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Lactic acid food fermentation in tropical climates

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Introduction

Lactic acid fermentation is widely applied in tropical climates as a low-cost method of enhancing food quality, safety and shelf-life (Cooke et al. 1987; Nout & Motarjemi 1997). In many developing countries, the manufacture, preservation and distribution of foods are still seriously restrained by the lack of required technological infrastructure. Whereas refrigeration, freezing, and canning require cold-chains and industrial-scale processing to be economically feasible, lactic acid fermentation processes can be successfully carried out using simple means and at a smaller scale.

As most of such fermentation processes are carried out under non-sterile conditions, the microbiological composition of the resulting products is of a mixed nature. Whereas lactic acid bacteria are often predominant, other micro-organisms such as yeasts are usually present as well.

Lactic acid fermentation occurs during the preparation of a wide variety of foods, made from raw materials of plant and animal origin. Some ingredients, their fermented products, mode of consumption and references to their microbiological composition are presented in Table 1. Two examples will be discussed, namely the fermentation of cassava, and of mixtures of cereals and legumes.

Cassava

Cassava (*Manihot esculenta* Crantz) is a tuberous rootcrop indigenous to South America, which is now grown in many tropical countries. Its global annual production is estimated at $1.5 \cdot 10^{11}$ kg. Its good yield of starchy tubers, even from unfertile soils or under conditions of drought, combined with its ease of cultivation, makes it a very important source of dietary

energy for low-income farmers and consumers. One of the limitations of cassava is that once harvested, the tubers are highly perishable. In addition, potentially toxic cyanogenic glycosides (mainly linamarin) occur in variable concentrations in the fresh roots of bitter cassava cultivars. Immediate post-harvest processing is required to preserve and detoxify this important food source.

Agbéli mawè is the name used in the Republic of Bénin (West Africa) for a fermented dough made of cassava. A similar product is known as agbelima in Ghana. As a result of the fermentation process, the chemical and microbiological stability of the product is improved, the toxicity is eliminated and a characteristic flavour is formed. This product must be cooked prior to consumption. The fermentation process contributes to the desirable visco-elastic properties of the resulting paste, which is usually consumed with a gluey sauce.

The major unit operations for producing agbéli mawè are illustrated in Figure 1. Cassava tubers are washed, peeled and grated to pulp. The pulp is pressed to remove water to 50–55% moisture content, sieved to remove fibrous materials, and milled to a fine flour. This is kneaded into a dough that is kept during 1–3 days at ambient temperature (25–35 °C) allowing a natural fermentation to take place which is characterized by a succession of lactic acid bacteria.

Facultative heterofermentative *Lactobacillus plantarum* and obligate heterofermentative *Lb. brevis* and *Leuconostoc mesenteroides* occurred in all samples of Ghanaian agbelima (Amoa-Awua et al. 1996). The common occurrence of *Lb. plantarum* in fermenting cassava had also been reported earlier by Oyewole & Odunfa (1990). They studied the succession of lactic acid bacteria species (Figure 2) and observed that *Lb. plantarum* is predominant after 3 days of fermentation.

Figure 1. Processing of cassava, and preparation and consumption of agbéli mawè (adapted from Nago & Hounhouigan 1998, with permission).

Figure 2. Succession of lactic acid bacteria in fermenting cassava pulp (adapted from Oyewole & Odunfa 1990).

Traditional cassava fermentation processes are fairly adequate for detoxification, for preservation and for creating a tasteful product with a desirable visco-elastic texture when cooked.

Several authors found that *Lb. plantarum* strains are able to degrade linamarin (Oyewole & Odunfa 1992, Giraud et al. 1993). The degradation of linamarin is a 2-stage process catalyzed by β -glucosidase (linamarin $\rightarrow$ glucose + acetone cyanohydrin), followed by hydroxynitrile lyase (acetone cyanohydrin $\leftrightarrow$ acetone + HCN). The latter equilibrium reaction is pH-dependent, towards cyanohydrin at lower pH. The data (Table 2) recalculated from Westby & Choo (1994) and Giraud et al. (1993) show that detoxification of cassava pulp by the endogenous enzymes of cassava, is most rapid under sterile conditions (neutral pH) whereas slow acidification (natural fermentation) and rapid acidification (added *Lb. plantarum* starter) allow degradation of linamarin by β -glucosidase, but are increasingly detrimental to final detoxification of acetone cyanohydrin. In processes where cassava is not grated to pulp, bacterial cell wall degrading enzymes

cause softening (Amoa-Awua & Jakobsen 1995) and facilitate the linamarin degradation by endogenous β -glucosidase. In all cases, lactic acidification should be moderate initially in order to adequately detoxify the cyanohydrin moiety.

