h 0574

Trop. Sci. 1999, **39**, 220–226

Use of starter cultures of lactobacilli and yeast in the fermentation of mawè, an African maize product

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Abstract *Starter cultures of lactobacilli (*Lactobacillus fermentum, L. brevis *and* L. salivarius*) and yeast (*Candida krusei *and* Saccharomyces cerevisiae*) were tested singly or in combination for their ability to ferment mawè (maize porridge).* L. fermentum *and* L. brevis *also showed the ability to ferment mawè, with similar dynamics to the natural fermentation. The pH of the sample fermented with* L. salivarius *singly or in combination with either yeast, was significantly higher.* C. krusei *and* S. cerevisiae *used singly showed little activity in acid production.* C. krusei *in combination with lactobacilli had no significant effect on the final acidity, but favoured a fast growth of* L. fermentum *and* L. brevis. *The mawè fermented using a starter had less flavour than that bought as street food, but there was no significant preference for any mawè sample.*

Keywords: fermentation, lactobacilli, maize, starter cultures, yeast, porridge.

Introduction

Lactic acid bacteria and yeast are dominant organisms in the natural fermentation of most plant foods (e.g. Nout 1980; Adegoke and Babalola 1988). Their development seems to be stimulated by the presence of yeast, which provides soluble nitrogen compounds and factors such as B vitamins (Nout 1991) and CO_2, pyruvate, propionate, acetate and succinate (Leroi and Pidoux 1993). On the other hand, the acidic environment created by lactobacilli favours yeast growth. Also the alcohol produced by the yeast, the acids produced by the bacteria and the anaerobiosis induced by the fermentation suppress microbes such as filamentous fungi and the bacteria associated with food spoilage and poisoning (Nout *et al.* 1989; Mensah *et al.* 1991). Thus the association of lactic acid bacteria and yeast provides a pleasant taste and flavour to safe foods. The use of these micro-organisms as starters in the fermentation of cereals would control the fermentation process and minimize the variation in product quality encountered in natural fermentation (Sanni 1993). Starter cultures have been used in the fermentation of some African cereal foods and beverages (Nout 1980; Mbugua *et al.* 1984). This paper concerns the use of starter cultures in the fermentation of mawè, which is made from dehulled and partially degerminated white maize. The natural fermentation of mawè has been studied by Hounhouigan *et al.* (1993a,b,c, 1994).

Accepted 5 March 1999

Materials and methods

White maize cultivar Sékou 85 was provided by the Niaouli Agronomic Research Station, Bénin. Maize flour was produced by the commercial mawè process (Hounhouigan *et al.* 1993b). The wet flour (40–42% moisture) obtained was packed in polyethylene bags, and held at –50°C; samples for use were thawed overnight at 5°C. A suspension was made by adjusting the moisture content to about 93% (w/w), using a Mettler LP 15 infrared unit on a Mettler PE 3600 electronic balance. A series of samples of suspension (about 100 g) in 250 ml Erlenmeyer screw-cap flasks were shaken on a Janke & Kunkel RW 20 mixer, autoclaved for 15 min at 121°C, and left to cool to room temperature (26–28°C) before inoculation. Another series of similar but non-autoclaved samples of suspension was prepared for natural fermentation.

Cultures of lactobacilli (*Lactobacillus fermentum, L. brevis, L. salivarius*) and yeast (*Candida krusei* and *Saccharomyces cerevisiae*), previously isolated from mawè (Hounhouigan *et al.* 1993c), 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 an 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. This culture (0.1 ml) was added to 10 ml MRS broth, incubated at 30°C for 16 h and centrifuged at 3000 rpm for 10 min. The pellet was washed in 10 ml sterile peptone solution (1 g peptone, 8.5 g NaCl in 1 l distilled water, pH 7.2), centrifuged again and redistributed in peptone solution. This gave an inoculum with 10^9 cfu/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 above, to give an inoculum with 10^7–10^8 cfu/ml, as viable count in yeast glucose agar (Oxoid CM 545).

