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Antioxidant activities of buckwheat extracts

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Abstract

The antioxidant activities of buckwheat (*Fagopyrum esculentum* Möench) extracts were evaluated and compared with butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ) using a β-carotene bleaching assay, a 2,2-diphenyl-β-picrylhydrazyl (DPPH) assay and the Rancimat method. Buckwheat was extracted with solvents of different polarities. The methanol extract showed the highest antioxidant activity coefficient (AAC) of 627 ± 40.0 at 200 mg/l by the β-carotene bleaching method and longest induction time of 7.0 ± 0.2 h by the Rancimat method. The acetone extract showed the highest total phenolics of 3.4 ± 0.1 g catechin equivalents/100 g and the highest scavenging activity of $78.6 \pm 6.2\%$ at 0.1 mg/ml by the DPPH method. The properties of the extracting solvents significantly affected the yield, total phenolics and antioxidant activity of buckwheat extract.

Keywords: Antioxidants; Buckwheat; Free radical scavenging; Phenolic content

1. Introduction

Natural antioxidants from plant extracts have attracted increasing interest due to consumer concern about the safety of the synthetic antioxidants in food. Extracts of fruits, vegetables, cereals (Kähkönen, Hopia, & Heinonen, 2001; Tsushida, Suzuki, & Kurogi, 1994; Velioglu, Mazza, Gao, & Oomah, 1998) and their by-products, such as the cereal hulls (Amarowicz, Naczk, & Shahidi, 2000; Moure et al., 2000), apple peel (Wolfe, Wu, & Liu, 2003), and citrus peel and seed (Bocco, Cuvelier, Richard, & Berset, 1998), all showed effective antioxidant activity in a model system. Natural antioxidants were also tested in real food systems. For example, tea catechins significantly retarded lipid oxidation in chicken meat (Tang, Kerry, Sheehan, & Buckley, 2002).

Buckwheat (*Fagopyrum esculentum* Möench), unlike most cereals, is an alternative crop belonging to the Polygonaceae family. Buckwheat seed contains antioxidants, such as rutin, tocopherols and phenolic acids, and can thus be stored for a long time without apparent chemical changes (Dietrych-Szostak & Oleszek, 1999). Buckwheat contains more rutin than most plants. Rutin is a flavonol glycoside plant metabolite with antioxidative, anti-inflammatory and anticarcinogenic effects, and can also reduce the fragility of blood vessels related to hemorrhagic disease and hypertension in humans (Oomah & Mazza, 1996). Rutin and isovitexin are the only reported flavonoids of buckwheat seed. Buckwheat hulls contain rutin, orientin, vitexin, quercetin, isovitexin and isoorientin (Dietrych-Szostak & Oleszek, 1999).The total flavonoid concentrations of buckwheat seed and hull are 18.8 mg/100 g and 74 mg/100 g, respectively. Flavonoids isolated from buckwheat hulls showed radical scavenging activity when analyzed in the purified form (Watanabe, 1998; Watanabe, Ohshita, & Tsushida, 1997). Variation in antioxidant activity of buckwheat was mainly due to the cultivars and environment effects (Oomah & Mazza, 1996).

No single solvent could extract all the antioxidants in buckwheat with different polarities and solubilities. Aqueous mixtures of ethanol, methanol and acetone are commonly used to extract plants. Phenolics, as well as non-phenolic compounds (sugars, organic acid, proteins

