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Full Length Research Paper

Use of starter cultures of lactic acid bacteria and yeasts as inoculum enrichment for the production of *gowé*, a sour beverage from Benin

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***Lactobacillus fermentum*, *Weissella confusa, Kluyveromyces marxianus* and *Pichia anomala*, previously isolated during natural fermentation of traditional *gowé*, were tested as inoculum enrichment for controlled fermentation of *gowé*. The final product was subjected to chemical analysis and sensory evaluation. Growth of the lactic acid bacteria (LAB) and yeasts were verified by determination of colony forming units (CFU) and molecular biology techniques. A significant decrease in pH from 6.1 to 3.3, with a concomitant increase in titratable acidity (11 to 60 g/kg as lactic acid, dry weight), was observed after 24 h of fermentation when LAB was used either alone or in combination with yeasts. The LAB count increased significantly from 6.1 to 9.4 log CFU/ml, while the yeast count remained constant throughout fermentation. Repetitive-polymerase chain reaction (rep-PCR) assays performed on isolates during the fermentation confirmed the dominance of the added LAB strains. Sensory evaluation revealed that the product fermented for 7 h with *L. fermentum* alone or in combination with *K. marxianus* was as acceptable as the traditional product normally obtained after a minimum of 24 h of fermentation. Consequently, *gowé* can be obtained by controlled fermentation, using *L. fermentum* as inoculum enrichment, in a small scale industry.**

Key words: Sorghum, *Gowé*, fermentation, starter cultures, lactic acid bacteria, yeasts, inoculum enrichment

INTRODUCTION

Gowé is a fermented sorghum-based food, which is widely consumed in urban areas of Benin (Michodjèhoun-Mestres et al. 2005). *Gowé*, an indigenous sour beverage, is made from a blend of malted and non-malted sorghum flour that is produced by spontaneous fermentation involving mixed cultures of lactic acid bacteria (LAB) and yeasts. The fermentation process takes place in an environment with a moisture content varying between 52 and 87%. The sweet and sour dough obtained by decantation during fermentation needs to be cooked and further diluted in water to obtain the beverage (Vieira-Dalodé et al., 2007).

A recent study showed that the dominant microorganisms of g*owé* fermentation were the lactic a cid bacteria *Lactobacillus fermentum, Weissella confusa, Lactobacillus mucosae* and *Pediococcus acidilactici*, and the yeasts *Kluyveromyces marxianus* and *Pichia anomala* (Vieira-Dalodé et al., 2007). Several investigations have been carried out in order to understand the role of LAB and yeasts in traditional African fermented foods. Different species of LAB and yeasts have been used successfully as starter cultures to ferment traditional products from cereals (Lorri and Svanberg, 1993; Halm et al., 1996; Hounhouigan et al., 1999; Annan et al., 2003a,b; Mugula et al., 2003; Zorba et al., 2003), milk (Gran et al., 2003), as well as legumes and seeds (Ouoba et al., 2004). The significance of LAB and yeasts for aroma formation in fermented cereals has also been demonstrated (Halm et al., 1993; Halm et al., 1996; Annan et al., 2003a, b).

The objective of this study was to assess selected LAB and yeasts for their use either individually or in combination as inoculum enrichment of a non-sterilized substrate for controlled fermentation during *gowé* production. Rep-PCR was also used to verify the dominance of the LAB

