



Short communication

Phenolic content and antioxidant activity of extracts from whole buckwheat (*Fagopyrum esculentum* Möench) with or without microwave irradiationGeorge E. Inglett^{a,*}, Devin J. Rose^a, Diejun Chen^a, David G. Stevenson^{a,1}, Atanu Biswas^b^a Cereal Products and Food Science Research Unit, National Center for Agricultural Utilization Research, USDA, ARS, 1815 N. University Street, Peoria, IL 61604, USA²^b Plant Polymers Research Unit, National Center for Agricultural Utilization Research, USDA, ARS, 1815 N. University Street, Peoria, IL 61604, USA

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ABSTRACT

The purpose of this study was to extract phenolic compounds and antioxidant activity from buckwheat with water, 50% aqueous ethanol, or 100% ethanol using microwave irradiation or a water bath for 15 min at various temperatures (23–150 °C). Phenolic content of extracts increased with increasing temperature. In general, phenolic contents in microwave irradiated extracts were higher than those heated with a water bath. The highest phenolic content, 18.5 ± 0.2 mg/g buckwheat, was observed in the extract that was microwave irradiated in 50% aqueous ethanol at 150 °C. The highest antioxidant activities, 5.61 ± 0.04 – 5.73 ± 0.00 μ mol Trolox equivalents/g buckwheat, were found in the 100% ethanol extracts obtained at 100 and 150 °C, independent of heat source. These results indicate that microwave irradiation can be used to obtain buckwheat extracts with higher phenolic content and similar antioxidant activity as extracts heated in a water bath.

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1. Introduction

Nutritional studies have demonstrated potential benefits of buckwheat. Brindzova, Mikulasova, Takacsova, Mosovska, and Opatova (2009) found that dimethylsulfoxide extracts from saponified buckwheat flour showed antimutagenic activity in the Ames test against both direct- and indirect-acting mutagens in a concentration dependent manner. Aqueous buckwheat extracts fed for 20 d to rats assuaged renal dysfunction (Yokozawa, Kim, Nonaka, & Kosuna, 2002) and provided protection from oxidative stresses (Mukoda, Sun, & Ishiguro, 2001). Fifty percent aqueous ethanol extracts from buckwheat groats have shown potential to alleviate diabetes symptoms by reducing serum glucose levels in rats (Kawa, Taylor, & Przybylski, 2003), and rats fed buckwheat flour have experienced suppressed gallstone formation and hypercholesterolaemia (Kayashita, Shimaoka, & Nakajoh, 1995; Tomotake et al., 2006).

Many of the health benefits of buckwheat have been attributed to its high levels of phenolic compounds, which exhibit antioxidant

activity (Holasova et al., 2002; Wijngaard & Arendt, 2006). The primary antioxidants in buckwheat are rutin, quercetin, hyperin, and catechins (Morishita, Yamaguchi, & Degi, 2007; Quettier-Deleu et al., 2000; Watanabe, Ohshita, & Tsushida, 1997). Whole buckwheat contains 2–5 times more phenolic compounds than oats and barley, while buckwheat bran and hulls have 2–7 times higher antioxidant activity than barley, triticale, and oats (Holasova et al., 2002; Zdunczyk et al., 2006).

In most cereal grains, the majority of phenolic compounds are bound to cell wall components in the hull (Adom & Liu, 2002). In contrast, buckwheat contains a majority of phenolic compounds present in the free form, which are distributed throughout the entire grain (Hung & Morita, 2008; Quettier-Deleu et al., 2000). Thus, flour, hull, and whole buckwheat extracts exhibit high antioxidant activity (Holasova et al., 2002; Quettier-Deleu et al., 2000).

