on ice post-mortem). Each species sample was divided into three subgroups, two of which were blast frozen. One frozen batch was then allowed to thaw at room temperature overnight ('slow thaw') and the second frozen batch ('quick thaw') carefully thawed in a microwave oven before the cooking and testing session. The third sample ('fresh') was held at 0°C overnight.

Sensory evaluation

Each sample was rated for appearance, texture, flavour and overall acceptability using a seven point hedonic rating scale (7, like very much; 4, neither like nor dislike; 1, dislike very much). Judges were college staff and students, technicians, cleaners, kitchen assistants etc. drawn from a wide geographical area. Eighty per cent of the panellists were female with a preponderance of judges in the 18–22 age range, sixty-six judges took part in freeze-thaw tests and 30 in iced-age tests and each judge rated each sample. The judges were drawn from a wide geographical area but were not selected in a way that would ensure that they were exactly representative of typical consumers; however, in a more recent experiment (Mackie *et al.* 1985) the judgements of these assessors were found to be identical to those of eight other panels operating at different locations in the U.K. There is therefore no reason to believe that the results of this panel would not generalize to ordinary consumers. Samples were cooked between plates over boiling water and were presented as plain unseasoned samples as described previously (Hamilton & Bennett, 1983).

Analysis and presentation of data

Data produced by hedonic rating tests is non-parametric (although often analysed by parametric methods) and for this reason the Wilcoxon matched pairs signed rank test

of significance was used to compare the acceptability scores of each judge for each treatment and median panel scores rather than mean scores were used as the measures of central tendency. The percentage of judges expressing some degree of preference was obtained by summing the number of judges awarding scores of 7, 6 and 5 together with half the number awarding 4 and expressing this as a percentage of the total number of judges.

Results and discussion

(a) Freezing and thawing

Table 1 shows the mean ratings and standard deviation for acceptability data from sixty-six judges for the two species and three conditions. The ratings awarded by each assessor to each treatment were compared directly using the Wilcoxon matched pairs signed rank test of significance which showed, for whiting, no statistically significant differences between any of the treatments. In the case of cod the quick thawed sample was preferred over both the fresh and slow thawed samples. We had expected that thawing slowly would give lower acceptability scores due to the fish being held between 0°C and ambient for some time during thawing, but this was not found.

Table 1. Mean ratings and standard deviation awarded by 66 judges for overall acceptability

Species/conditions	Fresh (s.d.)	Quick thaw	Slow thaw
Cod	4.9 (1.25)	5.3 (1.14)	4.8 (1.4)
Whiting	4.4 (1.75)	4.4 (1.5)	4.9 (1.3)

The main objective of this part of the investigation was to determine if the method of storing samples used in these trials influenced acceptability. Although the data does not allow firm conclusions to be drawn on the general effects of freezing and method of thawing on acceptability, from our previous and current work it seems reasonable to conclude that the processes of freezing and slow thawing (i.e. our routine procedure) does not lead to significant reductions in acceptability to the consumer over that of the fresh product. It appears from these data and from the storage data of Connell & Howgate (1968) that the consumer will probably be as well satisfied with the frozen as with the fresh product.

(b) Ageing on ice

A preliminary series of age comparisons was carried out using triangle tests but significant differences were not established. We believe that the confounding factors introduced by working with non-homogeneous solid samples which can never be exact duplicates probably negates the usual advantages of difference tests.

Tables 2 and 3 show matrices of significance of differences established by applying the Wilcoxon test to the acceptability ratings for the various age-pairs of the two species. Median scores are also shown which were calculated by treating the scores as grouped data and interpolating within the central group. The tables show that storage differences of about 5 days in the case of whiting and 7 days in the case of cod are readily detectable and there is in both cases a progressive loss of acceptability with time stored on ice.

Table 2. Cod–matrix of significant differences between acceptability ratings at different ages

Age	3	5	8	13	15
3			***	***	***
5				*	***
8					***
13					***
Median	5.5	5.32	4.72	4.28	3.95

Table 3. Whiting–matrix of significant differences between acceptability ratings at different ages

Age	3	5	8	13	15
3			*	***	***
5				***	***
8				**	***
13					
Median	5.25	4.5	4.3	3.2	2.8

Figure 1 shows the median scores plotted against days on ice for cod and whiting respectively and Fig. 2 shows median ratings for appearance, flavour, texture and acceptability plotted against days on ice for cod.

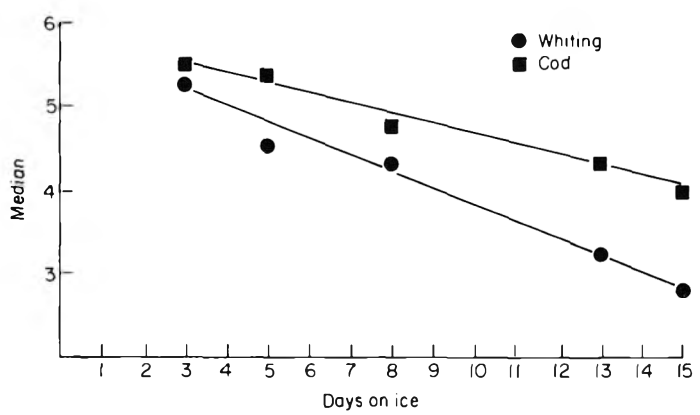

Figure 1. Median hedonic ratings of thirty judges for acceptability of fresh cod and whiting against time stored on ice.

The rating scale used has 4 as its neutral point (i.e., neither like nor dislike) therefore median scores greater than 4 indicate that a majority of judges expressed some degree of liking for the product. Figures 1 and 2 show a linear decline in acceptability with time on ice with the neutral point being reached at 15 days for cod and 8.5 days for whiting, i.e., at these times half the assessors expressed some degree of liking and half some degree of dislike. There is no real indication of any steps or plateaus which

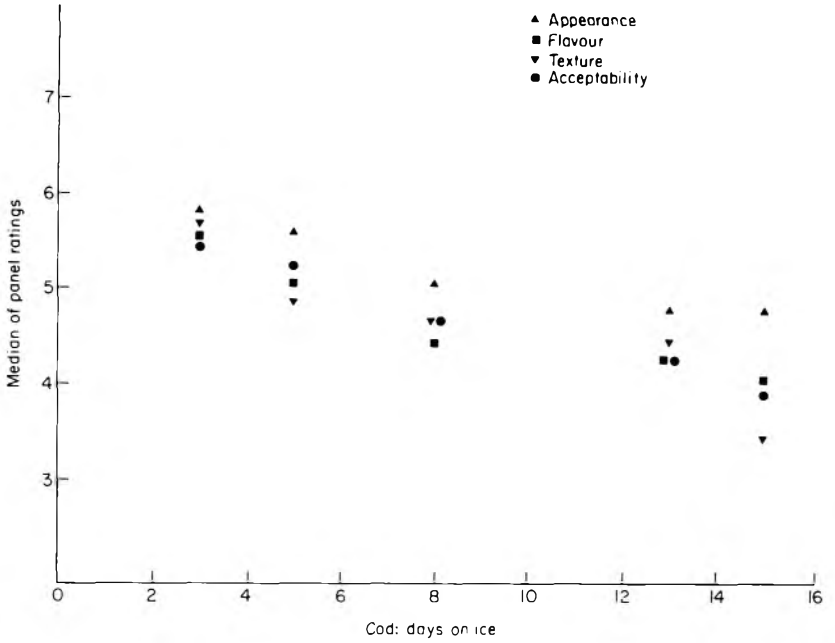

Figure 2. Median hedonic ratings of thirty judges for appearance, flavour, texture and acceptability of fresh cod against time stored on ice.

could be attributed to combinations of liked quality being lost while an independent growth in a disliked quanlity was occurring; neither is there any evidence to suggest a general liking for spoilt fish. An examination of raw data does show some high scores for 15-day fish but this seems more attributable to an inability of some assessors to discriminate rather than to a genuine preference. An advantage of examining data in this way is that panellists responses are simply categorized into either 'like groups' or 'don't like groups'. This procedure will reduce or eliminate any unconscious bias of panellists in response to the range of stimuli presented to them (Poulton, 1977).

The general consensus is that acceptability loss in fresh fish is initially attributable to a loss of some valued fresh flavour and subsequently to the development of disliked flavours by bacterial action. This should be at least a biphasic process and additionally bacterial growth is exponential rather than linear so it is not at all obvious how these various processes combine to produce the linear changes observed.

Figure 2 indicates that there is a broadly similar decline with age in the ratings for all the sensory characteristics. The four sensory characteristics were all rated for the same sample at the same tasting session. Appearance is scored before tasting takes place but texture, flavour and overall acceptability would be considered in turn by judges as they chew a single sample. Previous work (Hamilton & Bennett, 1983) established that the acceptability of fresh white fish could be explained entirely on the basis of flavour; texture was found to be neutral and appearance either neutral or negative. Judges were asked to consciously attempt to score the characteristics independently and although there is almost certain to be some sort of halo effect in experiments carried out in the manner described above, the very close correspondence of the scores for the four sensory characteristics at the various ages would not be expected on this basis alone.

The evidence is not conclusive but it does seem that there are changes which judges like less in appearance, and texture as well as flavour.

Fish is now being sold chilled and prepackaged and must be date-stamped, and the relevance of our findings to the retailer/processor should be discussed. Eating is an individual rather than a statistical experience and inspection of raw data suggests that the population contains various subgroups, e.g. discriminators, non-discriminators, dislikers of fish in any form, etc. It follows that there will never be a time when all the judges would like any sample. Howgate (1983) discusses the judgments a retailer might appropriately make in determining shelf life and suggests that most manufacturers would not be satisfied with a position where 50% of the population express some degree of disliking for their product. Data can be presented in a form which aids these judgments by plotting the percentage of assessors who expressed some degree of liking against time on ice and this is shown in Fig. 3. The retailer who wished, say, 70% of the

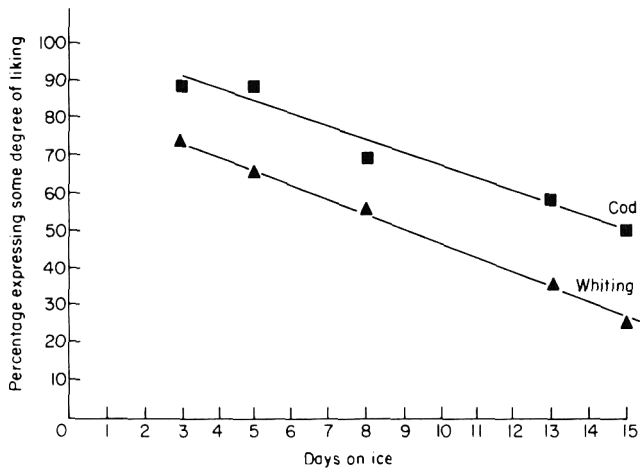

Figure 3. The percentage of judges expressing some degree of liking for fresh cod and fresh whiting against time stored on ice.

population to like his product would have to get it to them within 4 days of catching for whiting and 7 days for cod. The implications are clear but their implementation is not easy since fish not infrequently have spent this sort of time on ice before being landed at fish markets. The conclusions reached may be regarded as conservative since only plain steamed samples were tasted and acceptability was scrutinized much more intensively than would be the case where the fish formed part of a meal being consumed in a social environment. However the necessity for the industry to handle their product carefully, to chill it properly and to get it to the consumer promptly is underlined by these findings. The processor clearly needs a product of consistent quality if decisions regarding retail shelf-life are to be meaningful.

Acknowledgments

We are grateful to the Torry Research Station, Aberdeen for supplying fish samples and to Mr P. Howgate of that establishment for valuable discussions.

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Suitability of the Ottawa Pea Tenderometer to assess the quality of raw peas

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Summary

This paper gives an account of the relationship obtained between the force readings for samples of peas using the Martin Pea Tenderometer, the Ottawa Pea Tenderometer and the Instron 1140 used with an Ottawa Pea Tenderometer food cell, together with sensory quality of canned and frozen peas and alcohol insoluble solids. The effect of temperature on the readings obtained for the different instruments, and the effect of interchangeability of Ottawa Pea Tenderometer cells and components are also reported together with information concerning the influence of bruising and damage to a sample of peas.

Introduction

In the United Kingdom the Martin Pea Tenderometer (MPT) is the standard method for predicting the quality of canned or frozen peas from measurements made on the fresh peas. These measurements also enable the crop to be graded in order to establish the price received by a grower for a particular batch of peas. The importance of this can be realized when the size of the industry is considered, for example the estimated 1983 harvest of peas for processing was 197.5 thousand tonnes, and in 1981 the total estimated retail value of the packs of garden peas processed was 150 million pounds. Because of differences in the age and condition of factory machines it is possible to obtain different values from a given sample of peas, so it is important that the MPTs used are all calibrated to the same standard. However there are problems with calibrating MPTs, for example differing engineering tolerances and finding a suitable test material (Voisey & Nonnecke, 1971; Graham & Evans, 1957). The accepted method of standardizing the machines is to check against a designated 'Master' MPT using fresh peas. However, this procedure is time consuming and expensive to operate since the peas have to be transported from one factory to another to achieve cross-reference. This has resulted in a continuous search to find an alternative method of standardization which could be cheaper and more reliable. Ideally a machine, or even a texture cell, made to a reproducible standard would be suitable. The cells could be independently checked using mechanical calibration and this would remove the necessity of transporting fresh peas. A number of instruments, viz. the Food Technology Corporation (FTC) Shear Press and Maturometer, have been suggested but extensive trials have failed to produce reliable correlations (Atherton & Holdsworth, 1975). These instruments were initially designed to replace the MPTs completely but in the United Kingdom they have been tested and assessed for use in the standardization of tenderometers.

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Voisey & Nonnecke, (1972a) compared several mechanical methods of pea tenderness measurement to select design parameters for a new instrument. They concluded that a suitable method should produce results that correlate with those obtained from a correctly standardized and maintained MPT. Amongst the methods tested the wire extrusion cell appeared to offer some promise for further development. Further investigation found that a 30 cm^2 cell with a nine-wire grid was suitable (Voisey & Nonnecke, 1972b). The researchers recognized the need to manufacture the test cells and grids to close tolerances so that interchangeability of cells and components would be possible. To operate with this cell Voisey & Nonnecke (1973) developed an instrument to compress the sample and record the force used; this they called the Ottawa Pea Tenderometer (OPT). This instrument had the advantage, compared to the standard MPT, of having an independent method of calibration for the load cell.

From this work the OPT appeared to present a reliable alternative to the MPT, and consequently its suitability for the United Kingdom pea industry was investigated. Initially an OPT cell was used with an Instron 1140 universal texture measuring instrument because an OPT was not commercially available at that time. Subsequently an OPT was obtained and was included in the trials for comparative purposes.

This paper gives an account of the relationship obtained between the force readings for samples of peas using the MPT, OPT and Instron OPT cell together with sensory quality of canned and frozen peas and alcohol insoluble solids. The effect of temperature on the readings obtained for the different instruments and effect of the interchangeability of OPT cells and components are also reported together with information about the influences of bruising and damage of the peas.

Materials and methods

The experimental work was started in 1977 and was completed in 1983, using varieties of peas (Platinum, Scout, Waverex, Turon, Sprite, Dark Skinned Perfection (D.S.P.), Puget and Avola) which were then currently being used by the United Kingdom industry. The entire plants were harvested by hand and then the peas were separated from the haulm using a stationary plot viner, unless otherwise stated.

The relationship between sensory texture and instrumental measurement of texture was investigated using pea varieties (Platinum, Scout, Turon and Waverex) grown and then harvested as required, to give approximately ten distinct batches of peas within the range 80–180 MPT units. After vining, all samples of peas were stored out of direct sunlight, at ambient temperature for between 1 and 2 hr prior to use. Up to ten readings were then obtained from each of the MPT, OPT and Instron OPT cell. Further samples of peas from the same batches were either canned (1978–1982) or frozen (1980–1982). The procedure used to can the peas involved blanching in 1:3 tap/deionized water at 93°C for 3 min prior to cooling in cold water, followed by filling 285 g into UT (300×408 3/4) cans. A brine solution, containing 3.75% salt, 1.9% sugar and 0.2% 13T/64P green colour at 93°C, was then added to each can and the headspace adjusted. The cans were then seamed and processed for 15 min at 121.1°C and then pressure cooled. After drying, the cans were stored at ambient temperature until required for assessment. The frozen peas were prepared by blanching at 93°C for 1½ min in tap water prior to water cooling. The peas were then flow-frozen for 3 min at −37°C, and 230 g of peas were packed into polyethylene bags, which were sealed prior to storage at −24°C until required for assessment.

The effect of damaged peas on the MPT and OPT values was determined by using

batches of varieties (Puget, Avola and D.S.P.) harvested at varying levels of maturity in 1982 and 1983. The pea haulm was initially vined as above, but two thirds of the peas were then put through the viner a second time, and half of this sample was then put through the viner a third time. Up to ten readings were then obtained from the MPT and OPT for each sample.

The temperature correction factor for the OPT and the MPT was determined using peas of the varieties D.S.P., Scout and Avola (1982 and 1983). After harvesting and vining as described above, the peas were kept under water for 4 hr at 20–24°C. Samples of these peas were then transferred to other containers of water equilibrated at the desired test temperature in the range 4–30°C for 15 min. These peas were used to obtain three readings from the MPT and OPT. Prior to readings being carried out at each temperature, the OPT cells were equilibrated at the desired test temperature and the appropriate soaking water was poured through the MPT test chamber.

The interchangeability of OPT cells and components was tested several times during the years 1977–1981 and using the varieties Sprite and D.S.P.. Peas were kept under water for over 4 hr at a constant temperature of 21°C. Up to ten readings were then obtained using these peas for each variable being tested.

In addition to these planned experiments samples of peas were used, as they became available, to obtain comparative readings from the MPTs, OPT and Instron OPT cell from 1977 to 1983 (the varieties used were Freezer 47 (now called Banff), Hustler, Sprite, Platinum, Puget, Perfected Pea, Novella (now renamed Bikini), Dunston, Ceb 602, 39–77, Suprema, Tornado, 2991-75, Eaton, D.S.P., Avola, Dew, Aldot, Trapper, Dinos, Sela. Dawn, Fridgit, Norge, Dual, Aux 333-26 and Scout).

Instrumental texture measurements

The texture of fresh peas was measured using two types of instrument, firstly, by MPTs and secondly by an OPT cell in either an OPT or Instron machine. The readings were MPT units in the former, and force units in the latter. The MPT used for these trials was originally supplied to the Campden Food Preservation Research Association by Canners Machinery Ltd (CML) and has been maintained constantly as a 'Master' instrument. A second 'Master' MPT, supplied by the Food Machinery Corporation (FMC) and which has the same basic operation and type of food cell as the CML model, was included in some of the trials.

An OPT wire extrusion cell, designated as 30 cm^2, was used for all the trials, this cell has a removable grid containing nine wires of 2.36 mm diameter. The same basic design of OPT cell was used with the Instron and the OPT although the cell used with the Instron had longer support legs. The Instron used was a 1140 model operated with a 5000 N load cell, with a compression cross-head drive speed of 200 mm/min as this was the nearest equivalent speed to that of the OPT. The force readings obtained were recorded, using a strip chart, in Newtons and then recalculated into kg force. From 1980 onwards when the OPT was commercially available it was included in all comparative trials. The OPT was operated with a 4500 N load cell and at the preset compression cross-head drive speed of 180 mm/min. A digital display shows the readings in 2 kg units, these were recalculated into kg force. All instruments were maintained and used in accordance with the manufacturers instructions (Anon, 1972; Anon, 1974).

Analytical assessment

The percentage of alcohol insoluble solids (%AIS) present in the canned and frozen peas, was determined by the AOAC method (AOAC, 1980). This was used as an

independent method of texture assessment of the raw product using the established correlations between MPT readings and %AIS (Anthistle, 1961).

Sensory evaluation

The colour, flavour and texture of the canned peas were assessed by a skilled sensory evaluation team using a standard method (Anon, 1970). A similar method was used for the frozen peas, although the maximum number of points given for perfection of the attribute differs fer the canned and frozen methods, (Anon, 1975). For example colour and flavour of canned peas were each scored out of a maximum of 20 points, while frozen peas were scored out of a maximum of 10 points. Similarly texture assessment, which comprises an evaluation of firmness of skins and cotyledons, and mealiness (cloudiness of brine is also considered in canned samples) is scored out of 30 and 20 points respectively for canned and frozen peas.

Results and discussion

Overall correlations

The predictive regression equations and 95% confidence limits obtained from seven seasons work are given in Table 1. All data points were treated as paired comparisons but the pairing was not manipulated to obtain improved regression equations or confidence limits. From this table it can be seen that the comparison between the two 'Master' MPTs gave the lowest range of confidence limits viz. ±7.8 MPT units, while the ranges for other combinations were significantly greater. This was not expected as the OPT cell is very simple with few wearing surfaces or parts compared to the MPT, and the force recording systems of both the Instron and OPT should have greater accuracy than the MPT. Table 1 illustrates that the 95% confidence limits are greater for the relationship between the Instron OPT and the OPT than between the to 'Master' MPT. The figure of ±11.3 from Table 1 has been calculated using the relevant slope for the OPT/MPT relationship. The data points that are included to obtain Table 1 span the whole of the MPTs range of 80–180 MPT units.

Table 1. The predictive regression equations and 95% confidence limits for the relationship between the Martin Pea Tenderometer (MPT), Instron Ottawa Pea Tenderometer cell and Ottawa Pea Tenderometer (OPT)

Combination X/Y	Number of observations	Regression equation $y = mX+c$ m	c	Correlation coefficient*	95% Confidence limit (MPT units)
Instron OPT/MPT	2214	0.606	−12.6	0.949	±13.6
OPT/MPT	1235	0.538	− 3.3	0.965	±12.2
Instron OPT/OPT	711	1.038	− 0.4	0.972	±11.3
MPT/MPT	1129	1.000	− 0.03	0.977	± 7.8

*All correlations are significant at the 99.95% confidence level.

Peas with MPT readings within the range 95–105 are usually used for freezing, while canners prefer peas within the range 115–125. Therefore the results illustrated in Tables 2 and 3 were calculated for the two ranges. From the data it can be seen that the results

for the Instron OPT/OPT and MPT/MPT relationships for both maturity ranges gave similar confidence limits, as would be expected for similar cells, whereas for the Instron OPT or OPT with the MPT, the confidence limits were larger. Consequently replacement of MPTs by either OPTs or Instron OPTs would not lead to a loss of the standard but the use of an OPT or Instron OPT to standardize MPTs could introduce more errors than found by using the present 'Master' MPT system.

Table 2. The predictive regression equations and 95% confidence limits for the relationship between the Martin Pea Tenderometer (MPT), Instron Ottawa Pea Tenderometer cell and Ottawa Pea Tenderometer (OPT) for peas in the range 95–105 MPT units

Combination X/Y	Number of observations	Regression equation $Y = mX+c$		Correlation coefficient*	95% Confidence limit (MPT units)
		m	c		
Instron OPT/MPT	409	0.129	75.7	0.391	±5.6
OPT/MPT	179	0.159	69.0	0.519	±5.2
Instron OPT/OPT	112	0.253	146.7	0.525	±4.5
MPT/MPT	197	0.470	52.5	0.670	±4.6

*All correlations are significant at the 99.95% confidence level.

Table 3. The predictive regression equations and 95% confidence limits for the relationship between the Martin Pea Tenderometer (MPT), Instron Ottawa Pea Tenderometer cell and Ottawa Pea Tenderometer (OPT) for peas in the range 115–125 MPT units

Combination X/Y	Number of observations	Regression equation $Y = mX+c$		Correlation coefficient*	95% Confidence limit (MPT units)
		m	c		
Instron OPT/MPT	418	0.116	94.6	0.476	±5.3
OPT/MPT	178	0.099	97.0	0.462	±5.4
Instron OPT/OPT	72	0.232	178.1	0.521	±4.4
MPT/MPT	284	0.414	70.5	0.599	±4.7

*All correlations are significant at the 99.95% confidence level.

Effect of variety and season

The overall regression equation between the Instron OPT and MPT as used for Table 1 was:

$$\text{MPT} = 0.606 \times \text{OPT} - 12.6.$$

This equation was derived from 2214 data points. Voisey & Nonnecke (1973) suggested that the parameters in the equation varied among varieties. To investigate this further and the effect of season, pea varieties were used that had different characteristics viz. Scout is a medium to large-seeded variety, Turon and Waverex are small-seeded

varieties and Platinum is a pale, small to medium sized seeded variety. This work covers fourteen season/variety combinations, a total of 1104 data points representing 166 samples. Multiple regression analysis was used to fit an equation of the form:

$$\text{MPT} = (Vs+Ys+VYs+m)\times\text{OPT}+(Vi+Yi+VYi+c),$$

where VS = the effect of the particular variety on the slope of the regression; Ys = the effect of the particular season on the slope of the regression; VYs = the effect of the particular combination on the slope of the regression; Vi = the effect of the particular variety on the regression constant; Yi = the effect of the particular season on the regression constant; VYi = the effect of the particular combination on the regression constant; m = variety and year independent contribution to slope (Instron); and c = variety and year independent contribution to intercept (Instron).

Table 4 shows the results of analysis of variance on the regression analysis. For each of the factors in the equation the table shows: the degrees of freedom attributable to the factor; the sums of squares attributable to the factor; the mean square attributable to the factor; the variance ratio = mean square factor/mean square residual; and the significance level for the variance ratio (the confidence that inclusion of that factor really improves the prediction).

Table 4. Effect of season and variety on parameters of Martin Pea Tenderometer/Instron Ottawa Pea Tenderometer cell regression. Analysis of variance

Source	Degrees of freedom	Sums of squares	Means squares	Variance ratio *F* value	Level of significance (%)
Instron OPT (intercept and slope)	1	626568	626568	1770	99.99
Variety on intercept	3	3733	1244	3.51	98.00
Season on intercept	4	17786	4446	12.60	99.99
Variety on slope	3	83	28	0.08	N.S.
Season on slope	4	2593	648	1.83	N.S.
Variety and season on intercept	19	1692	89	0.25	N.S.
Variety and season on slope	19	572	30	0.08	N.S.
Residual	99	35040	354		
Total	152	688068			

The results show that inclusion of a factor to allow for the effect of variety on intercept improves the prediction slightly. There is very high confidence that season affects the intercept. There is no significant evidence that year or variety, singly or in combination, affect the slope, neither is there any evidence that the particular variety/year combination affect the intercept. The equation now becomes:

$$\text{MPT} = m\times\text{OPT}+Vi+Yi+c.$$

These conclusions are confirmed by Table 5 which shows the standard error of predictions from the various combinations of factors. It can be seen that the inclusion of variety makes a small difference to the predictive accuracy, while season makes quite a large difference. For comparison, Table 5 also includes the 'between replicate' standard deviations of the MPT and OPT measurements. The precision of the MPT estimated from OPT (6.0–7.5) seems very good when it is recalled that the Instron OPT is imprecise (4.6 MPT units) and so is the MPT (3.5 MPT units).

Table 5. Effect of season and variety on parameters of Martin Pea Tenderometer/Instron Ottawa Pea Tenderometer cell regression. Standard error of estimate

Factors included in addition to Instron OPT	Standard error of estimate (MPT units)
None	7.467
Variety	7.247
Season	6.167
Variety and season	6.040
MPT reproducible standard deviation	3.49 MPT units
Instron OPT reproducible standard deviation	7.6 Instron OPT units = 4.6 MPT units

Figure 1 depicts the OPT cell/MPT relationship for the variety Waverex for the seasons 1980, 1981 and 1982. Figure 2 depicts the OPT cell/MPT relationships for the varieties Scout, Turon and Platinum for the 1979 season. These variety/season combinations are illustrated as they showed the greatest variety/seasonal effect.

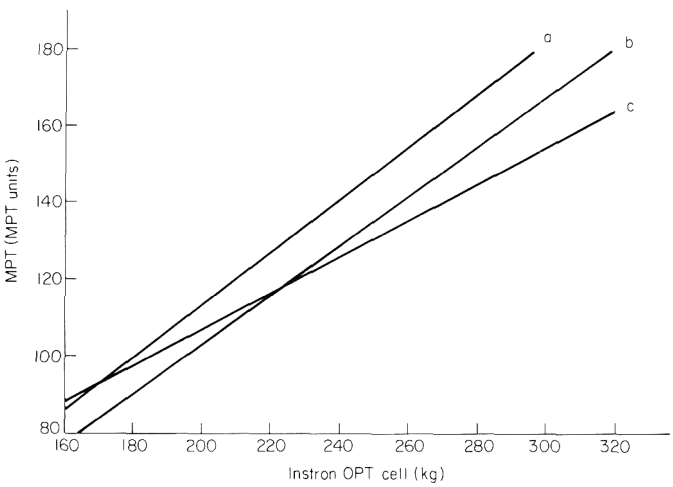

Figure 1. Instron Ottawa Pea Tenderometer (OPT) cell relationship with Martin Pea Tenderometer (MTP) readings for the variety Waverex in 1980(a), 1981(b) and 1982 (c). *X* axis Instron OPT cell kg; *y* axis MPT. MPT units.

