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Journal of Applied Bacteriology

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https://doi.org/10.1111/j.1365-2672.1992.tb03633.x

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Journal of Applied Bacteriology Symposium Supplement 1992, **73**, 136S–147S

Fermentative preservation of plant foods

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1. INTRODUCTION

Fermentation is an ancient method of food processing aimed at the prolongation of shelf-life and improvement of palatability. It may also improve digestibility and the nutritional value of food and feed. This article focusses mainly on the ecological aspects of fermentation for prolongation of shelf-life.

Fermented foods of plant origin are derived from a variety of raw materials of different chemical composition and biophysical properties (Table 1). Tuberous roots such as potatoes, cereals and tree-crops like breadfruit have a relatively high starch content. Legumes and oil seeds generally have a high protein content. Green vegetables, carrot, beet, tomato, olive, cucumber, okra and forage crops for animal feed silages have a high moisture content. Fruits contain high concentrations of reducing sugars. The preservation by fermentation of vegetables and cereals is mostly due to the action of lactic acid bacteria, often in combination with yeasts. However, other bacteria, e.g. *Bacillus* spp. or filamentous fungi, e.g. *Rhizopus* and *Aspergillus* spp. are equally important in the fermentation of legumes and oil seeds.

The economic importance of fermentative preservation is significant. For instance, in the USA approx. 300 000 tonnes of cucumbers (Humphries & Fleming 1989) and 200 000 tonnes of cabbage (Stamer 1988) are fermented annually; in The Netherlands millions of tonnes of forage crops are ensiled annually, whereas in tropical countries a variety of fermented plant foods are considered to be essential dietary items.

2. DRIVING FORCE

Each type of raw material possesses a unique combination of physical structure, chemical composition and natural microflora which influences the sequence of microbial development (Andersson *et al.* 1988a) and the activity of endogenous enzymes of the raw material during fermentation. The availability of fermentable substrate is the most important driving force for fermentation. In this section, sources of carbon and nitrogen, as well as the availability of minerals will be discussed.

2.1 Carbon

Cereal grains and their milled derivatives contain only 0·5–2·5% of freely available carbohydrates, mainly mono- and disaccharides (Becker & Hanners 1991). In raw (un-heated) cereals, however, starch and other polysaccharides may be mobilized by endogenous hydrolases, e.g. α- and β-amylases (Odunfa & Adeyele 1987). Figure 1 illustrates such auto-amylolysis taking place in maize and sorghum. Generation of such fermentable carbohydrates is of particular importance in lactic acid fermentations, as most lactic acid bacteria lack the ability to degrade starch and show only limited activity in pre-cooked cereals. There are exceptions, e.g. *Lactobacillus amylovorus*, which can adhere to and degrade cooked starch granules. The addition of lytic enzymes of malted cereals to cooked starch results in a

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Table 1 Approximate chemical composition of plant foods (per 100 g edible portion)*

	Roots	Cereals	Legumes	Oil seeds	Vegetables	Sugary fruits
Moisture (g)	50–80	8–13	12	3–6	85–95	85–91
Crude protein (g)	0·2–2	5–14	20–35	15–30	0·5–5	0·5–1·5
Crude fat (g)	<0·4	1–5	1–2	35–65	<1	<2
Carbohydrates (g)	17–37	62–80	40–60	10–35	1–6	5–25
Reducing sugars (g)	—†	0·5–2·5	2–4	5–10	1–4	5–20
Calcium (mg)	7–150	10–350	30–300	20–500	10–150	10–40
Phosphorus (mg)	60	90–400	400	90–600	10–100	8–50
Iron (mg)	0·5–2	0·5–5	5–10	1–10	0·5–5	0·2–2
Sodium (mg)	10	5–10	2–10	2–25	5–100	<5
Potassium (mg)	600	100–400	1000–1250	400–900	250–700	75–300
Manganese (mg)	—	—	—	500–800	500–800	—
Vitamin A (I.U.)	0–100	—	0–100	0–100	—	0–2000
β-carotene (mg)	—	—	0–0·15	—	<6	0–2·50
Thiamine (mg)	0·08–0·15	0·10–0·60	0·10–0·9	0·03–1·0	0·02–0·15	0·02–0·1
Riboflavin (mg)	0–0·06	0·05–0·15	0·10–0·4	0·01–0·30	0·03–0·30	0·02–0·1
Niacin (mg)	0·30–1·0	0·10–0·35	0·25–0·35	0·6–7	0·02–0·25	0·2–2
Ascorbic acid (mg)	0–20	—	—	<10	10–100	5–150

* Compiled from multiple literature sources.
† No data available.

significant improvement of the growth and acidification by lactic acid bacteria. Alternatively, starch-degrading yeasts (Khetarpaul & Chauhan 1990) or *Bacillus* spp. (Njoku & Okemadu 1989) may be used to generate reducing sugars.

