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# Bioactive compound content, antioxidant activity, deoxynivalenol and heavy metal contamination of pearled wheat fractions.

<u>Running title</u>: Functional compounds and contaminants in pearled wheat fractions

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deoxynivalenol, heavy metals.

**Abbreviations:** Cd, cadmium; DF, dietary fibre; DON, deoxynivalenol; dw, dry weight; FPA, free phenolic acids; Pb, lead; TAA, total antioxidant activity; TE, trolox equivalents.

### 1 Abstract

Wheat kernels are naturally rich in antioxidant compounds, that are mainly present in the outer bran layers and which are removed during milling. Unfortunately, several contaminants are concentrated in the external layers. The pearling process, which progressively and carefully debrans the outer layers of wheat, could provide new functional food ingredients. The aim of the current study was to determine the content of functional compounds and the mycotoxin and heavy metals contamination of fractions derived from the sequential pearling of wheat kernels.

The pearling consisted of consecutive passages of 3 wheat varieties to remove 5% of 9 the original grain weight. Totally, five consecutive fractions were obtained starting 10 from the outer layer until the inner kernel that designated as 0-5, 5-10, 10-15, 15-20, 11 12 20-25%, respectively. The remaining 75% of the inner kernel was also collected. Dietary fibre, free phenolic acid and total antioxidant activity decreased progressively 13 from the external to the internal layers. However, the 5-10% fraction was richer in  $\beta$ -14 glucan content than the external one (0-5%). Heavy metals were only found in the 15 most external fraction. Deoxynivalenol contamination decreased from the external to 16 the internal layers: 64% of total contamination of kernel was found in the 0-5 and 5-17 10% fractions. 18

The 10-15% kernel fraction offered the best compromise between high nutritional
value and low contamination risk.

### 1. Introduction

Increasing demands about healthier foods has intensified the interest of consumers 23 in phytonutrients (Liyana-Pathirana, Dexter & Shahidi, 2006). The addition of 24 antioxidants to food systems may increase the nutritional profile and the shelf life of 25 products, and thus reduce waste and nutritional loss by inhibiting and delaying 26 oxidation. Recently, much attention has been paid to replacing synthetic antioxidants 27 with natural alternatives. Cereals are an important source of bioactive compounds 28 and some of them, such as phenolic compounds (phenolic acids, lignans and 29 flavonoids) show a marked antioxidant activity (Liyana-Pathirana & Shahidi, 2006). 30 The increased consumption of plant-derived phenolics has been associated with a 31 reduced risk of degenerative and chronic diseases (Dykes & Rooney, 2007). 32 Moreover, a diet containing cereals improve the content of other bioactive 33 compounds, such as dietary fibre (DF) and micronutrients (Hemery, Rouau, Lullien-34 Pellrin, Barron & Abecassis, 2007). 35

The protective effects of cereal fibres depend on their solubility: soluble fibre, particulary  $\beta$ -glucans, can reduce blood cholesterol, while insoluble fibres shorten the transit time through the intestinal tract, decreasing the contact between carcinogens and the epithelial cells in the colon (Fardet, Rock & Rémésy, 2008).

Whole wheat flour is richer in protein, phenolic acids and DF than commercial white flour; thus, whole grain flour results in higher antioxidant activity than the refined flour (Liyana-Pathirana & Shahidi, 2007), since bran fractions are removed in traditional milling operations. Unfortunately, the outer layers of the wheat kernel are also the most subjected portions into contamination by natural, such as mycotoxins, principally deoxynivalenol (DON), or synthetic contaminants, such as heavy metals cadmium (Cd) and lead (Pb), and pesticides (Cheli et al., 2010). Thus, whole flour

chances more than white flour to cross the limits established by law for both natural 47 and synthetic contaminants. Moreover, whole grain foods are not so attractive to 48 consumers, because the higher bran content in whole grain flour reduces the sensory 49 value of the end-use products: the high fibre content is the main cause of the 50 negative technological properties of whole grain bread, with a reduction in loaf 51 volume, an increase in crumb firmness and a dark color (Zhang & Moore, 1999). 52 Therefore, a grain fractionation technology is needed in order to separate efficiently 53 the negative and positive aspects. This will let to produce new flour mixes and 54 ingredients with technologically optimized functional and nutritional attributes. 55

The pearling (debranning) of wheat, before roller milling, is becoming increasingly 56 accepted by wheat millers as a means of improving milling performance, since it 57 sequentially removes the outer kernel bran layers through an abrasive scouring and 58 increases the efficiency of the milling process (Dexter & Wood, 1996). The average 59 concentrations of DON and heavy metals are more efficiently reduced by pearling 60 than by milling (Cheli et al., 2010). Nevertheless, this process, which involves the 61 external layer of kernels, is responsible for the loss of high nutritional content. A 62 previous study demonstrated that the phenolic content, which is closely highly 63 correlated to the total antioxidant activity (TAA), progressively decreases as the 64 pearling progresses through the aleurone layer into the inner parts of the kernel 65 (Liyana-Pathirana et al., 2006). In fact, the typical grain fraction removed by pearling 66 (before milling) contains more than 40% of the total phenolic content of the whole 67 kernel (Beta, Nam, Dexter & Sapirstein, 2005). 68

However, the degree of pearling could be efficiently modulated in order to separate the external bran fractions, which are characterized by a high sanitary risk and coarse fibre, from the cereal fractions with potential high health benefits. An

alternative strategy to the use of whole flour, in order to maximize health benefits of wheat-based products, could be to enrich conventional flour with wheat bran fractions, obtained from sequential pearling, as they are characterized by a higher antioxidant activity and phytonutrient content, but lower risk from the contaminant content (Hemery et al., 2007). For this purpose, it is necessary to evaluate not only the distribution of the phytonutrients and TAA in wheat grain in more detail, but also the content of the contaminants in different and deeper pearled fractions.

The aim of this study was to determine the wheat kernel pearled fractions, obtained from progressive pearling, with the highest nutritional value, considering the free phenolic acids (FPA), DF and  $\beta$ -glucan contents and TAA, and the lowest natural and synthetic contaminant contents, in order to use them as functional food ingredients.

