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Research Article

Phytochemical Profiles and Antioxidant Activities of Four Gluten Free Grains

Sikha Bhaduri* & Khursheed Navder, P.

School of Urban Public Health, Hunter College, 2180 Third Avenue, NY, NY 10035, USA

***Correspondence to:** Dr. Sikha Bhaduri, School of Urban Public Health, Hunter College, 2180 Third Avenue, NY, NY 10035, USA.

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Abstract

Amaranth, Buckwheat, Chia and Quinoa are pseudocereal grains known to be rich in both macronutrients and micronutrients including vitamins, minerals and significant amounts of other bioactive components such as flavonoids and polyphenols. The total antioxidant capacity, phenolic composition and flavonoid content of these four gluten free grains (Amaranth, Buckwheat, Chia and Quinoa) investigated in the present study. Chia and Quinoa exhibited the highest total antioxidant activity (504.96 and 520.84 mg ascorbic acid/g dw of extract respectively) compared to Buckwheat, which had the highest total phenol content amongst all four grains. Buckwheat and Quinoa exhibited highest total flavonoid content (47.41 and 65.38 mg QE/g dw of extract) compared to other two. Highest antioxidant activity of Quinoa grains is possibly due to its highest amount of flavonoid content.

Introduction

Dietary polyphenols, the most studied group of plant secondary metabolites, play important roles in preventing and managing nutritional disease due to their antioxidant and anti-inflammatory activities. More than 8,000 polyphenolic compounds have been identified in various plant species so far. It has been reported that, they are excellent free radical scavengers, metal chelators, singlet oxygen quenchers, reducing agents and synergists with other antioxidants and therefore inhibit oxidation of biomolecules such as membrane lipids, proteins, LDL-cholesterol, and DNA to reduce associated disorders such as inflammation, atherosclerosis and carcinogenesis [1]. Polyphenolics are the largest group of natural antioxidants in the diet. They are reducing agents and their structure determines the biological properties such as bioavailability, antioxidant activity, and specific interactions with cell receptors. Polyphenols may be classified into different groups as a function of the number of phenol rings they contain and on the basis of structural elements that bind these rings to one another. Four main classes of polyphenols exist based on the nature of their carbon skeleton, namely, phenolic acids, flavonoids, stilbenes, and lignans. The most abundant antioxidants in whole grains are phenolic acids, which are predominantly found in the bran and in the germ. However, most of them are removed during processing to refined flours [2]. Recent studies have shown that bound phenolics may play a very important role in regulating inflammatory immune responses especially in improving gut health [3,4]. The effect of food processing on the bioaccessibility, bioavailability and bioactivity of phytochemicals in gluten free grains are not well studied [5].

Diet can play a significant role in the prevention of many chronic diseases, providing compounds useful for maintaining health beyond the necessary nutrients and energy. For instance, phenolic compounds are known to protect against coronary heart disease and carcinogenesis [6]. Epidemiological studies have shown that regular consumption of phenolic-rich foods such as cereals, legumes, oilseeds and their products/by-products can protect against the risk of cardiovascular diseases, type 2 diabetes, gastrointestinal cancers, and other health disorders [7]. Others like omega-3 fatty acids have been related to cardiovascular disease prevention, inflammation, hyperlipidemia, and cancer [8].

Whole pseudocereal grains such as Amaranth, Buckwheat, Chia and Quinoa are rich in a wide range of compounds e.g. flavonoids, phenolic acids, trace elements, fatty acids and vitamins with known effects on human health [9-12]. Amaranth is not an actual grain but a member of the Amaranthaceaea family. Its seeds have a robust nutty flavor and have been used as a staple by many ancient civilizations around the world. Buckwheat is not considered a "true" cereal but this gluten-free grain belongs to Polygonaceae family which is closely related to rhubarb. Common buckwheat is thought to have originated and cultivated in central and western China from a wild Asian species Fagropyrum cymosum for over 1,000 years. Today, however, larger producers of Buckwheat include the former Soviet Union, China, Brazil, Poland, France, Japan, United States, South Africa and Australia. Buckwheat possesses some phenolic compounds, such as rutin, quercetin, kaempferol-3-rutinoside and flavonol triglycoside, and a high antioxidant activity that helps to reduce the risk of major chronic diseases [13]. Buckwheat bread was found to have highest content of phenolic compounds compared to Amaranth and Quinoa [14]. Buckwheat and Chia grains are rich in flavonoids and omega-3 fatty acids [15].

