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Editorial

On the occasion of the first issue of the Journal since I took over the post of Editor, I would like to express appreciation of the work put into it by my immediate predecessors. Dr H. Liebmann was Editor from 1972, and, with advice from a small team of assistant editors, built up the Journal from four to six issues annually. In 1979 he was joined by a deputy, Dr W.F. Harrigan, and the team was not expanded further until 1984–1985. Dr Liebmann, after many years of dedicated service, retired in August last year. As an interim measure, a member of the Editorial Board, Prof. R.S. Hannan, became Acting Editor up to this issue, and I thank him for the efficient holding and transfer of the system to me. On behalf of the Institute, contributors and deputy editors, I would like to thank both of them and wish them a happy retirement from editing.

All areas of activity change with time, and research relevant to food technology is no exception. For many years the technology was often led by pragmatism, and, with notable exceptions, the underlying science followed. However, much current technology consists of the application of science-based knowledge in production and maintenance of high quality in an increasing variety of foods, most of which are processed in some way and many of which are manufactured. Furthermore, the areas in which food science and technology have impact and relevance are widening rapidly, for example back along the food chain to genetic specification and modification of seed and breed, or forward into distribution and marketing.

These trends have identified a need for reappraisal of the role of the *Journal of Food Technology*. Despite its title, it has always published both food science and technology, but with a conscious effort to concentrate on the latter. The reappraisal is not yet complete, but in future the Journal will accept contributions with more emphasis on fundamental aspects of the science upon whch the technology is based, provided the projected applications are at least discussed. It will continue to publish research on technology, but will demand a more rigorous science base than has sometimes been accepted.

The Journal will continue to provide a broadly-based vehicle for the international exchange of novel information relevant to food science and technology, unlike some of its recent, more specialized competitors. It will also provide short, topical, critical reviews. Topics covered range from raw materials to consumer acceptance, from physical and chemical properties to food engineering practices, and from quality assurance and safety to storage, distribution and even marketing. It covers the entire process leading to the food on the table, and provides information which will be of increasing interest to anyone in research, development, application, production or higher education related to food. Its circulation of over 2500 covers 89 countries worldwide.

The consideration of these and other factors will result in some changes in the Journal over the next few months. The first—revisions to 'Objects of the Journal' and 'Notes to Contributors'—will be published in the next issue. Prospective contributors should note that they will be increasingly applied to papers received during 1987, although allowances will be made for those from distant lands. Four types of contribution will be published: research papers, technical notes, short topical reviews and letters; and all but letters will be subject to defined peer review. Criteria will be defined and provision made for accelerated publication where justified.

Derek G. Land
Editor

Journal of Food Technology (1986) **21**, 419–436

Review: Lactic acid: considerations in favour of its acceptance as a meat decontamininant

F. J. M. SMULDERS, P. BARENDSEN, J. G. VAN LOGTESTIJN, D. A. A. MOSSEL AND G. M. VAN DER MAREL

Summary

Lactic acid occurs in a broad variety of foods consumed by man since times immemorial. At an appropriate pH it has bactericidal properties while not adversely affecting the sensory attributes of food. The suitability of lactic acid as a surface decontaminant for fresh meats, slaughter byproducts and poultry were studied with special reference to markedly reducing the contamination with enteropathogenic *Enterobacteriaceae* and *Campylobacter* spp. and extension of shelf life under refrigeration. Discoloration of meat surfaces does not occur at concentrations of approximately 1% v/v lactic acid, at pH = 2.4. Up to 2% does not cause off-flavours in meat. Such treatments result in a significant reduction of the bacterial flora, not only by means of a pH drop but also by a specific action of the acid in the undissociated form. Undesirable flora shifts favouring pathogenic microorganisms at the cost of microbial antagonists have not been observed. Since there are no indications that lactic acid decontamination of meats could in any sense endanger human health, public health authorities would seem well advised to allow meat processing plants to use lactic acid as a decontaminating agent, provided they adhere to the strict conditions of Good Manufacturing Practice (GMP).

Introduction

Despite its unconditional physiological acceptability (FAO/WHO, 1974) lactic acid has not yet legally been approved as a meat decontaminant in most European countries. It is likely that this reluctance is prompted by concern that allowing acids, or any bactericidal agent, to be used as meat decontaminants may lead to their use for masking hygienic failures. Thus it would result in a disregard of sanitary practices during and after slaughter.

Indeed, by far the most important factor in controlling the degree of initial contamination of fresh meat is hygiene (Childers, Keahey & Vincent, 1973; Gerats, Snijders & van Logtestijn, 1981; Smulders & Woolthuis, 1983). However, despite the use of increasingly sophisticated hygienic measures, carcasses are still found contaminated with pathogens including salmonellae and campylobacters (Mossel, 1984a). It would, therefore, be beneficial to use additional means of sanitizing carcasses and slaughter byproducts particularly at the end of the slaughterline. Similar to the situation in the dairy industry, such a terminal treatment would not only reduce, or even eliminate, pathogens but also further extend the keeping quality. Thus decontamination with lactic acid would not serve as a substitute for, but rather as an essential part of, strict longitudinally integrated hygiene programmes in the meat industry.

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This paper aims at overviewing relevant data on lactic acid, particularly those concerned with its decontaminating potency, and at reporting results of investigations demonstrating the utility of lactic acid for the red meat industry. The histological properties of poultry skin are entirely different from those of carcass surfaces. Hence surface decontamination of the former commodities will be dealt with in a separate paper (Van der Marel, Van Logtestijn & Mossel, 1986).

Some properties of lactic acid

Commercial availability

In a pure dry form lactic acid (2-hydroxypropionic acid) is a white powder with a melting point of 18°C (racemic lactic acid) to 26°C (lactic acid isomers). It is generally available in an aqueous solution of variable concentration and purity. The 'edible grade' L(+) lactic acid, meeting the specifications of the E.E.C. and Food Chemicals Codex (Holten, 1971, p. 500), is a slightly yellow liquid, generally as a 50 or 80% solution. The 'pharmaceutical grades' are colourless liquids of 88 or 90% concentration. These grades comply with the most common Pharmacapoeias. With increasing concentration the presence of intermolecular esters increases. At room temperature an equilibrium is only achieved within months. On dilution hydrolysis occurs, which is accelerated by heating (Holten, 1971).

The pK_a value of lactic acid is reported to be 3.857 at 22°C. Lactic acid corrodes stainless steel unless it is protected by a plastic film (Hamner, 1974) or is of high quality (e.g., 316 or 317). Plastics are used for packaging.

Optical activity

As lactic acid contains an asymmetrical carbon atom, it is optically active. The nomenclature related to this stereospecificity is rather confusing. The majority of authors use the terms L(+) and D(−). The (+) and (−) symbols indicate the direction of optical rotation, i.e., to the right and to the left, respectively. The capitals L and D indicate the configuration of the asymmetric carbon atom. This notation is most commonly used, although the R-S system (Roberts & Caserio, 1964) is a more systematic way of denoting configuration. Denoting the optical activity with small letters *l* (laevo = to the left) and *d* (dextro = to the right) is obsolete.

L(+) and D(−) lactic acid racemize only under extreme conditions such as heating for 1 hr at 120°C with an excess of potassium hydroxide. Some bacteria produce enzymes (racemases) which induce rapid racemization (Lockwood, Hunter & Zienty, 1965), but pure racemic lactic acid is rarely found. An exception to this rule is synthetic lactic acid prepared from lactonitrile (CH_3—CHOH—CN) via hydrolysis (Anon., 1964; Lockwood *et al.*, 1965).

Production

Homofermentative lactic acid bacteria produce mainly lactic acid, whereas the so-called heterofermentative lactic acid bacteria produce a variety of other metabolites as well. Consequently the former are most suitable for the production of lactic acid on an industrial scale, and in particular those genera which produce a high yield. The extent to which L(+) or D(−) lactic acid is synthesized depends on the bacterial strain, its age and the pH conditions.

Many processes have been described for the production of lactic acid using different substrates. Molasses, whey permeate, sulphite waste liquor, and hydrolysed starch can

be used (Inskeep, Taylor & Breitzke, 1952; Miall, 1978). Most of the manufacturers of commercial lactic acid, however, use more defined substrates such as sucrose and dextrose. A detailed description of such a process is given by Inskeep *et al.* (1952). Continuous fermentations of lactic acid have been mentioned (Whittier & Rogers, 1931; Childs & Welsby, 1966), but they have never reached a commercial scale.

The process of fermentative production of lactic acid can be separated into two steps: fermentation and the so-called down-stream processing. Most common is to ferment sucrose or dextrose with a strain of *Lactobacillus delbruckii*. The pH during fermentation can be kept constant by the addition of lime or by the addition of calcium carbonate in advance. In both cases the end product in the fermenter is calcium lactate. The solution is heated, made alkaline and after separation of cell debris and other impurities, lactic acid is liberated by the addition of sulphuric acid. The precipitated gypsum is separated, the supernatant further purified by ion-exchange resins, and concentrated to produce 'edible grade' lactic acid. 'Pharmaceutical grade' lactic acid is made after further purification by esterification, distillation and hydrolysis. It is possible to obtain high yields and also a high optical purity. Nanninga (1983) mentions a yield over 90%, and a L(+) isomer content of about 97%.

Lactic acid as a natural constituent of foods

Lactic acid plays an important role in the carbohydrate metabolism of higher and lower organisms. Mammals exclusively form L(+) ('physiological') lactic acid. Lower organisms such as bacteria and moulds produce both isomers in different proportions (Krusch, 1978) and higher plants may also form (DL) lactic acid (Merck-index, 1976).

Table 1 presents the lactic acid contents of some foods containing marked amounts together with the per capita consumption of these foods in The Netherlands. Lactic acid occurs in many foods obtained by fermentation. For over 2000 years bacteria have been used to convert lactose to lactic acid, and a variety of products have been generated

Table 1. Lactic acid content of some foods and annual per capita consumption in The Netherlands

Food	Lactic acid content (g/kg)	Per capita consumption (kg)*	Per capita lactic acid ingestion (g)
Pork	9	41.7	375.3
Beef	9	18.6	167.4
Cheese (Gouda)	13	12.5	162.5
Buttermilk	10	9.3	93.0
Poultry	10	9.0	90.0
Yogurt	10	6.9	69.0
Edible slaughter offals	9	3.9	35.1
Dry fermented sausage†	17	1.3	22.1
Sauerkraut	11	2.0	22.0
Horse meat	9	1.8	16.2
Veal	9	1.4	12.6
Mutton	9	0.5	4.5

*From Vreeman (1981) except where indicated otherwise.

†Computed from de Ketelaere *et al.* (1974) and The Netherlands Commodity Board for Livestock and Meat (1984).

varying in flavour, composition and consistency. In Western European countries this category includes buttermilk and yogurt. Both contain about 1% of lactic acid, of which up to 50% may be D(−) (Klupsch, 1982). Other well known examples of foods containing lactic acid resulting from fermentation are rye and wheat breads or mixed rye/wheat breads prepared from sourdoughs (Huber, 1977; Spicher & Lönner, 1985), cheese, sauerkraut and various types of fermented sausages. In all of these products both L(+) and D(−) lactic acid may be found in a ratio which is determined by the microorganisms used (Wagner, 1981. Table 59, p. 734), the fermentation conditions (pH, temperature, P_0), reaction time and substrate.

Lactic acid, almost always produced by fermentation, may also be added to foods. Well known examples in which lactic acid is an essential ingredient, are brined gherkins and olives, pickles, salads, dressings and some confectionery, dairy and meat products. In fresh meat lactic acid is present as a result of the anaerobic breakdown of carbohydrates, and its content is dependent on the energy stores of the animals at the time of slaughter. On average, meat contains 0.9% of lactic acid (Vreeman, 1981) (see Table 1), which contributes markedly to its flavour (Lawrie, 1979) and affects the keeping quality (Ingram, 1948).

Lactic acid as a meat decontaminant

Review of previous investigations

The bactericidal and bacteriostatic effects of lactic acid are well known and fermentation of foods is in fact one of the oldest ways to prevent microbial spoilage. The antimicrobial action of organic acids such as lactic acid depends on three factors: (i) the effect solely of pH; (ii) the extent of dissociation of the acid; and (iii) a specific effect related to the acid molecule (Ingram, Ottoway & Coppock, 1956). The relative importance of each of these contributions has been a point of discussion. Gill & Newton (1982) suggest that the pH fall is the most important of the three. Other investigators (Ingram *et al.*, 1956; Mossel & de Bruin, 1960; Grau, 1981; Rubin, Nerad & Vaughan, 1982; Eklund, 1983) consider the character of the acid molecule is also of significance, such that at a given pH the antimicrobial activity of an acid solution also depends on the character of the acid molecule.

Microorganisms multiply only within certain species-dependent pH ranges, and for each group of bacteria these are rather stable (Chung and Goepfert, 1970). Particularly, acid-forming bacteria, yeasts and moulds are more tolerant of acids as they do not interfere with the organisms' energy yielding biochemical activities, whilst some may even be metabolized (Corlett & Brown, 1980). An acid which decreases the pH of a food will extend the lag phase of acid sensitive microorganisms (Mountney & O'Malley, 1965; Greer, 1982; Smulders & Woolthuis, 1983, 1985), and will eventually result in the death of the organisms (Levine & Fellers, 1940; Van Netten, van der Zee & Mossel, 1984). The pH drop attained and the period during which it is maintained depend on the amount of acid and the buffering capacity of a food. After spraying hot calf carcasses with 1.25% (v/v) lactic acid, Woolthuis & Smulders (1985) found a surface pH fall of more than three units, but after 72 hr the pH had returned to its initial value. Immersion of pig livers in 0.2% (v/v) lactic acid effected a surface pH *circa* 0.6 units lower than controls, whereupon it took 5 hr to restore the initial pH value (Woolthuis *et al.*, 1984). Repeating a lactic acid treatment of broiler carcasses neither decreased the surface pH further nor enhanced the bacteriostatic and bactericidal effects (Van der Marel *et al.*, 1986).

A shift in pH within the species' specific growth range will hardly affect micro-organisms. However, when using weak acids and salts as preservatives, such pH shifts may cause considerable differences in growth inhibition and death (Ingram *et al.*, 1956). This is explained by the fact that the pH affects the extent of dissociation (Baird-Parker, 1980) and that undissociated weak acids are 10–600 times as effective as the dissociated form (Eklund, 1983). Undissociated acids penetrate the cell by means of diffusion and then dissociate to produce acidification of the 'cell interior' (Hunter & Segel, 1973). Effective growth inhibition by an acid only occurs when an appropriate amount of the undissociated molecule is present (Ingram *et al.*, 1956; Maeris, 1975). This amount may be obtained by either applying more acid or by lowering the pH. Most organic acids are effective only at low pH values (pH 5.5). However, at pH levels above 6 dissociated sorbic acid is reported to cause more than 50% of the growth inhibition of *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*; apparently the anion exerts some influence under these circumstances (Eklund, 1983).

Under the same conditions of pH and acid dissociation there are still differences in antimicrobial actions of various organic acids. Ingram *et al.* (1956) suggest that this 'specific effect' is related to (a) the potency to penetrate a cell, (b) the part of the cell which is attacked, and (c) the chemical nature of that attack. As regards (a) and (b) particularly the heteropolar nature of the molecule is important: heteropolar molecules are surface-active, i.e., will concentrate at the bacterial cell surface, interfere with its permeability and eventually result in acidification of the 'cell interior'. The moderately lipophilic part of the acid partly determines the penetration of the cell membranes. Wyss (1948) mentioned as possible points of impact of acids in the cell: competition with co-enzymes, inactivation of S-S enzymes and the suppression of fumarate oxidation in catalase-positive organisms.

Experimental part

Methods. As is current practice, most of our estimations of the bacteriostatic and bactericidal effects of lactic acid relied primarily on the use of *Enterobacteriaceae* as marker organisms. In view of their accurate taxonomic delineation, *Enterobacteriacea* may be regarded as suitable for this purpose (Mossel, 1982). Recently Gerats *et al.*, (1981) and Banks & Board (1983) have demonstrated that for pig carcasses and sausage meat a certain relationship exists between mesophilic *Enterobacteriaceae* colony counts and the extent of *Salmonella* contamination. Figure 1, redrawn from Gerats *et al.* (1981), illustrates this point. The validity of mesophilic *Enterobacteriaceae* as markers

Table 2. The lethal effect of exposure to 0.2 mol/l lactic acid at pH *circa* 2.5 for 2 min at room temperature (Van Netten *et al.*, 1982, 1984)

Class of pathogen	Number of strains tested	$\Lambda = \log_{10}N_0 - \log_{10}N_f$*
Salmonella spp.	4	4.3–5.2
Yersinia enterocolitica	2	3.8–5.1
Enteropathogenic *Escherichia coli*	4	2.2–4.1
Campylobacter jejuni	2	> 5.3
Mesophilic *Enterobacteriaceae*		2.2–2.8

*Λ, lethality. N_0 = initial CFU. N_f = final CFU.

for the elimination of *Salmonella, Yersinia,* enteropathogenic *Escherichia coli* and *Campylobacter* in studies on lactic acid decontamination is illustrated by Table 2. *Enterobacteriaceae* decrease by 10^2–10^3, salmonellae by 10^4–10^5. Hence an *Enterobacteriaceae* colony count as a lethality parameter does not overrate the reduction in *Salmonella* CFU nor that of any of the other enteropathogens.

In addition aerobic colony counts at 30°C were made to monitor the fate of the bacterial community structure of meats treated with lactic acid in more general terms.

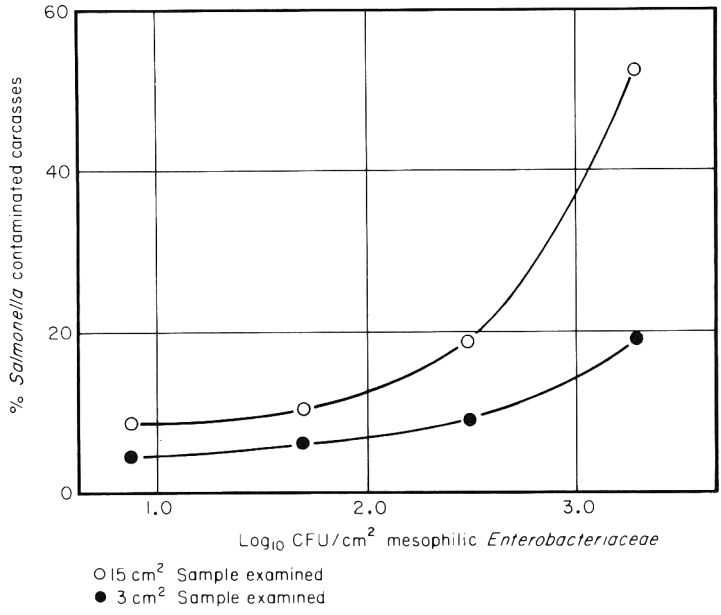

Figure 1. The relation between mesophilic *Enterobacteriaceae* and *Salmonella* on pig carcasses (n = 891). (Redrawn from Gerats *et al.*, 1981.)

In decontamination experiments relying on spraying proper control tests were carried out to account for the mechanical removal ('washing off') effects of showering (Woolthuis & Smulders, 1985).

Results. Tables 3 and 4 summarize our data on the effect of lactic acid on the bacteriological condition of carcasses and some slaughter byproducts. All data were collected in studies of the same experimental design, particularly with reference to (i) sampling by means of excision (Snijders *et al.*, 1984), (ii) use of identical culture media of previously validated performance (Mossel *et al.*, 1983), and (iii) consistent resuscitation (Mossel & Van Netten, 1984) and colony enumeration techniques. The data obtained should therefore be comparable. Resuscitation procedures were in all instances validated with the aid of destruction-repair curve analysis (Mossel & Van Netten, 1984; see Fig. 2). Liquid medium repair for about 1½ hr at ambient temperature (Mossel, Veldman & Eelderink, 1980) was invariably effective. For comparison, results of similar decontamination experiments by Patterson & Gibbs (1979), although determined with slightly different methodologies, have also been presented in Table 4.

Table 3. The immediate effect of lactic acid decontamination on the bacteriological condition of carcasses (in $\log_{10}$CFU/cm^2, except where indicated otherwise)

	Lactic acid		Bacteriological parameters				
			Enterobacteriaceae (1d,37°C)		Aerobic colony count (3d,30°C)		
	Concentration (% v/v)	pH/T	Initial count	Reduction*	Initial count	Reduction*	Reference
Beef	1.00	—/—	—	—	4.5	1.9	Snijders *et al.* (1979)
Veal	1.25	2.4/11°C	1.8	‡	3.7	1.2	Woolthuis & Smulders (1985)
	1.25	2.4/11°C	2.3	0.3	3.3	0.8	Smulders & Woolthuis (1985)
Pork	2.40	—/15°C	2.3	0.9	3.7	1.0	Labots *et al.* (1983)
	1.00	—/—	2.7	‡	4.7	0.9	Snijders *et al.* (1985)
Poultry†	1.00	—/15°C	4.2	1.2	5.8	0.9	Van der Marel *et al.* (1986)

* = Reductions are significant at least $P < 0.05$.
† = Lactic acid applied after evisceration, counts as $\log_{10}$CFU/g skin.
‡ = Reduction to below limit of detection ($\log_{10}$CFU/cm^2 = 1.3).
— = Not determined.

Table 4. The immediate effect of lactic acid decontamination on the bacteriological condition of some organs (in $\log_{10}$CFU/cm^2)

	Lactic acid		Bacteriological parameters				
			Enterobacteriaceae (1d,37°C)		Aerobic colony count (3d,30°C)		
	Concentration (% v/v)	pH/T	Initial count	Reduction*	Initial count	Reduction*	Reference
Beef heart†	1.00	—/7°C	—	—	5.5	0.8	Patterson & Gibbs (1979)
Beef liver†	1.00	—/7°C	—	—	4.6	1.9	Patterson & Gibbs (1979)
Calf brain	1.25	2.4/11°C	3.1	0.4	3.9	0.6	Smulders & Korteknie (1985)
Calf liver	0.20	2.8/11°C	§	§	3.5	0.8	Korteknie *et al.* (unpublished)
Calf tongues‡	2.00	2.3/11°C	1.5	§	2.9	3.0	Visser & Bijker (1985)
Pig liver	0.20	2.8/11°C	2.1	0.4	4.4	2.2	Woolthuis *et al.* (1984)

* = Reductions are significant at least $P < 0.05$.
† = Sampled by swabbing.
‡ = Centrifuged in the presence of 2.00% lactic acid.
§ = Reduction to below limit of detection ($\log_{10}$CFU/cm^2 = 1.3).

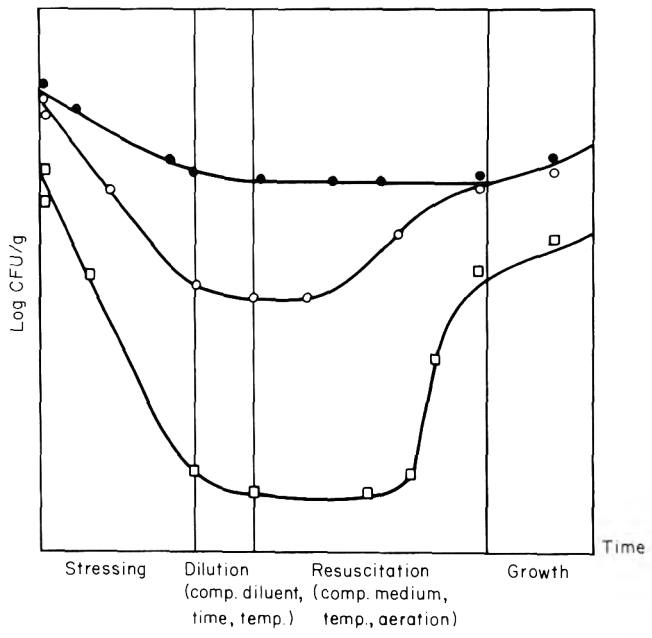

Figure 2. The recovery of sublethally stressed populations of microorganisms as affected by the intensity of stress and various conditions for recovery—the so-called destruction/repair curve: ●. optimal non-selective medium: O. optimal selective medium: □. suboptimal selective medium.

Discussion. Our studies demonstrated that the efficacy of treatment with lactic acid depends on several factors. These include the following:

(1) *The extent and nature of the initial contamination*

At high levels of initial contamination acceptable concentrations of lactic acid may not effect marked microbial lethality as theorized by Baird-Parker (1980). When the initial contamination was low, the lethality effect of lactic acid on aerobic colony counts, though significant, did not exceed 2 or exceptionally 3 log cycles (Snijders *et al.*, 1979; Smulders & Woolthuis, 1983; van Netten *et al.*, 1984; Smulders & Korteknie, 1985; Smulders & Woolthuis, 1985). However, after storage of lactic acid treated meat surfaces, a so-called delayed bacteriostatic effect was found (Smulders & Woolthuis, 1985). Gill & Penney (1985) also found that lamb cuts treated with 5% lactic acid and vacuum packaged in foil laminate remained unspoiled for 12 weeks of chilled storage.

The variable sensitivity of different taxa of microorganisms susceptible to lactic acid (*vide supra*) has been established in several experiments. Lueck (1980) showed the relative tolerance of yeasts and moulds to acids. This was confirmed in decontamination experiments with carcasses (Dezeure-Wallays & van Hoof, 1980). However, the possibility that this selective character of lactic acid decontamination might result in a flora shift towards pathogenic microorganisms at the cost of microbial antagonists was refuted (Snijders *et al.*, 1979; van Netten & Mossel, 1980; van Netten, Werdler &

Mossel, 1982; Woolthuis *et al.*, 1984). These findings were substantiated by Osthold *et al.* (1983) and Gill & Penney (1985). Van Netten *et al.* (1982) did observe a flora shift after lactic acid decontamination, but this resulted in a relative increase of the catalase-negative, Gram-positive bacteria which are known to be antagonists of enteropathogenic Gram-negative bacteria (Gilliland & Speck, 1972; Daly *et al.*, 1973; Gilliland & Speck, 1977).

A possible interaction by lactic acid utilizing acetic acid bacteria which can make meat surfaces alkaline (Aries, Cheney & Mossel, 1982) was not observed in our experiments. Grau (1980, 1981) reported an increased effect of lactic acid under anaerobic conditions. Grau also found that at that at low ultimate pH values (5.5) the muscle contains so much L(+) lactic acid that growth of Gram-negative fermentative bacteria is inhibited, whereas at an ultimate pH of 6.1 the amount of L(+) lactic acid is not sufficiently high to cause inhibition. Woolford (1975) suggested that lactic acid acts primarily against anaerobic bacteria. However, numerous authors (see Tables 3 and 4) also report significant reductions in aerobic colony count.

(2) *The concentration of the lactic acid solution*

Clearly lactic acid is generally found to exert a more marked antimicrobial effect in high than in low concentrations (Ockerman *et al.*, 1974; Snijders *et al.*, 1979; Woolthuis & Smulders, 1985). Moreover the buffer capacity of meats may be challenged more strongly by higher acid concentrations. The limit is what the product can tolerate in terms of loss of sensory properties.

(3) *Other factors*

As lactic acid exerts a stronger microbicidal effect at 35°C than at chill room temperatures (Goepfert & Hicks, 1969; Park & Marth, 1972) hot boning (Kastner, 1982) offers an excellent opportunity to benefit from lactic acid decontamination. As bacteria are not initially attached firmly to fresh meat surfaces (Firstenberg-Eden, 1981) the surfaces should be treated as soon as possible after slaughter.

Other extrinsic factors, including the accessibility and fat content of the substrate, as predicted by Jarvis & Burke (1977), were also observed to affect lactic acid decontamination.

Studies on the effects of lactic acid on the sensory quality of meat

Lactic acid may affect the colour and flavour of treated meat to a certain extent, depending on its concentration. Bleaching, which is frequently observed, is generally considered unacceptable. Dezeure-Wallays & van Hoof (1980) treated beef carcasses with an acid mixture (expressed by the authors as 0.15 meq cm^{-2}) containing lactic acid, sugars and antioxidants in a 2-week experiment. They observed a brown discoloration which was irreversible.

After studying the effects of several concentrations of lactic acid on beef carcasses Snijders *et al.* (1979) concluded that the application of a 1% v/v lactic acid spray resulted in only a slight reversible discoloration. On veal carcasses Woolthuis & Smulders (1985) observed no off-colours of fat or meat up to 1.25% v/v, and when deboned, in the course of which fat is trimmed off, concentrations of 2.00% v/v were used without noticeable bleaching of the meat (Smulders & Woolthuis, 1985). Higher concentrations resulted in an unacceptable bleaching of the lean and in considerable brown off-colours of the fat. Labots *et al.* (1983) reported an irreversible discoloration of pig carcasses

after treatment with 2.4% v/v lactic acid and Van der Marel (unpublished) noticed off-colours of poultry carcasses at 2.0% v/v. Surprisingly Ockerman *et al.* (1974) suggest a concentration of 12% v/v to be still acceptable for lamb. Colour stabilizing agents may counteract colour changes; Mulder & Krol (1975) found ascorbic and nicotinic acid useful for this purpose.

When applying lactic acid to decontaminate slaughter-byproducts specific commercial and domestic practices for each product should be considered. Tongues, for example, can be treated with high lactic acid concentrations as the top layer will be peeled off while being prepared for consumption, but livers increasingly discolour after immersion in lactic acid in concentrations higher than 0.20% v/v (Woolthuis *et al.*, 1984). Calf brain exhibits off-colours at 1.25% v/v whereas lower concentrations do not provide adequate decontamination (Smulders & Korteknie, 1985).

Adverse effects of lactic acid treatment of meat surfaces on the flavour of meat do not occur readily. Woolthuis & Smulders (1985) found no significant effect on veal longissimus chops treated with 2.00% v/v lactic acid when evaluated after 2 hr of incubation. Higher concentrations were identified by a consumer taste panel, although the flavour was not rated 'undesirable'. Gill & Penney (1985) found even 5% lactic acid to have no significant effect on flavour and odour of vacuum packaged lamb after storage periods of 3, 6, 9 and 12 weeks.

Investigations on the optimal mode of application of lactic acid in practice

The conditions under which lactic acid should be applied are different for carcasses, meat and slaughter-byproducts. We studied the following procedures.

Spraying is the most common way of applying lactic acid. We observed that carcasses may accumulate liquid in the subcutaneous fat when lactic acid is sprayed at high pressure. In the course of chilled storage this may occasionally lead to an undesirable carcass appearance. Even when sprayed at low pressure a lactic acid treatment may result in lower carcass grading scores; this occurs when many blood spots are present, since lactic acid tends to coagulate these, leaving rusty brown spots which are unattractive (Snijders *et al.*, 1979; Woolthuis & Smulders, 1985). Showering off these blood spots before decontamination may partly overcome this effect.

We applied lactic acid experimentally to pig carcasses at the slaughterline using *electrostatic dispersion* (Labots *et al.*, 1983). Whereas the washing effect may be less, this method allows a reduction of amount of lactic acid needed for effective decontamination (Snijders *et al.*, 1985).

Slaughter byproducts such as livers may be decontaminated by *immersion* for a certain period. In this procedure lactic acid very effectively reaches all locations, possibly including the interior of livers via the bile ducts (Woolthuis *et al.*, 1984). On the other hand lactic acid solutions used in this way were found to gradually lose part of their decontaminating potential, as lactate ions are easily bound by peptides and proteins which are progressively released into the immersion tanks (van Netten, unpubl.). For a similar reason we found it necessary to clean tongues thoroughly before lactic acid decontamination to dispose of the mucus covering the numerous papillae. To this end Visser & Bijker (1985) successfully applied centrifugation in the presence of 2% lactic acid. Delicate tissues such as brain require a more gentle treatment. Current stunning methods (Smulders, Korteknie & Woolthuis, 1985; Smulders & Korteknie, 1985) are a potential cause of contamination of brain with hair, skin and bone fragments

which substantially interfere with the decontaminating potency of lactic acid immersion in a bath, unless previously liberated from such debris.

Lactobacilli produce antibacterial agents as byproducts of their metabolism (Price & Lee, 1970; Reddy & Shahani, 1971; Upreti & Hillsdill, 1975; Gilliland & Speck, 1977; Talon, Labadie & Larpent, 1980; Geis, Singh & Teuber, 1983). Consequently *starter cultures* of lactic acid bacteria have been applied as *in situ* lactic acid producers, particularly in comminuted meat (Reddy, Henrickson & Olsen, 1970; Reddy, Chen & Patel, 1975; Raccach & Baker, 1978; Raccach *et al.*, 1979). Recently Murali *et al.* (1985) achieved suppression of the spoilage microflora on fresh mutton by applying cultures of *Lactobacillus plantarum, Lactobacillus bulgaricus* and *Streptococcus lactis* onto the meat surfaces. All cultures proved inhibitory to psychotrophic Gram-negative rods, bacteria of the coli-aerogenes group, staphylococci and proteolytic and lipolytic spoilers. This effect was primarily attributed to lactic acid production. More has to be learned about the effects of this mode of lactic acid decontamination on colour stability. Recently Gill & Penney (1985) reported that vacuum packaged lamb treated with a *Lactobacillus* culture and stored for 9 weeks at −0.5°C had lower odour and flavour scores than controls. Extended storage (12 weeks) resulted in the development of off-colours and unacceptable 'dairy' flavours.

Physiological aspects of the use of lactic acid as a decontaminant

The human body may be challenged with lactic acid of endogenous and exogenous origin. Endogenous lactic acid, formed by the body itself, is L(+) or 'physiological' lactic acid. It is constantly formed in large amounts in carbohydrate and amino acid metabolism and it is found in all tissues, liquids, secreta and excreta of the body (Wagner, 1981). The metabolism of lactic acid is carefully regulated by hormones such as adrenalin, insulin, glucagon and corticosteroids. Several measurements indicate that man produces 117–144 g lactate/24 hr/70 kg (Connor *et al.*, 1982).

Exogeneous lactic acid enters the body mainly by intestinal resorption through foods or intestinal flora. Exogenous lactic acid, which partly consists of D(−) lactic acid, is resorbed well by the intestines. Hyperlactaemia as a direct result of food intake is rarely seen in man. Moreover, blood concentrations up to 13–17 μmol/ml are easily buffered (Brecht, 1967). From Table 1 and from other data not listed (Vreeman, 1981) the annual per capita consumption in The Netherlands may be estimated at 1.2 kg.

L(+) lactic acid is not considered toxic (FAO/WHO, 1967). Doses up to 1500 mg/kg or more will be tolerated by most mammals (Holten, 1971). There are some indications, however, that infants have difficulties in metabolizing D(−) lactic acid (FAO/WHO, 1974). Ballabriga, Conde & Gallort-Catalla (1970) observed clinical symptoms of lactic acid acidosis in infants on a diet of 800 mg/kg body weight D(−) or DL lactic acid, which had been administered over a period of 12 days in succession. L(+) lactic acid alone did not elicit any such symptoms. The FAO/WHO report 'Evaluation of certain Food Additives' (1974) states, in summarizing, that it is not necessary to assess an ADI (acceptable daily intake) for lactic acid and lactate.

