Fermentation of maize (*Zea mays L.*) meal for mawè production in Bénin

Physical, chemical and microbiological aspects

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PROPOSITIONS

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1. The substitution of local foods by foreign imported foods, as a result of increasing income, population growth and foreign influences, is detrimental to the national food security system in Bénin. (This thesis).

2. One of the most important consequences of the development of the micro-enterprise food processing in Bénin is the diffusion of foods originally limited to some ethnical groups, throughout the whole urban population. (This thesis).

3. The commercial mawè process appears to be a technologically more advanced and effective method of mawè manufacture than the home process. (This thesis).

4. Obligately heterofermentative lactobacilli like *L. fermentum* and its biotype *cellobiosus* and *L. brevis* are responsible of acidification during the natural fermentation of mawè (This thesis).

5. The use of a computer-aided identification program based only on biochemical characteristics of microorganisms can result in false identifications (This thesis).

6. The most crucial factor in the search for creating an industrial base for African fermented foods and beverages will be governemental or political initiatives. Certain measures have to be taken to change the attitude of the people from foreign dependency to self-sufficiency.
   (Sanni, A.I., *Int. J. Food Microbiol.* **18**, 85-95, 1993)

7. The impoverishment of the conception of man caused by the omission of other human dimensions is precisely in line with the "scientific" conception of the universe as machine, and man as nothing but a cog within it.
   (Vancouver Declaration: Survival in the 21st Century, symposium organised by the Canadian Commission for Unesco, 15 September 1989).

8. In developing countries, obesity is considered a sign of excellent social and economical well being.

9. Development is science become culture (René Maheu, La civilisation de l'Universel, Paris: Laffont, 1966).

10. In developing countries, there is a large and widening gap between the small-scale producers need for solutions to their problems and the supply of innovations by scientists and others.

11. Top-down transfer of technology is inappropriate for agricultural innovation by small-scale farmers in developing countries. (Bunders J. & Broerse J.E.W., Appropriate Biotechnology in small-scale agriculture. Redwood Press Ltd. Melksham, UK, 1991).

12. The severity of the technical inspection of cars in Europe will improve the quality of those sold as secondhand in Africa. Suggestions should be made to set up also inspection of clothes, shoes, etc...

Propositions belonging to the thesis of D. Joseph Hounhouigan entitled "Fermentation of maize (*Zea mays L.*) meal for mawè production in Bénin".

Wageningen, The Netherlands, 15 March 1994.

A ma Chère Mère Gbodo: reçois à titre posthume, l'expression de ma profonde gratitude.

ABSTRACT

Hounhouigan, D.J. (1994) Fermentation of maize (*Zea mays L.*) meal for mawè production in Bénin: physical, chemical and microbiological aspects. Ph.D. thesis, Agricultural University Wageningen (99 pp., English, Dutch and French summaries)

Key words: maize, mawè, fermentation, *Lactobacillus*, *fermentum*, *cellobiosus*, *brevis*, *Candida*, *Saccharomyces*, proximate composition, physical and chemical characteristics.

Mawè is a sour dough made from partially dehulled maize meal, which has undergone natural fermentation for 1 to 3 days.

In this thesis, the processing methods, the characteristics of the products and the physical, chemical and microbiological changes during natural fermentation of two differently processed mawè (home and commercial processes) from Bénin were investigated.

The main difference between both processes is the removal of more hulls and germs from the commercial mawè. The latter was whiter than the home-produced mawè and had better swelling and thickening characteristics, but the nutrient loss was higher. This study showed that the physico-chemical changes occurring in the fermenting product depend on the processing method used.

Dominant microflora in mawè included obligately heterofermentative lactobacilli: *L. fermentum* and its biotype *cellobiosus*, *L. brevis*, and yeasts: *C. krusei* and *S. cerevisiae*. Ability of these organisms to ferment dehulled maize porridge was also tested and showed that fermentation can be carried out using a single starter culture of the *Lactobacilli*. The utility of the yeasts was not evident as far as their effect on acid production was concerned.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Fermentation is one of the oldest and most economical methods of producing and preserving foods (Steinkraus *et al.*, 1983; Cooke *et al.*, 1987; Chavan and Kadam, 1989), particularly in the tropical countries where the high temperature and high humidity, coupled with unsanitary conditions, favour food spoilage. Under these conditions, lactic fermentation inhibits spoilage and pathogenic microorganisms by a combination of factors including production of organic acids, hydrogen peroxide, antibiotic-like substances and lowering of oxidation-reduction potential (Cooke *et al.*, 1987; Nout *et al.*, 1989; Mensah *et al.*, 1991; Mbugua and Njenga, 1992; Nout and Rombouts, 1992). Lactic acid fermentation also improves the organoleptic properties of foods by producing a variety in flavours of the existing foods. Another advantage of lactic fermentation is a possible increase of the nutritional value or of the digestibility of the raw material used (Tongnual and Fields, 1979; Murdock and Fields, 1984; Chavan and Kadam, 1989).

While in developed countries, most fermented foods are produced under controlled conditions, in developing countries, such foods are processed under uncontrolled conditions, using village art methods and age-old techniques. Natural lactic fermentation of cereals, roots, tubers and legumes is a common practice of food processing in Africa. These fermented foods constitute a significant component of the diets, mostly in rural areas. Many of these foods are still unknown. Due to the increasing populations, the development of urban zones with all their constraints, and the cultural attachment of some urban groups of populations to these fermented foods, some of these products have become an important part of the diet for an increasing part of urban populations, mostly within the low income groups, as well as a source of income for many people including fermented food producers, vendors and related activity contractors such as millers and transporters. The present thesis deals with one of these lesser-known products, namely mawè in Bénin. Mawè is a sour dough made from maize, which constitutes the main cereal crop in Bénin. In this general introduction, emphasis is put on the importance of maize and maize-derived products in the human diet in Bénin, and the place of mawè as a maize-derived food.

Maize (*Zea mays L.*) is considered as the most important cereal crop of Africa. Originating from South America, this cereal was introduced in Africa during the 16th century by the Spaniards and the Portuguese (Adandé, 1984). The total production in this continent was estimated to exceed 39 million tonnes in 1989 and about 33 million tonnes in 1991 (FAO, 1992). The most important maize producing countries of the continent are South Africa and Egypt.

Maize is also the most produced cereal crop in Bénin (about 75% of the total cereal production). In 1991, its production was estimated to be about 446 000 tonnes, against a total of 138 000 tonnes for millets and sorghum. This production was twice the harvest of 1961 which was estimated to be 220 000 tonnes (Anonymous, 1992a). The adaptability of maize to different agro-ecological zones and the diversity of the processing methods used are the main reasons for its adoption as the basis of the diet by the majority of the population of Bénin. Especially in the Southern and Central part of the country with 70% of the national population, the pattern of food consumption is dominated by maize-derived products. In the rural areas of this part of the country, estimates show a maize consumption between 100 and 136 kg per year per person which corresponds to 58% of the energy intake (Anonymous, 1992b). In the North of the country, the pattern of food consumption is dominated by millets and sorghum. Cereal consumption was estimated to be more than 100 kg per year per inhabitant, with maize consumption estimated to be about 10 kg only per year per inhabitant. However, recently an increase of the consumption of maize has been noticed due to an increased production.

In addition to this increasing maize consumption in some parts of the country, there are other changes in food habits in Bénin. Important are those noticed in urban areas where the traditional food consumption pattern is influenced by imported foods as in many other developing countries. According to Nout (1992), the introduction of foreign "high-tech" processing concepts and food products like wheat bread, wheat-based or milk-based weaning foods, yoghurt and lager beer to tropical countries was followed by a rapidly increasing demand during the early post-independence period. The use of these expensive products provided status. Their adaptability to life in the cities which demands long shelf-life, ready-to-cook or ready-to-eat foods and their refined quality resulted in continued and increasing consumption.

In contrast, traditional indigenous foods processed by local methods lack appeal

because of unacceptable presentation due to lack of packaging and unhygienic practices, irregular quality, etc.. Furthermore, traditional technologies are laborious and time consuming, and thus incompatible with city life. A recent survey (Alexandre, 1991) in Cotonou, the largest city of Bénin, showed that the frequency of aklui consumption, a granulated maize porridge marketed as street food, decreases with an increasing level of income. This confirms the assumption that some traditional indigenous foods may be regarded as poor people's foods (Odunfa, 1985; Nout, 1992).

The substitution of local foods by foreign imported foods, as a result of increasing income, population growth and foreign influences, is detrimental to the national food security in Bénin at the long term. Upgrading traditional processes is a way to overcome this situation, to avoid the dependency of the urban populations on foreign foods.

The traditional food processing system of Bénin is diversified, maize being one of the major raw materials used. About 40 different ways of maize processing were recorded in Bénin (Nago, 1989). Besides traditional home-processing, a large variety of foods are produced and sold by food processing micro-enterprises. According to Nago *et al.*, (1990), this sector plays a strategic role in:

- national food security through the processing of local products;
- local food supply by the preparation of foods according to local customs;
- employment for women, food processing being their main source of income in urban areas.

Maize product manufacturers represent about 46% of all the food processing micro-enterprises present in the Southern and Central part of Bénin: among a total number of 19468 micro-enterprises recorded in this part of the country, 8934 were maize product manufacturers and/or sellers (Nago et al., 1990). More than 55% were located in Cotonou, where 10% of the total population of Bénin lives.

A recent survey carried out in the city of Cotonou by our laboratory confirmed the importance of maize product manufacturers in this city. This investigation comprised:

- counting all the millers trades, operating in town;
- conducting interviews in 40 randomly chosen mills;
- systematical and daily weighing of all the products to be ground in the chosen mills. Two weighing sessions were conducted per mill: the first session in March-April 1989, representing scarcity and the second in July-August 1989, representing abundance of cereals. Results of this survey are presented below.

In Cotonou, the cosmopolitan city of Bénin, like in the whole country and large parts of Africa, cereals used for human nutrition are milled using privately owned disc attrition mills (Premier 1A type). A total of 652 milling shops containing 659 similar mills were recorded in town. The millers are independent entrepreneurs who operate as grinding contractors. A fee is charged for grinding a volume unit of raw material, the price depending on the end-product to be obtained: in general, dry milling is more expensive than wet milling. According to Nago *et al.*, (1990), the introduction of mechanical mills has brought about revolutionary changes in the cereal processing system. It enabled the development of the micro-enterprise sector, which was limited by the low productivity of the early mortar technique. One of the most important consequences of this development is the acceptance of some foods, which where formerly considered as specific for some ethnical groups, by the whole urban population.

In Cotonou, about 126-146 tonnes of raw materials were milled daily for the production of local foods. Maize constituted 90-93%. Of the maize, 45% was processed into fermented foods through wet milling and 55% into whole maize flour through dry milling. The average daily consumption of all maize products was estimated at 300 g per inhabitant (or 90-110 kg per year). Consequently, daily consumption of fermented maize foods was estimated to be 135 g per inhabitant (or 40-50 kg per year, maize basis). These values are similar to those reported by Korthals Altes (1976), referring to a food consumption survey in the department "Ouémé" (Bénin).

The production of fermented foods from maize is arduous and time consuming, as compared to maize flour production. Consequently, their home production for family consumption is declining. This situation has allowed a few "initiates" to valorize their know-how of milling technology and has encouraged commercial production of fermented maize products by women. In Cotonou, 85% of maize-based fermented foods is produced commercially, against only 18% of maize flour.

The fermented maize dishes are derived from two types of intermediate products, namely ogi and mawè:

Ogi is a gruel obtained by fermentation of a suspension of wet-milled maize in water. This product has been described elsewhere (Akinrele, 1970; Akingbala *et al.*, 1987). According to Akinrele (1970), maize is commonly consumed in the form of an ogi-derived product amongst Yoruba in the Western region of Nigeria. Ogi is also known as akamu by

the Hausa of Nigeria (Akingbala *et al.*, 1987). A similar product is known in Kenya as uji and in South Africa as mahewu (Steinkraus *et al.*, 1983). About 26-32% of maize processed in Cotonou is used for ogi production. About 86% of the ogi production in the city is carried out by food processing micro-enterprises, the remaining 14% being produced for own consumption only. Ogi is marketed in the form of ready-to-serve dishes by women established on the markets or at the roadsides in the city.

The major dishes derived from ogi are:

- A gel of variable stiffness (akassa, kanan, gi, eko, kafa, lio, agidi). The major part (81%) of ogi produced in Cotonou is consumed as akassa or a similar product. Akassa is essentially a commercial product, more than 82% being produced to be sold. Akassa is consumed with a fish or meat stew.
- A porridge (koko), 7% of the ogi production. The production of koko is almost exclusively commercial. It is consumed as breakfast by adults and children and particularly appreciated by sick persons.
- A semi-solid gelatinized mass (akpan), which becomes a thirst-quenching beverage by adding water, ice, sugar and milk. Akpan, 7% of the ogi production in Cotonou, is always produced commercially.

Mawè, the other intermediate product, is a fermented dough. Doh (1970) described a similar product in Togo, also known as mawè. Based on our survey we estimate the processed maize in Cotonou used for mawè at 14-16%. Quantitatively it is less important than ogi, but it is suitable as a basis for the preparation of many dishes, including those obtained from ogi (Fig.1).

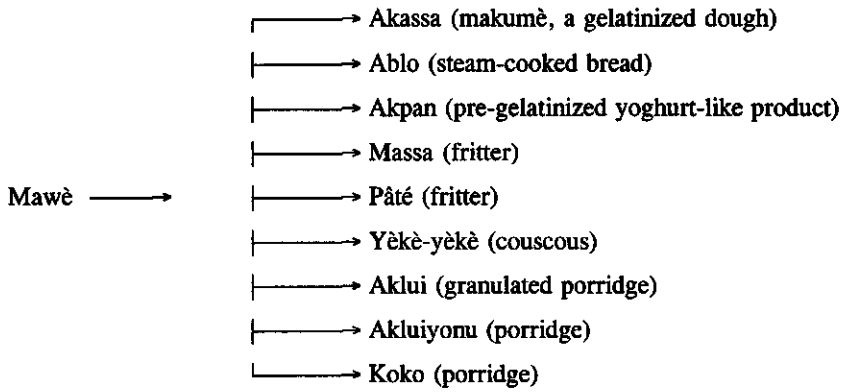

Figure 1. Main dishes obtained from mawè.

Dishes derived from mawè include gel-like products (akassa), pre-gelatinized semi-solid mass (akpan), porridges (koko, aklui, akluiyonu), fritters (massa, pâté), couscous (yèkè-yèkè) and steam-cooked breads (ablo).
Many other dishes include mawè as minor ingredient e.g. talé-talé (banana fritter).

Besides the traditional process here called **home process**, a new process has emerged to meet the quality requirement of the urban consumers of mawè. In this thesis, this new process is called the **commercial process**. About 83% of all the mawè produced in Cotonou is made by food processing micro-enterprises. Mawè is not only marketed as one of its ready-to-serve derivatives, but also as a ready-to-cook intermediate product. According to Nago *et al.*, (1990), this intermediary market of semi-finished mawè is a response to domestic food requirements in urban areas. Women in urban areas carry out a wide range of activities (salaried jobs, commercial and domestic activities), each of which competes for time allocation. Priority is given to income generation with more time allocated to it. The production of mawè for this new market is also justified by the necessity to satisfy the requirements of many consumers who prefer, for quality and hygienic reasons, to prepare their own dishes. The ease of packaging of mawè and its wide range of culinary applications have contributed to its success in this market where ogi is missing: 64% of the commercial production of mawè is sold as a ready-to-cook product. This growing market for semi-finished products has particularly contributed to the establishment of micro-enterprises producing mawè, and to the innovative improvements of the process.

AIM AND OUTLINE OF THIS THESIS

For the satisfaction of the market of the growing urban population, the traditional mawè process requires upgrading and optimization. As far as fermented products are concerned, four broad categories of priorities are recommended (Gaden *et al.*, 1992; Sanni, 1993):
- improving understanding of the fermentation processes,
- improving the technology,
- increasing the utilization of the processes,
- developing local capabilities.

The first two priorities are of concern in this thesis. Investigations are carried out to improve the knowledge base of traditional mawè processing. The different processes have been identified. The composition and microbiological and physical attributes of the resultant

mawè, as it was sold in urban markets or produced and consumed at home, were determined (Chapter 2). Yield and changes in acidity (pH and titratable acidity), in macro-nutrients composition, in colour and viscosity properties during the process were studied (Chapter 3). The various microorganisms involved in the fermentation of each process were isolated, identified and the changes occurring in the number of each type investigated (Chapter 4). In particular, characterization and frequency distribution of species of lactic acid bacteria involved in the process were outlined (Chapter 5). Representative species of lactic acid bacteria and yeasts were selected and studied alone or in combination for their role in the mawè porridge fermentation process (Chapter 6).

In a general discussion (Chapter 7), the main knowledge base of the traditional mawè processing was reviewed. Comparisons were made with similar products found in West-Africa and some suggestions were made for further prospects for the development of the mawè process.

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CHAPTER 2

COMPOSITION AND MICROBIOLOGICAL AND PHYSICAL ATTRIBUTES OF MAWÈ, A FERMENTED MAIZE DOUGH FROM BENIN

ABSTRACT

Home-produced and commercial mawè samples in urban Bénin were investigated. Titratable acidity was in the range 1.2-1.4% (w/w as lactic acid), but home-produced mawè appeared to be consumed at a slightly higher pH (4.2) than commercial mawè (3.8). Average protein contents of commercial and home-produced mawè were 8.2 and 9.2%, crude fat contents 1.0 and 2.3%, ash contents 0.6 and 1.1%, crude fibre 0.4 and 0.7%, respectively on a dry weight basis. Total aerobic mesophilic and lactic acid bacteria counts were similar for both products but yeast count was slightly higher and Enterobacteriaceae count lower in commercial mawè. Particle size analysis showed major fractions as $< 45\ \mu m$ in all samples. The higher viscosity of the commercial product indicates that it might be superior for making gel-type foods, e.g. agidi.

INTRODUCTION

Fermented cereal foods are produced for daily consumption in most African countries. In Bénin, they are obtained from two types of intermediate fermented products:

- **ogi**: a gruel obtained by fermentation of a suspension of maize in water (Oke, 1967; Akinrele, 1970) is consumed as a gel of variable degree of stiffness, having different local names (akassa, kanan, gi, eko, kafa, lio, agidi), as a semi-solid product (akpan), or as a liquid porridge (koko).

An abridged version of this chapter has been published as:

Composition and Microbiological and Physical Attributes of Mawè, a Fermented Maize Dough from Bénin
D.J. Hounhouigan, M.J.R. Nout, C.M. Nago, J.H. Houben and F.M. Rombouts
Int. J. Food Sci. & Technol. **28** (1993) 513-517.