### 84 **2. Materials and methods**

### 85 2.1 Wheat grain pearling

Three commercial winter wheat varieties (Triticum aestivum L.), Bolero, Bologna and 86 Taylor, were collected from homogeneous lots of each cultivar, cultivated in the 87 2010-2011 growing season in Alessandria (44° 57' N, 8° 29' E; altitude of 121 m; in 88 a deep and acid loamy soil - Aquic Frugiudalf) and stored in vertical silos. All the 89 compared cvs were seeded after an autumn ploughing (30 cm) and disk harrowing to 90 prepare a proper seedbed. Planting was held in 12 cm wide rows at the end of 91 October at a seeding rate of 450 seeds m<sup>-2</sup>. For Bologna and Taylor cvs, a total of 92 180 kg N ha<sup>-1</sup> was applied to wheat fields as granular ammonium nitrate fertilizer. On 93 the other hand, field cultivated with cv Bolero received a total of 140 kg N ha<sup>-1</sup>. The 94 95 amount of ammonium nitrate was split equally between tillering and stem elongation stages for each cv. None fungicide was applied at wheat heading to control Fusarium 96 Head Blight (FHB). Wheat fields were harvested in early-mid July with a combine-97 98 harvester and kernels of each cv were stored separately. The wheat varieties were characterized by hardness, color and technological qualities (Tab. 1), on the basis of 99 the ISQ method for quality classification of common wheat, proposed by Foca et al. 100 (2007). As far as FHB infection and DON contamination is concerned, Bologna cv is 101 classified as moderately resistant, while cv Bolero and Taylor are classified as 102 moderately susceptible (Mayerle, Pancaldi, Haidukowski, Pascale & Ravaglia, 2007). 103 Moreover, the environmental conditions from anthesis to harvest observed in the 104 growing area were slightly favorable to FHB and to DON contamination. 105

106 Six fractions of kernels from each variety were obtained through incremental 107 pearling, following the approach proposed by Beta et al. (2005). The pearling

consisted of consecutive passages of wheat and pearled wheat in an abrasive-type 108 grain testing mill (TM-05C model, Satake, Tokyo, Japan) at a constant speed of 55 109 Hz. The pearling process was monitored by time control. After each assay, the 110 laboratory pearler was thoroughly cleaned by means of dust aspiration and 111 compressed air, to minimize equipment contamination. Initially, a 500 g portion of 112 each unprocessed wheat was sub-sampled from a 5 kg sample, and the remaining 113 4.5 kg was pearled. Starting from unprocessed grain, kernels were initially pearled to 114 remove 5% of the original grain weight, and this resulted in a first fraction (0-5%). 115 The remaining kernels were then pearled to remove a second fraction of 5% (5-10%). 116 The pearling process was continued until a third, fourth and fifth fraction (designed 117 10-15%, 15-20%, 20-25%, respectively) plus a residual 75% of the kernel (25-118 100%), were collected. 119

A total of seven samples were obtained from each variety: the whole unprocessed wheat and the 0-5%, 5-10%, 10-15%, 15-20%, 20-25%, 25-100% fractions, obtained through the pearling process. The whole wheat samples and the residual 75% of the unprocessed kernels were milled using a laboratory centrifugal mill (ZM-100; Retsch, Haan, Germany) with a 1 mm opening. Then, both the milled and pearled samples (500 g) were ground to pass through a 0.5 mm screen and stored at -25°C before the chemical analyses.

127

### 128 2.2. Chemicals

Total Dietary Fibre and Mixed-Linkage β-Glucan kits for enzymatic determinations
were supplied by Megazyme (Megazyme International Ireland Ltd, Wicklow, Ireland).
Methanol (HPLC grade) and formic acid (50%, LC–MS grade) were purchased from
Sigma–Aldrich (Milan, Italy). Water was obtained from Milli-Q instrument (Millipore

Corp., Bedford, MA, USA). Antibody-based immunoaffinity columns were supplied by
 VICAM (Waters Corporation, Watertown, MA, USA). All the other chemicals and
 solvents were of a reagent-grade level and were also purchased from Sigma–Aldrich
 (Milan, Italy).

137

### 138 2.3 Chemical analyses

### 139 **2.3.1. Proximate composition analysis**

The moisture, protein, ash, total DF and  $\beta$ -glucan contents were determined on 140 ground whole kernels and their pearled fractions. The moisture content, determined 141 142 in order to express the results on a dry weight (dw) basis, was obtained using a 143 Sartorius MA30 thermo-balance (Sartorius AG, Goettingen, Germany). The total nitrogen content and total protein content (conversion factor: 5.70) were obtained 144 according to the Kjeldahl method, using Kjeltec system I (Tecator, Sweden). The ash 145 coBontent was determined in a muffle furnace according to the AOAC (1990) 146 procedure. The total dietary fibre was measured using the Megazyme total dietary 147 fibre analysis kit, according to the enzymatic-gravimetric method proposed by 148 Prosky, Asp, Schweizer, DeVries, and Furda (1988); the determination was 149 performed employing the Fibertec 1023 system (FOSS Italia S.p.A., Padova, Italy). β-150 glucan determination was performed using the Megazyme mixed-linkage β-glucan 151 assay kit, according to the instructions provided by the producer. 152

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### 154 **2.3.2. Extraction of free phenolic acids (FPA)**

Prior to the extraction of the FPA, samples were ground in a oscillatory mill (Mixer Mill MM440, Retsch GmbH, Hann, Germany) and sieved to obtain fine flours (particle size < 250 μm). Fifty milligrams of each sample were suspended in 1 mL of</li>
a MeOH/H<sub>2</sub>O 80:20 (v/v) mixture, vortexed for 10 seconds and then extracted in an
ultrasonic bath (Bransonic 1510, output 42 kHz, Branson Ultrasonics, USA) for 2
minutes. The extracts were centrifuged at 14000 rpm for 1 min (Microcentrifuge 5417
R, Eppendorf Italia, Milan, Italy) and pellets were extracted another two times,
according to the method described above. Supernatants were collected and used for
the chromatographic analyses. All the samples were extracted in triplicate.

164

### 165 **2.3.3. Determination of free phenolic acids by means of RP-HPLC/DAD**

Phenolic acid separation was performed using the Shimadzu LC-20 A Prominence 166 HPLC system (Shimadzu Italia, Milan, Italy) equipped with an LC-20AB pump 167 system, a SIL-20-A auto-injector, a CTO-20A column oven, and a SPD-M20A diode 168 array detector. The used column was an Ascentis RP-amide (150 x 2,1 mm i.d., with 169 a particle size of 3 µm, Supelco, Bellefonte, PA, USA) which was maintained at 27 170 °C. The mobile phase consisted of water/formic acid 0.1% (v/v) (eluent A) and 171 methanol/formic acid 0.1% (v/v) (eluent B), and the following elution programme was 172 used: isocratic 2.5% B (10 min), from 2.5% to 12% B (25 min), from 12% to 100% B 173 (31 min), from 100% to 2.5% B (2 min), isocratic 2.5% B (5 min), The total running 174 time was 73 min and was conducted at a constant flow-rate of 400 µL/min. 175 Chromatograms were recorded at two different wavelengths (280 and 330 nm). 176

The phenolic acids were tentatively identified through a comparison with the retention times and UV-vis spectra of individual standard molecules (gallic acid, protocatechuic acid, syringic acid, *p*-hydroxybenzoic acid, caffeic acid, chlorogenic acid, ellagic acid, ferulic acid); the quantification was performed on the basis of calibration curves (6 different concentration levels; linearity range:  $0.5 - 5.5 \ \mu g \ mL^{-1}$ ) obtained using the

corresponding standards. The previously described phenolic extracts were directly
 injected into the chromatographic system (injection volume: 15 μL).