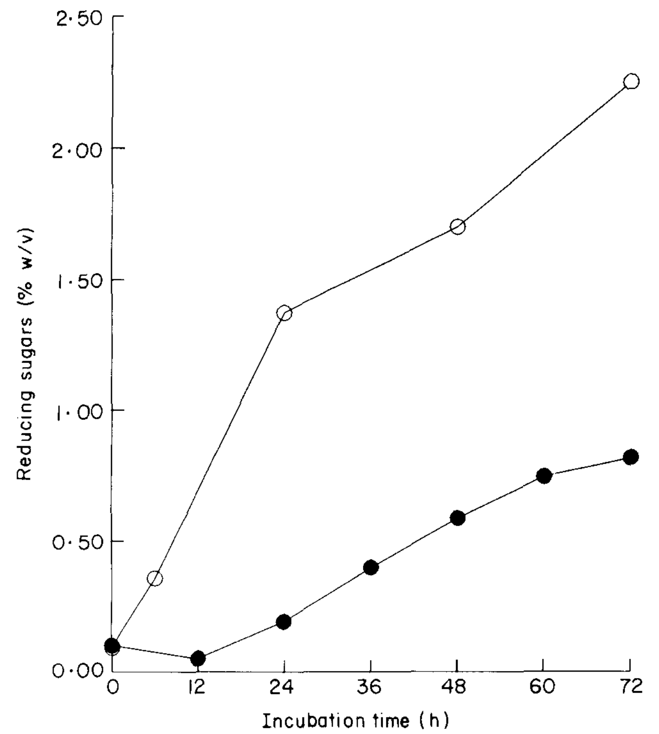

Fig. 1 Auto-amylolysis in cereals (based on data of Nout (1980) and Odunfa & Adeyele (1987)). ○, Maize; ●, sorghum

In vegetables the sugar content is sufficiently high to support fermentative processes. Fresh vegetables contain mainly fructose, glucose and saccharose, in total approx. 1–4% (Table 1), depending on the degree of maturity. Other sources of carbon include pectic substances and organic acids. A variety of fungi and bacteria have pectolytic activity; from a food quality point of view, however, the degradation of pectic substances and subsequent loss of firmness is regarded as undesirable. Organic acids, particularly malic acid, can be assimilated, e.g. by *Lact. plantarum*; in cucumbers this may lead to undesirable accumulation of CO_2. In the presence of citrate, *Lact. plantarum* can also degrade lactic acid to form acetic acid (Lindgren *et al.* 1990). Extraction of the fermentable matter from the plant cell may be achieved by physical disruption of the cellular structure, or by applying an osmotic gradient. In grass silage, physical disruption without salting is adequate. In some grasses the concentration of water-soluble carbohydrates is as high as 18%, so that diffusion ceases to be a limiting factor (Heron *et al.* 1988). The extraction of fermentable sugars from vegetables by osmotic gradient is limited by diffusion rates. In cabbage and other leafy vegetables, shredding is combined with the application of 1–2% salt (NaCl), but in whole cucumbers, olives, onions, and carrots, 4–18% salt is required to achieve adequate extraction of fermentable sugars (Fleming 1991). In legumes, oil seeds and sugary fruits the availability of fermentable matter is not limiting, and can be achieved by soaking or crushing.

Table 2 Epiphytic microflora on plant foods (log N/g)

	Roots	Cereals	Legumes and oil seeds	Vegetables
Bacteria				
total aerobic	4–6	4·5	5–7·7	4–6
Enterobacteriaceae	1·7	3·5–5	3·5	3–3·5
lactic acid bacteria	2·7	2·6–4·3	4·2	0·7–4
Yeasts and moulds	2·4	2·5–3·2	2·5	0·3–4·6

Compiled from multiple literature sources.

2.2 Nitrogen

Although most plant materials contain enough organic nitrogen to support fermentation, its availability may be limited by protein- or α-amino-complexing substances, e.g. phytic acid and phenolic compounds. Degradation of the latter, combined with proteinase and peptidase activity, increases the level of free amino acids and NH_3 nitrogen (Njoku & Okemadu 1989). Poolman & Konings (1988) concluded that the rate of active transport of amino acids into the cell of (dairy) lactococci is not a limiting factor for growth at pH values below 7 (see Marshall, this Symposium, pp. 127S–135S).

2.3 Minerals

Microbial activity is regulated by mineral availability. Mineral ions of metabolic importance include Ca^{2+}, Fe^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, P^{3+} and Zn^{2+}. Naturally-occurring phytic acid chelates minerals and reduces their availability. Degradation by microbial or endogenous phytase increases Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, P^{3+} and Zn^{2+} availability. Calcium is involved in the ethanol tolerance of *Saccharomyces cerevisiae*; Fe^{2+} is generally required for bacterial growth, except by lactic acid bacteria; Mn^{2+} is part of the defence against endogenous O_2^- in plant-associated *Lactobacillus*, *Pediococcus* and *Leuconostoc* spp. (Daeschel *et al.* 1987); and Zn^{2+} stimulates α-amino N-assimilation.

3. EPIPHYTIC MICROFLORA

Many foods of plant origin are fermented without prior heat treatment. Consequently, the epiphytic microflora found on the raw material (Table 2) is present at the start of the fermentation.

Strictly aerobic epiphytic bacteria include *Bacillus*, *Flavobacterium* spp., Pseudomonadaceae and moulds. Facultative anaerobes of importance are the Enterobacteriaceae, lactic acid bacteria and yeasts.

