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Phenols, lignans and antioxidant properties of legume and sweet chestnut flours

Alessandra Durazzo *, Valeria Turfani, Elena Azzini, Giuseppe Maiani, Marina Carcea

National Research Institute on Food and Nutrition, Via Ardeatina 546, 00178 Roma, Italy

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ABSTRACT

Total phenols (TPC) and antioxidant properties were determined in chick-pea, green and red lentils and sweet chestnut flours, in both aqueous-organic extracts and their residues, by the Folin Ciocalteau method and by the FRAP assay, respectively. Plant lignans were quantified in flours by means of HPLC. In addition, the FRAP of plant lignans (secoisolariciresinol, lariciresinol, isolariciresinol, pinoresinol, matairesinol), their mixture and enterolignans (enterodiol and enterolactone) were determined. In all flours, the highest TPC values were found in the residue. Specific and varietal significant differences were observed in all parameters. The highest TPC (737.32 and 1492.93 mg/100 g d.w.) and FRAP (140.32 and 101.25 μmol/g d.w.) values were reached by green lentils in both aqueous-organic extract and residue, respectively. Sweet chestnuts had the highest total lignans (980.03 μg/100 g d.w.). It was also found that the plant lignans standards have a higher antioxidant activity than enterolignans standards and that matairesinol has the highest activity.

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1. Introduction

The food industry is interested in the improvement of the nutritional quality of traditional products such as bread, pasta and breakfast cereals by using, together with wheat, vegetable flours of different origin (Chavan, Kadam, & Reddy 1993; Dewettinck et al., 2008; Sacchetti & Pinnavia, 1999).

Several studies have investigated the enrichment of bread and other cereal based foods with legume flours (Bojnanska et al., 2012; Dalgetty & Baik, 2006; Hawkins & Johnson, 2005; Johnson, Thomas, & Hall, 2005; Okoye, Nkwocha, & Agbo, 2008; Pittaway, Ahuja, Robertson, & Ball, 2007) due mainly to the complementary aminoacids composition of cereals and legumes and their relatively low cost (Amjad, Iqtidar, Nadia, & Khan, 2006; Fenn, Lukow, Humphreys, Fields, & Boye, 2010; Messina, 1999; Rysová et al., 2010).

In several recent investigations chestnut flour has been proposed as a good substitute for cereal flour in cereal based products with different nutritional attributes (Demirkesen, Mert, Sumnu, & Sahin, 2010; Sacchetti, Pinnavaia, Guidolin, & Dalla-Rosa, 2004; Vazquez, Gonzalez-Alvarez, Santos, Freire, & Antorrena, 2009).

Chestnuts represent a good source of antioxidants, calcium and an important source of phenolic compounds (Barreira, Ferreira, Oliveira, & Pereira, 2008; Barreira, Ferreira, Oliveira, & Pereira, 2010; Bolling, Chen, McKay, & Blumberg, 2011; de Vasconcelos et al., 2010; Ribeiro et al., 2007; Vazquez et al., 2008; Yildiz, Ozcan, Calisir, Demir, & Er, 2009).

Within the frame of a project aimed at developing cereal based products for the Italian population with improved nutritional quality, the study of some bioactive components (polyphenols and lignans) as well as their antioxidant properties was undertaken in legume flours obtained from species commonly grown in Italy and in sweet chestnuts (which are also extensively grown in Italy) in order to assess their potential nutritional quality. The above raw materials are easily available, cheap and their flavour is known and liked by the Italian population.

Polyphenols exist as easily extractable compounds (free) and as less extractable types (bound) (Bravo, Albia, & Saura-Calixto, 1994; Bravo, Manas, & Saura-Calixto, 1993). The distinction between free and bound antioxidants represents a key element for a better understanding of the potential health benefits of foods of vegetable origin. Recent investigations have demonstrated that polyphenols content of plant foods have been underestimated: significant amounts of bioactive compounds remain in the residue from extraction as non extractable polyphenols (Arranz, Saura-Calixto, Shaha, & Kroon 2009; Arranz, Silván, & Saura-Calixto, 2010; Pérez-Jiménez & Torres, 2011).

