UNIVERSITÀ DEGLI STUDI DI TORINO

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Bioactive compound content, antioxidant activity, deoxynivalenol and heavy metal contamination of 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.

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 the original grain weight. Totally, five consecutive fractions were obtained starting from the outer layer until the inner kernel that designated as 0-5, 5-10, 10-15, 15-20, 20-25%, respectively. The remaining 75% of the inner kernel was also collected. Dietary fibre, free phenolic acid and total antioxidant activity decreased progressively from the external to the internal layers. However, the 5-10% fraction was richer in β-glucan content than the external one (0-5%). Heavy metals were only found in the most external fraction. Deoxynivalenol contamination decreased from the external to the internal layers: 64% of total contamination of kernel was found in the 0-5 and 5-10% fractions.

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

The protective effects of cereal fibres depend on their solubility: soluble fibre, particulary β-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 and synthetic contaminants. Moreover, whole grain foods are not so attractive to consumers, because the higher bran content in whole grain flour reduces the sensory value of the end-use products: the high fibre content is the main cause of the negative technological properties of whole grain bread, with a reduction in loaf volume, an increase in crumb firmness and a dark color (Zhang & Moore, 1999). Therefore, a grain fractionation technology is needed in order to separate efficiently the negative and positive aspects. This will let to produce new flour mixes and ingredients with technologically optimized functional and nutritional attributes.

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

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 β-glucan contents and TAA, and the lowest natural and synthetic contaminant contents, in order to use them as functional food ingredients.

2. Materials and methods

2.1 Wheat grain pearling

Three commercial winter wheat varieties (*Triticum aestivum* L.), Bolero, Bologna and Taylor, were collected from homogeneous lots of each cultivar, cultivated in the 2010-2011 growing season in Alessandria (44° 57' N, 8° 29' E; altitude of 121 m; in a deep and acid loamy soil - Aquic Frugiudalf) and stored in vertical silos. All the compared cvs were seeded after an autumn ploughing (30 cm) and disk harrowing to prepare a proper seedbed. Planting was held in 12 cm wide rows at the end of October at a seeding rate of 450 seeds m^{-2}. For Bologna and Taylor cvs, a total of 180 kg N ha^{-1} was applied to wheat fields as granular ammonium nitrate fertilizer. On the other hand, field cultivated with cv Bolero received a total of 140 kg N ha^{-1}. The 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 Head Blight (FHB). Wheat fields were harvested in early-mid July with a combine-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 the ISQ method for quality classification of common wheat, proposed by Foca et al. (2007). As far as FHB infection and DON contamination is concerned, Bologna cv is classified as moderately resistant, while cv Bolero and Taylor are classified as moderately susceptible (Mayerle, Pancaldi, Haidukowski, Pascale & Ravaglia, 2007). Moreover, the environmental conditions from anthesis to harvest observed in the growing area were slightly favorable to FHB and to DON contamination.

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

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.

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

133 Corp., Bedford, MA, USA). Antibody-based immunoaffinity columns were supplied by

134 VICAM (Waters Corporation, Watertown, MA, USA).   All the other chemicals and

135 solvents were of a reagent-grade level and were also purchased from Sigma–Aldrich

136 (Milan, Italy).

137

138 ## 2.3 Chemical analyses

139 **2.3.1. Proximate composition analysis**

140 The moisture, protein, ash, total DF and β-glucan contents were determined on

141 ground whole kernels and their pearled fractions. The moisture content, determined

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

144 nitrogen content and total protein content (conversion factor: 5.70) were obtained

145 according to the Kjeldahl method, using Kjeltec system I (Tecator, Sweden). The ash

146 coBontent was determined in a muffle furnace according to the AOAC (1990)

147 procedure. The total dietary fibre was measured using the Megazyme total dietary

148 fibre analysis kit, according to the enzymatic-gravimetric method proposed by

149 Prosky, Asp, Schweizer, DeVries, and Furda (1988); the determination was

150 performed employing the Fibertec 1023 system (FOSS Italia S.p.A., Padova, Italy). β-

151 glucan determination was performed using the Megazyme mixed-linkage β-glucan

152 assay kit, according to the instructions provided by the producer.

