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Effect of thermal processing on antioxidant properties of purple wheat bran

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Abstract

Antioxidant activity of purple wheat bran, heat-treated purple wheat bran, and purple wheat bran muffins was evaluated to determine the impact of thermal processing on potential health benefits. The purple wheat bran and muffin samples were analyzed for total phenolic content, anthocyanin content and free radical scavenging activity using peroxyl (oxygen radical absorbance capacity assay) and 2,2-diphenyl-1-picrylhydrazyl (DPPH assay) radicals. Total phenolic content and oxygen radical absorbance capacity (ORAC) values of sample extracts were significantly affected by various extracting solvents. The conditions selected for heat treatment did not markedly change antioxidant activity of purple wheat bran. However, there was a significant reduction in total phenolic contents, ORAC values and total anthocyanins during processing of purple wheat bran- or heat-treated purple wheat bran-enriched muffins. On the contrary, muffin extracts still remained excellent in DPPH radical scavenging activity.

Keywords: Antioxidant properties; Purple wheat bran; Heat treatment; Muffin

1. Introduction

Consumption of foods containing rich antioxidant activity substances, such as grains, vegetables, and fruits, may prevent many diseases and promote good health (Temple, 2000; Willet, 1994). Grains in particular, are a major source of antioxidants in our daily diets. The main antioxidative components in grain are classified as phenolic compounds such as anthocyanins, tannins, and ferulic acid, and other substances (Martínez-Tomé et al., 2004). Beneficial effect of antioxidants on promoting health is believed to be achieved through several possible mechanisms, such as directly reacting with and quenching free radicals, chelating transition metals, reducing peroxides, and stimulating the antioxidative defense enzyme system (Zhou & Yu, 2004a). Wheat is one of the most important grains and many studies indicated that wheat contains phytochemical substances that display in vitro good antioxidant activity (Baublis, Decker, & Clydesdale, 2000; Beta, Nam, Dexter, & Sapirstein, 2005; Mpofu, Sapirstein, & Beta, 2006; Onyeneho & Hettiarachchy, 1992; Yu et al., 2002; Zhou & Yu, 2004b). Our previous study also indicated that Chinese black-grained wheat had high antioxidant activity compared to white and blue wheat genotypes (Li, Shan, Sun, Corke, & Beta, 2005). Hence, wheat with a high level of antioxidant activity should have potential for use as an excellent dietary source of antioxidants for disease prevention and health promotion.

However, antioxidant properties of grains will be affected by processing temperature during food thermal processing (Dewanto, Wu, & Liu, 2002). Moreover different solvent systems used for sample extraction also have an effect on the estimation of antioxidant capacity (Zhou & Yu, 2004a). The main objectives of this study were to investigate the effect of heat treatment of purple wheat bran

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and to determine the influence of thermal processing during production of purple wheat bran muffins on antioxidant properties using three solvent systems.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picryhydrazyl radical (DPPH·), 2,2′-azobis (2-methylpropionamide) dihydrochloride (AAPH), and ferulic acid were purchased from Sigma–Aldrich (St. Louis, MO). Trolox and fluorescein were purchased from Fisher Acros Organics (New Jersey, USA) for use with oxygen radical absorbance capacity assay. All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Sample description

Bran derived from purple wheat (*Triticum* spp. L) was used for the investigation. Samples of purple wheat bran, heat-treated purple wheat bran, purple wheat bran muffins and wheat bran muffins (control) were supplied from Infra-Ready Products (1998) Limited. Purple wheat bran was baked at 177 °C for 20 min to obtain heat-treated bran samples. The ingredients used to produce purple wheat bran muffins and control muffins were as follows: 2 cups bran, 1 1/2 cup sugar, 2 1/2 cups flour, 2 1/2 teaspoons baking soda, 1 teaspoon salt, 1/2 cup oil, 2 cups buttermilk, 2 eggs (beaten), and 2 tablespoons molasses. The muffins were baked at 177 °C for 7–12 min in three batches to monitor the stability of antioxidant activity. Only the average values of the three batches of muffins were reported.

