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The influence of sorghum grain decortication on bioethanol production and quality of the distillers' dried grains with solubles using cold and conventional warm starch processing

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HIGHLIGHTS

- Decortication increased cold starch processing productivity of sorghum grain.
- Nutrient losses from decortication resulted in decreased ethanol yield.
- Cold process enzymes requirements were decreased by 11.7% using decorticated grains.
- DDGS from cold processing exhibited decreased neutral and acid detergent fibre.

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ABSTRACT

Very high gravity hydrolysis-fermentation of whole and decorticated sorghum grains were compared using conventional and cold hydrolysis methods to assess the extent by which decortication could minimize enzymes dosages and affect the quality of the distillers' dried grains with solubles (DDGS). All processing configurations achieved ethanol concentrations between 126 and 132 g/L (16.0–16.7% v/v), although decortication resulted in a decreased ethanol yield. Decortication resulted in a decreased volumetric productivity during warm processing from 1.55 to 1.25 g L^{-1} h^{-1}, whereas the required enzyme dosage for cold processing was decreased from 250 to 221 μl/100 g_{starch}. Cold processing decreased the average acid detergent fibre (ADF) from 35.59% to 29.32% and neutral detergent fibre (NDF) from 44.04% to 32.28% in the DDGS compared to the conventional (warm) processing. Due to lower enzyme requirements, the use of decorticated grains combined with cold processing presents a favourable process configuration and source of DDGS for non-ruminants.

1. Introduction

With bioethanol currently produced mainly from food crops (Demirbas, 2011), the use of dedicated non-food energy crops such as sorghum grain is believed to be a promising alternative (Sanchez and Cardona, 2008). Particularly in water scarce regions (Rooney et al., 2007) such practices would contribute to job creation through energy crops cultivation (Department of Minerals and Energy, 2007).

The conventional (warm) process for conversion of starches in cereal grains to ethanol involves the liquefaction, saccharification and fermentation of the starch slurry (Bothast and Schlicher, 2005). Liquefaction, typically performed at temperatures around 90 °C to gelatinize the starch making it hydrolysable by conventional amylases, requires the equivalent of 10–20% of the energy content of ethanol produced (Gray et al., 2006; Robertson et al., 2006).

An alternative strategy to reduce the energy demand of the current conventional process is the low temperature (cold) hydrolysis process. It involves the application of a mild heat treatment, below the gelatinization temperature of the starchy material, in combination with the utilization of raw starch hydrolysing enzymes (RSHE), capable of hydrolysing non-gelatinized (uncooked, granular) starch (Van Zyl et al., 2012). Apart from the reduced energy requirement, another benefit is the prevention of nutrient degradation during high temperature liquefaction through the Maillard reactions (Galvez, 2005).

The bran from sorghum grain is known to contain components such as tannins and flavonoids that are inhibitory to amylases (Awika and Rooney, 2004; Sales et al., 2012). As these components

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are located in the bran, their removal from the grain through a decortication process can significantly reduce their content in the starch slurry for hydrolysis-fermentation (Awika et al., 2005), with possible benefits such as a reduction in the required enzyme dosages. Furthermore, a conversion process with an initial decortication step prior to milling will benefit from the lower viscosities and peak viscosities of the mashes (Wu et al., 2007), and higher protein contents of distillers' dried grains with solubles (DDGS), the main co-production of cereal-ethanol plants (Corredor et al., 2006).

The removal of the fibrous seed coat of cereal grains, known as debranning, dehulling or decortication, depending on the type of grains, has been studied extensively (Sikwese and Duodu, 2007; Wang et al., 1999). The effect of bran components on starch hydrolysis by amylases has also been described (Sales et al., 2012; Alvarez et al., 2010). However, very few studies have described the effect of debranning/decortication on the required enzyme dosages and performance of the ethanol production, particularly in very high gravity (VHG) slurries. The relationship between debranning and the final ethanol concentration in fermentation has been investigated (Corredor et al., 2006; Perez-Carrillo et al., 2008), although VHG fermentations, cold processing and ethanol productivities were not considered.

The dried distillers' grains with solubles (DDGS) have become a feed ingredient of choice in ruminant production systems because they contain proteins in adequate amounts, energy and important ions (phosphorus, calcium) (Singh et al., 2005). The continuous increase in first generation bioethanol production from cereals grains is expected to result in increasingly larger amount of DDGS being available (Liu, 2011) which could lead to an overproduction of DDGS. Hence diversifying the market for DDGS has become an important issue.

