

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<sup>a\*</sup>, Amedeo Reyneri<sup>a</sup>, Monica Locatelli<sup>b</sup>, Jean Daniel Coïsson<sup>b</sup>, Massimo  
6 Blandino<sup>a</sup>

7

8 **AFFILIATIONS**

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

11 <sup>b</sup> 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](mailto:debora.giordano@unito.it)

16

17 **AUTHORS' E-MAIL ADRESSES**

18 Debora Giordano: [debora.giordano@unito.it](mailto:debora.giordano@unito.it)

19 Amedeo Reyneri: [amedeo.reyneri@unito.it](mailto:amedeo.reyneri@unito.it)

20 Monica Locatelli: [monica.locatelli@uniupo.it](mailto:monica.locatelli@uniupo.it)

21 Jean Daniel Coïsson: [jeandaniel.coisson@uniupo.it](mailto:jeandaniel.coisson@uniupo.it)

22 Massimo Blandino: [massimo.blandino@unito.it](mailto: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  $\beta$ -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,  $\beta$ -glucans, Arabinoxylans, Phenolic acids, Antioxidant capacity,  
42 Xanthophylls

43

44

## 45 1. INTRODUCTION

46 Wheat-based products are central dietary components worldwide owing to their good  
47 nutritional and organoleptic qualities. Nevertheless, the application of alternative cereal  
48 types and processing technologies for the production of foods rich in bioactive compounds  
49 has drawn the attention of both researchers and industrialists in the last few years (Abdel-  
50 Aal et al., 2002; Blandino et al., 2013; Delcour, Rouau, Courtin, Poutanen & Ranieri, 2012;  
51 Giordano et al., 2017; Taylor & Awika, 2017). Since the beginning of the twentieth century,  
52 cereal breeders focused their effort on the development of interspecific hybrids in order to  
53 obtain new cereals with increased phytochemical contents and improved agronomic  
54 performances and technological qualities. In this sense, tritordeum is a potentially interesting  
55 candidate. Hexaploid tritordeum is the amphidiploid cereal derived from the cross between  
56 a South American wild barley (*Hordeum chilense* Roem. et Schultz.) and a cultivated durum  
57 wheat (*Triticum turgidum* ssp. *durum* Desf.). Besides being used as a genetic bridge for  
58 transferring useful barley traits to wheat, tritordeum has been subjected to a breeding  
59 program to become a new hullless small cereal crop (Martín, Alvarez, Martín, Barro &  
60 Ballesteros 1999). Previous studies have shown that tritordeum is more suitable for  
61 breadmaking than for pasta making (Martín et al., 1999). At present, limited information is  
62 available on the content and the composition of phenolic acids in tritordeum (Eliášová &  
63 Paznocht, 2017; Navas-Lopez, Ostos-Garrido, Castillo, Martín, Gimenez & Piston, 2014).  
64 Nevertheless, several studies showed that this novel cereal is characterized by a high  
65 content of carotenoids, which give it a strong yellow color (Mellado-Ortega & Hornero-  
66 Méndez, 2012 and 2016; Paznocht et al., 2018), and tocots (Lachman, Hejtmánková, Orsák,  
67 Popov & Martinek, 2018), suggesting its potential use for the production of health-valued  
68 foods. Even if not suitable for celiac disease sufferers, tritordeum showed lower levels of  
69 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 m<sup>2</sup>), planting was performed in 12 cm  
95 wide rows at a seeding rate of 450 seeds/m<sup>2</sup> 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

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

107

## 108 **2.2 Analysis of grain quality parameters**

109 Grain yield (t/ha) was calculated on a plot basis. Thousand kernel weight (TKW) was  
110 determined on two 200-kernel sets of each sample, using an electronic balance. Test weight  
111 (TW) was determined by means of a Dickey-John GAC2000 grain analysis meter (Dickey-  
112 John Corp., Auburn, IL), using the supplied program, after validation with reference  
113 materials.

114

## 115 **2.3 Grain pearling**

116 Nine pearled fractions of the kernels of each cultivar were obtained through the incremental  
117 pearling of the cereals tested following the approach proposed by Beta, Nam, Dexter &  
118 Sapirstein (2005). The pearling consisted of consecutive passages of kernels or pearled  
119 kernels in an abrasive-type grain testing mill (Model TM-05C, Satake, Tokyo, Japan).  
120 Starting from unprocessed grain samples, the kernels were initially pearled to remove 5%  
121 of the original grain weight, and this resulted in a first fraction (0-5% w/w). The remaining  
122 kernels were then pearled to remove a second fraction of 5% (5-10% w/w). The pearling  
123 process was repeated to remove a third, fourth, fifth, sixth, seventh, eighth fraction (designed  
124 fractions of 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40% w/w). The pearling process  
125 was performed at a constant speed and the estimation of the time necessary in order to  
126 remove 5% of kernel weight at each pearling passage was experimentally quantified for  
127 each cultivar. The pearling process was then monitored by means of a time control, and  
128 after each pearling session, the laboratory pearler was cleaned thoroughly to minimize  
129 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  $\beta$ -glucan  
158 contents were determined by means of the Megazyme total dietary fiber analysis kit and the  
159 Megazyme mixed-linkage  $\beta$ -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  $\mu$ 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  $\mu$ 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  $\mu$ L of

181 ethyl acetate. After centrifugation at 10,600 x g for 2 min the upper layer was transferred in  
182 a clean microcentrifuge tube. The extraction was repeated twice, and the combined  
183 supernatants were evaporated to dryness under a nitrogen stream and then reconstituted in  
184 100 µL of 80:20 (v/v) methanol:water solution.

