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Research Article

The Relationship between Antioxidant Activity and Total Phenolic Content in Cereals and Legumes

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Abstract: The antioxidant activities and total phenolic content of 4 cereals (buckwheat, barley, wheat and oat) and 4 legume seeds (faba bean, azuki bean, soybean and mung bean) were determined. The total phenolic content (TPC), determined according to the Folin-Ciocalteu method, for cereal samples varied from 15.4 to 52.5 mg Gallic acid equivalent/g of dried extract, while for legume samples varied from 19.1 to 23.8 mg Gallic acid equivalent/g of dried extract. Antioxidant activities were comparatively assessed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity, ferric ion-reducing antioxidant power (FRAP) and the thiobarbituric acid (TBA) method. The tested plant extracts showed promising antioxidant and free radical scavenging activity, thus justifying their traditional use. Among examined cereals all the applied methods, except TBA method, have shown that buckwheat have the highest antioxidant activity, while among examined legumes results varied depending on the method used.

Keywords: Antioxidant activity, cereals, legumes, total phenolic content

INTRODUCTION

Cereals and legumes are an important source of macronutrients. Legumes are rich and economical source of proteins, complex carbohydrates (dietary fiber), minerals and vitamins, while cereal grains provide significant quantities of carbohydrates, proteins and selected micronutrients to the animal and human diet (Shahidi, 2009). Those plants also contain a wide range of chemical classes with antioxidant activity (Amarowicz *et al*., 2004; Mohd Zin *et al*., 2007; Strazzullo *et al*., 2007). Cereal grains are rich in phenolicacids phytosterines, saponins and phytoestrogens and flavonoids are present in small quantities (Senter *et al*., 1983). It has been suggested that these antioxidants may contribute to the health benefits of cereal-based foods in reducing the incidence of aging-related chronic diseases including heart diseases and some types of cancer (Miller *et al*., 2000). Legume seeds are also rich in various active phytochemicals, e.g., isoflavones, coumestrol, phytate, saponins, lecithin, phytosterols and vitamin E (Prakash *et al*., 2007). Epidemiological studies have shown correlations between the consumption of legumes (and there isoflavones such as daidzein, genistein, daidzinandgenistin from soybean) and decreasing incidence of several diseases, for example, cancer, aging and cardiovascular diseases (Russo *et al*., 2006). The chemical composition and bioavailability of nutrients varies between species and varieties of plants and may be affected by forms of processing as feed and food (Johnson *et al*., 2013; Luo and Xie, 2012).

Although synthetic antioxidants such as butylatedhydroxytoluene (BHT), butylatedhydro xyanisole (BHA) and Terc-Butylhydroquinone (TBHQ), as well as Propyl Gallate (PG) have widely been used in retarding lipid oxidation, their safety has recently been questioned due to toxicity and possible carcinogenicity (Gujral *et al*., 2012a). Thus, food manufacturers are considering the possibility to achieve antioxidative effect from natural sources (Aguilera *et al*., 2011) and development of safer natural antioxidants from extracts of plant materials may provide interest (Gujral *et al*., 2012b). Food antioxidants such as amino acids, peptides, proteins, flavonoids and other phenolics compounds might play a significant role as physiological and dietaryantioxidants, thereby augmenting the body's natural resistance to oxidative damage (Shahidi, 2009).

The objective of this study was to determine and compare the four commercial cereals (buck wheat, barley, wheat and oat) and four commercial legume seeds (faba bean, azuli bean, soybean and mung bean) for their free radical scavenging capacity against 2, 2-diphenyl-1-picryhydrazylradical (DPPH), their Ferric Reducing Antioxidant Power (FRAP), ability for inhibition of lipid peroxidation (TBA test) and for their Total Phenolic Contents (TPC). Nevertheless, these products were chosen because of their widespread availability and use in China. Studying such

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commercially prepared products may provide useful information to consumers and also incentive to food manufacturers to promote the consumption and production of value-added foods for improving human health.

MATERIALS AND METHODS

Materials: The cereal samples used in this study included buckwheat, barley, wheat and oatwere collected from local market of the same batch in Nanjing, Jiangsu Province, P.R. China and legume samples included faba bean, azuli bean, soybean and mung bean were manufactured by "Organic produces" China. The compounds 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Thiobarbituric Acid (TBA) and Gallic acid were purchased from SUNSON (Beijing China), Folin-Ciocalteu reagents was purchased from Merck and Co. Inc. (New York, USA) and all other chemicals and solvents were the highest commercial grade and used without further purification.