Microbial properties including energy metabolism, lag time, maximum specific growth rate and ability to adhere

Table 3 Major lactic acid bacteria in naturally fermented products of plant origin

Obligate homofermentative	Recorded occurrence	Facultative heterofermentative	Recorded occurrence	Obligate heterofermentative	Recorded occurrence
Enterococcus faecium	2 4				
Lactobacillus acidophilus	1 2				
lactis	1 2			*Lactobacillus brevis*	1 2 3
		Lactobacillus bavaricus	1	*brevis* var. *lindneri* II	2
		casei	1 2 4	*büchneri*	2
		coryniformis	2	*cellobiosus*	1 2 3
		curvatus	1	*confusus*	1 2
delbrückii ssp. *delbrückii*	2			*coprophilus*	3 4
delbrückii ssp. *lactis*	3			*fermentum*	1 2
leichmannii	1			*sanfrancisco*	2
				Leuconostoc dextranicum	1 2
		plantarum	1 2 3 4	*mesenteroides*	1 2 3
salivarius	1	*saké*	1	*paramesenteroides*	1 2
Streptococcus spp.	3 4				
bovis	2				
Enterococcus faecalis	1 2				
Streptococcus thermophilus	1				
Pediococcus acidilactici	1 2				
damnosus	1 2				
pentosaceus	1 2 3				

Occurrence: 1, vegetables; 2, cereals; 3, root crops; 4, oil seeds and legumes.
Compiled from multiple literature sources.

Table 4 Major yeasts in naturally fermented products of plant origin

Species	Recorded in	
Fermentative		
Candida famata		2
guilliermondii	1	2
lambica		2
krusei		2
milleri		2
sake	1	
Hansenula sp.	1	
anomala		2
Saccharomyces sp.	1	2
cerevisiae		2
dairensis		2
exiguus		2
Torulaspora delbrückii		2
Torulopsis sp.	1	
holmii		2
Oxidative		
Aureobasidium spp.	1	2
Candida sp.	1	2
curvata		2
Cryptococcus spp.	1	2
Debaryomyces sp.	1	
Geotrichum candidum	1	2
Pichia sp.	1	2
Rhodotorula spp.	1	2
rubra		2
glutinis		2
Sporobolomyces spp.	1	2
Trichosporon beigelii		2

Occurrence: 1, vegetables; 2, cereals.
Compiled from multiple literature sources.

or penetrate into the substrate are important factors that influence the chance of domination in a mixed microflora. Particularly, members of the Enterobacteriaceae (*Enterobacter* and *Klebsiella* spp.) dominate the early stages of fermentation because of their high specific growth rates at neutral pH. Their mixed acid metabolism results in acetic acid, formic acid and CO_2 and they are inhibited at pH $\leqslant 5{\cdot}5$.

Table 3 summarizes the epiphytic lactic acid bacteria occurring in food plants. Obligate homofermentative species produce lactic acid from all assimilable carbohydrates while obligate heterofermentatives produce a range of products including lactic acid. Facultative heterofermentatives, e.g. *Lact. plantarum* ferment glucose homofermentatively, whereas other carbohydrates can be fermented in a heterofermentative manner. From a functional point of view, heterofermentative lactic acid bacteria contribute to flavour development. In low-salt fermentations *Leuconostoc mesenteroides* is usually one of the first lactic acid bacteria to dominate due to its short lag and generation times (Stamer 1988). In cucumber fermentations, lactic acid bacteria enter the plant tissue through fissures and stomata but yeast cells are too large to penetrate (Daeschel *et al.* 1987).

Table 4 lists yeasts that occur frequently in fermented foods. The majority of epiphytic yeasts (mainly *Aureobasidium*, *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* spp.) do not ferment sugars (Dobolyi & Kecskés 1990), but several can use pectins as a carbon source. In pickled vegetables especially, fermentative yeasts play a crucial role in preservation through removal of fermentable substrate. In contrast, oxidative yeasts are associated with spoilage by degradation of organic acids, which causes an increase in pH and growth of spoilage-causing, acid-sensitive organisms.

Even under sub-optimum nutritional conditions, adequate colonization of the substrate and consequently biological stabilization can take place if commensalism or protoco-operation is possible. In particular, the fastidious lactic acid bacteria benefit from the presence of yeasts and it has been hypothesized that the extent of acidification in cereal lactic fermentation is regulated by yeast growth (Nout 1991). Also, in sourdough, growth of maltose non-fermenting yeasts is enabled by maltose-fermenting lactobacilli which, in turn, are stimulated by yeast peptides (Sugihara 1985).

4. PROCESS CONDITIONS

Figure 2 summarizes the principles and major operations involved in fermentative production of representative groups of plant foods. Heating and size reduction affect the epiphytic flora and the availability of substrates. Environmental factors determining the microbial activity will be discussed below.

