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Full Length Research Paper

Physical, chemical and microbiological changes during natural fermentation of "gowé", a sprouted or non sprouted sorghum beverage from West-Africa

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Gowé is a traditional Beninese fermented beverage prepared from sprouted and non-sprouted cereals. Due to urbanization, a new technique without any malting step has appeared in Southern Benin and is now widely used. The two techniques were compared using sorghum as the raw material. The physical, chemical and microbiological changes that occurred during a 72 h fermentation period were studied in both techniques. The dominant microflora was a mixed population of lactic acid bacteria and yeasts. The lactic acid bacteria population was higher in the traditional product at the start of fermentation (6.1 log cfu/g wet basis versus 4.5 in the modified process) because of the malting step. It then increased to 9.3 log cfu/g after 48 h fermentation. Enterobacteriaceae counts increased slightly during the first stage of fermentation but fell below the detectable level after 24 h in the traditional raw gowé and after 48 h in the modified raw gowé. The pH decreased from 6.3 to 3.4 in the traditional process and from 6.6 to 3.8 in the modified process, while the titratable acidity increased from 0.4 to 6.4% (w/w, lactic acid) and from 0.4 to 4.2%, respectively. Total soluble and reducing sugar content first increased, and then decreased with a concomitant increase in organic acids. The major organic acids were lactic acid and acetic acid. The fermentation process was thus more intense and faster in the traditional process, giving a significantly lower level of crude fat.

Key words: Traditional technology, beverage, sorghum, malting, fermentation, organic acids.

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a staple food in most parts of Africa, where it is the main source of carbohydrates and proteins for millions of people, mostly in the Southern Sahara. Various types of foods are prepared from sorghum whole kernels, for instance unfermented and fermented breads (chapati, roti, kisra) in Asia and East Africa and steamed foods (couscous), fermented and unfermented porridges and pastes (ugali, akamu, tô, eko, kamu, koko, ogi, etc.) and alcoholic and non-alcoholic beverages in Africa generally (Chilkunda and Paramahans, 2001).

Like other cereal products, sorghum products have poor nutritional value. This is due to their deficiency in lysine, threonine and tryptophan and to the presence of anti-nutritional factors (Salunkhe et al., 1977) such as tannins and phytates that interact with proteins, vitamins and minerals, thus restricting their bio-availability (Harland and Oberleas, 1986; Bhise et al., 1988). The

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above factors contribute to anemia and other nutritional diseases in developing countries where the consumption of sorghum products is high. Furthermore, sorghum proteins are less digestible than those of other cereals (Klopfenstein and Hoseney, 1995). Various simple techniques have been investigated to improve the protein digestibility and mineral availability of sorghum by reducing its tannin and phytate content. These include malting, fermentation (Kazanas and Fields, 1981; Chavan et al., 1988) and cooking (Khan et al., 1986). Fermentation is widely used traditionally for processing sorghum, and fermented products are well accepted and widely used as complementary foods (Tomkins et al., 1988). Their low pH confers the advantage of microbiological safety (Hamad and Fields, 1979). The malting of sorghum grains has been reported (Au and Fields, 1981; Ahmed et al., 1996; Lasekan et al., 1997) and is used to increase the water-soluble protein, lysine, methionine and soluble sugar content, diastasic activity and iron, calcium and phosphorus availability (Bhise et al., 1988). It should be noted, however, that sorghum plants contain appreciable amounts of dhurrin (a cyanogenic glycoside that yields hydrocyanic acid when hydrolyzed), and that immature plants contain exceptionally high levels of this cyanogen (Panasiuk and Bills, 1984).

Most Beninese households consume sorghum foods as unfermented paste (tô), fermented paste (akassa), and fermented beverages from sprouted grains (chakpalo, tchoucoutou and gowé). Gowé is a homogenous gelatinized, malted, fermented and cooked paste prepared from sorghum, millet or maize. It is consumed as a beverage after dilution in water and the addition of ice, sugar and sometimes milk. It is the preferred beverage of children, pregnant women, sick and old people. All types of sorghum, millet and white maize can be used to make acceptable gowé but colored sweet gowé is preferred. Raw gowé is cooked into a thin gel, the texture of which is between that of a thick and a stiff porridge. The texture is in fact a critical sensory attribute. Consumers want a gowé of intermediate texture, one that holds together but yields easily to finger pressure. When consumed, the gowé should not stick to the fingers, the teeth or the roof of the mouth, it should simply melt in the mouth. Gowé can be stored for two to three days at ambient temperature (and up to one week at +4°C) without reheating.

A survey conducted in the Cotonou area showed that gowé was traditionally produced in central Benin. The technique was introduced to Cotonou (Southern Benin) by small-scale producers. The traditional process (Figure 1) consists of three stages: malting (6 days), first fermentation (12 h) and second fermentation (24-48 h). The malting improves the nutritional quality of the product while the fermentation improves its safety. The major constraints of the traditional process are the time needed for malting and the skill required to carry it out, one that most urban producers do not have. Recent surveys in Cotonou showed that most producers use a modified technique, which consists of fermenting non-malted sorghum flour and adding commercial sugar to obtain the naturally sweet taste provided in the traditional process by malting. The time required to produce gowé using the modified technique (Figure 2) is 3 days, as against 6-8 days with the traditional process.