Relationship with sensory analysis

The relationship of sensory texture of canned peas with the instrumental methods could be depicted by the use of a straight line graph using linear regression techniques (Fig. 3). However using stepwise polynominal regression analysis the relationship deviated from linearity at high readings for the MPT. The relationship between sensory texture and instrumental textural measurement are therefore complex, probably due to the effects of variety and season. The relationships between sensory colour or flavour and the instrumental methods was not significant, however certain trends in the scores for colour and flavour could be identified.

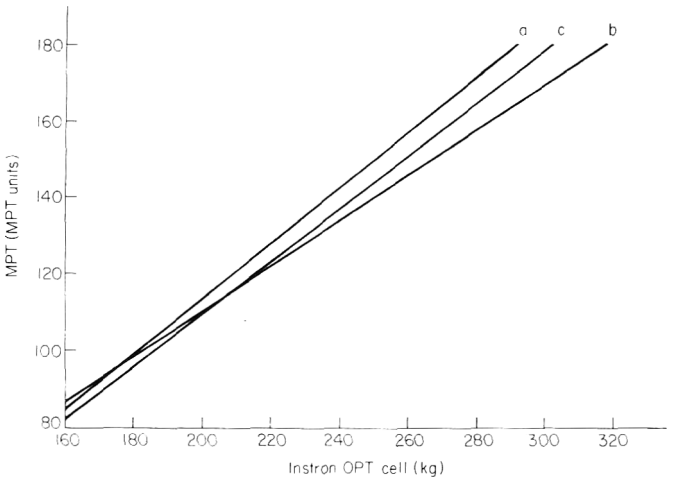

Figure 2. Instron Ottawa Pea Tenderometer (OPT) cell relationship with Martin Pea Tenderometer (MPT) readings for the season 1979, varieties Scout (a), Platinum (b) and Turon (c). *x* axis Instron OPT cell kg; *y* axis MPT. MPT units.

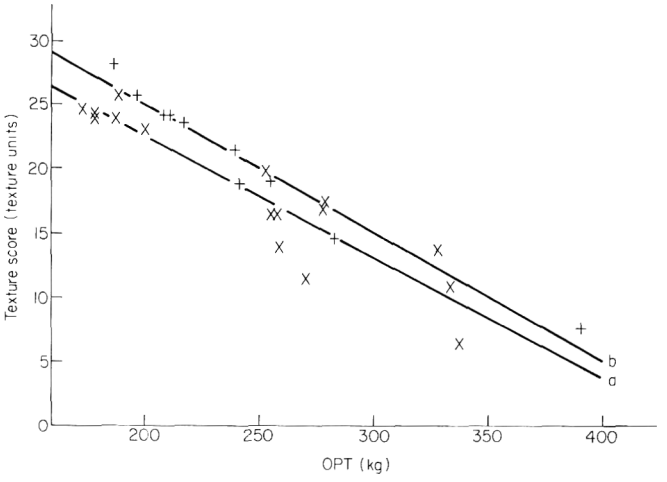

Figure 3. Ottawa Pea Tenderometer (OPT) relationship with sensory texture of canned peas for the season 1982, varieties Scout (a) and Waverex (b). *x* axis OPT kg; *y* axis Texture score. Texture units.

Although there was less data available the texture of frozen peas the results obtained were generally similar to those obtained from canned peas. There were seasonal and varietal effects, but all the instrumental methods were affected equally. The relationship with sensory colour and flavour were not significant although, again, the scores for colour and flavour tended to decrease with increasing texture reading. This is not too surprising in that the instruments used, (MPT, Instron OPT and OPT) only measure the texture of peas, whereas the increase in hardness (texture) is usually accompanied by colour and flavour changes. From the data obtained it was concluded that the OPT cell could be used for the prediction of final product quality with the same certainty as the other methods.

Relationship with analytical assessments

A highly significant correlation between %AIS, measured in canned pea samples, and MPT, Instron OPT and OPT readings were found for the raw peas. The relationship with sensory texture was similar to that obtained with the MPT, Instron OPT and OPT, however the 95% confidence limits were slightly wider. It would appear that measurements made on the raw peas, using a MPT, Instron OPT or OPT, would be as useful as assessing the percentage of %AIS present in the canned product. The relationship of %AIS and sensory texture of frozen peas was found to be slightly closer than that found between sensory texture and the mechanical methods of pea measurements. However, the relationship between %AIS and sensory texture of frozen peas gave wider confidence limits than those obtained for the relationship between %AIS and the mechanical methods. The relationship of MPT, Instron OPT and OPT with %AIS of canned and frozen peas did not appear to be affected by variety but appeared to be affected by season.

Effect of viner damage

The maturometer developed by Commonwealth Scientific Industrial Research Organisation, Australia, (Mitchell, Casimir & Lynch, 1961) has been shown to be significantly affected by harvesting or viner damage, this has reduced its use as an instrument to grade fresh peas (Rutledge, 1981). Although the OPT reading was not significantly affected by viner damage for immature peas it was affected when mature peas were damaged, even when there was no appreciable external damage to the peas. In comparison, viner damage to immature peas did not affect the MPT reading but damage to mature peas did. To assess the level of damage to the peas visually, the method given in the Campden Food Preservation Research Association specification for quick frozen peas was followed (Anon, 1978). Samples of the peas used in these experiments were frozen and used as a standard to enable the sensory assessment of the texture. The method followed is that given previously. However, poor agreement was found between sensory texture and the level of damage.

As the OPT reading was significantly affected by viner damage to mature peas it is possible that sub-standard mature peas could be graded as a better level because of the OPT reading. This situation might also arise when the MPT was used with immature peas, but mature peas were observed to be more susceptible to viner damage than immature ones. The work of Rutledge (1981) indicated that neither the OPT nor the MPT were affected by viner damage. However the peas used were of low maturity and so the results are consistent with the present work as the OPT readings tended to be affected by viner damage to more mature peas.

Temperature correction

The OPT readings were slightly more affected by differences in pea temperature than those of the MPT. However the OPT readings were reproducible, giving a consistent temperature correction factor of 1 OPT unit per 2°C. The readings from the MPT were less reproducible and the temperature correction factors found for the MPT ranged from 0.6 to 1.2 MPT units per 2°C. The usual correction factor used by the United Kingdom industry is 0.7 MPT units per 2°C.

Interchangeability of the OPT cells and components

Voisey & Nonnecke (1972b) indicated that variations in the construction of the OPT cell components should be small although the cumulative effect might be appreciable.

Although, the test results indicated that there were no significant differences in the readings obtained from OPT cells operating in different environments. They also showed the difference in readings between two wire grid frames to be 0.73% which increased to 2.6% after the wires became bent. He concluded that as the damage imposed on the wire grid was considerable, normal wear and tear would produce negligible errors.

The initial trials performed with the Instron comparing commercially available OPT cells showed 1–2% differences in the readings, which were highly significant for low maturity peas, while for more mature peas it was significant at the 10% level. The use of different plunger plates showed no difference in the readings and only a 1% difference in the readings obtained from different wire grids. One of the piston plates which had been slightly damaged on the corners did not affect the readings, while a previously unused cell box gave lower readings than a used cell box. When commerical OPT cells for the OPT were tested, no significant difference between the cell boxes or grids was found although a grid with bent wires that was included in the test did tend to give higher readings.

Thus, the commercial OPT cells and cell components tested appear to be interchangeable, although the reading obtained from the Instron or OPT might be affected if the cell is worn or damaged.

Conclusions

To be a suitable method of assessing the texture of raw peas the OPT or OPT cell must have a similar or better performance than the existing methods of texture assessment, namely the MPT and %AIS. It was found that the OPT cell relationship with sensory texture was similar to that of the MPT with sensory texture, thus the OPT cell could be used for the prediction of final product quality with the same degree of certainty as the MPT. The OPT correlation with %AIS was very similar to that obtained with the MPT, so that it would be possible to determine either the MPT or OPT cell reading on the raw peas from %AIS readings on the processed samples.

The OPT reading was significantly affected by viner damage to mature peas, thus sub-standard peas might be graded higher because of the low OPT reading obtained. The MPT was not affected by viner damage to mature peas but could give lower MPT readings than expected with viner damaged immature peas, however, immature peas are less susceptible to damage than mature peas so this may not be a problem. The OPT reading was affected only slightly more than the MPT by differences in pea temperature, but it was possible to determine a reproducible temperature correction factor for the OPT. The commercial OPT cell components were found to be interchangeable and damage to components did not give significantly different readings.

The trials performed have shown the OPT not to be an improvement over the MPT as a final product assessor, in certain areas viz. viner damage and temperature correction, the OPT performance was worse. The replacement of all MPTs by OPTs could be possible and this should not lead to a loss of the standard, but the use of an OPT to standardise existing MPTs could introduce more errors than using the present 'Master' MPT system.

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Influence of stabilizers on the salt balance and pH of buffalo milk and its concentrate

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Summary

The influence of three different concentrations (0.05%, 0.10% and 0.15%) of two stabilizers, disodium phosphate and trisodium citrate on the salt balance (concentration and molar ratios of salt constituents in the dissolved phase) and pH of buffalo milk and its 2:1 concentrate was determined. The disodium phosphate caused a significant shift in all the salt constituents (calcium, magnesium, phosphate and citrate) from the dissolved to the colloidal phase while the trisodium citrate produced a significant shift from the colloidal to the dissolved phase. Further, the phosphate caused a uniform decrease in the molar ratios of Ca/P and (Ca+Mg)/(P+Cit.) in the dessolved phase, while the citrate produced only a small and non-significant effect. Both salts caused a significant increase in pH which was progressive with increase in the concentration of added salts. Therefore, the primary effect of stabilizers in stabilizing or destabilizing the milk is a consequence of their influence on the pH and not on the mineral equilibrium of milk.

Introduction

The equilibrium of salt constituents between the dissolved and colloidal phases of milk is known to affect its heat stability (McMeekin & Groves, 1965). Therefore, any process or product variable which causes an alteration in mineral equilibrium is likely to influence the heat stability. One such variable is the addition of sodium salts of phosphate and citrate to milk during its processing. The stabilizing influence of these salts when added to bovine milk has long been known although the mechanisms of their action are still not clear (Verma, 1965). A decrease in the calcium ion activity in milk due to addition of sodium phosphate (Van Kreveld and Van Minnen, 1955; Kruk Palich & Sajke, 1978) and sodium citrate (Kruk *et al.*, 1978) has been reported. A decrease in dissolved calcium (Hardy *et al.*, 1984) and Ca/P ratio (Edmondson & Tarassuk, 1956; Verma, 1965) was also observed due to addition of sodium phosphate. A decrease in cations (calcium and magnesium) compared to anions (phosphate and citrate) due to addition of these salts may be the cause of stabilization of milk. However, studies in our laboratory (Tayal, 1983; Sindhu & Tayal, 1984) had revealed that instead of acting as stabilizers disodium phosphate and sodium citrate act as strong destabilizing agents when added to buffalo milk. The differential behaviour (in stabilizing or destabilizing milk) of these salts when added to milks from two species (bovine and buffalo) indicated that either the alteration in the mineral equilibrium (shift of constituents between dissolved and colloidal phases) due to their addition is different in the two milks or is not the cause of the stabilizing or destabilizing effect. The present investigation was carried out to determine the influence of disodium phosphate and sodium citrate on the pH and

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partitioning of four salt constituents; calcium, magnesium, phosphate and citrate, in buffalo milk and its 2:1 concentrate with the objective of understanding the mechanisms of their interaction.

Materials and methods

Milk samples. Pooled milk samples from Murrah breed buffaloes of the Institute herd were collected during morning milking in a plastic container.

Addition of salts to milk. Three concentrations (0.05%, 0.10% and 0.15%) of disodium phosphate ($Na_2HPO_4.2H_2O$) or trisodium citrate ($Na_3C_6H_5O_6.2H_2O$) were added to different sets of samples. All lots (control and salt added) were then prewarmed as described below.

Prewarming of milk. Milk was prewarmed at 85°C ± 1°C in an Erlenmeyer flask by dipping in boiling water with constant shaking, held at 85°C for 5 min and then cooled to room temperature by circulating cold water over the surface of the flask.

Concentration of milk. Five hundred gram samples of milk (control and salts added) were concentrated in 2:1 ratio at 55°C ± 1°C in a vacuum evaporator at a pressure of 0.3 mm Hg, using a 2 l Pyrex round bottom flask heated in a thermostatic temperature controlled waterbath.

Separation of dissolved and colloidal phases. Samples of fluid and concentrated milk were centrifuged at 35000 rpm (105000 × g) for 50 min at about 20°C in a preparative ultracentrifuge (Beckman Model L). The serum was drained as completely as possible, collected and used for the determination of dissolved salt constituents corrected for the volume of fat and casein.

Determination of salt constituents. Four salt constituents: calcium, magnesium, phosphate and citrate were determined in milk, concentrated milk and their serums as in our earlier procedures (Sindhu & Roy, 1973).

Determination of pH. The pH of milk and concentrate was determined electrometrically with a mains operated pH meter (Elico Model L-1-10) using a combination of glass and calomel electrodes.

Adjustment of pH. To study the influence of stabilizers on the heat coagulation time (HCT)/pH profile of buffalo milk and its concentrate 0.1% of disodium phosphate or trisodium citrate were added to samples of milk and these were then prewarmed as described above at 85°C. After cooling to room temperature sub-samples were adjusted to pH 6.4–7.2 at 0.1 unit intervals by adding either normal hydrochloric acid or normal ammonium hydroxide. A similar set of samples was subjected to pH adjustment after 2:1 concentration.

Determination of heat coagulation time (HCT). HCT of pH adjusted samples was determined at 130°C ± 1°C as in our earlier procedure (Tayal & Sindhu, 1983).

Statistical analyses of the data. Statistical analyses of the data for *F*-test was carried out on a programmable minicomputer (HCL-Micro-2200).

Results

Effect on partitioning. The effect of disodium phosphate and trisodium citrate on the mineral equilibrium (concentration and molar ratios of salt constituents in the dissolved phase) of buffalo milk and its concentrate are shown in Tables 1 and 2 respectively. Addition of disodium phosphate to buffalo milk before prewarming caused a significant decrease in the dissolved proportions of calcium, magnesium and citrate, even when added at the rate of 0.05%. If compensation is made for the amount of added phosphate there was also a significant shift of phosphate from the dissolved to the colloidal phase.

Addition of citrate, on the other hand, caused a significant increase in the dissolved constituents due to a shift from the colloidal to the dissolved phase (Table 2). The shift caused by addition of salts was similar in both the fluid milk and its concentrate, and progressive with the increase in the amount of added salts.

Effect on molar ratios. The two salts differed considerably in their influence on the molar ratios. Disodium phosphate caused a significant decrease in the molar ratios of Ca/P and (Ca+Mg)/(P+Cit.) in the dissolved phase while trisodium citrate caused a non-significant and irregular change (except in case of (Ca+Mg)/(P+Cit.) ratio where a uniform but non-significant decrease was observed). The non-significant influence on the molar ratios of cationic to anionic salt constituents due to addition of sodium citrate indicated that it caused a proportionate shift of both cationic and anionic salt constituents from the colloidal to the dissolved phase.

Effect on pH. Both disodium phosphate and trisodium citrate caused a significant increase in pH of fluid milk and its concentrate and the increase was progressive with the increase in concentration of added salts.

Effect on HCT/pH profile. The HCT/pH profile of one of the samples of buffalo milk and the effect on it of stabilizers is shown in Fig. 1a, and of concentrated milk in Fig. 1b. It is evident that the unadjusted pH of buffalo milk (6.8) lay on the alkaline side

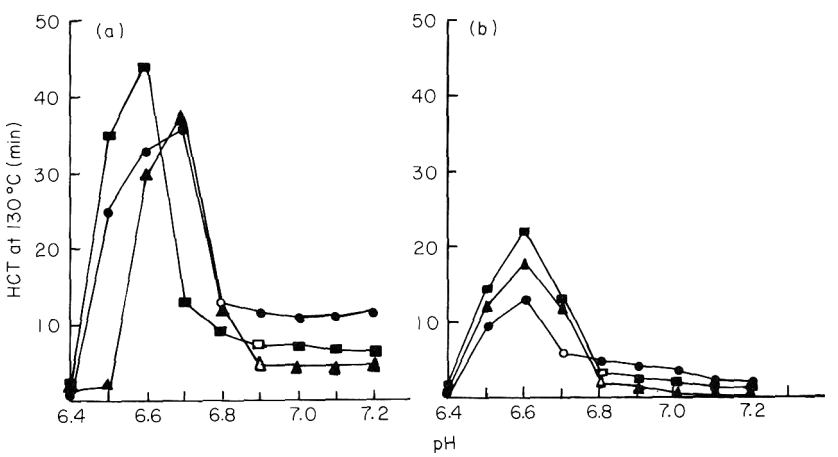

Figure 1. Effect of stabilizers on the HCT/pH profile of buffalo milk and its 2:1 concentrate. (a) Fluid milk, ● control, ▲ milk+0.1% $Na_3C_6H_5O_7 . 2H_2O$, ■ milk+0.1% $Na_2HPO_4 . 2H_2O$. (b) Concentrated milk, ● control, ▲ milk+0.1% $Na_3C_6H_5O_7 . 2H_2O$, ■ milk+0.1% $Na_2HPO_4 . 2H_2O$. Open symbols denote the unadjusted pH.

Table 1. Influence of disodium phosphate on the salt constituents (concentration and molar ratios in the dissolved phase) and pH of buffalo milk and its 2:1 concentrate*

S. No.	Parameter	Fluid milk				Concentrated milk			
		Control	0.05%	0.10%	0.15%	Control	0.10%	0.20%	0.30%
1	Calcium†	38.7	36.2	34.2	32.5	67.2	62.7	59.8	57.2
		(19.8)	(18.5)	(17.5)	(16.6)	(17.2)	(16.1)	(15.3)	(14.6)
2	Magnesium†	10.1	9.6	9.2	8.7	18.6	17.9	17.2	16.7
		(52.4)	(49.9)	(47.7)	(45.3)	(48.3)	(46.3)	(44.7)	(43.1)
3	Phosphate†	33.5	39.8	45.5	51.3	54.2	65.0	72.3	78.1
		(32.7)	(38.8)	(44.5)	(50.1)	(26.5)	(31.7)	(35.3)	(38.7)
4	Citrate†	162.0	146.9	134.0	126.7	242.6	222.1	198.9	182.2
		(73.9)	(67.0)	(61.1)	(57.5)	(55.30)	(50.6)	(45.4)	(41.6)
5	Ca/P	0.9	0.7	0.6	0.5	1.0	0.8	0.6	0.6
6	(Ca+Mg)/(P+Cit.)	0.7	0.6	0.6	0.5	0.8	0.7	0.7	0.6
7	pH	6.8	6.85	6.90	6.95	6.6	6.65	6.70	6.75

*Results represent the average of five samples.

†Concentration of salt constituents is in mg/100 ml milk or concentrate. Values in parenthesis are for the percents of salt constituents in dissolved phase out of total 195.3, 19.3, 102.4 and 219.3 mg of calcium, magnesium, phosphate and citrate respectively/100 ml of milk or/50 ml of concentrated milk.

Table 2. Influence of sodium citrate on the salt constituents (concentration and molar ratios in the dissolved phase) of buffalo milk and its 2 : 1 concentrate*

		Fluid milk				Concentrated milk			
S. No.	Parameter	Control	0.05%	0.10%	0.15%	Control	0.10%	0.20%	0.30%
1	Calcium†	41.0	45.1	48.6	51.7	73.2	81.6	89.5	97.1
		(21.0)	(23.1)	(24.7)	(26.6)	(18.8)	(21.0)	(23.0)	(24.9)
2	Magnesium†	10.1	10.5	11.0	11.3	19.4	20.3	21.1	22.0
		(51.3)	(53.5)	(55.7)	(57.5)	(49.2)	(51.6)	(53.7)	(55.7)
3	Phosphate†	35.3	31.7	34.9	39.9	55.9	57.9	64.5	72.3
		(32.1)	(28.9)	(31.8)	(36.4)	(24.6)	(26.4)	(29.4)	(32.9)
4	Citrate†	175.9	199.7	223.3	248.2	246.2	317.1	351.1	390.0
		(80.3)	(91.1)	(101.9)	(112.4)	(56.2)	(72.3)	(80.1)	(89.0)
5	Ca/P	0.9	1.1	1.1	1.0	1.1	1.1	1.1	1.0
6	(Ca+Mg)/(P+Cit.)	0.7	0.8	0.7	0.7	0.8	0.8	0.8	0.8
7	pH	6.80	6.85	6.90	6.95	6.55	6.60	6.65	6.70

*Results represent the average of five samples.

†Concentration of salt constituents is in mg/100 ml milk or concentrate. Values in parenthesis are for the percents of salt constituents in dissolved phase out of total 194.7, 19.7, 109.8 and 219.1 mg of calcium, magnesium, phosphate and citrate/100 ml of milk or/50 ml concentrate.

of the pH of maximum stability (6.7) in the HCT pH profile. Similarly for concentrated milk the unadjusted pH was 6.7 and the pH of maximum stability 6.6. Addition of either disodium phosphate or trisodium citrate caused a considerable decrease in the heat stability of buffalo milk or its concentrate at unadjusted pH but it was restored by decrease in the pH.

Discussion

As described in the results section, addition of disodium phosphate caused a significant decrease in dissolved calcium, magnesium and other salt constituents of milk and concentrated milk due to a shift from the dissolved to the colloidal phase. With bovine milk a decrease in ionic and dissolved calcium was also observed by various workers (Edmondson & Tarassuk, 1956; Verma, 1965; Van Kreveld & Van Minnen, 1955; Kruk *et al.*, 1978; Hardy *et al.*, 1984). Moreover, addition of sodium citrate to cow milk resulted in an increase in the dissolved calcium (Tessier & Rose, 1958) as also observed in the present work (Table 2). Therefore, buffalo milk does not behave differently from bovine milk in a qualitative manner with respect to the influence of disodium phosphate and sodium citrate on the distribution of salt constituents.

With reference to the mechanisms of interactions of disodium phosphate and sodium citrate in stabilizing or destabilizing milk, Hardy *et al.* (1984) concluded from a study of evaporated milk that 'mineral equilibrium in the evaporated milk influence heat stability probably by differences in the concentration of soluble calcium. The effect of stabilizers addition can be attributed to this phenomenon.' However, in the present investigation one of the salts (disodium phosphate) decreased the dissolved calcium and magnesium while the other (trisodium citrate) increased them, but both behaved similarly in their influence on the heat stability (Fig. 1a and b). Both salts caused a considerable decrease in heat stability (when determined at unadjusted pH) of buffalo milk and its 2:1 concentrate. Therefore, the mechanisms of stabilization or destabilization of milk by the addition of these salts may not be attributed to the alterations in mineral equilibrium.

The other factor which is influenced by addition of these salts is pH. Both significantly increased the pH of buffalo milk and its concentrate and this in turn could have caused destabilization, since maximum stability of buffalo milk and its 2:1 concentrate was located in the acidic side of the normal pH in the HCT/pH profile: pH 6.7 compared with 6.8 for milk and pH 6.6 compared with 6.7 for concentrated milk.

In our earlier study the same trend was observed with the addition of sodium citrate. Acidifications of stabilizer containing milk or its concentrate resulted in an increase in the heat stability, supporting the hypothesis that disodium phosphate or trisodium citrate destabilizes buffalo milk and its concentrate due to their influence on pH.

On the other hand, these salts would cause stabilization of bovine milk because in a majority of samples of bovine milk the pH of maximum stability lies on the alkaline side of the normal pH. Sweetsur & Muir (1980) also observed that the primary effect of stabilizers is a consequence of their influence on the pH of milk and suggested that Na_2HPO_4, Na_3 Citr. and $NaHCO_3$ should be used if the pH of milk falls on the acidic side of the maximum heat stability while NaH_2PO_4 or $CaCl_2$ should be used, if the natural pH is alkaline to the maximum. It can be concluded that stabilizers altered the heat stability of milk due to their influence on pH.

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Determination of sorbic acid diffusivity in model food gels

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Summary

The diffusional behaviour of sorbic acid in model food gels is discussed. The apparent diffusivity (D_a) of sorbic acid in a aqueous gel containing 1.5% w/w agar, when determined by monodimensional diffusion (infinite gel columns) was found to be 8.8, 8.9 or 9.2×10^{-10} m²/sec at 25°C depending on the method of calculation (eye fitting, eye fitting graphical method or computerized fitting method) and 8.7×10^{-10} m²/sec when determined by tridimensional diffusion (gel cubes immersed into an appropriate solution).

The two methods displayed a good agreement; the tridimensional diffusion method had slightly worse repeatability than the monodimensional method (coefficient of variation $\leq$ 10% instead of 5.5% for the infinite gel columns) but has the advantage of being simple and rapid (1–3 hr instead of 24 hr). For the tridimensional diffusion method, D_a values of sorbic acid measured by inward and outward diffusion in gel cubes of different compositions were found to be not significantly different, which suggests an absence of partition effects between the immersion solution and the gel.

D_a values appear to be slightly dependent on the gelling agent concentration (1.5, 2.5 and 4% w/w agar) in gel containing 40% w/w glycerol and 60% water. D_a values of sorbic acid decreased when the temperature decreased. An apparent activation energy of 18 KJ/mol was found for the diffusion of sorbic acid. This value was similar to the activation energy for the change in viscosity of the solution occluded in the gel network. D_a values were influenced by the concentration of the diffusant, with a slight decrease when initial concentration of sorbic acid increased, following a linear relationship. The product $D.\eta$ when η is the viscosity of the solution occluded in the gel was approximately constant.

Introduction

Solute diffusion phenomena play an important role in several food unit operations such as dehydration (Daudin, 1983), osmotic treatments (Karel, 1976) and leaching processes (Schwartzberg & Chao, 1982). Solute diffusion also takes place during the storage of foods.

Migration of food additives, in particular of antimicrobial additives, has a special interest in a number of cases. Sorbic acid is being used increasingly as one of the numerous 'hurdles' employed in the preservation of intermediate moisture foods (Troller & Christian, 1978) of low pH foods, or of shelf stable products (Leistner, Rödel & Krispien, 1981; Leistner, 1985). It is important to predict and to control sorbic acid

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migration between phases during: (a) food treatments (e.g., penetration of sorbic acid during the processing of dried prunes, or loss during cooking of fabricated foods); (b) storage of composite foods (e.g., dairy products or cakes with pretreated fruits); (c) storage of foods in contact with wrapping materials or films containing sorbic acid (absorption by dairy products covered with paper saturated with sorbic acid); and (d) storage of foods coated with an external edible layer of high concentration of sorbic acid (Guilbert, 1985). In these two last cases, maintaining a local high effective concentration of sorbic acid may allow, to a considerable extent, a reduction of the total amount in the food for the same antifungic effect.

Extensive studies on diffusion of solutes in food gels have been done (Belton & Wilson, 1982; Brown & Chitumbo, 1975; Nakanishi *et al.*, 1977; Busk & Labuza, 1979; Nicolas & Duprat, 1983). Diffusion of solutes has also been used to characterize the structure of gels (Busk & Labuza, 1979; Laurent, 1967). A complete review of diffusion phenomena in gels was recently written by Muhr & Blanshard (1982).

The laws of mass transfer by molecular diffusion, as applicable to foods or to food gels are discussed by Crank (1975), Karel (1975), Loncin (1976; 1980) and Crank *et al.* (1981).

The correlation between diffusivity (D), radius of the diffusing molecule or particle (R), absolute temperature (T) and viscosity (η) of the diffusion medium is given, for large molecules in dilute solutions, by the Stokes-Einstein equation:

$$D = \frac{k \cdot T}{6 \cdot \pi \cdot \eta \cdot R}, \text{ where k is the Boltzmann constant.} \quad (1)$$

However only macroscopic or overall viscosity can directly be measured but small molecule migration is controlled by microscopic or local viscosity of the occluded solvent (Schwartzberg & Chao, 1982). This is probably one of the main reasons why the Stokes-Einstein equation is not always valid for diffusion in solutions or in gels (Turq, Brun & Chemla, 1973).