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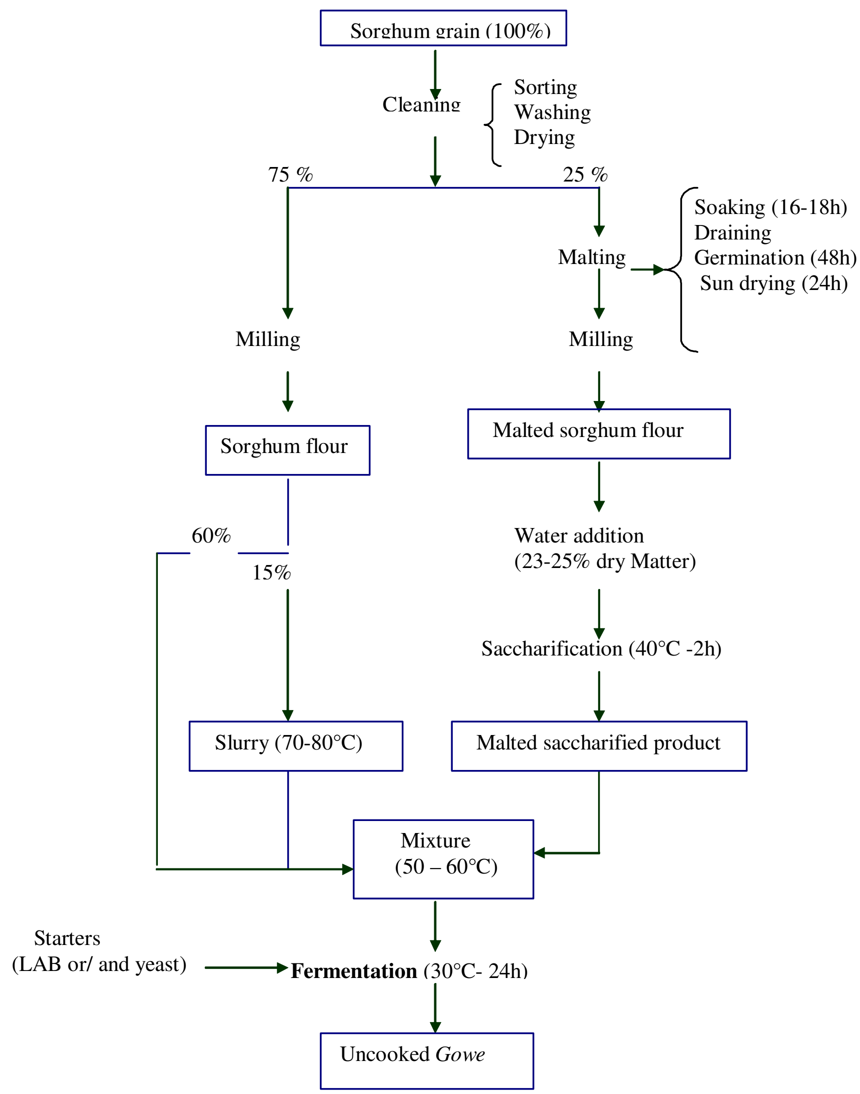

Figure 1. Procedure used for the production of gowé in the laboratory using starter cultures

and yeast strains used. The quality of the final product was evaluated by chemical analysis and sensory evaluation.

MATERIALS AND METHODS

Gowé production

Gowé was produced in the laboratory with a red variety of sorghum (*Sorghum bicolor* (L) Moench) purchased at the local market in Cotonou, Benin. A slight modification of the traditional process, as outlined in Figure 1, was used. The modification entailed mashing of the sorghum malt slurry at 40℃ for 2 h in order to facilitate breakdown of starch into fermentable sugars. This saccharification was carried out before the mash was mixed with non-malted sorghum flour. Samples from the same batch of malted and non-malted sorghum flours were used for all experiments. The flours were kept at -20℃ and thawed overnight at 4℃ before they were used.

Cultures and preparation of inocula

The LAB and yeast cultures used are shown in Table 1. The LAB cultures *L. fermentum* L025 and *W. confusa* L015, and the yeast cultures *K. marxianus* Y815 and *P. anomala* Y1238, previously obtained from naturally fermented *gowé* (Vieira-Dalodé et al., 2007), were respectively kept in MRS and MYGP broth containing 30% (v/v) glycerol at -80℃. The LAB were grown by streaking onto MRS agar (Oxoid) and incubated at 30℃ for 48 h. Subsequently, one colony was picked, transferred to a tube containing 10 ml of MRS broth (Oxoid) and incubated at 30℃ for 24 h. From this culture, 0.1 ml was added to 10 ml MRS broth, incubated at 30℃ for 16 h and centrifuged at 3000 x *g* for 10 min. The pellet was washed in 10 ml of sterile peptone water (5 g peptone, 8.5 g NaCl and 1000 ml of distilled water, pH 7.2), centrifuged and resuspended in peptone water to give a concentration of about 10^9 cells/ml, as verified by microscopy using a Thomas counting chamber. The yeasts were grown by streaking onto MYGP (Oxoid)

Table 1. Starter cultures used as inoculum enrichment for the fermentation of *gowé*