A survey on legislative aspects

Legislation in the major meat producing countries as to the authorization of decontaminating agents in general is very unclear. All meat inspection laws contain some paragraph indicating that it is prohibited to treat meat with foreign substances or with

Table 5. The interpretation of the legislation on the use of lactic acid as a meat decontaminant in the European Economic Community and in some other major meat producing countries (July 1985)*

Country	Allowed	No decision	Prohibited
Belgium	X		
Denmark		X	
Federal Republic of Germany	X		
France			X
Greece		X	
Ireland		X	
Italy		X	
Luxemburg			X
Portugal		X	
Spain		X	
The Netherlands			X
United Kingdom		X	
Argentina		X	
Australia		X	
New Zealand			X
United States		X	

*Based on data provided by the local agricultural authorities.

additives, unless these are specifically listed. A survey on the relevant legislation within the European Economic Community as well as in four major meat producing other countries is presented in Table 5.

Until recently the responsibility to allow the import of meat having been treated with lactic acid was left to the individual member states (European Economic Community, 1964). However, the amendment to this directive (European Economic Community, 1983) which came into force January 1985, states under Article 5 that every member state "shall ensure that the following meat is not sent from its territory to that of another member state: . . . (sub d): fresh meat from animals to which substances likely to make the meat dangerous or harmful to human health has been administered and on which the Scientific Veterinary Committee has expressed its opinion". . . . Up to this moment the desirability of admission of lactic acid as a meat decontaminant has not officially been discussed by the EEC authorities. Consequently particularly meat exporting countries are reluctant to authorize any meat decontaminant, even for 'intramural' use.

Only Belgium has conditionally, i.e., for a certain experimental period, allowed the use of lactic acid (as well as ascorbic and citric acid) in aqueous solutions of a maximal concentration of 1 mg/kg for the purpose of decontaminating beef, sheep and poultry carcasses (Belgisch Staatsblad, 1983). Unintentionally, the use on pig carcasses was not included in the announcement in the Statute-Book. In May 1985, the Belgian High Council for Public Health received a recommendation from an expert committee to authorize the use of lactic acid also for pork (Prof. J. van Hoof, The University of Ghent, Belgium, pers. comm.).

In the Federal Republic of Germany the Acts referring to the use of additives for meat products will also allow the use of lactic acid for decontamination purposes (Bartels, 1977). The Bundesanstalt für Fleischforschung at Kulmbach indicates as a

(theoretical) exception meat intended for the production of minced meat, since the 'Hackfleisch-verordnung' (minced meat regulations) prohibits the use of any additive (Prof. H. Linke, pers. comm.).

In Australia the use of acetic acid as a decontaminant has been allowed. Similarly U.S.D.A. legislators are considering authorizing its use (Dr R.E. Engel, pers. comm.). The marketing of meats from these countries within the European Community may thus lead to unintentional ambiguity. Therefore the European authorities may in the near future wish to pronounce upon a possible authorization of lactic acid. Such a decision will require thorough consideration of the underlying microbiological and physiological data.

It is our opinion that the use of lactic acid should be allowed provided the plants conform to a number of specific requirements of GMP. Possibly an authorization under license is a suitable way to ensure appropriate use.

Retrospect

Our studies reported in this paper and those of other research groups similarly discussed suggest that the use of L(+) lactic acid in an appropriate concentration and at a suitable pH allows effective decontamination of meat surfaces. The observed low numbers of enteric CFU's cannot be achieved, not even by the most sophisticated system of integrated hygiene (Gerats *et al.*, 1981), nor by physical means. Hence lactic acid decontamination of carcasses, organs, and/or cuts followed by packaging will allow one to bring specific pathogen-free meats to the consumer's table—a long overdue challenge for the food industry.

The amount of L(+) lactic acid added in this way to the average daily dietary intake does not affect the latter significantly. Hence no health risks whatsoever need to be feared. Proper technology is available to apply lactic acid in such a way that the sensory properties of meat so treated are not adversely affected. The concern that terminal lactic acid decontamination may prompt relaxation of hygiene measures along processing lines finds no substantiation whatsoever in five decades experience in the dairy industry, where systematic pasteurization has been introduced in the early 1930s (Mossel, 1984b).

In conclusion health authorities are therefore well advised not to raise any hurdles to the application of lactic acid decontamination, obviously provided sanitary practices are at least maintained as they have been so far. On the other hand not allowing lactic acid decontamination means unnecessarily exposing the public to enteropathogenic organisms which will inevitably result in the order of 10^4 victims of food-transmitted enteritis per 10^6 population per annum (Mossel, 1984a). The choice seems indeed to be an easy one.

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References

Anonymous (1964). Synthesis process brightens lactic acid picture. *Chemical Engineering*, **71**, 82–84.

Aries, V., Cheney, P.A. & Mossel, D.A.A. (1982). Ecological studies on the occurrence of bacteria utilizing lactic acid at pH-values below 4.5. *Journal of Applied Bacteriology*, **52**, 345–351.

Baird Parker, A.C. (1980). Organic acids. In: *Microbial Ecology of Foods. I. Factors Affecting Life and Death of Microorganisms* (edited by J.H. Silliker). Pp. 126–135. New York: Academic Press.

Ballabriga, A., Conde, C. & Gallort-Catalla, A. (1970). Metabolic response of prematures to milk formulas with different lactic acid isomers or citric acid. *Helvetica Paedriatrica acta*, 25–34.

Banks, J.G. & Board, R.G. (1983). The incidence and level of contamination of British fresh sausages and ingredients with salmonellas. *Journal of Hygiene (Camb.)*, **90**, 213–223.

Bartels, H. (1977). Wirkstoff zum Besprühen der Fleischoberfläche frischer Slachttierkörper. *Fleischwirtschaft*, **57**, 11.

Belgisch Staatsblad (1983). *Officiële berichten*, *11739*, 29 September 1983.

Brecht, K. (1967). Muskelphysiologie. In: *Physiologie* (edited by W.D. Keidel). Pp. 262–294. Stuttgart: G. Thieme Verlag.

Childs, G.C. & Welsby, B. (1966). Continuous lactic acid fermentation. *Process Biochemistry*, **1** (8), 441–444.

Childers, A.B., Keahey, E.E. & Vincent, P.G. (1973). Sources of salmonellae contamination of meat following approved livestock slaughtering procedures. *Journal of Milk and Food Technology*, **36**, 635–638.

Chung, K.C. & Goepfert, J.M. (1970). Growth of *Salmonella* at low pH. *Journal of Food Science*, **35**, 326–328.

Connor, H., Woods, H.F., Ledingham, J.G.G. & Murray, J.D. (1982). A model of L(+) lactate metabolism in normal man. *Annals of Nutrition and Metabolism*, **26**, 254–263.

Corlett, D.A. & Brown, M.H. (1980). pH and acidity. In: *Microbial Ecology of Foods. I. Factors Affecting Life and Death of Microorganisms* (edited by J.H. Silliker). Pp. 92–111. New York: Academic Press.

Daly, C., Lachance, M., Sandine, W.E. & Elliker, P.R. (1973). Control of *Staphylococcus aureus* in sausage by starter cultures and chemical acidulation. *Journal of Food Science*, **38**, 426–430.

Dezeure-Wallays, Br. & Hoof, J. van (1980). Effect of lactic acid sprays on beef carcass contamination. *Proceedings European Meeting of Meat Research Workers, Vienna, N18*, 316–319.

Eklund, T. (1983). The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. *Journal of Applied Bacteriology*, **54**, 383–389.

European Economic Community. (1964). Council directive of 26th June 1964 on health problems affecting intracommunity trade in fresh meat (64/433 EEC). *Official Journal of the European Communities*, No. C 189/34.

European Economic Community. (1983). Council Directive of 7th February 1983 amending Directive 64/433 EEC on health problems affecting intracommunity trade in fresh meat (83/90 EEC). *Official Journal of the European Communities*, No. L 59/14.

FAO/WHO. (1967). Toxicological evaluation of some flavouring substances and non nutritive sweetening agents. *FAO Nutrition Meetings Report Series* no. 44a WHO/Food Add/68.33.

FAO/WHO. (1974). Toxicological evaluation of some food additives. 17th Report on the joint FAO/WHO Expert Committee on Food Additives. *FAO Nutrition Meetings Report Series* no 53. Rome. *WHO Technical Report Series*, Geneva, no. 539, 461–465.

Firstenberg-Eden, R. (1981). Attachment of bacteria to meat surfaces: a review. *Journal of Food Protection*, **44**, 602–607.

Geis, A., Singh, J. & Teuber, M. (1983). Potential of lactic acid streptococci to produce bacteriocin. *Applied and Environmental Microbiology*, **45**, 205–211.

Gerats, G.E., Snijders, J.M.A. & Logtestijn, J.G. van (1981). Slaughter techniques and bacterial contamination of pig carcasses. *Proceedings 27th European Meeting of Meat Research Workers, Vienna*, 198–200.

Gill, C.O. & Newton, K.G. (1982). Effect of lactic acid concentration on growth on meat of Gram negative psychrotrophs from a meat works. *Journal of Applied and Environmental Microbiology*, **43**, 284–288.

Gill, C.O. & Penney, N. (1985). Modification of in-pack conditions to extend the storage life of vacuum packaged lamb. *Meat Science*, **14**, 43–60.

Gilliland, S.E. & Speck, M.L. (1972). Interactions of food starter cultures and food-borne pathogens: lactic streptococci versus staphylococci and salmonellae. *Journal of Milk and Food Technology*, **35**, 307–310.

Gilliland, S.E. & Speck, M.L. (1977). Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative cultures. *Journal of Food Protection*, **40**, 820–823.

Goepfert, J.M. & Hicks, R. (1969). Effect of volatile fatty acid on *Salmonella typhimurium*. *Journal of Bacteriology*, **97**, 956–958.

Grau, F.H. (1980). Inhibition of the anaerobic

growth of *Brochotrix thermosphacta* by lactic acid. *Applied Environmental Microbiology*, **40**, 433–436.

Grau, F.H. (1981). Role of pH, lactate and anaerobiosis in controlling the growth of some fermentative Gram negative bacteria on beef. *Applied Environmental Microbiology*, **42**, 1043–1050.

Greer, G.G. (1982). Mechanism of beef shelf life extension of sorbate. *Journal of Food Protection*, **45**, 82–83.

Hamner, N.E. (1974). Metals section. In: *Corrosion data survey*. Publ. National Association of Corrosion Enginners, Houston (Texas). P. 110.

Holten, C.H. (1971). *Lactic Acid*. Weinheim: Verlag Chemie GmbH.

Huber, H. (1977). Die Technologie der Teigführungen auf dem Roggen-mehl-Sektor, *Die Brotindustrie*, **5**, 180–192.

Hunter, D.R. & Segel, I.H. (1973). Effect of weak acids on amino acid transport by *Penicillium chrysogenum*: Evidence for proton or charge gradient as the driving force. *Journal of Bacteriology*, **113**, 1184–1192.

Ingram, M. (1948). Fatigue musculaire, pH et prolifération bactérienne dans la viande. *Annales de l'Institut Pasteur*, Lille, **75**, 139–146.

Ingram, M., Ottoway, F.J.H. & Coppock, J.B.M. (1956). The preservative action of acid substances in food. *Chemistry and Industry*, **42**, 1154–1163.

Inskeep, G.C., Taylor, G.G. & Breitzke, W.C. (1952). Lactic acid from corn sugar—a staff-industry collaborative report. *Industrial and Engineering Chemistry*, **44**, 1955–1966.

Jarvis, B. & Burke, C.S. (1977). Practical and legislative aspects of the chemical preservatives of food. In: *Inhibition and Inactivation of Vegetative Microbes* (edited by F.A. Skinner & W.B. Hugo). Pp. 345–367. New York: Academic Press.

Kastner, C.L. (1982). Hot processing-Overview. In: *Meat Science and Technology International Symposium Proceedings* (edited by K.R. Franklin & H.R. Cross). Pp. 148–168. Lincoln-Nebraska, Nov 1–4, Publ. National Live Stock and Meat Board, Chicago (Ill.).

Ketelaere, A. de, Demeyer, D., Vandekerckhove, P. & Vervaeke, I. (1974). Stoichiometry of carbohydrate fermentation during dry sausage ripening. *Journal of Food Science*, **39**, 297–300.

Klupsch, H.J. (1982). L(+) und D(−) Milchsäure in Sauermilchprodukten. *Deutsche Milchwissenschaft*, **33**, 268–272.

Krusch, U. (1978). Ernährungsphysiologische Gesichtspunkte der L(+) und D(−) Milchsäure in Sauermilchprodukten. *Kieler Milchwirtschaftliche Forschungsberichten*, **30**, 341–346.

Labots, H., Logtenberg, H., Stekelenburg, F.K. & Snijders, J.M.A. (1983). Study into the possibilities to reduce the *Salmonella* colony count on pig carcasses under practical conditions. Central Institute for Nutrition and Food Research, TNO, Zeist, The Netherlands, Report T 83-183/110 264. (In Dutch.)

Lawrie, R.A. (1979). *Meat Science* (3rd Edn.). Pergamon International Library of Science, Technology, Engineering and Social Studies. Robert Maxwell, M.C. Oxford. P. 358.

Levine, A.S. & Fellers, C.R. (1940). Action of acetic acid on food spoiling microorganisms. *Journal of Bacteriology*, **39**, 449–515.

Lockwood, L.B., Hunter, D.E. & Zienty, M. (1965). Lactic acid. *Annals of the New York Academy of Sciences*, **119**, 854–867.

Lueck, E. (1980). *Antimicrobial Food Additives*. P. 265. Berlin: Springer Verlag.

Maeris, B.J. (1975). Mechanism of benzoic acid uptake by *Saccharomyces cerevisiae*. *Applied Microbiology*, **30**, 503–506.

Merck index (9th edn.). (1976). Edited by M. Windholz. (NJ), U.S.A.: Merck & Co. Inc. Rohway.

Miall, L.M. (1978). Primary products of metabolisms. In: *Economic Microbiology, Vol. II* (edited by A.H. Rose). Pp. 95–98. New York: Academic Press.

Mossel, D.A.A. (1982). Marker (index and indicator) organisms in food and drinking water. Semantics, ecology, taxonomy and enumeration. *Antonie van Leeuwenhoek*, **48**, 609–611.

Mossel, D.A.A. (1984a). Intervention as the rational approach to control diseases of microbial etiology transmitted by foods. *Journal of Food Safety*, **6**, 89–104.

Mossel, D.A.A. (1984b). Seventy-five years of longitudinally integrated microbiological safety assurance in the dairy industry in The Netherlands. *Netherlands Milk and Dairy Journal*, **37**, 240–244.

Mossel, D.A.A. & Bruin, A.S. de (1960). The survival of *Enterobacteriaceae* in acid liquid foods at different temperatures. *Annales de l'Institut Pasteur*, Paris, **11**, 65–72.

Mossel, D.A.A. & Van Netten, P. (1984). Harmful effects of selective media on stresed microorganisms: Nature and Remedies. In: The Revival of Injured Microorganisms (edited by M.H.E. Andrew & A.D. Russell). Pp. 329–369. London: Academic Press.

Mossel, D.A.A., Veldman, A. & Eelderink, I. (1980). Comparison of the effects of liquid medium repair and the incorporation of catalase in MacConkey type media on the recovery of *Enterobacteriaceae* sublethally stressed by freezing. *Journal of Applied Bacteriology*, **49**, 405–419.

Mossel, D.A.A., Bonants-van Laarhoven, T.M.G., Ligtenberg-Merkus, A.M.Th & Werdler, M.E.B.

(1983). Quality assurance of selective culture media for bacteria, moulds and yeasts. An attempt at standardisation at the international level. *Journal of Applied Bacteriology*, **54**, 313–317.

Mountney, G.J. & O'Malley, J. (1965). Acids as poultry meat preservatives. *Poultry Science*, **44**, 582–586.

Mulder, S.J. & Krol, B. (1975). Der Einfluss der Milchsäure auf die Keimflora und die Farbe frischen Fleisches. *Fleischwirtschaft*, **55**, 1255–1258.

Murali, H.S., Leela, R.K., Sankaran, R. & Sharma, T.R. (1985). Effect of some lactic cultures on the natural microflora of mutton. *Chemie Mikrobiologie Technologie der Lebensmittel*, **9**, 19–23.

Netherlands Commodity Board for Livestock and Meat (1984). Annual Statistical Report 1983. Publ. P.V.V., P.O. Box 5805, 2280 HV Rijswijk (ZH), The Netherlands.

Nanninga, G.L. (1983). Fermentative production of lactic acid. *Antonie van Leeuwenhoek*, **49**, 86.

Ockerman, H.W., Borton, R.J., Cahill, V.R., Parett, N.A. & Hoffman, H.D. (1974). Use of acetic and lactic acid to control the quantity of microorganisms on lamb carcasses. *Journal of Milk and Food Technology*, **37**, 203–204.

Osthold, W., Shin, H.K., Dresel, J. & Leistner, L. (1983). Verbesserung der Haltbarkeit von Slachttierkorpern, herbeigefuhrt durch Oberflachenbehandlung mit einem Säurespray. *Fleischwirtschaft*, **63**, 603–605.

Park, H.S. & Martin, E.H. (1972). Inactivation of *Salmonella typhimurium* by sorbic acid. *Journal of Milk and Food Technology*, **35**, 532–539.

Patterson, J.T. & Gibbs, P.A. (1979). Vacuum packaging of bovine edible offal. *Meat Scicence*, **3**, 209–222.

Price, R.J. & Lee, J.S. (1970). Inhibition of *Pseudomonas* species by hydrogen peroxide producing Lactobacilli. *Journal of Milk and Food Technology*, **33**, 13–16.

Raccach, M. & Baker, R.C. (1978). Lactic acid bacteria as an antispoilage and safety factor in cooked, mechanically deboned poultry meat. *Journal of Food Protection*, **41**, 703–705.

Raccach, M. & Baker, R.C. and Regenstein, J.M. & Mulnix, E.J. (1979). The potential application of microbial antagonism to extended storage stability of a flesh type food. *Journal of Food Science*, **44**, 43–46.

Reddy, G.V. & Shahani, K.M. (1971). Isolation of an antibiotic from *Lactobacillus bulgaricus*. *Journal of Dairy Science*, **54**, 748.

Reddy, S.G., Henrickson, R.L. & Olson, H.C. (1970). The influence of lactic cultures on ground beef quality. *Journal of Food Science*, **35**, 787–791.

Reddy, S.G., Chen, M.L. & Patel, P.J. (1975). Influence of lactic cultures on the biochemical, bacterial and organoleptic changes in beef. *Journal of Food Science*, **40**, 314–318.

Roberts, J.D. & Caserio, M.C. (1964). *Basic Principles of Organic Chemistry*. Pp. 588–593. Menlo Park, California: W.A. Benjamin Inc.

Rubin, H.E., Nerad, T. & Vaughan, F. (1982). Lactate acid inhibition of *Salmonella typhimurium* in yogurt. *Journal of Dairy Science*, **65**, 197–203.

Smulders, F.J.M. & Korteknie, F. (1985). Control of the bacteriological condition of calf brain. II. Effects of lactic acid decontamination and frozen storage. *International Journal of Food Microbiology*, **2**, 293–299.

Smulders, F.J.M. & Woolthuis, C.H.J. (1983). Influence of two levels of hygiene on the microbiological condition of veal as a product of two slaughtering/processing sequences. *Journal of Food Protection*, **46**, 1032–1035.

Smulders, F.J.M. & Woolthuis, C.H.J. (1985). The immediate and delayed microbiological effects of lactic acid decontamination of calf carcasses. The influence on conventionally versus hot boned and vacuum packaged cuts. *Journal of Food Protection*, **48**, 838–847.

Smulders, F.J.M., Korteknie, F. & Woolthuis, C.H.J. (1985). Control of the bacteriological condition of calf brain. I. Impact of improving hygiene. *International Journal of Food Microbiology*, **2**, 169–176.

Snijders, J.M.A., Schoenmakers, M.J.G., Gerats, G.E. & Pijper, T.W. de (1979). Dekontamination slachtwärme Rinderkörper mit organischen Säuren. *Fleischwirtschaft*, **59**, 656–663.

Snijders, J.M.A., Janssen, M.H.W., Gerats, G.E. & Corstiaensen, G.P. (1984). A comparative study of sampling techniques for monitoring carcass contamination. *International Journal of Food Microbiology*, **1**, 229–236.

Snijders, J.M.A., Logtestijn, J.G. van, Mossel, D.A.A. & Smulders, F.J.M. (1985). Lactic acid as a decontaminant in slaughter and processing procedures. *Veterinary Quarterly*, **7**, 277–282.

Spicher, G. & Lönner, C. (1985). Die Mikroflora des Sauerteiges XXI. Mitteilung: Die in Sauerteig schwedischer Bäckereien vorkommenden Lactobacillen. *Zeitschrift für Lebensmittel- Untersuchung und -Forschung*, **181**, 9–13.

Talon, R., Labadie, J. & Larpent, J.R. (1980). Characterization of the inhibitory power of *Lactobacillus* of meat origin. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene, I Abt. Originale B*, **170**, 133–142.

Upreti, G.C. & Hillsdill, R.D. (1975). Production and mode of action of lactocin 27: bacteriocin from a homofermentative *Lactobacillus*. *Antimicrobial Agents and Chemotherapy*, **7**, 139–145.

Van der Marel, G.M., van Logtestijn, J.G. & Mossel, D.A.A. (1986). Decontamination of

broiler carcasses by lactic acid. *International Journal of Food Microbiology*. (In press.)

Van Netten, P. & Mossel, D.A.A. (1980). The ecological consequences of decontaminating raw meat surfaces with lactic acid. *Archiv für Lebensmittelhygiene*, **31**, 190–191.

Van Netten, P., Werdler, M.E.B. & Mossel, D.A.A. (1982). Assessment of the ecological impact of lactic acid decontamination of fresh meat surfaces. *Journal of Applied Bacteriology*, **53**, XII.

Van Netten, P., Zee, H. van der & Mossel, D.A.A. (1984). A note on catalase enhanced revovery of acid injured cells of Gram negative bacteria and its consequences for the assessment of the lethality of L-lactic acid decontamination of raw meat surfaces. *Journal of Applied Bacteriology*, **57**, 169–173.

Visser, I.J.R. & Bijker, P.G.H. (1985). The microbial flora of variety meats, vacuum packaged immediately after slaughter. *Antonie van Leeuwenhoek*, **51**, 605–606.

Vreeman, G. (1981). *Survey of sales of lactic acid and lactates in The Netherlands in 1979 and 1980, classified to marketing structures and functions*. C.V. Chemie Combinatie Amsterdam, P.O. Box 21, 4200 AA Gorinchem, The Netherlands. (In Dutch.)

Wagner, K.H. (1981). Stand der Erkentnisse uber links- und rechtsdrehende Milchsäure und deren Ernährung-physiologische Bedeutung. *Deutsche Milchwirtschaft*, **32**, 733–742.

Whittier, E.O. & Rogers, L.A. (1931). Continuous fermentation in the production of lactic acid. *Industrial and Engineering Chemistry* (industrial section), **23**, 532–534.

Woolford, M.K. (1975). Microbiological screening of food preservatives, cold sterilants and specific antimicrobial agents as potential silage additives. *Journal of the Science of Food and Agriculture*, **26**, 229–237.

Woolthuis, C.H.J. & Smulders, F.J.M. (1985). Microbial decontamination of calf carcasses by lactic acid sprays. *Journal of Food Protection*, **48**, 832–837.

Woolthuis, C.H.J., Mossel, D.A.A., Logtestijn, J.G. van, Kruijf, J.M. de & Smulders, F.J.M. (1984). Microbial decontamination of porcine liver with lactic acid and hot water. *Journal of Food Protection*, **47**, 220–226.

Wyss, O. (1948). *Advances in Food Research*. P. 373. New York: Academic Press.

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Effect of chilled storage of radiation-pasteurized chicken carcasses on the eating quality of the resultant cooked meat*

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Summary

Chicken carcasses were γ-irradiated with about 3.7 kGy whilst at 0°C, and stored at from +1° to +2°C. Sensory panels found that the eating quality of leg meat was satisfactory for at least 1 week, and decreased after about 3 weeks. Breast meat changed, but was satisfactory for about 3 weeks, decreasing in quality after about 4 weeks.

Introduction

The progress of food irradiation has been the theme of several international conferences. It has been extensively reviewed (e.g., Desrosier & Rosenstock, 1960; Urbain, 1978, 1983) and has reached the popular press (Begley & Bruno, 1983). Following a long study of its public health aspects, clearance for its use has been given in several countries, with product and dosage limitations: in Israel, poultry carcasses may now be treated with up to 7 kGy (Israel Official Gazette, 1982).

The commercial distribution of refrigerated but unfrozen chicken carcasses requires them to have a shelf life measured in weeks rather than days. This in turn depends on such clearly objective measures as microbiological quality, and also on subjective measures such as appearance, colour and odour. These are necessary qualifications at the point of sale, but they do not provide any guarantee *per se* of the eating quality. The present purpose was to estimate the storage time possible for such irradiated-and-refrigerated chicken carcasses, whilst maintaining a satisfactory eating quality.

'Quality' is an elusive term, although everyone is quite sure that they know what they mean when they use the word (Van Arsdel, Copley & Olsen, 1969). A pragmatic approach was adopted in this study, and each assessor was free to integrate in his own way all his sensory reactions regarding the cooked product (appearance, odour, texture, taste, etc.) into an overall 'quality' composite (Kramer & Twigg, 1962; Chichester *et al.*, 1969): none registered any difficulty in doing so.

Early work in our own laboratories indicated that (raw) whole chicken carcasses γ-irradiated with 2.5 kGy and stored at +1°C had a shelf life of perhaps 11–16 days, as judged by the acceptability of their odour. Irradiation at from 2 to 4.5 kGy reduced the initial total aerobic mesophilic count by a factor of from 10^3 to 10^4; during subsequent storage at +4°C, the total count gradually rose to its initial value after about 30 days.

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The pathogenic bacteria more sensitive to irradiation, *Salmonella*, *E. coli*, coagulase-positive *Staphylococcus aureus* and sulphite-reducing *Clostridium* had been reduced to below the enumeration level. This improved bacterial status is, however, quite distinct from sensory acceptability (Desrosier & Rosenstock, 1960).

It was also found that the pH of untreated chicken leg meat generally increases on storage at +4°C by perhaps 0.5 unit in a month, probably as a result of microbiological activity. Irradiation at 2 and 3 kGy did not retard this increase, whereas 3.75 and 4.5 kGy did. For the present trials, therefore, the dose used was approximately 3.7 kGy.

Materials and methods

Broiler chicken carcasses were prepared and packed commercially in polyester trays at two industrial slaughterhouses and in accordance with Orthodox Jewish practice (Regenstein & Regenstein, 1979). Leg meat and breast meat were packed separately, on the bone. For each of the two series of trials here reported, some of the trays of meat were deep-frozen and stored at −18°C. Other trays of the same meat were irradiated as follows whilst at 0°C, and stored at from +1° to +2°C: leg meat: 3.6±0.2 kGy and breast meat: 3.8±0.5 kGy.

For the first taste panel in each series, unfrozen and unirradiated meat from the same batch was used as a further comparison, after overnight refrigeration. Fresh batches of similar meat had to be used for the subsequent taste panels, as preliminary tests had indicated that chilled storage alone, with neither freezing nor irradiation, would allow a shelf life at acceptable quality for only about 4 days.

The frozen meat was thawed in refrigerated air overnight. It was then cooked in water with the addition of mild (background) spicing (Bartov, Basker & Angel, 1983). Chilled irradiated meat was prepared similarly, as was the untreated meat. After cooking, the products were allowed to cool, manually deboned, cut into bite-sized pieces, and refrigerated overnight at +4°C. They were presented at room temperature to a taste panel as a (2 *versus* 2 *versus* 2) trihedral test (Basker, 1981). The panels were composed principally of veterinarians, researchers, technicians and clerks at our respective institutions, with previous experience in taste panel procedures; they were not preselected in any way.

The panellists were requested to (a) attempt identification of the sample pairs, (b) rank their preferences for the various pairs, (c) rate the hedonic qualities on a 13-point pictorial (Smiley) scale including a neutral rating and (d) score the overall eating qualities on a scale of from zero (lowest possible) to 10 units. The replies to the three last questions were considered only when correct sample-pair identifications had been made; the correct identifiers at each session thus constituted sub-panels of discriminators.

For each taste panel session, the statistical significance of the number of correct identifications of the sample pairs was determined (Basker, 1981). The significance of the preference rank totals at each panel session was determined according to Kahan *et al.* (1973). Except for one panel session, the non-parametric pictorial ratings were compared by the Dwass-Gabriel procedure (Sokal & Rohlf, 1981); in the remaining session (leg meat, day 23), the number of correct identifiers of one product was too low for this procedure to be applied: by default, the ratings of the three possible product-pairs at this session were compared by the Kolmogorov-Smirnov two-sample test (Siegel, 1956; Pearson & Hartley, 1972). The quality scores were compared by Duncan's (1955) multiple range test. The storage times reported below refer to the number of days between irradiation and cooking, and between freezing and thawing.

Results and discussion

Leg meat

After 1 day of storage, the panel as a whole (48 assessors) did not distinguish significantly among the three products. The sub-panel showed a slightly greater preference ($0.01 < P < 0.05$) for the irradiated product; no significant differences were found among their non-parametric pictorial ratings or quality scores.

After 8 days' storage, the panel as a whole (35 assessors) again did not distinguish among the three products. The sub-panel preferred the untreated product ($P < 0.01$), but no significant differences were found among their non-parametric pictorial ratings or quality scores.

After 20 days' storage, the panel as a whole (23 assessors) distinguished among the three products ($0.001 < P < 0.01$). The sub-panel showed a slightly lower preference ($0.01 < P < 0.05$) for the irradiated product; no significant differences were found among their non-parametric pictorial ratings, but the quality score of the irradiated product was lower ($P < 0.05$) than that of the two other products.

After 23 days' storage, the panel as a whole (42 assessors) distinguished between the irradiated product and the two others ($0.001 < P < 0.01$). The sub-panel showed a lower preference ($P < 0.01$) for the irradiated product; no significant differences were found among their non-parametric pictorial ratings, but the quality score of the irradiated product was lower ($P < 0.05$) than that of the frozen product, with the untreated product in an intermediate position. After 29 days' storage, small mould colonies were detected on the irradiated product, and the trial was terminated.

Overall, the quality scores of the frozen product were found to be stable over the 23-day storage period, i.e., they were not found to be significantly correlated with the storage time. The quality scores of the untreated product fluctuated somewhat, reflecting its different origins. The quality scores of the irradiated product showed a significant decrease between 8 and 20 days' storage, by the multiple range test (Duncan, 1955). The change in the eating quality of the chilled, irradiated product can be calculated as a function of storage time:

$$\text{Quality score} = 7.32 - 0.13\,(\text{days}), \quad (1)$$
$$(N_c \times 45,\ r = -0.38,\ \text{d.f.} = 43,\ 0.001 < P < 0.001)$$

where N_c is the total number of correct identifiers. The pictorial ratings can be grouped to show a similar change:

$$\text{Better-than-neutral ratings}\ (\%) = 86.3 - 2.1\,(\text{days}), \quad (2)$$
$$(N_p = 4,\ r = -0.996,\ \text{d.f.} = 2,\ 0.001 < P < 0.01)$$

where N_p is the number of panels, and the standard error of the estimate is 2.3.

Breast meat

After 1 day of storage, the panel as a whole (43 assessors) did not distinguish among the three products. The sub-panel showed a slightly greater preference ($0.01 < P < 0.05$) for the frozen product; no significant differences were found among their non-parametric pictorial ratings or quality scores.

After 12 days' storage, the panel as a whole (45 assessors) distinguished among the three products with varying significance: for the frozen product, $0.01 < P < 0.05$; for the irradiated product, $0.001 < P < 0.01$; and for the untreated product, $P < 0.0001$.

The sub-panel showed no preference among the three products; no significant differences were found among their non-parametric pictorial ratings or quality scores.

After 21 days' storage, the panel as a whole (47 assessors) distinguished among the three products: for the frozen and the irradiated products, $0.001 < P < 0.01$; and for the untreated product, $0.0001 < P < 0.001$. The sub-panel showed preference ($P < 0.01$) for the frozen product, the irradiated product occupied an intermediate position, and the untreated product was least preferred ($P < 0.01$). The non-parametric pictorial ratings of the untreated product were lower than those of the frozen ($P < 0.01$) and irradiated ($0.01 < P < 0.05$) products. The quality score of the frozen product was higher, and that of the untreated product was lower, than that of the irradiated product ($P < 0.05$).

After 28 days' storage, the panel as a whole (40 assessors) distinguished the irradiated product from the two others ($0.001 < P < 0.01$). The sub-panel showed no preferences among the three products; no significant differences were found among their non-parametric pictorial ratings or quality scores. After 35 days' storage, small mould colonies were detected on the irradiated product, and the trial was terminated.

Overall, the quality score fluctuations of the frozen and untreated products closely resembled those for leg meat. The quality score of the irradiated (breast meat) product showed a significant decrease between 21 and 28 days' storage, by the multiple range test (Duncan, 1955). The change in the eating quality of the chilled, irradiated product can be calculated as a function of storage time:

$$\text{Quality score} = 7.82 - 0.08\,(\text{days}), \quad (3)$$
$$(N_c = 60, r = -0.36, \text{d.f.} = 58, 0.001 < P < 0.01).$$

The pictorial ratings can be grouped to show a similar change:

$$\text{Better-than-neutral ratings}\,(\%) = 82.4 - 0.8\,(\text{days}), \quad (4)$$
$$(N_p = 4, r = -0.92, \text{d.f.} = 2, 0.05 < P < 0.10)$$

where the standard error of the estimate is 5.2.

Conclusions

Chicken leg meat, irradiated with 3.6 kGy and stored at +2°C, was found to retain satisfactory eating quality for about 2 weeks, with the quality decreasing appreciably during a further week's storage. Chicken breast meat, irradiated with 3.8 kGy and stored at +1°C, was found to retain satisfactory eating quality for about 3 weeks, with the quality decreasing appreciably during a further week's storage.

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References

Bartov, I., Basker, D. & Angel, S. (1983). Effect of dietary vitamin E on the stability and sensory quality of turkey meat. *Poultry Science*, **62**, 1224–1230.

Basker, D. (1981). Further polyhedral taste panel difference tests. *Journal of Food Quality*, **4**, 229–232.

Begley, S. & Bruno, M. (1983). A high-energy food fight. *Newsweek*, **51** (13), 72.

Chichester, C.O., Ayres, J.C., Goldblith, S.A., Joslyn, M.A., Milner, R.T., Powers, J.J. & Schweigert, B.S. (Eds) (1969). *101 Problems in Food Science and Technology*. 29. U.S. Dept of Health, Education and Welfare, Washington, D.C.

Desrosier, N.W. & Rosenstock, H.M. (1960). *Radiation Technology*. Westport, CT: Avi Publishing Company, Inc.

Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*, **11**, 1–42.

Israel Official Gazette (1982). {Public Health Ordinance (Preservation of Foodstuffs by Radiation) (Amended)}. *Collection of Regulations*, **4354**, 1073. (In Hebrew.)

Kahan, G., Cooper, D., Papavasilio, A. & Kramer, A. (1973). Expanded tables for determining significance of differences for ranked data. *Food Technology*, **27** (5), 63–69.

Kramer, A. & Twigg, B.A. (1962). *Fundamentals of Quality Control for the Food Industry*. P. 10. Westport, CT: Avi Publishing Company, Inc.

Pearson, E.S. & Hartley, H.D. (1972). *Biometrika Tables for Statisticians*. Vol. 2. Cambridge: Cambridge University Press.

