



Sorghum flour fractions: Correlations among polysaccharides, phenolic compounds, antioxidant activity and glycemic index



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ABSTRACT

Nutrients composition, phenolic compounds, antioxidant activity and estimated glycemic index (EGI) were evaluated in sorghum bran (SB) and decorticated sorghum flour (DSF), obtained by a rice-polisher, as well as whole sorghum flour (WSF). Correlation between EGI and the studied parameters were determined. SB presented the highest protein, lipid, ash, β-glucan, total and insoluble dietary fiber contents; and the lowest non-resistant and total starch contents. The highest carbohydrate and resistant starch contents were in DSF and WSF, respectively. Phenolic compounds and antioxidant activities were concentrated in SB. The EGI values were: DSF 84.5 ± 0.41 ; WSF 77.2 ± 0.33 ; and SB 60.3 ± 0.78 . Phenolic compounds, specific flavonoids and antioxidant activities, as well as total, insoluble and soluble dietary fiber and β-glucans of sorghum flour samples were all negatively correlated to EGI. RS content was not correlated to EGI.

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1. Introduction

Diabetes has increased rapidly in recent years. In 2013 the diabetes prevalence was 382 million people, and this number is expected to rise to 592 million by 2035 (Guariguata et al., 2014). Moreover, diabetes has been strongly associated to obesity and carbohydrate intake (Bray & Popkin, 2014; Cheng et al., 2013). Owing to an improper balance of glucose homeostasis, the glycemic raising capability of foods is an efficient dietary approach for maintaining a healthy weight and preventing chronic illness such as diabetes (Brand-Miller, Stockmann, Atkinson, Petocz, & Denyer, 2009). High glycemic index (GI) foods cause a rapid and large release of glucose and are associated with increased risk of diabetes, whereas foods with low GI contain slowly digested carbohydrates and cause slower and lower increase of the blood glucose level (Bhupathiraju et al., 2014; Brand-Miller et al., 2009).

In this context, sorghum grains (*Sorghum bicolor* (L.) Moench) have been reported to have lower starch digestibility than maize and other cereals (Ezeogu, Duodu, & Taylor, 2005). Studies with

sorghum phenolic extracts and sorghum products have assigned the low starch digestibility with types and content of phenolic compounds. Sorghum genotypes with high phenolic and tannin levels are associated to enzyme inhibition and starch molecule interaction. These facts impair starch digestibility, increase resistant starch and decrease GI of foods (Lemlioglu-Austin, Turner, McDonough, & Rooney, 2012; Mkandawire et al., 2013; Siller, 2006).

Sorghum grains decortication is an abrasive method to remove the grain's pericarp, testa layers and part of the germ. Phenolics compounds and dietary fiber are concentrated in the bran, whereas starch is the main component in grits (endosperm). The distinct composition between both products guides sorghum bran to be used as a functional ingredient to enhance the consumption of healthy food, while decorticated grains produce lighter products to attend other food industry fields (Aboubacar, Yazici, & Hamaker, 2006; Awika, McDonough, & Rooney, 2005; Buitimea-Cantúa et al., 2013; Dlamini, Taylor, & Rooney, 2007).

However, an efficient separation is required to ensure the healthy and economic use of sorghum fractions, such as the knowledge about the nutrient and phenolics distribution and how this processing could affect the estimated glycemic index of sorghum flour fractions. Therefore, this study aimed to evaluate sorghum

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bran and decorticated sorghum flour, obtained by a rice-polisher, as well as whole sorghum flour for nutrient composition, phenolic compounds and antioxidant activity. Furthermore, the estimated glycemic index was correlated with polysaccharides, phenolic compounds and antioxidant activity of the three samples.

2. Materials and methods

2.1. Sorghum sample

Sorghum genotype SC 21 with brown pericarp and pigmented testa was selected among 100 genotypes of the Embrapa Maize and Sorghum panel, due to its anthocyanin and tannin contents. The grains were grown in the experimental field of Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil, in February 2012. The experimental plots were composed of two rows of three meters, with spacing of 0.50 m between rows. The fertilization at planting consisted of the application of 300 kg/ha of formulated 08-28-16 (NPK). After 25 days of planting, fertilization with 50 kg ha⁻¹ nitrogen was performed.

After harvesting, the sorghum grains were dried in an oven at 40 °C (Famen, Model 315 SE) overnight, selected manually and sieved to remove dirt and impurities.

2.2. Sorghum flour, decortication and particle size distribution

Whole sorghum flour (WSF) was obtained by grinding cleaned grains in a laboratory impact mill (Marconi MA 630/1) and passing through a 0.850 mm sieve (Moraes et al., 2012). Sorghum bran (SB) and decorticated grains flour (DSF) were obtained by decorticating process. Approximately 100 g were decorticated in a rice-polisher (Zaccaria, Modelo PAZ 1 DTA) for 4 min according to Awika et al. (2005). After processing, collected decorticated grains were milled and sieved in a 0.850 mm sieve and the bran was similarly collected and sieved. The percent of decorticated grains, bran removal and processing loss were calculated. The samples were kept at -20 °C until analyses.