Extraction and Sample Preparation: Cereals and legume grains (100 g) were sop up with distillate water for 24 h, then filtered and milled with 400 mL of distillate water. Plant mush was next sterilized in anautoclave for 1 hour and cooled out for 24 h. The sample was extracted with 70% (v/v) ethanol (700 mL) for three hours using a magnetic paddle and then centrifuged 10 min on 4500 rpm. Extract was poured to a lab dish and residue was extracted again with70% (v/v) ethanol (300 mL) for another three hours using a magnetic paddle, following centrifugation for 10 min on 4500 rpm, the extract were combined. Volumes of extracts were approximately 1200 mL and kept in a refrigerator until drying.

Before drying samples were concentrated to 100 mL with rotavapor (temperature 50°C, pressure 50-150 mbar). Concentrated extracts were dried on SprayDryer after water dilution. Inlet temperature and pump were adjusted to 120-125°C and 15-20%, respectively, leading outlet temperature of 60-63°C. Dried samples were kept in hermetically closed dishes in a freezer (-22°C) until further analysis.

Methods:

Determination of the Total Phenolic Content: The total phenolic contents of cereals and legumes were determined according to Xu and Chang (2007) with slight modifications. After adding Folin-Ciocalteau reagent and sodium carbonate to aliquots of samples, the mixtures were set in a 40ºC water bath for 20 min. The absorbance was measured at 740 nm using a spectrophotometer (Unico, Shanghai, China) and totalphenolic contents were expressed as milligrams of garlic acid equivalents per grams of defatted sample.

Determination of DPPH radical Scavenging Activity: The antioxidant activity was determined by DPPH assay according to Llorach *et al*. (2008) with some modifications. Aliquot of 200 mL sample mixed with 3.8 mL DPPH solution (200 mM in methanol) was incubated in dark at room temperature for 60 min, then its absorbance at 517 nm was measured by a spectrophotometer. Scavenging ability of the sample to DPPH radical was determined according to the following equation:

Antioxidant Activity (AA) was expressed as percentage inhibition of DPPH radical by using below equation:

$$AA = 100-[100\times(A_{sample}/A_{control})]$$

where,
A_{sample} = The absorbance of the sample at t = 60 min
$A_{control}$ = The absorbance of control

FRAP method: FRAP assay was measured according to the procedure described by Benzie and Strain (1996). The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mMHCl, 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37ºC. A 900 µL FRAP reagent was mixed with 90 µL water and 30 µL of the extract. The reaction mixture was incubated at 37ºC for 30 min and the absorbance was measured at 593 nm.

Thiobarbituric Acid Test (TBA): Thiobarbituric acid tests were performed according to the method of Afanasev *et al*. (1989) to determine the malonaldehyde formation from lipid peroxidation. Lipid peroxidation was measured in liposome rimifon "Lipotech 10" (0.3 g lecithin/mL). The mixture was containing 20 µL $FeSO_4$ (0.075 M), 50 µL liposome, 10 µL of test samples of various concentrations (1-10% w/v), 20 µL L-ascorbic acid (0.1 M) and 3.9 mL phosphate buffer (pH 7.4). Mixture was left in thermostat on 37ºC for one hour and then mixed with 0,2 mL ethylenediaminetetraacetic acid (EDTA) (0.1 M) and 1,5 mL TBA reagent (3 g thiobarbituricacid, 120 g trichloroacetic acid and 10.4 mL perchloric acid in 800 mL trace element water). After heating on 100°C for 15 min and centrifugation (10 min, 3000 rpm), the absorbance of the supernatant was measured at 532 nm, against blank (distilled water).Inhibition of lipid peroxidation in percent (%) was calculated by the formula:

Statistical analyses: Data were analysed with SPSS (Statistical Package for the Social Sciences) 13.0 for windows. The mean and standard deviation of means were calculated. The data were analysed by one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate means. Significance was accepted at a probability $p<0.05$.

RESULTS AND DISCUSSION

Total phenolics and antioxidant activities of plant materials: The antioxidant activities and total phenolics of four examined cereals and 4 examined

Table 1: Total phenolics and antioxidant activities of legumes and cereals

Sample name	TPC[1] (mg GAE/g d.e.)	DPPH (IC50)[2] (μg/mL)	FRAP[3] (nmol Fe^{2+}/mg d.e.)	TBA[4] (%)
Faba bean	23.8±0.04	134.4	27.41±0.76	52.4±0.53
Azuki bean	20.7±0.05	129.1	24.89±0.48	37.6±0.45
Soybean	20.6±0.03	>200	13.43±0.83	47.8±0.63
Mung bean	19.1±0.04	143.7	30.12±0.85	45.6±0.48
Buckwheat	52.5±0.05	68.50	53.68±0.72	43.3±0.59
Barley	18.3±0.04	>200	21.12±0.51	47.9±0.72
Wheat	18.2±0.06	>200	17.28±0.49	52.3±0.62
Oat	15.4±0.06	>200	13.99±0.42	55.1±0.48