Experiment number	Type of starter used*
1	*Lactobacillus fermentum* L025
2	*Weissella confusa* L015
3	Non inoculated sample (Natural fermentation)
4	*Kluyveromyces marxianus* Y815
5	*Pichia anomala* Y1238
6	*Weissella confusa* L015 + *Kluyveromyces marxianus Y815*
7	*Weissella confuse* L015 + *Pichia anomala* Y1238
8	*Lactobacillus fermentum L025* + *Kluyveromyces marxianus Y815*
9	*Lactobacillus fermentum L025* + *Pichia anomala* Y1238

*All strains isolated by Vieira-Dalodé et al. (2007)

agar and incubated for 48 h at 25°C. Subsequently, one colony was transferred to a tube containing 10 ml of MYGP broth and incubated for 24 h at 25°C. The cultures were prepared, as described above for LAB, and resuspended in peptone water to give a concentration of about 10^7cells/ml.

Gowé fermentation

The LAB and yeast strains were inoculated individually or in combination, as shown in Table 1. A 200 ml sample of the mixture of malted and non-malted sorghum flour (Figure 1) were aseptically inoculated into a 250 ml flask to give a concentration of 10^7 cells/ml and 10^5 cells/ml for LAB and yeast, respectively. To avoid disturbing the fermentation during sampling, one flask was prepared for each sampling time. The inoculated samples were mixed by shaking the flask manually, which was then covered and incubated at 30°C for 24 h. An uninoculated sample was included as a control.

Sampling

Sampling was done at 0, 4, 8, 12 and 24 h of fermentation. At each time point, 10 g of sample was aseptically taken from the flask for microbiological analysis, while 50 g was kept at 4°C for pH, titratable acidity and dry matter measurements within 6 h after sampling. The remaining sample was frozen for further analysis.

Determination of dry matter, total sugars, pH and titratable acidity

Water content was determined as described (AACC, 44-15 A, 1984), while the Luff-Schoorl method (Lees, 1968) was used for the measurement of total sugar content. The pH was determined using a digital pH meter (Hanna Instruments) calibrated with buffers at pH 4.0 and 7.0. The titratable acidity, expressed as lactic acid, was performed by titrating 10 g of sample using 4 g/l NaOH and phenolphtalein (Merck) as indicator.

Microbiological analysis

Enumeration and isolation of the added microbial strains

A 10 g sample was homogenized in 90 ml of sterile peptone water with a stomacher (Lab Blender 400) for 1 min at normal speed. One ml from each of the ten-fold dilutions of the homogenized samples was inoculated onto MRS agar for enumeration of LAB. To enumerate yeasts, MYGP supplemented with 100 mg of chloramphenicol (Oxoid) and 50 mg of chlortetracycline (Sigma) per 1000 ml, was used. The MRS agar plates were incubated for 3 - 5 days at 30°C after overlaying with more agar, while the MYGP agar plates were incubated at 25°C for 3 days. For each sample, five different colonies of LAB or yeasts were picked at random for each sampling time and purified by successive streaking onto the same agar medium. A total of 300 LAB strains and 300 yeast strains were isolated and respectively kept on agar slants of MRS and MYGP for molecular typing (rep-PCR).

Repetitive-PCR (rep-PCR)

Rep-PCR was performed on isolates used for inoculum enrichment, as well as the predominant LAB and yeasts isolated from the fermentation trials, including the control. Microbial DNA was extracted using InstaGene™ (BioRad) according to the instructions of the manufacturer. The rep-PCR was performed using primer GTG5 (5’-GTG GTG GTG GTG GTG-3’) according to the method described by Nielsen et al. (2007). Amplified fragments were separated by electrophoresis on a 1.5% agarose gel in 1 x TBE (10 x TBE: 108 g Tris base/l, 55 g boric acid/l and 40 ml of 0.5M EDTA, pH 8.0). The gel was electrophoresed at 120 V for 4 h, stained with ethidium bromide and photographed using a digital camera. The PCR band patterns of the LAB or yeasts used were visually compared to those isolated from the fermenting samples.

Sensory evaluation of *Gowé*

The sensory properties of *gowé* obtained by controlled fermentation was compared to those of the control sample by a taste panel familiar with *gowé*. For the control sample, the 24 h product was used, whilst for those produced by controlled fermentation, samples with a pH between 4.0 and 3.9 and titratable acidity between 30.2 and 40.2 g/kg lactic acid (d.w.) were used. These were inoculated samples that had only been fermented for 7 h. The 30 panelists rated appearance, taste (sourness and sweetness) and aroma on a hedonic scale ranging from one to five (1 represented “dislike extremely” and 5 “like extremely”). The overall acceptability was also assessed.

