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Progressive pearling of barley kernel: chemical characterization of pearling fractions and effect of their inclusion on nutritional and technological properties of wheat bread.

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1 **Abstract**

2 Two hulled varieties were sequentially pearled for 1 to 8 cycles, each with 5% removal. The derived
3 fractions were analyzed for their bioactive compound content. The dietary fibre (DF) decreased
4 from the external to the internal layers, while β -glucans showed an inverse trend. Deoxynivalenol
5 contamination was concentrated in the outer layers. Total antioxidant activity (TAA) was higher in
6 the 15-25% fractions, which was used to prepare bread. Five mixtures of refined wheat flour with
7 an increasing replacement of this pearled barley fraction were compared with a control for the
8 bioactive compound content, the rheological and physical bread properties. The inclusion of pearled
9 fraction up to a 10% substitution lead to a clear enhancement of the DF and TAA, with only minor
10 detrimental effect on physical parameters. Selected by-products of barley pearling could be
11 proposed as functional ingredients for bakery products rich in DF and TAA.

12

13

14 **Keywords:** barley, pearling, antioxidant activity, bioactive compounds, dietary fibre, β -glucan,
15 phenolic compounds, bakery products.

16

17 **Introduction**

18 Today there is an increasing demand of functional foods, products fortified with special constituent
19 that possess advantageous physiological effects (1). Cereals offer multiple beneficial effects related
20 to the content of several non-digestible carbohydrates and antioxidant compounds (2), allowing to
21 design novel cereal foods or ingredients that can target specific populations. Moreover, bakery
22 products, including bread, provide an ideal matrix by which functionality can be delivered to the
23 consumer in an acceptable food.

24 The majority of consumed cereal foods are prepared with refined flour, mainly due to texture and
25 taste perception reasons, but in this form they contain less health-promoting compounds that are
26 present in the whole grain raw material. Thus, in developing functional bakery products it is
27 decisive to realize a food which deliver the appropriate level of bioactive compounds, but which
28 also meets the consumer's requirements in terms of appearance, taste and texture (3).

29 Among cereals, barley (*Hordeum vulgare* L.) is gaining a growing interest as functional ingredient,
30 due to its high nutritional significance (4). Barley grain provides high levels of dietary fibre (DF),
31 with general and specific health benefits. Moreover, barley is particularly rich in important soluble
32 DF component, a non-starch unbranched polysaccharide composed of (1→4) and (1→3) linked β -
33 D-glucopyranosyl units, simply known as β -glucan (5,6). Health claims for barley have been
34 approved by Food and Drug Administration (7) and EFSA (8).

35 The protective effects of cereal fibres depend on their solubility: soluble fibre, particularly β -
36 glucans, can reduce blood cholesterol and regulate blood glucose levels for diabetes management,
37 while insoluble fibres shorten the transit time through the intestinal tract, reducing the risk of
38 colorectal cancer and can positively affect weight control, through their satiety effect (9). In
39 addition to DF content, barley is an important source of other bioactive compounds, and some of
40 them, in particular phenolics, such as phenolic acids, lignans and flavonoids, show a marked

41 antioxidant activity (10,11).

42 Although much is known about the nutritional and health benefits of barley consumption, much less
43 is reported about the use of its grain components in terms of processing and food product
44 development. The principal uses of this cereal are as livestock feed and, secondary, as grain for
45 malting and brewing. Traditional food processing of barley produces pot and pearled barley, flakes
46 and flour by roller-milling pearled barley. The inedible hull is strongly attached to the pericarp, thus
47 barley grains are generally pearled, through an abrasive scouring process that gradually removes
48 hull, bran, with seed coat (testa and pericarp) and aleurone layers, and germ. The pearled barley
49 usually represents $\approx 60\text{-}70\%$ of the total grain weight, but may be adjusted depending on for what
50 purpose is to be used the final product (12). The by-products of the pearling process ($\approx 30\text{-}40\%$
51 w/w) are mainly used in animal feed, although the abraded material is a potential natural source of
52 phytochemicals (13). The main challenge in adding these by-products into bakery products is to
53 find new processing strategies in order to incorporate more of the bioactive compounds in flour,
54 while still removing those parts of grain which confer inferior technological quality or affect safety
55 (3). DF additions in baking, in general, leads to important reductions of loaf volume and a different
56 texture of the breads obtained, thus the optimal proportion of DF adding in each different food
57 should be determined also for the sensory attributes.

58 The cereal grain fractionation technology, which could be applied easily also with a selective dry
59 pearling process, is receiving high attention for their capacity to separate efficiently negative and
60 positive aspects, in order to produce new ingredients and flour mixes with technologically
61 optimized functional and nutritional attributes (14,15). In previous works (16,17) the sequential
62 pearling of wheat kernel and the incorporation into bread formula of selected pearled fractions have
63 been studied.

