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1. INTRODUCTION

Fermented foods from plants are to be found all over the world. Many have their origins in ancient times. Fruits, vegetables, cereals, rootcrops, legumes and oilseeds are all used for the production of fermented foods. An important result of the fermentation of fruits and vegetables is that products are obtained which can be stored and used as a food supply in between harvests. Cereals and seeds can best be stored as dry products, and shelf-life extension has probably never been the primary aim of fermentation of such commodities. For both categories of fermented foods, i.e. those derived from fruits and vegetables and those derived from cereals and other seeds, however, the bioconversions caused by fermentation contribute very much to the character and organoleptic properties of the fermented products. In many cases also fermentation contributes to the digestibility and nutritional value of the final products. For example, soya beans can be stored relatively well in the dry state, but, as such, they are not readily consumable by humans even when cooked. When converted to tempe they are a base material for several delicious, easily digestible and nutritious food items, which provide many millions of people with a valuable and affordable source of proteins.

Some of the most important fermented foods of plant origin are listed in Table 1. It is obvious that these cannot be treated in sufficient detail in one contribution. Thus a choice has been made. South-East Asian tempe and African fermented cereal doughs are considered in detail. Some research developments in Europe with respect to using fermentation principles in minimally-processed foods are also discussed. Microbial associations as well as food safety aspects of these products will be emphasized.

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2. TEMPE

Tempe is a traditional Indonesian fermented food made from soya beans. A large number of other leguminous seeds, cereals or by-products may occasionally be used, but yellow-seeded soya beans are the preferred raw material (Nout and Rombouts 1990). Fungi, particularly *Rhizopus* spp., play an essential role in tempe fermentation. A production scheme is presented in Fig. 1. Variations to this general scheme exist. Soya beans are soaked (hydrated) and dehulled, before or after soaking, to yield moist cotyledons. The soaking of beans may take 12–48 h. This allows for bacterial proliferation. Steaming or boiling in excess water favours the subsequent fermentation process as well as improving the digestibility of the finished product. After draining, cooling and superficial drying the cotyledons are inoculated with fungal spores. Traditionally the inoculum was produced by the growth and sporulation of the tempe moulds on plant leaves which were dried and used as inoculum. Nowadays spore preparations are produced from mould growing on starchy substrates such as rice. The inoculated bean mass fashioned to particular sizes is wrapped in plant leaves. Alternatively the mass is put in trays to a depth of 4–6 cm and covered with leaves or plastic film. Incubation time depends on temperature and may vary between 20 and 50 h at 37 and 25°C, respectively. During this time mycelium proliferates and binds the bean cotyledons into a coherent cake having a whitish, creamy colour and a clean, mushroomy or nutty flavour. This bean-cake is the normal form in which tempe is sold. For consumption it is sliced and deepfried in oil or boiled in soup.

Table 1 Fermented plant foods (cf. Campbell-Platt 1987)

Country or region	Primary product	Type of fermentation	Fermented product
Europe	Grapes	Alcoholic	Wine
Global	Barley, other cereals	Alcoholic	Beer
Global	Cereals	'Alcoholic'	Bread
Japan	Rice	Alcoholic	Sake
Africa	Maize, other cereals	Lactic	Mageu, uji, ogi, kenkey, mawè
Mexico	Maize	Lactic	Pozol
Africa	Cassava	Lactic	Gari
Japan, South-East Asia	Soya beans	'Moulds', lactic, alcoholic	Shoyu (Soya sauce)
Japan, South-East Asia	Soya beans, cereals	'Moulds', lactic, alcoholic	Miso
Indonesia	Soya beans	'Moulds', lactic	Tempe
Europe, USA	Cabbage	Lactic	Sauerkraut
China, Korea	Cabbage	Lactic	Kimchi
Europe, USA	Cucumbers	Lactic	Pickles
Europe, USA	Olives	Lactic, alcoholic	Fermented olives
Africa	Locust beans	*Bacillus* spp.	Dawadawa

2.1 Microbial associations

The microbial composition of traditionally prepared tempe may vary considerably. Bacterial counts are in the order of 10^8–10^9 cfu g^{-1} and yeasts occur in numbers up to 1×10^7 cfu g^{-1} (Mulyowidarso *et al.* 1990). The most commonly encountered bacteria include lactic acid bacteria, Enterobacteriaceae and *Bacillus* spp. *Trichosporon beigelii*, *Geotrichum candidum*, *Clavispora* (*Candida*) *lusitaniae*, *Candida maltosa* and *C. intermedia* are the most commonly found yeasts (Samson *et al.* 1987; Mulyowidarso *et al.* 1990).

Invariably fungal mycelium has developed profusely in good quality tempe and *Rhizopus* and *Mucor* spp. are isolated most frequently. In fact Ko and Hesseltine (1961) showed that good quality tempe can be made by using an inoculum of certain species of *Rhizopus*, including *Rhizopus oligosporus*, *Rh. stolonifer*, *Rh. oryzae* and *Rh. arrhizus*. *Rhizopus oligosporus* appears to be the principal fungus in Indonesian tempe (Hesseltine *et al.* 1963). This species is often the only one to be used to produce spores as an inoculum for tempe production in countries outside South-East Asia.

