AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Bioactive compound and antioxidant activity distribution in rollermilled and pearled fractions of conventional and pigmented wheat varieties

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1634531> since 2022-01-07T14:39:03Z

Published version:

DOI:10.1016/j.foodchem.2017.04.065.

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in FOOD CHEMISTRY, 233, 2017, 10.1016/j.foodchem.2017.04.065..

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>), 10.1016/j.foodchem.2017.04.065.

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/1634531>

TITLE

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

RUNNING TITLE

Bioactives in milled and pearled fractions of conventional and pigmented wheats

AUTHORS

Debora Giordano¹, Monica Locatelli², Fabiano Travaglia², Matteo Bordiga², Amedeo Reyneri¹, Jean Daniel Coïsson², Massimo Blandino^{1*}

AFFILIATIONS

¹Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università di Torino, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy.

²Dipartimento di Scienze del Farmaco and Drug and Food Biotechnology Center, Università del Piemonte Orientale “A. Avogadro”, Largo Donegani 2, 28100 Novara (NO), Italy.

*Corresponding author: Massimo Blandino

Phone +39 011 6708895, massimo.blandino@unito.it

27 **ABSTRACT**

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

31 Roller-milling promoted the recovery of total dietary fiber, β -glucans, phenolic acids and
32 anthocyanins in the bran fraction which resulted also in a higher total antioxidant activity
33 than the refined flour. Conversely, lutein resulted mainly concentrated in the refined flour.

34 In the same way, the distribution pattern in the pearled fractions differ depending on the
35 bioactive considered. The study highlights that a careful selection of the most appropriate
36 fractionation process should be performed to produce flours rich in bioactive compounds.

37 Roller-milling resulted useful for the production of refined flours rich in xanthophylls, with
38 particular emphasis to the yellow-grained wheats. Contrarily, pearling could be more useful
39 in the valorization of the health potential of anthocyanin-pigmented varieties.

40

41 **KEYWORDS**

42 Pigmented wheats, Grain-fractionation technologies, Total dietary fiber, β -glucans,
43 Antioxidant capacity, Phenolic acids, Carotenoids, Anthocyanins

44

45

46

47

48

49

50

51

52

53 1. INTRODUCTION

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

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

75 Nevertheless, the use of wheat varieties that are naturally rich in bioactive compounds
76 should be combined with a suitable grain processing technology, in order to preserve the
77 bioactives and to produce functional ingredients. Previous studies, performed on
78 conventional common wheat varieties, have shown that the bioactive compounds are

79 mainly concentrated in the outer layers of the grain, and that their distribution within the
80 kernel differs according to the class of nutrients (Sovrani et al., 2012). Consequently, the
81 conventional roller-milling process, which promote the removal of the outer layers of the
82 kernel in the bran fraction, causes a great decrease in the nutritional value of the refined
83 flour (Siebenhandl et al., 2007). Another fractionation technology that was proposed as an
84 alternative strategy to valorize the outer layers of the kernel was the pearling process
85 (Hemery, Rouau, Lullien-Pellerin, Barron & Abecassis, 2007). Wheat millers are
86 increasingly using the pearling process before roller milling, since it improves the efficiency
87 of the milling process by removing the outer layers of the kernel through an abrasive
88 scouring (Campbell, Webb, Owens & Scanlon, 2012). Moreover, the degree of pearling
89 could be carefully modulated in order to separate the outermost fractions, which could be
90 characterized by a higher content in contaminants and coarse fiber, from the intermediate
91 fractions, which offer potentially high health benefits (Sovrani et al., 2012; Blandino et al.,
92 2015).

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

98 2. MATERIALS AND METHODS

99

100 **2.1 Grain samples**

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

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

109

110 **2.2 Analysis of the kernel traits**

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

115

116 **2.3 Wheat grain roller-milling and pearling**

117 Grain samples were processed in order to obtain both roller-milled and pearled fractions.
118 The roller-milled fractions were obtained using a laboratory-scale mill (Labormill 4RB,
119 Bona, Monza, Italy), after tempering, according to the grain variety. After milling, two
120 fractions were analyzed: the bran and the refined flour. On average, the milling yield
121 (relative amount of refined flour) was $48 \pm 1\%$.
122 Six pearled fractions of the kernels were obtained through the incremental pearling of the
123 wheat varieties, according to the approach described by Sovrani et al. (2012). The pearling

124 consisted of consecutive passages of kernels or pearled kernels in an abrasive-type grain
125 testing mill (Model TM-05C, Satake, Tokyo, Japan). Starting from unprocessed grain
126 samples (5 kg), the kernels were initially pearled to remove 5% of the original grain weight,
127 and this resulted in a first fraction (0-5% w/w). The remaining kernels were then pearled to
128 remove a second fraction of 5% (5-10% w/w). The pearling process was repeated to
129 remove a third, fourth and fifth fraction (designed fractions of 10-15%, 15-20%, 20-25%
130 w/w). The pearling process was performed at a constant speed (55 Hz), thus the
131 estimation of the time necessary in order to remove 5% of kernel weight at each pearling
132 passage was experimentally quantified for each variety. The pearling process was then
133 monitored by means of a time control, and after each pearling session, the laboratory
134 pearler was cleaned thoroughly to minimize equipment contamination. The residual 75% of
135 the kernel (25-100% w/w) was also collected.

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

143

144 **2.4 Chemical analyses**

145 **2.4.1 Chemicals**

146 Acetonitrile (CHROMASOLV®Plus, $\geq 99.9\%$), dichloromethane (CHROMASOLV®,
147 $\geq 99.9\%$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT,
148 $\geq 99.0\%$), ethanol (CHROMASOLV®, $\geq 99.8\%$), ethyl acetate (CHROMASOLV®, $\geq 99.8\%$),
149 formic acid ($\geq 95.0\%$), hexane (CHROMASOLV®, $\geq 97.0\%$), ($\pm$)-6-hydroxy-2,5,7,8-

150 tetramethylchromane-2-carboxylic acid (Trolox, 97%), hydrochloric acid (HCl, 37.0%),
151 methanol (CHROMASOLV®, ≥99.9%), potassium hydroxide (KOH, 90.0%), sodium
152 hydroxide (NaOH, ≥98.0%), *tert*-butyl methyl ether (MTBE, CHROMASOLV®, ≥99.9%)
153 and phenolic acid standards (caffeic acid ≥98%, chlorogenic acid ≥95%, ellagic acid ≥95%,
154 *p*-hydroxybenzoic acid ≥99%, *p*-coumaric acid ≥98%, protocatechuic acid ≥97%, sinapic
155 acid ≥98%, syringic acid ≥95%, *trans*-ferulic acid ≥99% and vanillic acid ≥97%) were
156 purchased from Sigma-Aldrich (St. Louis, Missouri, US). Anthocyanin standards (cyanidin-
157 3-O-glucoside, delphinidin-3-O-glucoside and peonidin-3-O-glucoside) and carotenoid
158 standards (lutein, zeaxanthin, β -cryptoxanthin and β -carotene) were purchased from
159 Extrasynthese (Lyon, France).

