Debranning of purple wheat: recovery of anthocyanin-rich fractions and their use in pasta production

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Keywords: ~~Purple Wheat, Debranning, Pasta, Bioactive compounds~~

Pigmented wheat; Antioxidant capacity; bioactive compounds; enriched-pasta

Abbreviations: CB, bran from conventional milling; CF, refined flour from conventional milling; F1, fraction removed with the first step of debranning; F2, fraction removed with the second step of debranning; FRAP, Ferric reducing-antioxidant power; IDF, insoluble dietary fiber; M-CB, CB-enriched wheat mixture; M-F1, F1-enriched wheat mixture; M-F2, F2-enriched wheat mixture; P-C, control pasta from refined wheat flour; P-CB, CB-enriched wheat pasta; P-F1, F1-enriched wheat pasta; P-F2, F2-enriched wheat pasta; SDF, soluble dietary fiber; TDF, total dietary fiber.

Abstract

Debranning is a pre-milling treatment that partially removes the external coats and the aleurone layer of the kernel, allowing the selective recovery of bioactive compounds, such as fiber and phenolic compounds. A two-step debranning process was applied to purple wheat, a naturally antioxidant-rich variety, that removed 9.7% of the material. Debranned fractions from the first (F1; 3.7% of the whole grain) and the second (F2; 6.0% of the debranned grain after the first step) step were used separately to produce fiber-enriched pasta. Bran from conventional milling (CB) was also used as a control. F1 and F2 had a higher or comparable content in total and soluble fiber than CB. Moreover, both samples exhibited a higher ferric reducing-antioxidant power (FRAP) than CB, whereas the highest amount of anthocyanins was found in F1 (695±64 μg/g). When compared with CB-enriched pasta, samples enriched with either F1 or F2 had similar FRAP values (2.6±0.1 and 2.3±0.2 μmol Fe(II)/g for pasta with F1 and F2, respectively), and a higher amount of anthocyanins (67.9±0.9 and 60±1 μg/g for pasta with F1 and F2, respectively), while retaining a fair cooking quality.

1. Introduction

Whole wheat grain is a good source of dietary fiber and antioxidants which can promote health benefits towards several chronic diseases usually associated with oxidative stress (Yu, 2008). Although most of the cultivated cultivars are white- or red- grained, some varieties – such as purple and blue wheat grains - have drawn the attention of researchers and food industry due to their high content in anthocyanin pigments and to their antioxidant properties (Zeven, 1991; Escribano-Bailón, Santos-Buelga, & Rivas-Gonzalo, 2004; Abdel-Aal, Young, & Rabalski, 2006).

Anthocyanins are the largest group of water-soluble natural pigments that provide many fruits, vegetables, and cereal grains with red, violet, and blue color (Escribano-Bailón et al., 2004; Mazza & Miniati, 1993). These bioactive compounds not only scavenge free radicals, they also have a detoxifying effect towards heavy metals (Jan et al., 2015). Various fruits and vegetables are good sources of anthocyanins (Mazza & Miniati, 1993). However, all of these foods are less frequently consumed in comparison with cereal products. Consequently, blue and purple grains would be potential candidates for the development of bioactive food ingredients. At present, these grains are underutilized, and their contribution to the human diet is very little. For this reason, only limited data are available about their functional characteristics.

Currently, blue and/or purple corns are used for the production of naturally colored blue tortillas. As for wheat-based products, anthocyanins-rich biscuits (using whole purple wheat; Pasqualone et al., 2015), muffins (using bran from purple wheat; Li, Pickard, & Beta, 2007) and pasta (from purple durum wheat; Ficco et al., 2016) have been recently studied, focusing on the effects of processing conditions on the antioxidant properties.

In the case of pasta from durum purple wheat, the technological process led to a dramatic decrease in nutritionally-relevant antioxidant compounds (Ficco et al., 2016), suggesting that greater attention needs to be paid to optimize either the extrusion or drying conditions and to ensure their preservation. Furthermore, it can be considered the effect of the partial leaching of bioactive compounds into the cooking water.