2.3. Sample extraction

Muffin products were first dried at 40 °C in a forced-air oven (Blue M Electric Company, USA) for 10 h. Then wheat bran and muffins were ground into fine powder using the sample mill (Krups Type 202, Germany). Methanol (100%), methanol:HCl (36.5–38%) (99:1, v/v), and ethanol (95%):HCl(1 N) (85:15, v/v) were used as solvents for extraction of the finely ground samples. Since different extraction solvents affect estimation of antioxidant activity (Zhou & Yu, 2004a), the three solvents were chosen based on literature related to phenolic assays. Methanol and methanol: HCl (99:1, v/v) are commonly used to obtain crude extracts for determination of phenolic content (Beta, Rooney, Marovatsanga, & Taylor, 1999). For anthocyanin analysis, ethanol:HCl(1 N) (85:15, v/v) was selected as the extraction solvent according to Abdel-Aal and Hucl (2003). Samples (1.0 g) of purple wheat bran or heat-treated purple wheat bran were extracted in 20 ml each of the three different extraction solvents. Sample (4.0 g) of purple wheat bran muffins or wheat bran muffins were extracted in 20 ml each of methanol or methanol:HCl (99:1, v/v). Samples (1.0 g) of purple wheat bran muffins or wheat bran muffins were extracted in 20 mL of ethanol:HCl(1 N) (85:15, v/v). The samples were shaken at 300 rpm in a rotary shaker (Fermentation Design Inc., Allentown, PA) at ambient temperature. The extraction time was 3 h in methanol system or methanol:HCl (99:1, v/v) system and 1 h in ethanol:HCl(1 N) (85:15, v/v) system. Samples in 50 mL tubes were then centrifuged at 5 °C at 10,000 rpm (SS-34 Rotors, RC5C Sorvall Instruments) for 15 min. The supernatant fluids were kept at −20 °C in the dark until further analysis for antioxidant properties.

2.4. Total phenolic content

Total phenolic content (TPC) of extracts was determined using the Folin–Ciocalteau reagent according to modified procedures (Beta et al., 2005; Gao, Wang, Oomah, & Mazza, 2002; Mpofu et al., 2006; Singleton & Rossi, 1965). An extract (200 μL) was added to 1.9 mL of freshly diluted 10-fold Folin–Ciocalteau reagent (BDH Inc., Toronto, ON). Sodium carbonate solution (1.9 mL) (60 g/L) was then added to the mixture and mixed. After 120 min of reaction at ambient temperature, the absorbance was measured at 725 nm against a blank of distilled water. Ferulic acid was used as an equivalent standard. All analyses were performed in duplicate.

2.5. Oxygen radical absorbance capacity assay

An improved oxygen radical absorbance capacity (ORAC) assay, using fluorescein (FL) as the fluorescent probe, was used according to Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) Dávalos, Gómez-Cordovés, and Bartolomé (2004). ORAC assay measures the ability of antioxidative compounds in tested materials to inhibit the decline in fluorescence induced by AAPH radical. Briefly, a Precision 2000 Automated Microplate Pipetting System (BIO-TEK Instruments, Inc.) was used to automatically transfer solutions to a 96-well flat bottom polystyrene microplate (Corning Incorporated, Corning, NY, USA) according to programmed procedures. An FL$_\times$800 microplate reader (Bio-Tek Instruments, Inc., Winooski, VT) equipped with fluorescence filters for an excitation wavelength of 485/20 nm and an emission wavelength of 528/20 nm and controlled by KC4 3.0 software was used. In the first step, 120 μL of fluorescence working solution was transferred to each well in a 96-well microplate, then 20 μL of buffer solution (blank), Trolox standard, diluted samples and 20 μM rutin control transferred to designated wells of the 96-well microplate, respectively. After that, the 96-well microplate was incubated at 37 °C for 20 min. In the second step, 60 μL of AAPH solution was transferred to each well of the 96-well microplate. After covering by means of an adhesive sealing film, the 96-well microplate was immediately transferred to the FL$_\times$800 microplate fluorescence reader, and the fluorescence was measured every minute for 50 min at 37 °C. Peroxyl radical was generated by AAPH during measure-

ment, and fluorescein was used as the substrate (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003). All the reaction mixtures were prepared in the measured plate in duplicate, and at least three independent assays were performed for each sample.