160

161 **2.4.2 Proximate composition analysis**

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

170

171 **2.4.3 Determination of the Total Antioxidant Activity (TAA)**

172 TAA was determined employing DPPH radical scavenging method (direct measurement
173 on solid samples), as previously described in (Sovrani et al., 2012). Samples were
174 opportunely weighed (0.5-20 mg, to obtain a final inhibition percentage in the 35-65%

range); then, 700 μ l of water and 700 μ l of a DPPH methanolic solution (100 μ M) 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 minute at 17530 g, and the absorbance was measured at 515 nm after exactly 30 minutes. A control solution, without the ground sample, was tested under the same conditions, in order to calculate the DPPH inhibition percentage of the samples. The final results were expressed as mmol of Trolox equivalents (TE)/kg of sample (dw) through a calibration curve (linearity range: 2.8 - 33.5 nmol; r^2 : 0.982).

183

2.4.4 Extraction of the free and bound phenolic acids and quantification by means of RP-HPLC/DAD

Fifty milligrams of each sample were suspended in 1 mL of a methanol:water 80:20 (v/v) mixture, vortexed for 10 sec, and then extracted in an ultrasonic bath (Bransonic 1510, output 42 kHz, Branson Ultrasonics, Danbury, CT) for 2 min. The extracts were centrifuged at 17530 g for 2 min, and pellets were extracted another two times, according to the method described above. The supernatants were collected and used for the chromatographic analyses of the free phenolic acids.

The pellet remaining after the extraction of the free phenolic acids was hydrolyzed for 3.5 hours under a nitrogen stream, and in continuous agitation, by adding 10 mL NaOH 1 M. After acidification to pH 2.3 with HCl, the bound phenolic acids were extracted with 20 mL of ethyl acetate. The extraction was repeated three times. The combined supernatants were evaporated to dryness, and then reconstituted in 2 mL of methanol.

The extracts were centrifuged at 17530 g for 2 min, and analyzed using an HPLC system (Shimadzu LC-20A Prominence, Shimadzu Italia, Milan, Italy), coupled to a SPD-M20A diode array detector. Separations were carried out using a 150 x 2 mm, 5 μ m, Luna C18(2) column (Phenomenex, Torrance, CA), protected by a guard column containing the same

201 phase; the column temperature was set at 30°C. The mobile phase consisted of 0.1% v/v
202 formic acid in water (solvent A) and 0.1% v/v formic acid in methanol (solvent B), and the
203 following operating gradient was used: 0-30 min, 5-17.5% B; 30-40 min, 17.5-30% B; 40-
204 45 min, 30-100% B. Finally, the mobile phase was brought to 5% B in 10 minutes, and this
205 was followed by 15 minutes of equilibration. The mobile phase flow rate was 0.4 mL/min,
206 and the injection volume was 20 µL for the free phenolic acid extracts and 1 µL for the
207 bound phenolic acid extracts. The hydroxycinnamic acids (ferulic acid, caffeic acid, *p*-
208 coumaric acid, sinapic acid, chlorogenic acid) and hydroxybenzoic acids (vanillic acid, *p*-
209 hydroxybenzoic acid, protocatechuic acid, ellagic acid, syringic acid) were identified using
210 the retention times and UV/Vis spectra of their respective standards. Phenolic acid
211 standards were prepared and diluted to different concentrations, in order to obtain
212 calibration curves for quantification purposes. The hydroxycinnamic acids and
213 hydroxybenzoic acids were quantified at 330 nm and 280 nm, using ferulic acid and
214 protocatechuic acid as reference compounds, respectively (injection volume: 1 µL; linearity
215 range: 0.05-5 µg/mL for hydroxycinnamic acids [5-300 µg/mL in the case of bound ferulic
216 acid]; 0.5-10 µg/mL for hydroxybenzoic acids).

217

218 **2.4.5 Extraction of the anthocyanins and quantification by means of RP-HPLC/DAD**

219 Each sample (1 g) was extracted using 8 mL of ethanol acidified with HCl 1 N (85:15, v/v)
220 for 30 min. The absorbance was measured after centrifugation at 17530 g for 2 min at 540
221 nm, as reported by Siebenhandl et al. (2007). The total Anthocyanin Content (TAC) was
222 expressed as mg cyanidin-3-O-glucoside (Cy-3-glc) equivalents/kg of sample (dw).

223 The identification and quantification of the individual anthocyanins was performed using
224 the previously described chromatographic system. Separations were carried out using a
225 250 x 4.6 mm, 4 µm, RP SynergiTM 4 µm Max-RP 80 Å LC Column (Phenomenex,
226 Torrance, CA), protected by a guard column containing the same phase; the column

temperature was set at 30 °C. The mobile phase consisted of water:formic acid:acetonitrile [87:10:3, v/v; (solvent A)] and water:formic acid:acetonitrile [40:10:50, v/v; (solvent B)], and the following operating gradient was used: 0-20 min, 6-20% B; 20-35 min, 20-40% B; 35-40 min, 40-60% B; 40-45 min, 60-90% B; 45-50 min isocratic 90% B. Finally, the mobile phase was brought to 6% B in 0.5 minutes, and this was followed by 22.5 minutes of equilibration. The mobile phase flow rate was 0.5 mL/min, and the injection volume was 15 µL. Cyanidin-3-O-glucoside, delphinidin-3-O-glucoside and peonidin-3-O-glucoside were identified using the retention times and UV/Vis spectra of the individual authentic standard molecules; delphinidin-3-O-rutinoside, cyanidin-3-O-rutinoside and peonidin-3-O-rutinoside were identified on the basis of literature data (Abdel-Aal, Young, & Rabalski, 2006; Abdel-Aal, Abou-Arab, Gamel, Hucl, Young, & Rabalski, 2008; Knievel, Abdel-Aal, Rabalski, Nakamura & Hucl, 2009; Liu, Qiu & Beta, 2010). Quantification of the individual compounds was performed at 520 nm, using glucoside forms of anthocyanins (rutinosides were quantified as equivalents of the corresponding glucosides); calibration curves were obtained at six different concentration levels (injection volume: 5 µL; linearity range: 0.005-10 µg/mL).

243

244 **2.4.6 Extraction of the carotenoids and quantification by means of RP-HPLC/DAD**

Each sample (0.3 g) was extracted for 6 min at 85°C with 95% ethanol, containing 1 g/L BHT. The extracts, including solids, were hydrolyzed with 125 µL of KOH (1g/mL) at 85°C for 10 min, chilled on ice and 3 mL of cold deionized water was then added. This was followed by the addition of 3 mL of hexane, containing 1 g/L BHT. The test tubes were then vortexed and centrifuged at 1200 g for 10 minutes. The extraction was repeated four times, and the combined supernatants were evaporated to dryness under a nitrogen stream, and then reconstituted in 75 µL of dichloromethane.

