Distribution of bioactive compounds in pearled fractions of tritordeum

TITLE

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ABSTRACT

Hexaploid tritordeum is the amphidiploid cereal derived from the cross between wild barley and durum wheat. The present study compares two cultivars of tritordeum with other cereals grown in the same experimental area to weigh up its potential use as ingredient for health-valued foods. Tritordeum shows 2.5-fold higher concentration of lutein than common wheat and barley, and 1.2-fold higher than durum wheat, while the concentration of β-glucans is 5 folds lower than the one observed for barley. Based on the distribution of bioactive compounds in pearled fractions, the use of whole-grain flours seems the best way to exploit the antioxidant potential of tritordeum. Nevertheless, the internal layers of the kernel of this cereal are characterized on average by high concentrations of antioxidants (32.0 mg/kg and 518 mg/kg soluble and cell wall-bound phenolic acids, respectively), making tritordeum interesting also for the production of refined flours rich in bioactive compounds.

KEYWORDS

Tritordeum, Barley, Wheat, β-glucans, Arabinoxylans, Phenolic acids, Antioxidant capacity, Xanthophylls

1. INTRODUCTION

Wheat-based products are central dietary components worldwide owing to their good nutritional and organoleptic qualities. Nevertheless, the application of alternative cereal types and processing technologies for the production of foods rich in bioactive compounds has drawn the attention of both researchers and industrialists in the last few years (Abdel-Aal et al., 2002; Blandino et al., 2013; Delcour, Rouau, Courtin, Poutanen & Ranieri, 2012; Giordano et al., 2017; Taylor & Awika, 2017). Since the beginning of the twentieth century, cereal breeders focused their effort on the development of interspecific hybrids in order to obtain new cereals with increased phytochemical contents and improved agronomic performances and technological qualities. In this sense, tritordeum is a potentially interesting candidate. Hexaploid tritordeum is the amphidiploid cereal derived from the cross between a South American wild barley (*Hordeum chilense* Roem. et Schultz.) and a cultivated durum wheat (*Triticum turgidum* ssp. *durum* Desf.). Besides being used as a genetic bridge for transferring useful barley traits to wheat, tritordeum has been subjected to a breeding program to become a new hulless small cereal crop (Martín, Alvarez, Martín, Barro & Ballesteros 1999). Previous studies have shown that tritordeum is more suitable for breadmaking than for pasta making (Martín et al., 1999). At present, limited information is available on the content and the composition of phenolic acids in tritordeum (Eliášová & Paznocht, 2017; Navas-Lopez, Ostos-Garrido, Castillo, Martín, Gimenez & Piston, 2014). Nevertheless, several studies showed that this novel cereal is characterized by a high content of carotenoids, which give it a strong yellow color (Mellado-Ortega & Hornero-Méndez, 2012 and 2016; Paznocht et al., 2018), and tocols (Lachman, Hejtmánková, Orsák, Popov & Martinek, 2018), suggesting its potential use for the production of health-valued foods. Even if not suitable for celiac disease sufferers, tritordeum showed lower levels of gluten immunogenic epitopes than wheat (Vaquero et al., 2018).

70 The aim of the present study was to provide new insight about tritordeum. Tritordeum was
71 compared with other small cereals such as barley, durum wheat and common wheat
72 cultivated side by side in the same experimental area, in order to avoid any environmental
73 influence. The comparison was carried out by means of field experiments in which both grain
74 yield and kernel traits were evaluated. Moreover, kernels were compared for their
75 phytochemical composition, and then pearled to analyze the distribution pattern of bioactive
76 compounds in progressive pearled fractions.
77

2. MATERIALS AND METHODS

2.1 Experimental design

The present study compared:

- two cultivars of tritordeum (*xTritordeum martinii* A. Pujadas, nothosp. nov.) registered in the CPVO (Community Plant Variety Office) as Aucan and Bulel (Agrasys S.L., Barcelona, Spain);
- a hulled and six-row cultivar of barley (*Hordeum vulgare* L., cv. Ketos - Limagrain Italia S.p.A, Fidenza, Italy);
- a durum wheat cultivar (*Triticum turgidum* ssp. *durum* Desf., cv. Saragolla - Syngenta Italia, S.p.A, Milano, Italy);
- a common wheat cultivar (*Triticum aestivum* ssp. *aestivum* L., cv. Illico - Syngenta Italia) classified as bread-making-quality wheat (Foca et al., 2007).

All the cereal cultivars were cultivated side by side on the same field in northwestern Italy (Cigliano, Piedmont; 45°31'97"N, 8°4'77"E) in a completely randomized block design with four replications. Field trials were carried out during the 2015-2016 growing season, according to the ordinary crop management program applied to barley and wheat in the growing area. The plot size was 7x1.5 m (10.5 m^2), planting was performed in 12 cm wide rows at a seeding rate of 450 seeds/m^2 on 6 November 2015, following an autumn plowing (30 cm) and disk harrowing to prepare a proper seedbed. The previous crop was maize. The nitrogen fertilization performed during the harvest season was in accordance to the agronomic management usually carried out in the North of Italy for the cultivation of barley, durum and common wheat. 170 kg N/ha were provided to plots of tritordeum and durum wheat, split in 50 kg N/ha at the tillering stage (Growth stage - GS 23), 80 kg N/ha at the beginning of stem elongation (GS 31), 40 kg N/ha at the heading stage (GS 55). 120 kg N/ha were provided to plots of barley and common wheat, split in 60 kg N/ha at the tillering stage (GS 23) and 60 kg N/ha at the beginning of stem elongation (GS

31). Harvesting was carried out with a plot combine-harvester on 21 June for the barley cultivar and on 4 July 2016 for the tritordeum and wheat cultivars, according to their crop cycle.

2.2 Analysis of grain quality parameters

Grain yield (t/ha) was calculated on a plot basis. Thousand kernel weight (TKW) was determined on two 200-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 Grain pearling

Nine pearled fractions of the kernels of each cultivar were obtained through the incremental pearling of the cereals tested following the approach proposed by Beta, Nam, Dexter & Sapirstein (2005). 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, 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, fifth, sixth, seventh, eighth fraction (designed fractions of 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40% w/w). The pearling process was performed at a constant speed and the estimation of the time necessary in order to remove 5% of kernel weight at each pearling passage was experimentally quantified for each cultivar. 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 60% of the kernel (40-100% w/w) was also collected

and milled through a laboratory centrifugal mill (Model ZM-100, Retsch, Haan, Germany) equipped with a 1-mm sieve. The same milling process was performed for the unprocessed grain samples in order to obtain a wholemeal flour. Prior to chemical analyses, all the samples were ground to a fine powder (particle size < 300 µm) with a cyclotec 1093 sample mill (Foss, Padova, Italy), and stored for 2 weeks at -25°C until the beginning of the analyses.

2.4 Chemical analyses

2.4.1 Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT, ≥99.0%), ethanol (CHROMASOLV®, 99.8%), ethylacetate (CHROMASOLV®, 99.8%), 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%), *trans*-β-Apo-8'-carotenal, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and phenolic acid standards (caffeic acid ≥98%, *p*-coumaric acid ≥98%, *t*-ferulic acid ≥99%, *p*-hydroxybenzoic acid ≥99%, sinapic acid ≥98%, syringic acid ≥95% and vanillic acid ≥97%) were purchased from Sigma-Aldrich (St. Louis, Missouri, US). 3,5-Dichloro-4-hydroxybenzoic acid (DHB) was purchased from Thermo Fisher (Waltham, Massachusetts, US), while xanthophylls standards (lutein ≥95% and zeaxanthin ≥98%) were purchased from Extrasynthese (Lyon, France).

