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Phenolic content and antioxidant capacity of supercritical carbon dioxide-treated and air-classified oat bran concentrate microwave-irradiated in water or ethanol at varying temperatures ☆

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Abstract

Oat bran concentrate (OBC) was defatted with supercritical carbon dioxide (SCD), then microwave-irradiated (MI) at 50, 100 or 150 °C for 10 min in water, 50% or 100% ethanol, and extract pH, soluble solids, phenolic content (PC) and antioxidant capacity (AC) were analysed. OBC was air-classified into five fractions and MI in water at 150 °C. OBC without SCD and microwave irradiation was extracted at 22 °C. Most effective temperature during microwave irradiation for maximising extraction of PC and AC was 150 °C. Defatted OBC in 50% ethanol and MI at 150 °C extracted greatest PC and AC. SCD treatment slightly reduced PC and AC. OBC extracted in water or 50% ethanol at 22 °C without microwave irradiation had similar PC and AC than OBC MI at 150 °C, but much higher levels were observed for latter heat treatment using absolute ethanol. Air-classification shows potential to enhance PC and AC. Published by Elsevier Ltd.

Keywords: Phenolics; Antioxidant capacity; Oat bran concentrate; Supercritical carbon dioxide; Microwave irradiation; Solvents

1. Introduction

Oat bran has been widely shown to provide a vast range of human health benefits such as serum cholesterol lowering (Chen, He, Wildman, Reynolds, & Whelton, 2006), reduced coronary heart disease (Berg et al., 2003), reduced symptoms of diabetes (Tapola, Karvonen, Niskanen, Mikola, & Sarkkinen, 2005), reduced blood pressure (He, Streiffer, Muntner, Krousel-Wood, & Whelton, 2004), cancer prevention (Adom & Liu, 2002) and obesity (Zduńczyk et al., 2006). The primary component of oat bran implicated for these health benefits is β-glucans, however, oat phenolics and other antioxidant compounds also provide health benefits as demonstrated for barley (Madhujith & Shahidi, 2007).

The antioxidant capacity of oats is largely due to the presence of tocopherols, tocotrienols, phytic acid, flavonoids, and non-flavonoid phenolic compounds such as avenanthramides (Peterson, 1995). Oat antioxidants have

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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been reported to inhibit low-density lipoprotein oxidation and promote scavenging of reactive oxygen species (Chen et al., 2004). Avenanthramides have been implicated in inhibiting initiation and development of artherosclerosis (Liu, Zubik, Collins, Marko, & Meydani, 2004). Cholesterol-lowering effect of oats due to binding of bile acids has been shown to involve lignin and β-glucan, synergistically (Sayar, Jannink, & White, 2006). The distribution of antioxidants within oat kernels is important in implementing technologies to develop oat products with enhanced antioxidant capacity. Oat germ, the high-lipid compartment of kernels has concentrated levels of tocopherols (α and γ isomers), whereas tocotrienols are concentrated in endosperm and absent from germ (Peterson, 1995). In wheat, higher total phenolic content was observed in the germ, and there were no differences in antioxidant capacity among milling fractions (Liyana-Pathirana & Shahidi, 2006). Free phytochemical extracts of oat kernels have been reported to have higher antioxidant capacity than corn, wheat or rice, but wheat was higher for bound phytochemicals (Adom & Liu, 2002). Oat bran has been shown to be less effective at free-radical scavenging compared with other cereal brans such as from wheat, barley and rye (Martínez-Tomé et al., 2004), but in contrast oat has been reported to have greater antioxidant capacity than the same three cereals, with highest activity observed for soluble fibre fractions (Lehtinen & Laakso, 1997). Total phenolic content has been reported to be higher in oat hulls than groats (Xing & White, 1997) and avenanthramides have also been reported to be higher in oat hulls that have high-lipid content, but total antioxidant capacity was higher in oat groats (Bryngelsson, Mannerstedt-Fogelfors, Kamal-Eldin, Andersson, & Dimberg, 2002).

