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Antioxidant activity, avenanthramide and phenolic acid contents of oat milling fractions

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ABSTRACT

Avenanthramide (AV) 2a, 2p and 2f as well as five phenolic acids (vanillic, caffeic, p-coumaric, ferulic, and *p*-cinnamic) were quantified by LC–MS in six oat milling fractions (medium bran (MB), fine bran (FB), low bran (LB), whole oat groat flour (WF), 15% (15HG) and 20% high glucans). AVs content ranged from 323.7 to 775.5 μg/g while the free phenolic acids content was much lower 103.5–194.6 μg/g. The extraction procedure used greatly increased the concentration of identified molecules compared to previous data. FB had the highest content of both groups of phenols while WF contained the lowest. The peroxyl radical scavenging assay showed that granulation had no effect of on scavenging power as MB (31.8 ± 2.3 μM TE/g) and FB (27.7 ± 2.1 μM TE/g) had similar activities. Radical scavenging data correlated with AVs content but not with free phenolic acids, demonstrating that AVs were the main antioxidant compounds. The identity of quantified molecules was confirmed by mass spectrometry.

1. Introduction

Phenolic and polyphenolic compounds are naturally occurring secondary metabolites present in cereals, fruits, and vegetables. These metabolites protect the plant against ultraviolet radiation or pathogen attack. In food, these molecules can contribute to oxidative stability but also to bitterness, astringency, or color. Previous research works have shown that consumption of grain products confer some protection against the development of chronic diseases such as cardiovascular diseases, cancers, and diabetes (Slavin et al., 1997, Banerjee and Rimm, 2003). Cereals are reported to protect by altering serum cholesterol profiles, exerting antioxidant and antithrombic actions, and through their favorable effects on the vascular system and insulin sensitivity (Anderson and Hanna, 1999). The protective effect of cereals is attributed to the presence of both dietary fibers (i.e. glucans) and polyphenols. As antioxidants, polyphenols act to prevent free radical damage to lipids, proteins, DNA, RNA, and cellular organelles (Peterson, 2001).

Polyphenolic compounds in oats include phenolic acids, flavonoids and a unique group of amides referred to as avenanthramides (AVs) (Collins, 1989). There are likely more two dozen AVs in oats but the structure of only five have been completely elucidated (Shahidi and Naczk, 2003). The most abundant ones are N-[3′,4′-Dihydroxy-(E)-cinnamoyl]-5-hydroxyanthranilic acid (AV2c), N-[4′-Hydroxy-3'-methoxy-(E)-cinnamoyl]-5-hydroxyanthranilic acid (AV2f), N-[4′-Hydroxy-(E)cinnamoyl]-5-hydroxyanthranilic acid (AV2p). These three main AVs have been found to possess strong *in vitro* radical scavenging activity (Emmons et al., 1999). An AV mixture was reported to down regulate the expression of adhesion molecule and inhibited the production of pro-inflammatory cytokines IL-6, and IL-8 as well as monocyte chemoattractant protein in human aortic endothelial cells (Liu et al., 2004). AV2c inhibited serum-stimulated smooth muscle cell proliferation (Nie et al., 2006); decreased exercise-related free radical

List of abbreviations: 15HG, 15% high glucans; 20HG, 20% high glucans; AV, Avenanthramide; CA, caffeic; CiA, cinnamic; FA, ferulic acid; FPA, free phenolic acids; FB, fine bran; LB, low bran; LC–MS, liquid chromatography – mass spectrometry; MB, medium bran; ORAC, oxygen radical absorbance capacity assay; pCA, p-coumaric; TE, Trolox equivalents; TPC, total phenolic content; VA, vanillic; WF, whole oat groat flour.

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production in soleus muscle, malondialdehyde content in heart, and increased the activity of the antioxidant enzyme superoxide dismutase in skeletal muscle, liver, and kidneys in rats (Ji et al., 2003). In humans who consumed AV-enriched oats, the main AVs were bioavailable and the concentration of the endogenous antioxidant peptide glutathione increased by 21% (Chen et al., 2007). Total AVs can be up to 0.8% dry weight in bran-rich oat mill fractions (Collins, 1986). Steaming, autoclaving, and drum drying of oats showed that concentrations of *p*-coumaric acid and vanillin were unaffected during the steam-polishing step, meanwhile, there was an increase in ferulic acid and a decrease in caffeic acid (Bryngelsson et al., 2002). On the other hand, AV-2c and 2f were not significantly affected during preparation of rolled oats, while there was a reduction of AV2p (45%) in steam rolled oats compared to raw groats. Although the content of phenolic compounds in oats are affected by growing condition and processing, the effect of particle size or glucan content has not yet been investigated. The objective of this work was then to determine the content of phenolic molecules in six commercial oat milling fractions of different granulation, bran and glucan contents in addition to their antioxidant activity.

2. Methodology

2.1. Materials and chemicals

2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), mono- and dibasic potassium phosphates, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), rutin trihydrate, gallic acid, FerroZine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt), ethylene diamine tetraacetic (EDTA), potassium phosphate monobasic and dibasic, were obtained from Sigma–Aldrich Ltd (Oakville, ON, Canada). Methanol, ethyl acetate, hexane and fluorescein were purchased from Fisher Scientific Co. (Nepean, ON, Canada).