Statistical analysis

The fermentation trials were carried out in duplicate. Sensory evaluation was also performed in duplicate. For data analysis, Multivariate Analysis of Variance (MANOVA) was performed using Statistica 7.1 (StatSoft, France). The effect of the different cultures used was compared to the natural product using Dunnett’s test (Gouet and Philipeau, 1992).

Table 2. Changes in pH during *gowé* production using starter cultures as inoculum enrichment

Fermentation	pH								
Time (h)	L	W	C	K	P	WK	WP	LK	LP
0	6.16 (0.11)	6.16 (0.11)	6.30 (0.40)	6.29 (0.41)	6.16 (0.26)	6.10 (0.09)	6.08 (0.11)	6.13 (0.15)	6.18 (0.04)
4	4.64 (0.57)	4.54 (0.43)	6.28 (0.23)	6.14 (0.35)	6.26 (0.34)	4.54 (0.74)	4.35 (0.52)	4.13 (0.34)	4.38 (0.08)
8	3.63 (0.10)	3.70 (0.25)	5.56 (0.58)	5.62 (0.27)	5.48 (0.08)	3.62 (0.01)	3.57 (0.03)	3.53 (0.14)	3.69 (0.13)
12	3.50 (0.06)	3.35 (0.05)	4.14 (0.26)	4.28 (0.12)	4.05 (0.02)	3.35 (0.01)	3.27 (0.01)	3.36 (0.01)	3.47 (0.01)
24	3.24 (0.03)	3.25 (0.13)	3.22 (0.40)	3.29 (0.35)	3.26 (0.16)	3.24 (0.04)	3.19 (0.03)	3.20 (0.14)	3.31 (0.03)
LSD starter effect	1.47								
LSD time effect	5.06								
LSD starter time effect	0.64								
Standard deviation of the residual	3.20								

L : *Lactobacillus fermentum ;* W *: Weissella confusa ;* C: control; K *: Kluyveromyces marxianus ;* P *: Pichia anomala*; WK , WP: Starters composed of *W. confusa* and *K. marxianus* or *P. anomala.* LK LP: starters composed of *L. fermentum* and *K. marxianus* or *P. anomala*. ( ): standard deviation.
LSD: Least significant difference at 0.05 level

RESULTS

Changes in pH, titratable acidity and total sugars

The pH varied significantly ($p<0.05$) during the fermentation according to the enrichment cultures used (Table 2). The use of *Lactobacillus fermentum* and *Weissella confusa* accelerated *gowé* fermentation. When used individually or in combination with *K. marxianus* or *P. anomala*, a significant decrease ($p<0.05$) in the pH was observed after 4 h of fermentation. After 4 h of fermentation, the lowest pH value (4.13) was observed when *L. fermentum* was used in combination with *K. marxianus*, and was similar to the pH value (4.15) observed after 12 h of spontaneous fermentation (Vieira-Dalodé et al., 2007). After 8 h of fermentation, when the two LAB were used individually or in combination with the yeasts, the pH decreased to values in the range of 3.70-3.53. The titratable acidity increased significantly after 4 h of fermentation when *L. fermentum* and *W. confusa* were used as enrichment cultures individually or in combination with the yeasts (Table 3). The highest titratable acidity value (29.4 g/kg d.w.) at that time was observed when *L. fermentum* was used in combination with *K. marxianus*, which was higher than the titratable acidity value (25.1 g/kg d.w.) obtained after 12 h of the secondary fermentation in the traditional process (Vieira-Dalodé et al., 2007). *W. confusa* used in combination with *K. marxianus* gave a titratable acidity value (25.2 g/kg d.w.) that was similar to the value observed after 12 h of the fermentation in the traditional process. For both the pH and titratable acidity, a significant correlation was found between the enrichment inoculum used and the fermentation time.

The total sugar content was significantly affected by the enrichment inoculum used (Table 4). The sugar content before inoculation (15.28 g/100g) was similar to the value obtained after 12 h of the primary fermentation in the traditional process (15.54 g/100g) (Vieira-Dalodé et al., 2007). Total sugar content increased significantly with the fermentation time, despite an increase in the microbial counts.