153

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

155 Prior to the extraction of the FPA, samples were ground in a oscillatory mill (Mixer

156 Mill MM440, Retsch GmbH, Hann, Germany) and sieved to obtain fine flours

157 (particle size < 250 μm). Fifty milligrams of each sample were suspended in 1 mL of

158 a MeOH/H$_2$O 80:20 (v/v) mixture, vortexed for 10 seconds and then extracted in an

159 ultrasonic bath (Bransonic 1510, output 42 kHz, Branson Ultrasonics, USA) for 2

160 minutes. The extracts were centrifuged at 14000 rpm for 1 min (Microcentrifuge 5417

161 R, Eppendorf Italia, Milan, Italy) and pellets were extracted another two times,

162 according to the method described above. Supernatants were collected and used for

163 the chromatographic analyses. All the samples were extracted in triplicate.

164

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

166 Phenolic acid separation was performed using the Shimadzu LC-20 A Prominence

167 HPLC system (Shimadzu Italia, Milan, Italy) equipped with an LC-20AB pump

168 system, a SIL-20-A auto-injector, a CTO-20A column oven, and a SPD-M20A diode

169 array detector. The used column was an Ascentis RP-amide (150 x 2,1 mm i.d., with

170 a particle size of 3 μm, Supelco, Bellefonte, PA, USA) which was maintained at 27

171 °C. The mobile phase consisted of water/formic acid 0.1% (v/v) (eluent A) and

172 methanol/formic acid 0.1% (v/v) (eluent B), and the following elution programme was

173 used: isocratic 2.5% B (10 min), from 2.5% to 12% B (25 min), from 12% to 100% B

174 (31 min), from 100% to 2.5% B (2 min), isocratic 2.5% B (5 min), The total running

175 time was 73 min and was conducted at a constant flow-rate of 400 μL/min.

176 Chromatograms were recorded at two different wavelengths (280 and 330 nm).

177 The phenolic acids were tentatively identified through a comparison with the retention

178 times and UV-vis spectra of individual standard molecules (gallic acid, protocatechuic

179 acid, syringic acid, *p*-hydroxybenzoic acid, caffeic acid, chlorogenic acid, ellagic acid,

180 ferulic acid); the quantification was performed on the basis of calibration curves (6

181 different concentration levels; linearity range: 0.5 – 5.5 μg mL^{-1}) obtained using the

182 corresponding standards. The previously described phenolic extracts were directly

183 injected into the chromatographic system (injection volume: 15 µL).

184

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

186 The TAA was determined adapting the classical DPPH radical scavenging method

187 (Locatelli, Gindro, Travaglia, Coisson, Rinaldi & Arlorio, 2009) to the QUENCHER

188 approach (direct measurement of antioxidant activity on solid samples suggested by

189 Gökmen, Serpen and Fogliano, 2009). Exactly 10 milligrams of ground whole kernels

190 and pearled fractions (particle size < 250 µm) were weighted, then 700 µL of

191 methanol and 700 µL of a DPPH$^•$ methanolic solution 100 µM were added. The

192 samples were vortex-mixed and the reaction was then carried out in the dark under

193 stirring at 20 °C and 1000 rpm (Thermomixer comfort, Eppendorf, Milan, Italy) for 25

194 min. The samples were promptly centrifuged for 1 min at 14000 rpm (Microcentrifuge

195 5417 R, Eppendorf Italia, Milan, Italy) and the absorbance at 515 nm was then

196 measured after exactly 30 min of reaction (on attainment of the steady state), using a

197 Kontron UVIKON 930 Spectrophotometer (Kontron Instruments, Milan, Italy). A

198 control solution (700 µL of methanol and 700 µL of DPPH$^•$ methanolic solution 100

199 µM) was tested under the same conditions, in order to calculate the DPPH$^•$ inhibition

200 percentage of the samples. The final results were expressed as mmol of trolox

201 equivalents (TE) per kg of sample (dw) through a calibration curve (linearity range:

202 4–60 nM; r^2 = 0.982).

