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Production and characterization of functional biscuits obtained from purple wheat

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ABSTRACT

Purple wheat contains higher levels of anthocyanins than conventional wheat cultivars. The aim of this work was to produce anthocyanin-rich biscuits from purple wheat, and to characterize the final product. Control biscuits, having the same formulation but obtained from a non-pigmented wheat cultivar, were used for comparisons. Purple biscuits showed a level of total anthocyanins of 13.86 mg/kg cyanidin 3-*O*-glucoside and exhibited higher antioxidant activity than control. The volatile compounds profile of purple biscuits showed lower levels of lipid-derived carboxylic acids and higher levels of alcohols and aldehydes than control biscuits, indicating a lower oxidative degradation of the lipid fraction. In particular, the ratio (lipid-derived alcohols + aldehydes)/acids accounted for 5.9 in purple and 3.0 in control biscuits. The sensory score for friability and the spread ratio of purple biscuits accounted for 2.6 and 6.0, respectively.

1. Introduction

In recent years, the interest towards functional properties of foods has increased progressively and a relevant role has been played by antioxidant compounds, able to scavenge free radicals. Anthocyanins are antioxidant flavonoid compounds present in fruits and vegetables, as well as in the pigmented varieties of many cereals (Mazza & Miniati, 1993). Anthocyanin-pigmented wheats have been discovered in the 19th century in Ethiopia. More recently, many cultivars of purple-, red-, blue- and black-grained wheat have been developed (Zeven, 1991).

Several researches have been aimed to study pigmented wheats. The composition of the anthocyanin fraction has been found to be characterized by cyanidin 3-*O*-glucoside and peonidin 3-*O*-glucoside in purple-grained wheats, while delphinidin 3-*O*-rutinoside and delphinidin 3-*O*-glucoside prevail in blue wheats (Abdel-Aal & Hucl, 2003; Abdel-Aal, Young, & Rabalski, 2006; Ficco et al., 2014; Liu, Qiu, & Beta, 2010). The antioxidant activity of purple cultivars, determined as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity, has been quantified in the range between 5.87 and 8.57 μmol Trolox/g (Li, Pickard, & Beta, 2007; Liu et al., 2010), while higher values have been found in dark blue wheat (Hu, Cai, Li, Corke, & Kitts, 2007). Moreover, grain pigment distribution has been assessed pointing out that purple pigments are present in the pericarp, whereas blue pigments are located in the aleurone layer (Zeven, 1991).

In spite of the interest of producers for obtaining whole-grain food specialties with potentially functional properties, marginal attention has been paid to evaluate the performance of pigmented wheats during manufacturing. A single study regarded the evaluation of processing effect on the antioxidant properties of purple bran added in muffins (Li et al., 2007).

Biscuits could represent a good candidate for the addition of functional ingredients because they are popular, daily consumed, bakery items and have long shelf-life. Many functional biscuits have been formulated, mainly with antioxidant and/or prebiotic properties (Hassan, Rasmy, Foda, & Bahgaat, 2012; Mohsen, Fadel, Bekhit, Edris, & Ahmed, 2009). However, although not perceived as fatty foods by consumers, biscuits contain a relevant level of fats (Caponio, Summo, Clodoveo, & Pasqualone, 2008; Caponio, Summo, Delcuratolo, & Pasqualone, 2006). Attempts to improve their nutritional profile have been directed to reduce fats, sugars and energy level (Pasqualone, Bianco, & Paradiso, 2013; Sudha, Srivastava, Vetrimani, & Leelavathi, 2007; Taylor, Fasina, & Bell, 2008).

In previous papers, the authors proposed the production of functional biscuits enriched with phenolics and anthocyanins extracted from grape marc (Pasqualone et al., 2014a; Pasqualone et al., 2013). The levels of fat and sugar were minimized, and extra

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virgin olive oil, known for its positive healthy features, was used instead of shortenings. The aim of this work was to obtain anthocyanin-rich biscuits directly starting from purple wheat whole meal, and to characterize the final product. Again, particular attention was put in lipid choice: exclusively extra virgin olive oil was used and its amount was kept low.

2. Materials and methods

2.1. Sample preparation

Purple wheat line *CItr 14629* (*Triticum turgidum* ssp. *durum* (Desf.) Husnot), derived from an Ethiopic landrace kindly provided by the United States Department of Agriculture (USDA), and conventional durum wheat cv. *Ciccio* were grown in 2012 at the experimental field of University of Bari – DISSPA Dept. (Valenzano, Bari, Italy). Grains, harvested at full ripening, were milled to wholemeal flour by means of 1093 Cyclotec Sample Mill (Tecator Foss, Hillerød, Denmark) equipped with a 1-mm sieve. Sucrose and extra virgin olive oil were purchased at local retailers. Three independent biscuit-making production trials were performed. The biscuit-making process consisted of: (i) kneading for 3 min sucrose (350 g), extra virgin olive oil (160 mL) and sodium bicarbonate (8 g) by an electric mixer with flat beater (Kitchen Aid, Antwerp, Belgium), then adding 1 kg wholemeal flour of cv. *Ciccio* or *CItr 14629* line and kneading for 3 min, and finally adding water (250 mL) and kneading for 3 min; (ii) sheeting the dough to a thickness of 5 mm and cutting by a round biscuit cutter, 50 mm internal diameter; (iii) baking in electric oven (Smeg SI 850 RA-5 oven, Smeg S.p.A., Guastalla, Italy) for 20 min at 160 °C. Biscuits were finely crushed in a mortar for subsequent analyses, apart for colorimetric and sensory determinations.