Since the purple pigments are likely to be located in the outer layers of the pericarp (Zeven, 1991), most of the anthocyanins are removed along with fiber during milling in the case of refined semolina (Ficco et al., 2016). On the other hand, whole-grain products appear less palatable than those that are refined, due to the high bran content, which affects their technological and sensory properties (Heiniö et al., 2016). Pearling or debranning has demonstrated to be an effective strategy to recover the bioactive compounds in the external layers of barley or other grain kernels (Beta, Nam, Dexter, & Sapirstein, 2005). This pre-milling process applies abrading forces to separate the outer region from the inner part of the kernel, and results in a gentler and effective fractionation of the wheat kernel layers with respect to the conventional roller milling (Bottega et al., 2009; Delcour, Rouau, Courtin, Poutanen, & Ranieri, 2012).

In view of developing functional, grain-based ingredients, such as anthocyanin-rich flours, and foods (i.e. pasta), the present work aims at: (i) thoroughly evaluating the effect of debranning pre-treatment on dietary fiber and anthocyanins content, and on the antioxidant capacity of the various milling fractions of purple wheat in comparison with the conventional roll-milling process; (ii) producing pasta with high fibre and anthocyanins content along with retaining a good cooking quality

2. Materials and Methods

2.1 Wheat sample and wheat fractions

Commercial purple common wheat was provided by Molino Quaglia S.p.A. (Vighizzolo D'Este, Italy) and processed as shown in Fig. 1. A part of it was milled in a lab-scale mill (Labormill, BONA, Monza, Italy) to produce refined flour, middlings, and bran. Another part of purple wheat underwent two debranning steps prior to conventional milling. Kernels were first hydrated (adding 2 g of water to 100 g of wheat) to make the seed coats less brittle and prevent kernel breakage (Bottega et al., 2009). Debranning was carried out by using two sequential steps in a laboratory debranning machine equipped with an abrasive stone element (NAMAD Impianti,

Roma, Italy). The first step allowed the collection of the fraction F1, corresponding to a debranning level of 3.7% of whole grain; the second passage removed the fraction F2, corresponding to a debranning level of 6%. Both fractions were stored at 4 °C until analysis. Debranned grains were then milled in a lab-scale mill (Labormill, BONA, Monza, Italy) to obtain refined flour, middlings, and bran.

Particle size distribution of bran and debranning fractions was determined by mechanical sieving 50g of sample on Sieve Shaker (EFL 300, Endecotts Ltd, London, UK), equipped with six sieves with sieve aperture sizes of 2 mm, 1 mm, 500 μm, 250 μm, 125 μm, and 40 μm.

2.2 Microstructure approaches

Microscopy images of wheat kernels were obtained by using a stereo microscope Zeiss Axio Zoom V16 (Carl Zeiss AG; Oberkochen, Germany). Debranning fractions F1 and F2 and milling by-products CB and DB were observed by using a light Olympus BX50 microscope (Olympus, Tokyo, Japan) after staining with 1 g/L Toluidine blue in water, which is a generic dye for plant tissues. Samples were layered on the glass slide, covered with a coverslip and a small drop of staining was left to permeate in between. For each sample, at least ten observations (magnification: 4x) were made in order to obtain a semi-quantitative analysis of particle size.

2.3 Chemical analysis

Ash (AOAC 942.05), protein (AOAC 960.52), total starch (AOAC 996.11), and total (TDF), and insoluble (IDF) dietary fiber (AOAC 991.43) were determined according to official methods (AOAC, 2005). Soluble (SDF) dietary fiber was determined as the difference between TDF and IDF.