Air-classification of ground oat kernels has not been investigated for its potential to obtain fractions with enhanced phenolic and antioxidant activity. However, air-classified amaranth flour was found to have increased phenolic content and antioxidant capacity (Gamel, Linßen, Mesallam, Damir, & Shekib, 2006). Other milling procedures of cereals have also been studied. Oat pearlings were found to have higher antioxidant capacity and phenolic compounds than groat flour, aspirations from flaking process, trichomes and bran (Peterson, Emmons, & Hibbs, 2001) and similar trends were observed for outer-layer pearlings of wheat (Beta, Nam, Dexter, & Sapirstein, 2005). Roller mills and sieving were used to separate dehulled oats into a bran (>420 μm) and starch-rich (<420 μm) fractions in which the former had higher antioxidant capacity (Gray et al., 2000), with similar findings for wheat (Liyana-Pathirana & Shahidi, 2007a, 2007b). Germ, bran and aleurone regions of milled wheat has been reported to be fraction with highest phenolic content and antioxidant capacity (Adom, Sorrells, & Liu, 2005). Antioxidant capacity and tocol content varied in rice bran collected from different milling breaks and highest activity of oryzanol was observed in outer bran layers (Lloyd, Siebenmorgen, & Beers, 2000). Steeping oat groats and subsequent germination prior to milling was reported to increase avenanthramide concentration (Bryngelsson, Ishihara, & Dimberg, 2003). Alkaline treatments of oat fibre have been demonstrated to extract soluble fibre with higher antioxidant capacity (Lehtinen & Laakso, 1998).

Various solvents have been employed to evaluate their potential to extract phenolic compounds and other components contributing to antioxidant activity. Eight solvent combinations involving ether, diethyl ether, petroleum ether, chloroform, dichloroethane and methanol found methanol extracts to possess greatest antioxidant activity (Duve & White, 1991). Comparison of various aqueous ethanol, methanol or acetone mixtures found more phenolics were extracted from wheat flour and bran using successive acidified methanol/water (50:50, v/v, pH 2) and acetone/water (70:30, v/v) than 70:30 (v/v) of either ethanol:water or methanol:water (Pérez-Jiménez & Sauro-Calixto, 2005). Acidified aqueous methanol effectively extracted more total phenolics and exhibited greater antioxidant capacity from blueberries than acidified acetonitrile, or acidified mixture of methanol and acetone (Kalt et al., 2001). Evaluation of 70% methanol or ethanol, absolute ethanol and 50% acetone found 50% acetone was most effective at extracting phenolic antioxidants from wheat bran (Zhou & Yu, 2004). Whole oats in 80% methanol extracted substantially higher total phenolic compounds (17.6 vs. 1.5 μg catechin/mg lyophilizate, respectively) and exhibited higher antioxidant capacity (0.08 vs. 0.03 μmol Trolox/lyophilizate, respectively) than water extracts (Zieliński & Kozłowska, 2000), while both 80% methanol or ethanol was found to be efficient at extracting phenolic compounds from barley (Bonoli, Verardo, Marconi, & Caboni, 2004; Madhujith, Izydorczyk, & Shahidi, 2006). Methanol extraction of milled oat groats yielded higher total phenolics than isopropanol (156 vs. 104 mg/kg, respectively) (Auerbach & Gray, 1999). Methanol has been reported to extract lower molecular weight phenolic compounds compared with hexane and aqueous acetone (Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998). Combination of supercritical carbon dioxide and ethanol was found to extract greater antioxidant activity from marjoram than with either alone, or in combination with hexane (Vági et al., 2005).

There have been no studies on the effects of microwave irradiation on phenolic content and antioxidant capacity of ground oats. However, some antioxidant compounds are likely to be heat labile. Steaming and flaking oat groats has been reported to decrease tocotrienols, caffeic acid and some avenanthramides, but increase ferulic acid and vanillin, whereas autoclaving whole grains increased content of tocopherols, tocotrienols, and acids of vanillin, ferulic and *p*-coumaric, but degraded avenanthramides (Bryngelsson, Dimberg, & Kamal-Eldin, 2002). Drum-drying of whole meal or rolled oats resulted in decreases in all tocols and phenolic compounds but avenanthramides were unaffected (Bryngelsson et al., 2002). Extrusion cooking up to 200 °C increased phenolic content of oats by two to three times (Zieliński, Kozłowska, & Lewczuk, 2001).