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and pigments), can be present in the extracts (Macheix, Fleuriet, & Billot, 1990). Polyphenols, including *p*-hydroxybenzoic, syringic, vanillic and *p*-coumaric acids, exist in the bran-aleurone layer of buckwheat seed in free and bound forms and the latter could be liberated by either alkaline or acid hydrolysis. Buckwheat seed have been extracted with ethanol (Watanabe, 1998) or 80% methanol (Oomah & Mazza, 1996) to separate the antioxidant compounds. Methods to determine antioxidant activity were generally based on the inhibition of certain reactions by the presence of antioxidants (Arnao, Cano, & Acosta, 1999). Oxidizing reagents could be organic radical producers or metal ions (Schlesier, Harwat, Bohm, & Bitsch, 2002). Antioxidants react with free radicals or peroxide. The mechanisms to measure antioxidant activity may be divided into several types: (1) The delay of radical generation and the ability to scavenge a radical, such as total radical-trapping antioxidant parameter (TRAP) assay (Ghiselli, Serafini, Maiani, Azzini, & Ferro-Luzzi, 1995; Pellegrini et al., 2003) and photochemiluminescence (PCL) (Gahler, Otto, & Bohm, 2003; Popov & Lewin, 1999); (2) Free radicals a reagent is reduced by antioxidants and the absorbance at certain wavelengths is decreased, such as 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993; Re et al., 1999), DPPH (Brandwilliams, Cuvelier, & Berset, 1995) and *N*,*N*-dimethyl-*p*-phenylendiamine (DMPD) assay (Fogliano, Verde, Randazzo, & Ritieni, 1999), while the ferric reducing ability of plasma (FRAP) assay (Benzie & Strain, 1996; Pulido, Bravo, & Saura-Calixto, 2000) measures increased absorbance of the formed ferrous ions. Antioxidant activity estimation is testing system-dependent. The specificity and sensitivity of one method does not lead to complete examination of all phenolic compounds in the extract. A combination of several in vivo and in vitro tests could provide a more reliable assessment of the antioxidant activity, such as six methods used for the antioxidant activity of beverages (tea and juices) (Schlesier et al., 2002) and three antioxidant activity methods used for phenolic acid, fruit and vegetable extracts (Cervellati, Renzulli, Guerra, & Speroni, 2002; Galato et al., 2001; Proteggente et al., 2002).

The objective of our research work was to investigate the effect of extracting solvents on the yield, total phenolics and antioxidant activity of buckwheat. Antioxidant activity of buckwheat extract was compared to synthetic antioxidants, such as BHA, BHT and TBHQ.

2. Materials and methods

2.1. Materials

Buckwheat (*F. esculentum* Möench) was purchased from a local grower. Whole buckwheat was ground to pass a 1 mm screen and stored at 4 °C before experiment. Sodium tungstate, phosphomolybdic acid and tannic acid were purchased from Aldrich Chemical Company (Milwaukee, WI). The compounds, α, α-diphenyl-β-picrylhydrazyl, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), β-carotene and lard were obtained from Sigma Chemical Company (St. Louis, MO).

2.2. Extraction

Twenty grammes of the powdered buckwheat sample were weighed and put into a 500 ml bottle. Three hundred ml of acetone, butanol, 96% ethanol, ethyl acetate or methanol, were added to each bottle, respectively. After 1 week of storage at room temperature, the supernatant and the sediment were separated by vacuum-filtration. The residue was extracted a second time described as the first extraction. The first and second extraction solutions were combined and dried by vacuum-evaporator. The dried extract was weighed and the yield was calculated based on the wet weight of buckwheat seed.

2.3. Determination of total phenolics content

The total phenolics of buckwheat extracts were determined according to the AOAC method 952.03 with revision (AOAC, 1995). One litre of Folin–Denis reagent contained 100 g sodium tungstate, 20 g phosphomolybdic acid and 50 ml acid phosphate in water. Folin–Denis reagent reacted with buckwheat extract in the presence of sodium carbonate solution. After half an hour of incubation, the absorbance was determined at 760 nm using a spectrophotometer. Catechin was used to set up the standard curve.

2.4. Determination of antioxidation activity by β-carotene bleaching method

The oxidative losses of β-carotene in a β-carotene/linoleic acid emulsion were used to assess the antioxidation ability of the buckwheat extracts (Moure et al., 2000). Two milligramme of β-carotene were dissolved in 10 ml of chloroform and 1 ml β-carotene solution was mixed with 20 mg of purified linoleic acid and 200 mg of Tween 40 emulsifier in a round-bottom flask. Then, chloroform was removed in a rotary vacuum evaporator. Distilled water (50 ml) was added to the flask and the mixture was stirred in a sonicator. Buckwheat extract (2 mg/ml) or the synthetic antioxidant (BHA, BHT or TBHQ) were added to 5 ml of the β-carotene/linoleic acid emulsion and tested with two final concentrations of 100 and 200 mg/l. Control contained 0.2 ml water and 5 ml β-carotene/linoleic acid emulsion. An absorbance at 470 nm was immediately recorded after adding the sample to the emulsion, which was regarded as $t = 0$ min. The vials

were capped and placed in a water bath at 50 °C. The absorbance of the emulsion at 470 nm was determined every 15 min until 120 min. A second emulsion (B), consisting of 20 mg of linoleic acid, 100 mg of Tween 40 and 50 ml of water, was also prepared. Water (200 μl) with 5 ml emulsion B was used to zero the spectrophotometer. Antioxidant activity coefficient (AAC) was calculated according to the following equation:

$$AAC = (A_{A(120)} - A_{C(120)})/(A_{C(0)} - A_{C(120)}) \times 1000,$$

where $A_{A(120)}$ is the absorbance of the antioxidant at 120 min, $A_{C(120)}$ is the absorbance of the control at 120 min, and $A_{C(0)}$ is the absorbance of the control at 0 min.