No information is available on the processing of sorghum to produce gowé, as this takes place in Benin. The present study deals with the physical, chemical and microbiological changes that occur during processing, using the traditional and the modified techniques as applied in small-scale urban production.

MATERIALS AND METHODS

Pilot-scale experiments for processing sorghum into gowé were carried out using the skills of a local producer.

Materials

A local red variety of sorghum (*Sorghum vulgare* (L.) Moench) was purchased from a market in Southern Benin. The grains were cleaned by removing foreign *objects* and broken kernels. They were immediately washed in water and sun-dried. The grains were then divided in two parts. One part was milled into flour to pass through a 0.5 mm sieve, using a community plate disc mill. The flour was stored at ambient temperature (28-30°C). The second part, stored at ambient temperature (28-30°C), was used for malting.

Malting

The dried grains were steeped overnight in a plastic container in 3 volumes of tap-water at room temperature (28-30°C), without changing the water. The soaked grains were washed and drained. They were uniformly spread on a wet piece of thin cloth placed in a traditional wicker basket, then covered with another piece of cloth to reduce dehydration. The grains were kept wet by frequent spraying with tap-water. Germination was done at room temperature (28-30°C), over a 48-*h* period. The germinated grains were sun-dried (4 days) but the roots were not removed, in accordance with local practice. The malt was milled in the community plate disc mill to get a fine flour, passing through a 0.5 mm sieve.

Preparation of fermented malted raw gowé

The traditional process was reproduced by a local gowé producer at pilot scale (Figure 1). 1 kg of malted sorghum flour was mixed with 2 kg of non-malted sorghum flour and 3 L of water. The sample was divided between 7 lidded plastic buckets marked with different fermentation times (0, 4, 8, 12, 24, 48 and 72 h). The first fermentation was carried out at room temperature (28-30°C). After 12 h fermentation, non-acid thick porridge (prepared by mixing 1 kg of whole sorghum flour and 10 L of water and cooking at 100°C for 10 min) was added to the samples in the buckets marked 12, 24, 48 and 72 h. The temperature of the mixture was 50-60°C when added to the samples and decreased to room temperature (28-30°C) during the following (or second) fermentation period.