2.4.2 Proximate composition analysis

The moisture content, determined in order to express all the results on a dry weight (dw) basis, was obtained by oven-drying at 105 °C for 24 h. The moisture values are reported as Supplementary Material in Table S1. The total protein content (conversion factor: 5.70) was obtained according to the Kjeldahl method by means of a Kjeltec system I (Foss Tecator

156 AB, Höganäs, Sweden) (Sovrani et al., 2012). The ash content was determined in a muffle
157 furnace according to the AOAC (1990) procedure. The total dietary fiber (TDF) and β-glucan
158 contents were determined by means of the Megazyme total dietary fiber analysis kit and the
159 Megazyme mixed-linkage β-glucan assay kit, respectively. Total arabinoxylans were
160 extracted according to Rouau and Surget (1994) and quantified by means of colorimetric
161 determination (Douglas, 1981; Kiszonas, Courtin & Morris, 2012) through a D-xylose
162 calibration curve (range: 0.05 – 0.5 mg/mL; $y = -2.2213\ x^2 + 2.7996\ x + 0.0968$, $R^2 = 0.9978$).
163
164 **2.4.3 Extraction of the soluble and cell wall-bound phenolic acids**
165 The extraction of soluble (free and conjugated) and cell wall-bound phenolic acids was
166 performed according to the procedure proposed by Li, Shewry and Ward (2008) and
167 Nicoletti, Martini, De Rossi, Taddei, D'Egidio and Corradini (2013) with some modifications.
168 DHB was used as internal standard to ensure that losses due to the extraction method were
169 accounted for. Three individual extractions were carried out for each sample (n=3) for both
170 soluble and cell wall-bound phenolic acids.
171 *Extraction of soluble phenolic acids*
172 One hundred and twenty-five milligrams of each sample were added with 50 μL DHB (1
173 mg/mL) and then extracted with 1 mL of 80:20 (v/v) ethanol:water solution. The mixtures
174 were vortexed for 30 sec, and then sonicated (35 kHz, Sonorex Super RK 156 BH, Bandelin
175 Electronic, Berlin, Germany) for 10 min, maintaining the temperature at 4°C to avoid starch
176 gelatinization. Samples were centrifuged at 10,600 x g for 10 min, and a second extraction
177 was carried out with 80:20 (v/v) ethanol:water solution. The pellet was discarded, while the
178 supernatants were collected and then evaporated to dryness under a nitrogen stream.
179 Samples were hydrolyzed with 2 M NaOH (400 μL) for 2 h under continuous stirring at 4°C.
180 After acidification to pH 2 with HCl, soluble phenolic acids were extracted with 500 μL of

ethyl acetate. After centrifugation at 10,600 x g for 2 min the upper layer was transferred in a clean microcentrifuge tube. The extraction was repeated twice, and the combined supernatants were evaporated to dryness under a nitrogen stream and then reconstituted in 100 µL of 80:20 (v/v) methanol:water solution.

Extraction of cell wall-bound phenolic acids

Samples (125 mg) were extracted two times with 80:20 (v/v) ethanol:water in order to remove soluble phenolic acids. Mixtures were vortexed before being sonicated for 10 min. Samples were then centrifuged at 10,600 x g for 10 min, and the supernatant was removed and discarded. Fifty microliters of the internal standard solution (2 mg/mL) were added to the remaining pellet prior to hydrolysis 4 h under continuous stirring at 4°C, by adding 2 M NaOH (400 µL). After acidification to pH 2 with HCl, the bound phenolic acids were extracted with 800 µL of ethyl acetate and then centrifuged at 10,600 x g for 2 min. The extraction was repeated another time. The combined supernatants were evaporated to dryness under a nitrogen stream, and then reconstituted in 200 µL of 80:20 (v/v) methanol:water solution.

2.4.4 Quantification of soluble and cell wall-bound phenolic acids by means of RP-HPLC/DAD

The phenolic extracts were filtered through a 0.2 µm filter and then analyzed by means of a high performance liquid chromatograph Agilent 1200 Series (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 1200 Series diode array detector. The chromatographic method was developed starting from the one proposed by Shao, Hu, Yu, Mou, Zhu & Beta (2018). Separations were carried out using a 150 x 4.6 mm, 5 µm, Gemini RP-18 column (Phenomenex, Torrance, CA, USA); the column temperature was set at 35 °C. The mobile phase consisted of 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in methanol (solvent B). The following operating linear gradient was used: 0-22 min, 9-42% B; 22-27 min, 42-90% B; 27-32 min, 90% B. Finally, the mobile phase was brought to

9% B in 3 min, and this was followed by 16 min of equilibration. The flow rate of the mobile phase was 1 mL/min. Phenolic acids were identified using the retention times and the UV/Vis spectra of their respective standards. Solutions of individual phenolic acid standards were also prepared and diluted to different concentrations to obtain calibration curves for quantification purposes. Retention time, detection wavelength and the principal parameters of the calibration curves are reported as Supplementay Material in Table S2.

2.4.5 Extraction of xanthophylls and quantification by means of RP-HPLC/DAD

The extraction of xanthophylls was performed has previously reported in Giordano et al. (2017). Each sample was analyzed in triplicate and *trans*-β-Apo-8'-carotenal was used as internal standard to ensure that losses due to the extraction method were accounted for. Samples (0.3 g) were 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 (0.8 g/mL) at 85°C for 10 min, chilled on ice. Fifty microliters of the internal standard solution (4.5 μg/mL) were added prior the addition of 3 mL of cold deionized water. 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 1,200 g for 10 minutes. The extraction was repeated four times, and the combined supernatants were evaporated to dryness under a nitrogen stream, and then dissolved in 150 μL of methanol:MTBE (1:1 v/v).

The chromatographic method was developed starting from the one proposed by Moros, Darnoko, Cheyran, Perkins & Jerrel (2002). Separations were carried out using a 100 x 4.6 mm, 3 μm, C30 carotenoid YMC column (YMC Co., Kyoto, Japan); the column temperature was set at 35°C. The mobile phase consisted of methanol:MTBE:water [81:15:4, v/v; (solvent A)] and MTBE:methanol [91:9, v/v; (solvent B)]. The following operating linear gradient was used: 0-3 min, 5-15% B; 3-7 min, 15-40% B; 7-8 min, 40-100% B; 8-13 min,

100% B. Finally, the mobile phase was brought to 5% B in 1 minute, and this was followed by 10 minutes of equilibration. The flow rate of the mobile phase was 1 mL/min. Xanthophylls were identified using the retention times and the UV/Vis spectra of their respective standards (lutein and zeaxanthin). Individual xanthophyll standards were also prepared and diluted to different concentrations to obtain calibration curves for quantification purposes. Retention time, detection wavelength and the principal parameters of the calibration curves are reported as Supplementay Material in Table S3.

2.4.6 Determination of DPPH radical scavenging activity (AC_{DPPH})

DPPH radical scavenging activity (QUENCHER procedure – direct measurement on solid sample, Gökmen, Serpen & Fogliano, 2009) was carried out as reported in Giordano et al. (2017). The DPPH radical scavenging activity was expressed as mmol of Trolox equivalents/kg of sample (dw) through a calibration curve (linearity range: 0.5-5 µg/mL; y=18.573x-1.3947, R^2: 0.999). The analysis was carried out in triplicate (n=3).

2.4.7 Determination of antioxidant capacity by means of the FRAP assay (AC_{FRAP})

Tre FRAP (Ferric Reducing Antioxidant Power) assay adapted into QUENCHER method was performed as described by Serpen, Gökmen and Fogliano (2012). Briefly, FRAP reagent was prepared by mixing the aqueous solution of 10 mM TPTZ and 20 mM ferric chloride in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10 (v:v:v). Samples (2 mg) were analyzed by adding FRAP working solution (2 mL). The reaction was carried out under stirring at 1,000 rpm (PCMT Thermoshaker, Grant Instruments, Cambridge, UK). After exactly 120 min from the first introduction of FRAP solution onto solid samples, centrifugation was performed for 1 min at 20,800 x g, and the absorbance was measured at 593 nm. The final results were expressed as mmol Trolox equivalents/kg of sample (dw)

through a calibration curve (linearity range: 0.2-8 μg/mL; y=0.1663x+0.0078, R^2: 0.998). The analysis was carried out in triplicate (n=3).

2.4.8 Statistical analyses

One-way analysis of variance (ANOVA) was applied in order to compare wholemeal flours on the basis of cereal cultivar and, different pearled fractions within the same cereal cultivar. The REGW-Q test was performed for multiple comparisons. A 0.05 threshold was used to reject the null hypothesis.

Statistical analyses were carried out by means of SPSS for Windows statistical package, Version 25.0 (SPSS Inc., Chicago, Illinois).

