ISSN 0022–1163

Volume 20 Number 3 June 1985

Journal of Food Technology

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

JOURNAL OF FOOD TECHNOLOGY

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

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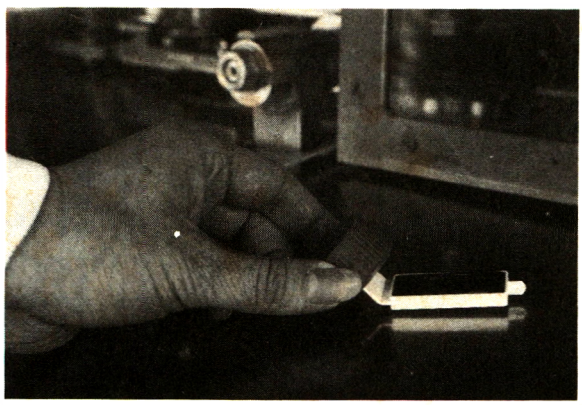

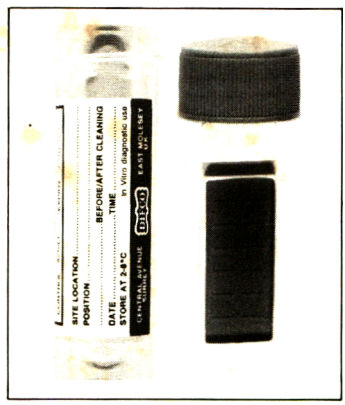

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Enzymically hydrolysed and bacterially fermented fishery products

J. D. OWENS*AND L. S. MENDOZA†

Summary

The classification of the different types of enzyme hydrolysed and bacterially fermented fishery products is discussed and a classification based on the conditions of enzymic hydrolysis and the involvement/non-involvement of bacterial fermentation is proposed. Brief descriptions are given of the processes used to make representative products of each of the different categories. The potential health hazards associated with preserved fishery products is evaluated. The ecology of lactic acid bacteria in the fermented products and the factors affecting the fermentations are considered.

Introduction

Mackie, Hardy & Hobbs (1971) defined fermentation as the 'transformation of organic substances into simpler compounds by the action of enzymes or microorganisms'. In the literature on preserved fishery products the term fermentation is commonly used to describe virtually any process that involves a period of anaerobic storage. Nevertheless, since the conditions for growth of microorganisms are markedly more restricted than the conditions under which enzymes may be active, it is useful to maintain a clear distinction between microbial and non-microbial phenomena. It would, therefore, seem preferable to keep to the more precise and widely accepted biochemical definition of fermentation as an ATP-generating metabolic process in which organic compounds serve both as electron donors and as electron acceptors (Stanier, Adelberg & Ingraham, 1976). Hence, only those products involving the deliberate fermentative growth of microorganisms should be described as fermented and products whose manufacture primarily involves the activity of indigenous or added enzymes are better referred to as enzyme hydrolysed. In cases where the hydrolysis is purely due to indigenous enzymes this may properly be described as autolysis and will be so referred to in this paper.

Preserved fishery products involving enzymic hydrolysis and/or bacterial fermentation in their preparation embrace a great diversity of traditional products, including many salt cured products, Southeast Asian fish sauces, fish pastes and shrimp pastes, European marinaded herring and herring titbits, Southeast Asian bacterially fermented shrimp and fish sauces, as well as recent innovations such as fish protein hydrolysates and fish silage. This article considers both enzyme hydrolysed and microbially fermented fishery products of Southeast Asia as well as some related European products. Most attention is given to the fermented products since their lower salt content gives them a greater potential for expanded future use.

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Categories of products

There is an enormous diversity of aquatic animals and processes used to make traditional products and most often they are classified according to the method of preservation as dried, salted, pickled or fermented. But it is also apposite to recognize just two major categories; enzyme hydrolysed and bacterially fermented, in order to direct attention to the nature of the biologically induced changes that are involved in the production of widely differing products. The classification given in Table 1 attempts to demarcate useful categories on the basis of the conditions under which enzymic hydrolysis occurs and the involvement or non-involvement of microbial fermentation. Any classification is, of course, arbitrary and it is likely that the processing of bacterially fermented products will involve some autolysis by indigenous enzymes and that microbes may make a contribution to the enzymically hydrolysed products.

The range of aquatic animals processed by enzymic hydrolysis and/or fermentation includes freshwater and marine fish and shrimps, fish visceral mass, squid, sea urchin roe, sea cucumber visceral mass, crab roe, molluscs, and others (Tanikawa, 1971). Although only fish and shrimp products are referred to by name below, since these are

Table 1. Types of enzyme hydrolysed and fermented fishery products classified according to the controlling parameter(s) during processing

Category	Conditions of processing				Additional treatments
	Salt (% w/w)	pH	Temp. (°C)	Enzyme added	
A. Products primarily involving enzymic hydrolysis					
I. Hydrolysis in > 20% salt					
(i) traditional clear sauces	> 20	6–7*	20–35	–	filtration
(ii) traditional unfiltered sauces				–	
(iii) accelerated with added enzyme				+	
(iv) traditional salted wet fish				–	
II. Hydrolysis in salt + drying					
(i) traditional fish/shrimp pastes	13–20	6–7*	20–35†	–	drying
(ii) traditional dried salted fish	5–25	6–7*	20–35	–	
(iii) dried liquefied fish protein	0	3–7§	40–50	+	
III. Hydrolysis at low temperature					
(i) traditional Scandinavian anchovies and herring titbits	10–14	6–7*	−2–12‡	–	store < 2°
IV. Hydrolysis at low pH values					
(i) traditional marinaded herring	10–14	~ 4.3	10–12	–	store ~ 0°
(ii) acid fish silage	0	< 4	10–30	–	
B. Products preserved by microbial fermentation					
I. Fermented with added carbohydrate					
(i) traditional fermentations with added salt	~ 3	‖	20–30	–	
(ii) bacterial fish silage, usually without added salt	0	‖	10–30	–	
II. Fermented without added carbohydrate					
(i) traditional fermented herring	8–12	6–7	?	–	store < 5°

*Indigenous pH value of fish flesh.

†Range of mean daily maximum and minimum temperature of some coastal stations in Southeast Asia.

‡Range of mean daily maximum and minimum temperature from November to March of some coastal stations in Scandinavia.

§Value used depends on enzyme.

‖Final pH, 3.5–4.5.

quantitatively the most common raw materials, similar processes exist for other aquatic animals or parts thereof.

The salt concentrations given are weight dry salt (often solar salt of unspecified purity is used in Southeast Asian products), % wet weight of total ingredients. This is not equivalent to the concentration of sodium chloride in the liquid phase. Since sodium chloride concentration is such an important factor in the processing of many of the products it would be desirable to also give salt concentrations in the liquid phases (Zaitsev *et al.*, 1969) but this information is not generally available.

Descriptions of typical processes

The reviews of Amano (1962), Hess (1953), Mackie *et al.* (1971), Orejana (1983), Steinkraus *et al.* (1983), Tanikawa (1971), Veen (1965) and Westenberg (1951) give information about many of the different products made in Southeast Asia and Japan from aquatic animals and only brief descriptions of the manufacturing processes for some representative products will be given here. However, apart from reports by French workers on the products of Indochina (Westenberg, 1951), there is a dearth of really precise descriptions of the methods of manufacture for many of these products and there are virtually no reports of detailed surveys of the variant procedures used in the manufacture of single products.

A. Products involving the action of indigenous or added enzymes

The traditional methods for making fully or partially enzyme hydrolysed products represent successful solutions to the problem of how to control the enzymic activity, in order to obtain the desired degree of textural change and flavour development without undue risk of bacterial spoilage or toxin formation. The major variables that are manipulated to this end are listed below.

(i) Enzyme concentration. Since autolytic enzymes are present at much higher concentrations in the viscera and head than in other tissues (Backhoff, 1976; Westenberg, 1951; Zaitsev *et al.*, 1969) their concentration during processing is influenced by the timing and completeness of beheading/evisceration, or by using whole uneviscerated fish if maximum enzyme activity is required. Alternatively, the amount of indigenous enzymes can be reduced by beheading and evisceration and controlled amounts of other enzyme sources, such as papaya or pineapple fruits, added.

(ii) Enzyme activity. The activity of the enzymes is regulated by manipulating environmental factors such as temperature, salt concentration and water content. The classification of enzyme processed products adopted here and the descriptions of representative processes given below attempt to emphasize the role(s) of traditional treatments in regulating enzyme concentration and activity.

I. Products made by enzymic hydrolysis in the presence of more than 20% salt

(i) Traditional high salt clear fish sauces. Included here are Vietnamese nuoc-mam, Philippine patis and Thai nam-pla. The process for the manufacture of nuoc-mam is outlined in Fig. 1. The essential features of the processes for these high salt clear sauces are the use of whole uneviscerated fish, salt concentrations greater than 20%, anaerobiosis, and a slow hydrolysis over a period of months at ambient temperatures of 20–35°C followed by filtration to remove undigested residues.

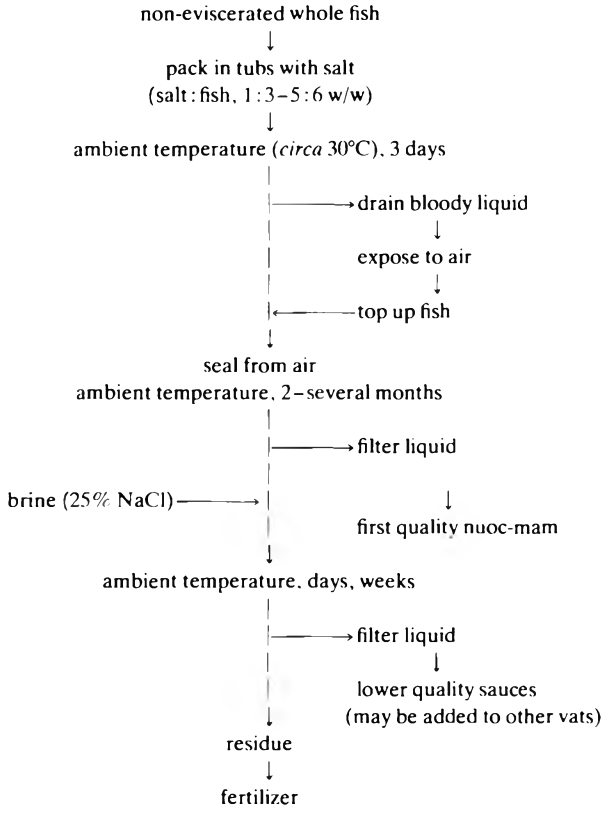

Figure 1. Flow diagram for production of Vietnamese nuoc-mam: an enzyme hydrolysed, high salt, clear fish sauce (based on Westenberg, 1951: Steinkraus *et al.*, 1983).

The use of uneviscerated fish ensures maximum enzyme activity and the combination of high salt concentration and anaerobiosis will totally inhibit any microbial growth once the salt has fully penetrated the tissues. Rose (1918) and others (see Amano, 1962) have shown that salt concentrations greater than 20% are required to prevent excessive conversion of organic nitrogen into ammonia. Sauces made with 20 or 25% salt had less than 10% of total nitrogen present as ammonia whereas in sauces with 14 and 8% salt ammonia represented 24 and 31%, respectively, of the total nitrogen (Rose, 1918). However, it is not clear whether these differences were due to the effects of salt concentration on the activities of fish enzymes or a consequence of microbial growth. Certainly, it is now well established that microbes are not involved in the formation of these high salt sauces other than as possible spoilage organisms prior to salting or complete penetration of the salt into the tissues (Beddows, Ardeshir & Wan, 1979; Orejana & Liston, 1979; Orejana & Liston, 1981; Saisithi *et al.*, 1966; Shimudu, 1934). However, it is often claimed that such microbial development contributes to the aroma of the final product. There is some evidence for this in the case of nuoc-mam, where the development of the characteristic aroma was attributed to the growth of a particular *Clostridium* species (Westenberg, 1951) but Orejana & Liston (1979) found no significant chemical or organoleptic differences between Philippine fish sauces made with radiation-sterilized or with non-sterile fish.

(ii) Traditional high salt unfiltered fish sauces. The traditional enzymic hydrolysis of fish does not result in a total conversion to liquid products but leaves a proportion of undigested material. Hence, there are a number of traditional sauces which are made in exactly the same way as the clear sauces but without filtration. These sauces are relatively thick suspensions of undegraded fish flesh together with liquefied protein and 20–26% salt. Examples are Philippine bagoong (Canonizado, 1978) and Malaysian budu (Beddows *et al.*, 1979).

(iii) Protein liquefaction processes using added enzymes. In the production of the vast majority of traditional high salt fish sauces the enzymic hydrolysis is due to enzymes from the fish. However, in certain parts of Vietnam nuoc-mam is produced from eviscerated fish to which fresh pineapple juice is added because of the lower content of fish enzymes (Chevey, 1931). In Japan koji (a preparation of cooked rice bearing growth of *Aspergillus oryzae*) is used as a source of proteases in some traditional products (Tanikawa, 1971).

The use of commercial proteolytic enzymes to accelerate the traditional process has been investigated (Beddows & Ardeshir, 1979; Dougan & Howard, 1975) but they do not give flavours comparable with the traditional autolysis and their use has not, therefore, been adopted by producers (Canonizado, 1978).

(iv) Traditional wet salted fish. The use of beheaded and eviscerated fish slows down autolysis and the fish retains its shape for much longer. In Kampuchea beheaded, eviscerated and descaled fish is packed into jars with salt (proportions not stated) and kept as a family store of preserved fish to be used as required (Westenberg, 1951).

In Thailand a similar product is made with *Scomber neglectus* which is exported, packed wet in crates, to Malaysia and Indonesia (Hess, 1953; Veen, 1965).

II. Products involving enzymic hydrolysis and drying

(i) Traditional fish/shrimp pastes involving autolysis and drying. Malaysian belacan is a shrimp paste made by alternating periods of anaerobic storage, when autolysis

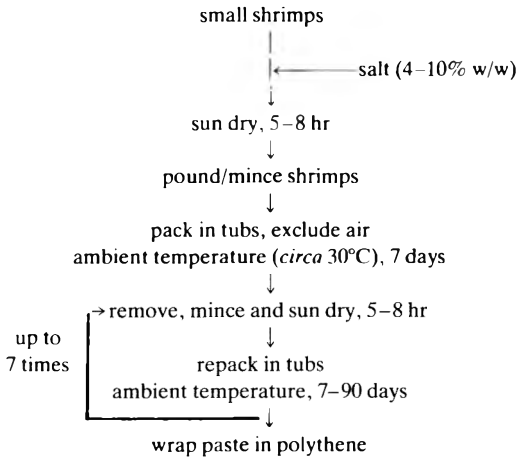

Figure 2. Flow diagram for production of Malaysian belacan: a partially autolysed, salted and dried shrimp paste (from Adnan & Owens, 1984; Steinkraus *et al.*, 1983).

occurs, with periods of sun drying to remove water and maintain the material in a pasty consistency (Fig. 2; Adnan & Owens, 1984; Steinkraus *et al.*, 1983). The product is a thick paste containing 13–20% salt and a sufficiently low water activity of 0.67–0.71 to preclude microbial growth. Adnan & Owens (1984) could find no evidence of any role by microorganisms in the production of belacan. Similar shrimp pastes are made in other Southeast Asian countries and the Kampuchean fish paste product, pra-hoc, probably belongs in this category (Westenberg, 1951).

(ii) Traditional dried salted fish. The processing of some dried salted fish includes treatments designed to promote limited enzymic hydrolysis and the development of a desirable flavour and texture (Zaitsev *et al.*, 1969). In Kampuchea beheaded fresh fish is suspended in bamboo baskets in lakes or rivers for 12–16 hr, during which time some autolysis occurs. They are then eviscerated, washed, salted for 6–12 hr until salt penetration is complete, washed again and finally sun dried (Hess, 1953). The second washing is used only for first grade product and presumably serves to reduce the final salt concentration in the dried fish and increase its market value (Ling, 1953).

Since full penetration of salt into fish flesh takes a number of hours (Klaveren & Legendre, 1965; Zaitsev *et al.*, 1969), it is likely that some autolytic (and possibly bacterial) changes are involved in the production of most kinds of dried salted fish.

(iii) Dried, liquefied fish protein. Modern processes for the enzymic hydrolysis of fish proteins (Fig. 3), using proteases of plant or microbial origin, hold the potential of allowing the protease to be tailored to the particular fish protein and kind of product desired. Under the right conditions liquefaction takes place in a matter of hours and the hydrolysate can be concentrated and spray dried for long term storage (Kinumaki, 1978; Mackie, 1974; Ritchie & Mackie, 1982; Windsor & Barlow, 1981). However, apart from a report of a French company manufacturing milk replacer, apparently by papain digestion of fishery waste (Pigott, 1982), these processes have not yet found much successful commercial application.

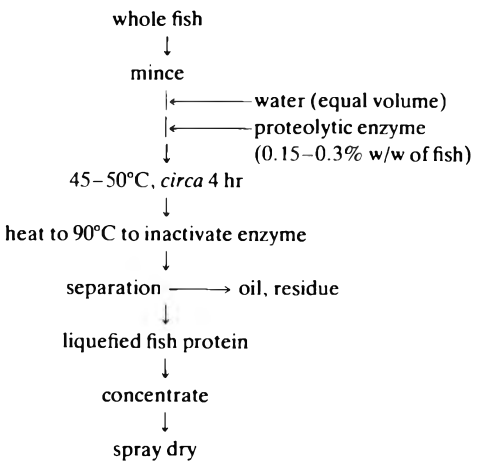

Figure 3. Flow diagram for production of dried enzymically hydrolysed fish protein (from Kinumachi, 1978).

III. Products involving enzymic hydrolysis at low temperatures

(i) Traditional semi-preserved Scandinavian anchovies and herring titbits. These products are made from sprats (*Clupea sprattus*) and herrings (*C. harengus*) by packing eviscerated, partially eviscerated or uneviscerated fish with salt (10–14%), sugar (5%), spices (and sometimes also nitrate, benzoate or other chemical preservatives) into barrels or directly into tins or bottles. They are stored at winter ambient temperatures until the flesh gains a soft smooth consistency due to the action of proteolytic enzymes primarily from the fish (Alm, 1965; Jul & Kondrup, 1953; Zaitsev *et al.*, 1969). Alternatively, the barrels may be stored in cold stores at temperatures of 4–6°C for up to 1.5 years and the fish then filleted and packed into cans or bottles with a sauce containing about 4% salt, 35% sugar, 10–12% acetic acid and sometimes a chemical preservative (Knochel & Huss, 1984). They concluded that the ripening process is predominantly autolytic and that the microbial flora only plays a minor role in flavour development. The products are not heat treated and must be stored under refrigeration (preferably at 2°C) to reduce further enzymic hydrolysis and to prevent undesirable bacterial development.

IV. Products involving enzymic hydrolysis at low pH values

(i) Traditional marinaded herring. Herring fillets are immersed in a marinade containing 10–14% salt and 5–7% acetic acid at 10–12°C for 3–7 days (Fig. 4). When the fillets are mature they are packed in barrels or cans, covered with a more dilute solution of 2–4% salt and 1–2% acetic acid and sealed. No heat treatment is used and they must be stored under refrigeration (preferably near 0°C) to retard further hydrolysis and to avoid bacterial spoilage. The major changes are partial proteolysis of the fish flesh by indigenous enzymes with a suggested involvement of bacteria in aroma formation (Jul & Kondrup, 1953; Meyer, 1965; Zaitsev *et al.*, 1969).

(ii) Acid fish silage. Acid fish silage is prepared by autolysis of whole fish or parts of fish at low pH values to make a liquid animal feedstuff (Backhoff, 1976). Generally formic acid at about 3% (w/w) is used as acidifying agent to lower the pH to 4.0 or less, but strong mineral acids or a mixture of formic and propionic acids can be used (Arufudin, Kompiang & Raa, 1978; Windsor & Barlow, 1981). At temperatures between 10 and 30°C liquefaction occurs in 2–10 days. Commercial manufacture is practised to some extent in Scandinavia but the high water content precludes long

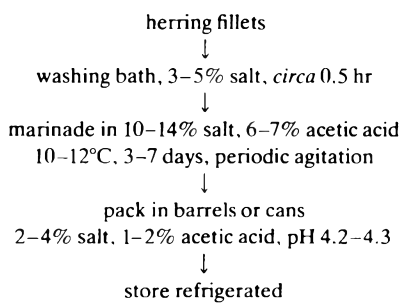

Figure 4. Flow diagram for production of marinaded herring: a semi-preserved product made by partial autolysis at low pH value (based on Meyer, 1965).

distance transport and for this reason the production of fish meal is preferred if the scale of operation is large enough for this to be economic.

B. Products preserved by bacterial fermentation

The most commonly fermented animals are fish and shrimps but a great variety of aquatic animals are used in different traditional products (Amano, 1962; Tanikawa, 1971). The majority of these are prepared with added carbohydrate, but there are some products described which do not involve added carbohydrate, or which have carbohydrate added only after an initial fermentation. Hence it is convenient to recognize two classes of fermented products.

I. Products made by bacterial fermentation with added carbohydrate

(i) Traditional products made with added carbohydrate and salt. Typically, beheaded and eviscerated fish is given an initial heavy salting, stored for a period of time to allow salt penetration, and then the fish is washed and mixed with cooked rice and a source of amylase. Salt may be added to the mixture at a rate of 3–10% or may be derived only from salt remaining in the salted fish. Presumably the initial salting achieves a more rapid inhibition of spoilage bacteria than would the immediate fermentation of fresh fish and might also allow some desired autolysis and flavour development. The use of beheaded and eviscerated fish, besides cleaning the fish of gut contents, will ensure that the enzymic hydrolysis is relatively slow.

A general feature of shrimp, as opposed to fish fermentations, is that whole small shrimps are used, with or without an initial salting, and usually without the addition of an external amylase source.

Japanese fermented fish, funasushi, is made by mixing eviscerated fish with their weight of salt and storing them under weights for 1–2 months. The salted fish is washed, drained, and packed in barrels with an equal amount of cooked rice containing some koji (*Aspergillus oryzae* on cooked rice). The koji provides a source of amylase (Steinkraus *et al.*, 1983). A vigorous bacterial fermentation ensues and lasts for about 10 days. The fish is allowed to mature for another 1.5–2 months before being eaten (Matsuchita, 1937; Tanikawa, 1971).

Orillo & Pederson (1968) investigated the lactic acid fermentation of Philippine fish sauce (burong dalag) with formulations containing about 30% fish flesh, 65% cooked rice, 3% salt and an unstated amount of angkak (a deep red to purple coloured rice obtained by growing *Monascus purpureus* on cooked rice). The prepared fish flesh was sprinkled with the dry salt, mixed with the cooked rice and angkak, packed tightly into containers and sealed from contact with air. The pH value fell to 4.0 in 24 hr and the fermentation was complete in 7–10 days. Angkak is added to confer colour on the product but since it contains amylase (Steinkraus *et al.*, 1983) it is possible that it also serves as an amylase source.

Philippine fermented shrimp sauce, balao-balao, (Malaysian cencalok is similar) is made from whole small shrimps which are mixed with 20% their wet weight of salt, stood for 2 hr, drained, mixed with cooked rice in the ratio one part shrimps to 4.8 parts cooked rice by weight, and 3% salt added to the whole. The mixture is packed in containers and fermented at ambient temperatures (28–32°C) for about 7 days. No koji, angkak or other external amylase source is added (Arroyo *et al.*, 1978). Vatana & Rosario (1983) investigated changes in soluble and amino nitrogen in fermented rice-

shrimp mixtures and concluded that the main source of proteolytic enzymes was the shrimps.

(ii) Bacterially fermented fish silage. Fish silage prepared by bacterial fermentation and intended for use as animal feedstuff is similar to fermented fish-carbohydrate mixtures for human consumption except that salt is usually not added. Nilsson & Rydin (1965) proposed that fish be fermented along with cereal meals as starch source and malt meal as amylase source. A mixture of small baltic herring with 20% barley meal and 2% malt meal, ensiled at 28°C for 4 weeks, yielded a silage at pH 4.3 and containing 5.0% acid as lactic acid. Stanton & Yeoh (1977) used a similar approach but substituted Malaysian ingredients, namely tapioca starch instead of cereal meal and ragi (a mixed culture of amylolytic moulds grown on rice) for malt. They found that, in the absence of added salt, it was necessary to use ratios of fish to tapioca of 3:2 or less to obtain a satisfactory, non-putrid preserved product. Why Nilsson & Rydin were able to prepare acidic silages with fish:carbohydrate ratios as high as 4:1 whereas Stanton & Yeoh could not is unknown. It might be due to differences between the fish used but could also be due to other factors, such as the rate of release of sugars from the starch.

Lingren & Pleje (1983) studied the fermentation of a mixture of 80% herring offal, 10% cereal grains and 10% molasses. They inoculated the mixture with an actively growing starter culture of *Pediococcus acidilacti* and *Lacobacillus plantarum* (2×10^8 lactic acid bacteria/g) and obtained a rapid fermentation with the pH falling to below 4.5 within 30 hr at 24°C. Important contributing factors to the rapidity of the fermentation were, presumably, the large inoculum and the immediate availability of fermentable sugar.

To date the preparation of fermented fish silage for feeding to animals does not appear to have found commercial application.

II. Products made by bacterial fermentation without added carbohydrate

One of the few European fermented fish products is surstromming which is made on the north Baltic coast of Sweden (Alm, 1965; Schmidt-Nielsen & Bohmer, 1937). Fresh whole herring is immersed in saturated brine for 30–40 hr, with continuous stirring for the first 3–4 hr. It is then eviscerated and packed into barrels with fresh brine and a vigorous gas-forming fermentation occurs that lasts for about 2 weeks. Alm gives the brine concentration as about 18% but Schmidt-Nielsen & Bohmer reported salt concentrations in the range 8.3–12.7% both in the brine and the fish flesh. The herring is then packed in cans and covered with the brine from the fermentation barrels and sealed. The final product has a pH of 5.9–6.4 and a characteristic odour said to be due to methylmercaptan. No heat treatment is used and the cans must be stored under refrigeration. No carbohydrate is added to the fish and information on the nature of the fermentation and of the bacteria involved does not appear to be reported. However, it is conceivable that the bacteria involved might be similar to the obligately anaerobic, Gram negative, gas and hydrogen sulphide producing bacteria described by Knochel & Huss (1984) as being responsible for spoilage of herring titbits.

A related product is possibly Kampuchean mam-chao (fish paste-glutinous rice mixture). The initial stages of preparation involve packing salted, beheaded and eviscerated fish (salt:fish ratio between 1:30 and 1:5 depending on taste) in jars under weights for 20–30 days. The weights presumably serve to express air and brine from the fish. The resulting fish paste is mixed with fermented rice (a mixture of cooked glutinous rice with 3% angkak, 2% palm sugar, inoculated with a yeast preparation and incubated

overnight), repacked into jars, covered with dilute brine and kept for another 1–3 months (Westenberg, 1951). No information is available on any microbial involvement, but it seems that the salt concentration during the initial storage is sufficiently low to permit growth of bacteria. The second storage period with added carbohydrate probably allows a lactic fermentation to occur.

Scales of production and significance of enzyme hydrolysed and fermented fishery products

Sauces and pastes made from fish, shrimps and other aquatic animals are traditional products in all the countries of Southeast Asia as well as in Korea, Japan and parts of China. They were at one time widely manufactured around the shores of the Mediterranean and a common item of commerce in Europe (Mackie *et al.*, 1971) but the European representatives are now largely restricted to the Scandinavian anchovies, herring titbits and marinaded herring products.

Information on current production levels is rather limited. Fisheries Statistics for West Malaysia (Wan, 1978) suggest that of the total fish catch in 1976, more than 70% was consumed fresh and the rest was preserved in various ways. Of the estimated 37 175 t of preserved products, dried salted fish represented 6165 t, shrimp paste (belacan) 960 t, high salt fish sauce (budu) 13 t and fermented shrimp sauce (cencalok) 1 t. The remainder of the preserved products was constituted of dried anchovies, dried prawns, fish meal and fertilizer. Annual production of fish sauce and paste in the Philippines was estimated to use not less than 100 000 t of fish in the manufacture of 12.5 million litres of sauce (Canonizado, 1978). In 1960 the production of Scandinavian anchovies was 5250 t and of titbits and fermented herring fillets 11 000 t (Alm, 1965). Japanese production of autolysed and fermented marine foods was about 13 000 t in 1968 (Tanikawa, 1971).

It is evident that the high salt enzyme hydrolysed products are made in much larger quantities than the low salt bacterially fermented ones. This may be a reflection of consumer preference for their flavours but it is also possible that the greater difficulty of manufacture and higher failure rate of the bacterial fermentations make these products less attractive to the manufacturers (personal communication). In addition, manufacturers no doubt appreciate that products containing near saturated concentrations of salt are stable almost indefinitely, even under tropical conditions, whereas those with lesser concentrations require more care in their storage and handling if spoilage is to be avoided.

None of these products are major items of diet but in the countries of Southeast Asia they are very widely consumed as condiments to add flavour to otherwise rather bland rice-based diets. Intake of most of them is limited by their high salt content and no one eats a great daily volume but almost everyone consumes a little every day (Amano, 1962). Their contribution to protein nutrition is not great, but since it is the poor who consume the greatest quantities the nutritional contribution for them may be valuable. For example, it has been estimated that nuoc-mam at one time provided 7.5% of the average protein intake for the Vietnamese (Amano, 1962). Although various claims have been made regarding the nutritional advantages of these products compared with fresh fish, Veen & Steinkraus (1970) have concluded that this is not an important consideration. A much more significant factor in the nutritional evaluation of preserved fish products is the extent to which nitrogenous compounds have been converted to amines or ammonia and thus rendered unavailable to human and non-ruminant animal nutrition (Amano, 1962; Mackie *et al.*, 1971).

Health hazards associated with preserved fishery products

The major potential hazard associated with proteinaceous foods of near neutral pH value is from the growth of food poisoning bacteria. Of particular concern with non-heat treated foods offering anaerobic conditions is the possibility of growth and toxin production by *Clostridium botulinum*. Hence, any method of fish preservation needs to ensure both that potential infectious and toxin-forming bacteria shall not grow in the product during processing and that the possibility of their growth in the final product is excluded. In addition, the possible presence of parasitic worms and of physiologically active amines needs to be considered.

A number of parasitic worms may be contracted by eating raw fish, partially cooked fish or uncooked fermented fish products (Healey & Juranek, 1979) but the relative importance of enzyme hydrolysed and fermented fishery products was not reported. Physiologically active amines, such as histamine formed by the bacterial decarboxylation of histidine, may be produced in amounts sufficient to cause poisoning in certain fishes (Eitenmiller, Orr & Wallis, 1982). However, to what extent such amines are problems or potential problems with enzyme hydrolysed or fermented fish is not clear.

Fish and fish products have been associated with a major proportion of the botulism outbreaks in Japan, with seventy-three outbreaks, 411 cases and ninety-nine deaths from 1951–1974 (Sakaguchi, 1979). Preserved fishery products were also involved in a substantial proportion of the botulism outbreaks occurring in Poland and Scandinavia, and in the USSR salt cured sturgeon fillets used to be responsible for many cases of botulism prior to the introduction of improved hygienic standards and temperature control (Zaitsev *et al.*, 1969). The enzyme hydrolysed and/or fermented fish products most often incriminated in *C. botulinum* type E poisonings are isushi (fermented fish-rice) and kirrikomi (salted, partially autolysed fish) in Japan, salmon egg cheese (fermented crushed salmon roe) among Eskimos and Indians in Canada and Alaska, and rakefish (a fermented fresh water trout product similar to surstromming) in Scandinavia (Bartl, 1972; Sakaguchi, 1979). With the exception of kirrikomi and the salt cured sturgeon all of these are bacterially fermented products.

The intensities at which various environmental factors are singly able to prevent the growth of the main food poisoning bacteria under otherwise optimum conditions are shown in Table 2. It is evident that neither the highly salted nor the fermented fish products will support the growth of any of these bacteria once they are prepared due to their salt content or low pH value. In practice, environmental parameters at non-optimal levels interact to cause growth inhibition at less extreme values than those given in the table. For example, *C. botulinum* type E is inhibited from growing by pH values less than 5.0–5.4 at 29°C but at 5°C it is unable to grow at pH values below 6.2 (Riemann, Lee & Genigeorgis, 1972). However, the practical exploitation of these interactions with complete security requires more data than is presently available.

Although these preserved fishery products might potentially be sources of any of the food poisoning bacteria listed in Table 2 (Shewan, 1962) there appears to be little evidence to suggest that they are important sources, except in the case of botulism (Bartl, 1972). Hence, the discussion that follows is concerned mainly with botulism but also includes a brief consideration of the possible risks from the relatively salt tolerant staphylococci.

Control of Clostridium botulinum

The growth of *C. botulinum* is accompanied by the formation of a toxin which is

Table 2. Maximum sodium chloride concentrations and minimum temperatures, pH values and water activities for growth of food poisoning bacteria under otherwise optimal conditions (from Bergdoll, 1979; Bryan, 1980; Genigeorgis & Riemann, 1979)

	Conditions allowing growth/toxin production			
	Minimum values			
Bacterium	Temperature (°C)	pH value	a_w	Maximum salt concentration (% w/w)
Bacillus cereus	7	4.4–5.0	0.93–0.95	7.5
Clostridium botulinum				
types A and B	10–12	4.8	0.94–0.95	10
type E	3.3	5.0	0.97	5
C. perfringens	15–20	5.0	0.95–0.96	4–6
Salmonella species	5.2	4.1–5.5	0.95	8
Staphylococcus aureus				
growth: aerobic	6.7	4.3	0.86	16–18
anaerobic	6.7	4.7	0.90	14–16
toxin: aerobic	10	4.3	0.90–0.93	12–13
anaerobic	10	6.5	0.90–0.93	12–13
Vibrio parahaemolyticus	3–13	4.8	0.94	8–10

stable in acidic conditions but relatively easily destroyed by cooking (Sakaguchi, 1979). Since ingestion of this toxin is apt to be fatal the aim in the processing of foods is to reduce the possibility of *C. botulinum* growing to as near zero as possible.

The post processing environment offered by all the products discussed in this paper are such as to prevent any growth of *C. botulinum*. Hence, the control of botulism in these products depends upon eliminating the risk of botulinum growth during processing, and especially during the early stages before inhibitory levels of salt or acid have fully penetrated the tissues.

Theoretical and practical aspects of the kinetics of salt penetration into tissues have been studied extensively by Russian workers and their results are reviewed by Zaitsev *et al.* (1969). The information given includes an equation for calculating rates of salt penetration which yields predictions that are in fairly good agreement with experimentally determined rates for small and medium sized fish. Experimental data on rates of penetration into anchovies with a surface to weight ratio of 4 and immersed in 20% brine showed that the aqueous phase concentration of sodium chloride inside the fish was approximately 10% after 12 hr and 18% after 24 hr.

Lerke (1973) considered the risks of *C. botulinum* growth in relation to the preparation of pickled seafood cocktails. He measured rates of acid penetration into crab leg muscles and the times for production of detectable levels of botulinum toxin in crab meat homogenates inoculated with 10^4 spores and incubated at different temperatures. He used the data to calculate a botulism safety factor, being the difference between the time required for the interior pH of crab leg muscle to reduce to 4.8, and the minimum time for detectable toxin formation in crab meat homogenate (Table 3). At 24°C this margin was only 7.5 hr which, it was suggested, was not enough when dealing with so dangerous a pathogen as *C. botulinum*. Lerke, therefore, made two recommendations: (i) that the initial pH of the pickle should be 3.8 or less in order to set up an adequate pH gradient to ensure that the interior pH of tissues is reduced to below 4.8 as rapidly as practicable; and (ii) that after preparation cocktails should be held at 10°C or less for 24

hr to allow acid penetration to occur while growth of botulinum is restricted by the low temperature. At least with respect to botulism it is not necessary to keep the products cool after this time. If such recommendations are observed the risk of growth of *C. botulinum* in these products should be completely eliminated.

