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Free and bound minor polar compounds in oats: Different extraction methods and analytical determinations

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ABSTRACT

The extraction capacity of several solvent mixtures and comparison between alkaline and acid hydrolysis to extract free and bound minor polar compounds in oat samples were carried out. The extraction yield of each method was evaluated by correlating several spectrophotometric indices (absorption at 320 nm and total phenolic compounds by the Folin–Ciocalteu method) with HPLC-MS and the antioxidant activities of the oat extracts (DPPH radical method). The results showed that methanol and alkaline hydrolysis reported the highest recovery of polar compounds, and spectrophotometric indices overestimated the content of these compounds. Antioxidant compounds of five Romanian oat samples, named Comun, Jeremy, Mures, Lovrin 1, Lovrin 27-T were determined. Large intervarietal differences in phenolic compounds and their antioxidant activities among oat varieties were detected. Total minor polar contents content was in the range of 1179.47 and 1897.18 mg/kg of seed. According to the literature, we found that the majority of the minor polar compounds existed in bound form.

1. Introduction

A plant-based diet protects against chronic oxidative stress-related diseases. Dietary plants contain variable chemical families and amounts of antioxidants (Naczk and Shahidi, 2006). Over the past several years, nutritional antioxidants have attracted considerable interest in the popular press as potential treatment for a wide variety of disease states, including cancer, chronic inflammatory diseases and aging (Benzie, 2003).

Whole grains contain main health promoting components such as vitamins, minerals and phytochemicals which include phenolic compounds (Dykes and Rooney, 2007).

Cereal phenolic compounds are concentrated mainly in outer layers of the grain (bran). In traditional processes, the bran is removed either by roller milling or pearling (debranning). In recent years, significant changes to milling technology have occurred (Gray et al., 2000). For this, different physical separation techniques, such as sieving, pearling and air classification, were used to produce flours naturally enriched in bioactive compounds. This industry change has given us an excellent opportunity to add value to this by-product of the milling process, but only if the requisite research is performed that we can understand how to obtain antioxidant-enriched milling streams (McKevith, 2004).

Oats and its products are rich in many bioactive ingredients, such as: water-soluble β-glucans, compounds showing antioxidant activity (tocols, phenolic compounds), polyunsaturated fatty acids, especially α-linoleic acid, and phytosterols (for example β-sitosterol, Δ5-avenasterol). In the last decades, the natural or manufactured products from oats (*Avena sativa* L.) have been largely considered a source of high-value bioactive components associated with human health benefits.

Phenolic compounds present in oats may contribute to functional and nutritional properties of the grain. Phenolic acids derived from hydroxybenzoic and hydroxycinnamic acids occur throughout the plant kingdom including cereals. Early studies showed that the phenolic acids in oats had antioxidant properties in vitro (Peterson, 2001; Shewry et al., 2008) and in vivo (Ryan et al., 2007). Literature showed different phenolic acid composition in oat samples depending on the cultivar and agronomic treatments (Dimberg et al., 2005; Emmons and Peterson, 2001; Xu et al., 2009). The major phenolic acids in oat were ferulic, p-coumaric, caffeic, vanillic, hydroxybenzoic acid and their derivatives (Emmons et al., 1999; Emmons and Peterson, 2001; Kováčová and Malinová, 2007; Mattila et al., 2005).

Abbreviations: Ac, extraction with acetone/water 4/1 v/v; Et, extraction with ethanol/water 4/1 v/v; HI, hydroxycinnamic index; Met, extraction with methanol/water 4/1 v/v; TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic compound by Folin–Ciocalteu reagent.

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Moreover, several oat-specific hydroxycinnamic acid derivatives have been identified as avenanthramides (AVAs). These compounds have been found only in oats (Dimberg et al., 1993; Dimberg et al., 2005; Meydani, 2009). The three most abundant AVAs in oat (Bratt et al., 2003) are 2c, 2p, and 2f, number 2 indicates 5-hydroxyanthranilic acid, and letters p, c and f indicate the kind of hydroxycinnamic acids as p-coumaric, caffeic, and ferulic acids, respectively. Some authors have demonstrated that the AVAs have a good antioxidant activity (Fagerlund et al., 2009), and it can be 10–30 times greater than other phenolic antioxidants such as vanillin and caffeic acid (Dimberg et al., 1993). Okazaki et al. (2007) described the presence of dimeric compounds of AVAs in oat. Moreover, some flavonoids were isolated in oat samples (Wenzig et al., 2005).

Phenolic compounds in oat, as well in other cereals, are either in free or bound forms. Generally, the free phenolic compounds were extracted with methanol, ethanol or acetone, whereas the bound phenolic compounds are ester-linked to cell wall polymers and were extracted by alkaline or acid hydrolysis (Naczk and Shahidi, 2006).

The phenolic compounds and other minor polar compounds have already been quantified in oats; however quantitative data are highly variable and difficult to compare due to differences in extraction methods and analysis that have been performed. For this reason, different extraction methods and some analytical techniques were carried out to establish the appropriate method to determine the free and bound minor polar content in oat. The set up methods have been used to determine the minor polar compounds in five Romanian cultivars of oat.

