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Original article

Dextrin, sugar and organic acid profiles of spontaneous and modified gowe: a malted and fermented sorghum beverage from Benin

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Summary Gowé is a traditional Beninese fermented red sorghum beverage made from a blend of malted and precooked nonmalted flour. The main objective of this work was to evaluate the degradation of starch during the production of gowé and to characterise the organic acid and sugar profiles of the product obtained using starter cultures. Inoculation was performed with strains of *Lactobacillus fermentum* or *Weissella confusa* and of *Kluyveromyces marxianus or Pichia anomala* used singly or in combination. Size exclusion chromatography revealed that starch was hydrolysed into high molecular weight dextrins (DP over 35), oligosaccharides and glucose, mainly due to the malting and precooking steps. Glucose was the main free sugar, and lactic acid was the main organic acid. The choice of the inoculation strain directly influenced the acidity but also indirectly the sugar content of the resulting gowé and will thus affect consumer acceptability.

Keywords Gowé, organic acid, sorghum, starch degradation, sugar.

Introduction

Essential dietary nutrients are provided by cereals in many developing countries. The bioavailability of these nutrients is limited by the antinutrients that are often present in the cereals. Fermentation is one of the oldest natural methods used to improve the nutritional quality of cereals. Indeed, fermenting cereals for a limited period of time increases protein, amino acid and starch availability and reduces antinutrients (Chavan & Kadam, 1989; Asiedu *et al*., 1993; Aka *et al*., 2008; Muyanja *et al*., 2010). Several metabolic activities take place during fermentation which lead to starch hydrolysis and to the production of organic acids (Nago *et al*., 1998; Michodjehoun-Mestres *et al*., 2005; Tou *et al*., 2007).

Gowé is a traditional fermented sorghum soft cooked paste made from a blend of malted (25%) and unmalted (75%) red sorghum flour. It is sold wrapped in vegetable leaves and has a short shelf life (3–4 days). It is consumed after dilution with water as a sweet, slightly acidic drink (Adinsi *et al*., 2014). The malt is first fermented for 12 h; this step is actually a saccharification step (starch hydrolysis) (Vieira-Dalode *et al*., 2007). The nonmalted flour is then partly cooked and mixed with the saccharified mash. The mixture undergoes the secondary fermentation for 24 h and then is cooked. An accelerated saccharification process (at 40 °C for 2 h) was recently proposed (Vieira-Dalode *et al*., 2008). The main microbiota involved in gowé fermentation are the lactic acid bacteria (LAB) *Lactobacillus* spp., *Weissella* spp. and *Pediococcus* spp., and the yeasts *Kluyveromyces* spp., *Pichia* spp. and *Candida* spp. (Vieira-Dalode *et al*., 2007). The malting and fermentation steps involved in the preparation of gowé cause several changes in the physical–chemical characteristics of gowé resulting in a specific taste, aroma and texture which determine its acceptance by consumers. Controlled fermentation may have an effect on different metabolic activities and consequently on the organic acid and sugar profiles of the product. Bako Seïdou *et al*. (2011) thus demonstrated that water content during the saccharification and fermentation steps has a great impact on the quality of gowé, particularly on the reducing sugar content. In addition, we hypothesised that the accelerated saccharification process may enhance the degradation of starch. It has been shown that accelerated saccharification and a shorter secondary fermentation period combined with

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inoculation with the main lactic acid bacteria and yeasts found in traditional *gowé* (*Lactobacillus fermentum* and *Weissella confusa* together with *Kluyveromyces marxianus* and *Pichia anomala)* produced a *gowé* that was as acceptable as the traditional one (Vieira-Dalode *et al*., 2008). The main objective of this work was to evaluate the degradation of starch during the production of gowé and to evaluate the effect of specific lactic acid bacteria and yeasts on the organic acid and sugar profiles of the product.