Table 3. Estimated botulism safety factors in the preparation of seafood cocktails using pickling sauce of pH 3.7 at different temperatures (from Lerke, 1973)

Pickling temperature (°C)	Maximum time for internal tissues to reach pH 4.8* (hr)	Minimum time for detectable botulinum toxin at optimum pH†	Botulism safety time (difference)
24	12.5	20 hr‡	7.5 hr
10	18	7 d	6.3 d
5.5	23.5	> 30 d	> 29 d

*Interior of crab leg muscles about 10 mm across smallest dimension immersed in pickle of tomato paste, acetic acid and water (pH 3.7, meat : sauce, 4 : 6).

†In crab muscle homogenates inoculated with 10^4 spores of *C. botulinum* type E.

‡Determined at 30°C.

It is obvious that similar criteria and recommendations are directly transferable to the preparation of marinaded herring products, and the traditional procedures of marinading at low temperature should ensure that these products are entirely free from the risk of *C. botulinum* growth.

Similarly the use of low temperatures for the processing of Scandinavian anchovies and herring titbits should ensure that salt penetration is complete before *C. botulinum* has an opportunity to multiply to hazardous levels.

However, the traditional Southeast Asian salted fish products made at ambient temperatures in warm climates would appear to be much more at risk. The role of kirrokimi in Japanese botulism would tend to confirm that the risk is a real one, though possibly lessened in cases where the product is cooked before being consumed.

Many of the traditional processes make use of dry salting, which presumably maximizes the concentration gradient and the rate of penetration of salt into the interior of the tissues. It is also possible that the lowering of the internal water activity due to abstraction of water by the salt may make a more rapid contribution to the restriction of microbial growth in interior tissues than achieved by the diffusion of salt into tissues. Nevertheless, due to the difficulties of obtaining completely homogenous salting, to remove any risk of botulinum growth it can be concluded that it would be desirable for these products to be cooled during the initial salting. Alternatively, for fish destined to be liquefied, the use of comminuted fish would permit more reliable mixing with salt and less risk that some material might remain unsalted for a substantial period of time. Certainly the practice of holding fish in baskets in lakes or rivers in tropical climes for 12–16 hr, as is done for certain Kampuchean dried salted fish (Westenberg, 1951) cannot be condoned.

The products with the greatest difficulties, and the worst record with respect to botulism, are the bacterially fermented ones. All of the traditional products include some salt but it is not clear whether the concentration established in the brine is

sufficient on its own to inhibit growth of *C. botulinum*. Eklund (1982) reported that in hot processed smoked fish stored at 25°C the concentrations of sodium chloride sufficient to inhibit growth and toxin production by *C. botulinum* were 3.8–4.2% for type E and 8.1% for type A. If the brine concentration in fermented fishery products is adequate to achieve this inhibition then an initial period at a low temperature could secure complete protection from risk of botulism. If the brine concentration is not sufficient on its own to provide complete safety from botulinum growth, then the assurance of safety of these products depends upon the rapid microbial production of acid and depression of the pH value so as to inhibit the growth of *C. botulinum* before it has time to multiply to hazardous levels. It is not possible to dictate circumstances that will ensure total safety from botulinum growth since the maximum time that can be allowed for acidification depends on many interacting factors that affect the growth of both *C. botulinum* and the acid producing lactic acid bacteria. Some aspects of these interactions are considered in detail below. The use of low temperatures to restrict growth of *C. botulinum* does not seem feasible since it would also slow down the growth of the lactic acid bacteria. Kanzawa & Iida (1957) suggested that acetic acid be added to the fish early in the preparation of isushi to obtain a more certain rapid acidification. However, apart from likely inhibitory effects on the lactic acid bacteria, this would also need to be combined with an initial cool period for complete security. The only other way in which safety from botulism could be assured is by the use of compounds inhibitory to *C. botulinum* but not to lactic acid bacteria. At present no such compounds are commercially available and permissible in foods.

Control of Staphylococcus aureus

S. aureus is more salt tolerant than other food poisoning bacteria and produces a toxin which is relatively stable to both acid and cooking (Bergdoll, 1979). However, unlike the case with *C. botulinum*, toxin production by *S. aureus* is not so closely linked to growth and the conditions allowing toxin formation are more restricted than those permitting growth (Table 2). Its high salt tolerance would suggest that it could be an important problem in many of these salted fish products, but staphylococcal food poisoning by them has only been reported infrequently (Bartl, 1972; Shewan, 1962). It is not known whether this is due to poor reporting or to *S. aureus* being unable to compete effectively with natural mixed microbial populations under anaerobic conditions and thus not growing to toxic levels in salted fishery products. In either case the control of *S. aureus* in preserved fishery products depends upon the same general principles as for *C. botulinum*, namely of ensuring that conditions are rendered inhibitory to its growth sufficiently rapidly during processing that toxigenic populations cannot develop.

Conditions for lactic acid bacterial fermentation

The preservation of foods in a safe and wholesome condition by natural lactic acid fermentation depends upon rapid growth and acid production by lactic acid bacteria and suppression of competing microbes by low pH values, weak organic acids, and in some cases other antimicrobial factors. The major influences on the growth of lactic acid bacteria and the rate at which the pH value of the ferment declines and competitors are suppressed are: (i) availability of fermentable carbohydrate; (ii) availability of organic growth factors; (iii) anaerobiosis; (iv) temperature; (v) sodium chloride concentration; (vi) concentration of organic acids and pH value; (vii) carbon dioxide

concentration; (viii) production of other inhibitory compounds; (ix) buffering capacity of the substrate; (x) initial numbers of lactic acid bacteria; and (xi) initial numbers of competing microbes.

Carbohydrate source. Since fish flesh is very low in free carbohydrate it is necessary to add some in order to get a good acid-producing fermentation. Most Southeast Asian fermented fishery products include cooked rice as carbohydrate source but before it can be utilized by the bacteria the rice starch must be broken down to simpler sugars. The susceptibility of starch granules to enzymic hydrolysis is affected by the source and condition of the starch granules as well as by the source of the amylase. Cooking causes gelatinization of starch and renders it much more susceptible to the action of amylolytic enzymes (Kulp, 1975). The use of glutinous rice in some fermented products may reflect differences in susceptibility to certain amylases. In some products roasted rice is used but it is not clear whether roasting increases susceptibility to enzymic attack or is done to add flavour.

In many traditional processes mould preparations, such as koji, are added as a source of amylase. It is possible that the mould colouring agent, angkak, added to other fermentations also serves as an amylase source. Some fermentations, such as those of whole-shrimp rice mixtures, do not have any external source of amylase added and presumably the starch is hydrolysed by amylases in the shrimps or produced by the bacteria. Relatively few lactic acid bacteria have been reported to produce amylases (Champ *et al.*, 1983; Dunican & Seeley, 1962; Nakamura, 1981; Seeley & Dain, 1960) and it is not presently known whether such bacteria are involved in these traditional starch-based fermentations. Lindgren & Refai (1984) isolated amylolytic lactic acid bacteria from fish silage but, as the formulation included malt, it is unclear as to whether the bacterial amylolytic activity was important.

While lactic acid bacteria normally require fermentable carbohydrates to be able to grow, some are able to generate energy from L-arginine in the presence of low concentrations of glucose (Bauchop & Elsden, 1960). Jonsson, Clausen & Raa (1983) isolated a *Lactobacillus plantarum* strain from fish with such an ability and suggested that this ability might explain why lactic acid bacteria become dominant in certain stored fish.

Organic growth factors. The vitamins, amino acids and other organic growth factors required for growth by the lactic acid bacteria derive from the fish tissues and are seemingly available in adequate amounts.

Anaerobic conditions. The early exclusion of oxygen is an important factor in reducing the growth of obligately aerobic Gram negative spoilage bacteria during the initial stages of the fermentation before acidic conditions are established. The maintenance of anaerobic conditions in, and especially at the surface of, the final fermented product is necessary to exclude the growth of obligately aerobic moulds and yeasts. These are well able to tolerate the acidic conditions and their growth may lead to depletion of the organic acids and consequent rise in the pH value of the fermented material with serious implications for the keeping quality and safety of the product.

Temperature. Temperature can have a considerable influence on the composition of microbial populations and the final flavours of natural fermentations such as sauerkraut (Pederson, 1971) but its effects on Southeast Asian fish fermentations does not seem to

have been investigated. Certainly, the high ambient temperatures will promote rapid growth of all kinds of microbes, including spoilage and food poisoning organisms as well as the lactic acid bacteria.

Salt concentration. Salt serves the dual functions in these fermentations of withdrawing water and nutrients from the fish flesh, and of aiding the lactic acid bacteria in their competition with spoilage and poisoning bacteria. As already mentioned, the concentration of sodium chloride to which the microflora is exposed is that existing in the liquid phase and this concentration is usually not known. In the absence of this information it is difficult to estimate what effects salt has on the competition between lactic acid bacteria and other microbes. However, the presence of sodium chloride in diverse kinds of lactic acid food fermentations, including sauerkraut, fermented sausages, and fish sauces suggests that the concentrations are sufficient to aid the lactic acid bacteria but there is no conclusive data on the extent of the advantage conferred. Arroyo *et al.* (1978) investigated the effect of 3, 6, 9 and 12% salt (w/w, dry salt/wet rice-shrimp) on the fermentation of rice-shrimp mixtures and showed that acidification was more rapid the lower the salt concentration but did not directly examine the effects on the microflora.

It is noteworthy that some lactic acid fermentations, such as those for yoghurt and other fermented milk drinks, Nepalese fermented mustard, rape or radish leaves (Karki *et al.*, 1983) and grass silage, do not have salt added. Why these fermentations should normally proceed satisfactorily (i.e. under traditional non-aseptic conditions) in the absence of salt whereas tradition decrees that it is essential for others is not immediately evident. It would be useful to know the answer to this question since it is desirable to reduce the amount of salt in many of these fermented products.

Concentration of organic acids and pH value. The growth of lactic acid bacteria is necessarily accompanied by the excretion of lactic acid and, in some circumstances, of acetic acid (Kandler, 1983) and a consequent lowering of the pH value of the medium. Since the lactic acid bacteria are exceptionally tolerant among the bacteria to low pH values in the presence of weak organic acids, they rapidly dominate anaerobic, sugar and nutrient rich environments. The toxicity of weak organic acids is related to the concentration of undissociated acid molecules, which is a function of the total acid concentration, the pKa of the acid and the pH value of the system (ICMSF, 1980). For the effective inhibition of spoilage and food poisoning bacteria it is therefore important that the pH value in natural fermentations should fall as rapidly as possible to levels at which a significant proportion of the acid is present in the undissociated form. With lactic acid, having a pKa of 3.87, this corresponds to a pH value of below about 4.9.

Carbon dioxide concentration. Ingram (1975) suggested that tolerance towards high concentrations of carbon dioxide is a decisive ecological factor for the lactic acid bacteria. The majority of other bacteria are substantially less tolerant (ICMSF, 1980). Thus the early production of carbon dioxide in natural food fermentations by gas-forming heterofermentative lactic acid bacteria could be a factor in the rapid suppression of spoilage and food poisoning bacteria.

Production of other inhibitory compounds. Some lactic acid bacteria produce compounds beside lactic acid, acetic acid and carbon dioxide that are antagonistic to competing organisms. While the role of some, such as nisin, in the organisms ecology, is

relatively well established (Hurst, 1981) the significance of many other compounds produced by various lactic acid bacteria is much less clear. Nevertheless, it is possible that some of these compounds may be important in restricting some competitors under certain conditions.

Buffering capacity of the substrate. The preservation of foods by natural lactic acid fermentation depends upon the rapid establishment of acidic conditions so that spoilage organisms do not have time to make significant growth. It is obvious that a major influence on the rate of decline of pH value must be the buffering capacity of the food. Foods with a low buffering capacity, such as most vegetables, will have their pH value lowered rapidly by a relatively small amount of growth and acid production. Proteinaceous foods, with much higher buffering capacities, will require a much greater amount of growth and acid production to effect the same fall in pH value. For example, sauerkraut at a pH value of 3.4–3.6 contains only 1.5–1.7% acid as lactic acid (Pederson, 1971) whereas the fish silage of Nilsson & Rydin (1965) contained 5% lactic acid at a pH value of 4.3. If the buffering capacity at pH values about 5 to 6 is especially high it is likely that considerable spoilage can occur before sufficient acid is produced to lower the pH. It is possible that the high ratios of rice to fish in some of the traditional products may reflect a need to dilute out the buffering capacity of the fish flesh, rather than being a simple adulteration of fish with less costly rice.

Initial numbers of lactic acid bacteria. Traditional fermentations depend on naturally occurring microbes in the substrate or built up on equipment as a source of inoculum, followed by the provision of suitable conditions for the proliferation of the desired types. The inoculation of sufficiently high numbers of appropriate kinds of lactic acid bacteria into rice-fish ferments would certainly serve to ensure rapid acidification but, with the present rudimentary knowledge of the biochemical ecology of these fermentations, it is not at present possible to identify appropriate strains.

Initial numbers of competing microbes. It is axiomatic that the higher the initial numbers of potential spoilage organisms or food poisoning bacteria, the greater is the probability of their causing undesirable changes or producing infectious or toxigenic populations before their multiplication is suppressed by the lactic acid bacteria.

Future developments and research

Although the highly salted, enzyme hydrolysed fishery products are presently more popular than the fermented ones, their high salt content means that they can only be eaten in relatively small amounts and generally, in Southeast Asia, they serve as condiments. For this reason, and because of current concern about the possible relationship between high salt intake and high blood pressure, it does not seem desirable to advocate a large expansion in the use of these methods of fish preservation. On the other hand, the traditional bacterially fermented fishery products contain relatively low concentrations of salt and would appear to offer greater opportunities for increased usage. Thus the need is for a fish fermentation method that will give a rapid and assured pH drop, using cheap ingredients with a minimal level of sodium chloride and simple techniques. In addition, of course, the product must be organoleptically attractive. The requirement for economy presupposes that the carbohydrate source will be starch, such as rice, cassava, tapioca or sago. In order to put such a system on a sound scientific basis more information is needed on:

(i) Starch utilization in these fermentations, particularly with regard to the sources and types of amylases involved and the routes of utilization of the resultant sugars. Presently boiled rice is the most commonly used starch source but the use of a dry, uncooked starch would reduce costs and allow easier use of fermentation processes on board small boats.

(ii) The roles of sodium chloride in the fermentation and the extent to which the concentrations used can be reduced or replaced by other compounds while still ensuring that the products are entirely safe from risk of botulism or staphylococcal poisoning. Chayovan *et al.* (1983) investigated the partial replacement of sodium chloride by potassium chloride in certain autolysed fish sauces. Fleming (1982) mentions the use of calcium chloride to lower the salt requirement in vegetable fermentations but investigation of a similar approach with bacterial fish fermentations has not been reported.

(iii) The characteristics of suitable strains of lactic acid bacteria for inoculation into fish-starch mixtures.

(iv) The interactions of the environmental parameters that influence the development of lactic acid bacteria, spoilage bacteria and food poisoning organisms. In particular, information is needed in order to define the circumstances which will assure freedom from the risk of botulism and staphylococcal poisoning.

(v) Factors influencing the organoleptic properties and methods for the consistent manufacture of products with desirable properties. It seems likely that some degree of enzymic hydrolysis might be desirable to form flavour compounds in products otherwise processed by bacterial fermentation.

Conclusions

Although enzyme hydrolysed and fermented fishery products have been investigated for over 50 years, very few studies have got beyond the stage of rather basic descriptions of the processes. It is to be hoped that future research will concentrate upon specific questions whose answers will provide a rational basis for the greater exploitation of these natural food preservation methods. Currently, there is some interest in the possible expansion of the use of bacterial fermentation for food preservation since there is a need, especially in the tropics, for reliable, cheap, and safe preservation methods for highly perishable protein foods. Bacterial fermentation is cheaper than canning or refrigeration and is readily adaptable to both large and small scales of operation, including relatively simple procedures that might be utilized on board small fishing boats. Thus if reliable techniques for the manufacture of safe and flavourful fermented fishery products can be established, they can potentially make a large contribution to protein nutrition in many lesser developed countries.

Acknowledgments

The authors wish to thank Mr J. D. Legan for many helpful comments on the manuscript and L. S. Mendoza is grateful for financial support from the German Agency for Technical Cooperation.

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(Received 8 August 1984)

Journal of Food Technology (1985) **20**, 295–303

Biochemical changes in fermenting African locust bean (*Parkia biglobosa*) during 'iru' fermentation

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Summary

The enzymic activities and biochemical changes in the principal food constituents of African locust bean were investigated. The reducing sugar level increased from 63 mg/g to 134 mg/g during the first 24 hr but subsequently decreased. Amylase activity was not detected. The lipase activities were detectable with the peak at 48 hr after the start of fermentation. The most significant activity during the fermentation is the rapid and steady increase in the quantity of free amino acids throughout the fermentation. This is due to a consistently active proteinase activity by the fermentative microorganisms. The number and quantities of each amino acids analysed also increased in the fermented beans. Glutamic acid, valine, aspartic acid and alanine were rapidly liberated during the fermentation. The changes observed are compared with the fermentation of other protein rich seeds.

Introduction

Fermented African locust bean is an important vegetable protein in the Guinea savanna zone of West and Central Africa. It is called 'iru' by the Yorubas of south-western Nigeria while the Hausas who inhabit most of the northern parts of West Africa call it 'dawadawa'. During its preparation African locust bean seeds (*Parkia biglobosa* Jacq. Benth, syn. *P. clappertoniana*) are boiled and left to ferment in calabash trays for up to 60 hr depending on the environmental conditions. The fermentation which is by chance inoculation is by various subspecies of the *B. subtilis* group and *Staphylococcus* spp. During the short period of fermentation heat is evolved and the pH increases (Odunfa, 1981a).

The locust bean itself is made up of protein, 39–47%; oil, 31–40% and carbohydrate 11.7–15.4% (Campbell–Platt, 1980). Although 'iru' is a food condiment, it is used in poor rural families in West Africa as a low cost meat substitute and it contributes to their protein and calories intake. Simmons (1976) found that the average daily per capita intake of 'dawadawa' among some Hausas of Northern Nigeria constitutes 1.4% of the daily calories intake and 5% of the total protein intake.

Information is available on the predominant microorganisms in the fermenting beans (Odunfa, 1981a) and its nutrient status (Eka, 1980). It is also known that flatus forming oligosaccharides, notably stachyose and raffinose, decrease significantly during fermentation thereby improving the product nutritionally (Odunfa, 1983a). There is, however, no information on the biochemical changes that occur during the fermentation. The present paper is aimed at providing this information.

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Materials and methods

Preparation of 'iru'

Samples of cooked locust beans and fermented beans at 12, 24, 36, 48 and 60 hr were collected from a local 'iru' maker who prepares 'iru' in the traditional way. The steps involved in the preparation are shown in Fig. 1.

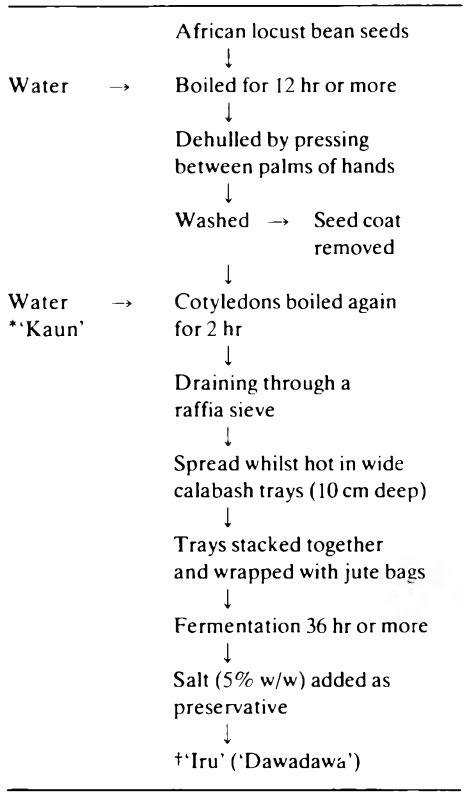

Figure 1. Flow sheet for 'iru' production.

*Native rock salt containing K_2CO_3 and $KHCO_3$ may be added to aid softening; †sticky, dark brown, strong-smelling beans with greyish outer layer.

Preparation of extracts

For chemical analysis, approximately 5 g of sample was weighed into a 100 ml conical flask and 50 ml ethanol water mixture (50:50 vol./vol.) was added and ground in a mortar. The suspension was then washed with 5 ml petroleum ether to extract the oil, centrifuged at 5000 rpm in an MSE high speed-18 refrigerated centrifuge at 5°C for 30 min. The clear supernatant was used for analyses. To extract the enzymes, the mash was ground with mortar and pestle in an appropriate buffer, after which the suspension was centrifuged at 5000 rpm and was centrifuged as stated above. The supernatant was stored in a deep-freezer at −20°C. Assays and analyses were carried out on duplicate fermentations and for each sample three determinations were made at each time interval.

Determination of soluble reducing sugars

The reducing sugars were determined using the dinitrosalicylic reagent method (Sumner & Howell, 1935). Washed extract (1 ml) was diluted ten-fold; 1 ml of the resultant solution was added to 2 ml of dinitrosalicylic acid reagent. After 5 min in a boiling water bath, 20 ml of water was added and the optical density of the solution was measured with a Pye Unicam SP6 250 spectrophotometer at 550 nm. The total concentration was determined from a standard curve prepared using known concentrations of maltose.

Determination of free amino acids

The total (free) amino acids were determined by the ninhydrin colorimetric analysis method of Rosen (1957). The extract was suitably diluted and to 1 ml of this was added 0.5 ml cyanide-acetate buffer and 0.5 ml of 3% ninhydrin solution in Methyl Cellosolve. The mixture was heated for 15 min in a 100°C water bath. Thereafter 5 ml isopropyl-alcohol water mixture was added and shaken vigorously. After cooling, the colour was read in a colorimeter at 570 nm. The concentration of amino acids was calculated from a standard curve based on known concentrations of leucine. Each determination was done in triplicate.

Identification of free amino acids

A total of 20 g each of fermented and unfermented samples was weighed out and ground in 100 ml water. After stirring for 1 hr, the suspensions were filtered through Whatman No. 1 filter paper. $(NH_4)_2SO_4$ was added to each suspension to precipitate the proteins which was filtered off. The filtrate was evaporated to dryness in a rotary evaporator and each sample was redissolved in 10 ml of citrate buffer pH 2.2. The quantities of individual amino acids were measured on a Beckman/Spinco 120 automatic amino acid analyser.

Enzyme assays

α-Amylase. The extracting buffer was 1 M potassium hydrogen phosphate, pH 6.5. The assay procedure described by Bernfeld (1955) was used: 2 ml of the extract was mixed with 1 ml of 1% starch solution and incubated for 1 hr at 40°C. The reaction was stopped by adding 3 ml dinitrosalicylic acid reagent (DNS). The mixture was heated in a boiling water bath for 5 min, cooled in cold water, and then diluted with 18 ml water. The optical density of the resultant solution was measured at 550 nm, using an SP6 250 spectrophotometer. The blank was similarly treated except that the DNS was added before adding the starch solution. The amount of the reducing sugars formed was calculated from a standard curve prepared with known concentrations of maltose.

Proteinase. The extracting buffer was 0.1 M sodium hydrogen phosphate, pH 6.5. The assay method used was that of Yong & Wood (1977a). It has been found useful for analysing proteinases in the presence of reducing sugars normally found in food substances: 5 ml of the extract was added to 10 ml of 2% solution of light soluble casein (BDH) and incubated at 35°C for 30 min. The reaction was terminated by adding 10 ml of 10% trichloroacetic acid (TCA) solution. The mixture was filtered through Whatman No. 1 filter paper. The optical density of the filtrate was measured at 275 nm. The blank contained the same mixture but with the TCA added simultaneously with the enzyme extract.

Enzyme activity was expressed in terms of an arbitrary unit called an XS unit, and defined thus: 'An enzyme extract which under the stated experimental conditions produced a filtrate with an optical density of 0.500 when measured in a 10 mm path length cell, had a strength of 36 XS units per gram.'

Lipase. The extracting buffer was 0.1 M sodium acetate-acetic acid mixture (pH 5.5). The assay procedure was a modified form (Yong & Wood, 1977a). Five ml of the extract added to a reaction mixture containing olive oil, 1 ml; sodium taurocholate, 0.4 g; $CaCl_2$, 1 ml of 0.1 M solution; acetate buffer, 6 ml. The mixture was incubated at 35°C for 1 hr. The reaction was terminated by adding 40 ml absolute alcohol. The mixture was then titrated with 0.02 M potassium hydroxide, using phenolphthalein as an indicator.

The blank was a mixture of the assay medium and 5 ml distilled water. The difference between the titre of the blank and that of the reaction mixtures gave the amount of alkali required to neutralize the liberated fatty acids and is expressed as oleic acid. The unit of enzyme is that amount of enzyme which liberates 1.0 mg of oleic acid per min.

Results

The reducing sugar level increased duirng the first 24 hr but subsequently decreased (Fig. 2). The quantity of soluble amino acids was found to increase rapidly throughout the course of fermentation (Fig. 3). Table 1 shows the free amino acids in unfermented and fermented locust beans. The number and quantities of amino acids analysed increased in the fermented beans. Glutamic acid, valine, aspartic acid and alanine were

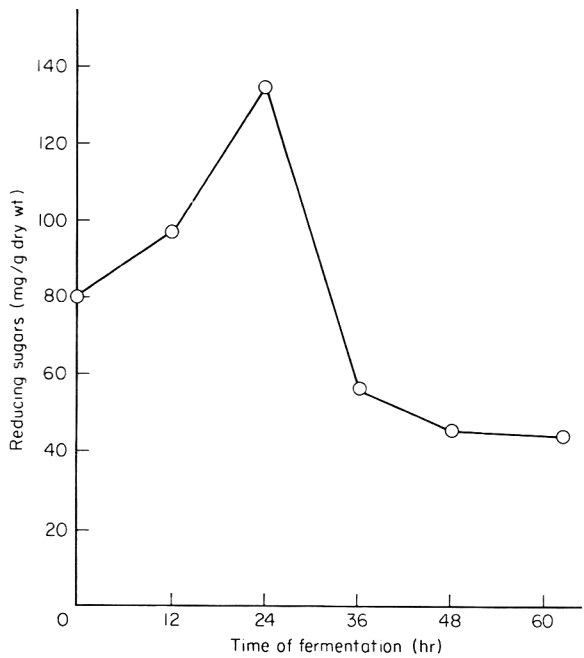

Figure 2. Changes in reducing sugar levels during 'iru' fermentation.

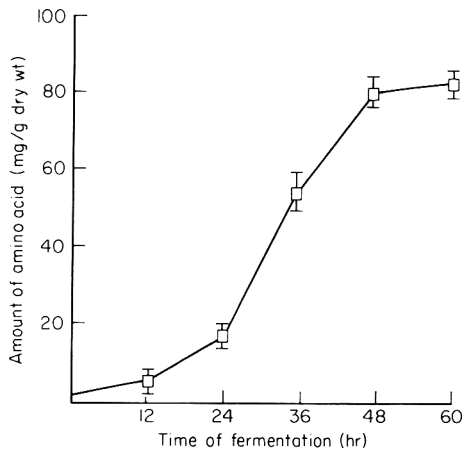

Figure 3. Changes in total amino acids during 'iru' fermentation.

rapidly liberated during the fermentation. Arginine and cysteine were absent in both the unfermented and fermented beans, while the 'iru' was very low in histidine, threonine and serine. Of the three extracellular enzymes assayed, only proteinase showed a high activity culminating in a peak at 36 hr (Fig. 4). Amylase activity was not detectable throughout the course of fermentation. The lipase activity showed an unsteady increase during the course of fermentation attaining a peak at 48 hr (Fig. 5).

Table 1. Free amino acids in the extracts of unfermented and fermented locust beans in mg/100 g samples

Amino acids	Unfermented beans	Fermented locust bean
Lysine	0.42 ± 0.03	2.29 ± 0.04
Histidine	—	0.39 ± 0.02
(Ammonia)	—	off scale
Arginine	—	—
Aspartic acid	1.86 ± 0.08	7.91 ± 0.40
Threonine	0.03 ± 0.01	0.37 ± 0.02
Serine	—	0.15 ± 0.01
Glutamic acid	2.45 ± 0.34	11.90 ± 1.10
Proline	0.08 ± 0.03	1.52 ± 0.03
Glycine	0.04 ± 0.01	0.87 ± 0.02
Alanine	0.35 ± 0.04	9.93 ± 1.18
Cysteine	—	—
Valine	0.38 ± 0.05	14.75 ± 2.4
Metheonine	—	0.85 ± 0.15
Isoleucine	0.23 ± 0.02	5.97 ± 0.54
Leucine	0.84 ± 0.04	6.67 ± 0.48
Tyrosine	0.24 ± 0.02	1.05 ± 0.05
Phenylalanine	0.85 ± 0.01	0.94 ± 0.03

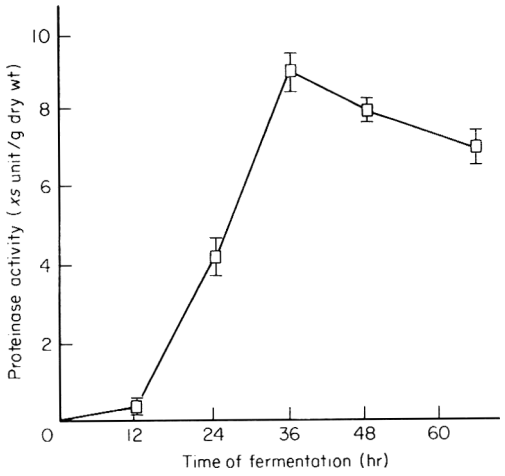

Figure 4. Proteinase activities during 'iru' fermentation.

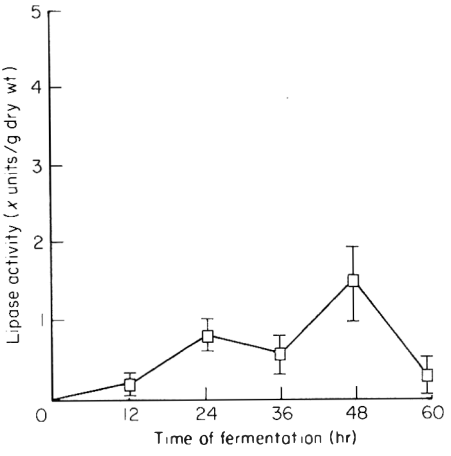

Figure 5. Lipase activities during 'iru' fermentation.

Discussion

The most important biochemical change during 'iru' fermentation is the protein hydrolysis. This is due to a consistently active proteinase activity resulting in rapid amino acid production. High proteinase activity has frequently been reported in the fermentation of similar protein-rich seeds as in the production of Japanese miso (Shibasaki & Hesseltine, 1962), soy-sauce (Wang & Hesseltine, 1970) 'tempeh' and 'ontjom', Indonesian mould fermented food products (Wang & Hesseltine, 1965; Steinkraus, Lee & Buck, 1965) and Nigerian 'ogiri', a fermented melon product (Odunfa, 1983b). The strong ammonia smell characteristic of the latter stages of 'iru' fermentation may also be an end product of proteinase activity as illustrated by Whitaker (1978).

The initial rise in the reducing sugar level may not be due to the amylase activity. They might be produced from the hydrolysis of oligosaccharides present in the unfermented bean (Odunfa, 1983a). These sugars are easily utilizable by the *Bacillus* spp. and *Staphylococcus* involved in the fermentation. Although carbohydrates constitute 11–15% of the unfermented beans no starch has been reported in the fermented beans (Watson, Dako & Amoakwa-Adu, 1975). Eka (1980) also provided information on the decrease in the carbohydrate level between the unfermented locust beans and the fermented beans.

Although oil constitutes 31–30% of the locust bean, lipase activity is fairly low especially when compared with 'tempeh' fermentation (Steinkraus *et al.*, 1975) and 'natto' (Kiuchi & Ohta, 1976). This is desirable since high amounts of fatty acids in foods generally imparts an objectionable taste and could cause rancidity to develop. Free fatty acids in foods have also been found to inhibit trypsin activity as well as several glycolytic enzymes (Wang *et al.*, 1975). However, some levels of fatty acids have been known to produce characteristic flavours of fermented vegetable proteins such as in 'tempeh' (Shibasaki & Hesseltine, 1962). The unsteady increase in lipase activity may be attributed to increase in pH earlier reported during 'iru' fermentation (Odunfa, 1981a). This increase has been shown to enhance lipase activity in *B. licheniformis* and *Staphylococcus* species (Jonsson & Snygg, 1974). The subsequent decrease after 48 hr may be due to destruction of lipase by increase in proteolytic activity (Jonsson & Snygg, 1974). The source of lipase in the fermentation is attributable to *Staphylococcus* species, in which lipolytic activities are well-known (Franklin & Sharpe, 1963; Vadehra & Harmon, 1969; Mates & Sudakevitz, 1973).

The liberation of soluble amino acids during the 'iru' fermentation is significant. Soluble nitrogenous constituents have been identified as one of the most important factors in the quality and taste of 'shoyu', a similar Japanese vegetable protein sauce from soy bean (Yokotsuka, 1981). The presence of glutamic acid is particularly important; the salt of this amino acid, monosodium glutamate, is used universally as an additive to enhance the flavour of soups. The sodium chloride traditionally added to 'iru' may therefore, in addition to its preservation action, combine with this liberated glutamic acid to enhance the flavouring potential.

The increase in glutamic acid in 'iru' may be explained in part by the ability of the fermenting microorganisms, notably *Bacillus subtilis, B. pumilus* and *B. natto* to accumulate glutamic acid extracellularly (Dulaney, 1967; Chattopadhay & Banergee, 1973).

The spectrum of liberated acids is similar to that obtained for 'natto' from soybean (Sakurai, 1960); the highest was glutamic acid followed by leucine, threonine, valine and alanine in descending order. Hence glutamic acid, valine and alanine were both liberated in high amounts in both 'iru' and 'natto'. The similarity may be due to the fact that both are fermented by similar microorganisms, strains of *B. subtilis*.

The enzymic activities in the fermenting locust beans are somewhat related to the enzymic properties of the microbial isolates from 'iru'. Of forty-five strains of *B. subtilis, B. licheniformis* and *B. pumilus* isolated from 'iru', all were proteolytic, forty were lipolytic and thirty-one were amylolytic (Oyewole, 1984); these characteristics were obtained on appropriate agar plates culture. As in the fermentation of 'ogiri', a Nigerian melon product, the most significant change during the fermentation is the increase in soluble products particularly amino acid; these increases improve the digestibility of locust bean, which in its unfermented state is not normally used as food.

Acknowledgment

The author is grateful to the International Foundation of Science, Sweden for the award of a grant to carry out this study.

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(Received 16 February 1984)

Journal of Food Technology (1985) **20**, 305–309

Effect of granularity on the characteristics of extruded rice snack

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Summary

Rice flours of different granularity over 542 μm (30 mesh), 542–286.5 μm (30–50 mesh), 286.5–175 μm (50–80 mesh) and throughs of 175 μm (80 mesh) were processed using a Wenger X-5 extruder with a 1/8 inch die and at an exit temperature of 95°C. With increased fineness of the flour, the expansion ratio, water absorption index and water solubility index of the product increased from 1.4, 4.91 and 0.016 to 2.6, 7.16 and 0.027, respectively. The extent of gelatinization of the starch component increased from 36.1% to 55.1% as the flour particle size became finer. This was also supported by amylograph paste viscosity data. The organoleptic evaluation of samples at the equilibrated moisture content showed that 43% ERH corresponding to 6.56% moisture and 11.3 kg breaking strength of the product was critical for acceptable textural properties of the product.

Introduction

Extrusion cooking has been accepted as one of the most useful technologies during the recent years in the field of food processing (Chiang & Johnson, 1977). The characteristics of the extruded products are monitored by several factors, namely design of extruder, feed rate, particle size and composition of raw materials, screw speed, extruder barrel temperature, die size, etc. (Taranto *et al.*, 1975; Smith, 1976; Park, 1976; Chiang, 1977; Seiler & Seibel, 1978; Mega & Cohen, 1978, Mottern, Spadaro & Gallo, 1969; De-Muelenaere & Buzzard, 1969). The effects of several factors other than particle size on the physico-chemical characteristics of extruded products have been studied comparatively in detail. Therefore, the present study was undertaken to study the effect of particle size on the physico-chemical characteristics of rice snacks.