4.1 (An)aerobiosis

Anaerobic conditions are created by consumption of oxygen by plant and microbial respiration, while oxygen access is prevented either by submersion under gentle pressure (Andersson *et al.* 1988a), in soil-covered pits (Aalbersberg *et al.* 1988) or in anaerobic tanks flushed with nitrogen gas (Humphries & Fleming 1989). The aim of anaerobiosis is to inhibit potential spoilage by acetic acid bacteria, oxidative yeasts including *Candida* spp. (Middelhoven & Van Baalen 1988) and moulds. However, the complete absence of O_2 is not always optimum, since it has been shown recently that in lactic acid fermentations with, e.g. *Lact. fermentum*

	Root crops	Cereals			Legumes	Vegetables		
Raw material	Cassava	Maize	Maize	Maize	Soya bean	Grass	Cabbage	Cucumber
Preparatory	Peel	Grain	Flour	Dry grits	Dehull	Cut	Trim	Wash
	Wash	Soak	Soak	Cook	Soak			
	Grate	Grind		Cool	Decant	Chop	Shred	Tank
						Heap		
Salting	—	—	—	—	—	—	Dry salt	Brine
		Sieve		Add bran	Cook			(Acidify)
		Sediment			Cool			Purge
								Buffer
Inoculation	(Starter)	—	(Starter)	Starter	Starter	(Starter)	(Starter)	(Starter)
	Pressurize				Package	Pressurize	Pressurize	
						Cover	Cover	
Fermentation	Ferment	Ferment	Ferment	Ferment	Ferment	Ferment	Ferment	Ferment
Maturation	—	—	—	—	—	Store	Store	Store
Processing	Roast/fry	Boil/steam	Boil/steam	—	Cook/fry	Airing	Pasteurize	Pasteurize
Product	Gari	Ogi	Uji	Mageu	Tempe	Silage	Sauerkraut	Pickles

Fig. 2 Fermentative processing of representative plant foods. —, Not applied; ( ), optional

(Vahvaselkä *et al.* 1990), and in mixed olive fermentations subtle aeration improves the speed of the process and the quality of the product.

4.2 Salts

Sodium chloride applied either directly or as a brine serves multiple functions as a food ingredient, amongst which is the inhibition of micro-organisms. The antimicrobial action of NaCl comprises a non-specific a_w reduction effect and an additional inhibitory effect. NaCl does not specifically inhibit spoilage or pathogenic organisms; lactic acid bacteria and yeasts are also inhibited. *Leuconostoc* spp. are the least salt-tolerant, whereas lactobacilli and pediococci have similar salt tolerance. For instance, in cucumbers the optimum salt concentration for lactic acid bacteria is 5%; at this concentration, lactic acid bacteria dominate over yeasts and *Enterobacter* spp., whereas at higher salt levels (10–16%) lactic acid bacteria are strongly inhibited and are, in turn, dominated by yeasts and *Enterobacter* spp. (Stamer 1988). Salt and acidity have cumulative inhibitory effects. For example, inhibition of *Propionibacterium acnes* in olive brines required 11% salt at pH 7·0, 9% at pH 5·1 and no salt at pH 3·5 (Fleming *et al.* 1989).

Reduction of salt consumption by reducing salt concentrations or by using salt substitutes in vegetable fermentations is of consumer health interest. In cucumber juice, NaCl and KCl were found to be more selective in favour of *Lact. plantarum* compared with $CaCl_2$ and $MgCl_2$; sulphates and phosphates were unsuitable (Naewbanij *et al.* 1986).

4.3 Water

The availability of water, expressed as water activity (a_w) or, more precisely from a thermodynamics point of view, water potential, is influenced by the total moisture content, the presence of hydrating substances including salts, proteins and sugars, and temperature. Xerotolerance and osmotolerance are governed by different mechanisms (Ushio & Nakata 1989). The inhibitory effect of reduced a_w interacts with pH, atmospheric composition and temperature. Yeasts usually require less available water than lactic acid bacteria (Rehacek *et al.* 1982).

4.4 Temperature

Most fermentations of plant material take place at ambient temperatures. Exceptions are fermentations with thermophilic *Bacillus* spp. (Odunfa 1986) or *Lact. delbrückii* (Haggblade & Holzapfel 1989) that take place at 45°–55°C. Seasonal or geographical temperature differences contribute to the dominating microflora in, e.g. sourdough, silages and sauerkraut. For example, *Leuc. mesenteroides* and the majority of yeasts are favoured at 20°–30°C, whereas most lactic acid bacteria grow more rapidly at 30°–35°C (Andersson *et al.* 1988a). Although acidification has no sharp temperature optimum, higher temperatures tend to increase the rate of acidification. On the other hand, lower temperatures favour growth (Vahvaselkä & Linko 1990) and may still achieve lower final pH and higher titratable acidity in the long run.

4.5 Inhibitors

Naturally- and widely-occurring substances, e.g. phytic acid and polyphenols (Niwa *et al.* 1987) inhibit growth by

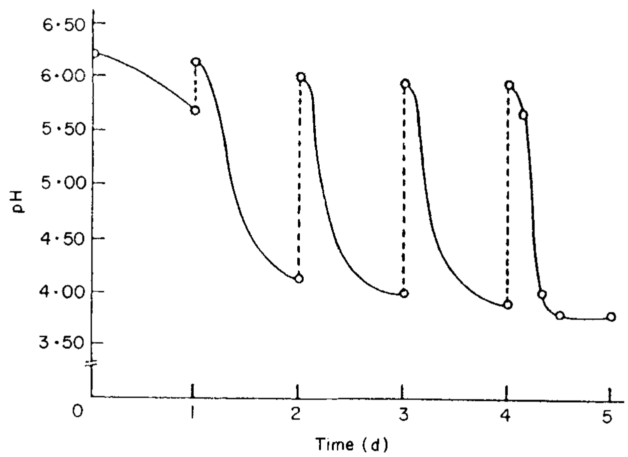

Fig. 3 Lactic fermentation of sorghum by daily back-slopping (inoculation rate 3%; incubation at 30°C) (Nout *et al.* 1988)

reducing the availability of minerals and/or nitrogenous substrates. Other natural inhibitors include phenolic (e.g. caffeic) acids, proteins, organic acids, essential oils, pigments and oleuropein, some of which are highly concentrated in spices, herbs, onion and garlic (Beuchat & Golden 1989).