Recently, phenolic compounds and other antioxidants were extracted from wheat bran using solvents during microwave irradiation, and methanol was found to be more effective than acetone or hexane (Oufnac et al., 2007). Grape extract derived from heating at 60 °C exhibited higher antioxidant activity than at 22 °C (Pinelo, Rubilar, Sineiro, & Nuñez, 2005).

The effects of microwave irradiation on phenolic compounds and antioxidant capacity have been investigated in other crops. Microwave heating of apple mash from 40 °C to 70 °C resulted in an increase in phenolic and flavonoid compounds (Gérard & Roberts, 2004). Microwave irradiation was found to be more effective than dioxane-HCl at liberating bound phenolic compounds from cereal stem cell walls (Provan, Scobbie, & Chesson, 1994). Microwaved broccoli inflorescences severely degraded phenolic content and antioxidant capacity (Zhang & Hamauzu, 2004), with similar findings for potatoes (Tudela, Cantos, Espín, Tomás-Barberán, & Gil, 2002), but other studies of green vegetables and herbs found phenolic content and antioxidant capacity increased or remained unchanged after microwave irradiation (Türkmen, Sarı, & Velioğlu, 2005). Phenolics and antioxidant capacity were increased in fava beans exposed to microwave irradiation (Randhir & Shetty, 2004). Microwave vacuum drying of carrots converts *trans*-lycopene to *cis* isomer that has higher antioxidant activity (Mayer-Miebach, Behsnilian, Regier, & Schuchmann, 2005).

In this study we investigate the effectiveness of water, ethanol and their equal mixture, in conjunction with microwave irradiation, to extract phenolic and other antioxidant compounds from ground oat bran concentrate without and with air-classification. Developing methods that concentrate the antioxidant components of oats would greatly facilitate production of nutraceuticals or food ingredients that enable consumers greater access to the health benefits of oats.

2. Materials and methods

2.1. Supercritical carbon dioxide treatment and air-classification

Oat bran concentrate (OBC) (Quaker, Cedar Rapids, IA) had lipids removed by supercritical carbon dioxide extraction (SCD), which used 50 g of OBC powder placed in a stainless steel high-pressure cell (9.5 cm long with 1.6 cm i.d.) and extracted using supercritical carbon dioxide at 85 °C and 68.9 MPa. Five-hundred litres expanded carbon dioxide was used for each extraction at a flow rate of 2.8 L/min. Operating conditions have been described in greater detail previously (King, Johnson, & Friedrich, 1989). Defatted OBC was pin-milled at 14,000 rpm using an Alpine model 160Z pin mill (Augsburg, Germany). The ground material was then air-classified with successive set points of 15, 18, 24 and 30 μm using a Pillsbury laboratory model air-classifier (Minneapolis, MN) with turbo separator running at 3500 rpm as diagramed by Wu and Doehlert (2002). Defatted OBC without pin-milling, and pin-milled OBC without air-classification were also analysed.

2.2. Microwave irradiation and pH measurement

OBC defatted from SCD treatment was microwave irradiated using the sophisticated microwave (Ethos 1600, Milestone Inc., Monroe, CT) that allows highly accurate control of pressure, temperature and microwave power. A 2% solution of SCD-treated OBC (w/w) in deionised water, 50% ethanol or absolute ethanol (50 g total weight) was placed in a sealed 100 mL perfluoroalkoxy Teflon reactor vessel and microwave-irradiated for 15 min at 50, 100 and 150 °C. SCD-treated OBC was additionally separated into five fractions by air-classification and a 2% solution (w/w) of each fraction in deionised water was microwave-irradiated for 15 min at 150 °C. Each treatment was replicated three times which involved three vessels inserted into a carousel and microwave-irradiated at the same time. Each microwave run only had the one treatment replicated three times because the microwave sensors only monitor one vessel, and microwave power required to maintain temperature varies due to solvent used and percentage of solids. A Teflon stirrer bar was placed in each reactor vessel with a stirring rate of 320 rpm used. pH of microwaved extracts was measured after cooling to 25 °C using an Accumet AB15 plus pH meter (Fisher Scientific, Pittsburgh, PA) that had been calibrated for pH 4 and 7.