Data processing to obtain ORAC values was performed according to previous reports (Huang et al., 2002). Briefly, the area under the curve (AUC) is calculated according to the following equation:

$$\mathrm{AUC} = 0.5 + f_1/f_0 + f_i/f_0 + \cdots + f_{49}/f_0 + 0.5(f_{50}/f_0) \quad (1)$$

where f_0 = initial fluorescence reading at 0 min and f_i = fluorescence reading at time i min.

The relative Trolox equivalent ORAC value is calculated according to the following equation:

$$\begin{aligned}\text{Relative ORAC value} &= [(\mathrm{AUC}_{\mathrm{sample}} - \mathrm{AUC}_{\mathrm{blank}})/(\mathrm{AUC}_{\mathrm{Trolox}} - \mathrm{AUC}_{\mathrm{blank}})] \\ &\times (\text{molar mass of Trolox/sample weight}) \\ &\times \text{sample dilution factor} \quad (2)\end{aligned}$$

where molar mass of Trolox is 250.3.

Relative ORAC values were expressed as mg of Trolox equivalent/g of sample.

2.6. Measurement of total anthocyanins

Total anthocyanins were determined according to the pH-differential method (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005; Guisti & Wrolstad, 2001). Samples were extracted by using ethanol:1 N HCl (85:15, v/v) in brown bottle, to avoid light-induced changes. Clear extract (1 mL) was placed into 25 mL volumetric flask, made up to a final volume with pH 1.0 buffer (1.49 g of KCl/100 mL water and 0.2 N HCl, with a ratio of 25:67), and mixed. Another 1 mL of extract was also placed into a 25 ml volumetric flask, made up to a final volume with pH 4.5 buffer (1.64 g of sodium acetate/100 mL of water) and mixed. Absorbance was calculated as $\Delta A = (A_{510\,\mathrm{nm}} - A_{700\,\mathrm{nm}})\mathrm{pH}_{1.0} - (A_{510\,\mathrm{nm}} - A_{700\,\mathrm{nm}})\mathrm{pH}_{4.5}$ with a molar extinction coefficient for cyanidin 3-glucoside of 26,900. Results were calculated using the following equation and expressed as milligrams of cyanidin 3-glucoside equivalents per gram of dry basis weight:

$$\text{Total anthocyanins (mg/g)} = (\Delta A/\epsilon L) \times \mathrm{MW} \times D \times (V/G)$$

where ΔA is absorbance, ϵ is cyanidin 3-glucoside molar extinction coefficient (26,900), L is cell path length (1 cm), MW is the molecular weight of anthocyanins (449.2), D is a dilution factor, V is the final volume (mL), and G is the sample weight (g).

All determinations were carried out in at least duplicate.

2.7. DPPH· scavenging activity

DPPH· method was used for evaluating radical scavenging activity of samples according to Brand-Williams, Cuvelier, and Berset (1995) and Yu et al. (2002), with some modifications. The method involves the reaction of the antioxidants with the stable DPPH· in 95% ethanol. Samples were extracted by using ethanol:1 N HCl (85:15, v/v) in brown bottle, to avoid light-induced changes. A 60 μm DPPH· working solution was freshly made in 95% ethanol prior to measurement. Sample extract (200 μL) was reacted with 3.8 mL of DPPH· working solution for 60 min. The absorbance was measured at 515 nm against a blank of pure 95% ethanol at 0, 5, 10, 20, 30, 40, 50, and 60 min, and used to estimate the level of free radical scavenging ability. The chemical kinetics of antioxidant activity of samples was also recorded. Antioxidant activity was calculated as follows:

$$\%\ \text{DPPH radical scavenging activity} = (1 - [A_{\mathrm{sample}\,t}/A_{\mathrm{control}\,t=0}]) \times 100.$$

All analyses were carried out in duplicate.