Lactic acid bacteria and yeast counts

The LAB count increased significantly ($p<0.05$) after 4 h of fermentation and during the fermentation process when the LAB were used individually or in combination with the yeasts (Figure 2). After 24 h of fermentation the counts in the inoculated sample (9.23 - 9.62 log CFU/ml) were not higher than the counts observed in the traditional process (9.67 log CFU/ml). When *L. fermentum* and *W. confusa* were used individually, the mean count of the yeast (2.08 - 2.03 log CFU/ml) was signifycantly lower (Figure 3). After the accelerated saccharification preceding the use of the cultures, the yeast count was reduced to a non-detectable level.

Predominance of added cultures

DNA amplification by rep-PCR of the isolates randomly obtained from the inoculated samples yielded patterns that were compared visually to profiles of the inoculum cultures used (Figure 4). Profiles of 90 DNA patterns of strains isolated from samples inoculated with *L. fer-*

Table 3. Changes in titratable acidity (as g/kg lactic acid, dry weight) during *gowé* production using starter cultures as inoculum enrichment.

Fermentation	**Titratable acidity (as g/kg lactic acid, dry weight)**								
Time (h)	**L**	**W**	**C**	**K**	**P**	**WK**	**WP**	**LK**	**LP**
0	10.6	10.5	11.1	11.1	11.5	10.2	10.6	10.9	10.2
	(0.2)	0.2)	(0.1)	(0.6)	(0.5)	(0.1)	(0.1)	(0.3)	(0.1)
4	21.6	23.2	11.1	12.1	09.3	25.2	26.4	29.4	24.1
	(0,7)	(0,7)	(0,5)	(0,6)	(0,3)	(0,9)	(0,3)	(0.7)	(0.4)
8	44.5	41.5	16.5	17.5	16.9	42.6	46.4	49.2	41.8
	(0.7)	(0.8)	(0.8)	(0.6)	(0.5)	(0.1)	(0.4)	(0.4)	(0.3)
12	50.4	57.6	30.2	32.3	33.3	55.6	57.7	57.0	52.5
	(0.5)	(0.7)	(0.4)	(0.2)	(0.6)	(0.1)	(0.2)	(0.1)	0.1)
24	62..5	66.3	56.5	61.5	56.4	68.1	65.9	72.9	67.2
	(0.6)	(0.1)	(0.1)	(0.3)	(0.2)	(0.2)	(0.1)	(0.3)	(0.2)
LSD starter effect	2,56								
LSD time effect	8,83								
LSD starter time effect	1,08								
Standard deviation of the residual	9.19								

L : *Lactobacillus fermentum ;* W *: Weissella confusa ;* C: control ; K *: Kluyveromyces marxianus ;* P *: Pichia anomala*; *;* WK , WP: Starters composed of *W. confusa* and *K. marxianus* or *P. anomala.* LK, LP: starters composed of *L. fermentum* and *K. marxianus* or *P. anomala;* ( ): standard deviation

LSD: Least significant difference at 0.05 level.

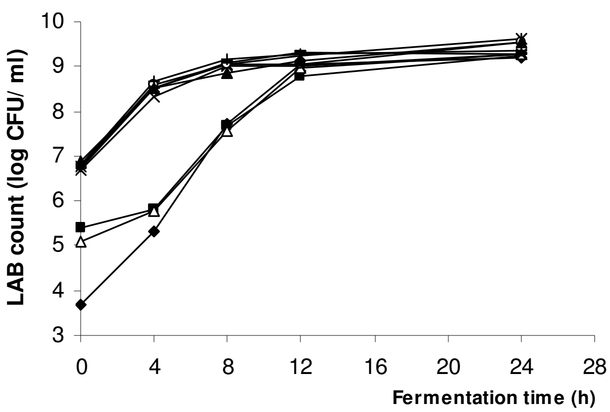

Figure 2. Laboratory count during gowé fermentation using starter cultures. *Lactobacillus fermentum* (x) *; Weissella confusa* (*) *;* control (♦) ; *Kluyveromyces marxianus* (▪) *; Pichia anomala* (Δ)*;* Starters composed of *W. confusa* and *K. marxianus* (▲); Starters composed of *W. confusa and P. anomala*(-) *;* starters composed of *L. fermentum* and *K. marxianus* (□); starters composed of *L. fermentum and P. anomala*(+) .