2.4 Anthocyanins Content

About 2 g of milling fractions, debranning fractions, or cooked pasta - after freeze drying - were defatted by overnight soaking in 30 mL petroleum ether, which contributed to improving the efficiency of anthocyanins extraction. Fractions were extracted with 15 mL of solvent solution for 16 h at room temperature with continuous stirring. The solvent solution was prepared with ethanol (65 mL), water (35 mL) and HCl (0.1 mL). The mixture was centrifuged at 10000 *x g* for 10 min, the supernatant recovered and the solid residue was twice re-extracted using 15 mL of the same solvent. A further extraction step did not increase anthocyanin recovery. Hence, the three supernatants of the first, second and third extraction steps were eventually pooled together. All extractions were performed in duplicate.

Total monomeric anthocyanins were measured according to the pH differential method (Lee, Durst, & Wrolstad, 2005). Samples were diluted 10 times to a final volume of 2 mL, respectively with 0.03 mol/L potassium chloride buffer, pH 1.0; or with 0.4 mol/L sodium acetate buffer, pH 4.5, respectively. The absorbance of each sample was measured at 520 nm against distilled water as a blank. Correction at 700 nm was carried out to eventually correct haze. The concentration of each anthocyanin was calculated and expressed as micrograms of cyanidin 3-O-glucoside equivalents per gram of dry product.

2.5 Ferric reducing-antioxidant power (FRAP)

The FRAP assay was performed on milling and debranning fractions and on pasta extracts (see previous paragraph), according to a previously-described procedure (Benzie & Strain, 1996). Briefly, FRAP reagent was prepared by adding: 25 mL of 0.3 mol/L acetate buffer, pH 3.6; 2.5 mL of 0.01 mol/L 2,4,6-tripyridyl-s-triazine in 0.04 mol/L HCl; and 2.5 mL of 0.02 mol/L $FeCl_3$. The reaction mixture contained 0.4 mL of each extract prepared as described above, diluted with the same solvent solution used for anthocyanins content determination, and 3 mL of FRAP reagent. The increase in absorbance at 593 nm was evaluated after 4 min of incubation at 37 °C against a blank, where extract was not added. For each extract, 2 to 4 different dilutions were assessed in duplicate.

141 A solution of $FeSO_4 \cdot 7H_2O$ was used for calibration. Results were expressed as micromoles of Fe(II)
142 sulfate equivalents per gram of dry product.
143
144 **2.6 Dough rheology**
145 Taking into account the antioxidant capacity and the anthocyanins content, the CB, F1, and
146 F2 fractions were respectively used for producing wheat mixtures enriched in bioactive
147 components. These fractions were added to commercial flour from common wheat (sample C;
148 Molino Quaglia, Vighizzolo d'Este, Italy; protein: 13.5 g/100 g; alveographic W: 380 * 10^{-4} J;
149 alveographic P/L: 0.65) in such amounts to reach the same total fiber content (8.5 g/100 g) in each
150 mixture, a quantity generally higher than that found in commercial wholegrain pasta (6-6.5 g/100
151 g). Consequently, CB, F1 and F2 were added at 20.4, 14.2, and 21.1 g/100 g, respectively. The
152 related mixtures were labeled as M-CB, M-F1, and M-F2.
153 Gluten aggregation properties of mixtures were measured by using the GlutoPeak
154 (Brabender GmbH and Co KG, Duisburg, Germany), according to Marti et al. (2014). An aliquot of
155 sample (9 g) was dispersed in 10 mL of distilled water, while keeping water temperature at 35 °C.
156 The paddle was set to rotate at 3000 rpm and each test was run for 5 min. Maximum Torque (BE –
157 Brabender Equivalent) and Peak Maximum Time (s) – which is the time required to achieve
158 maximum torque development - were considered. Measurements were performed at least in
159 triplicate.
160
161 **2.7 Pasta-making**
162 Distilled water was added to the flour mixtures containing 20.4 g/100 g CB, 14.2 g/100 g
163 F1, and 21.1 g/100 g F2 in order to obtain dough with 32 g/100 g final moisture. Samples were
164 hand-mixed at room temperature for 10 min. After mixing, the dough was covered with plastic
165 stretch-film and kept at 4 °C for 30 min. A home dough-sheeter (SP50, Imperia 1932, Moncalieri,
166 Italy) was used to prepare pasta. The thickness of the sheet was gradually reduced by five

consecutive steps through decreasing roll gaps (respectively of 6 mm, 4 mm, 2 mm -twice, folding the sheet - and 1 mm). Pasta dough was shaped into "tagliatelle" (length: 300 mm; width: 13.5 mm; thickness: 1.0 mm) and dried in a cell (M710 Thermostatic oven, Fratelli Galli, Milano, Italy) at 40 °C for 18 hours and stored at 4 °C until analysis.