Materials and methods

Sample preparation

*S*orghum [*Sorghum bicolor* Moench] grains were from a local red variety largely used for preparing gowé. They were purchased at the local market in Cotonou. Malt was produced in the laboratory as previously described (Michodjehoun-Mestres *et al*., 2005). Gowé was produced using two different processes. The first one was the traditional procedure described by Vieira-Dalode *et al*. (2007); the malted flour was wetted (47% water content) and first fermented for 12 h (saccharification step). Raw and pasted (70–80°C) nonmalted sorghum flour were mixed with the saccharified dough, and hot (50–60 °C) water was added. The mixture (77% water content) was then fermented for 24 h (secondary fermentation step), at ambient temperature without inoculation. The second process was an experimental modified process (Vieira-Dalode *et al*., 2008). An accelerated saccharification was performed by leaving the malted mash at 40 °C for 2 h, followed by fermentation. The mash was naturally fermented (control) or enriched with inoculum obtained from naturally fermented gowé (Vieira-Dalode *et al*., 2007). Pure cultures of *L. fermentum* L025, *W. confusa* L015, *K. marxianus* Y815 and *P. anomala* Y1238 were grown in MRS and MYGP medium (for lactic acid bacteria and yeasts, respectively) then diluted in peptone water for inoculation (Vieira-Dalode *et al*., 2008). The inoculation level was of 10^7 and 10^5 cells mL^{-1} for lactic acid bacteria and yeasts, respectively.

Gowé was produced in the laboratory. Samples were taken at 0, 4, 8, 12 h of the saccharification step (primary fermentation) and 12 and 24 h after the beginning of secondary fermentation in the traditional process. Samples were taken 0, 12 and 24 h after the beginning of secondary fermentation in the modified process. Each sample (100 g) was freeze-dried and stored at −20 °C for further analysis.

Proximate analysis

The dry matter, crude fat, protein and ash contents were determined in duplicate using AOAC methods, 27.005, 27.006, 27.007, 27.009, respectively (Anon, 1984). Carbohydrates were determined by difference.

Size exclusion chromatography

Starch and dextrin profiles were determined by size exclusion chromatography on a Superdex Peptide 10/300GL (GE-Healthcare Bio-Sciences, Uppsala, Sweden) column. Standard solutions of normal and waxy maize starches, maltoheptaose, maltohexaose, maltopentaose, maltotetraose, maltotriose, maltose and glucose were used for calibration. The samples (50 mg of freeze-dried gowé or starches, 10 mg of glucose and oligosaccharides) were solubilised in 1 mL of 1 M NaOH, after dispersion in 200 μL under strong agitation. The mixture was gently shaken for 2 h before 9 mL of ultra-pure water was added. One millilitre was filtered through a 5-μm pore size filter and directly collected in a vial. Twenty microlitres were injected and eluted with 0.05 M NaOH at 0.6 mL min^{-1} using a LC-9A HPLC pump (SHIMADZU, Vitry sur Seine, France). Detection was by refractometry (RID-6A; Shimadzu). Solubilisation and gel permeation chromatography were performed in duplicate.

Determination of organic acids and free sugars

Organic acid and sugar contents were determined by HPLC using an Aminex HPX 87H column (Biorad, Hercules, CA, USA) as previously described (Michodjehoun-Mestres *et al*., 2005). Extraction was performed on 25 mg of freeze-dried gowé with 1 mL of 5 mM H_2SO_4 solution. The suspension was dispersed vigorously then gently shaken for 1 h and centrifuged at 10 000 $\times g$ for 5 min. The supernatant was filtered through 0.45-μm pore size filter and injected onto the HPLC system. The extraction and HPLC were performed in duplicate.

Statistical analyses

All analyses were carried out in duplicate on one single set of samples. ANOVA, mean comparison tests (using Newman–Keuls mean comparison test at probability level of 0.05) and principal component analysis (PCA) were performed with Statistica 7 (StatSoft, Tulsa, OK, USA).

Results

Changes in proximate composition during traditional preparation of gowé

Crude fat content decreased significantly from 3.8% to 2.8% during processing (Table 1), whereas protein content increased from 10.3% to 11.2% during the

Table 1 Proximate composition of traditional gowé during natural fermentation (% db)

	Saccharification (Primary fermentation)			Fermentation* (Secondary fermentation)		
	0 h	8 h	12 h	12 h	24 h	SDR†
Ash	1.78	1.80	1.80	1.83	1.81	0.09
Crude fat	3.8	3.24	3.14	2.82	2.77	0.12
Proteins	10.3	10.5	11.24	10.94	10.97	0.10
Carbohydrates	84.5	84.5	83.8	84.4	84.5	0.3

*Fermentation started (0 h) after the gruel was added to the saccharified mash.
†Standard deviation of the residual.

primary fermentation and remained statistically unchanged during the secondary fermentation. Carbohydrate content decreased significantly from 67.1 to 32.2 ($P < 0.05$) during the fermentation process. No significant variation ($P > 0.05$) in ash was observed during the process, mean value of 1.8% (db).