- **mawè**: a fermented dough, also known in Togo (Doh, 1970), is used for cooked dishes including gels (ogi-like products), steam-cooked bread (ablo), or porridge (koko, aklui, akluiyonu). Although less mawè is produced than ogi, it is sold both as ready-to-serve and as domestic ready-to-cook products in urban areas. The difference between the two processes for mawè production, i.e. the traditional "home process" (Fig.1) and the "commercial process" (Fig.2) is outlined below:

Maize is cleaned by winnowing, washed in water and crushed in a plate disc mill (Premier 1A type). The crushed maize is screened by metal or nylon sieve with 0.5 x 0.5 mm apertures (commercial process), or through a palm-fibre sieve ("sassado") with 2 x 2 mm or 2 x 4 mm apertures (home process). Grits and hulls are separated by gravity on the sieve and the fine endosperm fraction collected in a bowl. In the commercial process which takes place entirely in the milling shop, the grits are washed by rubbing in water, the germs and remaining hulls floated off and discarded, water decanted and the sedimented endosperm grits blended with the fine endosperm fraction. In the home process, the grits are not washed, but home-dehulled. The resulting grits and fine fraction are moistened, held for 2-4 h and milled to a dough. The kneaded dough is then covered with a polyethylene sheet and allowed to naturally ferment to a sour dough and held in the fermentation bowl or wrapped in paper or polyethylene.

These methods of mawè production show some similarity with the dry-milling approach for ogi manufacture (Banigo *et al.*, 1974).

The present study was carried out to determine the relevant chemical, microbiological and physical characteristics of home-produced and commercial mawè for product quality improvement.

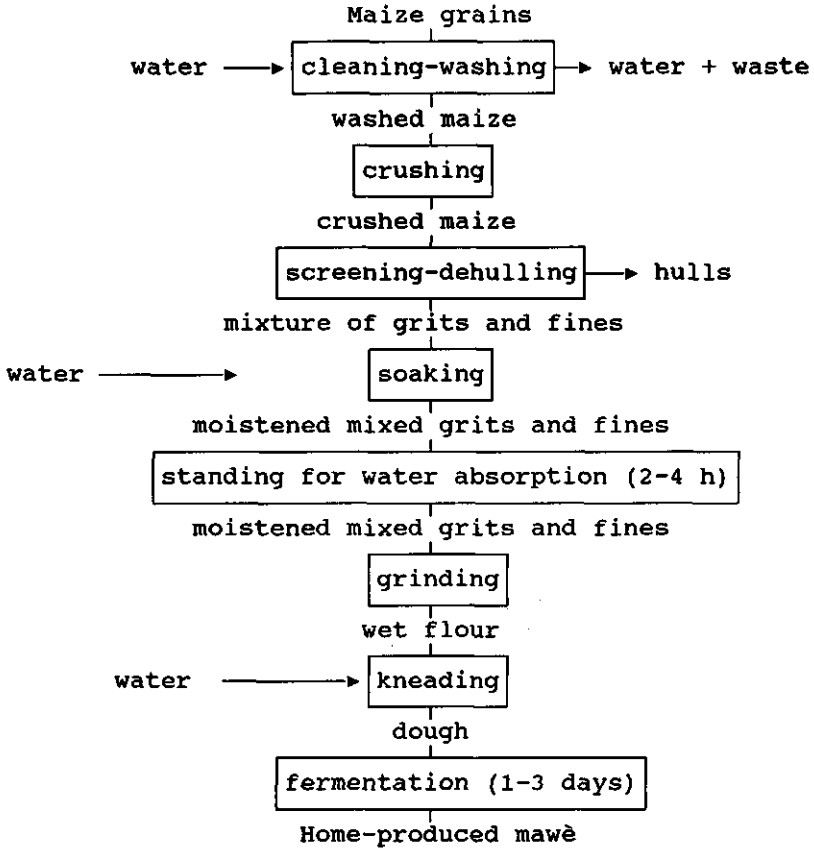

Figure 1. Flow diagram of mawè production: home process

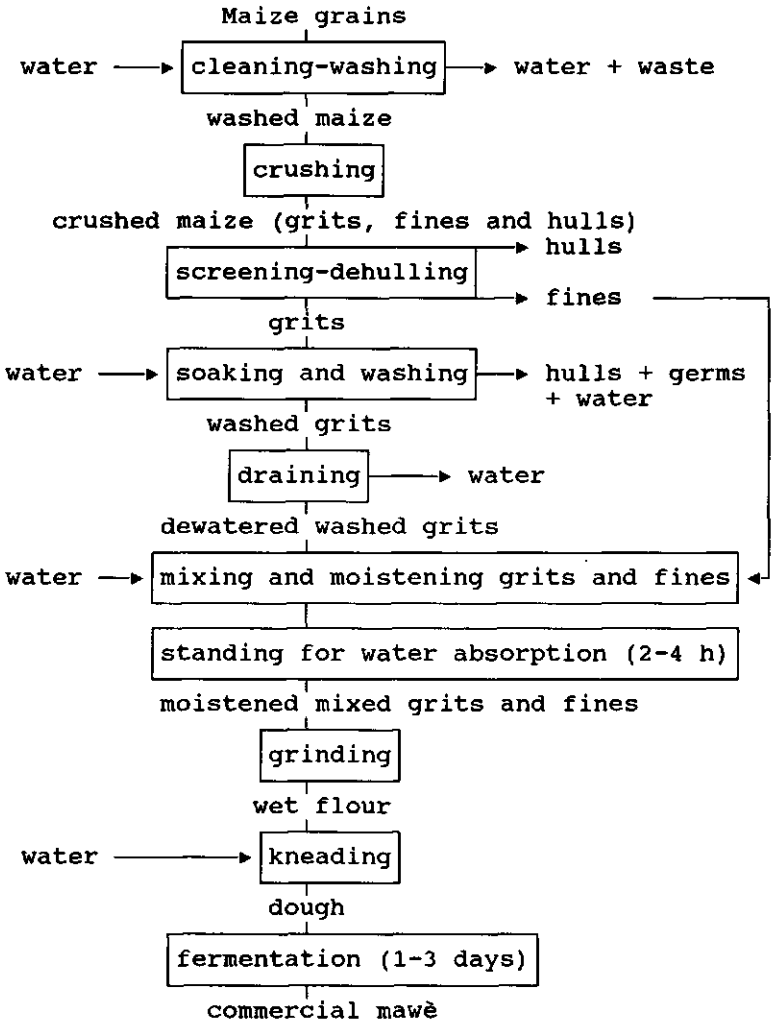

Figure 2. Flow diagram of mawè production: commercial process.

MATERIALS AND METHODS

Samples (2 kg) of home-produced mawè were collected from 20 homes, 15 samples of commercial mawè were collected at markets, and 15 samples obtained directly from producing mills. Samples in their original packaging (bowl, paper or polyethylene bag) were immediately brought to the laboratory where sub-samples were taken for microbial culture, pH, titratable acidity and colour measurements. The remainder was packed in freezer bags and held at -50°C for proximate analysis, viscosity and particle size measurement.

Chemical Analyses

Titratable acidity and pH were measured immediately (Nout *et al.*, 1989), as was moisture (method 44-15A, AACC, 1984). Thawed mawè (50 g) was dried at 60°C for 48 hours, ground and dried to constant weight at 103°C. The dried sample was vacuum-sealed in laminated aluminium bags for proximate analysis. Protein, fat and ash content were determined using AACC methods 46-11A, 30-25 and 08-01 (AACC, 1984). Crude fibre content was determined as described by Osborne and Voogt (1978). Carbohydrate was calculated by difference.

Microbiological analyses

Duplicate sub-samples of mawè (10 g) were homogenized with 90 ml sterile peptone-physiological salt solution (5 g peptone, 8.5 g NaCl, 1000 ml distilled water, pH 7.0 $\pm$ 0.2) and decimal diluted. All cultures were in pour plates. Total aerobic mesophilic counts were made after incubation (3 d, 30°C) on Plate Count Agar (PCA; 5 g tryptone, 2.5 g yeast extract, 1 g dextrose, 15 g agar N°1, 1000 ml distilled water, pH 7.0 $\pm$ 0.2) with a PCA overlay to avoid surface spreading. Lactic acid bacteria counts were made after 3-5 d at 30°C on MRS medium (Oxoid CM 361) containing 0.1% (w/v) natamycin (Delvocid, Gist-Brocades, Delft, The Netherlands), with an overlay of the same medium. *Lactobacillus* spp. counts were made after 3 d at 30°C on Rogosa agar (Oxoid CM 627) with overlay. Yeasts and filamentous fungi (moulds) were counted after 3-5 d at 25°C on Yeast Extract Glucose Agar (Oxoid CM 545) to which 0.01 % (w/v) sterile oxytetracycline (Oxoid SR 73) was added after autoclaving. Enterobacteriaceae were counted after 20-24 h at 37°C on Violet Red Bile Glucose Agar (VRBG, Oxoid CM 485) with overlay.

Physical analyses

Colour of mawè samples was measured with a Minolta CR 210b portable chromameter, using chromaticity coordinates L^*, a^* b^* and ΔE (illuminant D65; CIE 1976). The instrument was standardized with a standard white tile ($Y = 94.8$, $x = 0.315$ and $y = 0.3324$).

Particle size was determined as described by Sefa-Dedeh (1989): 100 g of mawè sample was shaken for 30 min through 355, 250, 125, 90, and 45 μm screen sieves, on an Endecotts test sieve shaker (Endecotts, London, UK). A continuous jet of water was sprayed on to the sample. The solids content of the sample on each sieve was determined by drying and the corresponding percentage of the sample (dry weight basis) calculated. The solids content of the throughs from the bottom sieve (45 μm) was determined by difference.

Viscosity of mawè thawed overnight at +5°C was measured with a viscograph (Pt 100, 700 cmg sensitivity cartridge, Brabender OHG Duisburg, Germany) on 8% dry matter slurries (450 g). The mixture was heated at 1.5°C min^{-1} from 30°C up to 92°C, held at 92°C for 15 min and then cooled at 1.5°C min^{-1} to 50°C (Adeyemi, 1983). The viscograms were evaluated as described by Banigo *et al.*, (1974).

RESULTS AND DISCUSSION

In mawè resulting from both processes, the average moisture contents were 45-47% and did not differ significantly. Titratable acidity of both home-made and commercial mawè samples were similar (1.2-1.4% w/w as lactic acid), but home-made mawè had a slightly higher pH (Table 1). Both values were similar to that found in highly acceptable ogi (Steinkraus *et al.*, 1983) and Ghanaian maize dough (Plahar and Leung, 1983). As expected, crude protein, crude fat, crude fibre and ash contents of home-produced mawè were higher than those of commercial mawè since more hulls and germs are retained during the home production. Process conditions influence ogi composition in a similar way (Banigo *et al.*, 1974; Adeyemi *et al.*, 1987).

The dominating micro-organisms in mawè (Table 2) were lactic acid bacteria (mainly *Lactobacillus* spp.) and yeasts. Enterobacteriaceae counts were lower than 400 and 3600 cfu/g in commercial and home-produced mawè, respectively. Commercial market mawè had a slightly higher yeast count with no real differences in counts of total aerobic mesophilic and lactic acid bacteria.

In general, mawè is a white dough produced from white maize varieties. Most commercial mawè is visually whiter than home-produced mawè, although all the measured values were very similar (Table 3).

Table 1. Chemical characteristics of mawè.

	Home-produced mawè	Commercially produced mawè	
	(collected from homes) n=20	(fresh from the mill) n=15	(sold at the market) n=15
pH	4.2 ± 0.4[1]	3.9 ± 0.3	3.8 ± 0.3
Titratable acidity (% w/w, as lactic acid)	1.2 ± 0.2	1.1 ± 0.3	1.4 ± 0.5
Moisture content (%)	46.8 ± 2.7	45.9 ± 1.5	45.1 ± 1.2
Crude protein[2] (% dwb)[3]	9.2 ± 0.9	8.3 ± 0.8	8.2 ± 1.0
Crude fat (% dwb)	2.3 ± 0.9a[4]	0.9 ± 0.4b	1.0 ± 0.6b
Crude fibre (% dwb)	0.7 ± 0.1a	0.4 ± 0.1b	0.4 ± 0.1b
Ash (% dwb)	1.1 ± 0.2a	0.6 ± 0.1b	0.6 ± 0.1b
Soluble carbohydrate (% dwb)	86.7	89.8	89.8

[1] Averages and standard deviations.

[2] N x 6.25.

[3] dwb = dry weight basis.

[4] a, b: Averages with different letters are significantly different (Student's t-test; $p<0.05$).

Table 2. Microbiological composition of mawè (Log_{10} c.f.u./g).

	Home-produced mawè	Commercially produced mawè	
	(collected from homes) n=20	(fresh from the mill) n=15	(sold at the market) n=15
Total aerobic mesophilic count	9.0 ± 0.4[1]	8.8 ± 0.4	8.8 ± 0.3
Lactic acid bacteria	9.0 ± 0.6	8.9 ± 0.4	8.9 ± 0.3
Lactobacillus spp.	9.0 ± 0.4	8.9 ± 0.4	8.9 ± 0.3
Yeasts[2]	5.8 ± 0.8	6.4 ± 0.9	6.9 ± 0.5
Enterobacteriaceae	≤ 3.6	≤ 2.5	≤ 2.3

[1] Averages and standard deviations.

[2] Moulds were not detected.

Table 3. Colour parameters of mawè

	Home-produced mawè	Commercially produced mawè	
	(collected from the homes) n=20	(fresh from the mill) n=15	(sold at the market) n=15
L^*	78.7 ± 2.1[1]	78.0 ± 2.5	79.7 ± 1.3
a^*	-1.0 ± 0.4	-1.0 ± 0.4	-1.1 ± 0.3
b^*	9.8 ± 0.9	9.3 ± 0.9	9.2 ± 1.8
ΔE	20.8 ± 2.1	22.0 ± 3.2	19.0 ± 1.4

[1] Averages and standard deviations.

Particle size analysis showed major fractions as $< 45\mu m$ in all samples (Table 4).

Brabender viscosity profiles of mawè are similar to those for sorghum ogi (Adeyemi, 1983) and ogi from different varieties of maize (Adeyemi *et al.*, 1987). The average temperature of gelatinization was about 73°C for the commercial market mawè and 75°C for home-produced mawè. Viscosity during heating and cooling, stability and gelatinization index were all slightly higher in commercial than in home-produced mawè (Table 5). According to Banigo *et al.*, (1974), this would make commercial mawè more suitable than home-produced mawè for the preparation of gel-type products (e.g. agidi).

Table 4. Particle size distribution of mawè (weight %)

Particle size (μm)	Home-produced mawè	Commercial mawè
	n=20	n=30
$x \geq 355$	5.5 ± 3.1[1]	2.8 ± 1.4
$250 \leq x < 355$	4.9 ± 1.6	4.2 ± 1.5
$125 \leq x < 250$	13.3 ± 3.0	12.9 ± 3.0
$90 \leq x < 125$	6.7 ± 3.3	6.1 ± 1.5
$45 \leq x < 90$	7.7 ± 3.1	10.2 ± 3.5
$x < 45$	61.9 ± 6.3	63.8 ± 6.5

[1] Averages and standard deviations.

Table 5. Viscograph characteristics of traditional mawè

	Home-produced mawè	Commercially produced mawè	
	(collected from the homes) n=20	(fresh from the mill) n=15	(sold at the market) n=15
T_g (°C)	74.9 ± 2.2	73.2 ± 1.3	72.6 ± 0.9
M_g (min)	30.0 ± 1.5	28.8 ± 0.9	28.4 ± 0.7
M_n (min)	41.4 ± 2.5	40.2 ± 0.5	39.7 ± 0.8
M_n-M_g (min)	11.4 ± 3.1	11.4 ± 0.8	11.3 ± 0.5
V_m (BU)[1]	221 ± 61	333 ± 68	383 ± 48
V_r (BU)	197 ± 47	269 ± 51	291 ± 36
V_c (BU)	338 ± 84	456 ± 87	492 ± 62
V_m-V_r (BU)	24 ± 17	64 ± 30	92 ± 44
V_c-V_m (BU)	117 ± 38	123 ± 32	109 ± 53
V_c-V_r (BU)	141 ± 44	187 ± 38	201 ± 28

[1] BU, Brabender unit.

T_g, gelatinization temperature; M_g, time to reach T_g; M_n, time to reach V_m; M_n-M_g, Ease of cooking; V_m, maximum viscosity during heating; V_r, viscosity after 15 min at 92°C; V_c, viscosity after cooling to 50°C; V_m-V_r, stability of the starch; V_c-V_m, setback value; V_c-V_r, index of gelatinization.

CONCLUSION

Colour, fineness and acidity are the major quality criteria in mawè production. Commercial mawè is thought to be whiter and finer than home-produced mawè, but no real differences were found. As significantly more hulls and germs are retained in the home-made mawè, it has more protein, crude fat, crude fibre and ash. The microflora of both products is fairly similar and consists mainly of *Lactobacillus* spp. and yeasts. Traditional mawè varies widely, and is not of uniform composition; factors responsible include the maize cultivars used,

actual process conditions and environmental conditions during the fermentation, all of which affect the nature and activity of dominating microorganisms and the biochemical transformations taking place.

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CHAPTER 3

CHANGES IN THE PHYSICO-CHEMICAL PROPERTIES OF MAIZE DURING NATURAL FERMENTATION OF MAWÈ

ABSTRACT

The physical and chemical changes that occurred during a 72-h fermentation period were studied in two differently processed maize doughs from Bénin, referred to as home-produced and commercial mawè. The pH decreased from 6.1 to 3.5 in the commercial process and from 6.2 to 3.6 in the home-style process, whereas the titratable acidity increased from 0.2 to 1.7% (w/w, lactic acid), and from 0.3 to 2.3%, respectively. Home-produced mawè had significantly higher levels of crude fat, crude fibre and ash compared with the commercial mawè, as a consequence of the difference in the processing methods. No marked changes in proximate composition occurred during subsequent fermentation. Commercial mawè was whiter than home-produced mawè, and this whiteness increased with increasing fermentation time. Fermentation significantly increased the swelling and thickening capabilities of mawè, which were more pronounced in the commercial than in home-produced samples. Overall, the commercial mawè process appeared to be a technologically more advanced and effective method of mawè manufacture than the home process.