The samples of the maize flour suspension were inoculated with 1 ml of lactobacilli or yeast inoculum. In mixed fermentation, 1 ml of each inoculum was used. The samples were shaken vigorously by hand and incubated at 30°C for 24 h in a Memmert incubator. At time 0 and after 6, 12, 18 and 24 h, samples were taken for the enumeration of lactobacilli or yeast. The rest of each sample was kept in polyethylene bags at –50°C for analysis. As the control, autoclaved samples of the maize suspension were incubated at 30°C for 24 h. Experiments were carried out in triplicate.

The extent of fermentation was assessed by the pH and titratable acidity on frozen samples, after thawing overnight at +5°C. About 30 g of sample accurately weighed was mixed with 20 ml of distilled water, and the pH was measured using a Hanna 8417 pH meter. The titratable acidity was determined on this mixture diluted with 50 ml distilled water. Analyses were in triplicate. Lactic acid bacteria and yeast counts were performed on 10 g of the samples drawn after different fermentation periods, as described by Hounhouigan *et al.* (1993a). Results from different treatments were compared using analysis of variance (Snedecor and Cochran 1989).

In the sensory evaluation, three groups of four coded samples of porridge were presented to a panel of 27 untrained judges, familiar with mawè. For each group, a sample of traditional

mawè porridge sold in the town as street food was used as reference. Multiple comparison and Friedman (Watts and Elias 1991) tests were used to assess the samples according to flavour and to determine the most preferred sample respectively.

Results

L. fermentum and *L. brevis* used as single inocula were effective in fermenting mawè porridge (Table 1). The pH of the samples inoculated with these heterofermentative lactobacilli decreased from 6.1 to 4.0–4.3 after 6 h of fermentation. In all samples inoculated with these lactobacilli and in the non-autoclaved sample fermented naturally, the pH fell to about 3.4–3.6 after 24 h of fermentation, with a corresponding increase of titratable acidity. *L. salivarius* was less effective, as the pH was 4.3 after 24 h of fermentation, although the titratable acidity was similar to the other lactobacilli. *C. krusei* and *S. cerevisiae* were not very active. There was no significant difference between the lactobacilli when used singly or in combination with yeast, as judged from the rate of the decrease of the pH (data not shown), or from the final pH. When used singly, *L. salivarius* showed less growth than *L. brevis* and *L. fermentum* (Figure 1). *L. brevis* grew better in combination with *C. krusei* than singly or with *S. cerevisiae* (Figure 2). *L. fermentum* also grew quickly in combination with *C. krusei* (Figure 3). The use of *C. krusei* or *S. cerevisiae* enhanced the growth of *L. salivarius* only later in the fermentation (Figure 4). *C. krusei* had more effect on growth than *S. cerevisiae* in combination with any of the lactobacilli (Figures 5–7).

Table 1. pH and titratable acidity of mawè after 24 h of fermentation

Starter culture	pH	Titratable acidity (% w/w as lactic acid, dwb)
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
Lactobacillus fermentum	3.6c	0.14abc
L. fermentum + *C. krusei*	3.5c	0.13abc
L. fermentum + *S. cerevisiae*	3.9c	0.11c
L. brevis	3.4c	0.15ab
L. brevis + *C. krusei*	3.5c	0.13abc
L. brevis + *S. cerevisiae*	3.8c	0.11bc
L. salivarius	4.4d	0.14abc
L. salivarius + *C. krusei*	4.3d	0.15abc
L. salivarius + *S. cerevisiae*	4.4d	0.13abc

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

Sensory evaluation tests showed that the porridges fermented using starter had less flavour than the traditional mawè porridge bought in the town, despite a similar pH (3.5) and titratable acidity (0.17%).