2.8. Statistical analysis

Data were reported as means of measurements and subjected to analysis of variance. Least significant difference (LSD) was calculated using Fisher's protected LSD test at $P = 0.05$. Quantitative results were generally expressed on a dry weight basis (dwb).

3. Results and discussion

In order to evaluate total phenolic content and antioxidant properties of muffins prepared from wheat bran and other ingredients, the antioxidant values are based on dry basis of the whole muffins and not the fraction of bran used to prepare the muffins. Since the same levels of wheat bran were used in muffin formulations, the contribution of other ingredients to antioxidant activity was also of interest in this study.

3.1. TPC

Total phenolic contents, expressed as mg of ferulic acid equivalent/g of sample, are shown in Table 1. The results indicated that there were marked effects of solvent systems on estimation of TPC of each sample. The best extraction efficiency was found when ethanol:HCl was used as extracting solvent. For instance, TPC of purple wheat bran in methanol, methanol:HCl, and ethanol:HCl solvent was 3.34, 5.98, and 7.70 mg/g, respectively. Methanol:HCl solvent was also a good extractant compared to methanol solvent. Antioxidant properties of wheat bran extracts in four solvent systems including 50% acetone (v/v), 70% methanol (v/v), 70% ethanol (v/v), absolute ethanol were evaluated by Zhou and Yu (2004a), and extracting solvent significantly altered estimation of the antioxidant properties of wheat bran. Although there were differences in each extracting solvent, TPC of purple wheat bran and heat-

Table 1
TPC of extracts from bran and bran-containing muffins[a]

Name	EM-equiv. of ferulic acid (mg/g)	EMH-equiv. of ferulic acid (mg/g)	EEH-equiv. of ferulic acid (mg/g)
Purple wheat bran	3.34b	5.98b	7.70a
Heat-treated purple wheat bran	3.68a	6.98a	7.05b
Purple wheat bran muffin	0.26c	0.52c	1.56c
Wheat bran muffin (control)	0.35c	0.56c	1.14d
LSD	0.11	0.15	0.19

[a] LSD, least significant difference at $P = 0.05$ level of probability. Sample means containing similar letters in the same column are not significantly different. EM, extracts in methanol solvent. EMH, extracts in methanol:HCl solvent. EEH, extracts in ethanol:HCl solvent.

treated purple wheat bran were of a similar magnitude. The results indicated that effect of heat treatment on TPC of purple wheat bran was limited, $\leqslant 1$ mg/g. Dewanto et al. reported that the total antioxidant activity of sweet corn was elevated by 44% after thermal processing (Dewanto et al., 2002). TPC of methanol (3.68 mg/g) and methanol:HCl (6.98 mg/g) extracts of heat-treated purple wheat bran were slightly higher compared to levels found in untreated purple wheat bran extracts (3.34 and 5.98 mg/g in methanol solvent and methanol:HCl solvent, respectively). However, TPC of heat-treated bran was slightly lower than that of purple wheat bran extract (7.05 and 7.70 mg/g, respectively) in ethanol:HCl solvent. The TPC of bran extracts of Chinese black-, Dongjian purple-, and Wu blue-grained wheat in methanol solvent were lower (2.42, 2.29 and 1.42 mg/g, respectively) (Li et al., 2005) in comparison to the purple wheat bran.

