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Effect of processing on buckwheat phenolics and antioxidant activity

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Abstract

The effect of processing on functional compounds in buckwheat was investigated. Extractions of buckwheat flour were carried out before and after roasting or extrusion. Folin–Ciocalteu assays indicated that processing did not cause any change in total phenolic content in buckwheat flour. Roasted (200 °C, 10 min) dark buckwheat flour exhibited an increase in non-polar compounds as well as polar compounds whereas extrusion exhibited increase only in polar compounds. Antioxidant activity test (DPPH) showed that roasting at 200 °C for 10 min decreased the antioxidant activity slightly whereas extrusion (170 °C) did not cause any change. The results suggest that processing conditions can be optimized to retain the health promoting compounds in buckwheat products.

Keywords: Buckwheat; *Fagopyrum esculentum*; Roasting; Extrusion; Phenolic

1. Introduction

Buckwheat (*Fagopyrum esculantum* Moench) is a dry fruit that belongs to the *Polygonaceae* family (Tanaka et al., 2002). Although it is not a cereal grain, it is usually grouped with other cereals because of similarity in cultivation and utilisation. Buckwheat is a native of Northern Europe and Asia (Fortin, 1996; Li & Zhang, 2001; Pomeranz, 1983). Buckwheat is classified as whole grain in the pyramid servings database of the United States Department of Agriculture (Cleveland, Moshfegh, Albertso, & Goldman, 2000).

Phenolic compounds in buckwheat have been shown to possess antioxidative activity (Halosava et al., 2002). Tian, Li, and Patil (2002) have identified four main flavonolglycosides in methanol extracts, namely, rutin, quercetin, kaempferol-3-rutinoside, and a trace quantity of a flavonol triglycoside. Watanabe (1998) has isolated catechins along with rutin in buckwheat. Buckwheat was cultivated as a rutin source but later was discontinued due to the discovery of other highly concentrated sources of rutin such as dimorphandra, which is a legume plant. Rutin has been shown to exhibit antioxidative, antihemorrhagic, and blood vessel protecting properties (Baumgertel, Grimm, Eisenbeib, & Kreis, 2003).

Several researchers have investigated health benefits of buckwheat. According to Prestamo, Pedrazuela, Penas, Lasuncion, and Arroyo (2003) buckwheat could be used as a prebiotic food because it was found to increase lactic acid bacteria in rat intestine. Kim et al. (2003) claimed that buckwheat grain extract could be used in the treatment of allergic inflammation. Buckwheat has been shown (Kawa, Taylor, & Przybylski, 2003) to reduce the serum glucose level in rats due to high content of D-*chrio*-inositol (D-Cl), a component of an insulin mediator.

Proteins in buckwheat flour do not have any toxic prolamins to Celiac patients (Aubrecht & Biacs, 2001; Im, Huff, & Hsieh, 2003). Buckwheat protein suppresses gallstone formation and cholesterol level more strongly than soy protein isolate (Kayashita, Shimaoka, & Nakajyuh, 1995; Tomotake et al., 2000). Protein digestibility of whole buckwheat grain is relatively low (<80%) due to high content of crude fiber and tannin, a protease inhibitor (Ikeda,

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Sakaguchi, Kusano, & Yasumoto, 1991; Pandya, Smith, Yarwood, Gilroy, & Richardson, 1996). Interestingly, buckwheat has been recognized as a common food allergen in Korea and Japan (Park et al., 2000; Tanaka et al., 2002; Taylor & Hefle, 2001) but not in North America where milk, egg, peanut, tree nut, fish, shellfish, soy and wheat are at the top of the list of allergies, probably due to less popularity of buckwheat products.

It is well known that thermal processing causes chemical changes in food products. It is important to understand the effect of processing on functional components like phenolic compounds in buckwheat because it is thermally processed one way or the other before consuming. Few researchers have studied the effect of processing on a few functional components in buckwheat. According to Im et al. (2003), rutin, a flavonoid, in buckwheat grit cakes is affected by temperature and heating time adversely. Orsak, Lachman, Vejdova, Pivec, and Hamouz (2001) observed an increase in rutin content at low dose of γ radiation (50 Gy) and decrease at higher doses (100 Gy) in buckwheat seedlings.