Of more positive importance is the impact of lactic acid bacteria on the sensory qualities and preservation of cassava. Important sensory properties of fermented cassava are its flavour and the visco-elastic properties of the cooked paste, made from the fermented pulp. The aroma compounds of agbelima were mainly low molecular weight alcohols, 1-propanol, isoamyl-alcohol, 3-methyl-1-butanol, ethyl acetate and acetoin (Amoa-Awua et al. 1996).

Whereas the swelling capacity of the cassava starch is not affected by lactic acid fermentation, improved visco-elastic properties of the cooked product are achieved (Moorthy & Mathew 1998). Presently, the mechanism for this effect has not been convincingly demonstrated. It is hypothesized that amylolytic enzyme activity, chemical depolymerization of native starch, or extraction of amylose from native starch are involved. Amylolytic strains of *Lb. plantarum* (Giraud et al. 1993, Olasupo et al. 1996) and *Lb. manihotivorans* (Morlon-Guyot et al. 1998) were reported. A study of the effect of natural lactic fermentation on physicochemical characteristics of cassava starch (Mestres & Rouau 1997) revealed that fermentation and mild drying did not affect the crystallinity of native starch granules or their gelatinization properties. However, Numfor et al. (1995) observed significant decrease of hardness, gumminess and adhesiveness (stickiness) of fermented cooked cassava. Probably, leaching and enzymatic degrada-

Table 1. Examples of lactic acid fermented foods of tropical climates

Ingredients	Fermented product (Geography)	Mode of consumption	References
Plant origin: Cereals:			
maize	kenkey (Ghana)	stiff, cooked dough	Nche et al. 1996
maize	pozol (Mexico)	sour beverage	Nuraida et al. 1995
maize, sorghum	uji (Kenya)		Mbugua & Njenga 1992
maize	busaa (Kenya)	sour-alcoholic	Nout 1980
millet	jnard (India)	beverage (beer)	Tamang et al. 1996
Starchy roots and stems:			
cassava	agbéli mawè (Bénin), agbelima (Ghana)	cooked paste from fermented pulp	Nago & Hounhouigan 1998 Amoa-Awua et al. 1996
cassava	gari (Nigeria)	fermented, toasted and dried pulp	Kemdirim et al. 1995
cassava	peujeum (Indonesia)	sour-sweet-alcoholic softened root	Arihantana & Buckle 1987
radish tap root	sinki (India, Nepal)	soup base, pickle	Tamang & Sarkar 1993
young bamboo shoots	mesu (India, Nepal)	pickle	Tamang & Sarkar 1996
Leafy vegetables:			
cabbage, garlic, peppers	kimchi (Korea)	pickles	Cheigh & Park 1994
rayo ¹ / radish / cauliflower	gundruk (India, Nepal)	soup base	Yonzan & Tamang 1998
Pulses and beans:			
black gram and rice	idli (India, Sri Lanka)	steamed cake	Reddy et al. 1986
Bengal gram and rice / wheat	dhokla (India)	steamed cake	Ramakrishnan 1979
Animal origin: Milk:			
cow or buffalo	dahi (India)	yoghurt	Balasubramanyam & Varadaraj 1994
Meat:			
pork or beef	chorizo (Mexico)	dry sausage	Santos et al. 1997
Fish:			
fish, salt, roasted rice	pla-ra (Thailand)	fish paste	Tanasupawat et al. 1998

¹*Brassica campestris* L. var. *cumifolia* Roxb.

Table 2. Changes in cyanogens in cassava pulp at 30°C.

Incubation (h)	Irradiated (sterile) (Westby & Choo 1994)				Natural Fermentation (Westby & Choo 1994)				<i>Lb. plantarum</i> A6 (high linamarase and acidifying activity) (Giraud et al. 1993)			
	Total	Linamarin	Cyanohydrin	HCN	Total	Linamarin	Cyanohydrin	HCN	Total	Linamarin	Cyanohydrin	HCN
0	100	88	-	12	100	88	-	12	100	77	8	15
6	53	6	29	18	76	-	18	58	77	-	31	46
12	47	-	26	21	71	-	24	47	77	-	54	23
24	41	-	24	17	53	-	29	24	77	-	62	15
48	35	-	21	14	47	-	29	18	77	-	62	15
72	26	-	18	7	41	-	24	17	77	-	63	14
96	23	-	12	11	41	-	24	17	74	-	65	9

Values represent % of levels of total cyanogens at start, i.e. 170 and 325 mg HCN.kg⁻¹ dry matter, for Westby & Choo (1994) and Giraud et al. (1993), respectively.