In general, polar organic solvents are most effective at producing extracts high in phenolic compounds and antioxidant activity (Przybylski, Lee, & Eskin, 1998). In buckwheat, 80% methanol was found to extract 64 times more phenolic compounds and 4 times the antioxidant activity than water (Zielinski & Kozłowska, 2000). In another study investigating effectiveness of methanol, ethanol, butanol, acetone, and ethyl acetate at extracting phenolic compounds from buckwheat flour, acetone extracted the most phenolic compounds, while methanol extracts had highest antioxidant activity (Sun & Ho, 2005). In contrast, one study reported similar levels of phenolic compounds and greater antioxidant activity in

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aqueous extracts from buckwheat flour compared with 80% methanol extracts (Gallardo, Jimenez, & Garcia-Conesa, 2006).

No studies have reported the effects of heat treatments during extraction on the phenolic content or antioxidant activity of buckwheat extracts. Therefore, in this study, we investigated the effectiveness of extracting phenolic compounds and antioxidant activity from buckwheat with water, 50% aqueous ethanol, and 100% ethanol using microwave irradiation or a water bath at various temperatures.

2. Materials and methods

2.1. Buckwheat preparation and experimental design

Whole buckwheat (Minn-Dak Growers Ltd., Grand Forks, ND) was ground using a Fritsch rotor speed-mill (Idar-Oberstein, Germany) and sieved through a 1 mm screen (8 mesh). In triplicate, ground buckwheat flour (1 g) was suspended in 50 mL of water, 50% aqueous ethanol, or 100% ethanol, and then extracted at 23, 50, 100, or 150 °C using microwave irradiation, or 23, 50, and 100 °C using a water bath as the heat source. Water and ethanol were chosen for environmental reasons and possible food applications. A temperature of 150 °C in the water bath was not used due to limitation of conditions. The slurries were then centrifuged (1500g for 10 min) and analysed for total phenolics and antioxidant activity as described below. The effect of extraction temperature, solvent type, and heating method were then compared to determine which conditions resulted in the highest level of total phenolics and antioxidant activity.

2.2. Microwave irradiation

Microwave irradiation took place in 100 mL perfluoroalkoxy Teflon reactor vessels, equipped with lids that contained temperature monitors. A stir bar was placed in each reactor vessel, which was then inserted into the microwave oven (Advanced Microwave System, Ethos 1600, Milestone Inc., Monroe, CT) with a stirring rate of 320 rpm. Conditions within the reaction vessel were monitored with Easy Wave Software (Version 3.5.4.1, Milestone Inc., Monroe, CT), which controlled pressure and microwave power to reach and maintain set temperatures. Come-up time was 5 min and treatment time was 15 min. For the extraction at 23 °C (room temperature) the reaction vessels were placed in the microwave and stirred without applying any microwave irradiation. After microwave treatment, samples were allowed to cool and then transferred to a test tube for centrifugation followed by total phenolics and antioxidant activity evaluation.

2.3. Water bath

Ground whole buckwheat was suspended in solvent as described above for microwave irradiation, and then heated in a water bath at the desired temperature for 15 min. Samples were agitated every 5 min during extraction using a vortex mixer. Following treatment, tubes were cooled and then transferred to a test tube for centrifugation followed by total phenolics and antioxidant activity evaluation.

2.4. Quantification of total phenolics and antioxidant activity of buckwheat extracts

Phenolic content was determined using the Folin–Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999), with minor modifications. Briefly, to 100 µL of extract, 7.9 mL of water, and 0.5 mL of Folin–Ciocalteu reagent (F-9522,

Sigma Aldrich, St. Louis, MO, USA) were added, mixed on a vortex mixer, and 1.5 mL of 1.85 M Na₂CO₃ was added after 8 min. Absorbance of samples was measured at 765 nm after 2 h and gallic acid (220761000, Acros Organics, Morris Plains, NJ, USA) was used as a standard.

Antioxidant activity was determined by reacting 3 mL of extract with 3 mL of 200 µM 2,2-diphenyl-1-picryl-hydrazyl (DPPH; D-9132, Sigma Aldrich) as described by Sensoy, Rosen, Ho, and Karwe (2006). Cloudiness occurred after mixing the reagent with some of the buckwheat extracts; therefore, during the final 10 min of the 40 min reaction time, the tubes were centrifuged for 10 min at 3000 rpm prior to reading the absorbance at 515 nm. Results were expressed as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox; 238813, Sigma Aldrich) equivalents.

