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Fuel ethanol production from commercial grain sorghum cultivars with different tannin content

María Belén Ramírez, Mario Daniel Ferrari, Claudia Lareo*

Depto. Bioingeniería, Facultad de Ingeniería, Universidad de La República, J. Herrera y Reissig 565, CP 11300, Montevideo, Uruguay

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ABSTRACT

The two-step enzymatic starch hydrolysis of three commercial sorghum grain cultivars (Flash 10 Plus, 8419 and Flash 1) containing different tannin content (<0.2%, 1.1% and 2.0% respectively) was evaluated, in order to determine the most promising process for the production of ethanol. High viscosities were obtained for suspensions of the three cultivars (6440–8071 cP). The hydrolysis efficiency values were two and three times greater in the cultivars with higher tannin content (8419 and Flash 1, respectively) when a gelatinization step previous to starch hydrolysis was performed, reaching similar values for the three cultivars (87%–88%); for the low-tannins cultivar a lower improvement was observed. The addition of a protease, pepsin, which could disrupt the interaction between the protein and starch, did not improve the starch hydrolysis efficiency. Simultaneous saccharification and ethanol fermentation (SSF) assays were carried out. The removal of tannins improved the performance of the SSF, suggesting that this treatment may have eliminated inhibitors and also contributed to a partial depolymerization or loss of crystallinity of starch. For a 1:3 solid–liquid ratio, an ethanol concentration of 107 g/L at 68 h, a fermentation efficiency of 100% and an industrial yield of 390 L/t of sorghum (dry basis) were obtained.

1. Introduction

Grain sorghum (*Sorghum bicolor* (L.) Moench) has great potential for the production of bioethanol. It is a cereal rich in starch similar to corn which has important agronomic advantages, such as ability to grow in a wide range of soil types and climates, efficient in water use and drought tolerant. However, limited research has been conducted on its performance for bioethanol since it generally shows relatively lower ethanol yield compared to maize and its lower susceptibility to hydrolysis, especially after heat–moisture treatments (Perez-Carrillo et al., 2012; Zhao, 2008).

It has been reported that the key factors that affect the performance and efficiency of ethanol fermentation of grain sorghum, include starch content, starch digestibility, the amount of extractable proteins, protein-starch interactions, viscosity of the sorghum grain suspensions, content of phenolic compounds (tannins), amylose-amylopectin ratio (du Preez et al., 1985; de Jong et al., 1987; Mullins and NeSmith, 1988; Mullins and Lee, 1991; Wu et al., 2006, 2007; Wang et al., 2008; Yan et al., 2010; Zhao, 2008). Starch and proteins are the major components of sorghum (Vermerris, 2008). The starch–protein interaction reduces the susceptibility of the starch to enzymatic hydrolysis, and thus it affects the conversion efficiency and yield of ethanol (Rooney and Pflugfelder, 1986; Zhao, 2008). The addition of proteases during starch liquefaction of sorghum has been reported to have a positive effect on starch digestibility and bioethanol yields (Perez-Carrillo et al., 2012; Zhang and Hamaker, 1998). Also, the ratio amylose/amylopectin affects the starch hydrolysis: the resistance to the hydrolysis is higher for the cultivars with high amylose content (Wu et al., 2006).

Unlike other major cereals, grain sorghum contains tannins which protects it from both bird and mould attack, increasing its agronomical yield. Tannins interact with proteins, including amylolytic enzymes, via hydrogen bonding coupled with hydrophobic interactions. This association of tannins with proteins has been recognized as having adverse effects on the ethanol conversion rates (de Jong et al., 1987; Mullins and Lee, 1991; Wang et al., 2008; Wu et al., 2007). Sorghum tannin interactions with proteins can be prevented by grain pre-processing steps, such as chemical treatment (Adetunji et al., 2015; Ali et al., 2009; Beta et al., 2000). Zhao (2008) reported that the presence of tannins can produce high viscosities in grain sorghum flour suspension and

* Corresponding author.
E-mail address: clareo@fing.edu.uy (C. Lareo).

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that the sorghum cultivars most difficult to hydrolyse have high viscosity peaks. This fact restricts the use of high ratios of grains to water, which are necessary to achieve a high final ethanol concentration and to reduce energy consumption of distillation. One promising technology which would reduce the manipulation of viscous material and the costs due to the high energy demand of starch-based ethanol production is known as granular starch hydrolysis. In that process, the starch hydrolysis is performed at temperatures below its gelatinization temperature (Robertson et al., 2006; Cinelli et al., 2015).

Therefore, sorghum cultivars with higher product yields either starch or grain per hectare, not necessarily produce higher ethanol yields per amount of raw material processed. The industrial performance also depends on the bioavailability of starch, its features, its interaction with other components of the grain and the presence of inhibitors (Zhao et al., 2008, 2009).

In this work, commercial cultivars of grain sorghum, which differ in their content of tannins, were studied in order to determine the most promising process for the production of ethanol. The two-step enzymatic starch hydrolysis and simultaneous saccharification and ethanol fermentation (SSF) were evaluated. The effect of tannin removal on the fermentation performance was also studied.