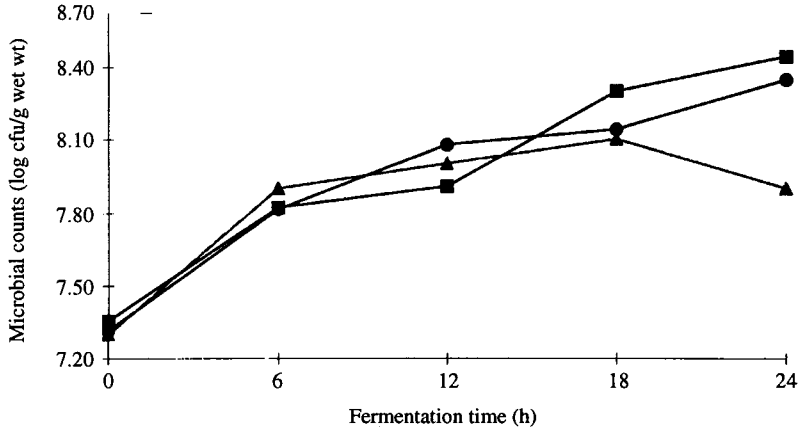

Figure 1. Growth of lactobacilli during fermentation of mawè. ● = *L. fermentum*; ■ = *L. brevis*; ▲ = *L. salivarius*.

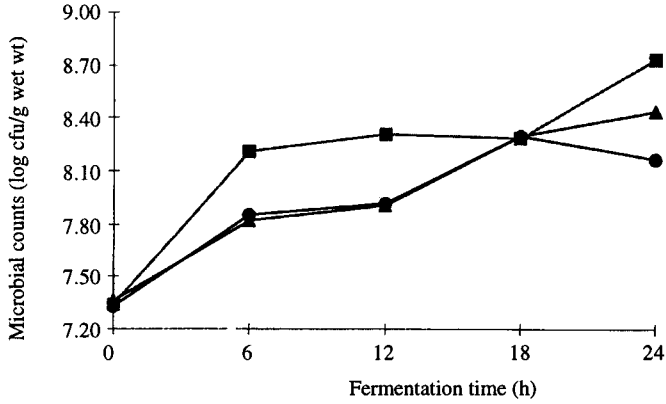

Figure 2. Growth of *L. brevis* singly or with yeast during fermentation of mawè. ▲ = *L. brevis*; ■ = *L. brevis* (in combination with *C. krusei*); ● = *L. brevis* (in combination with *S. cerevisiae*).

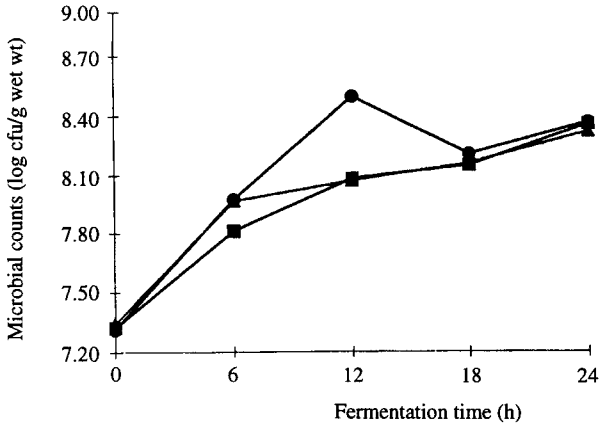

Figure 3. Growth of *L. fermentum* singly or with yeast during fermentation of mawè. ■ = *L. fermentum*; ● = *L. fermentum* (in combination with *C. krusei*); ▲ = *L. fermentum* (in combination with *S. cerevisiae*).

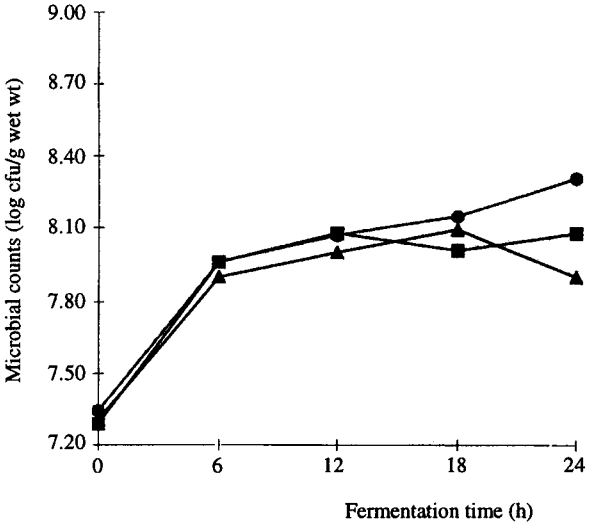

Figure 4. Growth of *L. salivarius* singly or with yeast during fermentation of mawè. ▲ = *L. salivarius*; ■ = *L. salivarius* (in combination with *C. krusei*); ● = *L. salivarius* (in combination with *S. cerevisiae*).