3. RESULTS AND DISCUSSION

3.1 Field experiments, grain yields and chemical composition of the wholemeal flours

The cultivation of tritordeum for the production of health-valued foods is increasing in Italy. The present study compared two cultivars of tritordeum (cvs. Aucan and Bulel) selected in Southern Spain, with three cultivars of barley, durum wheat and common wheat. All cereals tested were grown under the same environmental conditions (Supplementary material - Figure S1) in an experimental area located in the North-West of Italy. The two cultivars of tritordeum showed a grain yield of 4.5-5.0 t/ha (Table 1), about two times higher than the one observed by Villegas et al. (2010) in different Mediterranean regions located in Spain, Lebanon and Tunisia, with higher drought stress. Nevertheless, in comparison to both barley and wheat, tritordeum presented minor yield, showing on average significantly lower TKW (39.4 g) than both durum and common wheat (47.9 and 46.8 g, respectively). As far as the test weight was concerned tritordeum did not differ significantly from durum wheat (72.7 vs 72.9 kg/hL), while a significant higher value was recorded for the common wheat cultivar. Both TKW and TW observed for tritordeum were in accordance with previous studies performed on this cereal (Alvarez, Ballesteros, Sillero & Martín, 1992; Martín et al., 1999), highlighting that at present the cultivars of this new cereal resulted in lower values than wheat for these grain qualitative parameters.

The wholemeal flour of tritordeum was characterized by the highest protein content (14.3% dw). The two varieties of tritordeum showed a TDF content similar to the one of durum and common wheat. Cv. Bulel showed a significantly higher TDF (14.7% dw) than cv. Aucan (12.2% dw). As expected, the highest TDF was observed in the wholemeal flour of barley, because of the presence of the hulls covering the grain. The content of β-glucans of tritordeum was higher than the one of durum wheat (0.652% dw vs 0.389% dw). Nevertheless, both Aucan and Bulel cvs. showed a β-glucan content 24% lower than common wheat and 5 folds lower than the six-row barley cultivar. Similar concentrations of

β-glucans were observed previously for other 5 tritordeum lines grown in Cordoba (Rakha, Saulnier, Åman & Andersson, 2012), confirming the low β-glucan content of this novel cereal. Contrarily, the content of total arabinoxylans in tritordeum was significantly higher than the one observed in all the other cereal tested, and the highest concentration was observed in the cv. Aucan (2.15% dw).

The antioxidant capacity, determined by means of the DPPH and FRAP assays and performed directly on solid samples (Gökmen et al., 2009), was the highest in the wholemeal flour of barley (11.6 and 35.3 mmol Trolox eq/kg dw, respectively). The wholemeal flour of tritordeum did not differ significantly from durum and common wheat. Nevertheless, significant differences were observed in the concentration antioxidant compounds, such as phenolic acids and xanthophylls.

Limited information is available about the concentration and the composition of phenolic acids in tritordeum (Eliášová & Paznocht, 2017; Navas-Lopez et al., 2014). The present study measured the concentration of individual phenolic acids across soluble (free and conjugated phenolic acids) and cell wall-bound fractions. Like other cereals, the content of cell wall-bound phenolic acids of tritordeum was higher than that of soluble phenolic acids. Durum wheat showed the highest SPA (Soluble Phenolic Acids) content but the lowest concentration of total CWBPAs (Cell Wall-Bound Phenolic acids), while an opposite trend was observed in barley. Tritordeum showed a concentration of soluble phenolic acids 1.9 folds higher than barley, but 42% lower than durum wheat. An opposite trend was observed for cell wall-bound phenolic acids: tritordeum showed a concentration of CWBPAs 1.6 folds higher than that of durum wheat, but 32% lower than barley. The concentration of SPAs and CWBPAs in tritordeum was 33% higher and 12% lower than common wheat. As reported in Figure 1A, which shows the chromatogram at 280 nm of soluble phenolic acids of cv. Bulel, the main soluble phenolic acids detected in tritordeum were sinapic acid, followed by ferulic, vanillic, syringic, *p*-hydroxybenzoic and *p*-coumaric acid. On the contrary, ferulic acid was

319 the predominant component of cell wall-bound phenolic acids (Figure 1B), followed by
320 sinapic, *p*-coumaric, caffeic, syringic, vanillic and *p*-hydroxybenzoic acids. Moreover, the
321 concentration ratio of these compounds varies according to the cereal species, and the
322 phenolic acid profile of tritordeum was clearly closer to the one observed for the durum and
323 common wheat cultivar than that of barley (Figure 1C, D). As far as the two main phenolic
324 acids are concerned, in the soluble fraction, the sinapic/ferulic (S/F) acid ratio was 3 both in
325 tritordeum and durum wheat. S/F ratio decreased to 2 in common wheat and to 1 in barley.
326 Concerning the cell wall-bound fraction, tritordeum and durum wheat showed a F/S ratio of
327 18 and 15, respectively. Higher F/S ratios where observed in both barley (67) and common
328 wheat (23). It is worth noting that the barley cultivar tested in the present study showed a
329 soluble and cell wall-bound phenolic acid profile totally different from the one observed in
330 tritordeum and wheat: vanillic acid and *p*-coumaric acids were 23 and 7% of SPAs,
331 respectively; while cell wall-bound *p*-coumaric acid was even higher than sinapic acid
332 because of the presence of the hulls around the kernel (Butsat & Siriamornpun, 2010).
333 Previous studies showed that tritordeum is characterized by a high proportion of lutein
334 esterified with fatty acids (Atienza, Ballesteros, Martín & Hornero-Méndez, 2007; Rodríguez-
335 Suarez, Mellado-Ortega, Hornero-Méndez & Atienza, 2014; Mellado-Ortega and Hornero-
336 Méndez, 2018). The esterification is supposed to increase lutein stability during storage and
337 at high temperatures, thus improving lutein retention through the food chain. All the samples
338 analyzed in the present study were subjected to saponification with KOH in order to obtain
339 free xanthophylls before chromatographic analysis. The concentration of lutein observed in
340 the tritordeum cultivars tested in the present study was similar to the one detected by
341 Mattera, Hornero-Méndez and Atienza (2017). The highest lutein concentration was
342 detected in the wholemeal flour of cv. Bulel (6.14 mg/kg dw); on the contrary the cv. Aucan
343 showed a significant lower content of lutein (4.54 mg/kg dw), which did not differ significantly
344 from durum wheat (4.58 mg/kg dw). The lowest concentration was detected in common

345 wheat and barley, characterized by a lutein content 3 folds lower than the one detected in
346 the cv. Bulel. The concentrations of zeaxanthin detected in the two cultivars of tritordeum
347 tested in the present study were lower than the one detected in other lines of tritordeum
348 (Paznocht et al., 2018). According to previous studies which showed that *H. chilense* has a
349 higher concentration of zeaxanthin than tritordeum (Mellado-Ortega and Hornero-Méndez,
350 2015), the cultivar of barley tested in the present study showed a concentration of
351 zeaxanthin about 3 folds higher than tritordeum.
352
353 **3.2 Distribution of dietary fiber components in pearled fractions**
354 As demonstrated by several studies (Beta et al., 2005; Giordano et al., 2017; Liyana-
355 Pathirana, Dexter & Shahidi, 2006), bioactive compounds are unevenly distributed in the
356 grains and the distribution pattern depends on both the type of cereal and the class of
357 nutrient considered. Tritordeum and other small cereals can be commercialized in different
358 ways from whole-grain to refined flour. Nevertheless, at present no one has analyzed the
359 distribution of bioactives in the pearled fractions of tritordeum, thus exploring alternative
360 ways of using this cereal for the production of health-valued foods.
361 The distribution of ash, protein and dietary fiber components observed in the present study
362 is shown in Table 2. In accordance with previous studies (Fardet, 2010; Zanoletti et al.,
363 2017), TDF decreased progressively from the external to the internal layers of both
364 tritordeum, barley and wheat kernels. As expected, the highest concentration was observed
365 in the first two pearled fractions of barley, characterized respectively by 83.0 and 79.6% of
366 TDF, as they correspond mainly to the hulls which cover the kernel. Unlike TDF, the
367 distribution of β-glucans differed depending on the cereal species. The two cultivars of
368 tritordeum tested were closer to durum wheat in terms of distribution of β-glucans, showing
369 the highest β-glucan concentration in the intermediated layers of the kernel (from 10-15% to
370 20-25% pearled fractions). A different distribution pattern was observed in the common

wheat cultivar, which showed the highest content of β-glucans in the 5-10% pearled fraction and a gradual decrease moving toward the endosperm. In agreement with previous studies (Blandino et al., 2015), the concentration of β-glucans in barley was the lowest in the outermost pearled fractions and the highest in the residual pearled kernel (3.94% w/w). Contrary to TDF and β-glucans, total arabinoxylans were uniformly distributed in the pearled fractions of tritordeum. A similar distribution pattern was also observed in durum and common wheat. On the contrary, barley showed a gradual decrease of total arabinoxilans from the 0-5% to the 25-30% pearled fraction.

