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Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties

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ABSTRACT

Objective: To investigate the changes in total phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. **Methods:** The changes in total phenols, total flavonoids, tannins, phenolic acids compounds, flavonoid components, vitamin E, β-carotene and antioxidant activity during soaking of sorghum grains were determined. **Results:** Total phenols, total flavonoids, tannins, vitamin E, β-carotene and antioxidant activity in raw sorghum were ranged from 109.21 to 116.70, 45.91 to 54.69, 1.39 to 21.79 mg/100 g, 1.74 to 5.25, 0.54 to 1.19 mg/kg and 21.72% to 27.69% and 25.29% to 31.97%, respectively. The above measured compounds were significantly decreased after soaking. *p*-Hydroxybenzoic acid, vanillic acid, syringic acid and cinnamic acid represent the major phenolic acids in Dorado variety. While ferulic acid, *p*-coumaric acid, gallic acid and caffeic acid represent the major phenolic acids in Shandaweel-6. On the other hand, protocatechuic acid represents the major phenolic acids in Giza-15. Regarding flavonoids components, Dorado was the highest variety in kampferol and naringenin while Shandaweel-6 was the highest variety in luteolin, apigenin, hypersoid, quercetin and christen. Finally, Giza-15 was the highest variety in catechin. Phenolic acids, flavonoid compounds and antioxidant activities were decreased after soaking. **Conclusions:** Sorghum varieties have moderate quantities from total phenols, total flavonoids, tannins, phenolic acids compounds, flavonoid components, vitamin E, β-carotene and antioxidant activity which decreased after soaking.

1. Introduction

Sorghum [*Sorghum bicolor* (*S. bicolor*) L. Moench] is a crop that is widely grown all over the world for food and feed. It is one of the main staples for the world's poorest and most insecure people in many parts of the developing world[1,2].

Phenolic compounds in sorghum occur as phenolic acids, flavonoids and condensed tannins. Sorghums phenolic acids are located in the pericarp, testa, aleurone layer, and endosperm[3]. The most abundant phenolic acids in sorghum are ferulic acid, *p*-coumaric acid and vanillic acid, which are predominant in bran layer of grains[4]. Sorghums with white, yellow, red, or brown color pericarp may or may not have tannins depending upon the presence of a pigmented testa[5]. Most sorghum does not contain condensed tannins, but all contain phenolic acids[6]. Compared to other cereal crops, sorghum has unique chemical component of tannin including type II sorghum (tannins present in pigmented testa) and type III sorghum (tannins present in pigmented testa and pericarp), while non-tannin sorghum is classified as type I[7]. The tannins in sorghums have the highest levels of antioxidants compared to cereals[8]. The evidence of possible benefits of tannins in the diet has led to research that focuses on sorghum tannins and health[9,10].

Free radicals may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues and cells[11]. This oxidative stress may be related to many disorders such as cancer, atherosclerosis, diabetes and liver cirrhosis[12].

Epidemiological studies have suggested that increased consumption of whole grains, fruits and vegetables is associated with reduced risks of chronic diseases[13-15]. This association may be attributed to the natural antioxidants

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from plant foods such as vitamin C, tocopherol, carotenoids, polyphenolics and flavonoids which prevent free radical damage by modulating the effects of reactive oxidants. Also, some plants are promising sources of potential antioxidants and may be efficient as preventive agents in the pathogenesis of some diseases. It can be also used in stabilizing food against oxidative deterioration[16-18].

Sorghum phenolic compounds have been shown to have antioxidant activity. These phenolic compounds possess structural features favorable for radical scavenging and/or metal chelation, which would enable them to be effective antioxidants. A potential therefore exists to use sorghum bran as a cheap source of natural antioxidants to prevent the development of oxidative rancidity in edible oils and other lipid food systems[5]. Some varieties of sorghum are recognized as important sources of dietary antioxidants because of the phenolic compounds found in the grain[19,20]. Many cereal and grain legumes are soaked before further processing. During soaking, water enters the kernel by molecular absorption, capillary absorption and hydration. Soaking gives a volume increase in the grain[21]. Soaking, fermentation and germination are three biological processes of significant impact on phytate and phenolic compounds. Several studies demonstrated that germination and fermentation affect condensed phenolic compounds[22].

