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The interaction between starch hydrolysis and acidification kinetic determines the quality of a malted and fermented sorghum beverage

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ABSTRACT

Gowé is a traditional fermented Beninese soft cooked paste made from a blend of malted and non-malted cereals that is diluted with water/ice and sugar just before consumption as a thirst-quenching drink. Major differences in the processes used for the preparation of gowé, which includes natural lactic fermentation, result in variable quality. Acidity and free sugar content have been linked to the process parameters but also to the type of strains that can be used for inoculation. The aim of this study was to investigate the starch degradation mechanism in relation with the activity of degrading enzymes during the preparation of gowé and enzyme impact on the quality (sugar content and viscosity) of the final product. Our results point to a key role for malt α-amylase and its susceptibility to acidic conditions in the sequence of the preparation process and in the final quality of gowé. Pre-cooking and inoculation speeds up and increases acidification of the product thereby favoring its safety, but reduces the final free sugar content and increases the final viscosity of gowé, which are both organoleptic defects of the product.

1. Introduction

Lactic fermentation is a food processing technology that meets the challenges of the specific food security problems encountered in Africa as it can improve food safety and micronutrient availability (Oyewole, 1997; Svanberg and Lorri, 1997). Gowé is a traditional fermented Beninese soft cooked paste made from a blend of malted and non-malted cereals; it is diluted with water/ice and sugar just before consumption as a thirst-quenching drink. Consumers prefer gowé as a slightly sour, but sugary and light textured beverage (Adinsi et al., 2015).

Major variations in the preparation of gowé are observed (Adinsi et al., 2014); the traditional process always includes natural lactic fermentation (24–48 h) of malt/non malted mixture, but it can be performed directly or after a saccharification step (steeping of malt for less than 12 h) and/or a partial pre-cooking on non-malted grain. These variations result in variable quality of the final product (Adinsi et al., 2014); consumers generally prefer a gowé prepared with the saccharification step. After fermentation, lactic acid is the main organic acid that is produced by the microorganisms and glucose is the main free sugar that originates from the hydrolysis of starch. It has thus been shown recently that the inoculation with *Lactobacilli* and/or yeasts directly influence the acidity but also indirectly the sugar content of the resulting gowé (Vieira-Dalodé et al., 2015). This interaction between the acidification process linked to bacterial growth and the sugar profile linked to the activity of the amylases has not been studied yet whereas the balance between the acid and sugary taste is of primary importance for the acceptability of the final product.

The aim of this study was to investigate the starch degradation mechanism in relation with the activity of the degrading enzymes during the fermentation of gowé and the impact of the degradation of starch on the quality (sugar content and viscosity) of the final product. The effect of inoculation and of pre-cooking on starch degradation and on gowé quality was also studied.

2. Materials and methods

2.1. Material

Grains of red sorghum [*Sorghum bicolor*, L. Moench], traditionally used for preparing gowé, were purchased on the local market in

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Cotonou; it was a tannin free cultivar as assessed by ISO procedure (ISO, 1988). Grains were sieved through a 2 mm sieve. Impurities were removed by hand and the grain was stored in a cold room (4 °C) until use.

Raw sorghum flour was prepared by grinding the grains in a Laboratory Mill 3100 (Perten Instruments Hägersten, Sweden) equipped with a 0.5 mm sieve.

2.2. Inoculum preparation

Lactobacillus fermentum strain 7.4 was isolated from a fermented pearl millet gruel, *ben-saalga* (Songre-Ouattara et al., 2008), and stored in MRS broth containing 30% (v/v) glycerol at −80 °C until use. It was grown on MRS broth at ambient temperature for one night and then centrifuged at 10,000 g for 5 min. The pellet was washed with 9 g NaCl in 1 l water, centrifuged again, and then resuspended in 9 g NaCl in 1 l water. The cell concentration was assessed by absorbance at 600 nm.

2.3. Malting

The grains were soaked in deionized water at 30 °C for 15 h. Excess water was drained off and the grains were placed in semi-hermetic plastic boxes and were left to germinate in an air-conditioned cabinet (30 °C, 98% relative humidity) for 72 h. The grains were then dried in a forced air dryer (60 °C, 40% relative humidity) for 2 h. The dried malt (12% water content, wet basis) was then manually degermed and ground in a Laboratory Mill 3100 (Perten Instruments Hägersten, Sweden) equipped with a 0.5 mm sieve.