There are two stages in tempe manufacture in which microbial proliferation occurs: the soaking stage and the incubation stage following inoculation with spores of *Rhizopus* spp.

2.2 The soaking process

The soaking (hydration) process may last 12–48 h at a temperature of *ca* 28°C; natural acidification occurs at this time. In the early stages of soaking the microflora is quite diverse and includes several members of the Enterobacteriaceae: *Citrobacter diversus*, *Pantoea*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Kl. ozaenae*. These disappear with increasing acidification and are replaced with principal micro-organisms contributing to natural acidification, viz *Lactobacillus casei*, *Enterococcus faecium* and *Streptococcus dysgalactiae* (Mulyowidarso *et al.* 1989). Sufficient acidification during the soaking stage is important from the point of view of food safety. Although the soaking stage is followed by boiling and draining, the outgrowth of pathogenic micro-organisms during fungal fermentation is reduced if beans are sufficiently acidified. This is particularly true for *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. All of these occasionally occur at unacceptably high levels in commercial tempe (Samson *et al.* 1987). Challenge tests (Tanaka *et al.* 1985) have shown that *Salmonella typhimurium*, *Yersinia enterocolitica* and *Clostridium botulinum* could grow out in under-acidified beans. This shows the need for adequate heating (deep frying or boiling) for the safe consumption of tempe.

Sufficient acidification in the soaking stage is important both from the point of view of food safety and failure of tempe fermentation due to excessive development of *Bacillus* spp. and Enterobacteriaceae. It has been shown by Nout *et al.* (1987) that a reliable and accelerated acidification (pH 4·3) can be achieved by adding (back-slopping) a part (about 5–10% v/v) of the previous soakwater to subsequent batches of beans. At temperatures varying from 19 to 37°C, *Lact. plantarum* and *Saccharomyces dairensis* became the dominant organisms. Beans prepared in this way yielded a

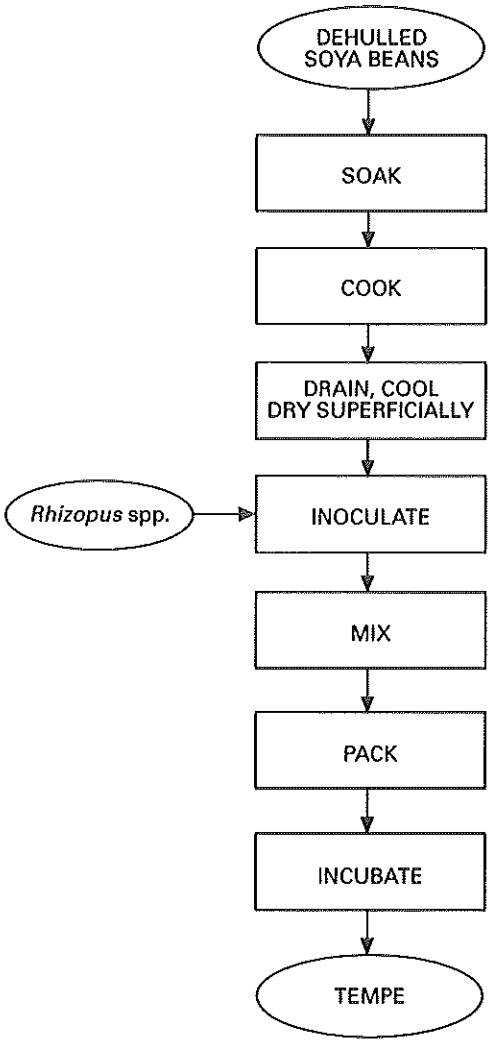

Fig. 1 Flow diagram for tempe production

good quality tempe with a normal pH of 6·1 following fermentation with *Rh. oligosporus*. With accelerated acidification the concentration of acetic acid should not rise above 0·3% (w/w), as this would inhibit fungal spore germination (De Reu *et al.* 1995b); normally the concentration of acetic acid in cooked and drained beans does not exceed 0·1% (w/w).

2.3 The fungal fermentation process

Various methods exist for the inoculation of soya beans with sporangiospores of *Rhizopus* spp., ranging from the traditional Indonesian 'usar' leaves (heavily sporulated *Rhizopus* spp. grown on soya beans wrapped in *Hibiscus* leaves; Nout *et al.* 1992) to spore suspensions of a single fungal species, usually *Rh. oligosporus*. *Rhizopus* comes to dominance even with mixed fungus inocula, mainly because of its quick growth in this environment (Nout and Rombouts 1990). Inoculation density, approximately 1×10^4 cfu g^{-1} inoculated bean mass, should be such that fungal development proceeds at the correct rate. Failures of fungal fermentation due to suboptimal inoculation are associated with the excessive proliferation of bacteria and yeasts. As the fungus has a respiratory metabolism, considerable heat is developed during growth—up to 2500 kJ kg^{-1} fermented dry matter (Aidoo *et al.* 1982). Indeed over-inoculation may lead to overheating due to excessive fungal activity; heat inactivation of the fungal mycelium with the concomitant proliferation of *Bacillus* spp. results in a foul smelling, slimy product.

