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# Microbiological changes in *mawè* during natural fermentation

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**Lactic acid bacteria increased from $3.2 \times 10^6$ and $1.6 \times 10^7$ c.f.u./g (wet wt) to $2 \times 10^9$ and $1.6 \times 10^9$ 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 $1.3 \times 10^5$ to $2.5 \times 10^7$ c.f.u./g after 48 h of fermentation before decreasing, whereas in the home-produced *mawè* it increased from $2.5 \times 10^4$ to $3.2 \times 10^7$ 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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## References

Adegoke, G.O. & Babalola, A.K. 1988 Characteristics of microorganisms of importance in the fermentation of fufu and ogi, two Nigerian foods. *Journal of Applied Bacteriology* **65**, 449–453.

Akinrele I.A. 1970 Fermentation studies on maize during the preparation of a traditional African starch-cake food. *Journal of the Science of Food and Agriculture* **21**, 619–625.

Christian, W.F.K. 1970 Lactic acid bacteria in fermenting maize dough. *Ghana Journal of Science* **10**, 22–28.

Fields, M.L., Hamad M., & Smith, D.K. 1981 Natural lactic acid fermentation of corn meal. *Journal of Food Science* **46**, 900–902.

Halm M., Lillie A., Sørensen A.K & Jakobsen M. 1993 Microbiological and aromatic characteristics of fermented maize doughs for kenkey production in Ghana. *International Journal of Food Microbiology* **19**, 135–143.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M. Houben, J.H. & Rombouts, F.M. 1993a Composition and microbiological and physical attributes of *mawè*, a fermented maize dough from Bénin. *International Journal of Food Science and Technology* **28**, 513–517.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M. Houben, J.H. & Rombouts, F.M. 1993b Characterization and frequency distribution of species of lactic acid bacteria involved in the processing of *mawè*, a fermented maize dough from Bénin. *International Journal of Food Microbiology* **18**, 279–287.

Hounhouigan, D.J., Nout, M.J.R., Nago, C.M. Houben, J.H. & Rombouts, F.M. 1993c Changes in the physico-chemical properties of maize during natural fermentation of *mawè*. *Journal of Cereal Science* **17**, 291–300.

Leroi, F. & Pidoux, M. 1993 Characterization of interactions between *Lactobacillus hilgardii* and *Saccharomyces florentinus* isolated from sugary kefir grains. *Journal of Applied Bacteriology* **74**, 54–60.

Lodder, J. & Kreger van Rij, N.J.W. (eds) 1984 *The Yeasts. A Taxonomic Study*, 3rd edn. Amsterdam: Elsevier Science.

Mbugua, S.K. 1982 Microbiological and biochemical aspects of uji (an East African sour cereal porridge) fermentation, and its enhancement through application of lactic acid bacteria. *Dissertation Abstracts International* **42**, 3178.

Mbugua, S.K. 1984 Isolation and characterization of lactic acid bacteria during the traditional fermentation of uji. *East African Agricultural and Forestry Journal* **50**, 36–43.

Mbugua, S.K. & Njenga, J. 1992 The antimicrobial activity of fermented uji. *Ecology of Food and Nutrition* **28**, 191–198.

Mensah, P., Tomkins, A.M., Drasar, B.S., & Harrison, T.J. 1991 Antimicrobial effect of fermented Ghanaian maize dough. *Journal of Applied Bacteriology* **70**, 203–210.

Nout, M.J.R. 1980 Microbiological aspects of the traditional manufacture of busaa, a Kenyan opaque maize beer. *Chemie Mikrobiologie Technologie der Lebensmittel* **6**, 137–142.

Nout, M.J.R. 1991 Ecology of accelerated natural lactic fermentation of sorghum-based infant food formulas. *International Journal of Food Microbiology* **12**, 217–224.

Odunfa, S.A. & Adeyele, S. 1985 Microbiological changes during the traditional production of ogi-baba, a West African fermented sorghum gruel. *Journal of Cereal Science* **3**, 173–180.

Snedecor, W.G. & Cochran W.G. 1989 *Statistical Methods*, 8th edn. Ames: Iowa State University Press.

Wacher, C., Cañas A., Cook P.E., Barzana E. & Owens J.D. 1993 Sources of microorganisms in *pozol*, a traditional Mexican fermented maize dough. *World Journal of Microbiology and Biotechnology* **9**, 269–274.

Wood B.J.B. 1981 The yeast/*Lactobacillus* interaction. A study in stability. In *Mixed Culture Fermentation*, eds Bushell, M.E. & Slater, J.H. pp. 137–150. London: Academic Press.

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