3.3 Distribution of soluble and cell wall-bound phenolic acids in pearled fractions and their antioxidant capacity

In accordance with previous papers (Liyana-Pathirana et al., 2006; Giordano et al., 2017; Blandino et al., 2013) SPAs gradually decreased moving from the outermost pearled fractions towards the innermost one. Interestingly, as shown in Table 3, the relative proportion of these compounds vary not only according to the cereal species, but also depending on the pearled fraction. The main soluble phenolic acid observed in the pearled fractions of tritordeum was sinapic acid, which represent on average more than 60% of SPAs in each fraction. The concentration of sinapic acid decreased moving towards the internal layers of the kernel, in fact the lowest content was observed in the residual pearled kernel. A similar distribution pattern was observed for ferulic acid. Nevertheless, the S/F ratio was different depending on the pearled fraction and decrease from 4 to 2 moving from the 0-5% pearled fraction to the 40-100% residual pearled kernel. All the other phenolic acids detected represented less than 10% of SPAs regardless of the pearled fraction, and their concentration usually decreased from the outermost to the innermost kernel layers. The common and durum wheat cultivars tested showed a similar distribution pattern of phenolic acids in their pearled fractions, even if the proportion of individual compounds was a bit

different (i.e. sinapic acid represent from 61 to 70% of SPAs in durum wheat, while in common wheat only from 51 to 58%). The barley cultivar showed a characteristic phenolic acid profile and distribution. The 0-5% and 5-10% pearled fractions, which mainly corresponds to the hulls, showed not only a low concentration of SPAs (47.9 and 63.6 mg/kg dw, respectively), but they also differed completely from the other fractions for their phenolic acid profile: ferulic acid represents 27% of SPAs, *p*-coumaric acid 20%, vanillic acid 20%, sinapic acid 17%, syringic acid <10%, *p*-hydroxybenzoic acid <10%. Contrary to all the other cereals, the phenolic acid profile observed from the 10-15% pearled fraction to the residual pearled kernel was not characterized by a clear prevalence of sinapic acid. In fact, in the 10-15% pearled fraction sinapic acid was only 31% of SPAs, while ferulic and vanillic acids represented 20 and 32% of SPAs, respectively. The same phenolic acids were 20, 44 and 22% of SPAs, respectively, in the 40-100% residual pearled kernel. The highest content of SPAs and of the three main soluble phenolic acids was observed in the 15-20% pearled fraction, then a significant and gradual decrease of the concentration of these compounds was observed at each pearling step.

The content of CWBPAs decreased from the outermost to the innermost layers of the kernels regardless of the cereal species (Table 4). Both cv. Aucan and cv. Bulel showed a peculiar distribution pattern of cell wall-bound phenolic acids in their pearled fractions. In fact, tritordeum showed a higher retention of CWBPAs in the residual pearled kernel when compared to both the durum and common wheat cultivar. As far as the 0-5% and 5-10% pearled fractions are concerned, the concentration of CWBPAs of tritordeum was on average 39% lower than the one observed in the same fractions of the common wheat cultivar, whereas in the residual pearled kernel the concentration of CWBPAs was 36% higher in tritordeum. The high content of cell wall-bound phenolic acids in the internal layers of the kernel of tritordeum makes both whole-grain and refined flour, derived from this novel

422 cereal, interesting ingredients for the production of functional foods, especially given the role
423 that cell wall-bound phenolic acid may have on human health (Fardet, 2010).
424 Contrary to soluble phenolic acids, genotypes showed less variation in the relative
425 percentage of individual cell wall-bound phenolic acids in each pearled fraction. Ferulic acid
426 was the main cell wall-bound phenolic acid in all the pearled fractions, representing more
427 than 80% of CWBPAs. The only exception was observed in the 0-5% and 5-10% pearled
428 fractions of barley. In these two fractions, characterized by the highest CWBPA content
429 (5027 and 5857 mg/kg dw, respectively), sinapic acid was not detected, while ferulic and *p*-
430 coumaric acids were more than 98% of CWBPAs (49% both). A high concentration of *p*-
431 coumaric acid (30% of CWBPAs) was observed also in the 10-15% pearled fraction,
432 probably due to the presence of hull residues (Hernanz et al., 2001; Nordkvist, Salomonsson
433 & Åman, 1984).
434 Although phenolic acids are among the main antioxidant compounds of cereals (Adom &
435 Liu, 2002; Beta et al., 2005), many other compounds may have antioxidant properties
436 (Cömert & Gökmen, 2017), therefore extraction-independent procedures in association with
437 the DPPH and FRAP assays were carried out for the analysis of the antioxidant capacity of
438 the pearled fractions (Figure 2A and B). As expected, both methods highlighted the higher
439 antioxidant capacity in the outer layers of the kernel regardless of the cereal species. Even
440 if the 0-5% and 5-10% pearled fractions of the barley cultivar showed the highest
441 concentration of CWBPAs, their antioxidant activity was lower than other barley fractions.
442 Concerning the residual pearled kernel, the 40-100% residue of barley was characterized
443 by the highest antioxidant capacity (AC_{DPPH}: 5.36 mmol Trolox eq/kg dw; AC_{FRAP}: 9.89 mmol
444 Trolox eq/kg dw) in comparison to the other cereals tested (AC_{DPPH}: 2.40 mmol Trolox eq/kg
445 dw; AC_{FRAP}: 3.81 mmol Trolox eq/kg dw, average values), even if it was not the one
446 characterized by the highest levels of both SPAs and CWBPAs, confirming that several
447 compounds may influence the antioxidant potential of a raw material. The antioxidant

capacity of the residual pearled kernel of tritordeum was higher than the same fraction of both durum and common wheat. In particular, cv. Aucan showed an AC_{FRAP} equal to 4.89 mmol Trolox eq/kg dw, which was 54% and 40% higher than observed in the same fraction of durum and common wheat, respectively. The antioxidant capacity was also higher than that observed in the cv. Bulel (+34%), suggesting an intraspecific variability.

3.4 Distribution of xanthophylls in pearled fractions

As observed for the wholemeal flour, lutein was the main xanthophyll detected in each pearled fraction regardless of the cereal species. Tritordeum showed higher levels of lutein than barley, durum wheat and common wheat in all the pearled fractions (Figure 2C). Moreover, the comparison of the two cultivars of tritordeum showed that cv. Bulel was characterized by higher concentration of lutein than cv. Aucan, with the exception of the 0-5% fraction. The residual pearled kernel of cv. Bulel showed a lutein content even 48% higher than observed in the same fraction of cv. Aucan, confirming that differences may occur among tritordeum genotypes for their lutein content (Atienza et al., 2007). Mellado-Ortega and Hornero-Méndez (2018) showed that carotenoids are homogeneously distributed among the germ fraction (7.1% of the grain weight) and the residual kernel (92.9% of the grain weight) of tritordeum. The pearling process carried out in the present study highlights that an unevenly distribution of lutein occurs moving towards the innermost layers of kernels of tritordeum. In fact, after an initial increase in the concentration of lutein moving from the outermost pearled fraction to the intermediated ones, a significant decrease in the concentration was observed in the residual pearled kernel (-26% cv. Aucan; -10% cv. Bulel). A similar distribution pattern was observed in barley (27% drop in the residual pearled kernel). Contrarily, both the durum and common wheat cultivars did not show any significant decrease in their lutein content after the last pearling step. Therefore, even if cv. Aucan showed from 26 to 48% more lutein than cv. Saragolla from the 0-5% to the 35-40% pearled

474 fractions, in the residual pearled kernel it was 11% lower (3.93 vs 4.4 mg/kg dw,
475 respectively).
476 In accordance with previous studies (Atienza et al., 2007; Mellado-Ortega and Hornero-
477 Méndez, 2012 and 2018), the concentration of zeaxanthin in tritordeum was the highest in
478 the intermediate pearled fractions and a gradual decrease was observed moving towards
479 the internal layers of the kernel (Figure 2D). A similar distribution pattern was observed in
480 all the other cereals tested, and, as expected, barley showed the highest concentration of
481 zeaxanthin (2.52 mg/kg dw in the 15-20% fraction).
482