2.3. Determination of solids and phenolic content

After microwave irradiation, vessels were allowed to cool before samples were removed and centrifuged at $2570\times g$ for 10 min, and phenolic content of supernatant was determined. For solids determination, 5 mL of microwave-irradiated extract was placed in a tared aluminium dish, with water-extracted samples placed directly into oven at 100 °C for 3 h, while 50% and absolute ethanol-extracted samples were evaporated in fumehood prior to being placed at 100 °C for 3 h. Aluminium pans were subsequently removed from oven and cooled in a desiccator prior to being reweighed to calculate total solids. Phenolic content was determined based on the Folin-Ciocâlteu's colourimetric method as described previously (Yu & Zhou, 2004). To 100 μL of sample, 7.9 mL of deionised water and 0.5 mL of Folin-Ciocâlteu's reagent (Sigma Aldrich, St Louis, MO, product number F9252) were added, vortexed, and 1.5 mL of 1.85 M Na_2CO_3 were added after 8 min. Absorbance of samples was measured at 765 nm wavelength after 2 h and gallic acid was used as a standard. Each replicate of each treatment was measured in triplicate.

2.4. Antioxidant capacity measurements

Antioxidant capacity was determined by reacting 3 mL of the microwave-irradiated extracts, after centrifugation

at 2570×*g* for 10 min, with 3 mL of 200 μM 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Şensoy, Rosén, Ho, and Karwe (2006). Absorbance was measured at 515 nm wavelength after 40 min reaction in dark and 10 min centrifugation at 2570×*g*. Calibration curve was determined using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as an external standard and antioxidant capacity was expressed as Trolox equivalents. Each replicate of each treatment was measured in triplicate. To determine the effects of supercritical carbon dioxide and microwave irradiation on the solids, phenolic content and antioxidant capacity, analyses were conducted at 22 °C, with mixing for 15 min, on three replicates each of OBC in all three solvent mixtures studied without and with supercritical carbon dioxide extraction (no microwave irradiation).

2.5. Statistical analysis

All statistical significance tests were calculated using SAS (SAS Institute Inc., 1999), utilizing ANOVA followed by a multiple comparison, the Tukey difference test (Tukey, 1993), with a 5% level used to determine significance.

3. Results and discussion

3.1. Effect of supercritical fluid extraction and absence of microwave irradiation

The effect of supercritical fluid extraction at room temperature, and therefore absence of microwave irradiation, on the solids, phenolic content and antioxidant capacity of oat bran concentrate (OBC) extracted in water, absolute ethanol and their equal mixture is shown in Table 1. Although the majority of phenolic compounds extracted after supercritical carbon dioxide (SCD) extraction were present in untreated OBC, there were significantly less phenolic compounds in extracts derived from all three solvent treatments after SCD extraction. An identical trend was observed for antioxidant capacity of OBC extracted without and with SCD. SCD extraction has previously been reported to be poor at extracting phenolic compounds from pistachio hulls (Goli, Barzegar, & Sahari, 2005). In contrast, SCD was found to be very effective in extracting phenolics from cocoa hulls (Arlorio et al., 2005), herbs (Vági et al., 2005) and grape seeds (Murga, Ruiz, Beltrán, & Cabezas, 2000).

OBC, without or with SCD, extracted in water or 50% ethanol at 22 °C, without microwave irradiation, was just as effective as SCD-treated OBC that had been microwave-irradiated at 150 °C for extracting higher levels of antioxidant activity (Tables 1 and 2). However, using absolute ethanol, considerably higher levels of antioxidant activity were obtained when microwave-irradiated at 150 °C compared with non-microwave irradiation at 22 °C. Extraction of phenolic compounds showed a different trend. Compared with extraction at 22 °C, enhancement in extracted phenolic compounds from OBC was not observed with microwave irradiation at 150 °C in absolute ethanol, but phenolic content was doubled extracting with 50% ethanol at 150 °C with microwave irradiation.