Within the phytochemicals, lignans are of increasing interest due to their potential anticarcinogenic, antioxidant, estrogenic and antiestrogenic activities (Adlercreutz et al., 2007; Miur, 2010).

They are a class of secondary plant metabolites that belong to the group of diphenolic compounds derived from the combination of two phenylpropanoid C6-C3 units at the β and β′ carbon atoms. They have a chemical structure like the 1,4-diarylbutan. The range of their structures and biological activities is broad. They are derived from the shikimic acid biosynthetic pathway (Imai, Nomura, & Fukushima, 2006). The main sources of dietary lignans are

* Corresponding author. Tel.: +39 0651494651; fax: +39 065149550.
E-mail address: durazzo@inran.it (A. Durazzo).

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oilseeds (e.g. flax, soy, rapeseed, and sesame), whole-grain cereals (e.g. wheat, oats, rye, barley), legumes and various vegetables and fruit (particularly berries) (Milder, Arts, van de Putte, Venema, & Hollman, 2005; Penalvo et al., 2008; Smeds et al., 2007; Thompson, Boucher, Liu, Cotterchio, & Kreiger, 2006).

After ingestion, plant lignans are deglycosylated and partly converted to the enterolignans by colonic bacteria (Rowland, Wiseman, Sanders, Adlercreutz, & Bowey, 2000; Setchell & Adlercreutz, 1988). Presumably the enterolignans are re-conjugated to glucuronides and sulfates in the intestinal wall and in the liver. Enterolignans have a similar structure to the human hormone oestrogen and so may have estrogenic/anti-estrogenic effects. Several epidemiological studies have shown a potential protective effect of the enterolignans against hormone-dependent cancers, especially breast cancer, and against cardiovascular diseases (Buck, Zaineddin, Vrieling, Linseisen, & Chang-Claude, 2010; Prasad, 2009).

Therefore, in this work, total polyphenols contents, lignans (isolariciresinol, lariciresinol, secoisolariciresinol, pinoresinol, matairesinol) as well as their antioxidant properties are reported for chick-pea, green and red lentils and sweet chestnuts flours.

2. Materials and methods

2.1. Samples

Three commercial chick-pea (*Cicer arietinum* L.) and sweet chestnut (*Castanea sativa* Mill) flours were purchased at a local supermarket as well as the same number of green and red lentils (*Lens culinaris* Medik). Three commercial soft wheat flours (0 type) were also bought and used as a comparison. The legume grains were ground in a refrigerated mill (Janke and Kunkec Ika Labortechnik, Germany) and the wholemeal flours were sieved to obtain a granulometry of 0.5 mm. Only for lignans analyses, flours were defatted with hexane and diethyl ether for 8 h in a Soxhlet apparatus.

2.2. Chemicals and standards

All solvents were of HPLC or Optima grade and purchased from Carlo Erba (Milan, Italy). Common reagents and standards were purchased from Sigma–Aldrich Srl (Milan, Italy), Extrasynthese (Genay, France), Chemical Research (Rome, Italy) and were of the highest available grade. Double-distilled water (Millipore, Milan, Italy) was used throughout the study.

2.3. Extraction for evaluation of total polyphenol content (TPC) and antioxidant properties

Total polyphenols were extracted as described by Hartzfeld, Forkner, Hunter, and Hagerman (2002) and Pérez-Jiménez and Saura-Calixto (2005), with minor modifications as follows. Aqueous-organic extracts (extractable polyphenols) and their residues (non-extractable polyphenols) were isolated and studied. In particular in residues, among non-extractable polyphenols, hydrolysable polyphenols (comprising hydrolysable tannins, phenolic acids and hydroxycinnamic acids that are released from the food matrix by strong acidic hydrolysis) were determined.