Inoculated malt was prepared using the same procedure, except that the soaking water was inoculated with *L. fermentum* at 10^6 cells/g. The pH did not vary significantly during steeping with the inoculum; it remained very close to 6.5.

2.4. Preparation of gowé

Several processes were used to prepare the gowé (Table 1):

1. We first followed the traditional procedure described previously (Michodjehoun-Mestres et al., 2005). A mixture of malt (60 g) and raw (30 g) flours was saccharified for 15 h (natural fermentation at 30 °C with a flour: deionized water ratio of 1:1), after which a paste (30 g of raw flour boiled in 300 mL water for 10 min) was added and fermentation was performed at 30 °C for 48 h. The fermented gowé was then boiled for 20 min under constant stirring. This product is hereafter referred to as “natural gowé”;
2. The same procedure was used for inoculated malt, which is hereafter referred to as “IM gowé”,
3. In this procedure, the gowé was inoculated with *L. fermentum* (3 10^6 cells/g) at the beginning of the saccharification step. With a 24 h fermentation step, the product was named “24I gowé” and with a 48 h step, the product was named “48I gowé”;
4. In the last procedure, gowé was prepared without the saccharification step. In this case, the paste was added to the malt directly and the raw slurry and the whole mixture was inoculated with *L. fermentum* at 3 10^6 cells/g; with a 24 h fermentation step, the product was named “24InS gowé” and with a 48-h fermentation step, the product was named “48InS gowé”.

Table 1
Sample processing and sampling conditions.

Sample name	Inoculation (step)	Saccharification duration (h)	Fermentation duration (h)	Sampling time (h)
Natural Gowé	No	15	48	15/22/39/63
IM Gowé	Malting	15	48	15/22/39/63
24I	Saccharification	15	24	15/22/39
48I	Saccharification	15	48	15/22/39/63
24InS	Fermentation	0	24	7/24
48InS	Fermentation	0	48	7/24/48

The six types of gowé (Table 1) were prepared in duplicate. The pH was monitored throughout the process. Samples were taken after 7 h of the beginning of the saccharification step and after 7 h of the beginning of the fermentation step. In addition, samples were collected at 0, 24 and 48 h of fermentation. Total time from the beginning of the process was counted for labeling the samples (Table 1). Dry matter and titratable acidity were measured using fresh samples of gowé and sub-samples were kept at −20 °C for further analyses.

2.5. Biochemical characteristics

Dry matter content was determined using the AACC Method 44-15A (AACC, 1981). Titratable acidity was determined by neutralization with 0.1 M NaOH until a pH of 8.5 was obtained; results were expressed as lactic acid equivalent. Organic acids and sugar contents were determined by HPLC using the procedure described by Michodjehoun-Mestres et al. (2005), except that elution was performed at 35 °C. Alpha- and beta-amylase activities were assessed using Megazyme methods K-Cera 08/05 and K-Beta3 10/10 (Megazyme International, Wicklow, Ireland).

Starch content was determined by gel permeation chromatography using the modified procedure of Vieira-Dalodé et al. (2015). Starch was solubilized by 1 M NaOH at room temperature for 5 days under constant gentle stirring. The suspension was diluted ten times with water and stirred gently for two more days. The solution was then filtered through a 5 μm pore size sieve and 100 μl was injected on a Superdex Peptide 10/300 GL column (GE-Healthcare Bio-Sciences, Uppsala, Sweden). Elution was performed at 0.8 ml/min by 0.05 M NaOH at room temperature. A refractive index detector (RID 6A, Shimadzu, Vitry/Seine, France) was used for detection; starch components were eluted within two peaks close to the void volume and free sugars close to the total volume of the column.

2.6. Rheological measurement

A 28-g sample of fermented cooked gowé was placed in a can in a Rapid Visco Analyzer (Perten Instruments Hägersten, Sweden). The gowé was cooked under continuous stirring (160 rpm) using an RVA super 3 with the following temperature profile: 50 °C for 5 min, heating to 95 °C at 12 °C/min, plateau at 95 °C for 2 min 30 s, cooling down to 50 °C at 12 °C/min and plateau at 50 °C for 3 min. Apparent viscosity was measured at the end of the run.

2.7. Statistical analyses

All chemical analyses were performed in duplicate and rheological measurements in triplicate. Mean values were calculated and ANOVA and mean comparison tests were performed between gowé product types (6) taking into account process duplicates using Statistica 7 (StatSoft, Tulsa, USA).

3. Results

3.1. Malt characteristics

After steeping, the water content was close to 40% (wb) and remained at this level throughout the germination step. Mold