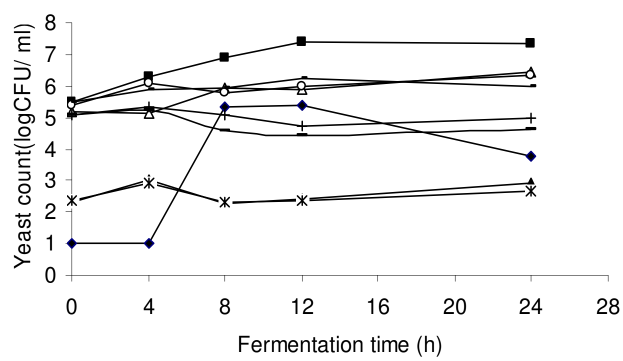

Figure 3. Yeast count during gowé fermentation using starter cultures. *Lactobacillus fermentum* (▲) *; Weissella confusa* (*) *;* control (♦); *Kluyveromyces marxianus* (▪) *; Pichia anomala* (Δ)*;* starters composed of *W. confusa* and *K. marxianus* (-) ; starters composed of *W. confusa and P. anomala* (_) *;* starters composed of *L. fermentum* and *K. marxianus* (□); starters composed of *L. fermentum* and *P. anomala.* (+)

me*ntum* individually or in combination with a yeast were compared to the *L. fermentum* profile*.* The same analysis was performed when *W. confusa, K. marxianus* or *P. anomala* was used individually or in combination. The percentages of the isolate profiles similar to the inoculum are given in Table 5. Among the two LAB tested, *L. fermentum* showed a higher percentage of identity than *W. confusa.* This higher percentage is an indication of stronger dominance over the wild LAB compared to *W. confusa.* Among the yeasts, *K. marxianus* showed a higher percentage of identity than *P. anomala*. In general, the LAB performed better than the yeasts.

Sensory evaluation

The mean scores given for each attribute by the 30 panelists are presented in Table 6. Regarding the overall acceptability, all inoculated products were given a score of more than 3, showing that the panelists liked the inoculated samples. For all of the sensory characteristics

Table 4. Changes in total sugars (g/100g) content during *gowé* production using LAB and yeasts as inoculum enrichment

Fermentation Time (h)	Total sugar								
	L	W	C	K	P	WK	WP	LK	LP
0	16.04 (0.11)	16.8 (0.10)	15.28 (0.04)	14.8 (0.06)	14.05 (0.10)	16.13 (0.06)	14.99 (0.04)	16.72 (0.08)	15.17 (0.12)
4	20.35 (0.10)	17.49 (0.08)	18.53 (0.06)	16.95 (0.03)	19.87 (0.06)	18.59 (0.08)	21.33 (0.06)	15.06 (0.11)	17.56 (0.10)
8	19.33 (0.06)	19.61 (0.10)	16.75 (0.03)	18.65 (0.06)	19.49 (0.10)	19.79 (0.07)	17.64 (0.03)	21.29 (0.08)	22.18 (0.08)
12	21..2 (0.08)	22.61 (0.08)	23.68 (0.04)	21.13 (0.04)	25.75 (0.08)	20.01 (0.10)	18.76 (0.08)	20.25 (0.81)	22.12 (0.08)
24	28..23 (0.10)	24..3 (0.07)	31.74 (0.07)	34.03 (0.06)	29.78 (0.08)	25.39 (0.11)	25.76 (0.06)	26.98 (0.10)	29.41 (0.06)
LSD starter effect	9.53								
LSD time effect	21.33								
LSD starter*time effect	3.18								
Standard deviation of the residual	0.95								

L : *lactobacillus fermentum ;* W *: Weissella confusa ;* C: control ; K *: Kluyveromyces marxianus ;* P *: Pichia anomala; ;* WK , WP: Starters composed of *W. confusa* and *K. marxianus* or *P. anomala.* LK, LP: starters composed of *L. fermentum* and *K. marxianus* or *P. anomala.* ( ): standard deviation
LSD: Least significant difference at 0.05 level

Table 5. Percentage of isolates identical to the LAB and yeasts used as inoculum enrichment during controlled fermentation of gowé

Starter cultures	*Number of isolates checked by rep PCR	Number of identical isolates	Percentage of identity (%)
L. fermentum	90	77	85
W. confusa	90	66	75
K. marxianus	90	53	58
P. anomala	90	25	27

*Number of isolates (LAB or yeast) obtained from inoculated samples which profiles were compared to the starters used.

and overall acceptability, the samples inoculated only with *L. fermentum* or with *L. fermentum* in combination with *K. marxianus* were given a score similar to the product obtained by traditional fermentation.