Starch degradation during traditional preparation of *gowé*

Figure 1 shows the typical size exclusion chromatograms obtained from unfermented gowé (malt), gowé at the end of the saccharification step, after the gruel was added, at the end of the fermentation step and after final cooking. In addition, the glucose chromatograms and several oligosaccharide standards are shown together with the calibration curve calculated from the standards. Glucose and oligosaccharide peaks (with a degree of polymerisation up to 5) can clearly be seen on the gowé chromatograms; glucose, maltose and maltotriose were the main peaks. The second peak (P2) was eluted at a time corresponding to a degree of polymerisation (DP) of 35, that is a molecular weight of 6000, according to the calibration curve. According to the documentation provided by the manufacturer, this corresponded to the exclusion limit of the column (molecular weight around 10 000 for proteins). A chromatogram of pure amylose (molecular weight over 10^6) also presented the same single peak, thus confirming the exclusion limit of the column. Polymers eluted in peak 2 could thus have had any molecular weight above 6000. In the samples of gowé, an earlier peak (P1) was observed; it was due to the elution of amylopectin, as pure waxy starch was eluted with this single peak. The separation of amylopectin was unexpected as its molecular weight (up to 10^8) is far higher than the exclusion limit of the column. This may have been due to electrostatic repulsion between amylopectin and the gel used in the column, as already observed for dextrans in certain elution conditions (Merienne *et al.*, 2000) which may accelerate elution of the amylopectin.

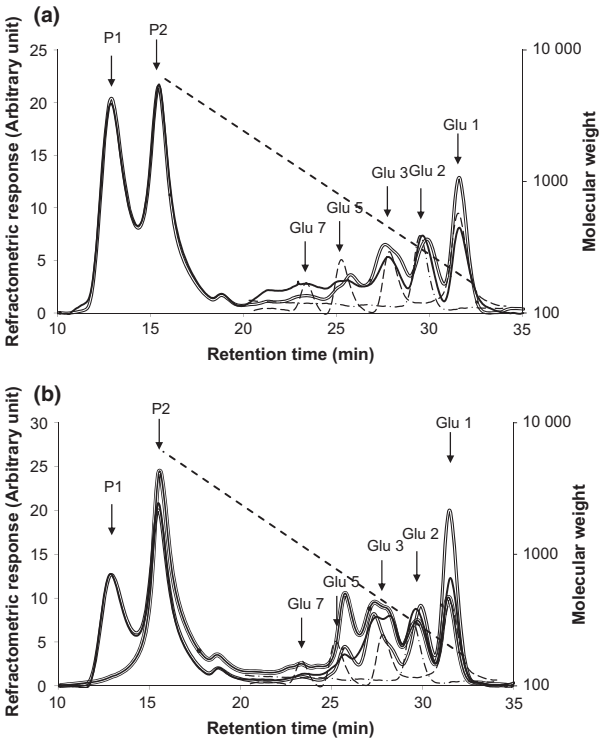

Figure 1 Size exclusion chromatograms of malt (a, solid line) traditional *gowé* after saccharification (a, double solid line), just after addition of the paste (b, solid line), after 24 h fermentation (b, double solid line) and after cooking (b, triple solid line), and oligosaccharide standards (dashed lines), Glu1-7, glucose and glucose oligomers (1–7 glucose residues).

With normal maize, P1 and P2 were observed and the area of the first peak was twice as large as that of the second, as expected from the relative amounts of amylopectin and amylose. In the gowé samples, the area of the second peak was bigger than that of the amylopectin peak; this meant that amylopectin was partly hydrolysed during processing and gave rise to high molecular weight polymers, which were co-eluted with amylose. We consequently collated the results (Table 2) of peak 1 (amylopectin), peak 2 (amylose and high DP dextrins) and the total area between 20 and 34 min (oligosaccharides and glucose).