Chemical additives may be used to control fermentations. For example, nitrite can be used to inhibit lactobacilli and leuconostocs in pickles to produce a mildly acid product (Miyao & Ogawa 1990); in silages, Na- or Ca-acrylate can be used to selectively inhibit heterofermentative lactic acid bacteria, and Fe(II)-scavengers, e.g. 2,2'-dipyridyl have been shown to suppress non-lactic acid bacteria (yeasts, clostridia and Enterobacteriaceae) (Bruyneel *et al.* 1990).

5. NATURAL FERMENTATION

Natural fermentations are initiated without the addition of micro-organisms and their control is limited to maintenance of external environmental conditions. A full microbial succession takes place. This has the advantage of developing complex ('rich') flavours but the drawback of being prone to off-flavours or failures. Natural vegetable fermentations undergo successive stages including an initiation phase dominated by Gram-positive and Gram-negative bacteria. Primary fermentation is dominated by lactic acid bacteria and yeasts. Subsequently, a secondary fermentation takes place during which residual fermentable sugars are depleted by fermentative yeasts. Spoilage flora, e.g. propionibacteria and clostridia, may develop at this stage and degrade lactic acid, especially if the pH is too high or the salt and acid concentrations are too low. Finally, a post-fermentation stage occurs, during which oxidative yeasts, moulds and bacteria can develop, especially in open storage vessels (Fleming 1991). Natural fermentations of cereals, roots and oil seeds undergo similar microbial successions. Tables 3 and 4 list the major lactic acid bacteria and yeasts involved in natural fermentations. Other micro-organisms of importance include *Corynebacterium* and *Micrococcus* spp. in, e.g. cassava and *Bacillus*, *Pseudomonas* and *Staphylococcus* spp. in oil seeds and legumes.

6. 'CONTROLLED' NATURAL FERMENTATION

In order to speed up the fermentation and increase its predictability, process control is required. Two principles should be distinguished, i.e. ecological *vs* environmental control.

Ecological control involves enrichment of starter organisms in the food by natural selection. 'Back-slopping', i.e. using inoculum from a previous batch of product, as is practised in sourdough, soyabean soaking for tempe manufacture (Nout & Rombouts 1990) and lactic fermentations of cereal–legume mixtures (Nout 1991) results in highly competitive and well-adapted multiple strain starters. Figure 3 illustrates the favourable effect of 'back-slopping' on the effectiveness of sorghum acidification.

Environmental control involves choosing pretreatments and incubation conditions to ensure and maintain the artificial dominance of starter organisms over epiphytic micro-organisms. The type of starter may vary from certain fermented foods, e.g. curdled milk or yoghurt (containing a variety of lactic acid bacteria, Enterobacteriaceae and yeasts), to more specific starters, e.g 'ragi' tablets (containing *Amylomyces* and *Endomycopsis* spp.) for tapé fermentation (Ardhana & Fleet 1989), or 'usar' leaves (containing *Rhizopus* spp.) for tempe fermentation (Nout & Rombouts 1990). Washing, cooking, disinfection, packaging and temperature control all contribute to process predictability.

7. PURE CULTURE FERMENTATION

Single or mixed cultures may be added to raw materials to achieve dominance over the epiphytic flora. This principle and practise are widely applied in, e.g. silages that use *Lact. plantarum*, *Lact. brevis*, *Pediococcus acidilactici* and *Bacillus pumilus* (Hendrick *et al.* 1991), and in vegetable fermentations (cabbage, carrots, cassava and okara). Pure cultures are selected according to criteria that include homo- or heterofermentation and CO_2 production, rate of production of organic acids and their configurations, temperature range, flavour production, acid tolerance, salt tolerance and cell sedimentation. Mixed starters consisting of lactic acid bacteria and fermentative yeasts are quite successful in achieving biological stability by assimilating all available fermentable carbohydrates (Daeschel *et al.* 1988).

Temporary dominance over the epiphytic microflora requires large inocula and supportive process control. Operations including washing, chlorination, exchange of internal gases with O_2 (Andersson *et al.* 1988a), brine pH control by buffers, e.g. Na- or Ca-acetate to assist in 'full curing', purging of produced CO_2, and the use of anaerobic tanks, all help to sustain the temporary dominance of the added starter culture (Fleming 1991). In silages, *Lact. plantarum* must be added at at least twice the level of the epiphytic *Enterobacter cloacae* (Bruyneel & Verstraete 1986) or 10^5–10^6 cfu/g (Heron *et al.* 1988). Excessive domination, however, may lead to flavour defects, e.g. when a pure culture of homofermentative *Lact. bavaricus* is used to enhance the L(+) lactic acid content in sauerkraut, the flavour-producing *Leuc. mesenteroides* cannot develop sufficiently. Figure 4 illustrates the effect of control measures in cucumber fermentation on the rate and extent of acid production, pH decrease, sugar utilization and CO_2 production. Another approach to ensure dominance of starter cultures is to use organisms which produce antibiotics. Harris *et al.* (1990) suggested using a mixed culture for sauerkraut fermentation. This consisted of a nisin-resistant *Leuc. mesenteroides* and a nisin-producing *Lactococcus lactis* to maintain dominance over the nisin-sensitive epiphytic *Lact. plantarum*.