Temperature, pO_2 and pCO_2 are critical parameters in the fungal fermentation. The Indonesians have learned empirically to control these factors by choosing the dimensions of the blocks of soaked beans and the manner of packaging (banana leaves or perforated polyethylene film). If the traditional fermentation is to be carried out in a modern solid-state operation these factors also have to be considered. Spore germination of *Rh. oligosporus* is optimum at pH 4·0 and 40°C; optimum growth occurs at 40°C. The fungus cannot grow under anaerobic conditions but, as its K_s value for oxygen is $<1\%$ (v/v), oxygen becomes a limiting factor only at $<1\%$ (v/v) (Nout and Rombouts 1990; De Reu *et al.* 1995a). Carbon dioxide stimulates the growth rate at low oxygen concentrations: 0·043 h^{-1} at 0·5% (v/v) oxygen and 0% (v/v) carbon dioxide and 0·096 h^{-1} at 0·5% (v/v) oxygen and 5% (v/v) carbon dioxide (De Reu *et al.* 1995a). Growth yield is also increased, even at carbon dioxide concentrations up to 20% (Seaby *et al.* 1988). At 0·5% (v/v) oxygen and 35% (v/v) CO_2, however, growth is inhibited (De Reu *et al.* 1995a). The stimulatory effect of carbon dioxide on growth may be due to carbon dioxide fixation to pyruvate by pyruvate carboxylase, yielding oxaloacetate (Gadd 1988).

Recently, De Reu *et al.* (1993) described a rotating drum reactor for controlled dynamic solid-substrate fermentation. In contrast to other rotating drum fermenters, this reactor has options for the measurement and control of multiple parameters (cf. Fig. 2 for temperature control). The system was tested on soya bean fermentation with *Rh. oligosporus*. A granular product was obtained and, in spite of discontinuous rotation, fungal growth continued and a product was obtained comparable with normal tempe. Further model studies showed that both aeration and heat removal were necessary for optimization of large-scale solid-substrate fermentations, such as for tempe manufacture (De Reu *et al.* 1995b).

2.4 Bioconversions

Rhizopus oligosporus produces a variety of carbohydrases, lipases, proteases, phytases and other enzymes. Pectinases,

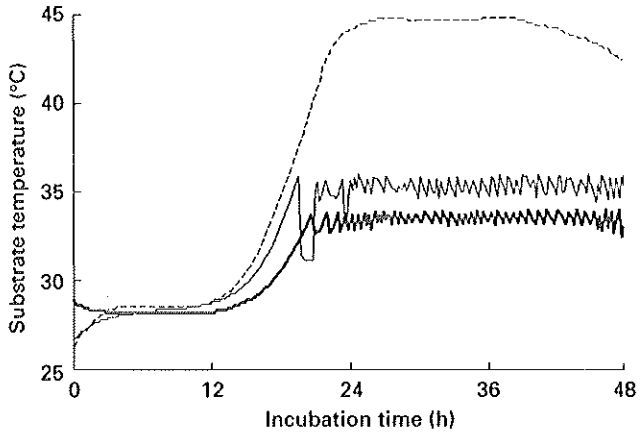

Fig. 2 Temperature changes in soya beans fermented with *Rhizopus oligosporus* in a rotating drum reactor. Ambient temperature, 30°C; air flow temperature, 29°C, relative humidity, 95%. ----, Static incubation; ———, discontinuous rotation with temperature set at 34°C; ———, discontinuous rotation with temperature set at 36°C (De Reu *et al.* 1993)

xylanases, arabinanases and cellulases are involved in cell wall degradation, resulting in tissue maceration and softening (Sarrette *et al.* 1992). Other fungal carbohydrases, including α-galactosidase and invertase, are important in the hydrolysis of oligosaccharides such as raffinose and stachyose which occur naturally in soya beans. As these oligosaccharides cause flatulence in humans, removal from beans by endogenous enzymes (Mulyowidarso *et al.* 1991) or fungal enzymes during fermentation is desirable.

There is considerable turnover of lipids during tempe fermentation (De Reu *et al.* 1994). Fatty acids bound to glycerol decrease by *ca* 50%. This decrease is not matched with a concomitant increase in free fatty acids. Indeed the latter are apparently an important substrate for fungal growth. The effects of tempe fermentation on total nitrogen content and amino-acid composition are rather small, but proteolysis results in increases of free amino acids, peptides and ammonia (Nowak and Szebiotko 1992).

The changes in carbohydrates, lipids and proteins improve the digestibility of tempe *vis-à-vis* soya beans. Digestibility is also favoured by the removal of various anti-nutritional factors during the tempe-making process. In addition to the oligosaccharides noted above, soya beans contain protease inhibitors, haemagglutinins (lectins), tannins, favism-inducing factors such as vicine and convicine and Ca, Mg, Zn and Fe-chelating phytates. Most of these are removed or inactivated by soaking and boiling. Phytates are hydrolysed by bacterial and fungal phytases. Fungal fermentation also leads to an increase of vitamin content, notably riboflavin (B_2), nicotinic acid, pantothenic acid, pyridoxin (B_6), folates and biotin; thiamine (B_1) may be reduced by about 50% (Nout and Rombouts 1990). The presence of cyanocobalamin (B_{12}) is important when tempe is used in vegetarian diets. It is practically absent in soya beans and in the dietary context no physiologically active B_{12} is produced by *Rh. oligosporus*. Its production in tempe is ascribed to *Kl. pneumoniae* and *Cit. freundii* (Keuth and Bisping 1993), both of which may possibly be carried over from the soaking process and grow during fungal fermentation.