203

204 **2.3.5. DON contamination**

205 The DON content was analysed using a high performance liquid chromatography

206 (HPLC-MS-MS) method (range 20-1000 µg kg^{-1}). Samples of 25 g each were

207 extracted with 100 mL of water in a blender at a high speed for 30 minutes; the entire

208 extract was then filtered and collected. Antibody-based immunoaffinity columns

209 (DON test[TM] WB Columns VICAM) were utilised for cleanup of the sample extracts.

210 Before the sample was loaded, the column was conditioned with 1 mL of deionized

211 water. 1 mL of the sample was loaded on the previously conditioned immunoaffinity

212 column at a rate of approximately 1-2 drops s^{-1}. The column was washed with 5 mL

213 of distilled water. DON was eluted from the column with 2 mL of methanol. DON was

214 quantified by the injection of 10 μL of diluted eluate into the HPLC-MS-MS system,

215 which consisted of a Varian 212-LC Chromatography Pump and a 310-MS TQ Mass

216 Spectrometer. The analytical column was a reverse Varian Polaris C18-A (100 x 2.00

217 mm, 3 μm) while the mobile phase was a mixture of methanol and water fed at a flow

218 rate of 0.2 mL min^{-1}.

219

220 **2.3.6. Heavy metal content**

221 The Cd and Pb analyses were performed according to the method of the Italian

222 Organization for Standardization (UNI EN 14083, 2003). Aliquots of the samples (500

223 mg on a dry-matter basis) were dissolved in 5 mL of concentrated nitric acid and 2

224 mL of 30% v/v hydrogen peroxide, then heated under reflux in a stoppered quartz

225 vessel placed in a microwave oven. The solution was diluted to 25 mL in a volumetric

226 flask with ultra-pure water. Samples were analysed by graphite furnace atomic-

227 absorption spectrometry (GFAAS; Analyst 700, Perkin Elmer Corporation, USA). All

228 the metal concentrations were determined by autosampler injection of the aqueous

229 solution into a graphite furnace.

230

2.4. Statistical analysis

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

3. Results

3.1. Bioactive compounds in the whole kernel

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

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 β-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.

15

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

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, protocatechuic and caffeic acid) present in the pearled wheat fractions in their free form is reported in table 3. Among the standard molecules employed for the chromatographic analysis, ellagic acid was not clearly recognized in the samples, while gallic acid was only identified in the 0-5% fraction (30, 26 and 48 µg kg^{-1} for the Bolero, Bologna and Taylor cvs, respectively). Ferulic acid was the predominant phenolic acid, followed by chlorogenic and caffeic acids. *p*-Hydroxybenzoic, syringic and protocatechuic acids were found in lower concentrations and were not detected in all the pearled fractions.

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

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.

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

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

362 The β-glucans, components of the soluble fibre, were higher in the middle fractions

363 (5-20%), and peaked in the 5-10% pearled fraction, while lower in the more external

364 layers (0-5%) had a lower β-glucan content. Several authors have reported that the

365 aleurone layer in common wheat contains higher levels of β-glucans than whole grain

366 (Hemery et al., 2007). A reduction of 12% in the β-glucan content of unprocessed

367 wheat grain has been observed after pearling processes (Dexter & Wood, 1996).

368 Barley is rich in β-glucans and the highest concentration of β-glucans was found in

369 the middle fractions, followed by bran, while the lowest content were found in the

370 flour (Sullivan, O'Flaherty, Brunton, Gee, Arendt & Gallagher, 2010).

371 The sequential removal of the external layers through pearling resulted in a decrease

372 in FPA with concurrent lower TAA values. The highest observed concentration of

373 FPA in the outer kernel fractions (0-15%) confirms the data reported by Beta et al.

374 (2005) on the concentration of the total phenol compounds in the wheat kernel.

375 Liyana-Pathirana et al. (2006) reported that total bran-rich fractions possess higher

376 total phenolic compounds and TAA than starch-rich fractions. Some authors have

377 shown that the aleurone layer is richer in antioxidant compounds than the other bran

378 tissues, mainly due to its high content of phenolic acids (Buri, von Reding & Gavin,

379 2004). This diversity in the phenolic composition between the bran layers probably

380 reflects differences in the biosynthetic and turnover mechanism, related to

381 arabinoxylan synthesis, as suggested by Parker et al. (2005). Liyana-Pathirana and

382 Shahidi (2006) reported that soft wheat has a higher total phenolic content and TAA

383 than hard wheat. In agreement with these results, in the present study it has been

384 found that Bolero, a soft variety, has a higher total FPA content than the Bologna

385 (medium hard) and Taylor (hard) cvs.