Regenstein, J.M. & Regenstein, C.E. (1979). An introduction to the kosher dietary laws for food scientists and food processors. *Food Technology*, **33** (1), 89–99.

Siegel, S. (1956). *Nonparametric Statistics*. New York: McGraw-Hill Book Co. Inc.

Sokal, R.R. & Rohlf, F.J. (1981). *Biometry* (2nd edn.). P. 437. New York: W.H. Freeman and Co.

Urbain, W.M. (1978). Food irradiation. *Advances in Food Research*, **24**, 155–227.

Urbain, W.M. (1983). Radurization and radicidation: meat and poultry. In: *Preservation of Food by Ionizing Radiation* (edited by E.S. Josephson & M.S. Peterson), Vol. 3. Pp. 1–11. Boca Raton, FL: CRC Press, Inc.

Van Arsdel, W.B., Copley, M.J. & Olson, R.L. (1969). *Quality and Stability of Frozen Foods*. P. 3. New York: Wiley-Interscience.

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Studies on size distribution of skipjack tuna (*Katsuwonus pelamis*): effect on chemical composition and implications for its utilization

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Summary

Size and composition of skipjack tuna in Nigeria's Exclusive Economic Zone were investigated between May 1982 and January 1983. Monthly average length±standard deviation varied between 41.65±2.64 cm in January 1983 and 46.60±3.02 cm in September 1982. Corresponding average weight varied between 1.34±0.18 and 1.90±0.37 kg. After logarithmic transformation of the variables (length and weight), a correlation coefficient of 0.901 was obtained. Frequency distribution of length classes enabled three size groups (small, medium and large) to be distinguished. Moisture, protein and ash content of fillets were not significantly affected by the size of the fish, but lipid content increased significantly ($P < 0.05$) with size. Widest variations were observed in salt content, which decreased significantly ($P < 0.05$) with increase in size. All chemical components were significantly affected ($P < 0.05$) by seasonal variation. The implications for the fishery and utilization of this resource are discussed.

Introduction

The intrinsic quality of fish can affect its suitability for processing. According to Connell & Hardy (1982) it includes chemical composition, texture, and the degree of parasitization. Many factors have been shown to influence body composition (Love, 1970). Large variations occur in proximate composition both from species to species, and from fish to fish of the same species. Stansby (1962) suggested that these variations, often ascribed to geographical and seasonal factors, are in fact primarily due to the feed ingested, the metabolic rate and the mobility of the fish. In addition to the quality and quantity of food and environmental factors, the chemical composition is also known to be dependent on size and change with ontogenesis (Parker & Vanstone, 1966; Ehrlich, 1972; 1974). Most reports (Phillips, Livingstone & Poston, 1966; Groves, 1970; Gras *et al.*, 1967a and b) have related body composition to age, size, season and diet. This work reports a preliminary investigation on the size distribution, and its effect on the chemical composition, of skipjack tuna; one of the neglected and under-exploited fish resources in Nigeria's Exclusive Economic Zone (EEZ)—200 nautical miles off Nigerian coastal waters. The effect of seasonal variation on the chemical composition of this species is reported and potential utilization considered.

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Materials and methods

Skipjack tuna (*Katsuwonus pelamis*) were caught using the 'Pole and line' method. Seven trips were made (from May 1982 to January 1983) within Nigeria's EEZ. Sampling was carried out on the vessel taking cognisance of the difference in sizes of the catches. Some 500–700 pieces were sampled in each trip and were brine-frozen at sea.

Size distribution

(a) Length and weight measurement. Each fish was weighed and fork length measured from the tip of the upper jaw to the posterior tip of the shortest caudal ray (Miyake & Hayasi, 1978).

(b) Size class (length group/frequency measurement). The fish were divided into length frequency classes, on a monthly basis, according to the procedure described by Miyake & Hayasi (1978).

Chemical composition

For the purpose of chemical analysis the consignment for each month was divided into the following size grades:

Size grade	*Length*
1	35.0–42.9 cm
2	43.0–49.9 cm
3	50.0 cm or more

Ten fish from each size grade were randomly selected, washed with jets of clean fresh water, beheaded, eviscerated and filleted. Individual fillets were then homogenized in a Kenwood blender and triplicate samples used for analyses.

Moisture and ash were determined by the method of A.O.A.C. (1975), lipid by the method of Bligh & Dyer (1959), nitrogen by the micro-kjeldahl method (protein = 6.25×nitrogen) and salt content by the silver nitrate method of A.O.A.C. (1970). Data from each of the ten fishes of each size grade were then pooled and used to calculate the average for each constituent and size grade.

Statistical analysis

Mean values and standard deviations for length, weight and chemical composition were calculated. All results of chemical composition with respect to the size grades and the months were subjected to a two-way analysis of variance using the IBM Computer (SPSS, 1976 version). Treatment means were compared using the Duncan's multiple range test (Steel & Torrie, 1960). Regression analyses were carried out to establish the relationship between the transformed length and weight using the equation:

$$\log Y = a + b \log X$$

where Y = length (dependent variable)

and X = weight (independent variable).

Results and discussion

Size distribution

The average length and weight of skipjack tuna caught between May 1982 and

January 1983 are presented in Table 1. Average length was 44.20±3.15 cm* for May samples and increased gradually to 46.60±3.02 cm for September. It then declined to 42.88±1.80 cm in November (1982) and 41.65±2.64 cm in January (1983).

Table 1. Length and weight of skipjack tuna

	Length (cm)		Weight (kg)	
Month	Average*	Range	Average*	Range
May	44.20±3.15	40.0–59.0	1.42±0.45	1.09–3.00
June	45.17±3.13	40.5–55.0	1.77±0.39	1.14–3.70
July	45.92±2.14	42.0–51.9	1.74±0.25	1.18–2.40
August	45.96±3.26	36.0–50.8	1.83±0.35	1.20–3.08
September	46.60±3.02	38.5–51.5	1.90±0.37	1.25–2.40
November	42.88±1.70	40.0–50.0	1.34±0.18	1.00–2.10
January	41.65±2.64	38.0–54.0	1.39±0.33	1.00–3.10

*Mean±standard deviation.

Average weights showed a similar pattern of variation to average lengths and after logarithmic transformation of the dependent (length in cm) and independent (weight in g) variables, the regression equation was: $\log Y = 0.873+0.242 \log X$, with a correlation coefficient (r) of 0.901. The strong positive relationship indicated that the size of skipjack can adequately be described either by the length or the weight.

The present results in relation to the average length of skipjack are similar to those of Choo (1977) who reported an average length of 47.60 cm for skipjack caught in the East Central Atlantic. A better picture of the size distribution in Nigeria's EEZ is shown in histogram form in Fig. 1. In May (1982) length groups 42.0–43.9, 44.0–45.9 and 46.0–47.9 cm accounted for more than 70% of the total catch. In June and July dominant length groups also included 48.0–49.9 cm. However, while length groups 42.0–49.9 cm comprised more than 80% of the total catch in June, groups 44.0–49.9 cm comprised more than 80% of the total catch in July. In August, dominant length groups were 46.0–49.9 cm, comprising more than 65% of the total catch, and group 48.0–49.9 cm comprised more than 51% of the total catch for September. Relative to other months, smaller size groups were obtained in November (1982) and January (1983). Size groups between 40.0 and 43.9 cm comprised more than 77 and 67% of the total catches for these 2 months respectively.

In summary skipjack showed a tendency towards larger sizes between July and September, and towards smaller sizes in November and January (and probably through February and March, if the data is extrapolated from January to May). Growth has been described (Weatherley, 1972; Ehrlich, 1972; Denton & Yousef, 1976) as a labile process affected by a host of nutritional, physiological, biotic and climatic factors which vary in space and time. Interplay of such factors clearly become favourable for skipjack to grow and flourish during the period between July and September. This is confirmed by the data in Table 2 which showed the highest catch by weight during August and September.

*Figures preceded by ± in the text are standard deviations.

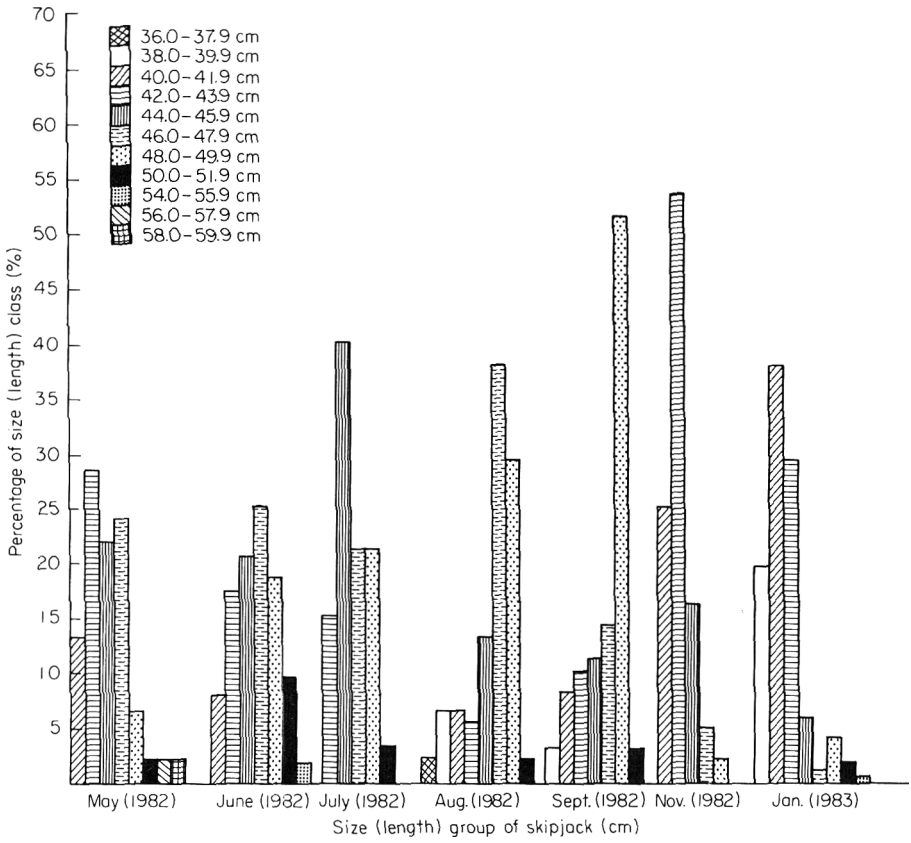

Figure 1. Histogram of monthly relative frequencies of size classes (by length) of skipjack tuna.

Table 2. Monthly catch of skipjack tuna during the survey period

Month	Total catch by vessel (metric tonne)	Amount of skipjack in total catch (metric tonne)
May	53.00	49.99
June	46.20	37.20
July	54.01	39.59
August	116.60	73.44
September	124.60	92.39
November*	75.90	55.89
January*	62.70	51.50

*Amount represented the catch for a trip lasting for more than 1 month.

Size is an important technological property of a fish. The seasonal distribution in the sizes of skipjack within Nigeria's EEZ may be of considerable significance to its fishery and subsequent utilization. Three distinct groups can be distinguished for fishing according to the period or season of the year: medium sizes 43–45 cm in May and June, large sizes 46–50 cm between July and September, and relatively small sizes between

November and probably March when conditions are becoming unfavourable and more suited to fishing for other species.

As observed by Connell & Hardy (1982) the small-sized fish are thinner and softer and may give a low yield of poorer quality flesh; filleting also gives a lower yield, and is labour intensive and costly in terms of machinery. Recovery of the flesh in the form of recovered mince, using deboning machines, presents problems for developing countries due to the relatively lower level of technological sophistication and prohibitive cost of imported machinery. Size of fish is, therefore, an important economic consideration. Skipjack fishery for large size could be carried out between July and September when catches could be sold profitably either as fresh, frozen or as fillets, and when conditions became unfavourable (between November and February), tuna fishery may be combined with fishery for other pelagics which can be used for canning.

Effect of size and seasonal variation on chemical composition

Data on proximate composition and salt content of skipjack tuna are presented in Table 3. Moisture, protein and ash content were not significantly affected by the size of the fish. Protein content increased slightly with size but the increases were not significant.

Lipid content increased significantly with size, consistent with the studies of Parker & Vanstone (1966) on Rainbow and Brook trout and related to the sexual maturity of large-sized skipjack tuna (Brown, 1957).

Growth rate is highest in early life and declines with age, which is manifested in an increase in body lipid and definite but slower increase in body protein (Thompson, 1963). The variations observed in the lipid and protein content of the different size grades of skipjack in the present study may be related to this phenomenon.

Wide variations were also obtained in the salt content of skipjack tuna. A decrease ($P < 0.05$) in salt content with increase in size, indicated that smaller-sized fish absorbed more salt per unit area than large fish during brine-freezing. The lower levels of salt in larger fish, with its possible effect on consumer acceptability, is a further reason in favour of fishing for larger sizes.

There was a significant ($P < 0.05$) seasonal effect on all the chemical components analysed. Protein content showed a non-significant decrease from May to September but increased significantly ($P < 0.05$) between November and January to levels of 24.95 ± 1.92 and $25.57 \pm 1.95\%$ respectively.

Throughout the survey period an increase in lipid content of the muscle corresponded with a decrease in moisture content. Lipid levels were lowest between November and January and moisture levels highest. Highest levels of lipid were obtained between July and September. These results confirm the inverse relationship between body fat and water content already established in fish (Stansby, 1962; Brown, 1957; and Lagler, Bardach & Miller, 1962).

Seasonal cyclical changes in flesh composition have been observed in all species. Connell (1980) reported that the fat content of herring flesh can change from below 1 to over 25% between the starvation period after spawning and the pre-spawning period. During this pre-spawning period, a corresponding reduction in water content is also observed. In the present study, the period between July and September may correspond to the feeding period of skipjack tuna in preparation for spawning.

Connell (1980) also reported that during this period the fat in most pelagic species is laid down in a very thin layer under the skin and that fish in this condition are difficult to brine successfully. This may have accounted for the significantly lower salt content in

Table 3. Percentage proximate composition* and salt content of the fillet of skipjack tuna as affected by size and seasonal variation

	Size grade														
	Moisture			Protein			Lipid			Ash			Salt		
Month	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
May	72.11	70.83	70.99	23.74	23.95	24.32	1.66	1.79	2.30	2.14	2.20	2.42	2.01	1.66	2.06
Average	71.31±0.99a			24.00±1.80ab			1.88±0.35a			2.25±0.09a			1.91±0.91a		
Range	70.01–73.22			20.94–25.94			1.60–2.20			2.01–2.48			1.66–2.36		
June	71.64	71.38	71.34	22.46	22.83	22.86	1.84	1.90	2.01	2.06	2.18	2.37	2.00	1.90	1.86
Average	71.45±0.78a			22.72±1.64b			1.92±0.25a			2.20±1.3a			1.92±0.80a		
Range	70.23–72.31			20.00–24.10			1.48–2.11			1.96–2.42			1.82–2.40		
July	71.92	71.21	71.12	22.25	22.35	22.45	2.01	2.40	2.56	2.22	2.34	2.45	2.08	1.47	1.03
Average	71.42±1.19a			22.35±1.55b			2.32±0.41b			2.34±0.21a			1.53±0.64b		
Range	69.17–72.43			19.32–24.02			1.95–2.75			2.04–2.50			1.01–2.50		
August	70.32	71.06	69.73	23.16	23.44	23.55	2.24	2.36	2.75	2.36	2.05	2.35	1.95	1.53	1.40
Average	70.37±1.00a			23.38±1.95b			2.45±0.46b			2.25±0.15a			1.63±0.29b		
Range	69.17–71.76			20.31–25.90			1.95–2.95			2.00–2.85			1.02–1.99		
September	70.34	69.56	68.89	22.29	22.55	22.53	2.46	2.59	3.17	1.58	1.76	1.72	1.17	0.89	0.97
Average	69.60±0.65ab			22.45±1.45b			2.74±0.51c			1.69±0.18b			1.01±0.38d		
Range	68.43–70.60			19.98–23.80			2.23–3.58			1.42–1.95			0.68–1.47		
November	75.25	74.50	73.66	25.36	24.21	25.29	1.49	1.60	1.68	1.52	1.70	1.75	1.29	1.05	0.98
Average	74.47±0.21c			24.95±1.92ac			1.59±0.23d			1.66±0.80b			1.11±0.29cd		
Range	73.23–75.65			22.13–27.78			1.40–1.79			1.47–1.87			0.77–1.43		
January	74.96	74.86	73.99	25.81	24.97	25.93	1.50	1.52	1.60	1.93	1.83	1.69	1.79	1.37	1.14
Average	74.60±0.70c			25.57±1.95c			1.54±0.19d			1.74±0.05b			1.43±0.21bc		
Range	72.58–75.81			21.10–27.49			1.41–1.67			1.65–1.99			0.83–1.99		
Average for the whole survey period	72.36	71.88	71.35	23.59	23.47	23.85	1.99X	2.02XY	2.30Y	1.94	2.01	2.11	1.75X	1.41XY	1.35Y

*Figures preceded by ± are standard deviations.

X,Y means followed by the same letter in the same horizontal line are not significantly different.

a, b, c, means followed by the same letters in the same vertical line are not significantly different.

the fillet of skipjack tuna between July and September. The high fat content between July and September for this species is also desirable for canning, for highest quality with excellent appearance, succulence and flavour.

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References

A.O.A.C. (1970). *Official Methods of Analysis*. 11th edn. Washington, D.C.

A.O.A.C. (1975). *Official Methods of Analysis*. 12th edn. Washington, D.C.

Bligh, E.E. & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911.

Brown, M.E. (ed.) (1975). *The Physiology of Fishes*, Vol 1. New York: Academic Press.

Choo, W.I. (1977). Report on the Korean bait-boat fishery in the Atlantic Ocean, 1975. ICCAT Collective Vol VI (SCRS-1976) (1): 69–71.

Connell, J.J. (1980). *Control of Fish Quality*, 2nd edn, Chapter 2, London: Fishing News Book Ltd.

Connell, J.J. & Hardy, R. (1982). *Trends in Fish Utilization*. A Buckland Foundation Book. Surrey, U.K.: Fishing News Books Ltd.

Denton, J.E. & Yousef, M.K. (1976). Body composition and organ weights of rainbow trout (*Salmo gairdneri*). *Journal of Fish Biology*, **8**, 489–499.

Ehrlich, K.F. (1972). *Morphological, behavioural and Chemical Changes During Growth and Starvation of Herring and Plaice Larvae*. Ph.D. Thesis, University of Stirling, Scotland.

Ehrlich, K.F. (1974). Chemical changes during growth and starvation of larval Pleuronectes platessa. *Marine Biology*, **24**, 33–48.

Gras, J., Reynaud, R., Gamoty, L., Frey, J. & Henry, J.C. (1976a). Biochemical study of fish. 1. Six-monthly variations in the water and protein content of the muscle tissue of rainbow trout (*Salmo gairdneri* Rich.). *Experientia*, **23**, 430–431.

Gras, J., Reynaud, R., Gamoty, L., Frey, J. & Henry, J.C. (1967b). Etude biochimique des poissons: 2 Variations semestrialles del la teneuren glycogene, en lipides totaux et en mineraux du tissu musculaire de la truite arc-en-ciel (*Salmo gairdnerii* Rich.). *Experientia*, **23**, 431–432.

Groves, T.D.D. (1970). Body composition changes during growth in young sockeye (*Oncorhynchus nerka*) in fresh water. *J. Fish. Res. Bd. Can.*, **27**, 929–942.

Lagler, K.F., Bardach, J.E. & Miller, R.R. (1962). *Ichthyology*. New York: John Wiley and Sons.

Love, R.M. (1970). *The Chemical Biology of Fishes*, Vol. I. London: Academic Press.

Miyake, M. & Hayasi, S. (1978). *Field Manual for Statistics and Sampling of Atlantic Tunas and Tuna-like Species*, 2nd edn. Madrid, Spain: International Commission for the Conservation of Atlantic Tunas.

Parker, R.R. & Vanstone, W.E. (1966). Changes in the chemical composition of central British Columbian pink salmon (Salmo salar) in early life. *J. Fish. Res. Bd. Can.*, **23**, 1353–1384.

Phillips, A.M. Jr., Livingstone, D.L. & Poston, H.A. (1966). The effect of changes in protein quality, calorie sources and levels upon the growth and chemical composition of brook trout. *Fish Res. Bull., N.Y.*, **29**, 6–7.

Stansby, M.E. (1962). Proximate composition of fish. In: *Fish in Nutrition* (edited by Eirik Heen & Rudolf Krueuzer), p. 55. London: Fishing News (Books) Ltd.

Steel, R.G.D. & Torrie, J.H. (1960). *Principles and Procedures of Statistics*. New York: McGraw-Hill Book Co.

Thompson, D.W. (1963). *On Growth and Form*. Cambridge: Cambridge University Press.

Weatherley, A.H. (ed.) (1972). *Growth and Ecology and Fish Populations*. London: Academic Press.

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Optimization of colour in commercial port blends

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Summary

Simple quantitative descriptive analysis coupled with ratio scaling techniques were used by a consumer panel for the objective assessment of fifteen ports, derived from blending Ruby, Tawny and White ports in varying proportions, for redness, brownness and intensity of colour. Deviation of the amounts of these attributes from those in the consumer's concept of an ideal port, both for Ruby and Tawny drinkers, was determined in relation to blend composition and L*, a* and b* values. Inverse simultaneous regression procedures showed the ports which best satisfied the two consumer subsets (Ruby and Tawny drinkers), in respect to all the sensory attributes, had chroma values of 23.0 and 21.8, and hue angles of 37.3 and 45.1° respectively. This corresponded to L*, a* and b* values of 86.13, 18.24 and 13.9 for the Ruby drinkers, and 92.1, 15.3 and 15.4 for the Tawny drinkers.

Introduction

With all products, whether wines or foods in general, it is essential to know just what it is the consumer is expecting when purchasing; what he or she considers acceptable as well as desirable. Such information is essential for effective marketing, product development and efficient research.

Conventional market research techniques, in general, tell the operator which of a set of products is preferred, hence providing information on the direction of the effect of altering some variable, but giving little indication of the magnitude of the effect, or how close the best of the products are to the customer's ideal. In attempts to provide better information on why one product is preferred to another, and the relative acceptability of products, a number of approaches have been developed. Vector and surface response models have been used to provide better information on the direction of acceptance and the position of ideal products (Williams, Langron & Arnold, 1983; Schiffman, Reynolds & Young, 1981). Hedonic information has also been linked with objective profile or similarity data, again to provide detailed information on the nature of ideal products and the direction in which a given product has to be changed to become more acceptable (Williams, 1983; A.A. Williams, unpublished results).

Although such profile descriptions coupled with consumer ratings, provides a great deal of information about a product and what is understood by quality, it is costly and time-consuming. The possibility of bypassing the trained panel, getting the consumer to provide profile information for a series of products and then using the same profile language to characterize their ideal product along similar lines to that described by Moskowitz & Chandler (1977) and Moskowitz, Stanley & Chandler (1977) offers a number of interesting possibilities. To be able to operate profiling type approaches with the consumer, however, it is necessary for the consumer to understand the idea of

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scaling and either to use a free choice approach in respect to terminology (Williams & Langron, 1984), or to use terms which convey the same meaning to all participants.

The following paper explores the possibility of obtaining information directly from the consumer using ratio scaling techniques and simple profiling procedures. It uses as a vehicle for the exercise blends of Tawny, Ruby and White ports, and attempts to find in terms of physical colour measurements the optimum value for each of the individual colour parameters being assessed, for both Tawny and Ruby port drinkers.

Materials and methods

Materials

The ports to be assessed were made by blending a Ruby, a commercial Tawny and a White port supplied by John Harvey & Sons Ltd. (Table 1).

Table 1. Composition of ports used in investigation into port colour

Blend no.	% Ruby	% Tawny	% White	Bottle no
1	100	0	0	7
2	75	25	0	8
3	50	50	0	3
4	25	75	0	6
5	0	100	0	11
6	75	0	25	2
7	50	0	50	14
8	25	0	75	9
9	0	0	100	1
10	0	75	25	12
11	0	50	50	10
12	0	25	75	15
13	25	50	25	4
14	50	25	25	13
15	25	25	50	5

Environment

Assessments took place in a secluded part of a stand at the 1979 Bristol Wine Fair. The surroundings and the table at which the samples were evaluated were white, and illumination was by tungsten filament spot lighting.

Assessors

Participants (fifty-eight) were selected from visitors to the above Fair. Only people who drank more than four glasses of port per month were selected. Demographic information and information on drinking habits are summarized in Table 2.

Sensory assessments

Introduction of concepts. Unlike trained panellists, the general public have little experience of scoring or the potential range of attributes in a product before being

Table 2. Demograpic and alcoholic beverage consumption data on assessors

Surveying Ports (as percentage)

No. of Respondents: 58

Socio-economic group	I	II	IIIN	IIIM	IV	V	No response
	6.2	39.6	29.3	10.3	8.6	1.7	5.2
Age range:	18–24	25–34	35–44	45–54	55–64	65+	No response
	22.4	62.2	17.2	8.6	13.7	1.7	3.4
Sex:	Male	Female	No response				
	75.8	20.7	3.4				
Marital status:	Married	Single	Others	No response			
	65.5	27.6	1.7	5.2			

General drinking habits:

	Beer (pts)	Cider	Ordinary wines	Sparkling wines	Sherry	Port	Vermouth	Spirits
4 or more glasses per week	44.8	3.4	56.8	6.8	17.2	24.0	1.7	29.3
1–3 glasses per week	12.0	10.3	32.7	8.6	24.0	34.0	13.7	29.3
1–3 glasses per month	15.5	13.8	6.9	13.7	24.0	29.0	15.5	5.2
Less frequently but on regular basis	6.9	22.4	17.2	27.5	12.0	12.0	15.5	13.8
On special occasions only	0	5.2	1.7	20.6	5.2	0	1.7	5.2
Never	20.6	44.8	1.7	17.2	17.2	0	51.7	17.2

Port drinking habits:

	Ruby	Tawny	White	Unidentified
16 or more glasses per month	12.5	19.6	1.8	0
4–15 glasses per month	19.6	23.2	5.4	0
1–3 glasses per month	32.1	17.8	5.4	8.9
Less frequently but on regular basis	7.1	21.4	17.8	3.4
On special occasions only	8.9	5.4	10.7	3.6
Never	26.8	12.5	59.9	83.9

Port preferences:	Ruby	Tawny	No preference
	34.5	50	15.2

asked to profile a set of samples. Hence, it was decided to use ratio scaling in which all attributes were scored with respect to the first sample presented, with no limitations on the order of presentation. Experiments conducted with people not associated with scientific sensory analysis indicated that they could readily comprehend the idea of ratio scaling and could apply it meaningfully to assessing attributes, in particular those associated with appearance (A.A. Williams unpublished results). To introduce the idea

of ratio scaling, participants in the Survey were first presented with a shape drawn on card and asked to give it a number indicating its size (they were instructed that it did not matter which number they used). The first shape was removed and they were then presented with a second shape and asked to give it a number indicating its size relative to the first shape. They repeated the exercise with three other shapes, each time being asked to give the shape a number indicating its size in relation to the others.

To introduce the idea of using the established scale to score an imaginary rather than a real product, participants were next asked to give the number which would correspond to the size (cross sectional area) of their ideal apple and also that of the largest apple they had ever come across.

Assessment of ports. Following the above exercise, participants were presented in turn with five ports (approximately 40 ml in a 4 oz standard wine glass), selected according to a balanced design from the fifteen blends. They were asked to use the same concepts as they had used with the shapes, giving a number to each of the attributes, redness, brownness and intensity of colour, which corresponded to its intensity in the first port. The remaining ports were then assessed in turn, the numbers being given to the attributes in each case reflecting the amounts relative to the first port assessed. Unlike the procedure when the shapes were presented, ports already assessed were not removed and could be retained for comparison purposes.

Participants were next asked to imagine their ideal port and, using the same attributes and scales, give the numbers which they believed this port would have. They were also asked to give the numbers which would correspond to the maximum amount of the same attributes they had come across in any port (not necessarily the same port).

Analysis

L*, a*, b* colour values, maximum absorbance (520 nm) and absorbance at 420 nm were measured using a Pye Unicam SP8-100 spectrophotometer with tristimulus colour measurement attachment scaled in 1976 CIELAB uniform colour space (Wyseki & Stiles, 1982). Cell path length was 1 mm and values were calculated for standard illumination A (tungsten filament) to match more closely that used during the actual sensory assessment. Observations were taken using a 1 mm cell in order to represent as closely as possible the thickness of the liquid in a tilted glass through which the majority of assessors looked when evaluating the colour of the ports.

Treatment of results

Scores for each of the three colour attributes for each port assessed by an individual were subtracted from those given by that participant for his or her ideal. The difference was standardized by dividing by the figure given for the maximum for that attribute in any port by that assessor. The maximum value was used in preference to the ideal value, as it was felt that as all assessors were regular port drinkers they should have similar experience of product ranges and that this figure would be the more uniform of the two.

Distances from the ideal for each port from assessors indicating a preference for Tawny ports and Ruby ports were pooled to provide two sets of means; one for assessors preferring Tawny ports and one for those preferring Ruby ports, for each attribute. This information was used to plot blend composition diagrams indicating blend regimes which deviated least from individuals' ideal Tawny and Ruby ports.

The deviations from ideal for each attribute were also regressed against L*, a* and b* values, hue angle ($\tan^{-1} b^*/a^*$), and chroma $(a^{*2}+b^{*2})^{1/2}$, factors being built into the

model to take into account port preference and any interaction which may exist between preferences and the scoring of the ports. The most suitable regression equations were then equated to zero (ideal value) to provide a series of simultaneous equations. These were solved to give the best overall ideal values allowing for the error structure in each of the individual equations using an inverse simultaneous regression procedure similar to that described by Williams (1959).

Results

Of the fifty-eight people interviewed, forty-nine provided satisfactory information and were used in the following analysis. The consensus deviation of each of the attributes (redness, browness and intensity of colour) for both Ruby and Tawny ports are presented in Figs 1 and 2. The shaded area in all figures corresponds to the blend composition closest to the ideal value for each of the attributes; the common areas of overlap and hence that blend region which best satisfies all criteria are shown in Figs 1(d) and 2(d).

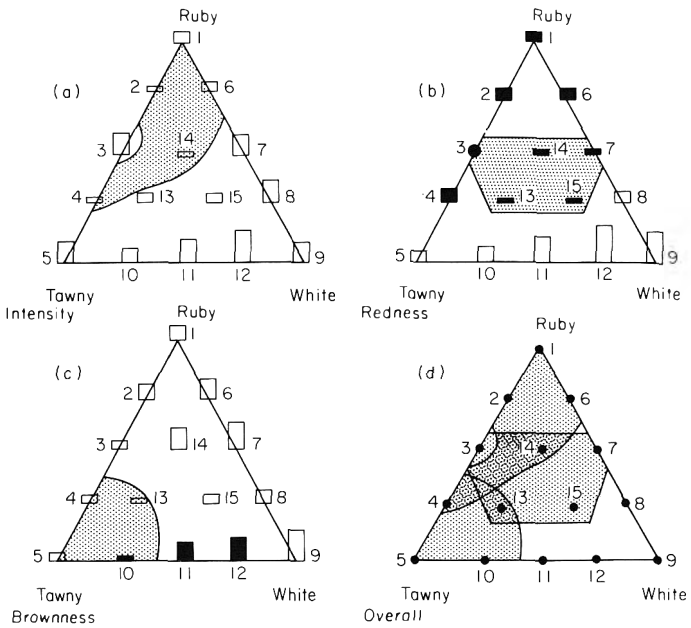

Figure 1. Deviation of ports from ideal (a) intensity of colour, (b) redness, (c) browness and (d) for overall assessors, preferring tawny ports ■ = greater than ideal, □ = less than ideal, ● = ideal.

The best Tawny port from the point of view of colour obtainable within the blends, would appear to be between blend 3 (50% Ruby, 50% Tawny, 0% White), blend 4 (25% Ruby, 75% Tawny, 0% White), blend 13 (25% Ruby, 50% Tawny, 25% White), blend 14 (50% Ruby, 25% Tawny, 25% White), i.e., between 33–37% Ruby, 57–63% Tawny and 0–10% White and corresponds to L* values between 89.26 and 92.56, a* values between 8.01 and 12.88, and b* values between 8.77 and 11.33.

The best Ruby port, on the other hand, would appear to be near the top left of the triangle (Fig. 2(d)) around blend 2 (75% Ruby, 25% Tawny) with L*, a*, b* values of 87.53, 14.42 and 11.80.

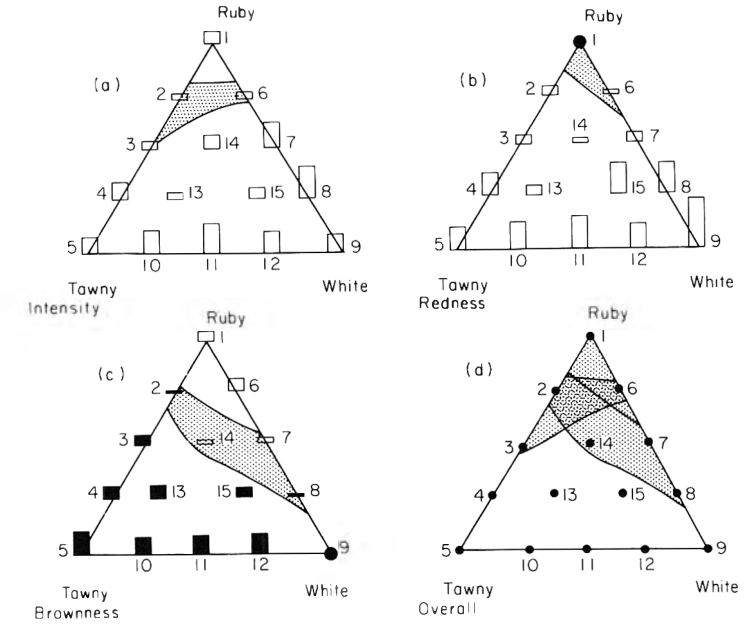

Figure 2. Deviation of ports from ideal (a) intensity of colour, (b) redness, (c) browness and (d) for overall assessors, preferring ruby ports: ■ = greater than in ideal, □ = less than in ideal, ● = ideal.

Only in the case of the Tawny ports does one find a blend which shows equal minimal deviation from ideal values for all attributes, and even in this case no attribute is satisfied completely.