INTRODUCTION

Mawè is a sour dough, which has undergone natural fermentation for 1 to 3 days. It is made from dehulled maize, and, on this basis, is different from the whole maize dough used to prepare Ghanaian kenkey[1]. Mawè is used for the preparation of a variety of dishes

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in its area of origin, Bénin and Togo. Depending on its intended use and on individual taste preferences, consumers choose mawè on the basis of its sourness, whiteness and fineness.

An investigation carried out in the Bénin urban area showed that ready-to-use mawè had a titratable acidity of 1.2-1.4% (w/w, as lactic acid), with pH values of approximately 4.2 in home-produced mawè and 3.8 in commercial mawè[2]. These degrees of sourness are generally accepted for the preparation of stiff gels (akassa, agidi, eko) and porridge (koko). On the other hand, less sour mawè is preferred for steamed cooked bread (ablo), and more acid mawè is used for the preparation of porridge for sick people. In Bénin, mawè is a substitute for ogi (a fermented maize gruel fermented as a suspension) for akassa and porridge preparation.

The physical and chemical changes during the processing of cereal grains into ogi have been investigated[3-5]. As information on the physical and chemical changes during the processing of maize to mawè is lacking, this study was carried out to evaluate these changes.

EXPERIMENTAL

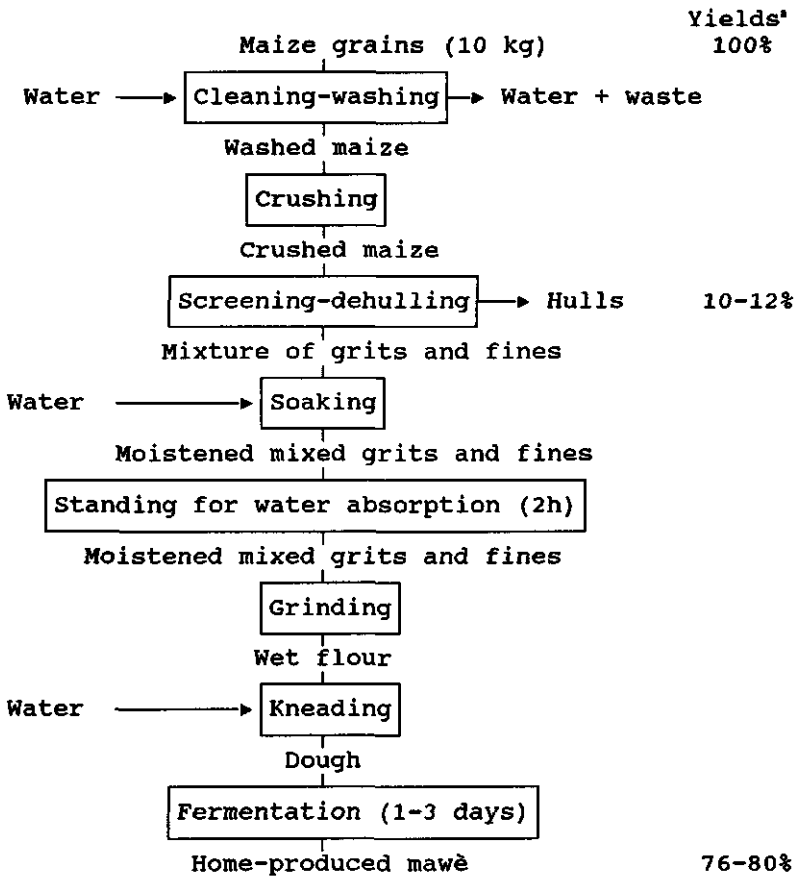
Mawè production

Home-produced mawè and commercial mawè were produced as illustrated in Figs 1 and 2, respectively. The processing was carried out by a local mawè producer, using the maize cultivar Sékou 85 provided by the International Institute of Tropical Agriculture, Bénin. The dough resulting from each process was divided between six plastic buckets with lids. Fermentation was at room temperature (28-32°C) and was carried out for 72 h. Each process was carried out in duplicate.

Sample treatment, packaging and preservation

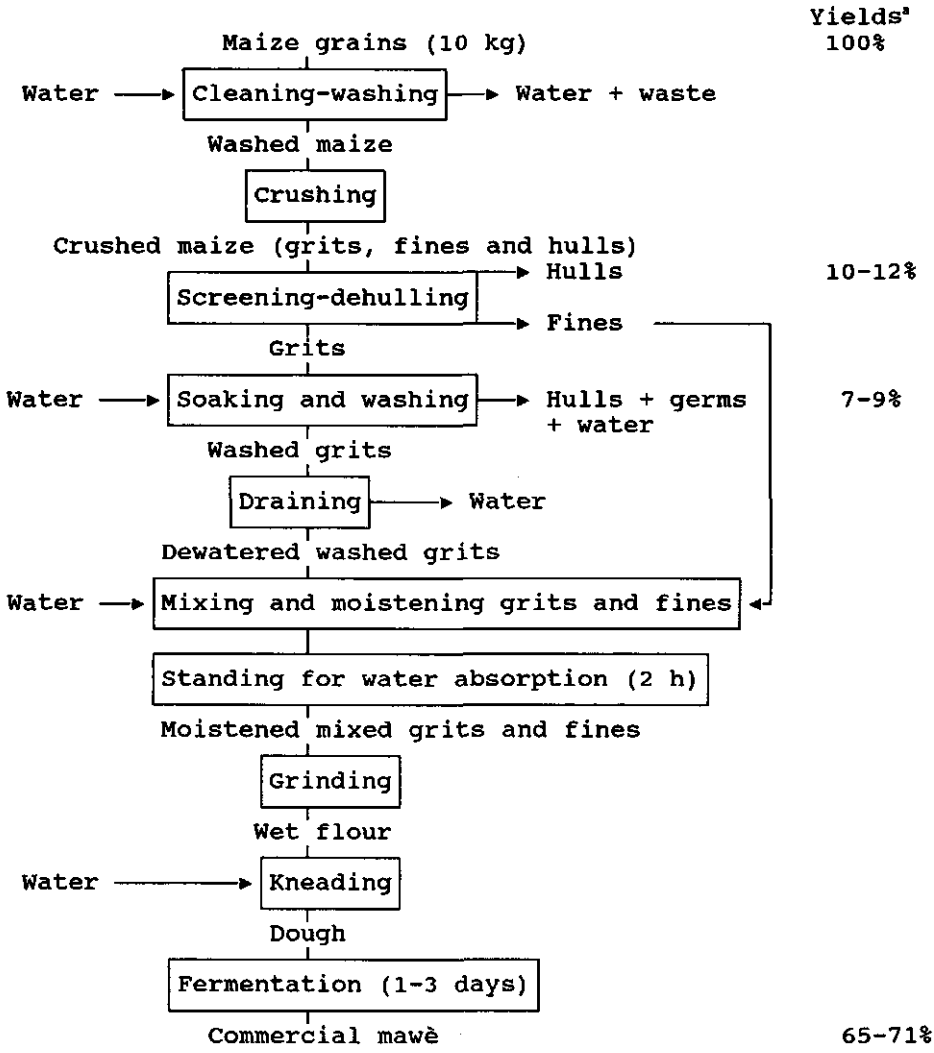
After 0, 6, 12, 24, 48 and 72 h fermentation, one bucket of mawè from each process was taken for analysis. Sub-samples (5 g) were used for moisture determination. Portions (100 and 20 g) were used for measurement of colour and determination of pH and titratable acidity, respectively. The remainder of the sample was packed in polyethylene bags, and held at -50°C for later analysis: frozen samples of mawè were thawed overnight at 5°C for viscosity measurement or dried in an air-oven at 60°C for 48 h,

ground in a Retsch ZM1 mill and dried further to constant weight at 103°C. The dried sample was vacuum-sealed in laminated aluminium foil bags until determination of proximate composition.



* Yields expressed on dry-weight basis.

Figure 1. Flow diagram for mawè production: home-style process



[a] Yields expressed on dry-weight basis.

Figure 2. Flow diagram for mawè production: commercial process

Chemical analysis

pH and titratable acidity were measured by the method of Nout *et al.*[6], modified as follows: the sample (10 g) was mixed with distilled water (20 ml) and the pH measured using a Hanna 8417 pH meter (Hanna Instruments, Limena, Italy). Subsequently, this suspension was mixed with distilled water (70 ml) for determination of the titratable

acidity. Moisture, crude protein, crude fat and ash contents were determined using AACC[7] methods 44-15 A, 46-11 A, 30-25 and 08-01, respectively. Crude fibre content was determined as described by Osborne and Voogt[8]. Carbohydrate content was calculated by difference.

Physical analysis

The colour of mawè samples was measured with a Minolta CR-210 portable chromameter, using chromaticity co-ordinates L^*, a^*, b^* and ΔE (illuminant D65, CIE 1976). The instrument was standardized with a standard white tile ($Y = 94.8$, $x = 0.3150$ and $y = 0.3324$). The pasting characteristics were measured using a Brabender Pt 100 Viscograph (Brabender OHG Duisburg, Germany). A 700 cmg sensitivity cartridge was used. Slurries containing 10.0% (w/v) dry matter were analyzed. The total weight of slurry in the Viscograph bowl was 450 g. The mixture was heated at 1.5°C/min from 30°C up to 92°C, held at 92°C for 15 min and then cooled to 50°C. The Viscograms were interpreted as described by Banigo *et al.*[9].

Statistical analysis

Samples from different processes and fermentation periods were statistically compared using analysis of variance[10] and Duncan's Multiple Range Test[11].

RESULTS AND DISCUSSION

Yield of mawè

The yield of mawè from three replications of each process ranged between 76 and 80% for the home-style process and between 65 and 71 % for the commercial process (dry-weight basis). The lower yield in the commercial process was caused mainly by the thorough washing of grits in water to remove hulls and germs, and to the concomitant loss of extractable dry matter in the washing water. In the home-style process, this operation was not applied: the mixture of grits and fines obtained after screening-dehulling was soaked in water without washing (Figs 1 and 2).

A significant ($P<0.05$) increase in the moisture content of mawè was observed during the fermentation period (Table I). This is caused by the combined effects of dry matter consumption and production of water during aerobic and anaerobic catabolism by

yeasts and heterofermentative lactic acid bacteria[2,12].

TABLE I. Influence of the fermentation time on the moisture content of mawè[a]

Fermentation time	Moisture content (% w/w, fresh weight)	
(h)	Home-produced mawè	Commercial mawè
0	45.5	46.0
6	45.4	46.0
12	46.1	46.2
24	46.3	46.8
48	47.2	48.4
72	48.3	48.2

[a] Mean of two independent determinations. Replicates were within 3% of the mean.

pH and titratable acidity

The changes in pH and titratable acidity in both types of mawè are shown in Figs 3 and 4, respectively. The titratable acidity of home-produced and commercial mawè increased from 0.3% and 0.2% (w/w, calculated as lactic acid), at the start of the fermentation (t_0), to 2.3% and 1.7%, respectively, after 72 h fermentation (t_{72}). The pH of home-produced mawè still remained the highest.

In the commercial process, water-extractable sugars and proteins are partly lost during the washing of the grits. In the home-style process, the greater availability of fermentable sugars led to enhanced production of organic acids (Fig. 4). But the home-produced mawè also had a higher protein content and consequently a higher buffering capacity[13]. The latter was responsible for the higher pH compared to the commercial product.

In the commercial product, the lower titratable acidity was, nevertheless, sufficient to create an extracellular pH[14] that limited further growth. The buffering effect of the higher protein content in the home-produced mawè necessitated larger amounts of titratable

acidity to reach an inhibitory extracellular pH. In fact, after 72 h fermentation, the home-produced mawè had not yet stabilized. A buffering action by proteins was observed also in ogi[3] and Ghanaian maize dough[15].

Our findings confirm that the extent of fermentation of cereal products cannot be evaluated merely on the basis of their pH. Consequently, evaluation of fermentation rates requires monitoring of both pH and titratable acidity. Earlier experiments[2] showed that at the moment of use, market samples of commercial mawè had a titratable acidity of 1.4 ± 0.5% (w/w, as lactic acid) and home-produced mawè contained 1.2 ± 0.2% titratable acidity. These levels of acidity were achieved within 24 h of fermentation in commercial mawè or within 12 h of fermentation in home-produced mawè under the conditions of the present study.

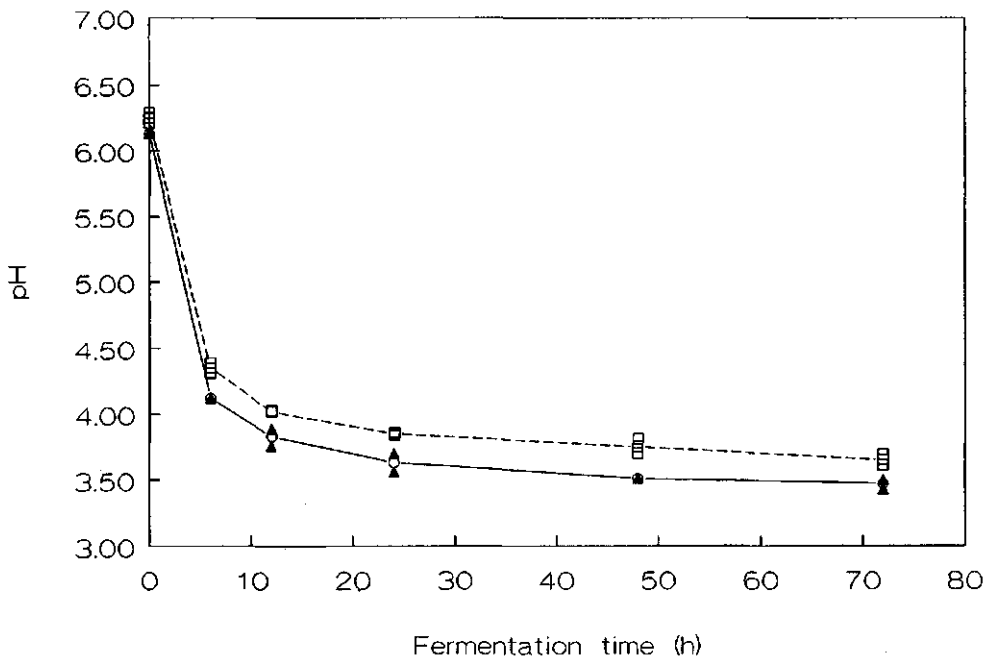


Figure 3. The influence of fermentation time on the pH of mawè.
Home-produced mawè (- □ -), commercial mawè (— ▲ —)

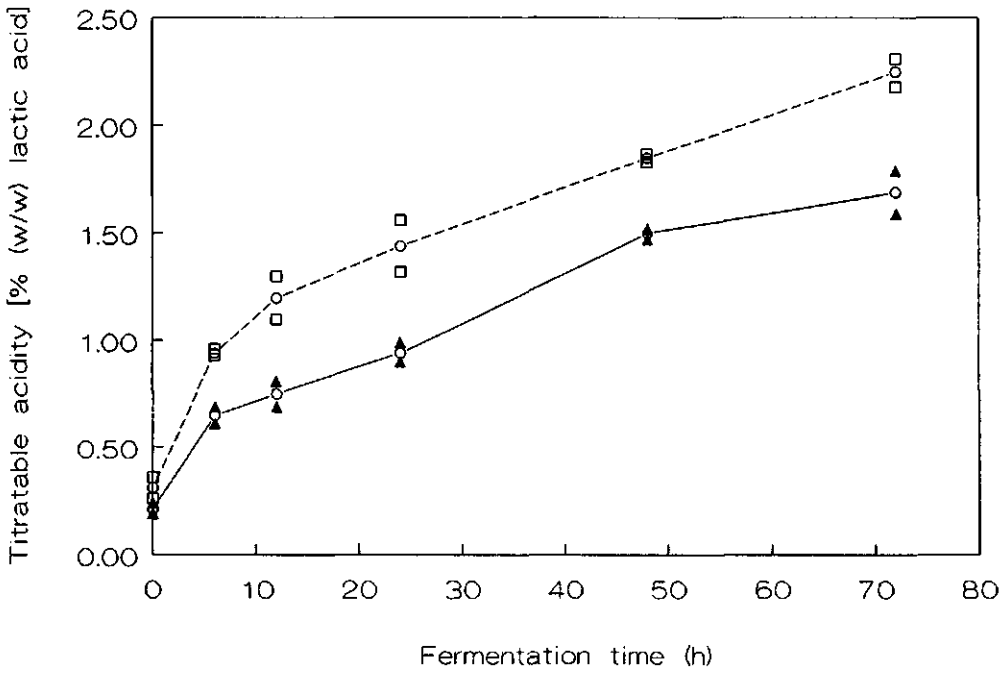


Figure 4. The influence of fermentation time on the titratable acidity of mawè.
Home-produced mawè (- □ -), commercial mawè (— ▲ —)

Proximate composition

From Table II, it can be seen that the processing method had only a slight effect on the crude protein content but the crude fat, ash, crude fibre and carbohydrate contents of mawè were influenced significantly ($P<0.01$). The maize cultivar Sékou 85 sample used contained about 11.2% crude protein, which was reduced to about 10.3% on conversion to unfermented commercial mawè (about 8% loss in t_0 sample) and remained constant on conversion to unfermented home-produced mawè.

A substantial loss of protein (40-50%) was reported during the processing of maize to ogi[3,16]. Compared with the unprocessed maize, the crude fat content was reduced by about 80% in commercial mawè and by 22% in home-produced mawè; the ash content by about 67% in commercial mawè and 13% in home-produced mawè; and the crude fibre content by about 72% in commercial mawè and 50% in home-produced mawè. No marked changes in proximate composition occurred during subsequent fermentation. The only

difference between the commercial process and the home process was the washing of the grits before milling, which may explain the difference in proximate composition between both types of mawè.

TABLE II. Proximate compositions of maize raw material, and of mawè at the start (t_0) and after 24 h (t_{24}) and 72 h (t_{72}) of fermentation[a]

	Maize (raw material)	Home-produced mawè			Commercial mawè		
		t_0	t_{24}	t_{72}	t_0	t_{24}	t_{72}
(% dwb)[b]							
Crude protein[c]	11.2	11.3a[d]	11.2a	11.3a	10.3a	10.5a	10.7a
Crude fat	4.9	3.8a	3.8a	3.9a	1.0b	1.1b	1.2b
Crude fibre	1.8	0.9a	0.9a	1.0a	0.5b	0.5b	0.6b
Ash	1.5	1.3a	1.3a	1.3a	0.5b	0.5b	0.5b
Carbohydrate[e]	80.6	82.7a	82.8a	82.5a	87.6b	87.4b	87.0b

[a] Mean of two independent determinations.

[b] dwb = dry weight basis.

[c] N x 6.25

[d] a, b, Means with the same letters are not significantly different ($P<0.05$).

[e] Calculated by difference.