2.5 Research options

In the tempe process soya beans or other leguminous seeds, which are not readily consumable, are converted to an attractive, easily digestible proteinaceous food with an attractive vitamin content. Tempe is an essential component in the diet of many millions of people in South-East Asia. The international interest in tempe technology and tempe consumption is increasing. Obvious objectives for further research in tempe technology are: improvements in the rather wasteful soaking process and developments in solid-state fermentation. The latter must take into account the need for dynamic control of temperature and composition of the gas atmosphere. Safety and health are also important aspects. The traditional non-aseptic process should be studied further in order to define and control systems that avoid infections and intoxications from this commodity. More studies, particularly with humans, need to be done in order to validate various claims made about nutritional and health benefits of tempe as a food or food ingredient.

3. AFRICAN FERMENTED CEREAL PRODUCTS

Cereals have been part of man's diet from the earliest times. In the Middle East and Europe preparation of cereals for consumption has evolved into bread-making. However, in Africa different practices have developed. Large quantities of sorghum, millets and particularly maize are soaked, wet milled and then fermented, either as a gruel (suspension), e.g. Nigerian ogi, Kenyan uji and South African mageu, or a dough, e.g. Ghanaian kenkey and Beninese mawè. Both can be cooked as a sour porridge. Additionally they are ingredients for a variety of other foods such as steam-cooked bread, fritters and couscous. Of these indigenous fermented products, ogi and mageu have been most extensively studied (Onyekwere *et al.* 1989; Holzapfel 1989). In fact, production of mageu (or mahewu) has developed into a large-scale commercial operation in South Africa and neighbouring countries (Holzapfel 1989). Until recently, relatively little attention was paid to some of the other products, such as kenkey and mawè. They essentially

differ from ogi or uji and mageu in being produced as fermented doughs rather than slurries.

3.1 Kenkey

A production scheme for Ghanaian maize kenkey is given in Fig. 3. Until recently the production of kenkey was mainly operated as a very small household affair, mostly by women. Larger production units with capacities of several tons of fermented dough per week are now in operation (Halm *et al.* 1993). Out of preference kenkey is made from maize, a cereal introduced into West-African countries by the Portuguese in the 16th century. Maize is cleaned, soaked for 2 d, and milled coarsely to a whole meal. It is made into a dough and allowed to ferment naturally for up to 4 d. Part of the dough is slurried and cooked to gelatinize the starch. A sticky paste known as aflata is produced. The aflata is mixed with the remainder of the dough, kneaded and portioned (300–500 g) into balls. These 'dumplings' are shaped and wrapped in maize sheaths (Ga kenkey) or banana leaves (Fanti kenkey) and boiled in water. In urban areas the product is sold in this ready-to-eat form.

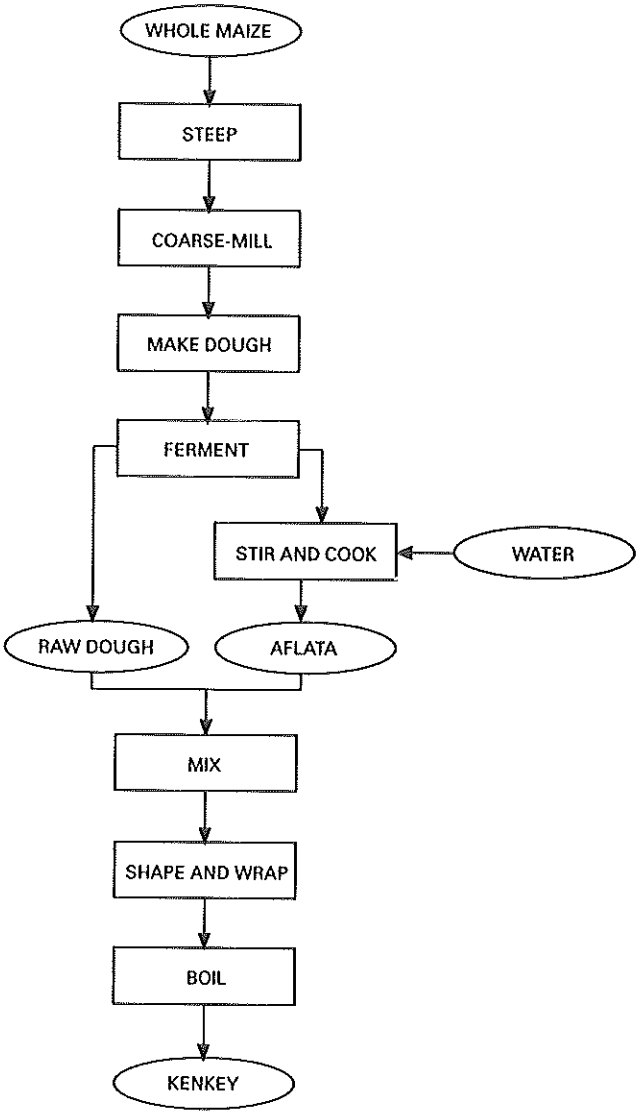

Fig. 3 Traditional kenkey process (adapted from Nche *et al.* 1994a)