In the malt, the area of peak 2 was similar to that of peak 1, indicating that at least amylopectin (peak 1) was partly degraded during malting and gave rise to high molecular weight dextrins. In addition, dextrins with intermediate DP (between 6 and 15) were observed between 20 and 25 min, and the levels of maltotriose, maltose and glucose were similar. Very few changes occurred after the saccharification step; only a slight reduction in the intermediate dextrins and an increase of the peak of glucose were observed. In contrast, the addition of the cooked paste at the end

Table 2 Changes in carbohydrate polymers of traditional gowé during natural fermentation (proportion of areas in SEC profiles)

	Saccharification (Primary fermentation)				Fermentation* (Secondary fermentation)				
Chromatogram peak (elution time)	**0 h**	**4 h**	**8 h**	**12 h**	**0 h**	**12 h**	**24 h**	**After cooking**	**SDR†**
Peak 1 (12 min)	0.33c	0.33c	0.32c	0.34c	0.24b	0.22b	0.22b	0a	0.02
Peak 2 (16 min)	0.39ab	0.37ab	0.34a	0.35a	0.41ab	0.40ab	0.39ab	0.69c	0.02
Oligosaccharide peak (20–34 min)	0.28a	0.30a	0.34a	0.31a	0.34a	0.38ab	0.37ab	0.31a	0.03

SEC, size exclusion chromatography. Mean values of duplicates. Values with the same letters in the same line are not significantly different at 0.05 level using Newman–Keuls mean comparison test.

*Fermentation started (0 h) after the paste was added to the saccharified mash.

†Standard deviation of the residual.

of the saccharification step triggered dramatic changes. The area of peak 1 significantly decreased while that of peak 2 increased (Table 2). Intermediate dextrins disappeared. No further changes in peaks 1 and 2 were observed during fermentation, but glucose increased and became predominant. At the end of the fermentation, glucose and oligosaccharides represented 37% of the refractive index response. After final cooking for 45 min, as applied in the traditional procedure, the amylopectin peak almost completely disappeared whereas peak 2 was almost unchanged.

Organic acids produced during the preparation of gowé

Lactic acid was the main organic acid produced during fermentation of gowé in the traditional process (Table 3). Acetic acid was detected but only at trace level and could not be quantified. Lactic acid increased exponentially from 3.0 g kg^{-1} to 15.3 g kg^{-1} at the end of the secondary fermentation (24 h).

In the modified process (Table 4), irrespective of the starter culture used, lactic acid was also the main organic acid; the concentration of lactic acid reached maximum after 24 h. When the yeasts (*K. marxianus and P. anomala*) were used singly, the concentration was lower (around 20 g kg^{-1}) and close to that of the control in the traditional process. When lactic acid bacteria (*L. fermentum and W. confusa*) were used singly, it was higher (over 25 g kg^{-1}). A positive effect on lactic acid production was observed when *P. anomala* was used in combination with lactic acid bacteria (the level of lactic acid was higher than that observed with lactic acid bacteria alone), but the interaction with *K. marxianus* was negative. Lactic acid content was higher at the beginning and end of the fermentation for the modified process without inoculation (control, Table 4) than for the traditional process (Table 3).

Changes in the profile of free sugars during the preparation of gowé

Maltose and glucose were the main free sugars whatever the process used (Tables 3–4). Glucose was the main free sugar from the beginning of the process. Glucose content increased dramatically during the first 12 h of the second fermentation, then remained almost constant, or increased slightly depending on which starter culture was used. Final glucose content ranged between 190 and 277 g kg^{-1} in the modified process depending on the starter culture (Table 4), vs. 240 g kg^{-1} in the

Table 3 Organic acid and sugar contents (g kg^{-1} db) of traditional *gowé* during natural fermentation

	Saccharification (g kg^{-1} db) (Primary fermentation)				Fermentation* (g kg^{-1} db) (Secondary fermentation)			
Compounds	**0 h**	**4 h**	**8 h**	**12 h**	**0 h**	**12 h**	**24 h**	**SDR†**
Organic acids								
Lactic acid	3.0b	2.9b	2.1a	6.1c	1.9a	10.1dc	15.3e	0.2
Sugars								
Maltose	63.6a	61.3a	62.6a	99.8c	63.6a	82.0b	61.7a	2.2
Glucose	71.9a	86.1a	94.6a	119.3a	119.7a	197.0b	240.1c	10.1

Mean values of duplicates. Values with the same letters in the same row are not significantly different at 0.05 level using Newman–Keuls mean comparison test.