386　　Fardet et al. (2008) reported that ferulic acid represents approximately 46-67% of the

387　　total phenolic acids in wheat, and that it is found associated with polysaccharides,

388　　mainly arabinoxylans, in aleurone cell walls. Our results show that ferulic acid

389　　occurred in about 65% of the total FPA analyzed. Liyana-Pathirana and Shahidi

390　　(2007) reported that although ferulic acid is dominant in cereal grains, caffeic acid

391　　shows a higher antioxidant activity. This information explains the similar TAA levels

392　　of the three varieties examined, although Bolero has a higher total FPA and ferulic

393　　acid content, while Taylor, with a lower total FPA content, showed a 4-times higher

394　　caffeic acid concentration. Moreover, many insoluble components of foods may exert

395　　antioxidant properties and esterified phenolic acids in particular can contribute to the

396　　total antioxidant activity of cereals (Serpen, Gökmen, Pellegrini & Fogliano, 2008).

397　　The direct procedure used in this work to determine TAA allowed the contribution of

398　　both the soluble (e.g. free phenolic acids) and insoluble (e.g. bound phenolic acids)

399　　antioxidants to be measured. Moreover, the use of an aqueous methanolic solution

400　　(methanol/water 50:50, v/v) to perform the DPPH$^{\bullet}$ method (so adding to the solid

401　　samples 700 µL of water and 700 µL of DPPH$^{\bullet}$ methanolic solution; see Materials

402　　and methods section) allowed to obtain a TAA about 20% greater than that obtained

403　　by the method using pure methanol as solvent. These additional results (data not

404　　shown) confirm that the use of water as one of the solvents in the mixture helps

405　　radicals to diffuse better into the particles of the sample, thus increasing the

406　　interaction between the radicals and the antioxidants (Gökmen et al., 2009). Even if

407　　an increase of TAA was observed employing aqueous methanolic solutions, the

408　　relative antioxidant activity of the samples confirmed results obtained using pure

409　　methanol, thus indicating a general TAA decrease during the progressive pearling

410　　process.

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

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

436   The external pearled wheat fractions also resulted in the highest ash content. House

437   et al. (2003) and Rehman, Ahmand, Bhatti, Shafique, Ud Din and Murtaza (2006)

438   reported a 29% and 19% lower ash content after removal of 15% of the mass grain of

439   barley and wheat, respectively. Dexter and Wood (1996) reported that the pearling

440   process on durum wheat significantly reduced the ash content by 36% and 16% in

441   pearled kernels and semolina, respectively.

442   In addition, Laca, Mousia, Diaz, Webb, and Pandiella (2006) and Bottega et al.

443   (2009) established that the number of bacteria and moulds present in wheat grains,

444   and located in the outer pericarp, can be conspicuously reduced by pearling.

445   The TAA, the β-glucan, the total FPA and the DON contents (mean values of the

446   three wheat cultivars) are summarised in Fig. 1, with the objective of showing the

447   kernel fractions that offer the best compromise between high nutritional value and

448   low contamination risk. The 10-15% pearled fraction has shown to greatly reduce the

449   DON content, compared to the outer fractions, and has not been contaminated by

450   heavy metals. At the same time, this pearled fraction preserved an acceptable high

451   nutritional content, since it maintained high protein and β-glucan concentrations, and

452   the loss of total FPA content and TAA was not so high compared to the richer

453   external layers. Moreover, the total DF in this pearled fraction remained high, while

454   only the coarse fibre was removed with the outermost bran layer (Esposito, Arlotti,

455   Bonifati, Napolitano, Vitale & Fogliano, 2005). This could constitute an important

456   technological aspect, since bread containing coarse particle size bran is considered

457   less acceptable in sensory quality than breads containing medium-fine particle size

458   bran (Zhang & Moore, 1999). As far as the major chemical components of wheat

459   kernel is concerned, Bottega et al. (2009) and Singh and Singh (2010) reported that

460   pearling level lower than 10% guaranteed low starch and protein losses in the waste

461 and, at the same time, noticeably reduced the detrimental components of kernel

462 (ash, microbial contamination).

