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Characterization and frequency distribution of species of lactic acid bacteria involved in the processing of mawè, a fermented maize dough from Benin

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Lactic acid bacteria involved in the natural fermentation of both home-produced and commercial mawè were investigated during a 72 h fermentation period. *Lactobacillus* spp. constitute the majority (94%) of the strains of the lactic acid bacteria isolated, among which 89% represent the Betabacterium group. They include *L. fermentum* (biotype *cellobiosus*) (41%), *L. fermentum* or *L. reuteri* (19%), *L. brevis* (26%), *L. confusus* (less than 2%), *L. curvatus* (less than 1%) and *L. buchneri* (less than 1%). Other isolated lactic acid bacteria were *L. salivarius, Lactococcus lactis, Pediococcus pentosaceus, Pediococcus acidilactici* and *Leuconostoc mesenteroides*. Several species were detected at the early stage of fermentation, but the final stage was dominated by *L. fermentum* (biotype *cellobiosus*) and *L. fermentum* or *L. reuteri* totalling 90% of the isolated strains. The trend was the same for both home-produced and commercial mawè. No strains of *L. plantarum*, generally reported as dominating lactic acid bacteria at the final stage of fermentation of most plant foods, were isolated.

Key words: Maize; Mawè; Fermentation; Lactobacilli

Introduction

Natural fermentation of cereal grains is a common practice of food processing in West Africa. One of the most popular of these foods, especially in Benin and Togo, is mawè, a dehulled maize dough used to prepare many dishes. Production and quality attributes of mawè have been investigated (Hounhouigan et al., 1992).

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Two major methods of mawè production were found: **(i)** the home-production method, used to prepare mawè for family consumption; **(ii)** the commercial method, used to satisfy the demand of ready-to-cook mawè in urban areas.

Commercial mawè is produced by skilled women in privately owned milling shops. A total of 659 plate disc mills ('Premier 1A' type) were recorded in Cotonou, the capital city of Benin. They are mainly used to process cereals. Many milling shops are specialized in commercial mawè production and have a daily processing capacity of about 500–1000 kg of maize each. Depending on the demand, the batch sizes vary between 60 and 120 kg of mawè which have approximately one week of shelf life.

Lactic acid bacteria are common in fermented cereal foods (Akinrele, 1970; Christian, 1970; Nout, 1980; Fields et al., 1981; Mbugua, 1984; Gashe, 1985; Odunfa and Adeyele, 1985). No studies of the microflora responsible for the fermentation of mawè have been reported. Previous investigations showed that the dominating lactic acid bacteria in this product were *Lactobacillus* spp. which accounted for 10^9 cfu/g of the product (Hounhouigan et al., 1992).

The present report describes the frequency distribution, the succession and the characteristics of different species of lactic acid bacteria isolated during a period of 72 h of fermentation of both home-produced and commercial mawè.

Materials and Methods

Mawè preparation

Commercial mawè was produced as follows: Sekou 85 variety of maize (10 kg) provided by the International Institute of Tropical Agriculture in Benin was cleaned by winnowing, washed in water and crushed. The crushed maize was sieved through 0.5 mm screens. The fine endosperm fraction passed through the sieve and was collected in a pan. The grits and the hulls were separated by gravity on the sieve. The hulls were discarded and the grits were collected and washed thoroughly in water. The bran floating on the surface of the washing water was discarded. The washed grits and the fine fraction which were previously collected were mixed, moistened by adding water, left to stand for 2 h and ground finely.

In the home-production method, the grits were not washed; after crushing, the material was sieved through 2–4 mm screens to remove the hulls. The resulting grits and fine fraction were moistened and left to stand for 2 h before milling.

The resultant flour from each process was kneaded with additional water. The final moisture content of the dough was adjusted to about 46% (w/w,), using a Mettler LP 15 infrared unit installed on a Mettler PE 3600 electronic balance. The resulting dough from each process (about 11–12 kg) was equally distributed in six plastic buckets, covered with a polyethylene sheet and allowed to ferment spontaneously for 72 h at room temperature (28–32°C). The production was carried out in a local milling shop by a mawè producer. Duplicate experiments were carried out for each process.

