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Bioactive compound and antioxidant activity distribution in roller-milled and pearled fractions of conventional and pigmented wheat varieties

Debora Giordano [a], Monica Locatelli [b], Fabiano Travaglia [b], Matteo Bordiga [b], Amedeo Reyneri [a], Jean Daniel Coïsson [b], Massimo Blandino [a,*]

[a] Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università di Torino, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy
[b] Dipartimento di Scienze del Farmaco and Drug and Food Biotechnology Center, Università del Piemonte Orientale "A. Avogadro", Largo Donegani 2, 28100 Novara (NO), Italy

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ABSTRACT

In this study, the chemical composition of pigmented wheats (yellow, purple and blue types), and the distribution of the bioactive compounds in their roller-milled and pearled fractions, were compared with conventional wheats (red and white types).

Roller-milling promoted the recovery of total dietary fiber, β-glucans, phenolic acids and anthocyanins in the bran fraction, which resulted also in a higher total antioxidant activity than the refined flour. Conversely, lutein resulted mainly concentrated in the refined flour. In the same way, the distribution pattern in the pearled fractions differ depending on the bioactive considered. The study highlights that a careful selection of the most appropriate fractionation process should be performed to produce flours rich in bioactive compounds. Roller-milling resulted useful for the production of refined flours rich in xanthophylls, with particular emphasis to the yellow-grained wheats. Contrarily, pearling could be more useful in the valorization of the health potential of anthocyanin-pigmented varieties.

1. Introduction

Whole grain cereals are an important source of bioactive compounds, and increasing evidence from clinical and epidemiological studies suggests that the regular consumption of wheat, as whole grain, might reduce the risk of developing chronic diseases (Belobrajdic & Bird, 2013; Borowicki, Stein, Scharlau, & Glei, 2010; Fardet, 2010).

Phenolic compounds occur abundantly in cereals, and are related to the antioxidant activity of the grain (Adom & Liu, 2002). Phenolic acids are the main phenolic compounds in cereals, and they constitute one of the major and most complex groups of phytochemicals (Li, Shewry, & Ward, 2008). In addition to phenolic acids, other bioactive compounds with antioxidant activity, such as anthocyanins and carotenoids, may also be present in cereal kernels. Anthocyanins and carotenoids both are classified as pigments, and are responsible for the characteristic blue-purple and yellow-orange hue of kernels, respectively. The content of these phytochemicals is generally limited in conventional white- or red-grained wheat varieties (Carson & Edwards, 2009), while they occur more consistently in the so-called pigmented varieties. These types of common wheat varieties, which are characterized by purple, blue or yellow grains, are actually produced in small amounts, but growing interest has recently been shown in the genetic development of novel pigmented varieties (Jaafar et al., 2013; Martinek, Škorpík, Chrpová, Fučík, & Schweiger, 2013). In fact, these unconventional varieties might be important sources of biologically active phytochemicals, and as a result, they could be valuable raw materials for the production of functional foods (Ficco et al., 2016; Li, Pickard, & Beta, 2007; Pasqualone et al., 2015).

Nevertheless, the use of wheat varieties that are naturally rich in bioactive compounds should be combined with a suitable grain processing technology, in order to preserve the bioactives and to produce functional ingredients. Previous studies, performed on conventional common wheat varieties, have shown that the bioactive compounds are mainly concentrated in the outer layers of the grain, and that their distribution within the kernel differs according to the class of nutrients (Sovrani et al., 2012). Consequently, the conventional roller-milling process, which promote the removal of the outer layers of the kernel in the bran fraction, causes a great decrease in the nutritional value of the refined flour

* Corresponding author.
E-mail address: massimo.blandino@unito.it (M. Blandino).

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(Siebenhandl et al., 2007). Another fractionation technology that was proposed as an alternative strategy to valorize the outer layers of the kernel was the pearling process (Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007). Wheat millers are increasingly using the pearling process before roller milling, since it improves the efficiency of the milling process by removing the outer layers of the kernel through an abrasive scouring (Campbell, Webb, Owens, & Scanlon, 2012). Moreover, the degree of pearling could be carefully modulated in order to separate the outermost fractions, which could be characterized by a higher content in contaminants and coarse fiber, from the intermediate fractions, which offer potentially high health benefits (Blandino et al., 2015; Sovrani et al., 2012).

The aim of this work was to characterize the chemical composition of roller-milled and pearled fractions obtained from common wheat varieties, characterized by different kernel colors (red, white, yellow, purple and blue), in order to evaluate the best fractionation-technology able to valorize the health potential of pigmented varieties in the production of functional ingredients rich in bioactive compounds.

2. Materials and methods

2.1. Grain samples

The present study has analyzed the milled and pearled fractions of five wheat varieties (*Triticum aestivum* L.). These wheat varieties included:

- PR22R58: red-grained wheat, provided by Pioneer Hi-Bred Italia S.r.l (Italy);
- Whitebear: white-grained wheat, provided by C&M Seeds (Ontario);
- Bona Vita: yellow-grained wheat, provided by Osivo a. s. (Slovakia);
- Rosso: purple-grained wheat, provided by Saatbau (Austria);
- Skorpion: blue-grained wheat, provided by the Agricultural Research Institute Kromeriz, Ltd. (the Czech Republic).

