The use of starter cultures in the fermentation of *bushera*: a Ugandan traditional fermented sorghum beverage

C. M. B K. Muyanja, T. Langsrud[1] and J.A. Narvhus[1],

Department of Food Science and Technology, Makerere University, P.O. Box 7062, Kampala, Uganda.
[1]Department of Food Science, Agricultural University of Norway, P.O. Box 5036, N-1432 Ås, Norway.

Abstract

Weissella (*W*) *confusa* MINF8, *Lactobacillus* (*Lb*) *plantarum* MINF227, *Lb. paracasei* subsp. *paracasei* MINF98, *Lb. fermentum* MINF99 and *Lb. brevis* MINF226, all previously isolated from *bushera* were evaluated as single strain starters for sorghum *bushera* production. Production of *bushera* was according to the traditional procedure except that the raw materials were steamed at 98°C for 45 minutes before inoculation. Fermentation was carried out at 30°C and analyses for pH, sugars, organic acids, volatile organic compounds and viable counts were made after 0, 4, 8, 12, 48, 72 and 96 h. Traditional *bushera* was also prepared using germinated sorghum flour as inoculum. The starter cultures increased during the fermentation of *bushera* from about 7 log cfu ml^{-1} to 8.5 - 9 log cfu ml^{-1}, while in spontaneously fermented *bushera* LAB increased from 6 to 9 log cfu ml^{-1}. No yeasts or coliforms were detected in starter fermentations due to the steaming step. However, in spontaneously fermented *bushera*, coliforms increased from about 6 to 8.4 log cfu ml^{-1} within 12 h, but thereafter decreased to <2 log cfu ml^{-1} after 48 h. Yeast numbers gradually increased during spontaneous fermentation from 4.5 to 7.1 log cfu ml^{-1}. Compared to the other starter strains, *W. confusa* MINF8 possessed a superior ability to utilise sugars. In addition, an increase in maltose and glucose levels due to starch degradation was observed after 48 h. All strains reduced the pH from 6.5 to below 4.0, except for *Lb. fermentum* MINF99 (pH 4.07 after 96 h). *W. confusa* MINF8 produced the lowest pH (3.6) which was similar to that of spontaneously fermented *bushera* (pH 3.6). The lactate content of spontaneously fermented *bushera* was 0.89 % while that produced by different starter cultures was in the range 0.34 - 0.66%. The added starters produced 0.002 - 0.14 % ethanol whereas spontaneously fermented *bushera* contained 1.04 %. The rapid acidification, superior utilisation of sugars, and hydrolysis of starch with increasing sugar level by *W. confusa* MINF8 during fermentation indicated its potential for use as a starter culture.

Keywords: Sorghum, fermented *bushera*, *Lactobacillus*, *Weissella*

Introduction

Lactic acid bacteria (LAB) are widely used as starter cultures in the production of fermented food and feed (Knorr, 1998). Fermentation of milk, meat, vegetables and cereals results in products with changed chemical composition and taste and they also have an extended shelf life (Knorr, 1998). Natural fermentation of plant materials results from activities of a lactic microflora that eventually predominates over a large and varied epiphytic flora. Lactic acid bacteria have been reported as being the dominant organisms in a majority of naturally fermented foods (Akinrele, 1970; Nout, 1980; Odunfa and Adeyele, 1985; Halm *et al.*, 1993; Hounhouigan *et al.*, 1996; Holzapfel, 1997). The fermentation process of *bushera* has been described and LAB have been shown to dominate the fermentation (Muyanja *et al.*, 2002). In brief, traditional sorghum *bushera* is produced by mixing germinated sorghum flour with boiled water. The mixture is left to cool at ambient temperature and then germinated sorghum flour is added to initiate fermentation.

Fermentation is carried out at ambient temperature in clay pots for 1-4 days. The fermentation is often carried out under poor hygienic conditions and there is therefore a need to improve the safety and quality of *bushera*. Sanni (1993) and Kimaryo *et al.* (2000) indicated that the use of starter cultures containing microorganisms isolated from fermented cereal foods for fermentation could suffice to control the process and thereby alleviate the variations in organoleptic characteristics that occur in natural fermentation and thereby also enhance the shelf life and quality. The potentials of using starter cultures for fermentation on a household scale are summarised by Holzapfel (1997). The use of starter cultures can greatly reduce the fermentation time to hours instead of days and improve its acceptability. The objective of the study was to assess the selected strains of *Lactobacillus* and *Weissella* isolated from traditionally produced *bushera* for their performance as single strain starter cultures for production of *bushera* in comparison with the natural fermentation.

Material and methods

Materials
 Germinated sorghum flour used to produce *Bushera* was purchased from local households and markets in Rukungiri district in Uganda.

Source and preparation of starter strains
The following strains previously isolated from traditionally fermented *bushera* were used; *Lactobacillus* (*Lb*) *brevis* MINF226, *Lb. fermentum* MINF99, *Lb. plantarum* MINF227, *Lb. paracasei* subsp. *paracasei* MINF98 and *Weissella* (*W*) *confusa* MINF8. *W. confusa* MINF8 was shown to be able to ferment starch in the API 50CHL system (Muyanja et al., 2002). Each culture was grown in 250 ml of MRS broth at 30°C for 18 hours and then centrifuged at 6000 rpm for 10 minutes. The cell pellets obtained were resuspended in 25 ml Ringers solution containing 10% glycerol and stored at -80°C until used. The procedure attained a frozen culture concentration of approx. 9 log cfu ml^{-1} ascertained as viable count on MRS agar after freezing.