Changes in colour

Colour parameters recorded during mawè fermentation are given in Table III. The fermentation period and the processing method both affected the colour parameters L^* (luminosity), a^* (greenness-redness) and ΔE (total colour difference with standard white tile) significantly ($P<0.01$). Commercial mawè was brighter than home-produced mawè, and the L^* values increased with increasing fermentation time, while a^* and ΔE values decreased correspondingly.

The results suggest that the luminosity (whiteness) of the product became more

intense during the course of the fermentation. On the other hand, a high correlation ($r \geq 0.96$) was observed between L^* value and the moisture content of the samples obtained at different fermentation times. Furthermore, unfermented samples at different levels of moisture content showed a similar correlation between their respective L^* values and moisture contents (results not included). This would suggest that the increase in the L^* values (or in the luminosity) of the product was due mainly to the increase of the moisture contents during the course of the fermentation.

TABLE III. Influence of fermentation time on the colour parameters of mawè[a]

	Home-produced mawè				Commercial mawè			
	L^*	a^*	b^*	ΔE	L^*	a^*	b^*	ΔE
0 h	78.0	-1.8	11.8	22.6	81.0	-2.2	9.2	18.7
6 h	78.4	-2.0	11.5	22.1	81.1	-2.4	8.8	18.5
12 h	78.9	-2.1	11.4	21.6	81.8	-2.3	8.7	17.9
24 h	79.2	-2.2	11.3	20.9	82.5	-2.4	8.4	16.8
48 h	79.9	-2.3	11.4	20.4	83.4	-2.6	8.1	15.9
72 h	80.8	-2.3	11.3	19.6	83.7	-2.6	8.4	15.7

[a] Mean of two independent determinations.

L^*, luminosity parameter. Replicates were within 1.5% of the mean.

a^*, greenness-redness parameter. Replicates were within 9% of the mean.

b^*, blueness-yellowness parameter. Replicates were within 8% of the mean.

ΔE, total colour difference with standard white tile. Replicates were within 5% of the mean.

Viscograph characteristics

The pasting characteristics, which developed during the fermentation of both types of mawè, are listed in Table IV. Fermentation time had no significant effect on the gelatinization temperature (T_g), nor on the time to reach T_g (M_g). On the other hand,

maximum viscosity (Vm), viscosity after 15 min at 92°C (V_r), viscosity after cooling to 50°C (V_e), time to reach V_m (M_n), stability value of the starch (V_m-V_r), setback value (V_e-V_m) and index of gelatinization (V_e-V_r), all increased with fermentation time. The biggest difference was observed between the unfermented sample and the fermented samples. This is in agreement with the report of Banigo *et al.*[9] that fermentation markedly increased the swelling and thickening characteristics of ogi.

Adeyemi and Beckley[4] also found that the peak viscosity of soured maize flour was much higher than that of the unsoured maize flour. In contrast, Sefa-Dedeh[17] found that the fermentation of maize dough drastically decreased the viscosity characteristics of Ghanaian-type corn dough. Adeyemi[18] also found that fermentation of sorghum flour samples reduced their peak viscosities. The composition of the fermented substrate, which will be affected by the nature of the raw material used and the processing method, probably affected the behaviour of the paste during the heating, holding and cooling cycles. Adeyemi and Beckley[4] suggested that the content of damaged starch granules, the *alpha*-amylase activity and the pH of the product would be factors having a more important influence than the presence of acetate ions, as suggested by Banigo *et al.*[9].

There was a significant ($P<0.01$) increase in the ease of cooking (M_n-M_g) of the fermented product, concomitant with the increased time to reach maximum viscosity (M_n). This finding contrasts with previous results on maize ogi[4], and corroborates the need for more research in order to understand the pasting behaviour of fermented cereal products.

It can be seen also that the processing method affected the gelatinization temperature (T_g), significantly ($P<0.01$), the time to reach T_g (M_g), the ease of cooking (M_n-M_g), the maximum viscosity (V_m), the viscosity after 15 min at 92°C (V_r), the viscosity after cooling to 50°C (V_e), the stability of the starch (V_m-V_r) and the index of gelatinization (V_e-V_r). The viscosity and the ease of cooking values were higher with mawè obtained by the commercial process whereas T_g and M_g values were lower.

These results are in accordance with those obtained previously[2]. For Nigerian ogi and Ghanaian maize dough, high gelatinization temperatures have been attributed to greater particle size[17,19,20], as well as to reduced starch content[19,20]: this may explain the behaviour of home-made mawè during heating. The higher crude fibre and crude fat contents of home-made mawè may have reduced the viscosities observed in this product, as reported for ogi obtained from dry milled maize compared with the viscosities of ogi from steeped whole maize[20].

TABLE IV. Influence of fermentation time on the pasting behaviour of mawè[a]

	Home-produced mawè				Commercial mawè			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
T_g(°C)	72.3	73.2	72	72.8	69.9	70.9	71.3	71
M_g(min)	28.2	28.8	28.6	28.5	26.7	27.3	27.5	27.5
M_n(min)	38.7	40.2	40.1	39.8	36.6	40.5	40.3	39.8
M_n-M_g(min)	10.5	11.4	11.5	11.3	9.9	13.2	12.8	12.3
V_m(BU)[b]	254	344	396	415	233	560	661	681
V_r(BU)	226	293	311	324	148	424	426	426
V_e(BU)	493	630	686	708	392	898	918	983
V_m-V_r(BU)	28	51	85	91	85	136	235	255
V_e-V_m(BU)	239	286	290	293	159	338	257	302
V_e-V_r(BU)	267	337	375	384	244	474	492	557

[a] Mean of two independent determinations. [b] BU = Brabender unit.

T_g, replicates were within 2% of the mean.

M_g, M_n, replicates were within 3% of the mean.

V_m, V_r, V_e, replicates were within 10% of the mean.

T_g, Gelatinization temperature; M_g, time to reach T_g; M_n time to reach V_m; M_n-M_g, ease of cooking; V_m, Maximum viscosity during heating; V_r, Viscosity after 15 min. at 92°C; V_e, Viscosity after cooling to 50°C; V_m-V_r, Stability of the starch; V_e-V_m, Setback value; V_e-V_r, Index of gelatinization.

CONCLUSION

The commercial process can be considered a technologically more advanced and more effective method of mawè production, compared with the home process. Its adoption was dictated by the market demand for a product as white and fine as possible, having maximum swelling and thickening characteristics. Considering that the loss of protein is

low during commercial mawè processing, and in view of the superior pasting behaviour, commercial mawè may be considered to be of superior quality.

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CHAPTER 4

MICROBIOLOGICAL CHANGES IN MAWE DURING NATURAL FERMENTATION

ABSTRACT

Two types of dough from dehulled maize, referred to as home-produced and commercial mawè, respectively, were investigated during a 72 h fermentation period for changes in their microbial composition. Lactic acid bacteria counts increased from 3.2 x 10^6 and 1.6 x 10^7 c.f.u./g (wet wt) of home-produced mawè and commercial mawè respectively, to 2 x 10^9 and 1.6 x 10^9 c.f.u./g after 12-24 h of fermentation. These populations were dominated by obligate heterofermentative *L. fermentum* (biotype *cellobiosus*), *L. fermentum* and *L. brevis*. In commercial mawè, the yeast count increased from 1.3 x 10^5 c.f.u./g to 2.5 x 10^7 after 48 h of fermentation before decreasing while in the home-produced mawè it increased from 2.5 x 10^4 to 3.2 x 10^7 c.f.u./g after 72 h of fermentation; dominating yeasts included *C. krusei* (mainly), *C. kefyr*, *C. glabrata* and *S. cerevisiae*. Enterobacteriaceae counts increased slightly during the initial stage of the fermentation, but decreased below the detection level after 24-48 h. *E. cloacae* was found mostly in commercial mawè and *E. coli* mostly in home-produced mawè.

INTRODUCTION

Fermentation is a widely used method for food crops processing all over the world. In Africa, most of the traditional cereal-based fermented foods are processed by natural fermentation. Investigations have been carried out to inventorize the microorganisms involved in the fermentation of these products (Akinrele, 1970; Christian, 1970; Nout, 1980; Fields *et al.*, 1981; Mbugua, 1984; Odunfa and Adeyele, 1985; Adegoke and Babalola, 1988). In

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most cases, lactic acid bacteria and yeasts played an important role in the fermentation process. These microorganisms and species of Enterobacteriaceae were also detected in home-produced and commercial mawè (Hounhouigan *et al.*, 1993a) where they can attain about 10^9, 10^7 and 10^3-10^4 c.f.u./g of mawè, respectively. Natural fermentation of mawè results in a product of variable quality. Development of controlled fermentation is necessary for the manufacture of a product of constant and reproducible quality. This requires knowledge of type and impact of the microorganisms involved in the fermentation of the product. In a previous paper we attempted to characterize the lactic acid bacteria isolated from mawè (Hounhouigan *et al.*, 1993b). The present report deals with the microbiological changes in mawè taking place during natural fermentation and identifies the predominant microorganisms involved.

MATERIALS AND METHODS

Sample preparation

Home-produced and commercial mawè were produced as described earlier, using maize cultivar Sékou 85 (10 kg for each process) provided by The International Institute of Tropical Agriculture-Bénin (Hounhouigan *et al.*, 1993b). Duplicate experiments were carried out under traditional conditions of mawè manufacture.

Isolation and purification of microorganisms

Samples (10 g) of mawè from each process were taken under aseptic conditions at different fermentation time intervals (0, 6, 12, 24, 48, and 72 h), homogenized with 90 ml sterile peptone physiological salt solution and decimal dilutions were made into pour plates as described previously (Hounhouigan *et al.*, 1993b). Enumeration of total aerobic mesophilic bacteria, lactic acid bacteria, lactobacilli, yeasts and Enterobacteriaceae was carried out as described by Hounhouigan *et al.*, (1993a). Yeast strains were randomly picked from selected plates to obtain representative strains from different fermentation time intervals and the isolates were purified by successive sub-culturing on Yeast Extract Glucose Agar plates (Oxoid CM 545) incubated at 25°C for 3-5 days. After microscopic examination, purified cultures were grown on slants of the same medium and stored at +5°C, prior to identification. Randomly selected colonies of Enterobacteriaceae were isolated from plates of different fermentation time intervals and purified on Tryptone Soya Agar plates (Oxoid

CM 131) incubated at 37°C for 18-24 hours followed by Gram-staining and microscopic examination. Stock cultures were grown on slants of the same medium and stored at +5°C, for further identification.

Tests of identification

Grouping of yeast isolates was done on the basis of fermentation profiles on ATB 32C or ID 32C strips (API system S.A., Montalieu Vercieu, France). Preliminary identification was according to Lodder and Kreger-van Rij (1984) and the identity was confirmed by the Centraalbureau voor Schimmelcultures Yeast Division (Delft, The Netherlands). Identification of the Enterobacteriaceae was performed using the RapiD 20E system (API system S.A., Montalieu Vercieu, France).

Statistical analysis

Samples from different processes and fermentation periods were statistically compared using analysis of variance (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Tables 1 and 2 present the microbiological composition of home-produced and commercial mawè respectively.

Concentrations of total aerobic mesophilic bacteria and lactic acid bacteria were not significantly different between both types of mawè during fermentation period, but concentration of yeasts were significantly different ($P < 0.05$). High counts of total aerobic mesophilic bacteria, lactic acid bacteria and yeasts at the initial stage of the fermentation were probably due to the commercial mill used, acting as an inoculant during wet milling (Wacher *et al.*, 1993). The highest counts of aerobic mesophilic bacteria and lactic acid bacteria were recorded between 12 and 24 hours of fermentation. The yeast counts increased until 48 h in commercial mawè before decreasing, but kept increasing in home-produced mawè. This supports our previous observation that home-produced mawè has not yet stabilized after 72 h of fermentation (Hounhouigan *et al.*, 1993c).

Enterobacteriaceae showed a slight increase during the early stages of the fermentation, but decreased to below detection level after 1 day in commercial mawè or 2 days in home-produced mawè.

Table 1. Changes in the microbial counts (Log_{10} c.f.u./g wet wt) during fermentation of home-produced mawè.

Fermentation time (h)	pH	Total aerobic mesophilic bacteria	Lactic acid bacteria	Lactobacilli	Yeasts	Enterobacteriaceae
0	6.25	6.5	6.5	6.3	4.4	2.5
6	4.35	9.1	9.2	9.0	4.8	3.8
12	4.02	9.1	9.2	9.1	4.9	3.2
24	3.85	9.3	9.2	9.3	6.5	3.4
48	3.75	9.0	9.1	9.1	7.3	< 1.7
72	3.65	9.0	9.2	9.1	7.5	< 1.7

Table 2. Changes in the microbial counts (Log_{10} c.f.u./g wet wt) during fermentation of commercial mawè.

Fermentation time (h)	pH	Total aerobic mesophilic bacteria	Lactic acid bacteria	Lactobacilli	Yeasts	Enterobacteriaceae
0	6.13	7.2	7.2	7.2	5.1	3.2
6	4.12	9.0	9.0	8.9	5.2	3.6
12	3.83	9.2	9.2	9.0	6.2	3.2
24	3.63	9.1	9.2	9.2	7.2	< 1.7
48	3.51	8.8	9.0	8.9	7.4	< 1.7
72	3.47	8.5	8.8	8.7	6.5	< 1.7

The predominant lactic acid bacteria isolated from mawè were identified (Hounhouigan *et al.*, 1993b). Most of them (89% of the isolates) were of the obligately heterofermentative Betabacterium group. They include *Lactobacillus fermentum* (biotype *cellobiosus*), *L. fermentum* (it could be also *L. reuteri*) and *L. brevis*, which accounted for about 85% of the strains isolated. 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 yeasts identified in both types of mawè are listed in Table 3. They were dominated by *Candida* species which include *C. krusei* mainly, *C. kefyr* and *C. glabrata*. Other yeast isolates were identified as *Saccharomyces cerevisiae*.

Table 4 summarizes thirty strains of Enterobacteriaceae which were isolated from home-produced and commercial mawè. 6 strains out of 10 isolated from commercial mawè were identified as *Enterobacter cloacae* while 19 strains out of 20 isolated from home-produced mawè were identified as *Escherichia coli*. Other species identified include *Klebsiella pneumoniae* and *Serratia odorifera* isolated from commercial mawè. *E. coli* species are generally considered as an indicator of contamination of faecal origin. Their presence in mawè could be attributed to the faecal contamination of the maize used, but their low number in commercial mawè could be due to the extent of washing of the grits, which was not applied in home-produced mawè.

Lactic acid bacteria, yeasts and Enterobacteriaceae grew together during at least 12 to 24 h fermentation of mawè, contributing to the characteristics of the final product by the production of organic acids, alcohol (ethanol), CO_2 and other volatile flavour compounds. It has been suggested that microbial amylases play an important role in the production of fermentable sugars from maize immersed in water (Akinrele, 1970). According to Nout *et al.*, (1980), the multiplication of *Lactobacillus* sp. in the souring maize is favoured by the production of fermentable sugars from the auto-amylolysis of maize. Unpublished data from our laboratory showed that sugar concentrations (mostly glucose and maltose) can increase from about 1.8-2.6% to about 3.0-4.3% (g/100 g commercial mawè) in 24 h fermentation before decreasing in the following phase of fermentation.

In addition, the development of lactic acid bacteria is also stimulated by the presence of yeasts which provide soluble nitrogen compounds and factors e.g. B-vitamin (Nout, 1991). Other yeast metabolites e.g. CO_2, pyruvate, propionate, acetate and succinate were shown to stimulate lactobacilli in kefir (Leroi and Pidoux, 1993).

Table 3. Species of yeasts isolated from mawè.

Species	Home-produced mawè	Commercial mawè
	Number of isolates	Number of isolates
Candida krusei	17	14
Candida kefyr	5	2
Candida glabrata	3	2
Saccharomyces cerevisiae	2	10
Total	27	28

Table 4. Species of Enterobacteriaceae isolated from mawè.

Species	Home-produced mawè	Commercial mawè
	Number of isolates	Number of isolates
Enterobacter cloacae	1	6
Escherichia coli	19	1
Klebsiella pneumoniae	-	1
Serratia odorifera	-	1
NI*	-	1
Total	20	10

* NI: Not identified.

On the other hand, the acidic environment created by lactobacilli is favourable for yeast growth (Wood, 1981). This protobiotic association of lactic acid bacteria and yeasts has been noticed in several cereal foods. *C. krusei* and *S. cerevisiae* were found together with lactic acid bacteria during the fermentation of busaa, a Kenyan opaque maize-millet beer (Nout, 1980). Odunfa and Adeyele (1985) found *Lactobacillus* sp. and *Lactococcus lactis* together with *C. krusei* and *Debaryomyces hansenii* during the fermentation of ogi-baba, a West African fermented sorghum gruel. Adegoke and Babalola (1988) found *S. cerevisiae* together with *L. fermentum*, *L. brevis* and *Enterococcus faecalis* in the fermentation of ogi, while Akinrele (1970) found that corynebacteria, *S. cerevisiae*, *E. cloacae* and *L. plantarum* were prominent in ogi. More recently, Halm *et al.*, (1993) found obligately heterofermentative lactobacilli closely related to *L. fermentum* and *L. reuteri*, in association with yeasts dominated by *Candida* sp. and *Saccharomyces* sp. in fermented maize dough from Ghana.

It is not clear whether Enterobacteriaceae can be considered as functional microorganisms in the mawè fermentation. As the acidic environment created by lactic acid bacteria is not favourable for their growth, their numbers decrease strongly after the first day of mawè fermentation. A negative aspect of Enterobacteriaceae is that coliform species were reported to be responsible for off-flavours and flavour instability in Kenyan uji (Mbugua 1982). Taking into consideration the very low levels of Enterobacteriaceae in mawè it seems unlikely that they would be responsible for the remarkable off-flavours noticed particularly in the home-produced version. These off-flavours, combined with undesirable sour taste beyond 24 h fermentation due to a high titratable acidity (Hounhouigan *et al.*, 1993c), renders home-produced mawè less appreciated than commercial mawè in urban areas.

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CHAPTER 5

CHARACTERIZATION AND FREQUENCY DISTRIBUTION OF SPECIES OF LACTIC ACID BACTERIA INVOLVED IN THE PROCESSING OF MAWÈ, A FERMENTED MAIZE DOUGH FROM BENIN

ABSTRACT

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.

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 Bénin and Togo, is mawè, a

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dehulled maize dough used to prepare many dishes. Production and quality attributes of mawè have been investigated (Hounhouigan *et al.*, 1993). 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 Bénin. 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.*, 1993).

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 hours of fermentation of both home-produced and commercial mawè.