252 The extracts were analyzed by means of a high performance liquid chromatograph Agilent
253 1200 Series (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 1200
254 Series diode array detector. Separations were carried out using a 250 x 4.6 mm, 3 μ m,
255 C30 carotenoid YMC column (YMC Co., Kyoto, Japan); the column temperature was set at
256 25°C. The mobile phase consisted of methanol:MTBE:water [81:15:4, v/v; (solvent A)] and
257 MTBE:methanol [91:9, v/v; (solvent B)]. The following operating linear gradient was used:
258 0-45 min, 0-50% B; 45-60 min, 50-100% B. Finally, the mobile phase was brought to 0% B
259 in 10 minutes, and this was followed by 15 minutes of equilibration. The flow rate of the
260 mobile phase was 1.0 mL/min, and the injection volume was 5 μ L. Carotenoids were
261 identified using the retention times and the UV/Vis spectra of their respective standards
262 (lutein, zeaxanthin, β -cryptoxanthin and β -carotene). Carotenoid standards were also
263 prepared and diluted to different concentrations to obtain calibration curves for
264 quantification purposes (linearity range: 0.1-100 μ g/mL for lutein and zeaxanthin; 0.3-40
265 μ g/mL for β -cryptoxanthin and β -carotene). The quantifications were performed at 450 nm.

266

267 **2.5 Color analyses**

268 The chromatic analyses were performed using a Minolta Chroma Meter reflectance
269 spectrophotometer (Model CR-400, Minolta Co., Osaka, Japan). A 45 mm diameter Petri
270 dish was loosely filled with a ground subsample, and the dish was then tapped gently until
271 the sample was levelled and no gaps were apparent through the base of the dish. The L^* ,
272 a^* and b^* color values were determined directly by the instrument.

273

274 **2.6 Statistical analyses**

275 All the analyses were performed at least in triplicate checking that the variation coefficient
276 was lower than 10%. One-way analysis of variance (ANOVA) was applied in order to

277 compare the contents of the bioactive compounds in wholegrain flours on the basis of the
278 wheat genotype, and in the different roller-milled and pearled fractions of each wheat type.
279 The REGW-Q test was performed for multiple comparisons. A 0.05 threshold was used to
280 reject the null hypothesis.
281 Statistical analyses were carried out by means of SPSS for Windows statistical package,
282 Version 22.0 (SPSS Inc., Chicago, Illinois).

283

284 3. RESULTS AND DISCUSSION

285 3.1 Kernel traits and the bioactive compound contents of the wholegrain flours

286 Although the most commonly cultivated wheat varieties are the red- and white-grained
287 ones, the application of pigmented wheat varieties for the production of functional foods
288 has drawn the attention of both researchers and the food industry in the last few years
289 (Ficco et al., 2016; Li et al., 2007; Pasqualone et al., 2015; Yu & Beta, 2015; Zanoletti et
290 al., 2017). In this study the chemical composition of pigmented wheat varieties (yellow,
291 purple and blue types) was analyzed and compared with conventional wheat varieties (red
292 and white types). The compared cultivars showed differences both in their physical
293 characteristics, in the chemical content and in the color of their wholegrain flours (Table 1
294 and 2).

295 The wholegrain flour of pigmented wheat varieties showed levels of total dietary fiber
296 (TDF), β -glucans, total antioxidant activity (TAA), free (FPAs) and cell wall-bound
297 (CWBPA)s phenolic acids similar to the ones of conventional wheat varieties, as well as
298 significant concentrations of anthocyanins and xanthophylls, which were absent or limited
299 in the red- and white-grained varieties. As far as the anthocyanin-pigmented varieties are
300 concerned, the purple type showed a higher overall total anthocyanin content (TAC) than
301 the blue one. The main carotenoid detected in all the samples was lutein, and this was
302 followed by zeaxanthin, while β -cryptoxanthin and β -carotene were not detected. The
303 concentration of lutein was on average 6.6 times higher than that of zeaxanthin, and the
304 yellow variety was the one characterized by the highest content of this xanthophyll.

305 The chromatic characteristics of the wholegrain flours of both the conventional and
306 pigmented varieties were determined in order to demonstrate that the analyzed grains
307 differed in their color. As expected, the different bioactive compound profiles resulted in
308 significant differences in the lightness (L^*), and in the red (a^*) and blue (b^*) indexes of the
309 wholegrain flour (Table 2). The wholegrain flour of the white variety was characterized by

310 the highest lightness value and the lowest redness one. Differently, the purple and the blue
311 varieties showed the lowest lightness value, and were characterized by the highest
312 redness and blueness values, respectively. The wholegrain flour of the yellow variety was
313 characterized by the highest yellowness, in accordance with its high lutein content.

314

315 **3.2 Bioactive compound distribution in the roller-milled fractions**

316 The analysis of the roller-milled fractions clearly shows that the bioactive compounds are
317 unevenly distributed in the kernels of both conventional and pigmented wheat varieties
318 (Table 3). In particular, roller-milling promoted the recovery of proteins, TDF, β -glucans,
319 FPAs and CWBPAs in the bran fraction. Consequently, regardless the variety considered
320 the bran fraction resulted in a TAA 10 and 3 times higher than the one of the refined flour
321 and the wholegrain flour, respectively. This result was in accordance with previous studies
322 that mainly ascribe TAA of wheat grains to phenolic compounds (Beta, Nam, Dexter &
323 Sapirstein, 2005). As far as the individual phenolic acids are concerned, only free and
324 bound ferulic acid and bound ellagic acid were identified in the refined flour, while a more
325 complex profile was observed in the bran fraction (the main phenolic acids quantified are
326 reported in Table S1). Considering the two anthocyanin-pigmented varieties, their bran
327 fraction was characterized on average by a total anthocyanin content 23 and 2 times
328 higher than the one of the refined flour and the wholegrain flour, respectively. Not many
329 data are currently available on the distribution of carotenoids in wheat kernels. Previously,
330 it has been observed that lutein was concentrated more in the endosperm than in the bran
331 of einkorn and durum wheat, while the opposite trend has been observed in common
332 wheat (Abdel-Aal, Young, Rabalski, Hucl & Fregeau-Reid, 2007). Conversely, Siebenhandl
333 et al. (2007) observed a higher concentration of total carotenoids in the refined flour than
334 in the bran fraction of a blue-grained wheat variety. In the present study, lutein was always
335 mainly concentrated in the refined flour than in the bran fraction, with the only exception

336 being the blue variety, which did not show any significant difference between its roller-
337 milled fractions. As far as the yellow-grained variety is concerned, the concentration of
338 lutein observed in the refined flour is slightly higher (+12%) than the one observed in the
339 wholegrain flour. Zeaxanthin, whose concentration was always lower than that of lutein,
340 showed an opposite distribution pattern.