*Fermentation started (0 h) after the gruel was added to the saccharified mash.

†Standard deviation of the residual.

Table 4 Sugar and organic acid profiles (g kg^{-1}) of modified *gowé* during fermentation using starter cultures added after saccharification

Starter Sugar/acid	Time (h)	C	L	W	K	P	WK	WP	LK	LP	SDR
Maltose	0	136 cd	112b	93.0a	102.2ab	148.2d	114.4b	120.7bc	103.8ab	134.7 cd	4.2
	12	91.1c	76.7b	73.9b	83.7c8210.1	87.1c	53.2a	53.2a	50.1a	97.2d	1.9
	24	61.0b	40.4a	40.5a	44.2a	73.0c	63.6b	47.6a	48.0a	45.8a	1.9
Glucose	0	126d	113cd	209f	87a	84a	108bc	117bc	97ab	162e	4
	12	236bc	241bc	251bc	224b	237bc	184a	267c	249bc	227b	10
	24	255bc	242bc	233b	190a	277c	275c	253bc	234b	250bc	7
Lactic acid	0	8.0	4.5	3.9	5.2	3.3	3.4	3.0	3.4	7.8	0.9
	12	8.2b	20.8e	24.0f	7.8b	10.5c	19.2d	25.0 g	23.8f	6.9a	0.2
	24	21.4b	28.9e	25.6d	18.4a	23.8c	19.2a	29.2e	23.1c	31.2f	0.5

Mean values of duplicates. Values with the same letter in the same line are not significantly different at 0.05 level using Newman–Keuls mean comparison test.

L: *Lactobacillus fermentum*; W: *Weissella confusa*; C: natural fermentation (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*.

traditional process (Table 3); values were quite similar when the fermentation was natural for the traditional and improved ('control' in Table 4) processes.

Maltose content increased significantly at the end of saccharification, then decreased when the cooked paste was added (0 h secondary fermentation; Table 3). The values observed at 0 h of fermentation gave a picture of the effect and of the reproducibility of the modified saccharification process. Maltose content was twice higher in the modified process (136 g kg^{-1} for the control sample, Table 4) than in the traditional one (64 g kg^{-1}, Table 3) while glucose content was very similar (126 vs. 120 g kg^{-1}). However, the modified saccharification process appeared to be poorly reproducible; the coefficients of variation of glucose and maltose contents were 33% and 15%, respectively.

The level of maltose did not change significantly at the end of the secondary fermentation, after a transitory increase in the traditional process. However, it decreased continuously during the fermentation step in the modified process in both the control and in the inoculated samples, irrespective of the starter culture used (Table 4). At the end of fermentation, the maltose content of the control and of some of the samples inoculated with yeasts (P and WK) was similar to that of traditional gowé (around 60 g kg^{-1}), whereas most of the inoculated samples had a significantly lower maltose content (around 40 g kg^{-1}). With the modified process, the sum of maltose and glucose contents significantly increased after 12 h of fermentation from 241 g kg^{-1} to 309 g kg^{-1}, mean values for the different types of inoculum, then decreased slightly but significantly, to reach a mean value of 297 g kg^{-1} at the end of fermentation (Table 4).

Discussion

The proximate composition of gowé varied during fermentation. The significant decrease in crude fat during the fermentation process is in accordance with several studies in which a reduction in fat was observed in germinated sorghum (Asiedu *et al.*, 1993; Michodjehoun-Mestres *et al.*, 2005). Protein content increased. These results are in agreement with those of previous studies (Poutanen *et al.*, 2009) reporting that the action of enzymes during fermentation causes hydrolysis and solubilisation of grain macromolecules, such as starch, proteins and cell wall polysaccharides. This resulted in a reduction in dry matter and an increase in proteins. In addition, Akingbala *et al.* (1981) reported that during fermentation of ogi from sorghum, most samples showed clear evidence of extensive enzymatic breakdown of starch granules and of the protein matrix.