There were no differences in TPC between purple wheat bran muffins and control wheat bran muffins in methanol and methanol:HCl solvent; however, differences were found using ethanol:HCl as solvent. TPC for purple wheat bran muffin was 0.26 mg/g in methanol, 0.52 mg/g in methanol:HCl, and 1.56 mg/g in ethanol:HCl. TPC of purple wheat bran muffins was much lower compared to untreated or heat-treated purple wheat bran. This indicated that thermal processing conditions of muffins and dilution by other ingredients markedly reduced TPC of untreated or heat-treated purple wheat bran. TPCs for control wheat bran muffin was 0.35 mg/g in methanol, 0.56 mg/g in methanol:HCl, and 1.14 mg/g in ethanol:HCl.

Phenolic compounds in wheat bran are the main contributors to its *in vitro* total antioxidant capacity. High TPC is generally regarded as an indication of high total antioxidant capacity. Polarity difference of acidic extracting solvents, such as methanol:HCl and ethanol:HCl solvents, should be the main reason of better extraction efficiency compared to methanol solvent. It is possible that slight hydrolysis of polyphenolic compounds will occur under acidic extracting conditions. Nine types of phenolic acids were found present in hydrolysates of wheat bran, including gallic, *p*-hydroxybenzoic, caffeic, syringic, *p*-coumaric, vanillic, gentistic, *o*-coumaric, ferulic acids (Li et al., 2005). Polyphenolic compounds in grain extracts were a mixture of free phenolic acids, soluble phenolic esters and insoluble-bound phenolic acids (Krygier, Sosulski, & Hogge, 1982).

3.2. ORAC

ORAC values of sample extracts are shown in Table 2. ORAC values of untreated and heat-treated purple wheat bran were 25.31 and 24.35 mg/g in methanol solvent, 52.49 and 58.54 mg/g in methanol:HCl solvent, and 89.15 and 84.44 mg/g in ethanol:HCl solvent, respectively. Although there were differences between untreated and heat-treated purple wheat bran in methanol and methanol:HCl solvent, ORAC values were of a similar magnitude in each extracting solvent. However, no difference in ORAC value was found between untreated and heat-treated purple wheat bran for ethanol:HCl extracts. This was an indication that antioxidant capacity of purple wheat bran was not adversely affected under the conditions used for heat treatment.

ORAC values were similar for purple wheat bran muffins and control wheat bran muffins in each extracting solvent. ORAC values of purple wheat bran muffins and control wheat bran muffins were markedly low possibly due to dilution of antioxidant components and their interaction with other ingredients during baking. ORAC values of purple wheat bran muffins and control wheat bran muffins were 2.87 and 3.03 mg/g in methanol solvent, 4.25 and 4.22 mg/g in methanol:HCl solvent, 23.01 and 23.09 mg/g in ethanol:HCl solvent, respectively.

ORAC assay measures the relative potencies of antioxidants in preventing biological molecules from free radical attacks (Zhou & Yu, 2004a) and a high ORAC value is an

Table 2
$ORAC_{FL}$ values of extracts from bran and bran-containing muffins[a]

Name	EM-equiv. of Trolox (mg/g)	EMH-equiv. of Trolox (mg/g)	EEH-equiv. of Trolox (mg/g)
Purple wheat bran	25.31a	52.49b	89.15a
Heat-treated purple wheat bran	24.35b	58.54a	84.44a
Purple wheat bran muffin	2.87c	4.25c	23.01b
Wheat bran muffin (control)	3.03c	4.22c	23.09b
LSD	0.84	3.89	6.89

[a] LSD, least significant difference at $P = 0.05$ level of probability. Sample means containing similar letters in the same column are not significantly different. EM, extracts in methanol solvent. EMH, extracts in methanol:HCl solvent. EEH, extracts in ethanol:HCl solvent.

indication of high antioxidant capacity in sample extract. Estimation of ORAC values was also markedly affected by extracting solvents. ORAC values in ethanol:HCl solvent were greater than those obtained in methanol and methanol:HCl solvent. High ORAC values in ethanol:HCl solvent indicated that ethanol:HCl solvent is a suitable solvent for extracting antioxidants from selected samples. It is important to note the effects of various solvent systems on the estimation of antioxidant capacity and their extraction efficiency of antioxidant compounds present in the sample. A recent report showed that ORAC values of wheat extracts in 50% acetone system were 3–20-fold greater than that in ethanol system (Zhou, Laux, & Yu, 2004).

