

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Distribution of bioactive compounds in pearled fractions of tritordeum

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1720446> since 2019-12-27T12:59:04Z

Published version:

DOI:10.1016/j.foodchem.2019.125228

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)

1 **TITLE**

2 **Distribution of bioactive compounds in pearled fractions of tritordeum**

3

4 **AUTHORS**

5 Debora Giordano^{a*}, Amedeo Reyneri^a, Monica Locatelli^b, Jean Daniel Coïsson^b, Massimo
6 Blandino^a

7

8 **AFFILIATIONS**

9 ^a Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università degli Studi di
10 Torino, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy.

11 ^b Dipartimento di Scienze del Farmaco, Università degli Studi del Piemonte Orientale “A.
12 Avogadro”, Largo Donegani 2, 28100 Novara (NO), Italy.

13

14 *Corresponding author: Debora Giordano

15 Phone +39 011 6708833, debora.giordano@unito.it

16

17 **AUTHORS' E-MAIL ADRESSES**

18 Debora Giordano: debora.giordano@unito.it

19 Amedeo Reyneri: amedeo.reyneri@unito.it

20 Monica Locatelli: monica.locatelli@uniupo.it

21 Jean Daniel Coïsson: jeandaniel.coisson@uniupo.it

22 Massimo Blandino: massimo.blandino@unito.it

23

24

25

26 **ABSTRACT**

27 Hexaploid tritordeum is the amphidiploid cereal derived from the cross between wild barley
28 and durum wheat. The present study compares two cultivars of tritordeum with other cereals
29 grown in the same experimental area to weigh up its potential use as ingredient for health-
30 valued foods. Tritordeum shows 2.5-fold higher concentration of lutein than common wheat
31 and barley, and 1.2-fold higher than durum wheat, while the concentration of β -glucans is 5
32 folds lower than the one observed for barley. Based on the distribution of bioactive
33 compounds in pearled fractions, the use of whole-grain flours seems the best way to exploit
34 the antioxidant potential of tritordeum. Nevertheless, the internal layers of the kernel of this
35 cereal are characterized on average by high concentrations of antioxidants (32.0 mg/kg and
36 518 mg/kg soluble and cell wall-bound phenolic acids, respectively), making tritordeum
37 interesting also for the production of refined flours rich in bioactive compounds.

38

39

40 **KEYWORDS**

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

43

44

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 hullless 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 tocopherols (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

78 2. MATERIALS AND METHODS

79 2.1 Experimental design

80 The present study compared:

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

90 All the cereal cultivars were cultivated side by side on the same field in northwestern Italy
91 (Cigliano, Piedmont; 45°31'97"N, 8°4'77"E) in a completely randomized block design
92 with four replications. Field trials were carried out during the 2015-2016 growing season,
93 according to the ordinary crop management program applied to barley and wheat in the
94 growing area. The plot size was 7x1.5 m (10.5 m²), planting was performed in 12 cm
95 wide rows at a seeding rate of 450 seeds/m² on 6 November 2015, following an autumn
96 plowing (30 cm) and disk harrowing to prepare a proper seedbed. The previous crop was
97 maize. The nitrogen fertilization performed during the harvest season was in accordance
98 to the agronomic management usually carried out in the North of Italy for the cultivation
99 of barley, durum and common wheat. 170 kg N/ha were provided to plots of tritordeum
100 and durum wheat, split in 50 kg N/ha at the tillering stage (Growth stage - GS 23), 80 kg
101 N/ha at the beginning of stem elongation (GS 31), 40 kg N/ha at the heading stage (GS
102 55). 120 kg N/ha were provided to plots of barley and common wheat, split in 60 kg N/ha
103 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

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

135

136 **2.4 Chemical analyses**

137 **2.4.1 Chemicals**

138 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT, ≥99.0%),
139 ethanol (CHROMASOLV®, 99.8%), ethylacetate (CHROMASOLV®, 99.8%), hexane
140 (CHROMASOLV®, 97.0%), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
141 (Trolox, 97%), hydrochloric acid (HCl, 37.0%), methanol (CHROMASOLV®, 99.9%),
142 potassium hydroxide (KOH, 90.0%), sodium hydroxide (NaOH, ≥98.0%), *tert*-butyl methyl
143 ether (MTBE, CHROMASOLV®, 99.9%), *trans*-β-Apo-8'-carotenal, 2,4,6-Tris(2-pyridyl)-s-
144 triazine (TPTZ) and phenolic acid standards (caffeic acid ≥98%, *p*-coumaric acid ≥98%, *t*-
145 ferulic acid ≥99%, *p*-hydroxybenzoic acid ≥99%, sinapic acid ≥98%, syringic acid ≥95% and
146 vanillic acid ≥97%) were purchased from Sigma-Aldrich (St. Louis, Missouri, US). 3,5-
147 Dichloro-4-hydroxybenzoic acid (DHB) was purchased from Thermo Fisher (Waltham,
148 Massachusetts, US), while xanthophylls standards (lutein ≥95% and zeaxanthin ≥98%) were
149 purchased from Extrasynthese (Lyon, France).