We evaluated the degradation of starch by SEC using a refractive index detector for the quantitation. The refractive index detector is a universal detector which quantifies not only starch components but also proteins, amino-acids, etc. It consequently overestimates the starch and oligosaccharide components. However, starch and sugars are the main components of the grain that are soluble in alkali and the refractive response indeed mainly reflects starch and sugar content; for example, the area of glucose and oligosaccharides represented 37% of the total area of the size exclusion chromatogram at the end of the fermentation (Table 2), a value close to the sum of glucose and maltose contents, measured by HPLC (31%, Table 3). SEC revealed that starch degradation after malting was relatively low but significant. Residual starch content, including high molecular weight dextrins (which were not separated from amylose), was 72%; starch contents ranging from 70% to 33% have been measured after germination, depending on the germination conditions (Sripriya *et al.*, 1997; Elmaki *et al.*, 1999). Glucose, maltose and maltotriose were the main degradation products together with low molecular dextrins (DP 6-15), but their levels were very low. Very few

studies have been published on starch degradation products after malting. Nirmala *et al.* (2000) reported the presence of glucose, maltose and maltotriose. Conversely, Bertoft & Henriksnas (1983) reported a high molecular weight (over 10^6) fraction in barley malt accounting for 15% of the malt. Similarly, Lauro *et al.* (1999) observed the progressive disappearance of original amylopectin and the appearance of high molecular dextrin (31×10^6) when native starch was hydrolysed with bacterial α-amylase. We also observed an increase in peak 2 after malting, but were unable to conclude on the molecular weight of this fraction: it could have had any molecular weight above 6000. Nevertheless, high molecular dextrins in peak 2 represented a relatively high proportion of starch; the area of peak 2 is twice lower than that of peak 1 in native starch which means that a third of the amylopectin fraction has been partly degraded during malting.

Starch degradation was low during the saccharification and fermentation steps; the degradation of intermediate dextrins into glucose was the main process observed. This may be linked to the action of β-amylase and α-glucosidase (Taylor & Dewar, 1994; Agu & Palmer, 1997); starch hydrolysis with pure α-amylase indeed led directly to oligosaccharides (with DP between 3 and 7) without any production of maltose and glucose (Nirmala & Murahkrishna, 2003). Most starch degradation occurs when precooked (gelatinised) starch is added just before the fermentation step and during the final cooking. In the former case, gelatinised starch is added to a mash which surely displays high amylase activity (due to the previous germination process), providing ideal conditions for starch hydrolysis to proceed. In the second case, starch degradation occurs at the beginning of cooking when starch gelatinisation occurs and before heat degradation of α-amylases. α-amylases in cereals, particularly those in tropical cereals, are indeed known to be stable up to 70–75°C (Nirmala & Muralikrishna, 2003; El Nour & Yagoub, 2010). At least amylopectin was rapidly degraded, surely by malt amylases, as soon as heat treatment was applied (peak 1 decreased). The degradation of starch during the preparation of gowé is a complex phenomenon: starch appears both in the native and gelatinised state (in the added cooked paste), and several enzymes (α- and β-amylases, α-glucosidases) can be involved. The degradation of the native part of starch and particularly of amylopectin appeared to be limited throughout the fermentation process (nonsignificant decrease in peak 1); but the small dextrins continued to be hydrolysed (probably by β-amylases and α-glucosidases) throughout the process, giving rise to a high glucose content in the final product (Tables 3 and 4). The free sugar profile was quite different from that observed during mashing for beer production when starch is normally completely gelatinised and that gives rise to high levels of maltose, glucose (with glucose: maltose ratio of 1:6; Ogu *et al.*, 2006) and intermediate dextrins (DP 3-8) (Bathgate & Bringhurst, 2011). It is also quite different from the sugar profile obtained by the action of sorghum malt amylases on corn starch at 65 °C (Ba *et al.*, 2013); high molecular dextrins (DP of 100) and intermediate dextrins (mean DP 7) were recovered with very high maltose content. This may be linked with the fermentation process during the preparation of gowé, which indeed causes a dramatic drop in pH (Vieira-Dalode *et al.*, 2007), and thus can change the activity of the enzymes present in the mash.