MATERIALS AND METHODS

Mawè preparation

Commercial mawè was produced as follows: Sékou 85 variety of maize (10 kg) provided by the International Institute of Tropical Agriculture in Bénin 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 hours 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 hours 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 hours 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 24 and 48 hours, 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.*, 1993). 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.

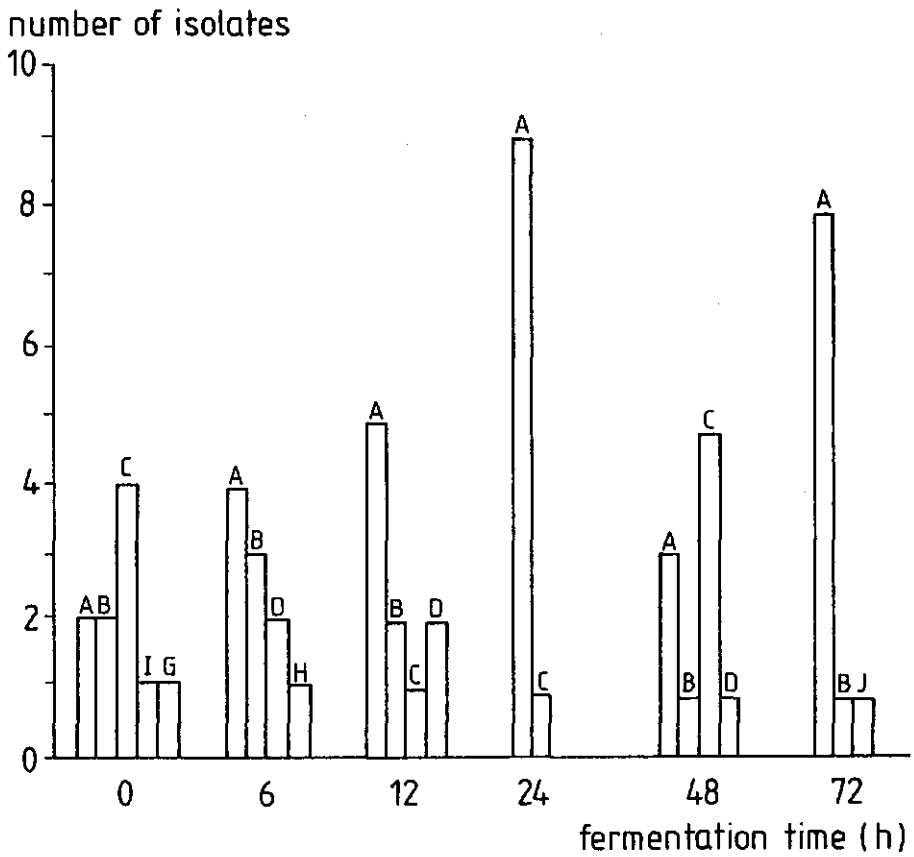

Figure 1. Frequency distribution and succession of lactic acid bacteria isolated from home-produced mawè.

A = *L. fermentum*, (biotype *cellobiosus*); B = *L. fermentum*; C = *L. brevis*; D = *L. salivarius*; G = *Leuconostoc mesenteroides*; H = *Lactococcus lactis*; I = *L. lactis*; J = *L. buchneri*.

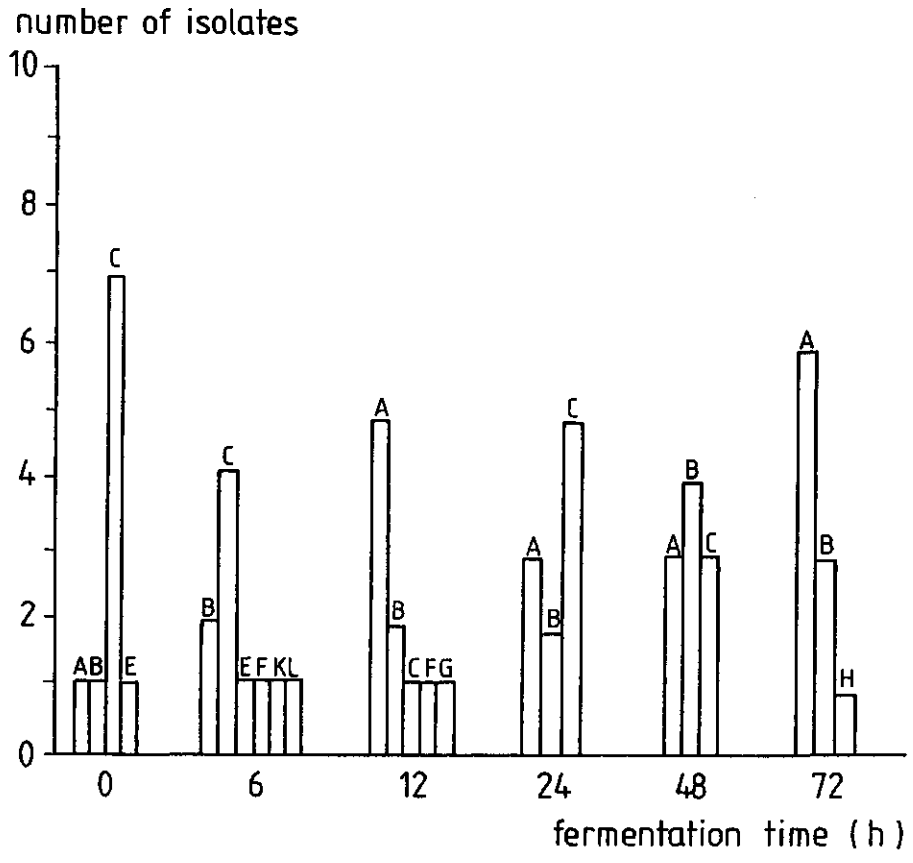

Figure 2. Frequency distribution and succession of lactic acid bacteria isolated from commercial mawè.

A = *L. fermentum* (biotype *cellobiosus*); B = *L. fermentum*; 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.

able 1. Characteristics of lactic acid bacteria isolated during mawè fermentation

ME OF SPECIES*	A	B	C	D	E	F	G	H	I	J	K	L
MBER OF STRAINS	49	23	31	5	2	2	2	2	1	1	1	1
RMENTATION OF:	PERCENTAGE OF STRAINS GIVING POSITIVE REACTIONS											
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
CELLOBIOSE	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
ß 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 GLUCOSAMINE	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
OWTH AT 15°C	39	26	100	0	100	100	100	100	0	100	100	100
OWTH AT 45°C	96	91	77	100	0	50	0	100	100	0	0	100
H, FROM ARGININE	100	100	100	0	100	100	50	100	0	100	100	100
ORPHOLOGY												
BACILLI	100	100	100	100	100				100	100	100	
COCCI						100	100	100				100
OMOFERMENTATION				100		100		100	100			100
ETEROFERMENTATION	100	100	100		100		100			100	100	

* A = *L. fermentum* (biotype *cellobiosus*); B = *L. fermentum*; 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*.

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 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 mol% 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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CHAPTER 6

USE OF STARTER CULTURES OF LACTOBACILLI AND YEASTS IN THE FERMENTATION OF MAWÈ PORRIDGE

D.J. Hounhouigan, M.J.R. Nout, C.M. Nago, J.H. Houben, and F.M. Rombouts.

ABSTRACT

Starter cultures of lactobacilli (*L. fermentum*, biotype *cellobiosus*, *L. fermentum* and *L. brevis*), and yeasts (*C. krusei* and *S. cerevisiae)* were tested singly or in combination for their ability to ferment dehulled maize flour, made into mawè porridge at 7% dry matter basis. All the species of lactobacilli showed a similar ability to ferment the porridge (pH from 6.1 to 3.4-3.6, and titratable acidity from 0.04 to 0.14-0.15% (w/w, as lactic acid, after 24 h). *C. krusei* and *S. cerevisiae* used singly showed little activity in acid production. The use of *C. krusei* in combination with lactobacilli had no significant effect on the final acidity. *S. cerevisiae* appeared to reduce the acidity of the product when used in combination with any of the species of lactobacilli tested.

INTRODUCTION

Lactic acid bacteria and yeasts have been reported as dominant organisms in the natural fermentation of most plant foods (Akinrele, 1970; Nout, 1980; Odunfa and Adeyele, 1985; Adegoke and Babalola, 1988; Halm *et al.*, 1993). They are involved in the fermentation of cereal foods and beverages in quite stable symbiotic association. For example, it has been suggested that the development of lactic acid bacteria is stimulated by the presence of yeasts which provide soluble nitrogen compounds and factors e.g. B-vitamin (Nout, 1991). According to Leroi and Pidoux (1993), yeast metabolites e.g. CO_2, pyruvate, propionate, acetate and succinate stimulated lactobacilli in kefir. On the other hand, the acidic environment created by lactobacilli is favourable for yeast growth, while the alcohol produced by the yeasts, the acids produced by the bacteria and the anaerobiosis induced by

the fermentation contribute to the suppression of other microbes present in the system. These microbes include the filamentous fungi and bacteria associated with various forms of food spoilage and poisoning, and the diarrhea causing pathogens (Wood, 1981; Wood and Hodge, 1985; Nout *et al.*, 1989; Mensah *et al.*, 1991; Mbugua and Njenga, 1992). As a result of the fermentation products formed, association of lactic acid bacteria and yeasts provides a pleasant taste and flavour to safe foods for the benefit of many people worlwide. In a practical application, the use of starter cultures containing these microorganisms to ferment cereal foods would be an adequate way to control the fermentation process and to overcome problems of variations in organoleptic characteristics and thus, in product quality encountered in the natural fermentation (Sanni, 1993). Attempts have been made to use starter cultures for the fermentation of some african cereals foods and beverages (Nout, 1980; Mbugua, 1984).

Mawè is a fermented dough made from dehulled white maize. Mawè processing has been investigated and the different species of lactic acid bacteria and yeasts identified (Hounhouigan *et al.*, 1993a,b,c), but the role of species in each group of microorganisms needs to be emphasised for the identification of the functionally most effective in mawè processing. This study is a preliminary attempt to clarify the role of species of lactic acid bacteria and yeasts in the fermentation of mawè, using dehulled maize porridge as a substrate.

MATERIALS AND METHODS

Sample preparation

White maize cultivar Sékou 85 provided by the "Station de Recherche Agronomique de Niaouli, Bénin" was used to produce maize flour following the commercial mawè process as described earlier (Hounhouigan *et al.*, 1993b). The wet flour with 40-42 % moisture content obtained after the final grinding was packed in polyethylene bags, and held at -50°C for later experiments: frozen samples of flour were thawed overnight at 5°C. A 7% dry matter suspension was made by adjusting the final moisture content to about 93% (w/w), using a Mettler LP 15 infrared unit installed on a Mettler PE 3600 electronic balance. About 100 g of the suspension (non-fermented mawè porridge) was distributed in 250 ml erlenmeyer flasks with screw-caps when mixing the batch suspension with a RW 20 mixer (Janke & Kunkel GMBH & Co.KG, Ika-Werk Staufen). The erlenmeyer flasks containing samples

were autoclaved for 15 min at 121 °C and left to cool to room temperature (26-28 °C) before inoculation.

Preparation of starter cultures

Cultures of lactobacilli (*L. fermentum* biotype *cellobiosus* strains LF 4809 and LF 0603, *L. fermentum* strain LC 4809, *L. brevis* strain LC 4805), and yeast cultures (*Candida krusei* strain YF 2401 and *Saccharomyces cerevisiae* strain YF 2405), previously isolated from mawè (Hounhouigan *et al.*, 1993a,b) were used. Lactobacilli were cultivated by streaking on MRS agar plates (Oxoid CM 361) and incubated at 30°C for 3 days, using anaerobic jars and anaerobic system envelope with palladium catalyst (BBL, Gas Pak Plus, Becton Dickinson). One colony was picked and transferred to a tube containing 10 ml MRS broth (Oxoid CM 359), and incubated at 30°C for 24 h. 0.1 ml of this culture was used to inoculate 10 ml MRS broth and incubated at 30°C for 16 h. This culture was centrifuged (3000 rpm, 10 min), the pellet was washed in 10 ml sterile peptone physiological salt solution (1g peptone, 8.5g NaCl in 1000 ml distilled water, pH 7.2), centrifuged again and redistributed in peptone physiological salt solution. This procedure achieved an inoculum containing 10^9 c.f.u./ml, checked as viable count in MRS agar. *C. krusei* and *S. cerevisiae* were cultivated by inoculating tubes containing 10 ml Sabouraud Liquid Medium (Oxoid CM 147) incubated at 30°C for 24 h. These cultures were centrifuged and washed as described previously. This procedure achieved an inoculum containing 10^7-10^8 c.f.u./ml, as viable count in yeast glucose agar (Oxoid CM 545).

Fermentation

Samples (about 100 g of autoclaved mawè porridge) were inoculated with 1 ml of lactobacilli inoculum or 1 ml of yeast inoculum. Mixed fermentation by lactobacilli and yeast was performed using 1 ml of each inoculum for the same sample. Inoculated samples were shaken vigorously by hand for homogenization and incubated at 30°C for 24 h (Memmert incubator, 854 Schwabach W. Germany). At 0 and after 6, 12, 18 and 24 h of fermentation, non autoclaved samples and samples inoculated with lactobacilli were taken out and kept in polyethylene bags at -50°C for further analysis of pH, titratable acidity, reducing sugar and total sugar. Other samples inoculated either with mixed culture, or yeast and sterile samples were taken out after 24 h of fermentation and frozen for analysis of pH and titratable acidity. Experiments were carried out in triplicate.

Chemical analysis

Assessment of the extent of fermentation was carried out by analysis of pH, titratable acidity, reducing sugar and total sugar on frozen samples, after thawing overnight at +5°C.

pH and titratable acidity: Method described by Nout *et al.*, (1989) was modified as follows: suspension was made with about 30 g of sample mixed with 20 ml of distilled water, and the pH was measured using a Hanna 8417 pH meter (Hanna Instruments, Limena, Italy). The titratable acidity was determined on the previous suspension mixed with 50 ml distilled water.

Reducing sugar and total sugar were determined before and after inversion respectively, using Luff Schoorl method as described by Lees (1968).

Statistical analysis

Samples from different treatments were statistically compared using analysis of variance (Snedecor and Cochran 1989) and Duncan's multiple-range test (Duncan, 1955).

RESULTS AND DISCUSSION

Changes in pH and titratable acidity

All the species of lactobacilli used as single inocula were effective in fermenting mawè porridge, as can be seen in figs. 1 and 2. After 6 h of fermentation, the pH had decreased from 6.1 to 4.3 in most cases except for *L. brevis* strain LC 4805 which lowered the pH to 4.0, although the difference was not significant. In all samples inoculated with *Lactobacillus* species and in the non autoclaved sample fermented naturally, the pH fell to about 3.4-3.6 after 24 h of fermentation, with a concomittant increase of titratable acidity from 0.03-0.04% to 0.14-0.17%. Calculated on dry weight basis, this titratable acidity is almost similar to that recovered in the natural fermentation of mawè, as studied earlier (Hounhouigan *et al.*, 1993a). This ability of the species of lactobacilli used, to ferment mawè porridge contrasts with the results obtained in fermenting Kenyan uji using strains of *L. cellobiosus* and *L. fermentum*. These organisms failed to successfully ferment sterile uji slurry, judged from a poor decrease in pH (Mbugua, 1984).

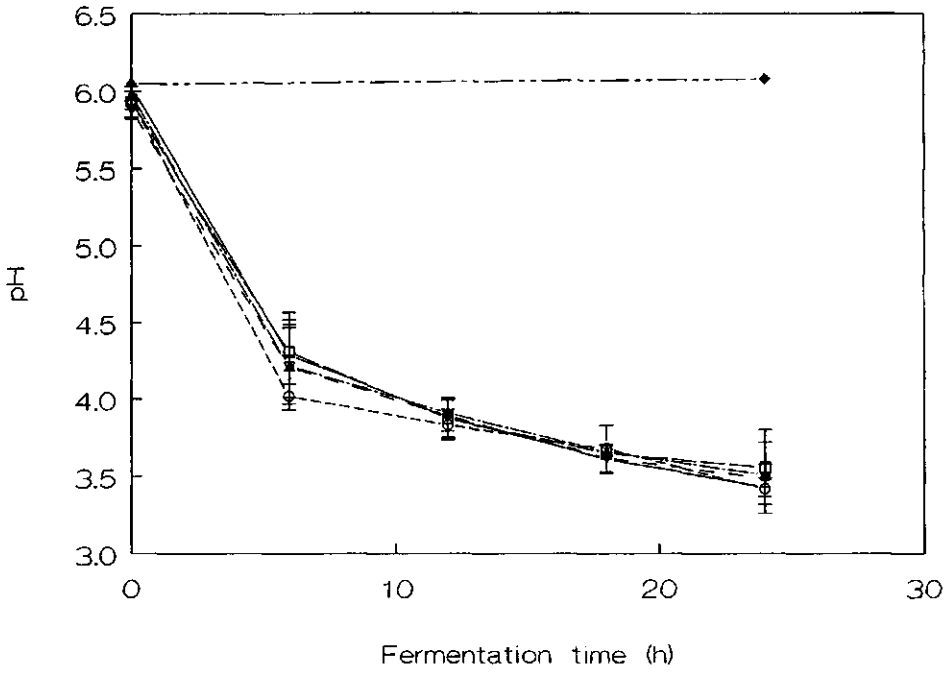

Figure 1. Effect of single starter cultures of lactobacilli on the pH during fermentation of mawè porridge.

- + - Natural fermentation; - ○ - *L. brevis* (LC 4805); - ▲ - *L. fermentum* (*biotype cellobiosus* strain LF 4809); - ∇ - *L. fermentum* (*biotype cellobiosus* strain LF 0603); - □ - *L. fermentum* strain LC 4809; - ◆ - Sterile sample (control).