2.2. Basic chemical and physical determinations

Moisture content was determined at 105 °C by means of an automatic moisture analyzer (Radwag Wagi Elektroniczne, Radom, Poland). Protein content ($N \times 5.7$) was determined according to the AACC approved method 46-11.02 (AACC, 2000). Dry gluten content and gluten index were determined by means of Glutomatic (Perten Instruments, Hägersten, Sweden) as in the AACC method 38-12.02 (AACC, 2000). Gluten index was expressed as the ratio of the wet gluten remaining on the sieve after centrifugation to the total wet gluten weight. Colorimetric evaluations of red index (a^*), yellow index (b^*) and brown index (BI, defined as 100-L^*) were carried out under D65 illuminant by using a spectro-colorimeter CM-700d (Konica Minolta Sensing, Osaka, Japan) equipped with a pulsed xenon lamp. To analyze wholemeal flour it was used the granular materials attachment CR-A50 (Konica Minolta Sensing, Osaka, Japan) to obtain a smooth surface suitable for color readings. Spread ratio (W/T) induced by baking was determined as the ratio between width (W) and thickness (T) of biscuits, according to the AACC method 10-50.05 (AACC, 2000).

Quality indices of extra virgin olive oil (free fatty acids, peroxide value, and UV spectrophotometric constants at 232 nm and 270 nm) were determined according to the EC Regulation No. 2568/91 (European Commission., 1991) and subsequent amendments and integrations. All determinations were carried out in triplicate.

2.3. Determination of total anthocyanin compounds (TAC)

TAC were extracted by adding 10 mL of 85:15 (v/v) methanol/1 M HCl to 1 g of sample (adjusting the pH to 1.0), then keeping on orbital shaker at 500 rpm, for 30 min, in the dark, centrifuging at 12,000×*g* for 5 min and recovering the supernatant. The pellet was re-extracted with 5 mL of acidified methanol in the same conditions and, after centrifuging at 12,000×*g* for 5 min, the two supernatants were pooled and concentrated to 5 mL under a stream of nitrogen. The absorbance of the solution was determined at 535 nm by a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). A calibration curve was previously set up by using solutions of the standard cyanidin 3-*O*-glucoside (Phytoplan, Heidelberg, Germany), the predominant anthocyanin in purple wheat, at concentrations from 1 to 50 mg/kg ($y = 0.0372\ x + 0.0101$; $R^2 = 0.9997$). All determinations were carried out in triplicate.

2.4. Determination of anthocyanin profile

The analyses of anthocyanin profile were performed by HPLC using a Waters 600 E apparatus (Waters, PA, USA) that included a quaternary pump, a photodiode array detector and an injection valve with a 10-μL loop. Samples, prepared as for determination of TAC, were filtered on a 0.45 μm nylon membrane and were injected into a Nova-Pak C18 column (150 × 3.9 mm, 4 μm particle size, Waters, Milford, MA, USA) maintained at 30 °C and eluted at a flow rate of 1 mL min^{-1} with 10% formic acid in water (solvent A) and acetonitrile (solvent B). The gradient program for solvent A was as follows: 0–1 min 95%, 1–22 min 60%, 22–27 min 30%, 27–35 min 30%, 35–36 min 95%. Detection was performed at 520 nm, and quantification was made according to external standard method on the basis of standard calibration curve obtained by the injection of solutions at different concentration of cyanidin 3-*O*-glucoside ($R^2 = 0.9991$). Tentative identification of anthocyanin compounds was achieved by combining elution pattern, UV–Vis spectra and data reported in literature (Abdel-Aal et al., 2006; Coletta et al., 2013). Results were expressed as mg/kg.

2.5. Determination of total phenolic compounds (TPC)

TPC were extracted by adding 1 mL of methanol to 0.1 g of sample, then purging with stream of nitrogen, keeping on orbital shaker at 250 rpm, for 2 h, in the dark, and centrifuging at 7000×*g* for 5 min. The recovered supernatant was subjected to Folin–Ciocalteu reaction as follows: the reaction mixture contained 100 μL supernatant, 500 μL Folin–Ciocalteu reagent (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) and 2 mL of 15% (w/v) sodium carbonate; the final volume was made up to 10 mL with distilled water. After 1 h in the dark, and centrifugation at 12,000×*g* for 3 min to precipitate any particles, the absorbance of the solution was measured at 765 nm by a Cary 60 UV–Vis spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA). A calibration curve was built by methanol solutions of ferulic acid (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) at concentrations between 0.1 and 2 g/L ($y = 0.0007\ x + 0.0089$; $R^2 = 0.9985$). The results were expressed as mg of ferulic acid equivalents per g, being ferulic acid the

Table 1
Basic quality characteristics of wholemeal flour obtained from purple (*CItr 14629* line) and conventional (cv. *Ciccio*) wheat (means and standard deviations; values expressed on dry matter).

Parameter	Wholemeal flour	
	Purple	Conventional
Moisture content (%)	$7.7^a \pm 1.0$	$7.9^a \pm 1.3$
Protein content (% d.m.)	$14.2^a \pm 0.3$	$13.7^a \pm 0.2$
Dry gluten content (% d.m.)	$11.0^a \pm 0.1$	$10.8^a \pm 0.1$
Gluten index	$68^a \pm 2$	$62^b \pm 2$

Means in the same row with different letters as superscripts are significantly different ($p < 0.05$).