150

151 **2.4.2 Proximate composition analysis**

152 The moisture content, determined in order to express all the results on a dry weight (dw)
153 basis, was obtained by oven-drying at 105 °C for 24 h. The moisture values are reported as
154 Supplementary Material in Table S1. The total protein content (conversion factor: 5.70) was
155 obtained according to the Kjeldahl method by means of a Kjelttec 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 μ L 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 μ L) for 2 h under continuous stirring at 4°C.
180 After acidification to pH 2 with HCl, soluble phenolic acids were extracted with 500 μ L 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

207 9% B in 3 min, and this was followed by 16 min of equilibration. The flow rate of the mobile
208 phase was 1 mL/min. Phenolic acids were identified using the retention times and the UV/Vis
209 spectra of their respective standards. Solutions of individual phenolic acid standards were
210 also prepared and diluted to different concentrations to obtain calibration curves for
211 quantification purposes. Retention time, detection wavelength and the principal parameters
212 of the calibration curves are reported as Supplementay Material in Table S2.

213

214 **2.4.5 Extraction of xanthophylls and quantification by means of RP-HPLC/DAD**

215 The extraction of xanthophylls was performed has previously reported in Giordano et al.
216 (2017). Each sample was analyzed in triplicate and *trans*- β -Apo-8'-carotenal was used as
217 internal standard to ensure that losses due to the extraction method were accounted for.
218 Samples (0.3 g) were extracted for 6 min at 85 °C with 95% ethanol, containing 1 g/L BHT.
219 The extracts, including solids, were hydrolyzed with 125 μ L of KOH (0.8 g/mL) at 85°C for
220 10 min, chilled on ice. Fifty microliters of the internal standard solution (4.5 μ g/mL) were
221 added prior the addition of 3 mL of cold deionized water. This was followed by the addition
222 of 3 mL of hexane, containing 1 g/L BHT. The test tubes were then vortexed and centrifuged
223 at 1,200 g for 10 minutes. The extraction was repeated four times, and the combined
224 supernatants were evaporated to dryness under a nitrogen stream, and then dissolved in
225 150 μ L of methanol:MTBE (1:1 v/v).

226 The chromatographic method was developed starting from the one proposed by Moros,
227 Darnoko, Cheyran, Perkins & Jerrel (2002). Separations were carried out using a 100 x 4.6
228 mm, 3 μ m, C30 carotenoid YMC column (YMC Co., Kyoto, Japan); the column temperature
229 was set at 35°C. The mobile phase consisted of methanol:MTBE:water [81:15:4, v/v;
230 (solvent A)] and MTBE:methanol [91:9, v/v; (solvent B)]. The following operating linear
231 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 μ g/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)

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

259

260 **2.4.8 Statistical analyses**

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

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

267 3. RESULTS AND DISCUSSION

268 3.1 Field experiments, grain yields and chemical composition of the wholemeal flours

269 The cultivation of tritordeum for the production of health-valued foods is increasing in Italy.
270 The present study compared two cultivars of tritordeum (cvs. Aucan and Bulel) selected in
271 Southern Spain, with three cultivars of barley, durum wheat and common wheat. All cereals
272 tested were grown under the same environmental conditions (Supplementary material -
273 Figure S1) in an experimental area located in the North-West of Italy. The two cultivars of
274 tritordeum showed a grain yield of 4.5-5.0 t/ha (Table 1), about two times higher than the
275 one observed by Villegas et al. (2010) in different Mediterranean regions located in Spain,
276 Lebanon and Tunisia, with higher drought stress. Nevertheless, in comparison to both barley
277 and wheat, tritordeum presented minor yield, showing on average significantly lower TKW
278 (39.4 g) than both durum and common wheat (47.9 and 46.8 g, respectively). As far as the
279 test weight was concerned tritordeum did not differ significantly from durum wheat (72.7 vs
280 72.9 kg/hL), while a significant higher value was recorded for the common wheat cultivar.
281 Both TKW and TW observed for tritordeum were in accordance with previous studies
282 performed on this cereal (Alvarez, Ballesteros, Sillero & Martín, 1992; Martín et al., 1999),
283 highlighting that at present the cultivars of this new cereal resulted in lower values than
284 wheat for these grain qualitative parameters.