184

### 185 **2.3.4. Determination of the total antioxidant activity (TAA)**

The TAA was determined adapting the classical DPPH radical scavenging method 186 (Locatelli, Gindro, Travaglia, Coisson, Rinaldi & Arlorio, 2009) to the QUENCHER 187 approach (direct measurement of antioxidant activity on solid samples suggested by 188 Gökmen, Serpen and Fogliano, 2009). Exactly 10 milligrams of ground whole kernels 189 and pearled fractions (particle size < 250  $\mu$ m) were weighted, then 700  $\mu$ L of 190 methanol and 700 µL of a DPPH methanolic solution 100 µM were added. The 191 samples were vortex-mixed and the reaction was then carried out in the dark under 192 stirring at 20 °C and 1000 rpm (Thermomixer comfort, Eppendorf, Milan, Italy) for 25 193 min. The samples were promptly centrifuged for 1 min at 14000 rpm (Microcentrifuge 194 5417 R, Eppendorf Italia, Milan, Italy) and the absorbance at 515 nm was then 195 measured after exactly 30 min of reaction (on attainment of the steady state), using a 196 197 Kontron UVIKON 930 Spectrophotometer (Kontron Instruments, Milan, Italy). A control solution (700 µL of methanol and 700 µL of DPPH' methanolic solution 100 198 µM) was tested under the same conditions, in order to calculate the DPPH inhibition 199 percentage of the samples. The final results were expressed as mmol of trolox 200 equivalents (TE) per kg of sample (dw) through a calibration curve (linearity range: 201  $4-60 \text{ nM}; r^2 = 0.982).$ 202

203

### 204 **2.3.5. DON contamination**

The DON content was analysed using a high performance liquid chromatography (HPLC-MS-MS) method (range 20-1000  $\mu$ g kg<sup>-1</sup>). Samples of 25 g each were

extracted with 100 mL of water in a blender at a high speed for 30 minutes; the entire 207 extract was then filtered and collected. Antibody-based immunoaffinity columns 208 (DON test<sup>™</sup> WB Columns VICAM) were utilised for cleanup of the sample extracts. 209 Before the sample was loaded, the column was conditioned with 1 mL of deionized 210 water. 1 mL of the sample was loaded on the previously conditioned immunoaffinity 211 column at a rate of approximately 1-2 drops s<sup>-1</sup>. The column was washed with 5 mL 212 of distilled water. DON was eluted from the column with 2 mL of methanol. DON was 213 quantified by the injection of 10 µL of diluted eluate into the HPLC-MS-MS system, 214 which consisted of a Varian 212-LC Chromatography Pump and a 310-MS TQ Mass 215 Spectrometer. The analytical column was a reverse Varian Polaris C18-A (100 x 2.00 216 mm, 3 µm) while the mobile phase was a mixture of methanol and water fed at a flow 217 rate of 0.2 mL min<sup>-1</sup>. 218

219

### 220 **2.3.6. Heavy metal content**

The Cd and Pb analyses were performed according to the method of the Italian 221 Organization for Standardization (UNI EN 14083, 2003). Aliguots of the samples (500 222 mg on a dry-matter basis) were dissolved in 5 mL of concentrated nitric acid and 2 223 mL of 30% v/v hydrogen peroxide, then heated under reflux in a stoppered guartz 224 vessel placed in a microwave oven. The solution was diluted to 25 mL in a volumetric 225 flask with ultra-pure water. Samples were analysed by graphite furnace atomic-226 absorption spectrometry (GFAAS; Analyst 700, Perkin Elmer Corporation, USA). All 227 the metal concentrations were determined by autosampler injection of the aqueous 228 solution into a graphite furnace. 229

### 231 2.4. Statistical analysis

All the analyses were performed in triplicate, with the exception of the heavy metal 232 content, performed in one replicate. The results are reported as the mean of the 233 three replicates; the coefficients of variation were < 10%. The analysis of variance 234 (One-way ANOVA) was applied for each variety to compare the protein, total DF, β-235 glucan, FPA, ash and DON contents and the TAA in the whole grain and in the 236 different pearled fractions. The residual normal distribution was verified using the 237 238 Kolmogorov-Smirnov test, while variance homogeneity was verified using the Levene test. Multiple comparison tests were performed according to the Student-Newman-239 Keuls test on treatment means. The SPSS for Windows statistical package, Version 240 17.0 (SPSS Inc., Chicago) was used for the statistical analysis. 241

### 243 **3. Results**

### 3.1. Bioactive compounds in the whole kernel

The protein, ash, total fibres, β-glucan and FPA contents, and the TAA determined 245 for the grain whole kernels are reported in Table 1. Except for the TAA, ANOVA 246 showed significant differences (P<0.05) between the wheat varieties used in this 247 study. According to the ISQ quality classification, the grain protein content of Bolero 248 cultivar was significantly lower than that of the Bologna and Taylor cultivars, 249 respectively. The total DF was significantly higher in the Bolero and Taylor cvs than 250 in the Bologna one. Compared to the other two varieties, Bolero showed significantly 251 lower and higher contents of  $\beta$ -glucans and FPA, respectively. 252

253

### 3.2. Bioactive compounds of the pearled fractions

The bioactive compounds content in the fractions obtained from the sequential wheat pearling is reported in Tables 2 and 3. ANOVA showed highly significant differences (P<0.001) for the proteins, total fibre and  $\beta$ -glucan content, and for the TAA determined in the different pearled fractions (Tab. 2).

The 10-15% fraction showed the highest protein content in all three varieties, while the concentration significantly decreased towards both the internal and the external layers. Only for the Bolero cultivar, there were no significant differences between the 5-10% and 10-15% fractions. The Bolero cv showed a significantly higher protein concentration in the more external layers (0-5%) than endosperm (25-100%), while no significant differences were observed between the 0-5% and 25-100% fractions of the Bologna and Taylor cultivars.