Materials and methods

A sample of raw milled Jaya rice was obtained from the local market. This was ground in a Fitz mill (Fitz Patrick Company, U.S.A.) to different granularity, overs of 542 μm, 542–286.5 μm, 286.5–175 μm and throughs of 175 μm. All the fractions were extruded in a Wenger X-5 extruder with a feed rate of 27.2 kg/hr, water flow 6.8 l/hr, screw speed, 500 rpm; die size, 3.125 mm diameter; and an extrusion exit temperature at 95°C. The extruded products were dried at 50–55°C for about 5–6 hr. A portion was milled using a pin mill and the remainder was used for the evaluation of physical properties.

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Physical properties

Expansion ratio, density, water absorption index (WAI), water solubility index were determined according to the methods described by Mercier & Feillet (1975). Paste characteristics were studied using the Brabender amylograph equipped with a 700 mg sensitivity cartridge with a sample to water ratio of 50 : 450.

Analysis

Amylose. This was determined by the method of Juliano (1971).

Degree of gelatinization. The principle of damaged starch determination was employed to determine the extent of gelatinization indicative of the degree of cooking that occurred in the extruded product. AACC (1976) method for determination of susceptibility of starch to enzymic digestion with fungal alpha amylase was followed (Novo, Denmark).

Reducing and non-reducing sugars. The AACC potassium-ferrycyanide method (1976) was followed.

Water-soluble proteins. A weighed quantity of sample (2 g) was dispersed in distilled water (80 ml) and extracted for 2 hr at room temperature using a mechanical shaker. The suspension was filtered through Whatman No. 42 filter paper after making up the volume to 100 ml. A measured aliquot (20 ml) of filtrate was taken for the determination of protein content using a micro Kjeldahl method (AACC, 1976).

ERH, moisture and breaking strength relationship. Equilibrium relative humidity was determined using Wink's weigh equilibrium method (1946). The breaking strengths of samples at the equilibrated moisture contents were measured using the Instron machine (Model 1140) with the following settings: chart speed, 20 cm/min; full scale load, 20 kg (changed to 50 kg in hard samples); cross head speed, 5 cm/min; and deformation, 3 mm. Ten randomly selected pieces of each sample were tested for breaking strength and the values reported are the average of ten values. ERH, moisture and breaking strength values were plotted to obtain the relationship.

The samples at the equilibrated moisture content were evaluated organoleptically to find out the critical ERH (i.e. the ERH at which the products lose their textural properties and become unacceptable).

Results and discussion

Physical properties. The expansion ratio, water absorption index (WAI) and water-solubility index (WSI) value of the extruded rice snack increased with the increase in fineness (Table 1), being lowest, i.e. 1.4, 5.2 and 1.7% respectively for the overs 542 μm to 2.6, 7.7 and 2.9, respectively for throughs of 175 μm sieve. In contrast, the density and breaking strength decreased from 0.97 to 0.46 and from 20 to 15.0 kg, respectively. This suggested the development of lighter texture with decreased granularity.

Paste characteristics. Results showing the effect of granularity on paste characteristics are summarized in Table 2. Gelatinization temperature of about 62°C remained unchanged up to 286.5–175 μm particle size but for the product of throughs of 175 μm the temperature decreased to 58°C. Gelatinization time was essentially the

Table 1. Effect of granulation on physical characteristics of extruded rice snack (EER, 95°C; 27.2 kg/hr)

Particle size (μm)	Moisture (%)	Expansion ratio*	Density (g/ml)	Breaking strength (kg)	WAI† (g sediment/ g of dry matter)	WSI‡
542	6.4	1.4	0.97	20.0	5.2	1.7
542–286.5	6.5	1.8	0.53	15.0	6.3	1.9
286.5–175	5.9	2.2	0.51	14.5	6.6	2.5
Through 175	6.3	2.6	0.46	15.0	7.7	2.9

*Ratio mean $\frac{\text{product diameter}}{\text{die diemeter}}$; †water absorption index; ‡water-solubility index.

Table 2. Effect of granulation and extrusion processing on paste characteristics of rice snack measured on a Brabender Amylograph

Particle size (μm)	Gelatinization temperature (°C)	Peak viscosity (A.U.)	Temperature at peak viscosity (°C)	Viscosity at 95°C (A.U.)
542	61.0	310	85.7	120
542–286.5	61.7	240	85.7	60
286.5–175	63.2	245	87.2	50
Throughs 175	58.0	230	81.4	—
Unprocessed rice powder (175)	75.2	1000	91.5	890

Table 3. Effect of granulation and extrusion processing on the amylose, damaged starch (DS), sugars and water-soluble proteins (WSP) in the product

Particle size (μm)	Amylose (%)	DS (%)	RS* (maltose/mg %)	Non-RS† (mg sucrose/10 g)	WSP (%)
542	26.2	36.1	107	33.1	0.69
542–286.5	26.2	39.5	110	33.2	0.46
286.5–175	25.5	44.0	106	38.3	0.34
Throughs 175	25.6	55.1	159	38.4	0.33

*Reducing sugars; †non-reducing sugars.

Table 4. ERH, moisture and breaking strength relationship of the extruded (EET, 95°C, 27.2 kg/hr) rice snack

RH (%)	Equilibrium moisture (%)	Break strength (kg)	Organoleptic evaluation
10	3,76	7.5	Crisp
23	5.04	8.2	Crisp
33	5.76	10.6	Crisp
43	6.56	11.5	Crisp
52	8.06	18.1	Tougher
67	10.54	20.2	Tougher and less plastic
75	12.04	16.2	Tough, plastic
86	14.84	15.1	Less tough, more plastic
97	30.64	0.8	More plastic

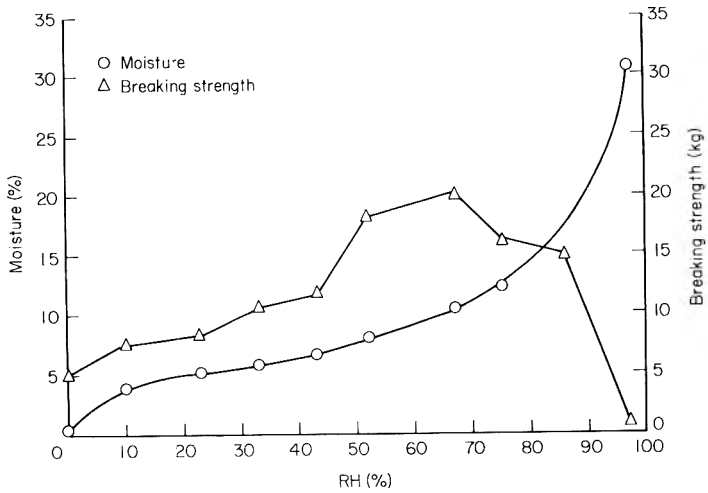

Figure 1. ERH, moisture and breaking strength relationship of rice snack extruded at 95°C EET, 27.2 kg/hr feed rate. O, moisture; Δ, breaking strength.

same for the products of various particle sizes. Peak viscosities and viscosities at 95°C decreased from 310 to 230 A.U. and 120 A.U. to base level, respectively, for the product of over 542 μm and that of throughs of 175 μm particle size.

Carbohydrate changes. Amylose content was decreased slightly with the increase in fineness from 26.2 to 25.6%. The extent of cooking as indicated by the damaged starch determination was affected greatly by the particle size of the raw material (Table 3). The DS increased from 36.1% for the product of 542 μm to 55.1% for that of the throughs 175 μm particle size (Table 3).

The reducing and non-reducing sugars were affected to a much lesser extent with a negligible increase. Water-soluble proteins decreased from 0.69% to 0.33% with the increase in fineness. However, the rate of decrease was higher in products of 542–286.5 μm particle size of the rice.

The increase in expansion ratio, WAI and WSI and the decrease in density, breaking strength, paste peak viscosity, viscosity at 95°C and water-soluble proteins with the decrease in particle size may be attributed to the greater heat penetration into the finer particles as compared with the coarser fractions as indicated by the damaged starch values. Mottern *et al.* (1969) reported a similar effect of different forms of rice on the characteristics of the extruded products.

ERH, moisture and breaking strength relationship. The results of equilibrium moisture contents corresponding to different ERHs and breaking strengths are summarized in Table 4. An absorption isotherm was developed by plotting equilibrium moisture contents against corresponding ERH. The effect of ERH on the breaking strength corresponding equilibrium moistures is shown in Fig. 1. As the ERH increased, the equilibrium moisture and breaking strength increased. However, the increase in moisture content above 52% ERH was steady. The breaking strength increased from 7.5 to 11.5 kg as the ERH increased from 10 to 43%. There was a further steep increase up to 18.1 kg at 52% ERH and a maximum of 20.2 kg at 67% ERH. Since there was loss of crispness at 52% ERH, the critical ERH for acceptable textural properties of product

was 43% as supported by the breaking strength data. The product became susceptible to microbial growth above 80% ERH. Thus, it is concluded that the critical ERH value of 43% obtained by organoleptic evaluation of the equilibrated moist samples corresponded to a breaking strength of 11.5 kg and moisture content of 6.56%.

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(Received 28 February 1984)

Journal of Food Technology (1985) **20**, 311–318

Control of a range of food related microorganisms by a multi-parameter preservation system

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Summary

Seventy-two process variables, based on a matrix of a_w, nature of controlling solute, pH, and the addition of sodium citrate and sodium benzoate were examined with a view to devising a food preservation system for ambient temperatures. Growth of a challenge 'cocktail' of *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Lactobacillus casei* and *Clostridium perfringens*, was assessed in a Brain Heart Infusion broth based model system. Each of the controlling factors had an effect on growth. The addition of sodium citrate (2% w/v) and sodium benzoate (2000 mg/l) enhanced the inhibitory effect. Reduction in a_w (1.00–0.94) and pH (7.0–5.5) in combination or as separate controlling factors increased any inhibition. Combinations of sodium chloride and glycerol were used as the controlling solute, and increasing the proportion of sodium chloride resulted in suppressed growth, irrespective of any change in a_w or pH. Combinations of sodium citrate and sodium benzoate with a pH less than 6.0 and an a_w less than 0.95 inhibited any growth during a 42 day incubation period at 37°C, irrespective of the controlling solutes studied.

Introduction

Chemical preservatives are used in a range of foodstuffs, and under appropriate conditions maintain keeping quality and control the growth of food poisoning and spoilage organisms (Jarvis & Burke, 1976). The use of these compounds is limited by their relative toxicities (Tilbury, 1980) and concomitant international legislation (Burke, 1980). Their effectiveness is dependent on their antimicrobial spectrum, stability in the food, solubility, partition coefficient and dissociation constant (Jarvis & Burke, 1976). The effectiveness of a preservative depends not only on its intrinsic properties, but also on its environment, such as the pH, water activity (a_w) and fat content of the food.

Humectants reduce the a_w of food and hence the microbial growth. The development of primarily humectant preserved (intermediate moisture) foods (Kaplow, 1970) has only found successful commercial application in pet foods (Brockman, 1973) as the concentration of humectant required to extend shelf life appreciably causes organoleptic or toxicological problems.

Interactions between chemical preservatives and pH (Roberts & Ingram, 1973) and chemical preservatives, pH, storage temperature and heating (Roberts, Gibson & Robinson, 1981) have demonstrated the potential of combining several factors in the preservation of cured meat products. Leistner & Rodel (1978) considered combining

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several sub-optimal parameters or 'hurdles' to control microbial growth. Few authors have reported on the microbial growth inhibition of humectant preservative combinations and have limited their data to a few species notably *Staphylococcus aureus* (Robach & Stateler, 1980; Fox & Loncin, 1982). The current study considers a range of food borne microorganisms commonly found in foods.

Preliminary studies with a range of different humectants and preservatives indicated that sodium citrate and sodium benzoate in combination with reduced a_w is a promising preservation system. Little or no information is available concerning the effect of the combination of reduced pH, reduced a_w and the use of citrate/benzoate as a food preservation system. Warren (1976) stated that a concentration of 0.001% undissociated citric acid inhibits the anaerobic growth of *Staph. aureus*. Restiano, Komatsu & Syracuse (1981) showed that citric acid was effective in increasing the inhibitory properties of 0.1% sorbic acid at pH 7 against a range of food related microorganisms. Huhtanen *et al.* (1983) demonstrated that citric acid enhanced the inhibitory properties of potassium sorbate against *Clostridium botulinum*. Leistner, Rodel & Krispien (1981) suggested that citrate could perhaps be used as one hurdle in a series for preserving shelf stable meat products. Webster & Cooke (1984) showed that a combination of sodium citrate and sodium benzoate could be used to inhibit the growth of *Staph. aureus* over a 3 day incubation period.

Recent work (Christian, 1981) has indicated that the nature of the controlling solute plays an important role in the antimicrobial effect of reduced a_w. This was investigated in the present work with regard to the use of different combinations of sodium chloride and glycerol. The effect of sodium citrate and sodium benzoate in combination with an organoleptically acceptable reduction in a_w and pH, on a range of common food spoilage and poisoning organisms is reported.

Materials and methods

Model system test solutions were prepared using a brain heart infusion broth base (BHI), (Lab M, Salford). A stock solution containing both tri-sodium citrate (Fisons, Loughborough) and sodium benzoate (BDH, Poole) was prepared by dissolving the chemicals in sterile BHI. This solution was then filter sterilized (Falcon Bottle Top Filter 0.45 m^{-6}, Becton Dickinson).

The water activity of the test solutions was adjusted by the addition of solutes, either glycerol (Fisons) or a combination of glycerol and sodium chloride (Fisons).

a_w and pH measurements

Water activity measurements were made at 25°C using an electronic hygrometer (Rotronic hydroskop D.T.). The accuracy of measurement was ±0.02 units. The initial pH of the test solution was adjusted using sodium hydroxide (Volucon, N/1, May & Baker) and hydrochloric acid (Volucon, N/1) and measurements were made using a pH meter (E.I.L.7010).

The citrate/benzoate stock solution was prepared to give a concentration in the growth medium of 2 and 0.2% respectively. Solutes were added to this stock solution at concentrations previously determined to give specific a_w values in the final solution. The initial pH of these stock solutions was then adjusted. Each solution was made to volume and filter sterilized (Gelman Acrodisc 0.45 m^{-6}) into sterile colorimeter tubes

(Corning), each containing a teflon coated stirrer bar. All test solutions were incubated for 24 hr at 37°C to check for sterility.

Inoculum

Stock cultures of *Staphylococcus aureus* (Bergdoll, MF31), *Bacillus subtilis* (NCTC 3610), *Pseudomonas aeruginosa* (NCTC 10332), *Streptococcus faecalis* (NCTC 775), *Lactobacillus casei* var. *rhamnosus* (NCTC 10302), and *Clostridium perfringens* (NCTC 8237) were maintained on BHI agar slopes at 4–5°C. Cultures of each organism were grown for 18 hr at 37°C in BHI broth and viable counts carried out. Approximately 2 ml of each culture was mixed and a 1:10 dilution prepared. 0.1 ml diluted mixture was used to inoculate each test solution to give a concentration of 10^5–10^6 microorganisms/ml. The inoculum was chosen to present a challenge to the preservation system and yet still be of a level found in meat products.

Experimental design

The experimental design is shown in Table 1. The four different a_w conditions (0.93–0.96) were obtained using four different combinations of sodium chloride and glycerol (Table 2) at four different pH values (5.5–7.0). In addition four pH treatments

Table 1. General experimental matrix

a_w 0.93	0.94	0.95	0.96	1.00		
*	*	*	*	*	5.5	pH
*	*	*	*	*	6.0	
*	*	*	*	*	6.5	
*	*	*	*	*	7.0	

Table 2. a_w controlling solutes

Experimental matrix no.	a_w	Controlling solutes
1	0.93	34% glycerol
	0.94	32%
	0.95	25.5%
	0.96	21.7%
2	0.93	24% glycerol and 4% NaCl
	0.94	21%
	0.95	16%
	0.96	12%
3	0.93	20% glycerol and 6% NaCl
	0.94	16%
	0.95	11%
	0.96	8%
4	0.93	16% glycerol and 8% NaCl
	0.94	11%
	0.95	8%
	0.96	5%

were investigated at a_w = 1 giving sixty-eight treatments *in toto*. All treatments contained sodium citrate (2%) and sodium benzoate (0.2%). In addition those combinations with a_w 0.95 and pH 6.0 were investigated in the absence of citrate/benzoate. Duplicate tubes for each test solution were prepared.

Incubation conditions

Test solutions were incubated at 37°C in a carousel fixed to a rotating motor. The carousel rotated over a magnetic stirrer at a speed of 1 rpm. Each test solution was therefore vortex stirred for 20 sec at intervals of 1 min. Preliminary experiments showed that evaporation of the test solutions was limited by saturating the atmosphere of the incubator with water.

Detection of growth

Growth was assessed using an absorptiometer (Corning), adjusted to zero against an uninoculated test solution. Visible growth was defined as having occurred when the values of percentage transmission decreased below 90%. This corresponds to an increase in viable numbers of cells of 10^7–10^8/ml. After incubation, final a_w and pH values were determined, and any dominant organisms identified.

Results

In a preliminary study (Webster & Cooke, 1984) sodium citrate (2% w/v) and sodium benzoate (2000 mg per l) were shown to have a marked inhibitory effect on the growth of *Staph. aureus*. The combination, although producing an a_w depression of only 0.01, at pH 5.5, 6.0 and 6.5 extended the lag phase from less than 1 hr to 3 days.

The effects of reduced pH, reduced a_w, nature of the controlling solute, and the presence or absence of sodium citrate and sodium benzoate, on the mixed inoculum, are summarized in Fig. 1. Each of the controlling parameters had an inhibitory effect on growth. Decreasing the pH, decreasing the a_w and increasing the proportion of sodium chloride as the controlling solute all increased the length of time to show growth. In the absence of sodium citrate and sodium benzoate the inhibitory effect was greatly reduced.

At a_w 1.0 (no humectants) growth occurred at all pH values; in less than 1 day at pH 7.0, 6.5 and 6.0 and at 2 days at pH 5.5. Changes in pH occurred with growth, and at pH 7.0, 6.5 and 6.0 the final pH was 9.15, 9.2 and 7.7 respectively. In the case of an initial pH 5.5 the final measurement was pH 5.6. No change in a_w occurred at any pH.

At fixed pH, decreasing the a_w increased the inhibitory effect e.g. at pH 7.0, using glycerol (A in Fig. 1) growth occurred in less than 1 day at a_w 1.0 and 0.96, 3 days at a_w 0.95 and no growth occurred during the 42 day experimental period at a_w 0.94 and 0.93.

Increasing the proportion of sodium chloride as the controlling solute (A–D Fig. 1) increased the inhibitory effect at all a_w values less than 1.0. At an a_w value of 0.96 and pH 7.0 growth occurred in less than 1 day using glycerol as the controlling solute, 2 days using glycerol plus 4% sodium chloride, 7 days using glycerol plus 6% sodium chloride and did not occur after 42 days using glycerol plus 8% sodium chloride.

Irrespective of controlling solute, at a_w less than 1.0, resultant growth was primarily *Strep. faecalis* in the presence of citrate/benzoate and *Staph. aureus* in the absence of citrate/benzoate. Both organisms caused a reduction in final pH and no change in a_w. After 36 hr at a_w 1.0 and pH 7.0 (control) the flora was mixed with all species

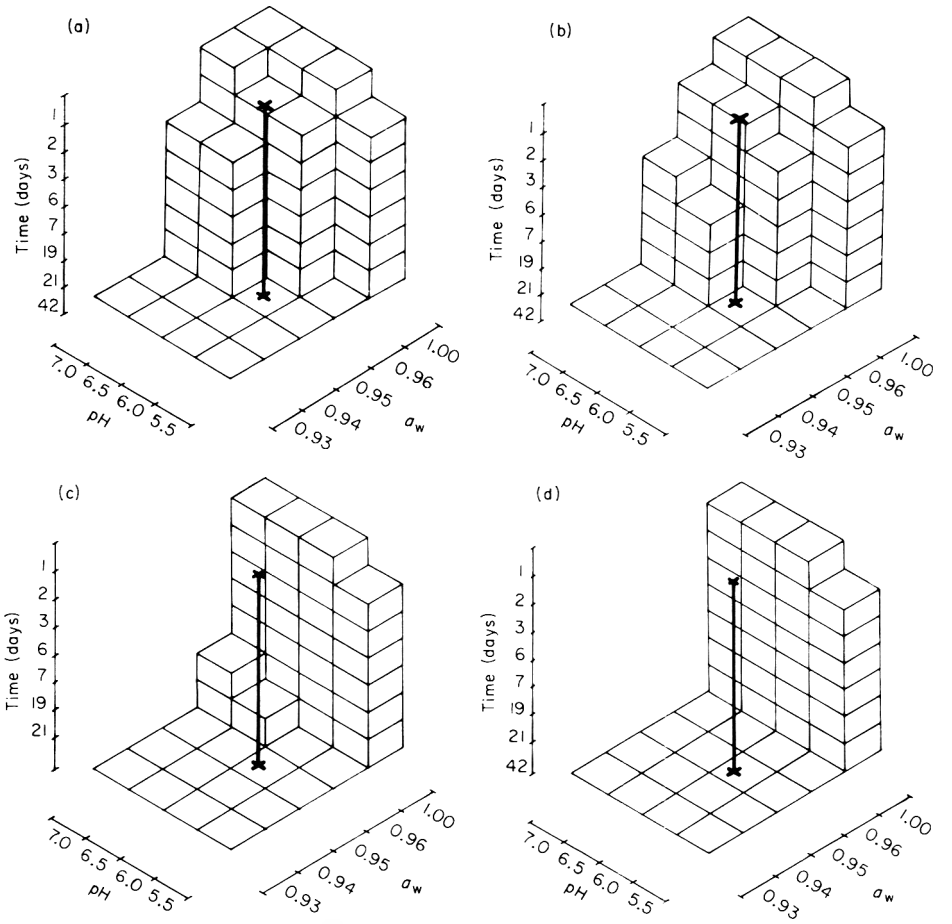

Figure 1. The effects of pH, a_w and the nature of the controlling solute, with and without sodium citrate and sodium benzoate, on the growth of a challenge cocktail of *Staph. aureus*, *B. subtilis*, *P. aeruginosa*, *Strep. faecalis*, *L. casei* var. *rhamnosus*, and *C. perfringens*. Controlling solutes: A, glycerol; B, glycerol + 4% NaCl; C, glycerol + 6% NaCl; D, glycerol + 8% NaCl. | , without sodium citrate and sodium benzoate.

represented, with a slight predominance of *P. aeruginosa*, *Strep. faecalis* and *C. perfringens*, which caused an increase in the pH but no change in a_w.

Discussion

The relatively drought resistant bacterium *Staph. aureus* is an indicator pathogen in reduced a_w products (Christian, 1980). An earlier study (Webster & Cooke, 1984) showed that a preservation couple of citrate/benzoate was bacteriostatic for *Staph. aureus* at a_w values of up to 0.99 at pH 6.5. Combining citrate/benzoate and a_w 0.94 at pH 7.0 (Fig. 1) inhibited the growth of a range of bacteria including *Staph. aureus* over an incubation period of 42 days, whilst reduced a_w alone did not.

Reduced pH and a_w alone have been shown to be relatively inefficient means of *Staph. aureus* control. Notermans & Heuvelman (1983) demonstrated *Staph. aureus*

growth at pH 5.2 and a_w 0.9. Fox & Loncin (1982) showed that growth of a range of organisms including *Staph. aureus*, *Streptococcus*, *Bacillus* and *Clostridium* occurred at a_w greater than 0.93 in combination with pH as low as 4.5. They found at the low a_w values the predominant organism was *Staph. aureus* with occasional colonies of *Bacillus* and *Streptococcus* at higher a_w values.

The present study showed that at reduced a_w alone growth was predominately *Staph. aureus*. Where growth occurred at reduced a_w plus citrate/benzoate it was predominantly *Streptococcus*. This indicates that in the absence of citrate/benzoate *Staph. aureus* is able to grow and competes favourably with the other less drought resistant organisms used in the inoculum. Once *Staph. aureus* is inhibited *Streptococcus* competes favourably and becomes the predominant organism. This agrees with the findings of Haas & Herman (1978) who showed staphylococci, streptococci and lactobacilli to be the most resistant bacteria in IM pet foods.

The antimicrobial activity of organic acids such as citric and benzoic has been thought to be due primarily to the undissociated acid (Rahn & Conn, 1940; Ingram, Ottoway & Coppock, 1956), and that the primary mode of action is acidification of the cell interior after passage of the undissociated molecule through the cell membrane (Baird-Parker, 1980). This is thought to reduce membrane potentials and inhibit substrate uptake (Freese, Chingju & Galliers, 1973). Reducing the pH increases the proportion of undissociated acid. Citric acid has a pKa value of 3.1 and sodium benzoate 4.2, and accordingly they should have little or no effect at neutrality. More recently Eklund (1983) showed that at pH values greater than 6 the dissociated acid accounted for more than 50% of the inhibition of microorganisms including *B. subtilis*, *E. coli*, *P. aeruginosa* and *Staph. aureus*. This may explain the observed bacteriostatic effect of citrate/benzoate even at pH 7, when combined with the additional load of sub-lethal reduction in a_w.

The nature of the a_w controlling solute has a significant effect on the inhibition of the challenge organisms. Increasing the proportion of sodium chloride as the controlling solute increases the length of time to show growth at all reduced a_w values and pH values tested (Fig. 1). In a study on sixteen species of non-halophilic bacteria, Marshall, Ohye & Christian (1971) showed that rods were more sensitive to sodium chloride than to glycerol and cocci were predominantly more sensitive to glycerol than to salt. Sperber (1983) showed that bacteria in general (the review did not include *Staph. aureus*) grow at lower a_w when adjusted with glycerol than with sodium chloride, potassium chloride, glucose or sucrose. The nature of the controlling solute has also been shown to affect spore germination (Jakobsen, Filtenborg & Bramsnaes, 1972). In general ionic solutes (e.g. sodium and potassium chloride) are more inhibitory than low molecular weight solutes such as glycerol and ethylene glycol.

It appears that inhibition at reduced a_w may be lower for permeating solutes such as glycerol than for relatively impermeable solutes such as sodium chloride. It is thought that non-halophilic cells are impermeable to sodium chloride and as a concentration gradient exists across the cytoplasmic membrane, the organism accumulates osmotically active compounds such as proline, glutamic acid and alpha-aminobutyric acid within the cell to reduce plasmolysis (Measures, 1975). Christian & Hall (1972) showed that this response could occur in the absence of protein synthesis provided that the appropriate solutes or solute precursors were present.

This could explain why increasing the proportion of sodium chloride increases inhibition (Fig. 1). The mechanisms for producing or accumulating osmotically active compounds are perhaps not necessary when compatible solutes are used. They are

therefore less inhibitory than impermeable solutes when the mechanisms are inhibited, perhaps by the presence of organic acids.

The use of a single preservation parameter for foods (physical or chemical) is not suitable in many cases, because of adverse effects on organoleptic quality, cost or toxicity. This promotes the application of the 'hurdle' concept (Leistner & Rodel, 1978). This paper presents data in model systems indicating that citrate/benzoate at slightly reduced a_w is a promising food preservation system. Current studies are proceeding in comminuted meat formulations.

Acknowledgments

The authors wish to thank Dr D. A. Ledward (Food Science section) for helpful discussion, and the Tropical Development and Research Institute for funding this research programme. *Staphylococcus* strain MF31 was kindly supplied by Professor S. Martin, University of Illinois, U.S.A.

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(Received 1 May 1984)

Journal of Food Technology (1985) **20**, 319–330

Protein production by *Schwanniomyces castellii* on starchy substrates, in liquid and solid cultivation

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Summary

In a preliminary screening, several yeast species were selected among which *Endomycopsis fibuligera* and *Schwanniomyces castellii* showed a high yield when inoculated in potato or potato peel media. Both these species were found to have an elevated protein percentage and a good amino acid composition. The effect of some cultural conditions on SCP (single cell protein) production by *S. castellii* grown on potato medium in a pilot plant 90 l fermenter was also investigated. Optimal conditions resulted in employing a substrate containing 80 g/l of potatoes, 5.0 g/l of cornsteep liquor, at 28°C, with a 3% inoculum.

S. castellii and *E. fibuligera* were also separately inoculated in solid and semi-solid substrates (potatoes, cassava flour, stale bread, maize flour). Under laboratory and semi-pilot plant conditions, carbohydrate conversion into protein was more effective on potato and cassava flour media.

Introduction

Many processes have been described for converting different forms of starchy substrates into single cell protein (SCP). First studies on biomass production from starch agricultural or industrial wastes and waste waters involved a preliminary hydrolysis stage of the substrate by chemical (Tong, Riel & Simard, 1973), enzymic (Schierbaum, Richter & Nordheim, 1968; Moreton, 1978; Musénge, Anderson & Holdom, 1982) or microbial means (Jarl, 1969; Lemmel, Heimsch & Edwards, 1979).

To avoid hydrolysis costs, some investigators have attempted to select microbial strains for the direct production of SCP on starch.

Among the amylolytic yeasts, the genera *Lypomyces, Schwanniomyces* and *Endomycopsis* have received major interest, which present a high yield on non-hydrolysed starch (Spencer-Martins & van Uden, 1977; Sá Correia & van Uden, 1981; Touzi *et al.*, 1982; Wilson, Khachatourians & Ingledew, 1982) and synthetize strong amylolytic enzymes (Moulin, Oteng Gyang & Galzy, 1979; Clementi *et al.*, 1980a; Oteng Gyang, Moulin & Galzy, 1980, 1981; Moranelli *et al.*, 1981; Wilson & Ingledew, 1982; Dhawale & Ingledew, 1983).

The purpose of this paper is to summarize all the results obtained in several of our studies on SCP production by yeasts grown on starchy substrates (Costamagna, Clementi & Rossi, 1978; Clementi, Rossi & Tuttobello, 1980b; Clementi *et al.*, 1980c; Rossi & Clementi, 1980; Clementi & Rossi, 1982).

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A preliminary screening has been conducted on sixty strains of Eumycetes, belonging to several species and genera. Selected yeasts *Schwanniomyces castellii* and *Endomycopsis fibuligera* were employed for biomass production in liquid, semi-solid and solid cultures, on various starchy substrates under laboratory and pilot plant conditions.

Materials and methods

Yeast strains

The yeasts employed in the preliminary screening belonged to the following genera and species (Lodder, 1970): *Hansenula (H. capsulata, H. anomala,* var. *anomala, H. subpelliculosa); Debaryomyces (D. cantarellii, D. castellii, D. hansenii, D. phaffii, D. marama); Trichosporon (T. cutaneum); Candida (C. humicola, C. intermedia, C. tropicalis, C. utilis); Torulopsis (T. colliculosa, T. molischiana, T. ernobii); Endomycopsis (E. fibuligera); Saccharomyces (S. diastaticus); Schwanniomyces (S. castellii); Rhodotorula (R. rubra, R. glutinis); Pichia (P. polymorpha).* All the strains, obtained from the I.M.A.T. collection, were maintained on Malt-agar Difco at +5°C.

Basal media composition; liquid media

Starch-YNB medium: 13.4 g/l Yeast Nitrogen Base Difco (YNB) and 20 g/l soluble starch BBL, pH 5.5; potato and potato peel-YE media: 100 g/l of peeled, broken potatoes or the same quantity of washed potato peels plus 5 g/l $(NH_4)_2HPO_4$, 5.0 g/l yeast extract (YE), 0.1 g/l AF Sogesil antifoaming, pH 5.5; potato and potato peel-CSL media: prepared as the preceding substrates, except that cornsteep liquor (CSL) was added instead of YE; and potato medium: 80 g/l of peeled, broken potatoes, boiled per 30 min and cloth filtered. plus 5 g/l $(NH_4)H_2PO_4$, 1 g/l $(NH_4)_2HPO_4$, 0.6 g/l antifoaming, pH 5.0–5.6. All the liquid media were autoclaved at 121°C for 20 min.

Semi-solid and solid media

Potato media: 400 or 600 g/l of peeled, broken potatoes, boiled per 30 min and cloth filtered, pH 5.2–5.9; cassava flour (tapioca) media: 200 or 400 g/l of tapioca, pH 5.2–5.9; stale bread media: 300 or 400 g/l of stale bread crumbs, pH 5.2–5.9; and maize flour media: 200 or 400 g/l of commercial maize flour, pH 5.2–5.9. Unless otherwise specified, solid and semi-solid substrates were mixed with 10 g/l cornsteep liquor (CSL), 20 g/l $(NH_4)_2SO_4$, then autoclaved at 121°C per 35 min.

Cultivations

Liquid culture conditions: strain selection was carried out on starch-YNB medium. The substrates, contained in Erlenmeyer conical flasks, filled to 1/5 of their capacity, were inoculated at 5%, with a 24 hr preculture on starch-YNB, and incubated at 28°C for 36 hr, with rotatory shaking (120 rpm). SCP production on the laboratory scale was carried out on starch-YNB medium and potato and potato peel media, in the same conditions described for strain selection. SCP production on the pilot scale was performed on potato medium, in 90 l capacity steel fermenters (ratio h/r = 2.5), with temperature regulation (unless otherwise noted) at 30°C. Agitation was set at 700 rpm by open vortex, aeration was 50 l/min. The substrate was inoculated at 3%, with a preculture on the same medium (modified by adding 0.5% CSL and 1% glucose). Semi-solid and solid culture conditions: laboratory scale fermentations were carried out on potato media under the same conditions described for liquid cultivations.

Semi-pilot scale fermentations were made on potato, cassava flour, stale bread and

maize flour media. The sterilized substrates were put into a bread making blender with a capacity of 20 kg (with rotating tank; 15 rpm, and spiral blender: 180 rpm). After pH adjustment to 4.5 with concentrated HCl, the media were inoculated at 5% with 24 hr preculture on starch-YNB, and maintained at 25°C for 48 hr without aseptical caution.

Analytical method

Duplicate samples were collected at intervals during cultivations in the different conditions tested. The cells, harvested by centrifugation at 1500×*g* and washed three times with twice-distilled water were employed for the following determinations:

(i) Dry matter: by weighing, after drying at 100°C to constant weight.

(ii) Total protein content: by the method of Lowry *et al.* (1951).

(iii) Crude protein content: by the semi-micro Kjeldahl method, multiplying total *N* value by 6.25.

(iv) Nucleic acid content: sample delipidation and nucleic acid extraction were carried out as described by Schneider (1945). Deoxyribonucleic acid was detected by diphenylamine reaction (using calf thymus DNA as a standard) (Dische, 1930) and ribonucleic acid by the orcinol reactions, using yeast RNA as a standard, after correcting experimental values for DNA interference (Mejbaum, 1939).

(v) Amino acid analysis: after acid hydrolysis (6 N HCl at 120°C per 24 hr), freeze dried cells were analysed by ion exchange chromatography as described by Spackman, Stein & Moore (1958), using an automatic analyser. Tryptophane was determined on separate samples after alkaline hydrolysis using the method of Knox *et al.* (1960). Cysteine and methionine were determined on performic acid oxidized samples (Moore, 1953).

(vi) Starch hydrolysis: the supernatants obtained by centrifugation as described above were analysed for residual starch content by measuring free and total reducing carbohydrates using the *o*-toluidine method (Boehringer-Mannheim), either directly or after incubation in the presence of amyloglucosidase from *Aspergillus* (BDH), for 60 min at 55°C (Costamagna, Clementi & Rossi, 1980).

(vii) Amylolytic activity: the glucose-producing activity of the supernatants was examined on starch, after incubation at pH 5.5 and 48°C, for 90 min (Clementi *et al.*, 1980a). One unit of amylolytic activity was defined as the quantity of enzyme which liberates 1 mg of glucose from the starch (8 mg) added in the test tube. There were at least four replicates.

Scanning electron microscope observations

Biomass production on different semi-solid and solid substrates was also controlled by a Philips scanning electron microscope (SEM). Observation and micrographs were made on samples collected during the fermentations, at two different magnifications (×15 and ×937) to observe the substrate structure modifications and the biomass growth, respectively.