[1]: TPC: Total Phenolic Content by Folin-Ciocalteu method; [2]: DPPH: Radical Scavenging Activity; [3]: FRAP: Ferric Reducing Ability of Plasma; [4]: TAB: Tiobarbituric Acid method

legumes are shown in Table 1. All plants showed a significant amount of total phenolics and more or less effective antioxidant activities (Table 1). Among cereals buck wheat had the highest amount of total phenolics, with highest DPPH radical scavenging activity and ability for Fe^{3+} reduction but it had the lowest lipid peroxidation inhibition. These data suggest that it might be more critical, in delaying the lipid peroxidation, to suppress the initiation of the radical chain reaction, than to terminate the radical chain reaction by quenching or removing the radicals generated during propagation of the radical chain. Among legumes faba bean had the highest amount of total phenolics and the highest lipid Peroxidation Inhibition Ability (TBA), but mung bean precede in ability for Fe^{3+} reduction (FRAP), while azuki bean precede in DPPH radical scavenging activity.

Legumes generally precedes in TPC, DPPH scavenging ability and FRAP comparing with cereals, with the exception of buckwheat, which had the highest phenolics content, DPPH radical inhibition activity and ferric reducing power among all examined plants. Furthermore, soybean and oat had similar FRAP numbers, while cereals in generally precedes in lipid peroxidation inhibition ability comparing with legumes.

Total phenolics content: As shown in Table 1, total phenolic contents in examined cereals were the highest in buckwheat 68.5 mg GAE/g dry extract. Similar phenolic content in buckwheat was reported by Velioglu *et al*. (1998). Lower total phenolic contents were found in wheat and barley (18.2 and 18.3 mg GAE/g d.e., respectively) and the lowest in oat 15.4 mg GAE/g dry extract. In the case of wheat and barley grain results reported by Zhou and Yu (2004) showed that the contents of TP were affected by the extraction solvents with the following order from high to low: acetone > ethanol>methanol, which can be used to explain lower amount of phenols in this plants. According to Zielinski and Troszynska (2000) way and length of extraction has great affect on TPC in rye, so it is very difficult to compare results in our work with those given by this set of authors. Generally it is difficult to compare our data with other data from literature, due to different methods of extraction, determination and plants results calculations applied by other authors.

Total phenolic content in examined legumes, as showed in Table 1, was the highest in faba beans 23.8 mg GAE/g dry extract. Slightly lower total phenolic contents were inazuki bean and soybean (20.7 and 20.6 mg GAE/g d.e., respectively) and the lowest in mung bean (19.1 mg GAE/g d.e.). According to Prakash *et al*. (2007) TPC for soybean showed wide variation from 6.4 to81.7 mg GAE/g of seed extract from different varieties. Also the biological activity of soybean poliphenoles may depend on the type of processing and storage conditions, knowing that degradation of the phenolics substances may be associated with the occurrence of oxidative reactions or decomposition of thermolabile compounds induced by heat and also with the possibility of losses of volatile substances during spray drying (Georgetti *et al*., 2008). For mung bean, similar phenolic content was reported by other authors (Georgetti *et al*., 2006), while considerable variation in TP are present also among the mung bean cultivars, which may be attributed to factors such as natural chemical composition, maturity at harvest, soil state and conditions of postharvest storage (Anwar *et al*., 2007).

DPPH radical scavenging activity: DPPH radical has been widely used in assessment of radical scavenging activity because of its ease and convenience. The scavenging effect of cereal extracts on DPPH radical is shown in Fig. 1. The weakest effect was noted for the extract of wheat with only 22.4% of inhibition within the highest sample concentration (200 μg/mL), followed by barley and oat (26.1 and 35.2%, respectively). In other investigations those extracts also possessed a weak activity to scavenge DPPH radical with this sample concentration (Yu *et al*., 2002b). Much stronger scavenging effects on DPPH radical were found for buckwheat extract (76.5%), leading to IC50 62.1 μg/mL. The DPPH radical scavenging effect observed in this study is in agreement with literature data reported by Sun and Ho (2005).

As shown in Fig. 2 and Table 1, among examined legumes, soybean showed the weakest effect (45.4% within highest sample concentration, IC50 higher than the highest concentration), followed by faba bean (56.1%, IC50 172.3 μg/mL), azuki bean (65.3%, IC50 127.4 μg/mL) and mung bean (68.7%, IC50 126.7 μg/mL). The DPPH radical scavenging effect observed in this study is in agreement with literature data applied by other authors (Prakash *et al*., 2007).

