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Effects of processing with dry heat and wet heat on the antioxidant profile of sorghum

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ABSTRACT

The effects of domestic processing with dry heat (*F2*-oven/milling; *F3*-milling/oven; *F4*-microwave oven/milling; *F5*-milling/microwave oven; *F6*-popped grains/milling) and wet heat (*F7*-cooking in water/drying/milling) on the antioxidant profile of sorghum flours (*F1*-raw flour) were evaluated. 3-Deoxyanthocyanidins and total phenolic compounds were stable to dry heat (retention between 96.1% and 106.3%) and reduced with wet heat. All processing with dry heat increased the vitamin E content (2,201.9–3,112.1 μg/100 g) and its retention, and reduced the carotenoids (4.78–17.27 μg/100 g). The antioxidant activity in processed flours with dry heat remained constant (*F3* and *F6*) or increased (*F2*, *F4* and *F5*) and decreased after processing with wet heat. Overall, the grains milled before processing in oven and in microwave oven retained more vitamin E and less carotenoids than those milled after these processing. In conclusion, dry heat did not affect the phenolic compounds and 3-deoxyanthocyanidins of sorghum, but increased the vitamin E and antioxidant activity, and reduced the carotenoids. The wet heat processing reduced all antioxidant compounds except carotenoids, which increased.

1. Introduction

Sorghum (*Sorghum bicolor* L.) is the fifth most cultivated cereal in the world (Waniska & Rooney, 2000). It is one of the most drought-resistant cereals and, due to the increasing world population and the decreasing availability of water, it represents an important crop for future use (Taylor, Schober, & Bean, 2006). In countries of Africa, Asia and Central America, sorghum is a staple food of the human feeding (Taylor, Schober, & Bean, 2006; Waniska & Rooney, 2000). However, it is underutilized in countries like the United States, Australia and Brazil, and is used mainly for animal feeding (Taylor, Schober, & Bean, 2006; Waniska & Rooney, 2000).

The sorghum grain is formed by the pericarp (outer covering), testa (layer between the pericarp and endosperm), the endosperm (storage tissue) and the germ (embryo) (Slavin, 2004; Waniska & Rooney, 2000). Overall, there are some pigments (carotenoids and anthocyanins) and phenolic compounds (phenolic acids, flavonoids and tannins) in the pericarp and testa of the grain (Slavin, 2004; Waniska & Rooney, 2000). There are also starch, proteins, minerals and B-complex vitamins in the endosperm, and lipids and some fat-soluble vitamins in the germ (Slavin, 2004; Waniska & Rooney, 2000).

Sorghum is an important source of bioactive compounds such as 3-deoxyanthocyanidins, tannins, vitamin E, carotenoids, and other antioxidants (Awika & Rooney, 2004; Kean, Bordenave, Ejeta, Hamaker, & Ferruzzi, 2011; Martino et al., 2012). These compounds reduce the action and damage caused by free radicals and thus promote benefits to human health (Valko et al., 2007).

However, like other cereals, sorghum grains need to be processed before human consumption, which may modify their chemical composition, and functional and nutritional value. Studies have demonstrated the effects of processing traditionally used in African and Asian countries (fermentation, germination and soaking) on some antioxidant compounds present in sorghum (Afify, El-Beltagi, El-Salam, & Omran, 2012; Jood, Khetarpaul, & Goyal, 2012; Rahman & Osman, 2011). However, few studies evaluated the effects of domestic processing that reflect the Western culture, such as heat treatment in conventional oven, cooking in water, and popped grains (Dlamini, Taylor, & Rooney, 2007; Moraes, 2011; Wu, Huang, Qin, & Ren, 2013), and the effects of microwave oven processing is unknown. Moreover, studies conducted so far evaluated a reduced number of bioactive compounds.

This study evaluated the effects of domestic processing with dry heat (cooking in conventional oven, microwave oven and popped

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grains) and wet heat (cooking in water) on the content of phenolic compounds, 3-deoxyanthocyanidins, vitamin E, carotenoids and antioxidant activity of sorghum.

2. Materials and methods

2.1. Raw sorghum

Sorghum with red pericarp, white endosperm and without tannin (BRS 310 genotype) developed by Brazilian Company of Agricultural Research (*EMBRAPA*)-Maize and Sorghum (Sete Lagoas, Minas Gerais, Brazil) was used in this study.

2.2. Standards and reagents

The standards of carotenoids (lutein and zeaxanthin), luteolinidin chloride, gallic acid and trolox were obtained from Sigma–Aldrich (St. Louis, MO, USA). The apigeninidin chloride was obtained from Chromadex (Santa Ana, CA, USA) and vitamin E standards (α, β, γ and δ-tocopherols and tocotrienols) were purchased from Calbiochem®, EMD Biosciences, Inc. (USA).