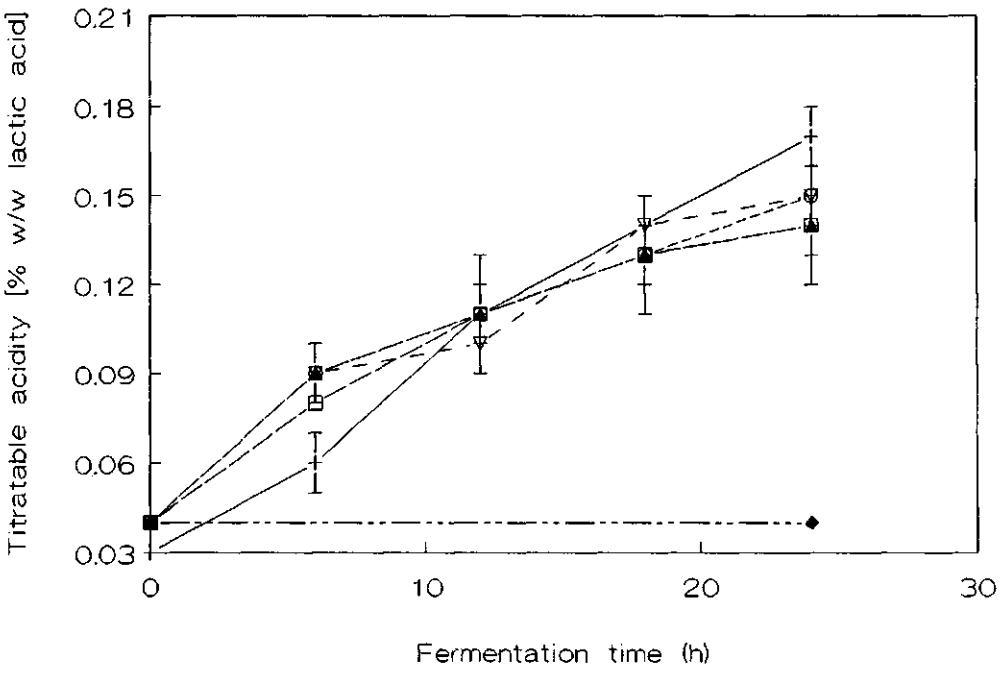

Figure 2. Effect of single starter cultures of lactobacilli on the titratable acidity during fermentation of mawè porridge.

- + - Natural fermentation; - ○ - *L. brevis* (LC 4805); - ▲ - *L. fermentum* (*biotype cellobiosus* strain LF 4809); - ∇ - *L. fermentum* (*biotype cellobiosus* strain LF 0603); - □ - *L. fermentum* strain LC 4809; - ◆ - Sterile sample (control).

Changes in the reducing and total sugar

The reducing sugar represented almost the total sugar present in the fermenting mawè porridge. Non autoclaved samples of mawè porridge showed significant successive increase and decrease of total sugar during the whole fermentation period, despite their utilization as substrate by microorganisms naturally present in the samples (Figs. 3 and 4). This is an indication that some amylolytic activities were developed in the naturally fermented mawè porridge, producing more sugar than required for metabolism. Amylolysis was reported to occur in fermenting cereal grains (Nout, 1980; Odunfa and Adeyele, 1987; Umeta and Faulks, 1988). Although fermenting microbes have been reported to possess amylolytic activities (Sen and Chakrabarty, 1986; Khetarpaul and Chauhan, 1990; Giraud *et al.*, 1991),

it seems that endogenous maize amylases played an important role in the release of sugar in the fermenting medium during natural fermentation (Nout, 1980; Odunfa and Adeyele, 1987; Nout and Rombouts, 1992). Autoclaving mawè porridge inhibits these endogenous maize amylase activities; this could explain the sharp and continuous decrease of the total sugar in the autoclaved and inoculated samples, compared to the non autoclaved and naturally fermented samples. However autoclaving had no influence on the availability of sugar for controlled fermentation of mawè porridge: enough fermentable sugar was available at the beginning of the fermentation to attain the titratable acidity of 0.15% (wet weight basis) or 2% (dry weight basis) corresponding with a pH of about 3.5 after 24 h of fermentation. Furthermore, due to the relatively important residual sugar content in the mawè porridge after 24 h of fermentation, fermentable sugar was certainly not the limiting factor to explain the apparently low titratable acidity in the fermented mawè porridge. Clearly, pH was the limiting factor. Due to its low protein content (0.7%, wet weight basis, calculated on the basis of a previous result, Hounhouigan *et al.*, 1993a), the fermenting medium had a low buffering capacity (Banigo and Muller, 1972). Consequently, a low titratable acidity was sufficient to create an extracellular pH that limited further bacteria growth and acid production (McFall and Montville, 1989).

There was no significant difference between species or strains of lactobacilli used, in their ability to reduce the sugar content of the samples, which confirmed the similarity of the acidity profiles illustrated by the Figs.1 and 2.

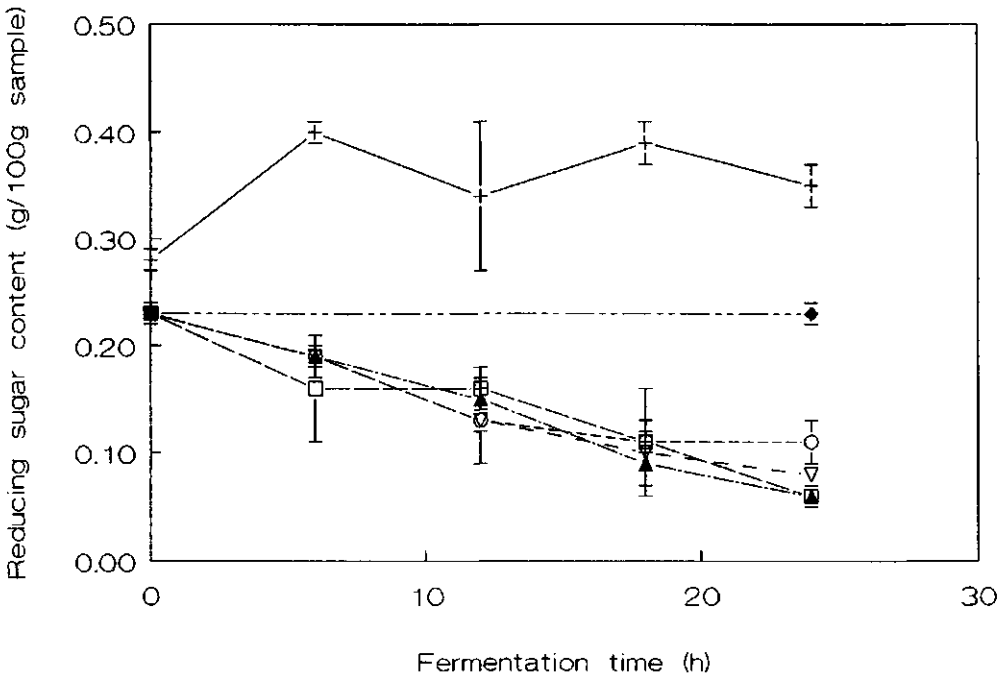

Figure 3. Effect of single starter cultures of lactobacilli on the reducing sugar during fermentation of mawè porridge.

- + - Natural fermentation; - ○ - *L. brevis* (LC 4805); - ▲ - *L. fermentum* (*biotype cellobiosus* strain LF 4809); - ▽ - *L. fermentum* (*biotype cellobiosus* strain LF 0603); - □ - *L. fermentum* strain LC 4809; - ◆ - Sterile sample (control).

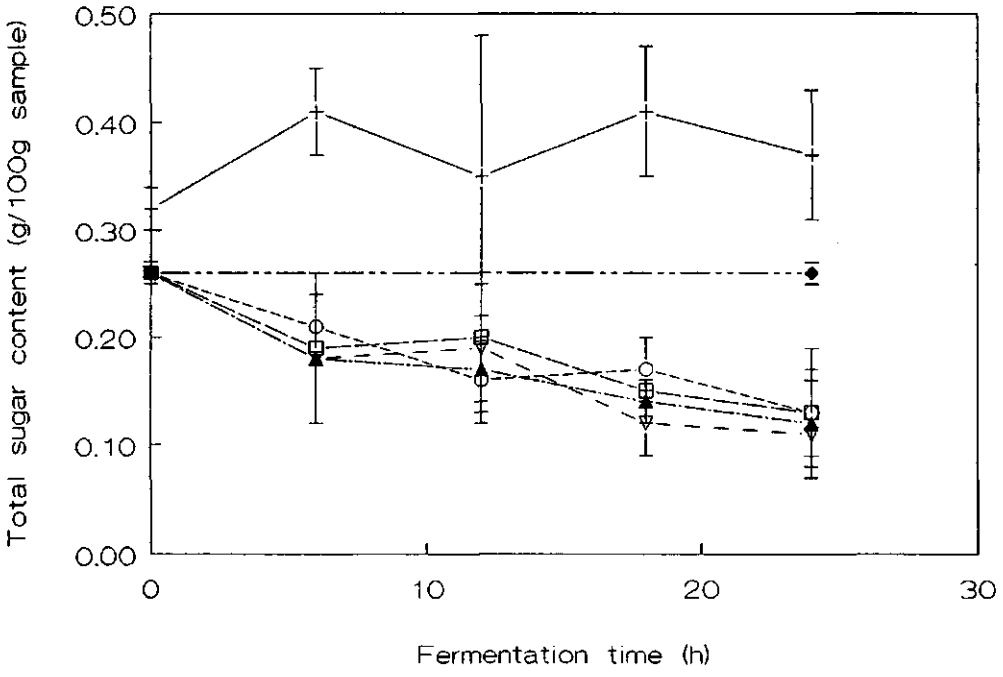

Figure 4. Effect of single starter cultures of lactobacilli on the total sugar during fermentation of mawè porridge.

- + - Natural fermentation; - ○ - *L. brevis* (LC 4805); - ▲ - *L. fermentum* (*biotype cellobiosus* strain LF 4809); - ∇ - *L. fermentum* (*biotype cellobiosus* strain LF 0603); - □ - *L. fermentum* strain LC 4809; - ◆ - Sterile sample (control).

Performances of different starter cultures used

Performances of starters used are listed in Table 1. It can be seen that *C. krusei* and *S. cerevisiae* were not very active in decreasing the pH or increasing the titratable acidity of the mawè porridge. On the other hand, there was no significant difference between the lactobacilli used alone and in combination with yeasts, in their ability to ferment the substrate. However, *S. cerevisiae*, used in combination with lactobacilli, was associated with lower acidity of the final product. Particularly, samples of mawè porridge fermented with *L. fermentum* (biotype *cellobiosus*) strain LF 0603, *L. fermentum* strain LC 4809 and *L. brevis* strain LC 4805 in combination with *S. cerevisiae* had a titratable acidity which was significantly lower than that of the naturally fermented mawè porridge. Similar effect of *S. cerevisiae* on the reduction of acidity was reported on nono, a nigerian milk fermented with

this species of yeast in combination with *Streptococcus diacetilactis* and *L. brevis* (Okagbue and Bankole, 1992).

It appeared from this investigation that fermentation of mawè porridge can be carried out using a single starter culture of *L. fermentum* biotype *cellobiosus* strains LF 4809 and LF 0603, *L. fermentum* strain LC 4809, *L. brevis* strain LC 4805. The presence of *C. krusei* in the fermenting medium had no significant effect on the acidity whereas the presence of *S. cerevisiae* was detrimental to the acid production. However, further experiments need to be carried out for a better knowledge on the compounds responsible of taste and flavour in the natural fermentation and the contribution of lactic acid bacteria and yeasts to the formation of these compounds.

Table 1. Effect of starter cultures on the pH and titratable acidity of mawè porridge*.

Starter culture	pH	Titratable acidity
Control (sterile sample)	6.1a**	0.04d
Naturally fermented sample	3.4c	0.17a
Candida krusei	5.6b	0.05d
Saccharomyces cerevisiae	5.5b	0.06d
L. cellobiosus (LF 0603)	3.5c	0.15abc
LF 0603 + *C. krusei*	3.5c	0.14abc
LF 0603 + *S. cerevisiae*	3.8c	0.11c
L. cellobiosus (LF 4809)	3.5c	0.14abc
LF 4809 + *C. krusei*	3.5c	0.14abc
LF 4809 + *S. cerevisiae*	3.7c	0.12abc
L. fermentum (LC 4809)	3.6c	0.14abc
LC 4809 + *C. krusei*	3.5c	0.13abc
LC 4809 + *S. cerevisiae*	3.9c	0.11c
L. brevis (LC 4805)	3.4c	0.15ab
LC 4805 + *C. krusei*	3.5c	0.13abc
LC 4805 + *S. cerevisiae*	3.8c	0.11bc

* Mean of 3 independent determinations.

** Means with the same letters are not significantly different ($P < 0.05$)

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CHAPTER 7

GENERAL DISCUSSION

In this thesis, two types of maize-based fermented dough from Bénin, referred to as home-produced and commercial mawè, were studied. Particular attention was paid the processing methods, the characteristics of the products, and the physical, chemical and microbiological dynamics of the fermentation. Some species of *Lactobacillus* and yeasts isolated from the naturally fermented mawè were also tested for their ability to ferment sterilized dehulled maize porridge.

In this general discussion, the characteristics of the product are reviewed in relation with the physico-chemical and microbiological changes occurring during the processing. Attempts will be made to compare mawè with similar products in the region of West Africa, and further prospects for mawè improvement will be outlined.

CHARACTERISTICS OF MAWÈ IN RELATION TO THE CHANGES OCCURRING DURING THE PROCESSING

Mawè is a sour dough made from partially dehulled maize meal, which has undergone natural fermentation for 1 to 3 days. Preliminary investigations (Chapters 1 and 2) showed that two processing methods are used for mawè production: the home process now used to produce mawè for own consumption and the commercial process used to produce marketed mawè. The main difference between both processes is a thorough washing of the maize grits to remove more hulls and germs from the commercial product; this operation is not applied in the home process. Using the maize cultivar Sékou 85, it was found that the latter yielded more mawè (76-80% dry weight basis) than the commercial process (65-71%). This washing operation contributed also to a loss of extractable dry matter in the washing water during processing of commercial mawè (Chapter 3).

Commercial mawè was whiter than home-produced mawè. This whiteness became more pronounced during the course of the fermentation and was significantly correlated with the increasing moisture content during fermentation (Chapter 3). It was suggested that this

increasing moisture content was caused by the combined effects of dry matter consumption and production of water during aerobic and anaerobic catabolism by yeasts and heterofermentative lactic acid bacteria (Chapters 4 & 5). The average moisture contents of products as they were collected in the home and the market were in the range 45-47% (wet weight basis) and did not differ significantly.

Mean proximate composition of market and home-collected mawè was, respectively, proteins: 8.2 and 9.2%; fat: 1.0 and 2.3%; ash: 0.6 and 1.1%; crude fibre 0.4 and 0.7% dry weight basis (Chapter 2). The processing method affected the proximate composition of the product, the loss of nutrients being more pronounced in the commercial than in the home process. Crude fat, ash and crude fibre contents were affected more drastically than protein content. The fermentation stage had no significant influence on the proximate composition of mawè (Chapter 3).

Titratable acidity of both types of mawè collected in the home and in the market, respectively, was in the range 1.2-1.4% (w/w as lactic acid), but the pH of home-produced mawè was slightly higher (pH = 4.2) than the pH of market mawè (pH = 3.8) (Chapter 2). These levels of acidity can be attained after 24 hours fermentation of commercial mawè and 12 hours fermentation of home-produced mawè. The monitoring of pH and titratable acidity during the course of the fermentation showed that, despite a significantly higher titratable acidity, the pH of home-produced mawè still remained the highest. This phenomenon has been attributed to the buffering action of soluble proteins on pH and confirms that the extent of fermentation of cereal products cannot be evaluated merely on the basis of their pH: monitoring of both pH and titratable acidity is required as suggested by Banigo & Muller (1972) (Chapter 3).

Pasting characteristics of fermenting home-produced and commercial mawè, as measured with a Brabender Pt 100 Viscograph, showed that fermentation time has no significant effect on the gelatinization temperature (T_g), nor on the time to reach the gelatinization temperature (M_g), but maximum viscosity during heating (V_m), viscosity after 15 min at 92°C (V_r) and viscosity after cooling to 50°C (V_c), all increase with fermentation time. The most significant change was noticed between the non-fermented samples and the fermented samples. The processing method affected also T_g, M_g, V_m, V_r and V_c values. Viscosity values were significantly higher in commercial mawè than in home-produced mawè while T_g and M_g values were significantly lower. It was concluded that fermentation increased the swelling and thickening capabilities of mawè, which were more pronounced in the commercial than in the home-produced samples (Chapter 3).

It would be of great importance to understand which factors determine the choice of technology in mawè processing. The commercial process has several disadvantages: it is time and labour consuming due mainly to the thorough washing of the grits before final milling. Furthermore, yields are lower and more nutrients are lost. However, it provides 83% of all the mawè produced in Cotonou (Chapter 1). This success of commercial mawè should be due to its appearance, texture, longer shelf-life and good thickening and swelling characteristics: in the market, consumers choose mawè on the basis of whiteness, fineness and sourness; home-produced mawè was less white and less fine than commercial mawè although the latter was not clearly established (Chapters 2 and 3). Furthermore, home-produced mawè had a high titratable acidity and consequently an undesirable sour taste beyond 24 h fermentation, and its greater fat content may predispose the product to rancidity during storage: this probably renders home-produced mawè unsuitable for a larger scale of production under the traditional conditions. On the other hand, commercial mawè had better shelf-life, and good thickening and swelling characteristics probably due to its fineness and its lower fat content (Sefa-Dedeh, 1989; Akingbala *et al.*, 1987).

On the basis of all these considerations, the commercial process can be considered a technologically more advanced and more effective method of mawè production for gel-type food preparation, compared to the home process.

However, mawè is also used to prepare porridge and weaning foods. The minimum nutrient loss and the relatively weak thickening and swelling characteristics of home-produced mawè are advantageous for its use as a basis for porridge and weaning foods of high caloric density.