463 On the basis of the data collected, it is possible to state that the sequential pearling

464 of wheat kernels confirms to be an interesting dry-fraction technology, which can

465 produce bran fractions with high concentration in aleurone and intermediate material

466 rich in phytochemicals. This material can be used to transform a flour by-product into

467 a high nutritional value food ingredient (Hemery et al., 2007). The present study can

468 be considered as a first contribution towards individuating the most useful pearled

469 grain fractions for this purpose, although more research, also using the measurement

470 of biochemical markers found in wheat grain tissue (Hemery et al., 2009) is still

471 necessary.

472

5. Conclusion

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

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.

Acknowledgements

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.

Cubadda, F., Raggi, A., Zanasi, F., & Carcea, M. (2003). From durum wheat to pasta: effect of technological processing on the levels of arsenic, cadmium, lead and nickel-a pilot study. *Food Additives and Contaminants, 20*, 353-360.

Dexter, J. E., & Wood, P. J. (1996). Recent applications of debranning of wheat before milling. *Trends in Food Science & Technology, 7*, 35-41.

Dikes, L., & Rooney, L.W., (2007). Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World, 3(52),* 105-111.

Esposito, F., Arlotti, G., Bonifati, A. M., Napolitano, A., Vitale, D., & Fogliano, V. (2005). Antioxidant activity and dietary fibre in durum wheat bran by-products. *Food Research International, 38*, 1167-1173.

528 Fardet, A., Rock, E., & Rémésy, C. (2008). Is the *in vitro* antioxidant potential of

529   whole- grain cereals and cereal products well reflected *in vivo*? *Journal of Cereal*

530   *Science, 48*, 258-276.

531 Foca, G., Ulrici, A., Corbellini, M., Pagani, M. A., Lucisano, M., Franchini G. C., &

532   Tassi, L. (2007). Reproducibility of the Italian ISQ method for quality classification

533   of bread wheats: an evaluation by expert assessors. *Journal of the Science of*

534   *Food and Agriculture, 87*, 839-846.

535 Gökmen, V, Serpen, A., & Fogliano, V. (2009). Direct measurement of the total

536   antioxidant capacity of foods: The "QUENCHER" approach. *Trends in Foods*

537   *Science & Technology, 20*, 278–288.

538 Hemery, Y., Rouau, X., Lullien-Pellrin, V., Barron, C., & Abecassis, J. (2007). Dry

539   process to develop wheat fractions and products with enhanced nutritional quality.

540   *Journal of Cereal Science, 46*, 327-347.

541 Hemery, Y., Lullien-Pellrin, V., Rouau, X., Abecassis, J., Samsom, M-F., Åman, P.,

542   von Reding, W., Spoerndli, C. & Barron, C. (2009). Biochemical markers: efficient

543   tools for the assessment of wheat grain tissue proportions in milling fractions.

544   *Journal of Cereal Science, 49*, 55-64.

545 House, J.D., Nyachoti, C. M., & Abramson, D. (2003). Deoxynivalenol removal from

546   barley intended as swine feed through the use of an abrasive pearling procedure.

547   *Journal of Agricultural and Food Chemistry, 51*, 5172-5175.

548 Italian Organization for Standardization (UNI EN), 2003. Method 14083. Foodstuff –

549   determination of trace elements - determination of Pb, Cd, chromium and

550   molybdenum by graphite furnace atomic absorption spectrometry (GFAAS) after

551   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.

555   Laca, A., Mousia, Z., Diaz, M., Webb, C., & Pandiella, S.S. (2006). Distribution of

556   microbial contamination within cereal grains. *Journal of Food Engineering, 72*,

557   332-338.