Laboratory preparation of bushera
 Sorghum *bushera* was prepared at the Department of Food Science, Agricultural University of Norway, according to the traditional procedure (Muyanja et al., 2002). Thirty grams (30g) of flour was weighed into glass bottles, mixed with distilled water (250ml) and steamed for 45 minutes at 98°C. During steaming samples were shaken to avoid lump formation. The samples were left to cool to room temperature and then inoculated with the selected strains at about 7 log cfu ml^{-1}. Germinated sorghum flour (7.5g) was added as a source of inoculum to initiate spontaneous fermentation of *bushera* (control samples). The samples were incubated at 30°C for 4 days. At 0 time and after 4, 8, 12, 24, 48 and 96 h of fermentation, samples were withdrawn to determine pH and viable counts and to analyse the content of sugars, organic acids and volatile compounds. The experiment was replicated three times.

Enumeration of LAB, yeasts and coliforms in bushera
 All serial dilutions were made in Ringer's solution (Oxoid, Unipath Ltd, UK). The LAB counts in *bushera* with single starters were determined by pour plating on MRS agar (Merck, Darmstadt, Germany) and incubating at 30°C for 48 h. MRS and M17 agar (Merck) were used for LAB counts in spontaneously fermented *bushera*. The yeasts were enumerated by surface plating on Rose Bengal Chloramphenicol agar (Oxoid). Plates were incubated at 25 °C for 3-6 days.Coliforms were enumerated by pour plating on Violet Red Bile agar (Oxoid). After solidification, the plates were placed in plastic bags and incubated aerobically at 37°C for 24 hr.

Measurement of pH
The pH of *bushera* during fermentation was determined using a Lab pH meter (PHM92, Lab pH meter, Radiometer analytical, Copenhagen, Denmark) with a combined pH electrode (Radiometer analytical), calibrated using standard buffer solutions (Merck) at pH 4.0 and 7.0.

Determination of ethanol and sugars in bushera
 Sugars were determined by high performance liquid chromatography (HPLC) according to the method described by Narvhus et al. (1998), Gadaga (2000) and Mugula (2001). The sugars determined (maltose, glucose and fructose) were detected by a Refractive Index detector (Series, 2000, Perkin Elmer, Norwalk, USA). Standard sugar solutions (Sigma, St Louis, MO, USA) were used for calibration.

Statistical analyses
The data were also subjected to analysis of variance using SAS/stat (SAS institute, Cary, NC, USA) to determine the significant difference between the various treatments.

Results

Enumeration of LAB, yeast and coliforms during bushera fermentation
In *bushera* with added starters, bacterial growth was monitored on MRS agar. An increase in viable numbers of one log cycle was observed during the first 4 h of fermentation for all strains (Figure 1a). The maximum numbers were found after 24 h for all starters. There was significant differences (P>0.05) in microbial counts among the starters. The poorest growth (8.2 log cfu ml^{-1}) was observed with *Lb. brevis* MINF226 whose growth rate was already reduced after 4 h, and the highest number (9.1 log cfu ml^{-1}) was with *Lb. paracasei* subsp. *paracasei* MINF98. Reductions in numbers were observed after 48 h for all starters except *Lb. paracasei* subsp. *paracasei* MINF98. No coliforms or yeasts were found in starter fermented *bushera*, which indicated the efficiency of the heat treatment

 In spontaneously fermented *bushera* the maximum numbers of bacteria, 9.0 log cfu ml^{-1}, was reached after 12 to 24 h on both M17 and MRS agar and then a slight decrease was observed (Figure 1b). During the first 4 h, an increase in viable numbers from 6.4 to 8.3 log cfu ml^{-1} was observed on M17, while the increase on MRS was from 5.9 to 6.7 log cfu ml^{-1}. However, the numbers were similar on both agars after 8 h. Coliforms increased to their maximum number, 8.4 log cfu ml^{-1} within the first 12 h of fermentation and then decreased to less than 2 log cfu ml^{-1} by 48 h. Yeasts increased from 4.3 to 7.1 log cfu ml^{-1} and remained constant after 72 h.
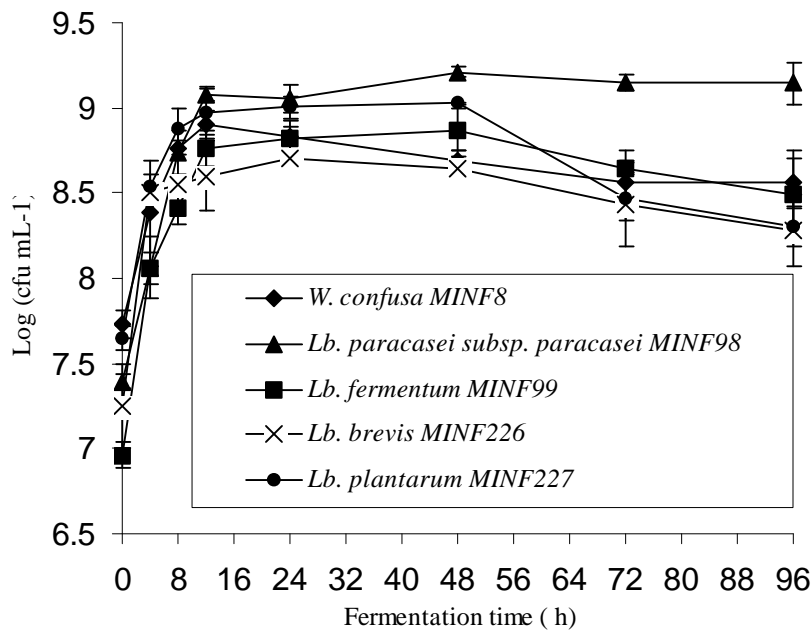
Fig. 1a. Changes in lactobacillus counts used as starters on MRS agar during fermentation of bushera made from germinated sorghum flour. Results given as averages and standard deviation indicated by bars.
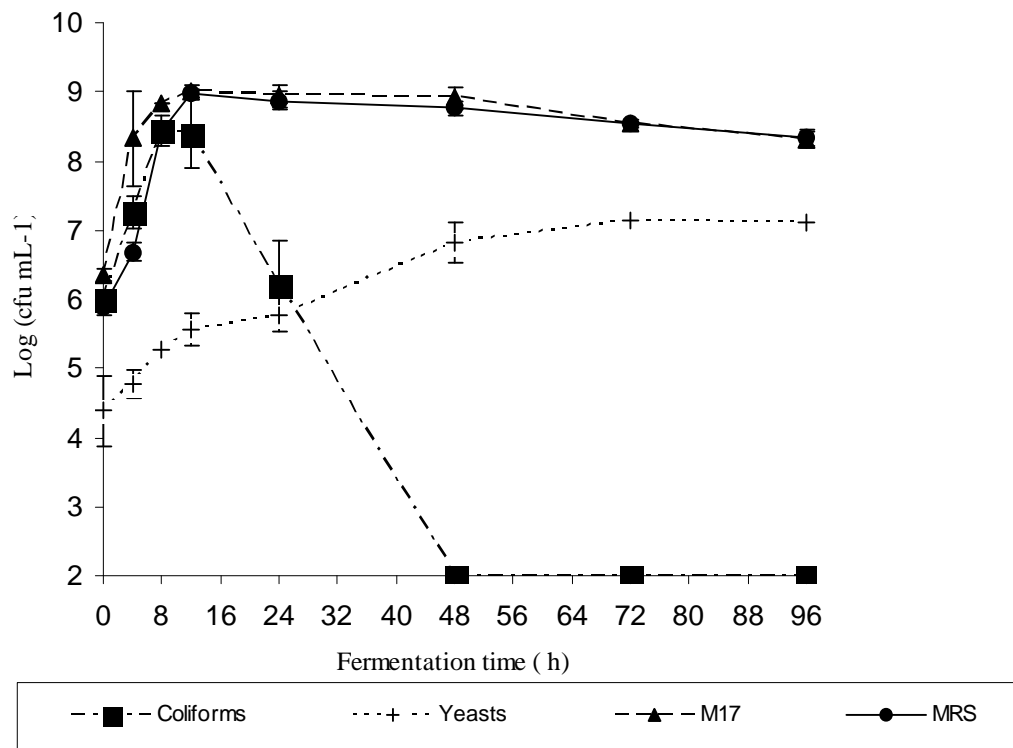