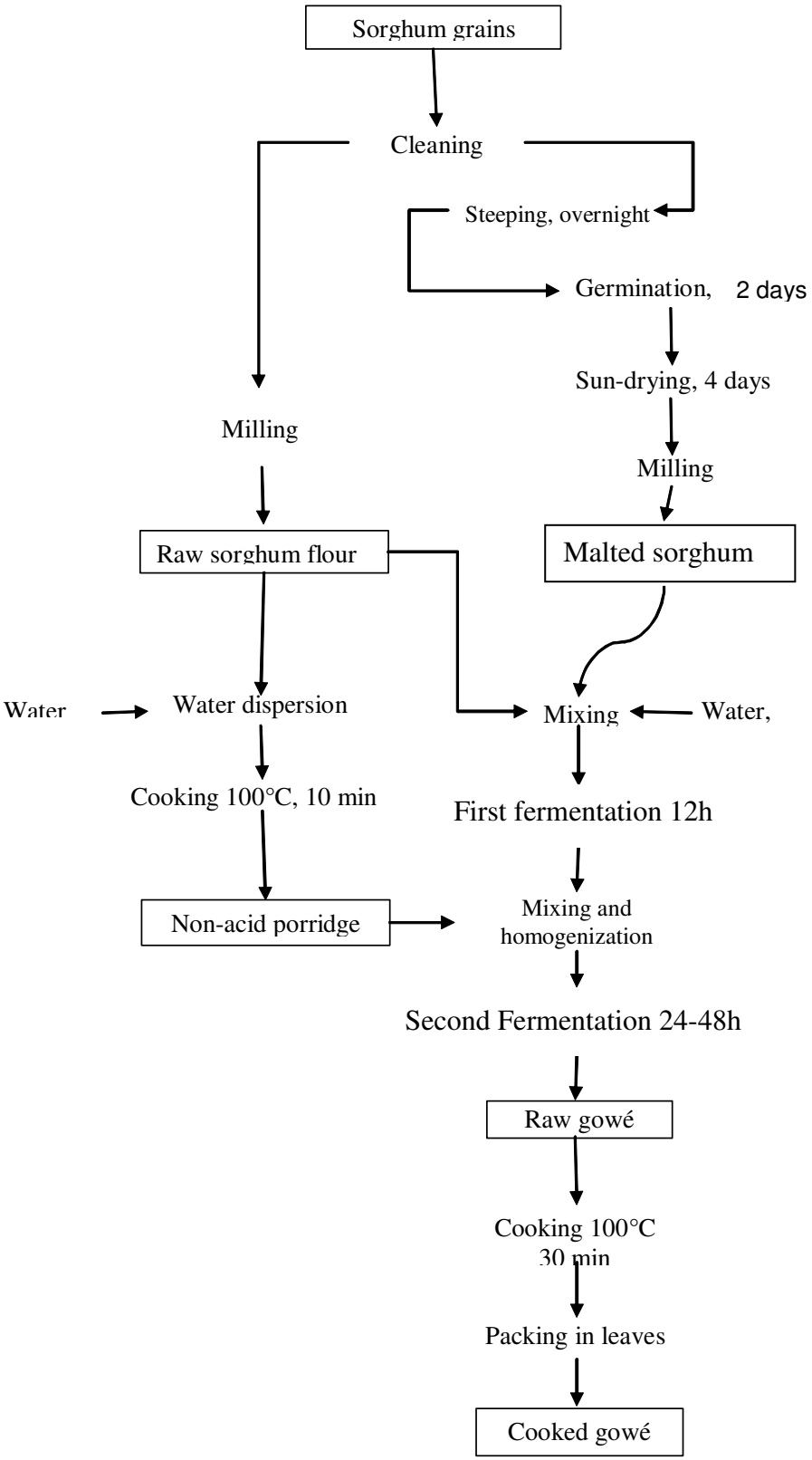


Figure 1. Flow sheet for the production of traditional gowé.

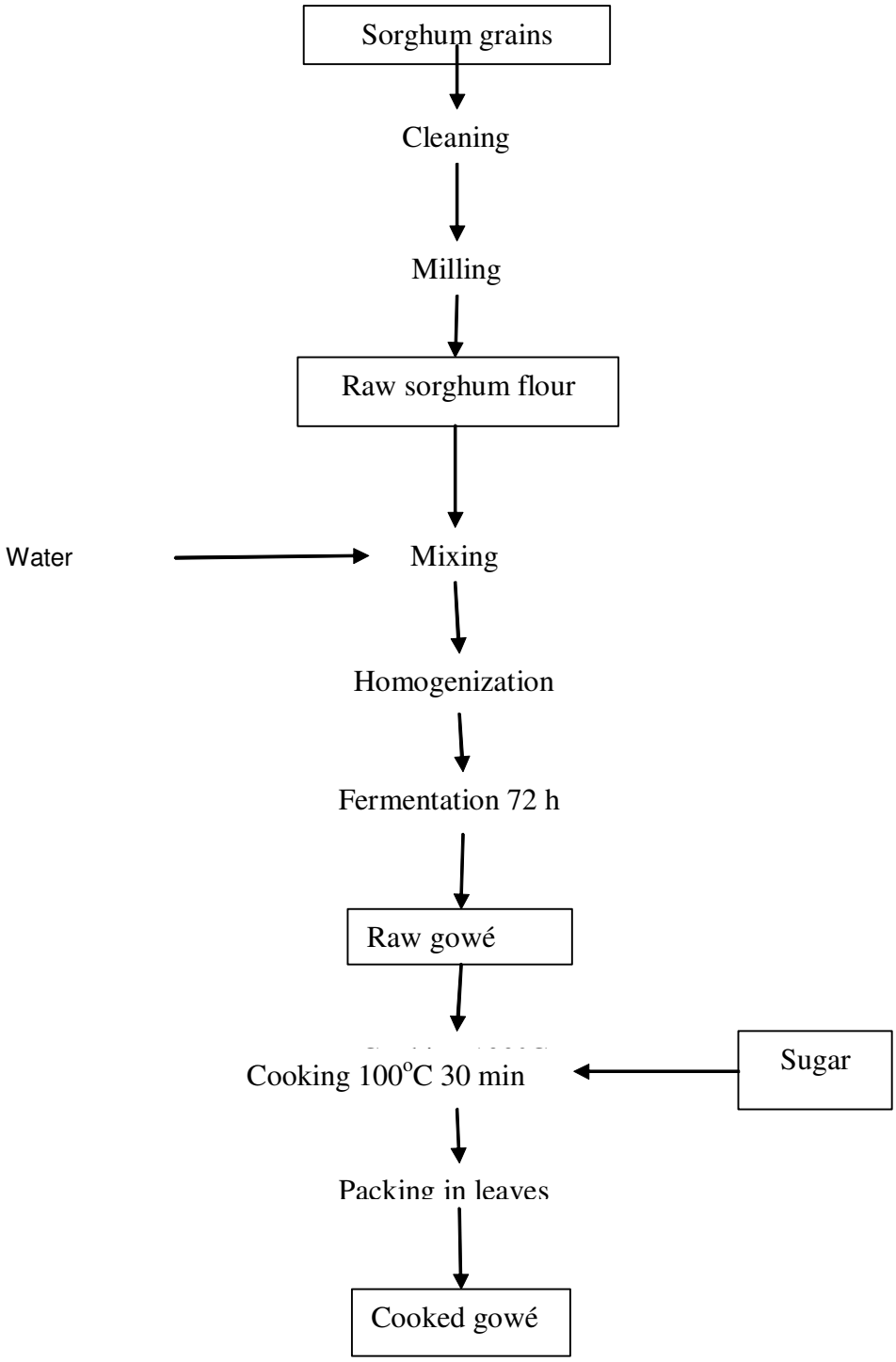


Figure 2. Flow sheet for the production of modified gowé.