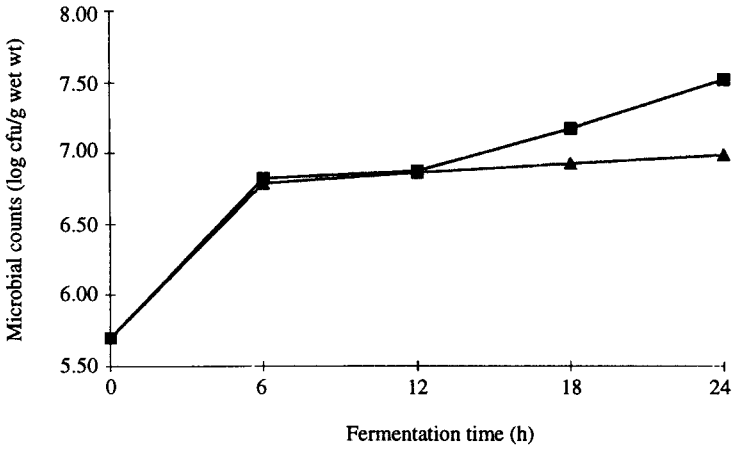

Figure 5. Growth of *C. krusei* and *S. cerevisiae* in combination with *L. brevis* during fermentation of mawè. ■ = *C. krusei*; ▲ = *S. cerevisiae*.

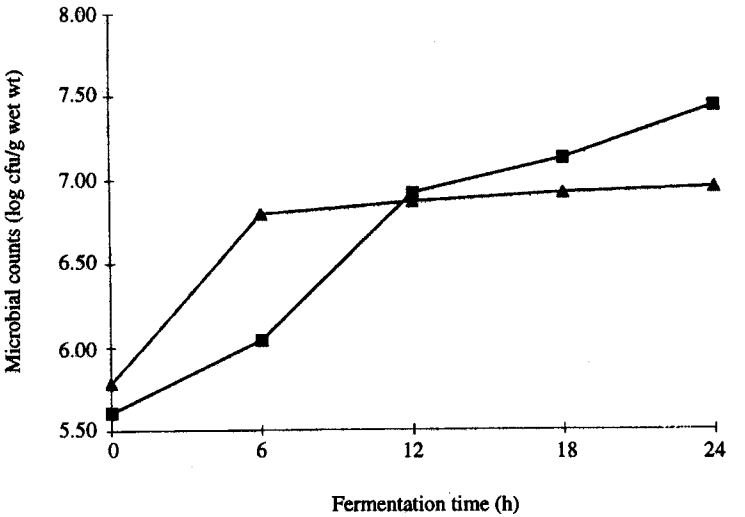

Figure 6. Growth of *C. krusei* and *S. cerevisiae* in combination with *L. fermentum* during fermentation of mawè. ■ = *C. krusei*; ▲ = *S. cerevisiae*.

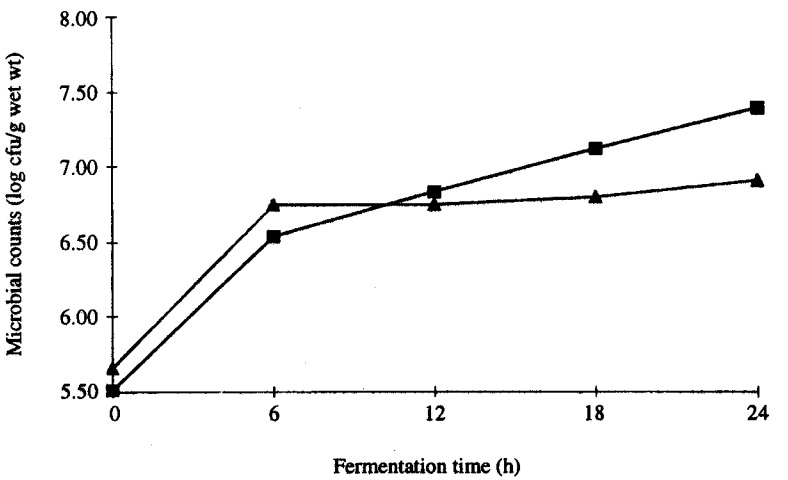

Figure 7. Growth of *C. krusei* and *S. cerevisiae* in combination with *L. salivarius* during fermentation of mawè. ■ = *C. krusei*; ▲ = *S. cerevisiae*.