Micro-organisms develop during both the soaking and fermentation stage. A detailed study of the microbial ecology of the kenkey process in a large commercial production unit was made by Halm *et al.* (1993) who confirmed and extended the findings made in earlier work. They found that, in the early stage of soaking, a mixed flora was present. It included lactic acid bacteria, catalase-positive Gram-positive bacteria, Gram-negative bacteria, yeasts and moulds. During steeping of the maize (24–48 h), natural selection led to a significant increase of lactic acid bacteria and a sharp reduction of catalase-positive bacteria, with concomitant reduction of pH from 5·9 to 4·2. After milling and dough production the pH had changed slightly (5·3) but it was invariably below 4·0 at the end of fermentation. During fermentation lactic acid bacteria increased rapidly to $>10^9$ cfu g^{-1}; yeasts increased to *ca* 10^6 cfu g^{-1} but their number decreased towards the end of fermentation. At this time, catalase-positive bacteria and moulds were no longer detectable.

At the advanced stage of fermentation, more than 96% of isolates were obligately heterofermentative lactobacilli with carbohydrate fermentation patterns most closely similar to those of *Lact. fermentum* and *Lact. reuteri*. Facultative anaerobic homofermentative cocci were found mainly in samples of home-made kenkey. These were identified with *Pediococcus pentosaceus* and *Ped. acidilactici*. *Candida krusei* and *Sacch. cerevisiae* were the most frequently isolated yeasts. Recently, the microflora of fermented sorghum dough was analysed. Over 99% of isolates were lactobacilli, mainly *Lact. fermentum*, *Lact. reuteri* and *Lact. amylovorus*. *Candida krusei* was the dominant yeast in this product (Hamad *et al.* 1992).

Major fermentation products were D- and L-lactic (14 g kg^{-1} dough) and acetic acid (1·8 g kg^{-1}). Other important aroma compounds, occurring in mg quantities kg^{-1} dough, were acetoin, 2,3-butanediol, propionic acid and butyric acid (Halm *et al.* 1993).

From a food safety point of view it has been established that fermented kenkey has considerable antimicrobial potential against enteric pathogens such as enterotoxic *E. coli* and *Shigella flexneri*. The cooking process to prepare porridge appeared to reduce this antimicrobial effect somewhat (Mensah *et al.* 1991). This was ascribed to dilution and evaporation of volatile fatty acids, such as acetic and

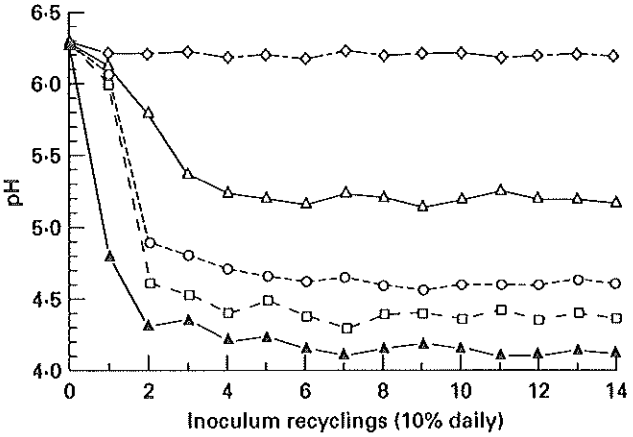

Fig. 4 Effect of moisture content on acidification of a sorghum-maize-soya mixture (Nout 1992). ◇, a_w 0·885; △, a_w 0·925; ○, a_w 0·940; □, a_w 0·970; ▲, a_w 0·990

propionic acids, or to the inactivation of other, and as yet unidentified antimicrobial factors. Similarly, Mbugua and Njenga (1991) and Simango and Rukure (1992) showed that *E. coli*, *Shigella dysenteriae*, *Salm. typhimurium*, *Campylobacter*, *Aeromonas* and *Staph. aureus* died off in fermenting uji and ready-to-drink uji. The development of moulds, including *Aspergillus flavus* and *Asp. parasiticus*, is a major problem in stored grains in Ghana. Aflatoxin concentrations in maize did not decrease during fermentation and contents of over 100 $\mu g\ kg^{-1}$ dough were found (Jespersen *et al.* 1994).

