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Determination of free and bound phenolic compounds and their antioxidant activity in buckwheat bread loaf, crust and crumb

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ABSTRACT

This study demonstrated the role of buckwheat flour in improving phenolic compounds in white wheat bread. Three bread samples were obtained by using different buckwheat levels (10, 20 and 30%) in formulations. HPLC-ESI-MS was used to detect the presence of free and bound phenolic compounds in bread loaf, crust and crumb. The phenolic profile changed thanks to the addition of buckwheat flour; in fact, flavan-3-ols and flavonols compounds (i.e. rutin, catechin, etc.) were identified in enriched buckwheat. As expected, the phenolic content increased proportionally to buckwheat flour quantity in bread formulations. The total free phenolic amounts ranged from 109 to 235 mg/kg d.w. in control bread and 30% enriched buckwheat bread, respectively. Bread crusts showed the highest total free and bound phenolic content; however, flavan-3-ols, flavonols and flavones are more concentrated in crumb than crust. Moreover, enriched breads showed higher *in vitro* antioxidant properties (evaluated by DPPH and ABTS assays) than control one.

1. Introduction

Wheat bread is considered to be a good source of energy for the human body. It is known that bread obtained with natural raw ingredients such as cereals and seeds, spices, herbs and parts of green plants, fruit or vegetable products and waste products from the food industry can be enriched in antioxidant compounds (Balestra, Cocci, Pinnavaia, & Romani, 2011; Blandino et al., 2013; Dziki, Rozyło, Gawlik-Dziki, & Swieca, 2014).

Foods based on wholegrain cereals, including bread, play an important role in human health and well-being. It has been demonstrated that the regular consumption of wholegrain cereals can contribute to reduce the risk of cardiovascular disease (CVD), type 2 diabetes mellitus and certain types of cancer, as well as several gastrointestinal pathologies (Gil, Ortega, & Maldonado, 2011). The healthy properties of whole grains are linked to the presence of bioactive compounds such as dietary fiber and phenolic compounds. Phenolic acids and flavonoids represent the most common form of phenolic compounds found in whole grains, and they are among the major and most complex groups of phytochemicals with a number of types that exist as soluble free compounds, soluble conjugates that are esterified to sugars and other low molecular mass compounds, and insoluble, bound forms (Zilic et al., 2011). Among cereals and pseudo-cereals, buckwheat represents a good source of bioactive compounds. These compounds are strictly related to the health benefits attributed to buckwheat including plasma cholesterol level reduction, neuroprotection, anticancer, anti-inflammatory, anti-diabetic effects, and improvement of hypertension conditions (Gimenez-Bastida, & Zielinski, 2015). Moreover, buckwheat is a gluten-free pseudocereal, for this reason, it can be used for gluten-free products formulation. As reported by Gimenez-Bastida, Piskula and Zielinski (2015), the buckwheat flour incorporation into a bread gluten-free experimental formula affected positively

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the technological quality of the product, enriching its protein and microelement contents.

Among the microelements, buckwheat is a source of several phenolic compounds such as flavonols, flavan-3-ols, propelargonidins and phenolic acids (Verardo et al., 2010; Inglett, Chen, Berhow, & Lee, 2011; Verardo, Gomez-Caravaca, Segura-Carretero, Caboni, & Fernandez-Gutierrez, 2011) with antioxidant activity. Recently, Stokić and co-workers (Stokìc et al., 2015) stated that buckwheat-enriched wheat bread had higher content of dietary fibers and phenolic compounds than wheat bread; moreover the same authors noticed that the consumption of buckwheat enriched bread caused a significant decrease in total cholesterol, LDL-cholesterol as well as the ratio of LDL/HDL cholesterol in statin treated patients.

Several studies have been developed to evaluate the phenolic content in buckwheat bread; however, to our knowledge, literature lacks of information about phenolic distribution in buckwheat bread. Because of that, and due to the health effects attributed to the phenolic compounds of buckwheat, the aim of this study was to study in depth the content of free and bound phenolic compounds in whole, crust and crumb of bread samples formulated with different level of buckwheat flour (10, 20 and 30%).

2. Materials and methods

2.1. Samples

Control bread and three bread samples obtained with wheat and different buckwheat flour (var. Lileja from Umbria, Italy) levels (10, 20 and 30% on wheat flour quantity) were formulated. All used ingredients were supplied by a local bakery company (Cesena, Italy). The list of ingredients and related amount used for each kind of bread are reported in Table 1. In particular, the wheat flour (type 0) used in this research, was a refined flour made from soft wheat (extraction rate ~ 700 g/kg). The physicochemical characteristics of the wheat flour used to develop the dough were: $W = 270\ 10^{-4}$ J; P/L 0.5; moisture: 140 g/kg; ash: 6.5 g/kg; dry gluten: 96 g/kg; protein content: 129 g/kg. The physicochemical characteristics of the buckwheat flour were instead: moisture: 120 g/kg; ash: 20 g/kg; protein content: 105 g/kg. Moreover, the buckwheat percentages used were chosen after preliminary trials, carried out in order to obtain products with good technological properties, comparable to those of a standard bread formulation (control bread).