483 **4. CONCLUSIONS**

484 This study highlights that tritordeum could be an excellent raw material for the production of
485 health-valued foods. The cultivation of tritordeum in a Continental region, located in the north
486 of Italy, resulted in a grain yield about two times higher than the one observed in
487 Mediterranean regions characterized by higher drought stress (Villegas et al., 2010).
488 Nevertheless, a significant gap in the yield was observed between tritordeum and all the
489 other cereal tested. Further studies are necessary to estimate the yield of tritordeum in a
490 wider range of locations, and to improve its yield by means of both breeding programs and
491 the optimization of the agricultural practices. Both the wholemeal flour and the pearling
492 fractions of tritordeum turned out to be interesting as far as total arabinoxylans, lutein and
493 phenolic acids are concerned. Therefore, tritordeum has several potential end-uses in the
494 production of health-valued foods. A better understanding of antioxidant value of different
495 pearled fractions will provide millers critical information to identify the best way to use
496 tritordeum for the production of health-valued ingredients or food products. As for other
497 cereals, the distribution of bioactive compounds in the pearled fractions points out that the
498 use of whole-grain flours of tritordeum is the best way to exploit its antioxidant potential,
499 since a reduction in the concentration of phenolic acids occur after removing the outer layers

of the kernel. Nevertheless, the high concentration of antioxidant compounds in the internal layers of tritordeum makes this cereal interesting also for the production of refined flour rich in antioxidant compounds, even if a highly refined flour could result in a reduction of the concentration of lutein.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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TABLES AND FIGURES

Table 1. Kernel traits and chemical composition of the wholemeal flours of tritordeum, barley, durum and common wheat.

Cereal	Cultivar	Grain yield	TKW	TW	Ash	Proteins	TDF	β-glucans	TAX	SPAs[1]	CWBPAs[2]	Lutein	Zeaxanthin	AC_{DPPH}	AC_{FRAP}
		t/ha	g	kg/hL	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mmol Trolox eq/kg	mmol Trolox eq/kg
Tritordeum	Aucan	5.0 ^{c}	40.3 ^{b}	72.7 ^{b}	1.66 ^{bc}	14.4 ^{a}	12.2 ^{c}	0.691 ^{c}	2.15 ^{a}	64.3 ^{b}	976 ^{b}	4.54 ^{b}	0.438 ^{c}	4.01 ^{b}	7.84 ^{b}
Tritordeum	Bulel	4.5 ^{c}	38.5 ^{b}	72.7 ^{b}	1.48 ^{c}	14.1 ^{a}	14.7 ^{b}	0.614 ^{d}	1.71 ^{b}	51.7 ^{c}	767 ^{c}	6.14 ^{a}	0.513 ^{bc}	3.98 ^{b}	8.25 ^{b}
Barley	Ketos	7.5 ^{a}	37.9 ^{b}	60.9 ^{c}	2.30 ^{a}	9.60 ^{d}	25.2 ^{a}	3.46 ^{a}	1.27 ^{c}	31.2 ^{d}	1283 ^{a}	2.13 ^{c}	1.41 ^{a}	11.6 ^{a}	35.3 ^{a}
Durum wheat	Saragolla	6.2 ^{b}	47.9 ^{a}	72.9 ^{b}	1.85 ^{b}	12.5 ^{b}	12.2 ^{c}	0.389 ^{e}	1.06 ^{d}	99.6 ^{a}	539 ^{d}	4.58 ^{b}	0.450 ^{c}	3.81 ^{b}	7.60 ^{b}
Common wheat	Illico	8.0 ^{a}	46.8 ^{a}	81.2 ^{a}	1.52 ^{c}	11.7 ^{c}	13.0 ^{bc}	0.853 ^{b}	1.35 ^{c}	43.6 ^{c}	985 ^{b}	2.20 ^{c}	0.612 ^{b}	3.43 ^{b}	8.05 ^{b}
SEM		0.3	1.2	0.6	0.05	0.08	0.3	0.015	0.05	2.4	34	0.18	0.026	0.15	0.69
P (F)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

TKW, thousand kernel weight; TW, test weight; TDF, total dietary fiber; TAX: total arabinoxylans; SPAs, soluble phenolic acids (free and conjugated forms); CWBPAs, cell wall-bound phenolic acids; AC, antioxidant capacity determined by means of the DPPH and FRAP assays.

Composition is 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 SPAs determined by means of RP-HPLC/DAD.

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

Table 2. Ash, protein, total dietary fiber (TDF), β-glucan and total arabinoxylans (TAX) content of the pearled fractions of tritordeum, barley, durum and common wheat.

Cereal (Cultivar)	Pearled fraction	Ash	Proteins	TDF	β-glucans	TAX
		%	%	%	%	%
Tritordeum (Aucan)	0-5%	2.51 ^{b}	14.3 ^{e}	34.0 ^{a}	0.650 ^{c}	2.21 ^{a}
	5-10%	2.71 ^{ab}	14.9 ^{d}	27.1 ^{b}	0.840 ^{b}	1.92 ^{a}
	10-15%	2.81 ^{a}	15.4 ^{c}	22.1 ^{c}	0.888 ^{b}	2.03 ^{a}
	15-20%	2.86 ^{a}	16.5 ^{a}	19.7 ^{cd}	0.912 ^{b}	2.01 ^{a}
	20-25%	2.50 ^{b}	16.2 ^{ab}	17.4 ^{de}	1.02 ^{a}	2.00 ^{a}
	25-30%	2.50 ^{b}	16.5 ^{a}	16.7 ^{ef}	0.882 ^{b}	2.14 ^{a}
	30-35%	2.19 ^{c}	15.9 ^{bc}	14.2 ^{fg}	0.869 ^{b}	2.08 ^{a}
	35-40%	2.06 ^{c}	15.9 ^{bc}	12.5 ^{g}	0.865 ^{b}	2.08 ^{a}
	40-100%	1.27 ^{d}	13.3 ^{f}	7.25 ^{h}	0.625 ^{c}	1.99 ^{a}
	SEM	0.07	0.1	0.53	0.025	0.07
	P (F)	<0.001	<0.001	<0.001	<0.001	0.151
Tritordeum (Bulel)	0-5%	3.11 ^{c}	14.1 ^{e}	36.0 ^{a}	0.619 ^{d}	1.61 ^{bc}
	5-10%	3.85 ^{a}	15.2 ^{d}	30.7 ^{b}	0.889 ^{b}	1.88 ^{a}
	10-15%	4.02 ^{a}	16.2 ^{bc}	29.0 ^{c}	0.992 ^{a}	1.67 ^{ab}
	15-20%	3.48 ^{b}	16.5 ^{b}	23.2 ^{d}	1.03 ^{a}	1.44 ^{cd}
	20-25%	2.79 ^{d}	16.9 ^{a}	18.2 ^{e}	0.992 ^{a}	1.36 ^{d}
	25-30%	2.70 ^{de}	16.4 ^{b}	16.4 ^{f}	0.894 ^{b}	1.41 ^{cd}
	30-35%	2.44 ^{e}	16.3 ^{b}	14.0 ^{g}	0.841 ^{bc}	1.74 ^{ab}
	35-40%	2.15 ^{f}	15.9 ^{c}	11.8 ^{h}	0.801 ^{c}	1.63 ^{bc}
	40-100%	1.18 ^{g}	13.4 ^{f}	9.42 ^{i}	0.488 ^{e}	1.63 ^{bc}
	SEM	0.07	0.1	0.30	0.018	0.05
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.01
Barley (Ketos)	0-5%	7.88 ^{a}	5.09 ^{g}	83.0 ^{a}	0.224 ^{f}	6.18 ^{a}
	5-10%	5.51 ^{b}	6.34 ^{f}	79.6 ^{b}	0.455 ^{e}	3.58 ^{b}
	10-15%	5.48 ^{b}	11.8 ^{d}	60.3 ^{c}	1.56 ^{d}	2.91 ^{c}
	15-20%	5.02 ^{c}	15.4 ^{a}	38.4 ^{d}	2.87 ^{c}	3.10 ^{c}
	20-25%	3.63 ^{d}	14.9 ^{a}	25.9 ^{e}	3.25 ^{b}	1.99 ^{d}
	25-30%	3.08 ^{e}	13.7 ^{b}	22.6 ^{e}	3.23 ^{b}	1.54 ^{e}
	30-35%	2.61 ^{f}	12.8 ^{c}	18.5 ^{f}	3.29 ^{b}	1.48 ^{e}
	35-40%	2.18 ^{g}	12.2 ^{d}	16.1 ^{f}	3.36 ^{b}	1.50 ^{e}
	40-100%	1.01 ^{h}	8.63 ^{e}	9.80 ^{g}	3.94 ^{a}	1.17 ^{e}
	SEM	0.08	0.14	0.62	0.038	0.10
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Durum wheat (Saragolla)	0-5%	2.78 ^{ef}	13.4 ^{bc}	31.7 ^{a}	0.387 ^{e}	0.862 ^{e}
	5-10%	3.35 ^{d}	13.6 ^{bc}	30.0 ^{a}	0.482 ^{d}	0.994 ^{cd}
	10-15%	3.75 ^{b}	13.7 ^{abc}	25.3 ^{b}	0.665 ^{abc}	1.02 ^{bcd}
	15-20%	4.18 ^{a}	14.3 ^{a}	21.4 ^{c}	0.709 ^{a}	1.08 ^{abc}
	20-25%	3.72 ^{bc}	14.0 ^{ab}	17.5 ^{d}	0.684 ^{ab}	1.17 ^{a}
	25-30%	3.47 ^{cd}	13.6 ^{bc}	14.7 ^{de}	0.684 ^{ab}	1.12 ^{ab}
	30-35%	2.92 ^{e}	13.3 ^{c}	13.0 ^{ef}	0.642 ^{bc}	1.06 ^{abc}
	35-40%	2.64 ^{f}	13.3 ^{c}	11.1 ^{f}	0.619 ^{c}	0.942 ^{de}
	40-100%	1.27 ^{g}	10.9 ^{d}	5.84 ^{g}	0.288 ^{f}	1.11 ^{ab}
	SEM	0.07	0.2	0.62	0.014	0.027
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Common wheat (Illico)	0-5%	3.23 ^{c}	10.4 ^{g}	58.0 ^{a}	1.20 ^{d}	1.31 ^{d}
	5-10%	4.13 ^{a}	15.0 ^{bc}	37.2 ^{b}	1.76 ^{a}	1.38 ^{cd}
	10-15%	3.61 ^{b}	15.9 ^{a}	27.2 ^{c}	1.60 ^{b}	1.55 ^{bc}
	15-20%	2.96 ^{d}	15.4 ^{ab}	18.6 ^{d}	1.41 ^{c}	1.69 ^{ab}
	20-25%	2.17 ^{e}	14.4 ^{cd}	16.1 ^{de}	1.18 ^{d}	1.74 ^{a}
	25-30%	2.13 ^{e}	14.1 ^{de}	12.9 ^{ef}	1.07 ^{e}	1.66 ^{ab}
	30-35%	1.80 ^{f}	13.5 ^{ef}	10.6 ^{fg}	0.960 ^{f}	1.55 ^{bc}
	35-40%	1.58 ^{f}	12.9 ^{f}	9.55 ^{fg}	0.959 ^{f}	1.58 ^{ab}
	40-100%	0.775 ^{g}	10.4 ^{g}	7.18 ^{g}	0.584 ^{g}	1.24 ^{d}
	SEM	0.057	0.2	0.60	0.018	0.04
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001