Results and discussion

SCP production in liquid culture

Strain selection: yeast growth on YNB-starch medium resulted as a function of genus, species and strain (Costamagna, Clementi & Rossi, 1978). The highest values of biomass yields were obtained for the species *H. capsulata, H. anomala* var. *anomala, H. subpelliculosa, D. cantarellii, D. castellii, E. fibuligera, S. castellii.*

Laboratory scale cultivation. When inoculated in potato or potato peel media, only

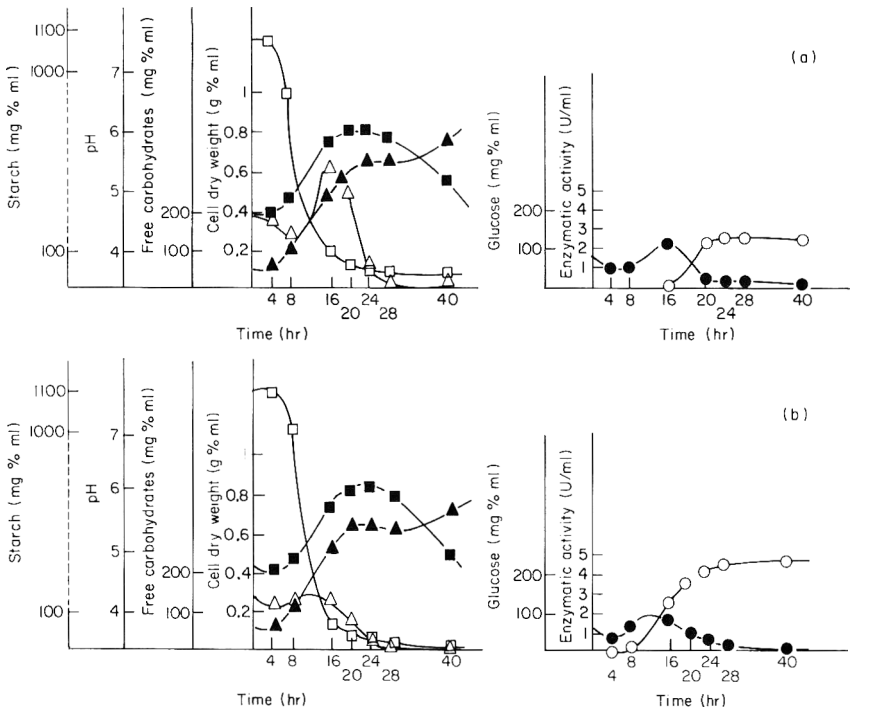

Figure 1. *S. castellii* growth and enzymatic activity on potato (a) and potato peel (b) -YE media, under laboratory conditions. pH (■ ■); cell dry weight (▲ ▲); residual starch (□ □); total soluble carbohydrates (△ △); free glucose (● ●); and amylolytic units (O O).

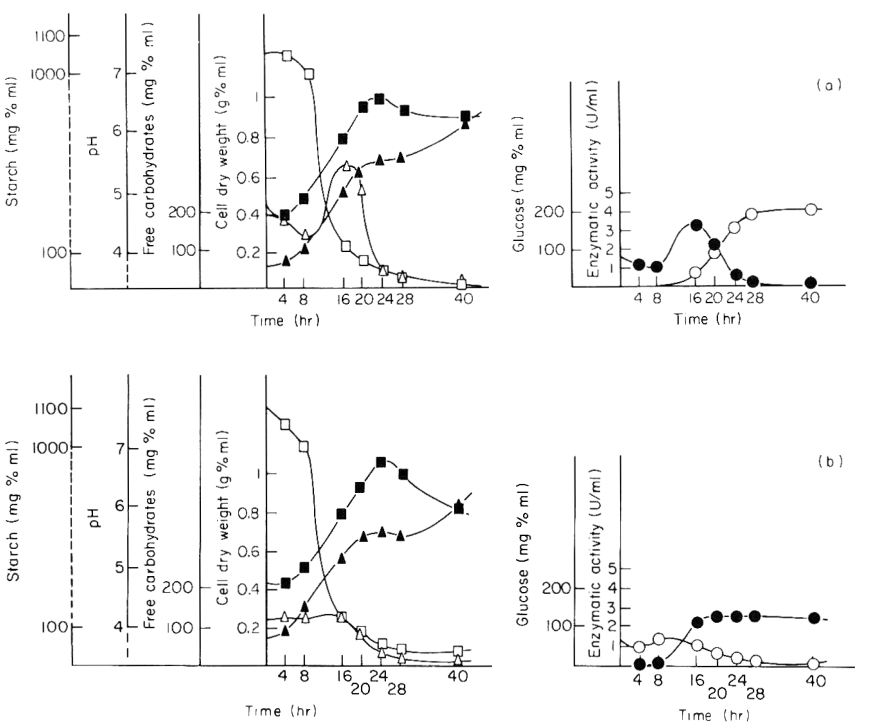

Figure 2. *S. Castellii* growth and enzymatic activity on potato (a) and potato peel (b) -CSL media, under laboratory conditions. pH (■ ■); cell dry weight (▲ ▲); residual starch (□ □); total soluble carbohydrates (△ △); free glucose (● ●); and amylolytic units (O O).

Table 1. Biomass composition and yields of *S. castellii* and *E. fibuligera* grown on different starchy substrates*

Substrates									
Starch-YNB			Potato-YE			Potato peel-YE			
S. castellii		*E. fibuligera*	*S. castellii*		*E. fibuligera*	*S. castellii*		*E. fibuligera*	
3754	2196	3812	3754	2196	3812	3754	2196	3812	
30.23	37.23	26.27	40.55	38.49	34.20	31.83	33.60	30.20	Total protein (g% DM)
—	—	—	48.75	—	40.19	—	—	—	Crude protein ($N \times 6.25$)
8.11 ±0.47	7.91 ±0.51	7.16 ±0.49	8.20 ±0.24	7.94 ±0.25	6.11 ±0.55	—	—	—	Total nucleic acids (% DM)
1538	1494	1168	3.13	3.12	31.57	0	1.44	28.84	Residual starch (as glucose mg% ml)
14.03	11.14	13.92	38.59	37.88	39.12	51.40	53.76	36.84	Yield†
44.19	36.72	38.36	38.70	37.99	40.37	51.82	54.41	38.83	Yield‡

*Original starch concentration in the media (as glucose mg% ml): starch-YNB = 1992; potato-YE = 935.04; potato peels-YE = 687.82.

†Yield = g of dry weight/g of initial soluble starch.

‡Yield = g of dry weight/g of consumed soluble starch.

the *E. fibuligera* strain 3812 and the *S. castellii* strains 3754 and 2196, showed a high growth rate. Figures 1 and 2 shed some light on *S. castellii* growth and amylolytic activity on potato and potato peel-YE or CSL media. The use of cornsteep liquor which is a very economic byproduct (Foda *et al.*, 1973), instead of the more expensive yeast extract, did not affect *S. castellii* growth and substrate utilization.

In Table 1 it may be seen that these strains present elevated values for yield and protein content, particularly *S. castellii* which also presented a carbohydrate conversion rate higher than *E. fibuligera*.

In Table 2 the detailed amino acid composition of *S. castellii* and *E. fibuligera* protein is compared with those of *Kluyveromyces fragilis*, 'Bel' yeast (Delaney, Kennedy & Walley, 1975) and FAO Standard (whole egg) (FAO/WHO, 1965). The comparison of the essential amino acids revealed, for both species examined, experimental values like those reported for the other yeasts. FAO reference protein presents higher values for several essential amino acids; however the only significant limitation for *S. castellii* and *E. fibuligera* biomass would appear to be in the methionine and cysteine content, as well as for *Candida utilis* and all the other yeast SCP (Delaney *et al.*, 1975).

Table 2. Amino acid composition of *S. castellii* and *E. fibuligera* biomass (grown on potato-YE medium), compared with reference proteins

Amino acids	*S. castellii* 3754*	*E. fibuligera* 3812*	*K. fragilis*§	'Bel' yeast§	Whole egg FAO ref§
Thr	4.21±0.21	3.58±0.38	5.3	5.9	5.1
Val	5.69±0.37	6.48±0.38	5.6	5.3	7.3
Iso-leu	4.75±0.11	4.51±0.30	4.8	4.5	6.6
Leu	7.00±0.08	8.48±0.18	8.1	7.4	8.8
Tyr	1.49±0.08	0.90±0.12	3.9	3.6	4.2
Phe	5.51±0.31	6.94±0.40	4.2	3.8	5.8
Lys	9.37±0.30	7.41±0.44	8.0	7.3	6.4
Trp†	1.19±0.04	1.23±0.26	1.7	1.4	1.6
Met‡	0.99±0.05	1.37±0.19	1.5	1.2	3.1
Cys‡	1.82±0.07	2.95±0.44	1.7	1.0	2.4
Asp	8.87±0.23	9.59±0.39	9.4	9.1	—
Ser	4.63±0.14	4.62±0.35	4.7	5.4	—
Pro	4.31±0.18	4.35±0.34	4.2	4.2	—
Glu	18.19±0.39	11.80±0.41	13.8	14.7	—
Gly	4.81±0.14	4.66±0.46	3.7	4.1	—
Ala	8.36±0.18	9.54±0.51	5.8	5.5	—
Arg	5.34±0.16	4.23±0.32	4.9	4.8	—
His	1.71±0.08	1.95±0.15	2.0	1.9	—
Total AA	98.28	94.74	93.3	91.1	—

*Mean of three separate hydrolysate.
†Separately determined by colorimetric analysis.
‡From performic acid-oxidized samples.
§(Delaney *et al.*, 1975).

Pilot scale cultivation. The influence of potato concentration in the substrate was examined by testing two different potato-CSL media containing 200 and 100 g/l of potatoes, in 90 l fermenters (Fig. 3). The use of a lower potato concentration greatly improved operative conditions, without affecting cell protein production (Clementi *et*

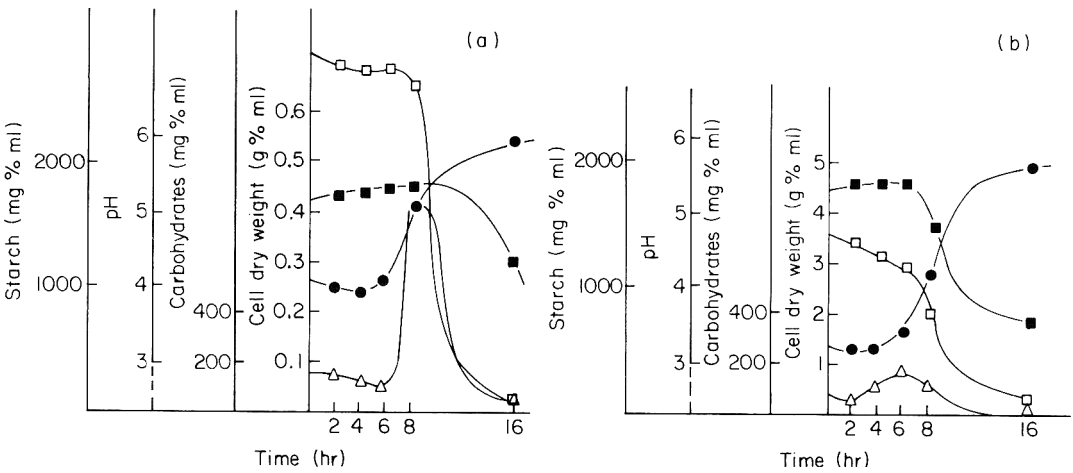

Figure 3. *S. castellii* growth on 20% (a) and 10% (b) potato-CSL media, in a pilot scale 90 l fermenter. Cell protein (● ●); pH (■ ■); residual starch (□ □); and total soluble carbohydrates (△ △).

al., 1980b). Therefore optimal condition was defined at 80 g/l concentration which was subsequently employed in all the pilot scale fermentations.

The effect of different cultural conditions (substrate composition, temperature, inoculum rate) on the growth of *S. castellii* 3754 on potato media was also tested (Clementi *et al.*, 1980c). The addition of organic N_2 sources to the basal medium greatly improved the growth: in the presence of 0.3% YE and 0.5% CSL, 8.16 and 8.07 g/l of cell dry matter were obtained respectively, much higher than the dry matter obtained on the basal medium (5.83 g/l). The biomass from basal medium also presented a protein content of 38.3 g% dry matter (DM), less than those from the integrated substrates (from 48.2 to 49.5 g% DM). On the other hand, no growth improvement was obtained by increasing the CSL amount added to the substrates (from 0.5 to 1.5%).

Temperature effect on growth was assayed in a range between 22.5 and 37.5°C. In the presence of the highest value tested, the growth was strongly inhibited, while only small differences were determined between 22.5 and 34°C (Fig. 4). The optimum temperature was defined around 30°C; however, a wide range of temperature values may be considered suitable for *S. castellii* growth (Fig. 4).

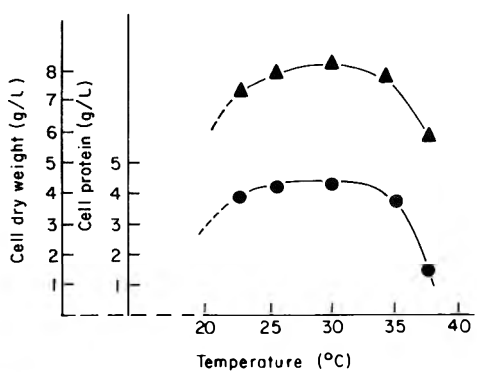

Figure 4. *S. castellii* growth at different temperatures, on potato-CSL medium, in pilot scale 90 l fermenter. Maximum cell protein (● ●); and dry weight (▲ ▲).

The influence of the inoculum concentration on biomass production was also examined, by inoculating different inoculum amounts (from 0.7 to 18%). Growth and carbohydrate conversion were not strongly affected by inoculum percentage: the only significant difference was observed in the presence of the highest or the lowest inoculum concentrations tested. Optimal condition was determined at 3% inoculum which ensured the maximum growth rate (9.87 g/l of cell dry matter) (Fig. 5).

SCP production in semi-solid and solid culture

Solid state fermentation of starchy substrates had been obtained by fungal growth, employing *Aspergillus niger* (Senez, 1978; Raimbault & Alazard, 1980). The growth of *S. castellii* and *E. fibuligera* on various semi-solid and solid starchy materials has been investigated either in flasks or on a semi-pilot scale (Rossi & Clementi, 1980; Clementi & Rossi, 1982).

Laboratory scale cultivations were carried out with the two species *S. castellii* 3754 and *E. fibuligera* 3812, separately grown on different potato media (Table 3). When basal medium was integrated with different concentrations of salts and nitrogen sources, for both yeast species, growth and starch utilization were affected by substrate composition (Table 4). From semi-solid substrates (potatoes 400 g/l), final products were obtained with a protein content higher than that from solid substrates (potatoes 600 g/l) (Table 4). In particular, the species *E. fibuligera,* grown on solid potato media was able to convert only 50% of the available carbohydrates into protein. This may be due to a poor oxygen availability in the substrate, because substrate aeration was obtained only through the spiral blender mixing.

Table 3. Semi-solid and solid substrate composition (g/l)

Components	Substrates							
	a	b	c	d	e	f	g	h
Potatoes	400.0	400.0	400.0	400.0	600.0	600.0	600.0	600.0
Yeast extract (YE)	—	0.5	5.0	—	—	0.5	5.0	—
Cornsteep liquor (CSL)	—	—	—	10.0	—	—	—	10.0
KH_2PO_4	—	2.0	10.0	2.0	—	2.0	10.0	2.0
K_2HPO_4	—	2.0	10.0	2.0	—	2.0	10.0	2.0
$(NH_4)_2SO_4$	—	2.0	15.0	2.0	—	2.0	15.0	2.0

Table 4. Protein enrichment of semi-solid and solid potato substrates, by *S. castellii* and *E. fibuligera* fermentation

	Total protein g% DM (dry material)		
		Final products	
Substrates	Initial composition	*S. castellii*	*E. fibuligera*
a	4.55	20.51	19.90
b	6.28	26.13	20.35
c	5.58	28.58	25.09
e	4.56	19.63	14.55
f	4.85	20.83	13.44
g	4.97	22.91	19.26

Table 5. Protein enrichment of different semi-solid and solid starchy substrates by *S. castellii* in a bread-making blender

Substrates	Protein*	Dry matter	Total carbohydrates	Protein/ DM
	Initial composition (g‰)			
Potatoes	4.6	119	126	38
Tapioca	5.1	151	141	34
Stale bread	13.6	150	132	91
Maize flour	10.7	137	132	78
	Final products (g‰)			
Potatoes	9.4	57	35	165
Tapioca	11.0	117	99	94
Stale bread	21.0	109	100	192
Maize flour	19.0	160	91	118

*(Lowry *et al.*, 1951.)

Protein enrichment of starchy substrates other than potatoes by *S. castellii* is summarized in Table 5. Protein content varied in the final products between 94 and 192 g‰ DM.

Semi-pilot scale cultivations. Several substrates assayed in laboratory conditions were also employed to define the conditions of protein enrichment by *S. castellii* on a semi-pilot scale, suitable for use on farms, for direct animal feeding. Carbohydrate conversion into protein was more effective on potato and cassava flour substrates. In particular, protein enrichment obtained under our experimental conditions is similar to that observed on some starchy solid substrates fermented by *Aspergillus niger*, in a

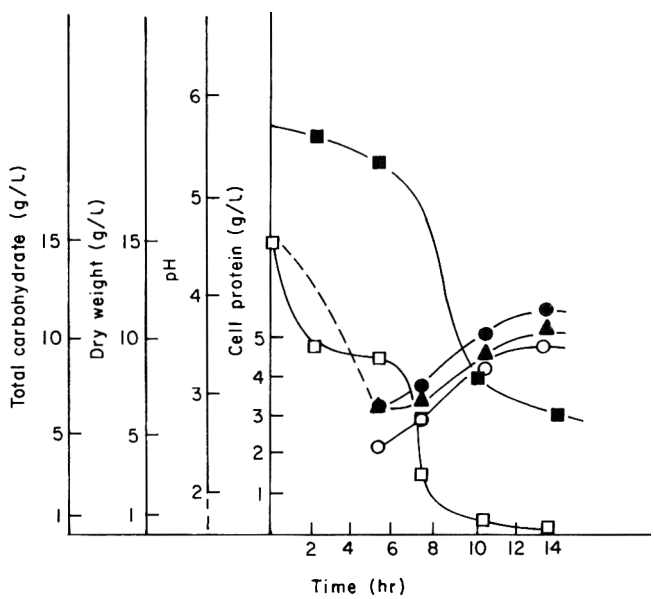

Figure 5. Typical fermentation diagram for *S. castellii* on potato-0.5% CSL medium, at 28°C, with a 3% inoculum: total dry weight (● ●); cell dry weight (▲ ▲); cell protein (O O); total soluble carbohydrates (□ □); and pH (■ ■).

bread-making blender (modified to allow substrate aeration by introducing humidified air) (Senez, 1978).

Figures 6 and 7 report the scanning electron micrographs of samples collected during potato medium fermentation. At magnifications of ×15 and ×937, the progressive decomposition of potato starch grain structure and the increase of biomass growth may be seen.

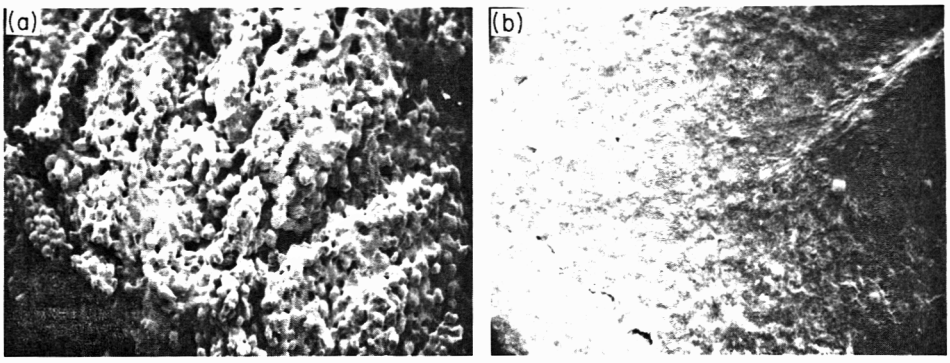

Figure 6. Scanning electron micrographs of solid potato medium modification, during fermentation by *S. castellii* (a = unfermented medium; b = 48 hr fermented medium; 15× magnification).

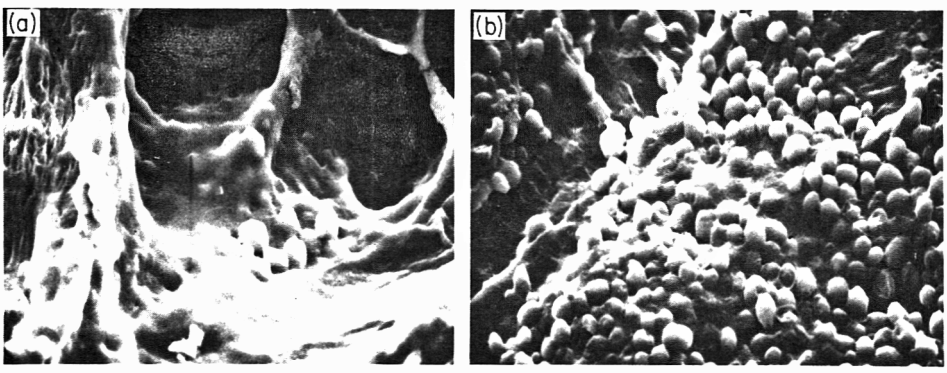

Figure 7. Scanning electron micrographs of *S. castellii* growth on solid potato medium (a = immediately following inoculum; b = after 48 hr; 937× magnification).

Figure 8 reports the scanning electron micrographs of unfermented and fermented cassava flour, maize flour and stale bread substrates, with some yeast cells observed in the 48 hr samples.

In conclusion, the yeast strain *S. castellii* 3754 was proved to be suitable for biomass production from non-hydrolysed starchy materials, even when compared with the better known species *E. fibuligera*. Optimal conditions were defined for SCP production on pilot scale submerged cultures. An effective protein enrichment was obtained on different types of semi-solid and solid starchy substrates.

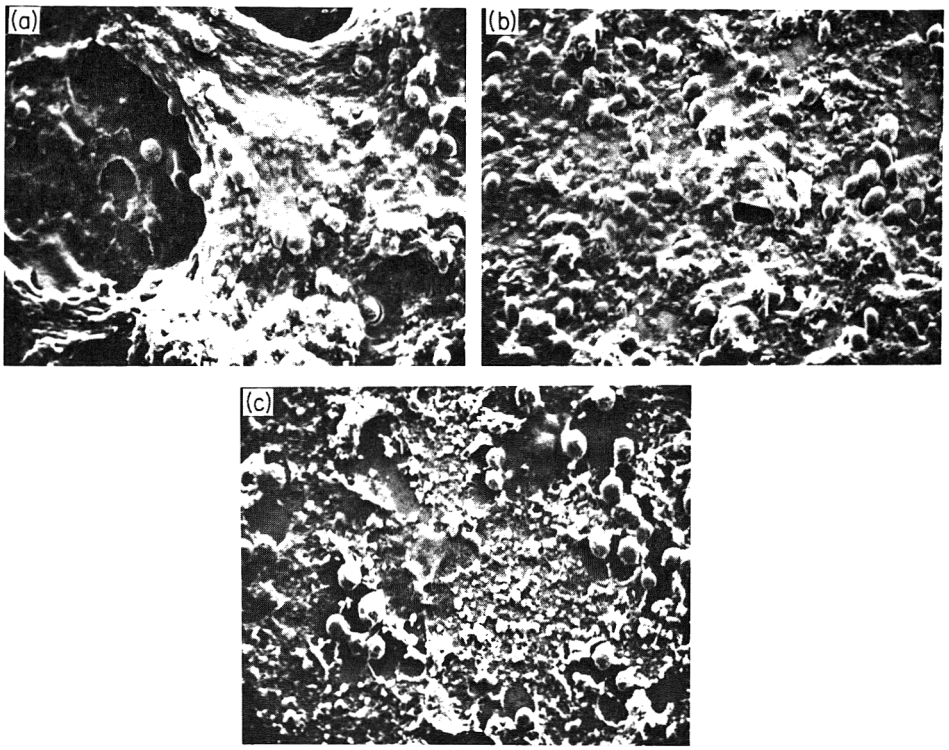

Figure 8. Scanning electron micrographs of *S. castellii* growth after 48 hr fermentation, on different solid media (a = cassava flour; b = stale bread; c = maize flour; 937× magnification).

Acknowledgment

This research was supported by the 'Consiglio Nationale delle Ricerche'.

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(Received 14 May 1984)

Journal of Food Technology (1985) **20**, 331–338

Influence of packaging on the shelf life of frozen foods. III. Ice cream*

R. AHVENAINEN AND Y. MÄLKKI

Summary

The effect of various packagings on the shelf life of ice cream in different storage conditions was studied. The storage temperature is of greater importance on the quality of ice cream than the package. However, a correctly chosen package can significantly prolong the acceptable storage time of ice cream, especially in retail conditions.

Introduction

Among frozen foods, ice cream is perhaps the most sensitive to high and fluctuating storage temperatures. Thus it is probable that packaging has an effect on the stability of ice cream in poor storage conditions. It has been known for some time that in externally aluminium foil laminated cardboard packages the temperature of ice cream stored in an open display cabinet is a few degrees centigrade lower than in the conventional cardboard packages. Also, in a recent comparative storage study (Broman, 1979), ice cream preserved its quality better in aluminium foil laminated packages than in PE-coated cardboard packages both in open display cabinets and in closed retail cabinets. The purpose of this study was to investigate the effects of various alternative packagings on the shelf life of ice cream stored in different storage conditions.

Materials and methods

Preparation and packaging

Vanilla ice cream was used as test material. The fat content of the ice cream was 12.6%, total solids 35.9% and specific gravity 0.53. The freezing temperature was −40°C. In order to obtain representative samples, both the packaging and freezing of the ice cream were carried out in industrial scale.

Test conditions

Storage tests were mainly performed in those three closed frozen food cabinets where the storage tests of carrot cubes and Baltic herring fillets were performed (Ahvenainen & Mälkki 1985a,b). The set temperatures of the cabinets were −12, −15 and −18°C. The actual product centre temperatures varied from −10.3 to −11.5°C at a nominal −12°C, from −13.3 to −14.1°C at −15°C and from −17.0 to −17.8°C at −18°C. The defrost performed twice a week raised the temperatures by about 0.2°C. The shelf life was also tested in an open display cabinet simulating retailing conditions. The set temperature of this cabinet was −15°C. The cabinet was the same as in the storage tests for carrot cubes (Ahvenainen & Mälkki 1985a), and the uniformity and

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*A contribution to the joint European COST 91 research project

fluctuations of the temperature and their dependence of the reflectance of the surface were presented in connection of that study. Reference samples were stored at −25–−27°C in industrial storage.

Packages tested

In the closed frozen food cabinets the storage life of ice cream was tested in four different cardboard and in two plastic boxes. Cardboard packages were made from: low density polyethylene (LDPE)-coated cardboard (inside coating); hot melt-coated folding boxboard (outside and inside coating), latex-coated (experimental) folding boxboard (outside and inside coating) or water-repellent (WR) folding boxboard (thin wax/polyethylene coating on the inside). The size of the cardboard packages was 0.5 l. The ends of the packages were not heat-sealed. The plastic boxes were made from polystyrene (PS) (220 μm) and high density polyethylene (HDPE) (700 μm). The size was 1.0 l for the PS box and 2.0 l for the HDPE box.

In the open display cabinet, the shelf life was tested in LDPE-coated and externally aluminium foil laminated cardboard boxes. The package of the reference sample was a printed LDPE-coated cardboard box, which is the most frequently used ice cream package in Finland.

Evaluation of samples

Quality changes during storage were followed principally by sensory evaluation, which was carried out at intervals of 1–6 weeks until the quality was unacceptable. For evaluation, ice cream was tempered at −12°C overnight. The appearance after opening a package and allowing the contents to melt for 30 min at room temperature (= melting) were evaluated by two or three trained persons using a five-point scale (5 = extremely desirable, 1 = extremely undesirable). The texture and taste of ice cream samples were evaluated by a ten-member panel also using a five-point scale. Final scores were calculated in the following way: 2×(appearance+melting)+8×(texture+taste) = max 100 points. At the beginning of the storage, the ice cream scored 93.5 points. According to the final scores the quality of the ice cream samples was divided into three quality grades. The limits were 80 points for first grade, 60 points for second grade and 40 points for third grade. The third grade ice cream sample was still acceptable but the quality was only fair.

Weight losses were determined by weighing five samples of each package before the storage and at the end of the storage. The changing of texture during storage was investigated by microscope. Also the colour and firmness both before and after storage were determined using instruments.

Water vapour permeability of packaging materials

For cardboard materials water vapour permeabilities were determined at +23°C and at −20°C by the Scan-P 22:68 method by the Finnish Pulp and Paper Research Institute, in order to determine the effect of material characteristics on the shelf life of ice cream.

Results

Permeability of packaging materials

WR-cardboard had the highest water vapour permeability, even at −20°C it was very high (Table 1). The permeability of other cardboard was normal for this kind of

Table 1. Water vapour permeabilities of the cardboard materials tested

Cardboard	Water vapour permeability ($mg/dm^2/24$ hr)	
	+23°C (RH 50%)	−20°C
Hot melt-coated	98	11
LDPE-coated	57	23
Latex-coated	210	38
WR-coated	4820	820

material. The lowering of temperature had the greatest effect on the permeability of hot melt-coated cardboard.

Sensory quality

The storage temperature had so great an effect on the quality of ice cream that even in the polystyrene box which showed the best protection in these experiments, the quality when stored at −18°C was already close to the limit of acceptability (54 points, limit of acceptability 40 points) after only 30 weeks (Tables 2 and 3). By contrast, the quality of the reference sample stored at −25–−27°C was fairly good after a storage of 30 weeks (hedonic points 77 in total, maximum 100).

Table 2. The effect of package on the shelf life of ice cream stored in closed frozen food cabinets at three different temperatures

Package	Shelf life (weeks)					
	I			II		
	−12°C	−15°C	−18°C	−12°C	−15°C	−18°C
Cardboard packages:						
Hot melt-coated	0–1	0–1	2–4	3–4	2–3	11–15
LDPE-coated	0–1	0–1	4–5	3–4	3–4	7–10
Latex-coated	0–1	0–1	4–5	3–4	3–4	7–10
WR-coated	0–1	0–1	2–4	2–3	2–3	4–7
Plastic packages:						
PS-box	< 3	< 3	< 4	3–4	3–4	11–15
HDPE-box	< 2	< 2	< 4	2–3	2–3	< 11
Reference (−25°C)	19			> 30		

I = shelf life in first quality grade and II = shelf life in second quality grade.

At temperatures of −12 and −15°C, the quality of ice cream remained in the first quality grade for less than 1 week and at −18°C for 4–5 weeks regardless of packaging (Table 2). At −12 and −15°C the package had no effect on the shelf life of ice cream stored in closed frozen food cabinets until the quality was in the third quality grade.

At −18°C the package was of importance on the storage life when the quality was in the second grade. At this temperature, among cardboard packages the hot melt-coated cardboard box was the best for quality, and plastic packages were comparable to the hot melt cardboard box (Table 2).

With regard to the shelf life of ice cream in the third quality grade, still acceptable, significant differences were observed in shelf lives in different packages even at −12°C (Table 3). The best package for the quality of ice cream under these circumstances was the polystyrene box. The shelf life of ice cream in this package was about 1.5 times that in the best cardboard package, the hot melt cardboard box. The package weakest from the quality of ice cream aspect was the WR-cardboard box. The acceptable time in this package was only half that of other cardboard packages.

Table 3. The effect of package on the shelf life of ice cream in the third quality grade (=acceptable storage time) in closed frozen food cabinets

	Shelf life (weeks)		
Package	−12°C	−15°C	−18°C
Cardboard packages:			
Hot melt-coated	8–10	11–16	22
LDPE—coated	6–8	11–16	13–17
Latex-coated	6–8	7–10	22
WR-coated	3–4	4–5	8–10
Plastic packages:			
PS-box	16	22–26	> 30
HDPE-box	16	19	22–36

In an open display cabinet at −15°C, the shelf life could be extended by using an aluminium foil laminated cardboard package by around 2 months, as compared to the LDPE-coated cardboard package (Table 4).

The losses of quality of ice cream were faster in the early part than in the later part of the storage. However, on the basis of the statistical calculations it was possible to plot mean overall scores from sensory evaluations for the different packages *versus* time so that straight lines with correlation ≥ 0.9 in most cases appeared. The slopes of the lines indicate a loss of quality per time unit over the whole storage period. Calculating

Table 4. The effect of package on the shelf life of ice cream stored in the top layer of an open display cabinet at a set temperature of −15°C

	Shelf life (weeks)		
Package	I	II	III
LDPE-coated cardboard box	< 2	4	10
Al-foil laminated cardboard box	< 2	6	16–18

I = shelf life in the first quality grade, II = shelf life in the second quality grade and III = shelf life in the third quality grade (= acceptable storage time).

confidence intervals of slopes (95% probability) showed statistically significant differences between packages existed.

On the basis of regression analyses, the polystyrene box showed the best protection of quality, the difference being fairly significant or significant compared to cardboard packages (Figs 1 and 2). At −18°C the quality deteriorated significantly faster in the WR-cardboard box than in other packages (Fig. 1). Also at higher temperatures the quality of ice cream packed in the WR-cardboard box deteriorated clearly faster than in the other cardboard packages, but not more significantly faster. However, the plastic packages tested protected the quality also at −12°C fairly significantly better than the WR-cardboard box. The reference sample stored at −25−−27°C kept its quality very significantly better than all the samples stored at −12−−18°C.

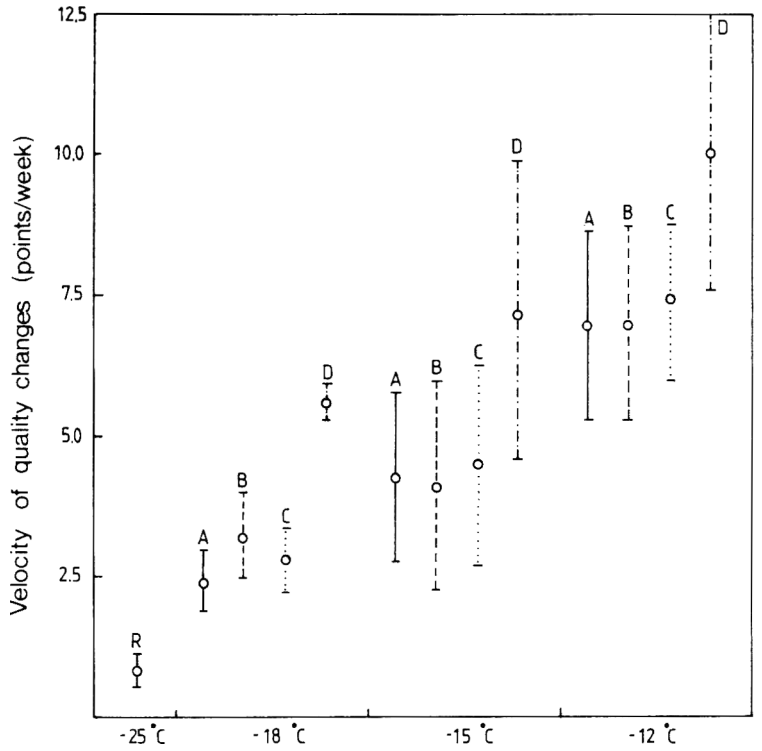

Figure 1. The effect of various cardboard packages on the rate of quality changes in ice cream stored in closed frozen food cabinets at three different temperatures. Vertical lines: confidence interval of the rate (95% probability). R = reference sample, A = hot melt-coated, B = LDPE-coated, C = latex-coated and D = WR-coated cardboard box.

The quality changes occurring in ice cream samples were in their nature different in different packages. However, appearance and melting suffered faster at all test temperatures and in all packages than texture and taste. The ice cream packed in the latex-coated and WR-coated cardboard boxes had already after storage of 1–2 weeks a strong taste of cardboard, especially at −12°C. Also the LDPE-coated cardboard box caused at this temperature a slight taste of cardboard, but only at the end of the storage. The ice cream packed in the hot melt-coated and aluminium foil laminated cardboard packages and in the plastic packages had no taste caused by the package.