#### 185 Extraction of cell wall-bound phenolic acids

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

195

#### 196 **2.4.4 Quantification of soluble and cell wall-bound phenolic acids by means of RP-** 197 **HPLC/DAD**

198 The phenolic extracts were filtered through a 0.2 µm filter and then analyzed by means of a  
199 high performance liquid chromatograph Agilent 1200 Series (Agilent Technologies, Santa  
200 Clara, CA, USA) coupled to an Agilent 1200 Series diode array detector. The  
201 chromatographic method was developed starting from the one proposed by Shao, Hu, Yu,  
202 Mou, Zhu & Beta (2018). Separations were carried out using a 150 x 4.6 mm, 5 µm, Gemini  
203 RP-18 column (Phenomenex, Torrance, CA, USA); the column temperature was set at 35  
204 °C. The mobile phase consisted of 0.1% acetic acid in water (solvent A) and 0.1% acetic  
205 acid in methanol (solvent B). The following operating linear gradient was used: 0-22 min, 9-  
206 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*- $\beta$ -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  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ 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,

232 100% B. Finally, the mobile phase was brought to 5% B in 1 minute, and this was followed  
233 by 10 minutes of equilibration. The flow rate of the mobile phase was 1 mL/min. Xanthophylls  
234 were identified using the retention times and the UV/Vis spectra of their respective standards  
235 (lutein and zeaxanthin). Individual xanthophyll standards were also prepared and diluted to  
236 different concentrations to obtain calibration curves for quantification purposes. Retention  
237 time, detection wavelength and the principal parameters of the calibration curves are  
238 reported as Supplementay Material in Table S3.

239

#### 240 **2.4.6 Determination of DPPH radical scavenging activity ( $AC_{DPPH}$ )**

241 DPPH radical scavenging activity (QUENCHER procedure – direct measurement on solid  
242 sample, Gökmen, Serpen & Fogliano, 2009) was carried out as reported in Giordano et al.  
243 (2017). The DPPH radical scavenging activity was expressed as mmol of Trolox  
244 equivalents/kg of sample (dw) through a calibration curve (linearity range: 0.5-5  $\mu\text{g/mL}$ ;  
245  $y=18.573x-1.3947$ ,  $R^2: 0.999$ ). The analysis was carried out in triplicate ( $n=3$ ).

246

#### 247 **2.4.7 Determination of antioxidant capacity by means of the FRAP assay ( $AC_{FRAP}$ )**

248 The FRAP (Ferric Reducing Antioxidant Power) assay adapted into QUENCHER method  
249 was performed as described by Serpen, Gökmen and Fogliano (2012). Briefly, FRAP  
250 reagent was prepared by mixing the aqueous solution of 10 mM TPTZ and 20 mM ferric  
251 chloride in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10 (v:v:v). Samples (2  
252 mg) were analyzed by adding FRAP working solution (2 mL). The reaction was carried out  
253 under stirring at 1,000 rpm (PCMT Thermoshaker, Grant Instruments, Cambridge, UK). After  
254 exactly 120 min from the first introduction of FRAP solution onto solid samples,  
255 centrifugation was performed for 1 min at 20,800 x g, and the absorbance was measured at  
256 593 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  $\beta$ -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  $\beta$ -glucan content 24% lower than  
292 common wheat and 5 folds lower than the six-row barley cultivar. Similar concentrations of

293  $\beta$ -glucans were observed previously for other 5 tritordeum lines grown in Cordoba (Rakha,  
294 Saulnier, Åman & Andersson, 2012), confirming the low  $\beta$ -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

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 were 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  $\beta$ -glucans differed depending on the cereal species. The two cultivars of  
368 tritordeum tested were closer to durum wheat in terms of distribution of  $\beta$ -glucans, showing  
369 the highest  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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

397 different (i.e. sinapic acid represent from 61 to 70% of SPAs in durum wheat, while in  
398 common wheat only from 51 to 58%). The barley cultivar showed a characteristic phenolic  
399 acid profile and distribution. The 0-5% and 5-10% pearled fractions, which mainly  
400 corresponds to the hulls, showed not only a low concentration of SPAs (47.9 and 63.6 mg/kg  
401 dw, respectively), but they also differed completely from the other fractions for their phenolic  
402 acid profile: ferulic acid represents 27% of SPAs, *p*-coumaric acid 20%, vanillic acid 20%,  
403 sinapic acid 17%, syringic acid <10%, *p*-hydroxybenzoic acid <10%. Contrary to all the other  
404 cereals, the phenolic acid profile observed from the 10-15% pearled fraction to the residual  
405 pearled kernel was not characterized by a clear prevalence of sinapic acid. In fact, in the 10-  
406 15% pearled fraction sinapic acid was only 31% of SPAs, while ferulic and vanillic acids  
407 represented 20 and 32% of SPAs, respectively. The same phenolic acids were 20, 44 and  
408 22% of SPAs, respectively, in the 40-100% residual pearled kernel. The highest content of  
409 SPAs and of the three main soluble phenolic acids was observed in the 15-20% pearled  
410 fraction, then a significant and gradual decrease of the concentration of these compounds  
411 was observed at each pearling step.