The total DF was predominant in the outermost layers (0-5%) for all three varieties. 266 Each successive pearling passage significantly decreased the fibre content towards 267 the inner layer. The 5-10% fractions of Bolero, Bologna and Taylor cultivars showed 268 a 37%, 35%, and 33% reduction in DF content compared to the corresponding 0-5% 269 fractions, respectively. The fibre content of Bolero cultivar in the 10-15% and 15-20% 270 fractions was 3.2 and 2.7 times higher than that of endosperm (25-100% fraction), 271 respectively. On the other hand, on average, the 10-15% and 15-20% fractions of the 272 Bologna and Taylor cvs had a 4.3 and 3.4 times higher content of total fibre than the 273 25-100% fraction, respectively. The 5-10% fraction contained the highest β-glucan 274 concentration for all of the three varieties tested. In the next inner pearling passage 275 (fraction 10-15%), a significant reduction in the  $\beta$ -glucan contents by 12%, 22% and 276 12% was perceived for the Bolero, Bologna and Taylor cvs, respectively. The β-277 glucan content significantly decreased from this fraction to the inner layers at each 278 subsequent pearling. Moreover, the outer fraction (0-5%) in all three varieties showed 279 a significantly lower concentration of  $\beta$ -glucans than the 10-15% fractions. Cv. Bolero 280 showed a significantly higher  $\beta$ -glucan content in the 15-20% fractions than in the 0-281 5% one, while the outermost layer of the Bologna cv showed a significantly lower 282 concentration of these compounds. No significant differences were observed for the 283 Taylor cv between the 0-5 and 15-20% fractions. 284

The highest TAA for the Bologna and Taylor cvs, was found in the outermost 0-5% fraction, then TAA decreased significantly after each progressive pearling towards the inner layers. On average, for these varieties, TAA was 14%, 23%, 41%, 56% and 87% lower than the 0-5% fraction, for the 5-10%, 10-15%, 15-20%, 20-25% and 25-100% fractions, respectively. The pearling passage between the 10-15% and 15-20% fractions resulted in the first important loss of TAA. On the other hand, there were no significant differences in TAA values between the 0-5% and 5-10% fractions of the
 Bolero cv. Then, from the 5-10% fraction, TAA decreased significantly after each
 progressive pearling towards the inner layers.

The content of some phenolic acids (ferulic, chlorogenic, p-hydroxybenzoic, syringic, 294 protocatechuic and caffeic acid) present in the pearled wheat fractions in their free 295 form is reported in table 3. Among the standard molecules employed for the 296 chromatographic analysis, ellagic acid was not clearly recognized in the samples, 297 while gallic acid was only identified in the 0-5% fraction (30, 26 and 48  $\mu$ g kg<sup>-1</sup> for the 298 Bolero, Bologna and Taylor cvs, respectively). Ferulic acid was the predominant 299 phenolic acid, followed by chlorogenic and caffeic acids. p-Hydroxybenzoic, syringic 300 and protocatechuic acids were found in lower concentrations and were not detected 301 in all the pearled fractions. 302

ANOVA showed highly significant differences (P<0.001) for all the free phenolic acids 303 detected in the different wheat fractions. The total FPA content for the three varieties 304 decreased from the outer fractions to the endosperm. The total FPA content for 305 Bologna cv significantly decreased at each successive pearling from the outer 306 fractions towards the inner layers. Each pearling fraction for Taylor cv showed a 307 significantly different total FPA concentration, with the exception of the 15-20% and 308 20-25% fractions. On the other hand, no significant differences were observed 309 between the 0-5% and 5-10%, or between the 15-20% and 20-25% fractions 310 obtained from Bolero cv. On average, the free ferulic acid and the total FPA were 311 312 27% and 30% lower in the 10-15% fraction than in the 0-5% one, respectively.

313

314 3.3. Ash, heavy metals and DON contamination of pearled fractions

The ash, heavy metal and DON contents of in the wheat fractions are reported in Table 4. ANOVA showed highly significant differences (P<0.001) for the ash content and the DON contamination.

The highest ash content for the Bolero and Taylor cvs was found in the 5-10% fraction, followed by the 0-5% one, then ash decreased significantly after each progressive pearling towards the inner layers. The ash concentration in the 0-5% and 5-10% fractions was not significantly different for the Bologna variety. The ash content of the three varieties was on average 5.4, 5.6, 4.5, 3.5 and 2.9 times higher in the 0-5%, 5-10%, 10-15%, 15-20% and 20-25% fractions compared to the endosperm residue (25-100%), respectively.

Among the heavy metals, Pb was not found in any of the pearled wheat fractions, while Cd concentration was only detected in the outermost layer (0-5%) of the Bologna and Taylor cvs.

Kernels from the Bolero and Taylor varieties resulted to be contaminated by DON, while the data for the Bologna cv were always below the detection limit. The highest DON contamination in both varieties was found in the outermost fraction (0-5%), and the DON content then decreased significantly after each progressive pearling towards the inner layers. On average, the DON content decreased by 49, 19, 9, 5 and 4 times in the 0-5%, 5-10%, 10-15%, 15-20% and 20-25% fractions compared to the endosperm residue (25-100%), respectively.

335

### **4. Discussion**

The presented data have shown clearly how the concentration of bioactive compounds is greater in the outer layers of wheat grain, but their distribution in each pearled fraction is different considering the classes of nutrients.

Shetlar, Rankin, Luman and France (1947) reported that outer pericarp, the inner pericarp, the testa and the aleurone layer, respectively represents 3.9, 0.9, 0.7, and 9.0% of the kernel weight. Therefore, according also to data reported by Bottega, Caramanico, Lucisano, Mariotti, Franzetti and Pagani (2009); Jerkovic, Kriegel, Brander, Atwell, Roberts and Willows (2010) and Singh and Singh (2010), pearling up 5% level on average removed most of the outer pericarp, while at 5-10% and 10-15% level the aleurone layers were removed.

The protein content is higher in the 10-15% fraction for all three varieties, confirming data reported by Jerkovic et al. (2010), who found a much greater concentration and diversity of protein functions in the microdissected intermediate layers (testa and nucellar tissue), corresponding to this pearled fraction, than in the other bran layers.

Sequential pearling has shown that, in common wheat, the total DF decreased 351 progressively from the external to internal layers. This reduction was higher in the 352 hard varieties (cvs Bologna and Taylor) than soft one (cv Bolero). In common wheat 353 bran, the values for total DF was almost 4 times higher that of the whole grain (Sidhu, 354 Al-Hooti & Al-Sager, 1999). Dexter and Wood (1996) reported that the pearling of 355 common wheat reduces the insoluble and soluble fibre content compared to 356 unprocessed grain by 57% and 30%, respectively. The outermost tissues are rich in 357 insoluble DF, while the aleurone layer in particular results in a high soluble DF 358 content (Parker, Ng & Waldron, 2005). Moreover, the fibre present in the most 359 external bran layers is relatively coarse, whereas the fibre near the aleurone layer is 360

361 finer (Noort, van Haaster, Hemery, Schols & Hamer, 2010).