Diffusivities are also frequently related to temperature by an Arrhenius type equation:

$$D = D_0 \cdot \exp -\frac{E_{diff}}{RT}, \quad (2)$$

where E_{diff} is the apparent activation energy for diffusion, R the universal gas constant, D_0 a constant and T the absolute temperature. Apparent activation energy for diffusion can be interpreted using the diffusion cage model (Eyring, 1936).

Two main approaches have been utilized for the determination of solute diffusivities: pseudo steady state diffusion through a porous diaphragm (Stokes, 1950; Robinson, Edmister & Dullien, 1965; Chandrasekaran & King, 1972; Navari, Gainer & Hall, 1971), and unsteady state diffusion from initially sharp boundary conditions (Belton & Wilson, 1982; Busk & Labuza, 1979; Favetto, Chirife & Bartholomai, 1981; Naesens, Bresseleers & Tobback, 1981; 1982). In the present study we describe two methods based on the second approach. The first (monodimensional diffusion in 'infinite' gelified columns) is described by Naesens *et al.* (1981) and can be considered as a reference method, but experiment and treatment of data are time-consuming. The second method which we propose here (tridimensional diffusion in agar gel cubes) is very rapid and simple (Guilbert, Giannakopoulos & Chertel, 1985).

These methods are strictly valid only when D is independent of concentration of diffusent. Because of the frequent concentration dependence of D, the experimental

concentration curves will generally differ slightly in shape from the theoretical curves. The diffusion coefficient determined will therefore be an apparent diffusion coefficient (D_a) rather than the true value of diffusivity at a particular concentration. However if diffusivity is determined in this way at several concentrations and extrapolated to zero concentration, the error so introduced may be expected to disappear, and the limiting value will be equal to the true value of diffusivity at infinite dilution (D_0) (Tanford, 1961). The influence of initial concentration of sorbic acid, of agar concentration and of temperature on the diffusivity of sorbic acid is reported here. A study of sorbic acid diffusivity in model food gels and in real foods, related to composition and water content is being published separately (Giannakopoulos & Guilbert, 1986).

Materials and methods

Diffusivity measurements

The diffusivity of sorbic acid was measured by two independent methods: (a) monodimensional diffusion from a semi-infinite agar gel column (containing an initially homogeneous concentration of sorbic acid) into a contiguous semi-infinite agar gel column free of sorbic acid; (b) outward diffusion from cubes of agar gel, containing an initially homogeneous concentration of sorbic acid placed in an 'immersion solution' free of sorbic acid, or inward penetration of sorbic acid from an 'immersion solution'. Thus the 'immersion solution' always contained the same components as the corresponding gel (except agar) and could contain sorbic acid. The concentrations of sorbic acid chosen were low, for solubility reasons, in order to keep concentration gradients as small as possible and to avoid significant counter-diffusion of solvent.

For the preparation of gels, 1.5, 2.5 or 4 g of agar (Merck) were dispersed and made up to 100 g with a aqueous solution of sucrose (Merck), glycerol (Merck), sodium chloride (Merck), maltodextrin (D.E. 20, Roquette) or glucose syrup (DE 46, Roquette) or appropriate concentration. Sorbic acid was eventually added to this mixture at a concentration of about 2.5, 6, 10, 20 or 40 μmol/g of gel. The mix was then placed into glass jars provided with hermetic lids, allowed to stand for 16 hr, and heated for 20 min in a boiling water bath. The clear solution was poured while hot into either glass tubes (16 mm i.d $\times$ 240 mm, closed at the bottom by a rubber plug), or cubic moulds (9$\times$9$\times$9 mm). Gelation took place upon cooling. After 12 hr at 25°C, the gel cubes were removed from the moulds and placed in 4 kg of 'immersion solution' which was stirred constantly. The gel columns were half removed from the tubes, cut through the middle with a razor blade in order to provide a smooth flat surface orthogonal to the length axis. A half (or 'semiinfinite') column containing sorbic acid was placed in contact end-to-end with another one free of sorbic acid. A drop of solution of the same composition as the gel was placed at the junction in order to avoid interface resistance. The 'junction' surface was then pushed back to a mark drawn in the middle of one of the glass tubes. The tube was closed and then stored at the desired conditions (25°C, for about 24 hr).

Two models have been used for the determination of diffusion profiles and the calculation of diffusivity values.

(a) Monodimensional diffusion in infinite columns. After 24 hr of diffusion at 25 $\pm$ 0.3°C the gel columns were removed from the glass tubes and cut into numerous slices (at least twelve on each side of the junction) of 1–2.5 mm thickness, using a razor blade. Sorbic acid concentration in each independent slice was determined and plotted

against the distance (x) from the junction surface. This distance was assessed from the weight of each slice. The diffusion profiles given correspond to an average profile calculated from three independent experiments.

In the case of isothermal monodimensional diffusion, for an extended source of infinite extent with the following initial and boundary conditions:

$$C = C_0, x < 0; C = 0, x > 0; t = 0$$

$$C_{(x,t)} = 1/2\, C_0\left(1\text{-erf}\frac{X}{2\sqrt{Dt}}\right) \tag{3}$$

where $C_{(x,t)}$ is the concentration of the diffusing substance at distance x from the junction surface at time t, C_0 the initial concentration, and D the diffusivity or diffusion coefficient. Function erf is the error function given by:

$$\text{erf}\,(u) = \frac{2}{\sqrt{\pi}}\int_0^u e^{-u^2}\,.\,du\,. \tag{4}$$

Convenient tables of the error function are found in Crank (1975).

The diffusivity can be estimated at any point of the experimental diffusion profile by means of equation (3) with tabulated values of equation (4). In our experiments D was calculated for the following noticeable values of C/C_0: 9/10, 3/4, 2/3, 1/4, 1/6 and 1/9. The average D value was taken into account, and the coefficient of variation characterizes the experimental variation and agreement with the theoretical model (equation (3)).

The diffusivities were also calculated from the experimental profiles using a least squares curve fitting method. According to this method a number of diffusion coefficients in the range of the expected value of D are selected. The best fit diffusion coefficient minimizes the sum (S) of the squared deviation between the experimental concentrations (C_e) and the calculated concentrations (C_c) at the different locations: $S = (C_e - C_c)^2$. The theoretical concentrations were calculated from the Gouyette equation (Gouyette, 1984). This method was implemented on a computer capable of plotting diffusion curves.

A graphical method to estimate the diffusivity was also used; the equation of the tangent at the inflection point of the sigmoid concentration profile was given by Jost (1957):

$$C = -\frac{1}{2}\frac{C_0}{\sqrt{\pi\,.\,D\,.\,t}}x + \frac{1}{2}C_0 \tag{5}$$

and diffusivities were calculated from the following relation at the intercept with the x axis (x_0)

$$x_0 = \sqrt{\pi\,.\,D\,.\,t\,.} \tag{6}$$

(b) Tridimensional diffusion in gel cubes. For outward diffusion from gel cubes, a cube containing an initially uniform concentration C_0 of a diffusing substance was immersed in a surrounding solution so as to keep $C = 0$ on all of the cube faces, for the whole duration of the diffusion experiment. The ratio of the average concentration of diffusing substance within the cube ($\bar{C}$, at $t > 0$) to the initial concentration C_0 is equal to the third power of the corresponding ratio obtained in conditions of monodimensional diffusion (Crouzat-Reynes, 1958; Newman, 1931). This is equivalent to the case of a slab of thickness e, with an initially uniform concentration C_0 of diffusing

substance, immersed in a surrounding solution so as to keep $C = 0$ on its two faces. The analytical resolution for a time t of diffusion is given by Crank (1975):

$$\frac{\bar{C}}{C_0} = \frac{8}{\pi^2} \sum_{n=0}^{n=\infty} \frac{1}{(2n+1)^2} \exp\left[-\frac{(2n+1)^2 \pi^2 D.t}{e^2}\right] \quad (7)$$

From the plot of the curve $\bar{C}/C_0 = f(D_a.t/e^2)$, it is possible to calculate the corresponding curve for tridimensional diffusion (Fig. 1), where the side s of the cube becomes the important dimension. Three values of $\bar{C}/C_0$ (between 0.10–0.80) at three different times of diffusion (generally 1/2, 1, or 2 hr) were taken in order to calculate the diffusivity, using curve a (Fig. 1). For penetration of sorbic acid into the gel cubes, initially free of sorbic acid, the data from curve b (Fig. 1) for inward diffusion was treated similarly.

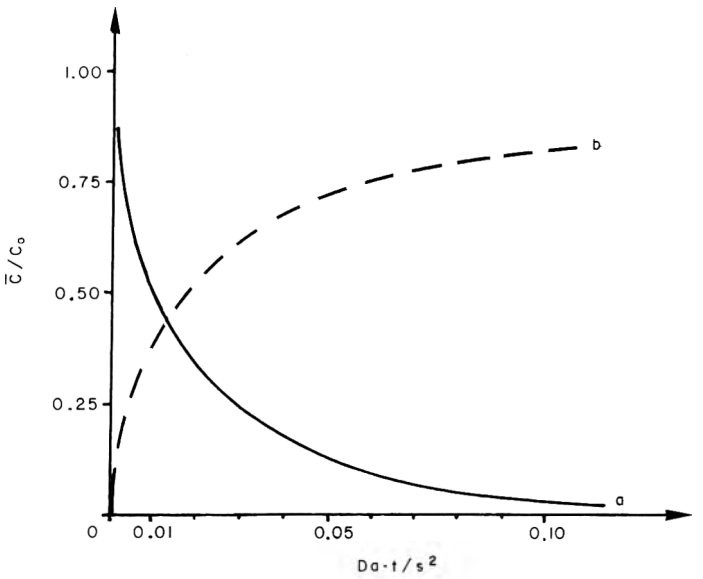

Figure 1. Ratio of average concentration ($\bar{C}$) of sorbic acid within a cube to the initial concentration (C_0) as a function of $D_a \times t/s^2$ (s is the side of the cube): (a) outward diffusion from gel cubes (——); (b) inward diffusion (– – – –).

D values for each diffusion time represent the average of six independent determinations on six separate cubes. The final D value was the average of the three values determined at three noticeable times.

Sorbic acid determinations

Sorbic acid (M.Wt = 112.13; pKa = 4.6) was determined in the gel cubes or slices by gas-liquid chromatography (G.L.C.). Concentrations are given in moles sorbic acid per g of gel. The G.L.C. analysis was carried out with a Girdel chromatograph 3000: injector temperature = 220°C; flame ionization detector temperature: 250°C; oven temperature: 170°C for 4 min, then a linear increase of 20°C/min to 230°C, followed by 230°C for 3 min (Glass column Chrom. W.A.W. 80–100 mesh with 10% FFAP and 1% H_3PO_4). The gel cube or slice was melted in 2 ml boiling 10^{-2}N hydrochloric acid. After

cooling to 30–40°C, sorbic acid was extracted by vigorously stirring with 1.5 ml diethyl ether containing lauric acid as internal standard (lauric acid is added to diethyl ether just before each series of extractions), and 10μl of the diethyl ether extract were injected in the chromatograph. The concentration of sorbic acid was calculated by comparing with standard solutions of sorbic acid.

Viscosity and gel strength measurements

The strength of 10×10×10 mm gel cubes was measured using an Instron 1140 texturometer equipped with a compression cell No. 2512–208 (5–50 N). The force necessary for gel rupture was taken as the gel strength. The viscosity of glycerol, sucrose or glucose syrups solutions was determined at 25°C using a double cylinder RV 12 Haake Rotovisko viscometer. The solutions tested exhibited newtonian behaviour.

Results and discussion

In our experiments it was convenient to define D values as apparent diffusivities (D_a), whereas intrinsic diffusivities regulate the basic process at the molecular level in Crank's terminology.

Monodimensional diffusion in infinite columns

The apparent diffusivities (25°C) of sorbic acid (in gels containing 98.5% water (gel nos 1–3), 40 or 70% glycerol (gel nos 4 and 6) or 44% sucrose (gel no. 5) determined by diffusion in infinite columns and calculated with three different methods are given in Table 1. The corresponding diffusion profiles after 24 hr of diffusion in the infinite columns are shown in Figs 2 and 3. These concentration profiles appear to follow closely equation (3), as D_a values were very similar when calculated for different values of C/C_0 (eye fitting method; variation coefficient $\leq$ 7.8%) and as the sums of the squared deviation are low (computerised fitting method which was estimated to be less than 5%

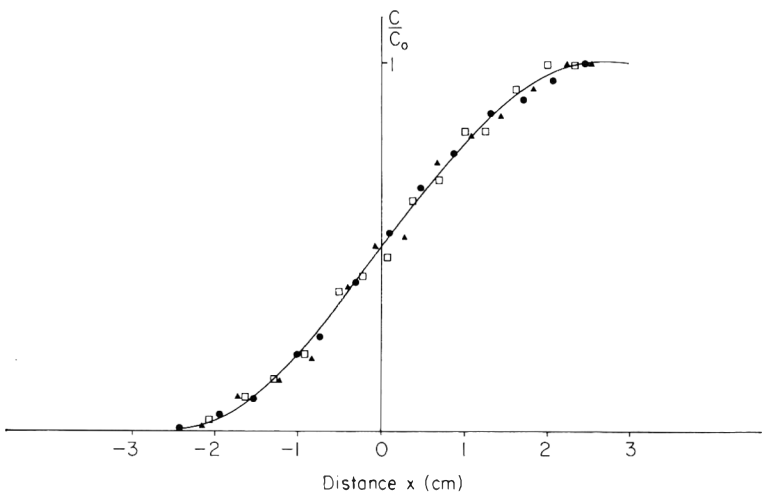

Figure 2. Concentration profiles of sorbic acid (25°C) in infinite gelified columns (1.5% agar and 98.5% water). Average of three independent experiments. Diffusion time: 24 hr. Initial sorbic acid concentration: 3.3 μmol/g of gel.

Table 1. Apparent diffusivities of sorbic acid at 25°C as determined by monodimensional diffusion in gel columns

	Gel composition (% w/w)						$D_a \times 10^{10}$ m²/sec		
	Water	Glycerol	Saccharose	Agar	a_w (25°C) of solution	Solution viscosity (10^{-3} Pa.s)	Eye fitting method	Computer fitting method	Eye fitting graphical method
1	98.5			1.5	1.0	1.5	8.73	9.35	8.90
2	98.5			1.5	1.0	1.5	8.11	8.66	8.70
3	98.5			1.5	1.0	1.5	8.40	8.42	8.00
4	58.5	40.0		1.5	0.88	4.0	1.55	1.63	1.60
5	54.5		44	1.5	0.95	7.6	1.36	1.61	1.10
6	28.5	70		1.5	0.64	21.9	0.60	0.50	0.59

Initial concentration of sorbic acid: 3.3 μmol/g of gel for gel nos 1, 2 and 3, 7.4 μmol/g of gel for gel no 5 and 13.4 μmol/g of gel for gel nos 4 and 6.

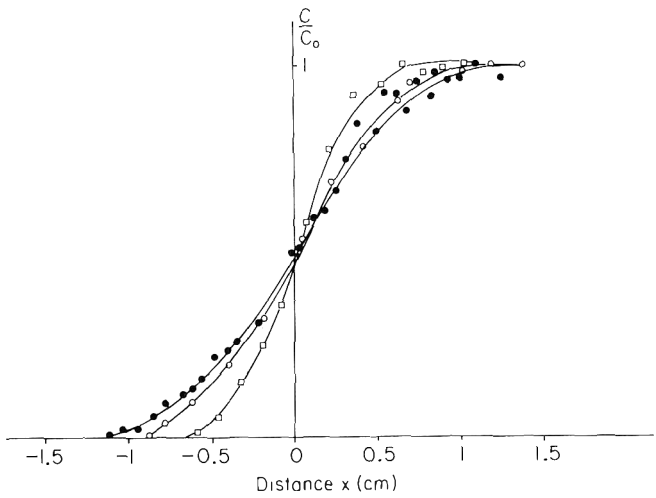

Figure 3. Concentration profiles of sorbic acid (25°C) in infinite columns of varying compositions. Diffusion time: 24 hr. Initial sorbic acid concentrations: 13.4 (gels containing glycerol) or 7.4 μmol/g of gel (gels containing saccharose). ●—● Glycerol 40%; water 58.8%; agar 1.5%; □—□ glycerol 70%; water 28.5%; agar 1.5%; ○—○ saccharose 44%; water 54.5%; agar 1.5%.

deviation). This appears to indicate that the mobility of sorbic acid in the agar gel follows Fick's second law of diffusion. The repeatability of the determination of D_a values is 5.5% (variation coefficient for a series of three independent measurements, gels nos 1, 2 and 3, Table 1 and Fig. 2) and independent of the calculation mode (based on eye or on computer fitting). The computer fitting method gives slightly higher D_a values than the eye fitting methods. However the eye fitting method is not recommended as it largely depends on the experimenter.

Tridimensional diffusion in gels cubes

During immersion in solutions of the same composition, gels lose only sorbic acid and no swelling is observed. Figure 4 shows sorbic acid removal from gelified cubes containing 0, 40 and 70% glycerol, as a function of immersion time in respectively water, 40 and 70% glycerol solution at 25°C. The initial concentration of sorbic acid in the gel cubes was 13.4 μmol/g of gel. It can be seen that the particular analytical solution of Fick's law (Fig. 1) was applied satisfactorily to the data, as D_a values were very similar when calculated for different immersion times. For example, particular D_a values of 2.8, 2.8, 2.9, 2.9, 2.7×10^{-10}m^2/sec for respectively 0.5, 1, 2, 4, 6 hr, were found for outward diffusion of sorbic acid in gel cubes containing 40% glycerol (Fig. 4). This confirms that an apparent 'fickian' behaviour is observed for sorbic acid diffusion in gels.

When measurements were obtained by outward diffusion from gel cubes (cubes initially containing sorbic acid), and by inward diffusion (immersion solution initially containing sorbic acid), similar D_a values were always observed. Table 2 shows D_a values measured by outward and by inward diffusion in gels cubes of different compositions. The possibility of important partition effects occurring between solution and gel, or of binding of the diffusent to the agar matrix, may therefore be eliminated since such effects would be a function of the mode of diffusivity measurement.

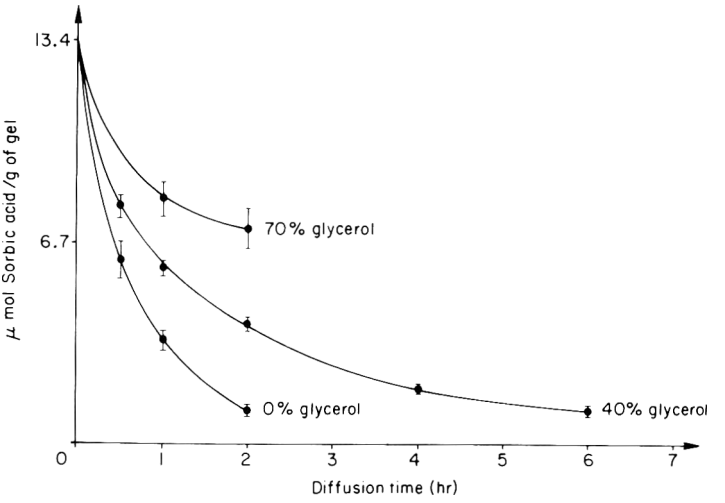

Figure 4. Average concentration profiles of sorbic acid (25°C). Outward diffusion in gel cubes containing various glycerol concentrations. Initial concentration of sorbic acid in the gel cubes was 13.4 μmol/g of gel. Concentrations are average from six independent experiments; standard deviations are given.

Table 2. Influence of diffusion mode on the diffusivity of sorbic acid in cube gels (25°C)

Gel composition (% w/w)						Outward diffusion from cube gels	Inward diffusion in cube gels
	Water	Glycerol	Maltodextrin chloride	Sodium	Agar	$\overline{D}_a \times 10^{-10}$ m²/sec	$\overline{D}_a \times 10^{-10}$ m²/sec
1	98.5				1.5	7.20 (6.4%)	7.02 (8.2%)
2	82.5		16		1.5	5.94 (8.1%)	6.12 (6.3%)
3	78.5			20	1.5	2.97 (4.8%)	3.42 (1.6%)
4	58.5	40			1.5	2.70 (7.2%)	2.68 (6.1%)

Apparent diffusivity values are the average of six independent determinations (with variation coefficient). Initial sorbic acid concentration was 13.4, 8.6, 10.7 and 13.4 μmol/g of gel respectively for gel nos 1–4.

Table 3 shows the effect of stirring rate of the solution of the apparent diffusivities of sorbic acid in a 40% glycerol gel at 25°C. Rotation speed varied from 120 to 360 rpm (corresponding to the modified Reynolds number of the impeller (N_{Re}) varying from 1940 to 5830) and measurements were made both by outward and by inward diffusion. It can be seen that stirring must be vigorous in order to minimize mass transfer resistances in the external solution (important external layer was suspected at 120 rpm (N_{Re} = 1940) (Table 3), but must not be so vigorous as to cause edge erosion of cubes. Edge erosion was easily observed after 30 min diffusion at 360 rpm (N_{Re} = 5830). On

Table 3. Influence of the stirring rate on the apparent diffusivity (D_a) of sorbic acid in gel cubes at 25°C

Rotation speed (rpm)	N_{Re}†	Outward diffusion D_a ‡	Inward diffusion D_a ‡
360	5830	3.33 (8%)	2.25 (4%)
250	4050	2.74 (8%)	2.61 (8%)
190	3080	2.70 (7%)	2.68 (6%)
150	2430	2.61 (8%)	2.7 (8%)
120	1940	2.43 (9%)	2.56 (10%)

*Gel cubes contained 58.5% water, 40.0% glycerol and 1.5% agar. Immersion solution contained 60% water and 40% glycerol (μ = 4 10^{-3} Pa.s; ρ = 1080 kg/m^3). Impeller diameter D = 6.10^{-2} m.

†Modified Reynolds number of the impeller ($N_{Re} = nD^2\rho/\mu$) with n = rotation speed (rps).

‡ D_a values $\times 10^{10}$ m^2/sec are the average of six independent determinations (with variation coefficient); initial concentration of sorbic acid: 13.4 μmol.

the basis of these results the rotation speed was fixed in order to have a $N_{Re} = 3000$ for all experiments.

It is interesting to compare the cube tridimensional diffusion method and the classical, but much more delicate and time-consuming reference method of monodimensional diffusion in infinite columns. The cube method displayed slightly poorer repeatability than the infinite columns method. For 'cube method', the coefficient of variation was always $\leq$ 10% for a series of six independent measurements (each value was the average of values calculated with three diffusion times) against 5.5% for the reference method.

The D_a value at 25°C for sorbic acid in an aqueous gel containing 1.5% agar, extrapolated at infinite diulution of sorbic acid was found to be 8.7×10^{-10} m^2/sec when determined by diffusion in gel cubes. This value was similar to the values determined by diffusion in infinite columns (extrapolated at infinite dilution) which were found to be 8.8, 8.9 or 9.2×10^{-10} m^2/sec depending on the calculation method. When determined by penetration into semi-infinite columns and extrapolated to infinite dilution, sorbic acid diffusivity had been found to be 8.4×10^{-10} m^2/sec (Guilbert *et al.*, 1985). These values are close to those reported by Schwartzberg & Chao (1982) for the diffusivity in water at 25°C of organic acids with similar molecular weights: D_a values of 8.2, 7.8 and 8.0×10^{-10} m^2/sec were found for valeric, caproic and tartaric acid, respectively. It also appears that the simple 'cube method' can be used after one single duration of immersion (for instance 1 hr). Important requirements of the 'cube method' are a sufficient gel rigidity (to avoid cube deformation during the diffusion experiment) and a homogeneous immersion solution liquid, with a composition sufficiently close to the gel composition (to avoid counter-diffusion phenomena).

Influence of the concentration of gelling agent

Diffusivities of sorbic acid in a 40% (w/w) glycerol solution gelled with varying amounts of agar (1.5, 2.5 or 4.0% (w/w)) were measured by outward diffusion from gel cubes (initial concentration 13.4 μmol/g of gel). Results are given in Fig. 5. D_a values

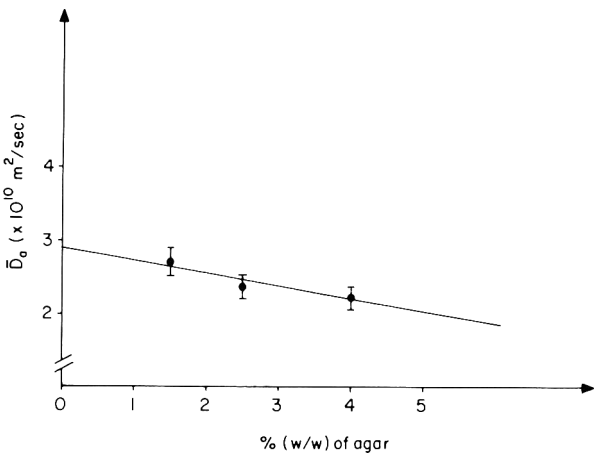

Figure 5. Average D_a of sorbic acid as a function of the agar concentration in the cubes (standard deviations are given). Initial concentration of sorbic acid was 13.4 μmol/g of gel.

appear to be slightly dependent on the agar concentration, while gel strength increased markedly when the agar concentration was raised (gel strengths of 10×10×10 mm cubes with 1.5, 2.5 or 4% agar were respectively 20, 47 and 55 *N*).

This indicates that the gel network tends to counteract the diffusion of small molecules such as sorbic acid, probably because the path length for diffusion is somewhat increased by the presence of the network. The fact that E_{diff} is unaffected by the presence of the agar (see later) is consistent with this explanation. Local, network-induced, increases in microscopic viscosity of the medium may also explain this behaviour (Brown & Chitumbo, 1974; Karel, 1976). If the variation is assumed to be linear, then the diffusivity value (D_0) extrapolated to zero agar concentration will be 2.89×10^{-10} m^2/sec and the proportionality coefficient (slope/D_0) −0.006 kg/g. Similar proportionality coefficients are obtained for salt diffusivities in agar gels: −0.004 kg/g for KNO_3 (Nicolas & Duprat, 1983); −0.003 kg/g for NaCl (Slade, Cremers & Thomas, 1966) and for K_2CrO_4 (Belton & Wilson, 1982). Higher proportionality coefficients were found for solutes with higher molecular weights, for example −0.06 kg/g for amaranth dye (Belton & Wilson, 1982).

Influence of temperature

The influence of temperature (5, 15, 25 or 40°C) on sorbic acid diffusivity in 40% (w/w) glycerol gel cubes (initial concentration 13.4 μmol/g of gel) is shown in Table 4 and in the Arrhenius plot in Fig. 6. Apparent activation energy (E_{diff}; equation (2)) for the diffusion of sorbic acid calculated from the slope (r = 0.996) was 18 kJ/mol. Activation energy for the change in viscosity (E_η; Andrade equation) of the occluded liquid (40% glycerol solution) was also determined to be 18 kJ/mol.

Similar apparent activation energies of 13.8, 11.6, 28.2, 23 and 18 kJ /mol were found respectively for the diffusion of CrO_4^{--} ions in aqueous agar gel (Patil & Adhypak, 1981), KNO_3 in aqueous agar gel (Nicolas & Duprat, 1983), carrot solutes (Selman, Rice & Abdul-Rezzak, 1983) glucose in hydroxy-methylcellulose gel (Brown & Chitumbo, 1975) and peroxidase in water (Lomcin, 1980).

Table 4. Apparent diffusivity of sorbic acid (initial concentration 13.4 μmol/g of gel) as a function of temperature (outward diffusion from cube gels)

T (°C)	D_a* × 10^{-10} m^2/sec	Solution viscosity 10^{-3} Pa.s
5	1.50 (9.8%)	6.71
15	1.94 (7.8%)	4.92
25	2.70 (7.2%)	4.00
40	3.57 (5.5%)	2.63

*D_a values are the average of six independent determinations in cube gels containing 58.5 (% w/w), 40 (% w/w) glycerol and 1.5 (% w/w) agar; (variation coefficients are given).