2.2. Analysis of the kernel traits

Thousand kernel weight (TKW) was determined on three 100-kernel sets of each sample, using an electronic balance. Test weight (TW) was determined by means of a Dickey-John GAC2000 grain analysis meter (Dickey-John Corp., Auburn, IL), using the supplied program, after validation with reference materials.

2.3. Wheat grain roller-milling and pearling

Grain samples were processed in order to obtain both roller-milled and pearled fractions.

The roller-milled fractions were obtained using a laboratory-scale mill (Labormill 4RB, Bona, Monza, Italy), after tempering, according to the grain variety. After milling, two fractions were analyzed: the bran and the refined flour. On average, the milling yield (relative amount of refined flour) was 48 ± 1%.

Six pearled fractions of the kernels were obtained through the incremental pearling of the wheat varieties, according to the approach described by Sovrani et al. (2012). The pearling consisted of consecutive passages of kernels or pearled kernels in an abrasive-type grain testing mill (Model TM-05C, Satake, Tokyo, Japan). Starting from unprocessed grain samples (5 kg), the kernels were initially pearled to remove 5% of the original grain weight, and this resulted in a first fraction (0–5% w/w). The remaining kernels were then pearled to remove a second fraction of 5% (5–10% w/w). The pearling process was repeated to remove a third, fourth and fifth fraction (designed fractions of 10–15%, 15–20%, 20–25% w/w). The pearling process was performed at a constant speed (55 Hz), thus the estimation of the time necessary in order to remove 5% of kernel weight at each pearling passage was experimentally quantified for each variety. The pearling process was then monitored by means of a time control, and after each pearling session, the laboratory pearler was cleaned thoroughly to minimize equipment contamination. The residual 75% of the kernel (25–100% w/w) was also collected.

The residual pearled kernels were milled by means of a laboratory centrifugal mill (Model ZM-100, Retsch, Haan, Germany) equipped with a 1-mm sieve. The same process was performed also for the unprocessed grain samples in order to obtain a whole-grain flour. Prior to the β-glucan analyses, all the samples were ground in an oscillatory mill (particle size <500 μm) (Mixer mill MM440, Retsch GmbH, Hann, Germany), and were also sieved (particle size <250 μm) to determine the total antioxidant activity. All the samples were stored at −25 °C, before the chemical analyses were performed.

2.4. Chemical analyses

2.4.1. Chemicals

Acetonitrile (CHROMASOLV®Plus, ≥99.9%), dichloromethane (CHROMASOLV®, ≥99.9%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT, ≥99.0%), ethanol (CHROMASOLV®, ≥99.8%), ethyl acetate (CHROMASOLV®, ≥99.8%), formic acid (≥95.0%), hexane (CHROMASOLV®, ≥97.0%), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%), hydrochloric acid (HCl, 37.0%), methanol (CHROMASOLV®, ≥99.9%), potassium hydroxide (KOH, 90.0%), sodium hydroxide (NaOH, ≥98.0%), *tert*-butyl methyl ether (MTBE, CHROMASOLV®, ≥99.9%) and phenolic acid standards (caffeic acid ≥98%, chlorogenic acid ≥95%, ellagic acid ≥95%, *p*-hydroxybenzoic acid ≥99%, *p*-coumaric acid ≥98%, protocatechuic acid ≥97%, sinapic acid ≥98%, syringic acid ≥95%, *trans*-ferulic acid ≥99% and vanillic acid ≥97%) were purchased from Sigma-Aldrich (St. Louis, Missouri, US). Anthocyanin standards (cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside and peonidin-3-*O*-glucoside) and carotenoid standards (lutein, zeaxanthin, β-cryptoxanthin and β-carotene) were purchased from Extrasynthese (Lyon, France).

2.4.2. Proximate composition analysis

The moisture content, determined in order to express the results on a dry weight (dw) basis, was obtained using a Sartorius MA30 thermo-balance (Sartorius AG, Goettingen, Germany). The total nitrogen content and total protein content (conversion factor: 5.70) were obtained according to the Kjeldahl method by means of a Kjeltec system I (Foss Tecator AB, Höganäs, Sweden). The ash content was determined in a muffle furnace according to the AOAC (1990) procedure. The total dietary fiber (TDF) and β-glucan contents were determined by means of the Megazyme total dietary fiber analysis kit and the Megazyme mixed-linkage β-glucan assay kit, respectively.

2.4.3. Determination of the total antioxidant activity (TAA)

TAA was determined employing DPPH radical scavenging method (direct measurement on solid samples), as previously described in (Sovrani et al., 2012). Samples were opportunely weighed (0.5–20 mg, to obtain a final inhibition percentage in the 35–65% range); then, 700 μl of water and 700 μl of a DPPH methanolic solution (100 μM) were added. The reaction was carried out in the dark under stirring at 20 °C and 1000 rpm (Thermomixer comfort, Eppendorf, Germany) for 25 min. The samples were promptly centrifuged for 1 min at 17530*g*, and the absor-