Preparation of fermented modified raw gowé

2 kg of whole sorghum flour was mixed with 5 L of water. The sample was divided between 7 lidded plastic buckets marked with different fermentation times (0, 4, 8, 12, 24, 48 and 72 h).

Sampling

After 0, 4, 8, 12, 24, 48 and 72 h of fermentation, one bucket of raw gowé from each of the two processes was taken for analysis. Sub-samples (10 g) were used for moisture determination. A 10 g

portion was used for microbiological analysis, while 20 g portions were used for pH and titratable acidity measurements. A 100 g portion was oven-dried at 105°C and ground for proximate analysis. And 20 g portions were frozen at –18°C and freeze-dried for sugar and organic acid content determination.

Chemical analyses

pH and titratable acidity were measured by the method of Nout et al. (1989) modified by Hounhouigan et al., 1993b). Moisture, crude protein, crude fat and ash contents were determined, using AACC methods 44-15A, 46-11A, 30-25 and 08-01, respectively (Anonymous, 1984). Crude fibre content was determined as described by Osborne and Voogt, 1978). Carbohydrate content was calculated by difference.

The fermentable sugars and organic acids were determined by HPLC analysis using an Altima C18 5U column (250 mm x 4.6 mm) standardized with standard solutions at 1 and 10 mg/mL of sugars (maltose, saccharose, fructose and glucose) and organic acids (acetate, citrate, formate, lactate, malate, oxalate, pyruvate and tartrate). Elution was performed at 60°C with diluted sulphuric acid (5 mM) at 0.6 ml/min. For the extraction, 250 mg of freeze-dried raw gowé was vigorously suspended in 1 mL of 5 mM H_2SO4 and stirred at ambient temperature (28-30°C) for 30 min. The suspension was then centrifuged at 7,000 rpm for 10 min. The supernatant was filtered through 0.45 µm pore size filters and 100 µL was then injected onto the HPLC system. Sugar detection was performed by refractometry and organic acid detection by UV absorption at 210 nm. The signals were recorded using Kroma system software (Bio-Tek Instruments).

Microbiological analysis

Sub-samples (10 g) from each bucket were homogenized with 90 mL of sterile peptone-physiological salt solution (5 g of peptone, 8.5 g of NaCl, 1000 mL of distilled water, pH 7.0 ± 0.2) and decimal diluted.

Enterobacteriaceae were enumerated in pour-plates of double-layered Violet Red Bile Glucose (VRBG, Oxoid CM 485) medium and incubated at 37°C for 24 h. Lactic acid bacteria were enumerated in pour-plates of double-layered de Man, Rogosa and Sharpe (MRS) medium (Merck N° 10661, Darmstadt, F.R.G.) and incubated at 30°C for 3 days. Yeasts and filamentous fungi were enumerated in pour-plates of Malt Extract Agar (MEA, Oxoid CM 59) with addition of 10% of sterile lactic acid after autoclaving and incubation at 25°C for 5 days. All media were prepared according to the manufacturer's instructions.

Statistical analysis

All experiments and analyses were conducted in duplicate. Two factorial (type of technique and length of fermentation) analysis of variance was performed using Statitcf software (Boigneville, France).

RESULTS

Microbiological changes during fermentation

The populations of total aerobic mesophilic bacteria were significantly ($P<0.05$) different between the two types of gowé at the start of fermentation (log cfu/g of 6.2 and 5.2 in the traditional and the modified raw gowé, respectively), but became similar after 24 h fermentation, reaching a maximum of log cfu/g 9.9 and remaining quite constant until the end of fermentation. Figures 3a, 3b and 3c present the kinetics of evolution of the population of lactic acid bacteria, yeasts and moulds and Enterobacteriaceae, respectively, during fermentation in both types of gowé. As for total aerobic mesophilic bacteria, higher counts of lactic acid bacteria, yeasts and moulds and Enterobacteriaceae were observed in the first stage of fermentation with traditional gowé. Lactic acid bacteria and yeast counts decreased significantly ($P<0.05$) in the traditional process due to the addition of hot porridge to the mash after 12 h fermentation, whereas a continuous increase in both types of microorganisms was observed in the modified process. However, lactic acid bacteria concentrations were similar in both types of gowé after 24 h fermentation while yeast counts were similar after 48 h fermentation.