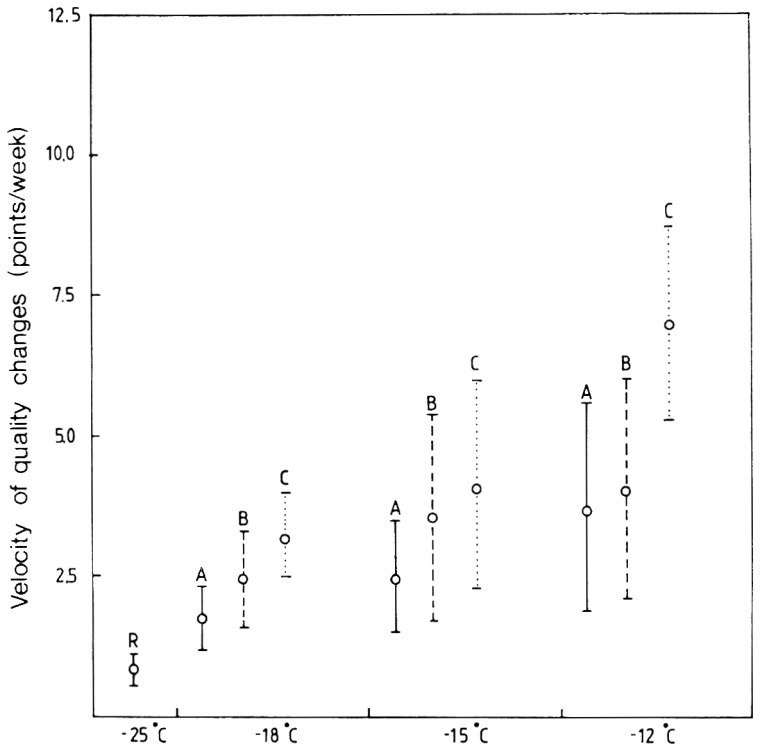

Figure 2. The effect of two kinds of plastic packages and the LDPE-coated cardboard box on the rate of quality changes in ice cream stored in closed frozen food cabinets. Vertical lines as in Fig. 1. R = reference sample, A = polystyrene box, B = HD-polyethylene box and C = LDPE-coated cardboard box.

The defects in quality of ice cream stored at −12°C differed from those of ice cream stored at −18°C. The texture of ice cream stored at −12°C was icy, and the appearance lumpy. The texture of ice cream stored at −18°C was doughy, tough, thick and fatty, although at the end of the storage the texture was somewhat icy. According also to microscope examinations, the texture of ice cream stored at −12°C was sandy and icy after 2 weeks storage (diameter of crystals ≥ 50–60 μm), while at −18°C crystals began to grow significantly only after 8 weeks of storage. However, ice cream packed in the WR-cardboard package was sandy and icy before the 8 weeks of storage had elapsed. The texture of ice cream packed in plastic packages was also doughy, tough, fatty and thick. Just in the latter part of the storage the texture was a little crystalline. The appearance was lumpy and the colour a little yellow.

The preservation of taste was longest in plastic packages. In the latter part of the storage, the taste in plastic packages was regarded as watery, while in cardboard packages defects in taste were primarily old taste and taste caused by cardboard. This difference might have been caused by the higher water evaporation from the cardboard packages.

Fluctuating temperature in the open display cabinet had a marked effect on the growing of crystals. The texture of ice cream stored in the open display cabinet at −15°C was sandy and icy before the storage had continued for 3 weeks, while in the closed frozen food cabinets at −15°C the texture was sandy after 8 weeks. The type of package

had only little effect on preventing crystals growing in ice cream stored in the open display cabinet.

Weight losses in cardboard packages

Weight losses of cardboard packages during storage were determined at the end of storage by weighing five packages of each sample. The losses were markedly dependent on storage temperature. At −18°C weight losses of three cardboard packages were only after 20 weeks as great as weight losses at −12°C after 11 weeks (Table 5). In the WR-cardboard box weight losses were significantly higher as a result of the higher water vapour permeability of WR-cardboard. Especially in the latter part of the storage ice cream in this package was collapsed, dry and had a skin on it. The smallest weight losses occurred in the LDPE-coated cardboard box, although the water vapour permeability of LDPE-coated cardboard was not the lowest. Weight loss is evidently also influenced by the tightness of sealing, possible fractures in the creasing and similar factors.

The hot melt-coated and latex-coated cardboard boxes had weight losses under 3%. In the three last-mentioned cardboard packages evaporation of water caused primarily the drying of corners, although just at the end of the storage also skin formation could be observed. Also the weight losses of packages stored in the top layer of an open display cabinet were small (Table 5).

Table 5. Weight losses of different ice cream cardboard packages stored in closed frozen food cabinets and in the top layer of an open display cabinet

Cardboard package	Weight losses (%)			
	In closed cabinets			In open display cabinet*
	−12°C*	−15°C†	−18°C‡	
Hot melt-coated	2.7	2.8	2.8	
LDPE-coated	0.7	1.0	0.8	1.7
Latex-coated	2.2	2.2	2.5	
WR-coated	12.7	11.2	19.1	
Al-foil laminated				0.8

*After 11 weeks.
†After 16 weeks.
‡After 20 weeks.

Conclusions

Although storage temperature has a greater effect on the shelf life of ice cream than package, and ice cream cannot be stored for a long time at temperatures above −25°C, the packaging does have a significant influence, especially under retailing temperatures and conditions and during subsequent storing at home. On the other hand, the storage temperature for ice cream in retail stores should be as low as possible and the temperature fluctuation as small as possible in order to prevent, among other things, the growing of crystals, and to guarantee the maintenance of quality as good (= in the first quality grade) for as long as possible.

At the retailing temperatures, no package tested in this study can keep ice cream in the first quality grade for longer than 4 weeks, even although the temperature

would not fluctuate. In open display cabinets shelf life is still shorter. When losses of quality to second and third quality grades are accepted, the shelf life at these temperatures can be extended by choosing tight closed plastic packages where the shelf life can be 1.5 times that of the most advantageous cardboard package, the hot melt-coated package, tested in dark vertical cabinets. Of those two cardboard packages tested in the open display cabinet the aluminium foil laminated package is clearly better for the quality of ice cream than the LDPE-coated package. It is probable that also in well coated cardboard packages the shelf life would be as long as in plastic packages if the closing of the package was tight. In this study the ends of cardboard packages were only folded but not heat-sealed, following the conventional practice in ice cream packaging.

In addition, the coating of the cardboard material must be impervious enough to prevent any off-flavour from the cardboard from migrating to the ice cream. According to this study, LDPE- and hot melt-coating are in this respect suitable for ice cream cardboard packages. The coating must also be so impervious that the water vapour permeability is very low. It seems that a permeability below 100 mg/dm^2/24 hr room temperature is sufficient to prevent significant weight losses and drying of ice cream.

Acknowledgments

The following institutions have sponsored this investigation: Ministry of Trade and Industry, Converta, Finnboard, Enso-Gutzeit Oy, Finnish Plastic Industries' Federation, Neste Oy, Finnish Food Research Foundation, Federation of Finnish Ice Cream Industries, Kesko Oy, SOK Cooperative Wholesalers and Tukkukauppojen Oy.

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(Received 14 June 1984)

Journal of Food Technology (1985) **20**, 339–349

Modified atmosphere packaging to extend the shelf life of tomatoes

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Summary

An experimental retail packaging system was used to compare the atmospheric composition and relative humidity which developed within packs sealed with a range of plastic films with different permeability properties, and to study the effects of these conditions on the rate of ripening (as indicated by colour change) and sensory quality of tomatoes. In packs sealed with several polyvinylchloride (PVC) films or with K-resin (a butadiene-styrene copolymer), an atmosphere containing 3–9% CO_2+3–9% O_2 developed within *circa* 3–4 days at 10 or 12.5°C. Ripening of part ripe fruit was retarded, but continued normally after the packs were perforated, with no obvious adverse effects on the smell, flavour or texture of the fruit. In packs sealed with less permeable films (e.g. cellulose acetate), the internal atmosphere equilibrated to 10–18% CO_2+ $< 2\%$ O_2, resulting in complete inhibition of ripening, a high incidence of rotting and tainting of the fruit. Very high (98% or above) relative humidity within sealed packs also encouraged fungal spoilage. The possible commercial applications and benefits of this type of modified atmosphere (MA) packaging are discussed

Introduction

After harvest, ripening continues and tomatoes become over-ripe very rapidly at ambient temperatures. This results in loss of quality, restricted shelf life and in some instances wastage of fruit during distribution and marketing. Although ripening changes can be retarded to some extent by cooling, storage of tomatoes at low temperatures is precluded by their susceptibility to chilling injury, which causes pitting, poor or uneven ripening and increased fungal spoilage (Tomkins, 1963; Ryall & Lipton, 1972; Dennis, 1981).

Since the experiments of Kidd & West (1932), many workers have demonstrated that the ripening of green or part-ripe tomatoes can be delayed and their storage life at higher temperatures (typically 10–20°C) extended in controlled or modified atmospheres, which are enriched in CO_2 and depleted in O_2; these findings are comprehensively reviewed by Isenberg (1979). When fruits such as tomatoes are enclosed within sealed packages, their respiration results in the development of a modified atmosphere. Delayed ripening and extended post-harvest life has been observed in tomatoes sealed inside polyethylene (Okubo, 1968; Saguy & Mannheim, 1975; Hobson, 1981), polyvinylchloride (PVC) (Henig, 1975; Saguy & Mannheim, 1975; Daun & Gilbert, 1974) and several other packaging films (Ayres & Peirce, 1960), although in many cases problems of adverse flavour or increased incidence of rotting in packaged fruit were reported. Nevertheless, several modified atmosphere (MA) pack-

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aging methods for tomatoes have been patented (Cummin *et al.*, 1974; Bedrosian & Schiffman, 1979).

The aims of the study described here were to compare the internal environmental conditions (particularly atmospheric composition) which developed within model retail pre-packs of tomatoes sealed with a wide range of plastic films, and to evaluate the effects of the conditions within these packs on the rate of ripening and quality of the fruit during simulated distribution and shelf life.

Materials and methods

Fruit

In most experiments, tomatoes, cv. Sonatine or Sonato were obtained from glasshouse crops in E. Norfolk, although imported Spanish tomatoes (cv. unknown) were used in some experiments during winter months. The calyces were removed before packing and, except in the case of imported fruit, tomatoes were packed on the day of harvest.

Experimental packaging system

Experimental packs consisted of a rigid relatively impermeable polyvinylchloride (PVC) tray 18.3×11.7×5.5 cm deep with a horizontal rim 0.5 cm in width, to which the test films were attached using double-sided adhesive tape and then over-sealed with adhesive cellulose tape ('Sellotape' brand) to prevent leaks. Table 1 shows the films tested, their polymer type or composition and thickness, together with the manufacturer's specifications of their gas permeabilities and water vapour transmission rates. Some films, particularly those which were most effective in delaying tomato ripening were used in many experiments, but several films were tested only once or twice. In each experiment, 4–6 packs were sealed with each film and a further 4–6 packs covered with perforated 15 μ polyvinylchloride (PVC) to allow free gaseous exchange with the room air were included as controls. Each pack contained 390±10 g (*circa* 14 oz) of tomatoes, usually six fruits, therefore giving similar ratios between the weight of fruit, the area of permeable film (214 cm^2) and the volume of free space (*circa* 550 ml) within the pack.

Storage of packs

Packs sealed with each of the experimental films were placed in constant temperature rooms at 10 or 12.5°C, although in experiments to study the effect of temperature replicate sets of packs sealed with each of three films were placed at 10, 12.5, 15 and 20°C (4–6 packs/treatment) for 1–3 (usually 2) weeks. All sealed packs were then perforated to permit free gaseous exchange and transferred to 20°C for a further 4–10 days to allow the fruit to ripen. The packs were kept in the dark, except when sampling or assessing the fruit.

Analysis of internal atmospheres

The concentrations of CO_2 and O_2 in sealed and control packs were monitored every 1–3 days by withdrawing 1 ml samples through the side of the rigid PVC tray using a hypodermic needle and syringe, after which the hole was immediately closed with a small piece of plastic insulating tape. These samples were then injected into a gas chromatograph (Pye Series 104) for analysis.

Table 1. Composition, thickness, gas permeabilities and water vapour transmission rates of packaging films used

Film	Manufacturer or supplier	Composition	Thickness (μ)	O_2 permeability ($ml/m^2.d.atmos.$)	CO_2 permeability ($ml/m^2.d.atmos.$)	Water vapour transmission rate ($g/m^2.d.$)
1. QMS cellophane	British Cellophane Ltd.	cellulose (coated with cellulose nitrate)	28	25–100 (20°C, 43% RH)	—	300–500 (25°C, 75% RH)
2.	British Cellophane Ltd.	polyethylene/ethylene methyl acrylate (EMA) co-extrusion	60	1750 (25°C)	8300 (25°C)	2 (25°C, 90% RH)
3.	British Cellophane Ltd.	polyethylene/ethylene vinyl acetate (ELVAX) co-extrusion	60	1750 (25°C)	8300 (25°C)	2 (25°C, 90% RH)
4.	DRG Ltd.	cellulose acetate	40	1500–3000 (20°C, 0% RH)	15000 (20°C, 0% RH)	200–400 (25°C, 75% RH)
5.	British Cellophane Ltd.	cellulose acetate	24	3200 (25°C, 75% RH)	—	240 (25°C, 75% RH)
6.	British Cellophane Ltd.	cellulose acetate	50	—	—	—
7.	Pakcel Converters Ltd.	polypropylene-oriented (PP)	15	2600 (21°C, 44% RH)	7500 (21°C, 44% RH)	3 (25°C, 90% RH)
8.	Transatlantic Plastics	polyethylene-low density (LDPE)	25	4200 (25°C, 75% RH)	20000 (25°C, 75% RH)	6 (25°C, 90% RH)
9. K-resin	Phillips Petroleum (UK) Ltd.	butadiene-styrene co-polymer	25	4400–4800 (23°C, 0% RH)	37100 (23°C, 0% RH)	140–190 (37°C, 90% RH)
10. VF-70	Borden (UK) Ltd.	polyvinylchloride (PVC)	17	6000 (23°C)	36000 (23°C)	90–100 (25°C, 75% RH)
11. PS-H	Borden (UK) Ltd.	polyvinylchloride (PVC)	25	7700	46000	50 (25°C, 75% RH)
12. RMF-61	Borden (UK) Ltd.	polyvinylchloride (PVC)	17	15000–20000 (23°C)	100000–120000 (23°C)	110–140 (25°C, 75% RH)
13. VHY	Borden (UK) Ltd.	polyvinylchloride (PVC)	15	20000 (23°C)	120000 (23°C)	150 (25°C, 75% RH)
14. Microporous film	Van Leer (UK) Ltd.	polypropylene	—	—*	—*	150 (23°C, 50% RH)

Most figures from manufacturers or suppliers (test conditions shown in brackets).
*Air permeability 60000000.

Measurement of relative humidity

The relative humidity (RH) levels within experimental packs was measured with a direct reading humidity probe (Model HMP13, Vaisala UK Ltd., Northampton), which was inserted within a tightly-fitting rubber bung into a circular hole cut in the rigid side of the PVC tray. The calibration of the probe was checked regularly over appropriate saturated salt solutions (O'Brien, 1948; Wexler & Hasegawa, 1954; Winston & Bates, 1960). The RH of the constant temperature room, in which the rates of weight loss from experimental packs were compared was measured at regular intervals with an Assman psychrometer (Casella & Co. Ltd.).

Fruit assessments

As an indicator of the degree of ripeness, the colour of the tomatoes was assessed on the day of packing and at intervals of 3–4 days during the experiments on an eight-point scale (Table 2). All colour assessments were made in natural daylight, but avoiding direct sunlight. In each experiment, care was taken to ensure that all fruits were packed at a similar colour, typically 'breaker', 'quarter-ripe' or 'half-ripe'.

All fruit was inspected for evidence of fungal infection at intervals during and at the end of each experiment; where possible, the causal organisms were identified by the appearance of the lesions. The fruit in each pack was weighed at the end of the experiments (before perforating the packs) and weight losses (%) per week determined.

Table 2. Tomato colour grading scale

Grade	Colour	
1	'Breaker'	(some orange colour visible at blossom end)
2	'Quarter-ripe'	(less than half of fruit surface coloured)
3	'Half-ripe'	(more than half of fruit surface coloured, but some green areas around calyx scar)
4	Light orange	
5	Dark orange	
6	Orange-red	
7	Red	(fully-ripe)
8	Dark red	(over-ripe)

Tomatoes from some treatments were assessed in the controlled conditions of a sensory testing laboratory by a panel of *circa* 20 experienced assessors. Fruit from sealed packs, together with fruit from perforated control packs or freshly picked tomatoes of the same colour were rated for smell, texture and flavour on the scale: excellent, very good, good, fair, poor, unacceptable. The mean ratings for each sample were determined and the characteristics of each sample described. Samples of some other treatments which were not considered suitable for presentation to a sensory panel were assessed for smell, texture and flavour by the authors.

Results

Changes in the composition of the internal atmosphere of packs

In ventilated control packs, the concentration of O_2 remained $> 20\%$ and the CO_2 concentration $< 0.5\%$, whereas in packs sealed with the microporous film, the O_2 concentration decreased to 19–20% and that of CO_2 increased to only *circa* 2% during

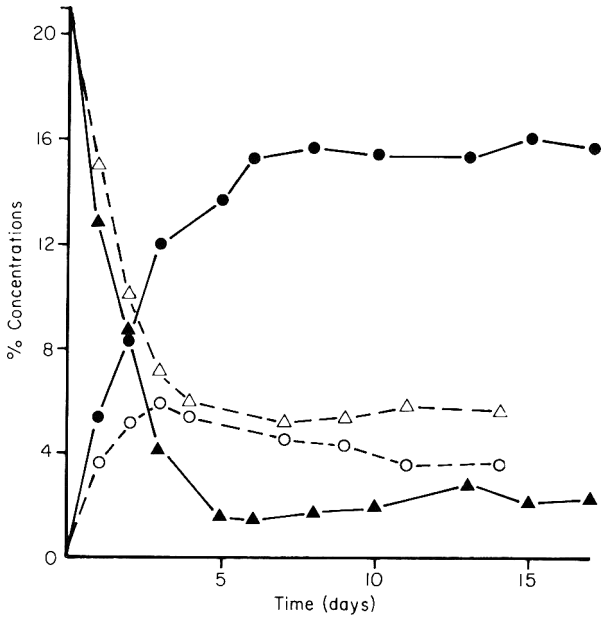

Figure 1. Changes in the concentrations of CO_2 (O, ●) and O_2 (△, ▲) within packs of tomatoes sealed with 25 μ K-resin (hollow symbols) or 15 μ polypropylene (solid symbols) and stored at 10°C.

the course of the experiments. In all other sealed packs, the concentration of O_2 decreased rapidly and that of CO_2 increased rapidly during the first few days of storage, after which a 'state of equilibrium' was attained and little or no further changes in the gas concentrations within the packs were detected (see Fig. 1). The approximate times

Table 3. Times (days) for concentrations of CO_2 and O_2 to equilibrate and equilibrium concentrations (%) in experimental packs of tomatoes sealed with different packaging films

Packaging film (storage temperature 10°C)	CO_2		O_2	
	Equilibrium time (days)	Equilibrium concentration (%)	Equilibrium time (days)	Equilibrium concentration (%)
Cellophane	2	14	2	5
*Polyethylene/EMA co-extrusion (60 μ)	2	10	2	2–3
*Polyethylene/ELVAX co-extrusion (60 μ)	2	12	2	4–5
Cellulose acetate (40 μ)	3	11	3	1–2
*Cellulose acetate (24 μ)	4–5	9–10	1–2	0.5–1.5
*Cellulose acetate (50 μ)	5	17–18	1–2	0.5–1.5
Polypropylene (15 μ)	3–5	15	3–5	2
Polyethylene (25 μ)	2–3	7–9	2–3	6–7
K-resin	4	4–5	2–4	4–6
VF-70	3–4	6–8	3–5	2–3
PS-H	3–5	3–5	3	3–4
RMF-61	3	3	3	9
VHY	3	2–3	3	9
Microporous film	1	2–3	1	19–20

*Stored at 12.5°C.
Mean data from 4–6 packs per treatment.

taken for the concentrations of O_2 and CO_2 to equilibrate and the approximate equilibrium concentrations in packs sealed with each of the experimental films and stored at 10 or 12.5°C are shown in Table 3.

In packs sealed with PS-H or VF-70 (PVC) or with K-resin (butadiene-styrene copolymer) the changes in the internal atmospheres and the equilibrium concentrations of O_2 and CO_2 were very similar at 10, 12.5, 15 and 20°C. Similarly, equilibrium times and concentrations of O_2 and CO_2 in packs sealed with PS-H and stored at 12.5 or at 20°C were not affected by the colour of the fruit (between 'breaker' and light orange) when packed.

Effects on colour change

The rate of colour change of tomatoes packed at a similar stage of ripeness and stored in perforated control packs at the same temperature varied from one experiment to another, and this variability was most evident in fruit packed at earlier stages of ripening. For example, fruit packed at colour grade 2 ('quarter-ripe') reached colour grade 6 (orange-red, a typical colour for pre-packed tomatoes on the supermarket shelf) after 10–15 days at 10°C or after 4–7 days at 20°C. Similarly, fruit packed at colour grade 4 (light orange) attained colour grade 6 after 8–10 days at 10°C or after 3–4 days at 20°C.

Tomatoes packed under microporous film changed colour at the same rate as fruit in control packs at 10°C. Colour change of tomatoes packed at colour grades between 1 ('breaker') and 4 (light orange) and stored at 10 or 12.5°C was retarded or inhibited in all other sealed packs. A progressive change in fruit colour was observed in packs sealed with the PVC films VHY, RMF-61, PS-H or VF-70, with K-resin or with 25 μ polyethylene, but this was noticeably slower than in the corresponding control packs (see Fig. 2). The extent of this delay was greatest with VF-70 and 25 μ polyethylene and least with RMF-61 and VHY. However, the rate of colour change of tomatoes in all of these

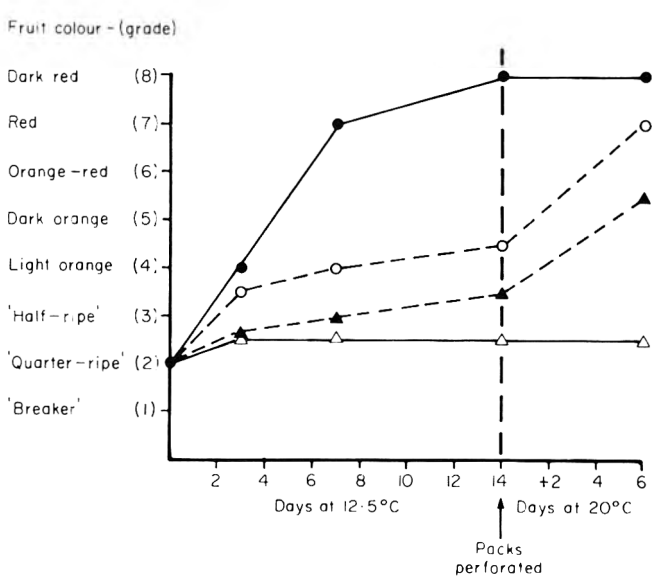

Figure 2. Colour change of tomatoes in perforated control packs (●—●) and in packs sealed with PS-H (O—O), K-resin (▲—▲) or 50 μ cellulose acetate (△—△) films stored for 14 days at 12.5°C, and after perforation for a further 6 days at 20°C.

sealed packs accelerated and the fruit 'ripened' fully and uniformly when the packs were perforated and transferred to 20°C (see Fig. 2). In contrast, tomatoes in pack sealed with 60 μ polyethylene co-extrusions, polypropylene, QMS cellophane or with any of the cellulose acetate films showed a slight change in colour during the first few days, but thereafter colour change was completely inhibited. Even when these packs were perforated, the tomatoes failed to ripen normally and showed only irregular colour change, although in many packs fruit colour was difficult to discern because of the high incidence of rotting (due mainly to *Penicillium* spp. or *Botrytis cinerea*).

In packs sealed with each of the three films, PS-H. VF-70 or K-resin, the rate of colour change of tomatoes packed at colour grade 2 ('quarter-ripe') was similar at 10, 12.5, 15 or 20°C. However, since ripening of the fruit in the corresponding control packs was more rapid at higher temperatures, the delay of ripening achieved by these sealed packs was therefore greater at higher temperatures (see Fig. 3).

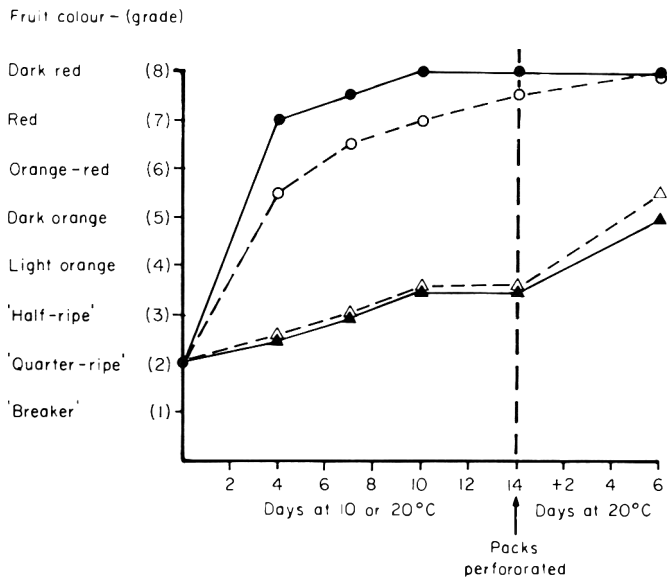

Figure 3. Colour change of tomatoes in perforated control packs (O. ●) and in packs sealed with VF-70 (△, ▲) stored for 14 days at 10°C (hollow symbols) or 20°C (solid symbols) and after perforation for a further 6 days at 20°C.

Internal relative humidities of packs and weight losses

The relative humidity levels and rates of weight loss by tomatoes within packs sealed with the experimental films and stored at 12.5°C, 65–73% RH are shown in Table 4.

Sensory assessments

In sensory assessments, fruit stored at 10 or 12.5°C for 7–14 days in packs sealed with the PVC films PS-H, RMF-61, VF-70 or VHY or with K-resin, followed by a further 4–6 days ripening in perforated packs at 20°C was found to have a similar or slightly better smell, flavour and texture than samples of fruit maintained at these temperatures in perforated control packs throughout. Mean scores and comments from sensory panels for fruit from PS-H, VF-70 and K-resin packs and from the corresponding control packs is shown in Table 5. However, all samples of fruit from sealed

Table 4. Internal relative humidities and weight losses (% per week) of tomatoes in packs sealed with different packaging films

Packaging film	Internal RH (%)	Weight loss (%)
Polypropylene	98–100	0.11
Polyethylene/EMA co-extrusion	98–100	0.12–0.15
Polyethylene/ELVAX co-extrusion	98–100	0.12–0.15
K-resin	89–92	0.14–0.18
Polyethylene (25 μ)	98–100	0.15
VF-70	90–93	0.4 –0.45
PS-H	89–93	0.8
Microporous film	87–90	0.8 –0.9
RMF-61	87–90	0.95–1.0
Control packs—perforated polyvinylchloride (15 μ)	85–88	1.0 –1.3
Cellulose acetate (50 μ)	83–86	1.5 –1.6
Cellophane	80	1.6 –2.0
Cellulose acetate (24 μ)	79–81	2.5

Packs stored at 12.5°C, 65–73% (mean 69%) RH.

or control packs was typically inferior in overall quality, particularly flavour to freshly picked vine-ripened tomatoes of a comparable colour.

Discussion

The rapid changes in the composition of the internal atmospheres and the equilibration of the concentrations of CO_2 and O_2 within sealed packs in these experiments closely resembles the patterns of internal atmosphere development within sealed packs of fruits and vegetables which have been discussed by Tomkins (1967) and Henig (1975). In general, the highest concentrations of CO_2 and the lowest concentrations of O_2 were found in packs sealed with the least permeable films and vice-versa, although the manufacturers' gas permeability specifications for the films were typically determined at higher temperatures (e.g. 20–25°C) than were used for these experiments. However, from these results, it is doubtful whether the correlation, particularly that for O_2, would be sufficiently close to enable prediction of the internal atmospheres of a pack from the CO_2 and O_2 permeabilities of the film, as proposed by Henig (1975). Comparison of the internal atmospheres in these packs with those reported by other workers, even in packs of tomatoes sealed with the same films, is difficult because of differences in pack construction (and therefore in the ratios between weight of fruit, internal volume and permeable surface area), temperature and other factors. Nevertheless, the concentrations of CO_2 and O_2 previously recorded in packs sealed with RMF-61 (Daun & Gilbert, 1974; Henig, 1975, Saguy & Mannheim, 1975) or with 25 μ polyethylene (Saguy & Mannheim, 1975) are similar to the equilibrium gas concentrations observed here.

The internal atmospheres developed within packs sealed with several PVC films, 25 μ K-resin or 25 μ polyethylene successfully retarded colour change of tomatoes without impairing the ability of the fruit to ripen subsequently in air; retardation of ripening has previously been reported in tomatoes sealed in polyethylene (Okubo, 1968; Saguy & Mannheim, 1975; Hobson, 1981; Anderson & Poapst, 1983) or over-wrapped with PVC

Table 5. Mean ratings* and descriptions given by sensory panels for samples of tomatoes cv. Sonatine stored in sealed and perforated control packs

Packaging film	Storage conditions	Panel assessments		
		Smell	Flavour	Texture
Experiment A				
PS-H	14 days in sealed pack at 12.5°C +4 days in perforated pack at 20°C	3.6 (low, no defects)	3.9 (low, but good acid/ sweet balance)	3.6 (flesh slightly soft, skins firm)
K-resin		3.3 (low, but fruity)	3.3 (low, fruity, sweet)	3.5 (flesh soft, skins not tough)
Control	14 days at 12.5° +4 days at 20°C	3.9	4.4 (acid)	4.6 (flesh soft, skins tough)
Experiment B				
K-resin	14 days in sealed pack at 10°C +4 days inperforated pack at 20°C	3.8 (low, slightly musty)	4.1 (low)	3.7 (skins slightly tough)
VF-70		3.7 (weak)	3.8 (low)	3.7 (skins slightly tough)
Control	14 days at 12.5° +4 days at 20°C	3.9 (low)	4.1 (fairly low, slightly sour)	3.9 (skins tough, flesh soft)
Experiment C				
PS-H	7 days in sealed pack at 10°C +6 days in perforated pack at 20°C	3.9 (weak)	4.1 (weak, slightly old)	4.0 (skins tough)
VF-70		3.6	3.6 (weak)	4.0 (skins tough)
Control	7 days at 10°C+6 days at 20°C	4.0 (weak)	4.6 (weak, acid, old)	3.8 (skins tough)

*Scale: 1, excellent; 2, very good; 3, good; 4, fair; 5, poor; 6, unacceptable.

films (Daun & Gilbert, 1974; Henig, 1975; Saguy & Mannheim, 1975). The modified atmospheres (3–5% CO_2+3–6% O_2) in the most effective packs sealed with PS-H or with K-resin are very similar to the optimum concentrations of CO_2 and O_2 recommended for longer term CA storage of tomatoes (Isenberg, 1979), including fruit of cv. Sonatine (Geeson & Browne, unpublished data). As reported by Henig (1975), excessively modified atmospheres, e.g. in those packs sealed with polypropylene or cellulose acetate films, caused severe physiological damage to the tomatoes, ripening was completely inhibited and did not resume when the packs were perforated, when many fruits rotted. This is contrary to the findings of Ayres & Peirce (1960), who concluded that cellulose acetate films were sufficiently permeable for packaging tomatoes.

As shown in Table 4, the rates of weight loss of tomatoes in packs sealed with each of the experimental films and stored in a room at 12.5°C, 65–73% RH was inversely related to the internal RH within the packs, and in turn, correlated quite closely with the water vapour transmission rates (WVTR) of the films (see Table 1) i.e. highest rates of weight loss and lowest RH's occurred in packs sealed with films having the highest WVTR's and vice-versa. As concluded by Saguy & Mannheim (1975), the RH within sealed packs also influenced the development of fungal spoilage. For example, although the atmospheric composition within packs sealed with 25 μ polyethylene was similar to that in some PVC-sealed packs, there was a much increased incidence of rotting in the polyethylene-sealed packs where the RH was very high. Extensive rotting of tomatoes within polyethylene packs has also been reported by Saguy & Mannheim (1975) and Hobson (1981). However, the experimental packs sealed with PVC films or with K-resin in which the internal RH was between 87 and 93%, similar to the optimum RH for CA storage of Sonatine tomatoes (Geeson & Browne, unpublished data) did not encourage fungal decay.

In these experiments, packaging treatments which delayed ripening of tomatoes for < 2 weeks had no obvious detrimental effects on the sensory quality (smell, flavour or texture) of the fruit. By contrast, those packs which developed excessively modified atmospheres resulting in total inhibition of ripening caused severe off-odours and off-flavours, which concurs with the earlier findings of Ayres & Peirce (1960) and Henig (1975).

The interval between harvesting and purchase of home grown tomatoes may be as little as 1–2 days when they are sold in a large supermarket, but is usually longer for fruit marketed through other retail outlets. This study has demonstrated that ripening of part-ripe tomatoes can be successfully delayed and that their shelf life could be extended by 1–2 weeks by enclosing the fruit in MA packs sealed with suitable films (e.g. K-resin, PS-H), which have appropriate permeability to CO_2, O_2 and to water vapour. The technique is apparently equally effective for tomatoes packed at various stages of ripening between 'breaker' and light orange, typical of the colour stages at which fruit is packed commercially. Similarly, MA packs were also equally effective in delaying ripening at temperatures between 10 and 20°C, comparable to those to which tomatoes would be subjected during distribution and marketing. This could be partly explained by increases in both the respiration rate of the tomatoes and in the gas permeabilities of the films with rise in temperature over this range. Having established optimum conditions of atmospheric composition and RH in experimental packs, the development of commercially practicable MA pre-packs of tomatoes in which these conditions are developed is currently being pursued (Geeson & Browne, 1983). Implementation of MA packaging for pre-packed tomato sales could extend shelf life both for the retailer and the consumer, improve fruit quality and reduce wastage and down-

grading of tomatoes which become over-ripe during distribution and marketing. The problems of over-ripening during transportation and distribution are most serious in the case of imported tomatoes, where the time interval between harvest and reaching the shops may be 5–8 days for fruit carried over land or as sea cargo. MA packaging in the country of origin could help to prevent the problem and also facilitate the use of these cheaper but slower methods of transport, rather than relying on airfreight.

Acknowledgments

The authors are indebted to the manufacturers and suppliers of packaging materials for their co-operation and the provision of samples, and to Mr P. K. Williamson, British Cellophane Ltd., Bridgwater, Somerset, for much helpful discussion and advice. Thanks are also due to Miss Nerys Griffiths and the FRI Sensory Testing Service and to Miss H. P. Miller for expert technical assistance.

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(Received 20 June 1984)

Journal of Food Technology (1985) **20**, 351–357

Storage behaviour of freeze dried watermelon juice powder

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Summary

The effects of water activity (a_w), sugar and citric acid addition on the keeping quality of freeze dried watermelon juice have been investigated. The stability of watermelon carotenoids increases with increase in a_w. Above 0.33 a_w caking limits the shelf life. Freeze dried watermelon has maximum stability between 0.22 and 0.25 a_w. The addition of sugar and citric acid has a beneficial effect on the stability of carotenoids and in retarding non-enzymic browning. The major pigment lycopene seems to be relatively more stable than β-carotene in watermelon juice powder during storage.

Introduction

Watermelon (*Citrullus vulgaris*) is widely cultivated throughout India for fruits. The sweet pulpy flesh of watermelon is highly relished and consumed fresh. The watermelon juice forms a cooling and refreshing beverage highly valued during summer. Juice is reported to have diuretic properties and is a good source of potassium and may be very useful for athletes and commandoes. Because of its low acidity (< 0.2%) and delicate flavour preservation of juice by conventional methods has not received much attention. In recent years, freeze drying has been successfully used to dehydrate fruit juices without loss of aroma and reconstitution characteristics. Therefore the possibilities of using this process for freeze drying of watermelon juice and the storage behaviour of freeze dried watermelon juice powder were investigated.