2.2. Samples

Medium bran (MB), fine bran (FB), low bran (LB), whole oat groat flour (WF), 15% high glucans (15 HG) and 20% high glucans (20HG) were donated by Richardson Milling (Portage La Prairie, Manitoba MB). The content of moisture, glucans, total fibers, proteins and ash (Table 1) as well as sieve analysis to determine particle size distribution was performed by Richardson Milling. The analysis used sieve numbers 10 (2.0 mm), 14 (1.4 mm), 20 (850 μm), 25 (710 μm), 30 (600 μm), 35 (500 μm), 40 (425 μm), 50 (300 μm), 60 (250 μm) and 100 (150 μm). Particle size percentage distributions were as follows: LB and WF #35 (2.6), #50 (25.2), #100 (17.2) and Thru #100 (55.2); MB #10 (0.8), #20 (61.5), #30 (32.1), #40 (5.0), Pan (0.6); FB #25 (7.5), #60 (92.0), # Pan (0.5); 15HG #50 (26.0), #60 (18.2), #100 (23.0), Pan (32.4); 20HG #50 (26.0), #60 (18.2), #100 (23.0), Pan (32.4).

2.3. Preparation of phenolic compounds

50 g of each sample were defatted by stirring for 1 h in hexane (1:4 w/v) and filtration on Whatman paper No. 1. The defatted oat samples were dried overnight under a fume hood, 50% aqueous methanol (1:8 w/v) was then added. Slurries were incubated at 50 °C for 2 h, 200 rpm (MaxQ 5000, Fisher Scientific, Nepean, ON, Canada). Extracts were recovered after centrifugation at 4000 g. To obtain free (i.e. not bound to sugars or proteins) phenolic compounds, the aqueous methanol solution was adjusted to pH 2.0 with 1 N HCl and extracted with ethyl acetate (EtOAc) 3 × 150 mL. The EtOAC fractions containing the phenols were dried at 40 °C on a Büchi 210 rotary evaporator (VWR Canada, Montreal, QC), reconstituted with 1200 μL methanol-H_2O (4:1), aliquot (100 μL each) into brown vials and stored at −80° C until HPLC and antioxidant analyses.

2.4. HPLC and LC–MS analysis

The system consisted of Waters 1525 Binary HPLC equipped with a vacuum membrane degasser, 2707 auto sampler model (maintained at 10 °C), 2998 photodiode array detector and Empower version 3 software (Waters, Milford, MA). The separation was performed on a Waters Nova-Pak® C18, 4 μm and 3.9 × 150 mm column. The injection volume was 20 μL and the flow rate was 0.8 mL/min. A mixture of 1% acetic acid in water (A) and 1% acetic acid in methanol (B) was used to elute the free phenolic acids and AVs from the column. The linear gradient conditions were 0.5 min 100% A, 5–10 min 80% A, 10–35 min 50% A, 35–40 min 20% A, 40–50 min 20% A. There was 10 min of equilibrium between injections. The peaks were monitored at 280 and identified by comparing their retention times and UV spectra with those of authentic standards. LC–MS was performed to confirm the identity of the AVs and they were quantified as ferulic acid equivalents (FAE). UV detector, column, gradient and flow rate were as above but methanol (solvent A) was replaced with acetonitrile and the couple LC was Alliance 2795 and phenolic acid authentic standards were used. Chromatograms from both systems were similar. The MS instrument was Quattro micro™ operating in electrospray positive mode: capillary voltage 3.0 kV, source temperature 100 °C, and desolvation temperature of 200 °C. The cone and desolvation nitrogen gas flows were 70 and 540 L/h, respectively, and the electron multiplier was set at 650 V. Data were collected from 100 to 900 Da and processed using MassLynx 4.0 (Waters, Milford, MA).

2.5. Determination of total phenolic content (TPC)

The TPC was determined according to literature (Verardo et al., 2011). 100 μL of phenolic extracts from the freezer was further diluted 50 times with methanol. To run the assay, 100 μL of each diluted sample, standard or methanol blank and 200 μL Folin reagent were vortex mixed in 2-mL microtubes, then 800 μL Na_2CO_3

Table 1
Nutritional content of oat flour samples: LB: low bran, WF: whole flour, MB: medium bran, FB: fine bran, 15HG: 15% high glucans, 20HG: 20% high glucans.

	MB	FB	LB	WF	15HG	20HG
Glucans	6.6 ± 0.4	8.3 ± 0.6	1.8 ± 0.1	4.0 ± 0.2	15.0 ± 0.7	20.0 ± 0.5
Total fibers	17.4 ± 1.0	21.0 ± 1.1	6.4 ± 0.5	12.7 ± 0.8	30.0 ± 1.2	39.0 ± 1.1
Carboyhydrates[a]	64.4 ± 3.4	60.9 ± 4.0	72.4 ± 1.8	69.3 ± 2.9	59.1 ± 3.6	60.8 ± 3.1
Proteins	15.6 ± 0.8	18.0 ± 1.0	10.1 ± 0.6	13.0 ± 0.9	21.0 ± 0.9	22.0 ± 0.9
Fat	8.1 ± 0.4	9.0 ± 0.5	6.4 ± 0.3	6.9 ± 0.4	10.0 ± 0.4	10.0 ± 0.3
Moisture	9.4 ± 0.6	9.0 ± 0.6	10.1 ± 0.5	9.0 ± 0.5	6.0 ± 0.2	3.0 ± 0.1
Ash	2.5 ± 0.2	3.1 ± 0.2	1.0 ± 0.1	1.8 ± 0.1	3.9 ± 0.2	4.2 ± 0.2

[a] Carboyhydrates = 100 − moisture − fat − protein − ash.