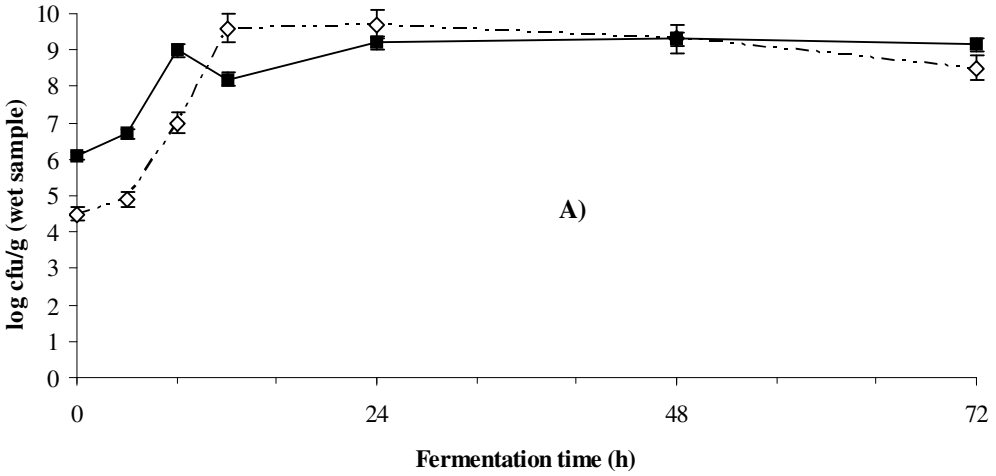
At the start of fermentation, Enterobacteriaceae counts were high in both types of gowé but decreased more sharply in the traditional than in the modified process. No Enterobacteriaceae was detected after 72 h fermentation in the traditional gowé, whereas the population in the modified process was 1.5 log cfu /g.

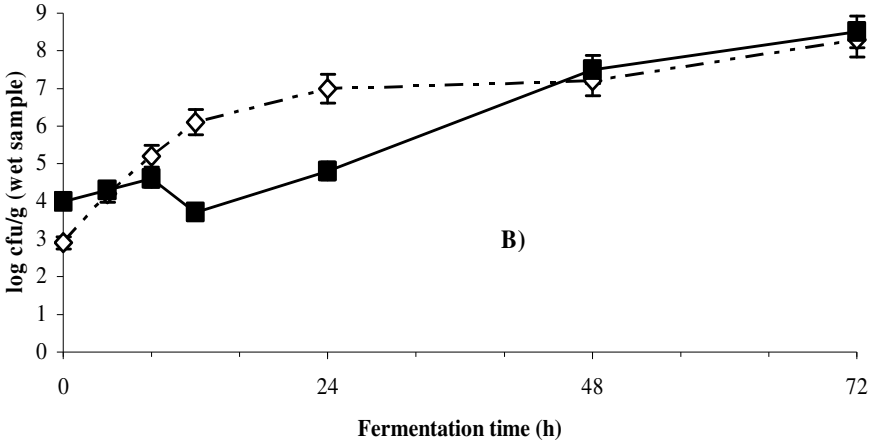
pH and titratable acidity

Titratable acidity (Figure 4) increased from 4.3 and 3.6 mg/g (w/w, calculated as lactic acid) at 0 h fermentation in the traditional and the modified gowé to 64.4 and 41.8 mg/g, respectively, after 72 h fermentation. A concomitant decrease in pH was observed (Figure 5). The pH decrease was significantly ($P<0.05$) higher in the traditional raw gowé between 4 and 12 h fermentation. Measurement of pH and titratable acidity of mash before and after addition of hot porridge at 12 h showed a significant decrease in acidity from 25 ± 0.2 to 16.3 ± 0.1 mg/g whereas pH increased from 4.39 ± 0.02 to 4.75 ± 0.02. Despite the reduction in acidity at this stage, the kinetics of acid production were higher in the traditional process involving malted sorghum than in the modified process, as can be seen from Figures 4 and 5. The pH stabilized at 3.4-3.5 in the traditional product and at 3.7-3.8 in the modified product after 48 h fermentation.

Changes in reducing sugars and organic acids

Sucrose, fructose, glucose and maltose were detected in both products, but in higher concentrations in the traditional product (Table 1). Sucrose and glucose could not be completely separated by the chromatographic system used. The proportion of glucose was nevertheless higher and both were assessed together as a glucose equivalent. Fructose was found at a low concentration at the start of fermentation (1.8 mg/g in the traditional raw