285 The wholemeal flour of tritordeum was characterized by the highest protein content (14.3%
286 dw). The two varieties of tritordeum showed a TDF content similar to the one of durum and
287 common wheat. Cv. Bulel showed a significantly higher TDF (14.7% dw) than cv. Aucan
288 (12.2% dw). As expected, the highest TDF was observed in the wholemeal flour of barley,
289 because of the presence of the hulls covering the grain. The content of β -glucans of
290 tritordeum was higher than the one of durum wheat (0.652% dw vs 0.389% dw).
291 Nevertheless, both Aucan and Bulel cvs. showed a β -glucan content 24% lower than
292 common wheat and 5 folds lower than the six-row barley cultivar. Similar concentrations of

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

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

304 Limited information is available about the concentration and the composition of phenolic
305 acids in tritordeum (Eliášová & Paznocht, 2017; Navas-Lopez et al., 2014). The present
306 study measured the concentration of individual phenolic acids across soluble (free and
307 conjugated phenolic acids) and cell wall-bound fractions. Like other cereals, the content of
308 cell wall-bound phenolic acids of tritordeum was higher than that of soluble phenolic acids.

309 Durum wheat showed the highest SPA (Soluble Phenolic Acids) content but the lowest
310 concentration of total CWBPAs (Cell Wall-Bound Phenolic acids), while an opposite trend
311 was observed in barley. Tritordeum showed a concentration of soluble phenolic acids 1.9
312 folds higher than barley, but 42% lower than durum wheat. An opposite trend was observed
313 for cell wall-bound phenolic acids: tritordeum showed a concentration of CWBPAs 1.6 folds
314 higher than that of durum wheat, but 32% lower than barley. The concentration of SPAs and
315 CWBPAs in tritordeum was 33% higher and 12% lower than common wheat. As reported in
316 Figure 1A, which shows the chromatogram at 280 nm of soluble phenolic acids of cv. Bulel,
317 the main soluble phenolic acids detected in tritordeum were sinapic acid, followed by ferulic,
318 vanillic, syringic, *p*-hydroxybenzoic and *p*-coumaric acid. On the contrary, ferulic acid was

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

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

379

380 **3.3 Distribution of soluble and cell wall-bound phenolic acids in pearled fractions and** 381 **their antioxidant capacity**

382 In accordance with previous papers (Liyana-Pathirana et al., 2006; Giordano et al., 2017;
383 Blandino et al., 2013) SPAs gradually decreased moving from the outermost pearled
384 fractions towards the innermost one. Interestingly, as shown in Table 3, the relative
385 proportion of these compounds vary not only according to the cereal species, but also
386 depending on the pearled fraction. The main soluble phenolic acid observed in the pearled
387 fractions of tritordeum was sinapic acid, which represent on average more than 60% of SPAs
388 in each fraction. The concentration of sinapic acid decreased moving towards the internal
389 layers of the kernel, in fact the lowest content was observed in the residual pearled kernel.
390 A similar distribution pattern was observed for ferulic acid. Nevertheless, the S/F ratio was
391 different depending on the pearled fraction and decrease from 4 to 2 moving from the 0-5%
392 pearled fraction to the 40-100% residual pearled kernel. All the other phenolic acids detected
393 represented less than 10% of SPAs regardless of the pearled fraction, and their
394 concentration usually decreased from the outermost to the innermost kernel layers. The
395 common and durum wheat cultivars tested showed a similar distribution pattern of phenolic
396 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

cereal, interesting ingredients for the production of functional foods, especially given the role that cell wall-bound phenolic acid may have on human health (Fardet, 2010).

Contrary to soluble phenolic acids, genotypes showed less variation in the relative percentage of individual cell wall-bound phenolic acids in each pearled fraction. Ferulic acid was the main cell wall-bound phenolic acid in all the pearled fractions, representing more than 80% of CWBPAs. The only exception was observed in the 0-5% and 5-10% pearled fractions of barley. In these two fractions, characterized by the highest CWBPA content (5027 and 5857 mg/kg dw, respectively), sinapic acid was not detected, while ferulic and *p*-coumaric acids were more than 98% of CWBPAs (49% both). A high concentration of *p*-coumaric acid (30% of CWBPAs) was observed also in the 10-15% pearled fraction, probably due to the presence of hull residues (Hernanz et al., 2001; Nordkvist, Salomonsson & Åman, 1984).

Although phenolic acids are among the main antioxidant compounds of cereals (Adom & Liu, 2002; Beta et al., 2005), many other compounds may have antioxidant properties (Cömert & Gökmen, 2017), therefore extraction-independent procedures in association with the DPPH and FRAP assays were carried out for the analysis of the antioxidant capacity of the pearled fractions (Figure 2A and B). As expected, both methods highlighted the higher antioxidant capacity in the outer layers of the kernel regardless of the cereal species. Even if the 0-5% and 5-10% pearled fractions of the barley cultivar showed the highest concentration of CWBPAs, their antioxidant activity was lower than other barley fractions. Concerning the residual pearled kernel, the 40-100% residue of barley was characterized by the highest antioxidant capacity (AC_{DPPH}: 5.36 mmol Trolox eq/kg dw; AC_{FRAP}: 9.89 mmol Trolox eq/kg dw) in comparison to the other cereals tested (AC_{DPPH}: 2.40 mmol Trolox eq/kg dw; AC_{FRAP}: 3.81 mmol Trolox eq/kg dw, average values), even if it was not the one characterized by the highest levels of both SPAs and CWBPAs, confirming that several 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

500 of the kernel. Nevertheless, the high concentration of antioxidant compounds in the internal
501 layers of tritordeum makes this cereal interesting also for the production of refined flour rich
502 in antioxidant compounds, even if a highly refined flour could result in a reduction of the
503 concentration of lutein.
504

505 **ACKNOWLEDGEMENT**

506 This research did not receive any specific grant from funding agencies in the public,
507 commercial, or not-for-profit sectors.