Fig. 1b. Changes in microbial counts in bushera made from germinated sorghum flour during spontaneous fermentation. Results given as average and standard deviation indicated by bars.
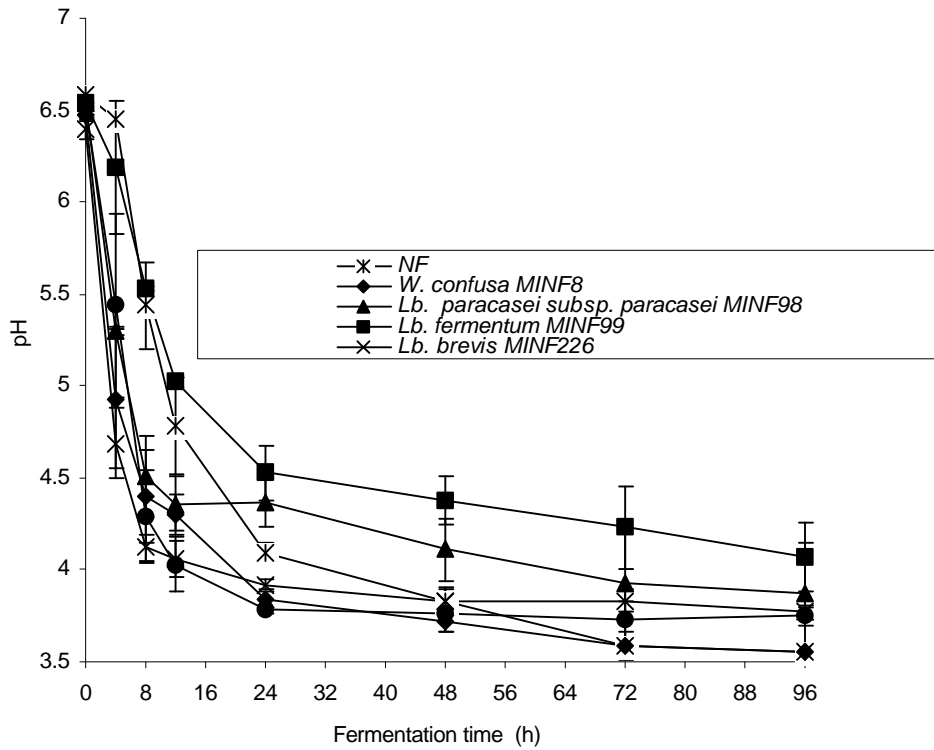
Fig. 2. Changes in pH of bushera made from germinated sorghum flour during spontaneous fermentation (NF) and with starters. Results givens as averages and standard deviation indicated by bars
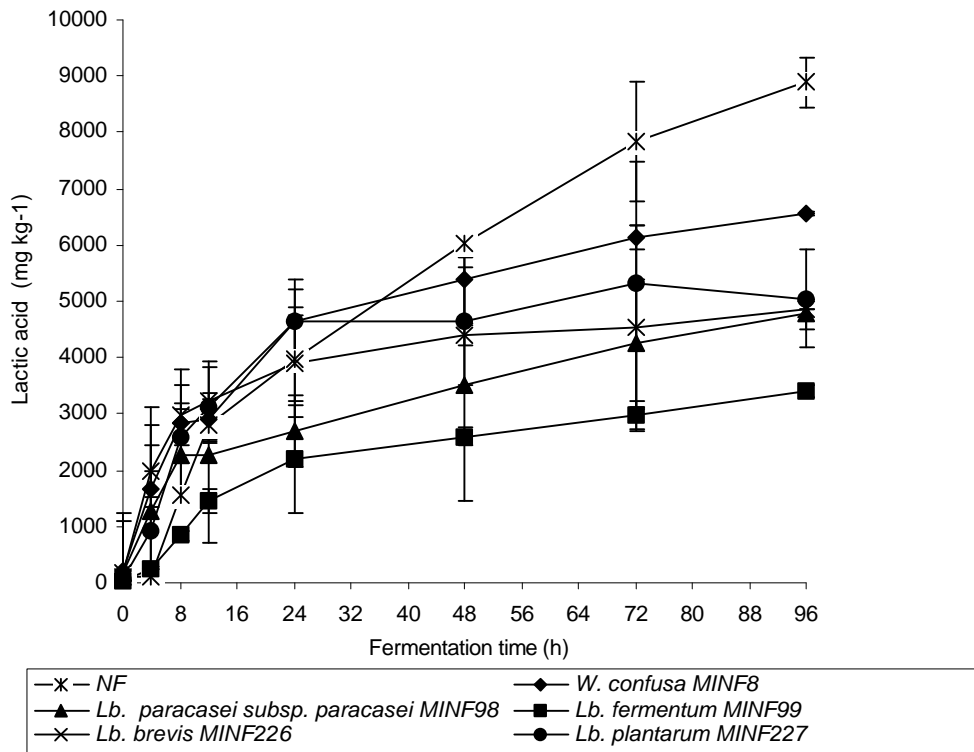