Inspection of the various regressions of redness, brownness and intensity of colour against L*, a*, b* values, hue angle and chroma (Table 3) provided the significant

Table 3. Colour measurements of port blends

Blend no.	E^{10}_{520}	E^{10}_{420}	Illuminant A, 10° obs, 1 mm cell path length L*	a*	b*	Hue°	Chroma	Tint†
1	2.90	2.45	86.14	16.11	12.08	36.9	20.1	0.84
2	2.50	2.25	87.53	14.42	11.80	39.3	18.6	0.90
3	2.20	2.05	89.26	12.38	11.33	42.5	16.8	0.93
4	1.85	1.80	90.99	10.27	10.73	46.3	14.9	0.97
5	1.50	1.60	92.62	8.03	10.14	51.6	12.9	1.06
6	2.05	1.85	89.52	11.84	9.78	39.6	15.4	0.90
7	1.65	1.60	91.21	9.03	8.12	42.0	12.1	0.97
8	0.95	1.00	94.88	4.48	5.60	51.3	7.2	1.05
9	0.30	0.55	97.65	0.81	3.05	75.1	3.2	1.83
10	1.05	1.25	94.36	5.83	8.13	54.4	10.0	1.09
11	0.75	1.00	95.56	4.30	6.52	56.6	7.8	1.33
12	0.50	0.75	96.80	2.54	4.81	62.2	5.4	1.50
13	1.40	1.50	92.56	8.01	8.79	47.7	11.9	1.07
14	1.85	1.75	90.45	10.78	9.77	42.2	14.5	0.95
15	1.05	1.15	94.05	6.21	6.96	48.3	9.3	1.09

† = $E^{10}_{420}/E^{10}_{520}$

Table 4. Regression coefficients (with standard errors) of colour values against visual attributes

Score	Constant	L*	Hue angle ($\tan^{-1} b^*/a^*$)	Chroma ($\sqrt{a^2+b^2}$)	% Variance accounted for
Redness	+1.0	—	−0.024 (0.091)	—	94
Brownness	−21.8	0.22 (0.062)	—	0.13 (0.042)	49
Colour intensity	−0.49	—	—	0.020 (0.0044)	58

Table 5. Regression coefficients (with standard errors) of hue angle and chroma against visual attributes for assessors preferring tawny and ruby ports

Attribute	Constant (A)	Constant (B)	Significance of difference (B−A) (%)	Coefficients: Hue angle	Coefficients: Chroma	% Variance accounted for
Redness	−1.05	−1.25	1	−0.027 (0.149)	—	84
Brownness	−1.88	−2.14	1	+0.035 (0.302)	0.026 (0.0088)	84
Colour intensity	−0.56	−0.52	5	—	0.023 (0.0039)	57

A = Assessors preferring ruby ports.
B = Assessors preferring tawny ports.

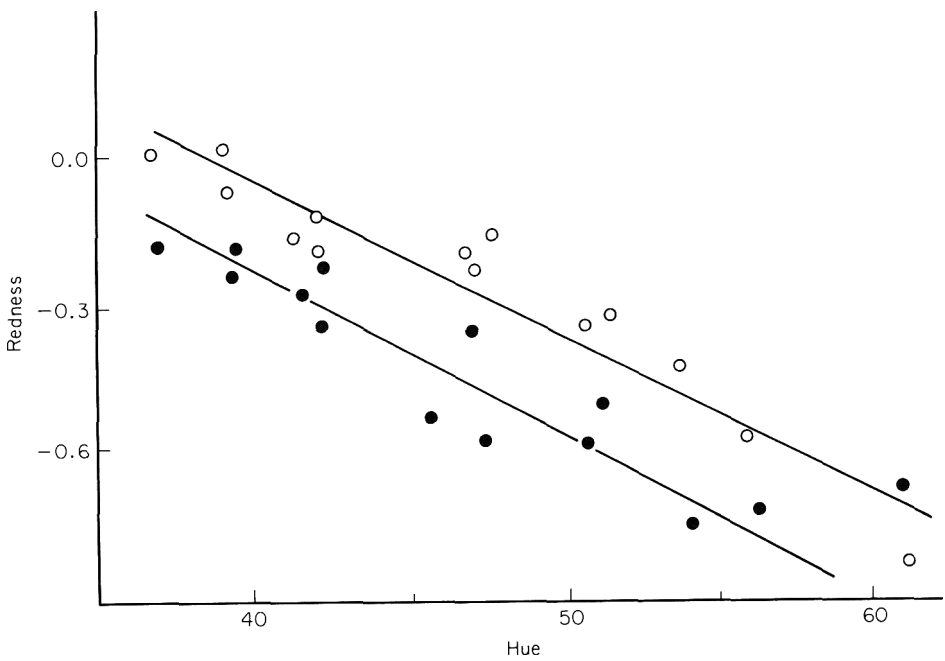

Figure 3. Plot of redness against hue angle for assessors showing preference for tawny (●) and ruby (O) ports.

relationships shown in Table 4. The inclusion of L* values or chroma in the equation for redness did not improve the fit. Likewise the inclusion of hue angle added nothing to the equation for brownness, nor L* values or the hue angle to the equation for colour intensity.

By excluding the White port, which appeared from inspection of the various regressions and the raw data to be anomalous, restricting the examination to L* values, chroma and hue angle, and adding a term to account for Tawny and Ruby preferences, the information shown in Table 5 was obtained, with scatter plots similar to those shown in Fig. 3. In none of these relationships did the L* value contribute significantly. Neither were any interaction terms involving the difference between assessors preferring Ruby and Tawny ports significant, indicating that all relationships reduced to parallel straight lines as shown in Fig. 3.

The information relating the deviation of the sensory attributes from the ideal value to the physical information could therefore be expressed as three simultaneous equations each including the three significant parameters, port preference, hue angle and chroma, shown below:

$$\begin{array}{lll} \text{Deviation of redness from ideal} = & 72.4+19.8\,X_0+0.23\,(X_1), & -2.63\,(X_2), \\ & (3.7) \quad (0.94) & (0.57) \end{array}$$

$$\begin{array}{lll} \text{Deviation of brownness from ideal} = & -189-25.0\,X_0+2.57\,(X_1), & +3.55\,(X_2), \\ & (3.5) \quad (0.84) & (0.53) \end{array}$$

$$\begin{array}{lll} \text{Deviation of intensity of colour from ideal} = & -54+\ 3.8\,X_0+2.28\,(X_1), & +0.02\,(X_2), \\ & (3.8) \quad (0.84) & (0.51) \end{array}$$

where X_0 = 0 from assessors preferring Ruby ports and
1 from assessors preferring Tawny ports.
X_1 = chroma, X_2 = hue angle. Standard errors of the regression are given in brackets.

The chroma and hue angle for the ideal ports for each of the two populations (the solution to this equation when the attribute value = 0) cannot be uniquely defined because solving any two of the above equations does not provide the same result as obtained from any other two (Fig. 4). The method of inverse simultaneous estimation (Williams, 1959) enables account to be taken of the error in each of the three regression equations and the best common solution to all three equations to be determined.

The approach consists simply of determining the values of chroma and hue angle that minimize the general distance of redness, brownness and intensity of colour from the origin. The generalized distance (D^2) is defined as $(\mathbf{a}'+\mathbf{X}'\mathbf{B}')\mathrm{S}^{-1}(\mathbf{a}+\mathbf{BX})$ where $\mathbf{a}+\mathbf{BX}$ is the vector expression of the three regressions, and S^{-1} is the inverse of the covariance matrix of the visual attributes.

In this case, S is calculated from the residuals of the attributes about the regression,

i.e.

$S = \mathbf{RNR}'$ where $\mathbf{R}$ = residual, = $\mathbf{y}-\mathbf{a}-\mathbf{BX}$, and
$\mathbf{N}$ = the diagonal matrix of the number of assessors on which each mean score is based.

Values of chroma (X_1) and hue (X_2) are then obtained by solving:

$$\frac{d(D^2)}{dX_1} = 0 \text{ and } \frac{d(D^2)}{dX_2} = 0.$$

These values, together with the values for redness, brownness and intensity, obtained by substituting them in the regression equations, are given in Table 6 and are marked on Fig. 4.

Table 6. Estimates of colour parameters for 'ideal' port

	Assessors preferring ruby ports	Assessors preferring tawny ports
Estimates of 'ideal' values		
Chroma	23.0	21.8
Hue angle	37.3	45.1
F- ratio (test of consistency)*	1.73	0.53
Score predicted by 'ideal' values:		
Redness (% total range)	−3.3	−2.4
Brownness (% total range)	−2.5	−1.8
Colour intensity (% total range)	2.8	2.0

*Degrees of freedom = 1, 21.

An F test for the departure of predicted ideal values of redness, brownness and intensity from the true values 0 showed this to be non-significant at the 5% level; therefore the ideal analytical values are consistent with the original regression coefficients. Operating the ratio in reverse the same information can be used to construct 95% confidence limits around the ideals. These are again presented in Fig. 4.

As the ideal ports in this instance lie on the extremes of the mean scores for the different ports and the estimate for the analytical values lies outside the range recorded, the errors are large and the ideal values should be treated with caution.

Using the figure for ideal hue angle and chroma, a* and b* values for the ideal ports may be calculated, these being 18.24 and 13.9 for the assessors who prefer Ruby ports, and 15.3 and 15.4 for those who prefer Tawny ports.

Examination of the correlation between L* values and chroma (0.984) clearly shows why the addition of L* values in the original regression equation provides little additional information and enables the ideal L* values corresponding to chroma values of 23.0 (assessors preferring Ruby ports) and 21.8 (assessors preferring Tawny ports) to be calculated and shown to be 86.13 and 92.1 respectively.

Discussion

Examination of the data as a whole, in particular the obvious trends in the blend diagrams and the % variance accounted for by the various regression equations of the

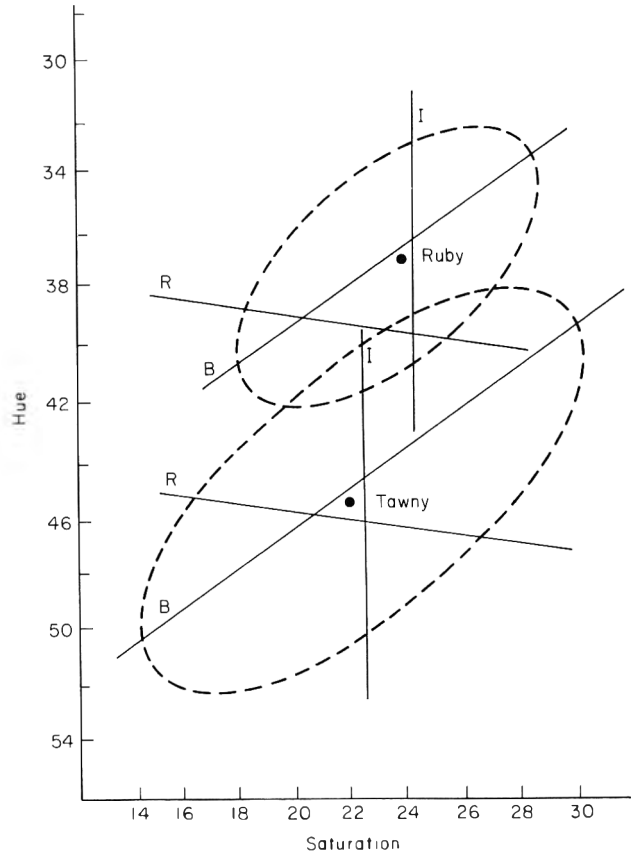

Figure 4. Regression lines for redness, brownness and intensity of colour for ideal ruby and tawny ports, ideal ports and confidence elipses: R = redness; B = brownness; I = intensity.

sensory scores on the analytical information, clearly confirmed that untrained assessors could provide meaningful quantitative objective information, particularly when ratio, rather than category type scales were employed. Whether such consistency would have been obtained, had hedonic information been asked for, is debatable. In this particular experiment, data from different assessors was pooled. This clearly necessitated each of the attributes being scored having a common underlying meaning to all assessors; in the case of appearance attributes they obviously did. With more comprehensive data assessors could have been treated as individuals and analytical parameters obtained for their individual ideal ports irrespective of the meaning they attached to attributes. Indeed, we could have used free choice profiling approaches (Williams & Langron, 1984) with no fixed terminology. Such ideals could be used to produce response surfaces, and a sub-population with common likes identified (Williams *et al.*, 1983).

The paper also shows clearly the problem which arises when more than one attribute is being scored, when it is inevitable that each attribute on its own will require a different set of parameters for optimization. By using inverse simultaneous regression the precision of the predictability of the sensory attribute from the physical measurement can be taken into account and the best ideal values to satisfy a number of criteria can be determined. In this context, the advantages of the mathematical approach compared with the purely visual approach of the blend diagram is obvious. Not only

does one know which are the preferred samples in any subset, but also how and by how much any need to be altered to approach the consumers' ideal product. This is particularly useful when the assessor's ideal samples appear to lie outside the range of blends provided. Prediction in such instances is obviously not as great as when the ideal falls within the experimental samples, but at least the operator is pointed in the correct direction.

In the case of the ports reported in this paper, the results have enabled the appearance of the consumer's ideal ports to be defined and have shown the manufacturer in which direction his product should be modified to satisfy better the majority of the population. As the ideals are based on averages, the responses of only forty-nine assessors, and lie outside the range of ports presented, the precision to which they can be predicted in this instance is relatively low.

Conclusion

The paper demonstrates the value of using magnitude estimation on objective attributes with the consumer and provides a mathematical approach for rationalizing apparently conflicting data when several attributes are being assessed. The examples used in this particular exercise dealt with appearance. The same methods of assessment, however, could be applied to aroma, flavour and texture and to any number of products, particularly if free choice profiling approaches were to be adopted.

Acknowledgments

The authors would like to thank Mr P. Bridle for providing the tristimulus values on these ports.

References

Moskowitz, H.R. & Chandler, J.W. (1977). New uses of magnitude estimation. In: *Sensory Properties of Foods* (edited by G.G. Birch., J.G. Brennan & K.J. Parker). Pp. 189–210. London: Applied Science Ltd.

Moskowitz, H.R., Stanley, D.W. & Chandler, J.W. (1977). The eclipse method; optimising product formulation through a consumer generated ideal sensory profile. *Canadian Institute of Food Science and Technology*, **10**, 161–168.

Schiffman, S.S., Reynolds, M.L. & Young, F.W. (1981). *Introduction to Multidimensional Scaling: Theory, Methods and Application*. Pp. 275–280. New York: Academic Press

Williams, A.A. (1983). Defining Sensory Quality in Foods and Beverages. *Chemical Industry*, 740.

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

Williams, A.A., Langron, S.P. & Arnold, G.M. (1983). Objective and hedonic sensory assessment of ciders and apple juices. In: *Sensory Quality in Foods and Beverages: Definition, Measurement and Control* (edited by A.A. Williams & R.K. Atkin). Pp. 310–324. Chichester: Ellis Horwood Ltd.

Williams, E.J. (1959). *Regression Analysis*. Pp. 166–172. London: J. Wiley and Sons.

Wyszeki, G. & Stiles, W.S. (1982). *Colour Science: Concepts and Methods Quantitative Data and Formulae* (2nd edn.). Pp. 166–168. New York: John Wiley and Sons.

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Simple enrichment procedure for the estimation of minor polyunsaturated fatty acids in food fats

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Summary

A simple, reliable and sensitive method, involving the partition of a mixture of fatty acid methyl esters (FAME) between hydrocarbon solvent and aqueous ethanolic silver nitrate, is described for the estimation of low concentrations of polyunsaturated fatty acids (PUFA) in fats and oils. It is suggested that this procedure could be used to give an indication of oxidative stability of food fats and of their potential for off-flavour development.

Introduction

The separation of lipids by silver ion chromatography has been reviewed by Morris (1966) and the technique is widely used for the separation of lipid components according to their degree of unsaturation. It is particularly useful for the separation of unsaturated compounds containing one or more double bonds with the *cis* configuration. Preliminary fractionation, by preparative column or thin-layer chromatography using silica gel impregnated with silver nitrate, followed by gas chromatography (g.c.) of the individual fractions, has proved invaluable for the separation and identification of components of complex lipid mixtures (e.g., natural triglycerides) or the methyl ester mixtures derived from them. This chromatographic fractionation according to the degree of unsaturation eliminates the problem of peak overlap often experienced in the g.c. analysis of a complex mixture of fatty acid methyl esters (FAME) on polar stationary phases. The polar cyanosilicone phases possessing high temperature stability now available allow rapid g.c. analysis of long chain polyunsaturated fatty acid esters (PUFAME) on packed columns.

This paper describes a simple non-chromatographic procedure to partition FAME mixtures between hydrocarbon solvent and aqueous ethanolic silver nitrate (Swoboda & Peers, 1976) followed by g.c. analysis of the methyl esters present in the two separated layers. The method has allowed estimations to be made of the PUFA content of food fats containing small ($< 0.2\%$) amounts of long-chain PUFA that would not have been detected by direct g.c. analysis involving no selective enrichment of PUFA. This method is accurate, sensitive and quick and it could be used to screen fats and oils for long-chain PUFA content and thus indicate the probability of oxidative instability and subsequent off-flavour development.

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Materials and methods

Vacuum packed bacon rashers were purchased from local supermarkets. Turkey depot fat was obtained from Mr T.C. Grey of the Poultry Group of this Institute. Mackerel oil was a gift from Dr J. Burt of the Torry Research Station, Humber Laboratory, Hull, U.K. The hexane solvent used for extraction was of HPLC grade; other reagents used were of analytical (AnalaR) grade.

Extraction procedures

Total lipid from wet tissue was extracted by the method of Bligh and Dyer as used by Parr & Swoboda (1976). The neutral lipids of previously dried (P_2O_5, vacuum) animal fatty tissues were obtained by Soxhlet extraction for 4 hr using hexane as solvent.

Preparation of esters

Fatty acid methyl esters (FAME) were prepared by transesterification of the extracted lipid using either sulphuric acid (0.5 M) or sodium methoxide (0.05 M) in methanol. The purity of ester mixtures was checked by thin layer chromatography and if necessary the mixtures were purified on a silica gel column eluting with increasing proportions of diethyl ether in hexane.

Silver nitrate partition

FAME (up to 1 g) dissolved in 2,2,4-trimethylpentane (10 ml) were shaken with an equal volume of silver nitrate solution (25% w/v in ethanol, water, 1:1) and allowed to stand for a few minutes to allow phase separation. The upper organic layer was removed and the lower aqueous layer diluted with water (10 ml) before extraction with hexane (3×10 ml portions). Both organic extracts were dried and filtered by passing them through cotton wool plugged Pasteur pipettes containing Na_2SO_4. After solvent removal the recovered esters from both upper and lower layers were weighed. To avoid losses, the initial and subsequent extractions of the aqueous silver nitrate layer were carried out in the same stoppered test tube (30–40 ml capacity). In this way recoveries of 97% or better were obtained. For g.c. analysis, solutions (about 5%) of the esters were prepared in 2,2,4-trimethylpentane containing BHT antioxidant (0.005% w/v); 0.5–1 μl was injected onto the column.

Gas chromatography (g.c.)

Either a Carlo Erba (HRGC 5300) or Pye Series 104 gas chromatograph, fitted with a flame ionization detector, was used. A coiled glass column (3 mm i.d.×2.5 m) was packed with 3% SP2310/2% SP2300 on 100–120 mesh Chromosorb WAW (Supelco Inc., U.S.A.). Operating conditions were as follows: oven and injector temperatures, 200°C; detector temperature, 250°C; and nitrogen carrier gas flow, 20 ml/min. Under these conditions methyl docosahexaenoate (22:6 *n*-3) had a retention time of approximately 60 min. The detector output data were processed by a computing integrator (Spectra Physics model SP 4270). As additional confirmation of identity, from equivalent chain length (ECL) values, ester mixtures were analysed on a column packed with 5% OV17 on 80–100 mesh Diatomite CLQ (J.J.'s Chromatography Ltd., U.K.).

Silver ion chromatography

Silver ion liquid chromatography (Ag-l.c.) was carried out on columns of Keiselgel 60 (E. Merck, Germany), 20–230 mesh, impregnated with silver nitrate according to the method of Christie (1973). FAME mixtures were thus separated according to their degree of unsaturation. Silver ion thin-layer chromatography (Ag-t.l.c.) was used to monitor fractions of mackerel fish body oil FAME eluted from Ag-l.c. columns. The mackerel oil FAME mixture thus provided an authentic source of long chain *n*-3 PUFAME including particularly the 18:4, 20:5, 22:5 and 22:6 esters.

Identification and composition of methyl ester samples

Individual esters were identified by ECL values (Jamieson, 1975), mass spectra (Kratos MS30, 70 eV) and chromatographic behaviour (Ag-l.c., Ag-t.l.c.) compared to authentic samples. ECL values were calculated from the linear relationship between the logarithms of retention times and chain lengths of saturated methyl esters. Methyl palmitate (ECL = 16.00) and stearate (ECL = 18.00), naturally present in the samples, were used as reference peaks for this purpose. The computing integrator was used to calculate and report these values together with the fatty acid composition (peak area %) of the samples.

After analysis of FAME present in the upper and lower phases from silver nitrate partition, the overall composition was calculated as follows:

$$\text{Overall \% of component 'A'} = \frac{U.A_u + L.A_L}{U+L},$$

where U,L are the weights of FAME in upper and lower phases respectively and A_u, A_L = % 'A' in upper and lower phases respectively.

Results and discussion

The results obtained using mackerel oil FAME in the silver nitrate partition procedure are listed in Table 1. They show that the recovery of FAME after partitioning was high ($> 97\%$) and that the major fatty acid composition after partition and recalculation was the same, within experimental error, as that obtained without partition (direct analysis). The preferential concentration of PUFAME in the lower phase allowed the measurement of minor components (e.g., 20:4 *n*-3, 22:4 *n*-3, 22:4 *n*-6) and eliminated the overlap of major component pairs such as 20:5 *n*-3 and 22:1. It was not possible however to eliminate the probable co-chromatography of some trace components (e.g., 20:3 *n*-3 and 20:4 *n*-6) with the particular g.c. column used.

Figures 1 and 2 are the chromatograms from fatty acid analysis of a bacon fat sample and a turkey depot fat sample before (a, direct analysis) and after (b, upper phase esters and c, lower phase esters) silver nitrate partition. The enrichment of PUFAME in the lower layers achieved by silver nitrate partition is clearly demonstrated. Polyunsaturated esters, particularly 22:5 and 22:6, were barely detectable by direct analysis because of their low concentrations and long retention times. After selective enrichment by silver nitrate partition these two particular PUFAME were detected as large peaks. The attenuation of the detector amplifier was set at the same value ($\equiv 2\times10^{-9}$ amp) for each chromatogram. The enrichment of PUFAME allowed the injection of up

Table 1. Results of mackerel oil analysis

Ester recovery:
Wt of esters used for partition = 219 mg
Wt of esters recovered from upper phase = 157 mg
Wt of esters recovered from lower phase = 56 mg

GC composition (area %) Upper phase *U*	Lower phase *L*	Calculated total (*U*+*L*)	Direct analysis	FAME Identity*
9.52	0.88	7.25	7.23	14:0
0.64	0.07	0.49	0.43	14:1
19.34	1.72	14.71	14.55	16:0
7.87	0.80	6.01	6.03	16:1
—	0.60	0.16	0.34	16:3
—	1.50	0.39	—	16:4 *n*-3
1.41	0.11	1.07	1.09	17:0
0.82	—	0.60	0.45	17:1
2.69	0.49	2.11	2.13	18:0
19.81	2.16	15.16	15.41	18:1
2.54	0.27	1.94	1.95	18:2 *n*-6
0.87	1.67	1.08	1.16	18:3 *n*-3
—	14.89	3.91	3.47	18:4 *n*-3
13.86	—	10.22	10.43	20:1
—	0.64	0.17	0.49	20:3 *n*-3, 20:4 *n*-6
—	1.63	0.43	—	20:4 *n*-3
—	30.27	7.96	A	20:5 *n*-3
19.70	—	14.52	23.84(-A)	22:1
—	1.20	0.32	—	22:4 *n*-6
—	0.34	0.09	—	22:4 *n*-3
—	2.62	0.69	1.57(-B)	22:5 *n*-3
—	38.14	10.03	9.43	22:6 *n*-3
0.93	—	0.69	B	24:1

*From ECL values (Jamieson, 1975).

to 50 μg (1 μl 5% w/v solution) of FAME onto the column and so avoided the use of high sensitivity settings of the detector amplifier. For many other bacon fat samples that we have analysed, long-chain PUFAME were only detected after silver nitrate enrichment.

To assess the possible error which might have been introduced by the partition procedure, an analysis of variance within- and between-partitions was carried out using a bacon fat sample with a very low long-chain PUFAME content. Triplicate g.c. analyses of each phase from triplicate partitions were carried out. The results of the statistical analysis are summarized in Table 2. For the long-chain (C_{20} and C_{22}) polyunsaturated fatty acids which were of primary interest, the F values indicated that there was no significant between-partition variation.

To test and demonstrate the accuracy of the method for the estimation of low concentrations of long chain PUFA, purified mackerel oil methyl esters were added to

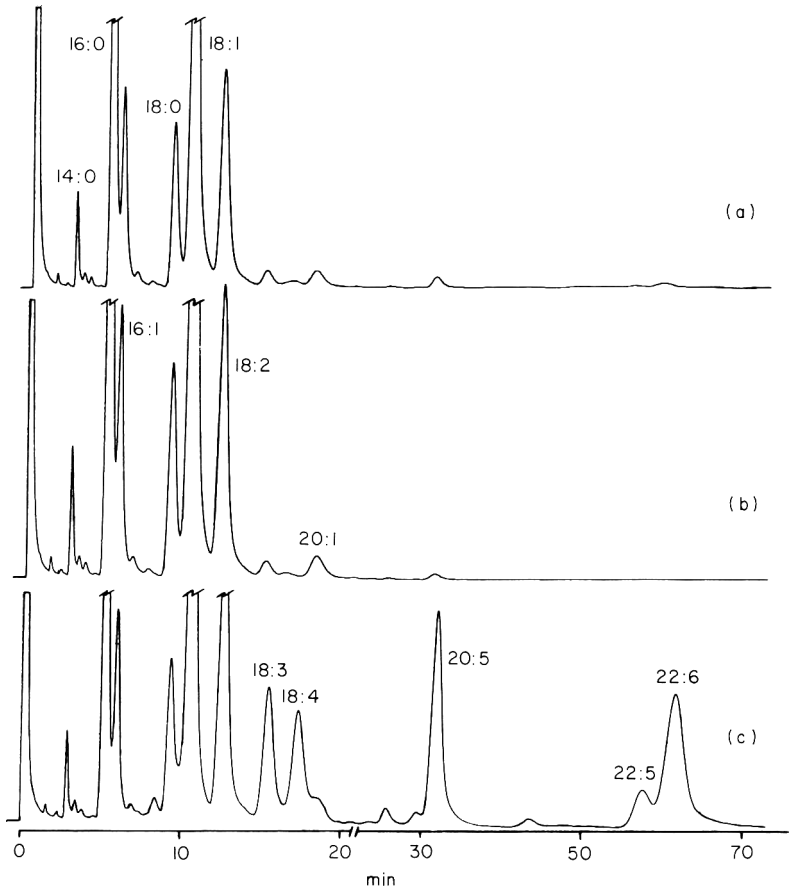

Figure 1. FAME analysis of bacon fat: (a) direct analysis, (b) and (c) upper and lower phases respectively after silver nitrate partition.

corn oil methyl esters to give four mixtures containing 22 : 6 *n*-3 PUFAME over a range of concentrations (0.1–1% of total FAME). Triplicate 1 g partitions were carried out on each mixture and duplicate g.c. analyses performed on the upper and lower phases obtained. The results for 20 : 5 *n*-3 and 22 : 6 *n*-3 PUFAME from the four mixtures are listed in Table 3.

For the determination of small amounts of long chain polyunsaturated fatty acids in food fats the method described is a simple reliable and sensitive procedure which does not require sophisticated g.c. equipment. With the increased nutritional and medical interest in these particular minor components of our diet we anticipate the method could find wide application.

Acknowledgments

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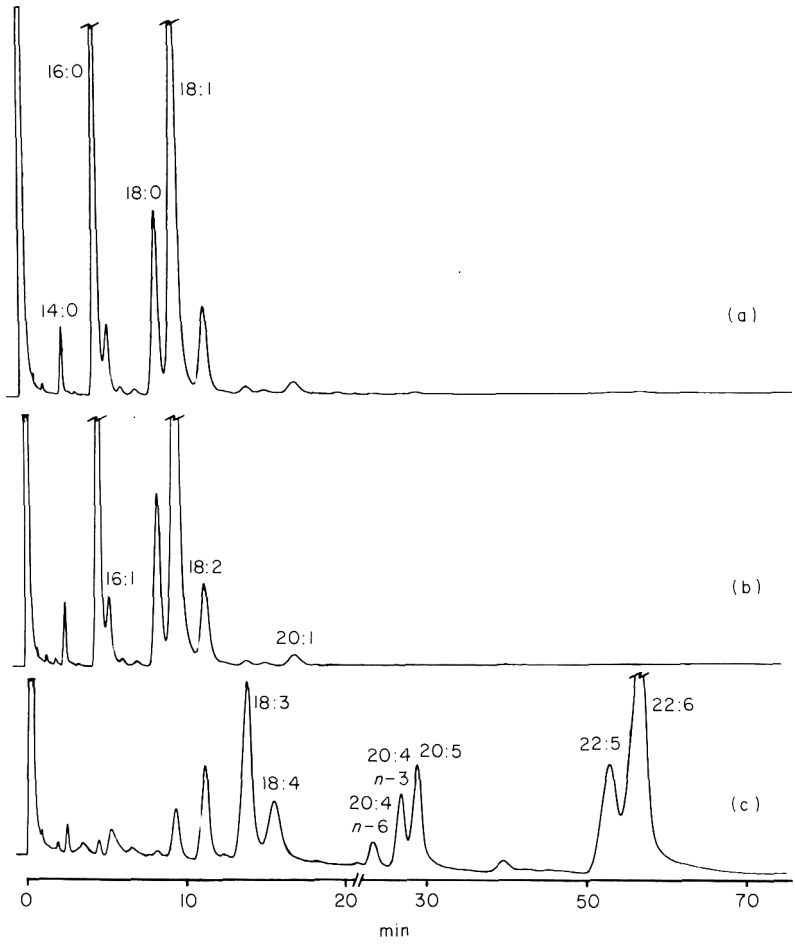

Figure 2. FAME analysis of turkey depot fat: (a), (b) and (c): see Fig. 1.

Table 2. FAME analysis of bacon fat. Analysis of variance within and between partitions

PUFAME	Mean % of Total FAME	s.e. of mean $*S_B/3$	s.d within-partitions S_W	s.d. between-partitions $\sqrt{\frac{S_B^2 - S_W^2}{3}}$	F value S_B^2/S_W^2
20 : 4 *n*-6	0.09	0.0058	0.009	0.0086	3.96
22 : 4 *n*-6	0.01	0.0007	0.002	0	1.05
22 : 5 *n*-3	0.10	0.0078	0.014	0.011	3.03
22 : 6 *n*-3	0.07	0.0061	0.010	0.088	3.24

$*S_B^2$, S_W^2 are the between- and within-partitions mean squares.

Table 3. PUFAME analysis of mackerel oil/corn oil methyl ester mixtures after silver nitrate partition

	20 : 5 *n*-3 (%)		22 : 6 *n*-3 (%)	
Mixture	Expected	Determined (±s.e.m.)*	Expected	Determined (±s.e.m.)*
1	0.09	0.097 (0.0067)	0.12	0.127 (0.0067)
2	0.16	0.153 (0.0033)	0.20	0.197 (0.0033)
3	0.40	0.407 (0.0088)	0.49	0.530 (0.0100)
4	0.81	0.807 (0.0120)	1.00	1.060 (0.0115)

*'Determined' values are the means from triplicate partitions.

References

Christie, W.W. (1973). *Lipid Analysis*. Oxford: Pergamon Press.

Jamieson, G.R. (1975). GLC identification techniques for long-chain unsaturated fatty acids. *Journal of Chromatographic Science*, **13**, 491–497.

Morris, L.J. (1966). Separations of lipids by silver ion chromatography. *Journal of Lipid Research*, **7**, 717–732.

Parr, L.J. & Swoboda, P.A.T. (1976). The assay of conjugable oxidation products applied to lipid deterioration in stored foods. *Journal of Food Technology*, **11**, 1–12.

Swoboda, P.A.T. & Peers, K.E. (1976). (*n*-3) Pentaenoic fatty acids in butterfat as precursors of metallic, fishy taint. *Chemistry and Industry*, 160–161.

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Frozen storage life of the Bolivian fish, sabalo (*Prochilodus platensis*)

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Summary

A supply of the Bolivian fish sabalo, *Prochilodus platensis*, was stored at −15 ° and −30 °C. Samples were taken at intervals for analysis and taste panel assessment. No significant change was observed in 20 months at either temperature in the pH or in the percentage of protein soluble in 5% sodium chloride. The fish remained acceptable to the taste panel throughout. Thus the storage life of the species is shown to be in excess of 20 months at both −15 ° and − 30 °C.

Introduction

Bolivia is a landlocked country, but it has substantial resources of fish from lakes in the high Andes and rivers of the Amazon and Parana basins. Despite these resources, and although fish is a popular food, consumption is at a fairly low level. A programme of practical measures to increase the supplies of fish has been launched, and to support this, iced storage trials were carried out on many local fish species (Nicolaides & Poulter, 1985). Most of the fish could be distributed in ice but since transport within the country is difficult the quality may deteriorate substantially during the handling operation. This is the case for the species known as sabalo, which is caught in the Pilcomayo River in the extreme south of Bolivia. The area is about 400 m above sea level, and the fish are taken to La Paz, 1000 km away and over 3000 m higher. Freezing might be useful in preserving sabalo, and this paper describes a study of the frozen storage life of the species.

Sabalo is the local name for *Prochilodus platensis* of the family Curimatidae. According to Nelson (1984), the species has a superficial resemblance to labeo, of the family Cyprinidae (carp).

Materials and methods

Sabalo were caught in traps in the Rio Pilcomayo near Villa Montes as they swam upriver during breeding migration, on 10 August 1983. The fish were placed in crushed ice while they were still respiring, but they were agitated and stressed due to the catching method. Torrymeter readings were taken on randomly selected fish. The fish were sent by air freight to La Paz where a sample was taken for determination of the pH and total volatile base content. The fish were sent in ice to London, where they arrived on 18 August. The whole fish were blast frozen individually and divided into two groups, for storage at −15° or −30 °C.

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Two sabalo were taken from each storage temperature at regular intervals over 20 months. An assessment of the quality was made by examining the eyes, gills, flesh, body cavity and skin. The fish were then weighed and filleted. Carcass analysis was also carried out on the first twenty fish used.

Six samples of muscle were taken from one skinned fillet from each fish. These samples were hermetically sealed in polyethylene bags and immersed in boiling water for 20 min. The cooked muscle samples were then presented to an experienced taste panel for assessment of texture, flavour and overall quality on a 10-point hedonic scale.

The remaining muscle was finely chopped with a scalpel after the dark muscle and large pieces of connective tissue had been removed. Samples were taken from this for analysis of pH, total nitrogen (crude protein), water, fat and ash, using the methods specified by Curran *et al.* (1980). The percentage of the protein extracted by 5% sodium chloride solution was determined as described by Cowie & Little (1966).

Results and discussion

The results of the carcass and proximate analysis of sabalo are given in Tables 1 and 2 respectively. The fish had an average weight of 1016 g (range 600–1950 g) and an average total length of 42.4 cm (36–52 cm). On capture, they gave Torrymeter readings of 11 or 12. Analysis of the fish muscle in La Paz after 5 days on ice gave a pH values in the range 6.43–6.74 and a total volatile base contents in the range 2.38–2.96 mg N/100 g.

Table 1. Carcass analysis of sabalo

Portion	Mean	Range
Fillet (%)	34.54	28.71–38.04
Skin (%)	7.49	5.78–9.88
Guts and gills (%)	8.05	5.27–11.90
Head (%)	20.38	16.29–25.76
Frame (%)	27.88	23.81–32.58

Analysis of twenty fish.

