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Antioxidant activities of barley seeds extracts

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Abstract

The antioxidant activities of different polarity solvents extracts from barley were evaluated by various antioxidant assays, including reducing power, free radical scavenging and lipid oxidation inhibition. Those various antioxidant activities were compared to standard antioxidants, butylated hydroxyluene (BHT) and ascorbic acid (Vit. C). The properties of the extracting solvents significantly affected the total phenolics, proanthocyanidins and antioxidant activities of barley extract. The highest contents of total phenolics and proanthocyanidins were obtained from extraction with 70% acetone. For three different solvent extracts, the antioxidant activities were in this order: 70% acetone extract > 70% ethanol extract ⩾ 70% methanol extract. Reducing powers of three extracts and their scavenging effects on 2, 2-diphenyl-1-picrylhydrazyl radicals were effective at amount of 200 μg. The 70% acetone extract of barley exhibited high antioxidant activity in linoleic acid system, which was not significantly ($P < 0.05$) different from BHT during the incubation time.

Keywords: Antioxidants; Barley; Extracts; Free radical scavenging

1. Introduction

During recent years people have been more concerned about the safety of their food and the potential effect of synthetic additives on their health. The two most commonly used synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have begun to be restricted because of their toxicity and DNA damage induction (Ito et al., 1986; Sasaki et al., 2002). Therefore, natural antioxidants from plant extracts have attracted increasing interests due to their safety.

Cereals are the most important foods in diet because they have high carbohydrate content that can provide energy. However, in recent years, researchers have also begun to study their other functional characters such as antioxidant profiles (Adom & Liu, 2002; Goupy, Hugues, Boivin, & Amiot, 1999; Peterson, Emmons, & Hibbs, 2001; Sun & Ho, 2005; Yu, Haley, Perret, & Harris, 2002a).

Barley is a widely consumed cereal among the most ancient cereal crops. Almost 80–90% of barley production is for animal feeds and malt, but now barley is gaining renewed interest as an ingredient for production of functional foods due to their concentration of bioactive compounds, such as β-glucans and tocols (Jadhav, Lutz, Ghorpade, & Salunkhe, 1998; Peterson, 1994). Moreover, there are many classes of phenolic compounds in barley, such as benzoic and cinnamic acid derivatives, proanthocyanidins, quinines, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds (Goupy et al., 1999; Hernanz et al., 2001; McMurrough & Madigan, 1996; McMurrough, Madigan, & Kelly, 1996). In fact, these phenolic compounds have attracted the attention of food and medical scientists because of their strong in vitro and in vivo antioxidant activities and their ability to scavenge free radicals, break radical chain reaction and chelate metals. Furthermore, high phenol consumption has been connected with a reduced risk of cardiovascular diseases and some cancers (Marja et al., 1999; Tapiero,

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Tew, Nguken Ba, & Mathe, 2002). The abundant contents of phenolic compounds in barley reveal that barley may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion.

The solvent extraction has been used widely to extract bioactive components from plants. It is noted that an extraction solvent system is selected according to the purpose of extraction such as the nature of interested components, the availability of reagents and equipments, cost and safety concerns and so on (Yu et al., 2002b). Aqueous methanol, ethanol and acetone separately or mixed are commonly used to extract phenolic compounds from materials (Kahkonen et al., 1999; Velioglu, Mazza, Gao, & Oomah, 1998; Zielinski & Kozlowska, 2000). The objective of our research was to investigate the total phenolic content and antioxidant activity of various solvent extract from barley using methanol, ethanol, and acetone. The antioxidants properties of these solvent extracts, including reducing power, scavenging effect on 2,2-diphenyl-1-picrylhydrazyl and inhibition of lipid oxidation were measured and compared to ascorbic acid (Vit. C) and synthetic antioxidants BHT.

2. Materials and methods

2.1. Materials

Barley was purchased from a local market. Whole barley was ground to pass through a 1.0 mm sieve, and then the powder was defatted for 24 h with *n*-hexane under continuous stirring at room temperature, and stored at 4 °C until used.

2.2. Chemicals and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteau reagent, linoleic acid and Standard of catechin were purchased from Sigma Co. (St. Louis, MO, USA). Butylated hydroxyluene (BHT) and ascorbic acid (Vit. C), food grade antioxidants, were purchased from the Guangzhou Chemical Company, PR China. All the other solvents and chemicals used were of analytical grade.

2.3. Extraction

Fifteen grams of the defatted barley powder were weighed and put into a 500 ml bottle. Two hundred milliliter of 70% acetone (v/v), 70% ethanol (v/v), and 70% methanol (v/v), were added to each bottle, respectively. After 4 h of extraction at 45 °C, the supernatant and the sediment were separated by vacuum filtration. The residue was re-extracted as the first extraction. The obtained extraction solutions were combined and concentrated to dryness by vacuum-evaporator at 45 °C. The dried extract was weighed and the yield was calculated. The antioxidant extracts were kept in dark at 4 °C until further analyses.

