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Effects of processing on phytochemical profiles and biological activities for production of sorghum tea

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ABSTRACT

The present study was undertaken to assess the changes of phytochemical profiles, antioxidant, α-glucosidase and α-amylase inhibitory activities of sorghum grain during the processes of sorghum tea production. Significant ($p<0.05$) changes of total phenolics (TPC), total flavonoids (TFC) and procyanidins (PAC) contents were found in sorghum grains during soaking, steaming and roasting processing. Significant ($p<0.05$) increases of ferulic (free) and *p*-coumaric acid (bound) were present in sorghum upon steaming processing. Roasting processing (150 °C, 1 h) caused significant ($p<0.05$) increases in phenolic acids, TPC, TFC and PAC compared with the soaking and steaming stages. Accompanied with the changes of phytochemicals of sorghum grain, there were complex changes of biological activities during the successive processes. Our study also showed that there were positive linear correlations between TPC, TFC, PAC and bioactivities of sorghum grain, however, PAC has the strongest correlation (0.979, 0.968 and 0.912, respectively, $p<0.0001$) with DPPH radical-scavenging activity, α-glucosidase and α-amylase inhibitory activities.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), the fifth leading cereal crop in the world, contains a higher content of polyphenols than wheat, barley, millet or rye (Ragaee, Abdel-Aal, & Noaman, 2006). Sorghum is typically consumed with the husk, bran, and germ fractions removed, however, the phenolic compounds are concentrated in the outer layers of the sorghum grain (pericarp and testa) (Beta, Rooney, Marovatsanga, & Taylor, 2000). There are high amounts of dietary fiber and bioactive phytochemicals, such as procyanidins, 3-deoxyanthocyanins, phytosterols, policosanols and phenolic acids in the whole sorghum grain (Awika, Mcdonough, & Rooney, 2005; Dykes, Peterson, Rooney, & Rooney, 2011). Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids. Ferulic, caffeic, *p*-coumaric, protocatechuic, *p*-coumaric, chlorogenic, sinapic and vannilic acids are commonly found in whole grains (Mattila, Pihlava, & Hellström, 2005).

Epidemiological studies have shown that increased consumption of whole grains and their products is associated with reduced risk of developing chronic diseases, such as cardiovascular disease, type II diabetes, obesity, and cancer (Awika & Rooney, 2004; Middleton, Kandaswami, & Theoharides, 2000; Okarter & Liu, 2010; Shetty, 1997). The recent evidence suggests that the complex mixture of bioactives in whole foods may be more healthful than individual isolated components (Brennan, 2009; Harris & Kris-Etherton, 2010; Liu, 2004). The health benefits have been attributed in part to the unique phytochemicals of whole grains (Liu, 2007). Sorghum tea produced by the whole sorghum grains maybe popular as one of healthy cereal tea beverages, such as Tartary buckwheat, black bean and barley tea, in China and worldwide.

Diabetes mellitus is a serious, complex chronic condition that is a major source of ill health all over the world (Yao, Chen, Wang, Wang, & Ren, 2008). One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolysing enzymes α-glucosidase and α-amylase in the digestive tract (Bhandari, Jong-Anurakkun, Hong, & Kawabata, 2008; Randhir & Shetty, 2007). The management of diabetes without any side effects is still a challenge to the medical system (Chakraborty & Rajagopalan, 2002), for example, the use of acarbose, a synthetic α-glucosidase inhibitor, associates with high frequency of gastrointestinal distress (Chiasson et al., 2002). From this point of view, more efforts have been made to explore effective and safe inhibitors of α-glucosidase and α-amylase from natural ingredients to expand healthy food alternatives to treat diabetes (Kim, Wang, & Rhee, 2004; Wang, Du, & Song, 2010).

Processing is a prerequisite for the consumption of whole grain. Previous literature showed that the technological processing (soaking, thermal, freezing and osmotic processing) can significantly affect physical tissue structure, particular substances levels, and functionality of grains (Wolosiak et al., 2010). Thermal processing has been shown to affect the antioxidant activity of foods (Dewanto, Adom, & Liu, 2002; Sikora, Cieślik, Leszczyńska, Filipiak-Florkiewicz, & Pisulewski, 2008). However, to our best knowledge, little information is available on the

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effect of processing on phytochemical profiles in cereal tea. This type of knowledge becomes especially important when the development of whole grain foods is considered. The present study was undertaken to investigate the effect of traditional processing techniques (soaking, steaming and roasting) on phytochemicals levels, antioxidant activity and type II diabetes related α-glucosidase and α-amylase inhibitory activities of sorghum tea based on the whole sorghum grains.

2. Material and methods

2.1. Processing of sorghum tea

S. bicolor (L.) Moench Jin Za 15, a red sorghum hybrid variety, was obtained from the Shanxi Academy of Agricultural Sciences (Taiyuan, China). The production of sorghum tea was performed through three successive processes, soaking, steaming and roasting. Four sorghum materials (including the raw and three processed material) were collected. The processing was repeated three times independently and three samples were collected at each processing point. The materials from each processing point (Fig. 1) were separately ground by a laboratory mill (ZN 08, Xingshi Technologies Inc., China), passed through an 80 mesh screen sieve.

2.1.1. Soaking

100 g raw whole sorghum grain was soaked in distilled water (1:8 w/v) for 1 h at room temperature. The soaked samples were then drained, washed and dried to constant weight at 50 °C. The material from this processing point was remarked as soaked sorghum grain.