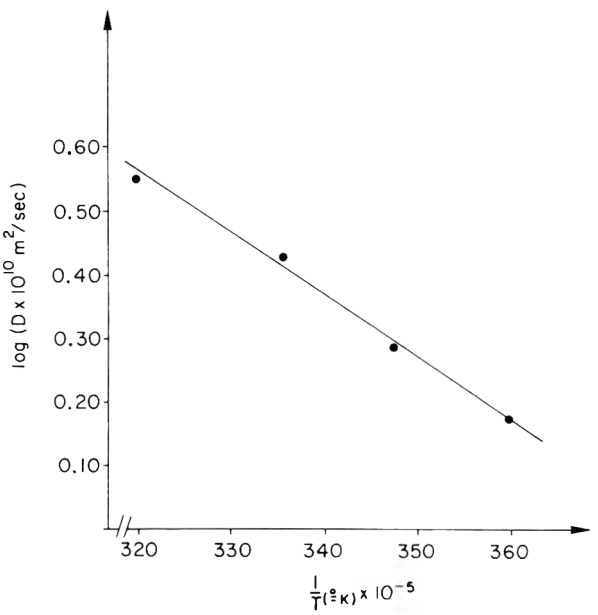

Figure 6. Arrhenius plot of sorbic acid diffusivity against temperature.

Loncin (1980) has shown (a) a strong increase in activation energy for the diffusion of nicotinamide and peroxidase with increasing solute (fructose) concentration in the diffusion medium, and (b) a similar evolution (with values very close to the E_{diff} values) of the activation energy for the change in viscosities. Similar conclusions were drawn by Chandrasekaran & King (1972) from measurements of the diffusion of water and ethyl alcohol in sucrose solutions. When using Brown & Chitumbo (1975) and Nicolas & Duprat (1983) results for diffusivities in gels of tritiated water or KNO_3 it was also found that $E_{diff} \simeq E_\eta$ (as for our own results on sorbic acid diffusivity). These results indicate that change in viscosity is of major influence on diffusivities when changing tem-

perature, and that E_{diff} is relatively independent of diffusent (with low mol. wt) and is unaffected by the presence of the gel network.

The Stokes-Einstein equation (equation 1) also applied well to our data (Table 4). The product $D.\ \eta$ was influenced by changes in temperature.

Influence of the initial concentration of sorbic acid

D_a values of sorbic acid in gels containing various concentrations of sucrose, glycerol or glucose syrups were determined for different initial concentrations of the diffusent (varying from 2 to 28 μmol/g of gel). Results are given in Fig. 7. D_a decreased as the initial sorbic acid concentration increased. In the range of concentrations studied the variations were assumed to be linear (correlation coefficient of the linear regression (r) better than 0.98).

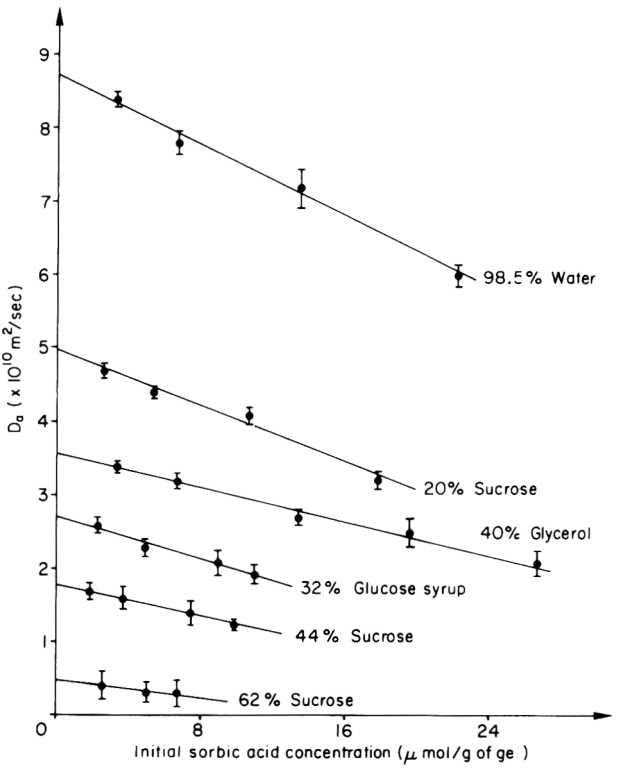

Figure 7. Influence of the initial concentration of sorbic acid on D_a values in gels with various compositions (standard deviations are given).

The diffusivity values (D_0) extrapolated to zero sorbic acid concentration are 8.74, 4.96, 2.71, 3.54, 1.8 and 0.45 $\times 10^{-10}$ m^2/sec respectively for gels containing 98.5% water, 78.5% water and 20% sucrose, 66.5% water and 32% glucose syrup, 58.5% water and 40% glycerol, 54.5% water and 44% sucrose, 36.5% water and 62% sucrose; the proportionality coefficients (slope (S1)/D_0) are respectively −0.014, −0.019, −0.026, −0.015, −0.030 and −0.053 $\times 10^{-6}$ g/mol.

Since the diffusivity depends upon the molecular interactions between the diffusent and the surrounding molecules in the solvent, it can be assumed that as the diffusent

concentration increases, the value of D_a will be influenced by diffusent-diffusent collisions (e.g. formation of aggregates) as well as by diffusent-solvent interactions. Examples of the same concentration dependance D are those of: acetic acid or glycerol+water systems (Crank, 1975), sucrose+water systems (Henrion, 1963), and ethanol+water systems (Robinson *et al.*, 1965).

It can also be observed that the slopes are higher (in absolute values) when the water contents are higher. The following linear relationship between the water contents (X) and the slopes (Sl) was found: Sl = 0.036–0.0016 X (with r = −0.996). This empirical relationship was from experimental observations and there is no clear fundamental approach to this phenomenon.

Further experiments are being carried out with model gels of various compositions, and with foods, in order to relate sorbic acid diffusivity to composition and water content.

Acknowledgments

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Hedonic rating of food: single or side-by-side sample presentation?

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Summary

Taste panels made hedonic ratings of the sensory properties of four grades of sultanas, evaluated as two pairs (Experiment 1), three chicken casserole products (Experiment 2), and two cola drinks (Experiment 3). In each experiment, two modes of sample presentation were compared: single presentation, where panellists rated only one sample per session; and side-by-side presentation, where the samples were presented simultaneously. In Experiment 1, hedonic ratings from single presentation suggested there were no significant differences between grades, whereas the side-by-side presentation did indicate significant differences. This discrepancy between modes of presentation was not noted in Experiments 2 and 3. Selective operation of methodological bias in category rating might possibly account for the findings.

Introduction

Since its introduction over 30 years ago (Peryam & Girardot, 1952), the hedonic scale has been used extensively in the sensory evaluation of food. This scale (and variants thereof) consists of a number of categories, the labels of which correspond to a 'liking continuum' (e.g. 'like extremely' through 'neither like nor dislike' to 'dislike extremely'), and as such it is readily understood by even the least experienced of respondents.

When the hedonic scale is used in the sensory laboratory, panellists are usually presented with a number of samples to evaluate at a single session. This *side-by-side* sample presentation is economical of time, both for panellists and the investigator; on the debit side, however, it may be prone to various context effects. For example, it has been noted that there is sometimes a preference for the sample tasted first (Filipello, 1956; Simone & Pangborn, 1957), and that this order effect is not always cancelled by balancing (McGill, 1979). Also, since side-by-side sample presentation facilitates perception of even the smallest differences, it is possible that the practical importance of these differences could be exaggerated (Amerine, Pangborn & Roessler, 1965; McBride, 1980).

According to Poulton (1977, 1979), the best way of precluding context effects is to allow each panellist just one judgment; that is, use a separate-groups design. As many authors have argued (Bayton & Thomas, 1954; Clements, Bayton & Bell, 1954; Filipello, 1956; McBride, 1980, McBride & Richardson, 1983; Simone & Pangborn, 1957), such a non-comparative approach has the advantage of more closely simulating the normal consumption of food, but it does suffer the disadvantage of requiring a large

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number of respondents. While such a design may be appropriate in a consumer survey, it is usually impractical in the laboratory where the experimenter often relies upon the cooperation of a small panel for sensory testing.

However, one way to minimize the possibility of obtaining context-dependent differences, yet at the same time retain economy of design, is to employ a compromise procedure: have the same panellists judge all samples, but present each panellest with only one sample per session. Provided there is a suffficient interval between sessions (McBride (1982) found 24 hr sufficient to preclude carry-over effects), then direct comparison between samples is prevented and context-dependent differences are nullified. This *single presentation* (monodic-sequential) approach has been used in the hedonic assessment of a number of products, including bread (Bell, 1956), orange juice (Clements *et al.* 1956), and a flavoured milk beverage (McBride, 1982).

But single presentation also suffers a disadvantage. As pointed out by Peryam & Girardot (1952), it is wasteful of laboratory and panellists' time. If, for example, there are four products to be evaluated and the intersession interval is 24 hr, then it will take 4 days to carry out an evaluation which, with multiple presentation, could be effected in a single session. Therefore, in order for it to be worthwhile considering the single presentation technique, the experimenter needs to known, *a priori*, if context effects are likely to present a problem. In a recent study with rats, Sunday, Sanders & Collier (1983) found that the mode of food presentation (single or side-by-side) markedly influenced food consumption. The present study explores the issue of context in human sensory evaluation, by comparing single and side-by-side presentation of three types of food under the same laboratory conditions.

The three experiments were carried out in a sensory evaluation laboratory which has been described elsewhere (Christie, 1964). Men and women employees of the CSIRO Food Research Laboratory served as panellists and participated voluntarily. Most had had some previous experience in the sensory evaluation of food.

Experiment 1: Materials and methods

Materials

Four grades of sultanas (cv. Thompson Seedless) served as the stimulus material. Sultanas are graded for quality, mainly on the basis of size, according to the 'crown' system. In the first test the two top quality grades of sultanas were used: five-crown (premium quality: large, plump fruit, light amber in colour); and four-crown (good quality: smaller and darker than five-crown). In the second test 12 months later, the grades used were three-crown (average quality: medium size, dark brown in colour) and one-crown (lowest quality: small, dark, shrivelled fruit). The testing was preliminary to a study on the shelf life of sultanas (McBride, McBean & Kuskis, 1984).

Response scale

The response scale consisted of nine categories with verbal descriptors at alternate points: 'very good' (9), 'good' (7), 'satisfactory' (5), 'poor' (3), and 'very poor' (1). The categories were not numbered on the response sheet.

Method

In the single presentation condition of the first test, twenty-one panellists were each presented with a 20 g sample of five-crown sultanas and required to rate colour, flavour,

texture and general acceptability on separate 9-point response scales. A completely different panel, also of twenty-one subjects, rated four-crown sultanas independently in exactly the same way.

In the side-by-side condition of the first test, conducted 4 days later, the two panels were combined (i.e., forty-two subjects in all); this combined panel then assessed the sensory properties of both grades of sultanas, five-crown and four-crown, the two 20 g samples being presented side-by-side. The same response scale was used.

The procedure in the second test, on one-crown and three-crown sultanas, was exactly the same except that each of the two panels in the single presentation consisted of twenty-four subjects; consequently forty-eight subjects took part in the subsequent side-by-side condition.

Results and discussion

Table 1 contains mean response scores for the colour, flavour, texture, and general acceptability of four-crown and five-crown sultanas, in both the single and side-by-side presentation conditions. Considering first the data from the single presentation condition, there is little difference between the scores for four-crown and five-crown sultanas on any of the four sensory properties; and in fact none of these differences is statistically significant [unpaired $t(40) \leq 0.96$]. In the side-by-side condition, however,

Table 1. Mean hedonic scores for the colour, flavour, texture, and general acceptability of four-crown and five-crown sultanas in the single and side-by-side presentation conditions

	Single[NS]		Side-by-side		
	4-crown	5-crown	4-crown		5-crown
Colour	7.1	7.1	6.6	***	7.5
Flavour	7.1	7.2	6.6	NS	6.9
Texture	7.1	7.4	6.6	NS	7.0
General acceptability	7.0	7.3	6.6	*	7.1

[NS]Scores within pairs are not significantly different.
***Scores within pair are significantly different, $P < 0.001$.
*Scores within pair are significantly different, $P < 0.05$.

the differences between five-crown and four-crown are more marked: they are now statistically significant for colour [unpaired $t(82) = 4.21$, $P < 0.001$] and for general acceptability [unpaired $t(82) = 2.23$, $P < 0.05$]. (Application of unpaired t-tests to the side-by-side data keeps the statistical analysis constant for both conditions). Of particular note is the shift in colour scores: no difference at all under single presentation, but a highly significant difference when presented side-by-side.

The results from the testing of one-crown and three-crown sultanas are given in Table 2. A similar pattern is evident. In the single-presentation condition there was no significant difference between the two grades on any of the sensory properties [unpaired $t(46) \leq 1.93$]. But in the side-by-side condition the difference is highly significant on all four sensory properties [unpaired $t(94) \geq 3.88$, $P < 0.001$], with the colour scores once again exhibiting the greatest shift.

Table 2. Mean hedonic scores for the colour, flavour, texture, and general acceptability of one-crown and three-crown sultanas in the single and side-by-side presentation conditions

	Single[NS]		Side-by-side***	
	1-crown	3-crown	1-crown	3-crown
Colour	4.8	4.8	4.5	6.3
Flavour	5.4	5.9	5.3	6.4
Texture	4.8	5.6	4.9	6.4
General acceptability	4.8	5.5	4.7	6.3

[NS]Scores within pairs are not significantly different.
***Scores within pairs are significantly different $P < 0.001$.

Inspection of the data showed that the shift in scores between the single and side-by-side presentation did not depend upon which sample panellists had received in the single presentation condition. For instance, those who received five-crown sultanas in the single presentation generated mean general acceptability ratings of 6.6 and 7.2 respectively for four-crown and five-crown sultanas in the subsequent side-by-side condition; those panellists who first received four-crown sultanas generated corresponding mean ratings of 6.5 and 7.0.

It appears, then, that in both tests the experimental context has influenced the ratings. When judged independently against the yardstick of past experience, panellists' scores indicate that there is no quality difference between four-crown and five-crown sultanas, nor between one-crown and three-crown sultanas. However, when in each case the samples are presented side-by-side, where every aspect can be scrutinized by direct comparison, panellists do recognize quality differences and rate accordingly.

The outcome of the side-by-side condition can most likely be explained in terms of what Poulton (1979) called a *stimulus equalizing bias:* panellists tend to use all of the response scale regardless of the actual range of stimuli presented. In Table 1, the scores for the premium quality five-crown sultanas have remained reasonably constant over presentation conditions, whereas the slightly inferior four-crown fruit have been rated lower in the side-by-side condition. In contrast, in Table 2 it is the scores for the low quality one-crown sultanas which have remained unchanged, whereas those for the superior three-crown fruit have increased (cf. Fig. 1B of Poulton, 1979).

For the food technologist, the critical question is which scores are 'correct'? Are the differences in the side-by-side condition real, or are they merely experimental artifacts? There is no single answer to this question; each mode of presentation has validity. For example, if sultanas are marketed in cardboard boxes which preclude any chance of sensory assessment before purchase, then the single presentation condition is more likely to mimic real-life assessment: when consumed, the sultanas will most likely be evaluated against a remembered, or notional, concept of sultana quality. On the other hand, if sultanas are marketed in transparent plastic packs, and several brands sit side-by-side on the supermarket shelf, then direct comparison of appearance before purchase is likely, and the side-by-side condition may be more realistic. The next experiment was conducted to check the generality of these findings, this time using three stimuli of a different food type.

Experiment 2: Materials and methods

Materials

A processed frozen food, chicken casserole, served as the test material. There were three product variants (A, B and C), each supplied by a different food processor. All samples of the one product were taken from the same production batch, and after delivery to the laboratory all material was stored in a constant temperature room at −15°C until required.

The products differed considerably: Product A contained large chicken pieces with tomato, onion and mushrooms; Product B was of the fricassee type, consisting of small chicken pieces with carrots and peas in a white sauce; and Product C contained large chicken pieces with potato, celery, carrot and turnip. Consequently, the three products were readily distinguishable on the sensory attributes of appearance, flavour and texture.

Response scale

The response scale consisted of thirteen categories with seven equidistant verbal descriptors: 'extremely good' (13), 'very good' (11), 'good' (9), 'satisfactory' (7), 'poor' (5), 'very poor' (3), and 'extremely poor' (1). The categories were not numbered on the response sheet.

Method

In the single presentation, one testing session was run per day over 3 consecutive days. The twenty-four panellists were divided randomly into three subpanels, eight in each, so that the order of evaluation could follow a Latin-square design.

In the side-by-side presentation, carried out 3 weeks later, there were two replicate sessions, held on consecutive days. The same twenty-four panellists were presented with the three products simultaneously, and the order of evaluation was balanced.

The products were prepared for evaluation by thawing at 5°C, then heating at 160°C in a convection oven for 45 min. Samples (about 150 g in each) were served hot, on white plates, together with eating utensils. All testing was carried out between 1130 and 1230 hr, immediately before the staff lunch break.

Panellists were instructed to taste the samples and to rate their appearance, flavour, texture, and general acceptability using separate 13-point response scales. Re-tasting was permitted.

Results and discussion

First, the data obtained in the single presentation were subjected to Latin-square analyses of variance, to check for possible order and sessions effects. There were no significant differences between the overall scores from the three subpanels [F (2, 2) ≤ 1.50], suggesting that the order of evaluation had no effect on the overall scores assigned; nor was there any difference in the overall mean score between the three sessions [$F(2, 2) \leq 1.55$], i.e., the overall magnitude of the scores assigned on the first day was no different from that of those assigned on the last. This freedom from order and sessions effects allowed the single presentation data to be treated as if obtained from a conventional complete-block experimental design in the subsequent analysis.

The main statistical analyses were carried out using the Genstat package on CDC Cyber 76 computer. Separate analyses of variance were performed on the four sensory

Table 3. Mean hedonic scores for the appearance, flavour, texture, and general acceptability of three chicken casserole products (A, B, C) in the single and side-by-side presentation conditions*

	Single			Side-by-side		
	A	B	C	A	B	C
Appearance	10.1b	8.3d	9.5c	10.8a	8.5d	10.3b
Flavour	10.3a	9.2b	8.8b	9.9a	9.4b	9.2b
Texture	10.3a	9.7ab	9.2b	10.4a	9.6b	9.1b
General acceptability	10.6a	9.2b	9.1b	10.3a	9.2b	9.4b

*Scores within each row which do not have a suffix in common are significantly different, $P < 0.05$.

attributes. Each analysis included data from both the single and side-by-side presentation.

The mean hedonic scores from the single and side-by-side presentation conditions are given in Table 3. The mean ratings lie mainly between 9 and 11 ('good' to 'very good' on the 13-point scale) indicating that all products were found to be acceptable.

Except for appearance, where overall the scores in the side-by-side presentation condition were higher [$F(1, 23) = 4.58, P < 0.05$], there was no significant difference between the overall mean scores in the single and side-by-side presentations.

There were significant differences between products on all sensory attributes [$F(2, 46) \geq 11.81$, $P < 0.001$]. Generally speaking, product A was preferred; except for appearance, however, there was no (preference) difference between products B and C (see Table 3).

But perhaps the most pertinent finding of the analysis is that in no case was there an interaction of presentation condition × product [$F(2, 46) \leq 2.00$]. Unlike Experiment 1, here the mode of presentation did *not* affect the relativity of the ratings: there is no evidence that the side-by-side presentation has accentuated the hedonic differences. The next experiment further explores the effect of presentation condition.

Experiment 3: Materials and methods

Materials

Cola drink served as the testing material. The two products, A and B, were produced by different manufacturers. Samples of each product were taken from the same production batch. Preliminary evaluation revealed the products to be similar in appearance, but qualitatively different in flavour.

Method

The response scale was as used in Experiment 2. The experimental design was also similar. In the single presentation condition, one session was run per day over 4 consecutive days. The panel of fifty was split and half of the panellists received the two samples in the order ABAB; the remainder, BABA (such replication affords a check on the reproducibility of the single presentation approach). Panellists were not, of course,

aware of the aim of the testing; as far as they were concerned, they were simply required to evaluate four samples over 4 days.

In the side-by-side condition 2 weeks later, there were two replicate sessions, held on consecutive days. The same fifty panellists received the two samples simultaneously and the order of evaluation was balanced.

Panellists received 100 ml of each sample, served at approximately 8°C, and were required to rate colour, flavour, and general acceptability on 13-point response scales. Re-tasting was permitted.

Results and discussion

Separate analyses of variance were effected on scores for colour, flavour, and general acceptability, once again using the Genstat package. The mean hedonic scores from the single and side-by-side conditions are given in Table 4.

Table 4. Mean hedonic scores for the colour, flavour, and general acceptability to two cola drinks (A and B) in the single and side-by-side presentation conditions*

	Single		Side-by-side	
	A	B	A	B
Colour	9.7a	10.0b	10.1b	10.1b
Flavour	8.3a	9.5b	8.4a	9.8b
General acceptability	8.3a	9.5b	8.5a	9.7b

*Scores within each row which do not have a suffix in common are significantly different, $P < 0.05$.

With regard to presentation condition, the overall rating for colour was slightly but significantly higher in the side-by-side presentation [F (1, 200) = 8.50, $P < 0.01$]; however, there was no difference on the other two sensory attributes [F (1, 200) $\leq$ 1.52].

There was a significant preference for product B on all sensory attributes: colour [F (1, 200) = 4.08, $P < 0.005$]; flavour [F (1, 200) = 83.12, $P < 0.001$]; and general acceptability [F (1, 200) = 83.06, $P < 0.001$] (see Table 4).

Consistent with the finding of Experiment 2, however, there was no evidence of a presentation condition × product interaction on any sensory attribute [F (1, 200) $\geq$ 2.47]. For instance, Table 4 shows that the difference between the mean scores for general acceptability is 1.2 scale points in both presentation conditions: the difference was not accentuated when the samples were presented side-by-side.

The experiment also indicated that the scores in the single presentation were reproducible and did not change over the 4 days of testing. At the very first session the mean general acceptability scores for A and B (each from twenty-five observations) were 8.2 and 9.8 respectively; this compares with the overall means from the single presentation condition of 8.3 and 9.5.

General discussion

The main question arising from this work is why the single and side-by-side presentations should produce different estimates in Experiment 1, but not in Experiments 2 and 3.

One possibility might be that, during the course of the single presentation in Experiments and 3, panellists to some extent learned the range of stimuli and were therefore making, at the later sessions, what were effectively comparative judgments (i.e., judgments more typical of a side-by-side condition). However, this can be dismissed. The scores in the single presentation conditions of Experiments 2 and 3 were stable: mean scores at the very first session were no different from those at the last. Reevaluation of a previous study in which the single presentation technique was employed (McBride, 1982), likewise showed no shift in the relativity of scores over sessions.

As another possibility, the operation of context effects might depend upon the extent to which panellists are familiar with the product under test. Although it is likely that panellists had experienced a considerable variety of chicken casseroles and cola drinks before the present experiments, they may not have experienced such variation in sultana quality. It could be argued that experience with a product results in the establishment of an internal quality scale for that product; thus, the greater experience with chicken casseroles and cola drinks would permit panellists to rate the quality of these products against their respective internal scales, uninfluenced by the experimental context.

But there is yet another possible explanation, of perhaps greater plausibility. In Experiment 1 the stimuli were effectively four grades of the one material; that is, the stimuli varied *quantitatively* along the continuum of sultana quality. Moreover, the dramatic shift in the colour scores between the two modes of presentation indicates that, in the side-by-side condition, this quality difference was readily apparent from visual inspection alone. In this respect, therefore, the samples were analogous to other univariate psychophysical stimuli previously shown to be susceptible to context effects, e.g. noisiness (Poulton, 1979), saltiness (Lawless, 1983), and sweetness (McBride, 1982). In a comparison of single *versus* paired presentation of wines, Filipello (1956) found that varying only the sweetness level of what were otherwise identical samples did produce context effects, but in no other cases did presentation condition affect the hedonic scores.

In contrast to Experiment 1, the stimulus materials used in Experiments 2 and 3 were, respectively, not as homogeneous; although significant preferences were obtained, in neither case did the samples vary quantitatively along a single, easily recognizable dimension. When panellists are required to assess products side-by-side, and the products do not vary quantitatively along a single, recognizable continuum, it is more likely that the judgment of each sample is made independently, unaffected by the context of the other samples. Consistent with this hypothesis, Anderson (1982) reported that side-by-side hedonic ratings of *different* types of vegetables were not affected by a shift in experimental context.

In conclusion, two of the three experiments in the present study have vindicated the use of side-by-side presentation. As noted earlier, side-by-side presentation is logistically attractive, and for this reason alone is likely to remain popular in the sensory laboratory. Nevertheless, the present study suggest that hedonic ratings of food may, under some circumstances, depend upon the way in which the samples are presented.

Just which mode of presentation affords the more valid assessment is for the investigator to decide.

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Prediction and measurement of volatile retention during extrusion processing

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Summary

The loss of volatiles during extrusion of a corn-based product was studied. Four organic flavour compounds were tested: n-butanol, octane, benzaldehyde and limonene. A method was developed to calculate the vapour pressure of the volatile at the extruder die exit temperature through use of the Henry's law coefficient. Two models were developed to predict loss of volatiles. The thermodynamic model assumed complete equilibrium between the solid and vapour phase. This model gave moderate to poor prediction of retention for n-butanol but was within 20–30% for limonene and benzaldehyde. A second model based on relative volatility with respect to water loss, as assessed by steam distillation, gave predictions which were good for both n-butanol and benzaldehyde. This latter model has potential for prediction of retention of polar flavour compounds.

Introduction

The growth of extruded snack foods has been well recognized (Scales, 1982). This success depends largely upon the quality of the extruded product including appearance, texture and flavour (Blanchfield & Ovenden, 1974). Recently, the prevalent views on nutrition and cost reduction have led to research to reduce calories, such as by decreasing the amount of fat-coating which is used to incorporate the flavours. This has brought about a need to modify the existing flavouring technology through an internal flavour incorporation technique rather than by surface application (Seiler, 1977). In the internal incorporation process the flavouring substances are mixed in with the raw materials such as maize (corn) or semolina before extrusion.

Lane (1983) found that better flavour perception for cheese and chicken flavoured extruded snacks was achieved by internal application as compared with surface application. Although the internal application technique is a technically viable concept, a very large number of experiments is usually required to arrive at a sufficiently satisfactory solution by this process. This is because most incorporated flavour compounds either cannot survive extrusion processing or are volatilized due to flash distillation with water vapour at the extruder die exit (Blanchfield & Ovenden, 1974). Palkert & Fagerson (1980) have studied the qualitative effects of extrusion processing and drying on the retention of volatiles in textured vegetable protein (TVP). However, no quantitative data on the volatiles lost during extrusion of cereal based snack foods have been published. The difficulties in studying the loss of volatiles during extrusion of foods as compared with other drying processes, such as freeze drying and spray drying, are

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mainly due to the lack of information such as the thermodynamic parameters (volatility and Henry's law coefficients) of volatile compounds in the food matrix, the physical binding state of volatile compounds in the food matrix, and the interactions between volatile compounds and the food matrix under extrusion conditions.

Accordingly, the objectives of this study were: (i) to examine the effect of extrusion processing variables such as temperature on the water binding properties of the matrix; (ii) to establish the basic thermodynamic volatility parameters for several organic compounds in the extruded matrix; (iii) to test a thermodynamic approach for prediction of the loss of volatiles during extrusion; and (iv) to test an engineering approach based on the volatility of compounds relative to water for prediction of the retention of volatiles after extrusion compared to actual retention.

Materials and methods

Materials

Degerminated yellow corn flour obtained from Krause Milling Company (Milwaukee, WI) was used. Five volatile compounds were tested: n-butanol, octane, benzaldehyde, cinnamaldehyde (all from Sigma Chemical Company, St. Louis, MO) and limonene (Universal Flavours Company, Indianapolis, IN). These were chosen to represent, respectively, a very polar compound; a non-polar compound, two typical aldehyde volatiles of moderate polarity, and a volatile oil. Benzaldehyde is typical of almond flavour and limonene is present in citrus and other flavours.

Cornmeal preparation and extrusion

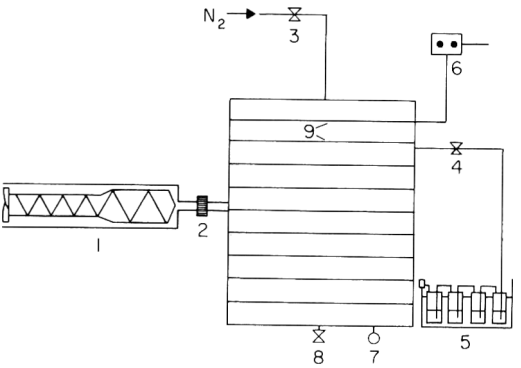
The moisture content of cornmeal (maize) was adjusted to the desired level (15–25%) in a twin-shell blender (G. S. Blakeslee & Company, Chicago, IL). After mixing, samples were packed in laminated bags and stored at 4°C overnight to allow moisture equilibration. Before each experiment, samples were analysed for moisture by the Karl Fischer method. The moisture sorption isotherm for the cornmeal and the extruded product were determined at 25, 30, 45 and 65°C by the method of Kaanane, Chen & Labuza (1985).