2. Materials and methods

2.1. Samples

Two commercial oat samples harvested in Gemmano farm in Rimini (Italy) were used for the set up of the extraction method. Five Romanian cultivars of oat (Comun, Jeremy, Mures, Lovrin 1, Lovrin 27-T) were cultivated in an experimental field under the same agronomic conditions in Timisoara (Romania) in 2009. Oat grains were briefly milled before extraction for 3 min at 15 °C to a particle size <0.6 mm using a laboratory mill (IKA A10-IKAWERKE GmbH &Co. KG, Staufen, Germany).

2.2. Extraction of free minor polar compounds

Every extraction trial was replicated four times ($n = 4$), the extracts were stored at −18 °C until used.

Four grams of sample were extracted by sonication with 40 mL of an organic solvent/water extraction mixture for 10 min to extract the free phenolic compounds. Three different extraction mixtures were used: 4/1 ethanol/water (v/v) (Et extract); 4/1 methanol/water (v/v) (Met extract); and 4/1 acetone/water (v/v) (Ac extract).

After centrifugation at 1000*g* for 10 min, the supernatant was removed and the extraction was repeated once more. The supernatants were pooled, evaporated at 40 °C with a vacuum evaporator, and reconstituted with 4 mL of 1/1 methanol/water (v/v).

2.3. Extraction of bound phenolic compounds

To determine the bound phenolic fraction, alkaline and acidic hydrolyses were carried out. Every extraction trial was replicated four times ($n = 4$) and the extracts were stored at −18 °C until used.

2.3.1. Alkaline hydrolysis

The residue of free polar compound extraction (from methanol extraction) was digested with 200 mL of 2 M NaOH at room temperature for 20 h by shaking under nitrogen gas atmosphere. The mixture was acidified to pH 2–3 by adding 10 M hydrochloric acid in a cooling ice bath and extracted with 500 mL of hexane to remove the lipids. The aqueous solution was extracted five times with 100 mL of 1/1 diethyl ether/ethyl acetate (v/v). The organic fractions were pooled and evaporated to dryness. The polar compounds were reconstituted with 4 mL of 1/1 methanol/water (v/v).

2.3.2. Acid hydrolysis

The residue of free polar compound extraction (from methanol extraction) was shaken with 24 mL of 96% ethanol and 120 mL of 25% hydrochloric acid at 65 °C for 30 min. Then, 40 mL of 96% ethanol and 50 mL of 1/1 diethyl ether/petroleum ether at 40–60 °C (v/v) were added to the digested samples. The organic fraction was discarded, and the residue was washed twice with 50 mL of 1/1 diethyl ether/petroleum ether 40–60 °C (v/v) to remove lipids. The aqueous fraction was washed five times with 100 mL of 1/1 diethyl ether/ethyl acetate (v/v). The organic fractions, containing the minor polar fraction of oat, were pooled and evaporated to dryness. The polar compounds were reconstituted with 4 mL of 1/1 methanol/water (v/v).

2.4. Spectrophotometric determinations

The spectrophotometric analyses were performed using a UV-1601 spectrophotometer from Shimadzu (Duisburg, Germany) and were replicated four times for each extract ($n = 4$) and three times for each calibration point ($n = 3$).

2.4.1. Determination of total phenolic compounds (TPC)

The TPC of the extracts was determined with the Folin–Ciocalteu spectrophotometric method (Singleton and Rossi, 1956). Briefly, 100 μL of each extract were shaken for 1 min with 500 μL of Folin–Ciocalteu reagent (Carlo Erba reagents, Rodano, Milano, Italy) and 6 mL of distilled water. After the mixture was shaken, 2 mL of 15% (w/v) Na_2CO_3 were added and the mixture was shaken once again for 0.5 min. Finally, the solution was brought up to 10 mL by adding distilled water. After 2 h, the absorbance at 750 nm (25 °C) was evaluated using glass cuvettes. The TPC was assessed by plotting the ferulic acid calibration curve (from 1 to 1000 μg/mL). The equation of the ferulic acid calibration curve was $A = 0.8326c + 0.0056$, and the correlation coefficient was $r^2 = 0.998$.

2.4.2. Hydroxycinnamic index (HI)

The HI was calculated according to the Maillard et al. (1996) method, with some modifications. Two-hundred microliters of each extract was diluted with 10 mL of methanol, and the absorbance was evaluated at 320 nm (25 °C) using quartz cuvettes. A ferulic acid calibration curve (1–250 μg/mL) was plotted to assess the HI. The equation of the ferulic acid calibration curve was $A = 3.914c - 0.0193$, and the correlation coefficient was $r^2 = 0.996$.

2.4.3. Evaluation of the antioxidant capacity of the extracts

To determine the antioxidant capacity (TEAC) of the extracts, the DPPH radical scavenging method was performed according to the Parejo et al. (2000) and Brand-Williams et al. (1995) methods. A 100 μL sample of each extract was added to 2.9 mL of 100 μM DPPH (Sigma, St. Louis, MO) solution in 4/1 methanol/water (v/v). A decrease of absorbance was determined at 517 nm in the 0–30 min range (at 25 °C). One hundred microliters of 1/1 methanol/water (v/v) added to 2.9 mL of DPPH solution was used to zero the