2.4. Determination of total phenolics (TP) content

The total phenolics content of the extracts was determined with the Folin–Ciocalteau method with little change (Bonoli, Verardo, Marconi, & Caboni, 2004). Briefly, 0.5 ml diluted extract solution was shaken for 1 min with 100 μl of Folin–Ciocalteau reagent and 6 ml of distilled water. After the mixture was shaken, 2 ml of 15% Na_2CO_3 was added and the mixture was shaken once again for 0.5 min. Finally, the solution was brought up to 10 ml by adding distilled water. After 1.5 h, the absorbance at 750 nm was evaluated using a spectrophotometer. The results were expressed as gallic acid equivalents.

2.5. Determination of proanthocyanidins (PA) content

The PA content of the extracts was determined according to the method of Sun, Ricardo-Da-Silva, and Spranger (1998). Briefly, 0.5 ml diluted extracts solution was mixed with 3 ml of 4% vanillin–methanol solution and 1.5 ml hydrochloric acid and the mixture was allowed to stand for 15 min. The absorbance at 500 nm was measured and the final results of PA content were assessed as catechin equivalents.

2.6. Reducing power

The reducing power was determined by the method of Oyaizu (1986). The extracts (50–200 μg) diluted in 1.0 ml distilled water with different concentrations were mixed with sodium phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. At the end of the incubation, trichloroacetic acid (2.5 ml, 10%) was added to the mixtures, followed by centrifuging at 3000 rpm for 10 min. The upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid (Vit. C) and butylated hydroxyluene (BHT) were used for comparison.

2.7. Free radical scavenging activity

Free radical scavenging capacity of barley extracts was evaluated according to the reported procedure using the stable 2, 2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) (Chen, Wang, Rosen, & Ho, 1999). Briefly, barley extracts (50–200 μg) diluted in ethanol (3 ml) was mixed with 3 ml $DPPH^{\cdot}$ solution in ethanol (200 μM). The final concentration of $DPPH^{\cdot}$ was 100 μM. The reaction mixture was shaken, and incubated in the dark. The absorbance of the solution was measured against a blank at 517 nm after 30 min. Inhibition of free radical DPPH in percent ($I\%$) was calculated by using the following equation: $I\% = [(A_0 - A_e)/A_0] \times 100$, where A_0 is the absorbance of the blank sample and A_e is the absorbance of the tested

sample. BHT and Vit. C dissolved in ethanol were also analyzed for comparison.

2.8. Ferric thiocyanate method (FTC)

The FTC method was adapted from Osawa and Namiki (1981). The extracts samples (200 μg) in ethanol (4 ml) were mixed with 2.5% linoleic acid in ethanol (4 ml), phosphate buffer (8 ml, 0.05 M, pH 7.0) and distilled water (4 ml), and kept in screw cap tubes under dark conditions at 40 °C. Aliquots (0.1 ml) were drawn from the incubation solution and mixed with 75% ethanol (9.7 ml) and 30% ammonium thiocyanate (0.1 ml). Precisely 3 min after addition of 0.1 ml 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red colour was measured at 500 nm each 24 h until reaching a maximum value. The control and standard were prepared as the sample. For the control, there was no addition of sample, and for the standard, 200 μg of sample were replaced by 200 μg of BHT and Vit. C.

2.9. Statistical analysis

Experimental results are mentioned as means ± SD of three parallel measurements. P values < 0.05 were regarded as significant. The statistical analysis was done by the Statistical Package for Social Science (SPSS 11.5).

3. Results and discussion

3.1. Yield

The extraction yield (Table 1) varied from 3.96% to 4.92% (w/w) depending on the extraction solvent with the following order: 70% methanol > 70% ethanol > 70% acetone. No significant ($P < 0.05$) difference was found only between the yield of 70% methanol extract and 70% ethanol extract. The result agreed with the yield from *Gevuina avellana* hulls (Moure et al., 2000) and buckwheat (Sun & Ho, 2005), which showed the same order of solvent.

Table 1
Yield and content of total phenolic and proanthocyanidins in extracts from barley

Sample	Yield (%)	Total phenolic (GE[A] mg/g flour)	Proanthocyanidins (CA[B] mg/g flour)
70% Acetone extract	3.96 ± 0.23^{a}	1.96 ± 0.06^{c}	1.15 ± 0.04^{c}
70% Ethanol extract	4.70 ± 0.13^{b}	1.75 ± 0.04^{b}	0.78 ± 0.08^{b}
70% Methanol extract	4.92 ± 0.22^{b}	1.32 ± 0.09^{a}	0.63 ± 0.06^{a}

Values within a column with different superscripts were significantly different ($P < 0.05$), a = the lowest value.
[A] GE is gallic acid equivalent.
[B] CA is catechin equivalent.