Discussion

The pH and titratable acidity of mawè porridge obtained using starter cultures were almost similar to those of mawè produced in the normal way (Hounhouigan *et al.* 1993b). The ability of the species of the heterofermentative lactobacilli to ferment mawè porridge is similar to that in the controlled fermentation of maize for kenkey production (Halm *et al.* 1993). In contrast, strains of *L. cellobiosus* and *L. fermentum* failed to ferment sterile uji slurry, judged from a poor decrease in pH (Mbugua *et al.* 1984). The low growth of *L. salivarius*

could explain why this species was not detected in commercial mawè and was found in only a few samples of home-produced mawè (Hounhouigan *et al.* 1993c). Better growth of *L. brevis* and *L. fermentum* when combined with *C. krusei* was probably due to the apparent symbiotic association between these organisms (Nout 1991; Leroi and Pidoux 1993).

Conclusions

Fermentation of mawè porridge can be carried out using a single starter culture of *L. fermentum* or *L. brevis*. The presence of *C. krusei* in the fermenting medium had no significant effect on the acidity.

Aknowledgements

We thank the Dutch-Beninese University Cooperation Programme and the International Foundation for Science for financial support, and Alphonse Kakai for assistance.

References

Adegoke G.O. and Babalola A.K. (1988) Characteristics of micro-organisms of importance in the fermentation of fufu and ogi, two Nigerian foods. Journal of Applied Bacteriology 65, 449–53.

Halm M., Lillie A., Sorensen A.K. and Jakobsen M. (1993) Microbiological and aromatic characteristics of fermented maize doughs for kenkey production in Ghana. International Journal of Food Microbiology 19, 135–43.

Hounhouigan D.J., Nout M.J.R., Nago C.M., Houben J.H. and 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, 513–17.

Hounhouigan D.J., Nout M.J.R., Nago C.M., Houben J.H. and Rombouts F.M. (1993b) Changes in the physicochemical properties of maize during natural fermentation of mawè. Journal of Cereal Science 17, 291–300.

Hounhouigan D.J., Nout M.J.R., Nago C.M., Houben J.H. and Rombouts F.M. (1993c) 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, 279–87.

Hounhouigan D.J., Nout M.J.R., Nago C.M., Houben J.H. and Rombouts F.M. (1994) Microbiological changes in mawè during natural fermentation. World Journal of Microbiology and Biotechnology 10, 410–13.

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

Mbugua S.K., Ledford R.A. and Steinkraus K.H. (1984) Application of pure lactic cultures on the fermentation of uji (an East African sour cereal porridge). Lebensmittel Wissenschaft und Technologie 17, 252–6

Mensah P., Tomkins A.M., Drasar B.S. and Harrison T.J. (1991) Antimicrobial effect of fermented Ghanaian maize dough. Journal of Applied Bacteriology 70, 203–10.

Nout M.J.R. (1980) Microbiological aspects of the traditional manufacture of busaa, a Kenyan opaque maize beer. Chemie Mikrobiologie Technologie der Lebensmittel 6, 137–42.

Nout M.J.R. (1991) Ecology of accelerated natural lactic fermentation of sorghum-based infant food formulas. International Journal of Food Microbiology 12, 217–24.

Nout, M.J.R., Rombouts F.M. and Havelaar A. (1989) Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic microorganisms. International Journal of Food Microbiology 8, 351–61.

Sanni A.I. (1993) The need for process optimization of African fermented foods and beverages. International Journal of Food Microbiology 18, 85–95.

Snedecor W.G. and Cochran W.G. (1989) Statistical methods, 8th edn. Ames: Iowa State University Press, pp. 38–83.

Watts B.M. and Elias L.G. (1991) Méthodes de base pour l'évaluation sensorielle des aliments. Ottawa: International Centre for Development Research, pp. 57–63.