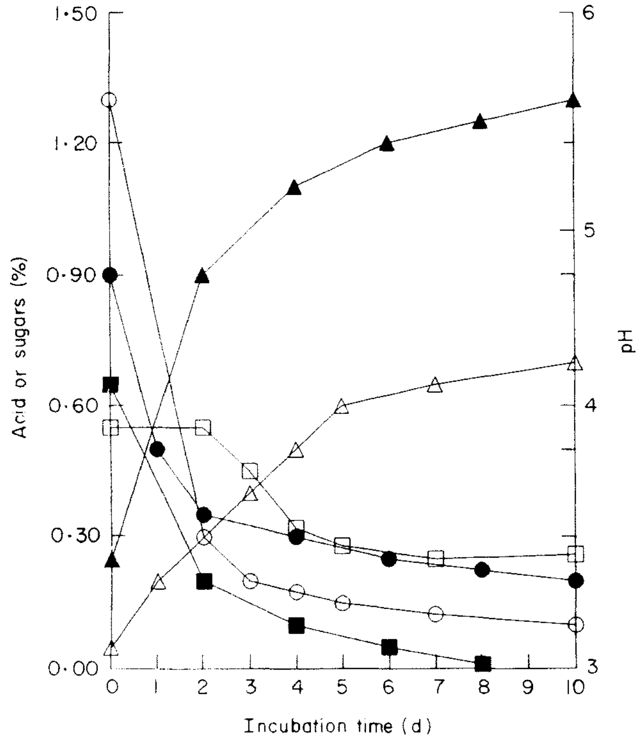

Fig. 4 Comparison of natural and controlled cucumber fermentation (based on data of Fleming (1984)). Open symbols, natural fermentation; closed symbols, controlled fermentation. ○, pH; □, sugar; △, acid

To increase the effect of pure culture starters, fermentations of pasteurized or sterilized substrate under aseptic conditions are desirable. Fermentation of pasteurized vegetable juice using mixtures of heterolactic bacteria results in pronounced flavour development. Preconditioning of cucumbers followed by fermentation with malate decarboxylase-deficient mutants of *Lact. plantarum* has the advantage of reduced gaseous spoilage. However, many of the commercial large-scale fermentations cannot be carried out profitably under such conditions as they require expensive energy and equipment.

8. IMPACT

In addition to the environmental factors mentioned above, the preservative effect of fermentation is attributed to accumulation of antimicrobial products (Table 5), and to microbial competition.

8.1 Anti-microbial metabolites of low molecular weight (<1000)

Weak organic acids, particularly lactic and acetic acids, probably account for most of the inhibitory effect on a broad spectrum of micro-organisms. As the formation of organic acids takes place gradually, this leaves opportunities for acid-sensitive organisms, e.g. Enterobacteriaceae to multiply during early stages of the fermentation. The inhibitory effect of undissociated organic acids is 10–600 times stronger than that of their dissociated molecules; the extent of dissociation is directly determined by the pH. Undissociated citric and lactic acid have the highest toxicity, but acetic acid is the most effective in moderately-acid products due to its high pK (4·75) value. Factors that affect the pH reduction in a product include the types and concentrations of lactic acid bacteria and fermentable carbohydrates, the rate of acid production and growth, the presence of inhibitory factors and the initial pH and buffering capacity of the food. The synergistic inhibitory effect of mixtures of lactic and acetic acids has been ascribed to the potentiation of acetic acid at the lower pH produced by lactic acid. The mechanism of inhibition studied in yeasts indicated that excessive energy is required to maintain the intracellular pH at the original level (Warth 1988) by continuous removal of protons and/or undissociated acid molecules; to this effect the cytoplasmic membrane must be intact (Shah & Jelen 1990). The intracellular pH (pH_i) at which cellular functions are inhibited varies with species and is different for growth or acid production. The acid resistance of *Lact. plantarum* might be the result of the low

Table 5 Antimicrobial products accumulating during fermentation

Substance	Produced by	Active against	Status of acceptance as food ingredient
Molecular weight <1000			
Acids			
lactic acid	All LAB	All micro-organisms	GRAS
acetic acid	Het. LAB	All micro-organisms, pH-dependent	
Alcohols	Yeasts Het. LAB	All micro-organisms	GRAS
Carbon dioxide	Het. LAB	Most micro-organisms aqueous: at pH $\geqslant 6$ gas: at 20–50%	GRAS
Diacetyl	*Lactococcus* spp.	Yeasts, G − bact: at 200 ppm Non-LAB, G+: at 300 ppm (butter flavour: 2–4 ppm)	GRAS
Hydrogen peroxide	All LAB	All micro-organisms	Not approved (USA) as additive
Microgard^{R}	*Propionibacterium shermanii*	Most G−, some yeasts	
Reuterin	*Lactobacillus reuteri*	Broad: G+, G−, fungi	FDA-approved
Bacteriocins (molecular weight $\geqslant 1000$)			
Lactacin B	*Lactobacillus acidophilus*	LAB	
Lactobacillin	*brevis*	LAB	
Brevicin	*brevis*	LAB	
Caseicin 80	*casei*	*Lactobacillus brevis*	
Bulgarican	*delbrückii* ssp. *bulgaricus*	Broad, incl. G−	
N.N.	*fermentum*	Broad G+, incl. *Listeria* spp.	
Lactocin 27	*helveticus*	Broad G+, incl. *Listeria* spp.	
Plantaricin A	*plantarum*	LAB	
Nisin	*Lactococcus lactis*	Broad G+, incl. *Listeria* spp., *Bacillus*, *Clostridium* spp.	GRAS
Diplococcin	*lactis* ssp. *cremoris*	Narrow: *Lactococcus lactis*, *Clostridium* spp.	
N.N.	*Leuconostoc dextranicum*		
N.N.	*gelidum*	LAB, *Listeria* spp.	
Pediocin	*Pediococcus acidilactici*	Broad G+	
N.N.	*pentosaceus*	Broad G+	