558   Lancova, K., Hajslova, J., Kostelanska, M., Kohoutkova, J., Nedelnik, J., Moravcova,

559   H., & Vanova, M. (2008). Fate of trichothecene mycotoxin during the processing:

560   milling and baking. *Food Additives and Contaminants, 25*, 650-659.

561   Liyana-Pathirana, C. M., Dexter, J., & Shahidi, F. (2006). Antioxidant properties of

562   wheat as affected by pearling. *Journal of Agricultural and Food Chemistry, 54*,

563   6177-6184.

564   Liyana-Pathirana, C. M., & Shahidi, F. (2006). Antioxidant properties of commercial

565   soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions.

566   *Journal of the Science of Food and Agriculture, 86*, 477-485.

567   Liyana-Pathirana, C. M., & Shahidi, F. (2007). The antioxidant potential of milling

568   fractions from breadwheat and durum. *Journal of Cereal Science, 45*, 238-247.

569   Locatelli, M., Gindro, R., Travaglia, F., Coïsson, .J. D., Rinaldi, M., & Arlorio, M.

570   (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).

573   Fusariosi e grano tenero: quali sono le varietà più resistenti. *L'Informatore Agrario,*
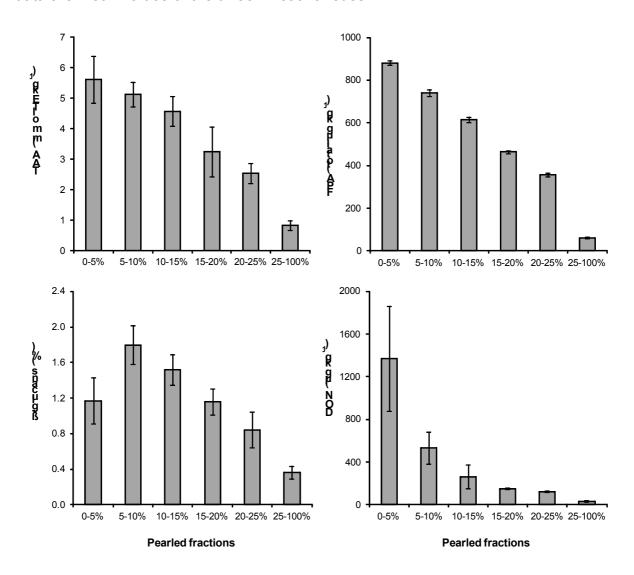
574   *32*, 45-49.

575 Noort, M. W. J., van Haaster, D., Hemery, Y., Schols, H. A., & Hamer, R. J. (2010).

576   The effect of particle size of wheat bran fractions on bread quality – Evidence for

577   fibre-protein interactions. *Journal of Cereal Science, 52*, 59-64.

578 Oliver, D. P., Gore, P. J., Moss, H. J., & Tiller, K. G. (1993). Cadmium in wheat-grain

579   and milling products from some Australian flour mills. *Australian Journal of*

580   *Agricultural Research, 44*, 1-11.

581 Parker, M. L., Ng, A., & Waldron, K. W. (2005). The phenolic acid and polysaccharide

582   composition of cell walls of bran layers of mature wheat (*Triticum aestivum* L. cv

583   Avalon) grains. *Journal of the Science of Food and Agriculture, 85*, 2539-2547.

584 Prosky, L., Asp, N. G., Schweizer, T. F., DeVries, J. W., & Furda, I. (1988).

585   Determination of insoluble, soluble and total dietary fibre in foods and food

586   products: interlaboratory study. *Journal of the Association of Official Analytical*

587   *Chemists, 71*, 1017–1023.

588 Rehman, S.U, Ahmad, M. M., Bhatti, I. A., Shafique, R., Ud Din, G. M., & Murtaza, M.

589   A. (2006). Effect of pearling on physic-chemical rheological characteristics and

590   phytate content of wheat-sorghum flour. *Pakistan Journal of Botany, 38*, 711-719.

591 Rios, G., Pinson-Gadais, L., Abecassis, J., Zakhia-Rozis, N., & Lullien-Pellerin, V.

592   (2009). Assesment of dehulling efficiency to reduce deoxynivalenol and *Fusarium*

593   level in durum wheat grains. *Journal of Cereal Science, 49*, 387-392.