Table 3. Bacterial flora of raw ingredients and fermented product of idli (From Ramakrishnan 1979)

Bacteria	% of total counts		
	Rice dal	Black gram dal	Fermented batter of rice and dal
<i>Leuconostoc mesenteroides</i>	33	69	43
<i>Lactobacillus fermentum</i>	17	-	14
<i>Lactobacillus delbrueckii</i>	8	7	14
<i>Enterococcus faecalis</i>	8	7	14
<i>Pediococcus acidilactici</i>	17	-	-
<i>Bacillus</i> sp.	17	15	-

Table 4. Microbial and physico-chemical changes during idli batter fermentation (from Soni & Sandhu 1991)

Parameters	Before fermentation	After fermentation
Bacteria (Log cfu.g ⁻¹)	6.3	10.9
Yeasts (Log cfu.g ⁻¹)	2.6	5.9
pH	6.6	4.9
Volume (ml)	200	410
Amylases (IU.ml ⁻¹)	16.0	22.0
Reducing sugars (mg.g ⁻¹)	3.6	6.0
Free amino acids (mg.g ⁻¹)	8.0	13.0
Crude protein (%) w/w)	12.0	12.4
Thiamine (mg.kg ⁻¹)	2.0	7.5
Riboflavin (mg.kg ⁻¹)	2.5	7.5

tion of amylose from native starch play an important and desirable role. Amylolytic lactic acid bacteria can possibly enhance this effect.

The production of organic acids and other antimicrobial components by lactic acid bacteria is well documented (Adams & Nicolaides 1997). Bacteriocin producing *Enterococcus faecium* (Olasupo et al. 1994b) and *Lactobacillus* spp. (Olasupo et al. 1994a) have been isolated from various African fermented foods. Olasupo et al. (1997) reported that bacteriocin producing *Lb.casei* strain 012 had a significant inhibitory effect on an enterotoxigenic strain of *E.coli* in an experimental fermentation of maize dough. The reported data do not permit a conclusion about the mechanism of this inhibition. All the same, there is ample evidence for the inhibitory and sometimes bac-

tericidal impact of lactic acid bacteria on susceptible bacterial pathogens, provided that lactic acid bacteria can dominate fermentations at an early stage. However, other pathogens e.g. enteric viruses and parasites appear to be less sensitive and may survive in lactic fermented foods in the absence of subsequent heat treatment (Adams & Nicolaides 1997). Thus the antimicrobial properties of lactic acid bacteria can improve the microbiological quality of the food, but only in combination with good hygienic practices.

Cereal-legume mixtures

From a nutritional point of view, it is advantageous to consume mixtures of cereals and legumes because this results in an improved balance of carbohydrates and proteins as well as a better balance of dietary essential amino acids (Nout 1993). Acidified and leavened foods like Indian idli, dosa and dhokla constitute an interesting group of products made from cereal-legume mixtures.

Idli is a popular food, prepared and consumed throughout India, particularly in southern parts, and in many places of Sri Lanka. It is relished mainly as a breakfast dish along with chutney (a batter containing fresh ground coconut and spices) and sambar, a thin spiced soup prepared from red gram dal (dehulled legume seeds), vegetables, tamarind juice, salt and herbs. An early history of the use of this traditional food dates back to 1100 A.D. (Ramakrishnan 1979). The ever-increasing popularity of idli is for its unique organoleptic quality (a typical sour flavour and spongy texture), nutritional quality and improved digestibility. For this reason, it is fed to infants as a weaning food, and as a main dish in diets in many South Indian hospitals.

The main ingredients used in the traditional preparation of idli are white polished rice (*Oryza sativa* L.) and black gram (*Phaseolus mungo* L.) dal. The proportion of these two ingredients may vary. In general, it consists of three parts of rice and one part dal. Rice and dal are washed several times with tap water, soaked separately in water at room temperature for 5-10 h before grinding in a stone mortar or other grinders. While rice is coarsely ground, the dal is ground to a fine smooth paste. The rice and dal slurries are mixed and stirred well manually to form a thick batter. Salt is added to taste. The batter, put in a closed container, is kept at a warm place to ferment overnight or longer. The fermentation period must allow a definite leaven-

Table 5. Examples of effects of lactic acid fermentation on anti-nutritional factors in cereals and legumes, and impact on *in-vitro* and *in-vivo* availability of nutrients.