3.2. Effect of temperature during microwave irradiation

Soluble solids, solids recovered, pH, phenolic content and antioxidant capacity of the defatted OBC after microwave irradiation at 50, 100 or 150 °C for 15 min are shown in Table 2. The effect of temperature on the pH of OBC extract after microwave irradiation depended on solvent. As temperature increased, OBC microwave-irradiated in water resulted in a significant decrease in pH, whereas in 50% ethanol there was no significant difference between 50 °C and 150 °C, and in absolute ethanol there was a significant increase (Table 2). Percentage of soluble solids increased with increasing temperature of microwave irradiation for OBC in all three solvents, with greatest differences observed in water. A similar trend was observed for recovered solids from OBC microwave-irradiated extracts for all three solvents with water exhibiting greatest differences. Microwave irradiation of defatted OBC in water or 50% ethanol at highest temperature tested (150 °C) was more effective at extracting phenolic compounds compared with

Table 1
Soluble solids, phenolic content and antioxidant capacity of oat bran concentrate (OBC) without and with supercritical carbon dioxide (SCD) extraction in deionised water, 50% ethanol or absolute ethanol at 22 °C with no microwave irradiation

Oat sample	Solvent	Soluble solids (%)	Phenolic content (mg/g)[A]	Antioxidant capacity (μmol/g)[A]
OBC	Water	0.16b[B]	9.2c	2.99c
OBC	50% ethanol	0.11c	9.6b	3.58a
OBC	100% ethanol	0.18ab	11.5a	1.22e
OBC/SCD	Water	0.19a	8.7d	2.88d
OBC/SCD	50% ethanol	0.12c	9.2c	3.48b
OBC/SCD	100% ethanol	0.04d	9.0c	0.80f
P[C]		<0.0001	<0.0001	<0.0001

[A] Phenolic content and antioxidant capacity expressed per gram of initial defatted OBC sample prior to microwave irradiation. Phenolic content is expressed as gallic acid equivalents and antioxidant capacity is expressed as μm Trolox.
[B] Values with different letters denote differences at the 5% level of significance for each comparison among treatments in the respective column.
[C] *P* represents the probability of *F*-statistic exceeding expected for each comparison among treatments in the respective column.

Table 2
Soluble solids, solids recovered, pH, phenolic content and antioxidant capacity of defatted oat bran concentrate microwave-irradiated at 50, 100 or 150 °C in deionised water, 50% ethanol or absolute ethanol

Temperature (°C)	Solvent	pH	Soluble solids (%)	Solids recovery (%)[A]	Phenolic content (mg/g)[B]	Antioxidant capacity (μmol/g)[B]
50	Water	6.67bc[C]	0.25d	12.6c	9.5d	1.95c
100	Water	6.71bc	0.58b	27.8b	11.0cd	2.14bc
150	Water	6.08e	1.03a	52.7a	14.8b	2.77b
50	50% ethanol	6.53cd	0.14def	7.6cde	10.8cd	2.83b
100	50% ethanol	6.83ab	0.19ed	10.1cd	12.1cd	2.53bc
150	50% ethanol	6.34d	0.42c	22.6b	20.1a	3.62a
50	100% ethanol	5.43f	0.03f	1.5e	10.5cd	0.71d
100	100% ethanol	5.99e	0.05f	3.0e	12.4bc	0.92d
150	100% ethanol	6.92a	0.10ef	4.9de	10.8cd	3.81a
P[D]		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[A] Expressed as percentage of solids remaining from initial defatted OBC powder.
[B] Phenolic content and antioxidant capacity expressed per gram of initial defatted OBC sample prior to microwave irradiation. Phenolic content is expressed as gallic acid equivalents and antioxidant capacity is expressed as μm Trolox.
[C] Values with different letters denote differences at the 5% level of significance for each comparison among treatments in the respective column.
[D] P represents the probability of F-statistic exceeding expected for each comparison among treatments in the respective column.