Materials and methods

Processing of juice

Good quality watermelons (*Citrullus vulgaris*) were obtained from the local market and cleaned under running water. The fruits were halved and the red flesh was separated from the rind by stainless steel knife. The flesh was passed through a stainless steel pulper having a 2 mm mesh sieve. The freshly prepared juice had a Brix of 6°, pH 5.1 and titratable acidity of 0.07%. The juice was divided into three portions. One portion was treated with cane sugar to increase the Brix to 15° while the second portion was treated with cane sugar and citric acid to bring Brix to 15° and titratable acidity to 0.25% (pH 3.8). The third portion was processed as such without any treatment. The juice was frozen at −30°C in a Hull freeze drier (Hull Corporation, Hatboro, U.S.A.) having built-in freezing facilities and dried by maintaining sublimation temperature at −25°C and maximum product temperature at 50°C. The vacuum in the chamber was

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maintained at 250 μm during initial sublimation phase and 100 μm during final stages of drying cycle.

Packaging and storage

Samples of freeze dried juice powder (50 g) were packed in paper (60 g/m^2)-Al foil (40 μm)-polyethylene (40 μm) laminate pouches and stored in an incubator maintained at 37 ± 1°C. For studying the sorption characteristics of watermelon juice powder, 10 g samples were stored in Petri dishes (8.5 cm diameter) over saturated salt solution in desiccators according to Rockland (1960).

Analysis

The moisture content was determined by heating in a vacuum oven at 60°C for 24 hr with a minimum of 25 inch vacuum. TBA value and browning intensity were measured as described previously (Arya *et al.*, 1979). The protein, fat and reducing and total sugars were determined by AOAC procedures (AOAC, 1975).

Total carotenoids were determined by extracting juice powder (0.2–0.4 g) successively with 10 ml methanol, followed by three 20 ml aliquots of acetone and 20 ml hexane. The combined extract was washed repeatedly with 5% sodium chloride solution to remove methanol and acetone. The volume of the hexane layer was made to 20 ml and the hexane extract was freed from traces of moisture by adding a pinch of anhydrous sodium sulphate. The absorbance was measured in a spectrophotometer (Shimadzu Graphicord-240) at 472 nm and the concentration of total carotenoid was expressed as μg lycopene per g of sample using $E^{1\%}_{472} = 3450$.

Separation of carotenoids

Total carotenoid extract (20 ml) was saponified using 10 ml of 10% methanolic potassium hydroxide and keeping the reaction mixture overnight at room temperature in the dark. Saponified mixture was treated with twice its volume of water. After thorough shaking, the hexane layer was quantitatively separated and the aqueous layer was re-extracted with hexane to remove traces of carotenoids. The combined hexane layer was washed with water to make it free from alkali, dried over anhydrous sodium sulphate and the total volume was made to 25 ml.

Carotenoids were separated by TLC on silica gel G plates activated at 120°C for 1 hr using a solvent system of petroleum ether (40–60°C) and benzene (95:5). The separated bands were scraped, quantitatively extracted with acetone and absorbance measured at 450 and 472 nm. From the absorbance values the concentrations of β-carotene and lycopene were calculated using $E^{1\%}_{450} = 2500$ for β-carotene and $E^{1\%}_{472} = 3450$ for lycopene. The concentration of minor carotenoids was expressed as β-carotene using $E^{1\%}_{450} = 2500$. The bands were identified by comparing the R_f values and absorption maxima with authentic compounds.

Results and discussion

The water sorption isotherms of freeze dried watermelon juice powder (protein 6.2%, fat 0.55% and total sugars 90%) both with and without added cane sugar are shown in Fig. 1. It may be observed that incorporation of cane sugar before freeze drying significantly reduced the equilibrium moisture content of juice powder at all values of a_w. At a_w levels of 0.33 and above, watermelon juice powder undergoes extensive caking and discoloration. But at 0.22 a_w and below, the product retained free flowing

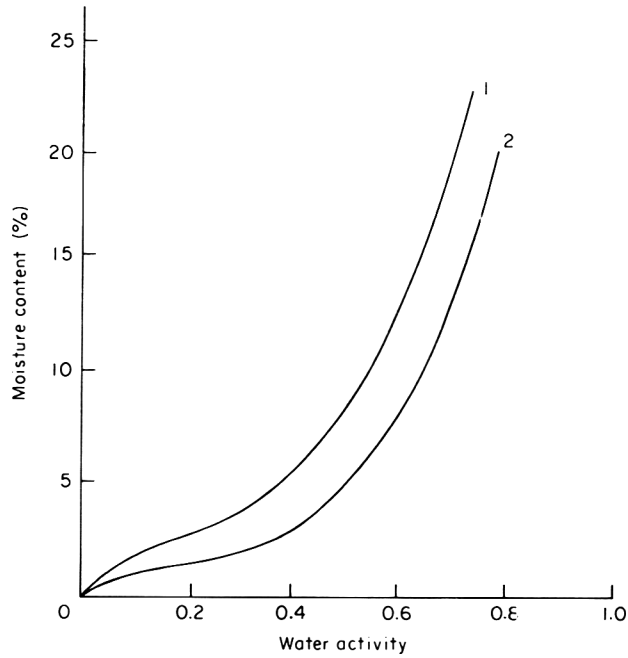

Figure 1. Adsorption isotherm of freeze dried watermelon juice powder. 1. Watermelon juice freeze dried powder: 2. Watermelon juice + sugar freeze dried powder.

characteristics and remained acceptable for more than 3 months at 37°C and 6 months at room temperature (15–35°C). At 0.22 a_w, watermelon juice powder equilibrates to 3.25% moisture compared to 1.75% moisture in the sugar fortified product. The observed caking phenomenon in freeze dried watermelon juice powder is of critical significance and may be considered as an important index in the establishment of the shelf life of the product under different environmental conditions of storage. The formation of hard cakes in freeze dried bananas even at low moisture levels has been observed previously (Lima & Cal-Vidal, 1983) and claimed to result from the structural modifications of sugars from the amorphous to crystalline state favouring release of free water (Junk, Harry & Pancoast, 1973).

Below 0.33 a_w shelf life of freeze dried watermelon juice powder is mainly governed by the changes in colour and flavour brought about by auto-oxidation of lipids and carotenoids and non-enzymic browning reactions. The freshly freeze dried watermelon juice powder had 547.5 + 10.0 μg/g total carotenoids as compared to 326.7 ± 5.0 μg/g in sugar fortified product when expressed on a total solids basis. This decrease in the concentration of carotenoids in sugar and sugar plus citric acid treated products resulted because of the increase in total solids as a result of addition of sugar with a corresponding decrease in the concentration of total carotenoids as a result of dilution. This decrease is not due to degradation of carotenoids because when expressed on the basis of watermelon juice solids alone, three types of juice powders had 547.5, 548.9 and 545.7 μg/g of total carotenoids indicating practically the same level. Total carotenoid level considerably decreased on storage in all the three types of powders. The rate of decrease was, however, substantially higher during the first month of storage and diminished considerably during subsequent months (Table 1). During the first month about 40% of the total carotenoids were lost from watermelon juice powder compared

to 21–23% loss in sugar and citric acid fortified products. After 6 months of storage the corresponding retentions were 50% in pure juice powder and 69–75% in sugar and citric acid treated products clearly showing the beneficial effect of sugar addition in minimizing the rate of degradation of watermelon carotenoids. Addition of citric acid, however, exhibited a very slight pro-oxidant effect compared to only sugar added product. TBA values of pure watermelon juice powder were relatively higher than the one having added sugar substantiating the above observation. TBA values of the watermelon juice powders did not increase to a significant extent during the first 3 months of storage; it registered a sharp increase after the fourth month but commenced to decline after the sixth month. The highest value attained was however larger in pure watermelon juice powder compared to sugar added product. A similar trend of changes in TBA values has been observed previously by Arya, Parihar & Nath (1972) in case of dehydrated rice and meat and by Seo (1976) in case of freeze dried meats. The beneficial effect of sugar in retarding auto-oxidation of safflower oil and sorbic acid has been reported previously (Sims, Fioriti & Trumbetas, 1978; Arya, 1980). In watermelon juice powder, added sugar may provide a coating to the fine pulp particles which may act as a barrier towards oxygen diffusion. Alternatively, it may also be argued that the

Table 1. Changes in total carotenoids, TBA value, browning index and sugars in freeze dried watermelon juice powder

Sample	Storage period (months)	Total carotenoids* ±10	TBA value† ±0.06	Browning index‡ ±0.04	Sugars§	
					Reducing ±0.8	Total ±1.0
A	0	547.5	0.34	0.20	61.5	89.3
	1	326.2	0.45	0.26	58.8	77.4
	2	300.8	0.32	0.21	55.5	78.2
	3	302.6	0.28	0.23	52.3	78.4
	4	300.0	0.94	0.35	51.8	78.8
	6	275.5	0.70	0.53	50.8	77.3
B	0	326.7 (548.9)	0.21	0.10	27.4	94.7
	1	259.3 (435.6)	0.30	0.11	24.8	90.3
	2	249.1 (418.5)	0.28	0.11	23.4	90.0
	3	251.1 (420.0)	0.29	0.15	23.2	90.1
	4	245.5 (412.4)	0.68	0.17	22.8	90.4
	6	245.0 (411.6)	0.68	0.27	22.4	89.8
C	0	324.8 (545.7)	0.20	0.10	26.8	89.8
	1	251.3 (422.2)	0.30	0.11	24.1	90.6
	2	240.4 (403.9)	0.27	0.10	23.3	89.4
	3	238.3 (400.3)	0.26	0.10	22.9	86.1
	4	224.5 (377.2)	0.86	0.10	22.8	86.5
	6	223.0 (374.6)	0.60	0.25	23.2	87.0

A, Freeze dried watermelon juice; B, freeze dried watermelon juice after adjusting to 15°Brix with sugar; and C, freeze dried watermelon juice after adjusting to 15°Brix with sugar and citric acid.

*Expressed as μg of lycopene/g of sample.

†Expressed as mg of malonaldehyde/kg sample.

‡Optical density of the aqueous extract at 420 nm.

§Expressed as gm/100 gm of juice powder.

Values given in the brackets are the μg of lycopene/gm of juice powder when expressed on pure watermelon juice solids.

effect of sugar may only be due to the dilution of reactants. Whichever may be the reason, addition of sugar before freeze drying significantly improves the retention of total carotenoids in juice powder besides its beneficial effects in the retention of volatile flavouring compounds (Flink & Karel, 1970a,b; Rulkens & Thijssen, 1972) during the freeze drying process.

The effect of a_w on the rate of degradation of carotenoids during storage is shown in Fig. 2. As is evident, degradation of carotenoids decreased considerably with rise in a_w. Also, the rate of degradation was significantly higher during initial stages of storage (1.5 months) and diminished significantly during subsequent months.

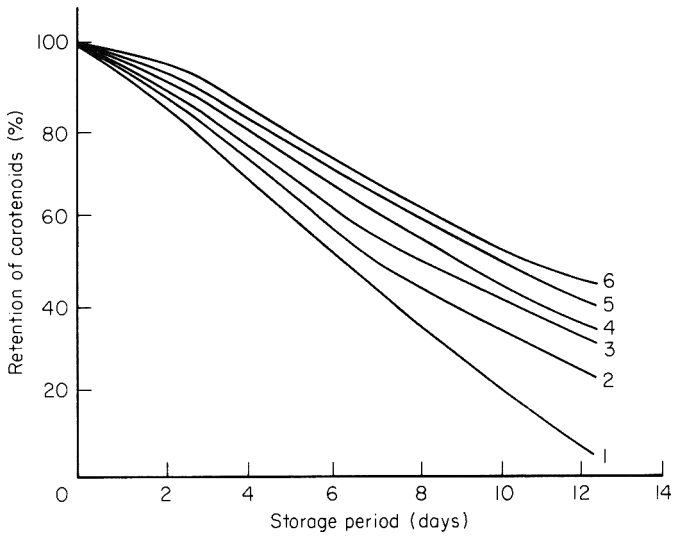

Figure 2. Effect of a_w on the stability of watermelon carotenoid extract in isolated systems. 1. 0.0; 2. 0.12; 3. 0.33; 4. 0.43; 5. 0.57; 6. 0.73 a_w.

Morgan (1967) has reported the compostion of carotenoids in watermelon juice. Lycopene (73.66%) was the major carotenoid besides neolycopene (7.64%), phytoene (2.1%), phytofluene (1.43%) and β-carotene (4.04%). In order to study the relative stability of different carotenoids in watermelon juice powder, total carotenoids were quantitatively separated by TLC on silica gel G in three fractions consisting of (i) β-carotene; (ii) lycopene; and (iii) other minor components. Relatively, the rate of degradation of lycopene was considerably lower than that of β-carotene in all the three types of watermelon fruit juice powders during storage (Table 2). After 3 months of storage only 57.6% of original β-carotene and 48.0% of other minor carotenoids were retained compared to 81.3% retention in case of lycopene. After 6 months of storage the relative percentages of retention were 31.8% of β-carotene, 48.5% of lycopene and 33% of other minor carotenoids clearly showing the better retention of lycopene in freeze dried watermelon juice powder. The same pattern was observed in sugar and citric acid added juice powders.

Falconer *et al.* (1964) have reported that 20–50% degradation of total carotenoids in dehydrated carrots was associated with the development of violet and hay-like odours. In freeze dried watermelon powder, however, no such odours were detected during storage. In the stored watermelon juice powder samples, oily, rancid and caramelized odours became noticeable after 4 months and this was accompanied by significant

Table 2. R_f values, absorption maxima, identification and concentration of individual carotenoids in freeze dried watermelon juice

R_f value	Absorption maxima (nm)	A (μg/g) after:			B (μg/g) after:			C (μg/g) after:			Identification
		0	3	6 months	0	3	6 months	0	3	6 months	
0.6	428 452 478	111.5 (100.0)	61.4 (57.6)	35.0 (31.5)	51.5 (100.0)	46.9 (90.7)	25.6 (49.6)	51.7 (100.0)	45.2 (86.9)	21.15 (57.7)	β-carotene
0.27	447 472 503	391.3 (100.0)	318.3 (81.3)	189.9 (48.5)	239.3 (100.0)	238.3 (99.7)	136.3 (56.9)	237.6 (100.0)	206.4 (86.9)	137.1 (57.7)	Lycopene
—	432 451 —	51.5 (100.0)	24.9 (48.0)	16.9 (33.0)	20.7 (100.0)	19.4 (93.6)	11.0 (53.1)	23.9 (100.0)	21.4 89.1)	19.2 (73.9)	Minor carotenoids unidentified*

A, B, C, see Table 1.

*Expressed as μg of β-carotene/g of sample.

Values in brackets indicate the percentage retention.

increase in TBA value and browning intensity of stored powders. This indicates the involvement of lipid peroxidation and sugar-amino interactions as the major causes of off-flavours in stored watermelon juice powder. The involvement of non-enzymic browning in the off-flavour development is also supported by the significant decrease in the concentration of reducing and total sugars in stored powder (Table 1). Addition of sugar and citric acid in watermelon juice before freeze drying significantly reduced the rate of browning and associated changes in reducing and total sugars. Similar observations were also made in freeze dried powders of mango, pineapple and mosambi in retarding the non-enzymic browning during storage (Ammu *et al.*, 1977).

From the foregoing discussion it is evident that watermelon juice powder is susceptible to caking, non-enzymic browning, carotenoids degradation and lipid peroxidation. Taking into consideration their relative rates, the zone between 0.20 to 0.25 a_w seems to be most suitable for maximum shelf life. This corresponds to a residual moisture content of 3.2 to 3.6% in the product. Also, addition of sugar in juice before freeze drying improves its keeping quality.

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(Received 27 July 1984)

Journal of Food Technology (1985) **20**, 359–366

Stability of watermelon carotenoid extract in isolated model systems

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Summary

The effect of water activity (a_w), fatty acid esters and adsorbents on the stability of watermelon carotenoids have been investigated. The rate of carotenoid degradation decreases with the increase in water activity. Methyl myristate, methyl palmitate, methyl stearate and methyl oleate enhanced the stability of watermelon carotenoids. In relative terms, the effect of methyl myristate and methyl palmitate was higher than that of methyl stearate and methyl oleate. Carotenoids were relatively more stable in starch, sugar and pectin systems compared to carboxymethyl cellulose and gelatin systems.

Introduction

Besides their provitamin A activity, carotenoids are responsible for attractive colours in a wide variety of fruits, vegetables and marine invertebrates. Being highly unsaturated, carotenoids are highly susceptible to auto-oxidative degradation during processing and storage and often their degradation becomes a rate limiting step in determining the shelf life of dehydrated and freeze dried foods. In complex food systems, many factors like moisture content, pro- and anti-oxidants, lipids and the nature of carotenoid influence the colour stability. Though many workers have investigated the role of various factors in the stability of carotenoids, both in foods and isolated systems, the conclusion of various studies are at variance (Arya *et al.*, 1979a,b; Arya, 1981; Baloch, Buckle & Edwards, 1977; Chou & Breene, 1972; Koloman & Gerhard, 1974; Lime, 1969; Martinez & Labuza, 1968; Quakenbush, 1963; Ramakrishnan & Francis, 1979a,b). Also, in most of the model system studies, synthetic β-carotene has been used for investigating the effect of various factors; other carotenoids have received very little attention. In the present study, effect of a_w, fatty acid esters and adsorbents has been investigated in the stability of watermelon carotenoids having lycopene as the major constituent.

Materials and methods

Reagents

All the solvents were of analytical reagent grade and used as such without further purification. Methyl myristate, methyl palmitate, methyl stearate, methyl oleate and β-carotene (synthetic) were from Sigma Chemicals Co. (U.S.A.). Microcrystalline cellulose, potato starch and sucrose were from E. Merck, Bombay; carboxy methyl cellulose was from BDH Chemicals Ltd. (England); pectin was from Marwel (Switzerland) and gelatin was from DIFCO Laboratories (U.S.A.).

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Extraction of carotenoids

Flesh (600 g) from fully ripened watermelon (*Citrullus vulgaris*, red variety) was blended with methanol-acetone (300–200 ml) in a laboratory blender. The mixture was filtered through a sintered funnel and the residue was washed repeatedly with acetone-petroleum ether (40–60°C B.P. range) mixture (500+150 ml) until completely freed from pigments. The carotenoids were transferred to petroleum ether layer by washing the extract with 5% sodium chloride solution and the petroleum ether extract was concentrated under vacuum and stored at −15°C.

Preparation of the isolated systems

Microcrystalline cellulose, carboxymethyl cellulose, pectin, sugar, starch and gelatin were passed through an ultracentrifugal mill (RETSCH, West Germany) having a 0.2 μm sieve to ensure uniformity in particle size in all the adsorbents. Suitable aliquots of watermelon carotenoid extract (0.3 mg when concentration expressed as lycopene/g adsorbent) were transferred to a 1 l round bottomed flask containing 60 g of the adsorbent. Sufficient solvent was added to form a uniform slurry and flask was swirled vigorously for thorough mixing. The slurry was dried in a rotary vacuum evaporator. The coloured powder was ground in a glass mortar to break the lumps and redried to remove the traces of solvent. To study the effect of fatty acid esters, weighed quantities (600 mg) of the methyl esters were dissolved in petroleum ether and added to the slurry before drying. Similarly β-carotene was also coated on different adsorbents.

Storage and analysis

Eight g of the coated samples were stored in Petri dishes (8.5 cm diameter) at room temperature (15–35°C) over saturated salt solutions in desiccators to obtain different a_w (Arya *et al.*, 1979a). Initially and periodically 250 mg of the samples were extracted with hexane and absorbence of the extract was measured at 450 and 472 nm using a Shimadzu Graphicord-240 Spectrophotometer. The concentration of the watermelon carotenoids was expressed as lycopene using $E^{1\%}_{472} = 3450$. In the case of β-carotene, the concentration was calculated using $E^{1\%}_{450} = 2500$.

Results and discussion

Absorption spectra of auto-oxidizing watermelon carotenoids at 0.12 a_w is shown in Fig. 1. An exactly similar pattern was observed at other a_w. In isolated systems the absorbence of extract at all the four maxima (362, 445, 472 and 502 nm) decreased significantly; but relatively decreases were considerably higher at 472 and 502 nm compared to 445 nm resulting in significant changes in the ratios of absorbences of the coloured pigment at 445/472 and 445/502 nm respectively (Table 1). The effect of a_w on the stability of watermelon carotenoids is shown in Fig. 2. It may be seen that moisture exerts a protective effect both below and above the monolayer region. The same effect has been observed by Arya *et al.* (1979a) in the case of β-carotene and Ramakrishnan & Francis (1979a) in the case of canthaxanthin and β-apo-8'-carotenal. However, papaya carotenoids were found to be more stable at 0.43 a_w in a microcrystalline cellulose model system (Arya, Natesan & Vijayaraghavan, 1983).

Addition of methyl esters of myristic, palmitic, oleic and stearic acids significantly retarded the degradation of watermelon carotenoids at all the three water activities (0.0, 0.33, 0.73). The typical pattern of degradation at 0.33 a_w is shown in Fig. 3.

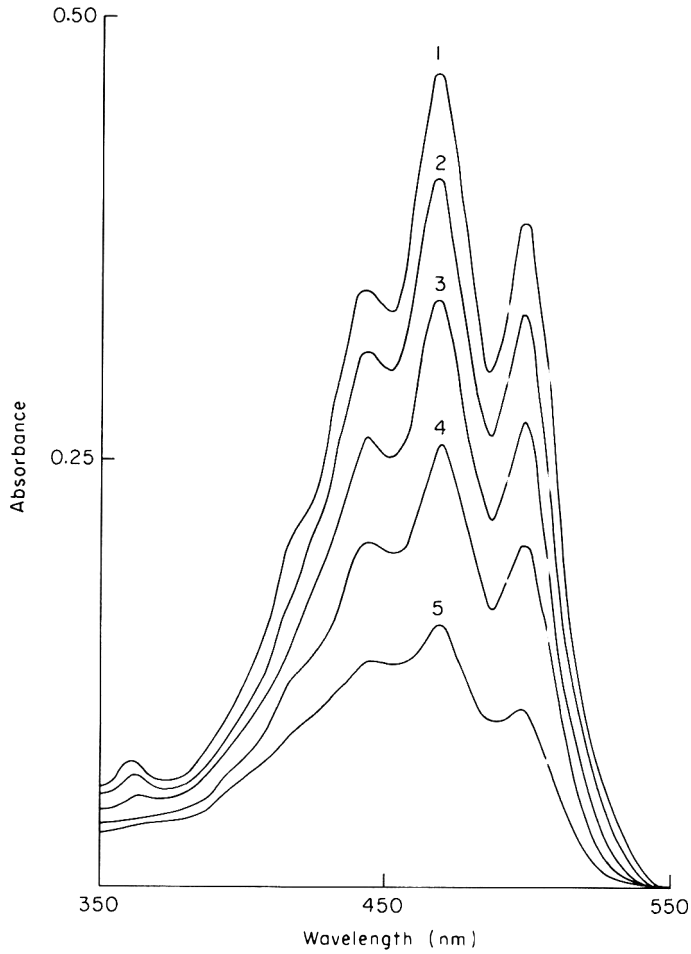

Figure 1. Absorption spectra of auto-oxidizing watermelon carotenoid extract impregnated on microcrystalline cellulose. 1, 2, 3, 4 and 5 are the absorption spectra of the hexane extract after 0, 1, 2, 3 and 5 days storage at 0.12 a_w.

Relatively, retardatory effect of methyl myristate and palmitate was significantly higher than that of methyl stearate and oleate. Previously, in the degradation of β-carotene, Arya *et al.* (1979a) have observed similar effects. Ramakrishnan & Francis (1979b), on the other hand, reported a pro-oxidant effect of saturated fatty acid esters in the auto-oxidation of β-carotene and cryptoxanthin.

Table 1. Changes in absorbance (A) ratio of watermelon carotenoids in isolated system stored at 0.12 a_w

Storage period (days)	A_{445}/A_{472}	A_{445}/A_{502}
0	0.74	0.90
1	0.76	0.93
2	0.77	0.96
3	0.78	1.01
5	0.88	1.26

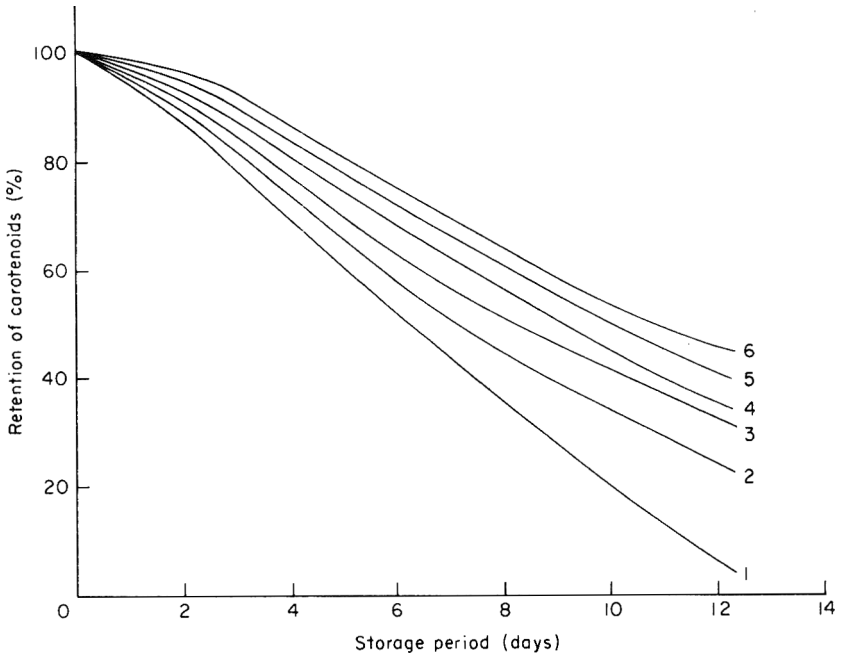

Figure 2. Effect of a_w on the stability of watermelon carotenoid extract in isolated systems. 1. 0.0; 2. 0.12; 3. 0.33; 4. 0.43; 5. 0.57; 6. 0.73 a_w.

Among the three saturated fatty acid esters, methyl stearate exhibited the least stabilizing effect at all the three a_w. Relatively, a lesser stabilizing effect of methyl stearate and tristearin was also observed in our previous studies on β-carotene degradation (Arya, Premavalli & Vijayaraghavan, 1982). This may be due to the lesser

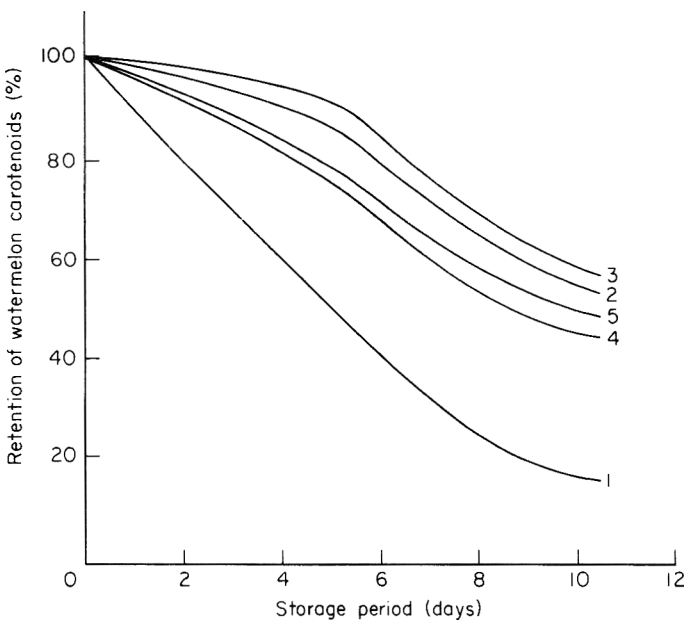

Figure 3. Effect of methyl esters on stability of watermelon carotenoid extract at 0.33 a_w. 1. Watermelon carotenoid extract (WC); 2. WC + methyl myristate; 3. WC + methyl palmitate; 4. WC + methyl stearate; 5. WC + methyl oleate.

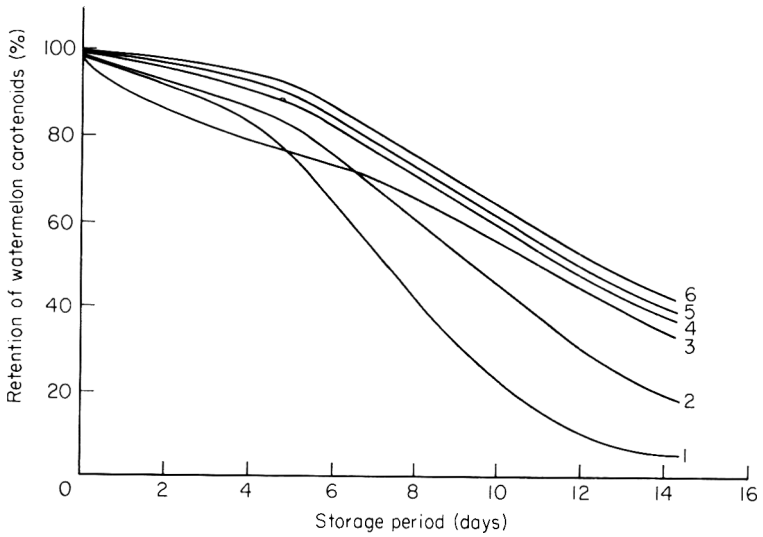

Figure 4. Effect of adsorbents on the stability of watermelon carotenoid extract at 0.0 a_w. 1. Watermelon carotenoid extract (WC) on gelatin; 2. WC on carboxy methyl cellulose; 3. WC on pectin; 4. WC on microcrystalline cellulose; 5. WC on starch; 6. WC on sugar.

solubility of carotenoids in methyl stearate or tristearin. During the course of our studies, it has been observed that stearic acid esters do not adhere well to microcrystalline cellulose particles but separate out during solvent evaporation. This is likely to reduce its dilution action and thereby its stabilizing effect towards carotenoids in the auto-oxidation process. It is also well known that in the winterization of oils, carotenoids mostly get concentrated in the liquid fractions. However, exact data on the solubility of carotenoids in different fatty acid esters are lacking in the literature.

In isolated systems, adsorbents have been reported to exert a pronounced effect on the stability of carotenoids. Ramakrishnan & Francis (1979a) have reported that carotenoids are more stable in a starch system than in a microcrystalline cellulose

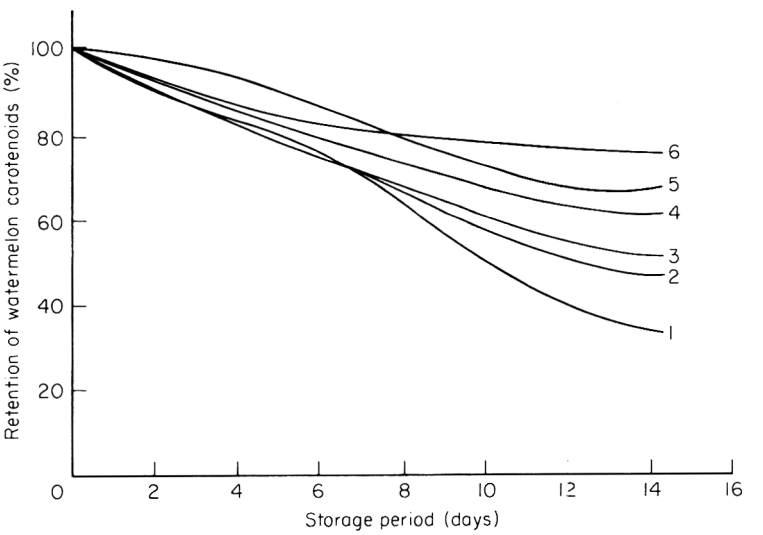

Figure 5. Effect of adsorbents on the stability of watermelon carotenoid extract at 0.73 a_w. 1, 2, 3, 4, 5 and 6 are same as in Fig. 4.

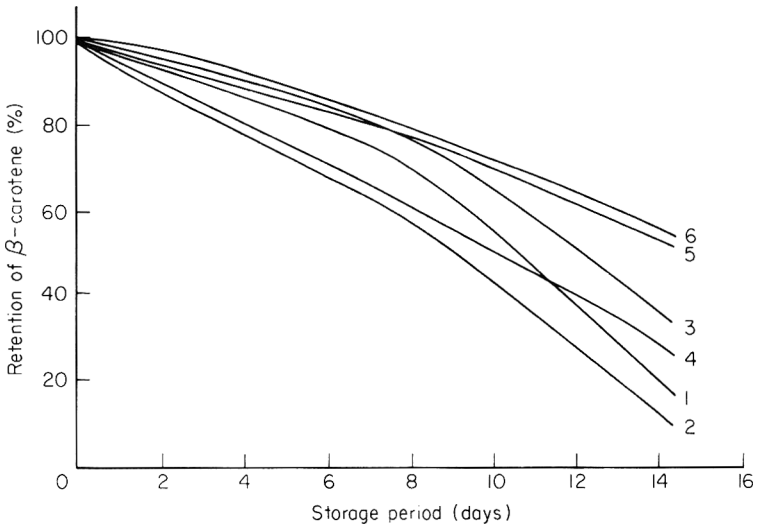

Figure 6. Effect of adsorbents on the stability of β-carotene at 0.0 a_w. 1. β-carotene (βC) on gelatin; 2. βC on carboxy methyl cellulose; 3. βC on pectin; 4. βC on microcrystalline cellulose; 5. βC on starch; 6. βC on sugar.

system. This effect was attributed to the higher moisture retention capacity of starch as compared to cellulose at a given relative humidity. Goldman, Horev & Saguy (1983), on the other hand, have reported that the nature of the adsorbent determines the concentration of adsorbed oxygen in the system which in turn governs the rate of decolorization of β-carotene. In the present study, all the adsorbents were ground in an ultracentrifugal mill having a 0.2 μm sieve before impregnation of watermelon carotenoids and β-carotene to minimize differences in total active surface area. The effect of different adsorbents on the stability of watermelon carotenoids at 0.0 and 0.73 a_w is depicted in Figs 4 and 5. Among the different adsorbents studied, sugar and starch

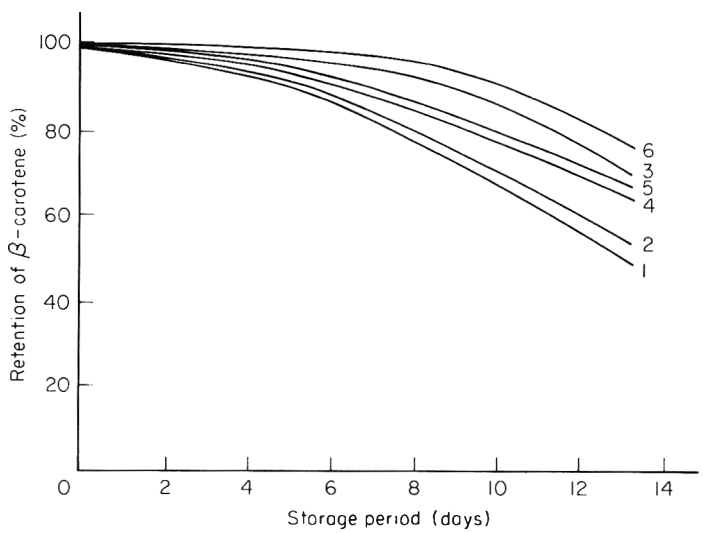

Figure 7. Effect of adsorbents on the stability of β-carotene at 0.73 a_w. 1, 2, 3, 4, 5 and 6 are same as in Fig. 6.