64 The effect of the inclusion of barley wholegrain or bran fraction were previously reported by
65 several authors, focusing separately for the nutritional benefits (18-20) or the technological

66 consequences (21-26). The addition of intermediate barley pearled fraction in bread-making has not
67 yet been considered.

68 The aims of this study were: i) to analyse the content in bioactive compounds and detrimental
69 factors in barley kernel, through a sequential pearling process, in order to use them as functional
70 food ingredients; ii) to comprehensively evaluate the nutritional enhancement and the technological
71 impact of the incorporation at different replacement levels of an intermediate pearled barley fraction
72 into bread formulation.

73

74 **Materials and methods**

75 *Field barley production*

76 Two commercial winter barley varieties were cultivated side by side on the same field, in a
77 medium-texture fertile soil located in Carignano, NW Italy (44°53'8.69"N, 7°41'16.75"E, 232 m
78 a.s.l.), during the 2011-12 growing season, according to the ordinary crop management program
79 applied in the growing area.

80 The compared barley varieties were: Trasimeno (Geo Seed, Grinzano di Cervere, CN, Italy) a two-
81 row cultivar and Ketos (Limagrain Italia Spa, Busseto, PR, Italy) a six-row cultivar. Both cultivars
82 have hulled grains.

83 Planting was conducted in 12 cm wide rows at a seeding rate of 450 seeds m⁻² on October 24, 2011.
84 The previous crop was maize for grain and the seed bed was set after ploughing (30 cm) and
85 harrowing. A total of 130 kg N ha⁻¹ was applied as a granular ammonium nitrate fertilizer, split
86 equally between tillering and stem elongation stages. Harvest was conducted with a combine-
87 harvester at June 23, 2012. Kernel samples of each cultivar were stored at 4°C until the beginning
88 of the pearling tests.

89

90 *Sequential grain pearling*

91 Nine fractions of kernels from each cultivar were obtained through incremental pearling, following
92 the approach proposed by Beta et al. (27). The pearling consisted of consecutive passages of barley
93 whole grain and pearled grain in an abrasive-type grain testing mill (TM-05C model, Satake,
94 Tokyo, Japan) at a constant speed of 1100 rpm and frequency of 55 Hz. The pearling process was
95 monitored by time control. After each assay, the laboratory pearler was thoroughly cleaned by
96 means of dust aspiration and compressed air, to minimize equipment contamination. Initially, a 500
97 g portion of each unprocessed barley was sub-sampled from a 5 kg sample, and the remaining 4.5
98 kg was pearled. Starting from unprocessed grain, kernels were initially pearled to remove 5% of the
99 original grain weight, and this resulted in a first fraction (0-5% w/w). The remaining kernels were
100 then pearled to remove a second fraction of 5% (5-10% w/w). The pearling process was continued
101 until other 6 fractions (designed 10-15%, 15-20%, 20-25%, 25-30%, 30-35% and 35-40%,
102 respectively) plus a residual 60% of the kernel (40-100%), were collected.

103 The whole grain samples and the residual 60% of the unprocessed kernels were milled using a
104 laboratory centrifugal mill (ZM-100; Retsch, Haan, Germany) with a 1 mm opening. Then, both the
105 milled and pearled samples (500 g) were ground to pass through a 0.5 mm sieve and stored at -25°C
106 before the chemical analyses.

107

108 *Substitution of flour with barley pearled fraction in bread making procedure*

109 A functional ingredient from the barley pearling was obtained, following the approach proposed in
110 a previous study focused on wheat (17). The two-row Trasimeno barley, which showed the lower
111 DON content and the higher content of β -glucans, was chosen to prepare an intermediate pearled
112 fraction, to obtain a functional ingredient preserving a high functional role, but without those parts
113 of grain that could confer inferior technological quality or affect safety. Considering that fibre,

114 proteins, minerals and antioxidant compounds are concentrated in the most external layers, but β -
115 glucans are presents in major concentration in the inner part of the barley kernel, the best
116 compromise to preserve all these functional compounds appeared to be the 15-25% (w/w) fraction.
117 In fact, the total and insoluble DF in the selected pearled fraction is still high, although the more
118 external coarse fibre, located in the hull and the more external endosperm layers, was removed, and
119 a good content of β -glucans is still provided. Starting from unprocessed grain, barley kernels were
120 initially pearled to remove 15% of the original grain weight, according with the procedure
121 previously reported, and this most external fraction was discarded. The remaining kernels were then
122 pearled to remove a second 10% fraction of the original grain weight (15-25%) and this fraction
123 was used to replace refined commercial flour for bread-making at different percentages.