2.5. Determination of antioxidant activity by the DPPH radical scavenging method

The antioxidant activity of the buckwheat extracts was measured in terms of hydrogen-donating or radical-scavenging ability, using the DPPH method (Brand-williams et al., 1995; Chen, Wang, Rosen, & Ho, 1999). Buckwheat extract in ethanol (3 ml) was mixed with 3 ml DPPH radical solution in ethanol (2.0×10^{-4} M). The final concentration of DPPH was 1.0×10^{-4} M. The reaction mixtures were shaken vigorously and incubated in the dark for 30 min. The absorbance of the solution was measured at 517 nm.

2.6. Inhibitory effect of buckwheat on lipid oxidation by the Rancimat method

Buckwheat extracts, BHA, BHT or TBHQ were added to 2 g of pure lard oil, giving a final concentration of 0.02% (w/w). A Metrohm 679 Rancimat instrument (Herisan, Switzerland) was used in the experiment. Air supply was maintained at 20 ml/min and the temperature was kept at 80 °C for 40 h. The induction time of the lard oil was recorded.

2.7. Statistical analysis

All analyse were run in triplicate. The average value and standard deviation were calculated using Excel. Analysis of variance (ANOVA) was used to evaluate the significant difference among various treatments with the criterion of $P = 0.05$ (Zar, 1996).

3. Results

3.1. Yield

The extraction yield (Fig. 1) varied from 2.8% to 5.0% (w/w) depending on the extraction solvent with the following order: Methanol > ethanol > butanol = acetone = ethyl acetate. The yield of methanol extract was 1.9 times that of butanol extract. Our result agreed with the yield from *Gevuina avellana* hulls, which showed the following order: Methanol > ethanol > acetone extract (Moure et al., 2000). Buckwheat was extracted twice to increase the yield. The yield of the second extraction was 16.5–36.2% (w/w) of the total extract.

3.2. Total phenol content

Total phenol content, expressed as g catechin equivalent/100 g buckwheat, was affected by the extracting

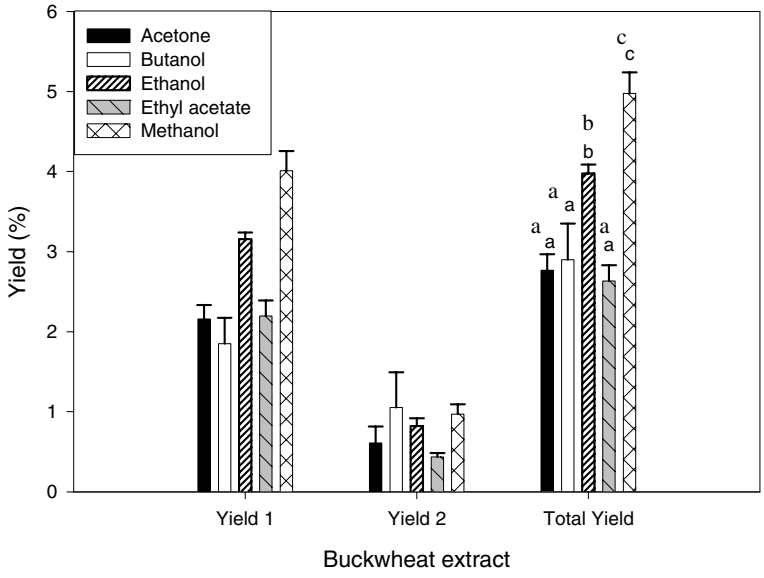

Fig. 1. Yield of buckwheat extract ($N = 3$, yield 1 was obtained from the first extract; yield 2 was obtained from the second extract; total yield was the sum of yield 1 and 2; values with different superscripts were significantly different, $P = 0.05$, a = the lowest yield).