Fig. 3. Changes in lactic acid content in bushera made from germinated sorghum flour during spontaneous fermentation (NF) and with starters. Results given as averages and standard deviation indicated by bars.

pH decreased rapidly with a simultaneous increase in lactic acid when *bushera* was fermented with added starter cultures at 30° C for 96 h (Figure 2 and 3). The initial pH was 6.5 and decreased rapidly within the first 24 h to levels ranging from 3.8 to 4.5. After 24 h, the pH reduction was slower and stabilised between pH 3.5 and 4.0 after 96 h depending on the starter used. There was significant differences (P<0.05) observed in pH between the starters used. The most rapid drop in pH was noted in *bushera* fermented with *Lb. brevis* MINF226, *Weissella confusa* MINF8 and *Lb. plantarum* MINF227. The pH reduction in spontaneously fermented *bushera* during the first 4 h was only 0.13 pH units. This was followed by a more rapid pH reduction down to approximately pH 4.0 after 24 h, which is comparative to those of the starters.

Changes in lactic acid.
Lactic acid was the main acid produced during *bushera* fermentation (Figure 3). There was a significant difference (P<0.05) in lactic concentration between the starters used. Rapid lactic acid production was observed in *bushera* with added starter within 4 h, with the exception of *bushera* with *Lb. fermentum* MINF99, whereas lactic acid production in spontaneously fermented *bushera* was delayed for at least 4 h. The highest amount of lactic acid produced with starter addition was with *W. confusa* MINF8. *Lb. fermentum* MINF99 produced least lactic acid. The largest amount (0.89 %) of lactic acid was produced in spontaneously fermented *bushera* while in single culture fermented *bushera* the lactic acid content varied between 0.34 –0.66% at the end of fermentation (96 h).

The changes in levels of glucose and maltose during fermentation are shown in Figures 4a and b. Glucose concentration was initially about 2000 mg kg^{-1} and markedly decreased during the first 24 h of fermentation with added starter. *W. confusa* MINF8 showed a superior utilisation of glucose between 0 and 24 h, and thereafter a small increase in glucose level was noted which was not observed with the other strains. *Lb. fermentum* MINF99 showed the slowest utilisation of glucose. For all starter fermented *bushera* other than *W. confusa* MINF8, glucose was reduced from approximately 2000 mg kg^{-1} to less than 200 mg kg^{-1} (Figure 4a). In comparison, the glucose levels of spontaneously fermented *bushera* increased markedly during the first 48 h (from 6136 to 29349 mg kg^{-1}), but then decreased to undetectable level after 96 h (Figure 4d). There was no significant difference (P>0.05) in utilisation of glucose after 48 h among the starters.

During spontaneous fermentation, maltose content increased during the first 48 h from 12181 to 50233 mg kg^{-1}, and then decreased rapidly during the following 48 h to 2826 mg kg^{-1} (Figure 4d). However, large variations in utilisation of sugars were observed between the different starters. A rapid decrease in maltose was observed

immediately in *bushera* fermented with *W. confusa* MINF8 whereas rapid utilisation by *Lb. plantarum* MINF227, *Lb. fermentum* MINF99 and *Lb. brevis* MINF226 was only observed after 8 h. In all *bushera* fermented with starters (except with *W. confusa* MINF8) maltose decreased from about 2100 mg kg^{-1} to less 500 mg kg^{-1} during fermentation (Figure 4b). *Lb. paracasei* subsp. *paracasei* MINF98 showed the slowest ability to utilise maltose. In comparison to the other strains used, *W. confusa* MINF8 showed a superior ability to utilise maltose during the first 24 h and then a marked increase in maltose content from 700 to 1400 mg kg^{-1} was observed between 24 and 96 h. There was no significant difference (P>0.05) in maltose levels after 72 h among the starters.