Chia seed is obtained most commonly from Salvia hispanica, a member of the mint family that grows abundantly in southern Mexico. Chia seeds contain a high amount of phenolic acids and vitamins [16,17], omega-3 fatty acids, antioxidants and other beneficial ingredients. They contain fat (25–32%), essential fatty acids 59.9–63.2% of alpha-linolenic acid and 18.9–20.1% of linoleic acid [18,19], protein (18.5–22.3%) and fibre (20.1–36.15%). Most of the species of Chia have homeopathic and horticultural importance as a source of many useful natural components like polyphenols, chlorogenic and caffeic acids, as well as flavonoids like myricetin, quercetin and kaempferol [20,21].

Quinoa is not actually a grain but the seeds of a broad-leafed plant from the Chenopodiaceae family which is a close relative of the weed, lamb's quarters. It has been consumed for thousands of years in South America and was a staple of the Incas. There are hundreds of varieties of Quinoa, ranging in color from white to red and purple to black. Different bioactive phytochemicals of Quinoa and Amaranth seeds including phenolics, betanins, and carotenoids have been shown to possess antioxidant, anti-inflammatory and other health promoting effects based on both in vivo and in vitro studies Increasing research has been focused on these non-essential nutrients including phytochemicals of Quinoa and Amaranth [22]. Many studies have shown that Quinoa seeds are a good source of bioactive polyphenols (flavonoids and phenolic acids) that might change the antioxidant status in the organism and prevent oxidative stress [23,24]. The United Nations FAO declared 2013 as "The International Year of Quinoa", promoting the planting, development and research on quinoa and their related products (A/RES/66/221).

Considering all above, the present study was undertaken to analyze phytochemical profile of four glutens free grains--Amarnath, Buckwheat, Chia and Quinoa.

Materials and methods

Preparation of extracts

The Amaranth, Buckwheat, Chia and Quinoa seeds (Bob's Red Mill, Milwaukie, Oregon) were powdered with a mechanical grinder to obtain a coarse powder, which were then subjected to extraction using a modified method with methanol [25]. Briefly, 2 g of grain powder were extracted with 20 mL of solvent into a 50 mL polyethylene centrifuge tube. The mixture was kept on a rotary shaker at speed 5 for 24 hours at room temperature. After 24 hours, the filtrate was centrifuged at 5000 g for 10 minutes, the supernatant was collected. The extraction was done at least three times with the residues and supernatants collected each time. All the collected supernatants were pulled out together and were filtered through Whatman No. 1 paper filter and concentrated to a dry mass with the aid of a rotary evaporator. Each dried extracts were dissolved in 1 mL dimethyl sulfoxide (DMSO).

Evaluation of antioxidant capacity

Determination of Total Antioxidant Activity (TAA)

Total Antioxidant Activity (TAA) was estimated by phosphomolybdenum assay method using Molybdate reagent Solution (1 mL each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 mL of distilled water and made up volume to 50 mL by adding distilled water) [26]. 100 μL of each extracts (and diluted ascorbic acids in different concentrations) were added to each test tube individually containing 3 mL of distilled water and 1 mL of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, the tubes were normalized to room temperature for 20-30 minutes and the absorbance of the reaction mixture was measured at 655 nm using a microplate spectrophotometer (Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, CA 94547, USA) against blank sample. Mean values from three independent samples were calculated for each extract. Ascorbic acid (AA) was used as positive reference standard and the means of three triplicate analysis of Total Antioxidant Activity was expressed as milligrams of ascorbic acid per gram of dry extract (mg ascorbic acid/gm dry extract).

Determination of Total Phenolic Content (TPC)

The amount of total soluble phenolic content in different grain extracts was determined according to Folin-Ciocalteu method with slight modifications [27]. Briefly, 10 μL of the sample extract or a series of gallic acid standards (0, 20, 40, 60, 80, and 100 mg/L) from the stock solution was mixed with 100 μL of Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, Mo., USA). After 10 minutes of incubation, 300 μL of 20% Na_2CO_3 solution was added and the volume was adjusted to 1 mL using deionized water. The mixture was incubated in dark for 2 hours at room temperature and the absorbance was measured at 750 nm using a microplate spectrophotometer (Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, CA 94547, USA) against blank sample. The total phenolic content was measured as gallic acid equivalents per gram of dry mass (mg GAE/gm dry extract) and the values were presented as means of triplicate analysis.