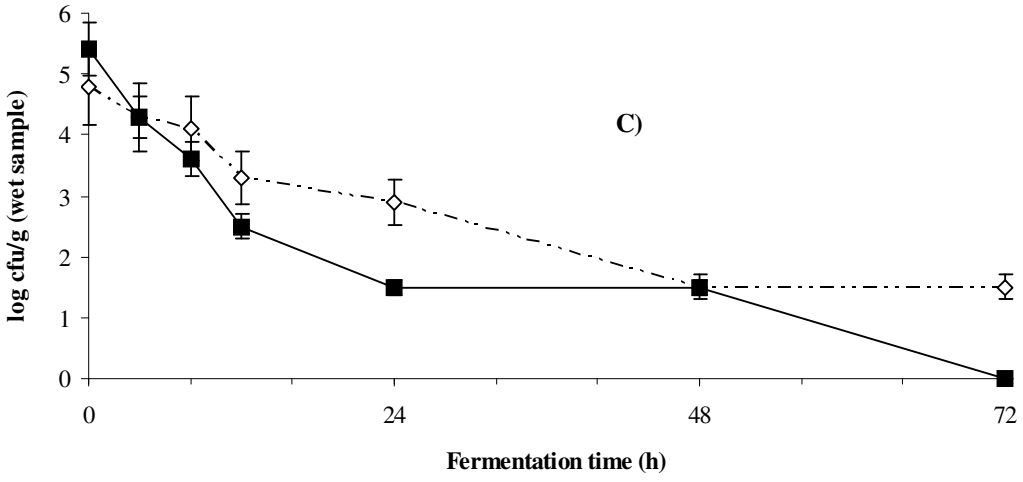


Figure 3. Changes in microbial counts (log cfu/g wet sample) during the fermentation of traditional (■) and modified (◊) raw gowé: A) lactic acid bacteria, B) yeasts and moulds, C) Enterobacteriaceae.

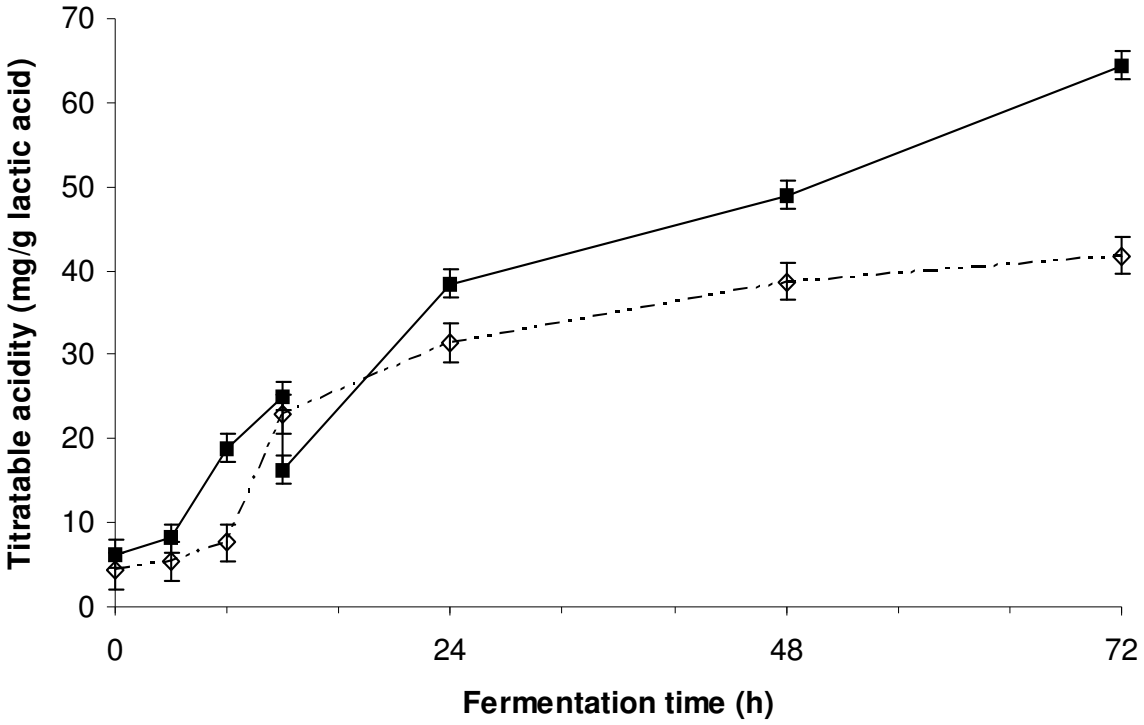


Figure 4. Changes in titratable acidity during the fermentation of traditional (■) and modified (◊) raw gowé.