The traditional kenkey process is slow and laborious, taking up to 6 d to complete. Lack of standardization results in variability in quality. Further, kenkey is a ready-to-eat product, but with a short shelf-life. Its supply is often irregular. An industrial-scale manufacturing process would allow for modifications that result in a more regular supply of a product of good quality with less hardship to workers. Wet-milling could be replaced at least partly by dry-milling (Nche *et al.* 1994b). Fermentation is essential in the kenkey process, being associated with the development of the typical aroma and textural properties of the final products (Halm *et al.* 1993; Nche *et al.* 1994b). Fermentation could be controlled more effectively, however, by recycling inoculum ('back slopping') or by using inoculants. The tedious aflata cooking step could be replaced by drum-drying part of the fermented dough. This would provide a dehydrated pre-gelatinized flour that could be reconstituted to give aflata. Indeed it would be possible to produce a shelf-stable dried kenkey product with satisfactory reconstitution properties (Nche *et al.* 1994b). Furthermore, it has been shown that fermentation of doughs with reduced water content ($a_w \sim 0{\cdot}96$) can be done satisfactorily by back-slopping (Fig. 4). This allows a significant reduction

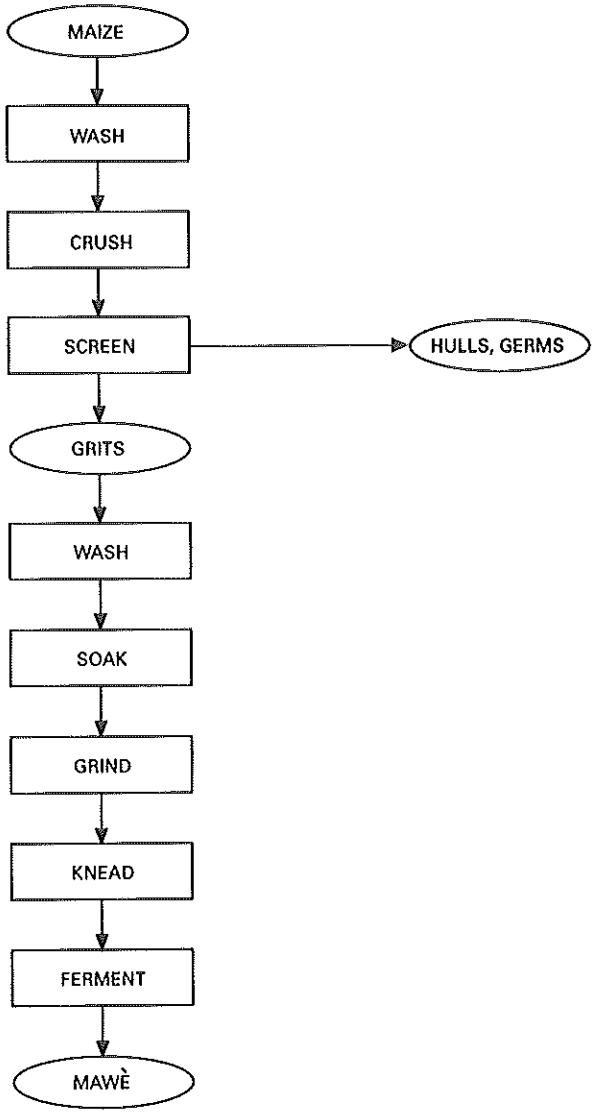

Fig. 5 Flow diagram for mawè production (adapted from Hounhouigan *et al.* 1993a)

in dehydration costs (Nout 1992). It has also been shown that traditional kenkey can be enriched with up to 10% (w/w) cowpea to improve its nutritional quality (Nche *et al.* 1994a).

3.2 Mawè

Mawè is a popular fermented maize product in Bénin and Togo. It is a fermented maize dough which is produced in addition to ogi, a maize gruel. Mawè dough serves as an intermediate in the preparation of many dishes such as 'akassa' (gelatinized dough), 'ablo' (steam-cooked bread), 'akpan' (gelatinized yoghurt-like product), 'massa' and 'pâté' (fritters), 'yèkè-yèkè' (couscous) and 'koko'

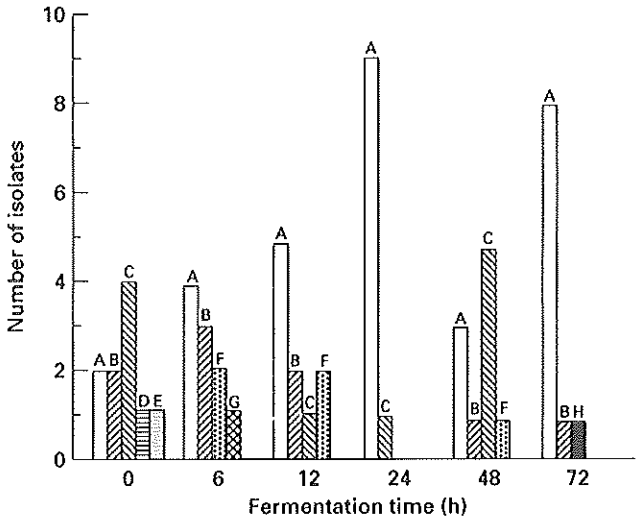

Fig. 6 Frequency distribution and succession of lactic acid bacteria isolated from home-produced mawè during fermentation (Hounhouigan *et al.* 1993c). A, *Lactobacillus fermentum*, biotype *cellobiosus*; B, *Lact. fermentum*; C, *Lact. brevis*; D, *Lact. lactis*; E, *Leuconostoc mesenteroides*; F, *Lact. salivarius*; G, *Lactococcus lactis*; H, *Lact. buchneri*