Pasta containing CB, F1, and F2 fractions was labeled as P-CB, P-F1, and P-F2, respectively. Pasta prepared from refined wheat flour (P-C) was produced in the same conditions described above and used as a control.

2.8 Pasta Quality

Pasta was cooked in distilled water (pasta:water ratio = 1:25) at the Optimum Cooking Time, according to the AACC method 16-50 (1995). Cooking losses (g of solid loss/100 g of dry pasta) were evaluated according to the AACC standard method (16-50, 1995). After cooking, pasta was drained for 1 min, and the weight increase was evaluated gravimetrically. Then, the cooking water was collected, brought back to the initial volume, and an aliquot (50 mL) was dried to constant weight at 105 °C. Cooking loss and water absorption values were replicated five times and the average values were used. Cooked pasta was freeze-dried, ground to a particle size lower than 500 μm with a lab-scale mill (IKA Universalmuhle M20, Janke and Kunkel GmbH & Co KG, IKA Laborteknic, Staufen, Germany) and stored at 4 °C until analysis.

2.9 Statistical Analysis

Analysis of variance (one-way ANOVA) was performed by using Statgraphic Plus v. 5.1 (StatPoint Inc., Warrenton, VA, USA). Different milling fraction or pasta samples were considered as factors for ANOVA. When a factor effect was found to be significant ($p \leq 0.05$) significant differences among the respective means were determined using Fischer's Least Significant Difference (LSD) test.

193 **3. Results and Discussion**

194 **3.1 Milling and debranning material**

195 **3.1.1 Microstructure features**

196 The debranning effects on purple wheat kernels are shown in Fig. 2. Both the first and
197 second abrasion steps promoted a heterogeneous removal of the external layers. Indeed, after the
198 first debranning, few grains appeared intact (Fig. 2B), as in the case of wheat before debranning
199 (Fig. 2A), whereas the purple bran layers were mostly removed from some grains, thus leaving
200 exposed the tissues below the bran layers exposed. Even after two debranning steps (Fig. 2C),
201 which removed about 10% of material, the mechanical abrasion of the kernel surface was non-
202 homogeneous. Nevertheless, the ventral-side of caryopsis seems to be more prone to abrasion,
203 probably due to its flat surface.

204 Microscopic features of bran from intact grain milling (CB), bran from debranned grain
205 milling (DB), F1 and F2 fractions are shown in Fig. 3. In CB, many particles were larger than 1000
206 μm (Fig. 3A). Milling of debranned grains resulted in a dramatic decrease in bran particle size,
207 down to about 600 μm in average (Fig. 3B).

208 Debranning operations led to the recovery of fractions with particles smaller than those for
209 CB (Fig. 3C, 3D). The amount of debranning fractions is usually expressed as debranning level and
210 considered as a marker of the debranning intensity. The higher the debranning level, the lower the
211 particle size of the removed material and the higher the starch amount, as shown in Table 1. In
212 particular, the average size of F1 was about 500-700 μm, while F2 particle size was in the 300-400
213 μm range. Furthermore, in F2 sample, fragments of aleurone layer and many starch granules were
214 recognizable (Fig. 3D).