Fructose content was initially lower than the other carbohydrates and also decreased during the fermentation (Figure 4c). *W. confusa* MINF8 and *Lb. plantarum* MINF227 showed the most efficient utilisation of fructose. *Lb. fermentum* MINF99 showed the slowest utilisation of fructose. Fructose was reduced from approximately 550 mg kg^{-1} to between 0 and 200 mg kg^{-1} during fermentation with the different starters. The initial level of fructose was higher in spontaneously fermented *bushera*. Fructose levels decreased from 1700 to 500 mg kg^{-1} during the fermentation period. There was a significant difference (P<0.05) in utilisation of fructose among starters.

After 96h, low levels (19 - 57 mg kg^{-1}) of ethanol were produced by *Lb. paracasei* subsp. *paracasei* MINF98, *Lb. plantarum* MINF227 and *Lb. brevis* MINF226, whereas by 24h, *W. confusa* MINF8 and *Lb. fermentum* MINF99 had produced 1406 and 1007 mg kg^{-1} respectively (Figure 5 and Table 1). Naturally fermented *bushera* showed a sharp rise in ethanol after 48h, reaching a maximum of 10370 mg kg^{-1} after 96 h.

Discussion
The germinated sorghum flour used to initiate the spontaneous fermentation contained lactic acid bacteria, coliforms and yeasts. As fermentation progressed, the numbers of LAB increased and reached maximum after 12 h. Yeast counts increased steadily throughout the fermentation. The number of coliforms decreased after 12 h and were < 2 log cfu ml^{-1} after 48 h. Similar results have been reported for other cereal-fermented foods (Sulma et al., 1991; Nout, 1991). The disappearance of coliforms may be attributed to the acid production by the dominating lactic acid bacteria. Steinkraus (1996) indicated that most coliforms are acid intolerant and are inhibited as low pH is achieved. The results of changes in pH and lactic acid concentration were similar to findings by other researchers who have examined other fermented foods (Sulma et al., 1991; Choi et al., 1994; Dziedzoave et al., 1996). During the first 4 h of spontaneous fermentation, no changes were observed in pH and lactic acid content. However, after 4 h production of
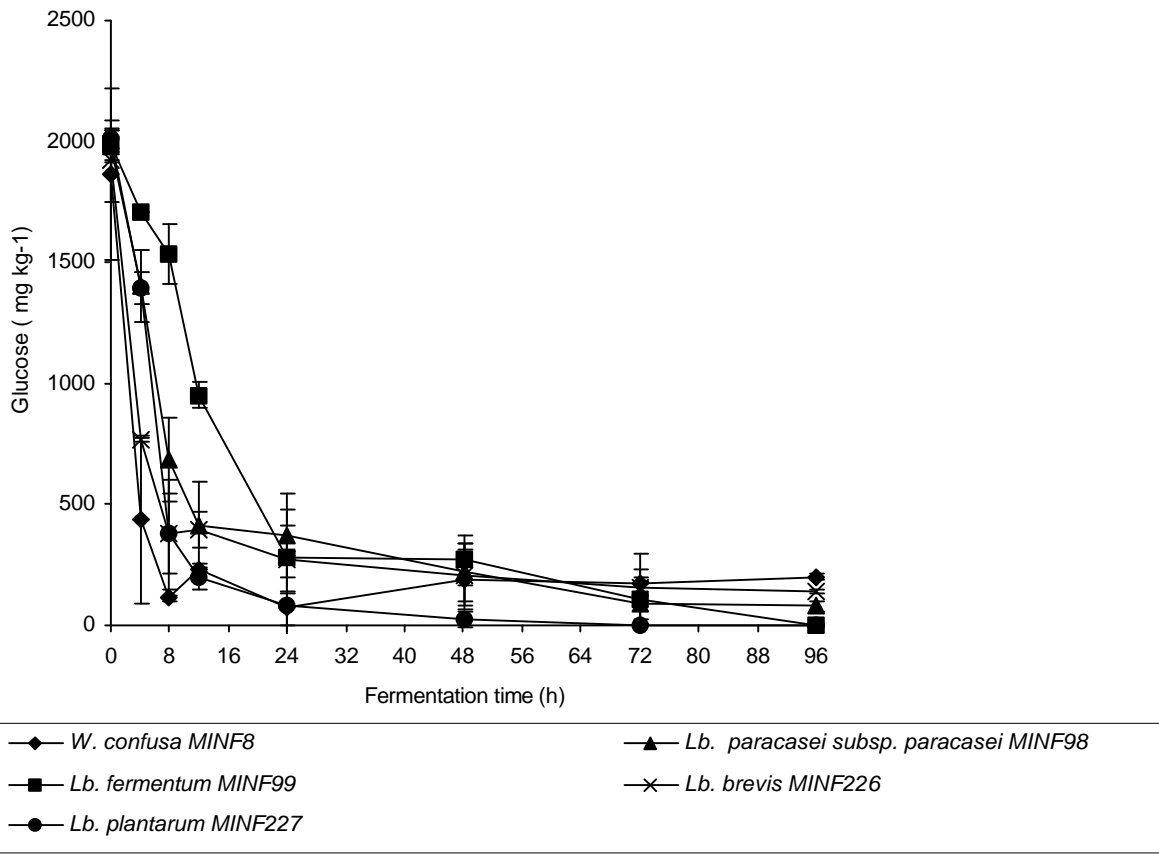
Fig. 4a. Changes in glucose content in bushera made from germinated sorghum flour
during fermentation with starters. Results given as averages and standard deviation indicated by bars.
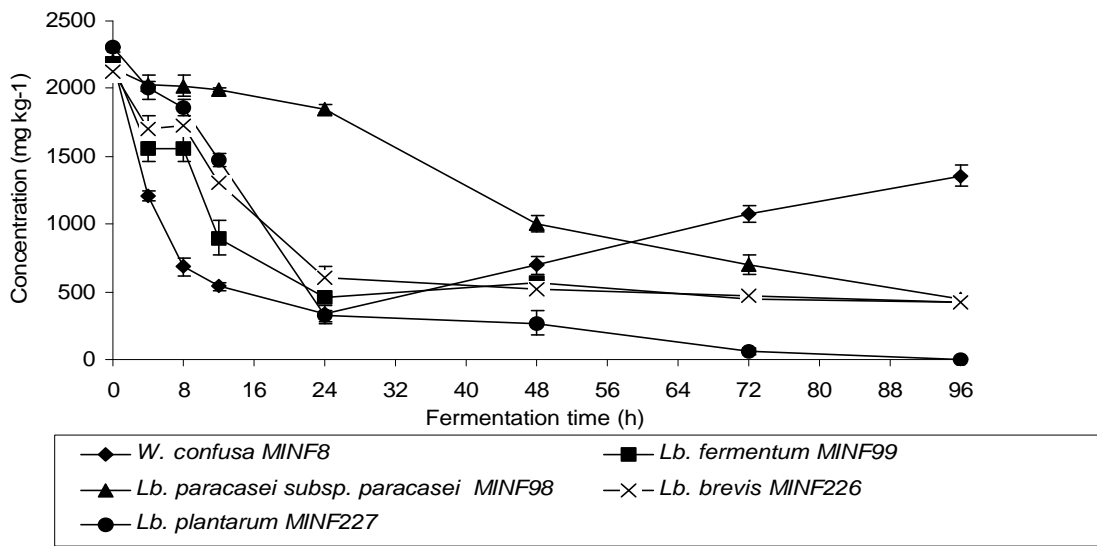