2.5. Data analysis

Data were analysed using SAS software (Version 8, SAS Institute, Cary, NC, USA) using a mixed model analysis of variance with Tukey's multiple comparison adjustment to determine significant differences between treatment combinations.

3. Results and discussion

3.1. Microwave irradiation

Because microwaves are notorious for uneven and unpredictable heating, temperature profiles for buckwheat heated in water at three different temperatures are shown in Fig. 1. Clearly, the Easy Wave Software was effective at maintaining the set parameters for each treatment, which were a come-up time of 5 min and treatment time of 15 min. Other treatment combinations showed similar behaviour.

3.2. Effect of extraction temperature on buckwheat extracts

Extraction temperature affected the phenolic content and antioxidant activity of buckwheat extracts, ranging from 1.43 to 18.45 mg total phenolics/g buckwheat (Table 1). Other researchers have reported less than 3.5 mg total phenolics/g of grain in aqueous methanol or acetone extracts from other cereal grains, including corn, wheat, oats, barley, and rice (Adom & Liu, 2002; Moore et al., 2005; Perez-Jimenez & Saura-Calixto, 2005; Verardo, Bonoli,

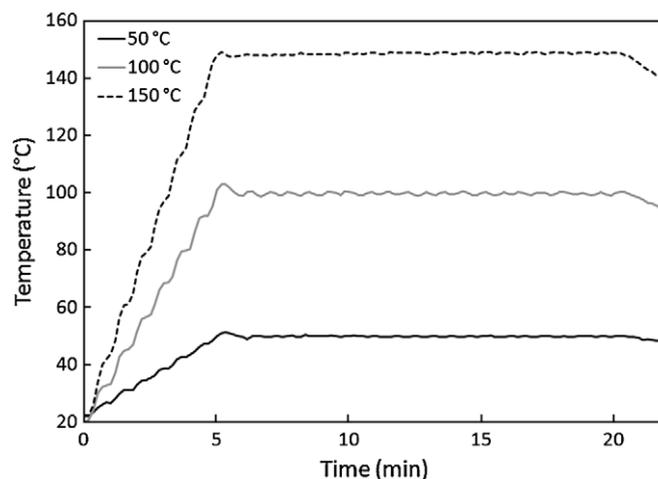


Fig. 1. Temperature profiles of three samples of buckwheat in water during microwave irradiation. Come-up time was set at 5 min for each sample; treatment times were for 15 min at the specified temperature.

Table 1

Phenolic content of water, 50% aqueous ethanol, or 100% ethanol extracts from whole buckwheat flour heated at various temperatures using microwave irradiation or a water bath; values are reported in mg gallic acid equivalents/g buckwheat flour as mean \pm standard deviation; $n = 3$; like capital-letter superscripts within row, or like lower-case superscripts within column indicate no significant difference ($p > 0.05$); nd = not determined.

Solvent	Temperature ($^{\circ}\text{C}$)			
	23	50	100	150
<i>Microwave</i>				
Water	3.90 \pm 0.08 ^{D,c}	6.19 \pm 0.10 ^{C,b}	8.52 \pm 0.07 ^{B,b}	13.2 \pm 0.2 ^{A,b}
50% Ethanol	7.14 \pm 0.11 ^{D,a}	8.02 \pm 0.11 ^{C,a}	11.2 \pm 0.2 ^{B,a}	18.5 \pm 0.2 ^{A,a}
100% Ethanol	2.00 \pm 0.05 ^{C,e}	2.35 \pm 0.09 ^{C,d}	3.89 \pm 0.15 ^{B,d}	6.47 \pm 0.24 ^{A,c}
<i>Water bath</i>				
Water	2.97 \pm 0.26 ^{B,d}	2.92 \pm 0.63 ^{B,d}	6.09 \pm 1.04 ^{A,c}	nd
50% Ethanol	5.36 \pm 0.31 ^{B,b}	5.32 \pm 0.27 ^{B,c}	9.24 \pm 0.67 ^{A,b}	nd
100% Ethanol	1.42 \pm 0.18 ^{B,f}	1.56 \pm 0.02 ^{B,e}	2.90 \pm 0.21 ^{A,d}	nd