The combination of sorghum decortication with cold hydrolysis processing, as means to improve the DDGS quality, has not been studied extensively. Corredor et al. (2006) have shown that 10% decortication of sorghum grains can increase the protein content of DDGS by up to 8%, while the concentrations of the phosphorus and calcium minerals were not significantly altered. The proportion of other important fibres such as neutral detergent fibre (NDF) and acid detergent fibre (ADF) in DDGS, which are good indicators of the amount of forage for ruminant and fibre digestibility for non-ruminants respectively, were not determined in that study (Corredor et al., 2006). In the animal nutrition system, a lower ADF content is preferred, particularly for non-ruminants, since this relates to the amount of least digestible fibre. NDF is of particular importance for ruminants, since it includes ADF as well as fibres that are digestible to them (Saha et al., 2010).

In this work, the effect of decortication on ethanol concentration, ethanol volumetric productivity and ethanol yield was assessed when using the conventional (warm) high temperature process and the cold conversion process. A response surface methodology was used to model the effect of variables on the desired responses, with experimental validation of the predicted optimum parameters, allowing comparison of different process configurations. The quality of the DDGS as affected by decortication and enzymatic hydrolysis methods (conventional and cold) was also assessed.

2. Methods

2.1. Raw materials

White sorghum grain (68% starch; w/w) was obtained from Agricol (Pty) Ltd (Brakenfell, Cape Town, South Africa). The grains were air-dried for 3 days, vacuum packed and stored at room temperature until needed. The moisture content of the stored grains was 8% (w/w). Before usage, the grains were milled using a Retsch mill (SM 100, Haan, Germany) to pass through a 0.5 mm and 2 mm screen for the cold and warm processing respectively. Sorghum grains were decorticated using a modified rice miller tester 65 (Grainman Corp, Miami, Florida, USA). Before decortication the grains were conditioned at room temperature (22 °C) to 16% moisture for 10 min. Grains were decorticated in batches of 500 g for 20 s. The starch content of decorticated grains was 73% (w/w).

For DDGS production, two sorghums varieties were compared. PAN 8816 obtained from Pannar Seed (Pty) Ltd (Greytown, South Africa) referred to as Sorghum 01 in this work. The second variety, referred to as Sorghum 02 in this work, is the one described in the previous paragraph.

2.2. Enzymes, microorganisms and reagents

Enzymes used for the conventional (high temperature) process were thermo-stable α-amylase from *Bacillus licheniformis*, called Termamyl SC (Novozymes, Bagsvaerd, Denmark) with a declared enzyme activity of 120 KNU/g (KNU, kilo novo units α-amylases – the amount of enzymes which breaks down 5.26 g of starch per hour at 37 °C, pH 5.6, 0.0043 M Ca^{2+}, reaction time 7–20 min) and the glucoamylase Saczyme (Novozymes), with a declared activity of 750 AGU/g (AGU, amyloglucosidase units – the amount of enzyme that catalyses the conversion of one μmol of maltose per minute at assay conditions of 37 °C, pH 5.0, substrate concentration 10 mg/ml, incubation time 30 min). For the cold process, acid stable α-amylase GC626 (Genencor, California, USA) and enzyme cocktail Stargen 002 (Genencor) were used. Stargen 002 is a blend of alpha- and gluco-amylases with declared activity 570 GAU/g (GAU, glucoamylase unit – the amount of enzyme that liberates one gram of glucose per hour from soluble starch at 60 °C, pH 4.2).

Ethanol red dry yeast (LEAF Technologies, Marcq-en-Baroeul, France) was used for the fermentation. The inoculum was freshly prepared for each fermentation. Ethanol red dry yeast (2.5 g) was rehydrated in 50 mL of 2% glucose solution at 33 °C for 25 min in a shaking incubator at 100 rpm. One mL of the broth was added to each flask as inoculum. Urea and calcium chloride for fermentation supplementation were obtained from Sigma–Aldrich (St. Louis, Missouri, USA).

2.3. Mash preparation and SSF procedures

Experiments were performed in pre-weighed 250 mL flasks using sorghum grain milled to pass through a 2 mm screen. Thirty five grams of whole sorghum grain flour (32.6 g for decorticated grains to maintain the same starch loading) was mixed with water and calcium chloride (5 mg/100 g slurry) to achieve final mass slurry of 100 g. The pH of the slurry was adjusted to 5.8 with 1 M H_2SO_4, before the adequate amount of α-amylase (Termamyl SC) was added (Table 1). The slurry was heated to 88 °C in a water bath for the required time (Table 1). An overhead stirrer was used to ensure adequate mixing throughout liquefaction. At the end of liquefaction, the mash was cooled to 30 °C by placing the flasks in water at ambient temperature, before the addition of the adequate

Table 1
Factors used in the central composite design and their levels using the conventional and cold processes.

	Factors	Levels		
		−1	0	1
Conventional process	Liquefaction time (min)	90	120	150
	α-amylase dosage (μl/100 g_{starch})	29	58	87
	G-amylase dosage (μl/100 g_{starch})	57	96	135
Cold process	Pre-saccharification time (min)	30	60	90
	Stargen dosage (μl/100 g_{starch})	128	256	384