Determination of Phenolic Acid Content (PAC)

Total content of Phenolic Acids of grain materials was determined by a spectrophotometric method [28] with Arnov's reagent (10.0 g sodium molybdate, 10.0 g sodium nitrite in 100.0 mL water) according to the procedure described somewhere (Polish Pharmacopoeia VI) [29]. Briefly, 1.0 ml of extract of different grains was pipetted into a 10.0 mL volumetric flask containing 5.0 mL water, followed by addition of 1.0 mL HCl (18 g/L), 1.0 mL Arnov's reagent and 1.0 mL NaOH (40g/L). The volume was made up to 10.0 mL with distilled water. Total Phenolic Acids Content was calculated according to the following formula:

$$(\%) = A \times 0.877/m$$

where A is the absorbance of the examined solution at 490 nm and m is the mass of the sample in grams. The results are averages of five measurements, expressed as caffeic acid equivalent (mg CAE/gm dry extract).

Estimation of Total Flavonoid Content (TFC)

Total Flavonoid Content (TFC) was determined by a modified aluminium chloride method using quercetin as standard [30]. Stock solution of grain extracts originally dissolved in DMSO was diluted with methanol. 10 mg of quercetin was dissolved in 100 mL methanol and then diluted to different concentrations using methanol to be used as standard. 0.1 mL of diluted extract or quercetin standard solution was mixed with 0.9 mL of distilled water in test tubes, followed by addition of 75 μL of a 5% sodium nitrite solution. After 6 min, 150 μL of a 10% aluminium chloride solution was added and the mixture was allowed to stand for further 5 minutes after which 0.5 mL of 1M sodium hydroxide was added to the reaction mixture. The reaction mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately at 510 nm in a spectrophotometer (Spectrnics 20, Spectronics, CA). The Total Flavonoid Content were determined as quercetin equivalents per gram of dry mass (mg QE/gm dry extract) and the values were expressed as means of triplicate analysis. Blank consist of all the reagents using 0.1mL of methanol in place of extract and quercetin.

Statistical analysis

Microsoft® Excel 2013 was used for statistical analysis, calculations and graphical plots.

Results and discussion

All four grains Amaranth, Buckwheat, Chia and Quinoa displayed a very high amount of Total Antioxidant Activity (TAA) ranging from 428.03 to 520.84 mg ascorbic acid/g dry extract (Figure 1). The Total Phenol Content (TPC) of these four grains are shown in Figure 2. Buckwheat was found to have highest Total Phenol Content 125.19 mg GAE/g dry extract [31]. The Phenolic Acid Content (PAC) was also highest in Buckwheat (680.22 mg CAE/g dry extract), compared to other three grains (Figure 3). Both Chia and Quinoa also had higher amounts of TPC (60.84 and 62.28 mg GAE/g dry extract respectively) and Phenolic Acid Content (289.58 and 250.92 mg CAE/g dry extract respectively) compared to Amaranth. Quinoa had the highest amount of Total Flavonoid Content (65.38 mg QE/g dry extract) when compared to the other grains (Figure 4). From these data, we speculate that the high Total Antioxidant Activity (520.84 mg ascorbic acid/g dry extract) of Quinoa grains is due to its high Total Flavonoid Content.