412 The content of CWBPAs decreased from the outermost to the innermost layers of the kernels  
413 regardless of the cereal species (Table 4). Both cv. Aucan and cv. Bulel showed a peculiar  
414 distribution pattern of cell wall-bound phenolic acids in their pearled fractions. In fact,  
415 tritordeum showed a higher retention of CWBPAs in the residual pearled kernel when  
416 compared to both the durum and common wheat cultivar. As far as the 0-5% and 5-10%  
417 pearled fractions are concerned, the concentration of CWBPAs of tritordeum was on  
418 average 39% lower than the one observed in the same fractions of the common wheat  
419 cultivar, whereas in the residual pearled kernel the concentration of CWBPAs was 36%  
420 higher in tritordeum. The high content of cell wall-bound phenolic acids in the internal layers  
421 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

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

453

### 454 **3.4 Distribution of xanthophylls in pearled fractions**

455 As observed for the wholemeal flour, lutein was the main xanthophyll detected in each  
456 pearled fraction regardless of the cereal species. Tritordeum showed higher levels of lutein  
457 than barley, durum wheat and common wheat in all the pearled fractions (Figure 2C).  
458 Moreover, the comparison of the two cultivars of tritordeum showed that cv. Bulel was  
459 characterized by higher concentration of lutein than cv. Aucan, with the exception of the 0-  
460 5% fraction. The residual pearled kernel of cv. Bulel showed a lutein content even 48%  
461 higher than observed in the same fraction of cv. Aucan, confirming that differences may  
462 occur among tritordeum genotypes for their lutein content (Atienza et al., 2007). Mellado-  
463 Ortega and Hornero-Méndez (2018) showed that carotenoids are homogeneously  
464 distributed among the germ fraction (7.1% of the grain weight) and the residual kernel  
465 (92.9% of the grain weight) of tritordeum. The pearling process carried out in the present  
466 study highlights that an unevenly distribution of lutein occurs moving towards the innermost  
467 layers of kernels of tritordeum. In fact, after an initial increase in the concentration of lutein  
468 moving from the outermost pearled fraction to the intermediated ones, a significant decrease  
469 in the concentration was observed in the residual pearled kernel (-26% cv. Aucan; -10% cv.  
470 Bulel). A similar distribution pattern was observed in barley (27% drop in the residual pearled  
471 kernel). Contrarily, both the durum and common wheat cultivars did not show any significant  
472 decrease in their lutein content after the last pearling step. Therefore, even if cv. Aucan  
473 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 concentration 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., & Sapistein, 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<sup>ch</sup>. *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  $\beta$ -glucan in triticale, 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->  
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., Coisson, 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 21<sup>st</sup> 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 <sup>1</sup>	CWBPA <sup>2</sup>	Lutein	Zeaxanthin	AC <sub>DPPH</sub>	AC <sub>FRAP</sub>
		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 <sup>c</sup>	40.3 <sup>b</sup>	72.7 <sup>b</sup>	1.66 <sup>bc</sup>	14.4 <sup>a</sup>	12.2 <sup>c</sup>	0.691 <sup>c</sup>	2.15 <sup>a</sup>	64.3 <sup>b</sup>	976 <sup>b</sup>	4.54 <sup>b</sup>	0.438 <sup>c</sup>	4.01 <sup>b</sup>	7.84 <sup>b</sup>
Tritordeum	Bulel	4.5 <sup>c</sup>	38.5 <sup>b</sup>	72.7 <sup>b</sup>	1.48 <sup>c</sup>	14.1 <sup>a</sup>	14.7 <sup>b</sup>	0.614 <sup>d</sup>	1.71 <sup>b</sup>	51.7 <sup>c</sup>	767 <sup>c</sup>	6.14 <sup>a</sup>	0.513 <sup>bc</sup>	3.98 <sup>b</sup>	8.25 <sup>b</sup>
Barley	Ketos	7.5 <sup>a</sup>	37.9 <sup>b</sup>	60.9 <sup>c</sup>	2.30 <sup>a</sup>	9.60 <sup>d</sup>	25.2 <sup>a</sup>	3.46 <sup>a</sup>	1.27 <sup>c</sup>	31.2 <sup>d</sup>	1283 <sup>a</sup>	2.13 <sup>c</sup>	1.41 <sup>a</sup>	11.6 <sup>a</sup>	35.3 <sup>a</sup>
Durum wheat	Saragolla	6.2 <sup>b</sup>	47.9 <sup>a</sup>	72.9 <sup>b</sup>	1.85 <sup>b</sup>	12.5 <sup>b</sup>	12.2 <sup>c</sup>	0.389 <sup>e</sup>	1.06 <sup>d</sup>	99.6 <sup>a</sup>	539 <sup>d</sup>	4.58 <sup>b</sup>	0.450 <sup>c</sup>	3.81 <sup>b</sup>	7.60 <sup>b</sup>
Common wheat	Illico	8.0 <sup>a</sup>	46.8 <sup>a</sup>	81.2 <sup>a</sup>	1.52 <sup>c</sup>	11.7 <sup>c</sup>	13.0 <sup>bc</sup>	0.853 <sup>b</sup>	1.35 <sup>c</sup>	43.6 <sup>c</sup>	985 <sup>b</sup>	2.20 <sup>c</sup>	0.612 <sup>b</sup>	3.43 <sup>b</sup>	8.05 <sup>b</sup>
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.