(porridge). While Ghanaian maize dough made from whole maize is used for kenkey, mawè is made from partially dehulled and de-germed white maize. It is found not only in rural areas but in cities, such as Cotonou, where it is still produced in households (home process) but more importantly in commercial enterprises from which mawè is sold as a ready-to-cook product. A simplified production scheme is depicted in Fig. 5. The commercial process differs from the home process mainly in that a more refined product is obtained (Hounhouigan *et al.* 1993a,b,c). In both processes the fermentation stage takes from 1 to 3 d, during which time lactic acid bacteria develop rapidly to numbers of $> 1 \times 10^9$ cfu g^{-1} and yeasts to 1×10^7 cfu g^{-1}. Enterobacteriaceae, present initially in varying numbers, disappear altogether (Hounhouigan *et al.* 1994). The succession of lactic acid bacteria during fermentation showed the same trend for both the home (Fig. 6) and the commercial process. A number of species of lactic acid bacteria were isolated in the early stages of fermentation in both but *Lact. fermentum* or *Lact. reuteri* and *Lact. fermentum* biotype *cellobiosus* rapidly attained dominance. Not a single strain of *Lact. plantarum* was isolated in the course of these fermentations, even although the pH dropped from an initial value of 6·1 to 3·5 within 3 d. The dominant yeast species included *C. krusei* (mainly), *C. kefyr*, *C. glabrata* and *Sacch. cerevisiae* (Hounhouigan *et al.* 1993c, 1994). These studies also confirmed the dominant role of obligately heterofermentative lactobacilli in association with *Candida* and *Saccharomyces* spp. in African fermented maize doughs and gruels.

4. FERMENTED VEGETABLES AND SALADS

A large variety of vegetables and fruits are preserved by brining and/or fermentation (Fleming 1991). In the case of brining only, blanched or fresh vegetables such as cucumbers, silver onions, peppers, etc. are immersed in a brine composed of sodium chloride, acetic and lactic acids and sugars in concentrations that do not allow the growth of fermentation or spoilage micro-organisms. When fermentation is sought, a brine usually containing only salt is used to favour the development of lactic acid bacteria. There are some interesting features in such traditional fermentations, for example the direct brining and fermentation of green olives (García García *et al.* 1992; Fernandez Gonzalez *et al.* 1993; Durán *et al.* 1994). Direct fermentation of green olives by lactic acid bacteria is not immediately possible because of the inhibition of these bacteria by polyphenols such as oleuropein. Oleuropein may be hydrolysed by lye (1% (w/w) NaOH) treatment. Such treatment cannot be used with Spanish type green olives, because of undesirable darkening of the fruit. Consequently, yeasts often become the dominant micro-organisms in brined green olives. They may cause spoilage, such as shrivelling and gas-pocket formation. A lactic fermentation is desirable for reasons of taste and quality. By carefully controlling the concentrations of sodium chloride and acetic acid in the brine, and by inoculation with *Lact. plantarum*, it was possible to induce lactic acid fermentation and to assure an acceptable shelf-life.

For various reasons there have been attempts recently to apply some of the traditional fermentation principles to products which are not normally available as fermented foods. Examples are mayonnaise-based salads, commodities composed of solid ingredients such as vegetables, potatoes, meat or fish and an acidic water-in-oil emulsion or sauce. The market share of large industrial producers has increased tremendously in the last two decades. For reasons of storage, distribution and display a certain minimum shelf-life is required. The microbiological shelf-life of these acidic, chilled products is limited mainly by the growth of lactic acid bacteria, yeasts and occasionally moulds. As these products contain unsaturated fats and oxygen and are displayed under intensive illumination in supermarkets their chemical shelf-life is limited by lipid oxidation. Much along the lines of set-yoghurt production (Fig. 7), we have recently developed a fermented variant of these salads. The procedure involves the addition of a thermophilic starter culture to the sauce, mixing and packaging the sauce and solid ingredients, followed by incubation (7 h at 42–45°C)

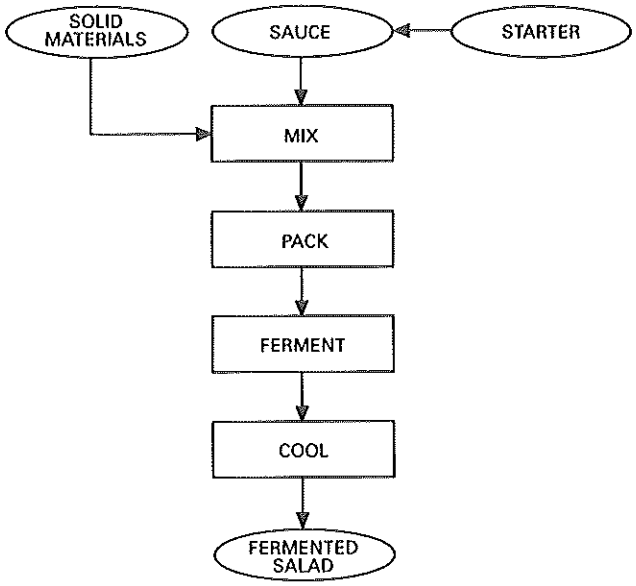

Fig. 7 Process for preparation of fermented mayonnaise-based salads

with subsequent cooling to $\leq 7°C$. Most suitable starter cultures were isolated from sugar-beet pulp and identified with *Lact. acidophilus*. These are homofermentative organisms which had a favourable temperature profile and a considerable oxygen-scavenging capacity (Bonestroo *et al.* 1992; Rombouts *et al.* 1993). The mayonnaise-based salads are inoculated at a level of 10^7 cfu g^{-1} and, in the 7 h at 42–45°C, the lactobacilli increase in numbers to 10^8–10^9 cfu g^{-1} and the pH changes from about 6 to close to 4. Lactic acid and acetic acids are produced (Table 2). Oxygen uptake in fermented salads was in the order of 100–120 mg kg^{-1} product while *Lact. plantarum* consumed only 40 mg kg^{-1} (Table 2). Various metabolic reactions in which oxygen is used can be carried out by lactic acid bacteria (Condon 1987) but it is not known which prevail in these lactobacilli.