341 In accordance with the composition and distribution of bioactive compounds, regardless
342 the variety considered the bran fraction showed lower lightness and higher redness values
343 than the refined flour (Table S2).

344 The present study clearly confirms that the roller-milling process results in the
345 concentration of the most nutritional compounds in the bran fraction of both conventional
346 and pigmented wheat varieties. This result is particularly relevant for the anthocyanin-
347 pigmented wheat varieties because of the removal of most of the anthocyanins in the bran
348 fraction. Several studies proposed the use of the wheat bran of both conventional (Sobota,
349 Rzedzicki, Zarzycki & Kuzawińska, 2015) and pigmented wheat varieties (Li et al., 2007)
350 for the production of cereal-derived products enriched in bioactive compounds. At the
351 same time, roller-milling removes the parts of the grain known or suspected to be
352 detrimental to foods, in relation to safety, processing and acceptability by the consumers
353 (Zhang & Moore, 1999; Cheli et al., 2010). Thus, especially for anthocyanin-pigmented
354 varieties, instead of reject or maintain totally the bran fraction, greater attention needs to
355 be paid to optimize the processing of kernels in order to ensure bioactive preservation and
356 at the same time the removal of the fractions suspected to be detrimental for food
357 production.

358 Contrarily to all the other bioactive compounds analyzed in the present study, lutein
359 resulted mainly concentrated in the refined flour than in the bran fraction. Thus, the
360 application of the conventional roller-milling process could be considered useful for the

361 production of refined flours naturally rich in xanthophylls, with particular emphasis to the
362 yellow-grained varieties.

363
364 **3.3 Bioactive compound distribution in the pearled fractions**

365 In agreement with data observed for the roller-milled fractions, the analyses performed on
366 pearled fractions showed an unevenly distribution of bioactive compounds in the grains of
367 both conventional and pigmented wheat varieties. Moreover, the distribution patterns in the
368 pearled fractions differ depending on the class of bioactive considered (Table 4).

369 In accordance with previous studies (Jerkovic, Kriegel, Brander, Atwell, Roberts & Willows,
370 2010; Sovrani et al., 2012), the protein and β -glucan contents were found to be higher in
371 the intermediate pearled fractions of all the varieties. Contrarily, the TDF, TAA, FPAs and
372 CWBPAs were generally detected at the highest concentration in the outermost layers of
373 the kernel, and decreased moving toward the inner layers. Individual phenolic compounds,
374 in both free and cell wall-bound form, generally presented the highest content in the first
375 two pearling fractions (corresponding to the 0-10% of the kernel weight). The main
376 compounds identified and their relative content was quite similar in the different wheat
377 varieties; the bound form of ferulic acid resulted the most representative (Table S3).
378 Similar distribution patterns were previously observed in the pearled fraction of
379 conventional wheat varieties (Sovrani et al., 2012). The TAA in the first two pearled
380 fractions, which correspond to the outermost layers of the kernels, resulted similar to the
381 one observed in the bran fraction and even 3 times higher than the one observed in the
382 wholegrain flour.

383 The distribution patterns of total anthocyanins in the purple and blue varieties are shown in
384 Figure 1. Anthocyanins were found to be located in specific layers of the kernel. In
385 particular, the purple-grained variety was characterized by the highest TAC in the 0-5%
386 fraction (579 mg Cy-3-glc equivalents/kg dw). The total anthocyanin content of this fraction

387 resulted about 6 times higher than the one observed in the wholegrain flour, and even 2
388 times higher than the one observed in the bran fraction. The second 5-10% pearled
389 fraction still showed a high TAC (425 mg Cy-3-glc equivalents/kg dw), about 4 and 1.5
390 times higher than the one observed in the wholegrain flour and in the bran fraction. The
391 blue-grained variety showed the highest total anthocyanin content in the 10-15% fraction
392 (211 mg Cy-3-glc equivalents/kg dw), about 3 and 1.5 times higher than the one observed
393 in the wholegrain flour and in the bran fraction. In accordance to previous studies (Bottega,
394 Caramanico, Lucisano, Mariotti, Franzetti & Pagani, 2009; Jerkovic et al., 2010; Shetlar,
395 Rankin, Lyman & France, 1947; Singh & Singh, 2010; Sovrani et al., 2012), pearling up to
396 the 5% level on average remove most of the outer pericarp, while at 5-10% and 10-15%
397 levels the aleurone layer is removed. Thus, the results were in agreement with previous
398 studies that showed that anthocyanins are mainly concentrated in the pericarp and in the
399 aleurone layer on purple- and blue-grained varieties, respectively (Abdel-Aal & Hucl, 1999;
400 Zeven, 1991).

401 The analysis of individual anthocyanins (Table 5) showed that the two varieties differ not
402 only for the total anthocyanin content, but also for the anthocyanin profile. The results were
403 in accordance with previous studies performed on other pigmented varieties (Bartl et al.,
404 2015; Knievel et al., 2009). The main anthocyanins in the purple wheat were peonidin and
405 cyanidin glycosides, while delphinidin glycosides were not detected. Contrarily, the main
406 anthocyanins detected in the blue-grained variety were delphinidin-3-O-rutinoside and
407 cyanidin-3-O-rutinoside, although cyanidin-3-O-glucoside, delphynidin-3-O-glucoside and
408 peonidin-3-O-rutinoside were also present. These results make possible assume that the
409 difference observed in the color of these two varieties could be probably ascribed not only
410 to different anthocyanin concentrations, but also to the different compositional profiles. In
411 fact, delphinidin glycosides, which are responsible for a characteristic blue color, were only

412 detected in the blue-grained variety, while cyanidin and peonidin glycosides were detected
413 above all in the purple-grained variety.

414 In accordance with the distribution observed in the roller-milled fractions, lutein was found
415 to be mainly concentrated in the internal layers of the kernel. Regardless the variety
416 considered, the concentration of lutein in the residual pearled kernel resulted similar to the
417 one observed in the refined flour. Contrarily, the distribution of zeaxanthin changed
418 according to which variety was considered.

419 Pearled fractions showed significant difference in their chromatic characteristics as
420 observed for the roller-milled fractions (Table S4).

421 Previous studies proposed the pearling process as an alternative technology in order to
422 select and valorize the intermediated fractions of conventional red-grained varieties, which
423 are rich in bioactive compounds, allowing at the same time the removal of the parts of the
424 grain that could be detrimental for technological quality and safety (Liyana-Pathirana, C.,
425 Dexter, J., & Shahidi F., 2006; Delcour, Rouau, Courtin, Poutanen & Ranieri, 2012;
426 Sovrani et al., 2012). The present study shows that this technology could be applied
427 indifferently both to conventional and pigmented wheat varieties in order to concentrate the
428 bioactive compounds, such as TDF, β -glucans and phenolic acids, that are generally
429 present in the outer layers of all the wheat kernels. Moreover, this study shows that the
430 use of the pearling process could be the best way in order to enhance the exploitation of
431 the health-promoting potential of purple- and blue-grained cereals, by means of a selection
432 of anthocyanin-rich fractions. The resultant fractions could be mixed with a refined flour to
433 incorporate as much as bioactive compounds in a final ingredient which could be used for
434 the production of both baked products (Blandino et al., 2013; Blandino et al., 2015) and
435 pasta (Zanoletti et al., 2017).