124 Five mixtures of refined commercial flour for bread-making with an increasing replacement rate
125 (5%, 10%, 15%, 20%, 25%) of the selected pearled barley fraction were obtained and employed for
126 bread preparation. Refined flour and the selected pearled barley fraction were accurately mixed
127 using a rotary laboratory blender (Beccaria S.r.l., Cuneo, Italy). The Chopin[®] alveograph
128 parameters of the commercial refined flour were: deformation energy (W) $325 \text{ J } 10^{-4}$ and curve
129 configuration ratio (P/L) 0.52. The particle size of the selected pearling fraction resulted similar to
130 that of refined commercial flour: in both cases more than 80% of the particles were $< 200 \mu\text{m}$.

131 The breads were prepared according to the AACCI Method 10-10.03 for 3 kg of flour (AACC,
132 2008). The formula contained salt (2% of flour weight), brewer yeast (3% of flour weight) and the
133 optimal water absorption, which was previously determined through the Mixolab[®] rheological
134 analysis. The dough was then mixed in a spiral mixer (Esmach[®], Bonpard Group, Vicenza, Italy)
135 and divided into three pieces of approximately 400 g/piece, to obtain three loaves, which were
136 placed in baking pans ($10.5 \times 6 \text{ cm}^2$ and 6.5 cm deep). The mixing time was different for the
137 mixtures of refined commercial and the selected pearled barley fraction, to obtain an equivalent
138 dough development. After fermentation (at 30°C and a relative humidity of 85% for 120 min), the

139 loaves were baked at 215°C for 45 min.

140 The three composite loaves for each replacement level were used as replicates for chemical and
141 technological analyses.

142

143 *Chemicals*

144 The total Dietary Fibre (DF) and Mixed-Linkage β -Glucan kits for enzymatic determinations were
145 supplied by Megazyme (Megazyme International Ireland Ltd, Wicklow, Ireland). The solvents
146 (HPLC or GC grade) and formic acid (50%, LC-MS grade) were purchased from Sigma-Aldrich
147 (Milan, Italy). The water was obtained from Milli-Q instrument (Millipore Corp., Bedford, MA,
148 USA). The antibody-based immunoaffinity columns were supplied by VICAM (Waters
149 Corporation, Watertown, MA, USA). The analytical standards (purity \geq 95%) and all the other
150 chemicals (reagent-grade level) were purchased from Sigma-Aldrich (Milan, Italy).

151

152 *Chemical analyses on pearling fractions and breads*

153 **Sample preparation**

154 Bread samples were ground in a laboratory mill (ZM-100; Retsch, Haan, Germany), and in the case
155 of DF, the total phenolic content (TPC) and the total antioxidant activity (TAA) determinations,
156 they were further freeze-dried (Heto Drywinner 8, Copenhagen, Denmark) and ground in an
157 oscillatory mill (Mixer Mill MM440, Retsch GmbH, Hann, Germany). Barley pearled fractions,
158 whole flours and freeze-dried ground breads were sieved (particles size $<250 \mu\text{m}$) prior to the TAA
159 analyses.

160 **Proximate composition**

161 Moisture, total proteins, ashes, dietary fibre (both total and insoluble) and β -glucan content were
162 determined as previously described (16,17). The conversion factors employed to calculate total

163 protein content were 5.83 and 5.70 for barley and for wheat flour and bread, respectively.

164 **Total phenolic content (TPC)**

165 TPC was determined on composite breads and on the flours employed to prepare them. Phenolic
166 extracts were prepared as previously reported (17), then 30 to 100 μL (according to the expected
167 concentration) were made to react with 100 μL of Folin-Ciocalteu reagent and 350 μL of sodium
168 carbonate (5%), adding distilled water to a total volume of 2900 μL . After 1 h incubation, the
169 absorbance was measured at 760 nm (Evolution 60S spectrophotometer, Thermo Scientific, Milan,
170 Italy). Results were expressed as ferulic acid equivalents through a calibration curve ($y = 0.0105x -$
171 0.0563 ; $R^2 = 0.994$; linearity range: 10–80 μg).

172 **Total antioxidant activity (TAA)**

173 TAA was determined employing DPPH \cdot and ABTS $^{++}$ methods (direct measurement on solid
174 samples), as previously described (16,17,28). DPPH \cdot antiradical activity was determined in both
175 methanolic (DPPH MeOH) and hydroalcoholic (DPPH H $_2$ O) solutions.

176 Concerning DPPH MeOH method, the samples were opportunely weighted (0.5 – 20 mg, in order
177 to obtain final inhibition percentage values <70%), then 700 μL of methanol and 700 μL of a 100
178 μM DPPH \cdot methanolic solution were added. Reaction was carried out in the dark under stirring for
179 25 min, then the samples were promptly centrifuged for 1 min at 14000 rpm (Microcentrifuge 5417
180 R, Eppendorf Italia, Milan, Italy) and absorbance was measured at 515 nm after exactly 30 min of
181 reaction (Evolution 60S spectrophotometer, Thermo Scientific, Milan, Italy). A control solution
182 (without sample) was tested under the same conditions, in order to calculate the DPPH \cdot inhibition
183 percentage.