Particle size distribution of the sorghum samples was calculated using #14, 20, 35, 60, 80 and 100 ABNT standard sieves (1.4; 0.85; 0.5; 0.25; 0.18 and 0.15 mm, respectively) and 50 g sample size. Results were reported as percentage retained on each sieve. Measurements for each sample were made in triplicate.

2.3. Proximate composition

Protein content was determined by the semi-micro Kjeldahl method and ash was quantified by means of sample incineration in a muffle furnace as described by the Association of Official Analytical Chemists (2002). Total lipids were determined by the Bligh and Dyer (1959) method, and moisture in an oven at 105 ± 1 °C. Soluble and insoluble dietary fiber were performed in accordance with the enzymatic gravimetric method (AOAC, 2002). Total dietary fiber was obtained by summing the soluble and insoluble dietary fiber. The available carbohydrate content was calculated by difference using the equation: 100 - (moisture + protein + lipid + ash + dietary fiber). The caloric value of samples was calculated using the Atwater conversion factors: 9 kcal per gram of lipid, 4 kcal per gram of carbohydrate and 4 calories per gram of protein.

2.4. Polysaccharides (resistant starch, non-resistant starch, total starch and β-glucan)

Resistant starch was measured using the assay kit from Megazyme (AACC Method 32-40.01) (AACC, 2010). Samples were

weighed out into screw cap tubes and sodium maleate buffer (100 mM; pH 6.0), containing pancreatic and amyloglucosidase (3 U mL⁻¹), was added. Samples were incubated for 16 h at 37 °C with agitation. The tubes were then treated with 50% ethanol and centrifuged for 10 min. The supernatants were decanted and the pellets re-suspended in 50% ethanol. This process was repeated twice and the pellets treated with potassium hydroxide (2 M) in an ice bath with stirring for 20 min. Sodium acetate buffer (1.2 M; pH 3.8), followed by amyloglucosidase (3300 U mL⁻¹) were added and the tubes incubated for 30 min at 50 °C. Aliquots (0.1 mL) were removed and treated with GOPOD reagent. These were incubated at 50 °C for 20 min and the absorbance read at 510 nm, against a reagent blank. Glucose solution (10 mg mL⁻¹) was used as standard.

Non-resistant starch was determined in the combined supernatant solutions obtained by centrifugation of the initial incubation and the supernatants obtained from the subsequent two 50% ethanol washings. The volume was adjusted to 100 mL with sodium acetate buffer (100 mM; pH 4.5). An aliquot (100 μL) of this solution was incubated with 10 μL of dilute amyloglucosidase solution (300 U mL⁻¹) in sodium maleate buffer (100 mM; pH 6.0) for 20 min at 50 °C. The reaction mixture was incubated with GOPOD for a further 20 min at 50 °C and the absorbance read at 510 nm against a reagent blank. Total starch was calculated by the sum of resistant starch and non-resistant starch.

β-Glucan content was determined using the assay kit from Megazyme (AACC Method 32-23.01) (AACC, 2010). Samples were treated with 50% (v/v) ethanol/water, then sodium phosphate buffer (20 mM; pH 6.5) was added and the samples incubated in a boiling water bath. The tubes were equilibrated at 50 °C, lichenase (50 U mL⁻¹) was added, and then tubes were further incubated for 60 min at 50 °C. Acetate buffer (200 mM; pH 4.0) was added and the tubes centrifuged for 10 min, after which aliquots were removed and treated with β-glucosidase (2 U mL⁻¹) for a further 10 min. The reaction mixture was incubated with GOPOD for 20 min. The absorbance was read at 510 nm against a reagent blank. All analyses were conducted in triplicates.

2.5. Sample extraction of total phenolic, flavonoids, anthocyanins and antioxidant assays

Samples of 0.5 g were extracted in 10 mL of 1% HCl/methanol (v/v) for 2 h under mechanical shaking at low speed (Marconi®) for total phenolic, flavonoids, anthocyanins and antioxidant determinations. Samples were then stored at -20 °C in the dark, overnight, to allow diffusion of compounds from the cellular matrix. Samples were then equilibrated at room temperature and centrifuged at 2790g for 10 min. Sample residues were rinsed with two additional 10 mL volumes of solvent with shaking for 5 min, centrifuging at 2790g for 10 min. The three aliquots were mixed and stored at -20 °C in the dark until analysis within 24 h (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003).

For condensed tannin assay, samples (0.2 g) were extracted for 20 min in 8 mL of 1% HCl/methanol (v/v) at 30 °C in a water bath shaker (Marconi®). Extracts were then centrifuged at 805g for 4 min and the supernatant was used for the assay (Herald, Gadgil, Perumal, Bean, & Wilson, 2014). All extractions and analyses were conducted in triplicates.

2.6. Colorimetric phenolics determination

Determination of total phenolic content of samples was performed using the Folin-Ciocalteu method as described by Singleton, Orthofer, Lamuela-Raventós, and Lester (1999). The samples and gallic acid standard curve (0.016–0.1 mg gallic acid mL⁻¹; $y = 3.0161x - 0.0216$; $R^2 = 0.9915$) were read at

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