Aqueous-organic extract. About 3–4 g of flours were placed in a test tube and 20 mL of acidic methanol/water (50:50 v/v, PH 2) were added. The tubes were vortexed at room temperature for 3 min, followed by 1 h shaking in a water bath at room temperature. The tube was centrifuged at 2500 g for 10 min, and the supernatant was recovered. Twenty millilitres of acetone/water (70:30, v/v) were added to residue, then vortexing, shaking and centrifugation were repeated. Both methanolic and acetonic extracts were combined and centrifuged at 3500 g for 15 min. The resulting supernatant was transferred into tubes and directly used for the determination of antioxidant capacity and total polyphenol content.

Residue. Residues were left in a ventilating and heating apparatus (max temperature 25 °C), until dryness. Briefly, 300 mg of the residue were mixed with 20 mL of methanol and 2 mL of concentrated sulfuric acid (18 M). The samples were gently stirred for 1 min and were shaken in a water bath at 85 °C for 20 h.

The samples were then centrifuged (3000*g* for 10 min), and the supernatant was recovered. After two washings with minimum volumes of distilled water and recentrifuging as necessary, the final volume was taken up to 50 mL. The tube was centrifugated at 3500*g* for 20 min and was transferred into tubes and directly used for the determination of antioxidant capacity and total polyphenol content.

2.4. Determination of total polyphenol content (TPC)

The TPC was determined in both aqueous-organic extracts and their residues using the Folin–Ciocalteau procedure (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, appropriate dilutions of extracts were oxidised with Folin–Ciocalteau reagent, and the reaction was neutralised with sodium carbonate. The absorbance of the resulting blue colour was measured at 760 nm against a blank after 2 h of reaction at room temperature. Gallic acid was used as the standard.

2.5. Antioxidant properties evaluation

The ferric reducing activity of a single compound in solution, a food extract or biological fluid can be measured by the FRAP (Ferric Reducing Antioxidant Power) assay. Antioxidant properties have been determined in both aqueous-organic extracts and their residues by the FRAP assay. The method followed Benzie and Strain (1996) and Pulido, Bravo, and Saura-Calixto (2000) through the use of a Tecan Sunrise® plate reader spectrophotometer. The method is based on the reduction of Fe^{3+} – TPTZ (2,4,6-tripyridyl-s-triazine) complex to ferrous at low pH. Briefly, 160 μl of working reagent prepared daily was mixed well with 10 μl of diluted sample and the absorbance was recorded at 595 nm after 30 min incubation at 37 °C. Methanolic solutions of known Trolox concentrations were used for calibration.

2.6. Extraction and quantification of lignans

Samples were extracted taking into account the methods of Obermeyer et al. (1995) and Milder et al. (2004). 12.0 mL of alkaline hydrolysis reagent (0.3 M NaOH in 70% MeOH) were added to about 1 g of samples and, after stirring, were incubated for 1 h at 60 °C. The samples were cooled down to room temperature and neutralised with 100% acetic acid (380 μL). The hydrolysate was centrifuged (3500*g*, 10 min). An aliquot (2 mL) of supernatant was transferred into a test-tube. The supernatant was evaporated until only water was left (600 μL).

Water (1.4 mL) was added together with 2 mL of sodium acetate buffer (0.05 M, pH 5.0) and then 400 μL of Helix Pomatia β-glucuronidase/sulphatase were added (β-glucuronidase/sulphatase S9626–10KU Type H-1, 0.7 G solid, 14200 units/G solid, Sigma, in 10 mL of acetate buffer). The samples were incubated in a shaker water bath at 37 °C for 24 h. Samples were evaporated to dryness and dissolved in the mobile phase. Quantitative analyses were performed on 50 μL extract using an ESA–HPLC system (ESA, Chelmsford, Ma, USA) (Durazzo et al., 2009). The HPLC system used consisted of an ESA Model 540 autoinjector, an ESA Model 580

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