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Microbiological changes in *mawè* during natural fermentation

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Lactic acid bacteria increased from 3.2×10^6 and 1.6×10^7 c.f.u./g (wet wt) to 2×10^9 and 1.6×10^9 c.f.u./g after 12 to 24 h of fermentation of home-produced *mawè* (a dough produced from dehulled maize) and commercial *mawè*, respectively. In commercial *mawè*, the yeast count increased from 1.3×10^5 to 2.5×10^7 c.f.u./g after 48 h of fermentation before decreasing, whereas in the home-produced *mawè* it increased from 2.5×10^4 to 3.2×10^7 c.f.u./g after 72 h of fermentation; the dominant yeasts were mainly *Candida krusei*, although *C. kefyr*, *C. glabrata* and *Saccharomyces cerevisiae* were also present. Enterobacteriaceae counts increased slightly during the initial stage of the fermentation, but decreased below the detection level after 24 to 48 h. *Enterobacter cloacae* was mostly found in commercial *mawè* and *Escherichia coli* mostly in home-produced *mawè*.

Key words: Enterobacteriaceae, fermentation, lactic acid bacteria, maize, *mawè*, yeast.

In Africa, most of the traditional cereal-based fermented foods are processed by natural fermentations. In most cases the microorganisms involved in these fermentations are lactic acid bacteria and yeasts (Akinrele 1970; Christian 1970; Nout 1980; Fields *et al.* 1981; Mbugua 1984; Odunfa & Adeyele 1985; Adegoke & Babalola 1988). These and Enterobacteriaceae have also been detected in home-produced and commercial *mawè* (Hounhouigan *et al.* 1993a), reaching about 10^9, 10^7 and 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 consistent quality. This requires knowledge of the microorganisms involved and their impact on the product. In a previous paper we characterized the lactic acid bacteria (LAB) isolated from *mawè* (Hounhouigan *et al.* 1993b). The present report deals with the microbiological changes in *mawè* during natural fermentation and identifies the predominant yeasts and enterobacteria involved.

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Materials and Methods

Sample Preparation

Home-produced and commercial *mawè* were produced in a local milling shop, as described earlier, using maize cultivar Sékou 85 (10 kg for each process) provided by the International Institute of Tropical Agriculture, Benin (Hounhouigan *et al.* 1993c). The dough (46% moisture content, wet wt basis) resulting from each process was divided equally between six plastic buckets, kneaded, covered with a polyethylene sheet and allowed to ferment spontaneously for 72 h at room temperature (28 to 32°C). Duplicate experiments were carried out for each process.

Isolation and Purification of Microorganisms

Samples (10 g) of *mawè* from each process were taken after 0 (kneading stage), 6, 12, 24, 48 and 72 h of fermentation, and each immediately homogenized in a stomacher (Lab-blender 400; Seward Medical, London UK) with 90 ml of sterile 0.5% (w/v) peptone, containing 0.85% (w/v) NaCl, pH 7.0 ± 0.2, and decimally diluted. Total aerobic mesophilic bacteria, LAB, lactobacilli, yeasts and Enterobacteriaceae were enumerated by the pour method as described previously (Hounhouigan *et al.* 1993a). Yeasts were randomly picked from plates at each of the sampling times and purified by streaking on yeast extract/glucose/agar plates (Oxoid CM 545) and incubating at 25°C for 3 to 5 days. After microscopic examination, purified cultures were grown on slants of the same medium and stored at 5°C. Randomly selected colonies of Enterobacteriaceae were isolated from plates at different time intervals between 0 and 24 h, purified on Tryptone/soya/agar plates (Oxoid CM 131) at 37°C for 18 to 24 h,

and identified approximately by Gram-staining and microscopical examination. Stock cultures were grown on the same medium and stored at 5°C for further identification.

Identification Tests

Yeast fermentation profiles were carried out on ATB 32C or ID 32C strips (API system S.A., Montalieu Vercieu, France). Preliminary identification was according to Lodder & 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 & Cochran 1989).

Results and Discussion

The microbial compositions of home-produced and commercial *mawè* are shown in Tables 1 and 2, respectively. The numbers of total aerobic mesophilic bacteria and lactic acid bacteria (LAB) were not significantly different between both types of *mawè* during the fermentation period, but the numbers of yeasts were significantly different ($P < 0.05$). High initial numbers of total aerobic mesophilic bacteria, LAB and yeasts were probably due to microorganisms in the commercial mill, acting as an inoculant during wet milling (Wacher *et al.* 1993). The vessels and the sieves used during processing also probably contributed. The highest counts of aerobic mesophilic bacteria and LAB were obtained 12 and 24 h of fermentation. The yeast counts increased until 48 h in commercial *mawè* before decreasing, but continued increasing in the home-produced *mawè*. This supports our previous observation that home-produced *mawè* does not stabilize microbiologically even 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 the detection level after 1 day in commercial *mawè* and 2 days in home-produced *mawè*.