<sup>1</sup> sum of the SPAs determined by means of RP-HPLC/DAD.

<sup>2</sup> sum of the CWBPAs determined by means of the RP-HPLC/DAD.

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

Cereal (Cultivar)	Pearled fraction	Ash %	Proteins %	TDF %	$\beta$ -glucans %	TAX %
Tritordeum (Aucan)	0-5%	2.51 <sup>b</sup>	14.3 <sup>e</sup>	34.0 <sup>a</sup>	0.650 <sup>c</sup>	2.21 <sup>a</sup>
	5-10%	2.71 <sup>ab</sup>	14.9 <sup>d</sup>	27.1 <sup>b</sup>	0.840 <sup>b</sup>	1.92 <sup>a</sup>
	10-15%	2.81 <sup>a</sup>	15.4 <sup>c</sup>	22.1 <sup>c</sup>	0.888 <sup>b</sup>	2.03 <sup>a</sup>
	15-20%	2.86 <sup>a</sup>	16.5 <sup>a</sup>	19.7 <sup>cd</sup>	0.912 <sup>b</sup>	2.01 <sup>a</sup>
	20-25%	2.50 <sup>b</sup>	16.2 <sup>ab</sup>	17.4 <sup>de</sup>	1.02 <sup>a</sup>	2.00 <sup>a</sup>
	25-30%	2.50 <sup>b</sup>	16.5 <sup>a</sup>	16.7 <sup>ef</sup>	0.882 <sup>b</sup>	2.14 <sup>a</sup>
	30-35%	2.19 <sup>c</sup>	15.9 <sup>bc</sup>	14.2 <sup>fg</sup>	0.869 <sup>b</sup>	2.08 <sup>a</sup>
	35-40%	2.06 <sup>c</sup>	15.9 <sup>bc</sup>	12.5 <sup>g</sup>	0.865 <sup>b</sup>	2.08 <sup>a</sup>
	40-100%	1.27 <sup>d</sup>	13.3 <sup>f</sup>	7.25 <sup>h</sup>	0.625 <sup>c</sup>	1.99 <sup>a</sup>
	SEM	0.07	0.1	0.53	0.025	0.07
P (F)	<0.001	<0.001	<0.001	<0.001	<0.001	
Tritordeum (Bulel)	0-5%	3.11 <sup>c</sup>	14.1 <sup>e</sup>	36.0 <sup>a</sup>	0.619 <sup>d</sup>	1.61 <sup>bc</sup>
	5-10%	3.85 <sup>a</sup>	15.2 <sup>d</sup>	30.7 <sup>b</sup>	0.889 <sup>b</sup>	1.88 <sup>a</sup>
	10-15%	4.02 <sup>a</sup>	16.2 <sup>bc</sup>	29.0 <sup>c</sup>	0.992 <sup>a</sup>	1.67 <sup>ab</sup>
	15-20%	3.48 <sup>b</sup>	16.5 <sup>b</sup>	23.2 <sup>d</sup>	1.03 <sup>a</sup>	1.44 <sup>cd</sup>
	20-25%	2.79 <sup>d</sup>	16.9 <sup>a</sup>	18.2 <sup>e</sup>	0.992 <sup>a</sup>	1.36 <sup>d</sup>
	25-30%	2.70 <sup>de</sup>	16.4 <sup>b</sup>	16.4 <sup>f</sup>	0.894 <sup>b</sup>	1.41 <sup>cd</sup>
	30-35%	2.44 <sup>e</sup>	16.3 <sup>b</sup>	14.0 <sup>g</sup>	0.841 <sup>bc</sup>	1.74 <sup>ab</sup>
	35-40%	2.15 <sup>f</sup>	15.9 <sup>c</sup>	11.8 <sup>h</sup>	0.801 <sup>c</sup>	1.63 <sup>bc</sup>
	40-100%	1.18 <sup>g</sup>	13.4 <sup>f</sup>	9.42 <sup>i</sup>	0.488 <sup>e</sup>	1.63 <sup>bc</sup>
	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 <sup>a</sup>	5.09 <sup>g</sup>	83.0 <sup>a</sup>	0.224 <sup>f</sup>	6.18 <sup>a</sup>
	5-10%	5.51 <sup>b</sup>	6.34 <sup>f</sup>	79.6 <sup>b</sup>	0.455 <sup>e</sup>	3.58 <sup>b</sup>
	10-15%	5.48 <sup>b</sup>	11.8 <sup>d</sup>	60.3 <sup>c</sup>	1.56 <sup>d</sup>	2.91 <sup>c</sup>
	15-20%	5.02 <sup>c</sup>	15.4 <sup>a</sup>	38.4 <sup>d</sup>	2.87 <sup>c</sup>	3.10 <sup>c</sup>
	20-25%	3.63 <sup>d</sup>	14.9 <sup>a</sup>	25.9 <sup>e</sup>	3.25 <sup>b</sup>	1.99 <sup>d</sup>
	25-30%	3.08 <sup>e</sup>	13.7 <sup>b</sup>	22.6 <sup>e</sup>	3.23 <sup>b</sup>	1.54 <sup>e</sup>
	30-35%	2.61 <sup>f</sup>	12.8 <sup>c</sup>	18.5 <sup>f</sup>	3.29 <sup>b</sup>	1.48 <sup>e</sup>
	35-40%	2.18 <sup>g</sup>	12.2 <sup>d</sup>	16.1 <sup>f</sup>	3.36 <sup>b</sup>	1.50 <sup>e</sup>
	40-100%	1.01 <sup>h</sup>	8.63 <sup>e</sup>	9.80 <sup>g</sup>	3.94 <sup>a</sup>	1.17 <sup>e</sup>
	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 <sup>ef</sup>	13.4 <sup>bc</sup>	31.7 <sup>a</sup>	0.387 <sup>e</sup>	0.862 <sup>e</sup>