2.1.1. Sponge preparation

The sponge was prepared with 775 g of flour, 352 g of water and 7.75 g of brewer's yeast. The main ingredients of the dough were kept constant: sponge (1136 g), salt (37 g), brewer's yeast (39 g), improver (5 g) and water (510 g).

All ingredients were mixed in a kneading professional machine (Tauro –Sigma s.r.l, Brescia, Italy). As a first step, refined wheat flour was mixed with water for 3 min at minimum speed of 40 rpm, after that, sourdough was added and mixed for other 7 min, at the same speed. After mixing, the obtained "sponge dough" was stored in a thermoclimatic chamber (Constant Climate Chambers with Peltier technology, model HPP 108/749, Hemmert, Germany) at 28 °C for 24 h, before to add it into the dough.

Table 1
Amounts (kg) of the different ingredients used for bread formulation.

Ingredients	Control	BB 10%	BB 20%	BB 30%
Wheat flour (Type 0)	1.25	1.125	1.00	0.875
Buckwheat flour	–	0.125	0.25	0.375
Water	0.83	0.83	0.83	0.83
Brewer's Sourdough	0.06	0.06	0.06	0.06
NaCl	0.06	0.06	0.06	0.06
Alpha-amylase	0.01	0.01	0.01	0.01
Sponge	1.84	1.84	1.84	1.84

2.1.2. Bread preparation

Bread enriched with buckwheat was obtained by mixing the sponge previously prepared, with the other ingredients: refined wheat flour, water, sodium chloride (NaCl), alpha-amylase and the different percentages of buckwheat. All ingredients were mixed for 7 min at minimum speed by using the same mixer used for the sponge preparation and let to leaven for 1 h at 32 °C and 70% of relative humidity in a thermoclimatic chamber (Constant Climate Chambers with Peltier technology, model HPP 108/749, Hemmert, Germany). The baking of bread samples was carried out in a professional oven for 20 min at 210 °C (FC61, Angelo Po e Grandi Cucine S.p.A, Carpi, Italy). After baking, bread samples were left to cool at room temperature for 2 h, before performing analysis. The volume of the resulting bread (mL/g) was 3.3 ± 0.9 for the control; 3.2 ± 0.5 for the bread obtained with the addition of 10% of buckwheat flour; 2.9 ± 0.1 for the product obtained with the addition of 20% of buckwheat flour and 2.5 ± 0.2 for the bread with the 30% of buckwheat flour.

Each type of bread was produced in triplicate. After baking, crust and crumb were separated from each bread sample, frozen in encoded plastic bags at −20 °C and then freeze-dried (Thermo HETO, powerdry LYOLAB 3000; Waltham, USA). Dried samples were ground to a fine powder in a blender mixer (Ika-Werke M20; Staufen, Germany) and used for the analyses.

2.2. Reagents and chemicals

HPLC-grade acetonitrile, ethanol and methanol were purchased from Labscan (Dublin, Ireland). Acetic acid analytical grade (assay > 99.5%) was purchased from Fluka (Buchs, Switzerland). Water was purified by using a Milli-Q system (Millipore, Bedford, MA, USA). Other reagents were purchased from VWR (Denver, CO, USA). Analytical standards were from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Extraction of free and bound phenolic compounds from control and buckwheat bread

To determine the free and bound phenolic fraction of bread samples, the method developed by Verardo, Arráez-Román, et al. (2011) and Verardo, Gomez-Caravaca, et al. (2011) was applied.

Briefly, 2 g of bread were extracted twice in an ultrasonic bath with a solution of ethanol/water (4:1 mL/mL). The supernatants were collected, evaporated and reconstituted with 2 mL of methanol/water (1:1 mL/mL). The extracts were stored at −18 °C until use.

To obtain the bound phenolic fraction, residues of free phenolic extraction were digested with 200 mL of 2 mol/L NaOH at room temperature for 4 h by shaking under nitrogen gas. The hydrolyzed solution was acified to pH 2–3 by adding 10 mol/L hydrochloric acid in a cooling ice bath and extracted with 500 mL of hexane to remove the lipids. The final solution was extracted five times with 100 mL of 1/1 diethyl ether/ethyl acetate (mL/mL). The organic fractions were pooled and evaporated to dryness. The phenolic compounds were reconstituted in 2 mL of methanol/water (1:1 mL/mL).

2.4. HPLC-ESI -MS analysis of phenolic compounds

HPLC analysis was performed by an Agilent 1100 series LC system (Agilent Technologies, CA, USA) consisting of a vacuum