184 The DPPH H $_2$ O method was performed as described for DPPH in methanolic solution, but adding
185 to solid samples 1400 μL of water and 1400 μL of DPPH \cdot methanolic solution.

186 ABTS $^{++}$ reagent was prepared as described in Re et al. (29) and further diluted in a mixture of
187 ethanol:water (50:50, v/v) to obtain an absorbance of 0.700 ± 0.020 at 734 nm. Samples were tested

188 in the ratio of 0.5 - 10 mg per 6 mL of ABTS^{•+} solution (inhibition percentage values <70%).
189 Reaction solutions were maintained under stirring for 25 min and then centrifuged at 14000 rpm for
190 1 min (Microcentrifuge 5417 R, Eppendorf Italia, Milan, Italy). Absorbance of samples and control
191 solutions were performed after exactly 30 min, then inhibition percentage values were calculated.
192 For all the methods, final results were expressed as mmol of trolox equivalents (TE) per kg of
193 sample (dw) through a calibration curve (DPPH: $y = 11.267 - 4.3171x$, $R^2 = 0.982$, linearity range: 1–
194 15 μg ; ABTS: $y = 2.666 - 1.9208x$, $R^2 = 0.986$, linearity range: 5–35 μg).

195 **DON contamination**

196 The DON deoxynivalenol (DON) content was analysed on barley fractions obtained through the
197 sequential pearling using a high performance liquid chromatography (HPLC-MS-MS) method
198 (range 80-4000 $\mu\text{g kg}^{-1}$) as previously described in Sovrani et al. (16). The percentage of recovery,
199 obtained using a Certified Reference Materials (CRM Trilogy[®] Analytical Laboratory, 2.1 mg kg^{-1}
200 $\pm 0.2 \text{ mg kg}^{-1}$) was 79% (Relative Standard Deviation = 6%). The limit of detection (LOD) and the
201 limit of quantification (LOQ) were 5 $\mu\text{g kg}^{-1}$ and 16 $\mu\text{g kg}^{-1}$, respectively.

202

203 *Bread-making technological quality analyses*

204 **Rheological properties of the flour**

205 The mixing and pasting behaviours of the control and different replaced flours was studied using a
206 Mixolab[®] analyser (Chopin Technologies, Paris, France), according to the ICC Standard Method
207 173 (30). The instrument allows specific information to be obtained about the behaviour of dough
208 constituents (starch, protein, water) by continuously measuring the torque (Nm) produced by the
209 passage of the dough between two mixing blades, subject to the dual stress of mixing and
210 temperature changes.

211 **Bread crust and crumb color**

212 The chromatic characteristics of the bread crust and crumb were determined using a Minolta
213 Chroma Meter (Model CR-400, Minolta Co., Osaka, Japan) reflectance spectrophotometer.
214 Standard illuminant C was used as the reference. The analysis was performed in triplicate at 3
215 different points for each loaf, for both bread crust and crumb. The color values of L*, a*, and b*
216 were determined directly by the instrument in accordance with Commission Internationale de
217 L'Eclairage methods (31).

218 **Combined acoustic-mechanical analysis of the bread crust**

219 A penetration test was carried out to assess the mechanical and acoustic properties of the bread
220 crust, using a TA-XT Plus Texture Analyzer (SMS-Stable Micro Systems, Surrey, UK), combined
221 with an AED Acoustic Envelope Detector supplied by the same manufacturer. Force and acoustic
222 emission acquisitions were made simultaneously using the Texture Exponent software (Stable
223 Micro Systems), with a data rate of 500 points per second during a compression/penetration test
224 (32). Each loaf was penetrated by a P/6 6-mm steel cylindrical probe, a deformation of 20 mm was
225 applied with a test speed of 1 mm s⁻¹ and an instrumental trigger of 0.05 N was used. The
226 microphone was placed at a fixed distance of 10 mm from the sample for the acoustic
227 measurements. In order to minimize the noise, the acoustic measurements were filtered through an
228 integrated 1-kHz high pass filter, and a 24 dB instrumental gain was applied.

229 The analysis was performed in triplicate at 3 different points for each loaf. The following
230 mechanical and acoustic parameters were determined from the force-distance and acoustic spectra
231 according to Piazza et al. (32): total energy (mJ), maximum acoustic emission (dB (SPL)), and
232 number of acoustic emission peaks using 15 dB (SPL) as peak threshold value (17).