Data are expressed on a dw basis. For each cereal cultivar, 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.

Table 3. The main soluble phenolic acids (free and conjugated forms) detected in the pearled fractions of tritordeum, barley, durum and common wheat.

Cereal (Cultivar)	Pearled fraction	Sinapic acid	Ferulic acid	Vanillic acid	*p*-Coumaric acid	SPAs[1]
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Tritordeum (Aucan)	0-5%	109 ^{a}	29.4 ^{a}	12.3 ^{a}	4.31 ^{a}	170 ^{a}
	5-10%	114 ^{a}	29.2 ^{a}	11.7 ^{a}	3.34 ^{b}	172 ^{a}
	10-15%	109 ^{a}	29.2 ^{a}	10.8 ^{b}	3.06 ^{c}	166 ^{a}
	15-20%	90.1 ^{b}	25.5 ^{b}	9.54 ^{c}	2.60 ^{d}	139 ^{b}
	20-25%	79.1 ^{c}	23.9 ^{bc}	8.78 ^{d}	2.28 ^{e}	125 ^{c}
	25-30%	80.0 ^{c}	26.2 ^{b}	9.30 ^{cd}	2.39 ^{de}	129 ^{bc}
	30-35%	63.9 ^{d}	22.0 ^{cd}	7.92 ^{e}	2.00 ^{f}	106 ^{d}
	35-40%	56.6 ^{e}	20.5 ^{d}	7.21 ^{f}	1.84 ^{f}	95.0 ^{e}
	40-100%	20.2 ^{f}	9.16 ^{e}	2.98 ^{g}	1.01 ^{g}	36.8 ^{f}
	SEM	1.6	0.58	0.16	0.07	2.6
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Tritordeum (Bulel)	0-5%	124 ^{c}	33.9 ^{b}	15.1 ^{a}	6.32 ^{a}	195 ^{b}
	5-10%	145 ^{a}	37.2 ^{a}	14.3 ^{b}	4.52 ^{b}	217 ^{a}
	10-15%	130 ^{b}	34.3 ^{b}	12.1 ^{c}	3.55 ^{c}	193 ^{b}
	15-20%	106 ^{d}	29.6 ^{c}	10.4 ^{d}	2.83 ^{d}	160 ^{c}
	20-25%	84.4 ^{e}	25.3 ^{d}	8.79 ^{e}	2.21 ^{e}	130 ^{d}
	25-30%	64.2 ^{f}	20.6 ^{e}	7.27 ^{f}	1.82 ^{f}	101 ^{e}
	30-35%	56.0 ^{g}	18.8 ^{f}	6.66 ^{g}	1.61 ^{g}	89.7 ^{f}
	35-40%	43.9 ^{h}	15.6 ^{g}	5.59 ^{h}	1.34 ^{h}	72.1 ^{g}
	40-100%	14.2 ^{i}	7.65 ^{h}	2.38 ^{i}	0.776 ^{i}	27.2 ^{h}
	SEM	1.3	0.37	0.11	0.041	1.8
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Barley (Ketos)	0-5%	6.28 ^{g}	12.8 ^{f}	9.60 ^{d}	10.7 ^{b}	47.9 ^{g}
	5-10%	12.8 ^{f}	17.3 ^{e}	12.3 ^{cd}	11.6 ^{a}	63.6 ^{ef}
	10-15%	39.7 ^{b}	26.1 ^{b}	41.3 ^{a}	7.95 ^{c}	129 ^{b}
	15-20%	60.1 ^{a}	32.1 ^{a}	43.4 ^{a}	4.84 ^{d}	158 ^{a}
	20-25%	41.8 ^{b}	25.2 ^{b}	31.2 ^{b}	3.39 ^{e}	114 ^{c}
	25-30%	31.4 ^{c}	22.2 ^{c}	14.3 ^{c}	2.43 ^{f}	79.8 ^{d}
	30-35%	24.1 ^{d}	20.0 ^{d}	13.5 ^{cd}	2.03 ^{fg}	67.0 ^{e}
	35-40%	18.6 ^{e}	17.9 ^{e}	10.6 ^{cd}	1.77 ^{g}	55.0 ^{fg}
	40-100%	3.22 ^{g}	6.92 ^{g}	3.41 ^{e}	0.668 ^{h}	15.7 ^{h}
	SEM	0.88	0.48	1.10	0.154	2.2
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Durum wheat (Saragolla)	0-5%	110 ^{e}	40.3 ^{d}	9.70 ^{d}	4.05 ^{a}	176 ^{de}
	5-10%	174 ^{c}	56.4 ^{c}	10.9 ^{c}	3.84 ^{a}	256 ^{c}
	10-15%	208 ^{b}	65.8 ^{b}	12.2 ^{b}	3.87 ^{a}	302 ^{b}
	15-20%	244 ^{a}	74.4 ^{a}	13.2 ^{a}	4.04 ^{a}	349 ^{a}
	20-25%	202 ^{b}	62.9 ^{b}	11.4 ^{bc}	3.54 ^{b}	291 ^{b}
	25-30%	165 ^{c}	52.2 ^{c}	9.84 ^{d}	3.04 ^{c}	240 ^{c}
	30-35%	128 ^{d}	41.5 ^{d}	8.09 ^{e}	2.57 ^{d}	188 ^{d}
	35-40%	102 ^{e}	34.1 ^{e}	6.95 ^{f}	2.20 ^{e}	153 ^{e}
	40-100%	23.6 ^{f}	9.79 ^{f}	2.22 ^{g}	0.907 ^{f}	38.8 ^{f}
	SEM	3.8	1.30	0.23	0.056	5.5
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Common wheat (Illico)	0-5%	97.8 ^{b}	38.8 ^{b}	15.1 ^{a}	5.34 ^{a}	174 ^{b}
	5-10%	105 ^{a}	45.7 ^{a}	14.0 ^{b}	4.44 ^{b}	184 ^{a}
	10-15%	71.4 ^{c}	36.4 ^{b}	11.0 ^{c}	3.00 ^{c}	134 ^{c}
	15-20%	57.5 ^{d}	30.7 ^{c}	9.15 ^{d}	2.28 ^{d}	110 ^{d}
	20-25%	44.9 ^{e}	25.1 ^{d}	7.32 ^{e}	1.87 ^{e}	87.1 ^{e}
	25-30%	36.7 ^{f}	21.5 ^{e}	6.12 ^{f}	1.50 ^{f}	72.0 ^{f}
	30-35%	35.3 ^{f}	20.3 ^{ef}	5.67 ^{g}	1.48 ^{f}	68.2 ^{fg}
	35-40%	31.4 ^{f}	17.9 ^{f}	4.91 ^{h}	1.22 ^{g}	60.1 ^{g}
	40-100%	15.0 ^{g}	6.72 ^{g}	1.76 ^{i}	0.520 ^{h}	25.7 ^{h}
	SEM	1.6	0.63	0.09	0.043	2.2
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001