2. Materials and methods

2.1. Materials

Three commercial sorghum cultivars with different tannin content were used in this study: low, ~0% (Flash 10 plus); medium, ~1% (8419); and high, ~2% (Flash 1). Sorghum grains were provided by INIA (La Estanzuela, Colonia, Uruguay). They were ground using a Bühler Miag disc mill (DLFV) to a mean particle size of 0.5 mm. Table 1 presents their composition.

2.2. Enzymes

The enzymes used were, α-amylase E-BLAAM100 (3000 U/mL), amyloglucosidase E-AMGDF100 (AMG) (3260 U/mL) from Megazyme (Ireland), and pepsin from Sigma–Aldrich (601 U/mg).

2.3. Starch hydrolysis studies

The sorghum suspension was prepared in a 250 mL Erlenmeyer flask with 29 g of flour and 130 mL of distilled water. The pH was adjusted to 6.0 ± 0.1. The liquefaction was initiated by the addition of 100 or 200 μL of α-amylase (5.7 and 11.4 μL/g of sorghum flour in dry base, respectively). The suspension was maintained at 75 °C during 180 min under agitation. After the starch liquefaction step, the suspension was cooled to 60 °C and the pH adjusted to 4.5 ± 0.1. Saccharification was conducted by adding 200 or 400 μL (11.4 and 22.8 μL/g, respectively) of amyloglucosidase (AMG). The suspension was maintained at 60 °C under agitation. Samples were taken at different times and the enzymatic reaction was stopped with 40% trichloroacetic acid or NaOH 1 M and immersion in an ice batch as described by Lareo et al. (2013) before to the sugar content determination. At least 2 replications of each assay were performed.

For the assays where a gelatinization step was performed before the liquefaction, the suspension was heated to 95 °C during 15 min under agitation, then cooled to 75 °C and α-amylase was added following the procedure described above.

2.4. Protease treatment

The sorghum suspension was prepared in a 250 mL Erlenmeyer flask with 29 g of flour and 130 mL of distilled water. The pH was adjusted to 2.0 ± 0.1, and 1 mL of pepsin (20 mg/mL) was added (1.1 mg of enzyme per gram of dry flour). It was incubated at 37 °C during 2 h. The pH was adjusted to 6.0 ± 0.1. The liquefaction was initiated by the addition of 200 μL of α-amylase (11.4 μL/g of sorghum flour in dry base, respectively). It was maintained at 75 °C for 90 min under agitation. After the starch liquefaction step, the suspension was cooled to 60 °C and the pH adjusted to 4.5 ± 0.1. Saccharification was conducted by adding 400 μL (22.8 μL/g) of AMG. The suspension was maintained at 60 °C under agitation and 150 rpm. Samples were taken at different times and the enzymatic reaction was stopped with 40% trichloroacetic acid or NaOH 1 M and immersion in an ice batch. Control assays without addition of pepsin were performed. The assays were carried out in duplicate.

For the assays where a gelatinization step was performed after the pepsin treatment and previous to the liquefaction step, the suspension was heated to 95 °C during 15 min under agitation, then cooled to 75 °C and α-amylase was added following the procedure described above.

2.5. Tannin removal

Two methods for tannin removal of whole sorghum grains were carried out: deactivation by formaldehyde (Daiber and Taylor, 1982) and alkaline treatment (du Preez et al., 1985). For both methods a solid:liquid ratio of 1:1 was used. For the first method, the material was steeped with formaldehyde 0.04% for 6 h at room temperature. For the second method, the material was steeped with NaOH 1%, for 30 min at 40 °C and 100 rpm. After the treatments the material was washed with distilled water, dried at 50 °C for 16 h, and milled using a Bühler Miag disc mill (DLFV). Control assays were performed steeping the sample with distilled water under the same experimental conditions. The assays were carried out in duplicate.

2.6. Simultaneous saccharification and fermentation (SSF)

Dry commercial baking yeast, *Saccharomyces cerevisiae* (Fleischmann) was used in fermentation assays. The inoculum was prepared in 500 mL Erlenmeyer flasks with 100 mL medium containing: 100 g/L glucose, 3 g/L yeast extract, 3 g/L malt extract and 5 g/L peptone. The pH was adjusted to 4.5 ± 0.1. It was incubated in an orbital shaker at 30 °C, 150 rpm for 10–12 h.

The sorghum suspensions were prepared in 250 mL Erlenmeyer

Table 1
Composition of sorghum cultivars.

Cultivar	Moisture (%)	Starch (% w/w db)	Amylose (% w/w db)	Protein (% w/w db)	Tannin (% w/w db)*	Ash (% w/w db)
Flash 10 Plus	11.46 ± 0.06	70.7 ± 0.9	11.5 ± 1.7	11.7 ± 0.5	⟨0.2	1.20 ± 0.00
8419	12.79 ± 0.02	68.4 ± 0.8	14.4 ± 0.9	11.0 ± 0.5	1.06 ± 0.02	1.34 ± 0.04
Flash 1	12.36 ± 0.04	68.0 ± 0.4	14.4 ± 1.0	12.1 ± 0.5	1.96 ± 0.24	1.24 ± 0.01

± Standard deviation of duplicates or triplicates.
Free simple soluble sugars (glucose + fructose + sucrose + maltose) were not detected (<0.5% w/w db).
*The tannin content is expressed as equivalents of catechin, detection limit 0.2%.