233 **Bread volume**

234 Loaf volume was determined 1 h after baking, by means of the rapeseed displacement method,
235 AACCI Standard 10-05-01 (33).

236 **Breadcrumb texture profile analysis**

237 Texture measurements were performed on two slices (20 mm thick), cut out from the central part of
238 the three replicated loaves for each mixture of refined flour and pearled fraction, 4 h after baking.
239 The bread slices were compressed in the central area using a SMS P/35 flat probe (Stable Micro
240 Systems) for a 50% deformation of the slice with a waiting time between the two bites of 30
241 seconds, using 1 mm s^{-1} as the speed test (17). An instrumental trigger of 0.05 N was applied. The
242 typical texture profile analysis parameters were determined from the Force-Distance curves and
243 calculated by the software: hardness (N), cohesiveness (adimensional), gumminess (N), springiness
244 (mm), chewiness (mJ), and resilience (adimensional) (34).

245

246 Statistical analysis

247 All the analyses were performed at least in triplicate. Results of bread samples were reported as
248 means of the three loaf replicates. The analysis of variance (ANOVA) was used to compare the
249 samples. The residual normal distribution was verified using the Kolmogorov-Smirnov test, while
250 variance homogeneity was verified using the Levene test. Multiple comparison tests were
251 performed according to the REGW-Q test on treatment means. The SPSS for Windows, Version
252 20.0 statistical package (SPSS Inc., Chicago), was used for the statistical analysis.

253

254 Results and discussion

255 *Bioactive compounds in the barley whole kernel*

256 The ash, protein, total and insoluble DF and β -glucan contents, the DON contamination and the
257 TAA determined for the grain whole kernels are reported in Table 1. Except for the TAA obtained
258 with DPPH methods, ANOVA showed significant differences between the barley varieties used in
259 this study. Trasimeno (two-row cultivar) reported a higher content of ash (+0.3%) and above all of
260 β -glucan (+1.6%), compared to Ketos (six-row). Conversely, cv. Ketos had higher total and

261 insoluble DF (+7%), and TAA obtained through ABTS method (+8%). As expected, the protein
262 content was higher in the six-row barley (+1%). The DON content was under the LOQ for the cv.
263 Trasimeno, while the average contamination of this mycotoxin in cv. Ketos kernel was 178 $\mu\text{g kg}^{-1}$.
264 The highest difference between the compared cultivars is related to the almost double content in β -
265 glucans recorded in the two-row cultivar compared to the six-row one, confirming previous
266 information (35,36).
267 Even if the relative content of some compounds is genetically regulated (as in the case of β -
268 glucans), their final absolute concentration is strongly dependent on pedo-climatic and agronomic
269 factors. For this reason, considering that the observed varietal differences are referred to a single
270 year and location of cultivation, further investigations should be done to improve the
271 characterization of these barley cultivars.

272

273 *Bioactive compounds of the barley pearled fractions*

274 A total of 10 samples was obtained from each barley variety: the whole unprocessed grain and the
275 0-5%, 5-10%, 10-15%, 15-20%, 20-25%, 25-30%, 30-35%, 35-40% and 40-100% fractions,
276 obtained through the pearling process. The two first pearling passages (0-5% and 5-10%) led to at
277 almost complete visual dehulling of barley kernel, according to previous works (15). The bioactive
278 compounds content in the fractions obtained from the sequential barley pearling is reported in
279 Tables 2. In both varieties, ANOVA showed highly significant differences ($P < 0.001$) between
280 kernel pearling fractions for all the considered parameters. The first pearling fraction (0-5%)
281 resulted in the highest content of total and insoluble DF. Sequential pearling has shown that both
282 total and the insoluble fibre decreased progressively from the external to internal layers, while β -
283 glucan content was growing toward the inner kernel layers in both varieties, according to several
284 authors (37-39). According with Liu et al. (15), the inner part of barley kernel, after the removing of
285 60% of grain weight, showed a β -glucan content 27% higher than the whole kernel. In the cultivars

286 considered in our study, the β -glucan content of the residual part of kernel after pearling (40-100%)
287 was 79% and 38% higher than that of whole kernel, for six-row and two-row barley, respectively.
288 Comparing the content of β -glucans of milling fractions of wholegrain or pearled barley, Sullivan et
289 al. (38) found the highest concentration in the middling fractions, followed by flour and, finally the
290 bran. Moreover, the authors reported that the barley pearling (10% w/w) clearly affected the content
291 of soluble and insoluble fibre in the milling fractions: the total DF of the grain was decreased
292 considerably by the pearling process. Conversely, there was little difference β -glucan content. In the
293 study carried out by Marconi et al. (40) (2000), the abrasive pearling fractions of hulled barley by
294 approximately 10%, 25% and 40% showed a DF removed of 35%, 55% and 71%, respectively, and
295 β -glucan content removed of 5%, 19% and 37%, respectively.