Marconi, & Caboni, 2008; Zhou et al., 2007). Therefore, extracts from buckwheat are potentially higher than those of other grains; however, the wide range in phenolic contents of buckwheat extracts obtained with different extraction temperatures demonstrates the importance of this parameter during extraction of phenolic compounds. Phenolic content increased with increasing temperature for all solvent types when comparing temperatures of 50 $^{\circ}\text{C}$ and above (Table 1).

Antioxidant activity ranged from 3.72 to 5.73 μmol Trolox equivalents/g buckwheat (Table 2). The antioxidant activities of the 100% ethanol extracts showed an increasing trend with increasing extraction temperature up to 100 $^{\circ}\text{C}$, while the opposite was true for the 50% aqueous ethanol extracts, and the water extracts did not show a trend with temperature. Similar antioxidant activities have been reported in aqueous methanol or acetone extracts from oats, rice, wheat, and millet (Stratil, Klejdus, & Kuban, 2007; Zhou et al., 2007). This suggests that while total phenolics extracted from cereal grains are strongly dependent on temperature, antioxidant activity may not be. This may be due to the instability of strongly antioxidant phenolic compounds at high temperatures (Liazid, Palma, Brigui, & Barroso, 2007; Makris & Rossiter, 2000).

3.3. Effect of solvent type on buckwheat extracts

Fifty percent aqueous ethanol extracted substantially more phenolic compounds than water and 100% ethanol when microwave irradiated or heated with water bath at all temperatures (Table 1). With respect to antioxidant activity, however, no consistent advantage of 50% aqueous ethanol over the other solvents was observed (Table 2).

Table 2

Antioxidant activity of water, 50% aqueous ethanol, or 100% ethanol extracts from whole buckwheat flour heated at various temperatures using microwave irradiation or a water bath; values are reported in μmol Trolox equivalents/g buckwheat as mean \pm standard deviation; $n = 3$; like capital-letter superscripts within row, or like lower-case superscripts within column indicate no significant difference ($p > 0.05$); nd = not determined.

Solvent	Temperature ($^{\circ}\text{C}$)			
	23	50	100	150
<i>Microwave</i>				
Water	4.49 \pm 0.05 ^{B,b}	3.90 \pm 0.15 ^{C,e}	4.89 \pm 0.01 ^{A,b}	4.72 \pm 0.19 ^{AB,b}
50% Ethanol	5.09 \pm 0.01 ^{A,a}	4.87 \pm 0.01 ^{B,c}	4.63 \pm 0.02 ^{C,c}	4.40 \pm 0.01 ^{D,c}
100% Ethanol	5.03 \pm 0.10 ^{C,a}	5.24 \pm 0.08 ^{B,ab}	5.73 \pm 0.00 ^{A,a}	5.67 \pm 0.01 ^{A,a}
<i>Water bath</i>				
Water	5.04 \pm 0.09 ^{A,a}	5.09 \pm 0.07 ^{A,b}	4.92 \pm 0.06 ^{A,b}	nd
50% Ethanol	5.32 \pm 0.01 ^{A,a}	5.38 \pm 0.03 ^{A,a}	4.92 \pm 0.08 ^{B,b}	nd
100% Ethanol	3.72 \pm 0.39 ^{B,c}	4.15 \pm 0.04 ^{B,d}	5.61 \pm 0.04 ^{A,a}	nd