508 The authors would like to thank Andrea Borio, Federica Gagliardi and Ilaria Fino (Università
509 degli Studi di Torino) and Stefania Monteduro (Università degli Studi del Piemonte Orientale)
510 for their precious help and cooperation in laboratory and field activities.

511

512 **CONFLICT OF INTEREST**

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

514 REFERENCES

- 515 Abdel-Aal, E. –S. M., Young, J. C., Wood, P. J., Rabalski, I., Hucl, P., Falk, D., & Frégeau-
 516 Reid, J. (2002). Einkorn: a potential candidate for developing high lutein wheat. *Cereal*
 517 *Chemistry*, 79, 455-457. <https://doi.org/10.1094/CCHEM.2002.79.3.455>.
- 518 Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and*
 519 *Food Chemistry*, 50, 6182-6187. <https://doi.org/10.1021/jf0205099>.
- 520 Alvarez, J. B., Ballesteros, J., Sillero, J. A., & Martín, L. M. (1992). Tritordeum: a new crop
 521 of potential importance in the food industry. *Hereditas*, 116, 193-197.
 522 <https://doi.org/10.1111/j.1601-5223.1992.tb00822.x>.
- 523 Atienza, S. G., Ballesteros, J., Martín, A., & Hornero-Méndez, F. (2007). Genetic variability
 524 of carotenoid content and degree of esterification among tritordeum (*x Tritordeum*
 525 *Ascherson et Graebner*) and durum wheat accessions. *Journal of Agricultural and Food*
 526 *Chemistry*, 55, 4244-4251. <https://doi.org/10.1021/jf070342p>.
- 527 Beta, T., Nam, S., Dexter, J. E., & Sapiststein, H. D. (2005). Phenolic content and antioxidant
 528 activity of pearled wheat and roller-milled fractions. *Cereal Chemistry*, 82, 390-393.
 529 <http://doi.org/10.1094/CC-82-0390>.
- 530 Blandino, M., Sovrani, V., Marinaccio, F., Reyneri, A., Rolle, L., Giacosa, S., Locatelli, M.,
 531 Bordiga, M., Travaglia, F., Coisson, J. D., & Arlorio, M. (2013). Nutritional and technological
 532 quality of bread enriched with an intermediated pearled wheat fraction. *Food Chemistry*,
 533 141, 2549-2557. <https://doi.org/10.1016/j.foodchem.2013.04.122>.
- 534 Blandino, M., Locatelli, M., Gazzola, A., Coisson, J. D., Giacosa, S., Travaglia, F., Bordiga,
 535 M., Reyneri, A., Rolle, L., & Arlorio, M. (2015). Hull-less barley pearling fractions: nutritional
 536 properties and their effect on the functional and technological quality in bread-making.
 537 *Journal of Cereal Science*, 65, 48-56. <https://doi.org/10.1016/j.jcs.2015.06.004>.

538 Butsat, S., & Siriamornpun, S. (2010). Phenolic acids and antioxidant activities in husk of
 539 different Thai rice varieties. *Food Science and Technology International*, 16, 329-336.
 540 <https://doi.org/10.1177/1082013210366966>.

541 Cömert, E. D., & Gökmen, V. (2017). Antioxidants bound to an insoluble food matrix: their
 542 analysis, regeneration behavior, and physiological importance. *Comprehensive Reviews in*
 543 *Food Science and Food Safety*, 16, 382-399. <https://doi.org/10.1111/1541-4337.12263>.

544 Delcour, J. A., Rouau, X., Courtin, C. M., Poutanen, K., & Ranieri, R. (2012). Technologies
 545 for enhanced exploitation of the health-promoting potential of cereals. *Trends in Food*
 546 *Science & Technology*, 25, 78-86. <https://doi.org/10.1016/j.tifs.2012.01.007>.

547 Douglas, S. G. (1981). A rapid method for the determination of pentosans in wheat flour.
 548 *Food Chemistry*, 7, 139-145. [https://doi.org/10.1016/0308-8146\(81\)90059-5](https://doi.org/10.1016/0308-8146(81)90059-5).

549 Eliášová, M., & Paznocht, L. (2017). Total phenolic content and antioxidant activity of
 550 tritordeum wheat and barley. *Agronomy Research*, 15, 1287-1294.