3.2. The contents of TP and PA

It is considered that the phenolic compounds contribute to overall antioxidant activities of barley extracts. Total phenolics and proanthocyanidins contents of the barley extracts from different solvents were examined and are presented in Table 1. Significant difference was detected among these extracts. The results showed that the contents of TP and PA were affected by the extraction solvents with the following order from high to low: acetone > ethanol > methanol. No correlation was found between the yield and the content of TP and PA. For example, although 70% methanol extract had lower content of TP and PA, it gave a higher yield than the 70% acetone extract. Acetone was superior to ethanol and methanol in extracting total phenolics, which agrees with the results from buckwheat (Sun & Ho, 2005). Variations in the TP and PA contents of three extracts are attributed to the polarities of different solvents using in the experiment. The results indicated that the polarity of 70% acetone was more selective to the phenolic compounds present in barley than other two solvents. The proanthocyanidins were sensitive to the extract solvents. PA content of acetone extract was 1.5 times and 1.8 times higher of those ethanol extract and methanol extract, respectively. In addition, PA account for about half part of total phenolics in barley extracts. It is predicted that the proanthocyanidins play an important role in barley extracts.

3.3. Reducing power

For measurement of the reductive ability, the Fe^{3+}–Fe^{2+} transformation in the presence of barley extracts samples was investigated. Fig. 1 shows the reductive capabilities of different solvent barley extracts compared with BHT and Vit. C. Increase in absorbance of the reaction mixture indicated the reducing power of the samples. As shown in Fig. 1, the reducing power of all the barley extracts increased with increasing amount of sample, and all of the amounts showed high activities. At amount of 200 μg, the reducing power of 70% acetone extract was closed to that of BHT. Reducing power of different solvent extracts of barley and standard compounds exhibited the following order: Vit. C > BHT > 70% acetone extract > 70% ethanol extract ≈70% methanol extract.

The reducing capacity of a compound may serve as a significant indictor of its potential antioxidant activity (Meir, Kanner, Akiri, & Hadas, 1995). The reducing properties are generally associated with the presence of reductones (Pin-Der, 1998). It is reported that the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom, or reacting with certain precursors of peroxide to prevent peroxide formation (Gordon, 1990). The data presented here indicate that the marked reducing power of barley extracts seem to be the result of their antioxidant activity. It is presumed that the phenolic compounds may act in a similar fashion

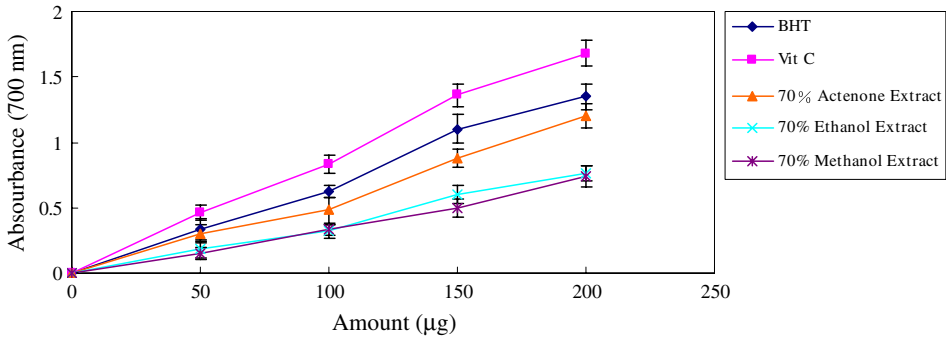

Fig. 1. Reducing power of barley extracts, BHT, and Vitamin C. Results are means ± SD of three parallel measurements.

as reductones by donating electrons and reacting with free radicals to convert them to more stable products and terminating the free radical chain reaction.

3.4. Free radical scavenging activity

The free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food products. DPPH· is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, Dins, Cunha, & Ameida, 1997). The reduction capability of DPPH· is determined by the decrease in its absorbance at 517 nm induced by antioxidants. Now DPPH· has been widely used in assessment of radical scavenging activity because of its ease and convenience.

The scavenging effect of different solvent extracts from barley on DPPH radical decreased in this order: 70% acetone extract > 70% ethanol extract ⩾ 70% methanol extract (Fig. 2). These results indicated that all the extracts had a noticeable effect on scavenging free radical. There was no significant ($P < 0.05$) difference between the scavenging activity of 70% methanol and 70% ethanol extracts at the same amount. The extract from 70% acetone had the strongest scavenging activity, which showed similar scavenging activity to BHT at the amount of 200 μg.