Table 1
Total phenolics in buckwheat extract ($N = 3$) and their antioxidant activity as indicated by the induction time of lard added the buckwheat extract determined using the Rancimat method ($N = 3$)

Sample	Total phenolics* (g catechin equivalents/100 g)	Induction time (hours)**
Control		2.8 ± 0.2^{a}
Acetone extract of buckwheat	3.3 ± 0.1^{n}	6.2 ± 0.1^{d}
Butanol extract of buckwheat	1.0 ± 0.2^{k}	4.6 ± 0.2^{c}
Ethanol extract of buckwheat	2.3 ± 0.2^{m}	5.0 ± 0.2^{c}
Ethyl acetate extract of buckwheat	0.9 ± 0.1^{k}	3.6 ± 0.1^{b}
Methanol extract of buckwheat	2.1 ± 0.1^{m}	7.0 ± 0.2^{e}
BHA		31.8 ± 1.0^{h}
BHT		16.8 ± 0.8^{g}
TBHQ		10.8 ± 0.6^{f}

* Values with different superscripts were significant different ($P = 0.05$), k = the lowest value.
** Values with different superscripts were significant different ($P = 0.05$), a = the lowest incubation time.

solvents with the following order from high to low: Acetone > methanol = ethanol > butanol = ethyl acetate (Table 1). Total phenol content of acetone extract was 3.9 times and 1.6 times those of ethyl acetate extract and methanol extract, respectively. Total phenolics of methanol extract was 2.1 g catechin equivalent/100 g, which was slightly lower than in the published data, i.e., 5.9 g/100 g for buckwheat methanol extract (Przybylski, Lee, & Eskin, 1998). No correlation was found between the yield and the total phenol content ($R^2 = 0.037$, $P = 0.22$). For example, methanol extract of buckwheat gave a higher yield than the ethanol extract although both extracts showed similar total phenol contents.

3.3. Antioxidant activity determined by β-carotene bleaching method

The absorbance of the emulsion decreased with time (Fig. 2). Buckwheat extract, BHA, BHT or TBHQ showed variant antioxidation activity. The decreasing rate of absorbance for emulsion sample with methanol extract added was significantly lower than the samples with the addition of other extracts. For BHA, BHT and TBHQ, the absorbance did not change apparently during the incubation.

The AAC values are summarized in Fig. 3. Due to the large standard deviation of the methanol and ethanol extracts, no significant difference was found among the five extracts at the concentration of 100 mg/l, however, significant difference existed among the following three extracts with values from high to low: Acetone > butanol > ethyl acetate. At the concentration of 200 mg/l, the order of AAC values was as follows: Methanol extract > ethanol extract = ethyl acetate extract = butanol extract > acetone extract. Increasing the concentration from 100 to 200 mg/l increased the AAC value. The methanol extract of buckwheat, at 200 mg/l showed the highest antioxidation activity, the AAC value of which was 67% of that of BHA and TBHQ. Synthetic antioxidants are permitted to be added to edible oils to a maximum level of 0.02%. Buckwheat extract could be used in food as a natural antioxidant to replace the synthetic antioxidant. Natural buckwheat extract is regarded safe and could be added to food at a higher concentration than 0.02%, resulting in an antioxidant activity comparable to that of the artificial antioxidants.

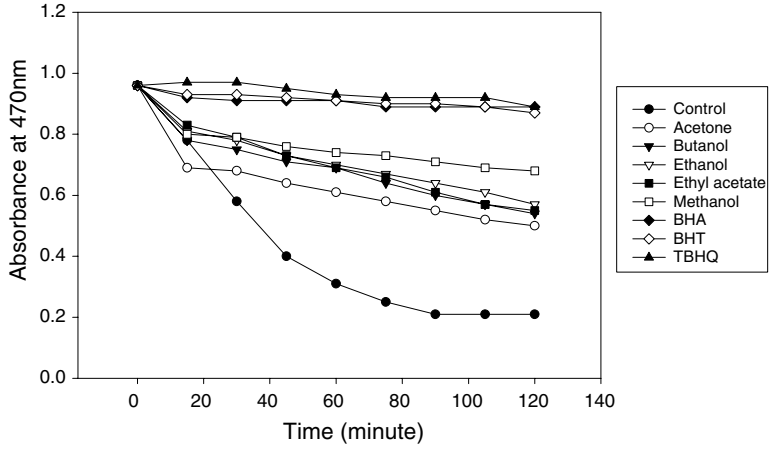

Fig. 2. Change of absorbance at 470 nm with time for buckwheat extract (200 mg/l) in β-carotene/linoleic acid emulsion.