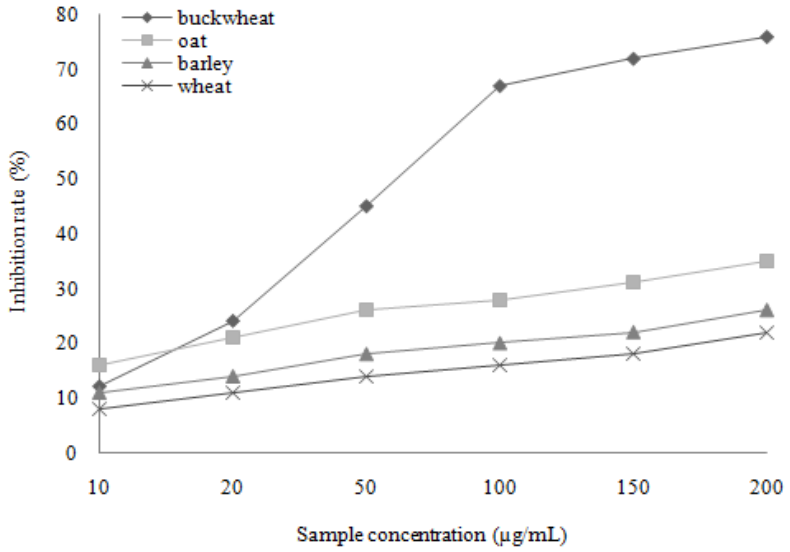

Fig. 1: DPPH radical scavenging activity for cereal samples

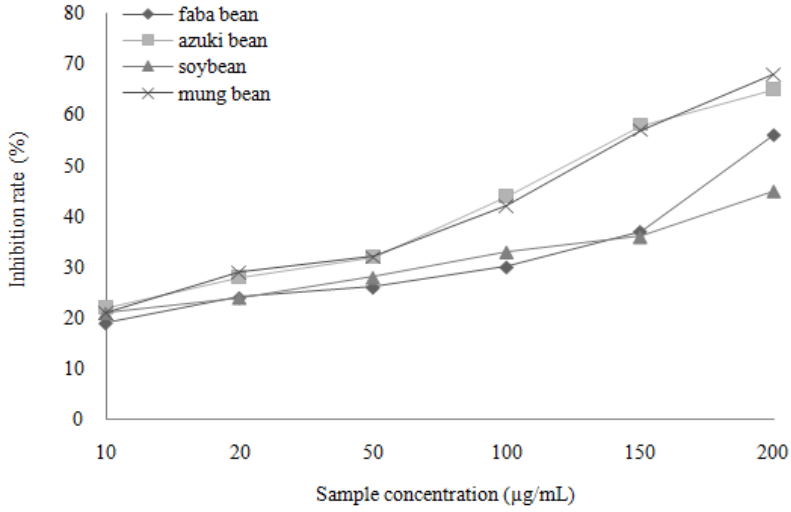

Fig. 2: DPPH radical scavenging activity for legume samples

It is obvious from Table 1 that there was lack of correlation between TPC and DPPH radical scavenging activities of plants, because those cereals which had higher TPC values were not necessary better in DPPH inhibition (Table 1 and Fig. 1). According to Brand-Williams *et al*. (1995) ferulic acid, the main phenolic acids in cerealgrains, showed a weak antiradical effect in experiments with DPPH radical, which can be used to explain this discordance among cereals. Also, although azuki bean and soybean among group of examined legumes showed similar TPC values (20.7 and20.6 mg GAE/g d.e.), their DPPH radical scavenging activities different remarkably, in the way that faba bean had the highest (IC50 134.4 µg/mL) while soybean showed the lowest DPPH inhibition (IC50 higher than the highest concentration of 200 µg/mL). This discordance can be explained by limitations of Folin-Ciocalteu method.

Although the Folin-Ciocalteu method is widely used to determine the total phenolic contents in botanical and biological samples, it has limitations. Other reducing agents, such as L-ascorbicacid and sulphur dioxide, may also react with the Folin-Ciocalteu agent and contribute to the total absorbance, which generally results in overestimated levels of total phenolic contents. In addition, individual phenolics compounds may have different reactivity with the Folin-Ciocalteu reagent, which could result in potential errors in the total phenolic content measurements (Yu *et al*., 2002b).