551 Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain
 552 cereals: what is beyond fibre? *Nutrition Research Reviews*, 23, 65-134.
 553 <https://doi.org/10.1017/S0954422410000041>.

554 Foca, G., Ulrici, A., Corbellini, M., Pagani, M. A., Lucisano, M., Franchini, G. C., & Tassi, L.
 555 (2007). Reproducibility of the Italian ISQ method for quality classification of bread wheats:
 556 an evaluation by expert assessors. *Journal of the Science of Food and Agriculture*, 87, 839-
 557 846. <https://doi.org/10.1002/jsfa.2785>.

558 Giordano, D., Locatelli, M., Travaglia, F., Bordiga, M., Reyneri, A., Coisson, J. D., &
 559 Blandino, M. (2017). Bioactive compound and antioxidant activity distribution in roller-milled
 560 and pearled fractions of conventional and pigmented wheat varieties. *Food Chemistry*, 233,
 561 483-491. <https://doi.org/10.1016/j.foodchem.2017.04.065>.

562 Gökmen, V., Serpen, A., & Fogliano, V. (2009). Direct measurement of the total antioxidant
 563 capacity of foods: the "QUENCHER" approach. *Trends in Food Science & Technology*, 20,
 564 278-288. <https://doi.org/10.1016/j.tifs.2009.03.010>.

565 Hernanz, D., Nuñez, V., Sancho, A. I., Faulds, C. B., Williamson, G., Bartolomé, B., &
 566 Gómez-Cordovés, C. (2001). Hydroxycinnamic acids and ferulic acid dehydrodimers in
 567 barley and processed barley. *Journal of Agricultural and Food Chemistry*, 49, 4884-4888.
 568 <https://doi.org/10.1021/jf010530u>.

569 Kiszonas, A. M., Courtin, C. M., & Morris, C. F. (2012). A critical assessment of the
 570 quantification of wheat grain arabinoxylans using a phloroglucinol colorimetric assay. *Cereal*
 571 *Chemistry*, 89, 143-150. <https://doi.org/10.1094/CCHEM-02-12-0016-R>

572 Lachman, J., Hejtmánková, A., Orsák, M., Popov, M., & Martinek, P. (2018). Tocotrienols
 573 and tocopherols in colored-grain wheat, tritordeum and barley. *Food Chemistry*, 240, 725-
 574 735. <https://doi.org/10.1016/j.foodchem.2017.07.123>.

575 Li, L., Shewry, P. R., & Ward, J. L. (2008). Phenolic acids in wheat varieties in the
 576 HEALTHGRAIN diversity screen. *Journal of Agricultural and Food Chemistry*, 56, 9732-
 577 9739. <https://doi.org/10.1021/jf801069s>.

578 Liyana-Pathirana, C., Dexter, J., & Shahidi, F. (2006). Antioxidant properties of wheat as
 579 affected by pearling. *Journal of Agricultural and Food Chemistry*, 58, 9235-9241.
 580 <https://doi.org/10.1021/jf060664d>.

581 Martín, A., Alvarez, J. B., Martín, L. M., Barro, F., & Ballesteros, J. (1999). The development
 582 of Tritordeum: a novel cereal for food processing. *Journal of Cereal Science*, 30, 85-95.
 583 <https://doi.org/10.1006/jcrs.1998.0235>.

584 Mattera, M. G., Hornero-Méndez, D., & Atienza, S. G. (2017). Lutein ester profile in wheat
 585 and tritordeum can be modulated by temperature: evidences for regioselectivity and fatty
 586 acid preferential of enzymes encoded by genes on chromosome 7D and 7H^{ch}. *Food*
 587 *Chemistry*, 219, 199-206. <https://doi.org/10.1016/j.foodchem.2016.09.133>.

588 Mellado-Ortega, E., & Hornero-Méndez, D. (2012). Isolation and identification of lutein
589 esters, including their regioisomers, in tritordeum (*x Tritordeum* Ascherson et Graebner)
590 grains. Evidences for a preferential xanthophyll acyltransferase activity. *Food Chemistry*,
591 135, 1344-1352. <https://doi.org/10.1016/j.foodchem.2012.05.046>.

592 Mellado-Ortega, E., & Hornero-Méndez, D. (2015). Carotenoid profiling of *Hordeum chilense*
593 grains: the parental proof for the origin of the high carotenoid content and esterification
594 pattern of tritordeum. *Journal of Cereal Science*, 62, 15-21.
595 <https://doi.org/10.1016/j.jcs.2014.12.005>.

596 Mellado-Ortega, E., & Hornero-Méndez, D. (2016). Carotenoid evolution during short-
597 storage period of durum wheat (*Triticum turgidum* conv. *durum*) and Tritordeum (*x*
598 *Tritordeum* Ascherson et Graebner) whole-grain flours. *Food Chemistry*, 192, 714-723.
599 <https://doi.org/10.1016/j.foodchem.2015.07.057>.