215

216 **3.1.2 Compositional Traits**

217 The compositional features of purple wheat, debranning fractions and milling products are
218 shown in Table 1. Lab-scale milling provided three fractions (flour, bran and middlings, see Fig.1)

219 characterized by a recovery yield, and by contents in starch, protein, ash, and fiber comparable to
220 those reported for common wheat (Lai & Lin, 2006). As expected, F1 and F2 showed different
221 compositions: F1 (debranning level = 3.7%) contained a lower amount of starch and protein than F2
222 (debranning level = 6.0%). On the other hand, the ash and fiber content progressively decreased as
223 debranning level increased from the first to the second debranning step, as evident in previous
224 findings (Bottega et al., 2009). In particular, the total starch content relates to the progressive
225 removal of the kernel layers that included the starchy endosperm (Bottega et al., 2009).
226 Interestingly, F1 contained only 12±2 g/100 g of starch; the amount of this component doubled in
227 F2, up to the level measured in the bran produced in the conventional milling process. The higher
228 protein amount in F2 compared to F1 (16.06±0.06 *vs* 12.60±0.04 g/100 g) confirmed the presence
229 of some aleuronic cells, as highlighted by microscopic observations (Fig. 3D). However, some
230 aleurone fragments were still present in F1 (Fig. 3C), although F1 (debranning level = 3.6%) is
231 most likely formed by the outer pericarp. Indeed, Shetlar, Rankin, Lyman, & France (1947) stated
232 that the outer pericarp and the aleurone layer represented 3.9 and 9.0% of the kernel weight,
233 respectively. However, a certain variability in wheat grain structure should be considered
234 (Pomeranz, 1988; Kent, 1983).

235 As for the total dietary fiber content, whole purple wheat exhibited comparable values to
236 those reported for other varieties (TDF: 11.6-17.0 g/100 g; Vitaglione, Napolitano, & Fogliano
237 (2008)). As expected, bran showed a high TDF content (43.5±0.4 g/100 g), composed of almost
238 insoluble macromolecules (96 g/100 g of TDF). Both F1 and F2 showed a remarkable amount of
239 TDF, confirming the data reported by Blandino et al. (2013) and Sovrani et al. (2012). The latter
240 found a TDF content of 58.0-61.5 g/100 g in the outermost layer (corresponding to 5% of
241 debranning level) of various common wheat varieties, whereas the amount of TDF in the second
242 pearling fraction (up to 10% of debranning level) ranged from 36.4 to 40.9 g/100 g. In our study, F1
243 exhibited a TDF content more than 40% higher than bran, but a similar IDF/SDF ratio. On the
244 contrary, sample F2 showed a comparable TDF content, but a slight higher SDF as compared with

bran, likely related to the high SDF content in the aleurone layer (Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007). Differences in both the composition and the particle size (Table S1, Fig. 3) of the various bran fractions may strongly affect their hydration properties and technological behavior (Marti et al., 2014).

As regards the composition of flour and middling before and after kernel debranning, the former did not exhibit any relevant modification, whereas the middle fraction obtained from debranned grain showed an unexpected enrichment in the soluble fiber, likely due to part of the aleurone cells that could have flown into this milling fraction, contributing to a higher amount of protein and total fiber amount.

3.1.3 Anthocyanins content and FRAP

The anthocyanins content and FRAP of purple wheat and its milling and debranning fractions are reported in Table 2. In the whole purple kernel the anthocyanins content was 52.2±0.4 μg/g, values in accordance with the data reported by Abdel-Aal et al. (2006) for purple wheat (38-96 μg/g). Conventional milling promoted the recovery of anthocyanins pigments in the bran fraction (Table 2), allowing to obtain 3-8 times more pigment than that collected in other pigmented cereals (*e.g.* black rice, red rice, blue wheat, black, brown, and red sorghum bran fractions) (Abdel-Aal et al., 2006). A single step of debranning, associated with the removal of 3.7% of the grains, was a useful strategy to concentrate anthocyanins, as in F1 sample the anthocyanin content increased significantly ($p \leq 0.05$) to 695±64 μg/g. The anthocyanins collected in the F2 were 295±7 μg/g, the same amount determined in bran. Consequently, F1 contained more than 50% of the total anthocyanins content of whole wheat grains. Studies on purple barley showed that the bran-rich fraction, corresponding to a pearling level of 10%, contained up to 75% of the anthocyanins in the kernel (Bellido & Beta, 2009).