DISCUSSION

An accelerated acidification was noticed in comparison with the spontaneous fermentation, especially when the LAB was used as starter cultures. A significant decrease in pH was observed with the fermentation time after inoculation of the different starters. For all combinations of LAB and yeasts, a lower pH value was obtained compared to the pH value (4.03) obtained after 12 h in the traditional process, as described by Vieira-Dalodé et al. (2007). Eight hours of fermentation with the LAB used individually or in combination with the yeast produced a decrease of the pH to values lower than observed after 12 h of the secondary fermentation in the traditional process. The decrease of pH was less when the yeasts were used individually. The use of *L. fermentum* to ferment some cereal-based foods like *mawè* (Hounhouigan et al., 1999), maize dough (Annan et al., 2003a, b) and *togwa* (Mugula et al*.,* 2003) decreased the pH to 3.5, 3.8 and 3.6, respectively, after 24 h of fermentation. For *gowé* fermented with *L. fermentum*, the pH value was 3.2 after 24 h of fermentation.

A significant increase in titratable acidity was observed during the fermentation when LAB were used individually or in combination with yeasts. Similar observations have been made by several authors (Hounhouigan et al., 1999; Mugula et al., 2003; Annan et al., 2003a, b). The values of 4.0 for pH and 25 g/kg for titratable acidity as lactic acid, obtained at the end of the process in the traditional fermentation (Vieira-Dalodé *et al*., 2007), were obtained before 8 h when the lactic acid bacteria were used in controlled fermentation. These results showed that using the LAB as inoculum enrichment drastically reduced the fermentation time during *gowé* production. The accelerated acidification observed constituted an asset that can contribute to the improvement of the final product quality, as observed in other studies (Holzapfel, 1997; Ross et

Table 6. Results of sensory evaluation performed on traditional gowé and *gowé* fermented for 7 h using different starter cultures, and scored by 30 panelists (1= dislike extremely, 5= like extremely)

Sensory characteristic	Traditional process	Saccharified (2h – 40℃) not inoculated	Saccharified (2 h – 40℃) + LAB		Saccharified (2 h – 40℃) + Combination LAB – Yeast			
	NF	C	L	W	WK	WP	LK	LP
Colour	4,11±0,12b	3,76±0,25a	4,20±0,03b	3,90±0,13b	3,81±0,07b	3,60±0,10a	4,35±0,07b	3,87±0,12b
Sour taste	4,14±0,30b	3,29±0,51a	4,04±0,08b	3,64±0,03a	3,67±0,34a	2,74±0,10a	4,05±0,13b	3,40±0,27a
Sweetness	4,17±0,26b	3,34±0,22a	3,90±0,15b	3,62±0,00a	3,38±0,27a	3,12±0,17a	4,13±0,03b	3,36±0,14a
Aroma	4,03±0,23b	3,39±0,41a	4,14±0,28b	3,60±0,24a	3,71±0,07b	3,10±0,13a	4,08±0,17b	3,47±0,12a
Overall acceptability	4,11±0,35b	3,23±0,41a	4,01±0,03b	3,52±0,13a	3,48±0,27a	3,00±0,00a	3,95±0,07b	3,34±0,06a

NF: Natural fermentation; C: Control; L: *Lactobacillus fermentum;* W*: Weissella confusa;* WK, WP: Starter composed of *W. confusa* and *K. marxianus* or *P. anomala.* LK, LP: Starter composed of *L. fermentum* and *K. marxianus* or *P. anomala;* The characteristics of the inoculated samples were compared to the naturally fermented product. Values with the same letters are not significantly different.