600 Mellado-Ortega, E., & Hornero-Méndez, D. (2018). Effect of lutein esterification on the
601 differential distribution of carotenoids in germ and endosperm fractions from tritordeum
602 grains. *Journal of Cereal Science*, 79, 462-468. <https://doi.org/10.1016/j.jcs.2017.12.006>.

603 Moros, E.E., Darnoko, D., Cheryan, M., Perkins, E.G., Jerrell, J. (2002). Analysis of
604 xanthophylls in corn by HPLC. *Journal of Agricultural and Food Chemistry*, 50, 5787-5790.
605 <https://doi.org/10.1021/jf020109l>.

606 Navas-Lopez, J. F., Ostos-Garrido, F. J., Castillo, A., Martín, A., Gimenez, M. J., & Piston,
607 F. (2014). Phenolic content variability and its chromosome location in tritordeum. *Frontiers*
608 *in Plant Science*, 5, 10. <https://doi.org/10.3389/fpls.2014.00010>.

609 Nicoletti I., Martini D., De Rossi A., Taddei F., D'Egidio M.G., Corradini D. (2013).
610 Identification and quantification of soluble free, soluble conjugated, and insoluble bound
611 phenolic acids in durum wheat (*Triticum turgidum* L. var. *durum*) and derived products by
612 RP-HPLC on a semimicro separation scale. *Journal of Agricultural and Food Chemistry*, 61,
613 11800-11807. <https://doi.org/10.1021/jf403568c>.

614 Nordkvist, E., Salomonsson, A.- C., Åman, P. (1984). Distribution of insoluble bound
 615 phenolic acids in barley grain. *Journal of the Science of Food and Agriculture*, 35, 657-661.
 616 <https://doi.org/10.1002/jsfa.2740350611>.

617 Paznocht, L., Kotíková, Z., Šulc, M., Lachman, J., Orsák, M., Eliášová, M., & Martinek, P.
 618 (2018). Free and esterified carotenoids in pigmented wheat, tritordeum and barley grains.
 619 *Food Chemistry*, 240, 670-678. <https://doi.org/10.1016/j.foodchem.2017.07.151>.

620 Rakha, A., Saulnier, L., Åman, P., & Andersson, R. (2012). Enzymatic fingerprinting of
 621 arabinoxylan and β -glucan in triticales, barley and tritordeum grains. *Carbohydrate Polymers*,
 622 90, 1226-1234. <https://doi.org/10.1016/j.carbpol.2012.06.054>.

623 Rodríguez-Suárez, C., Mellado-Ortega, E., Hornero-Méndez, D., & Atienza, S. G. (2014).
 624 Increase in transcript accumulation of *Psy1* and *e-Lcy* genes in grain development is
 625 associated with differences in seed carotenoid content between durum wheat and
 626 tritordeum. *Plant Molecular Biology*, 84, 659-673. [https://doi.org/10.1007/s11103-013-0160-](https://doi.org/10.1007/s11103-013-0160-y)
 627 [y](https://doi.org/10.1007/s11103-013-0160-y).

628 Rouau, X., & Surget, A. (1994). A rapid and semiautomated method for determination of
 629 total and water-extractable pentosans in wheat flours. *Carbohydrate Polymers*, 24, 123-132.
 630 [https://doi.org/10.1016/0144-8617\(94\)90022-1](https://doi.org/10.1016/0144-8617(94)90022-1)

631 Serpen, A., Gökmen, V., & Fogliano, V. (2012). Solvent effects on total antioxidant capacity
 632 of foods measured by direct QUENCHER procedure. *Journal of Food Composition Analysis*,
 633 26, 52-57. <https://doi.org/10.1016/j.jfca.2012.02.005>.

634 Shao, Y., Hu, Z., Yu, Y., Mou, R., Zhu, Z. & Beta, T. (2018). Phenolic acids, anthocyanins,
 635 proanthocyanidins, antioxidant activity, minerals and their correlations in non-pigmented,
 636 red, and black rice. *Food Chemistry*, 239, 733-741. <https://doi.org/10.1016/j.foodchem.2017.07.009>.

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

640 activity, deoxynivalenol and heavy metal contamination of pearled wheat fractions. *Food*
641 *Chemistry*, 135, 39-46. [https://doi.org/ 10.1016/j.foodchem.2012.04.045](https://doi.org/10.1016/j.foodchem.2012.04.045).

642 Taylor, J. R. N., & Awika, J. M. (2017). *Gluten-free ancient grains. Cereals, pseudocereals,*
643 *and legumes: sustainable, nutritious, and health promoting foods for the 21st Century.*
644 Duxford, UK: Woodhead Publishing, Elsevier.