Similarly to results obtain in a previous study (Michodjehoun-Mestres *et al.*, 2005)], the main free sugars produced during the preparation of gowé were glucose and maltose. During the secondary fermentation step, maltose content decreased significantly whereas glucose content increased, surely as a result of maltose hydrolysis by α-glucosidase, which is present in sorghum malt (Taylor & Dewar, 1994). This observation is consistent with that of Mugula *et al.* (2003) who reported maximum maltose content followed by a sharp increase in glucose content during the fermentation of *togwa*. Despite its use by microorganisms (revealed by the increasing production of lactic acid), the glucose balance was positive during the first 12 h of fermentation and then became equilibrated up to 24 h of fermentation. Similarly, Michodjehoun-Mestres *et al.* (2005) observed an increase in the concentration of glucose during the first 12 h of the secondary fermentation, followed by a considerable decrease after 36 h of fermentation.

Compared to the traditional process, the modified saccharification process enhanced maltose production: starch was more hydrolysed after 2 h at 40 °C than after 12 h at 25–28 °C (ambient temperature). This was probably due to the higher temperature (40 °C vs. 25–28 °C) but also to the higher water content (about 75% vs. 50% wet basis) in the modified process, both conditions that facilitate amylase activity.

Concerning the production of organic acids, several authors already reported a dramatic increase in lactic acid during the acidic fermentation of cereal-based products (Mugula *et al.*, 2003; Michodjehoun-Mestres *et al.*, 2005) accompanied by limited production of acetic acid. In another traditional gowé process, Michodjehoun-Mestres *et al.* (2005) found lactic and acetic acid contents of, respectively, 14.1 g kg^{-1} and 0.7 g kg^{-1}, after 12 h of fermentation. In the present study, we measured a lactic acid content of 10 g kg^{-1} in the traditional gowé and of between 6.9 and 25.0 g kg^{-1} in the modified process for the same period of fermentation. As expected, there was a clear increase in lactic acid content in the modified gowé when it was inoculated with lactic acid bacteria. Lactic

acid was, however, also presented in control and yeasts inoculated samples as natural lactic acid bacteria flora was present; it increased from 10^4–10^5 CFU g^{-1}, at the beginning of the fermentation, to 10^9 CFU g^{-1} after 12 h (Vieira-Dalodé *et al.*, 2007). A similar result has already been observed for titratable acidity (Vieira-Dalodé *et al.*, 2008). Lactic acid values represented half the total titratable acidity, revealing that other acids were also produced. In this study, the absence of acetic acid is in agreement with previous studies on similar cereal-fermented products such as *togwa* (Mugula *et al.*, 2003) and ogi (Nago *et al.*, 1998). Ethanol was not detected in this study either despite the fact, it can be produced during acidic fermentation of cereal products (Nago *et al.*, 1998; Tou *et al.*, 2006). Acetic acid and ethanol should have been produced in gowé at least when *L. fermentum,* (which is known to be heterofermentative lactic acid bacteria (Vandamme *et al.*, 1996; Atlas, 1997) or yeasts were added. The absence of these compounds may be due to the freeze-drying process which could have eliminated these relatively volatile compounds.

A PCA was performed on the sugar and organic acid profiles and on the level of acidity of the gowé. We combined the results of this study and those for total sugar content, pH, titratable acidity from a previous study (Vieira-Dalodé *et al.*, 2008). The results of 24 h of fermentation in the traditional process and of 12 h of fermentation in the modified process were used so as to be as close as possible to the conditions used for the sensory tests performed on moderately acidic gowé in the previous study, that is traditional gowé fermented for 24 h and improved gowé fermented for 7 h. The first two principal components explained 80.7% of total variance. The first axis represented 63.1% of the variation and was mainly linked to acidity (lactate and titratable acidity on one side, pH on the opposite side), but also to maltose and total sugar content (Fig. 2). Maltose content was indeed significantly (at the 1% level) inversely correlated ($r = -0.81$) with lactic acid content. As maltose originates from starch degradation, this should mean that higher acidity impedes starch degradation (amylase activity). The second axis was only linked to glucose content. The traditional gowé process was located right in the middle of the plane formed by the two-first axes of the PCA. The uninoculated (control, C) and the gowé inoculated with the yeast (*P. Anomala, P and K. Marxianus, K*) were located on the left of the plane and indeed exhibited low acidity levels and/or high maltose levels. The gowé inoculated with lactic acid bacteria (*L. fermentum,* L and *W. Confusa,* W) was located on the right due to its higher acidity and lower maltose content. Several lactic acid and yeast combinations (LK and WP) were located at the extreme right of the PCA plane, because they had the highest acidity levels. These combinations could favour rapid acidification during the preparation of gowé. This hypothesis is supported by Nout (Nout, 1991), who provided evidence that acidification is favoured by the synergistic metabolic activities of LAB and yeasts during the fermentation process. In contrast, the LP combination was located between L and P, which should mean there is no synergy between these two strains. WK was located alone due to its relatively low glucose content after 12 h of fermentation. The modified processes L and LK were located close to the traditional process and were judged by the panel to be the most similar to