LAB, Lactic acid bacteria; Het., heterofermentative; GRAS, generally recognized as safe; G−, Gram-negative bacteria; G+, Gram-positive bacteria; N.N., not named; FDA, United States Food and Drugs Administration.

pH$_i$ (4·6) at which it is inhibited, compared with 5·2 for *Lact. helveticus* (Vahvaselkä & Linko 1990) and 5·4 for *Leuc. mesenteroides* (McDonald *et al.* 1990). However, other mechanisms may also contribute to acid resistance. For example, at decreasing pH, *Lact. plantarum* tends to produce acetoin, rather than lactic acid, from pyruvate. McFall & Montville (1989) concluded that the ability to shift between the production of neutral (acetoin) and acidic (lactic acid) compounds may help *Lact. plantarum* to maintain pH homeostasis; similarly, Vahvaselkä & Linko (1990) found that lactate production by *Lact. plantarum* and *Lact. helveticus* was maximum at near-neutral pH (5·4–5·8). Acid production and growth of lactic acid bacteria are also directly influenced by a_w, and can be described by a non-linear Arrhenius model (Larsen & Anon 1989). Figure 5 illustrates the effect of water activity on the effectiveness of sorghum acidification by 'back-slopping'. At reduced a_w (0·92) growth and acidification occur at a significantly

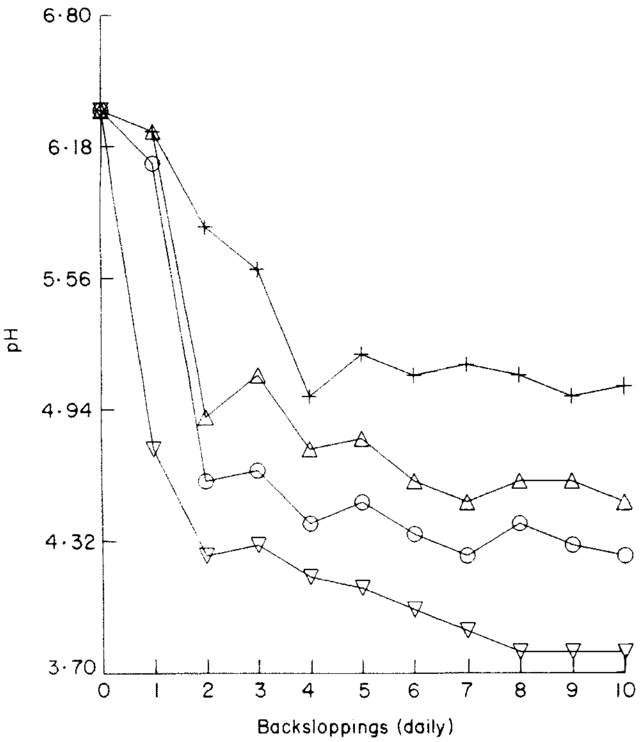

Fig. 5 Effect of water activity on sorghum acidification by daily back-slopping (Nout, unpublished). a_w: +, 0·92; △, 0·94; ○, 0·96; ▽, 0·99

reduced rate since considerable energy is diverted towards maintenance.

The inhibitory effect of alcohols on yeasts is associated with the increase of the anion and proton permeability of the plasmalemma, e.g. in *Sacch. carlsbergensis*, resulting in a loss of ΔpH ($pH_{out} - pH_{in}$) and membrane potential. C_6 alcohols have the stongest effect (Petrov & Okorokov 1990). The permeabilization was reduced by the addition of Mg^{2+} or Ca^{2+}. In general, large (> 10%) amounts of ethanol are required to achieve a significant preservative effect.

Considerable production of CO_2 can take place as a result of heterofermentative degradation of hexoses and pentoses and also from decarboxylation of, e.g. malic acid. The dissolved CO_2 is in equilibrium with HCO_3^- to which the antimicrobial effect was ascribed by Daniels *et al.* (1985). At $pH \leqslant 6$, however, only 2% of the aqueous CO_2 is present as HCO_3^- and consequently its antimicrobial effect in most fermentations should be negligible. In the gaseous phase, 2–5% CO_2 stimulates growth of lactic acid bacteria and fungi, whereas concentrations > 10% have a significant antimicrobial effect. Consequently, it may be expected that in hermetically-sealed conditions, CO_2 accumulation contributes to preservation.