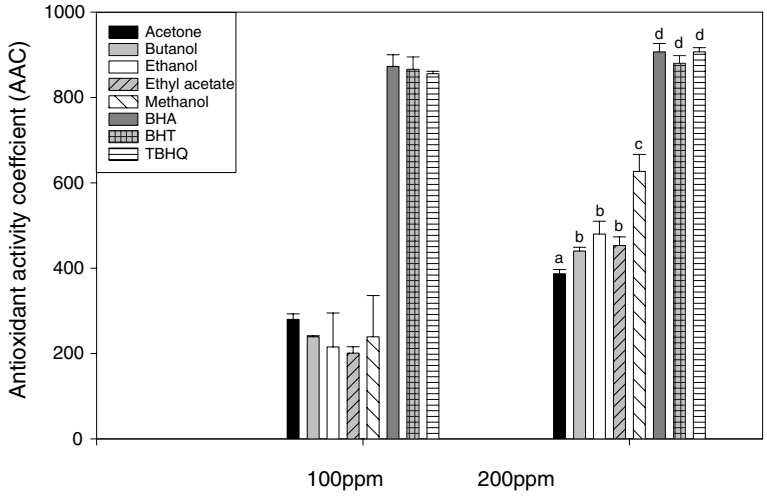

Fig. 3. Antioxidant activity of buckwheat extract analyzed by β-carotene bleaching method ($N = 3$, values with different superscripts were significantly different, $P = 0.05$, a = the lowest value of antioxidant activity).

3.4. Antioxidant activity determined by DPPH method

Fig. 4 shows the percentage inhibition of free radical by buckwheat extracts due to hydrogen donation from the antioxidant. The colour of the DPPH reagent was significantly reduced from purple to yellow. The order of antioxidant activity of 0.1 mg/ml buckwheat extract was: Acetone extract > ethanol extract = methanol extract ⩾ butanol extract = ethyl acetate extract. At 0.5 and 1.0 mg/ml, buckwheat extract showed similar scavenging activity to BHA, BHT or TBHQ.

3.5. Antioxidation activity determined by the Rancimat method

In Rancimat tests, buckwheat extract retarded the oxidation of lard and increased the induction time of lard peroxidation (Table 1). Longer induction time indicated higher antioxidation activity. Antioxidant activity of the sample decreased in the following order: BHA > BHT > TBHQ > methanol extract > acetone extract > ethanol extract > butanol extract > ethyl acetate extract. Methanol extract increased the induction time by 1.5 times compared to control.

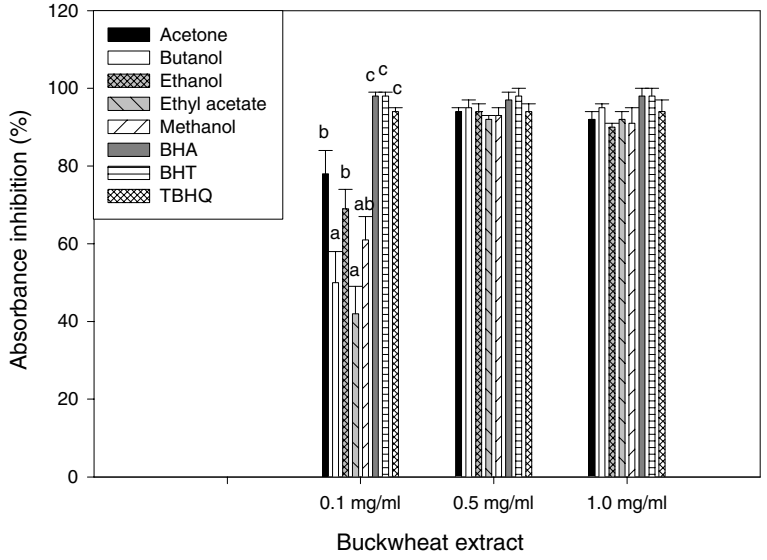

Fig. 4. Antioxidant activity of buckwheat extract at different concentrations analyzed by DPPH method ($N = 3$, values with different superscript were significantly different, $P = 0.05$, a = the lowest value of antioxidant activity).

4. Discussion

Solvent extraction is the most common method used in sample preparations from plants. The extraction yield depends on extraction solvent, time and temperature of extraction as well as on the chemical nature of the sample. At the same time and temperature of extraction, the solvent used and the chemical property of sample are the two most important factors. The yields of buckwheat extract was 1.5–4% (w/w). For potato peel, the yield was 0.5%, which was relatively low. The yield of lentil seeds and grape pomace were 23.3% and 42%, respectively (Moure et al., 2000).