	5-10%	3.35 <sup>d</sup>	13.6 <sup>bc</sup>	30.0 <sup>a</sup>	0.482 <sup>d</sup>	0.994 <sup>cd</sup>
	10-15%	3.75 <sup>b</sup>	13.7 <sup>abc</sup>	25.3 <sup>b</sup>	0.665 <sup>abc</sup>	1.02 <sup>bcd</sup>
	15-20%	4.18 <sup>a</sup>	14.3 <sup>a</sup>	21.4 <sup>c</sup>	0.709 <sup>a</sup>	1.08 <sup>abc</sup>
	20-25%	3.72 <sup>bc</sup>	14.0 <sup>ab</sup>	17.5 <sup>d</sup>	0.684 <sup>ab</sup>	1.17 <sup>a</sup>
	25-30%	3.47 <sup>cd</sup>	13.6 <sup>bc</sup>	14.7 <sup>de</sup>	0.684 <sup>ab</sup>	1.12 <sup>ab</sup>
	30-35%	2.92 <sup>e</sup>	13.3 <sup>c</sup>	13.0 <sup>ef</sup>	0.642 <sup>bc</sup>	1.06 <sup>abc</sup>
	35-40%	2.64 <sup>f</sup>	13.3 <sup>c</sup>	11.1 <sup>f</sup>	0.619 <sup>c</sup>	0.942 <sup>de</sup>
	40-100%	1.27 <sup>g</sup>	10.9 <sup>d</sup>	5.84 <sup>g</sup>	0.288 <sup>f</sup>	1.11 <sup>ab</sup>
	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 <sup>c</sup>	10.4 <sup>g</sup>	58.0 <sup>a</sup>	1.20 <sup>d</sup>	1.31 <sup>d</sup>
	5-10%	4.13 <sup>a</sup>	15.0 <sup>bc</sup>	37.2 <sup>b</sup>	1.76 <sup>a</sup>	1.38 <sup>cd</sup>
	10-15%	3.61 <sup>b</sup>	15.9 <sup>a</sup>	27.2 <sup>c</sup>	1.60 <sup>b</sup>	1.55 <sup>bc</sup>
	15-20%	2.96 <sup>d</sup>	15.4 <sup>ab</sup>	18.6 <sup>d</sup>	1.41 <sup>c</sup>	1.69 <sup>ab</sup>
	20-25%	2.17 <sup>e</sup>	14.4 <sup>cd</sup>	16.1 <sup>de</sup>	1.18 <sup>d</sup>	1.74 <sup>a</sup>
	25-30%	2.13 <sup>e</sup>	14.1 <sup>de</sup>	12.9 <sup>ef</sup>	1.07 <sup>e</sup>	1.66 <sup>ab</sup>
	30-35%	1.80 <sup>f</sup>	13.5 <sup>ef</sup>	10.6 <sup>fg</sup>	0.960 <sup>f</sup>	1.55 <sup>bc</sup>
	35-40%	1.58 <sup>f</sup>	12.9 <sup>f</sup>	9.55 <sup>fg</sup>	0.959 <sup>f</sup>	1.58 <sup>ab</sup>
	40-100%	0.775 <sup>g</sup>	10.4 <sup>g</sup>	7.18 <sup>g</sup>	0.584 <sup>g</sup>	1.24 <sup>d</sup>
	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 <sup>1</sup>
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Tritordeum (Aucan)	0-5%	109 <sup>a</sup>	29.4 <sup>a</sup>	12.3 <sup>a</sup>	4.31 <sup>a</sup>	170 <sup>a</sup>
	5-10%	114 <sup>a</sup>	29.2 <sup>a</sup>	11.7 <sup>a</sup>	3.34 <sup>b</sup>	172 <sup>a</sup>
	10-15%	109 <sup>a</sup>	29.2 <sup>a</sup>	10.8 <sup>b</sup>	3.06 <sup>c</sup>	166 <sup>a</sup>
	15-20%	90.1 <sup>b</sup>	25.5 <sup>b</sup>	9.54 <sup>c</sup>	2.60 <sup>d</sup>	139 <sup>b</sup>
	20-25%	79.1 <sup>c</sup>	23.9 <sup>bc</sup>	8.78 <sup>d</sup>	2.28 <sup>e</sup>	125 <sup>c</sup>
	25-30%	80.0 <sup>c</sup>	26.2 <sup>b</sup>	9.30 <sup>cd</sup>	2.39 <sup>de</sup>	129 <sup>bc</sup>
	30-35%	63.9 <sup>d</sup>	22.0 <sup>cd</sup>	7.92 <sup>e</sup>	2.00 <sup>f</sup>	106 <sup>d</sup>
	35-40%	56.6 <sup>e</sup>	20.5 <sup>d</sup>	7.21 <sup>f</sup>	1.84 <sup>f</sup>	95.0 <sup>e</sup>
	40-100%	20.2 <sup>f</sup>	9.16 <sup>e</sup>	2.98 <sup>g</sup>	1.01 <sup>g</sup>	36.8 <sup>f</sup>
	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 <sup>c</sup>	33.9 <sup>b</sup>	15.1 <sup>a</sup>	6.32 <sup>a</sup>	195 <sup>b</sup>
	5-10%	145 <sup>a</sup>	37.2 <sup>a</sup>	14.3 <sup>b</sup>	4.52 <sup>b</sup>	217 <sup>a</sup>
	10-15%	130 <sup>b</sup>	34.3 <sup>b</sup>	12.1 <sup>c</sup>	3.55 <sup>c</sup>	193 <sup>b</sup>
	15-20%	106 <sup>d</sup>	29.6 <sup>c</sup>	10.4 <sup>d</sup>	2.83 <sup>d</sup>	160 <sup>c</sup>
	20-25%	84.4 <sup>e</sup>	25.3 <sup>d</sup>	8.79 <sup>e</sup>	2.21 <sup>e</sup>	130 <sup>d</sup>