Diacetyl has a broad spectrum antimicrobial effect at concentrations of 300–1000 ppm but, since it produces a marked butter flavour at *ca* 5 ppm, its contribution to preservation is limited. The amount of H_2O_2 produced by lactic acid bacteria is variable and depends on the strain and availability of O_2 (Gibbs 1987). There is no published evidence of its preservative effect in fermented plant foods. Several non-proteinaceous, antimicrobial substances of low molecular weight are reported, other than organic acids or H_2O_2. For example, reuterin, a non-proteinaceous metabolite of glycerol produced by *Lact. reuteri*, has a broad-spectrum antimicrobial effect on Gram-negative and Gram-positive bacteria, yeasts and filamentous fungi.

8.2 Anti-microbial products of molecular weight ⩾1000

Strains of lactic acid bacteria, including *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Streptococcus* and *Leuconostoc* spp., are able to excrete antimicrobial proteins (bacteriocins). The mechanism of the antimicrobial activity of most bacteriocins starts with a more or less specific adsorption on to the host cell wall. In resistant cells, no reaction takes place, but sensitive cells will be killed quickly. This bactericidal effect has been associated with leakage of ions and also with lysis (Andersson *et al.* 1988b).

The majority of proteinaceous bacteriocins are inactivated by proteolytic enzymes including pronase, ficin and some by trypsin. They resist cooking and are active over a wide range of pH (Bhunia *et al.* 1988). The ability of lactic acid bacteria to produce bacteriocins is receiving increasing attention as it could improve the competitiveness of starters. Fermentation starter strains could be selected according to their ability to produce bacteriocins; alternatively, recombinant DNA techniques could be used to equip conventional starter strains with bacteriocin production ability. The objective of such biopreservation systems would be to suppress epiphytic spoilage flora *in situ*, or to prepare a 'natural' antimicrobial additive, e.g. the Microgard^R system.

For about 60 years, nisin has been known to be particularly inhibitory to Gram-positive spore-forming bacteria. So far, this is the only bacteriocin that has been given Generally Recognised As Safe (GRAS) status and that is commercially used as an additive in processed cheese, canned vegetables, sterilized milk products and beer and wine fermentations (Radler 1990).

At present, commercial application of bacteriocins or bacteriocin-producing starters is limited. Because of their specificity, bacteriocins may not inhibit all undesirable micro-organisms (yeasts are seldom affected); instead they may even suppress desirable lactic acid bacteria. Also, their production in foods is less than in laboratory media. Bacteriocins may also be inactivated by food constituents and in solid foods their diffusion is limited.

8.3 Competition

In addition to oxygen consumption and the consequent lowering of the redox potential, competition for nutrients contributes to the preservative impact of fermentations. For instance, in cucumber fermentations fermentative yeasts play an important role in preservation by competing for carbon sources. Sugar depletion by yeasts is maximum if very low pH values are avoided by using buffers (Na- or Ca-acetate) or assimilation of glucose with simultaneous decarboxylation of L-malate by lactic acid bacteria (Daeschel 1988). Competition for carbon sources has also been demonstrated in fungal-fermented foods. Although the preservative effects of *Bacillus* spp. in silages (Hendrick *et al.* 1991) and fermented foods (Odunfa 1986) are as yet poorly understood, competition probably plays an important role.

9. POST-FERMENTATION PROCESSING

The biological stability of fully fermented material depends on the combined preservative effects of anaerobiosis, metabolites, salt (if any) and depletion of substrates. If a fermentation is interrupted or completed by changing any of these conditions, the integrity of the preservative mechanism is lost. This has been shown to trigger development of oxidative yeasts and lactate degradation in silage (Middelhoven & Van Baalen 1988) followed by butyric spoilage and growth of acid-sensitive putrefactive autotrophs, e.g. *Clostridium tertium* (Fleming *et al.* 1989). Consequently, additional processing is required for shelf-life prolongation. This could involve, e.g. reduction of water activity by dehydration, or heating by baking, steaming or pasteurizing. An alternative to post-fermentation processing could be in-pack fermentation, such as practised with, e.g. sauerkraut and sorghum beer (Haggblade & Holzapfel 1989).

10. PROSPECTS

In industrially-manufactured consumer goods, the ability to control fermentation processes through better understanding of the ecological keyfactors is of prime importance; also, the quality characteristics and stability of consumer products will increasingly benefit from the use of mutant or recombinant DNA strains designed, e.g. for reduced gas production, production of bacteriocins and novel flavours.

In animal feed preservation in silages, important issues for research will be the control of the microbial population and nutritional status of the product.

New ways should be found to suppress or inactivate clostridia in silage, as this leads to an increase in their numbers in milk and consequently, the higher incidence of butyric acid fermentation failures in cheese manufacture. Another problem is the widespread growth of *Penicillium roqueforti* in silage with possible formation of mycotoxins.

Fermented foods play an important role in the diet in tropical developing countries. Particularly, fermentation processes contribute to the microbiological and chemical safety of cereal and root crop products (Cooke *et al.* 1987). Traditional sour-fermented cereal products prevent the multiplication of pathogenic bacteria that are responsible for, e.g. diarrhoea, one of the major causative factors of child mortality. Controlled lactic fermentation can be employed to ensure adequate and predictable acidification, e.g. in the manufacture of weaning foods.

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