In challenge tests several pathogens were added to the product prior to fermentation (Bonestroo *et al.* 1993a). Two to three generations of growth of *Listeria monocytogenes* Scott A and five to six generations of *Staph. aureus* were possible, after which both micro-organisms declined in numbers, disappearing altogether during storage at 7°C. No growth or survival of *B. cereus* could be detected. *Klebsiella pneumoniae*, a notorious spoilage organism in mayonnaise-based salads, disappeared rapidly during storage at 7°C. *Zygosaccharomyces bailii*, *Pichia membranaefaciens*, *Sacch. exiguus*, *Sacch. cerevisiae* and *Torulaspora delbrueckii* cause spoilage of mayonnaise-based salads. When present in numbers ≤ 100 cfu g^{-1} in salads prior to fermentation, they were unable to grow to spoilage levels in 3 weeks at 7°C (Bonestroo *et al.* 1993b).

The fermented products were organoleptically satisfactory, but differed from normal salads in having a less sour taste and a less muted taste of the other ingredients.

5. PERSPECTIVES

There is a large variation in the extent of development and industrialization of indigenous fermented foods of plant origin. Examples of highly industrialized fermentation processes are Japanese soya sauce manufacture (Fukushima 1989), European beer brewing and wine making and South African mageu (mahewu) production (Holzapfel 1989). On the other hand there are numerous fermentation processes which are still carried out at household or artisanal level.

Table 2 Production of lactic acid and acetic acid and uptake of oxygen in potato salads fermented with three different starter cultures*

		Fermented with *Lactobacillus*		
	Control† (acidified)	*plantarum* ATCC 8014	*acidophilus* strain 1	*acidophilus* strain 41
pH	3·9	3·9	4·1	4·2
Lactic acid (g kg^{-1})	3·8	4·0	3·1	1·3
Acetic acid (g kg^{-1})	0·2	0·4	0·8	1·4
Oxygen in salad (mg kg^{-1})‡	6·0	5·5	4·6	4·9
Oxygen in headspace (%)	20·7	15·0	7·0	4·7
Oxygen uptake (mg kg^{-1})	<1·0	40	101	120

* Fermentation was done in glass jars (100 g salad; 50 ml headspace) for 7 h at 42°C; the salads were subsequently stored for 10 d at 7°C and then analysed.

† Control salad was acidified with lactic acid from an initial pH of 6·0 to pH 4·0.

‡ Dissolved oxygen was measured in the geometrical centre of the product, according to Gemerden *et al.* (1989).

Some of these have been described in this contribution to the symposium. With a worldwide trend towards urbanization of rural populations, a need exists for industrializing many of the traditional indigenous fermented foods. In some cases, such as in tempe manufacture, scale-up requires the development of suitable solid-substrate reactors to alleviate heat and mass transfer limitations. In all cases, process development should lead to safe products of a high quality. A fundamental understanding of the microbial ecology of the natural, or spontaneous, fermentation processes is required to control these natural fermentations, and in a further stage to allow selection of adequate starter cultures. Recent examples of the successful introduction of starter cultures are the production of mageu with *Lact. delbrueckii*-type cultures (Holzapfel 1989) and fermentation of Spanish-type green olives with *Lact. plantarum* cultures isolated from brined olives (Durán *et al.* 1994). Ecological studies cited in this paper point towards a promising use of *Lact. fermentum* strains as starters in African fermented cereal doughs and gruels and *Lact. acidophilus* strains for fermented salads, in which oxygen scavenging would safeguard these products against rancidity. A further development could be the use of bacteriocin-producing starters in fermentations where specific pathogens, e.g. *L. monocytogenes*, *B. cereus* or *Staph. aureus*, cause public health problems.

The use of lactic acid bacteria in foods has also been advocated for purposes other than fermentation. In the 'Wisconsin process' (Hutton *et al.* 1991) the deliberate addition of *Ped. acidilactici* to low-acid refrigerated foods, such as bacon and chicken salads, was found to be effective in precluding botulinum toxin production with temperature abuse. Similarly, the use of protective cultures of lactic acid bacteria ('Schutzkulturen') was suggested by Cerny (1991) to safeguard mayonnaise-based vegetable and meat salads from becoming a public health hazard as a consequence of outgrowth of certain pathogenic contaminants with temperature abuse. These and other examples (cf. Gorris 1994) show that micro-organisms of traditional food fermentation may have the potential to be used as shelf-life and food-safety improving factors in the rapidly increasing range of minimally processed foods.

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