Table 2. Equilibrium moisture content of various adsorbents

Adsorbents	Equilibrium moisture (%)				
	(a_w) 0.0	0.33	0.43	0.57	0.73
1. Microcrystalline cellulose	0.69	2.14	3.45	3.56	4.81
2. Carboxymethyl cellulose	0.27	3.58	5.43	6.47	12.73
3. Potato starch	0.49	3.13	3.91	4.32	5.29
4. Gelatin	0.07	2.60	3.03	5.39	7.63
5. Pectin	0.13	3.36	3.61	5.77	8.41
6. Sugar	0.07	—	0.14	0.20	1.91

provided the maximum protection to watermelon carotenoids followed by microcrystalline cellulose, pectin, carboxymethyl cellulose and gelatin at both 0.0 and 0.73 a_w. In the case of β-carotene too, sugar provided maximum stability at both these a_w (Figs 6 and 7). In the case of other adsorbents, the pattern was slightly different from watermelon carotenoids. At 0.0 a_w starch provided more protection than pectin and microcrystalline cellulose, but at 0.73 a_w pectin exhibited slightly greater stabilizing action than starch. Carboxymethyl cellulose and gelatin imparted least stability to β-carotene among the various adsorbents studied. Data on equilibrium moisture contents of these adsorbents at different a_w are given in Table 2. Apparently the effect of different adsorbents does not seem to be related to their moisture retaining capacity alone. Probably, physico-chemical interactions of the adsorbents with the carotenoids may have a significant role in determining their overall effect in the auto-oxidation process besides the concentration of adsorbed oxygen and water. These interactions may play an important role in determining the stability of carotenoids in different food systems.

Acknowledgment

We wish to thank Dr T. R. Sharma, Director, Defence Food Research Laboratory, Mysore, for his encouragement and help during the course of investigation.

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(Received 26 July 1984)

Journal of Food Technology (1985) **20**, 367–374

Preservation of a perishable pomegranate product by radiation pasteurization

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Summary

The effect of gamma radiation on the quality characteristics of pomegranate kernels stored in plastic pouches at refrigerator temperatures was studied. Irradiation at doses of 0.2 and 0.4 Mrad markedly reduced the load of potential spoilage microorganisms. Fungal contaminants exhibited much higher radiation resistance than bacterial contaminants, which were eradicated at 0.4 Mrad. The predominant fungus isolated from non-irradiated pomegranate kernels was *Penicillium frequentans* while after irradiation at all doses tested kernels yielded the fungus *Sporothrix cyanescens*. An adverse effect of irradiation on colour was observed. The results of a taste evaluation panel did not indicate a significant difference between irradiated and control samples. It is concluded that the feasibility of irradiation for prolonging the storage life of packaged pomegranate kernels is questionable.

Introduction

Pomegranates are usually consumed as fresh fruit, with only a limited quantity being processed into juice, wines, etc. The special structure of the fruit is one of the main factors which limits industrial processing into food products. Recently, with the development of a machine which efficiently separates the edible part (kernels) from the fruit pericarp, a convenience product has been developed (N. Sandovsky, Y. Sarig & I. Regev, unpublished data). In the new product, the separated kernels are preserved at refrigerator temperatures in plastic pouches under a modified gas atmosphere. The storage life of this product (3–4 weeks at 1°C) is limited by off-flavours produced mainly by microbial metabolism; yeasts and acetic acid bacteria are the predominant spoilage organisms (Juven *et al.*, 1984). Attempts to extend the storage life of the new product by either thermal processing or freezing have failed due to adverse effects upon texture. Irradiation was tested in this study as a possible answer to the problem.

Materials and methods

Product preparation and storage conditions

Pomegranate (*Punica granatum,* var. Wonderful) kernels were manually separated from the whole fruit and placed on pre-formed polystyrene trays; the trays were overwrapped with a 0.09 mm thick nylon/saran/polyethylene laminate (oxygen transmission rate = 8 $cm^3/m^2/24$ hr; carbon dioxide transmission rate = 22 $cm^3/m^2/24$ hr

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at 23°C). The packages were either sealed without any treatment, or evacuated and flushed with nitrogen before sealing. The packages were stored at 1±1°C and after selected storage periods, samples (five packages each) were analysed; each container, once analysed, was discarded.

Sterile pomegranate juice

Juice was obtained by pressing the kernels through a very fine cloth. Juice for irradiation was further clarified by centrifugation (12000×g, 20 min) and then filter-sterilized. The juice had the following characteristics: 3.53 pH, 17.4 Bx and 0.83% acidity (expressed as anhydrous citric acid).

Irradiation

Irradiation was carried out at room temperature using a shipboard irradiator with a ^{60}Co source at a dose-rate of 0.1 Mrad/hr, with several doses ranging from 0.1 to 0.6 Mrad. One Mrad is equivalent to 10 kJ of energy absorbed per kg of matter, or 10 kGy.

Colour analysis

Spectrophotometric analyses were carried out on a UV-Vis Double Beam Spectrophotometer (Varian model DMS-90) and the visible spectrum (600–400 nm) was recorded with a strip chart recorder. Absorbance at 510 nm was used as a colour measure; this corresponds to the maximum wavelength of absorbance of the samples (Mishkin & Saguy, 1982). Transmission colorimetric measurements were expressed in L, a and b colour units using a Gardner colorimeter (Gardner Lab, Bethesda, MD) and 3.0 cm light pass. The colorimeter was calibrated with a dark red standard plate (L = 24.3, a = 17.6 and b = 4.9).

Taste evaluation

Taste preference was established on the irradiated kernel samples at 0, 10 and 20 days of storage, using the method described by Larmond (1970). Fifteen panellists were asked to evaluate the overall taste of the irradiated samples and to compare them with the control. The preference scale ranged from 0 (very poor) to 10 (excellent).

Microbial counts

A sample of *circa* 20 g was taken from each package, placed in a sterile polyethylene bag, and homogenized for 5 min with the Stomacher homogenizer with an identical weight of 0.3 mol/l sterile aqueous sucrose solution. Appropriate decimal dilutions of this mixture were prepared with 0.3 mol/l sucrose; 1.0 ml aliquots were plated in citric acid (CA) agar (Juven, 1979) with the addition of 100 μg/ml chloramphenicol (yeast and mould count) or 100 μg/ml cycloheximide (for bacterial count), or without any addition (for total aerobic microbial count). As total counts were approximately equivalent to the sum of fungal plus bacterial counts, they are not reported. All the plates were incubated at 20°C for up to 7 days. Logarithms of counts were calculated per ml of juice and then ascribed to the weight of kernels used to produce the juice.

In addition, sound kernels were taken in a sterile cabinet from each package and seeded in petri dishes (ten kernels per dish) containing either malt salt agar (4.5% malt agar, 4.5% NaCl/litre) or synthetic medium (SM) prepared according to Paster & Chet (1980). The plates were incubated at 27°C for 7–10 days and predominant fungi were isolated and identified.

Identification of radiation-resistant strains

Fungal cultures were identified to the species level with the assistance of the Commonwealth Mycological Institute (England).

Preparation of spore suspensions

Spore suspensions were prepared from 3–7 day old cultures grown in Petri dishes containing either CA agar (for *Sporothrix cyanescens*) or SM (for *Penicillium frequentans*). Distilled water was added to the dishes which were then shaken gently to get the spore suspensions. Final concentrations of 10^5–10^6 spores per ml were prepared in distilled water or pomegranate juice and the suspensions were exposed to the irradiation doses of 0, 0.1, 0.2, 0.3 and 0.6 Mrad. The number of colonies appearing was determined using the above described media after incubation at 27±1°C for 7 days. Logarithms of number of survivors were plotted against irradiation doses and decimal reduction values (D) were calculated from the dose-survival curves.

Results and discussion

Effect on colour

A loss in colour intensity was observed in kernels after treatment with doses of 0.2 Mrad and above (Fig. 1). The effect of irradiation on pomegranate colour stability appears to be exponential (Fig. 2). These results indicate that irradiation causes a significant change in the kernel colour, which may be explained by the high susceptibility of the juice anthocyanins. Hence, for practical purposes, irradiation of more than 0.1 Mrad is not recommended.

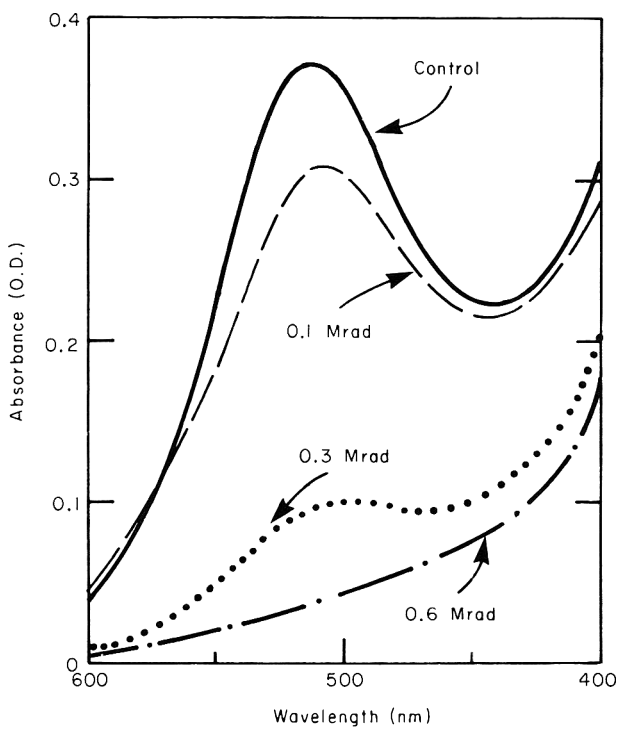

Figure 1. Effect of irradiation dose on a typical pomegranate juice spectrum.

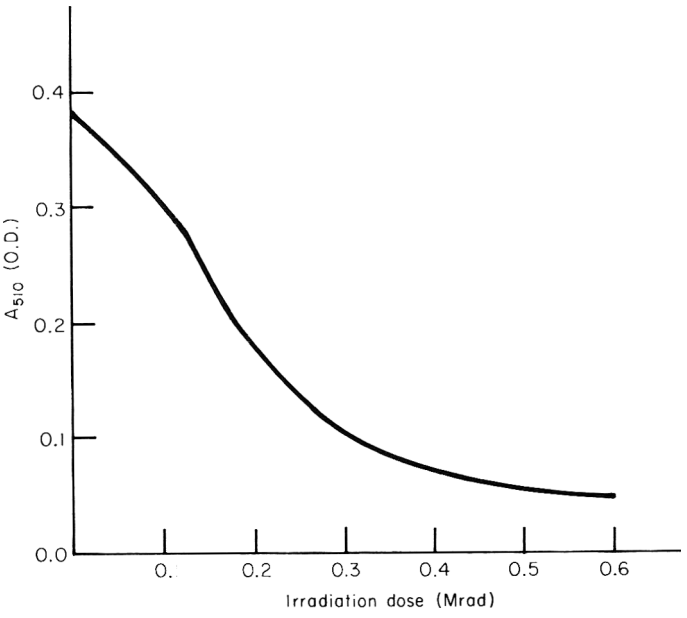

Figure 2. The effect of irradiation dose on the deterioration of pomegranate colour as expressed by the optical density at 510 nm.

Anthocyanins are known to be very sensitive to pH variation (Hrazdina, 1981). Hence, the marked discolouration was first related to possible pH increase. However, pH measurements showed an unchanged value (3.5 pH) due to irradiation or storage. An effect of irradiation on other fruit anthocyanins has been previously reported (Kiss & Farkas, 1972). It is desirable that the mechanism of discolouration of anthocyanin pigment caused by irradiation be elucidated.

Taste

The average overall score of the taste panel was 8.1 (at the beginning of the test) immediately after irradiation. The taste preference of the panellists for the irradiated samples showed that the judges were not consistent in their acceptance. Almost half of them preferred the irradiated series; hence no significant preference could be established. Yet this finding shows that irradiation of pomegranate kernels with up to 0.2 Mrad did not cause any off-flavours. Storage of the samples up to 20 days decreased the overall score, but no preference acceptance was found between irradiated and control samples.

Microbial changes in kernels

Figures 3 and 4 show the effect of irradiation (0.2 and 0.4 Mrad) on bacterial and fungal counts in pomegranate kernels packaged in either air or nitrogen and stored at 1°C.

About 4 $\log_{10}$ reduction in bacterial counts occurred in pomegranate kernels treated with a dose of 0.2 Mrad. In samples treated with 0.4 Mrad, no viable bacteria were recovered after irradiation, indicating a decrease of at least 4.5 to 5.0 logs. After 20 days' storage at 1°C, no change in bacterial counts was observed in samples treated with 0.2 Mrad and stored in air, whereas a small increase in counts occurred in samples stored under nitrogen. Within the same storage period, no viable bacteria were found in

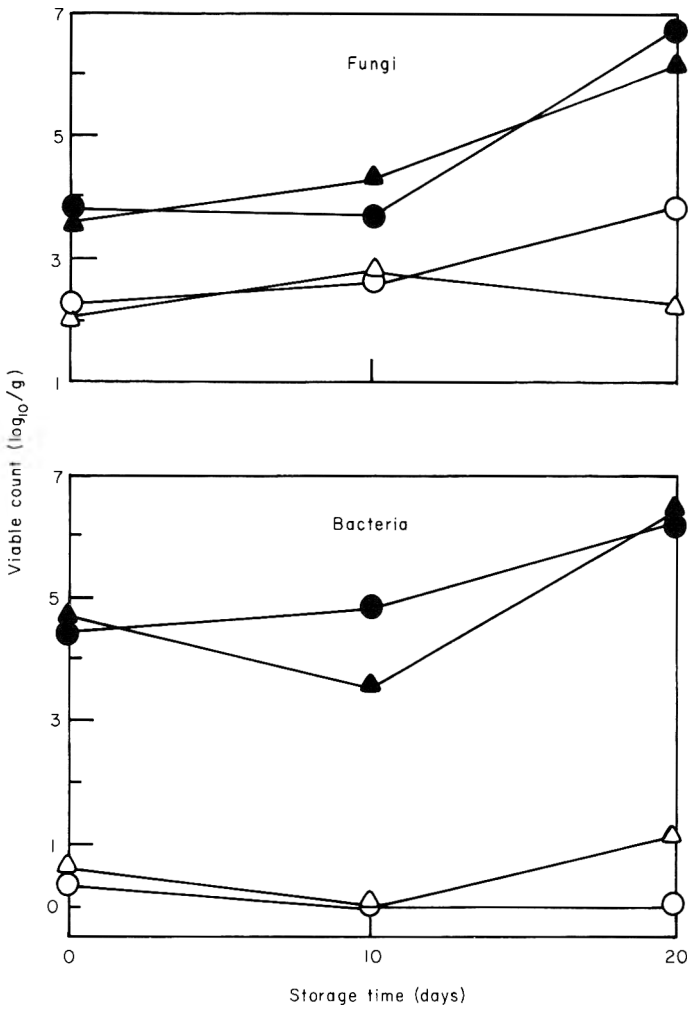

Figure 3. Bacterial and fungal counts in pomegranate kernels packaged in plastic pouches containing either air (O) or nitrogen (△) and stored at 1°C, after radiation treatment at 0.2 Mrad. Non-irradiated (black symbols); irradiated (clear symbols).

samples treated at 0.4 Mrad. In unirradiated samples, bacterial counts increased by 1.4–2.1 log after 20 days' storage at 1°C.

Fungal contaminants exhibited much higher radiation resistance than bacterial contaminants of the pomegranate kernels. A reduction in fungal counts of about 1.5 log was observed in samples irradiated at 0.2 Mrad, whereas a reduction of 1.3–2.3 log occurred in samples irradiated at 0.4 Mrad. After 20 days' storage at 1°C, very slight changes occurred in samples irradiated at 0.2 Mrad and stored in nitrogen, whereas a 2 log increase in fungal counts was observed in samples stored in air. In samples irradiated at 0.4 Mrad, an increase of about 1 log was found after an identical storage period. In unirradiated packages, fungal counts increased by 2–3 log after 20 days' storage at 1°C; changes in samples stored under air were usually higher than in those stored under nitrogen.

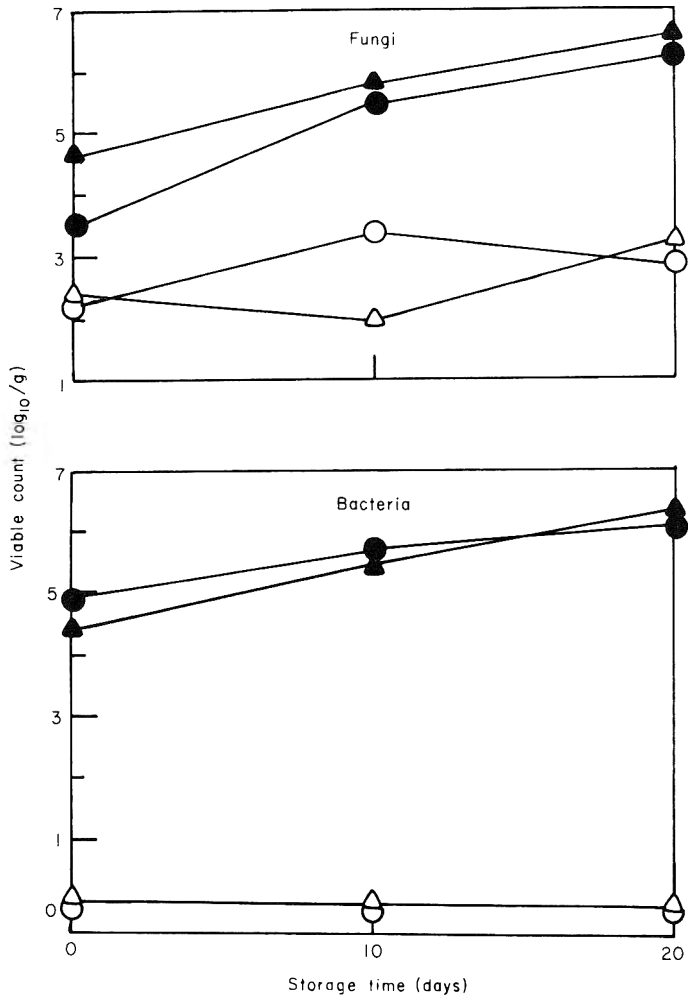

Figure 4. Bacterial and fungal counts in pomegranate kernels packaged in plastic pouches containing either air (O) or nitrogen (△) and stored at 1°C, after radiation treatment at 0.4 Mrad. Non-irradiated (black symbols); irradiated (clear symbols).

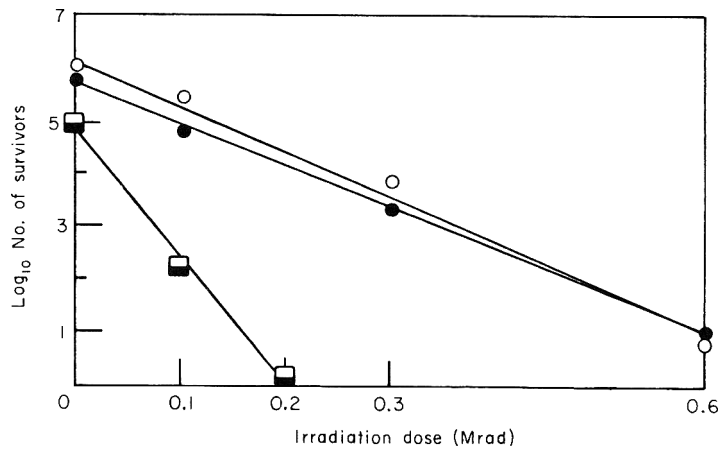

Figure 5. Dose-response curves for spores of *Sporothrix cyanescens* (O) and *Penicillium frequentans* (□) suspended in water (clear symbols) or pomegranate juice (black symbols).

Radiation resistance of fungal isolates

The predominant fungus isolated from non-irradiated pomegranate kernels was *Penicillium frequentans* and all the kernels seeded on the synthetic media yielded that fungus. Following irradiation, even with 0.6 Mrad, white colonies appeared on the kernels and they were identified as *Sporothrix cyanescens*. That fungus was predominant also when juice made from irradiated kernels was cultured on CA agar. No other fungi could be recovered from the irradiated kernels. Asexual spores of *S. cyanescens* had D values of 0.14 and 0.13 Mrad in distilled water and pomegranate juice, respectively, while those of *P. frequentans* had a D value of 0.04 Mrad (Fig. 5).

Dose survival curves of asexual spores of fungi show a wide range of resistance to irradiation. Comparison of our results with data published on the radiation resistance of various fungi (Sommer, 1973) indicates that the strain of *S. cyanescens* isolated in this study ranks among the most resistant fungal species. Information regarding the relative resistance of fungi to irradiation can indicate their ability to survive on irradiated agricultural products, thus pointing out their order of appearance after exposing the products to different doses. Indeed, a good correlation was found in our study between the resistance to irradiation of the predominant fungal spores as measured in water suspension, and their appearance on the irradiated kernels. Thus, even at the highest dose tested, viable spores of *S. cyanescens* were isolated.

A sizeable number of fruits has been irradiated for postharvest disease control and the subsequent shelf life extension. From the results of studies carried out with strawberries (Barkai-Golan, Temkin-Górodéiski & Kahan, 1967), peaches (Larmond & Hamilton, 1968), pome fruits (Sommer & Maxie, 1966), grapes (Maxie, Nelson & Johnson, 1964) and tropical fruits (Moy, 1973), it appears that moulds differ in their response to irradiation, and doses required to inhibit or delay fungal growth varied in the tested fruits.

Moreover, a major problem in applying radiation at the pasteurization dose level (0.2 to 0.3 Mrad or sometimes 0.5–0.6 Mrad) to fruits is that the host is often more sensitive to quality changes (softening, loss of flavour, etc.) than the pathogen (Moy, 1983).

Summing up the data of our experiments, it may be concluded that irradiation, at the low doses tested, markedly changed the colour of the kernels and did not destroy—although it significantly reduced—their load of potential spoilage microorganisms. In addition, the shelf life of the product was not prolonged significantly. It therefore seems that the feasibility of irradiation as a sole means of prolonging the storage life of packed pomegranate kernels is questionable.

Acknowledgment

The authors are grateful to H. Weisslowicz for technical assistance. This work was supported in part by funding from the Fruit Marketing Board.

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(Received 10 September 1984)

Journal of Food Technology (1985) **20**, 375–382

Effects of drying temperature and loading rates on cyanide elimination from cassava whole-root chips

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Summary

The effects of three oven drying temperatures (60, 70 and 80°C), each at three loading rates (10, 15 and 20 kg/m^2) on cyanide elimination from cassava whole-root chips were evaluated. Roots of two cassava cultivars, one with high (M Col 1684) and the other with intermediate (M Col 22) cyanide contents, were used. A total of twenty-seven drying experiments with chips of cv. M Col 1684 and eighteen with M Col 22 were evaluated in two factorial experiments 3×3×3, (temperature×loading×replicate) and 3×3×2, respectively. The main factor determining cyanide elimination was the loading rate rather than the drying temperature under these conditions. As the loading increased from 10 to 20 kg/m^2 the total and bound cyanide contents of dried chips decreased as a result of the higher cyanide losses. This effect was statistically significant only with the chips of the high-cyanide-containing cultivar but the same trend was observed with M Col 22. Artificial drying at the temperatures and loadings assayed allowed total cyanide losses of 81 and 69% for chips of cv. M Col 22 and M Col 1684, respectively; the residual cyanide content of dried chips of cv. M Col 1684 was three times the maximum permissible level set by the EEC for hydrocyanic acid in cassava products to be used as animal feeds. These experimental results are compared with previously published data on cyanide elimination.

Introduction

Cassava cultivars are cyanophoric, that is, capable of synthesizing and storing the cyanogenic glucosides in their edible leaves and roots (Nartey, 1978). There is, however, a wide range of root cyanide concentrations among the existing cassava cultivars (Joachim & Panditteseker, 1944; de Bruijn, 1971; Cooke, Howland & Hahn, 1978; Gómez *et al.*, 1980), which is reflected in the cyanide content of whole-root chips (Gómez *et al.*, 1983a).

Because high cyanide content limits the use of cassava products (Nestel & MacIntyre, 1973; Ermans *et al.*, 1980; Delange, Iteke & Ermans, 1982; Delange & Ahluwalia, 1983) and acyanophoric cultivars have not been found, it is important to sufficiently reduce the cyanide content by processing.

Sun drying on a concrete floor has proven to be more efficient than either sun drying on trays (Gómez & Valdivieso, 1983a; Gómez *et al.*, 1984); or oven drying at 60°C (Gómez & Valdivieso, 1984) in eliminating cyanide from cassava whole-root chips.

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Several processing parameters, such as chip geometry and size, loading per unit area of drying surface, temperature, windspeed, dehydration rates, etc., are important factors which could affect the efficiency of cyanide elimination.

Linamarase is reputed to be destroyed at temperatures above 72°C (Joachim & Panditteseker, 1944), thereby reducing the amount of cyanide eliminated (Joachim & Panditteseker, 1944; Cooke & Maduagwu, 1978). In these, and most other reports, chips prepared from peeled roots were used. In addition, only limited information on the effects of loading rate on cyanide elimination has been recorded (Gómez & Valdivieso, 1983b), and no report is available on any combined effects of temperature and loading rate during artificial drying.

The present study was undertaken to evaluate the effects of three temperatures (60, 70 and 80°C) each at three loading rates (10, 15 and 20 kg/m^2) and their interaction on cyanide elimination from cassava whole-root chips. Roots of two cassava cultivars, one with high (cv. M Col 1684) and the other with intermediate (cv. M Col 22) cyanide contents, were used.

Materials and methods

Roots of cassava plants of approximately 10–12 months of plant age were used in this study. The growth conditions of the two cassava cultivars, the processing and sampling procedures and the characteristics of the oven used were identical to those described by Gómez & Valdivieso (1984).

The experimental temperatures were monitored by the control thermostat of the oven (Despatch Model V-31, Despatch Industries, Inc., Box 1320, Minneapolis, Minnesota 55440). The dampers of the oven were adjusted to reach the operating temperature in the shortest time after the inititation of each drying period and they were maintained at fixed positions for each drying batch performed. Temperatures assayed were 60, 70 and 80°C; higher temperatures were not assessed because of the possible occurrence of non-enzymic browning (Cooke & Maduagwu, 1978). Wired-bottom trays of 0.54 m^2 each were used and, therefore, 5.4, 8.1 and 10.8 kg of fresh whole-root chips per tray were weighed in order to obtain the loading rates of 10, 15 and 20 kg/m^2, respectively.

At each drying, a total of ten trays, each with the same load of fresh chips, were sampled. Samples of fresh chips at the beginning of each drying and of dried ground chips at the end were taken for every two trays so that five samples of fresh and the corresponding five samples of dried chips were analysed. Dry matter (DM) contents were determined by drying the samples to constant weight at 60°C and cyanide (total and free) concentrations by the enzymatic assay (Cooke, 1978; 1979); bound cyanide or cyanogenic glucoside content was estimated by subtracting free cyanide from total cyanide. Samples of fresh chips were rapidly transferred (within 10–15 min after the beginning of the chipping operation) into 0.1 M phosphoric acid for cyanide analysis; samples of dried chips were ground through a laboratory mill and analyzed the day after the drying period. All cyanide concentrations are expressed on a DM basis. The mean value in each set of five samples, or per drying, was used as the experimental unit for each parameter.

Each cultivar was dried separately, at each temperature and loading rate combination; a total of twenty-seven dryings of fresh whole-root chips of cv. M Col 1684 and eighteen of cv. M Col 22 were evaluated. Henceforth, the 3×3×3 (temperature × loading × replicate) and 3×3×2 factorial experiments were separately

studied, and data were statistically evaluated by analysis of variance and Duncan's multiple range test when justified, using the Statistical Analysis System (1979).

Results

Table 1 shows the DM and cyanide concentrations in fresh chips of the two cassava cultivars, M Col 1684 and M Col 22. The DM contents of chips of cv. M Col 1684 were consistently lower than those of cv. M Col 22, and the total cyanide contents fell within the expected values for both the high- and intermediate-cyanide-containing cultivars (Gómez *et al.*, 1983a, Gómez & Valdivieso, 1983a; 1983b; 1984). In addition to the difference in total cyanide content, the proportion of free cyanide of fresh chips of cv. M Col 1684 tended to be higher than that of chips of cv. M Col 22 (21 ± 3 *versus* $15 \pm 2\%$), a trend similar to previously reported results (Gómez *et al.*, 1983a; Gómez & Valdivieso, 1984).

Table 1. Dry matter and cyanide contents in fresh whole-root chips of cassava cultivars M Col 1684 and M Col 22*

Parameter	M Col 1684	M Col 22
No. of mean values†	27	18
Dry matter (%)	32.55 ± 2.50	39.37 ± 1.39
Cyanide, DM basis		
Total (mg/kg)	1034 ± 160	540 ± 97
Bound (mg/kg)	822 ± 143	461 ± 84
Free (% of total)	21 ± 3	15 ± 3

*Each value represents the mean of the number of values indicated ± SE of the overall mean.

†Total number of observations: 135 for M Col 1684 and 90 for M Col 22.

The effects of the experimental treatments on cyanide elimination of whole-root chips were different for the two cultivars evaluated, as judged by the results of the separate statistical analyses. In the case of the intermediate-cyanide-containing cultivar (M Col 22), the only significant ($P < 0.05$) effect observed was that of drying temperatures on total and bound cyanide contents of dried chips (Table 2), but not on cyanide losses or on any other parameter. At the lower drying temperature (60°C), the total and bound cyanide contents of dried chips of cv. M Col 22 were higher ($P < 0.05$) than those obtained at the higher temperatures (70 and 80°C). Such a trend was also observed for cv. M Col 1684 although it was non-significant ($P > 0.05$) for the latter cultivar (Table 2). Drying temperatures, on the other hand, affected the final DM content, the free cyanide content and the free cyanide elimination in dried chips of cv. M Col 1684 (Table 2). No further effects of drying temperatures on cyanide elimination were found for either of the two cassava cultivars studied.

The experimental results indicated that the main factor influencing total and bound cyanide elimination was the loading rate rather than the drying temperature, notably with the chips of the high-cyanide-containing cultivar. The results as affected by loading rate, irrespective of the drying temperatures, are presented in Table 3. As the loading increased from 10 to 20 kg/m^2 the contents of total and bound cyanide decreased ($P < 0.05$) as a consequence of the higher ($P < 0.05$) cyanide losses (Table 3). Fresh

Table 2. Effects of drying temperature on cyanide elimination in cassava whole-root chips*

Parameter	Temperature (°C) 60	70	80	SD†
cv. M Col 1684 ‡				
DM of dried chips (%)	96.80[b]	97.23[b]	99.38[a]	1.19
Cyanide of dried chips				
Total (mg/kg)	348	312	296	58
Bound (mg/kg)	299	285	270	56
Free (% of total)	14[a]	9[b]	9[b]	4
Cyanide eliminated (%)				
Total	69	67	71	4
Bound	66	63	66	6
Free	78[b]	86[a]	88[a]	4
cv. M Col 22‡				
DM of dried chips (%)	95.13	94.67	97.21	1.55
Cyanide of dried chips				
Total (mg/kg)	123[a]	99[b]	85[b]	18
Bound (mg/kg)	111[a]	87[b]	77[b]	15
Free (% of total)	13	12	8	2
Cyanide eliminated (%)				
Total	79	82	82	3
Bound	78	81	82	4
Free	85	85	89	4

*Means on the same row with different letter superscripts are significantly different ($P < 0.05$).

†Standard deviation = square root of the error mean square.

‡Each value is the overall average of 9 and 6 means for cv. M Col 1684 and M Col 22, respectively.

chips loading at 20 kg/m^2 resulted in the lowest ($P < 0.05$) total and bound cyanide contents as compared to the two other loadings, as a result of the highest ($P < 0.05$) cyanide elimination. These trends were also observed, but to a lesser extent ($P > 0.05$) with the chips of cv. M Col 22 (Table 3). In both cultivars, the content of free cyanide in dried chips and the total amount of free cyanide eliminated were not affected ($P > 0.05$) by the loading rates assayed (Table 3).

The interaction of drying temperatures with loading rates evaluated did not affect ($P > 0.05$) the cyanide contents of dried chips or the cyanide losses. The only significant effect ($P < 0.05$) of the interaction was that on the drying time. At the lowest drying temperature (60°C) and loading rate (10 kg/m^2), the drying time was approximately 17 hr as compared to almost 21 hr at the highest temperature (80°C) and loading rate (20 kg/m^2). Since the dehydration rates were not monitored, it was not possible to terminate the drying periods when chips had a DM content of around 90%. In fact, the overall DM contents of dried chips were 97.80±1.58 and 95.67±2.24% for cv. M Col 1684 and M Col 22, respectively. The duration of the drying periods could be reduced to obtain final DM contents of around 90%.

In general, with the drying temperatures and loading rates assayed, dried chips of cv. M Col 22 had a final total cyanide content slightly higher than 100 mg/kg, which is the maximum permissible level of hydrocyanic acid content set by the Council of the

Table 3. Effects of loading rates on cyanide elimination in cassava whole-root chips*

Parameter	Loading rate (kg/m)			
	10	15	20	SD†
cv. M Col 1684 ‡				
Cyanide of dried chips				
Total (mg/kg)	369[a]	340[a]	247[b]	58
Bound (mg/kg)	333[a]	303[a]	218[b]	56
Free (% of total)	10	11	12	4
Cyanide eliminated (%)				
Total	63[c]	69[b]	75[a]	4
Bound	57[c]	66[b]	72[a]	6
Free	82	82	88	4
cv. M Col 22‡				
Cyanide of dried chips				
Total (mg/kg)	114	102	92	18
Bound (mg/kg)	102	91	81	15
Free (% of total)	11	10	11	2
Cyanide eliminated (%)				
Total	78	82	82	3
Bound	77	81	82	4
Free	85	88	86	4

*Means on the same row with different letter superscripts are significantly different ($P < 0.05$).

†Standard deviation = square root of the error mean square.

‡Each value is the overall average of 9 and 6 means for cv. M COl 1684 and M Col 22, respectively.

European Communities (cited by Ingram, 1975) as a quality standard for cassava products to be used as animal feeds. Only 10±3% of the total cyanide was present as cyanide free-like components (hydrocyanic acid and cyanohydrins). In the case of chips of cv. M Col 1684, the total cyanide content of dried chips was three times the maximum permissible level of hydrocyanic acid and 11±5% of the total cyanide was present as free cyanide. Under the experimental conditions assayed, the total cyanide loss for chips of cv. M Col 22 amounted to 81±4% of the initial cyanide content of fresh chips, whereas for cv. M Col 1684 this loss was of the order of 68±7%; similar values were observed for the bound cyanide losses. The content of free cyanide in dried chips as well as the free cyanide losses were similar for both cultivars.

Discussion

The results of the present study emphasize that loading rate is a critical parameter in chip drying rate and hence in cyanide elimination of cassava whole-root chips. Most of the reports on cassava drying (Joachim & Panditteseker, 1944; Correia, 1947; de Bruijn, 1971; Cooke & Maduagwu, 1978) did not mention the loading rate of cassava chips per unit area of drying surface; however, Cooke & Maduagwu (1978) did specify product drying rates. By increasing the loading, the drying time is prolonged and greater cyanide elimination is obtained apparently due to the extended periods in which

linamarase is active (Cooke & Maduagwu, 1978). This effect was clearly observed with the chips of the higher cyanide containing roots.

A previous report (Cooke & Maduagwu, 1978) has indicated that cassava bound cyanide was negligibly affected by drying at high temperatures (80–100°C), and drying at 46.5°C only removed about a third of the bound cyanide. The results of our study indicate that 80 and 65% of the initial bound cyanide content was eliminated from whole root chips of cv. M Col 22 and M Col 1684, respectively, at drying temperatures (60, 70 and 80°C) higher than 46.5°C. However, the main difference between the present study and some of the previous reports (Joachim & Panditteseke re, 1944; Cooke & Maduagwu, 1978) is that root peel or cortex was included as part of the whole root chips, which contain a higher linamarase activity than the peeled roots, and that loading was greater than that used by those workers, i.e. the drying rate was slower. Root peel usually has a higher cyanide concentration than root parenchyma (Joachim & Panditteseke re, 1944; de Bruijn, 1971; Cooke, 1978; Gómez *et al.*, 1980; Gómez & Valdivieso, 1984), but it is also a rich source of the enzyme linamarase (de Bruijn, 1971; Cooke, Blake & Battershill, 1978; Cooke & De La Cruz, 1982a). Although the total linamarase activity of the parenchyma tissue should exceed that of the peel because of the parenchyma's greater mass, the specific activity of the cyanide extracts of the peel enzyme has been found to be much higher than that of the parenchymal enzyme (Cooke, Blake & Battershill, 1978).