	25-30%	64.2 <sup>f</sup>	20.6 <sup>e</sup>	7.27 <sup>f</sup>	1.82 <sup>f</sup>	101 <sup>e</sup>
	30-35%	56.0 <sup>g</sup>	18.8 <sup>f</sup>	6.66 <sup>g</sup>	1.61 <sup>g</sup>	89.7 <sup>f</sup>
	35-40%	43.9 <sup>h</sup>	15.6 <sup>g</sup>	5.59 <sup>h</sup>	1.34 <sup>h</sup>	72.1 <sup>g</sup>
	40-100%	14.2 <sup>i</sup>	7.65 <sup>h</sup>	2.38 <sup>i</sup>	0.776 <sup>i</sup>	27.2 <sup>h</sup>
	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 <sup>g</sup>	12.8 <sup>f</sup>	9.60 <sup>d</sup>	10.7 <sup>b</sup>	47.9 <sup>g</sup>
	5-10%	12.8 <sup>f</sup>	17.3 <sup>e</sup>	12.3 <sup>cd</sup>	11.6 <sup>a</sup>	63.6 <sup>ef</sup>
	10-15%	39.7 <sup>b</sup>	26.1 <sup>b</sup>	41.3 <sup>a</sup>	7.95 <sup>c</sup>	129 <sup>b</sup>
	15-20%	60.1 <sup>a</sup>	32.1 <sup>a</sup>	43.4 <sup>a</sup>	4.84 <sup>d</sup>	158 <sup>a</sup>
	20-25%	41.8 <sup>b</sup>	25.2 <sup>b</sup>	31.2 <sup>b</sup>	3.39 <sup>e</sup>	114 <sup>c</sup>
	25-30%	31.4 <sup>c</sup>	22.2 <sup>c</sup>	14.3 <sup>c</sup>	2.43 <sup>f</sup>	79.8 <sup>d</sup>
	30-35%	24.1 <sup>d</sup>	20.0 <sup>d</sup>	13.5 <sup>cd</sup>	2.03 <sup>fg</sup>	67.0 <sup>e</sup>
	35-40%	18.6 <sup>e</sup>	17.9 <sup>e</sup>	10.6 <sup>cd</sup>	1.77 <sup>g</sup>	55.0 <sup>fg</sup>
	40-100%	3.22 <sup>g</sup>	6.92 <sup>g</sup>	3.41 <sup>e</sup>	0.668 <sup>h</sup>	15.7 <sup>h</sup>
	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 <sup>e</sup>	40.3 <sup>d</sup>	9.70 <sup>d</sup>	4.05 <sup>a</sup>	176 <sup>de</sup>
	5-10%	174 <sup>c</sup>	56.4 <sup>c</sup>	10.9 <sup>c</sup>	3.84 <sup>a</sup>	256 <sup>c</sup>
	10-15%	208 <sup>b</sup>	65.8 <sup>b</sup>	12.2 <sup>b</sup>	3.87 <sup>a</sup>	302 <sup>b</sup>
	15-20%	244 <sup>a</sup>	74.4 <sup>a</sup>	13.2 <sup>a</sup>	4.04 <sup>a</sup>	349 <sup>a</sup>
	20-25%	202 <sup>b</sup>	62.9 <sup>b</sup>	11.4 <sup>bc</sup>	3.54 <sup>b</sup>	291 <sup>b</sup>
	25-30%	165 <sup>c</sup>	52.2 <sup>c</sup>	9.84 <sup>d</sup>	3.04 <sup>c</sup>	240 <sup>c</sup>
	30-35%	128 <sup>d</sup>	41.5 <sup>d</sup>	8.09 <sup>e</sup>	2.57 <sup>d</sup>	188 <sup>d</sup>
	35-40%	102 <sup>e</sup>	34.1 <sup>e</sup>	6.95 <sup>f</sup>	2.20 <sup>e</sup>	153 <sup>e</sup>
	40-100%	23.6 <sup>f</sup>	9.79 <sup>f</sup>	2.22 <sup>g</sup>	0.907 <sup>f</sup>	38.8 <sup>f</sup>
	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 <sup>b</sup>	38.8 <sup>b</sup>	15.1 <sup>a</sup>	5.34 <sup>a</sup>	174 <sup>b</sup>
	5-10%	105 <sup>a</sup>	45.7 <sup>a</sup>	14.0 <sup>b</sup>	4.44 <sup>b</sup>	184 <sup>a</sup>
	10-15%	71.4 <sup>c</sup>	36.4 <sup>b</sup>	11.0 <sup>c</sup>	3.00 <sup>c</sup>	134 <sup>c</sup>
	15-20%	57.5 <sup>d</sup>	30.7 <sup>c</sup>	9.15 <sup>d</sup>	2.28 <sup>d</sup>	110 <sup>d</sup>
	20-25%	44.9 <sup>e</sup>	25.1 <sup>d</sup>	7.32 <sup>e</sup>	1.87 <sup>e</sup>	87.1 <sup>e</sup>
	25-30%	36.7 <sup>f</sup>	21.5 <sup>e</sup>	6.12 <sup>f</sup>	1.50 <sup>f</sup>	72.0 <sup>f</sup>
	30-35%	35.3 <sup>f</sup>	20.3 <sup>ef</sup>	5.67 <sup>g</sup>	1.48 <sup>f</sup>	68.2 <sup>fg</sup>
	35-40%	31.4 <sup>f</sup>	17.9 <sup>f</sup>	4.91 <sup>h</sup>	1.22 <sup>g</sup>	60.1 <sup>g</sup>
	40-100%	15.0 <sup>g</sup>	6.72 <sup>g</sup>	1.76 <sup>i</sup>	0.520 <sup>h</sup>	25.7 <sup>h</sup>
	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.