absolute ethanol (Table 2). Microwave irradiation, without solvents present, can affect phenolic content, possibly by liberating β-ether bound phenolic compounds from cell walls (Provan et al., 1994). Extrusion cooking of oats at 200 °C increased vanillic, ferulic and coumaric acids but decreased sinapic acid and unaltered syringic acid (Zieliński et al., 2001), and autoclaving oats resulted in enhanced α- and β-tocopherol, α- and β-tocotrienol, vanillin, ferulic and *p*-coumaric acid contents, but decreased avenanthramides (Bryngelsson et al., 2002). No significant differences were observed in phenolic content of extracts microwave-irradiated at 50 or 100 °C, regardless of solvent. Non-microwave heating of oat flour heated at 100 °C was also found to have no significant effect on phenolic content (Zadernowski, Nowak-Polakowska, & Rashed, 1999). Antioxidant activity of OBC extracts was significantly greater when microwaved irradiation temperature was increased (Table 2), irrespective of solvent. An increase in phenolic content and antioxidant capacity after microwave irradiation has also been reported for green vegetables (Türkmen et al., 2005). The increase in antioxidant capacity in microwaved carrots has been demonstrated to be due to isomerisation of *trans*-lycopene to *cis* configuration that possesses greater antioxidant capacity (Mayer-Miebach et al., 2005), but extent of isomerisation of phenolics and other antioxidant compounds in cereal grains exposed to microwave irradiation is unknown. Studies of phenolic containing foods exposed to microwave irradiation reported that microwave processing alters dielectric point of food, depending on frequency, temperature, bound to free water ratio and ionic conductivity, with all these factors potentially influencing phenolic structure, although the extent of isomerisation is unknown (Rastrelli, Piccinelli, De Simone, Barba, & d'Amore, 2004). Studies of 22 phenolic compounds (in methanol) exposed to microwave irradiation found little destruction up to 125 °C, 5–39% degradation at 150 °C, and two-thirds were degraded at 175 °C (Liazid, Palma, Brigui, & Barroso, 2007). It was also found that phenolic compounds with fewer substituents on aromatic ring had greater stability during microwave irradiation (Liazid et al., 2007). Therefore the phenolic compounds in OBC could predominantly have few substituents on aromatic rings, providing heat stability during microwave irradiation, and greater phenolic solubility may occur in water or ethanol that we studied compared with methanol studied by Liazid et al. (2007). Microwave irradiation in water at unknown temperatures has been reported to decrease phenolic content or antioxidant capacity of potatoes (Tudela et al., 2002), but this probably agrees with our findings, as phenolic compounds were presumably leached into solution rather than being thermally destroyed. Overall the results suggest that 150 °C is the most effective temperature during microwave irradiation for maximising extraction of phenolic compounds and obtaining greatest antioxidant activity.

3.3. Effects of solvent

The effects of solvent on the pH, soluble solids, solids recovered, phenolic content and antioxidant capacity of microwave-irradiated defatted OBC is shown in Table 2. At low microwave irradiation temperature (50 °C), pH of extract was significantly lower in absolute ethanol compared with water, but a reversal was observed at highest temperature tested (150 °C). Increasing ethanol concentration significantly decreased amount of soluble solids and solids recovered (Table 2). Extracts of defatted OBC microwave-irradiated at 50 or 100 °C in all three solvents tested had no significant difference in phenolic content, but at highest temperature tested there were significant differences (Table 2). Extracts of pistachio hulls with water or methanol at room temperature been reported to have no differences in phenolics content (Goli et al., 2005). Highest antioxidant capacity was observed for extracts microwave-irradiated in 50% or absolute ethanol at 150 °C. In contrast, lower temperatures during microwave irradiation

Table 3
Soluble solids, solids recovered, pH, phenolic content and antioxidant capacity of the five air-classified fractions of defatted oat bran concentrate microwave-irradiated at 150 °C in deionised water

Fraction	pH	Soluble solids (%)	Solids Recovery (%)[A]	Phenolic content (mg/g)[B]	Antioxidant capacity (μmol/g)[B]
<15 μm	6.10a	1.44a[C]	68.8a	14.1 b	2.32ab
15–18 μm	6.00b	1.43a	69.3a	14.5b	2.61ab
18–24 μm	6.00b	1.32a	64.9a	15.8ab	1.40b
24-30 μm	5.95b	1.34a	65.9a	17.5a	3.08a
>30 μm	5.94b	1.04b	52.8b	17.0a	3.00a
P[D]	0.003	0.003	0.003	0.002	0.02