296 A progressive decrease in the percentage of ash was observed, since the mineral components are
297 mainly distributed in the outer layers of the kernel (40). Since in ash are included useful elements
298 and heavy metals, such as cadmium and lead, it could be useful to preserve the good mineral form
299 present in cereals, removing the more contaminated fractions. In a previous experiment referring to
300 wheat sequential pearling, heavy metals were only found in the most external fractions (16).

301 Protein content was lowest at the initial surface removal layer, corresponding to the coarse hull
302 fraction, while the highest content was observed in the 15-25% and 10-20% fraction, for Ketos and
303 Trasimeno cv, respectively.

304 The pearling fractions 10-15% and 15-20% were those with the highest TAA. The next external
305 layers (0-5% and 5-10%), consisting mainly in the hull, and the next internal layers (20-25% and
306 25-30%) showed a 48% and 44% lower TAA, respectively.

307

308 *DON contamination of barley pearled fractions*

309 The DON contamination in the barley fractions are reported in Table 2. The content of this
310 mycotoxin was under the LOQ in all the compared pearling fractions of cv. Trasimeno, confirming

311 the data from wholegrain kernel, while for cv. Ketos the outermost kernel layers had the highest
312 DON contamination, which was 6 and 3 times higher compared to the whole kernel for 0-5% and 5-
313 10% fractions, respectively. These findings confirm that large concentration of DON accumulate in
314 the hulls of barley and the application of processing strategies able to remove carefully the hull lead
315 a significant reduction of DON levels (41).

316 As previously reported also for wheat (16), DON showed a decrease moving from the external to
317 the internal layers following a biphasic behavior: a high reduction was observed in the first pearling
318 steps and this was followed by a slower decrease. No significant differences were observed for
319 DON contamination between 25-30%, 30-35% and 35-40% fractions. House et al. (42) reported that
320 a grain mass loss of 15 % through barley pearling reduced the DON contamination of 34%.

321

322 *Characterization of the wheat flour and barley pearled fraction*

323 The two-row Trasimeno barley, which showed the lower DON content and the higher content of β -
324 glucans, was chosen to prepare an intermediate pearled fraction, to obtain a functional ingredient
325 preserving a high functional role, but without those parts of grain that could confer inferior
326 technological quality or affect safety. Considering that fibre, proteins, minerals and antioxidant
327 compounds are concentrated in the most external layers, but β -glucans are present in major
328 concentration in the inner part of the barley kernel, the best compromise to preserve all these
329 functional compounds appeared to be the 15-25% (w/w) fraction. In fact, the total and insoluble DF
330 in the selected pearled fraction is still high, although the more external coarse fibre, located in the
331 hull and the more external endosperm layers, was removed, and a good content of β -glucans is still
332 provided.

333 The refined wheat commercial flour and the selected pearled barley fraction were characterized for
334 their chemical composition (ash, protein, DF, β -glucan, TPC content) and TAA (Table 3). The

335 pearled barley fraction had a 10, 1.5, 11, 14 and 8 times higher content of ash, protein, DF, β -
336 glucans and TPC, respectively, than the refined commercial flour. Taking into account different
337 methods, the TAA of the pearled barley fraction was from 10 to 73 times higher than in the refined
338 wheat flour.

339

340 Rheological parameters of the replaced flours

341 Five mixtures of refined flour for bread-making with an increasing replacement of the selected
342 pearled barley fraction (5%, 10%, 15%, 20%, and 25%) were obtained and characterized for their
343 rheological properties; the refined flour (no replacement) was analyzed as control.

344 The progressive replacement of flour with the pearled barley fraction increased significantly the
345 amount of water required for the hydration process of the flour, while no differences have been
346 observed for the dough development time (DDT) at each replacement level. Compared to the
347 control without pearled fraction addition, the replacement at 5% level significantly reduce dough
348 stability and C2 point (protein strength), by 26% and 20%, respectively. A further reduction was
349 observed at 10% level, by 40% and 31% for both parameters, respectively. Conversely, at higher
350 level of replacement both these parameters increased slightly. Comparing the addition to white flour
351 of bran from different cereals at different replacement level, Sudha et al. (21) reported that the
352 barley bran (DF = 45%) led to the highest increase in water absorption (from 64% to 76%) and
353 reduction of dough stability (from 7.0 to 3.5 min), while the DDT increased more for wheat, rice
354 and oat bran. According to Rosell et al. (22), these differences are mainly related to the competition
355 for water of the added barley fibres with the flour proteins and starch and to the physical and
356 mechanical negative effect of fibre on the formation of the gluten network. Moreover, the increase
357 in water absorption of the dough is also related to the content in non-starch polysaccharides, in
358 particular β -glucans (43).