The physico-chemical changes in fermenting mawè were essentially the result of activities of fermenting microorganisms which used the nutrients available in the medium for their metabolic activities. As a result of these activities, these microorganisms produce organic acids and other aroma components. In mawè fermentation, we found that the microorganisms responsible of the fermentation were lactic acid bacteria and yeasts (Chapters 2 and 4). Maximum counts of lactic acid bacteria were recorded between 12 and 24 hours of fermentation ($\log_{10}$ N/g = 9.2-9.3) (Chapter 4). A total of 120 strains of lactic acid bacteria were isolated and identified (Chapter 5). *Lactobacillus* spp. constitute the majority (94%) of the lactic acid bacteria isolated, among which 89% were heterofermentative and included *Lactobacillus brevis*, *Lactobacillus fermentum* (biotype *cellobiosus*), *Lactobacillus fermentum* (it could be also *Lactobacillus reuteri*), *Lactobacillus confusus*, *Lactobacillus curvatus*, *Lactobacillus buchneri*. Other isolated species were *Lactobacillus salivarius*,

Lactococcus lactis, *Pediococcus pentosaceus*, *Pediococcus acidilactici* and *Leuconostoc mesenteroides*. *L. brevis*, *L. fermentum* (biotype *cellobiosus*), and *L. fermentum* represent 85% of the strains isolated from home-produced mawè and 86% of the strains isolated from commercial mawè. Seven different species of lactic acid bacteria were detected in each of the mawè types between 0 and 6 hours fermentation period, but the number of species was reduced after 72 hours fermentation, when the flora was dominated by *L. fermentum* (biotype *cellobiosus*) and *L. fermentum*, totalling 90% of the isolated strains at this stage. No strains of *L. plantarum*, generally reported as dominating lactic acid bacteria at the final stage of fermentation of most cereal foods (Akinrele, 1970; Nout, 1980; Mbugua, 1984), were isolated. In commercial mawè, the yeast count increased until 48 hours of fermentation ($\log_{10}$ N/g = 7.4) before decreasing, while in the home-produced mawè it continued to increase. From a total of 55 strains of yeasts isolated, we identified *Candida krusei* which was dominant, *Candida kefyr*, *Candida glabrata* and *Saccharomyces cerevisiae* (Chapter 4). As far as Enterobacteriaceae were concerned, their number increased slightly during the initial stage of fermentation, but they were reduced below the detection level after 24 hours fermentation in commercial mawè and after 48 hours fermentation in home-produced mawè. Six strains out of 10 isolated from commercial mawè were identified as *Enterobacter cloacae*, while 19 strains out of 20 isolated from home-produced mawè were identified as *E. coli*. Other species isolated were *Klebsiella pneumoniae* and *Serratia odorifera*. The high level of coliforms in home-produced mawè was probably due to the missing of the washing of the grits, an operation which was applied in the commercial process.

Starter cultures of lactobacilli (*L. fermentum*, biotype *cellobiosus*, *L. fermentum* and *L. brevis*) and yeasts (*C. krusei* and *S. cerevisiae*), were tested singly or in combination for their ability to ferment mawè porridge. All the species of *Lactobacillus* used showed a similar ability to ferment the porridge. *C. krusei* and *S. cerevisiae* used singly were not very active in acid production. *C. krusei* used in combination with *Lactobacillus* sp. showed no stimulating effect on acid production. *S. cerevisiae* seemed to reduce the acidity of mawè porridge when used in combination with any of the species of *Lactobacillus* tested. Further prospects for mawè improvement should focus deeply on the role of yeasts in mawè processing.

COMPARISON OF MAWE WITH SIMILAR PRODUCTS IN THE REGION OF WEST AFRICA

Mawè technology should be placed in the general basket of fermented cereals processes widespread in Africa, like ogi, and Ghanaian maize dough used to prepare koko and kenkey in West Africa, uji in East Africa, mahewu in South Africa (Steinkraus *et al.*, 1983; Odunfa, 1985). Unfortunately some of these products are still unknown. However, we could attempt to clarify some similarities and differences between some of these products described in literature, and particularly those from West Africa, e.g. ogi, mawè and Ghanaian dough.

Mawè is a solid-state fermented food (45-47% moisture content) made from partially dehulled white maize. Strictly speaking, there is no documented equivalent, described under different names in Africa. However, similar products obtained differently are described in literature. Ghanaian dough used to prepare koko and kenkey is also a solid-state fermented food, with 46-55% moisture content (Plahar and Leung, 1983), but made from whole maize (Christian, 1970). Another maize fermented product also well know in Bénin is ogi. Ogi is also well known in Nigeria where it has been thoroughly studied (Oke, 1967; Akinrele, 1970; Banigo and Muller, 1972; Adeyemi *et al.*, 1987).

In ogi and Ghanaian dough processing (Fig.1), the maize used is soaked for 1-3 days in water before wet milling for ogi (Steinkraus *et al.*, 1983), and for 12-48 h in water for Ghanaian dough (Christian, 1970; Plahar & Leung, 1983; Sefa-Dedeh, 1989; Halm *et al.*, 1993). The objective of this soaking operation is the softening of the grain for a fine milling, but probably also a means of cultivating desirable microorganisms (Akinrele, 1970). In mawè processing, the soaking period of the grain is limited to the washing time before crushing. The softening occurs probably during the water absorption step of the grits which lasts generally 2 to 4 hours depending on the hardness of raw material used. Certainly, the removal of the hulls and the smaller particle size of the grits enhance the rate of water absorption and the softening of the material. It is less probable that this softening period is sufficient to cultivate desirable organisms for fermentation. In mawè processing, the main source of microorganisms could be the commercial mill used, acting as inoculum source as reported by Wacher *et al.*, (1993) in traditional Mexican fermented maize dough.

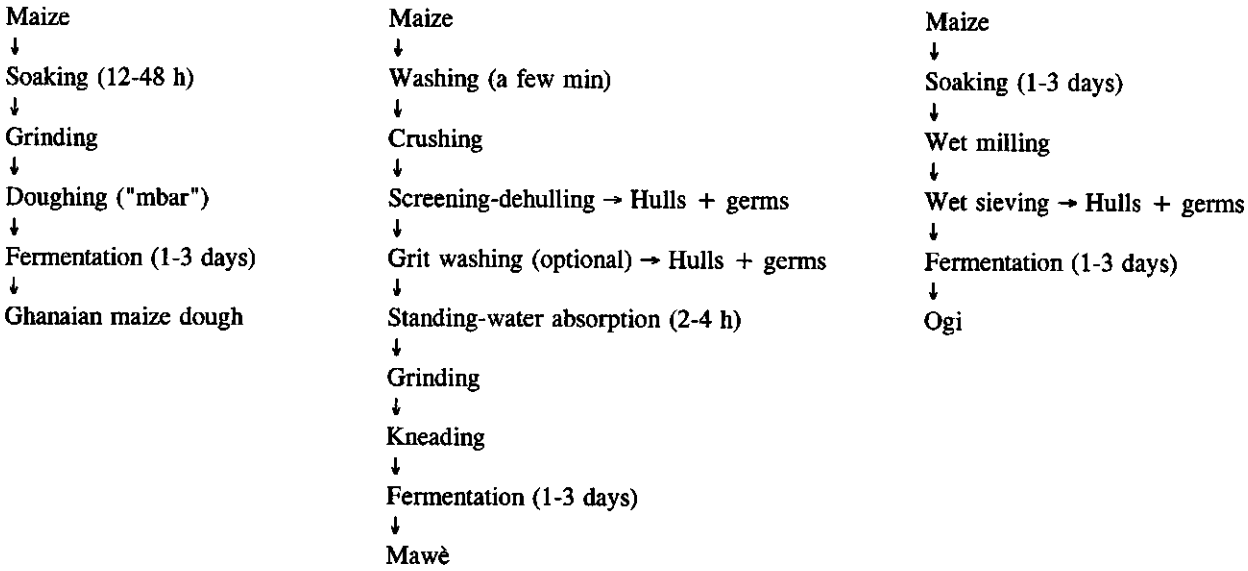

Figure 1. Flow diagram for Ghanaian dough, mawè and ogi production

The most significant difference between mawè and ogi on the one hand, and Ghanaian dough on the other hand is the removal of hulls and germs from the product (Fig.1). In Ghanaian dough processing, the fermentation takes place in a solid dough (like in mawè), but without prior sieving of the ground meal (Sefa-Dedeh, 1989). Then less material is discarded during the processing, whereas in mawè processing, 20 to 35% of material is lost as hulls, germs and soluble matter during the washing of the grits prior to the final milling. Adeyemi *et al.*, (1987) found a yield of ogi varying between 51.2 and 60.6% (on dry weight basis), depending on the variety of maize used, by the traditional wet-milling process. Consequently, the loss of material was about 39 to 49%. The same author reported a yield of 50% (50% loss) for an industrial pilot process. A maximum yield of 77% ogi (or 33% of material loss) was reported by Banigo *et al.*, (1974) using high lysine maize and a new, improved processing system similar in some aspects to mawè processing. Overall, varietal differences of maize affected yield of ogi (Adeyemi *et al.*, 1987) and could affect also yield of mawè.

In mawè processing, the removal of hulls and germs is carried out in one step by screening the crushed material (home process), or in two steps by screening the crushed material and by washing the grits (commercial process), before final milling. The meal obtained is kneaded into a dough and fermented. In ogi processing, the removal of hulls and germs is carried out by wet sieving after the only milling, and the product is fermented as a suspension in the sieving water.

As far as proximate composition was concerned, we found that protein content remained constant on conversion of Sékou 85 variety of maize to home-produced mawè and was reduced by 8% on conversion to commercial mawè (Chapter 3). Crude fat, ash and crude fibre were the most affected. Similar or more important losses were reported on ogi, depending on the processing method or the variety of maize used (Oke, 1967; Akingbala *et al.*, 1987; Adeyemi *et al.*, 1987).

From a technological point of view, mawè processing is time saving because it doesn't require days of steeping of raw material, but it is more energy consuming than ogi and Ghanaian dough. Another advantage of mawè on ogi is its obtention as a dough. In commercial practice, the dough is easier to handle and package than ogi. This characteristic would be also advantageous on stabilization by drying since it would be energy saving. However, it was reported that uncooked ogi was sold wrapped after removal of excess water in Nigeria (Steinkraus *et al.*, 1983).

Investigation on home-produced and commercial mawè samples (Chapter 2) showed that at the moment of use, pH and titratable acidity of mawè were almost similar to that

found in highly acceptable ogi (Steinkraus *et al.*, 1983) and Ghanaian maize dough (Plahar and Leung, 1983). This acidity is achieved by the activity of microorganisms, responsible of the fermentation. In mawè fermentation, we found that *Lactobacillus* sp. constitute the majority of the lactic acid bacteria present. Most of the strains isolated were obligately heterofermentative and included *L. fermentum*, biotype *cellobiosus*, *L. fermentum* and *L. brevis*, mainly. Yeasts were another group of microorganisms found in mawè; species of yeasts identified include *C. krusei* (the most important), *C. kefyr*, *C. glabrata* and *S. cerevisiae*. Enterobacteriaceae number was low and was dominated by *E. cloacae* in commercial mawè and *E. coli* in home-produced mawè. No strains of *L. plantarum* were isolated. Akinrele (1970) found *L. plantarum* in association with *Corynebacterium*, *Saccharomyces cerevisiae*, *Candida mycoderma* and *Enterobacter cloacae* in ogi fermentation. From this list, only *S. cerevisiae* and *E. cloacae* were found in mawè. Christian (1970) identified homofermentative *Pediococcus cerevisiae* and heterofermentative species which may include *Leuconostoc mesenteroides* and *L. fermentum* in Ghanaian dough. More recently, Halm *et al.*, (1993) found obligately heterofermentative lactobacilli closely related to *L. fermentum* and *L. reuteri*, in association with yeasts dominated by *Candida* sp. and *Saccharomyces* sp. in the same product. Our findings confirm the role of obligately heterofermentative lactobacilli in association with *Candida* sp. and *Saccharomyces* sp. in cereal foods fermented as a dough.

Organoleptic, nutritional and functional characteristics of intermediate ready-to-cook products like mawè, ogi or Ghanaian dough cannot be dissociated from the quality criteria of the various dishes as they have been developed empirically and transmitted from generation to generation by populations who are, at present, not easily influenced by innovation. Consequently, optimization and development of technology should focus also on the traditional appearance, taste, aroma and functional properties required for the reproducibility of these end-products through optimized processes which could be similar or different from the traditional processes. One justification to this assumption is that in Bénin, mawè is a substitute to ogi as far as end-products are concerned, despite the difference in processing method.

FURTHER PROSPECTS FOR MAWE IMPROVEMENT

In the present study, we found that fermentation of mawè porridge can be carried out using a single starter culture of *L. fermentum* (biotype *cellobiosus*), *L. fermentum* and *L. brevis*. The utility of *C. krusei* and *S. cerevisiae* was not evident as far as acid production was concerned. However, yeast species have been found to be as important and desirable as lactic acid bacteria in ogi fermentation (Akinrele, 1970; Banigo *et al.*, 1974). In particular, Akinrele, (1970) found that *Saccharomyces cerevisiae* and *Candida mycoderma* contributed to flavour acceptability of ogi. Further experiments need to be carried out for a better knowledge on the compounds responsible of taste and aroma in the natural fermentation of mawè, and the contribution of lactic acid bacteria and yeasts to the formation of these compounds.

It was found also that no marked changes in proximate composition occurred during the fermentation period. However changes in essential amino acids, particularly lysine, methionine and tryptophan and relative nutritive value due to natural fermentation of cereals have been reported (Oke, 1967; Hamad and Fields, 1979; Kazanas and Fields, 1981; Chavan and Kadam, 1989). This aspect could be investigated in mawè processing.

Another finding of importance which merits further study is related to the elimination of Enterobacteriaceae from fermenting mawè after 1 or 2 days of fermentation (Chapter 4). This is in accordance with that found in lactic fermentation and had been attributed to antimicrobial properties in some fermented foods (Mensah *et al.*, 1991; Mbugua and Njenga, 1992). In Africa, some of the most important factors responsible for morbidity among children under five years of age, are diarrhoea-causing organisms and malnutrition. Mawè is traditionally used as a weaning food. Studies should be carried out towards the antimicrobial properties of mawè and the possible use of these properties in soya-fortified mawè as weaning food.

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SUMMARY

Two differently processed maize-based fermented doughs from Bénin, referred to as home-produced and commercial mawè, were studied. Particular attention was paid to the processing methods, the characteristics of the products and the physical, chemical and microbiological changes during natural fermentation. Some species of *Lactobacillus* and yeasts isolated from the naturally fermented mawè were also tested for their ability to ferment sterilized mawè porridge.

Mawè is defined as a sour dough made from partially dehulled maize meal, which has undergone natural fermentation for 1 to 3 days. Two processing methods are used: the traditional home process presently still used to produce mawè for own consumption and the commercial process used to produce marketed mawè. The main difference between both processes is the thorough washing of the maize grits to remove more hulls and germs from the commercial product; this operation is not applied in the home process (Chapters 2 and 3).

With Sékou 85 variety of maize used as raw material, the home process yielded more mawè (76-80% dry weight basis) than the commercial process (65-71%), due to the washing of the grits which contributed also to the waste of soluble solids in the washing water during the processing of commercial mawè (Chapter 3).

Commercial mawè was whiter than home-produced mawè. This whiteness became more pronounced during the course of the fermentation and was significantly correlated with the increasing moisture content during fermentation (Chapter 3).

Mean proximate composition of commercial and home-produced mawè collected in the home and the market was, respectively, proteins: 8.2 and 9.2%, fat: 1.0 and 2.3%, ash: 0.6 and 1.1%, crude fibre 0.4 and 0.7%, on dry weight basis (Chapter 2). The higher fat content of home-produced mawè may predispose the product to rancidity during storage. The processing method affected the proximate composition of the product, the loss of nutrients being more pronounced in the commercial process than in the home process. The crude fat, ash and crude fibre contents were affected more drastically than the protein content. The fermentation stage had no significant influence on the proximate composition of mawè (Chapter 3).

Titratable acidity of both types of mawè collected in the home and in the market, respectively, was in the range 1.2-1.4% (w/w as lactic acid), but the pH of home-produced mawè was slightly higher (pH = 4.2) than the pH of market mawè (pH = 3.8) (Chapter 2). The monitoring of pH and titratable acidity during the course of the fermentation showed that these levels of acidity can be attained after 24 hours fermentation of commercial mawè and only 12 hours fermentation of home-produced mawè (Chapter 3).

Pasting characteristics of fermenting home-produced and commercial mawè, as measured with a Brabender Pt 100 Viscograph, showed that fermentation time has no significant effect on the gelatinization temperature (T_g), nor on the time to reach the gelatinization temperature (M_g), but maximum viscosity during heating (V_m), viscosity after 15 min at 92°C (V_r) and viscosity after cooling to 50°C (V_e), all increase with fermentation time. The most significant change was noticed between the non-fermented samples and the fermented samples. The processing method affected also T_g, M_g, V_m, V_r and V_e values. The viscosity values were significantly higher in the commercial mawè than in the home-produced mawè while T_g and M_g values were significantly lower. Fermentation increased the swelling and thickening capabilities of mawè, which were more pronounced in the commercial than in the home-produced samples.

The commercial process has several disadvantages: it is time and labour consuming; yields are lower, and more nutrients are lost. However, it provides 83% of all the mawè produced in Cotonou (Chapter 1). This success of commercial mawè should be due to its appearance, fineness, longer shelf-life and good thickening and swelling characteristics suitable for gel-type foods preparation. On this basis, commercial mawè process can be considered a technologically more advanced and effective method of mawè manufacture for gel-type foods preparation.

The low nutrient loss and the weak thickening and swelling characteristics of home-produced mawè are advantageous for its use as a basis for weaning food of high caloric density.

A total of 205 strains of microorganisms including 120 strains of lactic acid bacteria, 55 strains of yeasts and 30 strains of Enterobacteriaceae were isolated from both types of mawè during fermentation, examined and identified (Chapter 4 and 5).

Maximum counts of lactic acid bacteria were recorded between 12 and 24 hours of fermentation (Log_{10} N/g = 9.2-9.3). *Lactobacillus* spp. constituted the majority (94%) of the lactic acid bacteria isolated, among which 89% represent the obligately heterofermentative group; they included *Lactobacillus brevis*, *Lactobacillus fermentum* (biotype *cellobiosus*),

Lactobacillus fermentum (which all, represent 85% of the strains isolated from the home-produced mawè and 86% of the strains isolated from the commercial mawè), *Lactobacillus confusus*, *Lactobacillus curvatus*, *Lactobacillus buchneri*. Other isolated species were *Lactobacillus salivarius*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *Pediococcus acidilactici* and *Leuconostoc mesenteroides*. Seven different species of lactic acid bacteria were detected in each of the mawè types between 0 and 6 hours fermentation period, but the number of species was reduced after 72 hours fermentation, when the flora was dominated by *L. fermentum* (biotype *cellobiosus*) and *L. fermentum*, totalling 90% of the isolated strains at this stage.

In the commercial mawè, the yeast count increased until 48 hours of fermentation (Log_{10} N/g = 7.4) before decreasing, while in the home-produced mawè it continued to increase; isolated yeasts included *Candida krusei* which was dominant, *Candida kefyr*, *Candida glabrata* and *Saccharomyces cerevisiae*.

As far as Enterobacteriaceae were concerned, their number increased slightly during the initial stage of fermentation, but they were reduced below the detection level after 24 hours fermentation in the commercial mawè and after 48 hours fermentation in the home-produced mawè. Six strains out of 10 isolated from the commercial mawè were identified as *Enterobacter cloacae*, while 19 strains out of 20 isolated from the home-produced mawè were identified as *E. coli*. Other species isolated were *Klebsiella pneumoniae* and *Serratia odorifera*. The high level of coliforms in the home-produced mawè was probably due to the missing of the washing of the grits, an operation which was applied in the commercial process.