Bacterial isolation and purification

After 0, 6, 12, 24, 48 and 72 h of fermentation, one bucket of mawè (1.8–2 kg) from each process was taken from the fermenting lot for analysis. Ten grams of mawè were then sampled under aseptic conditions. The sample was homogenized with 90 ml sterile peptone-physiological salt solution (5 g peptone, 8.5 g NaCl, 1000 ml distilled water, pH 7.0 ± 0.2) and decimal dilutions were made. Dilutions were made into pour plates with MRS agar (Oxoid CM 361) with addition of 0.1% (w/v) natamycin (Delvocid, Gist-Brocades, Delft, The Netherlands), with an overlay of the same medium. Incubation was carried out for 3–5 days at 30°C. In general, colonies representing the square root of the number present were randomly selected from plates obtained from the highest countable dilution (Harrigan and McCance, 1976). The selected colonies were isolated by streaking on MRS agar and incubated at 30°C for 3–5 days, using anaerobic jars and anaerobic system envelope with palladium catalyst (BBL Gas Pak Plus; Becton Dickinson). The purity of the isolated organisms was checked by streaking again on MRS agar plates, followed by microscopic examination. Subsequently they were grown on slants of MRS agar and stored at 5°C, prior to identification.

Preliminary tests and biochemical characteristics

Preliminary tests included Gram staining on 18-h cultures, microscopic examination and catalase reaction, carried out according to the methods described by Harrigan and McCance (1976). Tests of aerobic, facultative aerobic and anaerobic growth were assessed using two inoculated tubes containing MRS broth (Oxoid CM 359) of which one was incubated aerobically at 30°C, and the other anaerobically in an anaerobic jar as described earlier. Homo- or heterofermentative assimilation of glucose was assessed using MRS broth with Durham tubes inserted.

Growth at 15 and 45°C was tested in MRS broth incubated in a Memmert incubator (854 Schwabach W. Germany) and in a Salvis water-bath (Bioblock, France) respectively, for 10 days. If growth was possible at 45°C, most strains showed turbidity after 24 h.

Ability of the isolates to ferment carbohydrates was studied using the API 50 CHL system (La Balme les Grottes, 38390, Montalieu Vercieu, France). The results were recorded after 2 and 48 h, as recommended by the manufacturer. Stock cultures were subcultured thrice in MRS broth before tests were performed. Production of ammonia from arginine was tested according to the method described by Harrigan and McCance (1976).

Computer analysis

Patterns of fermented carbohydrates were used for preliminary attempt of identification, using the computer-aided identification programme for lactic acid bacteria, as developed by Cox and Thomsen (1990). The bacteria were finally identified on the basis of the cell morphology, the nature of the fermentation pathway (homo- and heterofermentation) and the differential characteristics of the species, including the ability to grow at 15 and 45°C, and the ability to produce ammonia from arginine, according to Bergey's Manual (Kandler and Weiss, 1986).

Results

A total of 120 strains of lactic acid bacteria were isolated, examined and classified from both home-produced and commercial mawè. For this purpose, basic data including Gram-staining, morphology, gas production from glucose, growth at 15 and 45°C, and hydrolysis of arginine were essential in addition to the pattern of assimilated carbohydrates. Frequency distributions and succession of isolated dominating species in home-produced and commercial mawè are shown in Figs. 1 and 2, respectively. *Lactobacillus* spp. constitute the majority (94%) of the lactic acid bacteria isolated. This is in accordance with our previous results (Hounhouigan et al., 1992). Most of the *Lactobacilli* (89% of the isolated strains) were of the obligately heterofermentative Betabacterium group. They include *L. fermentum* (biotype *cellobiosus*), *L. fermentum* or *L. reuteri* and *L. brevis* which represent 85% of the strains isolated from home-produced mawè and 86% from commercial mawè. Other strains isolated were identified as *L. curvatus*, *L. confusus*, *L. buchneri*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Leuconostoc mesenteroides*, *L. lactis* and *L. salivarius*. The latter represents 4% of the isolated strains and was found only in home-produced mawè. A wide range of species of lactic acid bacteria were present at the beginning of the fermentation period. Seven different species were detected in each of the mawè types, between 0 and 6 h of the fermentation period. But the number of isolated species was reduced towards the end of the fermentation period where *L. fermentum* (biotype *cellobiosus*) and *L. fermentum* or *L. reuteri* accounted for 90% of the species isolated, *L. fermentum* (biotype *cellobiosus*) being the most important.