In this study, effects of roasting and extrusion on functional components, especially phenolic compounds of buckwheat, were assessed and the potential use of buckwheat flour in expanded–extruded products as a health promoter was investigated.

2. Materials and methods

2.1. Materials

Dark buckwheat flour: Dark buckwheat flour was purchased from Barry farm, Wapakoneta, Ohio, USA.

Whole buckwheat flour: Whole buckwheat grains (Barry farm, Wapakoneta, Ohio, USA) were milled using a Fitz Mill (Model D) or with a variable speed laboratory blender depending on the amount of flour needed.

White buckwheat flour: Hand separated groats from crushed whole buckwheat grain were milled using a laboratory blender to make flour.

Corn meal: Corn meal was purchased from Iowa Corn Processors, Iowa, USA.

Whole wheat flour: Whole wheat flour, produced by Uhlmann Company (Kansas City, Missouri, USA) was purchased from a local store.

High amylopectin starch: High amylopectin starch (99% amylopectin) was purchased from National Starch and Chemical Company (Bridgewater, New Jersey. USA).

2.2. Processes and analysis

All experiments were conducted in triplicate from the same batch of flour for all the subsequent analysis.

2.2.1. Roasting

Fifty grams of buckwheat flour was placed in a uniform thin layer (2–3 mm) in a 32×23 cm cookie pan prior to roasting. It was roasted in a pre-heated oven (Fisher Scientific, Isotemp® vacuum oven, model 282 A, USA) for 10 min at 200 °C.

2.2.2. Extrusion

Since buckwheat starch has higher amylose content (46%) (Qian, Rayas-Duarte, & Grant, 1998), it limits expansion during extrusion. Therefore, high amylopectin starch was mixed with dark buckwheat flour at 1:1 ratio before the extrusion. The starch was added to increase the amylopectin to amylose ratio in the final mix to enhance expansion during extrusion. The mixture was extruded with ZSK-30 twin-screw extruder (Coperion Corp., Ramsey, New Jersey, USA).

The extruder had two co-rotating, self wiping screws (30.7 mm diameter and 878 mm processing length; length to diameter ratio of 28.6) in a steel barrel with five zones. Each zone was heated by resistive electric heaters and cooled by tap water circulating in the jackets. Temperature of each zone could be controlled independently. The screw configuration used in extrusion experiments consisted of forward conveying elements, mild mixing elements, kneading elements, and reverse elements. The die had two circular orifices (3 mm diameter, 5 mm long). Flour was metered into the feed section of the extruder with a hopper feeder (K-Tron Corp., Pitman, New Jersey, USA). Water was injected into the feed section of the extruder using a triple action piston pump (US Electric Co., Milford CT). Throughput or the total mass flow rate (flour + water) was kept constant at 300 g/min during extrusion.

The ZSK-30 extruder is equipped with a torque indicator which shows % torque, which is proportional to the current drawn by the driver motor. A reading of 100% torque corresponds to the max allowable torque of 172 N m. The specific mechanical energy (SME), which is the net mechanical energy input divided by mass flow rate, was calculated from the measured torque reading as follows (Godavarti & Karwe, 1997):

$$\text{SME}\ \ (\text{kJ/kg}) = \frac{(\text{Total torque} - \text{Friction torque}) \times N \times (9.1)}{(172) \times (500) \times m_f},$$

where N is screw speed (rpm) and m_f is mass flow rate (kg/s). The drive motor has rated power of 9.1 kW at a rated screw speed of 500 rpm. The friction torque was measured at different screw speeds; with screws attached to the drive but without feeding any flour or water.