Starter cultures of lactobacilli: *L. fermentum* (biotype *cellobiosus*), *L. fermentum* and *L. brevis*, and yeasts: *C. krusei* and *S. cerevisiae*, were tested singly or in combination for their ability to ferment a mawè porridge. All the species of *Lactobacillus* used showed a similar ability to ferment the porridge. *C. krusei* and *S. cerevisiae* used singly were not very active in acid production. *C. krusei* used in combination with *Lactobacillus* sp. showed no stimulating effect on acid production. *S. cerevisiae* seemed to reduce the acidity of the mawè porridge when used in combination with any of the species of *Lactobacillus* tested.

Fields of further investigation on mawè processing have been outlined. They include knowledge on the compounds responsible of taste and aroma in the natural fermentation and the contribution of lactic acid bacteria and yeasts to the formation of these compounds, evaluation of micronutrient changes and antimicrobial activity during mawè processing.

SAMENVATTING

Mawè, een traditioneel gefermenteerd maisdeeg uit Bénin, wordt op twee verschillende manieren bereid, nl. in de huishouding en commercieel. Aandacht werd vooral besteed aan de produktiewijze, de produkteigenschappen en de fysische, chemische en microbiologische veranderingen die tijdens de natuurlijke fermentatie plaatsvinden. Tevens werden een aantal uit natuurlijk gefermenteerde Mawè geïsoleerde reincultures van *Lactobacillus* spp. en gisten onderzocht op hun vermogen gesteriliseerde maispap te fermenteren.

Mawè is een zuurdeeg bereid uit grof gemalen maismeel (gries) dat gedurende 1-3 dagen een natuurlijke fermentatie heeft ondergaan. In Bénin worden twee methoden toegepast: de traditionele huishoudelijke bereiding, en de commerciële methode t.b.v. de straat- en markthandel. Het belangrijkste verschil tussen beide methoden is de grondige wasstap van de maisgries tijdens het commerciële proces, waardoor meer zemelen en kiemen worden verwijderd uit de mais (Hoofdstukken 2 en 3).

Alle proeven werden gedaan met de lokale maisvarieteit "Sékou 85". De Mawè-opbrengst met het huishoudelijke proces (76-80% op droge stof basis) is aanmerkelijk hoger dan met het commerciële proces (65-71%); dit wordt vnl. veroorzaakt door het wassen van de gries waardoor behalve zemelen en kiemen ook oplosbare droge stof bestanddelen verloren raken (Hoofdstuk 3).

Commercieel bereide Mawè is witter dan thuisbereide Mawè. De graad van witheid werd hoger naarmate het fermentatieproces langer duurde, en was significant gecorreleerd aan de toename van het vochtgehalte (Hoofdstuk 3).

De gemiddelde samenstelling van op markten en in huishoudingen verzamelde praktijkmonsters van commerciële en thuisbereide Mawè was, repectievelijk: eiwit 8,2 en 9,2%, vet 1,0 en 2,3%, as 0,6 en 1,1%, vezel 0,4 en 0,7% op basis van drooggewicht (Hoofdstuk 2). Het hogere vetgehalte van thuisbereide Mawè maakt het produkt kwetsbaar voor ranzigheid tijdens bewaring. De afname van het nutriëntengehalte bij de commerciële Mawè ten opzichte van de thuisbereide Mawè was het gevolg van de gevolgde methode; vet-, as- en vezelgehaltes werden sterker verlaagd dan het eiwitgehalte. Het fermentatiegedeelte van het proces had geen significante invloed op de samenstelling van Mawè (Hoofdstuk 3).

Beide soorten Mawè bevatten 1,2-1,4% titreerbaar zuur (m/m als melkzuur). De pH van thuisbereide Mawè (pH 4,2) was echter hoger dan van commerciële Mawè (pH 3,8) (Hoofdstuk 2). Deze zuurgraad werd bereikt in 24 uur bij het commerciële proces, en in 12 uur in de huishoudelijke methode (Hoofdstuk 3).

Het verstijfselingsgedrag van fermenterende thuisbereide en commerciële Mawè werd onderzocht met een Brabender Pt 100 Viscograaf. De fermentatieduur had geen invloed op de verstijfselingstemperatuur (T_g) of op de benodigde tijd (M_g) om T_g te bereiken. De maximum verstijfselingsviscositeit (V_m), de viscositeit na 15 minuten bij 92°C (V_r) en de viscositeit na afkoelen tot 50°C (V_e) namen echter toe bij langere fermentatieduur. Het duidelijkste verschil werd gevonden tussen ongefermenteerde en volledig gefermenteerde Mawè. De bereidingswijze was ook van invloed op T_g, M_g, V_m, V_r en V_e. De viscositeitswaarden waren aanmerkelijk hoger in commerciële Mawè, met daaraan gekoppeld lagere waarden voor T_g en M_g. Door fermentatie nam het zwellend en verdikkend vermogen van de mais toe; in commerciële Mawè is dit effekt sterker dan in thuisbereide Mawè.

Het commerciële bereidingsproces heeft een aantal nadelen: het is tijds- en arbeidsintensief, het geeft een lagere Mawè-opbrengst, en het nutriëntenverlies is hoger dan bij het huishoudelijke proces. Gezien het feit dat 83% van de in Cotonou geproduceerde Mawè volgens het commerciële proces tot stand komt is het echter duidelijk favoriet. Dit kan worden toegeschreven aan het aantrekkelijke uiterlijk, de fijnkorreligheid, betere houdbaarheid en superieure verstijfselingseigenschappen die het bij uitstek geschikt maken voor de bereiding van traditionele maispuddingen. Het commerciële Mawè bereidingsproces kan daarom worden beschouwd als een technologisch verfijnder, en doeltreffender techniek om Mawè voor maispuddingen te produceren.

Daarentegen zijn het hoger nutriëntengehalte en de lagere verstijfselingsviscositeit in de thuisbereide Mawè eigenschappen die het geschikter maken voor toepassing als drinkbare kindervoeding met maximale energieinhoud.

In totaal werden 205 stammen van micro-organismen (120 melkzuurbacteriën, 55 gisten en 30 stammen van Enterobacteriaceae) geïsoleerd uit verschillende stadia van beide Mawè bereidingsprocessen, onderzocht en geïdentificeerd (Hoofdstukken 4 en 5).

De aantallen melkzuurbacteriën waren het hoogst tussen 12 en 24 uur fermentatieduur ($Log_{10}N/g$ 9,2-9,3). Het merendeel (94%) waren *Lactobacillus* spp.; binnen deze groep waren 89% obligaat heterofermentatief: voornamelijk *L. brevis*, *L. fermentum* (biotype *cellobiosus*), *L. fermentum* (de laatstgenoemde drie vertegenwoordigen 85% en 86% van de melkzuurbacteriën geïsoleerd uit respectievelijk thuisbereide en commerciële Mawè), *L.*

confusus, *L. curvatus* en *L. büchneri*. Andere melkzuurbacteriën waren *L. salivarius*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *P. acidilactici* en *Leuconostoc mesenteroides*. In de beginfase (0-6 uur fermentatie) van beide bereidingsprocessen werden 7 verschillende soorten melkzuurbacteriën aangetroffen, terwijl na 72 uur fermentatie het aantal soorten was afgenomen en de microflora werd beheerst (90% van de isolaten) door *L. fermentum* (biotype *cellobiosus*) en *L. fermentum*.

In commerciële Mawè nam tijdens de eerste 48 uur fermentatie het aantal gisten toe tot $Log_{10}N/g = 7,4$ waarna het weer afnam, terwijl in de thuisbereide Mawè de gisten doorgroeiden. De belangrijkste gistsoort was *Candida krusei*; verder werden *Candida kefyr*, *C. glabrata* en *Saccharomyces cerevisiae* aangetroffen.

Enterobacteriaceae vertoonden een lichte toename tijdens de beginfase van de fermentatie, maar hun aanwezigheid was na 24 uur (commerciële Mawè) en 48 uur (thuisbereide Mawè) niet meer aantoonbaar. Zes van de 10 isolaten uit commerciële Mawè betroffen *Enterobacter cloacae*, en 19 van de 20 isolaten uit thuisbereide Mawè waren *Escherichia coli*. Andere aangetroffen Enterobacteriaceae waren *Klebsiella pneumoniae* en *Serratia odorifera*. Het hoger aantal coli-achtigen in thuisbereide Mawè werd toegeschreven aan de afwezigheid van een wasstap in het bereidingsproces zoals toegepast in het commerciële proces.

Een aantal reincultures van lactobacillen (*L. fermentum* biotype *cellobiosus, L. fermentum* en *L. brevis*) en gisten (*C. krusei* en *S. cerevisiae*) werden afzonderlijk en als mengcultures onderzocht op hun vermogen gesteriliseerde maissuspensie te fermenteren. Alle afzonderlijk onderzochte *Lactobacillus* soorten hadden gelijkwaardig zuurvormend vermogen; de gisten *C. krusei* en *S. cerevisiae* produceerden individueel weinig zuur. In combinatie met lactobacillen had *C. krusei* geen stimulerend effect op de zuurvorming, terwijl *S. cerevisiae* in mengcultuur met lactobacillen tot een verlaagde zuurproduktie aanleiding gaf.

Verder onderzoek aan de Mawè-fermentatie zal nodig zijn, met name de smaak- en aromacomponenten in het natuurlijk gefermenteerde produkt en de bijdrage die hierbij door melkzuurbacteriën en gisten wordt geleverd zijn van belang, evenals de veranderingen in het nutriëntengehalte en de antimicrobiële activiteit van Mawè die tijdens het bereidingsproces kunnen plaatsvinden.

RESUME

Deux types de pâte fermentée de maïs (*Zea mays L.*) produits et consommées au Bénin, et appelés respectivement mawè domestique et mawè commercial, ont été étudiés au niveau des procédés technologiques employés, les caractéristiques des produits tels qu'ils sont utilisés dans les ménages ou vendus sur les marchés, leur dynamique physico-chimique et microbiologique de fermentation. Des souches de bactéries lactiques, de levures et d'Enterobacteriaceae ont été isolées des pâtes, caractérisées et identifiées. La capacité de certaines souches de bactéries lactiques et de levures à fermenter une bouillie stérilisée de maïs dépelliculé a été également étudiée.

Le mawè est défini comme une pâte acide faite à partir de la farine de maïs partiellement dépelliculée et qui a subi la fermentation naturelle pendant 1 à 3 jours. Deux procédés de production sont utilisés: le procédé domestique actuellement utilisé pour produire du mawè pour l'autoconsommation et le procédé commercial utilisé pour produire du mawè destiné à la vente. La différence principale entre les deux procédés est un lavage intense du gritz de maïs pour enlever plus d'enveloppes et de germes du produit commercial; cette opération n'est pas appliquée dans le procédé domestique (Chapitre 2 et 3).

Le rendement de production du mawè domestique (76-80%, base matière sèche) était plus élevé que celui du mawè commercial (65-71%); ceci est dû à l'enlèvement de plus de sons (composés d'enveloppes et de germes) et à la perte de matières solubles pendant le lavage du gritz lors de la production du mawè commercial (Chapitre 3).

Le mawè commercial était plus blanc que le mawè domestique. Cette blancheur devenait plus prononcée en fonction de la durée de fermentation et était corrélée de façon significative avec l'augmentation de la teneur en eau au cours de la fermentation (Chapitre 3).

La composition en macronutriments du mawè commercial et du mawè domestique collectés respectivement sur les marchés et dans les ménages était respectivement, protéines: 8.2 et 9.2%; matières grasses: 1.0 et 2.3%, cendres: 0.6 et 1.1%; fibres brutes 0.4 et 0.7%, sur la base du poids de matière sèche (Chapitre 2). Avec sa teneur élevée en matières grasses, le mawè domestique est plus prédisposé au rancissement pendant le stockage. La méthode de production affecte la composition en macronutriments du mawè, la perte en

macronutriments étant plus importante dans le procédé commercial que dans le procédé domestique. La perte en matières grasses, en cendres et en fibres brutes est plus importante que la perte en protéines. L'étape de la fermentation n'a pas un effet significatif sur la teneur en macronutriments (Chapitre 3).

L'acidité titrable des deux types de mawè collectés respectivement dans les ménages et sur les marchés était de l'ordre de 1.2-1.4% (p/p en % d'acide lactique), mais le pH du mawè domestique était légèrement plus élevé (pH = 4.2) que celui du mawè commercial (pH = 3.8) (Chapitre 2). L'étude de l'évolution du pH et de l'acidité titrable pendant la fermentation a montré que ces niveaux d'acidité peuvent être atteints en 24 h de fermentation du mawè commercial et seulement en 12 h de fermentation du mawè domestique (Chapitre 3).

L'analyse de la courbe de viscosité enregistrée au viscographe Brabender Pt 100 a montré que la durée de fermentation n'a pas d'effet significatif sur la température de gélatinisation (T_g), ni sur la durée pour atteindre la température de gélatinisation (M_g). Mais la viscosité maximale (V_m), la viscosité après 15 minutes à 92°C (V_r) et la viscosité au refroidissement à 50°C (V_c) augmentent avec la durée de fermentation. Le changement le plus significatif a été noté entre les échantillons non fermentés et les échantillons fermentés. Le procédé technologique influe sur la température de gélatinisation (T_g), la durée pour atteindre la viscosité maximale (M_g), la viscosité maximale (V_m), la viscosité après 15 minutes à 92°C (V_r) et la viscosité après refroidissement à 50°C (V_c). Les valeurs de viscosité sont plus élevées au niveau du mawè commercial qu'au niveau du mawè domestique tandis que T_g et M_g sont moins élevés. Selon certains auteurs, ces caractéristiques du mawè commercial en font une pâte plus apte pour la production des pâtes cuites du genre akassa.

Le procédé commercial présente plusieurs inconvénients: il est laborieux et dure plus longtemps que le procédé domestique, avec plus de perte de matière et de nutriments que dans ce dernier. Cependant, c'est un procédé qui fournit 83% du mawè produit à Cotonou. Ce succès du mawè commercial est probablement dû à son apparence, sa plus longue durée de conservation et ses caractéristiques de gonflement et de prise en masse adaptées à la préparation des pâtes gélifiées de type akassa. Sur cette base, le procédé de production du mawè commercial peut être considéré comme un procédé technologiquement plus avancé et plus effectif de production du mawè destiné à la préparation de ces pâtes que le procédé domestique.

La faible perte de nutriments et la faible capacité de gonflement et de prise en masse

du mawè domestique sont avantageuses pour son utilisation comme base de production des aliments de sevrage de haute densité calorique.

Les changements physico-chimiques qui interviennent dans le mawè sont le résultat de l'activité des microorganismes responsables de la fermentation. Deux cents cinq (205) souches de microorganismes comprenant 120 souches de bactéries lactiques, 55 souches de levures et 30 souches d'Enterobacteriaceae, ont été isolées des deux types de mawè en cours de fermentation, examinées et identifiées (Chapitres 4 et 5).

Le dénombrement maximum de bactéries lactiques a été enregistré entre 12 et 24 h de fermentation ($Log_{10}N/g$ = 9.2-9.3). *Lactobacillus* sp. constitue la majorité (94%) des bactéries lactiques isolées. La plupart des lactobacilles (89% des souches isolées) sont hétérofermentatifs et comprennent *Lactobacillus brevis*, *Lactobacillus fermentum* biotype *cellobiosus* et *Lactobacillus fermentum* (ce dernier pourrait aussi être *Lactobacillus reuteri*), *Lactobacillus curvatus*, *Lactobacillus confusus*, *Lactobacillus buchneri*. *Lactobacillus brevis*, *Lactobacillus fermentum* biotype *cellobiosus* et *Lactobacillus fermentum* représentent 85% des souches isolées du mawè familial et 86% de celles isolées du mawè commercial. D'autres espèces ont été isolées telles que: *Lactococcus lactis*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Leuconostoc mesenteroides*, *Lactobacillus lactis* et *Lactobacillus salivarius*. Au début de la fermentation, beaucoup d'espèces de bactéries lactiques étaient présentes: sept espèces ont été isolées dans chacun des deux types de mawè entre 0 et 6 heures de fermentation. Mais le nombre des espèces isolées est réduit au bout de 72 heures de fermentation où *L. fermentum* (biotype *cellobiosus*) et *L. fermentum* représentent 90% des souches isolées, *L. fermentum* (biotype *cellobiosus*) devenant l'espèce dominante.

En ce qui concerne les levures, leur nombre a augmenté jusqu'à 48 heures de fermentation dans le mawè commercial ($Log_{10}N/g$ = 7.4), pendant qu'il continuait à augmenter au-delà de 48 heures dans le mawè domestique. Les levures isolées des deux types de mawè sont: *Candida krusei* qui est prédominant, *Candida kefyr*, *Candida glabrata* et *Saccharomyces cerevisiae*.

Le nombre des Enterobacteriaceae a augmenté légèrement au cours des premières heures de fermentation, mais leur nombre était réduit en-dessous du seuil de détection au bout de 24 heures dans le mawè commercial, et au bout de 48 heures dans le mawè domestique. Six souches sur 10 isolées du mawè commercial ont été identifiées comme *Enterobacter cloacae*, tandis que 19 souches sur 20 isolées du mawè domestique ont été identifiées comme *Escherichia coli*. D'autres Enterobacteriaceae isolées sont: *Klebsiella pneumoniae* et *Serratia odorifera*.

Le taux élevé de coliformes dans le mawè domestique est probablement dû à l'omission du lavage du gritz au cours du processus domestique. Ce lavage est par contre très intense dans le processus commercial.

Des ferments de culture de lactobacilles: *L. fermentum* biotype *cellobiosus*, *L. fermentum* et *L. brevis*, et de levures: *C. krusei* et *S. cerevisiae*, ont été testés seuls ou en combinaison, pour leur aptitude à fermenter une bouillie stérilisée de maïs dépelliculé. Toutes les espèces de lactobacilles utilisées ont montré une aptitude similaire à fermenter la bouillie. *C. krusei* et *S. cerevisiae* utilisés seuls, ont montré peu d'activité dans la production d'acide. *C. krusei*, en combinaison avec les lactobacilles utilisés, n'a montré aucun effet stimulant sur la production d'acide. *S. cerevisiae* semble réduire la production d'acide dans les mêmes conditions.

Enfin, des domaines de recherche sur la technolnogie du mawè ont été proposés. Ces domaines incluent la connaissance des composés responsables du goût et de l'arôme dans le mawè fermenté naturellement et la contribution des bactéries lactiques et des levures à la formation de ces composés, l'évaluation des changements en micronutriments et de l'activité antimicrobienne au cours de la fermentation du mawè.

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