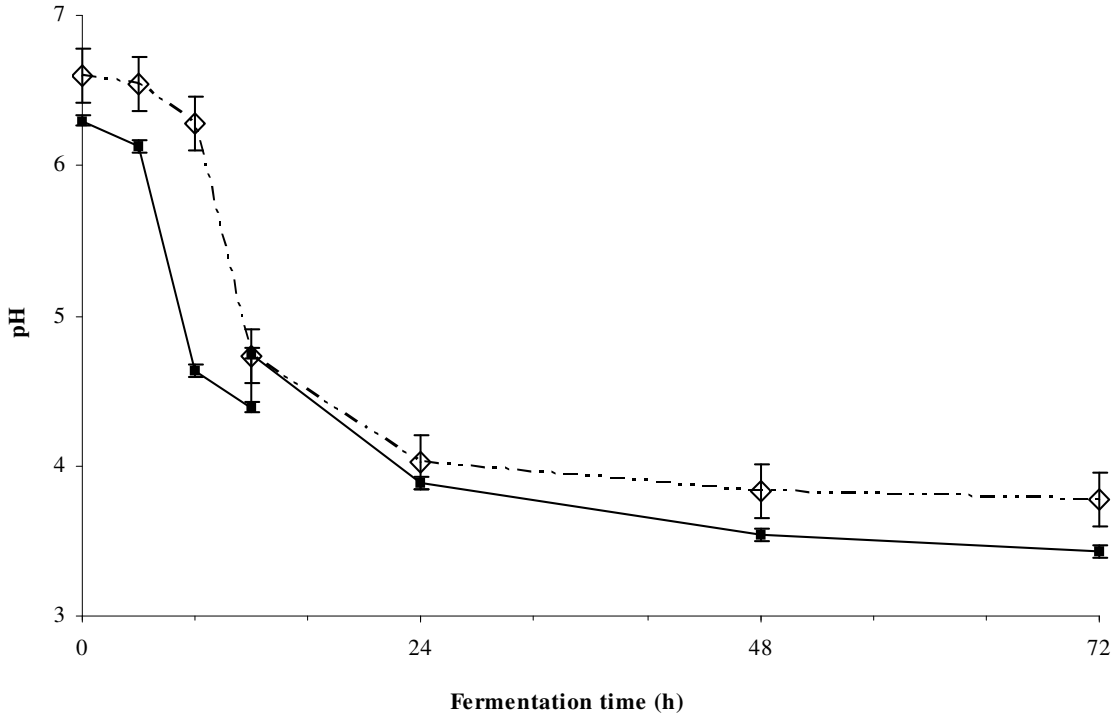


Figure 5. Changes in pH during the fermentation of traditional (■) and modified (◊) raw gowé.

Table 1. Sugar and organic acid identification during natural fermentation of traditional and modified gowé[a].

Fermentation Time (h)	Original raw gowé (mg/g db)[b]				Modified raw gowé (mg/g db)			
	Mal	Glu	Acet	Lact	Mal	Glu	Acet	Lact
0	38.5	36.1	0.0	0.1	14.5	21.4	0.0	0.0
8	46.3	72.9	0.6	6.5	16.4	42.2	0.4	2.8
12	35.0	92.2	1.0	9.7	6.5	36.5	0.9	6.4
12 with porridge	73.9	103.0	0.6	7.4	-	-	-	-
24	70.8	171.0	0.7	14.1	1.6	5.1	1.6	18.5
48	42.4	140.3	1.8	29.1	1.3	2.1	2.0	22.1
72	8.0	11.4	2.4	43.3	0.0	0.7	1.7	23.9

Mal = maltose; Glu = glucose; Acet = acetic acid; Lact = lactic acid [a]Mean of both experiments [b]db = dry basis.

Table 2. Influence of fermentaton time on the moisture content of traditional and modified raw gowé[a].

Fermentation time (h)	Moisture content (% w/w, fresh weight)	
	Original raw gowé	Modified raw gowé
0	53.7	72.9
4	54.0	73.6
8	54.8	74.2
12	55.9	74.7
12 with porridge	70.5	-
24	71.5	75.1
48	73.5	76.2
72	75.6	77.2

[a]Mean of both experiments.

gowé and 1.2 mg/g in the modified one) and decreased sharply to below the detection level after 8 h fermentation. Maltose and glucose were the most important sugars in the raw gowé at the start of fermentation. The addition of porridge to the mash after 12 h fermentation doubled the maltose content of the mixture in the traditional gowé, followed by a considerable increase in glucose at 24 h. Although these sugars decreased after 24 h, their level remained much higher in the traditional gowé than in the modified product. Acetic and lactic acids were the only organic acids detected. Apart from the slight decrease noted at 12 h in the traditional process after the addition of porridge, the increase in organic acids was the main feature of both processes, with higher final values in the traditional process. The lactic acid/acetic acid ratio was over 7 in both products after fermentation for 8 h and over 20 at the end of fermentation in traditional raw gowé.

Proximate composition

The moisture content was significantly ($P<0.01$) higher in the modified raw gowé than in the traditional raw gowé. It increased from 53.7 to 75.6 % in the traditional gowé and from 72.9 to 77.2 % in the modified product (Table 2) during fermentation. At 12 h fermentation, the moisture content of the traditional gowé increased from 55.9 ± 0.01 to 70.5 ± 0.04% due to the addition of the porridge, which had 90.3% moisture content, but still remained lower than that of the modified process. At the end of the fermentation period, the moisture content of both types of gowé was quite similar.

The protein, ash and fibre contents were respectively 9.7-10%, 1.7-1.8%, 2.1-2.3% (db, dry basis) in both types of raw gowé. The crude fat content decreased from 2.6% to 2.1% (db) during the fermentation of the traditional raw gowé but remained quite constant (from 3.0% to 2.9%, db) in the modified process.