The extruder used was a Brabender Model 2003 (C. W. Brabender Instruments, Inc., South Hackensack, NJ) with the following specifications: barrel length/barrel diameter (L/D) = 20:1; 2 zone heater/cooler collars and heat controlled die; screw compression ratio: 1.5:1; die nozzle: ¼ inch diameter (0.0098 m).

The operating conditions were set as follows: shear speed: 140 rpm, 155 rpm, 170 rpm; moisture content of feeding material: 15, 20, 25% (wet basis); zone temperature: zone I: 60°C; zone II: dependent upon zone III; zone III: 140, 150, 160°C.

The flavoured corn flour or ground extruded product for the volatility test was prepared by incorporating the model flavour system into the previously humidified corn flour or ground extruded product in the twin shell blender, with blending for at least 1 hr at 4°C. The concentration of each volatile compound in the final preflavoured sample was 50 ppm. The flavour system was prepared by dissolving each flavour component in absolute ethanol at a concentration of 4 mg/ml as a stock solution. The humidified pre-flavoured sample was then sealed in bags and conditioned overnight in the cold room before extrusion.

If the volatile compounds were to be collected from the vapour space after extrusion, a specially constructed volatile collection chamber was connected directly to the end of the exit die via a quick connection as shown in Fig. 1. During each run, the



1 Extruder
2 Quick connection
3 Pressure regulator
4 Flow rate regulator
5 Flavour recovery system
6 Temperature controller
7 Pressure gauge
8 Safety valve
9 Heating tape

Figure 1. Diagram of volatile collection device at end of extruder.

first 100–200 g of sample were collected. This chamber was made of stainless steel with temperature control heating tapes wrapped on the outside. The inner void volume is about 3000 ml. During the extrusion process, the solid fraction of the extruded product was retained in the chamber, while the volatile compounds which flashed off into the gas phase of the chamber were flushed out by nitrogen at a pressure of 5 psi (3.45×10^4 N/m^2) with a flow rate of 30 ml/min for 90 min. The gas passed into a recovery system composed of four collection tubes cooled by an ice/salt bath. The first tube is used to condense most of the water and water soluble volatiles, while the second and third collection tubes contain 20 ml each of ethyl ether. These were used to collect the remaining volatiles. The fourth tube was used as a precaution to ensure any further collection of volatile or solvent from the first three tubes. In all cases no liquid appeared in the fourth tube. An internal standard of nonane was added before the ether solutions were combined. After two ether extractions followed by anhydrous magnesium sulphate dehydration, the extracted fraction was analysed by gas chromatography. The extruded product collected from the chamber was analysed for moisture content and volatile concentration using the purge and trap method as described in the following

Table 1. Processing conditions for extruded products

System	Temperature (°C)	Moisture content (g H_2O/100 g sample)	Shear rate (rpm)
Extruded product 1	140	15	140
Extruded product 2	150	15	140
Extruded product 3	140	20	140
Extruded product 4	150	20	140

section. Due to problems of maintaining steady state flow in the extruder, out of the twenty-seven possible conditions (3 temperatures×3 shear rates×3 moistures) only four conditions achieved a sufficiently constant flow to allow the measured volatile retention in the solids to be compared with the predicted values. These conditions are shown in Table 1. In two of these studies (systems 2 and 3) the actual vapour volatiles at the exit were also collected so that a comparison could be made with the predicted vapourized volatiles.

Relative volatility

The head space analysis method used was a modification of Leahy & Reineccius (1984). Samples of approximately 10 g (flavoured humidified cornmeal or processed extruded product) were transferred into 140 ml serum bottles, covered with a Teflon cap liner (Arthur H. Thomas Company, Philadelphia, PA), a rubber septum and then an aluminium cap. This final cap was crimped tightly on the bottle top. Samples were placed in incubators at either 45, 65 or 85°C for four hours prior to GC analysis.

A Hewlett-Packard Model 5880 gas chromatograph equipped with a single flame ionization detector (FID) was used for the quantitative analysis of the head space volatile concentration as well as for the analysis of the volatile compounds collected during extrusion. For head space analysis, the GC was set up with a 2 ml head space sampling loop. When a sample was to be analysed, the fill line of the sampling loop was forced through the sample bottle septum. Pressure within the bottle forced head space vapours into the sampling system and filled the sampling loop. Changing the position of the six port valve then routed GC carrier gas through the sample loop, sweeping the head space into the GC for analysis.

The gas chromatographic conditions were as follows: 0.2 mm i.d.×12 m WCOT fused silica (Hewlett-Packard, Palo Alto, CA); stationary phase: OV-101; carrier gas: hydrogen at 35 cm/sec; split ratio: 1:60 (column flow:split flow); column temperature: initial temperature 40°C for 2 min, 40 to 160°C programmed at 15°C/min, final temperature 160°C for 1 min; injection port temperature: 225°C; detector temperature: 250°C; sample size (for direct liquid injection): 2 μl.

A cryogenic method was used to improve chromatographic resolution for all GC analysis. This was done by dipping the initial end of the capillary column in a liquid nitrogen bath for 2 min before sample separation. This preconcentration step gives a good separation between flavour carrier ethanol and n-butanol and also for each volatile compound. Calculations for liquid injection were based on an internal standard while those for head space analysis were based on an external standard (Anandaraman, 1984).

Purge and trap gas chromatography

A modification of the purge and trap method of Bangs & Reineccius (1980) was used. The amounts of volatile compound present in the extruded product were analysed using a Hewlett-Packard 7675A purge and trap sampler coupled to a Hewlett-Packard 5840 gas chromatograph equipped with an FID. A 20 min purge of the rehydrated sample (0.5 g of ground extruded product in 4 ml distilled water containing nonane as internal standard) at a helium purge rate of 100 ml/min was included. The purged organic volatiles were adsorbed on a 10 by 0.32 cm o.d. SS precolumn packed with Tenax (Hewlett-Packard Company, Avondale, PA). The concentrated organic volatiles from the Tenax precolumn onto the GC column were eluted by heating the precolumn to 180°C while flushing with helium (75 ml/min). The combined flow of

helium and volatiles was preconcentrated cryogenically and then split 60:1 and passed onto the capillary GC column (OV-101) as used in the head space analysis. The injection port and FID temperature was 200 and 280°C, respectively. The calculation of volatile concentration (nmol/g solids) based on the internal method is as in the previous section. All samples were run in triplicate with excellent replication (±5% of the peak areas).

Results and discussion

The adsorption isotherms at 25, 30, 45 and 65°C of cornmeal extruded at condition no. 4 (Table 1) are presented in Fig. 2. All give the characteristic S-shaped curve of normal water adsorption isotherms. It is evident that for any constant moisture content, an increase in temperature significantly increases the water activity as found for other foods (Kaanane *et al.*, 1985). Thus the corn flour loses water more easily as the temperature is raised. The extrusion process itself also decreases the ability of the cornmeal to bind water, as shown in Fig. 3, indicating more starch–starch interactions, as has been found by Iglesias & Chirife (1976) and Saravacos (1967). These interactions could form micro regions into which volatiles could be trapped, helping in retention even though the water is easier to remove. It can be noted that there were no significant differences between the isotherms in Fig. 3 among the extruded materials. This was tested with the GAB isotherm equation (van den Berg and Bruin, 1981; Bizot, 1983), using an HP9816 non-linear regression method. No difference was found at the 99% significance level.

Calculated heats of sorption from the isotherms at constant moisture are shown in Table 2 for cornmeal and two extruded products (Labuza, 1968). As noted in Table 2, as the moisture exceeds 14 g H_2O/100 g (about 12% wet basis) the excess heat drops to less than 0.42 kilocalories/mol. Thus, at the extrusion processing moistures used (> 15% wet basis), behaviour of the water vapour at the die exit should be very close to that of pure water. Since the die is above 100°C, the exit vapour pressure of water will be equal

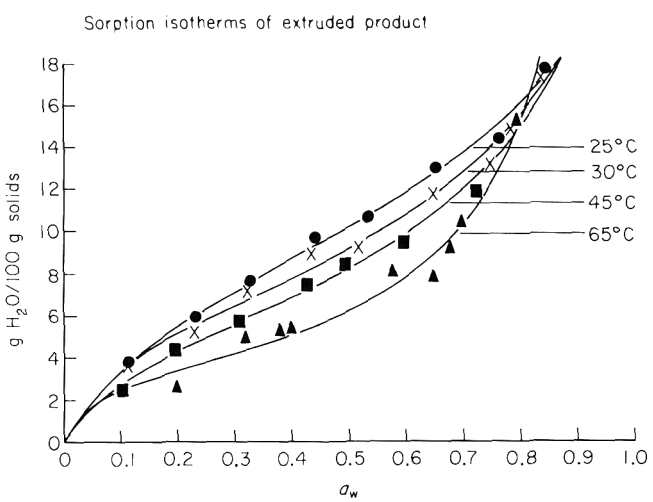


Figure 2. Moisture sorption isotherms of the extruded cornmeal product ● 25°, X 30°, ■ 45 ▲ 65°C.

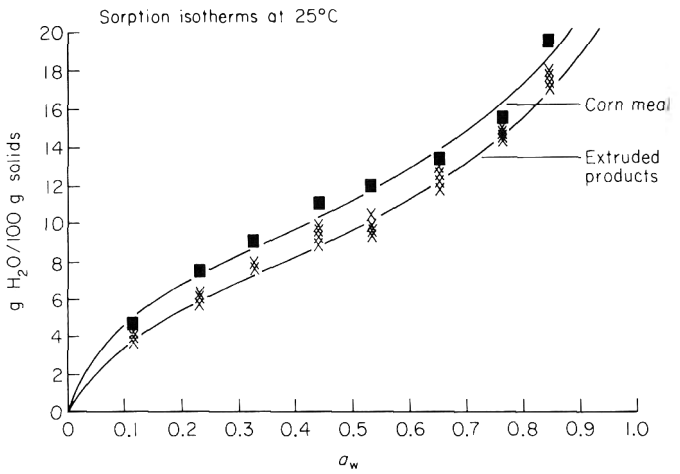


Figure 3. Comparison between moisture sorption isotherm of cornmeal and extruded product at 25°C.

to the vapour pressure of pure steam at the die temperature. This vapour pressure will drop quickly to that of the outside atmosphere as the vapour leaves the die area and mixes with air.

To predict the extent of volatile loss at the die exit, two approaches can be taken. One can simply use an engineering model assuming co-distillation of the volatiles with water at the die exit. This will be discussed later. In the other model, one can assume thermodynamic equilibrium of the volatile between the adsorbed phase and the vapour phase at the exit port. This model enables one to predict the amount of volatile compound which would be present in the head space at the extrusion temperature occurring at the die exit, based on data collected at lower temperatures. Table 3 shows the vapour concentration data for four volatiles using the volatility test described previously. In this test, a sample of either corn flour or ground extruded product at different moisture contents was mixed with a known amount of volatile, equilibrated at several temperatures, and the volatile content of the head space measured. The variance of this determination is within 10%. The corn flour represents the initial state of the corn matrix during extrusion, while the ground extruded product is used to simulate the state of the corn matrix after completely leaving the die exit where the

Table 2. Heat sorption as a function of moisture content

Moisture content (g H_2O/100 g solids)	Heat of sorption (kcal/mol)		
	Cornmeal	Extruded product 2	Extruded product 5
2	4.45	4.29	4.38
4	4.30	4.25	4.13
6	3.91	3.89	3.68
8	2.74	2.24	3.23
10	1.76	1.34	1.56
12	1.11	0.76	0.88
14	0.71	0.34	0.42
16	0.43	0.16	0.30

water and volatiles have flashed off. The true state of the corn matrix exactly at the flash off point during extrusion cannot currently be evaluated experimentally, but the actual condition must be between these two states. Using the log vapour pressure *versus* inverse T relation (Clausius–Clapeyron), the amount of compound volatilized at the extrusion temperature can be predicted for the higher die exit temperature (Adamson, 1976). As noted in Table 3, the amount of volatile present in the head space of extruded product is less than in that of unextruded corn flour for n-butanol, octane and limonene, but greater for benzaldehyde. The lower volatile desorption capacity of the extruded matrix for the first three compounds possibly indicates that the gelatinized starch exerts some effects by way of retarding the volatilization process. This might be due either to an increased viscosity of the gelatinized corn matrix, which might reduce volatilization to some degree as shown in a liquid system (King, 1983), or due to a stronger interaction between the volatile compounds and hydrated gelatinized starch, as observed by Maier (1975). Why benzaldehyde is not reduced in vapour pressure is not clear. Figure 4 shows that a linear relationship can be obtained when the amount of volatile compound present in the head space per unit weight of sample solids is plotted against the inverse of absolute temperature (Clausius–Clapeyron relation) on a semilog scale. The slope of each line corresponds to ΔH_s 'the composite heat of sorption' (heat of vapourization

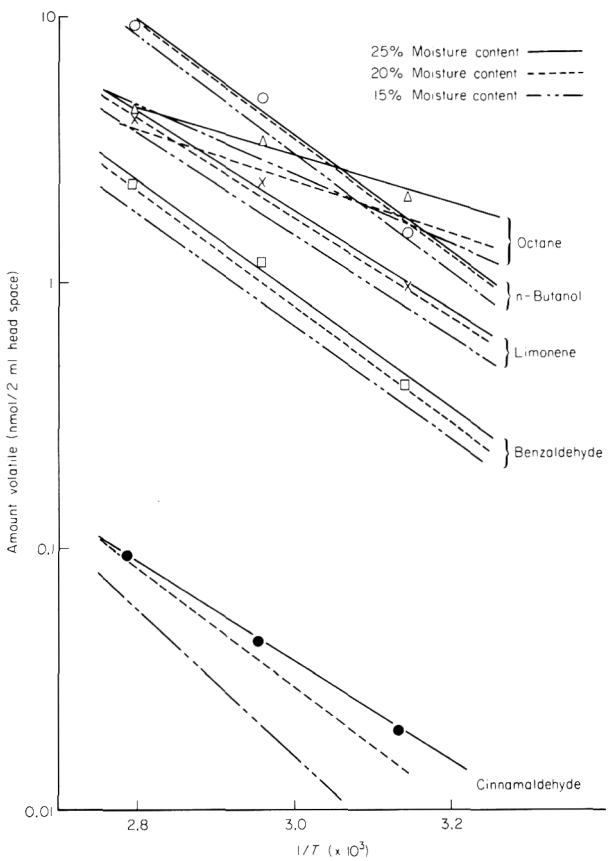


Figure 4. Volatile headspace concentration as a function of moisture content and temperature for the extruded product. Data points shown only for 25% moisture. Other lines drawn by linear regression and had a similar goodness of fit.

plus heat of sorption), of each volatile compound (Adamson, 1976). It should be noted that only the data points for 25% moisture are shown in Fig. 4, the other lines are the best fit by linear regression.

Table 3. Comparison of volatile concentrations in the head space between cornmeal and extruded product

		Amount of volatiles in the head space (nmol/g solids)					
		45°C		65°C		85°C	
Moisture content (wb)	Volatile compound	CM*	EX†	CM	EX	CM	EX
15%	n-Butanol	11.04	11.78	13.82	20.59	65.27	36.81
	Octane	13.51	4.94	23.47	9.88	39.45	13.75
	Benzaldehyde	2.49	4.36	7.53	11.28	14.65	21.66
	Limonene	5.99	5.76	16.22	13.59	29.60	24.95
20%	n-Butanol	14.13	9.62	45.11	17.94	83.04	37.62
	Octane	14.39	1.75	24.48	2.63	34.53	3.50
	Benzaldehyde	3.19	5.25	9.63	13.74	19.19	24.76
	Limonene	7.48	2.63	19.78	6.13	34.97	10.24

*CM = Cornmeal unextruded.
†EX = Cornmeal extruded.

The Henry's law coefficient (H) for each volatile compound was evaluated by dividing the amount of volatile compound in the head space by that remaining in the corn matrix at a specific moisture content and temperature. This coefficient should follow a van't Hoff relationship as a function of inverse absolute temperature. From the slope of this plot, the enthalpy change for desorption can be obtained.

Table 4 shows that the correlation coefficients for linear regression of the data either as a van't Hoff or Clausius–Claypeyron function are around 0.98–0.99. This indicates that both relationships show good fit. Since the amount of cinnamaldehyde present in the head space was very small and only two data points could be obtained, the comparison was made only on the other four volatile compounds.

Table 4 also shows the heat of vapourization of each volatile compound at its boiling point. Theoretically, the 'composite heat of sorption' for each volatile compound should be greater than that of vapourization, since the former includes both heat of vapourization and heat of sorption which is a function of the volatile concentration and of the state of the matrix (Adamson, 1976). As the volatile concentration increased, the enthalpy change of the sorption process tends to approach that of vapourization. In general, the heat of sorption is a positive value resulting from the interactions between volatile compound and the food components although the energetics of the sorption process includes both enthalpy and entropy factors (Adamson, 1976). However, except for benzaldehyde and n-butanol in unextruded corn flour, the values of the composite heat of sorption almost invariably are lower than that of the heat of vapourization. The actual heat of vapourization of a compound is a function of temperature which depends on the heat capacity of the component. Unfortunately no data exist for the dependence of the heat capacity of these volatile compounds on temperature, thus, the true heat of vapourization at the extrusion temperature may be less than listed in Table 4. Also the

error in the volatile entrapment and collection method is estimated to be a ±10% and thus will contribute to the observed difference.

Tables 3 and 4 also indicate that moisture affects the vapour concentration differently for each volatile. For n-butanol, for example, increasing the moisture content increased the volatility for the unextruded product except at 85°C but decreased the volatility in the extruded product, while ΔH_s remains constant in the unextruded and decreases in the extruded. No explanations can be given for this until more information is obtained on the matrix-volatile interactions.

Table 4 indicates that the composite heat of sorption for each volatile compound in the extruded product is lower than that of the unextruded cornmeal system. This suggests that the behaviour regarding the desorption of volatile compounds at the die exit is continuously changing during extrusion processing and thus the true vapour pressure
is also changing. The transition state between cornmeal and extruded product is impossible to evaluate experimentally. Thus, the prediction of volatile concentration in the head space at extrusion temperatures must be based on both physical states (i.e., feed state and extruded state) with extrapolation of the data using the $ln\ a_w$ *versus* $1/T$ function for extrusion conditions.

The fractional loss of volatile compounds during extrusion, where a near equilibrium is being achieved between vapour and liquid, has been described by King (1983). Using the data from the volatility tests in Tables 3 and 4 and extrapolating to 140 and 150°C, the amount of volatile compounds present in the head space at the extrusion temperature can be predicted if the composite heat of water sorption is assumed constant. To test this hypothesis, the actual amount of volatile compounds flashed during extrusion was collected from the collection chamber, as described before, and compared with the predicted values in the head space. The gas chromatogram of collected volatiles for one system extruded at 150°C is shown in Fig. 5. As seen from the GC profiles, minor amounts of side products are generated during extrusion processing, but these did not seem to interfere with the analysis.

Table 5 shows the mass balance data for the two systems in which not only steady state could be achieved but also where the volatile collection system at the end of the extruder worked adequately. For octane, 81% was lost from the system. This was due in part to the gas sweep in the collector, probably causing octane to be purged from the solids, and in part to the difficulty found in trapping octane on the Tenax GC column. This has also been reported by Sydor & Pietrzyk (1970) and Butler & Burke (1976). Thus, a comparison of predicted and actual retention for octane is not feasible. n-Butanol showed a 10–20% positive error (more found than put in), while the other errors in collection were from −7 to +34%. Prediction cannot be better than this. These differences would be due to errors in extraction and inherent GLC errors and are reasonable for the concentration range tested (Leahy & Reineccius, 1984).

Table 6 shows the thermodynamic model predictions for retention and volatilization based on extrapolation of the Henry's law coefficients and compares them with the actual retentions and volatilization. The predictions are based on both the cornmeal and extruded product volatility data, since the actual matrix will be somewhere in between at the extruder die exit. As noted above, based on mass balance error, the octane data show much less retention than expected, but this does not disprove the model. For butanol, which is a fairly polar substance, the actual % retention fell between the predicted retentions based on either extruded or non-extruded product with the value generally being closer to the extruded value, as expected. However,

Table 4. Clausius–Clapeyron* and van't Hoff† relations for volatile head space concentration as a function of moisture and temperature ($°K^{-1}$)

		Extruded cornmeal											
		10% H_2O			15% H_2O			20% H_2O			25% H_2O		
Compound	H_v‡	H_s	I	r^2	H_s	I	r^2	H_s	I	r^2	H_s	I	r^2
n-Butanol	10.5												
(bp = 117°C)*		5.76	11.6	0.996	6.44	12.6	0.998	7.70	14.4	0.982			
†		5.92	9.8	0.996	6.61	10.8	0.997	7.88	12.5	0.992			
Octane	9.97												
(bp = 125.7°C)*		6.95	12.4	0.990	5.83	10.9	0.972	3.33	6.8	0.996			
†		7.06	10.99	0.990	5.92	9.3	0.973	3.94	5.1	0.996			
Benzaldehyde	9.18												
(bp = 179°C)*		5.71	10.96	0.997	9.10	15.9	0.995	8.81	15.6	0.984			
†		5.83	9.5	0.997	9.27	14.4	0.995	8.99	14.1	0.990			
Limonene	9.47												
(bp = 165°C)*		7.86	14.6	0.993	8.32	14.9	0.996	7.72	13.2	0.984			
†		8.20	13.7	0.994	8.56	13.8	0.997	7.79	11.7	0.990			
		Unextruded cornmeal											
n-Butanol*					10.10	18.4	0.987	10.08	18.7	0.980	10.08	18.7	0.982
†					10.50	17.0	0.984	10.57	17.3	0.982	10.56	17.3	0.985
Octane*					6.06	12.2	0.999	4.97	10.6	0.992	4.24	9.7	0.980
†					6.36	11.1	0.999	5.19	9.2	0.993	4.46	8.4	0.981
Benzaldehyde*					10.08	16.9	0.988	10.19	17.3	0.990	9.81	16.9	0.991
†					10.20	15.4	0.984	10.34	15.8	0.991	9.98	15.4	0.991
Limonene*					9.08	16.2	0.989	8.77	15.9	0.987	8.39	15.5	0.990
†					9.40	15.3	0.990	9.12	14.9	0.989	8.77	14.6	0.992

*$\ln C = \frac{\Delta H_s}{R}(1/T) + I$ where C is in nmol volatile/g solids (van't Hoff relation).

†$\ln H = -\frac{\Delta H_s}{R}(1/T) + I$ where H is in nmol volatile per g solids (Clausius–Clapeyron relation).

‡Heat of vaporization in kcal/mol at boiling point from Chemical Engineers' Handbook (Perry & Chilton, 1973).

r^2 = Correlation coefficient.

Table 5. Mass balance for volatiles during extrusion processing

System no.	Initial moisture (%wb)	Extrusion temperature (°C)	Volatile	nmol/g solids				
				Initial concentration	Volatiles left in matrix	Volatiles collected in vapour	Total volatiles recovery	% Collection error*
2	15	150	n-Butanol	793	470	457	927	17%
			Octane	514	56	41	97	−81%
			Benzaldehyde	554	316	197	513	−7%
			Limonene	431	430	149	579	+34%
3	20	140	n-Butanol	843	512	425	937	+11%
			Octane	547	68	109	177	−70%
			Benzaldehyde	588	343	111	454	−23%
			Limonene	458	226	118	344	−25%

*(Total recovered - total initial)/initial.

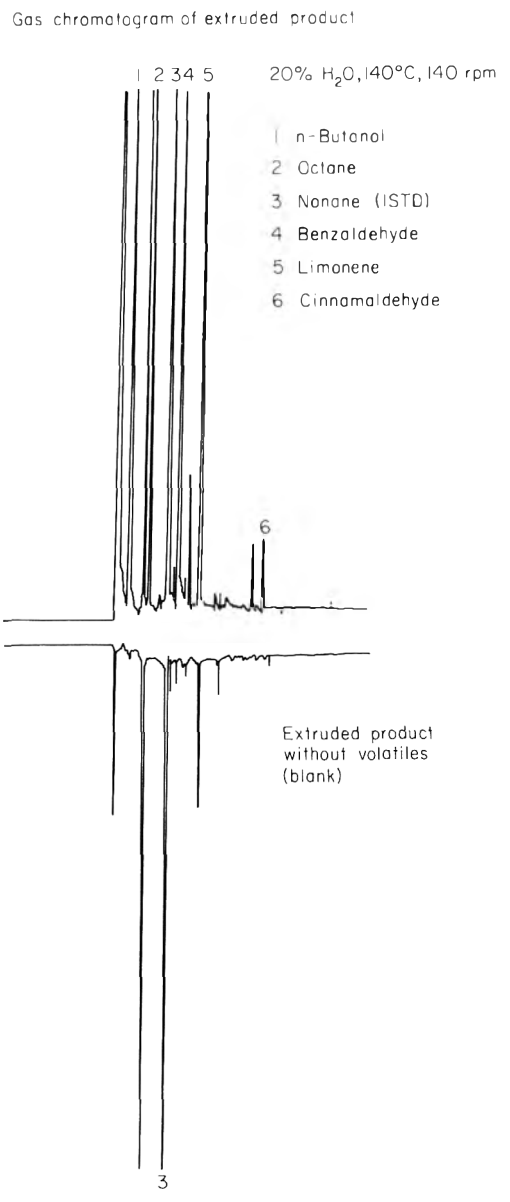


Figure 5. Typical gas chromatogram of volatiles in vapor collected at end of extruder compared to standards.

using the captured volatiles as the base, the predicted volatilization is greater than one expects, because of the 10–20% error in the mass balance. If the extruded product is used as the basis, actual retention is about 20% less than predicted and actual volatilization is about 40% more (these are not the same since the mass does not balance). For benzaldehyde, the actual retention is about 15–20% lower than that predicted for either matrix and is within ±5% for actual volatilization *versus* predicted volatiles. Finally, limonene shows large variations between experiments with errors of about 15% less, 45% more, 10–40% less and 0–40% more for experiments 1–4,

respectively, based on retention, while better results were found based on actual volatilization. The errors for any of these compounds are due to (i) the mass balance errors, (ii) the inability to accurately measure the true volatile vapour pressure at the (iii) the existence of non-equilibrium conditions. This latter problem also precludes accurate predictions in spray drying (King, 1983).

The second approach to prediction was to use an engineering model based on the volatility of volatile compounds relative to water. If the loss of volatile compounds during drying is mainly contributed by the fraction of volatile compounds dissolved in the aqueous phase, then the following relationship can be obtained (Thijssen & Rulkens, 1968):

$$(W/W_0) = (C/C_0)^{\beta} \tag{1}$$

where W and C are the amount of water and volatile compounds left in the matrix after drying and W_0 and C_0 are the amount of water and volatile compounds initially in the matrix. β is the relative volatility of volatile compound to water, and can be represented as follows (Loncin & Merson, 1979):

$$\beta = (P_v/X_v)/(P_{H_2O}/X_{H_2O}) \tag{2}$$

or

$$\beta = (P^{\circ}_v/P^{\circ}_{H_2O})\,(\gamma_v/\gamma_{H_2O}) \tag{3}$$

where P_v and P_{H_2O} are the vapour pressure of volatile compound and water, respectively and X_v and X_{H_2O} are, respectively, the molar fraction of volatile compound and water in a mixture. P°_v and $P^{\circ}_{H_2O}$ are the vapour pressure of volatile compound and water in the pure state, respectively, while γ_v and γ_{H_2O} are the activity coefficients for volatile compound and water, respectively. The idea of using relative volatility is widely accepted to predict the equilibrium state in a system with liquids or with a liquid and gas, although they can also be used with systems containing a solid phase (Null, 1965). Use of this simple volatility ratio avoids the complexity of evaluating true activity coefficients experimentally in a multicomponent system, as presented by the generalized Gibbs-Duhem equation (van Ness, 1964). The Gibbs-Duhem relationship shows that:

$$\Sigma\, X_i d_{\gamma_i} = (\Delta V/\mathrm{RT})\,\mathrm{d}P - (\Delta H/\mathrm{RT}^2)\,\mathrm{dT} \tag{4}$$

where ΔV and ΔH are, respectively, the changes of volume and enthalpy due to mixing of different substances present. Generally ΔV and ΔH are not known in complex systems.