The  $\beta$ -glucans, components of the soluble fibre, were higher in the middle fractions 362 (5-20%), and peaked in the 5-10% pearled fraction, while lower in the more external 363 layers (0-5%) had a lower  $\beta$ -glucan content. Several authors have reported that the 364 aleurone layer in common wheat contains higher levels of β-glucans than whole grain 365 (Hemery et al., 2007). A reduction of 12% in the  $\beta$ -glucan content of unprocessed 366 wheat grain has been observed after pearling processes (Dexter & Wood, 1996). 367 Barley is rich in  $\beta$ -glucans and the highest concentration of  $\beta$ -glucans was found in 368 the middle fractions, followed by bran, while the lowest content were found in the 369 flour (Sullivan, O'Flaherty, Brunton, Gee, Arendt & Gallagher, 2010). 370

The sequential removal of the external layers through pearling resulted in a decrease 371 in FPA with concurrent lower TAA values. The highest observed concentration of 372 FPA in the outer kernel fractions (0-15%) confirms the data reported by Beta et al. 373 (2005) on the concentration of the total phenol compounds in the wheat kernel. 374 Liyana-Pathirana et al. (2006) reported that total bran-rich fractions possess higher 375 total phenolic compounds and TAA than starch-rich fractions. Some authors have 376 shown that the aleurone layer is richer in antioxidant compounds than the other bran 377 tissues, mainly due to its high content of phenolic acids (Buri, von Reding & Gavin, 378 2004). This diversity in the phenolic composition between the bran layers probably 379 reflects differences in the biosynthetic and turnover mechanism, related to 380 arabinoxylan synthesis, as suggested by Parker et al. (2005). Liyana-Pathirana and 381 Shahidi (2006) reported that soft wheat has a higher total phenolic content and TAA 382 than hard wheat. In agreement with these results, in the present study it has been 383 found that Bolero, a soft variety, has a higher total FPA content than the Bologna 384 (medium hard) and Taylor (hard) cvs. 385

Fardet et al. (2008) reported that ferulic acid represents approximately 46-67% of the 386 total phenolic acids in wheat, and that it is found associated with polysaccharides, 387 mainly arabinoxylans, in aleurone cell walls. Our results show that ferulic acid 388 occurred in about 65% of the total FPA analyzed. Liyana-Pathirana and Shahidi 389 (2007) reported that although ferulic acid is dominant in cereal grains, caffeic acid 390 shows a higher antioxidant activity. This information explains the similar TAA levels 391 of the three varieties examined, although Bolero has a higher total FPA and ferulic 392 acid content, while Taylor, with a lower total FPA content, showed a 4-times higher 393 caffeic acid concentration. Moreover, many insoluble components of foods may exert 394 antioxidant properties and esterified phenolic acids in particular can contribute to the 395 total antioxidant activity of cereals (Serpen, Gökmen, Pellegrini & Fogliano, 2008). 396 The direct procedure used in this work to determine TAA allowed the contribution of 397 both the soluble (e.g. free phenolic acids) and insoluble (e.g. bound phenolic acids) 398 antioxidants to be measured. Moreover, the use of an aqueous methanolic solution 399 (methanol/water 50:50, v/v) to perform the DPPH' method (so adding to the solid 400 samples 700 µL of water and 700 µL of DPPH' methanolic solution; see Materials 401 and methods section) allowed to obtain a TAA about 20% greater than that obtained 402 by the method using pure methanol as solvent. These additional results (data not 403 shown) confirm that the use of water as one of the solvents in the mixture helps 404 radicals to diffuse better into the particles of the sample, thus increasing the 405 interaction between the radicals and the antioxidants (Gökmen et al., 2009). Even if 406 407 an increase of TAA was observed employing aqueous methanolic solutions, the relative antioxidant activity of the samples confirmed results obtained using pure 408 methanol, thus indicating a general TAA decrease during the progressive pearling 409 process. 410

The outermost kernel layers had the highest DON contamination which decreased 411 from the external to the internal layers. According to Lancova et al. (2008), the levels 412 of DON in bran can be two or more times higher than in whole wheat kernels, 413 indicating the concentration of this mycotoxin in the outer part of the kernel. In both 414 laboratory studies and industrial milling systems, the application of pearling before 415 milling has led to flours with lower DON contents (Cheli et al., 2010). Our data 416 confirm that DON decreases moving from the external to the internal layers following 417 a biphasic behavior: a high reduction was observed in the first pearling steps and this 418 was followed by a slower decrease. The residual grain after pearling of the first two 419 outer fractions (0-5 and 5-10%) contained around 64% of the total DON quantity of 420 the whole grains tested in this study. In previous experiment the DON contamination 421 was reduced after a 10% grain mass loss in durum wheat of 15% (Cheli et al., 2010) 422 and 45% (Rios, Pinson-Gadais, Abecassis, Zakhia-Rozis & Lullien-Pellerin, 2009). 423 For barley, a grain mass loss of 15%, reduced the DON contamination of 34% 424 (House, Nyachoti & Abramson, 2003) and in common wheat, with a 18% of mass 425 removal, DON contamination was reduced of 42% (Trenholm, Charmley, Prelusky & 426 Warner, 1991). 427

Moreover, heavy metals were only found in the most external fraction. Milling 428 reduced heavy metal contents in flour or semolina and increased their contents in the 429 by-products, derived principally from the pericarp layers (Oliver, Gore, Moss & Tiller, 430 1993). In the study of Cubadda, Raggi, Zanasi and Carcea (2003) the milling of 431 durum wheat determined an average reduction of 31% and 12% for Cd and Pb, 432 respectively. Cheli et al. (2010) reported that although no significant differences were 433 found between unprocessed wheat and pearled wheat, either in conventional milling 434 or in pearling before milling, Cd and Pb were concentrated in shorts and flour shorts. 435

The external pearled wheat fractions also resulted in the highest ash content. House et al. (2003) and Rehman, Ahmand, Bhatti, Shafique, Ud Din and Murtaza (2006) reported a 29% and 19% lower ash content after removal of 15% of the mass grain of barley and wheat, respectively. Dexter and Wood (1996) reported that the pearling process on durum wheat significantly reduced the ash content by 36% and 16% in pearled kernels and semolina, respectively.

In addition, Laca, Mousia, Diaz, Webb, and Pandiella (2006) and Bottega et al.
(2009) established that the number of bacteria and moulds present in wheat grains,
and located in the outer pericarp, can be conspicuously reduced by pearling.