Fig. 4b. Changes in maltose content of bushera made from germinated sorghum flour
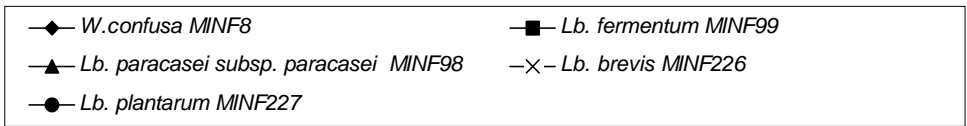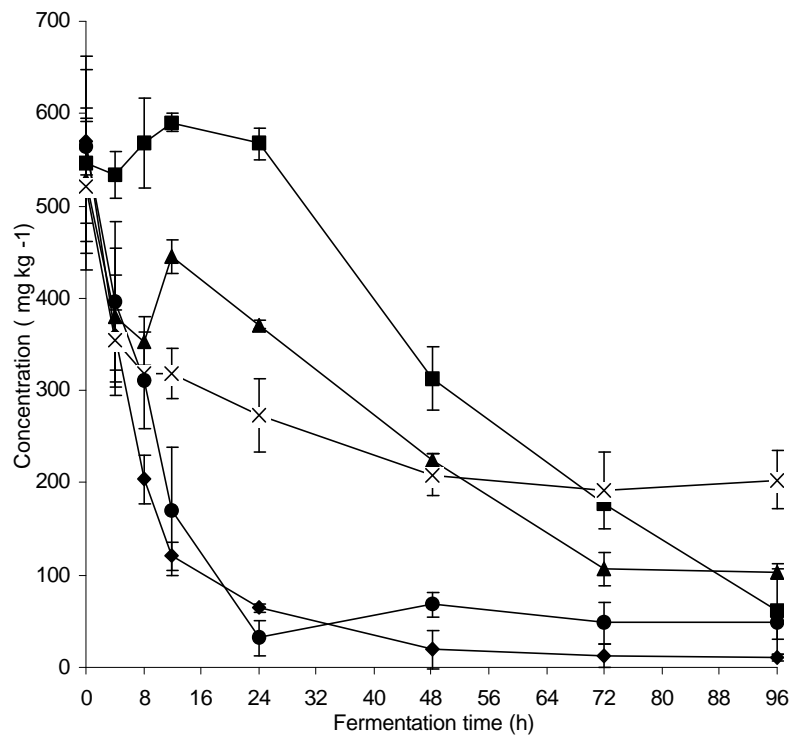during fermentation with starters. Results given as averages and standard deviation indicated by bars.

Fig. 4c. Changes in fructose content in bushera made from germinated sorghum flour during fermentation with starters. Results given as averages and standard deviation indicated by bars.
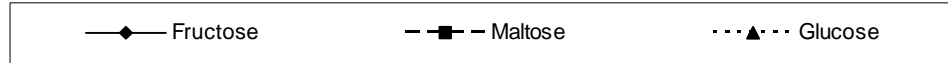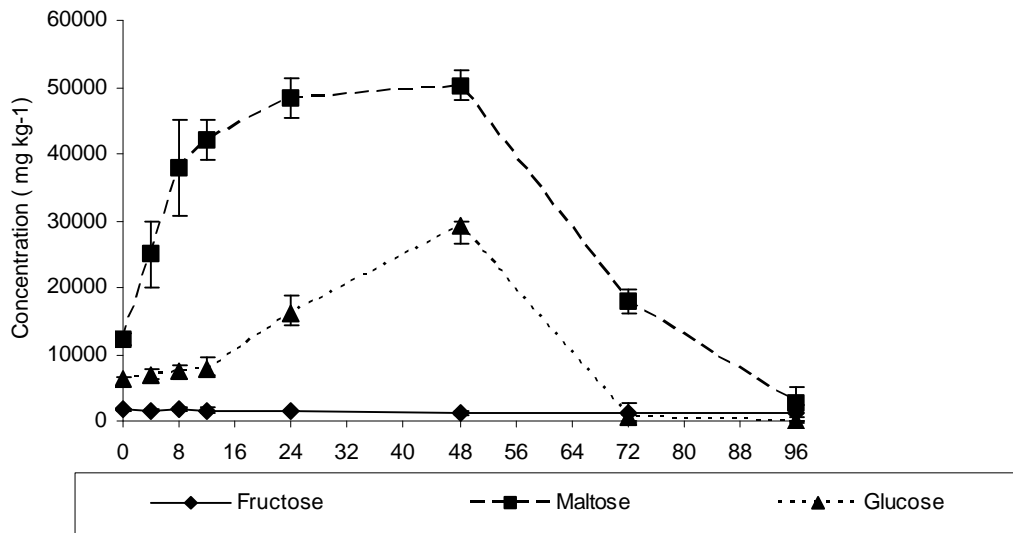