In tissue homogenates including whole-root homogenates, the autolytic conversion of bound to non-glucosidic (free) cyanide is rapid, but the slower conversion of the non-volatile cyanohydrin component to hydrocyanic acid has been shown to be the rate limiting step in the loss of total cyanide (Cooke & De La Cruz, 1982b). In the case of tissue pieces such as chips, however, in which the proportion of damaged tissue is relatively smaller than in the homogenates, the hydrolysis of bound cyanide determines the total cyanide loss (Cooke & Maduagwu, 1978). The results of the present study support this hypothesis and also show that the inclusion of the root peel as a rich source of linamarase enchances the hydrolysis of bound cyanide leading to higher cyanide elimination than those previously reported when root peel was not included (Joachim & Panditteseke re, 1944; Cooke & Maduagwu, 1978).

Harris *et al.* (1980) have demonstrated that hydrocyanic acid is bound to autolysis components in linseed meal homogenates and recommend continuous aeration during autolysis to remove the hydrocyanic acid as it is released. Since hydrocyanic acid *per se* is very volatile and the oven used in the present experiments was an air forced oven it is assumed that most of the actual free hydrocyanic acid was readily eliminated; therefore, it is suggested that the remaining free cyanide-like components of dried chips would be the non-volatile cyanohydrins or hydrocyanic acid bound to substrate components. This suggestion would be supported by the small variation of the free cyanide proportion found in dried chips as well as of the total elimination of free cyanide observed in this study. More detailed experimental evidence is required to confirm this suggestion.

The remaining high cyanide content of artificially dried chips of cv. M Col 1684 would pose nutritional problems when used for animal feeding. Results of a feeding trial with broilers fed diets with increasing levels of a cassava root meal containing approximately 300 mg/kg of total cyanide have shown some detrimental effects on animal performance (Gómez, unpublished data). Sun dried chips of high-cyanide-containing cultivars, such as M Col 1684 and CMC 84, however, had final cyanide levels which were low enough to allow normal animal performance and development when included in balanced diets for pigs and poultry (Gómez *et al.*, 1983). Further research is

needed in order to find adequate artificial processing procedures for high cyanide containing roots that will reduce the cyanide concentration to safe levels for animal nutrition. The results obtained so far (Gómez & Valdivieso, 1983a; 1983b; 1984; Gómez *et al.*, 1984) suggest that sun drying on a concrete floor should be the recommended method for drying whole-root chips for animal feeding.

It is interesting that despite the higher residual cyanide content, mainly present as bound cyanide, and the relatively lower amount of bound cyanide losses from chips of cv. M Col 1684 compared to those from M Col 22, the artificial drying of chips of M Col 1684, under the temperature and loadings tested, eliminated more total (716±145 *versus* 437±85 mg) and bound (583±136 *versus* 369±74 mg) cyanide than did that of chips of M Col 22. Since the experimental conditions were kept as similar as possible, these differences would suggest that, in addition to the processing factors, there may also be differences in the activity of the endogenous linamarase among the existing cultivars that would merit further research.

Acknowledgments

The authors are thankful to Dr R.D. Cooke for reviewing this manuscript, to Dr C. Wheatley for his editing of the manuscript, to Mr Eduardo Granados for his collaboration in the statistical analysis, to Ms Débora de la Cuesta and Teresa Salcedo for helping in the laboratory analysis and to Mrs Maruja Bejarano and Carmen Fuentes for stenographic work.

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(Received 19 September 1984)

Journal of Food Technology (1985) **20**, 383–388

Technical note: Influence of potassium sorbate on toxin production by *Clostridium botulinum* type A in model systems of cheese spread

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Introduction

A commercial cheese spread with onions caused an outbreak of *Clostridium botulinum* type A food poisoning in Buenos Aires, Argentina in 1974, involving six cases and three deaths (Amato de Lagarde, 1974). Magrini, Chirife & Parada (1983) made a survey of the water activity (a_w) and pH values of processed cheese spreads produced in Argentina; the a_w values of cheese spreads varied between 0.968 and 0.978 and the pH values ranged from 5.6 to 6.1. We recently reported (Briozzo *et al.*, 1983) that *C. botulinum* type A grew well and produced toxin in laboratory media having pH 5.7 and a_w adjusted to the above range, but no growth or toxin production was observed at or below a_w 0.949. It was also found that, in agreement with results obtained for media, toxin was produced in samples of processed cheese spread (a_w = 0.970; pH 5.7) incubated at 30°C. Although the labels of these products advocate refrigerated storage, they are often subjected to temperature abuse during distribution and storage. For this reason, it is important to find ways to prevent or reduce the risk of *C. botulinum* growth and toxin production in non-refrigerated processed cheese spread. Reduction of a_w will achieve this goal, but this is not always possible due to changes in flavour and texture of the cheese spread due to the increased solute concentration needed to depress a_w. Studies on *C. botulinum* growth in processed cheese have been reported by Wagenaar & Dack (1955), Grecz, Wagenaar & Dack (1965), Tanaka *et al.* (1979), Kautter *et al.* (1979; 1981), Tanaka (1982).

Sorbate is presently used as a permitted food additive in cheese spreads in the U.S.A. (Sofos & Busta, 1981); however, its use is not due to its antibotulinal properties. Sorbic acid has been long recognized as an effective fungistatic agent for foods (Sofos & Busta, 1981). In recent years, however, studies conducted in several laboratories demonstrated that sorbate (0.20–0.26%) has an effect in delaying growth and toxin production by *C. botulinum* in several products (Ivey & Robach, 1978; Sofos & Busta, 1981; Sofos & Busta, 1980; Sofos *et al.*, 1979; Sofos, Busta & Allen, 1979a; 1979b; Restaino, Komatsu & Syracuse, 1981). For this reason we investigated the effect of

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sorbate on toxin production by *C. botulinum* type A in 'cheese like' model systems whose a_w, pH, and chemical composition were adjusted to resemble that of process cheese spread produced in Argentina.

Materials and methods

Spore inoculum

Clostridium botulinum type A strain INM9 obtained from the Instituto Nacional de Microbiología 'Dr Carlos G. Malbrán', Buenos Aires, was used in all experiments. It was grown in 30 ml of Tarozzi medium (cooked meat broth, 20% peptone and 0.5% NaCl added with chopped meat pieces) at 32°C for 14 days; the spores were centrifuged and washed six times (with saline solution 0.85% w/v), resuspended in a sterile solution of NaCL 5% w/w (a_w = 0.970) and kept refrigerated until used. The first centrifugation was done at low rpm to separate meat particles, and the others at 3000 rpm during 15–20 min in a non-refrigerated centrifuge. The inocula was adjusted to yield between about 10^2–10^4 spores/ml of inoculated medium.

Model systems

The model system was prepared as follows. A basal medium (Briozzo *et al.*, 1983) was used in all experiments. It contained 25 g of trypticase (BBL Microbiology Systems), 5 g of yeast extract (Difco Laboratory, Detroit, Michigan), 5 g of glucose, 0.5 g of cysteine (Mallinckrodt Chemical Works, St Louis) and 1000 ml of distilled water. To

Table 1. Composition of the model systems used to investigate growth of *C. botulinum*

Ingredient	Weight (g) (a_w = 0.974)	(a_w = 0.967)
JoHa S-9 salt	4.16	5.24
NaCl	1.20	2.04
Na lactate	0.40	1.06
Casein	10.0	10.0
Tripticase	2.5	2.5
Yeast extract	0.5	0.5
Glucose	0.5	0.5
L-cysteine	0.05	0.05
Water	100	100
pH	5.7	5.7

adjust the a_w the following compounds were added to the basal medium: NaCl (Merck, Darmstadt, West Germany) casein (Sancor Coop. Lecheras, Buenos Aires, Argentina), sodium lactate (BDH Chemicals Ltd., Poole, England), and Joha S-9 emulsifying salt (mixture of polyphosphates and other salts, (Meyer, 1973) (Química Hoescht, Buenos Aires, Argentina). The a_w of the basal medium was 0.996 (Briozzo *et al.*, 1983) and it was reduced to 0.974 or 0.967 by regulating the concentration of the above

mentioned compounds. The amount of these salts required to obtain a particular level of a_w was calculated from literature data on their a_w-lowering behaviour (Robinson & Stokes, 1965; Chirife & Ferro Fontán, 1980; Briozzo *et al.*, 1983) and correcting for the a_w of the basal media (Chirife, Vaamonde & Scarmato, 1982). Water activity was also experimentally determined on each medium after sterilization and good agreement was always found between calculated and measured a_w values. The pH values of all media were brought to around 5.7 with HCl. Table 1 shows the composition of the 'cheese spread like' model system. Potassium sorbate (Fluka AG, Switzerland) was added to different levels (0.1%, 0.2%, 0.3% w/w).

The media were placed in screw-capped test tubes and inoculated with a heat-shocked spore suspension (10 min at 75°C). Anaerobiosis was secured by placing a layer of sterile vaspar on the surface of each medium. Ten tubes of media (pH 5.7) were used for each run at a given combination of a_w, sorbate and inoculum level, with exception of runs at $a_w = 0.974$ for which eight tubes were used for each sorbate level.

Incubation

The tubes containing the media were incubated at 32°C.

Toxin determination

The procedure employed for toxicity tests with the various media was the same as described before (Briozzo *et al.*, 1983) except that gelatin buffer (pH 6.2) was used (Duff, Wright & Yarinsky, 1956).

Determination of a_w

Water activity of media was determined with an electronic hygrometer Humicap HM 14 manufactured by Vaisala, Finland (Favetto *et al.*, 1983). The instrument was carefully calibrated with solutions of known a_w in the desired range (Chirife *et al.*, 1983).

Results and discussion

The a_w of the 'cheese-like' model systems was set at 0.967 or 0.974 since this range corresponds approximately with that reported for processed cheese spreads produced in Argentina (Magrini *et al.*, 1983). The results of the inoculation experiments were plotted as the accumulative number of tubes containing toxin/number of tubes inoculated, *versus* the incubation time at 32°C, and are shown in Fig. 1 for various combinations of a_w, inoculum size and added sorbate. A common behaviour of all systems studied was that the addition of sorbate produced a delay in toxin production of *C. botulinum* type A. In the systems having $a_w = 0.967$ sorbate addition also leads to a significant reduction in the titre of toxin produced, i.e. the minimal lethal dose per ml (MLD) sharply decreases (from 15000 to 17 MLD) with increasing sorbate concentration. In the system of higher a_w (0.974) inoculated with 1800 spores/ml the delay in toxin production seems to be of little practical value and the MLD was not affected by sorbate addition. However, in the systems of lower a_w (0.967) the delay in toxin

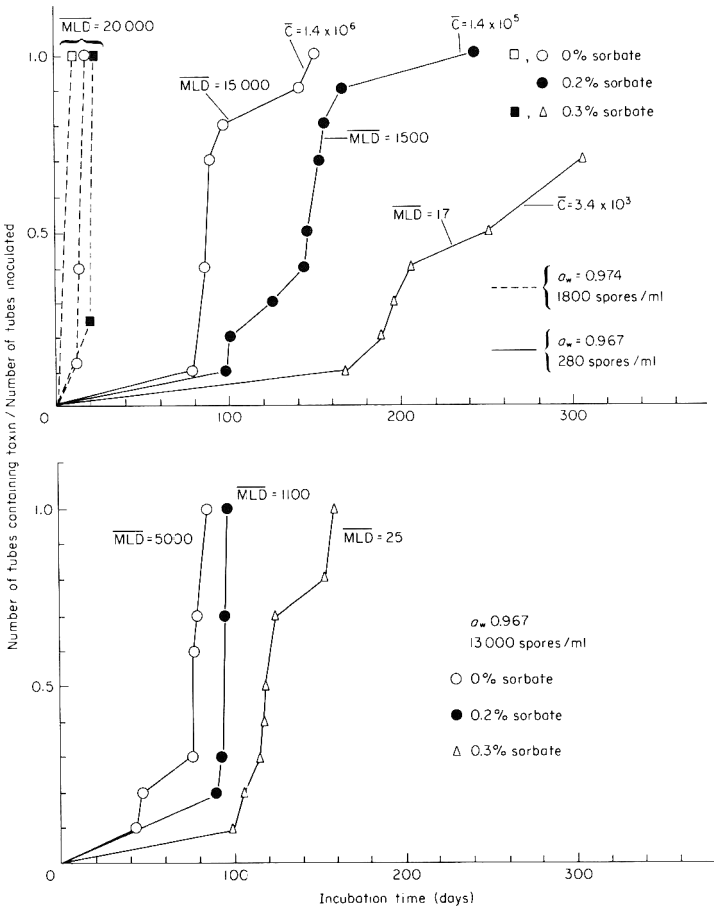

Figure 1. Effect of potassium sorbate on toxicity of model systems inoculated with *C. botulinum*; $\overline{MLD}$: minimal lethal dose per ml (average value for each run). Ten tubes of media (pH 5.7), were used for each run at a given combination of a_w, sorbate and inoculum level, with exception of runs at a_w = 0.974 for which eight tubes were used for each sorbate level. $\bar{c}$ (CFU/ml): average value of viable counts for each run.

production becomes much more important especially in the system with the lower inoculum (280 spores/ml). In this last case, the time for 50% of inoculated tubes to become toxic increased by about three-fold for the system containing 0.3% sorbate as compared to the control (0% sorbate). It appears that a combined effect exists between a_w level (even in a very narrow range as it is the case in this work), inoculum level and sorbate addition on the delay of toxin production by *C. botulinum*. In toxic samples of each system (control or sorbate added) viable number of bacteria were determined (Briozzo *et al.*, 1983) and this is also shown (for two sets of experiments) in the same Fig. 1; values reported ($\bar{c}$) are the average for all tubes of each particular run. It can be seen that in the system of a_w = 0.967 increasing sorbate concentration decreases the number of viable bacteria; the value observed for control samples (0% sorbate) was about 10^6 CFU/ml, but at 0.3% sorbate level it decreased to about 10^3 CFU/ml. It is noteworthy that the first signal of *C. botulinum* development in the present culture media, was gas production followed by coagulation of casein. Casein coagulation was not observed in samples of a_w = 0.967 containing 0.3% sorbate and low inoculum (280 spores/ml).

Cheese spreads produced in Argentina do not contain sorbate and this was confirmed by chemical analysis of several brands (data not shown).

The results of this work suggest that the use of sorbate at the 0.3% (w/w) level together with a slight reduction of a_w and pH may add an additional margin of safety against *C. botulinum* growth and toxin production in processed cheese spread subjected to temperature abuse. This is particularly true regarding the observed sharp decrease in the titre of toxin produced. However, the effectiveness of this procedure should be realistically assessed by inoculated-pack studies of the final formulated product.

Acknowledgments

The authors acknowledge financial support from Comisión de Investigaciones Científicas de la provincia de Buenos Aires and Instituto Nacional de Microbiología 'Dr Carlos G. Malbrán'.

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(Received 6 June 1984)

Journal of Food Technology (1985) **20**, 389–396

Book Reviews

Antimicrobials in Foods. Ed. by Alfred Larry Branen and P. Michael Davidson. (Food Sciences Series, Vol. 10).
New York: Marcel Dekker, 1983. Pp. x+465. ISBN 0 8247 7026 9. SFr. 187.

The title occasioned an initial shudder! When did 'antimicrobial' become a noun rather than an adjective? However, after recording a slight protest, I then settled down to the study of a book written by fourteen contributors working in the U.S.A. and Canada. The thirteen chapters are: Introduction; Sodium benzoate and benzoic acid; Phenolic compounds; Organic acids; Medium-chain fatty acids and esters; Sorbates; Sulphur dioxide and sulphites; Nitrite; Sanitizers; Halogens and surface-active agents; Dimethyl dicarbonate and diethyl dicarbonate; Nisin and other inhibitory substances from lactic acid bacteria; Antibiotic residues and their significance; and Naturally occurring and miscellaneous food antimicrobials. The recency of the references varies somewhat from chapter to chapter, but most cover the literature adequately up to and including 1981, and some to 1982.

Naturally, the discussion of regulatory and legal aspects centres on the North American scene. However, the scientific literature reviewed is drawn world wide, although with the notable exception of the chapter by C.S. Ough on DMDC and DEDC, the understandable emphasis is on English language journals or the translated editions of the Eastern European literature. References are given at the end of each chapter, rather than the more useful collation of all references at the end of the book, but a redeeming feature is the provision of a 24-page author index in addition to the subject index.

In the preface the editors state that flexibility was given to each author to allow the emphasis of those areas which they felt warranted special attention. Nevertheless, in each chapter comprehensive information is provided on the spectrum of antimicrobial activity, on applications, assay methods, mechanisms of action against microorganisms, toxicology, and legislation, with a large number of references on most of these aspects. Thus the book will prove extremely useful not only to students of food science and food technology, but also to very many research workers and to workers in the government regulatory agencies.

W. F. Harrigan

Improving Small-scale Food Industries in Developing Countries. Ed. by W. Edwardson and C.W. MacCormac. (IDRC-TS 48e).
Ottawa: International Development Research Centre, 1984. Pp. 167.
ISBN 088936 398 6.

Ever since Schumacher's slogan of 'small is beautiful', much effort has been spent around the world developing the so-called appropriate technology. This publication is the contribution by the Canadian Government's IDRC (International Development

Research Centre) to this field and, in particular, to the neglected study of small and medium scale food processors in developing countries. However, any reader glancing at the title and expecting a book giving development and design of food processing equipment will have been misled; this book sets out to illustrate the methodology of implementing improvements through management techniques.

The book is a summary of a Workshop sponsored by IDRC and held in June 1983 in Vancouver, where representatives from nine developing countries discussed the problems facing some of the small scale food industries in their countries, and their possible improvements. There are four principal sections apart from an introduction and conclusion, and as much as half the book devoted to appendices. The first section outlines the methology of examining the operation of the food industry in question, and identifying the priority problem areas. Standard techniques such as activity and flow charts, systems analysis and economic breakdown are developed and amply illustrated with examples in an appendix, which also includes advice on organizing the collection of data. The second deals with ways of improving the operation of the plant or its production by experimentation, the techniques of evolutionary operations and linear programming are discussed and fully illustrated by a case study in the appendix. The third section studies the economic evaluation of such improvement and ways of introducing them to the factory management. The fourth and largest section details two case studies to illustrate the principles discussed in the previous sections. One study involves the improvement of transparent noodle production in Thailand, and the other of soya sauce fermentation in Singapore. The advantages of a multidisciplinary approach are emphasized in implementing the study, although in both cases it was too early to know the final outcome of introducing the improvements. Examples from six other projects: breadmaking in Chile and Egypt, fish sauce in Malaysia, candied fruit in Jamaica, papadoms in Sri Lanka and fish crackers in Malaysia, are also briefly outlined along with a summary of the discussions which took place to suggest possible improvements. In addition, one of the appendices contains statistical data about the food industry in Chile, Egypt, Thailand and Malaysia.

The principal message which comes through is that the same techniques can be applied to small scale processors in developing countries as to large industries in developed countries provided there is a firm understanding of the social aspect of the enterprise, including an appreciation of the proprietor's problems and motivation.

The book is well written with few errors, although the small print and lack of a numbering system within sections causes some difficulty for the reader. On the face of it, this publication would appeal to a very limited readership. However I feel many food technologists involved in research and development would benefit by reading it, not to mention undergraduate students who should be aware of managerial methodology.

M. Woolfe

Sensory Analysis of Foods. Ed. by J.R. Piggott.
London: Elsevier Applied Science, 1984. Pp. x + 389. ISBN 0 85334 272 5. £38.00.

This book will inevitably be compared with a well-known text book on the sensory evaluation of food published in 1965 (Amerine, Pangborn and Roessler, Academic Press). The editor's purpose is 'to provide an overview of the present state of knowledge

and practice in sensory analysis'. It will be seen by most workers in the area as reflecting the progress of the last twenty years, in relation to the earlier volume.

It is organized along the same lines as Amerine *et al.*, but interesting differences emerge. Thus the opening chapters are devoted to basic consideration of the chemical senses (Plattig on The Sense of Taste; Maruniak and Mackay-Sim on The Sense of Smell) but whereas the earlier textbook devoted comparatively few pages to the other senses involved, this one has a separate chapter on Texture, Perception and Measurement (Brennan) and another on Colour Vision and Appearance Measurement (MacDougall). There follow three chapters on more practical aspects of sensory evaluation: Frijters on Sensory Difference Testing and the Measurement of Sensory Discriminability, Land and Shepherd on Scaling and Ranking Methods, and Powers on Current Practices and Application of Descriptive Methods. The last of these represents a considerable expansion of interest, compared with the earlier book. A chapter on Consumer studies of food habits by Meiselman follows the Amerine *et al.* pattern, but one concluding chapter on Statistical methods has now become two, the first by Smith (Statistical Analysis of Sensory Data) with a separate one on Multidimensional Scaling Methods by MacFie and Thompson.

The contributions of thirteen authors from four different countries could be expected to offer the advantage of ensuring wider coverage of the subject, at a more expert level, than any one author or team from a single research centre could have achieved alone. The disadvantage is that the quality varies considerably, and there is no unified overview of the separate facets of the subject matter. Each chapter stands alone demanding separate review.

It is a pity that certain areas that have been developed since the publication of Amerine *et al.* are inadequately discussed. The opening chapters, for example, tend to stress average threshold concentrations of stimuli, and make little reference to the increasing study of wide variations in human sensitivity and relationships between these and other characteristics. Chapter 7 makes some amends. It is a pity, too, that no room could be found for some contribution from the commercial side of the food industry. The last twenty years have seen, in the U.K. particularly, a massive increase in the industrial use of sensory methods, along with closer contact between scientific research interests and the more traditional, often artistic, approach of, for example, the wine and tea trades.

Here and there the student will be put off by unnecessary jargon, and indeed by avoidable errors. For example, in Chapter 8, he will have no difficulty with the incorrect reference to Table 2 within Table 15, which is itself confused with Table 16 in the text; but he may have more of a problem with the reference to a 'Dustbin' method of assessing food acceptance (Table 17) which is nowhere described in the text, and indeed could confuse it with the 'garbage' method of measuring food waste described later in the chapter.

Such minor hiccups, however, should not detract from the merits of some excellent contributions. Most, if not all, of the book will be of immense value to its intended readership—all those involved in the management and operation of sensory testing—and to those interested in food science and technology generally.

J. Harries

The Chemistry of Cereal Proteins. By R. Lásztity.
Boca Raton, Florida: CRC Press, 1984. Pp. vi+203. ISBN 0 8493 5140 5.

In this book Professor Lásztity sets out on the ambitious task of assembling current knowledge of cereal proteins in one volume, and presenting it in a unified way under chemical, biological and nutritional headings. The composition and structure of storage and cytoplasmic proteins are considered separately, and their nutritional value discussed. Inevitably the text is heavily biassed towards wheat proteins, which have been studied much more intensively than those of the other cereal grains because of their commercial importance and unique properties. The book is clearly aimed at workers active in the field and the author appends a short section setting out his views on the future aims of research in cereal proteins.

Bearing in mind Professor Lásztity's objectives as stated in the introduction, the book can be considered to be a reasonably successful attempt to achieve them. On the whole the literature coverage is good though not always as critical as one would have liked. The treatment accorded to different topics is variable and sometimes sketchy, for example the author's treatment of some of the physico-chemical aspects of wheat proteins where the discussion of the viscoelasticity of doughs and its modification by chemical methods is brief.

Unfortunately, there are numerous typographical errors in the text. In the sequences set out on p. 26 Glu appears ten times: in each case it should be Gln. On p. 56, five lines from the bottom of the page, there is the erroneous statement that 'the reduction of disulfide bonds causes an increase of the S—S linkage content', the reverse of what actually happens. On p. 10 methionine is misspelt in Table 5, and on p. 26, line 4, the name 'athins' is misspelt. These represent only a sample of the errors the reviewer noted. Furthermore, the English is sometimes strange and occasionally puzzling.

Apart from these defects, the book is well produced and printed and is easy to read. The subject index is comprehensive enough to facilitate the use of the book for reference purposes, though it is a pity that no author index is provided. In so far as it collects a great deal of information together in a single volume, it is a useful addition to the literature which most libraries will wish to add to their stock. Regrettably, however, its price is such that few individuals will feel able to purchase it.

D.W.E. Axford

Drugs and Nutrients: The Interactive Effects. Ed. by Daphne A. Roe and T. Colin Campbell. (Drugs and the Pharmaceutical Sciences, Volume 21).
New York: Marcel Dekker, 1984. Pp. xiv+601. ISBN 0 8247 7054 4. SFr. 283.

From first principles no one will be surprised to learn that drugs can affect the nutritional status of an individual, nor that one's nutritional status can influence the effects of the drug. Yet little attention is paid to this mutual interaction in the practice of medicine. This book is, however, not addressed to medical practitioners but rather to toxicologists and biochemists engaged in research.

The introduction almost excuses the gaps in medical practice by stating that even scientists have been slow to see the need to control the diets of experimental animals in testing drugs, and this criticism obviously applies to some of the earlier investigations of food additives and pollutants.

Amidst the vast presentation of information there is an explanation why certain widely used food additives had never been shown to cause harm in consumers yet have been banned as a result of animal experimentation. For example, azo dyes induce cancer of the liver but only when the diet is low in protein and low in riboflavin. Similarly, excessive intakes of the amino acid, methionine, can lead to an increase in the adaptive enzymes which catabolize the sulphur amino acids and lead to fatty infiltration of the liver if the subsequent diet is low in protein. Since the nutritional status and general health of individuals and the presence of a range of toxic materials in the environment can vary enormously, there might well be some people far more susceptible to a food additive, or indeed to any drug, than the average person. Consequently, any ill effects seen in experimental animals must be viewed with apprehension.

Recent developments in toxicology include a battery of short *in vitro* tests for carcinogenicity and mutagenicity which require prior activation of the compound under test since the substance reaching the target cells can be a metabolite of the ingested substance. The diet and the tissues can activate a foreign compound, and dietary factors can interact with cellular targets and influence detoxication and excretion.

Among the other topics dealt with is one that has long puzzled nutritionists: the effects, if any, of prolonged subclinical deficiencies of a nutrient. The present writer coined the term 'covert' malnutrition to mean that which becomes evident only under stress, and there is mention in this book of three reports of lower rates of drug detoxication (which may be beneficial or otherwise depending on the pharmacodynamics of the drug) in conditions of relatively low vitamin C status. However, several other reports fail to confirm this.

The book was written in response to a request for a state-of-the-art text on drug-nutrient interactions and its fifteen authoritative chapters with numerous references testify to its achievement. Apart from those working in the area it can be thoroughly recommended as a reference book to those involved in any way with 'chemicals in food'. The print in imitation typewritten characters is easy to read and there is a separate index of authors quoted in the various chapters. The broad coverage is indicated by the disciplines of the authors of each chapter which range from gastroenterology and food science to nutrition and poultry science.

A. E. Bender

Food Legislation of the UK: A Concise Guide. By D.J. Jukes.
London: Butterworths, 1984. Pp. iv + 128. ISBN 0 407 00357 6. £8.50.

Keeping up with the U.K. food legislation involves coping with large amounts of information and can be very expensive. Before the appearance of this Concise Guide, this reviewer was aware of only three good sources of the necessary information. One can buy the relevant Acts, Regulations, etc from Her Majesty's Stationery Office (though Her Majesty is less than completely helpful in indicating exactly what is relevant) or hunt for them, some time after promulgation, in Halsbury's 'Statutes of England' in a public library. In any case, for the practitioner there really is no substitute for having one's own copies of at least the major Acts and Regulations but every one will cost at least a pound or two, usually several. Next, one can use the excellent 'Guide to the Food Regulations in the U.K.' maintained by the Leatherhead Food R.A., fine if one works for an organization which is a member of that Research Association but their

Guide costs nearly £100 to non-members. Most thorough of all, one can buy Butterworths 'Law of Food and Drugs', giving the full text of the law and a detailed commentary, in six volumes costing £425, updated frequently for a further £180 per annum. So the first reaction to the appearance of this little guide is delight that it has been produced, especially at so comparatively small a cost. It is well produced, too, in good paperback, attractively printed and laid out and easy to use.

But, what do you get for your money? You get a good, accurate, 'snapshot' of the food law of the U.K. on the day the Concise Guide went to press. A 'snapshot' which shows the main features clearly but without any interpretation, and already dated. Between its publication and this review we have had a new Food Act, new Labelling Regulations and new Meat Product Regulations. By the time you buy your copy there may well have been more. If, like most practising food technologists, you have to be correct and up to date, this is not adequate. But if you are a student, or otherwise want a general 'snapshot' view, if you understand the limitations and you promise never to use the book unsupported in a real argument about what the law *is*, you should find it very good.

M. D. Ranken

Food Constituents and Food Residues: Their Chromatographic Determination.
Ed. by James F. Lawrence.
New York: Marcel Dekker, 1984. Pp. viii+617. ISBN 0 8247 7076. SFr. 277.

Several texts already exist on the application of individual chromatographic methods in food analysis. However, this substantial text edited by Dr J.F. Lawrence is different in that it attempts to cover all the most important chromatographic methods in selected areas of analysis. It is clearly a very ambitious task and a task that has been largely successful. The choice of analytical method for a particular determination is often very difficult and it is useful to have possible methods compared side by side in a single text. Unfortunately this concept does not seem to have been adopted throughout the book and there are three chapters that have been assigned to HPLC techniques alone. In chapter 2 HPLC of proteins and peptides is discussed, and although this technique will often prove the most effective, other simpler chromatography still deserves consideration. The same criticism can be made of chapter 4 (Carbohydrates) and chapter 7 (Synthetic food colours). Many of the other chapters do, however, discuss a wide range of methods as the title of the book would imply. Chapter 1 deals with lipid analysis in a workmanlike manner but could perhaps have included a wider coverage of the more recent liquid chromatographic separations of triglycerides. The chapter on vitamins is also written with considerable authority but some of the problems associated with efficient extraction and clean-up could have received greater coverage, as could the limitations of sensitivity of current HPLC systems, especially for B-group vitamins. Volatiles receive an in depth treatment in chapter 5 which consists of some 100 pages, the majority of which is used to describe extraction and concentration techniques prior to gas chromatography, only a few pages being allocated to identification procedures, e.g. mass spectrometry. The chapters following on phenolic compounds, mycotoxins, polynuclear aromatics, nitrosamines and pesticide residues all contain useful material and it is refreshing to see some critical appraisal of the currently available instrumentation, e.g. HPLC detectors for pesticide residue analysis. Chapter 12 deals solely

with contaminants in fish and shellfish and as the major part of the discussion is centred on pesticides, already discussed in chapter 11, this could have been omitted.

The editor quite correctly states in the preface that 'It is impossible in a volume such as this to include all natural or residual substances which may occur in foods'. However, with the present concern over synthetic food additives this topic should perhaps have received attention.

The overall standard of presentation of the book is acceptable with a high standard of illustrations. It would be helpful to have a list of the contents of each chapter, either at the beginning of the book or beginning of each chapter. There is a list of authors cited at the end of the book, which is incorrectly headed, the numbers in parentheses appear to be page numbers not reference numbers. The situation is further confused by the use of different reference systems in the chapters, two chapters use the Harvard system and the rest a numerical system. In a book as expensive as this it would have been hoped that inconsistencies such as this could be removed. In fact the author citation itself could have been removed to reduce the cost. There are a small number of typographical errors in the text. The standard of the cover and binding would be acceptable on a £5 novel but not on such an expensive book.

In conclusion this volume will prove to be a useful reference book, providing a good entry to the literature. It would have benefited from the inclusion of one or two more topics and more guidance from the editor/publisher as to the contents of each of the chapters, so that it truly covered *all* chromatographic techniques.

R. Macrae

Aspartame: Physiology and Biochemistry. Ed. by Lewis D. Stegink and L.J. Filer. New York: Marcel Dekker, 1984. Pp. xiii + 670. ISBN 0 8247 7206 7. SFr. 223.

The volume is a compilation of the biological research that was conducted to establish the 'safety-in-use' of the high intensity sweetener, aspartame (L-aspartyl-L-phenyl-alanine methyl ester). Although no other food additive has been investigated in such detail, the decision by the United States Food and Drug Administration to approve its use in foods and beverages was opposed by various consumer groups who continue to question its safety.

The subject matter describes (1) the discovery of aspartame, its sensory properties and applications and (2) the pre-clinical toxicology of the compound and that of its major breakdown product and specific studies in man or non-human primates. The latter include aspartame ingestion during pregnancy, its use by diabetics and individuals with phenylketonuria, the interaction between aspartame and monosodium glutamate, aspartame induced neurotoxicity and its effect on neurotransmitter activity in the brain. The absorption of peptides and amino-acids is reviewed as are the possible benefits of artificial sweeteners in preventing dental caries.

The standard of presentation is uniformly high, there is a comprehensive index and list of references and there are commendably few typographical errors. The publishers claim that 'it serves as an unequalled reference for nutritionists, food scientists, biochemists, dieticians, physicians, dentists and consumers' is surely an exaggeration. It is intended principally for toxicologists but as much of the information has been published in scientific journals, they may consider the cost excessive. Nevertheless, it affords a valuable insight into the regulatory process.

J. W. Daniel

Radiation Technology Handbook. By Richard Bradley.
New York: Marcel Dekker, 1984. Pp. xviii + 334. ISBN 0 8247 7271 2. SFr. 164.

In his preface the author states an intention to present the basic aspects of industrial radiation technology in a form useful to those chemists and engineers already practising in the field, and at the same time, offer a basic text to those who are just getting started. Judged by this objective some parts of the book are reasonably successful. Much of the text is easy to read and in 334 pages of double line spaced typescript reproduced by offset covers in greater or lesser detail a number of the subjects of interest to the user of industrial radiation processes. This coverage, however, is extremely uneven in detail and is clearly directed to the U.S. reader intending to use electron radiation for either the chemical modification of plastics material or to cure rubber used in the tyre industry.

The book would appear to have little to offer those who actually wish to operate processing plant or who require information about the commercially important and long established use of gamma radiation for the sterilization of medically related supplies. The extensive world wide literature on the use of radiation for the treatment of food and food components in agriculture and in the food industry for preservation purposes and to ensure that food reaches the consumer in the best possible condition is dismissed in only nine pages!

The text is generally free from typographical error and includes about 120 illustrations and tables. The former are frequently simple line drawings helpful to the person to whom the author has directed his text. The purpose of the tables is often less clear and the detail only relevant to the potential tyre manufacturer or those wishing to modify the properties of certain specific plastics. A glossary of radiation terms is included which is an arbitrary selection and many of the definitions are for commonly used words which may have a more specialized meaning in the radiation field. A brief index is included.

R. S. M. Frohnsdorff

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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References. Only papers closely related to the author's work should be included; exhaustive lists should be avoided. References in the text should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc. should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s) (b) year of publication in parentheses; (c) title of paper; (d) the title of the journal given in full and not abbreviated, set in italics (underlined once in typescript); (e) the volume number; (f) the first and last page numbers of the paper—e.g.

Steiner, E. H. (1966). Sequential procedures for triangular and paired comparison tasting tests. *Journal of Food Technology*, **1**, 41–53.

References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) place of publication and publisher—e.g.

Lawrie, R. A. (1979). *Meat Science*, 3rd edition. Oxford: Pergamon Press.

In the case of edited multi-author monographs, the editor(s) should be indicated in parentheses after the book title—e.g.

Hawthorn, J. (1980). Scientific basis of food control. In *Food Control in Action* (edited by P. O. Dennis, J. R. Blanchfield & A. G. Ward). Pp. 17–33. Barking, Essex: Applied Science.

Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. 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 one hundred or greater.

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

SI UNITS

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

Chemical formulae and solutions. The correct molecular formula should be used indicating whether the substance is anhydrous or hydrated. Solutions should be given in terms of molarity. The arithmetic basis of other expressions of concentration must be clearly defined.

Species names. In addition to common or trivial names of plants, animals or microorganisms, the species should normally also be indicated by use of the latin binomial at least once. This should be given in full and italicized (underlined once in typescript). In subsequent use, the generic name may be abbreviated to a single letter provided that this does not result in ambiguity.

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. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs, The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to figures'.

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in bold with an Arabic number, e.g. **Table 2.** Each table must have a caption in small letters. Vertical lines should not be used.

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Journal of Food Technology
Volume 20 Number 3 June 1985

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Printed by Adlard and Son Ltd, Bartholomew Press, Dorking