However, converting equation (2) by applying the definition of molar fraction, equation (5) can be obtained:

$$\beta = (P_v/P_{H_2O}) \times (1/C_v) \times (\mathrm{MW}_v/\mathrm{MW}_{H_2O}) \tag{5}$$

where C_v is the volatile concentration in the aqueous phase (g volatile/g H_2O) and MW_v and MW_{H_2O} are the molecular weight of volatile and water, respectively. Combining equation (1) and (5), equation (6) is obtained:

$$\ln(W/W_0) = \ln(m/m_0) = (P_v/P_{H_2O})(1/C_v)(\mathrm{MW}_v/\mathrm{MW}_{H_2O})\ln(C'/C'_0) \tag{6}$$

where m and m_0 are the final and initial moisture content, respectively and C' and C'_0 are the final and initial volatile concentration (g volatile/g solid), respectively.

Table 6. Comparison between the predicted volatile retention using Henry's law and measured retention and volatilization

System no.	Extrusion conditions	Volatile compound	Initial concentration (nmol/g solids)	Predicted volatile retention (nmol/g solids)		Measured volatile conc. in matrix (nmol/g solids)	Predicted retention based on (%)	
				CM*	EX†		CM	EX
1	15% H_2O	n-Butanol	793	332	692	510	42	85
	140°C	1-Octane	514	392	471	51	76	92
	140 rpm	Benzaldehyde	554	451	431	378	81	78
		Limonene	431	260	309	225	60	72
2	15% H_2O	n-Butanol	793	178	648	470	22	82
	140°C	1-Octane	514	369	463	56	72	90
	140 rpm	Benzaldehyde	554	417	394	316	75	71
		Limonene	431	209	276	430	48	64
3	20% H_2O	n-Butanol	843	250	690	512	30	82
	140°C	1-Octane	547	457	468	68	84	86
	140 rpm	Benzaldehyde	588	452	453	343	77	77
		Limonene	458	268	413	226	59	90
4	20% H_2O	n-Butanol	843	53	652	509	6	77
	150°C	1-Octane	547	444	463	48	81	85
	140 rpm	Benzaldehyde	588	405	414	315	69	70
		Limonene	458	213	401	347	47	88

System no.	Volatile	Actual retention (%)	Predicted volatile conc. in head space (nmol/g solids)		Collected by chamber (nmol/g solids)	Predicted volatile volatilized (%)		Volatiles volatilized (%)
			CM*	EX†		CM	EX	
2	n-Butanol	59	615	145	457	78	18	58
	1-Octane	11	145	51	41	28	10	8
	Benzaldehyde	57	137	160	197	25	29	36
	Limonene	99	222	155	149	52	36	35
3	n-Butanol	61	593	153	425	70	18	53
	1-Octane	13	90	79	109	16	14	21
	Benzaldehyde	58	136	135	111	23	23	20
	Limonene	49	190	45	118	41	10	27

*CM = Cornmeal unextruded.

†EX = Cornmeal extruded.

Table 7. Fraction of moisture retention as a function of extrusion processing conditions

Initial moisture content (g H_2O/100 g sample)	Shear rate (rpm)	Moisture retention (%) 140°C	150°C	160°C
	140	53.65	51.27	38.44
15	155	54.56	50.20	38.32
	170	56.12	48.17	40.15
	140	52.80	51.40	43.59
20	155	54.76	52.30	44.25
	170	53.48	53.25	45.16
	140	67.75	49.23	46.79
25	155	61.70	48.45	46.66
	170	67.81	50.71	47.69

Using equation (6), the final volatile concentration can be predicted if the loss fraction of water (m/m_0) and all the other parameters are known under a specific extrusion condition, especially at the metering zone of the extruder, where a near equilibrium between the volatile concentration in the extruded product and its respective vapour pressure P_v is assumed.

As stated previously, the Clausius–Clapeyron relation allowed us to preduct the vapour pressure of volatile compound at extrusion temperatures based on volatility data.

The vapour pressure of water (P_{H_2O}) at the extrusion temperature can be obtained from the sorption isotherms. As was shown, the excess heat of sorption of water decreased rapidly with increasing moisture content, approaching zero above 14% wet basis moisture, so the vapour pressure was assumed to be that of saturated steam. Table 7 shows the fraction of moisture retained as a function of extrusion processing conditions. These data indicate that loss of moisture at each feed moisture content is not affected by the shear rate used. Therefore the physical state change of the corn matrix caused by shear force during extrusion does not significantly affect the moisture loss.

Taking the amount of volatile compound or water which is volatilized into the head space at each temperature into account, the amount of volatile or water left in the matrix can be evaluated as long as the initial amount of volatile or water is known. This enables one to calculate the volatile concentration C_v (g volatile/g water) as presented in equation (6).

From the moisture retention data and the other parameters, the fraction of volatile retention for each incorporated volatile compound was calculated from equation (6). The comparison is shown in Table 8 using the volatile measurement in the extruded product. Some fraction of volatile is bound to the matrix network formed due to denaturation and gelatinization (Clark & Remson, 1978) which would decrease the head space concentration. Bangs & Reineccius (1980) proposed an enzyme digestion to evaluate these bound volatiles but this method showed nothing bound in our system when tested. However, at the temperature necessary for this test (20°C) the slow reaction rate of the enzyme may be limiting. Maier (1975) found a value of 0.5–0.1 mol/kg for the amount of volatiles bound to the protein zein at 23°C. Since corn flour at 12% water content contains 6% protein, we calculated that there would be 0.8–1.6% of volatiles bound at most. Thus we used a value of 1% bound in our calculations. As for

Table 8. Comparison between the predicted percent volatile retention using(C'/C'_0) using the engineering model and the experimental data

System no.	Moisture content (%)	Extrusion temperature	Volatile compound	Relative volatility		Moisture retention (%) m/m_0	Predicted C'/C'_0 based on model		Actual C'/C'_0 based on retention
				CM	EX		CM*	EX†	
1	15	140	n-Butanol	1.91	3.21	55	73	83	64
			Octane	1.41	0.41	55	65	23	10
			Benzaldehyde	1.02	1.30	55	56	63	68
			Limonene	2.93	1.78	55	82	71	52
2	15	150	n-Butanol	1.89	4.02	51	70	85	59
			Octane	1.31	0.36	51	60	15	11
			Benzaldehyde	1.10	1.37	51	54	61	57
			Limonene	3.49	1.87	51	82	70	99
3	20	140	n-Butanol	13.93	1.42	53	96	64	61
			Octane	1.25	0.09	53	60	0	13
			Benzaldehyde	1.92	1.91	53	72	72	58
			Limonene	4.43	0.70	53	87	40	49
4	20	150	n-Butanol	55.35	1.41	52	99	63	60
			Octane	1.11	0.07	52	55	0	9
			Benzaldehyde	2.17	2.02	52	74	72	54
			Limonene	5.36	0.68	52	89	38	88

*CM: = Cornmeal unextruded.
†EX: = Cornmeal extruded.

the fat in the corn flour, there is no report regarding its physical state, either free in lipid pools or bound to the matrix. In general, about 1–2% of fat is present in corn flour, which comes from the whole caryopsis during the milling process and it should be thoroughly distributed (Angold, 1979). Therefore, the effectiveness of this small amount of residual fat as a solvent to interact with volatile compounds at extrusion temperatures is probably small. Although partition coefficients between water and fat for each volatile may be available, they would not help since the state of the fat is unknown. Overall, the bound fraction of volatile compound in the corn matrix, probably is not so significant that it would affect the comparison between predicted values and actual retention and the value of 1% bound is probably adequate.

As expected, the comparison for octane is poor when based on the recovery from the gas phase, but not bad when based on retention in the extruded matrix. The results for limonene show retentions which are both larger and lower than predicted. Since the engineering model is based on polar compounds and little solubility in the lipid phase, the higher retention is to be expected. However, the data are variable. Given mass balance errors of about 20%, this would seem to be a very good method for predicting loss of polar volatile flavour compounds, better than the thermodynamic approach. Since both methods rely on the volatility data, it can be concluded that despite analytical errors, an engineering model has potential for predicting the extent of volatile loss during extrusion processing of cereals.

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Nomenclature

P' = final volatile concentration in matrix after extrusion (nmol/g solids),
C'_0 = initial volatile concentration in matrix before extrusion (nmol/g solids),
C_v = overall concentration in water (g/g matrix),
H = Henry's law coefficient (mmHg),
m = final moisture content of matrix after extrusion (g H_2O/g solid),
m_0 = initial moisture content of matrix before extrusion (g H_2/g solid),
MW_{H_2O} = molecular weight of water (g/mol) = 18,
MW_v = molecular weight of volatile compound (g/mol),
P_v = vapour pressure of volatile (atm),
P_{H_2O} = water vapour pressure (atm),
P°_v = vapour pressure of volatile at pure state (atm),
$P^\circ_{H_2O}$ = water vapour pressure at pure state,
W = amount of water left in matrix after extrusion (g),
W_0 = amount of water initially in matrix (g),
X_v = molar fraction of volatile in matrix,
β = relative volatility,
γ_v = activity coefficient of volatile compound,
γ_{H_2O} = activity coefficient of water.

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Comparison between the measured and predicted sterilization performance of a laboratory-scale, direct heated UHT plant

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Summary

The sterilizing efficiency of a small scale direct heating UHT sterilizer has been measured using *Bacillus stearothermophilus* spores suspended in water and milk. A special technique was used for accurate measurement of the correct concentration of heat resistant spores in the fluid before passage through the sterilizer. The results were compared with the values calculated from the thermal death kinetics of the same spore batch measured in the laboratory, together with the residence time distribution of the liquid in the sterilizer. The two sets of data were found to compare well for a wide range of operating temperatures, confirming that the performance of a continuous sterilizer in practice could be predicted, given accurate information for the spores and the equipment.

Introduction

The design of a good aseptic process relies on attaining an acceptably low spore concentration in the product while minimizing damage to its organoleptic and nutritional values by overprocessing. The spore concentration after heat treatment is a function of both the concentration of thermally resistant spores in the raw material and the sterilization efficiency of the Ultra High Temperature (UHT) sterilizer for those spores.

The sterilization efficiency of the sterilizer can be obtained in two ways. Firstly, a direct method by processing product which has been inoculated with a large number of heat resistant spores, then measuring the number that survive the treatment. This approach is impractical for commercial scale plants, however, owing to the high number of spores required and the subsequent contamination of the equipment. The second method is to calculate the sterilization efficiency using the thermal death kinetic data for the appropriate spores together with time-temperature data for the particular UHT plant.

Several attempts have been made to compare the performance of small UHT sterilizers using these two methods (Burton *et al.*, 1958, 1959, 1977; Cerf & Hermier, 1973). The two sets of data have rarely agreed and discrepancies between them have been attributed to a variety of different causes. One of the predominant difficulties lies in obtaining accurate thermal death kinetic data for spores at the temperatures used in UHT processing. Direct measurement is difficult owing to the methods which must be

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used, and extrapolation from thermal death data measured at lower temperatures into the UHT range gives different values depending on the basis used, i.e., constant activation energy or constant temperature coefficient (Q_{10} or z value). Another difficulty lies in the accurate measurement of the time-temperature profile for different fluids in the sterilizer.

Previous work at this Institute has measured the thermal death kinetic data for *Bacillus stearothermophilus* spores suspended in milk and water in the UHT temperature range using two different methods:

(a) in the laboratory, using a capillary tube method with computer correction for heating up time. This method gave data with a higher degree of accuracy than previously (Davies *et al.*, 1977; Perkin *et al.*, 1977).

(b) By measuring the sterilizing efficiencies of two experimental UHT sterilizers and their time-temperature profiles, then calculating the thermal death kinetics of the spores using this data (Burton *et al.*, 1977).

Discrepancies were observed between the data obtained using the two methods. At the lower temperatures, the decimal reduction times derived from the experimental data were greater than those measured using the capillary tube method, in all cases. At the upper end of the temperature range, however, the converse applied.

The purpose of this work was to investigate the possible reasons for these discrepancies. The factors which may be involved are associated either with the measurement of the sterilizing efficiency of the sterilizers or with the calculation of the thermal death kinetics from these data. In this paper, the factors affecting the accuracy of measurement of the sterilizing performance are discussed first, and a technique is described to give accurate values of the proportion of surviving spores for an experimental sterilizer over a range of operating temperatures. The inaccuracies involved in calculating the thermal death kinetic data for these spores from these data are then considered and comparisons made between data obtained using this method and the data measured using capillary tubes.

Theory

Measurement of the sterilization efficiency of a sterilizer

The evaluation of the proportion of surviving spores for a particular sterilizing process requires accurate measurement of both the initial and final concentration of heat resistant spores. Accurate measurement of the spore concentration by colony count requires the simultaneous germination and outgrowth of all viable spores when they are plated in a suitable medium. A proportion of a spore population is often reluctant to germinate under ideal conditions, even though they are viable. These spores, termed dormant spores, require activation before germination can occur. Many methods are available to achieve this. Exposure of the spores to sub-lethal temperatures, first reported by Curran & Evans (1944), can induce activation as can exposure to low pH, oxidizing reagents or a variety of chemicals (Gould & Hurst, 1969), and particularly calcium dipicolinate (Riemann & Ordal, 1961). The heat treatment received in a UHT sterilizer is much greater than that required for activation and hence all surviving spores are fully activated.

The conditions which give the maximum activation of spores have been shown to vary for different strains of spores and for different batches of the same strain, and have also been shown to alter as the spores age. Different optimum heat activation conditions have been reported for *B. stearothermophilus* spores usually in the temperature

range of 100 to 115°C (Gould & Hurst, 1969). Exposure to lower temperatures in the range 80–100°C has been shown to increase dormancy in these spores, which could then be reactivated by further exposure to higher temperatures (Finlay & Fields, 1962).

Prediction of sterilization performance

(a) Measurement of thermal death kinetic data. Equation 1 describes the death of microorganisms at a constant lethal temperature:

$$\text{Proportion of surviving spores} = \frac{N_t}{N_0} = 10^{-t/D} \quad (1)$$

Where N_t is the number of spores at time t, N_0 is the initial number of spores, t is the holding time at the lethal temperature, D is the decimal reduction time and is defined as the time taken for the number of organisms to fall to one tenth of the current value. It is constant at constant temperature. The conventional method for measuring the Decimal Reduction Time for a spore population at a constant temperature (Bigelow & Esty, 1920) involves immersing spore suspensions sealed in capillary tubes into a bath of heated fluid for a range of times and measuring the proportion of surviving spores for each sample. A graph of the log of proportion of surviving spores *versus* time of immersion is drawn and the slope of the graph gives the decimal reduction time.

Accurate thermal death data are not easy to obtain using this method at the temperatures required for UHT processing, where the D values are small. Decimal reduction times for *B. stearothermophilus* spores in water and milk were measured by Davies *et al.* (1977) using this method, for temperatures between 120 and 160°C. The graph of the log proportion of surviving spores *versus* time was found to be non-linear at the higher temperatures, with a pronounced 'shoulder' at the low immersion time end of the curve. Perkin *et al.* (1977) showed that the finite heating up time for the spore suspension in the capillary tube was responsible for this shoulder. Measurement of the rate of temperature rise at different radii in the capillary showed that the heating time was a large proportion of the immersion time for temperatures above about 135°C and, above 150°C the centre of the capillary might not even reach the bath temperature during immersion. The immersion times used at these higher temperatures had to be short in order to obtain a measurable number of surviving spores. The thermal death data from Davies *et al.* (1977) was then corrected to allow for the heating time curve at different radii in the suspension. This correction gave thermal death data for the spores that obeyed Arrhenius kinetics up to 147.5°C in both water and milk.

(b) Residence time distribution. The prediction of the sterilization performance of a UHT plant is greatly simplified if the plant is of the direct heating type and therefore has an insignificant heating and cooling time. The flow of liquid through the plant, however, produces a distribution of residence times within the high temperature zone, which has an effect on the sterilization performance.

The residence time distribution (RTD) can be measured by injecting a small plug of tracer into the liquid stream at the entrance of the zone under study. The variation of tracer concentration with time is then measured at the outlet and the area under the curve is normalized by dividing the tracer concentration values by the total area under the curve. These new values are defined as $E(t)$ values (Danckwerts, 1953). Mathematically,

$$\int_0^\infty E(t).\mathrm{d}t = 1. \quad (2)$$

The sterilization performance is a function only of the residence time distribution within the high temperature zone and the decimal reduction time for the spores at the operating temperature. The proportion of surviving spores is equal to the area under the spore survivor curve:

$$\text{Proportion of surviving spores} = \int E(t) \cdot 10^{-t/D}\, dt. \quad (3)$$

If the RTD of a sterilizer and the D value for the spores at the required temperature are known, the proportion of surviving spores can be predicted.

(c) Calculation of decimal reduction times from sterilizer data. The D value for the spores at the sterilizer operating temperature can be calculated if the proportion of surviving spores and the residence time distribution have been determined experimentally. This is achieved most accurately by an iterative method using equation 3, where the D value is estimated and repeatedly modified until the proportion of surviving spores calculated is the same as the experimental value.

Burton *et al.* (1977) calculated the D value by another method. For any sterilization process at a single temperature, a bacteriologically effective holding time ($\bar{t}_m$) for that process can be defined. By rearranging equation 1:

$$SE = \frac{-\bar{t}_m}{D} \quad (4)$$

where the sterilization efficiency of the plant (SE) $= \log_{10}(N_t/N_0)$.

The value used for the bacteriologically effective mean holding time was the mean residence time of the spore survivor curve. This was calculated from a graph of $(E(t) \cdot 10^{-t/D})$ versus time for each sterilizer operating temperature, using the appropriate D value derived from the capillary tube data. Using these values and the corresponding experimental sterilization efficiencies, the D values for the spores were calculated as a function of temperature.

Experimental

The spore suspension used was *Bacillus stearothermophilus* TH24 (NCDO 1096). The spore batch was the same as that used by Davies *et al.* (1977) and Burton *et al.* (1977), which had been prepared in 1976 and subsequently stored at 4°C in distilled water. The spores were enumerated after heat treatment by plating suitable decimal dilutions in BSA medium, drying the plates for 30 min at 37°C then incubating at 55°C for 48 hr before counting the colonies. The heat resistance of the spores was checked at 135°C and 142.5°C using the capillary tube method.

The initial spore concentration was evaluated in the laboratory in several different ways:

(a) by counting a suitable decimal dilution in a 0.02 mm deep spore counting chamber (Scientific Supplies Ltd, London) under a microscope. The count was reduced to allow for the proportion of phase dark spores, which was approximately 10%.

(b) by incorporating calcium dipicolinate in the BSA medium to activate the spores. Sodium dipicolinate was prepared by neutralizing 2, 6 pyridinedicarboxilic acid (Aldrich Chemical Co., Gillingham, Dorset) with sodium hydroxide. The resulting solution was filtered, evaporated and dried. The spores were activated by adding sterile solutions of first calcium chloride and then sodium dipicolinate to the molten BSA medium. The spores were then plated out immediately in the usual way using this

medium. The method was optimized by testing final levels of 50, 75 and 100 mmol calcium chloride and 40 and 50 mmol sodium dipicolinate in the medium.

(c) by heat activation. The method used by Davies *et al.* (1977) was to heat the spore suspension at 100°C for 30 min before plating out in BSA medium as usual. These heat-activated spores were also plated out in the BSA-calcium dipicolinate medium described above. Other time-temperature combinations were evaluated by immersing about 1 cm^3 of spore suspension sealed in an ampoule into a bath of heated polyalkylene glycol. Temperatures of 80°C, 100°C, 105°C and 110°C for times of up to 60 min were used. All heat activation was carried out in 1/4 strength Ringers solution.

(d) The initial spore count was also evaluated by repeatedly passing the suspension through the UHT sterilizer at a temperature between 139°C and 141°C. A sample was taken after every successive pass and a plate count performed. The effective initial count was determined from the intercept on a graph of plate count *versus* number of passes. The sterilizer was operated with distilled water after each pass to prevent contamination between successive samples. The operating temperatures of each pass were within 0.1°C.

A fresh batch of *Bacillus stearothermophilus* spores was prepared in 1982 from the original 1976 batch using the method described by Davies *et al.* (1977). Its initial count was determined using methods (a), (b) and (d) above, as well as after heat activation at 100°C for 30 min only.

Experimental sterilizer

A laboratory-scale UHT sterilizer of the infusion type was used. The sterilizer could be controlled to treat batches as small as 250 cm^3 in continuous flow. This enables a high spore concentration to be used in the inlet liquid, ensuring concentrations of surviving spores high enough to be measured by conventional colony counts. The equipment was one of the sterilizers used by Burton *et al.* (1977). A schematic diagram of the apparatus is given in Fig. 1.

The liquid is heated by forcing it from the feed reservoir using compressed air, through a spray nozzle into a chamber supplied with saturated steam at a pressure sufficient to give the required processing temperature. The liquid is heated very rapidly by condensing steam, falls to the bottom of the sterilizer and flows down the conical bottom into the sightglass section. After passing through the holding tube and orifice plate, the liquid is cooled rapidly by flash evaporation in a vacuum vessel. The sample can be collected by removing the vacuum and forcing the liquid, using compressed sterile air, through cooling coils into an aseptic collection apparatus. The equipment is described in greater detail by Perkin (1974).

Residence time studies

Although heating and cooling are virtually instantaneous, there is a wide distribution of residence times in the equipment. The RTDs for water and milk in this sterilizer over a range of operating temperatures have been measured by Heppell (1985). The curves obtained for the two liquids were significantly different, milk having a wider spread and shorter minimum residence time than water. Typical $E(t)$ curves are given in Fig. 2. A computer program was written to predict the proportion of surviving spores for the sterilizer at different operating temperatures using these $E(t)$ values and the appropriate D value for the spores to evaluate the function in equation 3. The

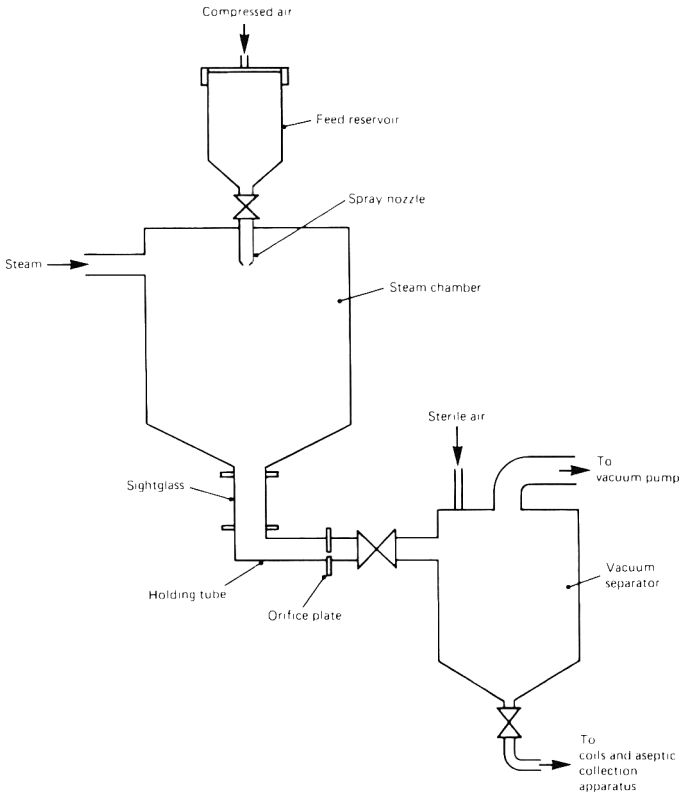

Figure 1. Diagram of laboratory-scale UHT sterilizer.

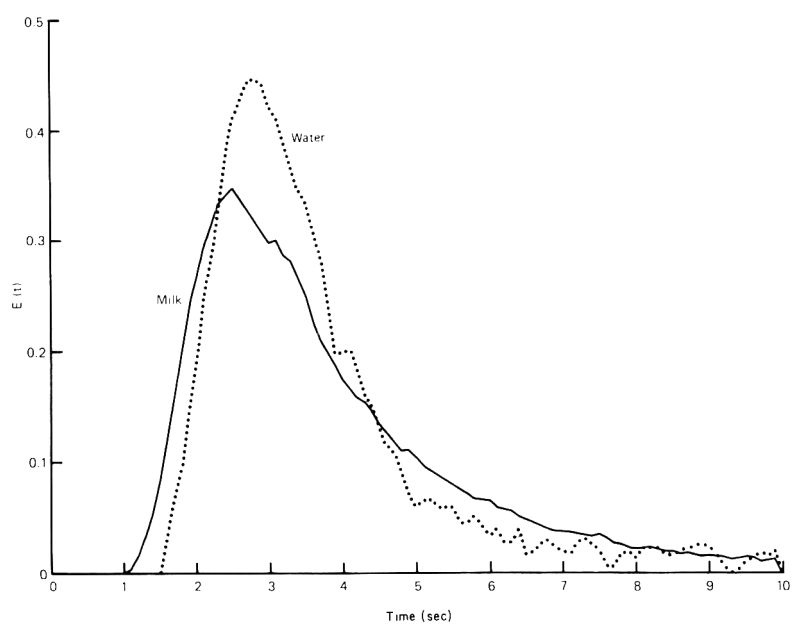

Figure 2. Typical residence time distribution curves as *E(t)* values *versus* time for milk and water in the experimental UHT sterilizer.

integration was performed using Simpson's Rule. The program was also used to calculate the mean of the spore survivor curve and to produce a graph of the predicted proportion of surviving spores against D value for the milk and water RTD data.

Experimental programme

The proportion of surviving spores was measured at temperatures between 138.7°C and 145.8°C for spores in water, and between 140.2°C and 147.9°C for spores suspended in milk, using the method described in (d) above to determine the initial concentration of heat resistant spores.

The D value of the spores for each temperature was calculated from the proportion of surviving spores by modifying the computer program above to take D values in an iterative process and calculate the corresponding proportion of surviving spores until the value obtained matched the experimental value.

Results and discussion

Determination of initial spore concentration

Any method which is used to determine the concentration of spores in a liquid must enumerate all those spores which are capable of germination and growth. The accuracy of the colony count method depends not only on providing ideal conditions for germination and growth of the spores, but also in ensuring that all dormant spores have been fully activated, allowing germination to occur. The different methods used in this work to determine the concentration of heat resistant spores in the feed liquid to the sterilizer all gave different values, as shown in Table 1. The values obtained are expressed as a

Table 1. Evaluation of initial spore concentration by plate counts: comparison of different treatments on the 1976 spore batch, expressed as a proportion of the microscopic count

Activation method	Original batch
Microscopic count	1.0
Calcium dipicolinate	0.80
No treatment	0.50
100°C for 30 min	0.17
Repeated pass	0.11

proportion of the microscopic count, which represents the maximum value that could be obtained by colony count assuming that each spore germinated and grew. The colony counts obtained without treatment of the spores showed that only 50% of them had germinated in the medium. Activation of the spores with calcium dipicolinate gave an increase in germination to 80%, but still left a discrepancy between the plate and microscopic counts. Heat activation of the spores at 100°C for 30 min, however, gave an unexpected decrease in the plate count compared to the untreated count. Further investigation of sub-lethal heat activation treatments, shown in Fig. 3, did not show any evidence of heat activation occurring but rather showed a decrease in plate count with increasing heating time, down to a value approximately 15% of the unactivated count.

The rate of decrease increased with increasing temperature. The use of calcium dipicolinate on spores heat treated at 100°C for 30 min did not increase the plate count.

The results of the sub-lethal heat treatment may be explained in two ways.

(a) The heat treatments had induced heat dormancy in the spores which resulted in a lower germination rate and thus lower plate count. In this case, activation by chemicals or by further heat treatment on these more dormant spores should have increased the count again. This did not occur, either by the use of calcium dipicolinate or by higher temperatures and longer holding times on the heat treated spores.

(b) The spore population had a mixed heat resistance where the minority, about 10%, had a high heat resistance but the resistance of the majority was low and thermal death occurred at temperaures just above 100°C. The increase in the rate of fall of plate count with increasing temperature, shown in Fig. 3, and the inability of further heat or chemical activation to increase the plate count of the heat treated spores, suggests that thermal death had taken place.