436

437

438 **4. CONCLUSIONS**

439 This study highlights that even if pigmented wheat varieties are an excellent source of
440 bioactive compounds, a careful selection of the most appropriate fractionation process
441 should be performed in order to produce flours naturally rich in bioactive compounds. In
442 fact, the application of the same process to different types of wheat varieties could lead to
443 conflicting results. The conventional roller-milling process could be useful to produce a
444 refined flour rich in lutein starting from yellow-grained varieties. On the contrary, the same
445 process applied to anthocyanin-pigmented varieties results in the loss of the main health
446 benefits associated to the use of these varieties, because of the removal of most of the
447 anthocyanins from the refined flour. The pearling process could be a much more useful
448 tool in order to valorize the latter varieties.

449 Further studies will be necessary for the development of supply chains based on
450 pigmented wheat varieties, in order to understand whether these varieties could be able to
451 compete with conventional ones in terms of yields and quality performance. Finally, pilot
452 and industrial scale tests should be performed in order to evaluate the potential application
453 of the pearling process to anthocyanin-pigmented varieties in order to produce new
454 ingredients with added value for consumer health.

455 **5. ACKNOWLEDGEMENT**

456 The authors would like to thank Francesca Vanara (Università of Torino), Valentina
457 Azzurra Papillo and Stefania Monteduro (Università del Piemonte Orientale “A.
458 Avogadro”), for their precious help and cooperation in the laboratory work.

459

460 **6. FUNDING**

461 This work was supported by the Regione Piemonte (Rural Development Programme
462 F.E.A.S.R. 2007/2013), as a part of the INNOBRAN project.

463

464

465

466

467

468

469

470

471

472

473

474

475

476

477

478

479

480

481 7. REFERENCES

- 482 Abdel-Aal, E. -S. M., Abou-Arab, A. A., Gamel, T. H., Hucl, P., Young, J. C., & Rabalski, I.
483 (2008). Fractionation of blue wheat anthocyanin compounds and their contribution to
484 antioxidant properties. *Journal of Agricultural and Food Chemistry*, 56, 11171-11177.
- 485 Abdel-Aal, E. -S. M., & Hucl, P. (1999). A rapid method for quantifying total anthocyanins
486 in blue aleurone and purple pericarp wheats. *Cereal Chemistry*, 76, 350-354.
- 487 Abdel-Aal, E. -S. M., Young, J. C., & Rabalski, I. (2006). Anthocyanin composition in black,
488 blue, pink, purple, and red cereal grains. *Journal of Agricultural and Food Chemistry*, 54,
489 4696-4704.
- 490 Abdel-Aal, E. -S. M., Young, J. C., Rabalski, I., Hucl, P., & Fregeau-Reid, J. (2007).
491 Identification and quantification of seed carotenoids in selected wheat species. *Journal of*
492 *Agricultural and Food Chemistry*, 55, 787-794.
- 493 Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and*
494 *Food Chemistry*, 50, 6182-6187.
- 495 AOAC (1990). *Official Methods of Analysis*, 15th ed., Arlington, VA: Association of Official
496 Analytical Chemists.
- 497 Bartl, P., Albrecht, A., Skrt, M., Tremlová, B., Ošťádalova, M., Šmejkal, K., Vovk, I., & Ulrih,
498 N. P. (2015). Anthocyanins in purple and blue wheat grains and in resulting bread:
499 quantity, composition, and thermal stability. *International Journal of Food Science and*
500 *Nutrition*, 66, 514-519.
- 501 Belobrajdic, D. P., & Bird, A. R. (2013). The potential role of phytochemicals in wholegrain
502 cereals for the prevention of type-2 diabetes. *Nutrition Journal*, 12, 62-74.
- 503 Beta, T., Nam, S., Dexter, J. E., & Sapiststein, H. D. (2005). Phenolic content and
504 antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chemistry*, 82, 390-
505 393.

506 Blandino M., Sovrani V., Marinaccio F., Reyneri A., Rolle L., Giacosa S., Locatelli M.,
 507 Bordiga M., Travaglia F., Coïsson, J. D., & Arlorio M. (2013). Nutritional and technological
 508 quality of bread enriched with an intermediated pearled fraction. *Food Chemistry*, 141,
 509 2549-2557.

510 Blandino, M., Locatelli, M., Sovrani, V., Coïsson, J. D., Rolle, L., Travaglia, F., Giacosa, S.,
 511 Bordiga M., Scarpino, V., Reyneri, A., & Arlorio, M. (2015). Progressive pearling of barley
 512 kernel: chemical characterization of pearling fractions and effect of their inclusion on the
 513 nutritional and technological properties of wheat bread. *Journal of Agricultural and Food*
 514 *Chemistry*, 63, 5875-5884.

515 Borowicki, A., Stein, K., Scharlau, D., & Glei, M. (2010). Fermentation supernatants of
 516 wheat (*Triticum aestivum* L.) aleurone beneficially modulate cancer progression in human
 517 colon cells. *Journal of Agricultural and Food Chemistry*, 58, 2001–2007.

518 Bottega, G., Caramanico, R., Lucisano, M., Mariotti, M., Franzetti, L., & Pagani M. A.
 519 (2009). The debranning of common wheat (*Triticum aestivum* L.) with innovative abrasive
 520 rolls. *Journal of Food Engineering*, 94, 75-82.

521 Campbell, G. M., Webb, C., Owens, G. W., & Scanlon, M. G. (2012). Milling and flour
 522 quality. In S. P. Cauvain (Ed.), *Breadmaking – Improving quality*, 2nd ed. (pp. 188-215).
 523 Cambridge: Woodhead Publishing.

524 Carson, G. R., & Edwards N. M. (2009). Criteria of wheat and flour quality. In K. Khan, &
 525 P. R. Shewry (Eds.), *Wheat. Chemistry and Technology*, 4th ed. (pp.97-118). Minnesota:
 526 AACC International. Inc.

527 Cheli, F., Campagnoli, A., Ventura, V., Brera, C., Berdini, C., Palmaccio, E., & Dell’Orto, V.
 528 (2010). Effects of industrial processing on the distributions of deoxynivalenol, cadmium
 529 and lead in durum wheat milling fractions. *LWT - Food Science and Technology*, 43, 1050-
 530 1057.

531 Delcour J. A., Rouau X., Courtin C. M., Poutanen K., & Ranieri R. (2012). Technologies for
 532 enhanced exploitation of the health-promoting potential of cereals. *Trends in Food Science*
 533 *& Technology*, 25, 78-86.