The objective of this study was to increase the efficient use of three white sorghum by studying the biochemical changes of total phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity after soaking.

2. Materials and methods

2.1. Samples and chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzotiazoline-6-sulphonic acid) (ABTS), sodium carbonate (Na_2CO_3), butylated hydroxytoluene (BHT), gallic acid, catechin, β-carotene and α-tocopherol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA), Folin-Ciocalteu reagent was purchased from LOBA Chemie, India. All other chemicals used were of analytical reagent grade.

Three white sorghum varieties (*S. bicolor* L. Moench), named Dorado, Shandaweel-6 and Giza-15 grown during the 2007 season, were used. Dorado and Giza-15 varieties were obtained from Central Administration for Seed Certification (CASC), Ministry of Agriculture and Land Reclamation (MOALR), Giza, Egypt. Shandaweel-6 variety was obtained from the Crops Research Institute, Agricultural Research Center (ARC).

2.2. Soaking of sorghum grains

Sorghum grains were soaked in distilled water for 20 h with a ratio of 1:5 w/v and the soaked water was changed twice. At the end of soaking period, the soaked water was discarded. The grains were rinsed twice with distilled water and the grains were dried in oven at (45±5 ℃). The grains were milled in a laboratory mill to obtain fine flour and kept at −20 ℃ until analysis.

2.3. Biochemical analysis

2.3.1. Determination of total phenols

Total phenols were determined colorimetrically as described by Matkowshi and Piotrowska[23]. Sample (1 g) was mixed with 10 mL 80% methanol in a dark bottle and shaking for 2 h. The color was developed by Folin-Ciocalteu reagent and sodium carbonate. A volume of 0.250 mL was mixed with 0.250 mL Folin-Ciocalteau reagent and 0.50 mL of 10% sodium carbonate (Na_2CO_3) and the volume was completed to 5 mL with distilled water. After incubation in dark at room temperature for 30 min, the absorbance of the reaction mixture was measured at 725 nm against blank. Gallic acid was used as a standard.

2.3.2. Determination of total flavonoids

Total flavonoids were determined according to the methods of Nabavi *et al*[24]. Sample (1 g) was mixed with 10 mL 80% methanol and shaking for 2 h. Total flavonoids extract (0.4 mL) was added to 4 mL of H_2O. Then 0.3 mL of 5% $NaNO_2$ was added. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. After 6 min, 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with distilled water. The color was measured at 510 nm against a blank reagent. Catechin was served as standard compound.

2.3.3. Determination of tannins

Tannins were determined as described by Price *et al*[25] followed with minor modification by Osman[26]. Sample (1.0 g) was mixed with 10 mL of 1% methanol/HCl solution in a in a dark bottle and shaking for 20 min at room temperature. Then the mixture was filtrated. The tannins in the supernatant were estimated by using 1 mL of supernatant and 5 mL of vanillin/HCl mixture (by mixing equal volumes of 2% vanillin in methanol and 8% methanol/HCl) in a test tube and kept for 20 min at room temperature. The formed color was determined at 500 nm. Catechin was used to prepare the standard curve.

2.3.4. Fractionation of phenolic acids and flavonoid compounds using HPLC

The phenolic acids and flavonoid compounds of the samples were extracted according to the method described by Goupy *et al*[27] and Mattila *et al*[28] by using HPLC instrument (Hewlett Packard, series 1050, country) composed of column C18 hypersil BDS with particle size 5 μm. The separation was carried out with methanol and acetonitrile as a mobile phase, flow with 1 mL/min. Quantification was carried out for a calibration based on the standards phenolic acid and flavonoid.

2.3.5. Determination of vitamin E as α-tocopherol using HPLC

The vitamin E was quantified according to the method described by Pykaa and Sliwiok[29] by using HPLC instrument (Hewlett Packard, series 1050, country) composed