594 Serpen, A., Gökmen, V., Pellegrini, N., & Fogliano, V. (2008). Direct measurement of

595   the total antioxidant capacity of cereal products. *Journal of Cereal Science, 48*,

596   816–820.

597 Shetlar, M. R., Rankin, G. T., Lyman, J. F., & France, W. G. (1947). Investigation of

598   the proximate chemical composition of the separate bran layers of wheat. *Cereal*

599   *Chemistry, 24*, 111-122.

600   Sidhu, J. S., Al-Hooti, S. N., & Al-Saqer, J. M. (1999). Effect of adding wheat bran

601   and germ fractions on the chemical composition of high-fiber toast bread. *Food*

602   *Chemistry, 67*, 365-371.

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

604   pasting and textural properties of common and durum wheat varieties. *Food*

605   *Research International, 43,* 2277-2283.

606   Sullivan, P., O'Flaherty, J., Brunton, N., Gee, V. L., Arendt, E., & Gallagher, E.

607   (2010). Chemical composition and microstructure of milled barley fractions.

608   *European Food Research and Technology, 230*, 579-595.

609   Trenholm, H. L., Charmley, L. L., Prelusky, D. B., & Warner, R. M. (1991). Two

610   physical methods for the decontamination of four cereals contaminated with

611   deoxynivalenol and zearalenone. *Journal of Agricultural and Food Chemistry, 39*,

612   356-360.

613   Zhang, D., & Moore, W. R. (1999). Wheat bran particle size effects on bread baking

614   performance and quality. *Journal of the Science of Food and Agriculture, 79*, 805-

615   809.

616

Figures

618 **Fig. 1.**

619 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

625

Tables

Tab. 1.

Technological characteristics and protein, ash, DF, β-glucan and FPA[a] contents and TAA levels of wheat varieties.

Variety	hardness	ISQ[b]	Colour	Proteins (%)	Ashes (%)	DF (%)	β-glucans (%)	FPA (mg kg^{-1})	TAA (mmol TE kg^{-1})
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
P (F)				< 0.001	0.013	0.015	0.038	0.003	0.126
sem[c]				0.09	0.05	0.12	0.03	0.43	0.05

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).

[a] total: sum of FPA determined by means of RP-HPLC/DAD

[b] Foca et al., 2007

[c] sem: standard error of mean

641 **Tab. 2.**

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

Variety	Pearling fractions	Proteins (%)	DF (%)	β-glucans (%)	TAA (mmol TE kg^{-1})
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
	P (F)	< 0.001	< 0.001	< 0.001	< 0.001
	sem[a]	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
	P (F)	< 0.001	< 0.001	< 0.001	< 0.001
	sem[a]	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
	P (F)	< 0.001	< 0.001	< 0.001	< 0.001
	sem[a]	0.14	0.53	0.05	0.14

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).

645 [a] sem: standard error of mean

646 **Tab. 3.** Free phenolic acid[a] content in pearled wheat fractions.

Variety	Pearling fractions	total (µg kg^{-1})	ferulic (µg kg^{-1})	chlorogenic (µg kg^{-1})	caffeic (µg kg^{-1})	syringic (µg kg^{-1})	p-hydroxybenzoic (µg kg^{-1})	protocatechuic (µg kg^{-1})
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
	P (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	sem[b]	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
	P (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	sem[b]	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
	P (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	sem[b]	46.2	32.1	3.8	10.2	6.4	1.7	0.4

647

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).

649   [a] total: sum of FPA determined by RP-HPLC/DAD. nd: not detected

650   [b] sem: standard error of mean

651

Tab. 4.

Ash, DON and Cd contents in pearled wheat fractions.

Variety	Pearling fractions	Ashes (%)	Cd (mg kg^{-1})	DON (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
	P (F)	< 0.001		< 0.001
	sem[a]	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
	P (F)	< 0.001		
	sem[a]	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
	P (F)	< 0.001		< 0.001
	sem[a]	0.05		20.8

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

nd: not detected. The quantification limit was 20 µg kg^{-1} for DON and 0.05 mg kg^{-1} for Cd.

[a] sem: standard error of mean