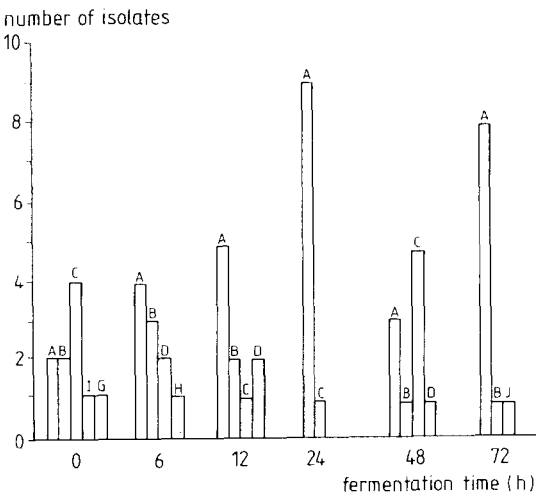

Fig. 1. Frequency distribution and succession of lactic acid bacteria isolated from home-produced mawè. A, *L. fermentum* (biotype *cellobiosus*); B, *L. fermentum* or *L. reuteri*; C, *L. brevis*; D, *L. salivarius*; G, *Leuconostoc mesenteroides*; H, *Lactococcus lactis*; I, *L. lactis*; J, *L. buchneri*.

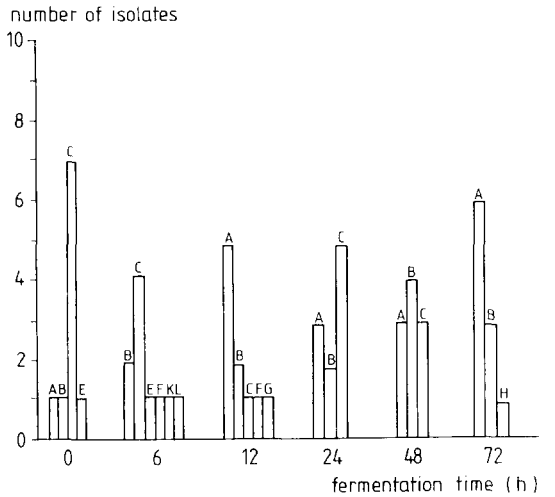

Fig. 2. Frequency distribution and succession of lactic acid bacteria isolated from commercial mawè. A, *L. fermentum* (biotype *cellobiosus*); B, *L. fermentum* or *L. reuteri*; C, *L. brevis*; E, *L. confusus*; F, *Pediococcus pentosaceus*; G, *Leuconostoc mesenteroides*; H, *Lactococcus lactis*; K, *L. curvatus*; L, *Pediococcus acidilactici*.

Characteristics of the strains of lactic acid bacteria isolated are summarized in Table 1. Most of the *L. fermentum* (biotype *cellobiosus*) strains identified were unable to ferment esculin. All the isolated strains of *L. fermentum* or *L. reuteri* lacked the power to ferment cellobiose. Most of the *L. brevis* fermented amygdalin, cellobiose, mannose, salicin, trehalose and grew at 45°C.