359 The dough amplitude was significantly affected only at 25% level, while the starch gelatinization
360 (C3 point) and amylase activity (C4) were already affected at 5% of substitution. A further
361 significant reduction of C3 value was observed with 15% level of replacement. Conversely,
362 increasing the replacement level of refined flour with the pearled barley fraction did not affect the
363 C5 point (starch retrogradation). Dough resistant to extension, elasticity and starch pasting
364 properties were reported to significantly affected by the 15% inclusion of barley middlings into the
365 bread formulation (18).

366 Overall, compared to literature data referring to the addition of wholegrain barley flour or other by-
367 product of barley processing (18,19,23), the substitution of wheat flour with the selected
368 intermediate pearling fraction led to a lesser dough weakening, thus leading to a lower reduction of
369 dough stability.

370

371 Bread technology properties

372 The previously described composite flours were employed to prepare 5%-, 10%-, 15%-, 20%- and
373 25%-substituted breads; a control bread was also prepared using the refined flour (without
374 replacement) for comparison. Bread samples were firstly analyzed for their technology properties:
375 crust color, bread crunchiness and volume, TPA test.

376

377 **Bread crust color and crunchiness**

378 ANOVA showed significant differences in the L* and b* for the crusts of bread made with different
379 replacement levels of the refined flour with the pearled barley fraction (Table 5). An increase in
380 substitution with the pearled fraction resulted in a moderate reduction in the L* (lightness) and the
381 blue-yellow component (b*). No significant changes were found in the redness (a*) values.

382 In a previous work, no significant differences in crust color were observed up to 10% blending with

383 wholegrain barley flour (20). Sumner et al. (37) reported that the removal of the outer kernel layers
384 resulted in an increase of lightness (L^*) of pearled barley grain and a reduction in the red (a^*) and
385 yellow (b^*) values, showing for an intermediate pearling fraction similar to that used in the present
386 study (15-24% w/w) values of L^* , a^* and b^* intermediate between the hull and the inner pearled
387 fractions. In the breads obtained at replacement levels of 5% and 10%, the lightness (L^*) of the crust
388 was reduced by 7% and 11%, respectively, in regards to the control test. In the previous study based
389 on pearled wheat fractions (17) the reduction of crust lightness at the same replacement levels was
390 found 6% and 12%.

391 The results of the mechanical and acoustic properties of the composite bread crust are reported in
392 Table 5. The total break energy, which is the energy necessary to break the crust and to continue the
393 compression until 20 mm of penetration, was already significantly reduced at the 5% replacement
394 level, and a descending trend was observed with the increasing of flour replacement level. A
395 decreasing trend was also evidenced for both the acoustic parameters evaluated (maximum acoustic
396 emission and number of acoustic emission peaks, using a threshold value of 15 dB (SPL)) while
397 increasing the replacement percentage. The maximum acoustic emission detected during
398 compression was lower at each substitution, with significant differences from the 15% replacement
399 level with respect to the control. A steep decrease of the number of acoustic emission peaks
400 parameter was found between the control and samples from 10% of replacement level, evidencing
401 the loss of crust crunchiness in the substituted samples. The mechanical and acoustic crust
402 evaluations showed a possible variation in the microstructure of the breads: this was also shown in
403 extruded breads and related to the water activity content and production parameters (44,45).

404

405 **Bread volume and breadcrumb texture profile analysis and color**

406 The increasing percentage of refined flour replacement with barley pearled fraction led to a
407 significant decrease of bread volume (Figure 1). This effect began already at 5% of replacement of

408 refined flour with the pearled fraction. At 10% replacement level the bread volume was reduced by
409 24% compared to the control made with only refined wheat flour. The same reduction was reported
410 by Gill et al. (24, 46) by including barley flour. At the same level of replacement, but using an
411 intermediate pearled fraction obtained from wheat, the volume was reduced by 8% compared to the
412 control (17). A higher impact on bread volume of the inclusion of barley and other cereal compared
413 to wheat was reported by Ragaee et al. (25): the inclusion of 20% rye, barley and oat wholegrain
414 flour led to a reduction in loaf volume by 18%, 27% and 28%, respectively. Conversely, the authors
415 did not find significant difference in control bread and the 20%-replacement of wholegrain wheat
416 flour. Gujral et al. (47) reported a reduction in loaf volume of 65% when 20% w/w of barley flour
417 was incorporated in wheat bread.

418 The TPA parameters obtained while analyzing the breadcrumb (Table 6) marked a variation in all
419 the parameters excluding resilience when the replacement level increases. In particular, the force-
420 related hardness parameter increased following the substitution increase, and the cohesiveness and
421 springiness slightly decreased. In general, the variation in hardness predominated over cohesiveness
422 and springiness in the variations of the derived parameters like gumminess and chewiness,
423 evidencing a more hard, gummy and difficult crumb to bite with the increased replacements. The
424 differences are particularly clear from the 10% substitution.