Table 2. Proximate analysis of sabalo

Portion	Mean	Range
Moisture (%)	79.06	77.40–80.96
Fat (%)	1.01	0.11–2.23
Total crude protein (%)	19.63	16.63–21.50
Total ash (%)	0.98	0.43–1.27

Duplicate analysis of twenty-two muscle samples.

The results of the storage trials are given in Table 3 and Figs 1–3. There was very little change during the 20 months' storage at either temperature. No off-odours were detected in the gills, body cavity, or flesh during the storage. The taste panel's scores for overall quality (Fig. 1) showed little change at either temperature, and the fish were still more than acceptable after 20 months. The panel's separate scores for flavour and texture were all close to the overall scores. There was no decrease in the percentage of extractable protein (Fig. 3), indicating that protein denaturation had not occurred to any extent; this is in agreement with the taste panel's assessments which showed no increase in toughness.

The frozen storage life of sabalo, more than 20 months at both − 15 and − 30 °C, is quite long. White fish from temperate or cold waters remains of good quality for 4 months at − 20 °C and 8 months at − 29 °C (FAO, 1973). These periods refer to fish

Table 3. Visual and olfactory changes in sabalo during storage at −15° and −30 °C.

	Change to	Observed after months at −15°	Observed after months at −30°
Eyes	Sunken, cloudy	on receipt	on receipt
	Sunken, bloody	1.5	1.5
Gills*	Red but slightly bleached	on receipt	on receipt
	Red/bleached	1	not observed
	Brown/bleached	12	not observed
	Bleached	20	not observed
Gill odour	Sweet/muddy	on receipt	on receipt
	Muddy	0.5	not observed
	Slightly bitter	9	9
	Malty	12	15
Flesh	Firm	on receipt	on receipt
	Fairly firm	0.5	1.5
	Softening	15	15
Body cavity	Firm	on receipt	on receipt
	Fairly firm	15	15
Skin	Shiny	on receipt	on receipt
	Less bright	1	9
	Dull	6	12
	Bloody mucus	20	20

*The gills contained very little mucus at any stage.

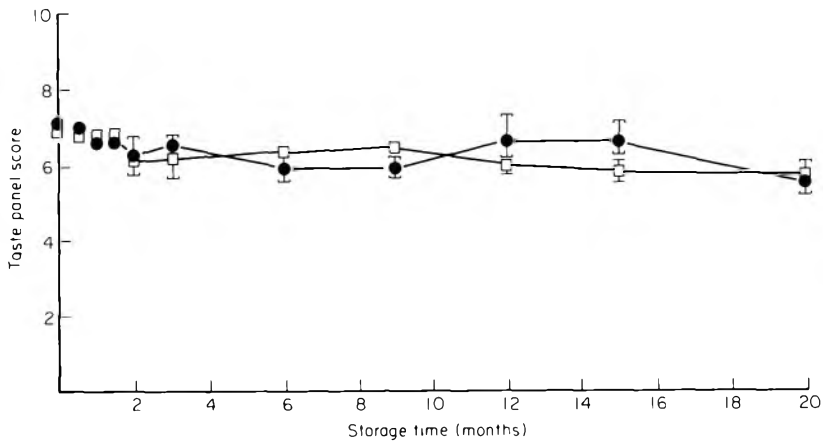

Figure 1. Taste panel score for overall quality. Changes in sabalo (*Prochilodus platensis*) during storage at − 15 °C (⊕) and − 30 °C (●) with ranges if beyond the point shown.

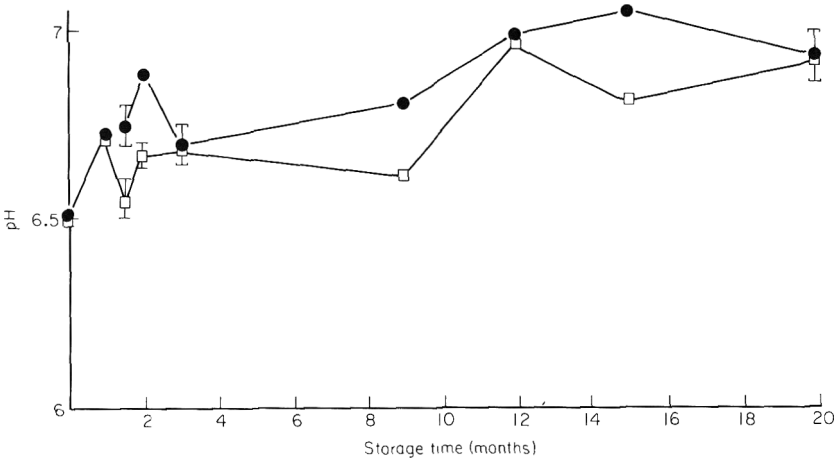

Figure 2. pH of minced fish muscle. Changes in sabalo (*Prochilodus platensis*) during storage at − 15 °C (□) and − 30 °C (●) with ranges if beyond the point shown.

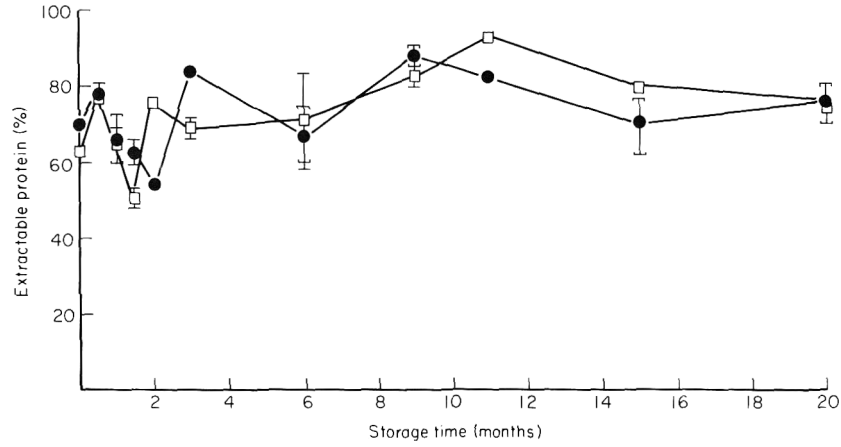

Figure 3. Extractable protein. Changes in sabalo (*Prochilodus platensis*) during storage at − 15 °C (□) and − 30 °C (●) with ranges if beyond the point shown.

frozen within 24 hr of catching; usually a delay in freezing shortens the storage life (Ames & Poulter, 1984), so the 8 days' delay in freezing these sabalo would have been expected to have this effect. The state of agitation and stress of the sabalo when caught could also have shortened the storage life (Bramstedt & Auerbach, 1961).

Conclusions

There was no noticeable change in quality during 20 months' storage at either − 15 ° or − 30 ° C, even though the handling of the fish during catching was not ideal and there was

a delay in freezing. Thus the storage life exceeds 20 months at both temperatures.

It is not likely that the fish would need to be kept for as long as this if freezing is used in the commercial distribution in Bolivia, so storage at −15°C would be quite acceptable. There is clearly no need to use the lower and more expensive temperature of −30°C as recommended by FAO.

It may be noted that while sabalo is popular with consumers in Bolivia, the fish is not likely to be very acceptable on an international market since the 'fillets' unavoidably contain many small bones.

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References

Ames, G.R. & Poulter, R.G. (1984). Frozen storage life of tropical fish species. Indo-Pacific Fisheries Commission Working Party on Fish Technology and Marketing, Melbourne.

Bramstedt, F. & Auerbach, M. (1961). Spoilage of freshwater fish. In: *Fish as Food* (edited by G. Borgstrom). Pp. 613–637. New York: Academic Press.

Cowie, W.P. & Little, W.T. (1966). The relationship between the toughness of cod stored at −29°C and its muscle protein solubility and pH. *Journal of Food Technology*, **1**, 335–343.

Curran, C.A., Nicolaides, L., Poulter, R.G. & Pors, J. (1980). Spoilage of fish from Hong Kong at different storage temperatures. 1. Quality changes in gold-lined sea bream (*Rhabdosargus sarba*) during storage at 0°C at 10°C. *Tropical Science*, **22**, 367–382.

FAO (1973). Code of Practice for Frozen Fish. Fisheries Circular No. 145 (Revision 1).

Nelson, J.S. (1984). *Fishes of the World*, 2nd edn. New York: Wiley Interscience.

Nicolaides, L. & Poulter, N.H. (1985). Preliminary studies on the ice storage characteristics and composition of Bolivian freshwater fish. 1. Altiplano basin fish; 2. Amazon and Parana basin fish. *Journal of Food Technology*, **20**, 437–449, 451–465.

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Sorbic acid diffusivity in relation to the composition of high and intermediate moisture model gels and foods

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Summary

The diffusivity at infinite diffusant dilution (D_0) of sorbic acid in high and intermediate moisture gels (with various substrates and water contents) and in three foods was evaluated. The determination of D_0 (25°C) was achieved by tridimensional diffusion in gels cubes or by monodimensional diffusion in infinite food columns. For the same substrate concentration by weight, D_0 values of sorbic acid in concentrated sugar solutions decreased slightly when the molecular weight of the sugar was increased. When a liquid substrate such as glycerol was used, D_0 values referred to equal concentrations by weight, were higher than in sugar solutions. The diffusion of sorbic acid is related, as a first approximation, to the water content rather than to the water activity of the diffusion medium.

Introduction

In the previous papers (Guilbert, Giannakopoulos & Cheftel, 1985; Giannakopoulos & Guilbert, 1986) a rapid and simple method for the measurement of sorbic acid apparent diffusivities (D_a) in model food gels (tridimensional diffusion in gels cubes) was developed. This method displayed good agreement with the reference method (monodimensional diffusion in infinite columns). It was also shown that the measured diffusivity was influenced by sorbic acid concentration. This concentration dependence was slight and therefore the experimental concentration profiles do not differ significantly in shape from the theoretical profiles when D_a is independent of the concentration of the diffusant. The sorbic acid diffusivity at infinite dilution (D_0) can be estimated, using a linear relationship between the D values and the diffusent concentration.

As has been shown previously (Guilbert *et al.*, 1985; Giannakopoulos & Guilbert, 1986) sorbic acid diffusivity is affected by the water content and by the water activity of the diffusion medium. When the water activity (a_w) and the water content of a food is markedly lowered, most residual water molecules are bound to food constituents and therefore few are available as a solvent or diffusion medium. As a result, the viscosity of the liquid phase becomes too high for diffusion to take place. Duckworth (1981) has suggested, with NMR studies, that the mobility of solutes in dehydrated foods is proportional to the a_w rather than to the water content. However in high and intermediate moisture foods the diffusivities are not a direct function of the water activity; as a first approximation and when 'substrates' of similar molecular weights are concerned, the diffusivities are proportional to the water content of the diffusion medium

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(Chandrasekaran & King, 1972; Schneeberger, Voilley & Weisser, 1978; Biquet & Guilbert, 1986). In addition, any liquid ingredient other than water might play a role in the process of migration (Duckworth, Allison & Clapperton, 1975; Guilbert *et al.*, 1985; Biquet & Guilbert, 1986).

Alternatively, the enhanced diffusion for water soluble constituents, at higher water contents may also be explained by a decrease in viscosity of the diffusion medium. In foods, however. the viscosity of the occluded solvent (which supports the diffusion (Schwartzberg & Chao, 1982)) cannot generally be measured, and the situation is complicated by heterogeneity of the structure (presence of membranes, skins, envelopes, layers, networks, pores, etc.) and by the occurrence of simultaneous water and solute transfers resulting from concentration gradients and osmotic forces.

This work attempted to study the relation between sorbic acid diffusivity and parameters such as water content, water activity and viscosity in high and intermediate moisture model food gels with various compositions and in selected foods (redcurrant jelly, processed cheese and chestnut cream). These foods have been taken as examples because (i) it can be useful to control sorbic acid transfers when they are used as ingredients in heterogeneous fabricated foods (e.g., fillings for baked goods) and (ii) it may be desirable to cover or to coat them with an external layer (edible film, gel or emulsion layers (Guilbert, 1985)) of high sorbic acid concentration (e.g., bite size foods).

Materials and methods

Diffusion experiments were carried out as described previously (Giannakopoulos & Guilbert, 1986). The apparent diffusivity of sorbic acid in model food gels was measured by tridimensional outward diffusion from cubes of agar gels (9×9×9 mm) containing an initially homogeneous concentration of sorbic acid (between 1.8 and 26.8 μmol/g of gel) and placed into an 'immersion solution' which contains the same components as the corresponding gel (except agar and sorbic acid). The model food gels were prepared as mentioned previously with 3% (w/w) agar and 97% (w/w) aqueous solution of polyethylene glycol (P.E.G. 600, Merck) or with 1.5% (w/w) agar and 98.5% (w/w) aqueous solution of sucrose (Merck), glucose syrup (DE 46, Roquette), maltodextrins (DE 20 and DE 6 Roquette) or glycerol (Merck) of various concentrations. For some experiments, part of the sucrose was replaced by gelatin (250 bloom, Rousselot) or by peanut oil mixed with surfactants (Tween 80 and Span 20, Prolabo). Apparent diffusivities (means of six independent determinations) were determined from the change of the sorbic acid concentration in gel cubes as a function of the immersion time (1/2, 1 and 2 hr) as mentioned previously.

The apparent diffusivity of sorbic acid in the foods was measured by monodimensional diffusion from a semi-infinite food column (containing an initially homogeneous concentration of sorbic acid) into a contiguous semi-infinite column free of sorbic acid. For the preparation of the food columns, the processed cheese (Bel; water content 44% (w/w), fat on dry basis 50% (w/w), $a_w = 0.96$), chestnut cream (water content 34%, $a_w = 0.90$) or redcurrant jelly (water content 37% (w/w), $a_w = 0.88$) was placed into glass jars with hermetic lids. Sorbic acid was eventually added to the food (three independent concentrations were used: 1.8, 4.5 and 8 μmol/g of food). The food was then heated for 30 min whilst stirring in a water bath (80°C). The food was poured while hot into glass tubes (16 mm i.d.×240 mm); after cooling, a half food column containing sorbic acid was placed in contact end to end with another one free of sorbic

acid. The tube was closed and then stored at the desired conditions (25°C, 4 days). Apparent diffusivities, (D_a) were determined from the experimental concentration-distance profiles using a least squares curve-fitting procedure as mentioned previously.

D_0 values were obtained by linear extrapolation at zero sorbic acid concentration, of the three independent D_a values (corresponding to the three sorbic acid concentrations as already mentioned).

Sorbic acid determinations (gas-liquid chromatography, Girdel 3000) and viscosity measurements (Haake Rotovisco RV12) were carried out as described previously. Water content measurements were made by Karl-Fisher titration (Baird and Tatlock AF 3H automatic titrator). Water activity measurements were made at 25°C using Sina hygrometers (EZFBA3 and EZFBA4; Nova Sina, Zürich) according to the recommendations of Bousquet-Ricard *et al.* (1980).

Results and discussion

The diffusivity of sorbic acid extrapolated to infinite diffusant dilution (D_0) in model gel cubes (containing various substrates with different water contents) and in foods (red-currant jelly, processed cheese and chestnut cream) are shown in Tables 1–3. Table 1 lists the components used and the concentrations to which each gel sample was formulated as well as the viscosity and the water activity of the corresponding aqueous solutions present without the agar gel matrix. The mean water contents, measured after the diffusion experiments are given for some of the gel samples. D_0 values given for each gel samples are obtained from diffusivity values determined by outward diffusion from gel cubes.

The mean water contents measured after the diffusion experiments are not significantly different from the corresponding formulated concentrations. This indicates that during the immersion into solutions of same compositions, gels lose only sorbic acid and no swelling phenomenon occurred. The model cubes characteristics (water contents, water activities) will therefore be similar to those at the beginning of the experiments.

It is evident from the results in Table 1 that D_0 values of sorbic acid sharply decreased when the concentration of substrates was increased. For the same substrate concentration (e.g., 20 or 40%) D_0 values are very similar in gels containing sucrose, glucose syrups or P.E.G. 600; somewhat higher in gels containing glycerol, and lower in gels containing maltodextrins. The sorbic acid diffusivity in gelified concentrated sugar solutions decreased slightly as the molecular weight of the sugar increased. These results are in agreement with those of Voilley & Simatos (1980) and Voilley & Le Meste (1985) for water and volatiles diffusion, and of Biquet & Guilbert (1986) for water diffusion. The latter workers also observed a decrease in diffusivity when the molecular weight of the substrate increased, both in P.E.G. solution and sugar solutions. Results obtained here with P.E.G. 600 must be interpreted taking into account the presence of 3% (w/w) agar (required to avoid the erosion of P.E.G. model cubes during the experiments). This higher agar concentration tended to counteract the sorbic acid diffusivity as mentioned previously (Giannakopoulos & Guilbert, 1986). The infinite dilution diffusivity values of sorbic acid are higher in glycerol gelified solutions than in sugar solutions. This effect is more pronounced at high glycerol concentrations. Similar results were reported by Guilbert *et al.* (1985) for sorbic acid diffusivity in glycerol and sodium chloride solutions, by Biquet & Guilbert (1986) for water diffusion, and by Voilley & Le Meste (1985) for diffusion of water and volatiles in glycerol, liquid P.E.G.

Table 1. D_0 values* (25°C) of sorbic acid in model gel cubes containing various substrates at different water contents

Gel composition (% w/w)												
Water	Agar	Sucrose	Glucose syrup DE46	Maltodextrin DE20	Maltodextrin DE6	Glycerol	P.E.G. 600	a_w (25°C) of solution	Solution viscosity (10^{-3} Pa.s)	Mean water† content (% w/w)	$D_0 \times 10^{-10}$ m²/sec
98.5	1.5							1	1.5	97.2	8.74
82.5	1.5		16					0.98	2.4		6.11
78.5	1.5	20						0.98	2.5	77.8	4.96
78.5	1.5			20				0.99	3.1		3.99
78.5	1.5				20			0.99	8.5		3.83
78.5	1.5					20		0.95	3.1	77.8	6.13
77.0	3.0						20	0.97	2.6		5.06
68.5	1.5	30						0.97	3.4		3.80
66.5	1.5		32					0.96	4.5		3.50
58.5	1.5			40				0.96	19.6		1.50
58.5	1.5					40		0.88	4.0		3.54
57.6	3.0						39.4	0.95	8.5		2.21
54.5	1.5		44					0.94	11.6		1.75
54.5	1.5	44						0.95	7.6	54.2	1.81
43.5	1.5					55		0.77	7.1		2.34
42.5	1.5		56					0.78	42.0	43	0.90
36.5	1.5	62						0.88	60.3		0.45
28.5	1.5					70		0.64	21.9		1.20

*Infinite dilution diffusivities (D_0) were obtained by extrapolation at zero sorbic acid concentration of three independent D_a values (for three sorbic acid concentrations included between 1.8 and 26.8 μmol/g of gel). D_a values used were determined by outward diffusion from gel cubes (average of six independent determinations).

†Water contents were measured at the conclusion of the diffusion experiments (average of each sample used for the corresponding D_0 determination).

and sugar solutions. Substrates such as glycerol are in the liquid state at room temperature and mix in all proportions with water; they are themselves good diffusion media and may contribute to the total pool of solvent at any given hydration level. Therefore they do not decrease markedly the diffusivity of diffusants, in spite of the increase in viscosity which they impart. This effect should be considered when a_w lowering agents in the liquid state are used to stabilize intermediate moisture foods (Duckworth *et al.*, 1975).

Table 2 presents the results obtained (with the same conditions as previously) for sucrose, and sucrose plus gelatine gels, and for sucrose solutions emulsified with oil and surfactants (gelified oil-in-water emulsion). D_0 values for sorbic acid were not modified by the replacement of part of the sucrose by food constituents such as gelatine (5 or 10%) or fat (13.5 or 21.5% peanut oil and emulsifiers).

Table 3 reports the infinite dilution diffusivity values of sorbic acid obtained by diffusion within infinite food columns as well as the water contents and the water activities of the foods. Sorbic acid diffusivity in processed cheese, chestnut cream and redcurrant jelly is very close to the diffusivity in sucrose gel with similar water content (model gel with 62% sucrose Table 1) although these foods contain components such as fat, proteins or starch.

Table 2. D_0 values* (25°C) of sorbic acid in model gel cubes containing sucrose and gelatin or oil at different water contents

Gel composition (% w/w)									
Water	Agar	Sucrose	Peanut oil	Span 20	Tween 80	Gelatin	a_w (25°C) of solution	Solution viscosity (10^{-3} Pa.sec)	$D_0 \times 10^{-10} m^2/sec$
98.5	1.5						1	1.5	8.74
73.5	1.5	20				5	0.98	53.7	4.41
68.5	1.5	30					0.97	3.4	3.80
68.5	1.5	20				10	0.97		3.93
65.0	1.5	20	8	4.4	1.1		0.98	3.84	2.50
57.0	1.5	20	16	4.4	1.1		0.98	4.92	2.24
54.5	1.5	44					0.95	7.6	1.81

*Infinite dilution diffusivities (D_0) are obtained by extrapolation at zero sorbic acid concentration of three independent D_a values (for three sorbic acid concentrations included between 1.8 and 26.8 μmol/g of gel). D_a values used are determined by outward diffusion from gel cubes (average of six independent determinations).

Table 3. D_0 values* (25°C) of sorbic acid in foods (processed cheese, redcurrant jelly and chestnut cream), as determined by monodimensional diffusion in infinite columns

	Water content % (w/w)	a_w (25°C)	$D_0 \times 10^{-10} m^2/sec$
Processed cheese	44	0.96	0.80
Redcurrant jelly	38	0.88	0.79
Chestnut cream	34	0.90	0.24

*Infinite diffusion diffusivities (D_0) are obtained by extrapolation at zero sorbic acid concentration of three independent D_a values (for three sorbic acid concentrations: 1.8, 4.5 and 8 μmol/g of food).

Influences of the water content, water activity and viscosity

As shown in Fig. 1, sorbic acid diffusivity (D_0) in various gelified concentrated solutions and in the foods, decreases as the water content (X) decreases (100 to 30% water) with an almost linear relationship. Regression lines are the following: $D_0 \times 10^{10} m^2/sec = 0.107\ X - 3.47$, with $r = 0.923$, or $D_0 \times 10^{10} m^2/sec - 0.134\ X - 5.04$, with $r = 0.984$ when calculated with all the products or with only the sucrose gels respectively. However there is no direct correlation between the water content and the sorbic acid diffusivity when the substrate is itself a good diffusion media (e.g., glycerol), when an important amount of high molecular component is added (e.g., maltodextrin with low DE), or when there is a change in the food structure (e.g., molecular obstruction to diffusent due to gelification (Giannakopoulos & Guilbert, 1986).

When plotted as a function of water activity (Fig. 2), the diffusivity appears to decrease very sharply where the water activity is lowered from 1 to 0.95–0.90 (depending on the kind of the substrate). The diffusivity was less affected upon further a_w reduction. Similar observations were made by Naesens, Bresseleers & Tobback (1982)

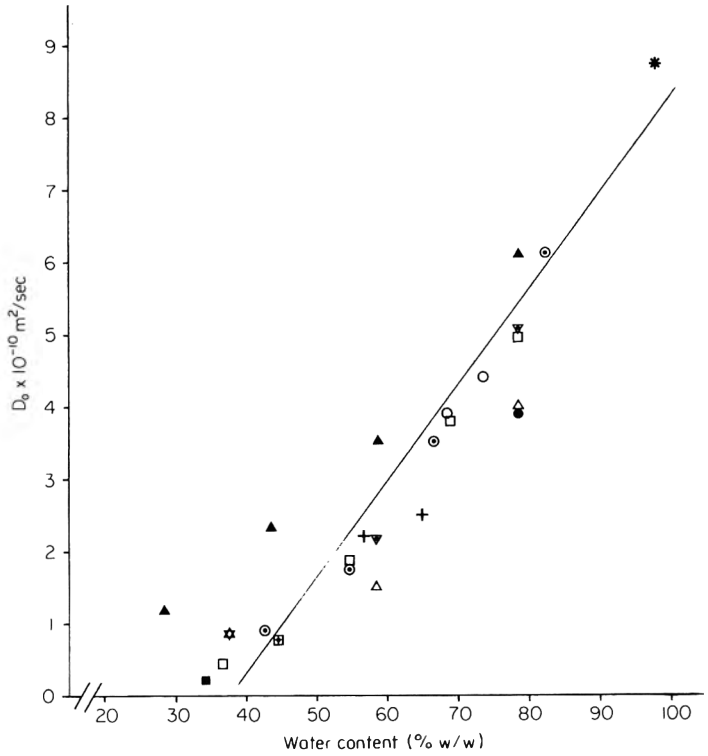

Figure 1. D_0 values of sorbic acid as a function of the water content of various gelified concentrated solutions and of redcurrant jelly, processed cheese and chestnut cream. Regression line given is calculated from the gelified concentrated sucrose solutions. ✱ Water, □ sucrose, ⊙ glucose syrup DE 46, △ maltodextrin DE 20, ● maltodextrin DE 6, ▲ glycerol, ▽ P.E.G. 600, ⊙ sucrose + gelatin, + sucrose + peanut oil, ⊞ processed cheese, ✡ redcurrant jelly, ■ chestnut cream.

for the diffusion of tripalmitin, and by Voilley & Le Meste (1985) for the diffusion of acetone in sugar and P.E.G. solutions.

As stated in the Stokes-Einstein or Wilke and Chang equation, the diffusivity should be inversely proportional to the viscosity. These equations are only strictly valid for large molecules in dilute solutions. However, they have been shown (Loncin, 1980) to apply correctly to the diffusion of nicotinamide and peroxidase in concentrated fructose solutions. Figure 3 reports the sorbic acid diffusivity values as a function of the inverse of the solution viscosities. All solutions exhibited a Newtonian rheological behaviour. The correlation between the diffusivity and 1/viscosity applies satisfactorily when concentrated sucrose or glucose syrup D.E. 46 are concerned; however, with maltodextrin solutions or when gelatin or fat is added to sucrose solutions, important changes in viscosity are not accompanied by comparable changes in diffusivities. In addition the measurement of viscosity of the liquid occluded into the food network is difficult.

Where various substrates with similar molecular weights at various concentrations (in the high or intermediate moisture range) are concerned, the diffusion of sorbic acid is related, as a first approximation, to the water content rather than to the water activity of the diffusion medium. Similar conclusions for the diffusivity of acetone and other organic solutes were drawn by Chandrasekaran & King (1972) with fructose, glucose

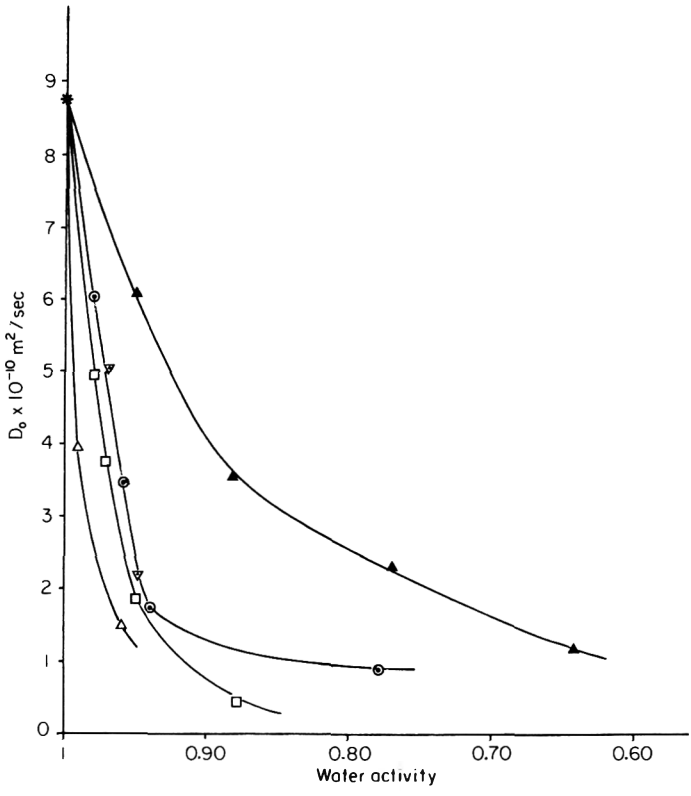

Figure 2. D_0 values of sorbic acid as a function of the water activity of the solution entrapped within the agar gels. ✱ Water, □ sucrose, Θ glucose syrup DE 46, △ maltodextrin DE 20, ▲ glycerol, ▽ P.E.G. 600.

and sucrose solutions and by Schneeberger *et al.* (1978) with sucrose and calcium chloride solutions.

The influence of water on the diffusion of solutes in foods is presumably very complex. The diffusion may be influenced by the extent to which the water is bound to food components (approximated by the a_w), by the total amount of water, as well as by changes in characteristics (e.g., physical state) of both the diffusant and the diffusion medium as related to hydration. In addition, the diffusion is influenced by the kind of substrate (molecular weight and physical state) and by the structure of the diffusion medium.

From a practical view point, the experimental linear relationship found between the sorbic acid diffusivity and the water content of gelified concentrated sucrose solutions can be used to approximate the sorbic acid diffusivity in processed cheese, chestnut cream and redcurrant jelly. We can expect that this empirical approximation should permit the prediction of sorbic acid diffusivity in various foods (with homogeneous structure).

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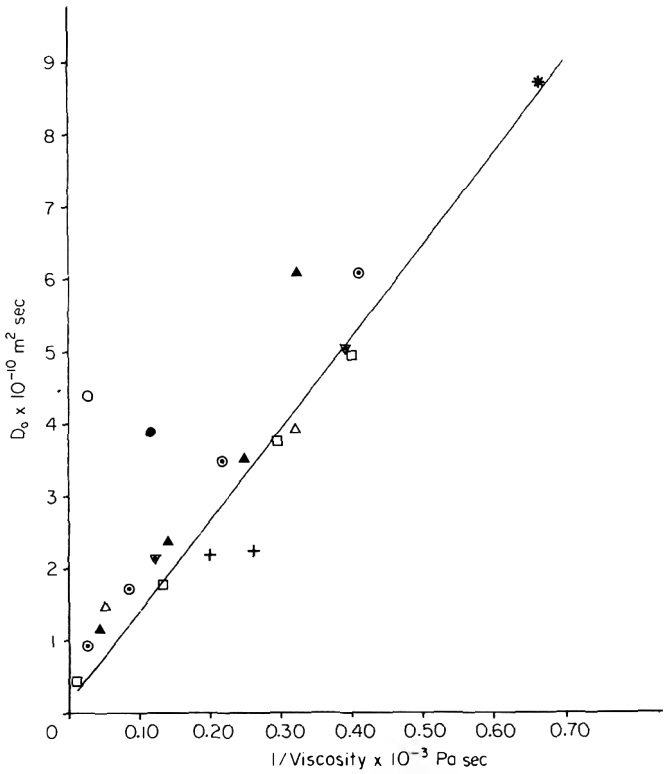

Figure 3. D_0 values of sorbic acid as a function of the inverse of viscosity of the solutions entrapped within the agar gels. Regression line given was calculated from the gelified concentrated sucrose solutions. ✱ Water, □ sucrose, ⊙ glucose syrup DE 46, △ maltodextrin DE 20, ● maltodextrin DE 6, ▲ glycerol, ▽ P.E.G. 600, O sucrose+gelatin, +sucrose+oil.

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References

Biquet, B. & Guilbert, S. (1986). Diffusivités relatives de l'eau dans des aliments modèles à humidité intermédiaire. *Lebensmittel Wissenschaft und Technologie*, in press.

Bousquet-Ricard, M., Quayle, G., Pham, T. & Cheftel, J.C. (1980). Etude comparative critique de trois méthodes de mesure de l'activité de l'eau des aliments à humidité intermédiaire. *Lebensmittel Wissenschaft und Technologie*, **13**, 169–176.

Chandrasekaran, S.K. & King, C.J. (1972). Multicomponent diffusion and vapor-liquid equilibria of dilute organic components in aqueous sugar solutions. *Journal of the American Institute of Chemical Engineers*, **18**, (3), 513–520.

Duckworth, R.B., Allison, J.Y. & Clapperton, J.A. (1975). The aqueous environment for chemical change in intermediate moisture foods. In: *Intermediate Moisture Foods* (edited by R. Davies, G.G. Birch and K.H. Parker). Pp. 89–99. London: Applied Science Publishers.

Duckworth, R.B. (1981). Solute mobility in relation to water content and water activity. In: *Water Activity: Influences on Food Quality* (edited by L.B. Rockland and G.F. Stewart). Pp. 295–317. New York: Academic Press.

Giannakopoulos, A. & Guilbert, S. (1986). Determination of sorbic acid diffusivity in model food gels. *Journal of Food Technology*, **21**, 339–353.

Guilbert, S., Giannakopoulos, A. & Cheftel, J.C. (1985). Diffusivity of sorbic acid in food gels at high and intermediate water activities. In: *Properties of Water in Foods in Relation to Quality*

and Stability (edited by D. Simatos and J.L. Multon). Pp. 343–356. Dordrecht: Martinus Nijhoff Publishers.

Guilbert, S. (1986). Technology and application of edible protective film. Symposium International Emballage et Conservation Produits Alimentaires. ECPA 85. REIMS, in press.

Loncin, M. (1980). Diffusion phenomena in solids. In: *Food Process Engineering*, Vol. 1 (edited by P. Linko, Y. Malkki, J. Olkky and J. Larinkari). Pp. 354–363. London: Applied Science Publishers.

Naesens, W., Bresseleers, G. & Tobback, P. (1982). Diffusional behaviour of tripalmitin in a freeze-dried model system at different water activities. *Journal of Food Science*, **47**, 1245–1249.

Schneeberger, R., Voilley, A. & Weisser, H. (1978). Activity of water in frozen systems. *International Journal of Refrigeration*, **1**, 201–206.

Schwartzberg, H.G. & Chao, R.Y. (1982). Solute diffusivities in leaching processes. *Food Technology*, **2**, 73–86.

Simatos, D., Le Meste, M., Petroff, D. & Halphen, B. (1981). Use of ESR for the study of solute mobility in relation to moisture content in model food systems. In: *Water Activity: Influences on Food Quality* (edited by L.B. Rockland and G.F. Stewart). Pp. 319–346. New York: Academic Press.

Voilley, A. & Simatos, D. (1980). Retention of aroma during freeze and air drying. In: *Food Process Engineering*, Vol. 1 (edited by P. Linko, Y. Malkki, J. Olkku and J. Larinkari). Pp. 371–384. London: Applied Science Publishers.

Voilley, A. & Le Meste, M. (1985). Aroma diffusion: the influence of water activity and of molecular weight of the other solutes. In: *Properties of Water in Foods in Relation to Quality and Stability* (edited by D. Simatos and J.L. Multon). Pp. 357–373. Dordrecht: Martinus Nijhoff Publishers.

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Comparison of selected food characteristics of three cultivars of bean *Phaseolus vulgaris*

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Summary

Three bean (*Phaseolus vulgaris*) cultivars were selected to study some of the physical properties of the seeds, hydration capacity, cooking time, phytic acid content, digestibility, colour changes as a function of soaking and cooking, and sensory properties. These cultivars were bayocel, canario 101 and flor de mayo. The water uptake was much lower for the cultivar with larger seeds. Some slight losses of phytic acid were found due to the cooking treatment; cooking improved the protein digestibility. The cultivar flor de mayo exhibited the most desirable sensory attributes.