3.3. Total anthocyanins

Total anthocyanins, expressed as cyanidin 3-glucoside equivalents, of sample extracts are shown in Table 3. The colour of extract solutions of purple wheat bran and heat-treated purple wheat bran was red in ethanol:HCl (pH 1.2). A red colour indicates the presence of anthocyanin compounds in acid media. Cyanidin 3-glucoside equivalents of purple wheat bran and heat-treated purple wheat bran extracts were 1.155 and 1.035 mg/g in ethanol:HCl (pH 1.2), respectively. There was no difference in total anthocyanin between untreated and heat-treated purple wheat bran. There was no obvious degradation of anthocyanins under the conditions selected for heat treatment of purple wheat bran. However, no anthocyanins were detected in muffins possibly due to degradation of anthocyanins in purple wheat bran during baking.

Anthocyanins have strong antioxidant capacity (Wang, Cao, & Prior, 1997). Kay and Holub reported that anthocyanins in grape juice had the ability to reduce *in vitro* oxidation of human low-density lipoprotein (Kay & Holub, 2002). Instability of anthocyanins during heat processing was reported, with losses of up to 92.5% in the thermal production of pekmez (Alasalvar, Al-Farsi, & Shahidi, 2005). Apart from heat, many other factors such as light, temperature, and storage, are also responsible for the degradation of anthocyanins during food drying and processing (Mazza & Miniati, 1993). All anthocyanins of black currant juice disappeared during a 9 weeks of storage at 37 °C, while 60% remained after 6 months of storage at 20 °C (Simard, Bourzeix, & Heredia, 1981). In this study, heat processing (at 177 °C for 7–12 min) led to complete destruction of anthocyanins during production of untreated or heat-treated purple wheat bran muffins. Anthocyanins present in purple wheat bran will be an important advantage of purple- compared to common-wheat grains if they can be retained during processing by use of appropriate conditions.

3.4. DPPH· scavenging activity

Only ethanol:HCl(1 N) (85:15, v/v) was used for the DPPH· assay. The other solvent systems were found to be unsuitable due to interference of a light yellow color obtained when DPPH radical reacted with methanol extracts. Instead of a clear solution, light yellow oily droplets could be seen on the walls of the cuvettes when the methanol extract was added to DPPH solution resulting in absorbance measurements that were unstable. DPPH· scavenging activities of extracts in ethanol:HCl solvent are shown in Table 4 and also expressed as equivalents of Trolox. DPPH· scavenging activity (71.31%) of heat-treated purple wheat bran was the highest among all the sample extracts. However, there were similar DPPH· scavenging activities and Trolox equivalents between purple wheat bran and wheat bran muffins. DPPH· scavenging activity of purple wheat bran extract was 63.17%. Increase in DPPH· scavenging activity was found during heat-treatment of purple wheat bran (at 177 °C for 20 min). There was no obvious loss in DPPH· scavenging activity during production of muffins containing purple wheat bran. Purple wheat bran muffins had good DPPH· scavenging activity after thermal processing. Reaction kinetics of sample extracts with DPPH· is shown in Fig. 1. The reaction rate was rapid in the first 30 min, but afterward, it became progressively slow and stable. Awika et al. (2003) reported that the reaction time with DPPH· could be up to 8 h, but after 8 h, the change in antioxidant activity for most of the sorghum extracts was very minimal.

Table 3
Total anthocyanin content of extracts from bran and bran-containing muffins[a]

Name	EEH-equiv. of cyanidin 3-glucoside (mg/g)	Colour in pH1.2 acid media
Purple wheat bran	1.155a	Red
Heat-treated purple wheat bran	1.035a	Red
Purple wheat bran muffin	nd	nd
Wheat bran muffin (control)	nd	nd
LSD	0.40	

[a] LSD, least significant difference at $P = 0.05$ level of probability. Sample means containing similar letters in the same column are not significantly different. EEH, extracts in ethanol:HCl solvent. nd – Not detected.