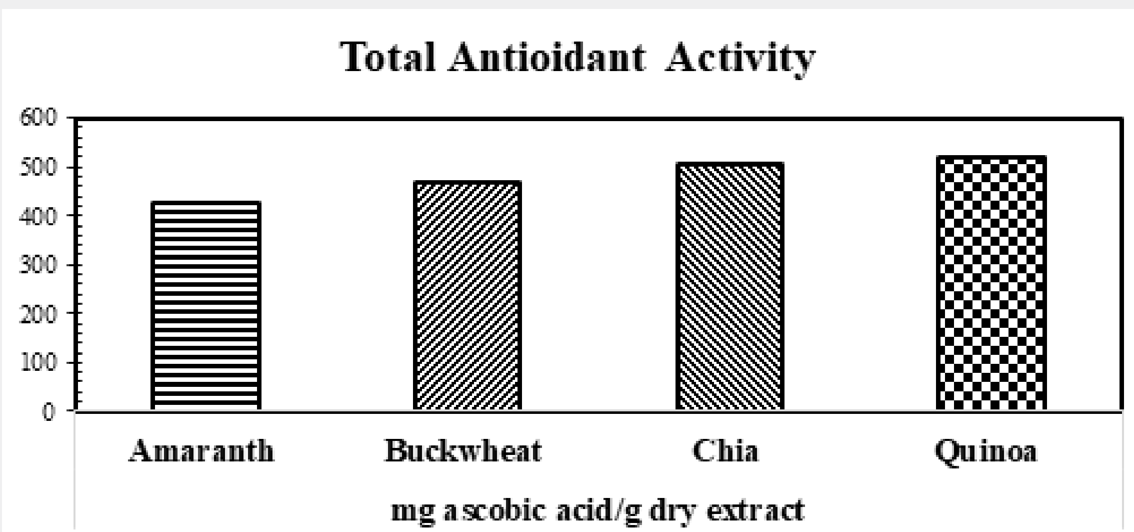

Figure 1: *TAA. Total Antioxidant Activities in mg ascorbic extract/g dry extract extracts of Amaranth, Buckwheat, Chia and Quinoa grains*

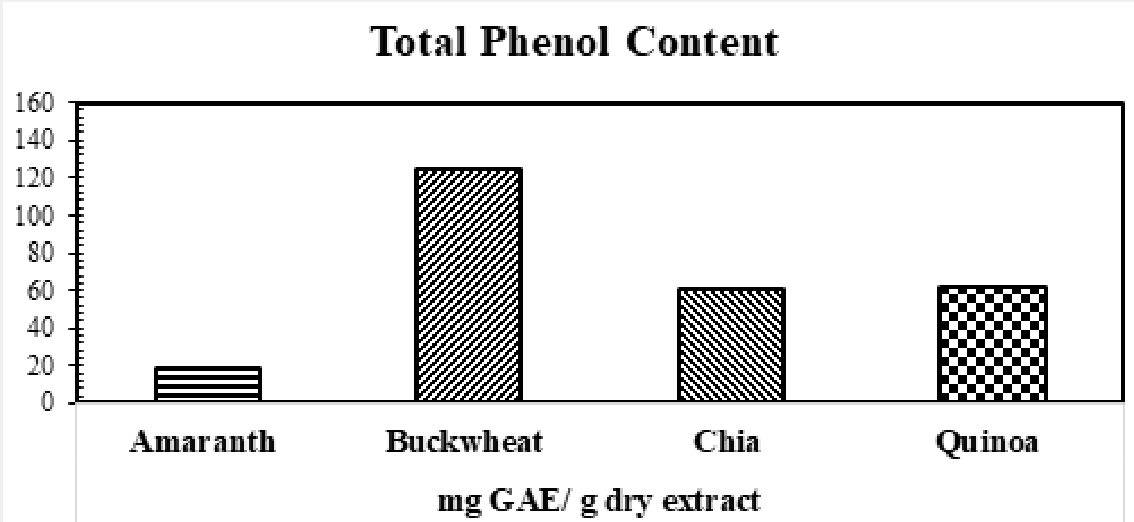

Figure 2: *TPC. Total Phenol Content in mg GAE/g dry extract of Amaranth, Buckwheat, Chia and Quinoa grains*