The evaluation of different extraction solvents produced valuable information about the use of buckwheat phenolics in food. Our results clearly showed that the extraction solvents significantly affected the phenolic content of the extract. Acetone extraction of buckwheat gained 1.6 times more total phenolics than methanol extraction. Acetone was superior to methanol in extracting total phenolics, which agrees with the results from berries (Kähkönen et al., 2001).

The relationship between the antioxidant activity and total phenolics of buckwheat extract was complex and difficult to describe by statistical tools. With the DPPH method, it seems that the inhibition percentage (antioxidant activity) and total phenolics correlated significantly ($R^2 = 0.96$, $P = 0.002$). However, there was no correlation found between the AAC value tested by the β-carotene bleaching method and total phenolics content ($R^2 = 0.016$, $P = 0.018$), nor between the antioxidant activity tested by the Rancimat method and total phenolics content ($R^2 = 0.50$, $P = 0.05$). The antioxidant activity of some vegetables and free phenolic compounds, showed a positive, but not strong, correlation ($R^2 = 0.57$) (Chu, Sun, Wu, & Liu, 2002). There are several reasons to explain the ambiguous relationship between the antioxidant activity and total phenolics: (1) Total phenolics content did not include all the antioxidants, such as ascorbic acid, carotenoid and tocopherol. Flavonols and hydroxycinnamic acids in berries accounted for only 31% of the antioxidant activity of the extract (Kähkönen et al., 2001); (2) The antioxidant activity of the fruit extracts was higher than those of most pure phenolics and vitamins on a weight basis (Vinson, Su, Zubik, & Bose, 2001). 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; (3) Different method to measure antioxidant activity with various mechanisms may lead to different observations. For example, the antioxidant activity of berry extract mainly related to anthocyanins in the method of the lower density lipoproteins (LDL) oxidation, but mainly correlated with hydroxycinnamates in the assay of liposome oxidation (Kähkönen et al., 2001). With the β-carotene bleaching method, peroxyl radical scavenging and metal inactivation were major antioxidation factors. However, the polarity of the compound and the physical state of the lipid system also affected the behaviour of antioxidants.

The antioxidant activity of buckwheat extract was affected by the extraction solvent and the analysis method (Moure et al., 2000). Methanol extract of buckwheat showed the highest antioxidant activity by two of the three antioxidant methods used in our research. When buckwheat was sequentially extracted with hexane, diethyl ether, ethyl acetate, acetone and methanol (Przybylski et al., 1998), methanol extract showed the highest antioxidant activity, which partially supports our results. Chemical methods with various mechanisms were available to determine the antioxidant activity (Koleva, Niederlander, & van Beek, 2001). The measured antioxidant activity of a sample depends on which free radical or oxidant is used in the assay (Cao, Sofic, & Prior, 1996). In our research, DPPH radical, peroxyl radical ($ROO^{\cdot}$) from oxidized linoleic acid or heated lard provided different rankings of the antioxidant activity of buckwheat extract. The highest antioxidant activity was from the methanol extract tested by the carotene bleaching method and the Rancimat method. Acetone extract showed the highest antioxidation activity tested by the DPPH method. Due to the nature of the assays and the variety of radicals applied, it is not possible to make an absolute comparison of different methods.

Natural antioxidants may have the potential to prevent the oxidation of lipid food. The antioxidant activity of buckwheat extract showed effective antioxidant activity when compared to artificial antioxidants. Using the Rancimat method, the induction time for methanol extract and TBHQ were 7.0 ± 0.2 h and 10.8 ± 0.6 h, respectively. For the carotene bleaching method, the AAC values of methanol extract and BHT at 200 mg/l were 626 ± 41 and 880 ± 12, respectively. For the DPPH method, the percentage inhibition of acetone extract and TBHQ at 0.1 mg/ml were $78.6 \pm 6.2\%$ and $94.1 \pm 2.6\%$, respectively. Natural antioxidant extract from galangal at 10% (w/w) was as effective as 0.1% tocopherol or 0.02% BHT in retarding lipid oxidation of raw beef.

In conclusion, the extracting solvent significantly affected the yield, total phenolic content and antioxidant activity of buckwheat extract. Methanol extract showed the highest antioxidant activity when determined by the carotene-bleaching method or the Rancimat method, while acetone extract showed the highest antioxidant activity when evaluated by the DPPH method. Methanol could be the optimal solvent for extraction of buckwheat antioxidant. The antioxidant activity of buckwheat showed promise as a food additive to replace artificial antioxidants.

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