Table 4
DPPH· scavenging % and equivalent of Trolox after 60 min reaction time[a]

Name	EEH-DPPH radical scavenging (%)	EEH-equiv of trolox (mg/g)
Purple wheat bran	63.17b	1.47b
Heat-treated purple wheat bran	71.36a	1.68a
Purple wheat bran muffin	63.49b	1.47b
Wheat bran muffin (control)	63.68b	1.47b
LSD	3.09	0.08

[a] LSD, least significant difference at $P = 0.05$ level of probability. Sample means containing similar letters in the same column are not significantly different. EEH, extracts in ethanol:HCl solvent.

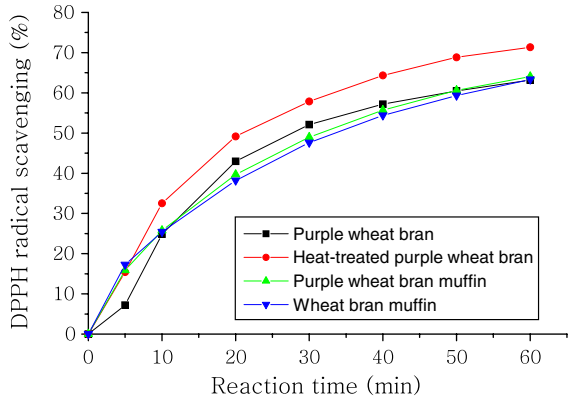

Fig. 1. Antioxidant activity kinetics of ethanol:HCl extracts with DPPH radical.

4. Conclusions

This study reported the antioxidant properties of purple wheat bran and its muffin products in three extracting solvent systems as well as the possible effects of heat processing on the antioxidant properties of purple wheat bran and muffin products. Levels of TPC and ORAC values in ethanol:HCl extracts were greater than in methanol and methanol:HCl extracts. The choice of extracting solvent will affect estimation of the antioxidant properties. The conditions selected for heat treatment did not markedly change antioxidant activity of purple wheat bran. However, there was a significant reduction in TPC, ORAC values and total anthocyanins during processing of purple wheat bran- or heat-treated purple wheat bran-enriched muffins. Surprisingly, muffin extracts still remained excellent in DPPH$^{\cdot}$ scavenging activity after thermal processing. The different moisture contents in wheat bran and muffins may also have been one of the factors affecting measurement of antioxidant activity even though baking was conducted at the same high temperature. The muffins contained other ingredients which likely interacted with the antioxidant components either interfering with their activity or extraction using the different solvent systems. The forms or levels of antioxidants in wheat bran and muffins were likely altered and our method evaluated total antioxidant activity in extracts. Future work will aim to identify individual compounds responsible for this antioxidant activity.

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References

Abdel-Aal, E.-S. M., & Hucl, P. (2003). Composition and stability of anthocyanins in blue-grained wheat. *Journal of Agricultural and Food Chemistry, 51*, 2174–2180.

Alasalvar, C., Al-Farsi, M., & Shahidi, F. (2005). Compositional characteristics and antioxidant components of cherry laurel varieties and pekmez. *Journal of Food Science*, 70, S47-52.

Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., & Shahidi, F. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolic of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *Journal of Agricultural and Food Chemistry, 53*, 7592–7599.

Awika, J. M., Rooney, L. W., Wu, X., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (*sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry, 51*, 6657–6662.

Baublis, A., Decker, E. A., & Clydesdale, F. M. (2000). Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals. *Food Chemistry, 68*, 1–6.

Beta, T., Nam, S., Dexter, J. E., & Sapirstein, H. D. (2005). Phenolic content and antioxidant activity of pearled wheat and roller mill fractions. *Cereal Chemistry, 82*, 390–393.