The predominant lactic acid bacteria isolated from *mawè* have been identified (Hounhouigan *et al.* 1993b). Most of them (89%) were obligate heterofermenters and included *Lactobacillus fermentum* (biotype *cellobiosus*), *Lactob. fermentum* or *Lactob. reuteri* and *Lactob. brevis*, all of which accounted for about 85% of the strains isolated. Other species identified were *Lactob. curvatus*, *Lactob. confusus*, *Lactob. buchneri*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *P. acidilactici*, *Leuconostoc mesenteroides*, *Lactob. lactis* and *Lactob. salivarius*.

The identity of the yeasts is shown in Table 3. They were dominated by *Candida* species, including *C. krusei* (mainly), *C. kefyr* and *C. glabrata*. *Saccharomyces cerevisiae* was also isolated.

Table 4 summarizes the identity of the *Enterobacteriaceae*. Six of the 10 strains from commercial *mawè* were identified as *Enterobacter cloacae* whereas 19 of the 20 strains from home-produced *mawè* were identified as *Escherichia coli*. Other species identified included *Klebsiella pneumoniae* and *Serratia odorifera*, both from commercial *mawè*. *Escherichia coli* is generally considered to be an indicator of faecal contamination. The presence of *Es. coli* in *mawè* may be due to faecal contamination of the maize used and their relatively low number in the commercial *mawè* could be due to the extent of washing of the grits, which does not occur in the preparation of home-produced *mawè*.

LAB, yeasts and Enterobacteriaceae grew together, at least during the 12 to 24 h fermentation period, contributing to the characteristics of the final product, probably by producing organic acids, ethanol, CO_2 and other volatile flavour compounds. It had 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 (1980), the multiplication of *Lactobacillus* spp. in souring maize is favoured by the production of fermentable sugars from the auto-amylolysis of maize. Sugar (mostly glucose and maltose) concentrations increased from approx. 1.8% to 2.6% to approx. 3.0% to 4.3% (w/w) in the commercial *mawè* in the first 24 h of fermentation and subsequently decreased (unpublished data). In addition, the development of LAB is stimulated by yeasts which provide soluble nitrogen compounds and other growth factors, e.g. the B-vitamins (Nout 1991). Yeast

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

*Values are means of two independent determinations. Replicates were within 11% of the mean for the 0 h samples and within 5% of the mean at the other time intervals.

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

*Values are means of two independent determinations. Replicates were within 11% of the mean for the 0 h samples and within 5% at the other time intervals.

Table 3. Identification of the yeasts isolated from *mawè*.

Species	No. of isolates from: Home-produced *mawè*	Commercial *mawè*
Candida krusei	17	14
Candida kefyr	5	2
Candida glabrata	3	2
Saccharomyces cerevisiae	2	10
Totals	27	28

Table 4. Species of *Enterobacteriaceae* isolated from *mawè*.

Species	No. of isolates from: Home-produced *mawè*	Commercial *mawè*
Enterobacter cloacae	1	6
Escherichia coli	19	1
Klebsiella pneumoniae	—	1
Serratia odorifera	—	1
Not identified	—	1
Totals	20	10

metabolites, e.g. CO_2, pyruvate, propionate, acetate and succinate, have been shown to stimulate lactobacilli in *kefir* (Leroi & Pidoux 1993). On the other hand, the acidic environment created by lactobacilli is favourable for yeast growth (Wood 1981). This association of LAB and yeasts has been noticed in several cereal foods. *Candida krusei* and *Sa. cerevisiae* were found with LAB during the fermentation of *busaa*, a Kenyan opaque maize-millet beer (Nout 1980). Odunfa & Adeyele (1985) found *Lactobacillus* spp. and *Lactococcus lactis* together with *C. krusei* and *Debaryomyces hansenii* during the fermentation of *ogi-baba*, a West African fermented sorghum gruel. Adegoke & Babalola (1988) found *Sa. cerevisiae* together with *Lactob. fermentum*, *Lactob. brevis* and *Enterococcus faecalis* in the fermentation of *ogi*, while Akinrele (1970) found that corynebacteria, *Sa. cerevisiae*, *Enterob. cloacae* and *Lactob. plantarum* were prominent in *ogi*. More recently, Halm *et al.* (1993) found obligately heterofermentative lactobacilli closely related to *Lactob. fermentum* and *Lactob. reuteri*, in association with *Candida* spp. and *Saccharomyces* spp., in fermented maize dough from Ghana.

It is unclear if Enterobacteriaceae function in the *mawè* fermentation. As the acidic environment created by LAB is not favourable for their growth, their number decreases strongly after the first day of fermentation. Similar antimicrobial effects have been found in other lactic fermentations and the inhibitors have been suggested to be antibiotic substances (Mensah *et al.* 1991; Mbugua & Njenga 1992). A negative aspect is that coliform species have been reported to be responsible for off-flavours and flavour instability in Kenyan *uji* (Mbugua 1982). Taking into consideration the very low numbers of Enterobacteriaceae in *mawè*, it seems unlikely that they are responsible for the remarkable off-flavours, noticed particularly in the home-produced version. These off-flavours, combined with the undesirable sour taste which develops beyond 24 h fermentation due to a high titratable acidity (Hounhouigan *et al.* 1993c), make home-produced *mawè* less desirable than commercial *mawè* in urban areas.

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