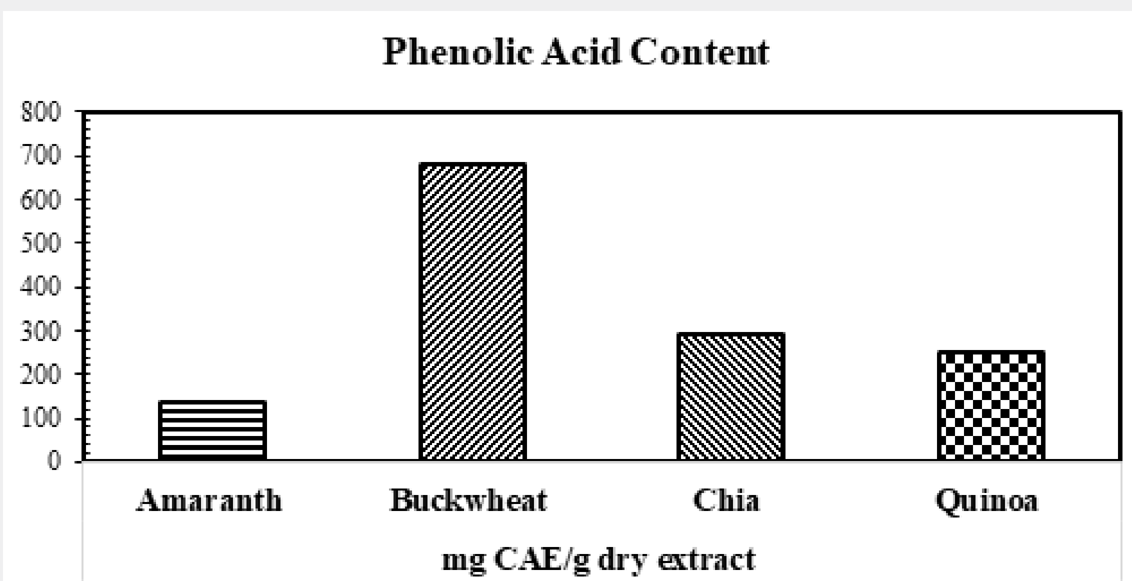

Figure 3: *PAC. Phenolic Acid Content in mg CAE/g dry extract of Amaranth, Buckwheat, Chia and Quinoa grains*

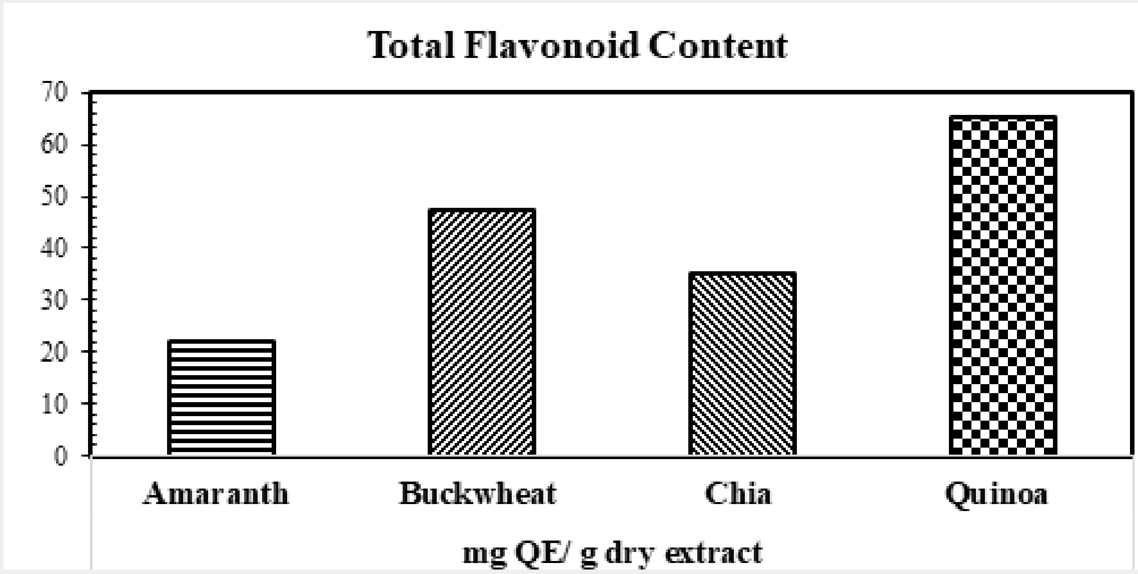

Figure 4: *TFC. Total Flavonoid Content in mg QE/g dry extract of Amaranth, Buckwheat, Chia and Quinoa grains*

Conclusion

The present study demonstrates that all four gluten free grains --Amaranth, Buckwheat, Chia and Quinoa--contain considerable amounts of total antioxidant activities. The higher total antioxidant activity of Buckwheat is possibly due to its high Total Phenol, phenolic acid and flavonoid contents. Similarly, the high total flavonoid content gives higher antioxidant activity to Quinoa giving it the potential to serve as an antiproliferative and antimicrobial agent. However, the exact mechanisms at play still remain unclear for the vital roles of these activities. Further investigations on gluten free grains are needed to identify the exact components responsible for their beneficial properties.

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