Fig. 4d. Changes in sugar content in bushera made from germinated sorghum flour during spontaneous fermentation. Results given as averages and standard deviation indicated by bars.
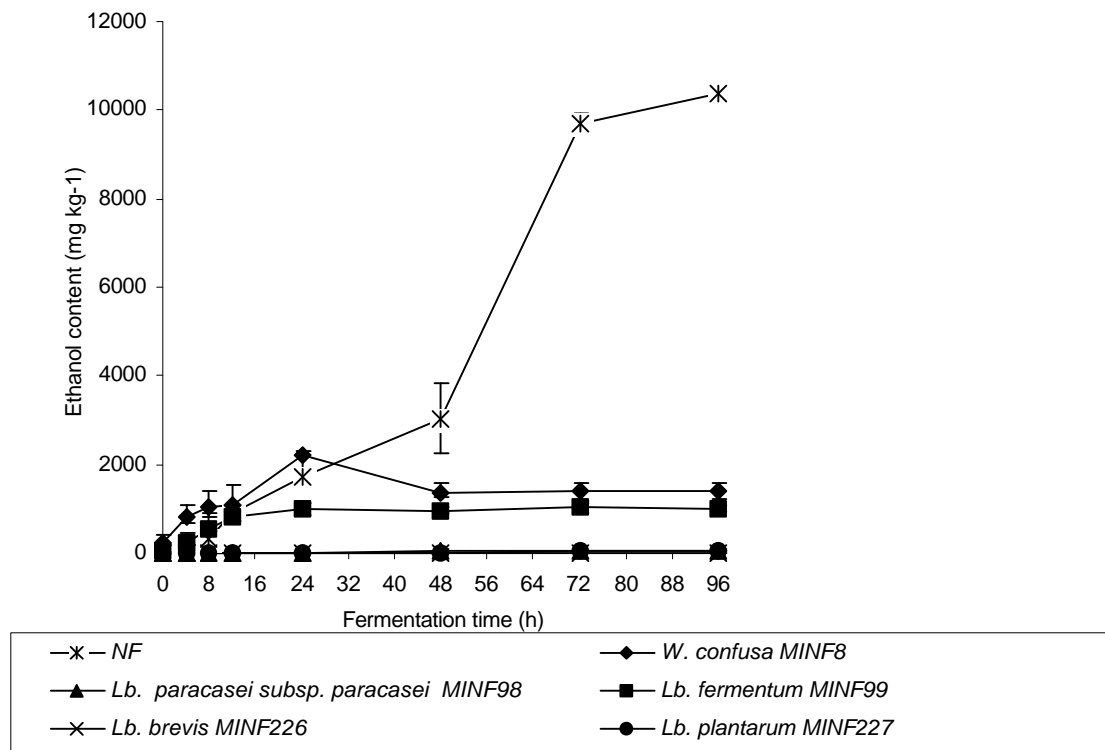
Fig. 5. Ethanol content in bushera made from germinated sorghum flour during spontaneous fermentation and with starters. Results given as average and standard deviation indicated by bars.

lactic acid started and pH was reduced. Nche et al. (1994) reported an increase in acidity and a decrease in pH in *kenkey* to be associated with an increase in LAB numbers.

The slower pH decrease and lactic acid production occurring in spontaneously fermented *bushera* compared with *bushera* with starters may be due lower initial numbers of LAB. In addition the LAB cells may have been stressed due to dry storage (i.e in the flour) and need repair before active acid production and cell division can commence. The large increase in total numbers on M17 agar after 4 h may be due to repair of cells or possibly growth of other bacteria.

Gobbetti (1998) reported that LAB multiply and produce lactic acid more slowly in mixtures with yeasts than in pure cultures. However, Khetarpaul and Chauhan (1990) reported a more significant decrease in pH and increase in acidity in millet fermented by a combination of lactobacilli and yeasts rather than with the use of single strain starter cultures. A similar phenomenon was observed in this study in the late stages (48-96 h) of spontaneous fermentation, where higher amounts of lactic acid were produced with increasing yeast numbers as compared with starter addition alone. It has also been reported that yeasts, for example *Saccharomyces cerevisiae*, have the ability to metabolise lactic acid in cultured milk (Gadaga, 2000). Reduction in lactic acid was not observed in this study.

Bushera fermented with *W. confusa* MINF8 attained the lowest pH (3.5), which was similar to that of spontaneously fermented *bushera*. Higher pH values were observed in *bushera* fermented with the other starters and this may be due to limiting amounts of fermentable sugars and/or their comparative acid intolerance. Among the lactobacilli, *Lb. plantarum* MINF227 showed the lowest pH (3.7). *Lb. plantarum* strains have been reported to be acid tolerant (Akinrele, 1970; Fleming and McFeters, 1981; Mbugua, 1984; Hounhouigan et al., 1993; Knorr, 1998) and exhibited the highest acid producing ability during the fermentation of starch suspension (Sanni *et al.*, 1994). This was confirmed in our experiments.