534 Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain
 535 cereals: what is beyond fibre? *Nutrition Research Reviews*, 23, 65-134.

536 Ficco, D. B. M., De Simone, V., De Leonardis, A. M., Giovaniello, V., Del Nobile, M. A.,
 537 Padalino, L., Lecce, L., Borrelli, G. M., & De Vita, P. (2016). Use of purple durum wheat to
 538 produce naturally functional fresh and dry pasta. *Food Chemistry*, 205, 187-195.

539 Hemery, Y., Rouau, X., Lullien-Pellerin, V., Barron, C., & Abecassis, J. (2007). Dry
 540 processes to develop wheat fractions and products with enhanced nutritional quality.
 541 *Journal of Cereal Science*, 46, 327-347.

542 Jaafar, S. N. S., Baron, J., Siebenhandl-Ehn, S., Rosenau, T., Böhmendorfer, S., &
 543 Grausgruber, H. (2013). Increased anthocyanin content in purple pericarp x blue aleurone
 544 wheat crosses. *Plant Breeding*, 132, 546-552.

545 Jerkovic, A., Kriegel, A. M., Brander, J. R., Atwell, B. J., Roberts, T. H., & Willows, R. D.
 546 (2010). Strategic distribution of protective proteins within bran layers of wheat protects the
 547 nutrient-rich endosperm. *Plant Physiology*, 152, 1459-1470.

548 Knievel, D. C., Abdel-Aal, E. -S. M., Rabalski, I., Nakamura, T., & Hucl, P. (2009). Grain
 549 color development and the inheritance of high anthocyanin blue aleurone and purple
 550 pericarp in spring wheat (*Triticum aestivum* L.). *Journal of Cereal Science*, 50, 113-120.

551 Li, W., Pickard, M. D., & Beta, T. (2007). Effect of thermal processing on antioxidant
 552 properties of purple wheat bran. *Food Chemistry*, 104, 1080-1086.

553 Li, L., Shewry, P. R., & Ward, J. L. (2008). Phenolic acids in wheat varieties in the
 554 HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, 56, 9732-
 555 9739.

556 Liu, Q., Qiu, Y., & Beta, T. (2010). Comparison of antioxidant activities of different colored
 557 wheat grains and analysis of phenolic compounds. *Journal of Agricultural and Food*
 558 *Chemistry*, 58, 9235-9241.

559 Liyana-Pathirana, C., Dexter, J., & Shahidi, F. (2006). Antioxidant properties of wheat as
 560 affected by pearling. *Journal of Agricultural and Food Chemistry*, 54, 6177-6184.

561 Martinek P., Škorpík M., Chrpová J., Fučík P., & Schweiger J. (2013). Development of the
 562 new winter wheat variety Skorpion with blue grain. *Czech Journal of Genetics & Plant*
 563 *Breeding*, 49, 90-94.

564 Pasqualone, A., Bianco, A. M., Paradiso, V. M., Summo, C., Gambacorta, G., Caponio, F.,
 565 & Bianco, A. (2015). Production and characterization of functional biscuits obtained from
 566 purple wheat. *Food Chemistry*, 180, 64-70.

567 Shetlar, M. R., Rankin, G. T., Lyman, J. F., & France, W. G. (1947). Investigation of the
 568 proximate chemical composition of the separate bran layers of wheat. *Cereal Chemistry*,
 569 24, 111-122.

570 Siebenhandl, S., Grausgruber, H., Pellegrini, N., Del Rio, D., Fogliano, V., Pernice, R., &
 571 Berghofer, E. (2007). Phytochemical profile of main antioxidants in different fractions of
 572 purple and blue wheat, and black barley. *Journal of Agricultural and Food Chemistry*, 55,
 573 8541-8547.

574 Singh, S., & Singh, N. (2010). Effect of debranning on the physico-chemical, cooking,
 575 pasting and textural properties of common and durum wheat varieties. *Food Research*
 576 *International*, 43, 2277-2283.

577 Sobota, A., Rzedzicki, Z., Zarzycki, P., & Kuzawińska, E. (2015). Application of common
 578 wheat bran for the industrial production of high-fibre pasta. *International Journal of Food*
 579 *Science and Technology*, 50, 111-119.

580 Sovrani, V., Blandino, M., Scarpino, V., Reyneri, A., Coïsson, J. D., Travaglia, F., Locatelli,
 581 M., Bordiga, M., Montella, R., & Arlorio, M. (2012). Bioactive compound content,

582 antioxidant activity, deoxynivalenol and heavy metal contamination of pearled wheat
583 fractions. *Food Chemistry*, 135, 39-46.

584 Yu, L., & Beta, T. (2015). Identification and antioxidant properties of phenolic compounds
585 during production of bread from purple wheat grains. *Molecules*, 20, 15525-15549.

586 Zanoletti, M., Parizad, P. A., Lavelli, V., Checchini, C., Menesatti, P., Marti, A., & Pagani,
587 M. A. (2017). Debranning of purple wheat: recovery of anthocyanin-rich fractions and their
588 use in pasta production. *LWT – Food Science and Technology*, 75, 663-669.

589 Zeven, A. C. (1991). Wheats with purple and blue grains: a review. *Euphytica*, 56, 243-
590 258.

591 Zhang, D., & Moore, W. R. (1999). Wheat bran particle size effects on bread baking
592 performance and quality. *Journal of the Science of Food and Agriculture*, 79, 805-809.

593

594 **FIGURE CAPTION**

595 **Figure 1.** The TAC in the pearled fractions of the purple (cv. Rosso) and the blue (cv. Skorpion) wheat varieties. The results are
596 expressed on a dw basis. The reported data are the means of three values; values with different letters differ significantly, according to
597 the REGW-Q test (the ANOVA level of significance is <0.001).

598

600 **Table 1.** Kernel traits and chemical composition of the wholegrain flours of conventional and pigmented wheat varieties.

Wheat type	Variety name	TKW g	TW kg/hL	Proteins %	Ash %	TDF %	β-glucans %	TAA mmol TE/kg	FPAs ¹ mg/kg	CWBPA ² mg/kg	TAC mg Cy-3- glc/kg	Lutein mg/kg	Zeaxanthin mg/kg
Red	PR22R58	43.6 c	75.6 d	9.1 e	1.27 c	14.1 a	1.01 a	6.65 a	1.10 d	889 c	n.d.	2.18 b	0.15 d
White	Whitebear	46.7 b	82.8 a	11.3 d	1.56 b	15.7 a	0.99 a	6.94 a	4.71 a	776 e	n.d.	1.07 e	0.37 a
Yellow	Bona Vita	37.0 d	79.0 c	15.1 a	1.48 b	12.8 a	0.96 a	6.61 a	4.33 ab	800 d	n.d.	3.62 a	0.34 b
Purple	Rosso	46.4 b	80.4 b	12.4 c	1.58 b	15.1 a	0.66 b	7.57 a	3.71 b	1071 b	102 a	1.67 c	0.34 b
Blue	Skorpion	50.1 a	74.6 e	13.0 b	1.91 a	12.7 a	0.53 c	6.47 a	2.35 c	1093 a	72 b	1.24 d	0.29 c
SEM		0.4	0.3	0.1	0.08	1.6	0.06	0.99	0.58	8	5	0.11	0.02
P (F)		<0.001	<0.001	<0.001	<0.001	0.109	<0.001	0.172	<0.001	<0.001	0.001	<0.001	<0.001

601 TKW, thousand kernel weight; TW, test weight; TDF, total dietary fiber; TAA, total antioxidant activity; FPAs, free phenolic acids; CWBPAs, cell wall-bound
602 phenolic acids; TAC, total anthocyanins.