2.2.3. High performance liquid chromatographic (HPLC) analysis

Isolation of phenolic compounds from raw and processed dark and white buckwheat flours was carried out by successive extractions with methanol (two successive 24 h extractions) and ethyl acetate (two successive 24 h extractions of the residue isolated from the previous methanol extract). After each extraction step, extracts were filtered and concentrated by a rotary evaporator. Final extracts were dissolved in methanol and subjected to chromatographic analysis after filtration with a 3 mm HPLC

certified 0.45 μm filter. Reverse phase (Supelco Discovery C_{18} HPLC column-25 cm × 10 mm, 5μm) HPLC (Waters GPC II Liquid chromatograph, Millipore Corporation, Milford, Massachusetts, USA) with absorbance detector, at 254 nm, (Waters 484 Tunable Absorbance detector) was used to analyse all extracts.

2.2.4. Total phenolic analysis

Total phenolics were determined colorimetrically using Folin–Ciocalteau reagent, as described by Emmons, Peterson, and Paul (1999) with some modifications. Total phenolic assay was conducted by mixing 4 mL of distilled water, 0.5 mL saturated Na_2CO_3, 0.25 mL Folin–Ciocalteu reagent (diluted with water 1:1 v/v) and 0.25 mL of methanol extract of buckwheat flour. Reagents were allowed to react at room temperature for 25 min. Sample tubes were centrifuged for 10 min at 5000 rev/min after the reaction. Absorbance of supernatants was measured at 725 nm. A standard curve was prepared with gallic acid (3,4,5-Trihydroxybenzoic acid, Sigma–Aldrich, St. Louis, MO).

2.2.5. DPPH radical scavenging activity test

Antioxidant activities of whole buckwheat flour, white buckwheat flour, corn meal, whole wheat flour and raw and processed dark buckwheat flour (with 50% amlylopectin) were determined with a stable radical, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as described by Tachibana, Kikuzaki, Hj-Lajis, and Nakatani (2001) with some modifications. Antioxidant activities were determined by reacting 3 mL of methanolic extract of grains with 3 mL, 200 μM DPPH (Sigma–Aldrich, St. Louis, Missouri, USA). Absorbance of the samples at 515 nm was measured after 40 min reaction at room temperature in dark. The calibration curve was determined using trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, from Sigma–Aldrich), a synthetic, hydrophilic vitamin E analogue, as an external standard with a range of concentrations from 0 to 1000 μM. Results were expressed as trolox equivalents.

3. Results and discussion

HPLC chromatograms of ethyl acetate extract of polar compounds for buckwheat flour indicated that roasting affected both polar and non-polar compounds. Roasted (200 °C, 10 min) dark buckwheat flour exhibited changes in the relative heights of non-polar and polar compounds (Fig. 1). It should be pointed out that we did not use the HPLC chromatograms for quantification of changes. They were used for qualitative comparison only, namely, to find out changes in the relative magnitudes of the peaks so as to know where major changes occurred. For quantification of changes in the concentration of a compound of interest, an internal standard would have to be used.

Extrusion only caused changes in polar compounds (Fig. 2). It has been known that mechanical energy supplied by extruder can have influence on formation of some complexes between the flour components and, on degradation of larger molecules such as starch. Increasing the specific mechanical energy of extrusion from 270 to 475 kJ/kg did not show any change in the chromatographic profile of dark buckwheat flour phenolic compounds. The profiles were similar to the one shown in Fig. 2(b).

Folin–Ciocalteu assay indicated that roasting (200 °C, 10 min) did not affect the phenolic content of either dark or white buckwheat flour significantly (Table 1). Antioxidant activity test (DPPH) showed that roasting decreased the antioxidant activity whereas extrusion did not cause any change (Table 2) in dark buckwheat flour (50% amylopectin). Extensive heat treatment has been known to cause degradation of flavonoids (Dietrych-Szostak & Oleszek, 1999). Although extrusion is a high temperature process that transforms raw ingredients into modified intermediate or finished products, the processing time at high temperature is very short (~10 s). Our study showed that roasting (200 °C, 10 min) caused degradation of antioxidant activity but extrusion (die temperature of 170 °C) did not, perhaps due to short processing time at high temperature.