One-hundred percent ethanol extracts contained the lowest phenolic content of all solvents at all temperatures independent of heating method (Table 1). On the other hand, 100% ethanol produced extracts with the highest antioxidant activity at the highest temperatures (Table 2). Improved antioxidant activity of buckwheat extracts using methanol or acetone compared with water have been reported (Przybylski et al., 1998; Sun & Ho, 2005; Zielinski & Kozłowska, 2000). Higher antioxidant activities in ethanol extracts compared with water have also been reported in microwave irradiated pistachio hulls (Goli, Barzegar, & Sahari, 2005). This suggests that 100% ethanol may select for those compounds that have high antioxidant activity. Another possibility is that buckwheat antioxidants may be more stable under high temperatures in 100% ethanol than in aqueous systems. This assertion is supported by the colour of the extracts (not shown). Rutin and quercetin, two of the most prevalent phenolic compounds in buckwheat (Morishita et al., 2007; Quettier-Deleu et al., 2000; Watanabe et al., 1997), are known to form brown degradation pigments when heated under aqueous conditions (Makris & Rossiter, 2000). The colour of extracts using 100% ethanol had only a slight brown tinge at all temperatures, while water and 50% aqueous ethanol extracts noticeably darkened (not shown).

3.4. Effect of heating method on buckwheat extracts

We observed some differences between heating methods during extraction. The phenolic content was significantly higher in microwave irradiated extracts at all comparable temperatures (23, 50, and 100 $^{\circ}\text{C}$), except the 100% ethanol extract at 100 $^{\circ}\text{C}$ (Table 1). Increases in phenolic content after microwave irradiation have been previously reported for other products (Turkmen, Sari, & Velioglu, 2005). Liazid et al. (2007) studied the effects of microwave irradiation on the stability of over 20 phenolic compounds, and found that all were stable for up to 20 min at 100 $^{\circ}\text{C}$. Therefore, microwave irradiation may disrupt the cellular structure of buckwheat more effectively than heating in a water bath, and thus liberate more phenolic compounds.

Conversely, microwave irradiation did not show a consistent advantage over water bath heating with respect to antioxidant activity (Table 2). Significantly higher antioxidant activities were found in microwave irradiated 100% ethanol extracts at 23 and 50 $^{\circ}\text{C}$ compared to the corresponding extracts that were heated in a water bath, while antioxidant activities of water extracts at 23 and 50 $^{\circ}\text{C}$ and 50% aqueous ethanol extract at 50 $^{\circ}\text{C}$ using microwave irradiation were significantly lower than the corresponding extracts that were heated using a water bath (Table 2). Thus, microwave irradiation may more effectively solubilise phenolic compounds with no antioxidant activity, such as vitexin and isovitexin, compared to heating in a water bath (Watanabe et al., 1997). Alternatively, microwave irradiation may induce degradation of phenolic compounds more than a water bath. The fact that we observed such darker extracts after microwave irradiation compared to water bath heating (not shown) supports this theory.

4. Conclusions

This report compared the effects of heating using microwave irradiation or water bath with different solvents on phenolic compounds and antioxidant activities of buckwheat extracts. Buckwheat extracts showed wide ranges of total phenolics and antioxidant activities; however, extracts containing higher phenolic content and comparable antioxidant activity to other cereal grains could be obtained under certain conditions. In general, higher extraction temperatures with microwave irradiation resulted in higher phenolic contents of extracts, but not necessarily higher

antioxidant activity. This was most likely due to the degradation of phenolic compounds with antioxidant activity during high temperature extraction. Fifty percent ethanol was the most effective solvent for extraction of phenolic compounds, while 100% ethanol produced extracts with the highest antioxidant activity. This may be a result of greater antioxidant stability in ethanol compared to aqueous ethanol systems. Further research on the relationship between phenolic content and antioxidant activity of buckwheat is necessary. In particular, studies on the stability of individual phenolic compounds with antioxidant activity in buckwheat, and their preservation under high temperature extraction conditions.

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