603 Data are expressed on a dw basis. Means followed by different letters are significantly different, according to the REGW-Q test (the ANOVA level of significance
604 is shown in the table).

605 n.d., not detected.

606 SEM, standard error of the mean.

607 ¹ sum of the FPAs determined by means of RP-HPLC/DAD

608 ² sum of the CWBPAs determined by means of RP-HPLC/DAD

609

610 **Table 2.** Chromatic characteristics of the wholegrain flours.

Wheat type	Color					
	<i>L</i> [*]		<i>a</i> [*]		<i>b</i> [*]	
Red			83.6	c	1.5	b
White			86.4	a	0.8	d
Yellow			84.3	b	1.4	b
Purple			78.8	e	3.1	a
Blue			80.7	d	1.0	c
SEM			0.4		0.1	
P (F)			<0.001		<0.001	

611 Means followed by different letters are significantly different, according to the REGW-Q test (the ANOVA
612 level of significance is shown in the table).

613 SEM, standard error of the mean.

614

615

616 **Table 3.** Chemical composition of the roller-milled fractions of conventional and pigmented wheat varieties.

Wheat type	Roller-milled fraction	Proteins		Ash		TDF		β-glucans		TAA		FPAs ¹		CWBPA ²		TAC		Lutein		Zeaxanthin	
		%		%		%		%		mmol TE/kg		mg/kg		mg/kg		mg Cy-3-glc eq/kg		mg/kg		mg/kg	
Red	Bran	12.9	a	3.94	a	31.6	a	1.44	a	21.71	a	4.35	a	2640	a	n.d.		1.85	b	0.28	a
	Refined flour	7.79	b	0.37	b	2.1	b	0.60	b	1.98	b	0.88	b	92	b	n.d.		2.41	a	0.09	b
	P (F)	0.02		0.02		0.6		0.05		1.13		0.25		6				0.11		0.01	
	SEM	<0.001		<0.001		0.001		<0.001		<0.001		<0.001		<0.001				0.002		<0.001	
White	Bran	14.5	a	3.79	a	26.6	a	1.29	a	20.57	a	12.22	a	2633	a	n.d.		0.99	b	0.49	a
	Refined flour	10.1	b	0.42	b	3.3	b	0.46	b	2.15	b	0.99	b	137	b	n.d.		1.12	a	0.11	b
	P (F)	0.04		0.03		0.5		0.02		0.83		0.18		1				0.04		0.01	
	SEM	<0.001		<0.001		0.001		<0.001		<0.001		<0.001		<0.001				0.007		<0.001	
Yellow	Bran	16.5	a	4.08	a	30.7	a	1.69	a	18.85	a	15.97		2818	a	n.d.		2.69	b	0.73	a
	Refined flour	14.7	b	0.25	b	3.5	b	0.43	b	1.97	b	n.d.		86	b	n.d.		4.11	a	0.13	b
	P (F)	0.1		0.07		0.5		0.02		0.58		-		7				0.23		0.01	
	SEM	0.004		0.001		0.001		<0.001		<0.001		-		<0.001				0.001		<0.001	
Purple	Bran	17.0	a	4.19	a	30.5	a	0.81	a	21.78	a	14.00	a	3314	a	271	a	1.60	b	0.76	a
	Refined flour	10.7	b	0.3	b	4.9	b	0.39	b	2.09	b	1.40	b	100	b	7	b	1.95	a	0.07	b
	P (F)	0.1		0.01		1.2		0.04		1.55		0.88		4		16		0.14		0.02	
	SEM	<0.001		<0.001		0.003		<0.001		<0.001		<0.001		<0.001		<0.001		0.021		<0.001	
Blue	Bran	15.3	a	4.47	a	33.6	a	0.66	a	21.69	a	17.42	a	3991	a	140	a	1.60	a	0.91	a
	Refined flour	12.6	b	0.39	b	3.9	b	0.35	b	2.01	b	0.68	b	111	b	11	b	1.28	a	0.05	b
	P (F)	0.04		0.02		1.4		0.05		1.95		0.65		7		3		0.24		0.17	
	SEM	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		0.134		0.002	

617 TDF, total dietary fiber; TAA, total antioxidant activity; FPAs, free phenolic acids; CWBPAs, cell wall-bound phenolic acids; TAC, total anthocyanins.

618 The results are expressed on a dw basis. Means followed by different letters are significantly different, according to the REGW-Q test (the ANOVA level of
619 significance is shown in the table).

620 n.d., not detected.

621 SEM, standard error of the mean.

622 ¹ sum of the FPAs determined by means of RP-HPLC/DAD

623 ² sum of the CWBPAs determined by means of RP-HPLC/DAD

Table 4. Chemical composition of the pearled fractions of conventional and pigmented wheat varieties.