3.5. Ferric thiocyanate method (FTC)

The FTC method was used to measure the peroxide level during the initial stage of lipid oxidation. Peroxides are formed during the linoleic acid oxidation, which react with Fe^{2+} to form Fe^{3+}. The latter ions form a complex with SCN^- and this complex has a maximum absorbance at 500 nm. The effects of various solvent extracts of barley, in preventing the peroxidation of linoleic acid, are shown in Fig. 3. After the incubation period (9 d), the formation of peroxides was stopped because of non-availability of linoleic acid. Also, the intermediate products had been converted to stable end-products resulting in the stoppage of oxidation of Fe^{2+}. Therefore, the absorbance at 500 nm was reduced. The oxidation of linoleic acid will be slow in the presence of antioxidants. The results showed that all extracts obtained from different solvent exhibited effective antioxidant activity. The antioxidant activity of ascorbic acid was similar with that of 70% methanol and 70% etha-

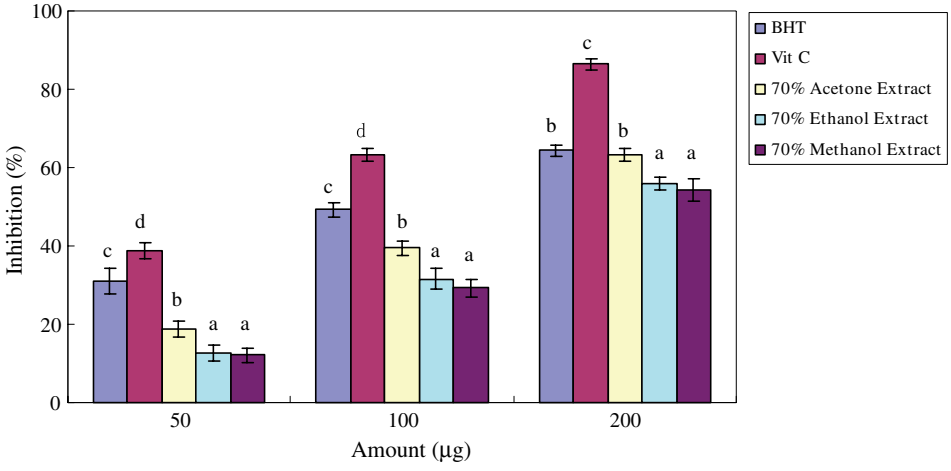

Fig. 2. Free radical scavenging activity of barley extracts analyzed by DPPH method. (Results are means ± SD of three parallel measurements, values with different superscripts were significantly different, $P < 0.05$, a = the lowest value of antioxidant activity.)

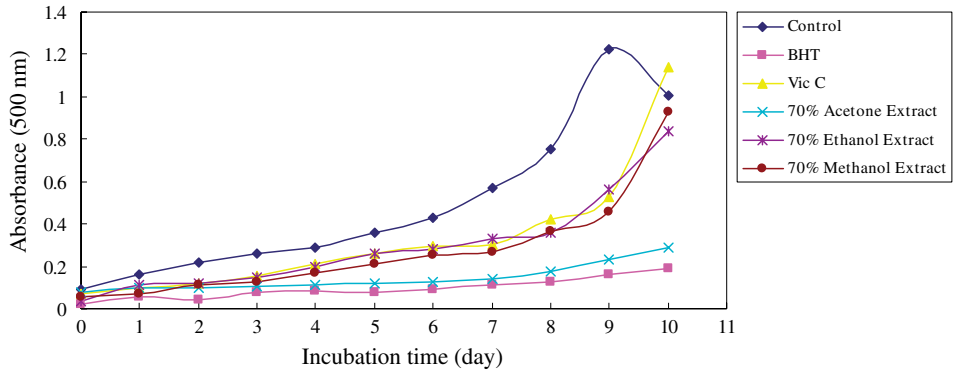

Fig. 3. Antioxidant activity of barley extracts as measured by the FTC method.

nol extracts. It is interesting to find that the 70% acetone extracts exhibited higher activity than other two extracts. There was no significant ($P < 0.05$) difference between antioxidant activities of the 70% acetone extracts and BHT during the incubation time. It is presumed that the 70% acetone extract owe its higher antioxidant activity to its higher phenolic compounds and proanthochanidins contents.

4. Conclusions

In this study we have shown that the extraction solvents significantly affect the total phenolics and proanthocyanidins contents of the extract. The results showed that 70% acetone extraction of barley gained more phenolic compounds, especially proanthocyanidins. The different solvent extracts from barley were found to possess antioxidant activity, including reducing power, radical scavenging activity determined by DPPH$^{\cdot}$ and the lipid peroxidation inhibition. The extract of 70% acetone can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the phenolic compounds or other components responsible for the antioxidant of extracts from barley are still unknown. Therefore, further studies would be in progress for isolation and identification of the antioxidant components in barley.

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