The TAA, the  $\beta$ -glucan, the total FPA and the DON contents (mean values of the 445 three wheat cultivars) are summarised in Fig. 1, with the objective of showing the 446 kernel fractions that offer the best compromise between high nutritional value and 447 low contamination risk. The 10-15% pearled fraction has shown to greatly reduce the 448 DON content, compared to the outer fractions, and has not been contaminated by 449 heavy metals. At the same time, this pearled fraction preserved an acceptable high 450 nutritional content, since it maintained high protein and  $\beta$ -glucan concentrations, and 451 the loss of total FPA content and TAA was not so high compared to the richer 452 external layers. Moreover, the total DF in this pearled fraction remained high, while 453 only the coarse fibre was removed with the outermost bran layer (Esposito, Arlotti, 454 Bonifati, Napolitano, Vitale & Fogliano, 2005). This could constitute an important 455 technological aspect, since bread containing coarse particle size bran is considered 456 457 less acceptable in sensory quality than breads containing medium-fine particle size bran (Zhang & Moore, 1999). As far as the major chemical components of wheat 458 kernel is concerned, Bottega et al. (2009) and Singh and Singh (2010) reported that 459 pearling level lower than 10% guaranteed low starch and protein losses in the waste 460

and, at the same time, noticeably reduced the detrimental components of kernel(ash, microbial contamination).

On the basis of the data collected, it is possible to state that the sequential pearling 463 of wheat kernels confirms to be an interesting dry-fraction technology, which can 464 produce bran fractions with high concentration in aleurone and intermediate material 465 rich in phytochemicals. This material can be used to transform a flour by-product into 466 a high nutritional value food ingredient (Hemery et al., 2007). The present study can 467 be considered as a first contribution towards individuating the most useful pearled 468 grain fractions for this purpose, although more research, also using the measurement 469 of biochemical markers found in wheat grain tissue (Hemery et al., 2009) is still 470 necessary. 471

### 473 **5. Conclusion**

Our study has confirmed the results of other previous reports, that investigated the 474 content of various contaminants and bioactive compounds in pearled wheat fractions 475 separately. Among the wheat varieties and the pearled fractions compared in this 476 experiment, the kernel fraction that offers the best compromise between high 477 nutritional value and low contamination risk is the 10-15% fraction. Furthermore, in 478 grain lots with a low contaminant presence in the outer part of the grains, the 5-10% 479 fraction could also be separated and recovered. On the basis of these results, the 480 pearling process could be an important way of valorizing the wheat bran layers of 481 kernel, as a natural source of bioactive compounds, separated from detrimental 482 components, in order to develop nutritionally enhanced ingredients and products. In 483 fact, the wheat bran layers, instead of being totally rejected or maintained as 484 happens in the traditional milling process for refined or whole flour, respectively, 485 could be progressively and carefully separated, through the pearling process. The 486 most external fractions, with higher risks because of the presence of natural and 487 synthetic contaminants, could then be discarded, while the fractions with a low 488 sanitary impact, but high nutritional value, could be reinserted into the flour or used 489 as a functional ingredient. 490

The replacement of wheat flour with the selected pearling fraction could be an important way of enriching wheat-based products in bioactive compounds and of reducing the sanitary risks associated to the use of bran layers.

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### 503 6. References

- AOAC (1990). Official Methods of Analysis (15th ed.). Arlington, VA: Association of Official Analytical Chemists.
- Beta, T., Nam, S., Dexter, J. E., & Sapirstein, H. D. (2005). Phenolic content and
   antioxidant activity of pearled wheat and roller-milled fractions. *Cereal Chemistry*,
   82, 390-393.
- Bottega, G., Caramanico, R., Lucisano, M., Mariotti, M., Franzetti, L., & Pagani, M.A.
  (2009). The debranning of common wheat (*Triticum aestivum* L.) with innovative
  abrasive rolls. *Journal of Food Engineering*, *94*, 75-82.
- Buri, R. C., von Reding, W., & Gavin, M. H. (2004). Description and characterization
  of wheat aleurone. *Cereal Foods World, 49*, 274-282.
- Cheli, F., Campagnoli, A., Ventura, A., Brera, C., Berdini, C., Palmaccio, E., &
  Dell'Orto, V. (2010). Effects of industrial processing on the distribution of
  deoxynivalenol, cadmium and lead in durum wheat milling fractions. *LWT-Food Science and Technology, 43*, 1050-1057.
- 518 Cubadda, F., Raggi, A., Zanasi, F., & Carcea, M. (2003). From durum wheat to 519 pasta: effect of technological processing on the levels of arsenic, cadmium, lead 520 and nickel-a pilot study. *Food Additives and Contaminants*, *20*, 353-360.
- 521 Dexter, J. E., & Wood, P. J. (1996). Recent applications of debranning of wheat 522 before milling. *Trends in Food Science & Technology, 7*, 35-41.
- 523 Dikes, L., & Rooney, L.W., (2007). Phenolic compounds in cereal grains and their 524 health benefits. *Cereal Foods World, 3(52),* 105-111.
- 525 Esposito, F., Arlotti, G., Bonifati, A. M., Napolitano, A., Vitale, D., & Fogliano, V.
- 526 (2005). Antioxidant activity and dietary fibre in durum wheat bran by-products.
- 527 Food Research International, 38, 1167-1173.

- Fardet, A., Rock, E., & Rémésy, C. (2008). Is the *in vitro* antioxidant potential of
  whole- grain cereals and cereal products well reflected *in vivo*? *Journal of Cereal Science, 48*, 258-276.
- 531 Foca, G., Ulrici, A., Corbellini, M., Pagani, M. A., Lucisano, M., Franchini G. C., &
- Tassi, L. (2007). Reproducibility of the Italian ISQ method for quality classification
- of bread wheats: an evaluation by expert assessors. *Journal of the Science of Food and Agriculture*, 87, 839-846.
- Gökmen, V, Serpen, A., & Fogliano, V. (2009). Direct measurement of the total
  antioxidant capacity of foods: The "QUENCHER" approach. *Trends in Foods Science & Technology*, 20, 278–288.
- Hemery, Y., Rouau, X., Lullien-Pellrin, V., Barron, C., & Abecassis, J. (2007). Dry
   process to develop wheat fractions and products with enhanced nutritional quality.
   *Journal of Cereal Science, 46*, 327-347.
- Hemery, Y., Lullien-Pellrin, V., Rouau, X., Abecassis, J., Samsom, M-F., Åman, P.,
- von Reding, W., Spoerndli, C. & Barron, C. (2009). Biochemical markers: efficient
  tools for the assessment of wheat grain tissue proportions in milling fractions. *Journal of Cereal Science, 49*, 55-64.
- House, J.D., Nyachoti, C. M., & Abramson, D. (2003). Deoxynivalenol removal from
  barley intended as swine feed through the use of an abrasive pearling procedure. *Journal of Agricultural and Food Chemistry, 51*, 5172-5175.
- Italian Organization for Standardization (UNI EN), 2003. Method 14083. Foodstuff –
   determination of trace elements determination of Pb, Cd, chromium and
   molybdenum by graphite furnace atomic absorption spectrometry (GFAAS) after
   pressure digestion. Milano. Italy: Ente Nazionale Italiano di Unificazione.