645 Vaquero, L., Comino, I., Vivas, S., Rodríguez-Martín, L., Giménez, M. J., Pastor, J., Sousa,
646 C., & Barro, F. (2018). Tritordeum: a novel cereal for food processing with good acceptability
647 and significant reduction in gluten immunogenic peptides in comparison with wheat. *Journal*
648 *of the Science of Food and Agriculture*, 98, 2201-2209. <https://doi.org/10.1002/jsfa.8705>.

649 Villegas, D., Casadesús, J., Atienza, S. G., Martos, V., Maalouf, F., Karam, F., Aranjuelo, I.,
650 & Nogués, S. (2010). Tritordeum, wheat and triticale yield components under multi-local
651 Mediterranean drought conditions. *Field Crops Research*, 116, 68-74.
652 <https://doi.org/10.1016/j.fcr.2009.11.012>.

653 Zanoletti, M., Parizad, P. A., Lavelli, V., Checchini, C., Menesatti, P., Marti, A., & Pagani, M.
654 A. (2017). Debranning of purple wheat: recovery of anthocyanin-rich fractions and their use
655 in pasta production. *LWT – Food Science and Technology*, 75, 663-669.
656 <https://doi.org/10.1016/j.lwt.2016.10.016>.

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	CWBPA 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 mg/kg	Ferulic acid mg/kg	Vanillic acid mg/kg	<i>p</i> -Coumaric acid mg/kg	SPAs ¹ 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.

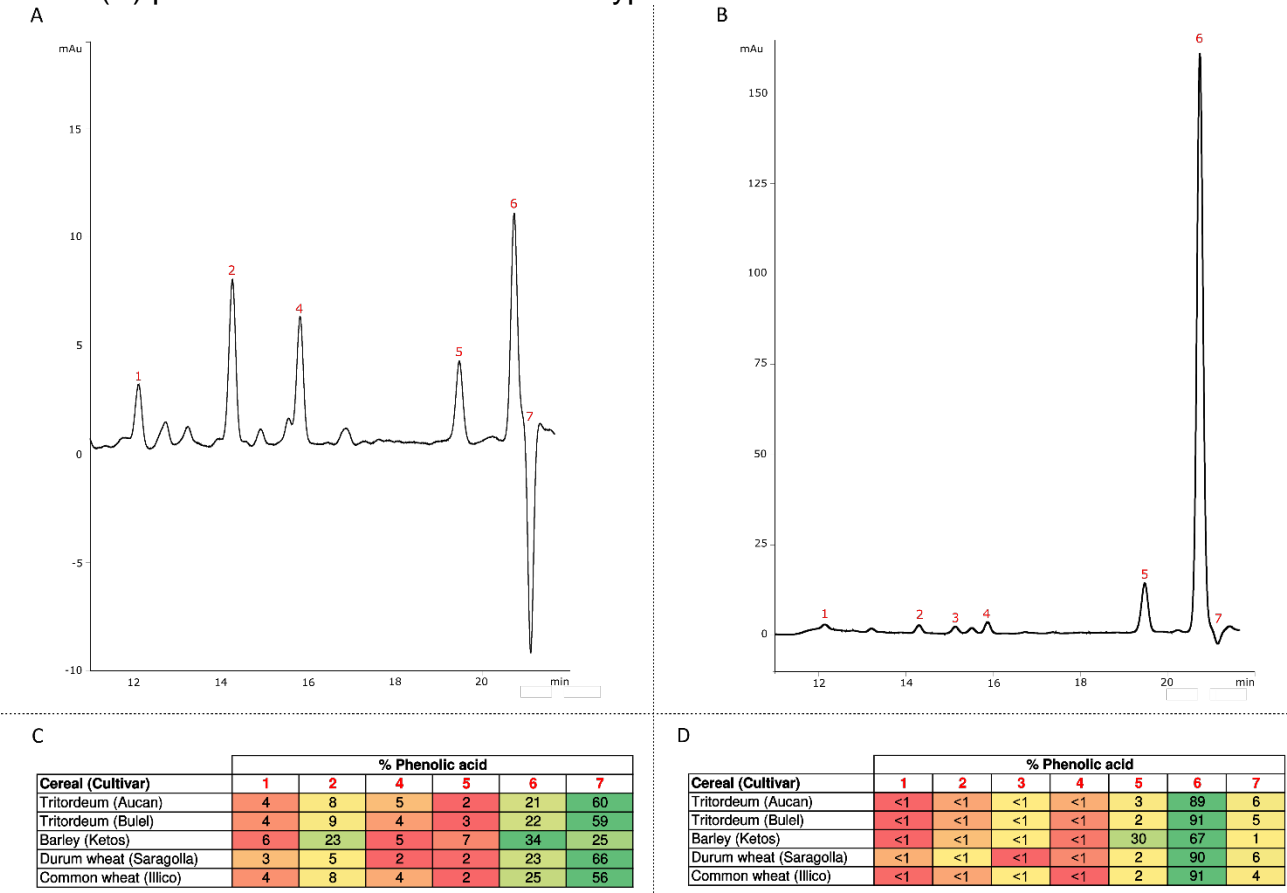
Cereal (Cultivar)	Pearled fraction	Ferulic acid mg/kg	Sinapic acid mg/kg	<i>p</i> -Coumaric acid mg/kg	Vanillic acid mg/kg	CWBPA ¹ mg/kg
Tritordeum (Aucan)	0-5%	1409 ^a	72.3 ^a	136 ^a	15.2 ^a	1669 ^a
	5-10%	1427 ^a	70.9 ^a	70.3 ^b	12.2 ^b	1614 ^a
	10-15%	1331 ^a	68.1 ^a	44.4 ^c	9.80 ^c	1483 ^b
	15-20%	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.