Wheat type	Pearled fraction	Proteins		Ash		TDF		β -glucans		TAA		FPAs ¹		CWBPAs ²		Lutein		Zeaxanthin	
		%		%		%		%		mmol TE/kg		mg/kg		mg/kg		mg/kg		mg/kg	
Red	0-5%	8.7	c	3.05	c	51.7	a	0.99	c	18.26	a	4.32	bc	2327	a	1.31	d	0.20	b
	5-10%	9.7	b	3.07	c	38.3	b	1.32	b	20.09	a	5.96	a	2086	b	1.35	d	0.21	b
	10-15%	10.9	a	3.17	b	32.9	c	1.52	a	19.40	a	5.43	ab	1679	c	1.64	c	0.24	ab
	15-20%	11.1	a	3.29	a	25.5	d	1.64	a	12.86	b	4.48	bc	1668	d	1.76	bc	0.22	ab
	20-25%	10.7	a	2.64	d	21.3	e	1.48	ab	10.55	b	5.30	ab	1419	e	2.03	b	0.25	a
	25-100%	9.0	c	1.17	e	9.5	f	0.60	d	3.65	c	3.71	c	449	f	2.55	a	0.15	c
	SEM	0.4		0.04		1.8		0.12		2.14		0.92		6		0.24		0.03	
	P (F)	<0.001		<0.001		<0.001		<0.001		<0.001		0.001		<0.001		<0.001		<0.001	
White	0-5%	11.0	d	5.04	b	65.1	a	1.19	c	22.62	a	12.90	a	3040	a	0.73	c	0.88	a
	5-10%	15.4	b	5.21	a	31.1	b	1.77	a	20.95	a	9.58	b	2892	b	0.94	b	0.90	a
	10-15%	15.9	a	4.14	c	26.9	bc	1.45	b	11.19	b	9.53	b	2026	c	1.05	ab	0.77	b
	15-20%	15.1	c	3.17	d	25.3	c	1.16	c	9.54	b	6.53	c	1697	d	1.10	a	0.70	b
	20-25%	15.4	b	2.58	e	18.5	d	0.99	d	7.21	c	6.50	c	1312	e	1.14	a	0.60	c
	25-100%	10.5	e	0.96	f	8.8	e	0.24	e	2.21	d	3.01	d	361	f	1.01	ab	0.20	d
	SEM	0.2		0.07		2.2		0.15		3.15		0.87		7		0.12		0.08	
	P (F)	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Yellow	0-5%	13.4	d	4.25	a	59.0	a	1.65	b	24.41	a	14.02	a	3088	a	1.37	c	0.78	b
	5-10%	20.6	b	3.82	b	35.1	b	2.26	a	20.34	b	9.67	b	3052	b	3.10	b	0.98	a
	10-15%	21.8	a	2.99	c	23.4	c	1.90	b	16.08	c	7.59	c	2199	c	3.65	ab	0.83	b
	15-20%	20.7	b	2.35	d	16.9	d	1.13	c	9.26	d	5.23	d	1533	d	3.74	a	0.61	c
	20-25%	20.8	b	1.95	e	13.8	de	1.01	c	6.41	e	3.88	d	1079	e	3.70	a	0.49	d
	25-100%	13.8	c	0.88	f	9.3	e	0.24	d	2.43	f	1.75	e	526	f	4.12	a	0.23	e
	SEM	0.2		0.07		2.5		0.23		3.11		1.24		20		0.49		0.06	
	P (F)	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Purple	0-5%	11.4	d	3.11	c	49.8	a	0.56	b	26.40	a	16.69	a	2632	b	1.12	b	0.75	a
	5-10%	12.5	c	3.60	a	42.0	b	0.83	a	23.63	b	16.28	a	2800	a	1.20	b	0.66	bc
	10-15%	14.6	b	3.62	a	34.3	c	0.84	a	17.48	c	11.38	b	2623	b	1.62	a	0.73	ab
	15-20%	15.2	a	3.49	b	26.0	d	0.82	a	12.03	d	8.98	c	1863	c	1.66	a	0.60	c
	20-25%	15.1	a	3.14	c	21.9	d	0.82	a	9.34	e	7.31	d	1797	d	1.62	a	0.49	d
	25-100%	12.5	c	1.47	d	12.1	e	0.22	c	3.15	f	2.63	e	733	e	1.59	a	0.19	e
	SEM	0.3		0.08		1.8		0.17		1.61		1.2		12		0.15		0.08	
	P (F)	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Blue	0-5%	9.9	f	3.57	c	59.9	a	0.52	c	23.11	a	11.16	a	5388	a	0.93	c	0.52	b
	5-10%	14.9	d	4.31	a	37.6	b	1.09	a	16.64	b	6.16	b	3452	b	1.15	b	0.66	a
	10-15%	17.7	b	4.59	a	27.8	c	1.03	a	15.25	b	5.10	bc	2913	c	1.36	ab	0.67	a
	15-20%	18.1	a	3.98	b	21.2	d	0.85	b	10.92	c	4.13	c	2243	d	1.51	a	0.63	a
	20-25%	17.5	c	3.32	c	15.8	e	0.82	b	8.04	d	5.13	bc	1890	e	1.46	a	0.51	b
	25-100%	12.5	e	0.98	d	8.0	f	0.24	d	2.48	e	2.51	d	622	f	1.32	ab	0.20	c
	SEM	0.2		0.27		1		0.17		2.92		0.95		11		0.18		0.09	
	P (F)	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	

TDF, total dietary fiber; TAA, total antioxidant activity; FPAs, free phenolic acids; CWBPAs, cell wall-bound phenolic acids; TAC, total anthocyanins.

The results are expressed on a dw basis. Means followed by different letters are significantly different, according to the REGW-Q test (the ANOVA level of significance is shown in the table).

SEM, standard error of the mean.

¹ sum of the FPAs determined by means of RP-HPLC/DAD

² sum of the CWBPAs determined by means of RP-HPLC/DAD

Table 5. Anthocyanin profile in the pearled fractions of the purple- and blue-grained wheat varieties.

Wheat type	Pearled fraction	mg/kg									
		Cy-3-glc		Cy-3-rut		Dn-3-glc		Dn-3-rut		Pn-3-glc	
Purple	0-5%	4.15	a	3.62	a	n.d.		n.d.		1.03	a
	5-10%	2.72	b	2.89	b	n.d.		n.d.		0.72	b
	10-15%	1.47	c	1.76	c	n.d.		n.d.		0.47	c
	15-20%	0.95	d	1.15	d	n.d.		n.d.		0.29	d
	20-25%	0.71	d	0.58	e	n.d.		n.d.		0.20	e
	25-100%	0.13	e	0.11	f	n.d.		n.d.		n.d.	f
	SEM	0.13		0.32						0.06	0.53
	P (F)	<0.001		<0.001		-		-		<0.001	<0.001
Blue	0-5%	1.32	c	5.62	c	1.47	c	4.62	c	n.d.	0.40 c
	5-10%	3.75	a	12.62	a	3.06	ab	10.26	a	n.d.	0.98 ab
	10-15%	3.79	a	12.85	a	3.34	a	10.85	a	n.d.	1.13 a
	15-20%	2.55	b	10.56	b	2.77	b	8.94	b	n.d.	0.92 b
	20-25%	2.69	b	10.33	b	2.85	b	9.17	b	n.d.	0.98 ab
	25-100%	0.18	d	1.15	d	0.37	d	1.15	d	n.d.	0.30 c
	SEM	0.33		1.08		0.32		0.81			0.12
	P (F)	<0.001		<0.001		<0.001		<0.001		-	<0.001

Cy-3-glc, cyanidin-3-O-glucoside; Cy-3-rut, cyanidin-3-O-rutinoside; Dn-3-glc, delphinidin-3-O-glucoside; Dn-3-rut, delphinidin-3-O-rutinoside; Pn-3-glc, peonidin-3-O-glucoside; Pn-3-rut, peonidin-3-O-rutinoside.

The results are expressed on a dw basis. Means followed by different letters are significantly different, according to the REGW-Q test (the ANOVA level of significance is shown in the table).

n.d., not detected

SEM, standard error of the mean.

22

23