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

- Laca, A., Mousia, Z., Diaz, M., Webb, C., & Pandiella, S.S. (2006). Distribution of
   microbial contamination within cereal grains. *Journal of Food Engineering*, *72*,
   332-338.
- Lancova, K., Hajslova, J., Kostelanska, M., Kohoutkova, J., Nedelnik, J., Moravcova,
  H., & Vanova, M. (2008). Fate of trichothecene mycotoxin during the processing:
  milling and baking. *Food Additives and Contaminants*, *25*, 650-659.
- Liyana-Pathirana, C. M., Dexter, J., & Shahidi, F. (2006). Antioxidant properties of wheat as affected by pearling. *Journal of Agricultural and Food Chemistry, 54*, 6177-6184.
- Liyana-Pathirana, C. M., & Shahidi, F. (2006). Antioxidant properties of commercial
   soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions.
   *Journal of the Science of Food and Agriculture, 86*, 477-485.
- Liyana-Pathirana, C. M., & Shahidi, F. (2007). The antioxidant potential of milling fractions from breadwheat and durum. *Journal of Cereal Science, 45*, 238-247.
- Locatelli, M., Gindro, R., Travaglia, F., Coïsson, J. D., Rinaldi, M., & Arlorio, M.
   (2009). Study of the DPPH-scavenging activity: Development of a free software for
- 571 the correct interpretation of data. *Food Chemistry*, *114*, 889–897.
- 572 Mayerle, M., Pancaldi, D., Haidukowski, M., Pascale, M., & Ravaglia, S. (2007).
- Fusariosi e grano tenero: quali sono le varietà più resistenti. *L'Informatore Agrario*,
  32, 45-49.

- Noort, M. W. J., van Haaster, D., Hemery, Y., Schols, H. A., & Hamer, R. J. (2010). 575 The effect of particle size of wheat bran fractions on bread quality – Evidence for 576 fibre-protein interactions. Journal of Cereal Science, 52, 59-64. 577
- Oliver, D. P., Gore, P. J., Moss, H. J., & Tiller, K. G. (1993). Cadmium in wheat-grain 578 and milling products from some Australian flour mills. Australian Journal of 579 Agricultural Research, 44, 1-11. 580
- Parker, M. L., Ng, A., & Waldron, K. W. (2005). The phenolic acid and polysaccharide 581 composition of cell walls of bran layers of mature wheat (Triticum aestivum L. cv 582 Avalon) grains. Journal of the Science of Food and Agriculture, 85, 2539-2547. 583
- Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W., & Furda, I. (1988). 584 Determination of insoluble, soluble and total dietary fibre in foods and food 585 products: interlaboratory study. Journal of the Association of Official Analytical 586 Chemists, 71, 1017–1023. 587
- Rehman, S.U, Ahmad, M. M., Bhatti, I. A., Shafique, R., Ud Din, G. M., & Murtaza, M. 588
- A. (2006). Effect of pearling on physic-chemical rheological characteristics and 589 phytate content of wheat-sorghum flour. Pakistan Journal of Botany, 38, 711-719.
- Rios, G., Pinson-Gadais, L., Abecassis, J., Zakhia-Rozis, N., & Lullien-Pellerin, V. 591

- (2009). Assessment of dehulling efficiency to reduce deoxynivalenol and *Fusarium* 592 level in durum wheat grains. Journal of Cereal Science, 49, 387-392. 593
- Serpen, A., Gökmen, V., Pellegrini, N., & Fogliano, V. (2008). Direct measurement of 594 the total antioxidant capacity of cereal products. Journal of Cereal Science, 48, 595 816-820. 596
- Shetlar, M. R., Rankin, G. T., Lyman, J. F., & France, W. G. (1947). Investigation of 597 the proximate chemical composition of the separate bran layers of wheat. Cereal 598 Chemistry, 24, 111-122. 599

Sidhu, J. S., Al-Hooti, S. N., & Al-Saqer, J. M. (1999). Effect of adding wheat bran
 and germ fractions on the chemical composition of high-fiber toast bread. *Food Chemistry*, 67, 365-371.

<sup>603</sup> Singh, S., & Singh, N. (2010). Effect of debranning on the physico-chemical, cooking,

- pasting and textural properties of common and durum wheat varieties. *Food Research International*, 43, 2277-2283.
- Sullivan, P., O'Flaherty, J., Brunton, N., Gee, V. L., Arendt, E., & Gallagher, E.

(2010). Chemical composition and microstructure of milled barley fractions.
 *European Food Research and Technology*, 230, 579-595.

- Trenholm, H. L., Charmley, L. L., Prelusky, D. B., & Warner, R. M. (1991). Two
   physical methods for the decontamination of four cereals contaminated with
   deoxynivalenol and zearalenone. *Journal of Agricultural and Food Chemistry, 39*,
   356-360.
- Zhang, D., & Moore, W. R. (1999). Wheat bran particle size effects on bread baking
   performance and quality. *Journal of the Science of Food and Agriculture, 79*, 805 809.

- 617 Figures
- 618 **Fig. 1.**

TAA and FPA, β-glucan and DON contents in pearled wheat fractions. The reported

620 data are mean values of the three wheat varieties.



621 622

623 The error bars indicate the standard deviation between the wheat varieties.

624

### 627 **Tables**

### 628 **Tab. 1.**

629 Technological characteristics and protein, ash, DF, β-glucan and FPA<sup>a</sup> contents and TAA levels of wheat varieties.

Variety	hardness	ISQ⁵	Colour	Proteins (%)	Ashes (%)	<b>DF</b> (%)	β-glucans (%)	FPA (mg kg <sup>-1</sup> )	<b>TAA</b> (mmol TE kg <sup>-1</sup> )
Bolero	soft	wheat for biscuits	white	12.6 c	1.8 ab	11.9 a	0.5 b	17.4 a	1.3 a
Bologna	medium-hard	superior breadmaking wheat	red	13.7 b	1.6 b	10.2 b	0.7 a	14.8 b	1.3 a
Taylor	hard	improver wheat	red	14.7 a	1.9 a	12.0 a	0.6 a	13.9 b	1.5 a
<i>P</i> (F)				< 0.001	0.013	0.015	0.038	0.003	0.126
sem <sup>c</sup>				0.09	0.05	0.12	0.03	0.43	0.05

- 631 Results are expressed on a dw basis for whole kernel. Means followed by different letters are significantly different (the level of significance is shown in the
- table).
- 633 <sup>a</sup> total: sum of FPA determined by means of RP-HPLC/DAD
- <sup>b</sup> Foca et al., 2007
- 635 <sup>c</sup> sem: standard error of mean
- 636
- 637
- 638
- 639
- 640