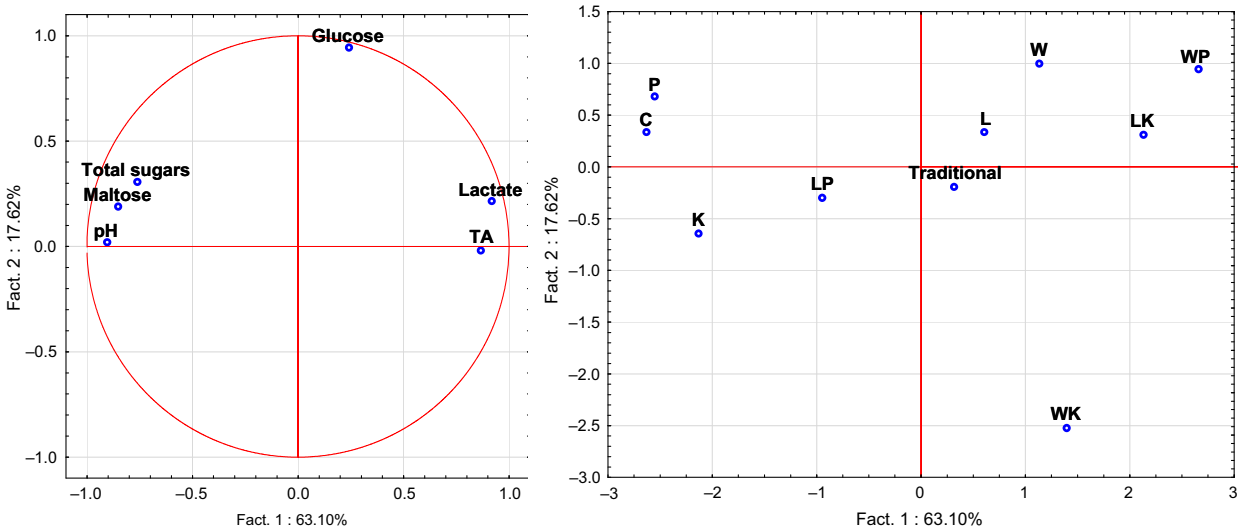

Figure 2 First plane of the principal component analysis of sugar and organic acid contents, and acidity.

traditional gowé (Vieira-Dalode *et al*., 2008). This confirmed that the balance between acidity and sugar is of major importance for consumer acceptability. As a negative correlation between maltose and acidity was observed, it appears that the choice of inoculation strain will thus have an effect not only on acidity but also on the sugar content of the resulting gowé and consequently on its acceptability.

Conclusion

The SEC experiments showed that the degradation of starch mainly occurred in two steps; during malting producing high molecular dextrins and oligosaccharides and during precooking releasing oligosaccharides and glucose. The latter degradation was surely due to the facilitated hydrolysis of gelatinised starch by the amylases in the malt. The precooking of part of the sorghum flour thus appears to be a crucial step in the preparation of gowé, and some variability in this step could explain the quite large variability observed in the level of glucose in gowé. It was shown in addition, that the choice of the inoculating strain will directly affect the level of lactic acid, and indirectly the level of maltose, due to the negative interaction between acidity and maltose. Further work is in progress to evaluate the amylase activity during the process of gowé.

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