F1 and F2 samples also exhibited the highest FRAP values, about 25 μmol Fe(II) eq/g (Table 2). In CB and DB samples, the antioxidant capacity was the halved. These results suggest

271 that the debranning of purple wheat is a more efficacious approach than conventional milling to
272 separate and collect anthocyanin-richer fractions with higher in antioxidant activity, thus
273 maximizing the potential health benefits of wheat-based products. The lack of differences in FRAP
274 values between F1 and F2 (Table 2) might be due to compensation between the removal of the outer
275 layers - where anthocyanins are accumulated - and a simultaneous passage of part of aleurone and
276 endosperm, regions richer that the pericarp in phenolic acids (Martini, D'Egidio, Nicoletti,
277 Corradini, & Taddei, 2015) and other compounds with antioxidant activity.

278 Besides antioxidant content, physical parameters of the wheat bran, such as the particle sizes
279 affecting the total surface area, could influence antioxidant solubility and, in turn, antioxidant
280 capacity in solid-liquid system. However, no remarkable effect of particle sizes on antioxidant
281 capacity is observed, when the measuring conditions resulted in plateau values to be reached
282 (Serpen, Gokmen, Pellegrini & Fogliano, 2008). For this purpose, in the present study, a long 3-step
283 antioxidant capacity was performed.

284 Interestingly, the antioxidant capacity of wheat bran increased when the medium particle
285 size decreased, due to the breakdown of the aleurone cell-wall (Zhou, Laux & Yu, 2004; Rosa,
286 Barron, Gaiani, Dufour & Micard, 2013). As shown in Fig. 3D, F2 contained disrupted aleurone
287 cells, which can account for its high antioxidant activity.

288

289 **3.2 Dough Rheology**

290 Based on the anthocyanins content in bran fractions of purple wheat, we focused on bran, F1
291 and F2 fractions for preparing bioactive compounds-enriched products. Each fraction was added to
292 commercial flour at different levels (20.4, 14.2, and 21.2 g/100 g, respectively) in order to have a
293 fiber content of 8.5 g/100 g in the final product. This percentage is higher than that one usually
294 present in commercial whole pasta from durum wheat semolina (Casiraghi et al., 2013), thus
295 allowing us to obtain a pasta with greater nutritional value.

296 The GlutoPeak Test is a new approach for testing gluten quality in common (Marti, Augst,

297 Cox, & Koehler, 2015) and durum (Marti, Seetharaman, & Pagani, 2013) wheat. The GlutoPeak
298 indices of bran-enriched flours are shown in Table 3. Dough enrichment in fiber generally increased
299 the maximum torque, likely due to the high fiber content and its water absorption capacity.
300 Peak maximum time relates to the time required for gluten to aggregate and to exhibit the
301 maximum spindle torque. The addition of any type of bran significantly ($p \leq 0.05$) decreased peak
302 maximum time, thus weakening the gluten network, as shown in previous studies (Marti et al.,
303 2014). However, M-F1 exhibited a significantly longer aggregation time, most likely due to the
304 lower replacement level, than either M-CB or M-F2. Indeed, no differences in the gluten
305 aggregation profile were found in flour enriched with either bran or aleurone fraction (Adams,
306 2015).
307
308 **3.3 Enriched pasta**
309 **3.3.1 Cooking quality**
310 The quality characteristics of pasta are summarized in Table 4. Despite the addition of fiber,
311 which has been demonstrated to decrease the cooking time (Aravind, Sissons, Egan, & Fellows,
312 2012), no differences in optimal cooking time were observed between the samples (4 min),
313 probably because of the low thickness (1 mm) and the high surface of the tagliatella-shape which
314 ensured a fast water diffusion and absorption.
315 During cooking, P-CB and P-F1 absorbed a similar amount of water which was significant
316 lower than that for P-CF and P-F2 (Table 4). The enrichment in IDF generally decreased the water
317 absorption because of the competition for water between bran and starch, resulting in a low starch
318 swelling capacity (Aravind et al., 2012).
319 Measurement of cooking loss is an important parameter in assessing overall pasta quality.
320 During pasta cooking soluble components, including starch fractions, proteins and non-starch
321 polysaccharides, leached into the cooking water, which becames cloudy and thick. For good-quality
322 pasta, the cooking loss should be lower than 4-5 g/100 g (Marti et al., 2013).