DISCUSSION

The high initial count of total aerobic mesophilic bacteria was in the same range as those measured by Sanni et al. (1994) on traditional ogi sorghum. Apart from the flora present at the surface of the sorghum grains, lactic acid bacteria and yeasts may also originate from contamination during milling. In addition, microbial flora

may have developed during the malting process, thus explaining the higher initial count in the traditional gowé.

A concomitant increase in lactic acid bacteria and yeasts was observed during fermentation. The association of lactic acid bacteria and yeasts has been noted in several cereal foods (Akinrele, 1970; Nout, 1980; Wood, 1981; Odunfa and Adeyele, 1985; Adegoke and Babalola, 1988; Halm et al., 1993; Hounhouigan et al., 1993ac). In fact, the development of lactic acid bacteria is stimulated by yeasts, which provide soluble nitrogen compounds and other growth factors (Nout, 1991). Yeast metabolites, e.g. CO_2, pyruvate, propionate, acetate and succinate have been shown to stimulate lactobacilli in kefir (Leroi and Pidoux, 1993). Although there was a suitable substrate for microorganism growth, the lactic acid bacteria population decreased after the end of the first day of fermentation in the traditional process. This was probably due to the thermal and dilution effect of the hot porridge at 12 h fermentation. The continuous growth of the yeast population at the end of fermentation indicated that the products were not yet stabilized. A similar observation was made by Hounhouigan et al. (1993b) on naturally fermented mawè. Enterobacteriaceae are common on fermenting plant material and have also been found in the natural fermentation of cereal products. The Enterobacteriaceae count (log cfu/g) decreased sharply during fermentation to less than 2 within 24 h in the traditional gowé and after 48 h in the modified raw gowé. A similar observation was made by (Nout, 1991) on fermented sorghum-based infant food, where the Enterobacteriaceae fell from 2.5 to 1.7 log cfu/g after 60 h of natural fermentation. The decrease in the Enterobacteriaceae population paralleled the pH decrease, which was in accordance with the death kinetic of Enterobacteriaceae due to low pH (<4.5) in similar fermented material (Nout et al., 1989; Hounhouigan et al., 1993c).

The moisture increase observed during fermentation was probably caused by the combined effects of dry matter consumption and water production during aerobic and anaerobic catabolism by yeasts and lactic acid bacteria as earlier described (Hounhouigan et al., 1993b). The processing method had a slight but non-significant effect on the crude protein, ash and fibre contents but the crude fat and carbohydrate contents were significantly ($P<0.05$) affected, mainly in the traditional process. Abasiekong (1991) found an increase in the protein content of a fermented sorghum product after 4 days of fermentation, but our study was unable to confirm the finding. The lower fat content of the traditional raw gowé may be due to some lipolytic activity of the malt.

During fermentation the fermentable sugars first increased, then decreased, as shown in Table 1. The transient increase in glucose and maltose despite consumption by an increasing population of microorganisms is probably the result of amylolytic activities producing larger amounts of sugars than the Microorganisms require for their metabolism. Amylolytic activities of lactic acid bacteria have been reported in fermenting cereal grains (Nout, 1980; Odunfa and Adeyele, 1987; Umeta and Faulks, 1988; Agati et al., 1998). The amylolytic activity of the malt no doubt contributes to this phenomenon, thus explaining the higher maltose and glucose content of the traditional gowé. Amylolytic activities can further explain the dramatic increase in maltose and glucose in the traditional gowé after the addition of the porridge (after 12 h fermentation), the latter providing a better substrate for amylases that will hydrolyze the gelatinized starch of the porridge much more quickly than the native starch of the uncooked flour. The sugar content fell as soon as amylolytic activity was insufficient to provide fermentable sugars for microorganism metabolism, i.e. after 24 h fermentation for traditional gowé but after only 8 h for the modified process. It should be noted that gowé is normally sold after 24 to 48 h of fermentation when the traditional product has a sugar (glucose + maltose) content of about 200 mg/g (db). By the end of fermentation, the sugar content of the modified gowé has fallen to almost zero and producers add 250 mg of sucrose per g of raw sorghum flour to restore the sweetness of the product.

Lactic acid was the main organic acid produced during fermentation, together with small amounts of acetic acid. The presence of these two organic acids is an indication that heterofermentative lactic acid bacteria are involved in the fermentation of traditional gowé, whatever the technique used. This confirms previous results related to the involvement of these microorganisms in the fermentation of cereal products. (Kheterpaul and Chauhan, 1990; Hounhouigan et al., 1993a,c).

In conclusion, this study showed that the traditional technique, which contains a malting step, is more time-consuming than the modified process but that it encourages more rapid fermentation, with a greater natural sourness and a stronger sugar taste. The microorganisms involved in gowé fermentation are mainly yeasts and lactic acid bacteria, some of which are heterolactic. Further research is needed to identify the species of lactic acid bacteria and yeasts involved in the process and to compare the nutritional quality of the traditional and modified products.

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