Discussion

Lactic acid bacteria have been associated with the spontaneous fermentation process of many plant foods. Fields et al. (1981) isolated *L. fermentum, L. cellobiosus* and *Pediococcus acidilactici* from naturally fermented corn meal. These species, as well as strains of *L. buchneri* and *Pediococcus pentosaceus* have also been isolated from traditional Kenyan uji (Mbugua, 1984), while *L. brevis* and *L. salivarius* have been isolated from busaa, a Kenyan sour maize beer (Nout, 1980). Adegoke and Babalola (1988) isolated *L. fermentum* and *L. brevis* from Nigerian fufu, while Oyewole and Odunfa (1990) found *L. cellobiosus, L. brevis, L. confusus, L. lactis* and *Leuconostoc mesenteroides* in this product. *Leuconostoc mesenteroides, L. brevis* and *L. fermentum* were isolated from fermenting tef dough (Gashe, 1985). Odunfa and Adeyele (1985) have isolated *Lactococcus lactis* from ogi baba. In many cases, *L. plantarum* was present in the fermenting product and dominated at the final stage of fermentation, due to its high acid tolerance (Akinrele, 1970; Nout, 1980; Mbugua, 1984; Kotzekidou and Roukas, 1986; Oyewole and Odunfa, 1990). No *L. plantarum* was isolated in the course of this

TABLE I

Characteristics of lactic acid bacteria isolated during mawè fermentation

	Percentage of strains giving positive reactions											
Name of species [a]:	A	B	C	D	E	F	G	H	I	J	K	L
Number of strains:	49	23	31	5	2	2	2	2	1	1	1	1
Fermentation of:												
Amygdalin	10	0	81	0	0	100	0	100	0	0	0	100
L-Arabinose	82	48	68	60	0	100	100	50	0	0	0	100
Cellobios	24	0	97	40	100	100	0	100	0	0	100	100
Esculin	24	0	100	0	100	100	0	100	0	0	100	100
Fructose	100	96	100	100	100	100	100	100	100	100	100	100
Galactose	76	96	100	100	100	100	100	100	100	0	0	100
Glucose	100	100	100	100	100	100	100	100	100	100	100	100
Gluconate	90	96	100	60	100	0	50	50	100	100	100	0
Lactose	61	65	71	100	0	0	0	100	100	0	0	0
Maltose	100	96	100	80	100	100	100	100	100	100	100	0
Mannitol	12	17	13	100	0	0	0	0	0	0	0	0
Mannose	71	74	100	100	100	100	100	100	100	0	100	100
Melezitose	0	4	3	0	0	0	0	0	0	0	0	0
Melibiose	94	100	97	100	50	50	100	100	100	0	0	0
Raffinose	90	100	100	100	0	0	100	100	100	100	0	0
Rhamnose	10	4	16	60	0	50	0	0	0	0	0	0
Ribose	98	96	100	60	100	100	50	50	100	100	100	100
Salicin	4	0	90	0	0	100	50	100	0	0	0	100
Sorbitol	8	4	13	80	0	0	0	50	0	0	0	0
Trehalose	80	78	97	100	50	100	100	100	100	0	0	100
D-Xylose	82	83	100	60	100	50	100	100	0	0	100	100
D-Arabinose	2	0	0	0	0	0	0	0	0	0	0	0
L-Xylose	2	0	0	0	0	0	0	0	0	0	0	0
β-Methyl-D-xyloside	2	0	0	0	0	0	0	0	0	0	0	0
α-Methyl-D-glucoside	2	0	6	0	0	0	0	0	0	0	0	0
N-Acetyl glucosoamine	31	26	100	100	100	100	100	100	0	0	100	100
Arbutin	2	4	84	0	0	100	50	100	0	0	0	100
Saccharose	100	100	97	100	100	0	100	100	100	100	0	0
Starch	0	0	3	0	0	0	0	50	0	0	0	0
Xylitol	0	0	0	40	0	0	0	0	0	0	0	0
Gentiobiose	24	4	97	0	100	100	50	100	0	0	100	100
D-Turanose	0	0	3	0	0	0	0	0	0	0	0	0
D-Tagatose	6	9	74	0	0	100	50	100	0	0	0	100
L-Fucose	0	0	0	0	0	0	0	0	0	0	0	100
D-Arabitol	2	9	0	80	0	0	0	0	0	0	0	0
L-Arabitol	0	0	3	0	0	0	0	0	0	0	0	0
5-Keto-gluconate	90	70	68	40	0	0	0	50	100	0	0	0
Growth at 15°C	39	26	100	0	100	100	100	100	0	100	100	100
Growth at 45°C	96	91	77	100	0	50	0	100	100	0	0	100
NH_3 from arginine	100	100	100	0	100	100	50	100	0	100	100	100