<sup>1</sup> 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.

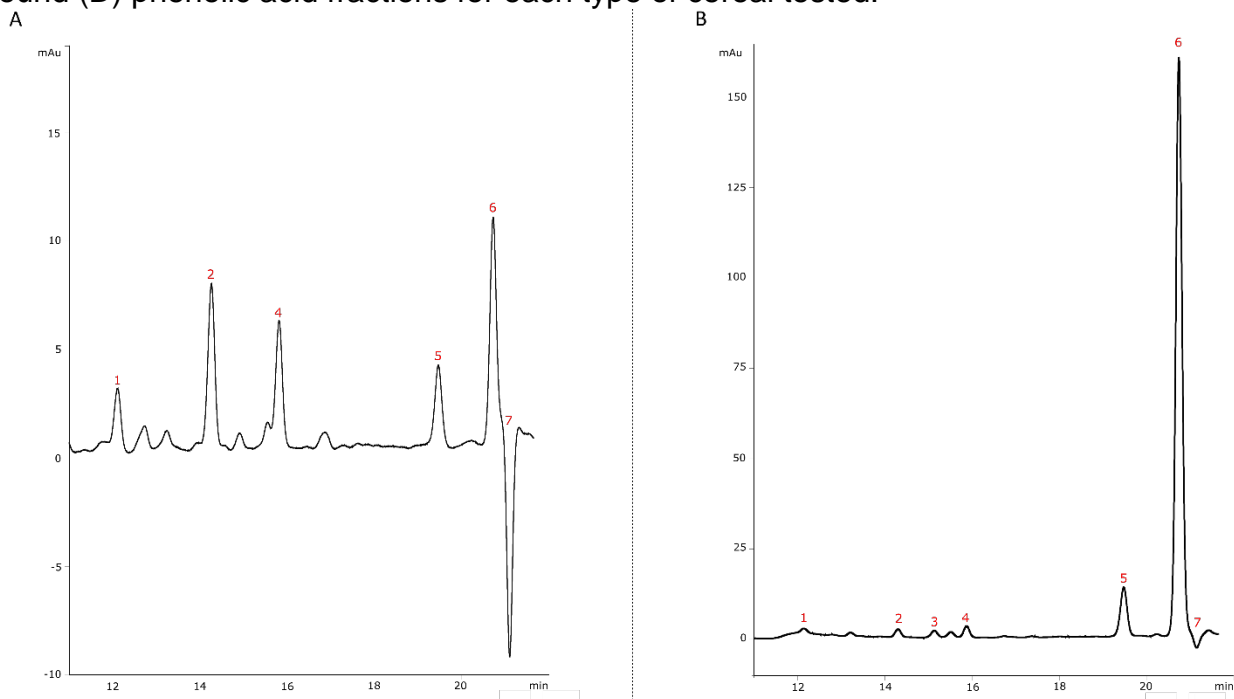
Cereal (Cultivar)	Pearled fraction	Ferulic acid	Sinapic acid	<i>p</i> -Coumaric acid	Vanillic acid	CWBPA <sup>1</sup>
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Tritordeum (Aucan)	0-5%	1409 <sup>a</sup>	72.3 <sup>a</sup>	136 <sup>a</sup>	15.2 <sup>a</sup>	1669 <sup>a</sup>
	5-10%	1427 <sup>a</sup>	70.9 <sup>a</sup>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

<sup>1</sup> 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.