Beta, T., Rooney, L. W., Marovatsanga, L. T., & Taylor, J. R. N. (1999). Phenolic compounds and kernel characteristics of Zimbabwean sorghums. *Journal of the Science of Food and Agriculture, 79*, 1003–1010.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28*, 25–30.

Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2004). Extending applicability of oxygen radical absorbance capacity (ORAC-fluorescein) assay. *Journal of Agricultural and Food Chemistry, 52*, 48–54.

Dewanto, V., Wu, X., & Liu, R. H. (2002). Processed sweet corn has higher antioxidant activity. *Journal of Agricultural and Food Chemistry, 50*, 4959–4964.

Gao, L., Wang, S., Oomah, B. D., & Mazza, G. (2002). Wheat quality: antioxidant activity of wheat millstreams. In P. Ng & C. W. Wrigley (Eds.), *Wheat quality elucidation* (pp. 219–233). St. Paul, MN: AACC International.

Guisti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV–visible spectroscopy. In R. E. Wrolstad, T. E. Acree, H. An, E. A. Decker, M. H. Penner, & D. S. Reid, et al. (Eds.), *Current protocols in food analytical chemistry* (pp. F1.2.1–F1.2.13). New York: Wiley.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry, 50*, 4437–4444.

Kay, C. D., & Holub, B. J. (2002). The effect of wild blueberry (*Vaccinium angustifolium*) consumption on postprandial serum antioxidant status in human subjects. *British Journal of Nutrition, 88*, 389–398.

Krygier, K., Sosulski, F., & Hogge, L. (1982). Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *Journal of Agricultural and Food Chemistry, 30*, 330–334.

Li, W., Shan, F., Sun, S., Corke, H., & Beta, T. (2005). Free radical scavenging properties and phenolic content of Chinese black-grained wheat. *Journal of Agricultural and Food Chemistry, 53*, 8533–8536.

Martínez-Tomé, M., Murcia, M. A., Frega, N., Ruggieri, S., Jiménez, A. M., Roses, F., et al. (2004). Evaluation of antioxidant capacity of cereal brans. *Journal of Agricultural and Food Chemistry, 52*, 4690–4699.

Mazza, G., & Miniati, E. (1993). *Anthocyanins in fruits, vegetables, and grains*. Boca Raton, FL: CRC Press.

Mpofu, A., Sapirstein, H. D., & Beta, T. (2006). Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *Journal of Agricultural and Food Chemistry, 54*, 1265–1270.

Onyeneho, S. N., & Hettiarachchy, N. S. (1992). Antioxidant activity of durum wheat bran. *Journal of Agricultural and Food Chemistry, 40*, 1496–1500.

Simard, R. E., Bourzeix, M., & Heredia, N. (1981). Facteurs affectant la degradation de cassis (*Ribes nigrum* L.). *Sciences des Aliments, 1*, 388.

Singleton, V. L., & Rossi, J. A. (1965). Calorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Temple, N. J. (2000). Antioxidants and disease: more questions than answers. *Nutrition Research, 20*, 449–459.

Wang, H., Cao, G., & Prior, R. L. (1997). Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural and Food Chemistry, 45*, 304–309.

Willet, W. C. (1994). Diet and health: what should we eat. *Science, 254*, 532–537.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., & Qian, M. (2002). Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry, 50*, 1619–1624.

Zhou, K., Laux, J. J., & Yu, L. (2004). Comparison of Swiss red wheat grain and fractions for their antioxidant properties. *Journal of Agricultural and Food Chemistry, 52*, 1118–1123.

Zhou, K., & Yu, L. (2004a). Effects of extraction solvent on wheat bran antioxidant activity estimation. *LWT-Food Science and Technology, 37*, 717–721.

Zhou, K., & Yu, L. (2004b). Antioxidant properties of bran extracts from Trego wheat grown at different locations. *Journal of Agricultural and Food Chemistry, 52*, 1112–1117.