TABLE I (continued)

	Percentage of strains giving positive reactions											
Name of species [a]:	A	B	C	D	E	F	G	H	I	J	K	L
Number of strains:	49	23	31	5	2	2	2	2	1	1	1	1
Fermentation of:												
Morphology												
bacilli	100	100	100	100	100				100	100	100	
cocci						100	100	100				100
Homo-fermentation				100		100		100	100			100
Hetero-fermentation	100	100	100		100		100			100	100	

[a] A, *L. fermentum* (biotype *cellobiosus*); B, *L. fermentum* or *L. reuteri*; C, *L. brevis*; D, *L. salivarius*; E, *L. confusus*; F, *Pediococcus pentosaceus*; G, *Leuconostoc mesenteroides*; H, *Lactococcus lactis*; I, *L. lactis*; J, *L. buchneri*; K, *L. curvatus*; L, *Pediococcus acidilactici*.

investigation on mawè which nevertheless acidifies from pH 6.1 (initial) to pH 3.5 (final product). The predominant lactic acid bacteria isolated in both types of mawè in this investigation belonged to the Betabacterium group, *L. fermentum* (biotype *cellobiosus*) being the most important. Oyewole and Odunfa (1990) reported *L. cellobiosus* to be the predominant lactic species in the unfermented cassava tubers for fufu production, but this species was not isolated beyond 36 h of fermentation. Kotzekidou and Roukas (1986) emphasized the domination of *L. cellobiosus* after 24 and 36 h of fermentation of okra, while *L. fermentum* and *L. cellobiosus* represented more than 50% of strains isolated in sorghum uji enriched at 45°C (Mbugua, 1984). Among heterolactic bacteria, the ability of *L. cellobiosus* to remove all detectable fermentable sugars from green beans and green bean juice, and to lower the pH to 3.52 has been reported (Chen et al., 1983a, 1983b). The present investigation confirms the predominant role of *L. fermentum* (biotype *cellobiosus*) in natural lactic fermentations.

Leuconostoc mesenteroides was recognized as initiating flora for many fermentation processes (Pederson, 1971; Gashe, 1985). Although this species was present in mawè, its number was very low. *L. brevis* was not isolated after 72 h of fermentation. This could be due to its inability to survive the high acidity produced in mawè after this fermentation period.

Strains of subgenus Betabacterium are not easily distinguishable; particularly the distinction between *L. brevis* and *L. buchneri* and between *L. fermentum* and *L. cellobiosus* is not clearly established from the biochemical characteristics of the species. In the present investigation, the only organism identified as *L. buchneri* was not able to fermented melezitose. On the other hand, three strains were identified as *L. brevis* although they ferment melezitose. Strains of *L. brevis* fermenting melezitose and strains of *L. buchneri* failing to ferment melezitose were reported (Kandler and Weiss, 1986; Mbugua, 1984). Presently, *L. cellobiosus* is considered as a biotype of *L. fermentum*. Furthermore, strains previously

classified as *L. fermentum* based on the sugar fermentation pattern have been found to be representative of two species, *L. fermentum* and *L. reuteri*, exhibiting a G + C content in the DNA of 52–54 and 40–42 mol%, respectively. However, *L. fermentum* seems to be more widespread in lactic acid fermented substrates (Kandler and Weiss, 1986).

The lactobacilli belonging to the obligately heterofermentative Betabacterium group form lactic acid, acetic acid, carbon dioxide and ethanol from hexoses via the hexose monophosphate shunt (Kandler, 1984); these components contribute to the taste and the flavour of the product.

These organisms are probably also responsible for the increase in volume and the porous structure in naturally fermented mawè, through gas (CO_2) production. This leavening action of heterofermentative bacteria was also reported by Christian (1970) in Ghanaian maize dough, and Gashe (1985) who considered the leavening action to be due to the activities of *Enterobacteriaceae* as well. The porous structure of the dough is desirable because it facilitates the disintegration of particles of mawè during the preparation of a granulated porridge (aklui).

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