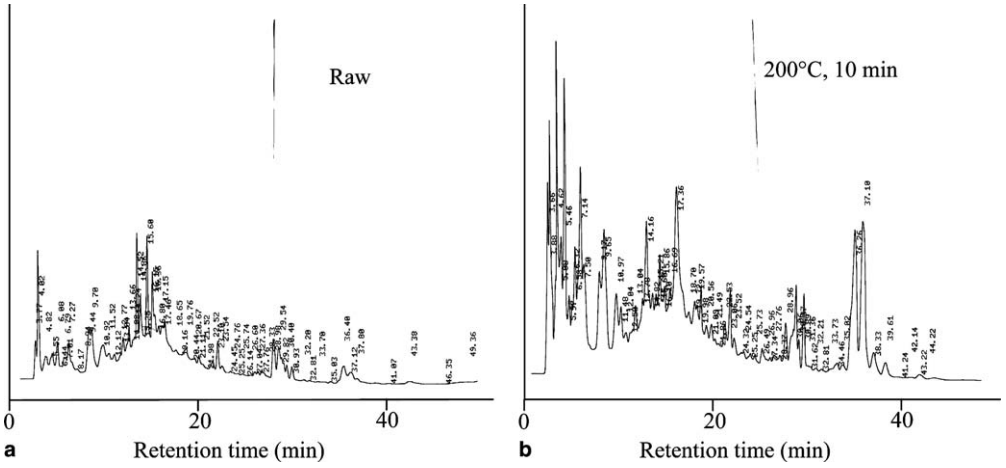

Fig. 1. HPLC chromatograms showing effect of roasting on dark buckwheat flour.

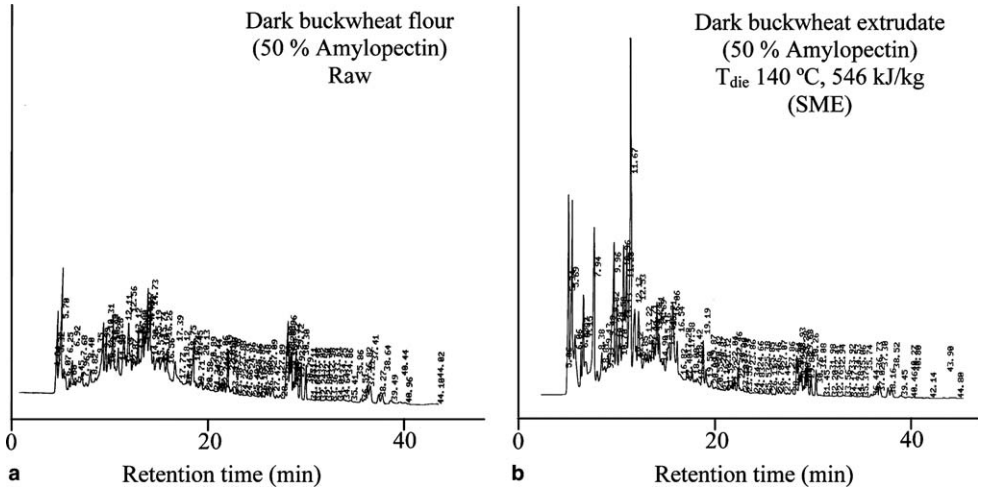

Fig. 2. HPLC chromatograms showing effect of extrusion on dark buckwheat flour.

Table 1
Comparison of total phenolic content in white and dark buckwheat flour before and after roasting

	Gallic acid equivalent/dry weight (mg/g)
White flour, raw	1.79 ± 0.65^{a}
White flour, 200 °C 10 min	2.83 ± 0.73^{a}
Dark flour, raw	10.47 ± 1.82^{b}
Dark flour, 200 °C 10 min	8.90 ± 2.12^{b}

Results are means ± SD ($n = 3$), $p < 0.05$; values of the same column, followed by the same letter (a,b) are not statistically different ($p < 0.05$).

Table 3
Antioxidant activity measured by DPPH test for selected grains

	Trolox equivalent/dry weight (μmol/g)
Corn meal	$1.97 \pm 0.19^{a,b}$
Whole wheat	1.68 ± 0.19^{a}
Buckwheat white	2.14 ± 0.07^{b}
Buckwheat whole	2.13 ± 0.05^{b}

Results are means ± SD ($n = 3$), $p < 0.05$; values of the same column, followed by the same letter (a,b) are not statistically different ($p < 0.05$).