Introduction

Beans (*Phaseolus vulgaris*) are an important source of protein calories, B-complex vitamins and minerals in Latin America. In Mexico, common beans are, with maize, the most strategic components of the daily diet; beans and lime-cooked maize have remarkable complementary nutritional qualities (Bressani *et al.*, 1962; Paredes-López & Saharópulos-Paredes, 1983). In spite of this, bean production is declining in Latin American countries.

The problems associated with bean consumption are well known, particularly those related to the presence of some anti-nutritional factors (Sgarbieri, Antunes & Almeida, 1979) and production of gastrointestinal distress (Olson *et al.*, 1982). When beans are prepared for consumption they are usually soaked and cooked—treatments that may reduce the proportion of unwanted components present in dry beans (Aw & Swanson, 1985; Kon, 1979; Olson *et al.*, 1982).

There is a programme in progress in our research centres for the development and selection of bean cultivars with good agronomic performance (i.e., high productivity, disease resistance, drought tolerance), simultaneously with improved nutritive value and functional qualities (Paredes-López *et al.*, 1985). This work was carried out to compare some of the food properties of three bean cultivars. These cultivars were selected because of their high yield and outstanding agronomic performance (INIA, 1985).

Materials and methods

Materials

The cultivars under study were seeded in plots at the experimental farm of the CIAB-Instituto Nal. de Investigaciones Agrícolas, Celaya, México. Bayocel and

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canario 101 were harvested, shelled and cleaned in the spring of 1984, whereas flor de mayo RMC was collected in the summer of the same year.

Moisture and protein determinations

The bean samples were ground in a Udy cyclone mill to pass a 100-U.S. mesh screen. The bean flours were kept in tightly covered glass jars at 4°C. Moisture content of the bean flour sample was determined on 3 g samples in triplicate by heating in an oven at 130°C for 1 hr (AACC, 1978). Protein (N×6.25) was analysed using samples of 0.5 g and following the Kjeldahl procedure (AACC, 1978). As suggested by other workers (CIAT, 1982), samples for water absorption, cooking and colour tests were incubated at 60°C to reach a moisture content of about 8%.

Physical characteristics of seeds

Weights of 100 randomly selected beans were determined in triplicate. Test weight (kg/Hl) determinations in triplicate were carried out according to Deshpande, Sathe & Salunkhe (1984). The length (L), width (W) and thickness (T) were measured on twenty-five randomly selected sound seeds for each cultivar with a vernier caliper.

Water absorption

The procedure described by Kailasapathy, Perera & MacNeil (1985) was followed, with small variations. One hundred seeds were soaked in three additional volumes of distilled water at room temperature (25±2°C). At predetermined time intervals, the submerged beans were drained, blotted and weighed. The increase in weight was reported as water uptake per 100 g of dry weight. Results are the average of three determinations.

Cooking conditions

After 16 hr of soaking, 100 seeds were cooked in three volumes of hot distilled water in an open vessel. By adding hot water, the total volume was kept constant throughout the heating period. Cooking time is the time required to cook 50% of the sample (Morris, 1963). Unless otherwise noted, determinations in triplicate were carried out at each soaking time and for each cultivar.

Phytic acid assay

Phytic acid content of whole seeds was determined by a combination of three methods. Extraction and precipitation of phytate were done according to the procedure of Doherty, Faubion & Rooney (1982). A 1 g sample of flour was extracted with 30 ml of 10% Na_2SO_4 in 1.2% HCl for 2 hr at 20°C with mechanical shaking. The slurry obtained was centrifuged at 5000 g for 15 min. Ten ml of supernatant were used for each determination. Phytate in the supernatant was precipitated as ferric phytate. The conversion of ferric phytate to ferric hydroxide was carried out according to Makower (1970). The precipitate (ferric hydroxide) was dissolved in 0.6 ml of 0.5 N HCl in a boiling water bath for 15 min. The solution was cooled, transferred and made to 50 ml volume with 0.1 N HCl. The ferric iron content was determined by the AACC (1978) method. The contents of iron and phosphorus were assumed to be in a 4:6 ratio in order to calculate phytate phosphorus content. Phytic acid content was calculated on the assumption that it contains, by weight, 28.2% phosphorus.

In vitro *digestibility*

In vitro protein digestibility of raw and cooked beans was determined by the multienzyme technique of Hsu *et al.* (1977). The enzyme peptidase and trypsin were from Sigma Chemical Co., St Louis, MO, and α-chymostrypsin from P.L. Biochemicals, Milwaukee, WI.

Surface colour

Colour was measured with the Hunterlab Model D25-2 colour and colour difference meter using the white tile as a standard ('L' = 91.2, 'a' = −1.0, 'b' = −1.7). About 100 g of beans were placed in an optically pure glass sample dish, covered to eliminate interfering light during colour measurements. To compensate for irregular surface effects, four readings per sample were taken by rotating the dish through 90° (Uebersax & Bedford, 1980).

Sensory evaluation

Eight panellists were selected by previous sensory tests from students and staff of the Unidad Irapuato. Panellists evaluated bean samples for appearance, colour, flavour, texture and adhesiveness. The hedonic scale ranged from 1 = like extremely to 9 = dislike extremely. Sensory evaluation was conducted in triplicate following the recommendations of the Canada Department of Agriculture (CDA, 1977) and IFT (1981).

Statistical analyses

Means were analysed statistically by analysis of variance using the randomized complete block design, and they were separated by Duncan's multiple range test.

Results and discussion

The moisture content of the bean samples ranged from 9.0±0.2 to 9.8±0.3%. The protein content of bayocel, canario and flor de mayo was 20.0±0.4, 21.9±0.1 and 23.1±0.2%, respectively. The 100 bean weight and test weight of canario were high compared to those of bayocel and flor de mayo (Table 1). Thus, the smaller bean types, bayocel and flor de mayo, will occupy more storage space per unit weight of beans.

Water uptake at 6 and 16 hr is shown in Table 2. Saturation was reached at 16 hr. The physical characteristics of the seeds appeared to be important for water absorption, as found by Deshpande *et al.* (1984); bayocel and flor de mayo had a larger surface area and this might partially account for the higher hydration levels relative to canario. Several workers (Powrie, Adams & Pflug, 1960; Ott & Ball, 1943; Sefa-Dedeh &

Table 1. Some physical characteristics of bean seeds*

Cultivar	100 bean weight (g)	Test weight (kg/Hl)	Seed dimensions (cm) Length (L)	Width (W)	Thickness (T)
Bayocel	30.77±0.66	78.4±0.6	1.10±0.09	0.73±0.04	0.53±0.12
Canario 101	40.69±1.34	79.1±0.2	1.44±0.08	0.73±0.05	0.58±0.05
Flor de mayo RMC	31.59±0.37	75.6±0.2	1.19±0.06	0.75±0.05	0.55±0.05

*Means±standard deviations.

Table 2. Hydration of bean seeds at two selected soaking times*

	Water uptake (%)	
Cultivar	6 hr	16 hr
Bayocel	72.9±1.6 a/g	105.9±6.7 a/f
Canario 101	31.9±3.7 b/g	68.0±10.9 b/f
Flor de mayo RMC	63.3±11.4 a/g	115.2±1.2 a/f

*Means within columns or within rows followed by the same letters are not significantly different at $P \leq 0.01$. At the left letters to be read in columns; at the right letters to be read in rows. Means±standard deviations.

Table 3. Cooking time of bean seeds at selected soaking times*

	Cooking time (min)		
Cultivar	0 hrs†	6 hr	16 hr
Bayocel	133 a/f	111±5 b/g	102±6 a/h
Canario 101	128 a/f	126±15 a/f	98±6 a/g
Flor de mayo RMC	135 a/f	128±9 a/g	104±11 a/h

*Means within columns or within rows followed by the same letter are not significantly different at $P \leq 0.01$. At the left letters to be read in columns; at the right letters to be read in rows.

†Average of duplicate determinations.

Table 4. Phytic acid content in raw and cooked beans*†

Cultivar	Raw beans	Cooked beans‡
Bayocel	1.8 b/f	1.5 b/g
Canario 101	2.3 a/f	2.0 a/g
Flor de mayo RMC	2.1 a/f	1.9 a/f

*Expressed in g/100 g sample, dry basis.

†Means within columns or within rows followed by the same letters are not significantly different at $P \leq 0.01$. At the left letters to be read in columns; at the right letters to be read in rows.

‡After 16 hr of soaking.

Table 5. *In vitro* digestibility of raw and cooked beans*†

Cultivar	Raw beans	Cooked bean‡	% Increase on cooking
Bayocel	66.7 a/g	76.0 a/f	13.9
Canario 101	67.4 a/g	75.2 a/f	11.6
Flor de mayo RMC	65.8 a/g	75.2 a/f	14.3

*Expressed in percentage.

†Means within columns or within rows followed by the same letters are not significantly different $P \leq 0.01$. At the left letters to be read in columns; at the right letters to be read in rows.

‡After 16 hr of soaking.

Stanley, 1979a, 1979b) have reported that the water absorption of seeds also depends on the microstructure of the seed coat, hilum, micropyle and protein content.

For the three cultivars the cooking time was decreased using 16 hr of soaking (Table 3). At this soaking time, there were no significant differences between the cooking times of the cultivars. Table 4 shows that bayocel contained the lowest amount of phytic acid. It is noteworthy that for all cultivars, cooking only slightly decreased the phytic acid level; other workers (Iyer *et al.*, 1980; Ologhobo & Fetuga, 1984) have reported remarkably high losses of phytic acid during soaking and cooking.

In vitro digestibility tests exhibited no significant differences within the three cultivars under study, either in a raw or cooked state (Table 5). However, it was observed that for all cultivars cooking marginally improved the protein digestibility. The digestibility levels of raw beans are in agreement with results reported by Deshpande *et al.* (1982). The Hunter colour of raw and cooked beans at selected soaking times was evaluated in terms of 'L', 'a' and 'b' values (Table 6). The 'L' value measures the amount of light reflected or transmitted by the object. The 'a' value measures redness and 'b' value yellowness, both in the positive portion of the scale. It was noted that in raw and cooked beans soaking tended to increase the 'L' values of all cultivars, with canario being the lighter in colour. Cooking produced a remarkable darkening effect in all bean samples. The red intensity did not follow a trend. For cooked beans, and for each particular cultivar, the 'b' values exhibited almost no variations at the selected soaking times. The sensory evaluation of cooked beans is presented in Table 7. For each cultivar, the soaking period produced in the sensory properties some changes of statistical significance. Flor de mayo bean at 16 hr was the cultivar with the most desirable sensory attributes to the eight assessors. Further research is in progress to determine, under different storage conditions, cooking kinetics, nutritional factors and sensory properties of various bean cultivars with outstanding agronomic performance.

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Table 6. Effect of soaking and cooking on the Hunter colour of beans*

	Hunter colour at selected soaking times								
	0 hr			6 hr			16 hr		
Cultivar	'L'	'a'	'b'	'L'	'a'	'b'	'L'	'a'	'b'
Raw beans†									
Bayocel	43.7±0 c/h	6.9±0.2 b/g	11.9±0 b/g	46.9±0.1 c/f	8.2±0.3 a/f	14.7±0.1 b/f	45.9±0.2 c/g	8.2±0.5 b/f	14.8±0.2 a/f
Canario 101	51.8±0.1 a/g	2.0±0.2 c/g	17.2±0.1 a/f	50.8±0.1 a/h	2.3±0.4 b/g	16.8±0.1 a/g	54.2±0.2 a/f	5.1±0.4 c/f	14.8±0.3 a/h
Flor de mayo RMC	44.7±0.2 b/h	9.5±0.2 a/f	9.3±0.1 c/h	50.1±0.1 b/g	7.6±0.1 a/g	12.8±0.1 c/g	51.2±0.1 b/f	9.8±0.2 a/f	14.3±0.1 b/f
Cooked beans†									
Bayocel	27.2±0 c/h	7.8±0.1 b/f	8.3±0 b/f	29.8±0 c/f	8.2±0.3 b/f	8.4±0.1 b/f	29.6±0.2 c/g	7.1±0.2 b/f	8.3±0.1 c/f
Canario 101	34.4±0.1 a/h	7.4±0.4 b/f	11.3±0.2 a/f	35.7±0.1 a/g	7.8±0.1 b/f	12.2±0.1 a/f	37.5±0 a/f	6.5±0.2 b/g	10.5±0.1 b/f
Flor de mayo RMC	32.3±0 b/h	8.8±0.3 a/g	11.0±0.1 a/g	35.2±0.1 b/g	10.0±0.1 a/f	12.3±0 a/f	36.8±0 b/f	10.4±1.2 a/f	12.3±0 a/f

*Statistic analysis for raw beans and cooked beans was carried out in a separate way.

†Means within columns or within rows followed by the same letters are not significantly different at $P \leq 0.01$. At the left letters to be read in columns. At the right letters to be read in rows. Means±standard deviations.

Table 7. Sensory evaluation of cooked beans at selected soaking times*†‡

	Cultivars								
	Bayocel			Canario 101			Flor de mayo RMC		
Properties	0	6	16	0	6	16	0	6	16
Appearance	56 a	51 a	54 a	36 b	35 b	45 ab	21 b	25 b	22 b
Colour	49 a	48 a	57 a	33 b	34 b	45 a	29 b	21 b	24 b
Flavour	44 a	48 a	48 a	18 b	21 b	36 a	40 a	29 ab	36 a
Texture	35 ab	43 ab	50 a	30 ab	28 ab	24 b	39 ab	28 ab	32 ab
Adhesiveness	36 a	35 a	35 a	36 a	44 a	45 a	48 a	50 a	45 a
Total	220	225	244	153	162	196	177	153	139

*Soaking times, 0, 6 and 16, in hr.

†The total score of the eight panellists is reported. Hedonic rating: 1 = like extremely; 9 = dislike extremely.

‡Means within rows followed by the same letters are not significantly different at $P \leq 0.01$.

References

AACC (1978). *Approved Methods*. St. Paul MN: American Association of Cereal Chemists.

Aw, T.-L. & Swanson, B.G. (1985). Influence of tannin on *Phaseolus vulgaris* protein digestibility and quality. *Journal of Food Science*, **50**, 67–71.

Bressani, R., Elias, L.G., Valiente, A.T. & Tejeda, C. (1962). All-vegetable protein mixtures for human feeding. VI. The value of combinations of lime-treated corn and cooked black beans. *Journal of Food Science*, **27**, 394–400.

CDA (1977). *Laboratory Methods for Sensory Evaluation of Food*. Ottawa, Ontario: Canada Department of Agriculture.

CIAT (1982). *Programa de frijol. Informe Anual*. Colombia, CA: Centro Internacional de Agricultura Tropical.

Deshpande, S.S., Sathe, S.K. & Salunkhe, D.K. (1984). Interrelationships between certain physical and chemical properties of dry bean (*Phaseolus vulgaris* L.). *Qualitas Plantarum Plant Foods for Human Nutrition*, **34**, 53–65.

Deshpande, S.S., Sathe, S.K., Salunkhe, D.K. & Cornforth, D.P. (1982). Effects of dehulling on phytic acid, polyphenols, and enzyme inhibitors of dry beans (*Phaseolus Vulgaris* L.). *Journal of Food Science*, **47**, 1846–1850.

Doherty, C., Faubion, J.M. & Rooney, L.W. (1982). Semiautomated determination of phytate in sorghum and sorghum products. *Cereal Chemistry*, **59**, 373–377.

Hsu, H.W., Vavak, D.L., Satterlee, L.D. & Miller, G.A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science*, **42**, 1269–1273.

IFT (1981). Sensory evaluation guide for testing food and beverage products. *Food Technology*, **35**(11), 50–59.

INIA (1985). *Guía para Cultivar Frijol de Riego en el Centro y Sur de Guanajuato*. Celaya, Gto., México: Instituto Nacional de Investigaciones Agrícolas, CIAB.

Iyer, V., Salunkhe, D.K., Sathe, S.K. & Rockland, L.B. (1980) Quick-cooking beans (*Phaseolus vulgaris* L.). II. Phytates, oligosaccharides, and antienzymes. *Qualitas Plantarum Plant Foods for Human Nutrition*, **30**, 45–52.

Kailasapathy, K., Perera, P.A.J. & MacNeil, J.H. (1985). Soaking studies of winged bean (*Psophocarpus tetragonolobus* L. DC) to process full-fat flour and determine it's shelf-life stability. *Journal of Food Science*, **50**, 773–776, 794.

Kon, S. (1979). Effect of soaking temperature on cooking and nutritional quality of beans. *Journal of Food Science*, **44**, 1329–1335.

Makower, R.V. (1970). Extraction and determination of phytic acid in beans (*Phaseolus vulgaris*). *Cereal Chemistry*, **47**, 288–292.

Morris, H.J. (1963). Cooking qualities of dry beans. *Sixth Annual Dry Bean Conference. Los Angeles, CA*.

Ologhobo, A.D. & Fetuga, B.L. (1984). Effect of processing on the trypsin inhibitor, haemagglutinin, tannic acid and phytic acid contents of seeds of ten cowpea varieties. *Tropical Agriculture* (Trinidad), **61**, 261–264.

Olson, A.C., Gray, G.M., Gumbmann, M.R. & Wagner, J.R. (1982). Nutrient composition of and digestive response to whole and extracted beans. *Journal of Agricultural and Food Chemistry*, **30**, 26–32.

Ott, A.C. & Ball, C.D. (1943). Some components of the seed coats of the common bean, *Phaseolus vulgaris*, and their relationship to water retention. *Archives of Biochemistry*, **3**, 189–192.

Paredes-López, O. & Saharópulos-Paredes, M.E. (1983). Maize: a review of tortilla production technology. *Bakers Digest*, **57**(5), 16–24.

Paredes-López, O., González, J., Arroyo, M.G., Montes, R. & Guevara, F. (1985). Estudios para la identificación de variedades de frijol de alto valor alimentario. *XVI Congreso Nacional de Ciencia y Tecnología de Alimentos. México, D.F.*

Powrie, W.D., Adams, M.W. & Pflug, I.J. (1960). Chemical, anatomical and histochemical studies on the navy bean seed. *Agronomy Journal*, **52**, 163–166.

Sefa-Dedeh, S. & Stanley, D.W. (1979a). Microstructure of cowpea variety Adua Ayera. *Cereal Chemistry*, **56**, 367–371.

Sefa-Dedeh, S. & Stanley, D.W. (1979b). The relationship of microstructure of cowpeas to water absorption and dehulling properties. *Cereal Chemistry*, **56**, 379–386.

Sgarbieri, V.C., Antunes, P.L. & Almeida, L.D. (1979). Nutritional evaluation of four varieties of dry beans. *Journal of Food Science*, **44**, 1306–1308.

Uebersax, M.A. & Bedford, C.L. (1980). Navy bean processing: effect of storage and soaking methods on quality of canned beans. *Research Report* 410. Michigan State University, Agricultural Experiment Station. East Lansing, MI.

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An attempt to elaborate the curing technology for broiler chicken gizzards

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Summary

The usefulness of several curing technologies for broiler chicken gizzards was investigated. The specific microstructure of the gizzard tissue is considered to be a crucial feature of this raw material, limiting the selection of the effective curing technology. Curing intact or diced gizzards in nitrate brine and the use of dry curing nitrate blend was unsuccessful, but the use of nitrite brine for curing both was acceptable. However, curing fresh broiler chicken gizzards during comminution in a bowl chopper with the nitrite brine was the most rapid and effective curing technology.

Introduction

At the present time an increasing proportion of broiler meat is being converted to further processed items such as rolls, fillets, frankfurters and other portion-controlled items. The increase in production volume in the poultry industry over the past 35 years has substantially contributed to the availability and utilization of poultry meat; in particular more giblets are available as sausage and patty ingredients, as fewer whole carcasses are being marketed with hearts, livers and gizzards as a package stuffed into the cavity of the carcass (Crawley, Sloan & Hale, 1980). Assuming that 3% of the dressed broiler is gizzard, hundreds of thousands of tons of this meat is available for further processing and at present under-utilized. Gizzards consumed in the intact muscle form are rather tough, with a unpopular, rubbery texture, which is different from that of skeletal muscle (Chen & Stinson, 1983). Mikulski & Stadelman (1976, 1977) studied this and evaluated papain as a marination solution for the gizzards in addition to piercing with a fork several times prior to cooking; this combination yielded a product with adequate tenderness.

The pickling of gizzards in vinegar and salt offers a poultry product with long shelf life and a good market potential (Chen, 1976; Arafa, 1977; Charoenpong & Chen, 1980). The quality, functional properties and microbial profile of poultry meat patties as affected by the incorporation of 10% broiler giblets were evaluated by Lyon *et al.* (1982) and Cox *et al.* (1983). Blackshear, Hudspeth & May (1966) assessed organoleptic properties of frankfurters made from giblet meats. Marsden (1982) reported that poultry frankfurters in the U.S.A. in 1980 accounted for over 8% of the frankfurter market and in some areas the market share was as high as 15–20%.

Parkes & May (1968) and Hudspeth & May (1969) considered edible byproducts of poultry, such as gizzards and hearts, a good raw material for comminuted sausage. Gizzard and heart protein binding value, binding and emulsifying coefficients are very good in comparison with protein of white and dark muscles, although the extractability of their salt-soluble protein is worse than the meat tissue. A review of factors affecting

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poultry meat emulsifying characteristics was made by Cunningham & Froning (1972). Thus the functional properties of selected poultry edible byproducts used as potential ingredients of sausage and/or of patties are quite well established. However as no information could be found in the available literature, studies were made to determine and evaluate the technological conditions for curing chicken broiler gizzards. To achieve this goal seven separate experiments were carried out.

Materials and methods

Fresh chicken gizzards obtained from the local industrial poultry processing plant were trimmed free from mucous, serous membranes and visible fat, and sub-divided into seven batches which were cured according to the experimental design shown in Table 1. The curing brines and/or curing dry blends employed are shown in Table 2.

Table 1. Experimental design

Experiment no.	Curing technology	Curing agent
Gizzards: Intact		
1	Brine curing	Nitrite
2	Brine curing	Nitrate
3	Dry curing	Nitrate
Gizzards: diced into cubes 15×15 mm		
4	Brine curing	Nitrite
5	Brine curing	Nitrate
6	Dry curing	Nitrate
Gizzards: comminuted		
7	Brine curing*	Nitrite

*Nitrite brine added during comminution in a bowl chopper of ground gizzards.

Table 2. Composition of curing brines and/or dry curing blends

Experiment no.	Grams					
	NaCl	KNO_3	$NaNO_2$	Sucrose	Na-Ascorb.	Water
1.4	80.0	0.0	0.20	5.0	5.0	909.8
2.5	80.0	2.0	0.0	5.0	5.0	908.2
7	20.0	0.0	0.20	5.0	5.0	100.0
3.6	98.8	0.2	0.0	0.5	0.5	0.0

Intact and diced gizzards were cured in brines for 24 hr at 4°C in weight proportions of one part of the raw material to two parts of brine; and also for 24 hr at 4°C, after thorough mixing with 2% w/w dry curing blend. Intact and diced gizzards, brine cured and/or dry cured, underwent thermal treatment at 90–95°C in a 2% common salt solution for 45 min and were then chilled under running tap water for 3 min and the excess water allowed to drip out for 3 min.

In experiment 7, to each 100 g of raw gizzards, ground twice using a grinder plate with 2 mm diameter holes, were added 100 ml of nitrite curing brine and 100 ml of ice water during comminution in a bowl chopper for 15 min. One hundred and fifty ml narrow glass containers were filled up with the comminuted mixture, left at room temperature for 20 min and then heated in a boiling water bath until the temperature of 78–80°C, monitored with Thermistor thermometers, was reached in a geometrical centre.

In order to evaluate the effectiveness of each of the curing procedures, and thus the applicability of the results of this study, dry matter, residual nitrite and nitrate, total haem- and nitroso pigments were determined. Percentage of haem pigments nitrosation i.e., the degree of nitrosation was calculated. Dry matter was determined by drying the sample at 105°C for approximately 24 hr. Residual nitrite and nitrate were determined according to the Polish Standard, PN-74A-82114. Total amounts of haem- and nitroso pigments as well as the degree of nitrosation were estimated according to Hornsay (1956).

Results and discussion

The amount of total haem pigments content for intact and diced gizzards cured in both nitrite and/or nitrate brine was on average 219 ppm of haematin, ranging from 216.8 to 240.4 ppm haematin. Differences were not significant ($P = 0.05$).

The total haem pigments content in broiler chicken gizzards is greater than in muscles of pigs, cattle and sheep. According to data collected by Pisula (1974) on myoglobin contents in selected muscles of slaughtered animals of different species and age, and using the conversion factor of Franke & Solberg (1971), the total pigment content expressed in ppm of haematin in beef longissimus dorsi approx. is 120–180 ppm and depends mainly on age, while in mutton it is approximately 190 ppm and in pig meat as low as 35–60 ppm.

Pisula's (1974) own results shows the amount of total pigments in pig meat as being in a range of 29–76 ppm haematin depending on the muscle investigated. Ziemiński *et al.* (1978) determined in beef longissimus dorsi 160–190 ppm of total pigments, expressed as the amount of haematin. Dowiercial, Muzyka & Pisula (1978) determined the total pigment in sheep M. adductor as 136±12.7 ppm haematin.

The content of haem pigments in the meat of various species of poultry (chicken broilers, turkeys, ducks and geese) was determined by Pikul, Niewiarowicz & Pospieszna (1982). The authors found 170 and 140 ppm haematin in very dark duck breast and thigh muscles respectively, i.e., a smaller quantity than determined for chicken gizzards, although a greater amount was determined by them for goose breast muscle (272 ppm).

Thus the gizzard meat tissue of chicken broiler is much richer in haem pigments than white and dark muscles of most species of poultry and the muscles of other species.

A significant difference ($P < 0.05$) in the amount of total pigments was observed for nitrate dry curing of intact gizzards and for curing using nitrite brine added during comminution. The determined amount of total pigments was approximately 233 and 240 ppm of haematin, respectively (Table 3). Our results indicate also that only approximately 4–5% of the pigment was eluted from the gizzard tissue into the brine.

The quantity of nitroso pigments depends strongly on the degree of comminution and/or on the curing agent used. Curing in nitrite brine was much more effective with regard to pigment nitrosation than curing in nitrate brine whether or not the raw

Table 3. Effectiveness of several curing technologies for chicken broiler gizzards

Traits		Gizzards: curing technology						
		Intact			Diced			Ground comminuted
Experiment no.		Brine $NaNO_2$ 1	Brine KNO_3 2	Dry KNO_3 3	Brine $NaNO_2$ 4	Brine KNO_3 5	Dry KNO_3 6	Brine $NaNO_2$ 7
Total pigments $n = 15$	$\bar{x}$	220.47^{ab}	222.53^{b}	232.87^{c}	217.33^{ab}	216.80^{a}	224.47^{b}	240.40^{d}
ppm haematine	s.e.	6.23	7.23	9.90	9.22	7.32	10.46	9.35
Nitroso pigments	$\bar{x}$	103.09^{e}	7.79^{a}	8.49^{b}	113.47^{f}	10.95^{c}	12.69^{d}	148.13^{g}
ppm, $n = 15$	s.e.	5.99	1.03	0.68	5.85	0.74	0.74	7.55
Degree of nitrosation	$\bar{x}$	47.27^{d}	3.50^{a}	3.65^{a}	52.37^{e}	5.02	5.67^{c}	61.35^{f}
(%) $n = 15$	s.e.	2.37	0.19	0.27	5.40	0.40	0.38	2.12
Residual nitrite	$\bar{x}$	12.57^{b}	1.19^{c}	1.20^{c}	12.10^{b}	1.17^{c}	1.18^{c}	18.67^{a}
ppm, $n = 15$	s.e.	0.91	0.04	0.06	0.97	0.04	0.07	1.33
Residual nitrate	$\bar{x}$	—	155.00^{ab}	172.00^{c}	—	135.67^{a}	163.00^{b}	—
ppm, $n = 15$	s.e.	—	10.69	9.78	—	19.99	10.76	—
Dry matter (%)	$\bar{x}$	28.47^{b}	28.43^{b}	30.67^{d}	27.42^{a}	27.44^{a}	29.95^{c}	31.37^{e}
$n = 15$	s.e.	0.28	0.27	0.36	0.32	0.28	0.18	0.21

All means within each row with different superscripts are significantly different ($P < 0.05$).

material was cured intact, diced and/or during comminution in a bowl chopper. However the quantity of nitroso pigments estimated for all the experimental variants of curing technique applied, differ from each other significantly ($P < 0.05$). The nitroso pigments in gizzards cured intact and/or diced in nitrite brine were 103.1 and 113.5 ppm, respectively; but when cured during comminution the value was 148.1 ppm i.e., the concentration of nitroso pigments was approximately 36% higher.

From these results it seems apparent that the diffusion of the curing ingredients, affecting the level of nitrosation of gizzard pigment, is strongly dependent on the specific histological ultrastructure of the gizzard meat tissue. According to Chen & Stinson (1983) the microstructure of raw gizzard is sponge-like, the fibres are shaped like flattened noodles and no fibre-like structure with band pattern is observed. Moreover they are of the opinion that the collagen of chicken gizzards might be different in quantity, quality or both when compared to that of chicken skeletal muscle. Also Forrest *et al.* (1975) indicated that smooth muscle fibres present in the greatest quantity in the gastrointestinal tract vary in size and shape and their sarcollemma forms membrane to membrane contact bridges with neighbouring fibres, each of which are additionally surrounded by a delicate network of reticular fibres that support and bind them in place. Scattered collagenous and elastin filaments, and an associated ground substance, occupy the narrow space between the fibres. Therefore, according to our findings, only fine comminution will result in fast conversion of the haem pigments of the gizzards into nitroso pigments, thus increasing the speed of the curing process.

In comparison with the results obtained for pigment nitrosation by curing during comminution in a bowl chopper, the nitrosation of the gizzard haem pigments during nitrate dry and/or brine curing was very ineffective. The process of nitrosation of intact and/or diced gizzards for dry nitrate curing was 17.4 and 11.7 times and for nitrate brine curing 19.0 and 13.5 times, less effective, respectively (Table 1).

The observed phenomena are also very well reflected in the degree of nitrosation expressed as a percentage of nitrosated haem pigments in relation to their total quantity. The estimated haem pigment conversion to the nitroso pigments, i.e., the determined degree of nitrosation, using nitrite brine for curing the gizzards during comminution in a bowl chopper (experiment 7) was 61%, for curing diced in nitrite brine, 52%, and for curing intact, 47%, respectively.

Dry nitrate curing of intact and/or diced gizzards resulted in only 3.7 and 5.7% of the pigment conversion and in nitrate brine in 3.5 and 5.0%, respectively. This reveals approximately the same ratio of curing effectiveness as was found for the quantity of the nitroso pigments i.e., 16.8, 10.8, 17.5 and 12.2 times less effective, respectively than when gizzards were cured during comminution. Regarding the results of pigment conversion no significant ($P > 0.05$) difference was observed for brine and dry nitrate curing of intact gizzards. Moreover, curing of intact gizzards for 24 hr in nitrate brine was 1.30 times less effective than curing during comminution, while 1.17 times less effective after dicing. The ratio of the nitrosation of the gizzard pigments expressed in the content of dry matter shows approximately the same effectiveness i.e., it was: 16.4, 10.5, 15.9, 10.7, 1.2 and 1.0 times less effective than the conversion of pigment during comminution of gizzards with nitrite brine.

The level of the residual nitrite was strongly influenced by the variations in curing technique. The greatest and statistically significant ($P < 0.05$) amount of free nitrite i.e., 18.7 ppm was found in scalded gizzard batter cured with nitrite brine during comminution in a bowl chopper (Table 3). Curing of intact and/or diced gizzards in nitrite brine does not significantly ($P > 0.05$) influence the amount of residual nitrite

determined. In both cases their quantity amounted to 12.6 and 12.1 ppm, respectively, being 32.7 and 35.2% smaller than free nitrite content in gizzards cured during comminution. As had been expected, the content of residual nitrite in batters of intact and/or diced gizzards cured in nitrate brine was very low and insignificant. A similar result, also insignificant, was observed when nitrate dry curing blend was used. The determined quantity of free nitrite was: 1.19, 1.17, 1.20 and 1.18 ppm, respectively. Cooking of intact and/or diced gizzards in 2% salt solution did not significantly ($P > 0.05$) influence the amount of free nitrite as their levels expressed as the content in dry matter were respectively as follows: 4.19, 3.80, 3.90 and 3.93 ppm.

Curing of intact gizzards in nitrate brine and diced in both nitrate dry blend and brine does not significantly ($P < 0.05$) influence the level of free nitrate determined i.e., 155, 163 and 136 ppm, respectively. A significantly ($P < 0.05$) larger quantity of residual nitrate was found in intact gizzards cured using dry nitrate blend. The result shows a very low level of nitrate denitrification during 24 hr curing of intact and/or diced gizzards using both nitrate dry blend or brine, which corresponds very well with the nitroso pigment content and the degree of nitrosation i.e., haem pigment conversion to nitroso pigments.

Conclusions

(i) Curing of raw chicken broiler gizzards using nitrite brine during comminution in a bowl chopper is the most effective procedure. The observed level of total pigment conversion to nitroso pigments is similar to that determined for breast and thigh meat. (ii) The structure of the gizzard meat tissue strongly decreases the diffusion of curing ingredients, particularly when dry curing is applied. The total amount of haem pigment in chicken broiler gizzards is greater than the quantity determined for beef, mutton and pig meat and for poultry meat tissue itself, both white and dark.

References

Anon. (1974). Polish Standard. PN-74A-082114. Determination of nitrate and nitrite.

Arafa, A.S. (1977). Pickled chicken gizzards: 1. Acceptability and proximate analysis. *Poultry Science*, **56**, 1014–1017.

Blackshear, D., Hudspeth, J.P. & May, K.N. (1966). Organoleptic properties of frankfurters made from giblet meats. *Poultry Science*, **45**, 733–736.

Charoenpong, C. & Chen, T.C. (1980). Qualities of pickled chicken gizzards as affected by salt and vinegar. *Poultry Science*, **59**, 537–542.

Chen, T.C. (1976). Chicken gizzards could be new gourmet treat. *Mississippi Agriculture and Forestry Experimental Station Research Highlights*, **39**, 1.

Chen, T.C. & Stinson, R.S. (1983). Scanning electron microscope studies on chicken gizzards structure as affected by cooking. *Poultry Science*, **62**, 2011–2016.

Cox, N.A., Bailey, J.S., Lyon, C.E., Thomson, J.E. & Hudspeth, J.P. (1983). Microbial profile of chicken patty products containing broiler giblets. *Poultry Science*, **62**, 960–964.

Crawley, S.W., Sloan, D.R. & Hale, K.J. Jr. (1980). Yields and composition of edible and inedible by-products of broiler processed at 6, 7 and 8 weeks of age. *Poultry Science*, **59**, 2243–2246.

Cunningham, F.E. & Froning, G.W. (1972). A review of factors affecting emulsifying characteristics of poultry meat. *Poultry Science*, **51**, 1714–1720.

Dowiercial, R., Muzyka, L. & Pisula, A. (1978). Zmiany fizykochemiczne zachodzące podczas peklowania mięsa baraniego. *Gospodarka Mięsna*, **30**, 17–20.

Forrest, J.C., Aberle, E.D., Hedrick, H.B., Judge, M.D. & Merkel, R.A. (1975). *Principles of Meat Science*. San Francisco: W.H. Freeman and Co.