2.1.2. Steaming

The soaked sample (50 g) was steamed at 200–220 °C for 20 min, then the sample was oven dried at 50 °C, and the sample from this processing point was remarked as steamed sorghum grain.

2.1.3. Roasting

The steamed sample (25 g) was roasted using the rotating oven (Jinan Food Machinery Co., Ltd., Jinan, China) at 150 °C for 1 h. The sample was moved to the temperature of 50 °C and dried to constant weight, then the roasted material was obtained.

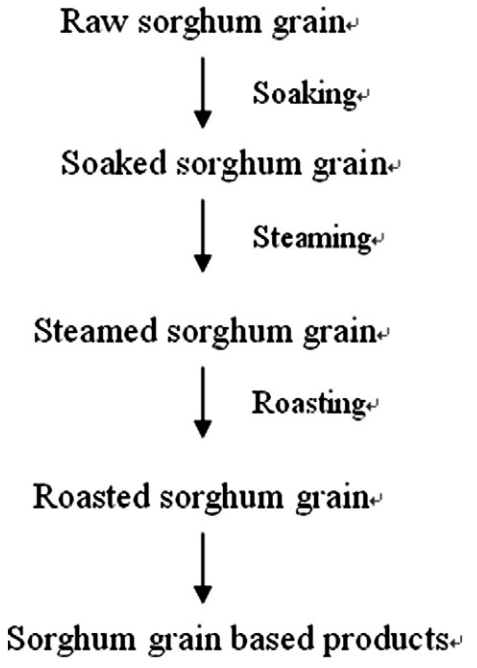

Fig. 1. Simplified flow diagram of processing procedures for sorghum grain based products.

2.2. Chemicals and reagents

Standards of *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, chlorogenic acid, syringic acid, *p*-coumaric acid, ferulic acid, Trolox, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), Folin–Ciocalteu phenolic reagent, trifluoroacetic acid (TFA, 99%), rat intestinal acetone powder, porcine pancreatic a-amylase and azure starch were purchased from Sigma–Aldrich (Shanghai, China). Gallic acid was purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Procyanidins standard (a mixture of oligomeric and polymeric procyanidin, HPLC grade, ≥95% pure) was purchased from JF-Natural (Tianjin, China). All the other chemicals used were analytical grade and obtained from Beijing Chemical Reagent (Beijing, China). High-performance liquid chromatography (HPLC) grade solvents were purchased from Fisher Chemicals (Shanghai, China).

2.3. Extraction of free and bound phenolic acids

Free phenolic acids in sorghum grains collected from different processing points were extracted according to the methods of Zhang, Zhang, Zhang, and Liu (2010) and Parra, Saldivar, and Liu (2007). Briefly, 3 g of sorghum flour was extracted twice with 45 mL of chilled acidified methanol (methanol and 1 M HCl 85:15, v/v) at 50 °C for 2 h. After centrifugation, the supernatants were concentrated under a reduced pressure rotary evaporator (Rotavapor R-210, Buchi Labrortechnik AG, Flawil,. Switzerland) at 50 °C to dryness. The evaporated samples were fleshed with nitrogen at room temperature, and then dissolved in 6 mL methanol. The extracts were stored at 4 °C until use. The residue from the free phenolic acids extraction was subjected to alkaline and acid hydrolysis to recover the bound phenolic acids as reported by Mattila et al. (2005) with minor modifications. Briefly, the residue was digested with 2 M NaOH at room temperature for 3 h. The solution was then adjusted to pH 2 with HCl, and liberated phenolic acids were extracted three times with 50 mL of cold diethyl ether. The diethyl ether layer was combined, evaporated to dryness at 50 °C, and dissolved in 6 mL methanol. The extracts were filtered through 0.45 μm syringe filter for HPLC analysis.

2.4. Determination of individual phenolic acids

Analysis of individual phenolic acid was performed by Shimadzu chromatographic system (Shimadzu, U.K. Ltd., Milton Keynes, U.K.) equipped with two pumps and diode array detector (DAD). A Thermo Hypersil BDS C18 column (4.6 mm×250 mm, Thermo Hypersil-keystone, Bellfonte, PA, USA) was used. The wavelength of the UV detector was set at 280 nm. The mobile phase was a mixture of solvent A (HPLC water containing 0.05% TFA) and solvent B (acetonitrile: MeOH: TFA = 30:10:0.05). The gradient elution was programmed as follows: from 10% to 12% B in 16 min; from 12% to 25% B in 9 min; from 25% to 50% B in 25 min; from 50% to 75% B in 8 min; from 75% to 10% B in 10 min. The flow rate was 1.0 mL/min, and the injection volume was 10 μL.

For the identification of phenolic acids in sorghum grain, the solution of each individual phenolic acid standard was prepared. The spiking and external standard methods were used for identification of peaks in the samples by comparing the increase of peak areas and retention time. All identified phenolic acids were quantified with external standards. Standard curves of phenolic acids were established by plotting peak areas against the concentrations of the standards from the averages of duplicate injections. Phenolic acid contents were expressed as micrograms per gram of sorghum grain (μg/g) on a dry weight basis.

2.5. Extraction of phenolic compounds

Total phenolic compounds were extracted as described previously, with some modification (Hung & Morita, 2008). Raw and processed