Antioxidant activity test with selected grains showed that white and whole buckwheat flour have slightly higher antioxidant activity from whole wheat and similar antioxidant activity with corn meal (Table 3). Several researchers have studied antioxidant activities of several grains and obtained interesting results. Velioglu, Mazza, Gao, and Oomah (1998) found higher antioxidant activity in buckwheat hulls compared to buckwheat groats (94.9% and 63.7%, respectively) and higher total phenolic content for hulls compared to groats (3900 mg/100 g to 726 mg/100 g, for hulls and groats, respectively). On the other hand, Przbylski, Lee, and Eskin (1998) observed pro-oxidant activity from buckwheat hulls in canola oil. In addition, according to Halosava et al. (2002) buckwheat groats had higher antioxidant activity in saturated lipid substrate than buckwheat hulls, oats, and barley. Zielinski and Kozlowska (2000) observed higher antioxidant activity for the same amount of total phenolic content of buckwheat groats compared to hulls. The results obtained by Velioglu et al. (1998) are similar to the results of Zielinski and Kozlowska (2000). Even though Velioglu et al. (1998) observed higher antioxidant activities for buckwheat hulls, the ratio of antioxidant activity to total phenolic content was lower compared to groats. This could be explained by having different content of phenolic compounds with different antioxidant activities. Folin–Ciocalteu reagent detects all phenolic groups present in the extracts, including those found in extractable proteins (Shahidi & Naczk, 1995). Reaction mechanism of the antioxidant and DPPH depends on structural conformation of the antioxidant (Bondet, Brand-Williams, & Berset, 1997). Arts et al. (2002) suggest that protein and flavonoids interaction may mask part of the antioxidant activity. This masking depends on both compounds, flavonoid and protein (Arts et al., 2002). Velioglu et al. (1998) also observed not a significant correlation between antioxidant and total phenolic content of some products, like anthocyanin-rich materials, which include blueberries, cherries, and red onion scales. According to Oomah and Mazza (1996) flavonoid content is not correlated well with the antioxidant activity for buckwheat whole seed and hulls. Quettier-Deleu et al. (2000) also concluded that white buckwheat flour had more antioxidant activity compared the hulls even though they showed similar total phenolic content. Their study showed that higher or individual flavanol content in the flour gave

Table 2
Antioxidant activity in raw and processed dark buckwheat flour (50% amylopectin) by DPPH (diphenylpicrylhydrazyl) test

	Trolox equivalent/dry weight (μmol/g)
Raw	2.14 ± 0.047^{a}
Extrudate	2.12 ± 0.003^{a}
Roasted	1.85 ± 0.077^{b}

Results are means ± SD ($n = 3$), $p < 0.05$; values of the same column, followed by the same letter (a,b) are not statistically different ($p < 0.05$).

higher antioxidant activity than higher flavonoid content in the hulls. Therefore, it is important to know exact composition of the extracts to understand antioxidant activities in buckwheat hulls and groat. In our study, even though dark buckwheat showed a higher total phenolic content than white flour (Table 1), they cannot be compared directly because dark buckwheat flour and white groats were bought separately. Therefore, the composition and the ratio of active compounds in buckwheat hulls and groats require further research.

Antioxidant activity of dark buckwheat flour was affected by processing at 200 °C for 10 min but not during extrusion with selected conditions. It is important to determine the effect of processing on functional compounds so that processing time and temperature can be optimized to keep functionality of active compounds. This needs extensive further research.

4. Conclusions

Total phenolic content was not affected by processing (200 °C, 10 min) in dark or white buckwheat flour. Extrusion caused changes in HPLC chromatograms of ethyl acetate extracts of dark buckwheat flour. Changing the specific mechanical energy in the range of 270–475 kJ/kg did not cause any relative change in HPLC chromatograms. Roasting (200 °C for 10 min) slightly reduced antioxidant activity whereas extrusion (170 °C) did not in dark buckwheat flour. Functionality of active compounds could be maintained by optimization of time and temperature during processing.

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