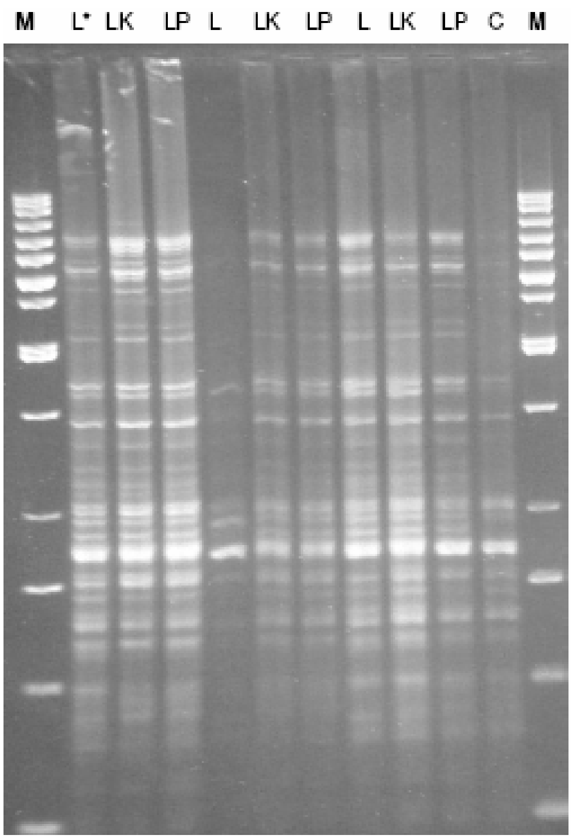

Figure 4. Rep-PCR of isolates from sample fermented with *L. fermentum*. M: Marker; *: Starters used; following rows are strains from samples inoculated with *L. fermentum* individually or in combination with *K. marxianus* or *P. anomala;* C: Control.

al., 2002). Growth of pathogens can also be avoided with the rapid acidification obtained (Nout et al., 1989; Michodjehoun-Mestres et al*.,* 2005).

Mashing the malt slurry at 40℃ for 2 h before fermentation produced a level of sugar content that could only be attained after 12 h of fermentation when mashing was was omitted, as in the traditional process.

Compared to the spontaneous fermentation a significant increase in the LAB count was observed when *L. fermentum* or *W. confusa* was used individually or in combination with yeasts. After 4 h of fermentation, the LAB count was about 8 log CFU/ml but in the traditional product it was about 6 log CFU/ml (Vieira-Dalodé et al., 2007). This increased number of LAB explains the significant decrease in pH and observed increase of the titratable acidity. The LAB count reached 9 log CFU/ml after 8 h. The same trend was observed when *L. fermentum* and other species of LAB were used to ferment *togwa (*Mugula et al., 2003). High viable counts of LAB are necessary to get the desired acid production, which affects the product shelf-life (Helland et al., 2004).

During the controlled fermentation, the yeast count did not increase significantly during the process. A significant variation was observed according to the starter used. The highest count was observed with *K. marxianus.* According to Nout (1991), the proliferation of yeasts in foods is favored by the acidic environment created by LAB, while the growth of bacteria is stimulated by the presence of yeasts that may provide growth factors such as vitamins and soluble nitrogen compounds.

The amylolytic activities of the sorghum malt used favored the production of higher amounts of fermentable sugars than what the microorganisms require for their metabolism (Michodjèhoun-Mestres et al. 2005). This may explain the significant increase of the total sugar content with the fermentation time, despite the increase in microbial counts.

Gowé can not be pasteurized before addition of the inocula as this would lead to gelatinization of the product. This condition explained the LAB and yeast counts obtained at 0h of the fermentation process. The Rep-PCR performed helped verification of the presence of the starters used. The percentage of similarity in profiles of the isolates obtained by Rep-PCR confirmed that *L. fermentum* and *W. confusa* was effective in fermenting the *gowé*. The colour, taste (sourness or sweetness) and aroma evaluated for the samples inoculated with the LAB used individually or in combination received a mean score of above 3. The *gowé* obtained by controlled fermentation was appreciated by the panelists. The product fermented with *L. fermentum* individually or combined with *K. marxianus* was as well appreciated as the *gowé* produced by natural fermentation.

Conclusion

An accelerated fermentation was obtained when *L. fermentum* was used individually or in combination with *K. marxianus* during the controlled fermentation of a mixed malted and non-malted sorghum flour for *gowé* production. *Gowé* obtained within 7 h of controlled fermentation was judged to be similar to the product obtained by spontaneous fermentation by sensory evaluation. This improved process required a 2 h saccharification of the malted flour at 40°C instead of 12 h of primary fermentation at 30°C. Such an improvement constitutes important progress in the traditional technology of *gowé* production.

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