Franke, W.C. & Solberg, M. (1971). Quantitative determination of metmyoglobin and total pigment in an intact meat sample using reflectance spectrophotometry. *Journal of Food Science*, **36**, 515–519.

Hornsay, H.C. (1956). The colour of cooked cured pork. 1. Estimation of the nitric oxide-haem pigments. *Journal Science of Food and Agriculture*, **7**, 534–540.

Hudspeth, J.P. & May, K.N. (1969). Emulsifying capacity of salt-soluble proteins of poultry meat. 2. Hearth, gizzards, and skin from broilers, turkeys, hens, and ducks. *Food Technology*, **23**, 99–100.

Lyon, C.E., Lyon, B.G., Thomson, J.E. & Hudspeth, J.P. (1982). Quality of broiler thigh meat patties as affected by the addition of giblets. *Poultry Science*, **61**, 1976–1981.

Marsden, J.L. (1982). Technological developments in the manufacture of poultry frankfurters. In: *Procedings 34th Annual Reciprocal Meat Conference of the American Meat Science Association*, June 21–24, 1981. National Live Stock and Meat Board, Chicago, 1982, 126–134.

Mikulski, J. & Stadelman, W.L. (1976). Tenderness changes in smooth muscles. *Poultry Science*, 55, 2066.

Mikulski, J. & Stadelman, W.J. (1977). Tenderness studies with smooth muscle. *Poultry Science*, **56**, 558–562.

Parkes, M.R. & May, K.N. (1968). Effect of freezing, evaporation and freeze-drying on emulsifying capacity of salt-soluble protein. *Poultry Science*, **47**, 1236–1240.

Pikul, J., Niewiarowicz, A. & Pospieszna, H. (1982). Gehalt an Hämfarbstoffen im Fleisch verschiedener Geflögelarten. *Fleischwirtschaft*, **62**, 900–905.

Pisula, A. (1974). Wpływ zawartości barwników hemowych w mięśniach na barwę mięsa wieprzowego. *Zeszyty Naukowe Akademii Rolniczej w Warszawie. Rozprawy Naukowe*, **42**, 1–74.

Ziemiński, R., Juszczak, J., Mielnik, J. & Jaremko, M. (1978). Ocena niektórych wskaźników jakości mięsa u bydla rzeźnego opasanego do ciężaru 600Kg. Roczniki Naukowe Zootechniki. *Monografie i rozprawy*, **12**, 127–136.

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Technical note: Sorption isotherms and monolayer moisture content of raw peas, and peas dehydrated after pressure cooking

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Summary

The moisture sorption isotherms of raw and pre-cooked Century, MP 888 and MP 889 peas at 10, 25 and 40°C were determined by the standard salt solution technique. Pea cultivars, although different in composition, did not differ in equilibrium moisture content. At 40°C and in the water activity range $0.50 \leq a_w \leq 0.90$, the sorption capacity of raw peas was higher than that of pre-cooked peas. All adsorption-desorption isotherms were of sigmoid shape and the quantity of sorbed water, at a given relative humidity, increased as the temperature was decreased. The monolayer moisture content of pre-cooked dehydrated peas stored at 40°C was 6.2%, with corresponding equilibrium relative humidity value of about 27%.

Introduction

Dehydration of water-soaked, pre-cooked peas has been suggested as a means of significantly reducing the time required to prepare dry peas for the table, and possibly increasing their consumption (Jayaraman, Gopinathan & Ramanathan, 1980). However, the equilibrium relationships between pre-cooked dehydrated peas and their surrounding water–air mixture, are not available for selecting the moisture and temperature conditions most suitable for the storage of the dehydrated products. Also it is difficult to interpret correctly the different mechanisms which contribute to the kinetics of the operations of dehydration. Consequently, the work presented here includes sorption isotherms of raw and pre-cooked dehydrated peas determined over a water activity range from 0.11 to 1.00 at 10, 25 and 40°C. The need for this information has been well established by several workers who have demonstrated the relationship between physical and chemical stability of food and modes of water binding (Loncin, Bimbenet & Lenges, 1968; Hill & Rizvi, 1982). Also, sorption isotherms of foods constitute an essential part of the theory of drying (King, 1968) and provide information directly useful to the design of drying equipment (Keey, 1980) and for the prediction of moisture transfer from packaged foods (Labuza & Contreras-Medellin, 1981).

Materials and methods

A commercial cultivar (Century), and two experimental yellow seeded pea lines (MP 888 and MP 889) grown at the Agriculture Canada Research Station, Morden in 1981 were used. The samples were stored in a cool room for 1–2 months and cleaned to

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remove extraneous matter and broken pieces prior to use. Chemical analyses were performed in triplicate by the following well established procedures: (i) protein by the Kjeldahl method (AOAC, 1980); (ii) crude fat by the Goldfisch extraction method; (iii) acid detergent fibre by the method of van Soest (1963); (iv) moisture content by the vacuum oven method (AOAC, 1980); and (v) elements by the atomic absorption method according to standard procedure (AOAC, 1980).

Equilibrium moisture contents were determined, over the range 11–100% relative humidity at 10, 25 and 40°C, using raw and pre-cooked dehydrated peas. For the determination of the adsorption isotherms of raw peas, samples were ground to a 50 mesh size in a laboratory mill (Weber Bros. Metal Works, Chicago, Ill.), placed in a pre-dried and pre-weighed aluminium moisture dish, dried in a vacuum oven at 70°C and 101 kPa vacuum for 24 hr and placed in vacuum desiccators containing salt solutions which gave different constant relative humidities (Rockland, 1960; Greenspan, 1977). The dessicators were kept in a constant temperature cabinet (±0.2°C) until equilibrium moisture content was reached. To obtain desorption isotherms of raw peas, the ground samples, equilibrated at 100% relative humidity, were placed in atmospheres of water activity in the range $0.11 \leq a_w \leq 1.00$ and kept there until equilibrium was reached. For the adsorption isotherms of pre-cooked dehydrated material, raw peas were soaked in water overnight at room temperature, drained, spread on trays and cooked in steam in a pressure cooker at a temperature of 118°C (82.7 kPa) for 15 min. The cooked samples were dried to a 5% moisture content in a batch fluidized-bed dryer (Lab-line Instruments, Inc., Melrose Park, Ill.), with air at 65°C, 2.9 m/sec velocity and at a bed depth of 10 cm.

The pre-cooked dried peas were then ground and exposed to various water activities as described for the raw samples. To obtain desorption isotherms, pre-cooked samples were mashed and placed in atmospheres of water activity in the range $0.11 \leq a_w \leq 1.00$, and kept there until equilibrium was reached. The moisture content of all equilibrated samples was determined by vacuum oven method (AOAC, 1980). BET parameters were calculated as described by Labuza (1968). Triplicate determinations were made on the equilibrium moisture content of each sample at each temperature.

Results and discussion

The mean values of equilibrium moisture of each sample were plotted against water activity. Data points obtained by this plotting were represented with a smooth curve through visual application of a statistical least square principle. Moisture sorption values shown in Table 1 were obtained from this smooth curve. The tabulated values were statistically analysed through the application of analysis of variance. According to this analysis, cultivars did not influence the moisture sorption. Thus, although the three cultivars of peas differed in key components such as protein, fat, fibre and calcium contents (Table 2) the equilibrium moisture contents did not differ.

Temperature had the normal effect predicted by the theory of physical adsorption, i.e., the quantity of sorbed water at a given relative humidity increased as the temperature was decreased. At 40°C and in the monolayer region of the isotherm, the equilibrium moisture content of pre-cooked dehydrated peas was higher than that of raw peas. However, at water activities above 0.50, the sorption capacity of pre-cooked peas was lower than that of raw peas. The practical implication of this finding is that when stored at high relative humidities pre-cooked peas should be more stable than raw peas.

Sorption behaviour of peas represents the integrated sorption properties of its main

Table 1. Moisture sorption isotherms of raw and pre-cooked dehydrated peas

Material	Temperature (°C)	Equilibrium moisture content (g H_2O/100 g dry matter) at the given water activity										
		0.05	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90	1.00
Adsorption												
Raw Century peas	10	3.0	5.5	9.0	11.8	14.6	17.4	20.4	24.5	30.4	38.5	52.0
Raw Century peas	25	2.3	3.9	5.9	7.5	9.5	11.5	14.0	17.5	22.0	29.0	50.0
Raw Century peas	40	1.8	2.9	4.8	6.5	7.7	9.5	11.6	14.5	19.1	26.0	55.0
Raw MP 888 peas	40	1.9	3.0	4.7	6.5	7.5	9.5	11.6	14.4	19.5	26.3	52.0
Raw MP 889 peas	40	1.8	2.9	4.8	6.5	7.6	9.4	11.6	14.3	19.0	26.5	58.0
Pre-cooked Century peas	40	2.1	3.6	5.8	7.2	8.2	9.1	10.6	13.3	17.5	24.6	57.0
Desorption												
Raw Century peas	40	2.5	4.0	5.5	7.0	7.8	9.5	11.5	14.6	19.0	25.9	60.0
Pre-cooked Century peas	10	3.0	5.1	8.5	11.3	14.1	17.1	20.7	34.7	29.6	38.6	58.0
Pre-cooked Century peas	40	2.3	3.7	5.4	6.6	7.5	8.5	10.4	13.0	17.2	28.0	57.0

components, starch (71% of the dry matter) and protein (29% of the dry matter). Thus, changes in the sorption properties of these polymers upon heating will affect the sorption behaviour of peas. It is known that starch and protein may undergo several changes upon heating, such as changes in degree of crystallinity, crosslinking, denaturation and interaction of the proteins with carbohydrates and lipids (Shimazu & Sterling, 1961; Iglesias & Chirife, 1976). Accordingly, the finding that pre-cooked dehydrated peas adsorbed more water than raw peas at low water activity but less in the water activity range $0.50 \leq a_w \leq 0.90$, can be attributed to one or several of the above mechanisms.

A comparison between the adsorption and desorption isotherms of raw peas and pre-cooked Century peas at 10 and 40°C, revealed a small but noticeable hysteresis loop

Table 2. Composition of raw and pre-cooked dehydrated peas

Constituent (% or ppm dry weight basis)	Raw Century	Pre-cooked and dehydrated Century	Raw MP 888	Raw MP 889
Water (%)	7.85	7.42	7.62	7.62
Ash (%)	3.21	3.01	2.82	3.05
Protein (%)	29.37	29.30	28.89	26.05
Fat (%)	0.92	1.32	0.93	0.72
Fibre (%)	6.16	6.26	6.14	7.77
Calcium (%)	0.08	0.75	0.09	0.12
Potassium (%)	1.12	1.16	1.09	1.19
Copper (ppm)	9.56	9.94	9.09	7.47
Zinc (ppm)	43.12	41.26	41.68	43.30
Iron (ppm)	73.35	86.41	69.28	83.36
Sodium (ppm)	22.23	21.6	19.49	59.54

Table 3. BET values for sorption data of raw and pre-cooked dehydrated peas

Material	Sorption temperature (°C)	Monolayer moisture content (kg H_2O/100 kg dry matter)	Surface area (m^2/g)	Heat of sorption (kJ/kg)
Adsorption				
Raw Century peas	10	10.7	373	2778
Raw Century peas	25	6.3	220	2826
Raw Century peas	40	5.7	200	2705
Raw MP 888 peas	40	5.4	187	2745
Raw MP 889 peas	40	5.6	197	2712
Pre-cooked Century peas	40	6.2	218	2792
Desorption				
Raw Century peas	40	4.8	167	2879
Pre-cooked Century peas	10	9.8	342	2657
Pre-cooked Century peas	40	5.6	197	2794

at low moisture levels of raw peas, but practically no hysteresis between the adsorption and desorption branches of the isotherms of pre-cooked peas.

Surface area of raw peas determined by the BET equation increased as the temperature was decreased, but again, did not vary significantly with cultivar (Table 3). Similarly, heat of sorption was unaffected by cultivar and temperature. The monolayer moisture content, on the other hand, decreased from 10.7 kg H_2O/100 kg dry matter for raw peas equilibrated at 10°C to 6.3 and 5.7 for samples equilibrated at 25 and 40°C, respectively. This effect has been reported for other food materials (Iglesias & Chirife, 1976; Mazza, 1980) and implies that an increase in temperature increases the water activity at a constant moisture content, making the product more susceptible to microbial, nutritional and aesthetic degradation (Rockland & Nishi, 1980).

The BET monolayer moisture content, surface area and heat of sorption of pre-cooked peas equilibrated at 40°C were higher than those of raw peas equilibrated at the same temperature. As starch and proteins are probably responsible for the binding of water in peas, a plausible explanation for this effect may be an increase in total number of active sites for water binding as a result of physical and/or chemical changes induced by cooking and/or dehydration.

Conclusions

The moisture sorption isotherms of raw peas and peas dehydrated after pressure-cooking were determined by the standard salt solution technique. These isotherms were then subjected to the BET analysis to examine the influence of cultivar, temperature and processing. The monolayer sorption values as well as heat of sorption and surface area were estimated from the isotherms.

References

AOAC (1980). *Official Methods of Analysis*. 13th edn. Washington: Association Official Analytical Chemists.

Greenspan, L. (1977). Humidity fixed point of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards-A. Physics and Chemistry*, **81A**, 89–96.

Hill, P.E. & Rizvi, S.S.H. (1982). Thermodynamic

parameters and storage stability of drum dried peanut flakes. *Lebensmittel-Wissenschaft und Technologie*, **15**, 185–190.

Iglesias, H.A. & Chirife, J. (1976). B.E.T. monolayer values in dehydrated foods and food components. *Lebensmittel-Wissenschaft und Technologie*, **9**, 107–113.

Jayaraman, K.S., Gopinathan, V.K. & Ramanathan, L.A. (1980). Development of quick-cooking dehydrated pulses by high temperature short time pneumatic drying. *Journal of Food Technology*, **15**, 217–226.

Keey, R.B. (1980). Theoretical foundations of drying technology. In: *Proceedings of International Symposium on Drying* (edited by A.S. Mujumdar). Pp. 1–22. New York: Hemisphere Publishing Company.

King, C.J. (1968). Rates of moisture sorption and desorption in porous, dried foodstuffs. *Food Technology*, **22**, 509–515.

Labuza, T.P. (1968). Sorption phenomena in foods. *Food Technology*, **22**, 263–272.

Labuza, T.P. & Contreras-Medellin, R. (1981). Prediction of moisture protection requirements for foods. *Cereal Foods World*, **26**, 335–343.

Loncin, M., Bimbenet, J.J. & Lenges, J. (1968). Influence of the activity of water on the spoilage of foodstuffs. *Journal of Food Technology*, **3**, 131–142.

Mazza, G. (1980). Thermodynamic considerations of water vapour sorption by horseradish roots. *Lebensmittel-Wissenschaft und Technologie*, **13**, 13–17.

Rockland, L.B. (1960). Saturated salt solution for static control of relative humidity between 5 and 40°C. *Analytical Chemistry*, **32**, 1375–1376.

Rockland, L.B. & Nishi, S.K. (1980). Influence of water activity on food product quality and stability. *Food Technology*, **34**(4), 42–59.

Shimazu, F. & Sterling, C. (1961). Dehydration in model systems: Cellulose and calcium pectinate. *Journal of Food Science*, **26**, 291–296.

van Soest, P.J. (1963). Use of detergents in the analysis of fibrous feeds. 2. A rapid method for the determination of fibre and lignin. *Journal of the Association of Official Agricultural Chemists*, **46**, 829–832.

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Technical note: Application of immobilized cells of *Kluyveromyces marxianus* for continuous hydrolysis to fructose of fructans in Jerusalem artichoke extracts

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Summary

Dead cells of *Kluyveromyces marxianus* having inulase (β-D-fructo fructanohydrolase E.C.3.2.1.7) activity were immobilized in alginate beads and used as a biocatalyst in a packed bed reactor and a stirred batch reactor. Fructosans of Jerusalem artichoke tubers, after extraction, were utilized for continuous or semi-continuous production of fructose. In a bed reactor packed with 100 ml of beads, a volumetric productivity of 36 g/l/hr total reducing sugars was obtained with 98% substrate conversion. When operated continuously for 30 days, a half life of 28 days was observed for the biocatalyst. Using artichoke extract containing 20% fructan solution, 98% conversion could be achieved in a batch reactor in 20 hr. Repeated cycling of beads resulted in considerable loss of catalyst from the reactor and subsequent loss in catalytic activity, thus giving a half life of only 14 days.

Introduction

Fructose is the sweetest known naturally occurring sugar compound. Fructose syrups are often used in the food industry to replace sucrose. For medical and nutritional reasons (Scheinin, Mekinen & Ylitalo, 1975), fructose syrups have gained tremendous popularity in both the food and beverage industry and are also useful in confectionery and pastry production to prevent desiccation and sugar crystallization (Guiraud *et al.*, 1983). High fructose containing syrups (HFCS) are currently manufactured by inversion of sucrose, or by isomerization of D-glucose (Bucke, 1980).

D-fructose syrups can also be prepared from fructans, which constitute the carbohydrate reserves of some agricultural plants (Fleming & GrootWassink, 1979). D-fructans of the inulin type are composed of linear chains of varying length of D-fructose residues with a terminal D-glucose unit, all linked by β-(2,1)-D-fructo-furanosidic bonds. The hydrolysis and processing of inulin sugars from the roots and tubers of, for example, Jerusalem artichoke, chicory, or dahlia produces syrups with a D-fructose content over 75% (Yandamme & Deryke, 1983). Chemical hydrolysis of inulin to fructose displays several drawbacks and is subject to a number of secondary reactions (Fleming & GrootWassink, 1979). Recent interest in high fructose syrup from inulin has concentrated on hydrolysis using microbial inulases (GrootWassink & Fleming, 1980; Yandamme & Deryke, 1983). Hydrolysis can be performed by the free enzyme

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(Guiraud & Galzy, 1981), or immobilized enzyme (Kim, Byun & Uhm, 1982) as well as by immobilized whole cells (Guiraud *et al.*, 1983).

Kluyveromyces marxianus was found to produce inulase (β-D-fructo fructanohydrolase E.C.3.2.1.7) with a high activity towards sucrose and inulin (Parekh & Margaritis, 1985). As much as 78% of the total inulase was found to be cell bound. Since hydrolysis of fructans is a single bioconversion reaction, on a continuous basis, the use of immobilized inulase is required. A non-viable immobilized whole cell preparation having inulase activity was evaluted for this purpose. The use of non-viable biocatalyst offers a number of advantages: (a) no extraction or purification of inulase, (b) greater reactor stability, (c) facilitated transfer of substrate to and from enzyme location due to mildly ruptured or permeabilized dead cells, and (d) unused substrate can be recycled. A review of the use of non-viable cells in general and its industrial application has appeared (Gestrelins, 1984). Presently the use of non-viable cells occurs in the industrial production of glucose isomerase, aspartate, and fumerate, and in lactose hydrolysis.

Our aim was to develop conditions for generating non-viable cells of *K. marxianus* containing inulase and to use immobilized whole cells for industrial application. A systematic literature survey reveals that there is no published information and data on the use of immobilized dead cell biocatalyst for hydrolysis of the fructan containing extract of Jerusalem artichoke. To our knowledge this is the first description of the continuous production of fructose using immobilized cells of *K. marxianus* in both a packed bed and a repeated batch stirred tank reactor.

Materials and methods

Preparation of substrate

The inulin-type sugars from Jerusalem artichoke tubers were extracted as described by Margaritis & Bajpai (1982). The extract was concentrated under vacuum to approximately 280 g/l total carbohydrates. The proteins from the turbid extract were removed by lowering the pH to 4.3 with 2 N sulphuric acid and holding for 2 hr at 50°C. After filtration the pale yellow supernatant obtained was adjusted to pH 5 with lime.

Micro-organisms and growth conditions

A culture of *Kluyveromyces marxianus* UCD (FST) 55-82 was cultivated in a 2 l conical flask, having 500 ml medium. The medium contained 10 g inulin and 5 g yeast extract per litre tap water, at pH 5.0. Cells were harvested after 18 hr, washed in 0.1 M acetate buffer (pH 5) and concentrated by centrifugation at 3000 rpm for 10 min to ten times the original concentration.

Preparation of non-viable biocatalyst

The thick slurry of yeast cells (50 ml) was suspended in 450 ml of 95% ethanol. The solvent cell mixture (500 ml) in a 2 l conical flask was agitated at 220 rpm (25°). Viability of the cells was periodically checked by plate counts. After all the yeast cells had been killed, they were centrifuged and washed twice with sterile 0.1 M acetate buffer. Preliminary studies (data not shown) indicated that free cells suspended in ethanol, and killed successfully, retained about 75% of the original inulase enzyme activity. The dead cells were immobilized in 1.8% alginate beads as described earlier (Margaritis & Bajpai, 1982).

Inulase assay

Inulase activity of the immobilized preparation was measured by determining the reducing sugars released from inulin by Somogyi's method (1952). Five ml of 4% (w/v) inulin in 0.1 M acetate buffer (pH 5) and an adequate amount of immobilized bead preparation were incubated for 10 min at 50°C. Periodically, samples (0.1 ml) were withdrawn and estimated for an increase in total reducing sugars. One unit of inulase activity is defined as the formation of 1 μmol of hexose per min. The immobilized preparation contained 994 units of inulase activity per g dry wt of cells.

Immobilized cell bioreactor system

The reactor column consisted of a borosilicate glass tube (1.9 cm i.d. and 15 cm long) fitted with a water jacket. The total working volume of the bioreactor was 130 ml and the column was packed with 100 ml of beads loaded to a cell density of 56 g dry wt/l bead volume. A high accuracy positive displacement peristaltic pump (Sybron-Brickman model 1P-12) was used to vary the liquid feed flow rate. The feed was sterilized at 121°C for 15 min in 20 l carboy bottles. Total sugars in the feed was determined by the anthrone method (Scott & Melvin, 1953). Effluent samples were collected at each dilution rate every 3 hr and assayed for total reducing sugars (Somogyi, 1952), glucose (Bergmeyer *et al.*, 1974), and free cell concentration in the liquid phase. A steady state was assumed when the level of total reducing sugars was constant in three successive samples. A temperature of 50°C was maintained during the operation of the reactor.

Batch reactor operation

Batch experiments were carried out at 50°C in a 200 ml Bellco jar fermenter with working volume of 100 ml comprising 40 ml of beads and 60 ml of artichoke extract. The reactor was stirred at 200 rpm and during the course of reaction, 2 ml samples were withdrawn and analysed for hydrolysis of fructosans and free biomass in the liquid phase. When 99% of the total sugars was hydrolysed, the spent liquid was drained and replaced with fresh extract to start a new batch run.

Results and discussion

Increasing the dilution rate over 1.5/hr (Fig. 1) gives rise to a decrease in the productivity of the reducing sugars and an increase in the unhydrolysed substrate. Complete conversion of 200 g/l total initial sugars was realized at the dilution rates below 0.5/hr in a packed bed column reactor, corresponding to a mean residence time of 2.3 hr. There was no significant leakage of free cells from the beads, eliminating cell recovery requirements. Also, the low cell release from the beads minimized the contamination of the product, and did not lower the catalytic capacity and efficiency of the reactor. The product obtained from the tuber extract after hydrolysis contained D-fructose and D-glucose as a mixture (D-fructose : D-glucose = 76 : 24 ratio), meaning that the average polymerization of the extracted Jerusalem artichoke sugars was particularly low. This was due to the late harvest date and abundant presence of low molecular weight polymers, sucrose and hexoses. Such a ratio was rather common (Kim *et al.*, 1982).

Operational stability

The packed bed was run continuously for 30 days at a constant dilution rate of 0.37/hr (flow rate 15 ml/hr). The volumetric productivity of reducing sugars remained constant (Fig. 2) at 74 g/l up to 24 volume exchanges (4 days) but decreased steadily

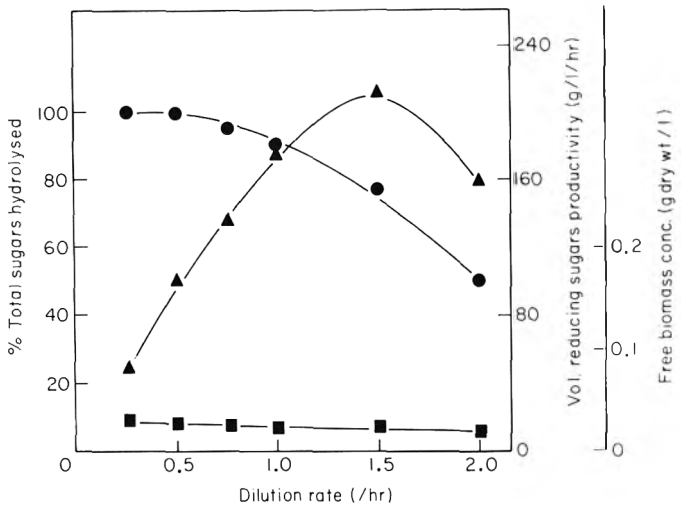

Figure 1. Hydrolysis of fructans of Jerusalem artichokes as a function of dilution rate in a packed bed column reactor containing immobilized cells of *K. marxianus*: (●) % sugars hydrolysed; (▲) volumetric of reducing sugars productivity; (■) free biomass.

thereafter. The data also showed that there was no significant increase in the effluent biomass during the 30 days of operation and in this period 55% loss in the original catalytic activity occurred (90 volume exchanges), corresponding to a half life of 28 days. Using viable immobilized whole cells of *K. marxianus* a half life of 22 days was obtained by Kierstan & Bucke (1977).

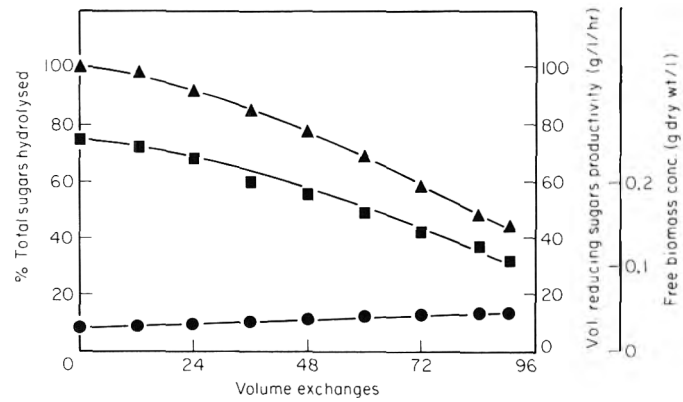

Figure 2. Continuous hydrolysis of fructans during operation at a fixed flow rate (15 ml/hr) in a packed bed column reactor: (▲) % sugars hydrolysed; (■) volumetric of reducing sugars productivity; (●) free biomass.

Batch stirred tank reactor

Figure 3 shows the time course of hydrolysis of artichoke extract in a stirred tank reactor (STR) during the first batch run. Ninety-eight per cent conversion was realized in 20 hr. The maximum hexose production rate was 24 g/l/hr and the free biomass concentration in the liquid was 0.075 g/l after the first run. The rate of production of

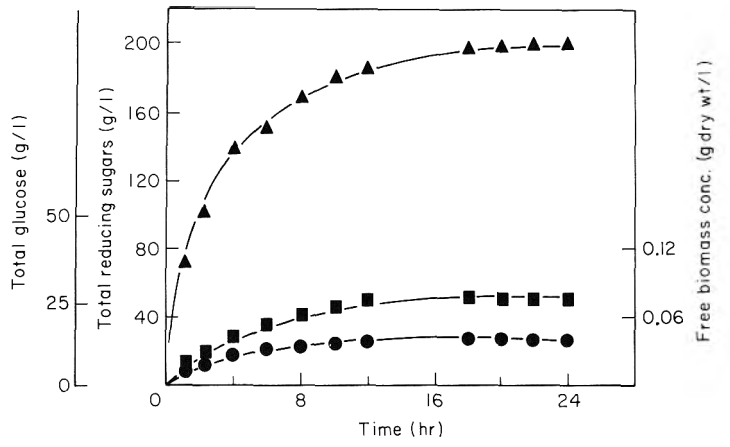

Figure 3. Time course of sugar released from artichoke tuber extract during batch operation in STR: (●) glucose; (▲) reducing sugars; (■) free biomass.

D-glucose and D-fructose from the tuber extract was faster at the initial stage of hydrolysis. It seems that polymers having a low degree of polymerization (DP) were hydrolysed more rapidly than those with a higher DP. This explanation was confirmed by a thin layer chromatographic analysis of the tuber extract (data not shown) and compared with standard inulin (M.W. 5000, from Jerusalem artichoke, Polysciences, Warrington, P.A.). Also, a low DP tuber extract will show less diffusional resistance than standard inulin for this immobilized cell system.

Operational stability

Using the same recycled immobilized cells repeatedly for sixteen batch runs (Fig. 4) the loss of the inulase activity was investigated. The decrease of activity was checked daily by measuring the released reducing sugars of the effluent. No significant change in steady state conversion was observed during the first 3 days. The decay in the activity seems to be a first order reaction after these times. Decay constant, K_d, was calculated

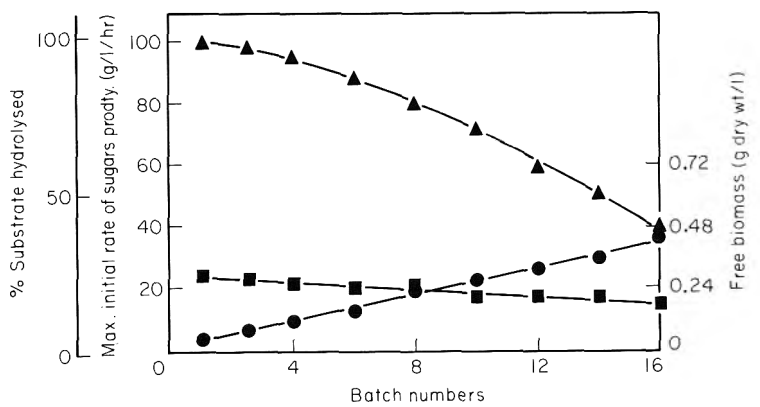

Figure 4. Decrease of conversion efficiency and productivity of fructan hydrolysis during repeated use of recycled immobilized cells of *K. marxianus*: (▲) % substrate conversion; (■) maximum initial rate of sugars productivity; (●) free biomass.

to be 0.05/day from the equation $K_d = (1/t)\ln(\% X_0/\% X_t)$, where X_0 is the initial conversion, X_t is the decreased conversion after operating time t, and K_d is the decay constant with 20% fructan sugars as feed substrate. The half life of immobilized biocatalyst in the repeated recycled batch reactor was 14 days.

As compared to the packed bed reactor, the batch STR had disadvantages: (a) the free biomass leakage is higher, thus increasing the cost of downstream processing, (b) the control of contamination has to be maintained, (c) the loss in the catalytic activity is higher in STR due to continuous loss of biocatalyst, (d) the sugar productivity is significantly higher in a packed bed reactor than in a normal batch or repeated batch process, and (e) the half life is almost half that of a packed bed reactor.

The experiments reported in this paper represent a preliminary feasibility study of fructan hydrolysis into high fructose solution using a novel biotechnological approach of utilizing non-viable immobilized cells with inulase activity. The study showed that substrates such as the fructans of the agricultural products, currently underutilized, could easily be adapted as inexpensive feedstock for the economic production of high fructose. The fructose content obtained by this biotransformation technique is much higher, and the production cost could be lowered if easily grown, high yield per hectare plants, like Jerusalem artichoke and chicory, are used. Under the operational conditions described, the bioconversion of inulin sugars to fructose could be effectively carried out in a packed bed bioreactor. The column operation did not cause any microbial contamination or any leakage, thus saving cost in downstream processing. The study lends support to the suggestion that immobilized dead cells having inulase activity may be exploited as an alternative means for production of enriched fructose syrups.

Acknowledgments

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References

Bergmeyer, H.U., Berut, E., Schmidt, F. & Stork, H. (1974). Determination of D-glucose. In: *Methods of Enzymatic Analysis* (edited by H.U. Bergmeyer and A. Gawhnk). Pp. 1196–1201. New York: Academic Press.

Bucke, A. (1980) In: *Recent Developments in the Production and Use of Glucose & Fructose Syrups*, **43**, p. 68, Barking, Essex: Applied Science.

Fleming, S.E. & GrootWassink, J.W.D. (1979). Preparation of high fructose syrup from tubers of Jerusalem artichoke. *CRC Critical Review in Food Science and Nutrition*, **12**, 1–28.

Gestrelins, S. (1984). Use of non-viable cells for industrial application. In: *CRC Immobilized Cells and Orangelles* (edited by Bo Mattison). **2**, p. 19. Boca Raton: CRC Press Inc.

GrootWassink, J.W.D. & Fleming, S.E. (1980). Non specific β-fructo furanosidase (inulase) from *K. fragilis*, batch and continuous fermentation. simple recovery and industrial properties. *Enzyme and Microbial Technology*, **2**, 45–53.

Guiraud, J.P. & Galzy, P. (1981). Enzymatic hydrolysis of plant extract containing inulin. *Enzyme and Microbial Technology*, **3**, 305–308.

Guiraud, J.P., Bajon, A.M., Chaurard, P. & Galzy, P. (1983). Inulin hydrolysis by an immobilized yeast-cell reactor. *Enzyme and Microbial Technology*, **5**, 185–190.

Kierstan, M. & Bucke, A. (1977). The immobilization of microbial cells, sub cellular organelles and enzyme in calcium alginate gels. *Biotechnology and Bioengineering*, **19**, 387–397.

Kim, W.Y., Byun, S.M. & Uhm, T.B. (1982). Hydrolysis of inulin from Jerusalem artichoke by inulinase immobilized on amino-ethyl cellulose. *Enzyme and Microbial Technology*, **4**, 239–244.

Margaritis, A. & Bajpai, P. (1982). Continuous ethanol production from Jerusalem artichoke

tubers—use of immobilized cells of *K. marxianus*. *Biotechnology and Bioengineering*, **24**, 1483–1493.

Parekh, S.R. & Margaritis, A. (1985). Inulase production by *K. marxianus* in batch culture. *European Journal of Applied Microbiology and Biotechnology* **22**, 446–448.

Scheinin, A., Mekinen, K.M. & Ylitalo, K. (1975). Turku sugar studies. V. Final report on the effect of sucrose, fructose, and xylitol diet in the caries incidence in man. *Acta. Adontologica Scandinavica* (Suppl. 70), **33**, 63.

Scott, T.A. & Melvin, I.M. (1953). Determination of dextran with anthrone. *Analytical Chemistry*, **25**, 1956.

Somogyi, M. (1952). Notes on sugar determination. *Journal of Biological Chemistry*, **195**, 19–23.

Yandamme, E.J. & Deryke, D.G. (1983). Microbial inuinases: fermentation process properties and application. *Advances in Applied Microbiology*, **29**, 139–176.

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Technical note: Kinetics of sorbic acid loss during storage of peaches preserved by combined factors

L. N. GERSCHENSON, S. M. ALZAMORA AND J. CHIRIFE

Summary

It was found that the loss of sorbic acid in stored peaches preserved by combined factors (water activity, pH, additives) followed first order reaction kinetics. Rate constants and activation energy (14 kcal/mol) were almost identical to those previously reported for sorbic acid loss in concentrated glucose solutions of identical water activity and pH.