C

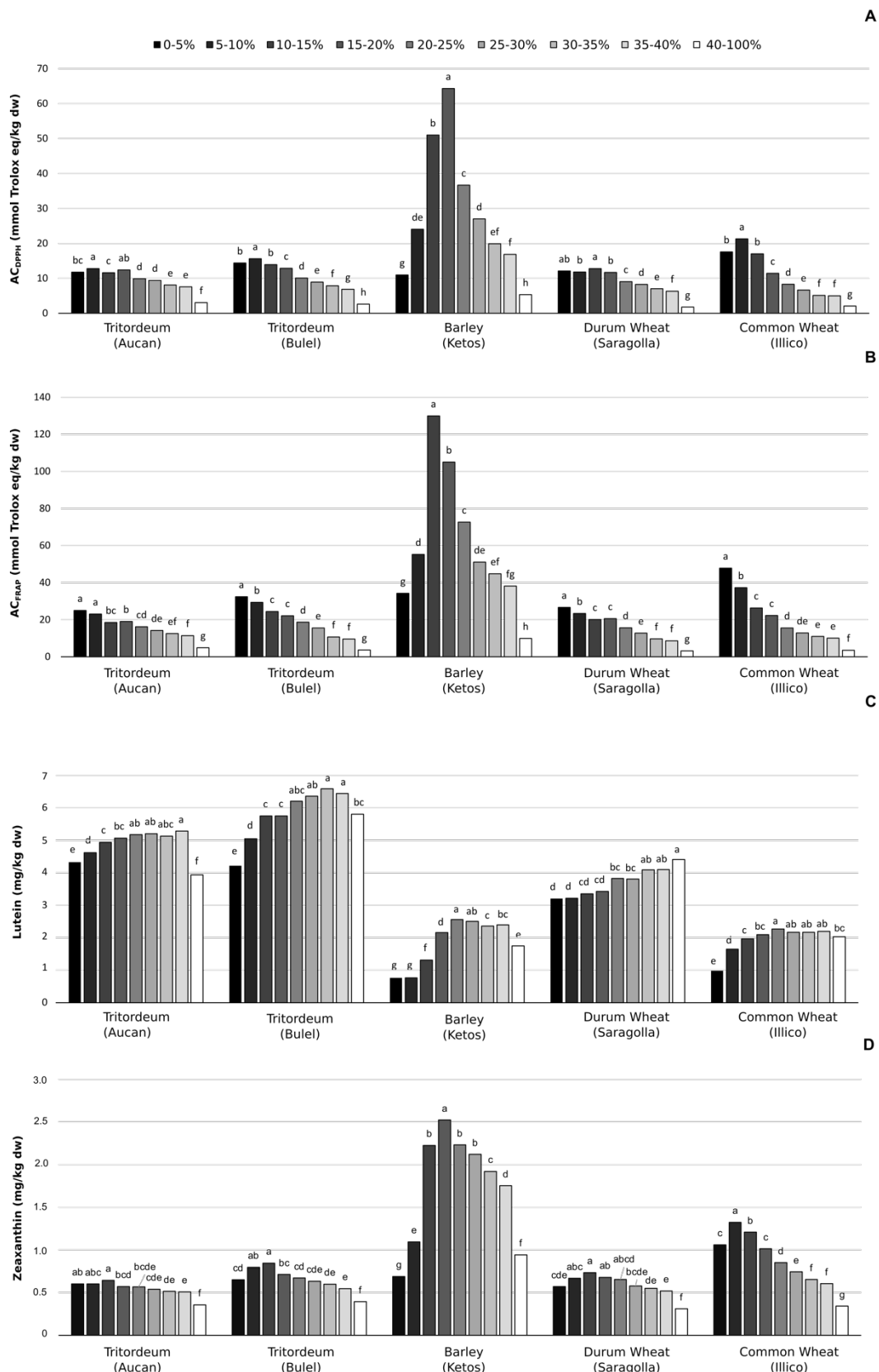
Cereal (Cultivar)	% Phenolic acid						
	1	2	4	5	6	7	
Tritordeum (Aucan)	4	8	5	2	21	60	
Tritordeum (Bulel)	4	9	4	3	22	59	
Barley (Ketos)	6	23	5	7	34	25	
Durum wheat (Saragolla)	3	5	2	2	23	66	
Common wheat (Illico)	4	8	4	2	25	56	

D

Cereal (Cultivar)	% Phenolic acid						
	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 perled 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.