[A] Expressed as percentage of solids remaining from initial defatted OBC powder.
[B] Phenolic content and antioxidant capacity expressed per gram of initial defatted OBC sample prior to microwave irradiation. Phenolic content is expressed as gallic acid equivalents and antioxidant capacity is expressed as μm Trolox.
[C] Values with different letters denote differences at the 5% level of significance for each comparison among treatments in the respective column.
[D] P represents the probability of F-statistic exceeding expected for each comparison among treatments in the respective column.

of OBC in absolute ethanol resulted in significantly lower antioxidant capacity than the other two solvent treatments. At 50 °C, defatted OBC microwaved in 50% ethanol had significantly higher antioxidant capacity than in water. The results indicate that phenolic compounds extracted from microwave-irradiated defatted OBC can be maximised using 50% ethanol, while both ethanol concentrations obtained the highest antioxidant activity. An increase in antioxidant activity with increasing per cent ethanol or methanol as solvent has been found for grapes (Pinelo et al., 2005), but we did not observe any enhancement of radical-scavenging activity with increased ethanol concentration. Although ethanol was not studied, fruit extracts have been reported to have higher antioxidant activity in methanol compared with water (Abdille, Singh, Jayaprakasha, & Jena, 2005) and methanol was found to effectively extract more oat antioxidants than diethyl ether, petroleum ether, chloroform and dichloromethane (Duve & White, 1991). Methanol was also found to be more effective than acetone or hexane for extracting phenolic compounds and antioxidant activity from microwave-irradiated wheat bran (Oufnac et al., 2007). However, a study on extracting antioxidants from oat fibre reported greater antioxidant activity from aqueous extracts with varying pH compared with methanol (Lehtinen & Laakso, 1998). From all combinations we tested, defatted OBC in 50% ethanol and microwave-irradiated at 150 °C would obtain an extract with the greatest amount of phenolics and antioxidant activity. This study focused on using solvents that would be considered by industry to be affordable and have low toxicity, but if we were prepared to be less restrictive, studies on wheat (Zhou & Yu, 2004) suggest that 50% acetone would provide extracts with considerably higher antioxidant activity.

3.4. Effect of air-classification

The effect of separating defatted OBC into five particle-size fractions prior to microwave irradiation at 150 °C in water is shown in Table 3. Extract pH of the finest fraction (<15 μm) after microwave irradiation was significantly higher than all other fractions, but differences were negligible. Soluble solids and solids recovered of extracts after microwave irradiation were both significantly lower in the coarsest fraction (>30 μm) compared with other fractions. Phenolics content was significantly higher in the two largest air-classified fractions ($\geqslant$24 μm) after microwave irradiation and antioxidant capacity tended to be greatest in the same two fractions. The larger air-classified fractions have been previously found to be derived from bran kernel region (Stevenson, Eller, Jane, & Inglett, 2007) and the bran of oats has been reported to have higher phenolics content and antioxidant capacity (Peterson et al., 2001). The larger fractions of air-classified cereal flour have been reported to have higher phenolic content for wheat bran (Antoine et al., 2004), beach pea hulls (Shahidi, Chavan, Naczk, & Amarowicz, 2001) and amaranth (Gamel et al., 2006). Smaller milled wheat particles have been reported to release greater amounts of antioxidant, but this accelerates losses during thermal treatments (Cheng et al., 2006), so this may partly explain why larger particle sizes have higher phenolic compounds and antioxidant capacity. Commercial milling of rice (Lloyd et al., 2000) also revealed higher phenolic content in bran, particularly the outer layers.

This study demonstrated that enhancement in extraction of phenolic compounds and antioxidant capacity from oat bran concentrate can be achieved by microwave-irradiating at 150 °C for 10 min in 50% ethanol. Supercritical carbon dioxide extraction may slightly decrease phenolic compounds and antioxidant capacity obtained while utilizing coarse fractions obtained from air-classification may help enrichment of phenolic compounds and antioxidant capacity.

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