Introduction

Recently, Sajur (1985) developed a simple process to achieve microbial stability of peaches stored at ambient temperature. This process was based on a combination of reducing water activity (a_w) through glucose incorporation, adjusting pH and addition of potassium sorbate and sodium bisulphite. The stabilized peaches were stored up to 4 months at 30°C without microbial deterioration. Sajur (1985) showed that sorbic acid was a key factor in achieving the microbial stability of peaches preserved as above. It is known that sorbic acid may be destroyed to a considerable extent during storage of foods (Bolin, King & Stafford, 1980; Vidyasagar & Arya, 1984). For this reason, present work was directed to studying the stability of sorbic acid in peaches stabilized against microbial deterioration by a combination of factors which included a_w lowering, pH reduction and antimicrobials addition.

Materials and methods

Fresh yellow peaches were obtained from a local supermarket. They were pulped and thoroughly mixed with glucose and water (60.17% w/w peaches; 30.69% w/w glucose; 9.03% w/w water) using a high speed mixer. Potassium sorbate (0.10% w/w) and sodium thiosulphate (150 ppm) were also added. The pH was adjusted to 3.5 with 50% w/w aqueous citric acid solution. The resultant a_w was 0.94 (Sajur, 1985). All chemicals used were reagent grade. Aliquots of 10 ml of peach suspension were placed in 100 ml stoppered polypropylene flasks which were stored, in the dark, at 20, 30 and 45°C (±0.5°C) in forced convection constant temperature ovens. The flasks were hermetically sealed to prevent evaporation. Headspace in each flask was 90% of total volume.

Sorbic acid was determined according to the AOAC (1980) method. It has been previously shown that this technique produces reliable results when used for sorbic acid determination in concentrated sugar (glucose) systems (Gerschenson, Alzamora, & Chirife, 1986). The same procedure was used to check the reliability of the method

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when used for the present system (preserved peaches) and similar results were obtained.

Results and discussion

The effect of storage time and temperature on sorbic acid degradation in preserved peaches is shown in Fig. 1. Sorbic acid retention decreased during storage following first order reaction kinetics. Destruction rates increased with increasing the temperature. It can be seen that the half life (time for 50% sorbic acid destruction) was about 284 days at 20°C and reduced to about 52 days when the storage temperature was increased to 45°C.

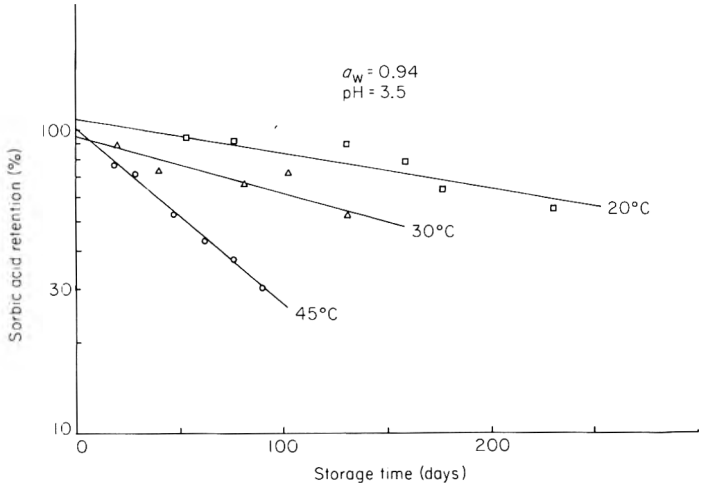

Figure 1. Kinetics of sorbic acid loss during storage of preserved peaches (a_w 0.94; pH 3.5). pH 3.5).

Table 1. Comparison of rate constants and activation energies for sorbic acid loss in preserved peaches and in concentrated glucose solutions of identical a_w 0.94 and pH 3.5

System	Storage temperature (°C)	Rate constant $k \times 10^3$ (day^{-1})	Activation energy E_a (kcal/mole)
Preserved peaches:	20	2.2±0.5	
	30	4.2±0.9	14±4
$NaHSO_3$ 150 ppm	45	13.3±0.5	
Concentrated glucose	20	2.5±0.4	
solution	30	4.2±0.7	13±3
	45	14.0±1.1	
Concentrated glucose	20	2.5±0.3	
solution	30	6.0±0.9	12±2
	45	13.4±0.8	
$NaHSO_3$ 150 ppm*			

*Gerschenson *et al.* (1986).

The temperature dependence of rate constants (k; calculated by linear regression analysis of data shown in Fig. 1) followed the Arrhenius model. Consequently, the activation energy (E_a) was calculated by linear regression analysis of the relationship ln rate constant-1/T. Table 1 compares the values of the rate constants for sorbic acid loss and the corresponding activation energy, with kinetic data previously reported for sorbic acid loss in concentrated glucose solutions adjusted to identical values of a_w and pH (Gerschenson *et al.*, 1986). It can be observed that the kinetic parameters (rate constant and activation energy) for sorbic acid loss in preserved peaches are almost identical to those obtained in concentrated glucose solutions with or without sodium bisulphite.

Various literature reports indicate that the loss of sorbic acid in preserved foods may be influenced by the nature of its components (Bolin *et al.*, 1980; Vidyasagar & Arya, 1984). However, the data obtained in the present work strongly suggest that the loss of sorbic acid in peaches preserved by a combination of lower a_w (through glucose addition) and pH reduction, might not be affected by the presence of non-glucose components.

Acknowledgments

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References

AOAC (1980). *Official Methods of Analysis* (13 edn.). Washington: Association Official Analytical Chemists.

Bolin, H.R., King, A.D. & Stafford, A.E. (1980) Sorbic acid loss from high moisture prunes. *Journal of Food Science*, **45**, 1434–1435.

Gerschenson, L.N., Alzamora, S.M. & Chirife, J. (1986). Stability of sorbic acid in model food systems of reduced water activity. I. Sugar solutions. *Journal of Food Science*, in press.

Sajur, S.A. (1985). Preconservación de duraznos por métados combinados. M. Sc. Thesis. Universidad Nacional de Mar del Plata.

Vidyasagar, K. & Arya, S.S. (1984). Degradation of sorbic acid in fruit squashes and fish paste. *Journal of Food Techology*, **19**, 447–454.

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Book Reviews

Diet-Related Diseases: The Modern Epidemic. By Stephen Seeley, David L.J. Freed, Gerald A. Silverstone and Vicky Rippere. Beckenham: Croom Helm, 1985. Pp. xi+272. ISBN 0 7099 3365 7. £9.95 (paperback)

Many articles and books have been written about the diseases of the affluent Western Society and how they are related to our diet. In their book *Diet-Related Diseases. The Modern Epidemic* Stephen Seeley, David Freed, Gerald Silverstone and Vicky Rippere take a somewhat controversial look at this subject.

The illustration of sweets, cakes, chips and a hamburger on the cover leads one to suppose this will be yet another book relating highly processed or so-called junk foods with forms of malnutrition in the 20th century. However, the team of authors which consists of an epidemiologist, medical immunologist, chemist and clinical psychologist, go to show how complex is the process of unravelling links with diseases, and throws some doubts on widely held beliefs such as those between fat intake and cardiovascular disease.

Each of the nine chapters is written by one or two of the authors and ends with a useful list of references. In the first chapter diseases known to be caused by diet are discussed, including ergotism due to fungus on cereals; liver cancer in Japan caused by the carcinogenic effect of butter yellow; and pink disease in babies and young children being due to mercurial poisoning caused by the high levels of mercury in teething powders. These make one realize how far and how rapidly knowledge about food ingredients has developed. In an interesting chapter of 'possible sources of food toxicants' the author Gerald Silverstone discusses both the mode of action and also sources of various adverse materials. The transfer of these materials into milk, meat and fish is also briefly discussed and a number of examples given.

Milk, rather than fat or cholesterol, is postulated as being atherogenic in a chapter on diet related diseases of the arteries. This is due, the author suggests, to its oestrogen content, and goes on to detail his arguments. Further controversial relationships of diet and disease appear in two further chapters by the same author. Meat and fish are shown to be more strongly correlated with colon cancer mortality than lack of fibre in the diet. Also in a chapter on cancer of the breast and prostate, there are correlated with both sugar intake and exposure to cadmium which is a zinc antagonist. Such controversial views remind one that the links between aspects of diet and disease are never clear cut.

Food allergies and food intolerance are covered by David Freed with a number of case histories. Also the difficulties of testing for food allergies are noted and the importance of ensuring that the treatment, i.e., an unbalanced exclusion diet, is not worse than the allergy itself. The psychologist Vicky Rippere discusses diet and mental illness including caffeinism, ascorbic acid deficiency causing mental illness, and adverse reactions to food additives. This book is worth reading for a different view on diet related disease which provokes though about how little is really known about this subject.

M. Blades

Starch Conversion Technology. Ed. by G.M.A. van Beynum and J.A. Roels. New York: Marcel Dekker, 1985. Pp. xi+362. ISBN 0 8247 7194 X. US $75.00.

Starch technology has undergone a mammoth change in the last 20 years or so. The importance of starch as a replenishable raw material, together with its end products, cannot be overstated. The Editors have carried out an admirable task in bringing together the current knowledge in this field.

The book is a compilation of ten chapters by various authors. Chapter 1 gives an overview of the political and economical aspects of starch conversion and an insight into the potential end products. Chapter 2 discusses the raw material, possible sources, composition and physical properties. Chapter 3 deals with the technology of corn wet-milling. Chapters 4–7 discuss in turn chemical modification, enzymic degradation, the technology of fuel alcohol production and enzymic isomerization. Chapter 8 discusses the very interesting subject of enzymic and microbial processes involved in the conversion of carbohydrates derived from starch, including the production of penicillins, amino acids and microbial polysaccharides. Chapter 9 is on the chemical modification of starch; isomerization and hydrogenation are discussed. There is also a very interesting section on the use of glucose as a chiral starting material in the synthesis of physiologically active compounds. A section on 'miscellaneous reactions and products' includes alkaline degradation, the formose reaction, alkaline degradation/hydrogenation, oxidative degradation, acid degradation and the synthesis of esters and acetals (surfactants). The book concludes with a chapter on the U.S. markets for starch-based products. I can only reiterate the Editors' disappointment that a comparison with the European strategy is not included.

Each chapter (but one) is well referenced, and all are complemented with many illustrations. The book will be a most useful addition to all those working in this area, both in academia and industry. The book is highly recommended; it is however expensive.

S.D. Dziedzic

Processing Aquatic Food Products. By Frederick W. Wheaton and Thomas B. Lawson. New York: John Wiley, 1985. Pp. xvi+518. ISBN 0 471 09736 5. £77.60.

Although the authors, two Professors of Agriculture Engineering, are not explicit on the point, this treatise seems to be written for professionals and students concerned with seafood processing in the United States. Certainly, its seventeen chapters contain a lot of technical detail, stronger in engineering than in chemistry or microbiology, and the statistics, regulations, examples of processing and of species are taken predominantly from the U.S.A. The background material is, however, drawn from a variety of well chosen international sources; indeed, some authors will detect in the book strong echoes of their own writings. The treatment is admirably thorough, systematic, and nicely blends theory and practice. With a few important exceptions the test is accurate. The descriptions and explanations are clear, and there are plenty of helpful illustrations, almost all of which are line drawings. Two interesting and useful chapters on waste production and management and on plant design have not appeared before in previous books of this kind.

However, the book is not without major faults. First it is unnecessarily repetitious. For example, applications of refrigeration are dealt with in some detail in three

different chapters. Secondly, some material could have been omitted without loss. For example, the chapter on fishing gear, though well done, is not used again. It is arguable that an understanding of the features of fishing vessels themselves, such as fishing decks, processing decks and fish rooms, none of which are dealt with separately, is more important than an understanding of fishing gear. Also, three detiled tables giving frictional coefficients of chopped water hyacinth, and others giving microelement compositions do not, in my view, serve any useful purpose. Thirdly, I am unhappy with the balance in some places. For example, we are treated to a photograph of a simple piece of equipment for turning over oysters but there are no illustrations of filleting machines, which are the single most important and complex pieces of fish processing equipment in use. Also, many details are given of the physical properties of fish oils but none of detailed fatty acid composition, which many would think more important: key references to, say, Ackman's work on fish oils are absent. Finally, there are some important omissions such as superchilling, the use of ozone, cooling grids in fish rooms on fishing vessels, automatic checkweighing and grading of fillets, trimming and V-cutting, curd and its prevention, methods of applying smoke flavours, microprocessor applications, seafood analogues made by kamaboko and extrusion technology, box washers, packaging machines (except MAP machines), demountable containers for fishing vessels and the organization of quality control.

The references are plentiful and mostly appropriate, though occasionally more recent ones would have been better, such as Liston on fish microbiology. Typographical errors are commendably rare: unfortunately, the preface is marred by a major transposition of text. There is a good index. Thus, with some improvements this handsomely produced book could be a very good one.

J.J. Connell

Analysis of Food Carbohydrates. Ed. by G.G. Birch. Barking: Elsevier Applied Science, 85. Pp. vii+311. ISBN 0 85334 354 3. £40.00.

Despite the title this book is, in my opinion, an edited review of the current state of the art of carbohydrate analysis plus some elementary chemistry and physics. The latter two topics should have been taken for granted, since the book is stated to be for scientists, technologists and analysts concerned with the food industry or research in similar and allied fields. I must emphasize that this is not a book of analytical methodology.

The editor avers that 'the book sets out to cover the main areas of modern food carbohydrate analysis, illustrating the sophisticated techniques now at our disposal' then goes on to mention properties, physiological characteristics and structural configuration of sugars, which is all very straightforward. I would query the statement 'Fibre may be an index of inadequate food processing (refining) but is now more generally accepted as an index of whole someness; maybe this could be taken up as the subject for a mini-symposium.

The next chapter by M. W. Kearsley gives a brief account of physical, chemical and biochemical methods, I believe that certainly the first two groups have been adequately covered by a well known volume on food analysis present in any food laboratory; the summary of biochemical methods is useful to those new to the field.

Having dealt at length with the use of polarimetry in five pages in the previous chapter, Chapter 3 is headed 'Monochromatic polarimetry' by Shallenberger and includes an elaborated description of mutarotation—a little judicious editing would not

have come amiss and it is to be debated whether this chapter ought to be included in a book of this calibre rather than in an undergraduate organic chemistry practical work.

The chapter on H.P.L.C. by Macrae is full of down-to-earth common sense but then I have seen very similar accounts in other recent reviews, some of which have been mentioned in this journal, although the table on applications of H.P.L.C. in Sugar Analysis would be useful to have in a methods file for easy reference.

The use of G.L.C. including derivitization, reviewed by Fothes is of value to somebody new to this branch of analysis and gives some examples of detection and quantitation but obviously the author did not intend his review to be read by practising analysts because, I am sure, he would not have ended his conclusion by saying 'Thus the actual procedure for optimum results is very dependent upon the mixture of carbohydrates to be determined and the nature of the samples to be analysed'.

Clode in Chapter 6, 'Carbohydrate analysis as an aid to Synthesis' describes again the many techniques stated previously. The high cost of H.P.L.C. is mentioned twice, but, since the advantages of speed, improved separations and less clean up are also admitted, then because time is money and the instruments can be used for many different determinations, it is not surprising that many laboratories dealing with food have H.P.L.C. as a normal piece of everyday equipment.

The next chapter by Rathbone dealing with N.M.R., covers 74 pages and will, no doubt, be of use to a new research worker. Personally, I would like to have seen some practical examples to illustrate its use in the structural analysis of food related carbohydrates.

The penultimate chapter on Food Glycosides by Dziedzic and Ireland includes an answer to the rhetorical question: 'What is a glycoside?' followed by some good old fashioned organic chemistry including a picture (the only one in the book) of the authors extracting chick pea using pilot plant!

The last chapter provides a short resumé of analysis of carbohydrates in the alimentary tract and its nutritional significance. This is the only part of the book where actual methods of analysis for dietary fibre fractions are described. However, these have been overtaken by some newer speedier methods recently published, since the references quoted end at 1983.

The book is well printed, easy to read, relatively free from printing errors, but better editing and co-ordination of the efforts of the individual authors might have removed anomalies and repetition. A more systematically organized review of the state of the art might have been of considerably greater utility than what is in reality a collection of disjoined articles of varying depth of coverage and specialization on somewhat divergent subjects.

With such illustrious authors of the individuals chapters, I feel an opportunity for an informative work has been lost and what has been produced is another 'me too' product which, at the price quoted, is rather expensive in these days of tight budgets.

S. Landsman

Radiation Disinfestation of Food and Agricultural Products. Ed. by James H. Moy. (Proceedings of an International Conference, Honolulu, Hawaii, November, 1983.) Honolulu, Hawaii: Hawaii Institute of Tropical Agriculture, 1985. Pp. xii+424.

This book gives the detailed proceedings of a conference held in Hawaii, 14–18 November 1983. It includes the text of 35 technical papers together with subsequent

verbatim comments. In the final section there is a panel discussion with the title 'Progress towards commercialisation'.

The sponsors and many of the attendees were from commercial and government institutions in the U.S. and there was a natural emphasis on those low dose applications, less than 1 kGy (0.1 Mrad), to be allowed by the initial F.D.A. legislation. Low doses are adequate for the control of insect contaminants in agricultural products and are considered in many of the papers. Due to the proposed F.D.A. ban on the use of the ethylene dibromide fumigant there is now a strong desire in the U.S. to use the irradiation process. Also included are six papers dealing with radiation plants and commercial processing and a further section deals with economics, marketing and consumer acceptance, all of which will be of more general interest.

Whilst this book cannot be recommended to the non-specialist reader, to those with a serious interest in the use of radiation for the disinfection of food and agricultural products it will be a useful addition to the bookshelf. Readers are reminded, however, that the processes covered form only a small part of the now clearly recognised potential for radiation in the food industries. The Codex Alimentarius at its 15th session in 1983 accepted the 1980 WHO/FAO/IAEA advice from an expert committee that the irradiation of food should be freely allowed up to a dose of 10 kGy (1 Mrad) and approved 'Recommended International General Standard for Irradiated Foods' to be circulated to all 122 member countries. It is also interesting to note that the U.S. Department of Energy was one of the joint sponsors of the conference as low dose radiation treatments have a potential for energy conservation, reduced costs and security of national energy resources. The confernce proceedings are remarkably well presented in bound form, free from typesetting errors and clearly printed.

R. Frohnsdorff

Keyguide to Information Sources in Food Science and Technology. By Syd Green. London: Mansell, 1985. Pp. viii+231. ISBN 0 7201 1748 8. £25.00.

I remember the whoops of joy from the librarian of the first food technology library with which I had frequent dealings, when Baker and Foskett's *Bibliography of Food* appeared, to remove some of the frustration from his task of hunting the more arcane bits of information which my colleagues and I always seemed to be wanting. That was in 1958 and according to the author of this new book 'nothing comparable in its detail and comprehensiveness has been published since'; until now. This Keyguide is an excellent guide to the greatly increased food science and technology literature of today, well ordered and very comprehensive.

Part I introduces the subjects of food science and technology and shows their relationships with other disciplines. It describes their history, the organization of education for them and career structures in them, all with abundant references to detailed source material. There follow chapters on the organization of information in the field, including guides to libraries and library classification schemes, on directories and similar publications, on the 'literature' proper and on language problems. Two most valuable chapters are entitled 'Keeping up with current publications and developments' and 'Special information and literature, and non-book materials'.

Part II is a Bibliography, arranged by food product groups (e.g., 'Beverages') or topics such as 'Food microbiology' or 'Food engineering, equipment and processing'. I tested this bibliography against my own knowledge of the literature of meat science and

technology: it did not retrieve everything which I consider useful. The missing items were of two kinds. The list of textboks is adequate but not exhaustive; that defect I find forgivable, for the alternative of including everything, if it were possible, would pose some problems of choice which I do not expect it to be in a librarian's power to decide. More difficult is the absence of two sets of annual Symposium proceedings which I consider most useful sources, but then I re-read Section 4.6 in which the author discusses the difficulties associated with conference proceedings in general, and decided that the absence of my favourite ones might also be forgiven, just.

Part III is a short directory of selected organizations in various parts of the world from which information may be sought. I found only one error which might directly mislead: a reference to the Food and Drugs Act 1955 which by the date of publication of this book had been replaced by the Food Act 1984.

But these are minor deficiencies to set against the great wealth of information and guidance to be found in this well organized and simply written book. It should be on the desk of every librarian and information officer concerned with food science and technology. It should be required reading for all serious students, especially those who have to write theses and literature reviews, who will find it invaluable. It is well laid out, clearly printed and robustly bound, so should survive hard use, and it is reasonably priced.

M.D. Ranken

Developments in Meat Science–3. Ed. by Ralston Lawrie. Barking: Elsevier Applied Science, 1985. Pp. x+227. ISBN 0 85334 361 6. £30.00.

This third volume in the *Developments in Meat Science* series continues the high standard set by its predecessors. The blurb on the cover begins "Despite the current movement against the consumption of flesh foods . . ." but the book contains no polemics on that topic, being devoted rather to thorough and scholarly reviews of particular aspects of meat science. The editor in his Preface says that the chapters are arranged in a sequence corresponding to the "history of the commodity", i.e., from live animals to finished meat products, but each chapter is about a selected topic within the sequence and there are no strong connections between them. The world-wide spread of the authorship of the chapters is notable.

The chapters are: (1) 'New and Improved Types of Meat Animals', by R. T. Berg and R. M. Butterfield of Edmonton, Canada. They consider the potential for increasing the production of meat by improvements in traditional meat animals, techniques such as selection of double-muscled animals or rearing of entire males, and introduction of new species.

(2) 'Myofibrillar Cytoskeletal Proteins of Vertebrate Striated Muscle', by K. Maruyama of Chiba in Japan. This review of recent advances in a field which becomes increasingly complex is most welcome to the present reviewer.

(3) 'Pre-Slaughter Stunning', by T. M. Leach, now at Murdoch, Western Australia, who reviews the very wide range of methods and conditions currently in use and assesses their various effects.

(4) 'Packaging Fresh meat', by A. A. Taylor of Langford, U. K., a general review of the principles underlying modern methods and of practical applications, mainly from the literature of the last decade or so.

(5) 'Irradiation Preservation of Meat and Meat Products', by P. S. Elias of Karlsruhe, West Germany. A good review of the science underlying the current debate about the authorization of this form of food preservation.

(6) 'High Pressure Technology and Meat Quality', by J. J. Macfarlane of Cannon Hill, Queensland, Australia. This is a most interesting summary of work which Macfarlane has pioneered for nearly 20 years, in which pronounced effects, mainly beneficial, can be produced in meat, but which still remains to be exploited commercially.

(7) 'Contaminants in Meat and Meat Products', by A. Ruiter of Utrecht in the Netherlands, a good review of contaminants arising from the environment, from treatment of the animals, during processing, from packaging or from toxin-producing moulds: the levels which may be found and their significance are discussed, but not methods of analysis. The standard of production of this volume is good. No significant typographical errors were discovered. The book, like its predecessors, will surely be found in all libraries with interests in meat science or technology, and on the shelves of most senior meat research workers.

Bothersome questions remain. How and why should No. 3 in a series of 'Developments' books be reviewed for a Journal such as this? Whose understanding or actions will be changed as a result of reading the review? If a reader learns of the existence of the book through seeing it reviewed, or thinks that a particular chapter will be worth reading, then goes off to find a copy somewhere—well and good, but there ought to be some more efficient means of getting that to happen. A few 'Developments' or 'Advances in . . .' series, but not all, are noted in Syd Green's *Keyguide to Information Sources in Food Science and Technology* (also reviewed here)—how else does the ordinary scientist or technologist learn of their existence? What should the IFST do to ensure that he *can* lean? To find an answer to that question seems to this reviewer to be more important than to review another book of reviews.

M. D. Ranken

Aseptic Processing and Packaging of Foods. (Proceedings of an IUFoST Symposium, Tylösand, Sweden, September, 1985.) Lund, Sweden: Lund Institute of Technology, 1985. Pp. 313. Price: SEK 300.

There have been several major international symposia on aseptic processing and packaging. The first was at Cahir, Ireland, in 1969; the second at Malmo, Sweden, in 1971; and the third at Raleigh, NC, U.S.A., in 1979. These symposia were primarily concerned with the processing of milk and milk products, since aseptic techniques were first widely applied in the dairy industry. The proceedings of these symposia referred to 'UHT processing', but the subjects covered were essentially the same as those for the present symposium, which is probably the first to have been influenced by the interest in aseptic processing of the food industry at large. The industrial sponsors and some of the organizers of the present symposium were closely involved with the earlier symposium in Malmo, so that continuity has been maintained.

The proceedings contain nine plenary lectures, and twenty-one papers, originating from ten countries. The plenary lectures appear to define certain topic areas, but in fact do this only loosely. Five topic areas contain only a single paper apart from the lecture. Some of the papers do not concern aseptic processing and packaging techniques, and

have presumably been included because of the organizers' need to accommodate all submitted papers.

Much of the material will be already familiar to those who have followed aseptic process development since its beginnigs in the dairy industry, and have studied the associated literature. There is, however, a new emphasis arising from the present interest of the food industry, e.g., in various references to the processing of particulates. The first plenary lecture, by A.C. Hersom, illustrates this. He remarks that current interest is due to commercial rather than technical developments, and the technology is relatively old. He then gives an excellent summary of the present position against a food industry background. B. Ecklund emphasizes the economic basis of the interest, showing for meat that aseptic processing is significantly less energy intensive than in-container sterilization or deep freezing, and about equivalent to refrigerated distribution.

The plenary lecture by H.G. Kessler on thermal processing of liquids contains much that is familiar, not least from his own book on Food Engineering and Dairy Technology, but he gives a good review of the field, particularly in relation to work done at Weihenstephen. The single associated paper, by A. Ellborg & C. Tragardh, presents a new and interesting approach to flow time distributions in liquid processing, illustrating the present recognition of such distributions as an important determinant of product quality.

S.A. Murray's plenary lecture is a useful summary of methods for processing liquids containing particles. It gives interesting information on two relatively new methods: ohmic heating (which was of course occasionally used for milk many years ago but now has a new lease of life) and the Jupiter system. D. Taeymans *et al.* return to the problem of residence time distributions in relation to scraped-surface heat exchangers, whilst N.J. Heppell points to the importance of surface heat transfer in the treatment of suspended particles, and gives interesting results from a novel experimental method involving mathematical modelling.

The problem of maintaining product sterility during processing and subsequent handling, by ensuring plant sterility and bacterial integrity, has been little discussed in the past. This important subject is introduced and considered in the plenary lecture by D.C. Kilsby & R. Metcalfe, and the paper by I. von Bockelmann.

Aseptic packaging and methods of pack sterilization are covered in four plenary lectures and four papers, which provide a useful summary although some of the material is well known. The plenary lecture by D.A. Herbert, the two papers by B. von Bockelmann, and the paper on surface sterilization by G. Czerny, can be recommended.

The final topic area is introduced by an excellent survey of product quality and shelf life by O. Cerf. which deals with microbiological and chemical effects during treatment and subsequent storage. The following papers are something of a mixture, of variable quality and dealing with an assortment of subjects and specific products, not always in an aseptic processing context.

The proceedings are clearly printed throughout, with few errors and these of little importance. Diagrams are clear and easy to follow. Since the reproduction is from ready-typed material, there are differences in the appearance of some of the papers, but this is unimportant in a publication of this kind. Many of the papers are extensively referenced, although some do not acknowledge preceding work. There is no index, but this is no disadvantage where the papers are clearly titled and many begin with a summary.

This area of work is covered poorly in textbooks, and those seeking information must search through published papers, with the risk of missing whole sections of the field. Symposium proceedings of this kind, in spite of the variable nature of their contents, are extremely valuable when used with discretion. Anyone involved with aseptic processing is likely to find something new and useful in this publication.

H. Burton

Food Chemistry and Nutritional Biochemistry. By Charles Zapsalis and R. Anderle Beck. New York: John Wiley, 1985. Pp. xxi+1219. ISBN 0 471 86129 4. £61.35.

This superbly produced book is undoubtedly a significant and worthwhile contribution to the numerous texts now available for students of food science. The integrated approach adopted by the authors, in which they attempt to balance the key principles underlying food chemistry, biochemistry and nutrition, has much to commend it, as it lends a coherence to the discipline of food science which is sadly lacking in a lot of traditional textbooks.

The book consists of three parts. Part 1 consists of thirteen chapters and details the basic chemistry of food constituents including water, proteins, vitamins, carbohydrates, lipids, natural colorants and flavours. Included in this section are detailed discussions of nutritional energetics, photosynthesis as it pertains to the production of primary food resources and colloidal food systems as they relate to industrial applications. Part 2 consists of nine chapters and outlines the integrated metabolism of food constituents; food toxicants and some basic concepts of food toxicology are also detailed. Also included in this section are surveys of some nutritional and aetiological factors that *may* be linked to atherosclerosis, hypertension, certain cancers and diabetes. Effects of hormonal control mechanisms on the overall scheme of nutritional biochemical processes are cited along with some common food–drug interactions. The third section, which consists of only one chapter, briefly surveys the potential of genetic engineering as a means of improving agricultural resources.

The authors are to be congratulated on both the way they have integrated these different aspects of food science and on the quality of each of the individual chapters. All the sections are very easy to read yet give, in most cases, a comprehensive introduction and survey of the topic under discussion. As might be expected in what is primarily an undergraduate text, the literature covered is not necessarily the most up-to-date but has been judiciously chosen for soundness and clarity of approach. However, in a few instances, such as in the discussion of the chemistry of the soya proteins and the aging process in meats, more up-to-date references to bring the student tc the forefront of knowledge would have been useful. Inevitably in a text of this size, errors have occurred, such as some of the statements concerned with the rigor process. These though are minor criticisms of an otherwise excellent book.

The book is remarkably free of typographical errors and has a most useful and extensive index, an essential requirement in what is basically a student text. In conclusion this text, although expensive, is in my opinion, *the* most useful book for students of food science to appear for many a year; I recommend it highly and feel sure it will be widely used by all students and teachers of food science.

D.A. Ledward

Methods of Vitamin Assay, 4th edn. Ed. by Jorg Augustin, Barbara P. Klein, Deborah A. Becker and Paul B. Venugopal. New York: John Wiley, 1985., Pp. xvi+590. ISBN 0 471 86957 0. £99.75.

When the *Book of Common Prayer,* a few years ago, ws 'translated' into the vernacular, there were many protests by the traditionalists. However, in this case the change in format from the 'classic bible' of 1966 is very welcome. The changes in analytical methodology have necessitated a new approach to this volume which is published for the Association of Vitamin Chemists of America, utilizing a four-editor and multi-contributor authorship of the volume.

Before going into details on the individual vitamins cited in this laboratory manual, I feel the first six chapters deserve special mention. Chapters 1 and 6 dealing with 'Method Choice and Development' and 'Sampling for Vitamin Analysis' respectively, could with advantage be part of any instructional course on analytical chemistry and indeed can be read with interest by all analysts, to remind them of the factors now embracing the state of the art. The other four chapters mentioned give a well written, concise, overall picture of biological, microbiological, chromatographic and automated vitamin assays, which would be a guide and help to persons new to the particular techniques and be helpful in considering which technique to use for a particular food matrix or pharmaceutical.

The bulk of the volume is composed of chapters dealing with the determination of individual vitamins covering Vitamin A, Carotenes, Vitamin D, Vitamin E, Vitamin K, Vitamin C, Thiamine, Riboflavin, Niacin, Pantothenic Acid, Vitamin B_6, Folacin and B12 together with consideration of Biotin and Choline, and a short discussion on multi-vitamin determination by H.P.L.C.

The volume has been well edited with all the chapters showing a general consistency in form and context. although the depth of treatment is variable. Each chapter commences with a historical and general consideration of the vitamin, occurrence and sources, metabolism, the chemistry, and forms found in foods, feeds and pharmaceuticals, followed by a general discussion of the analtical procedures and precautions, progressing to the detailed analytical methodology, which are well set out in an itemized form. Since the procedures are intended for North American readers, users in other countries will have to adapt the sources of standards, reagents and equipment to their own country.

Most of the methods cited are standard, classic, well tried and tested but it should be noted that the H.P.L.C. method is now frequently becoming the method of choice where the concentration present in the matrix makes this technique possible and a few examples are included.

The binding, general appearance and layout of the volume is good and the text very easily readable, with very few errors and a useful index, but perhaps it is one of my personal quirks that I feel that laboratory manuals should be looseleaf in structure with a periodical updating service—remember the last edition of this work was 1966—(now out of print) and the references generally are no later than 1981. Further, it would appear that the editors do not expect many overseas sales since there is a list of manufacturers and suppliers of reagents and equipment cited in the text all of North American domain.

Altogether, the four editors must be complimented on producing such a logical and coordinated text and it is obvious from the comments on the methods and techniques

for individual vitamins that each contributor has personally carried out the determinations.

The volume can be recommended for the library of any laboratory concerned with the determination of vitamins in any material, also teachers and students would do well to dip into parts for general analytical enlightenment and philosophy.

S. Landsman

Health and Hygiene

Journal of the Royal Institute of Public Health and Hygiene

Health and Hygiene is the journal of the Royal Institute of Public Health and Hygiene, a body which has interested itself in the subject over many decades. The Institute is interdisciplinary, including environmental health and health education, laboratory science, the scientific side of the food industry, as well as the teaching, nursing and medical professions. The content of the journal reflects these diverse interests. The articles are presented in an informal and informative style, designed to appeal to the journal's broad readership, and the subject matter goes beyond the confines of the medical and clinical professions.

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Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References.

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Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Verbs which contain the suffix ize (ise) and their derivatives should be spelt with the z. Statistics and measurements should always be given in figures, i.e. 10 min, 20 hr, 5 ml, except where the number begins the sentence. When the number does *not* refer to a unit of measurement, it is spelt out except where the number is greater than one hundred.

Abbreviations. Abbreviations for some of the commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular unless confusion is likely to arise.

gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	µg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

Figures. In the text these should be given Arabic numbers, e.g. Fig. 3. They should be marked on the backs with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of a figure the top should be marked with an arrow. Each figure must bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Line diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper and should be about four times the area of the final reproduction. Lines and lettering should be of sufficient thickness and size to stand reduction to one-half or one-third. Letters and numbers must be written lightly in pencil. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs. The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to Figures'.

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Page proofs will be submitted to the contributors for minor corrections and should be returned to the Editor within 3 days. Major alterations to the text cannot be accepted.

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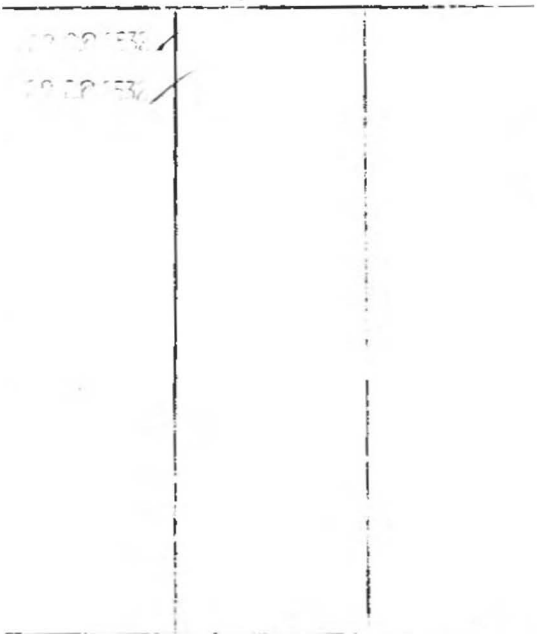

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