### 641 **Tab. 2.**

Variety	Pearling fractions	Proteins (%)	DF (%)	β-glucans (%)	<b>TAA</b> (mmol TE kg <sup>-1</sup> )
Bolero	0-5%	13.5 d	58.0 a	0.8 d	4.8 a
	5-10%	20.0 a	36.4 b	1.5 a	5.1 a
	10-15%	20.5 a	25.8 c	1.4 b	4.4 b
	15-20%	19.4 b	21.7 d	1.1 c	2.6 c
	20-25%	17.3 c	15.3 e	0.6 e	2.3 c
	Residue 25-100%	10.6 e	8.1 f	0.4 f	0.9 d
	<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001
	sem <sup>a</sup>	0.23	0.45	0.08	0.19
Bologna	0-5%	11.7 d	58.3 a	1.4 c	5.5 a
	5-10%	17.4 c	37.8 b	1.9 a	4.7 b
	10-15%	19.6 a	24.4 c	1.5 b	4.2 c
	15-20%	18.2 b	19.7 d	1.1 d	2.8 d
	20-25%	17.5 c	12.9 e	0.9 e	2.4 e
	Residue 25-100%	12.0 d	5.6 f	0.3 f	0.7 f
	<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001
	sem <sup>a</sup>	0.21	0.85	0.06	0.12
Taylor	0-5%	13.7 e	61.5 a	1.3 c	6.6 a
	5-10%	21.1 c	40.9 b	1.9 a	5.6 b
	10-15%	22.7 a	30.4 c	1.7 b	5.1 c
	15-20%	22.2 b	23.3 d	1.3 c	4.3 d
	20-25%	21.8 d	20.2 e	1.0 d	2.9 e
	Residue 25-100%	13.5 e	7.1 f	0.4 e	0.9 f
	<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001
	sem <sup>a</sup>	0.14	0.53	0.05	0.14

642 Protein, DF and β-glucan contents and TAA in pearled wheat fractions.

643

644 Results are expressed on a dw basis. Means followed by different letters are significantly different (the level of significance is shown in the table).

<sup>a</sup> sem: standard error of mean

Variety	Pearling fractions	<b>total</b> (µg kg⁻¹)	<b>ferulic</b> (µg kg⁻¹)	chlorogenic (µg kg⁻¹)	<b>caffeic</b> (µg kg⁻¹)	<b>syringic</b> (µg kg⁻¹)	<b>ρ-hydroxybenzoic</b> (μg kg⁻¹)	protocatechuic (μg kg <sup>-1</sup> )
Bolero	0-5%	959 a	620 a	95 c	60 b	77 a	65 a	12 a
	5-10%	876 a	517 b	188 a	78 a	57 b	29 b	6 b
	10-15%	718 b	435 b	160 b	67 b	38 c	14 c	4 c
	15-20%	483 c	313 c	105 c	36 c	29 d	nd d	nd d
	20-25%	396 c	260 c	82 d	32 c	23 e	nd d	nd d
	Residue 25-100%	94 d	53 d	29 e	4 d	8 f	nd d	nd d
	<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	sem <sup>b</sup>	156.0	39.4	5.5	3.8	3.1	1.8	0.6
Bologna	0-5%	788 a	538 a	71 b	67 a	43 a	25 a	18 a
-	5-10%	572 b	403 b	80 a	36 b	27 b	11 b	14 b
	10-15%	500 c	394 b	46 c	22 c	24 b	nd c	13 c
	15-20%	395 d	326 b	32 d	15 d	18 c	nd c	5 d
	20-25%	261 e	215 c	22 e	8 e	13 d	nd c	3 e
	Residue 25-100%	23 f	23 d	nd	nd f	nd e	nd c	nd f
	<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	sem <sup>b</sup>	29.1	29.9	3.1	1.5	1.8	0.5	0.5
Taylor	0-5%	999 a	464 a	57 b	211 a	146 a	67 a	6 a
	5-10%	773 b	411 ab	75 a	171 b	85 b	26 b	4 b
	10-15%	626 c	345 bc	73 a	124 c	57 c	24 bc	3 cd
	15-20%	511 d	289 cd	60 b	88 d	49 c	21 c	4 bc
	20-25%	416 d	234 d	52 b	66 e	41 c	20 c	3 d
	Residue 25-100%	68 e	43 e	13 c	5 f	7 d	nd d	nd e
	<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	sem <sup>b</sup>	46.2	32.1	3.8	10.2	6.4	1.7	0.4

## **Tab. 3.** Free phenolic acid<sup>a</sup> content in pearled wheat fractions.

648 Results are expressed on a dw basis. Means followed by different letters are significantly different (the level of significance is shown in the table).

- <sup>649</sup> <sup>a</sup> total: sum of FPA determined by RP-HPLC/DAD. nd: not detected
- 650 <sup>b</sup> sem: standard error of mean

### 652 **Tab. 4**.

Variety	Pearling fractions	Ashes (%)	Cd (mg kg⁻¹)	<b>DON</b> (µg kg⁻¹)
Bolero	0-5%	4.5 b	nd	1789 a
	5-10%	4.7 a	nd	636 b
	10-15%	4.0 c	nd	341 c
	15-20%	3.0 d	nd	157 d
	20-25%	2.5 e	nd	128 d
	Residue 25-100%	1.1 f	nd	28 e
	<i>P</i> (F)	< 0.001		< 0.001
	sem <sup>a</sup>	0.06		32.6
Bologna	0-5%	5.7 a	0.05	nd
	5-10%	5.6 a	nd	nd
	10-15%	4.4 b	nd	nd
	15-20%	3.3 c	nd	nd
	20-25%	2.5 d	nd	nd
	Residue 25-100%	0.8 e	nd	nd
	<i>P</i> (F)	< 0.001		
	sem <sup>a</sup>	0.06		
Taylor	0-5%	5.5 b	0.07	949 a
	5-10%	6.0 a	nd	426 b
	10-15%	5.0 c	nd	185 c
	15-20%	4.1 d	nd	147 cd
	20-25%	3.7 e	nd	118 d
	Residue 25-100%	1.2 f	nd	36 e
	<i>P</i> (F)	< 0.001		< 0.001
	sem <sup>a</sup>	0.05		20.8

### Ash, DON and Cd contents in pearled wheat fractions.

654

655 Results are expressed on a dw basis. Means followed by different letters are significantly

656 different (the level of significance is shown in the table).

nd: not detected. The quantification limit was 20  $\mu$ g kg<sup>-1</sup> for DON and 0.05 mg kg<sup>-1</sup> for Cd.

658 <sup>a</sup> sem: standard error of mean

659