Data are expressed on a dw basis. For each cereal cultivar, 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 SPAs determined by means of the RP-HPLC/DAD.

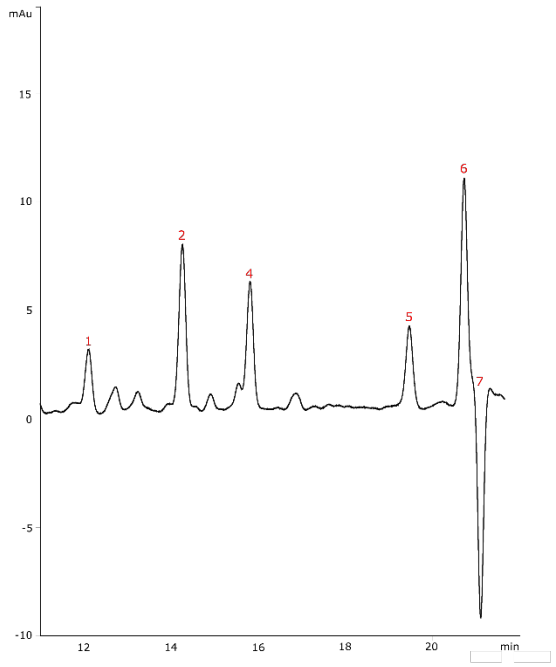
Table 4. The main cell wall-bound phenolic acids detected in the pearled fractions of tritordeum, barley, durum and common wheat.

Cereal (Cultivar)	Pearled fraction	Ferulic acid	Sinapic acid	*p*-Coumaric acid	Vanillic acid	CWBPAs[1]
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Tritordeum (Aucan)	0-5%	1409 ^{a}	72.3 ^{a}	136 ^{a}	15.2 ^{a}	1669 ^{a}
	5-10%	1427 ^{a}	70.9 ^{a}	70.3 ^{b}	12.2 ^{b}	1614 ^{a}
	10-15%	1331 ^{a}	68.1 ^{a}	44.4 ^{c}	9.80 ^{c}	1483 ^{b}
	15-20%	1204 ^{b}	56.6 ^{b}	33.4 ^{cd}	8.58 ^{d}	1327 ^{c}
	20-25%	1130 ^{bc}	51.9 ^{b}	28.5 ^{d}	7.08 ^{e}	1238 ^{cd}
	25-30%	1033 ^{cd}	43.4 ^{c}	26.1 ^{d}	6.58 ^{e}	1127 ^{de}
	30-35%	941 ^{de}	40.7 ^{c}	24.3 ^{de}	5.72 ^{f}	1027 ^{ef}
	35-40%	890 ^{e}	31.8 ^{d}	20.8 ^{de}	5.12 ^{f}	962 ^{f}
	40-100%	486 ^{f}	21.7 ^{e}	11.5 ^{e}	3.14 ^{g}	529 ^{g}
	SEM	28	1.4	3.1	0.17	30
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Tritordeum (Bulel)	0-5%	1406 ^{ab}	77.0 ^{ab}	96.9 ^{a}	14.9 ^{a}	1643 ^{a}
	5-10%	1586 ^{a}	83.3 ^{a}	42.8 ^{b}	10.5 ^{b}	1766 ^{a}
	10-15%	1519 ^{a}	65.5 ^{b}	34.7 ^{c}	8.47 ^{c}	1661 ^{a}
	15-20%	1294 ^{bc}	49.9 ^{c}	27.0 ^{d}	7.24 ^{d}	1403 ^{b}
	20-25%	1143 ^{cd}	45.3 ^{cd}	21.7 ^{de}	5.57 ^{e}	1235 ^{bc}
	25-30%	995 ^{de}	35.6 ^{de}	18.2 ^{ef}	4.88 ^{ed}	1069 ^{cd}
	30-35%	934 ^{e}	37.1 ^{de}	17.6 ^{ef}	4.63 ^{f}	1008 ^{d}
	35-40%	819 ^{e}	30.3 ^{ef}	14.7 ^{f}	4.06 ^{f}	880 ^{d}
	40-100%	470 ^{f}	20.2 ^{f}	7.43 ^{g}	2.92 ^{g}	506 ^{e}
	SEM	49	3.2	1.62	0.22	55
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Barley (Ketos)	0-5%	2398 ^{c}	n.d.	2564 ^{b}	25.3 ^{b}	5027 ^{b}
	5-10%	2976 ^{a}	n.d.	2804 ^{a}	30.5 ^{a}	5857 ^{a}
	10-15%	2729 ^{b}	50.7 ^{a}	1233 ^{c}	24.7 ^{b}	4098 ^{c}
	15-20%	1992 ^{d}	45.6 ^{a}	220 ^{d}	14.9 ^{c}	2326 ^{d}
	20-25%	1219 ^{e}	26.0 ^{b}	90.3 ^{de}	10.0 ^{d}	1374 ^{e}
	25-30%	923 ^{f}	19.3 ^{c}	72.0 ^{de}	7.58 ^{de}	1042 ^{ef}
	30-35%	793 ^{fg}	17.0 ^{cd}	62.4 ^{de}	6.26 ^{ef}	895 ^{f}
	35-40%	679 ^{g}	13.2 ^{d}	52.1 ^{de}	5.55 ^{ef}	765 ^{f}
	40-100%	305 ^{h}	6.69 ^{e}	16.5 ^{e}	3.37 ^{f}	338 ^{g}
	SEM	59	1.52	39.6	0.82	93
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Durum wheat (Saragolla)	0-5%	1059 ^{b}	56.3 ^{b}	75.0 ^{a}	13.8 ^{a}	1228 ^{b}
	5-10%	1123 ^{b}	63.5 ^{a}	35.8 ^{b}	10.6 ^{b}	1250 ^{ab}
	10-15%	1207 ^{a}	69.6 ^{a}	25.0 ^{c}	9.65 ^{b}	1326 ^{a}
	15-20%	1113 ^{b}	65.3 ^{a}	18.2 ^{d}	7.43 ^{c}	1217 ^{b}
	20-25%	929 ^{c}	51.7 ^{b}	14.5 ^{de}	6.18 ^{d}	1012 ^{c}
	25-30%	854 ^{d}	40.4 ^{c}	13.1 ^{ef}	5.57 ^{de}	922 ^{d}
	30-35%	725 ^{e}	32.8 ^{d}	10.8 ^{ef}	4.55 ^{ef}	781 ^{e}
	35-40%	599 ^{f}	27.6 ^{d}	9.04 ^{fg}	3.58 ^{f}	645 ^{f}
	40-100%	301 ^{g}	15.7 ^{e}	4.57 ^{g}	1.90 ^{g}	326 ^{g}
	SEM	17	1.6	1.28	0.26	20
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Common wheat (Illico)	0-5%	2561 ^{a}	84.8 ^{a}	116 ^{a}	22.1 ^{a}	2834 ^{a}
	5-10%	2492 ^{a}	78.7 ^{a}	68.3 ^{b}	13.3 ^{b}	2699 ^{a}
	10-15%	1587 ^{b}	42.4 ^{b}	40.8 ^{c}	8.71 ^{c}	1704 ^{b}
	15-20%	1412 ^{c}	43.2 ^{b}	36.2 ^{cd}	5.85 ^{de}	1516 ^{c}
	20-25%	1328 ^{c}	33.2 ^{c}	32.0 ^{d}	5.92 ^{d}	1415 ^{c}
	25-30%	1072 ^{d}	25.4 ^{cd}	25.5 ^{e}	4.80 ^{ef}	1140 ^{d}
	30-35%	914 ^{e}	23.9 ^{d}	21.3 ^{ef}	4.24 ^{f}	974 ^{de}
	35-40%	833 ^{e}	23.6 ^{d}	19.7 ^{f}	4.03 ^{f}	890 ^{e}
	40-100%	348 ^{f}	19.7 ^{d}	6.88 ^{g}	2.46 ^{g}	381 ^{f}
	SEM	39	2.0	1.36	0.27	43
	P (F)	<0.001	<0.001	<0.001	<0.001	<0.001