Generally, there are several reasons to explain the ambiguous relationship between the antioxidant activity and total phenolics:

- Total phenolic content did not include all the antioxidants, such as ascorbic acid, carotenoid and tocopherol.
- The synergism among the antioxidants in the mixture made the antioxidant activity, not only dependent on the concentration of antioxidant, but also on the structure and interaction among the antioxidants. That is why samples with similar concentrations of total phenolics may vary remarkably in their antioxidant activity.
- Different methods to measure antioxidant activity with various mechanisms may lead to different observations (Sun and Ho, 2005).

FRAP method: Ferric Reducing Antioxidant Power (FRAP) of examined cereals as showed in Table 1 was in correlation with total phenolic content. The highest FRAP number expressed innmol of Fe^{2+}/mg dry extract was in buckwheat (53.68 nmol Fe^{2+}/mg d.e.), followed by fairly lower FRAP in barley and wheat (21.12 and 17.28 nmol Fe^{2+}/mg d. e., respectively) and the lowest ferric reducing antioxidant ability in oat (13.99nmol Fe^{2+}/mg d. e.).

Among examined legume samples ferric reducing antioxidant power (FRAP) was the highest in mung bean (30.12nmol Fe^{2+}/mg d.e.), followed by faba bean (27.41nmolFe^{2+}/mg d.e.), azuki bean (24.89nmol Fe^{2+}/mg d.e.) and considerably lower insoybean (13.43nmol Fe^{2+}/mg d.e.) (Table 1). Difficulty in data comparing is even more obvious with FRAP method. In our work, results were expressed in nmol of Fe^{2+}/mg dry extract, but other authors reported their results in nmol or mmol Fe^{2+} in mg or g grains or flour (Halvorsen *et al*., 2002; Xand and Chang, 2007). Besides, extraction solvents and methods of samples preparations in other works were different and both is disproved to have influence on FRAP.

It is interesting that oat which had significant DPPH radical scavenging activity, showed the lowest ferric reducing power and also legumes which had significant DPPH radical inhibitor activity, such as soybean, showed lower ferric reducing power, comparing with legumes with lower DPPH scavenging activity and higher FRAP number, such as faba bean. It appears that care should be taken when using free radicals as the basis for generating an antioxidant activity, because the activity is very dependent on the specific free radical used (Cao *et al*., 1996). One should use different free radicals and calculate an antioxidant score as done by Cao *et al*. (1996) or one should preferably use the FRAP assay, which is based on a much less selective reduction.

Thiobarbituric Acid Test (TBA): As shown in Table 1, there is lack of correlation between TPC and the ability of lipid peroxidation inhibition in cereals and legumes. Plants with higher TPC values were not necessary better in inhibition of lipid peroxidation. Wheat extracts, for instance, had very high ability to inhibit lipid peroxidation in liposome but showed the lowest ability to directly react with and quench radical DPPH, while although azuki bean had the highest ability to directly react with and quench radical DPPH among examined legumes, it showed the lowest ability to inhibit lipid peroxidation in liposomes. This can be explained with complex mechanism of lipid peroxidation inhibition, which includes not only uncompounded phenols, but also high-molecular polyphenols and other nonphenolics antioxidants. In addition, these data may suggest that it might be more critical to suppress the initiation of the radical chain reaction, than to terminate the radical chain reaction by removing the radicals generated during propagation of the radical chain reaction (Yu *et al*., 2002a). According to results, oat had the greatest ability for inhibition of lipid peroxidation among examined cereals (55.1% within highest sample concentration (250 μg/mL)), followed by wheat and barley (55.2 and 47.9%, respectively) and the weakest result in TBA test was in buckwheat (43.3%) (Table 1). Among legumes faba bean had the greatest ability for inhibition of lipid peroxidation (52.4% within the highest sample concentration), followed by soybean and mung bean (47.8 and 45.6%, respectively) and the weakest results in TBA test was in azuki bean (37.6%) (Table 1).

CONCLUSION

This study indicated that cereals and legumes, used widely for human consumption, exhibited significant free radical scavenging activities, ferric reducing power, ability for inhibition of lipid peroxidation and total phenolic contents. These factors suggest that cereal- and legume-based foods could contain important dietary antioxidants and therefore warrant further research to determine whether these dietary antioxidants could be beneficial to human health. There were several significant differences among cereals and legumes in these characteristics, which warrant further study, especially in regard to their effects on human health. More research is needed to adequately know the composition of the extracts, to identify the antioxidant compounds in the extracts and to evaluate the potential use of cereal and legume products like natural antioxidants and therefore using for food supplements. In addition, identification of both biological (e.g., digestion) and food processing conditions that impact the distribution, stability and activity of wheat antioxidants is needed in order to be able to produce food products with maximum health benefits.

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