Food	Parameter	Not Fermented	Fermented	Starter	Reference
Degradation of anti-nutritional factors:					
maize:sorghum:ground-nut flour 30:30:35	Phytate (IP-6) (%dm)	0.87	0.41	<i>Lb.plantarum</i>	Nout (unpublished)
	IP-5	0.10	0.20		
millet flour	Polyphenols (%dm)	0.69	0.60	<i>Lb.brevis</i>	Khetarpaul & Chauhan 1991b
<i>In-vitro</i> availability:					
low-tannin sorghum	Fe solubility		40 - 50% increased	natural lactic fermentation	Svanberg et al. 1993;
	Protein digestibility (%)	61 - 79	70 - 82		Lorri & Svanberg 1993
high-tannin sorghum	Fe solubility		10 - 20% increased	natural lactic fermentation	Svanberg et al. 1993;
	Protein digestibility (%)	33 - 40	45 - 53		Lorri & Svanberg 1993
<i>In-vivo</i> availability:					
millet	Protein Efficiency Ratio (PER)	1.41	1.80 - 1.91	natural lactic fermentation	Khetarpaul & Chauhan 1991a
sorghum	PER	2.06	2.34	<i>Lb.plantarum</i>	Nout 1993

ing of the batter and development of a pleasant acid flavour. The fermented batter is poured in small cups or in a special idli pan having cups (8–10 cm dia), and steamed until the starch is gelatinized and the idli cakes are soft and spongy. The fermented batter is consumed the same day and there is no effort to preserve it.

Mukherjee et al. (1965) concluded that *Leuc. mesenteroides* is the micro-organism essential for leavening of the batter and also responsible, along with *Ent. faecalis*, for acid production in idli. Soni & Sandhu (1989) reported the involvement of *Lb. fermentum* and *Pediococcus cerevisiae* in addition to the aforementioned bacteria in idli batter fermentation.

Idli is a natural fermented food; no inoculum is added generally for fermentation. This is because the essential micro-organisms have been found to be naturally present in the ingredients (Table 3). When the product is made daily, it is often the practice of adding a bit of freshly fermented batter ('back-slop') to the newly ground one. In addition to lactic acid bacteria, the slightly acid environment favours the growth and activity of yeasts (Venkatasubbaiah et al. 1985). The principal yeasts in the fermenting idli batter are *Saccharomyces cerevisiae*, *Debaromyces hansenii*, *Hansenula anomala*, *Torulopsis candida* and *Trichosporon beigelii*.

The major functions of the fermentation include the leavening of the batter (Table 4) and the improvement of taste and nutritional value of idli. *Leuc.mesenteroides* is the main responsible for the production of CO₂ which results in about 2–3 times increase in the original volume of batter (Soni & Sandhu 1989).

Certain oligosaccharides such as raffinose, stachyose and verbascose are prevalent in legumes and are associated with abdominal distention and flatulence. Lactic acid fermentation reduces these flatulence factors.

Cereals and legumes have restricted bio-availability of nutrients as a result of the presence of anti-nutritional factors (ANF) such as phytates and polyphenols (tannins). Lactic acid bacteria are able to reduce their levels, and it has been demonstrated that this results in improved bio-availability of minerals and starch, as well as increased protein efficiency ratio (PER) (Table 5).

Not all ANF can be degraded by lactic acid bacteria. For example, lectins and protease inhibitors occurring in legumes can only be inactivated adequately by heat treatments. Thus, the steaming of fermented idli batter complements the impact of fermentation.

Research needs

Taking into account the increasing demand particularly by urban populations, for safe and affordable food of high quality, there are certainly prospects for industrialization of traditional fermentation processes. Commercial availability of ready-to-use fermented products saves much labour and time in the household.

Scaling-up and specialization require better control of fermentation and quality. This includes the choice of optimum raw materials, fermentation conditions and starters used. Concerning the development of starter cultures for use in larger-scale industrial processes, selection for, or genetic modification to introduce a number of properties could be valuable. Mention was made already of the partially understood effects on rheological properties. Optimization of flavour and texture would greatly enhance consumer appeal. For assurance of safety and maximum nutritional value, starter properties must include improved inhibition of acid-tolerant pathogens and the ability to degrade a number of potentially toxic or anti-nutritive food components such as lectins, protease inhibitors, allergens and mycotoxins. In addition the ability to degrade plant cell walls would contribute to detoxification and increased digestibility of foods of plant origin.

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