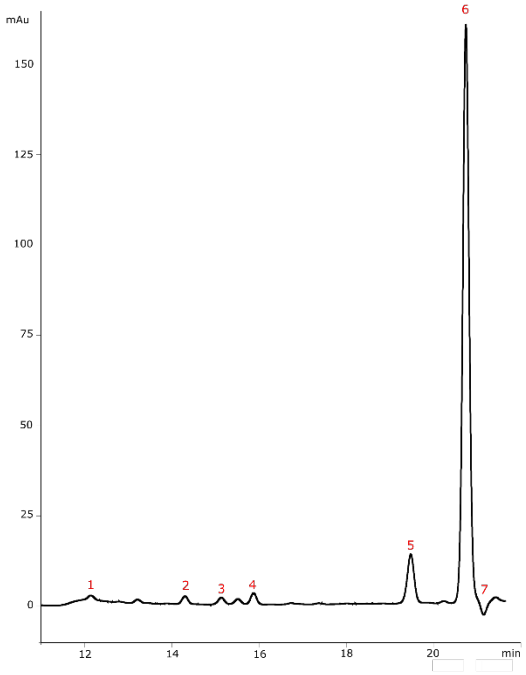
Data are expressed on a dw basis. For each cereal cultivar, means followed by different letters are significantly different, according to the REGW-Q test (the ANOVA level of significance is shown in the table).
SEM, standard error of the mean.
[1] sum of the CWBPAs determined by means of the RP-HPLC/DAD.

Figure 1. RP-HPLC/DAD chromatograms of soluble (A) and cell wall-bound phenolic acids (B) of the wholemeal flour of tritordeum (cv. Bulel). In the tables below is reported the distribution of individual phenolic acids (relative percentage) across soluble (C) and cell wall-bound (D) phenolic acid fractions for each type of cereal tested.

A



B



C

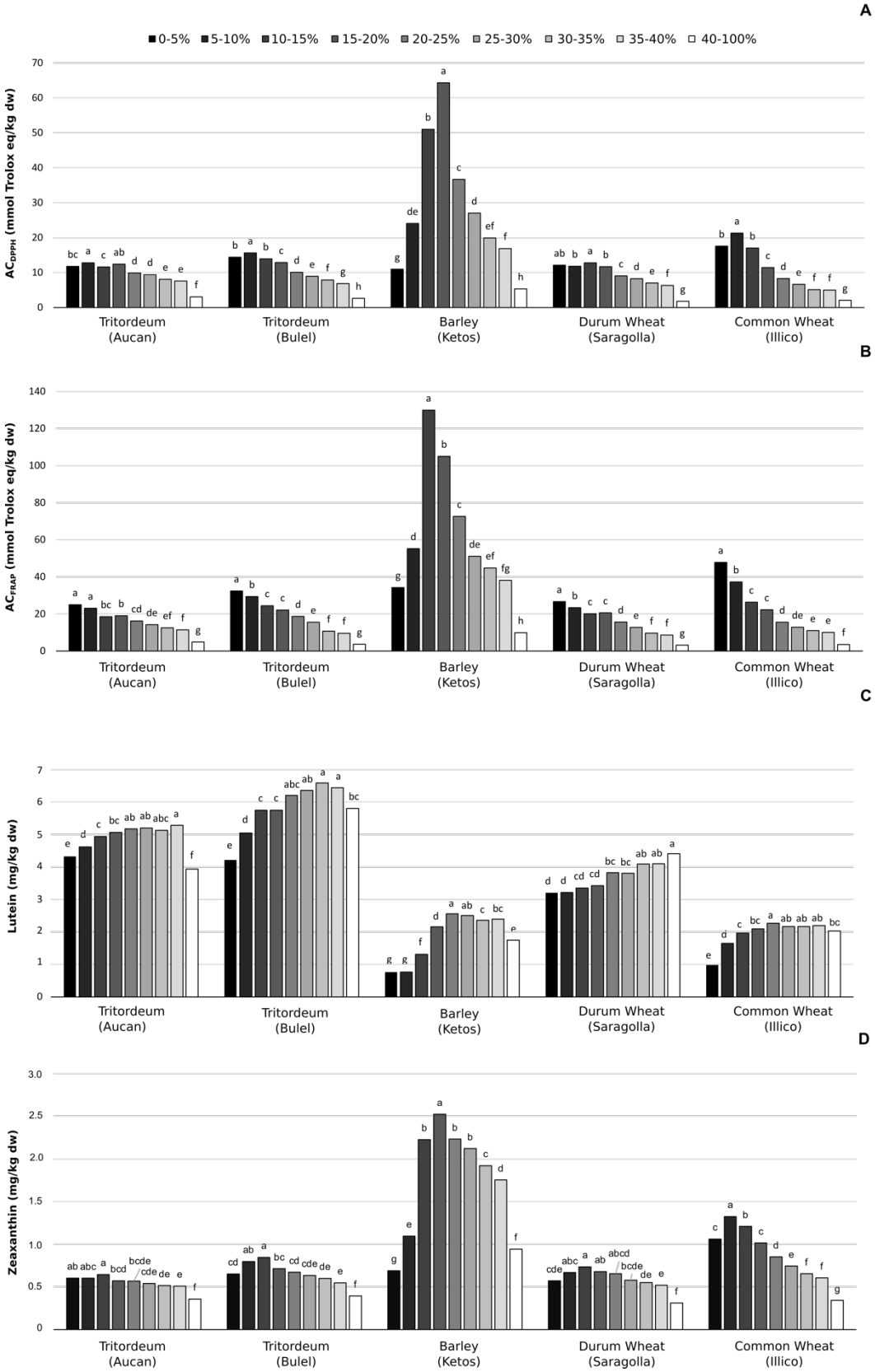
	% Phenolic acid					
Cereal (Cultivar)	**1**	**2**	**4**	**5**	**6**	**7**
Tritordeum (Aucan)	4	8	5	2	21	60
Tritordeum (Bulel)	4	9	4	3	22	59
Barley (Ketos)	6	23	5	7	34	25
Durum wheat (Saragolla)	3	5	2	2	23	66
Common wheat (Illico)	4	8	4	2	25	56

D

	% Phenolic acid						
Cereal (Cultivar)	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Tritordeum (Aucan)	<1	<1	<1	<1	3	89	6
Tritordeum (Bulel)	<1	<1	<1	<1	2	91	5
Barley (Ketos)	<1	<1	<1	<1	30	67	1
Durum wheat (Saragolla)	<1	<1	<1	<1	2	90	6
Common wheat (Illico)	<1	<1	<1	<1	2	91	4

The chromatograms reported are obtained at 280 nm: 1. *p*-Hydroxybenzoic acid; 2. Vanillic acid; 3. Caffeic acid; 4. Syringic acid; 5. *p*-Coumaric acid; 6. Ferulic acid; 7. Sinapic acid (quantified at 320 nm). The red to green gradient shows from the lowest to the highest relative percentage of phenolic acids within the same cereal.

Figure 2. Antioxidant capacity [AC, determined by means of DPPH (A) and FRAP (B) assays)] and xanthophyll [lutein (C) and zeaxanthin (D)] distribution in the pearled fractions of tritordeum, barley, durum and common wheat (the name of the cultivars is reported in brackets).



Data are expressed on a dw basis. For each cereal cultivar, bars overlooked by different letters are significantly different, according to the REGW-Q test.