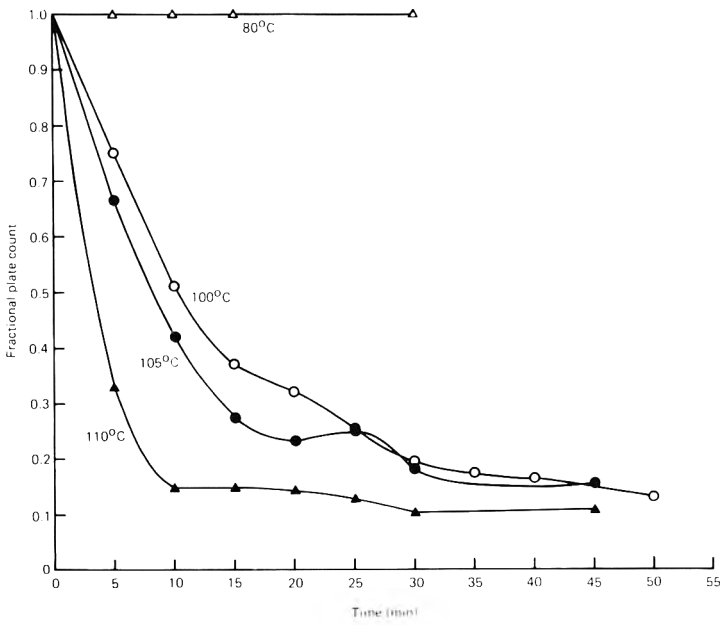

Figure 3. Effect of immersion time on plate count at different temperatures for untreated spore population.

Measurement of *D* values using the capillary tube method does not require an accurate determination of the initial spore concentration. The value is calculated from the slope of the line relating log (proportion of surviving spores) to time of immersion at a fixed temperature. An inaccuracy in the initial count will affect all the values of proportion of surviving spores to the same extent and therefore will not affect the slope of the line, assuming the inaccuracy is constant. The sterilizing heat treatments involved are more severe than would normally be required for activation and any surviving spores can be considered fully activated. In addition, spores with a low resistance will have been killed, and hence the plate count will represent the true concentration of surviving heat resistant spores. The *D* value is therefore only a function of the change

in plate count with time of immersion and is independent of activation requirements and he presence of relatively heat labile spores.

A method based on a similar principle that could be used for the experimental sterilizer would be of value in eliminating these initial count inaccuracies. This was achieved by repeatedly passing a spore suspension through the sterilizer under constant conditions and taking a plate count after each pass. From a graph of plate count *versus* number of passes through the plant, the initial concentration of heat resistant spores can be determined from the intercept and the proportion of surviving spores at that temperature from the slope of the line. An example is given in Fig. 4. This is impractical at operating temperatures greater than about 142°C as there must be a measurable number of surviving spores after at least three successive passes in order to get an accurate straight line. It must also be ensured that contamination of one pass with spores of the previous pass does not occur. The value for initial resistant spore concentration determined by this method was 11% of the microscopic count. This value compares with the results from the sub-lethal heat treatment experiments, where the plate count at the long immersion times approached a value about 15% of the unactivated count, which itself was 50% of the microscopic count.

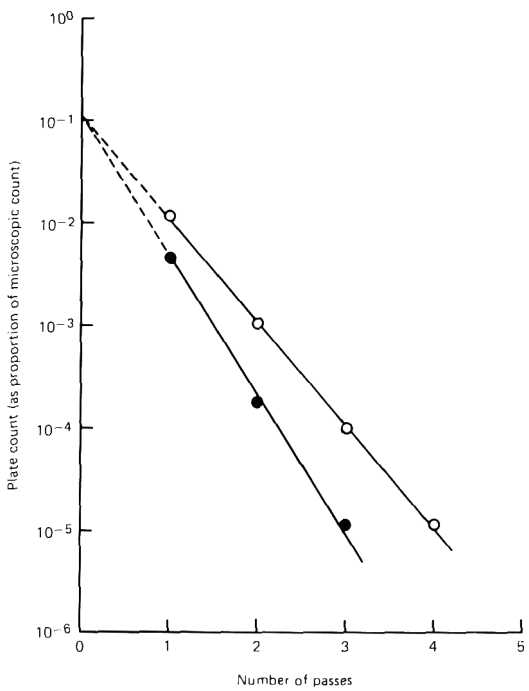

Figure 4. Evaluation of initial spore concentration from successive passes of a sample through the sterilizer at constant temperature.

For the remainder of this work, it was assumed that the concentration of the heat resistant spores in the feed liquid to the sterilizer was measured most accurately by this method, and was determined separately for each batch of inoculated liquid. The proportion of surviving spores was then measured in both milk and water for the sterilizer over a range of operating temperatures and the results are given in Figs 5 and

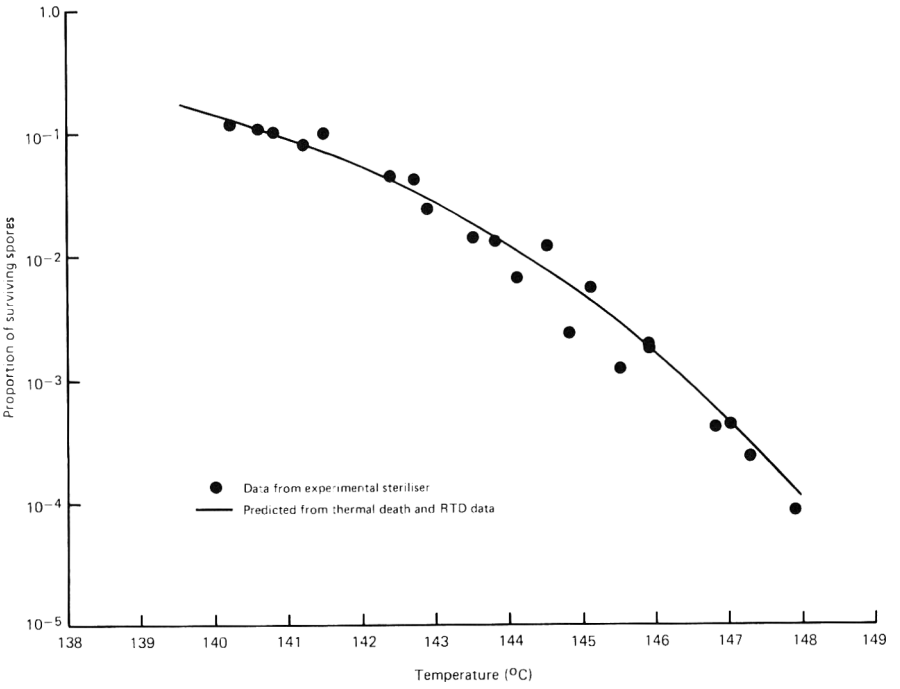

Figure 5. Comparison of the predicted and measured values for the proportion of surviving spores as a function of sterilizer operating temperature for spores suspended in milk.

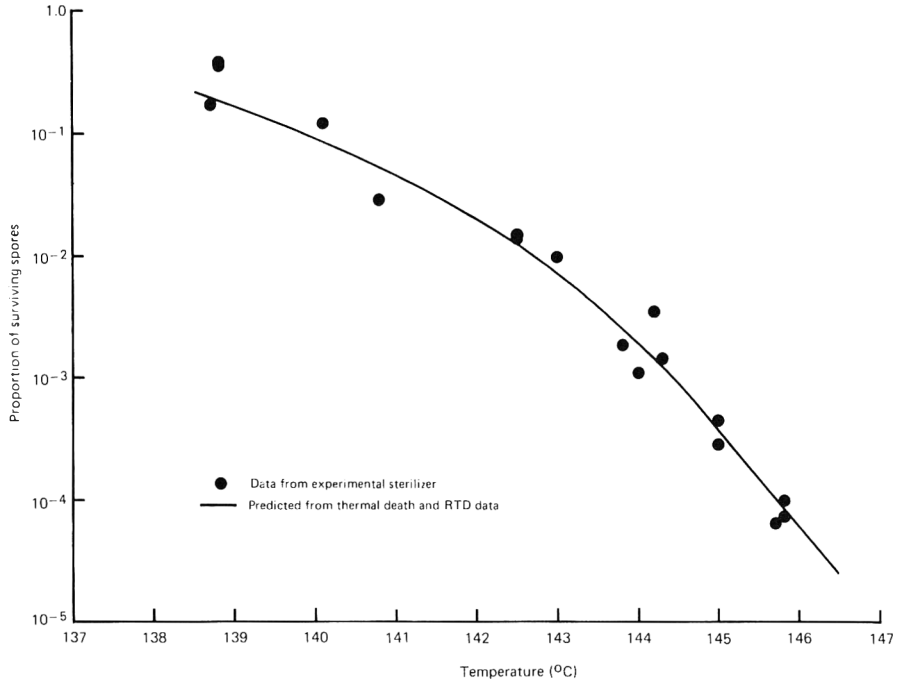

Figure 6. Comparison of the predicted and measured values for the proportion of surviving spores as a function of sterilizer operating temperature for spores suspended in water.

6, respectively. These diagrams also give the values predicted for the sterilizer using the thermal death kinetic data from Perkin *et al.* (1977) and the residence time distributions from Heppell (1985) The two sets of data can be seen to be a good agreement for both liquids.

Calculation of spore thermal death data

The effective decimal reduction time was calculated for each value of the proportion of surviving spores measured. This was achieved using the iterative computer method described above, using the appropriate residence time distribution given in Heppell (1985). The results are given as an Arrhenius plot in Figs 7 and 8 for spores suspended in milk and water, respectively. The best fit straight line for the capillary method from Perkin *et al.* (1977) is also given. The two sets of data can be seen to be a good agreement for both liquids.

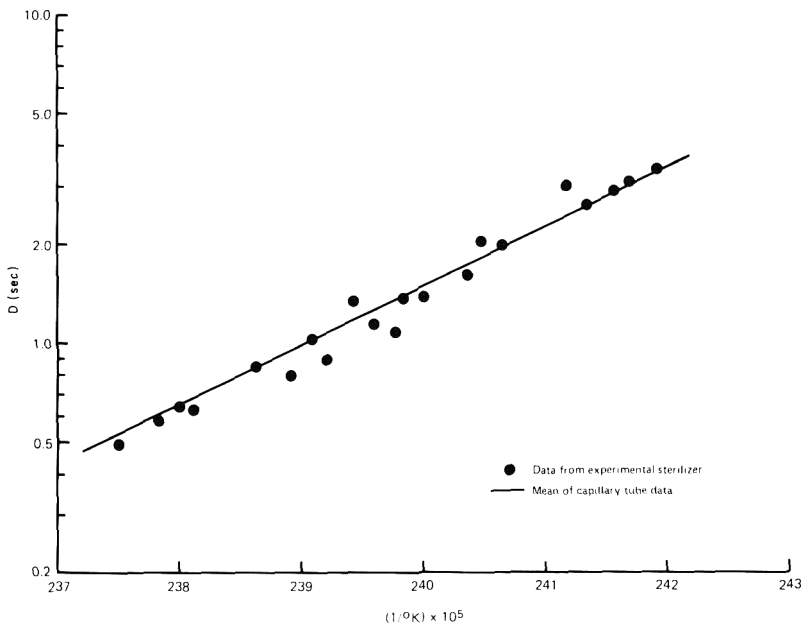

Figure 7. Comparison between decimal reduction times (D) from experimental sterilizer and capillary tube experiments for spores suspended in milk at different temperatures ($\frac{1}{T}$).

Application of these results to earlier work

The accuracy of the D value obtained using a small scale sterilizer obviously depends on the accuracy of the value used for the proportion of surviving spores. This itself is especially dependent on the value used for the initial spore concentration, as it is complicated by activation requirements. The use of non-optimum heat activation conditions will cause a constant error in the experimental value for the proportion of surviving spores. At the lower temperatures this error will become a large proportion of the predicted value and will cause a very large error in the D value calculated from it. Using the computer program described above, it can be shown that an error in the value for the proportion of surviving spores of a factor of two will give an error of about 10%

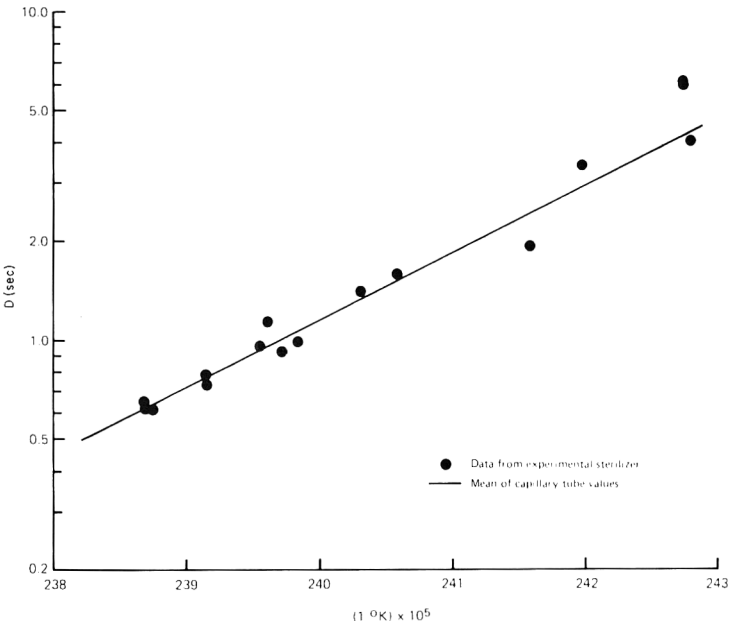

Figure 8. Comparison between decimal reduction times (D) from experimental sterilizer and capillary tube experiments for spores suspended in water at different temperatures ($\frac{1}{T}$).

in the D value at temperatures around 147°C but an error of 300% at 137°C. This error, however, will not affect the value from the capillary tube experiments.

The discrepancies found by Burton *et al.* (1977) between the D values determined by the two methods could largely be explained if the actual initial spore concentration used was a factor of two to three higher than that measured. The results given in Table 1, however, show that the standard heat activation conditions used overestimated the true initial count by about 55%. Activation conditions are known to alter as spores age, therefore a fresh batch of spores was prepared using this batch as inoculum. The effects of the different activation treatments on the plate count were studied. The results, given in Table 2, show that the microscopic count and the counts obtained by chemical

Table 2. Evaluation of initial spore concentration by plate count: comparison of different treatments on the subcultured spore batch, expressed as a proportion of the microscopic count

Activation method	Subcultured batch
Microscopic count	1.0
Calcium dipicolinate	0.97
No treatment	0.39
100°C for 30 min	0.34
Repeated pass	0.92

activation and repeated passes through the sterilizer all agree within experimental error. The count for heat activation at 100°C for 30 min, however, was only 35% of these. Although it is difficult to predict activation behaviour of a spore batch 8 years

previously, it seems likely that the earlier work underestimated the initial count, leading to the discrepancies found. During storage, a large proportion of the spores must have lost their heat resistance though the remaining spores had retained it, since the D values obtained using the capillary tube method in 1983 were the same as those measured by Davies *et al.* in 1977.

Although this factor would account mainly for the discrepancies found at the lower temperatures, there is a small but consistent error at the higher temperatures. This can be shown to be due to two other factors, the method used to calculate the D values from the sterilizer data, and the assumption of identical residence time distributions for water and milk in the experimental batch sterilizer.

The method for calculating the D value from the sterilizer data using equation 4 is accurate only if the value for the bacteriologically effective mean holding time (bemht) is accurate. This value usually lies between the mean residence times of the fluid in the sterilizer and of the spore survivor curve. Its position between these two values depends on the spread of the residence time distribution and particularly the minimum residence time. At low sterilizing temperatures, with a high proportion of surviving spores, the difference between these three values is small, but as the temperature rises, the difference between the values increases. The D value calculated using the mean of the spore survivor curve is then smaller than it should be. Using the computer program described above, the mean residence times of the fluid and spore survivors were calculated and compared with the b.e.m.h.t. calculated from the proportion of surviving spores and D value for the spores. These values are given in Table 3. It can be seen that using the mean of the spore survivor curve as the b.e.m.h.t. would cause the D value to be underestimated by 24% and 15.6% at 147°C, and 17.7% and 12.7% at 138°C for milk and water respectively.

Table 3. Comparison between the mean residence times of the fluid, the surviving spores and the bacteriologically effective mean holding time (bemht)

		Mean residence time (sec)		
Fluid	Temperature (°C)	Fluid	Spore survivors	Calculated bemht
Milk	138	3.29	2.65	3.3
	147	3.29	1.64	2.16
Water	138	3.27	2.88	3.27
	147	3.27	2.05	2.43

The other factor affecting the results concerns the residence time distribution used to calculate the D value from the sterilizer data. Burton *et al.* (1977) measured the RTD only for water in both sterilizers, and assumed it to be the same for milk, as the method used was not suitable for this fluid. Heppell (1985), used a different technique and showed a difference in distribution between the two curves for the laboratory infuser sterilizer only. The difference between the two curves would give an error in the D value calculated from this sterilizer of about 10% at 147°C, but negligible at the lower temperatures.

Overall, there are three factors that may be responsible for the discrepancies found

in this earlier work. An inaccurate evaluation of the initial spore concentration, an inacuracy in the method used to calculate the thermal death data from the plant data, and the difference in RTD for the two fluids in one of the experimental sterilizers. The first can give the pattern of results found at the lower temperatures. This cannot be said to have occurred with any degree of certainty, however, and would be extremely difficult to quantify, but there is evidence for it in the behaviour of the subcultured spore population. The other two factors have been shown to give errors mainly at the higher temperatures, and are of the correct magnitude to account for these discrepancies.

Conclusion

The design of a good aseptic process requires a knowledge of the sterilization efficiency of a particular UHT sterilizer for the microorganisms of interest. The difficulties encountered in directly measuring the sterilizing efficiency of a commercial scale sterilizer under different operating conditions means that great reliance is placed on the calculated value. The accuracy of the calculated value is of great importance, especially for low acid foods where a specified reduction in the concentration of organisms of public health significance must be achieved.

This work has demonstrated that, for a direct heating sterilizer, the calculation can be performed with confidence and that no fundamental differences have been observed between the kinetics of spore destruction in sealed capillary tubes and in continuous flow.

The acquisition of accurate data for the calculation is difficult however. The measurement of thermal death kinetic data in the correct temperature range and in the appropriate foodstuff is not easy for spores with a high heat resistance, such as those used in this study. For organisms with a lower heat resistance, such as *Clostridium botulinum*, the low number of survivors or the very short immersion times necessary for the capillary tubes, make the data more difficult to obtain accurately. In addition, an accurate temperature profile and residence time distribution are necessary for the relevant liquid in each high temperature section of the particular UHT plant under all operating conditions. These data are difficult to measure, especially for indirect heating systems, but if correct, then the calculation of the sterilizing efficiency should be accurate for any foodstuff in any type of sterilizer.

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Technical note: loss of nitrate from carrots during blanching

P. VAROQUAUX, F. VAROQUAUX AND L. TICHIT

Summary

Loss of nitrate from carrots during blanching was studied using several parameters, such as temperature of water, thickness of carrot slices, and volume ratio of carrots and water. It was found that the kinetics of nitrate diffusion fitted well to the Fick's general diffusion equation. Surface mass transfer coefficient (K) was related to temperature by an Arrhenius type reaction. The values of activation energy (E_a) and the constant K_0 were calculated as 74 kJ/mole and 2769×10^{-3} kg/m^2/sec. As expected the initial rates of diffusion were proportional to carrot surface and the efficiency of nitrate removal decreased with increasing blancher load. The proposed model may be used to determine the optimum blanching conditions of carrots in the baby food industry.

Introduction

The nitrate content of carrots varies widely. Recent reports showed that some carrots contained very high concentrations of nitrate ranging from 2000 to 7000 ppm as $NaNO_3$ on a fresh weight basis (Lemieszek-Chodorowska, 1979). High nitrate containing carrots give problems with baby foods since the Health Administration in France, as in most European countries, has set a maximum level of 50 ppm of $NaNO_3$ on a fresh weight basis in processed baby foods.

Auffray & Paufique (1976) have studied the effect of nitrogen fertilizers on the nitrate content of carrots and reported that the average nitrate content in fresh carrots can be reduced to below 300 ppm by properly managed cultural practices. Astier-Dumas (1976) was able to remove 60% of the nitrate in carrots during 35 min blanching in boiling water. However, similar lossess occurred in water soluble vitamins, sugars, and other nutrients. The effect of blanching on total loss and vitamin retention in vegetables has been reviewed by Alzamora, Hough & Chirife (1985). However very few authors measured nitrate diffusion in carrots. This paper reports the diffusion of nitrate from slices of carrot during blanching in relation to the temperature of water, size of carrot slices, and the relative proportions of carrots and blanch water.

Materials and methods

Materials

Carrots (TOUCHON cultivar) were obtained from the INRA's experimental plots in Montfavet. Harvested carrots of commercial maturity for canning were sorted (diameter 25/30 mm), hand peeled and then washed with distilled water. Washed and peeled carrots were cut into slices of 6 mm thickness. To obtain homogeneous samples, the slices from each carrot were divided into four groups, from which test samples were constituted.

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Blanching

Blanching was carried out in a rotating 1 litre flask (60 rpm) kept at a given temperature (±0.05°C) and with a condenser attached at the rotating outlet to prevent loss of water. The flask, containing 250 ml of distilled water, was allowed to equilibrate to the bath temperature and then the carrot slices were quickly introduced along with a sampling tube. Each experiment was duplicated. In spite of the extra care taken to obtain random and homogeneous samples, the nitrate content of each sample varied over a wide range: mean nitrate concentration of whole carrot samples was 7.28±1.3 mM (618.8±111.3 ppm). Therefore, in order to compare each set of experiments, it was necessary to express the diffusion as the ratio of nitrate concentration of the liquid/maximum nitrate concentration. To obtain the maximum nitrate concentration after each experiment, the whole flask content was blended in a Vorwerk blender for 3 min at maximum speed and the nitrate content of the filtered slurry was measured.

Nitrate determination

The analytical procedure of Volff, Noyelle & Gautrat (1974) was adapted to a Technicon II flow analyser (Fig. 1). The manifold was similar to that of Adamowicz, *et al.* (1980).

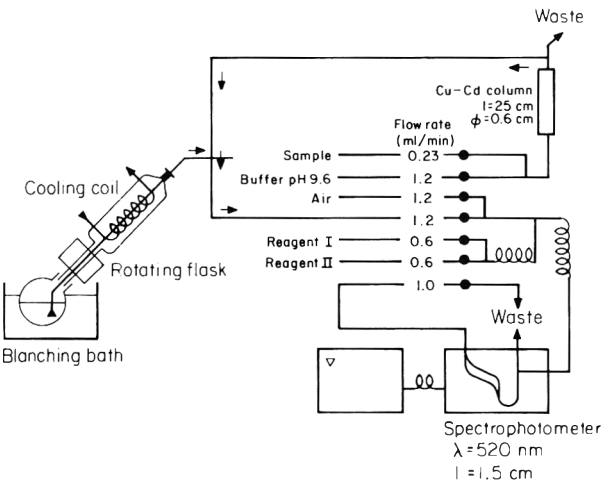

Figure 1. Flow diagram of nitrate analysis technique. (See Nitrate determination).

Results and discussion

Effect of temperature

The kinetics of nitrate diffusion at six temperatures are shown in Fig. 2. It was postulated that the apparent nitrate diffusion obeyed Fick's second law:

$$\frac{\partial N}{\partial t} = KA\,(C_i - C_e),$$

where: N = quantity of diffused nitrate (kg $NaNO_3$); K = surface mass transfer coefficient (kg/m²/sec); C_i = residual nitrate content in carrots (ppm $NaNO_3$), (volume of carrots V_i in 1; initial content C_0); C_e = nitrate concentration of the liquid phase (ppm

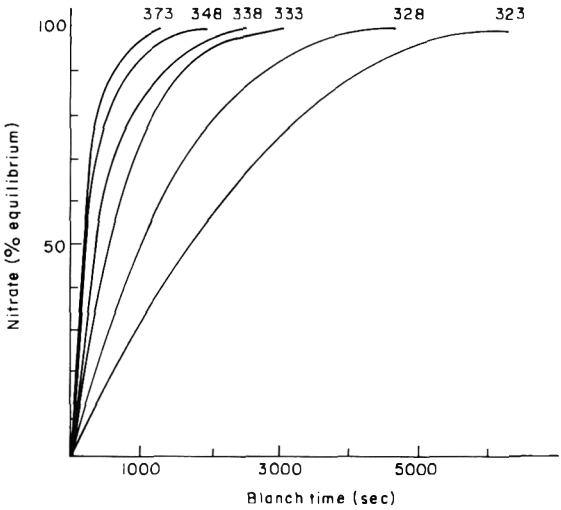

Figure 2. Influence of temperature on the kinetics of nitrate diffusion (percentage of the nitrate concentration in blanch water/nitrate concentration at equilibrium; TOUCHON cultivar; distilled water 0.25 l; 10 g of 6 mm thick pieces, 60 rpm; temperature in °K.

$NaNO_3$), (volume V_e in l); A = total surface area for mass transfer (m^2); t = blanch time in sec. In order to simplify, coefficient K was assumed to be constant throughout the process. The integration of this equation gives y %, the ratio of nitrate content in water/maximum nitrate content:

$$\text{equation 1 } y(\%) = 100\left(1-\exp\left[-\frac{V_e+V_i}{V_eV_i}KAt\right]\right)$$

and the initial rate of diffusion p (%/sec) is given by

$$\text{equation 2. } p(\%/\text{sec}) = 100\frac{V_e+V_i}{V_eV_i}KA.$$

All the parameters, except surface mass transfer coefficient K, are accurately measurable. This coefficient depends on specific diffusability of nitrate, and on the resistance of carrot tissues to nitrate diffusion. So K depends on the integrity of cell walls and tissue cohesion, and might be expected to increase during the blanching process. The influence of thermal destruction of carrot tissue on the rate of nitrate diffusion was not noticeable at high temperatures. Nevertheless kinetics at 323°K and 328°K are slightly sigmoid.

It was demonstrated in Fig. 3 that up to 353°K, K was related to temperature by an Arrhenius type equation $K = K_0 \exp(-E_a/RT)$ (logarithmic correlation coefficient $r = 0.9947$). The surface mass transfer coefficient could therefore be regarded as an apparent diffusion coefficient. The values of activation energy E_a and the constant K_0 were calculated from Fig. 3 as 74 kJ/mol and 2769×10^3 kg/m²/sec. The activation energy may be compared with the activation energies for other temperature dependent reactions occurring in carrots during heating. Selman, Rice & Abdul-Rezzak (1983) found a 28.2 kJ/mol E_a for solute content diffusion coefficient in carrots, and Paulus & Saguy (1980) reported an E_a of about 100 kJ/mol of texture change in carrots during cooking.

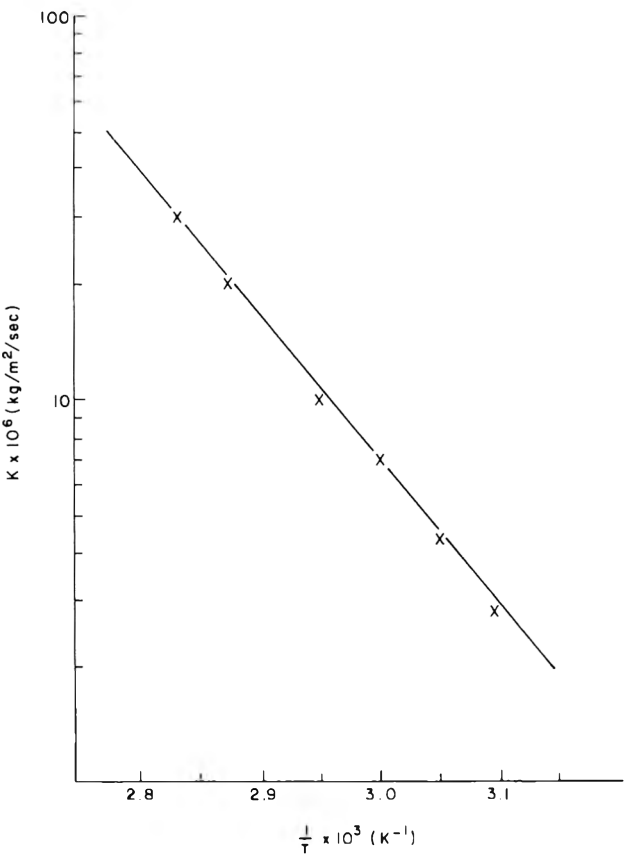

Figure 3. Surface mass transfer coefficient (K; kg m sec); *versus* the reciprocal of absolute temperature.

Effect of carrot size

In order to measure the effect of carrot size on the diffusion rate, carrots were cut into pieces of various length (from 0.0025 to 0.100 m) and blanching was performed at 328°K (Fig. 4) and at 373°K (Fig. 5). The initial apparent diffusion rates (at 328°K) are proportional to the carrot surface. This result is consistent with equation 2. The linear regression was:

p (%/sec) = 166.7×A−0.0197
with r = 0.9645.