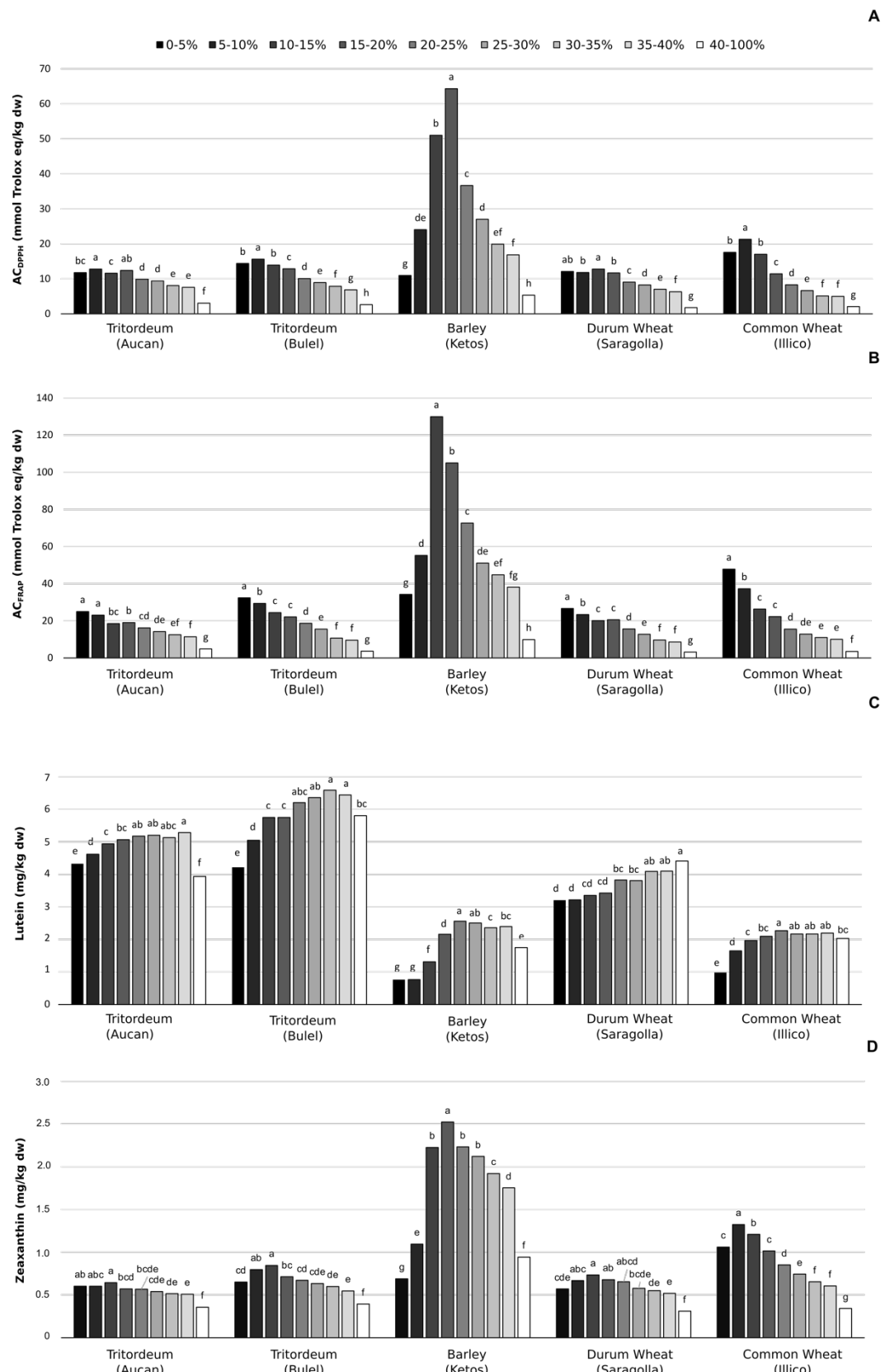
¹ sum of the CWBPAs determined by means of the RP-HPLC/DAD.

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.



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.