During spontaneous fermentation and fermentatiom with *W. confusa* MINF8 starch is probably continuously degraded, and an increase in maltose and glucose was observed in the later stages when active growth decreased. Nout (1980) indicated that microbial amylases play an essential role in the production of fermentable sugars from maize immersed in water. The pronounced starch degradation observed in the spontaneously fermented *bushera* is mainly caused by the indigenous sorghum amylases present in added germinated sorghum inoculum and possibly in addition, microbial amylases. Mosha and Svanberg (1993)

attributed the initial increase in reducing sugars to hydrolysis of starch and oligosaccharides present in unfermented substrates by amylases from germinated flour. The decrease of these reducing sugars with prolonged fermentation was attributed to utilisation by the fermenting microflora (Daeschel *et al.*, 1987; Khetarpaul and Chauhan, 1990). Further release of fermentable sugars by α and β- amylases and α-galactosidases may be reduced at low pH (Mbugua et al., 1983; Narendranath *et al.*, 1997). At this point the utilisation of the sugars becomes increasingly evident. The increased utilisation of maltose and glucose after 48 h coincided with the higher numbers of yeasts and a marked increase in ethanol production. The sugars are utilised by lactic acid bacteria with concomitant increase in lactic acid, ethanol, carbon dioxide and other metabolites (Mensah, 1997) and lactobacilli are mainly responsible for the acid production in fermented cereals (Akinrele, 1970; Ngaba and Lee, 1979; Hansen and Hansen, 1996). The production of high amounts of acid by *Lb. plantarum* strains has been attributed to their efficient use of carbohydrates (Oyewole and Odunfa, 1990) including the dextrins of the cereals after the depletion of the fermentable carbohydrates (Akinrele, 1970; Banigo and Muller, 1972; Sanni et al., 1994). In our experiments, *W. confusa* MINF8 seemed to utilise glucose, maltose and fructose simultaneously, while *Lb. plantarum* MINF227, *Lb. brevis* MINF226 and *Lb. paracasei* subsp. *paracasei* MINF98 preferentially utilised glucose before maltose. *Lb. plantarum* MINF227 and *W. confusa* MINF8 utilised fructose most efficiently. The higher pH of *bushera* fermented by *Lb. fermentum* MINF99 compared to other strains may possibly be explained by its inferior ability to metabolise glucose and fructose and it may also be the most acid sensitive strain used in this experiment.

The final pH of the *bushera* fermented by starter cultures was between pH 3.5 and 4.1. This pH range has been shown to be inhibitory to food poisoning bacteria (Chavan and Kadam, 1989; Mensah, 1997), *Enterobacteriaceae* and other gram-negative bacteria (Mbugua, 1985). Our results suggest that the use of a heating step and addition of starters to *bushera* would efficiently eliminate the coliforms or possibly other pathogens present in the raw materials and thence make a safer product.

Although the development of acidity is the primary and most vital characteristics of *bushera*, excessive sourness renders it unsuitable as a weaning food. Lactic acid has been reported to be a flavour enhancer (Banigo and Muller, 1972; Gobbetti and Corsetti, 1997). However, due to the sharp taste of lactic acid, it would be of interest to establish the levels at which it imparts desirable taste to fermented *bushera*. Alternatively, the excessive sourness can be overcome by choosing starter strains, which are inhibited at suitable pH, or by adding sweetening agents.

Traditionally, spontaneously fermented *bushera* is not fed to children if it has been fermented for more than 2 days.

However, during that period, the coliform level is at its highest, and this may jeopardise the safety of the product. Diarrhoea has been reported in children fed on 1 or 2 days spontaneously fermented *bushera* (Muyanja et al., 2003). Diarrhoeal diseases have been attributed to agents like *Escherichia coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio cholerae* that often result from contamination of foods (Holzapfel, 1997; Oyewole, 1997). In Latin American and Caribbean countries, diarrhoea was reported to have caused 5 million deaths of children under 5 years of age during 1960-1990 (Quevedo, 1997). At household level, this problem could be partly alleviated by heat treatment of young *bushera* after fermentation.

The selected strains used were as effective as spontaneous fermentation in the acidification of *bushera* to a pH which is sufficient to eliminate the coliforms. The faster pH drop by starter culture fermentation may suggest the possibility of challenging the spontaneous fermentation with the aim of eliminating coliforms in the early stages of fermentation. It has also been demonstrated that each strain used may affect the quality of *bushera* differently with respect to the levels of organic acids and volatile organic compounds produced. The higher pH, low lactic acid content, and inferior ability to utilise the sugars (glucose and fructose) suggested, however, that *Lb. fermentum* MINF99 would not be a good choice for a starter. The high levels of diacetyl produced at 8 h by *Lb. paracasei* subsp. *paracasei* MINF98 may make it a less suitable candidate for starter culture development. The faster pH drop and high lactic acid production by *W. confusa* MINF8, *Lb. plantarum* MINF227 and *Lb. brevis* MINF226 coupled with low ethanol production makes these strains more promising for starter culture development. *W. confusa* MINF8 used in this study may be a particularly interesting isolate and may be the best choice due to its ability to hydrolyse starch and its ability for rapid acid production. The amount of easily fermentable sugars (glucose and maltose) available in the fermenting mixture is crucial for the outcome of the fermentation. Nutritionally, the easily available sugars are desirable in *bushera* when used as a weaning food.

Conclusion

This study has demonstrated that selected strains can be used as single strain starter cultures to produce *bushera* with a pH similar to that of spontaneously fermented *bushera*. However, spontaneously fermented *bushera* differed markedly from starter *bushera* late in the fermentation period. For a small-scale production, *bushera* can be hygienically produced with consistent quality if such cultures are used, together with pre-fermentation heat-treatment. The different effects of LAB starter cultures on the product and may act as a guideline for the choice of potential starter. However, the starch metabolising starter *W. confusa* MINF8 shows particular promise. There is a need for sensory and nutritional evaluation of the *bushera* fermented by each strain.

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