The apparent diffusion coefficient D_a was estimated by the method developed by Selman *et al.* (1983). Values of D_a were only calculated by the diffusion curves of the smallest pieces (diameter 25–30 mm, thickness 2.2 mm). It was postulated that these thin pieces could be considered as slabs of infinite extent. The mean D_a values for 300, 600 and 900 sec at 328°K were respectively 7.7×10^{-10}, 10.48×10^{-10} and 10.75×10^{-10} m^2/sec. This is about four times greater than D_a value for total solute loss extrapolated from the results of Selman *et al.* (2.61×10^{-10} m^2/sec at 328°K).

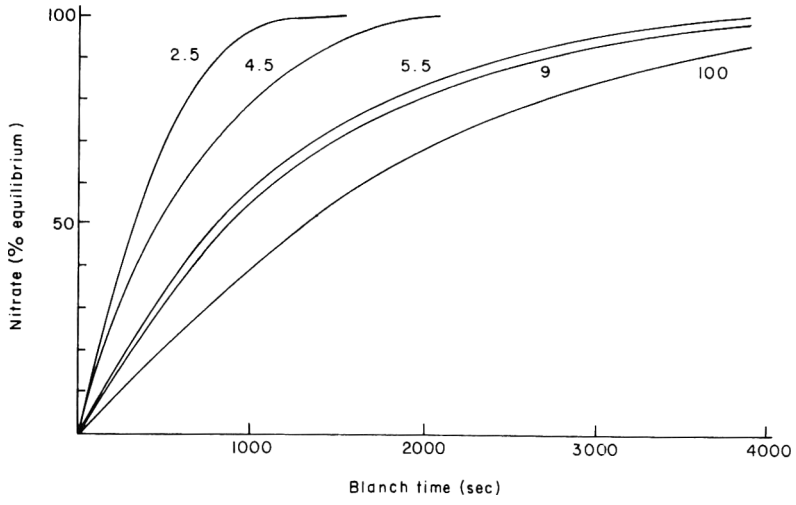

Figure 4. Influence of the carrot size on the kinetics of nitrate diffusion at 328°K (percentage of the nitrate concentration in blanch water/nitrate concentration at equilibrium; TOUCHON cultivar, distilled water 0.25 l; 10 g of given thickness in mm, 60 rpm).

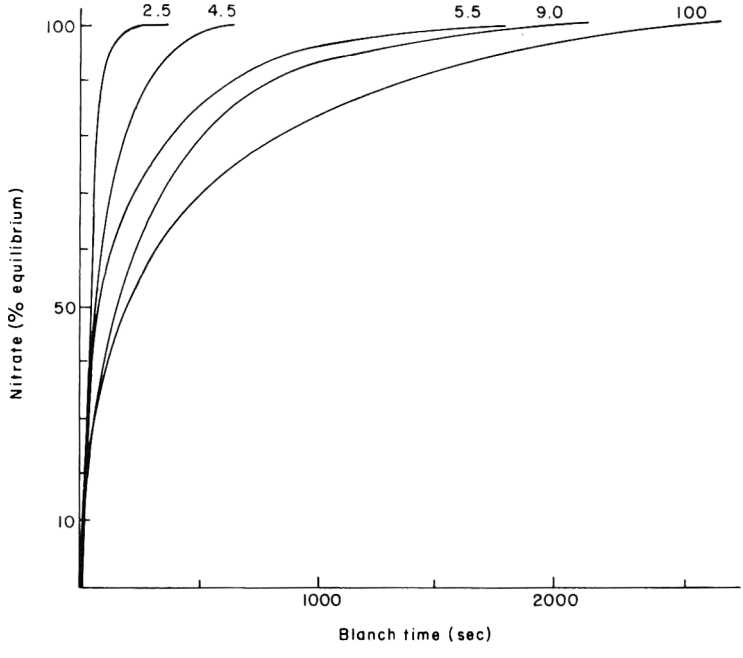

Figure 5. Influence of the carrot size on the kinetics of nitrate diffusion at 373°K (conditions as for Fig. 4).

Effect of the relative proportion of carrots and blanch water

In this experiment the volume of blanch water was kept constant (V_e = 0.250 l) and the amount of carrots was gradually increased from 0.0015 kg to 0.020 kg. Kinetics were

obtained at 328°K (Fig. 6) and at 373°K (Fig. 7). In this case the theoretical final nitrate concentration will increase when the blancher load increases:

$$C = \frac{V_i \times C_0}{V_i + V_e}.$$

The results in Figs 6 and 7 are consistent with equations 1 and 2.

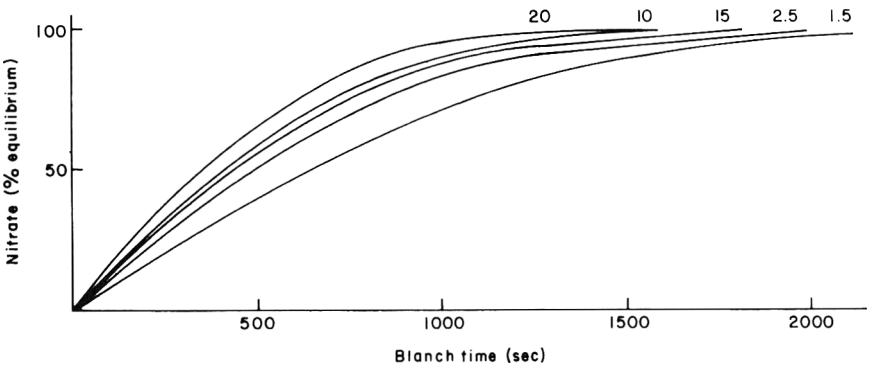

Figure 6. Influence of the blancher load on the kinetics of nitrate diffusion at 328°K (percentage of the nitrate concentration in blanch/water nitrate concentration at equilibrium; TOUCHON cultivar, distilled water 0.25 l; weight of carrot in g; 60 rpm).

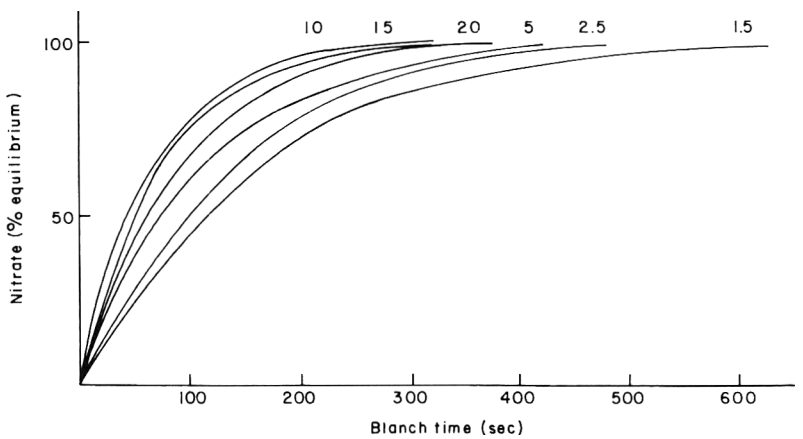

Figure 7. Influence of the blancher load on the kinetics of nitrate diffusion at 373°K (conditions as for Fig. 6).

Conclusion

The described procedures allow the experimental establishment of blanching conditions to control the residual nitrate concentration in carrots. A complete model of nitrate diffusion is not attainable due to the complexity of biological material. The

proposed model fits reasonably to the experimental data and could be used to predict nitrate loss during discontinuous blanching of carrots. Further investigations should be carried out in order to measure and modelize nitrate diffusion in a continuous process.

Acknowledgment

This research was supported by a grant-A.T.P. INRA no. 4347 'Stabilisation par blanchiment des produits végétaux'.

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Book Reviews

Photography for the Scientist. 2nd edn. Ed.by Richard A. Morton. Orlando, Florida: Academic Press, 1984. Pp. xviii+542. ISBN 0 12 508370 X. £76.00, US$98.00.

Photography for the Scientist is a comprehensive study of all aspects of photography from the basic developing and printing end through to the more specialized subjects of Photomacrography and Photomicrography etc. I found the book extremely easy to read and set out in a logical sequence giving clear and concise information and it is obviously written not to intimidate the reader. The chapters are dealt with in a thorough and straightforward way which allows the reader to select the area of interest and follow it up in greater depth from the copious references given. According to the Preface this book is intended for scientists who use photography in his or her research. Having worked many years with scientists in this respect my experience is that although they are prepared to go into the utmost detail in their own subject, it is less important in any topic secondary to their research. The point I am making is that the use of formulae throughout the book, though accurate, is not necessarily relevant to the scientist's research especially with the extensive references provided at the end of each chapter, unless he feels they are an integral part of his particular research.

I must congratulate the editor on putting together a book which is easily readable and has covered the subject in such depth that it can only be a tremendous help to the scientist and I am sure, will make him aware of the difficulty in recording his information. With regared to the authors, it must have been quite a challenge to be asked to write on one particular aspect of photography from the scientist's point of view and to know what is relevant or not in such a vast subject area and condense it into one chapter. On the whole I believe they have got the balance about right.

One criticism I would make is that the chapter on copying seems to be out of sequence. Copying is a relatively fundamental part of photography and I feel it should have preceded the more specialized areas of Photogrammetry, Infrared, Ultraviolet, Photomacrography and Photomicrography. Another criticism I have with regard to the chapter on Light Sources is that as a practising photographer it is vital to know certain aspects such as continuous spectrum, colour temperature etc., but I feel that the construction of various tungsten light sources is an unnecessary aspect to cover in this particular book.

The presentation of the book is excellent with a clear typeface on a gloss white paper, good diagrams and photographs, and a strong binding making this a very satisfying book to look at and read, and one I should be proud to have on my bookshelf.

J. Maclean

The Role of Fats in Human Nutrition. Ed. by F. B. Padley and S. Podmore in collaboration with J. P. Brun, R. Burt and B. W. Nicols. Chichester: Ellis Horwood, 1985. Pp. 210 ISBN 3 527 26219 9. £28.00.

During the last 20 years several national and international committees have considered the links between diet and heart disease. Of the many dietary recommendations, the

control of the level and type of fat has been highlighted consistently. This book, which is base on a Symposium organized by the Oils and Fats Group of the Society of Chemical Industry at the University of Reading, is particularly timely because of the publication in 1984, of the 'C.O.M.A.' report on 'Diet in Relation to Cardiovascular Disease' and the subsequent developments in consumer interest in the nutritional value of foods and the government proposals for the mandatory fat labelling of some of the major fat-containing foods in the U.K. diet.

The papers presented in this excellent and detailed book will provide the scientist and student with a wealth of information and will be a useful reference book to those interested and involved in the subject. The hardback book consists of fifteen chapters and 210 pages, taking the reader from the basic biochemical and physiological aspects of fat metabolism to the role of specific fats in the human diet and the changing nature of fat in the diet.

In Chapter 1, Professor James summarizes the rather sparse information from diet surveys over the last 100 years which shows that on average the proportion of energy derived from fat in the diet has doubled to a level of around 41%, which reflects the gradual reduction both in total energy intake and the contribution of energy from carbohydrate sources. Age factors and social class differences in fat intake, the changes in fat consumption since the Second World War and an interesting observation that the fat intake of females is consistently higher than males throughout life are discussed. Chapters 2 and 3 on the 'Biosynthesis of Fats' and 'Absorption and Metabolism of Fats' by M. I. Gurr and D. N. Brindley respectively, give thorough and comprehensive accounts of these subject areas. Both chapters assume that the reader is well acquainted with the sciences but the texts are very readable and flow well. The following chapter by R. T. Holman discusses the influence of the hydrogenated fats on the metabolism of the polyunsaturated fatty acids, and demonstrates that distinct chemical structures of fats and fatty acids will undoubtedly have unique effects on biological systems.

In Chapter 5, M. A. Crawford describes how different animal species respond differently to a deficiency of the essential fatty acids and presents an eloquent evolutionery argument for more attention to be paid to the meaning of the concept of essential nutrients using as examples a wide range of animals including man, the rat, monkey, zebra, and dolphin. In Chapter 6, A. G. Hassam discusses the role of evening primrose oil and the fatty acid γ-linolenic acid in human nutrition including their uses as a supplement in the management of some disease conditions. This is followed by a paper by T. A. B. Saunders on the importance of eicosapentaenoic and docosahexaenoic acids in the diet and of the need to study further the potential benefits of consuming large amounts of fish or smaller quanitites of fish oil supplements to reduce the incidence of acute myocardial infarction—perhaps a mackerel a day will keep the infarct away! In Chapter 8, J. J. Gottenbos describes the effects of a lower dietary intake of saturated fat and a higher intake of linoleic acid on plasma cholesterol levels, heart function, blood pressure and tendency to arterial thrombosis. S. M. Barlow and I. F. Duthie, in their Chapter 9 on 'Long-chain Monoenes in the Diet' highlight the difficulties in transposing the results of animal experiments to a public health interpretation. The paper discusses the observations of cardiac lipidosis in rats fed high fat diets containing rapeseed oil, the developments to produce a low erucic acid rapeseed oil and the lack of evidence in man that suggest that similar lessons to those in rats could be associated with the intake of C22:1 fatty acids in a balanced diet.

In Chapter 10, G. R. Thompson discusses the role of lipoproteins in the transport of fats in plasma. The composition of the plasma lipoproteins, the physiological and

pathological conditions which influence the levels in the bloodstream and the strong correlation between an increased plasma low density lipoprotein (LDL) - cholesterol level and coronary heart disease is reviewed.

Whereas the majority of papers describe the beneficial effects of diets rich in polyunsaturated fatty acids, A. D. Smith in Chapter 11 asks 'Can they be Harmful?' The author provides a brief survey of polyunsaturated fatty acids as co-carcinogens and their influence on the immune response. In Chapter 12, G. Billek discusses the oxidation of fats and oils during heating and the effects of heated fats in the diet. The final three chapters are discussion papers on the considerations involved in reducing and changing the nature of fat in the diet. M. J. Gibney discusses the 'Strategy and Philosophy of Dietary Intervention' in Chapter 13, and R. V. Crawford describes the patterns of refined fat usage and practical contraints in Chapter 14. This latter paper is particularly useful with summaries of the edible oil industry, the processing of fats and oils, the physical characteristics of fats and the reasons for their use in wide range of foodstuffs.

Finally, J. W. Marr reviews the practical issues of acceptance of dietary recommendations by the 'public' and demonstrates that there is a large gap between the theoretical manipulation of nutritional values to achieve diet recommendations and their practical interpretation. All in all, the book will be welcomed by those working in food science and nutrition and is highly recommended.

D. P. Richardson

Food Chemistry. 2nd edn. Ed. by Owen R. Fennema. New York: Marcel Dekker, 1985. Pp. x+991. ISBN 0 8247 7271 7 (hard covers), US$114.00; ISBN 0 8247 7449 3 (soft covers), US$59.50.

This is the second, revised and expanded edition of Part 1 of *Principles of Food Science*, published in 1976, which became *the* textbook for its subject. Its editor considers it to provide comprehensive coverage with the same depth and thoroughness that is characteristic of the better introductory textbooks of organic chemistry and biochemistry and thus to reflect a desirable maturation of the field of food chemistry.

The purpose of the book is as a textbook for upper division undergaduates and begining postgraduates with a sound background of organic chemistry and biochemistry. The organization remains the same, but the authors of several chapters are new, the chapter on food dispersions has been itself dispersed, and the indexing has been greatly improved. The selection of material has emphasized topics characterisitic of food chemistry and overlap with standard biochemistry texts has been reduced.

The book comprises: Introduction (O. R. Fennema & S. R. Tannenbaum, 21 pp.), water and ice (O. R. Fennema, 45 pp.), carbohydrates (R. L. Whistler & J. R. Daniel, 69 pp.), lipids (W. W. Nawar, 106 pp.), amino acids, peptides and proteins (J. C. Cheftel, J.-L. Cuq & D. Lorient, 125 pp.), enzymes (T. Richardson & D. B. Hyslop, 106 pp.), vitamins and minerals (S. R. Tannenbaum, V. R. Young & M. C. Archer, 68 pp.), pigments and other colorants (F.J. Francis, 40 pp.), flavours (R. C. Lindsay, 43 pp.), food additives (R. C. Lindsay, 59 pp.), undesirable or potentially undesirable constituents (G. N. Wogan & M. A. Marletta, 35 pp.), characteristics of muscle tissue (H. O. Hultin, 65 pp.), milk (H. E. Swaisgood, 37 pp.), eggs (W. D. Powrie & S. Nakai, 27 pp.), characteristics of edible plant tissues (N. F. Haard, 55 pp.), an integrated

approach to food chemistry: illustrative cases (T. P. Labuza, 26 pp.), and subject and chemical index (53 pp.).

Overall this is a very valuable book. The following comments must be read with that statement still in mind. Although domination of an American perspective is not unexpected, some mention of The Sale of Food and Drugs Act, 1875, should have been made. On the other hand, the editor is to be greatly commended for encouraging the use if 'enzymic', it being most atypical for Americans to have settled on the longer 'enzymatic' in the past. The treatment of non-enzymic browning is not of the depth appropriate for this book and the illustrated mechanism of inhibition of browning by SO_2 is antiquated (see B. L. Wedzicha *Chemistry of Sulphur Dioxide in Foods* Elsevier Applied Science London 1984). In dealing with enzymic is noticeable (e.g., W. Grosch, in I. D. Morton & A. J. Macleod, eds *Food Flavours* Elseveir Amsterdam, 1982). Among the methods for measuring resistance of lipids to oxidation, the Metrohm Rancimat should have a place. Books by W. W. Christie and by F. D. Gunstone & F.A. Norris, on *Lipid Analysis* and *Lipids in Foods*, Pergamon Press, Oxford, 1982 and 1983, respectively are worth at least a reference. The discussion of dietary fat and coronary heart disease is not sufficiently up-to-date and does not achieve the appropriate compromise between detail and brevity.

Winterization has been omitted. Considerable portions of the chapter on enzymes can be found in biochemistry textbooks. Flavours are dealt with in an unusual way, with emphasis on taste substances and pathways of formation of volatiles. There is a surprising omisson of esters and of a table summarizing character-impact compounds. The chapter on additives still includes the interesting discussion of chemical leavening systems, but also several new items such as natamycin, glyceryl esters, aspartame, Acesulfame K, masticatory substances and tracers. Even though undesirable constituents are being discussed, the reference to Butter Yellow seems inept, as does the reference to dyes derived from benzidine. The importance of SO_2 for biscuit flours warrants a mention. Hot deboning and electrical stimulation are dealt with briefly and, although there are intersting sections on the preparation of gel meat products and on modified (controlled) atmosphere storage, the latter is not linked to the section on packaging. The chapter on eggs is illustrated with interesting electron-micrographs and contains a useful section on functional properties. No formula is given of solanidine and its significance is not discussed. The final chapter is new and is concerned with shelf life prediction, though this cannot usually be made with great precision because of limitations due to cost and time. Knowledge of food processing should not have been missed as an intergral part of food science. Sections on singlet oxygen, extrusion, and potential applications of genetic engineering are noteworthy, as is the list of formulae of synthetic food dyes. The book is well produced overall, but the binding may not be strong enough to contain such a thick mass of paper. There are relatively few printer's errors, but several technical ones. Overall, in spite of some omissions and blemishes, this is a very valuable compilation, to which every serious food scientist will wish to have ready access. In terms of relevant information per unit of cost, it cannot compete with T. P. Coultate's *Food—The Chemistry of its Components*, the Royal Society of Chemistry, London, 1984 (pp. 197, £5.95), but the price of the soft cover version should not prove to be beyond the means of undergaduates.

H. E. Nursten

Nutritional Bioavailability of Calcium. Ed. by Constance Kies. (ACS Symposium Series 275) Washington, D.C.: American Chemical Society, 1985. Pp. vii+200. ISBN 0 8412 0907 3. US$45.95.

Calcium is the most abundant mineral in the body and apart from its obvious role in bone health it has important metabolic and regulatory functions. There is increasing interst in calcium availability and nutrition; for such a vital nutrient the dietary sources are relatively restricted and there is evidence that intake may be inadequate even in developed societies.

The book has two useful aspects: it deals with calcium availability from the nutritional rather than the metabolic viewpoint and there is considerable emphasis on the dietary interactions (for example with phosphorus, fat, protein, zinc and fibre as well as vitamin D) which are receiving much attention in the nutritional field. There is a good chapter on the perennially vexed question of the suitability of the rat as a model for man. The chapters show the usual variety of approaches found in collections of symposium papers. Some authors attempt to review the whole field, others concentrate on their own work. Clearly, these aspects will interest different sections of the readership. Most (though not all) authors have taken the opportunity to include the most recent references.

The book is well produced, although the range of typefaces (this is a camera-ready publication) results in some chapters being a pleasure to read whereas others require much concentration, depending on what printing equipment the authors' institutions can afford. I strongly recommend this book to the attention of anyone with an interest in calcium bioavailability, and especially those concerned with nutrient interactions.

B. Rolls

Food Microbiology and Hygiene. By P. R. Hayes. Barking: Elsevier Applied Science. 1985. Pp. xvi+403. ISBN 0 85334 355 1. £48.00.

This excellent book is broadly aimed at a wide range of professionals. It could serve as a basic text for Environmental Helath Officers and engineers who have a scientific background. However, it would also be a great interest to ancillary workers such as architects and lawyers who specialize in the food area and for senior management employed directly but who have no previous microbiological experience.

The text is written in a manner which could be understood by the interested lay person but does not oversimplify the basic microbiological principles and problems which are covered in the first four chapters of the book. Here food spoilage and poisoning are treated in detail, frequently giving the appropriate control measures. The chapter on microbiological examination is quite adequate in explaining the theory and simple methodology of testing to non-microbiologists, but relies on a bibliography for those who wish to obtain more detail.

There follow three chapters on the design, construction and layout of both factories and processing equipment containing sound practical advice together with clear diagrams and illustrations. The next chapter on quality assurance stresses the importance of control measures at every stage from raw materials inspection through storage and packaging to transport of finished goods.

The two chapters on cleaning and disinfection, materials and methods, should enable the reader to understand the theory of detergent and disinfectant formulation and to question a chemicals supplier on the suitability, safety and adequacy of products for their own particular purpose. Those difficulties commonly encountered when trying to balance the cost against the effectiveness of various methods are discussed.

An excessively long chapter on waste disposal is followed by a very short one on the hygiene and training of personnel. I would have preferred to see the detail of waste control curtailed with references provided in the bibliography for those requiring more information. An extension of the chapter on hygiene training for personnel could usefully have quoted current sources of training material. The final chapter on legislation compares the philosophies of law in some European countries and the U.S.A. It does not give extensive detail of U.K. food law and was prepared before the amalgamation of the various Acts into the Food Act 1984 which includes the power of closure of food premises and is not mentioned. An extension of this section would have been of use to those who find the interpretation of legal terminology difficult. However if the practical advice given throughout the preceding text is followed then the reader should have no difficulty in persuading Enforcement Officers that they are exercising due diligence within premises they influence.

Each chapter has a bibliography in addition to the quoted references which will be of particular use to those who do not have ready access to a good scientific library. The material included is, by and large, recent and would therefore serve as a useful update for those who left the educational world some time ago. Unfortunately the price may limit its distribution to company libraries rather than the briefcases of field workers where it belongs.

Sue Dix

Microwaves in the Food Processing Industry. By Robert V. Decareau. Orlando: Academic Press, 1985. Pp. xiii+234. ISBN 0 12 208430 6. £33.00.

This book is suitable for potential users of microwave heating technologies, either academic or industrial, and provides a detailed review of the historical development and commercial exploitation of the processes.

The topics covered in the publication are Dehydration, Freeze Drying, Meat Processing, Blanching, Baking, Tempering, Pasteurization and Sterilization. Each section is covered in some detail in terms of historical development and design considerations. Specific examples are included where appropriate such as the use of microwave techology for the drying of pasta, finish drying of potato crisps and vacuum drying of fruit juices. Microwave freeze drying is discussed as a technique which has not as yet reached commercial exploitation. The cooking of bacon and chicken are cited as examples of meat processing using microwaves. Microwave blanching of vegetables is discussed as a technique for both pre-package and in-package treatment, but the seasonal nature of the raw material is an economic handicap to its application. Bread and cake baking using a combination system of microwave and convection heating is described, but no present commercial uses are given. Microwave tempering of frozen products is the most widely used form of microwave heating in the food industry. Commercial systems used for the tempering of meat, fish and fuit are described.

Microwave heating as a tool for in-container sterilization or pasteurization is discussed in relation to in-pouch sterilization systems, such as the Multitherm system from Alfa Star, and an in-container pasteurization system for yoghurt. Two chapters have been contributed by R. E. Mudgett on the dielectric properties of foodstuffs and the modelling of microwave heating characteristics, thus broadening the scope and depth of the book. Many equations are presented to explain the behaviour of foodstuffs undergoing microwave heating.

Diagrams, graphs and photographs are included throughout the book where appropriate, and are a useful aid to understanding the text. A considerable amount of useful data is presented in the book, especially in the area of economic evaluation, which will be of interst to many readers. As a large proportion of the book is devoted to the historical development of microwave technology, many older references are cited. This however does not detract from the usefulness and scope of the reference section which lists over 400 articles.

P. Richardson

Amino Acid Composition and Biological Value of Cereal Proteins. Ed. by Radomir Lásztity and Mátá Hidvégi. Dordrecht: D. Reidel, 1985. Pp. xv+662. ISBN 90 277 1937 3. £53.50.

This book aims to review recent methodology and trends in the determination of the biological value and amino acid composition of cereal proteins and factors influencing their role in nutrition and animal feeding. There are thirty-nine papers divided into five sections: General Problems, Methodology of Protein Evaluation, Chemistry and Biochemistry of Cereal Proteins, Protein Preparations and Cereals—Composition and Nutritive Value and Cereals in Food and Feed. The contents are the edited proceedings of an International Symposium held in Budapest in 1983 with supplemental invited contributions which unfortunately are not speicfied. It requires considerable skill to edit a book produced in this way, particularly with direct reproduction of the original manuscripts. Sadly the editors have failed and would have served the interests of their contributors better had they published the papers given at the symposium alone and more quickly. Unfortunately they have sacrificed quality for quantity and although there is some interesting work reported it is difficult to find.

The general standard of editing is poor and can be illustrated by two examples from the editors' own contributions. They are co-authors of a paper (p. 421) in which the method used to determine trytophan is described in some detail. They state that this method was developed by a colleague from the same department. This colleague also describes this method in detail in the previous paper (p. 409). Dr Hidvegi is also co-author of a paper on the mathematical modelling of protein nutritional quality from amino acid composition (p. 205). This is three times longer than any other paper and takes up well over 10% of the book. It is full of lengthy quotations from original papers some of which are even referred to by using the first names of the authors. The time spent on this paper would have been better spent on avoiding the situation where one author of an eight page paper (Kies, p. 553) is allowed to take up four of these pages with references, half of which do not appear in the text. In keeping with the rest of the

book the index is repetitive, full of errors and overlong, due principally to the unnecessary inclusion of the amino acid, essential amino acid and individual amino acid composition of the cereals. Some of the figures appear to have been drawn free-hand but the print, in spite of the method or reproduction is generally easy to read.

There is considerable emphasis on the methodology of protein quality evaluation much of which is written in general terms rather than specifically relating to cereal proteins. There are examples of such papers in other sections, for example the paper by Hurwitz on the amino acid requirements of growing birds. The editors had hoped that these contributions would make the book a valuable source of information to scientists working with cereal proteins. The sponsors of the symposium, the Grain Trust, Hungary, expressed the hope that readers would get an overview of cereal research and production in Hungary. Only this latter aim has been achieved but at too high a price to be able to commend the book.

A. Williams

Tropical Science

an international journal of post-harvest science, technology and economics

Editor: Dr Philip C. Spensley

Tropical Science aims to disseminate information about advances in, and applications of, science and technology in making better use of the tropical environment and of the products that this environment can yield. The journal is international both in its sources of articles and its circulation. In particular it seeks to report studies related to the post-harvest stages in the exploitation of plants and animals, these including indigenous (wild) species as well as those arising in agriculture, animal husbandry, forestry, and fisheries (marine and fresh water). These resources find a wide variety of uses, for instance as food, beverages, fodder, flavourings or pharmaceuticals; as building materials or as sources of energy; as raw materials for industry (natural fibres, papermaking materials, essential oils, etc.) or for making handicrafts; and as exports for foreign exchange. The post-harvest stages include such operations as drying, processing, preservation, storage, packaging, transport, analysis and quality control, and marketing. *Tropical Science* also embraces investigations of the unwanted, destructive biological agencies of the Tropics — for example the tropical pests which damage plant and animal products, equipment, etc.

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

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3} m	hour	hr
micrometre	$\mu = 10^{-6}$ m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m^3		

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Contents

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