For the extraction of carotenoids, vitamin E, 3-deoxyanthocyanidins (3-DXAs) and total phenolic compounds (TPC), it was used analytic grade reagents purchased from VETEC (São Paulo, Brazil). For analysis of these compounds and antioxidant activity, it was used HPLC grade reagents (methyl alcohol, ethyl acetate, acetone, acetonitrile, hexane, isopropyl alcohol, acetic acid and formic acid) purchased from Tedia (São Paulo, Brazil) and analytical grade reagents (2,2-diphenyl-1-picrylhydrazyl radical and Folin–Ciocalteu) obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Preparation of sorghum flours

Seven types of sorghum flours were obtained using processing based on previous studies (Dlamini, Taylor, & Rooney, 2007; Moraes, 2011; Wu, Huang, Qin, & Ren, 2013). The processing varied according to the type of heat treatment (in conventional oven, microwave oven, popped grains and cooking in water) and the moment of the milling of the grains (before or after the heat treatment), as presented below:

(*F1*) *Raw flour*: milling of the grains.

(*F2*) *Oven/milling*: heat treatment of the grains in an conventional oven (121 °C, 25 min) followed by milling.

(*F3*) *Milling/oven*: previous milling of the grains followed by heat treatment of the flour in an conventional oven (121 °C, 25 min).

(*F4*) *Microwave oven/milling*: heat treatment of the grains in a microwave oven (middle power, 4 min) followed by selection of not popped grains (90% of the total) and milling of the selected grains.

(*F5*) *Milling/microwave oven*: milling of the grains followed by heat treatment of the flour in a microwave oven (middle power, 4 min).

(*F6*) *Popped grains/milling*: popped grains in a conventional popper followed by selection of popped grains (85% of total) and milling of the selected grains.

(*F7*) *Cooking in water/drying/milling*: cooking of the grains in 450 mL of water (100° C, 25 min) in a covered pot followed by drying of the grains in an oven (50° C, 180 min) until they presented moisture lower than 15% (Brasil, 1996) and milling of the dried grains.

The grains were milled in an analytical mill micro-rotor (Marconi, MA 090, Brazil) with the sieve of 850 mesh. Once obtained through the previously described processing, the moisture of the flours was determined by gravimetry after oven drying (Nova Etica, 4000, Brazil) at 105 °C. Next, the fresh flours were packed in polyethylene bags and stored in a freezer until analyzes.

2.4. Optimization of method for the extraction of antioxidant compounds in sorghum

The extracts preparation for the determination of TPC and antioxidant activity in sorghum flours was optimized. For this step, it was used only the raw sorghum flour.

2.4.1. Effect of extractor mean

The extractor mean able to extract the greatest possible amount of antioxidant compounds was determined using a system of sequential extraction composed by three extractor means of different polarities (ether mean: ethyl ether; alcoholic mean: ethanol; and aqueous mean: water) (Moreira & Mancini-Filho, 2003) (Fig. 1A). All the extracts were prepared by adding 1 part of milled sorghum to 20 parts of extractor (1:20, w/v). This suspension was stirred (180 rpm, 2 h) and centrifuged (2,790*g*, 15 min). Then, the supernatant (extract) was completed with the extractor to 25 mL, in a volumetric flask (Moreira & Mancini-Filho, 2003).

2.4.2. Effect of the extraction time

The lowest time able to extract the greatest possible amount of antioxidant compounds (ideal extraction time) was determined using the best extractor mean selected in the previous step (ethanol) and other extractor means similar to it and cited in literature (methanol, hydro-methanol and hydro-ethanol). The tested extraction times ranged from 1 to 8 h (Fig. 1B).

The extracts preparation was performed using the extraction protocol described in the item 2.4.1 (Moreira & Mancini-Filho, 2003). The TPC and antioxidant activity of the extracts obtained in the optimization steps (2.4.1 and 2.4.2) were determined using the methodologies described in the items 2.5.1 and 2.5.2.

2.5. Total phenolic compounds and antioxidant activity of processed sorghum flours

2.5.1. Extracts preparation

The extracts preparation was performed using the extractor and extraction time selected in item 2.4 and using the extraction protocol described in the same item (Moreira & Mancini-Filho, 2003).

2.5.2. Determination of total phenolic compounds

The TPC were determined using the Folin–Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventós, 1999). Aliquots of 0.5 mL of the extract, added of 0.5 mL of sodium carbonate 7.5% and 0.5 mL of Folin–Ciocalteu (diluted to 20% in water) were stirred in vortex and incubated at room temperature (30 min). Then, the absorbance was read in a spectrophotometer (Thermo scientific, Evolution 60S, USA) at 765 nm. The quantification was performed by standard curve obtained by the reading of the absorbance of solutions of gallic acid with different concentrations (0.01–0.10 mg/mL; $y = 24.888x + 0.0246$; $R^2 = 0.996$). The results were expressed in mg of gallic acid equivalents per gram of flour (mg GAE/g).

2.5.3. Determination of the antioxidant activity

The antioxidant activity was determined using the DPPH method (1,1-diphenyl-2-picrylhydrazil radical) (Bloor, 2001). Aliquots of 0.1 mL of the extracts obtained in item 2.5.1 were added of 1.5 mL of methanol solution of DPPH 100 μmol, followed by manual shaking for 1 min. After 30 min of rest, the absorbance was read in a spectrophotometer at 517 nm.

A standard curve was constructed using 50–100 μmol/L of trolox solutions ($R^2 = 0.9975$). The antiradical activity (%) was