323 Regardless of the number of debranning steps, both debranned fraction-enriched samples P-
324 F1 and P-F2 showed higher cooking losses than the control pasta (Table 4). This may be due to
325 changes in the elasticity of the gluten network because of the interference of the dietary fiber
326 content (Table 1). Indeed, several studies have shown that the addition of non-gluten flours in the
327 production of pasta decreases the gluten strength, and weakens the overall structure of pasta
328 (Tudorica, Kuri, & Brennan, 2002). Our results agree with those reported in literature regarding
329 fiber-enriched pasta (Marti, Fongaro, Rossi, Lucisano, & Pagani, 2011) and might be explained by
330 the use of a mild forming process (i.e. roll-sheeting) which does not impart extreme pressure and
331 stress to dough. On the contrary, conventional extrusion usually leads to damage or breakage of the
332 gluten structure in products characterized by a less tenacious protein network (i.e. common wheat
333 and wholegrain flours) (Pagani, Resmini, & Dalbon, 1999; Marti et al., 2011).
334
335 **3.3.2 Anthocyanins and FRAP**
336 In the case of pasta samples, anthocyanins content and FRAP values were considered only
337 for the cooked products, which are more important for the consumers' standpoint. Enriched wheat
338 pasta showed a relatively high antioxidant capacity, with P-F1 exhibiting no significant differences
339 in FRAP values from P-CB, despite the amount added was lower for F1 than for CB (Table 4).
340 Comparison between the antioxidant activity values of the samples and those referred to in the
341 literature is difficult, due to the various approaches used both for product preparation (sheeting *vs*
342 extrusion) and for the evaluation of the antioxidant capacity. The FRAP assay was chosen among
343 the electron-transfer based reactions (e.g. Folin-Ciocalteu assay, Trolox equivalence antioxidant
344 capacity, TEAC) because it is carried out at low pH, that allows the highest anthocyanin stability
345 (Mazza & Miniati, 1993).
346 The anthocyanin content values ranged from 28±2 to 67.9±0.9 μg/g for P-CB and P-F1, with
347 no significant differences between P-F1 and P-F2. The highest anthocyanins found in cooked P-F1
348 is in accordance with the FRAP values (Table 4). In addition, P-F1 and P-F2 enriched samples

showed a higher anthocyanins content (67.9±0.9 and 60±1 μg/g, respectively) than values found by Ficco et al. (2016) in pasta prepared from purple wholemeal semolina and ranging from 16.89 and 37.27 μg/g for cooked dried pasta and fresh pasta, respectively. During cooking, only P-CB and P-F1 showed a loss of anthocyanin content, whereas P-F2 was able to retain the anthocyanins during cooking. The effect of particle size of bran and debranning fractions (Table S1) on cooking behavior can not be neglected. Indeed, superior cooking quality has been observed in pasta from wheat with finer particle size (Hatcher, Anderson, Desjardins, Edwards, & Dexter, 2002).

4. Conclusions

Debranning can be considered a more efficacious processing than conventional milling to mechanically concentrate the bioactive compounds – mainly fiber and anthocyanins- present in the pericarp layers of purple wheat. The selective recovery of these fractions and their use in pasta could represent an interesting approach to encourage the diffusion and the use of wheat pericarp layers in making foods with functional properties. However, the presence of natural and synthetic contaminants in the most external layers pose a risk for consumer safety and need to be taken into serious consideration.

Acknowledgments

The authors would like to thank Prof. Stefania Iametti (University of Milan) for the helpful discussions and for the critical reading of the manuscript and Prof. Franco Faoro (University of Milan) for discussing the microscopic images.

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