425 The lower loaf volume and firmer crumb texture (hardness, gumminess, chewiness) with the
426 inclusion of the pearled barley fraction may be attributed to gluten dilution caused by addition of
427 DF, which interfered with the optimal gluten matrix formation during fermentation and baking (46).
428 Moreover, also non-starch polysaccharides could have profound impacts on dough properties during
429 bread-making, leading to lower loaf volume and increasing bread firmness (48). When added to
430 wheat flour, β -glucans from barley could tightly bind to high amounts of water in the dough,
431 suppressing the availability of water to develop gluten network and causing rupture of gas cells
432 (24,43).

433 Compared to the crust, for which an evolution in the lightness and some variations in the yellow
434 color were perceived, the color behaviour evidenced for breadcrumb was different. The breadcrumb
435 color evaluation showed an increase in both a* (red) and b* (yellow) color components with the
436 increase of the flour replacement, while the L* (lightness) value showed a non-homogeneous trend
437 between the substitutions. A similar reduction of crumb brightness was observed with the increasing
438 of barley whole flour substitution (24).

439

440 Chemical characterization of bread

441 The moisture, ash, DF, β -glucan and TPC content and TAA of bread increased linearly and
442 significantly as the refined flour was replaced with the selected pearled barley fraction, while the
443 protein content did not show a clear tendency (Table 7). In fact, while for the other parameters the
444 pearled barley fraction presented much higher values than the refined commercial flour, the protein
445 content was only 1.5 times higher. These results suggest that the contribution of barley fraction is
446 not sufficient to significantly improving the protein content in breads; for this reason, a linear
447 increase, as in the case of the other parameters, was not observed.

448 Compared to the control (0% of replacement), the 5%-substituted bread resulted in more than
449 double content of insoluble DF, β -glucans and TPC and, in the case of DPPH methods, also of
450 TAA. Moreover, at this replacement level a significant increase of moisture (+0.9%) and ash
451 (+0.14%) content in respect to the refined control was observed. At 10% replacement a further
452 significant increase of ashes, total and insoluble DF, β -glucans and TAA was observed, obtaining a
453 bread to being classified as “Source of Fiber” (DF > 3%) and very close to being a “Good Source of
454 Fiber” (DF > 6%) (49).

455 The supplementation of hulled barley wholegrain flour lead to generally lower increase of DF
456 compared to that reported in the present study: at a 15% level of substitution Dhingra and Jood (20)

457 reported an increase of 10% and 7% in total and insoluble fibre of bread as compared to control,
458 while no changes have been observed for the ash content. Compared to the wholegrain barley flour,
459 the intermediate pearled fraction led to a clearly higher increase also of TPC and TAA. In respect to
460 the control recipe, the bread enriched with 40% w/w of barley flour (26) presented a TPC and TAA
461 increase similar to that obtained in this study at 5% replacement level. This is consistent with the
462 fact that external layers of barley kernel and, particularly, the selected pearled fraction employed for
463 bread preparation, have higher TAA than whole barley.

464

465 Concluding, the results obtained in this work show that selected by-products of barley pearling
466 could be proposed as potential ingredients for the manufacture of functional food rich in DF and
467 antioxidant compounds. However, considering the technological impact on dough and bread
468 properties, the amount of their use in the formulation of bakery products need to be carefully
469 considered, taking into account the sensory acceptability of consumers and also the possible
470 application strategies to mitigate these undesirable effects.

471 The inclusion of this pearled fraction could be probably easily applied in other bakery products that
472 require lower leavening, such as biscuits, leading to lower technological changes. Otherwise, in
473 order to obtain enriched bread using these pearled barley fractions, it could be interesting to perform
474 specific enzymatic or physical treatments, or to modify the bread-making process with the use of
475 additives or sourdough fermentation.

476

477

478 **Abbreviations:** DDT, dough development time; DF, dietary fibre; DON, deoxynivalenol; dw,
479 dry weight; TPC, total phenolic compounds; TAA, total antioxidant activity; TE, trolox equivalents;
480 TPA, Texture Profile Analysis.

481

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489

490

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Tables

Table 1.

Ash, protein, DF and β -glucan content, TAA and DON contamination in barley kernel.

Barley cultivar	Ash (%)	Proteins (%)	DF		β -glucans (%)	TAA			DON ($\mu\text{g kg}^{-1}$)
			Total (%)	Insoluble (%)		DPPH MeOH	DPPH H ₂ O (mmol TE kg ⁻¹)	ABTS	
Ketos (six-row)	2.5 b	10.2 a	27 a	22 a	2.7 b	10.3 a	26.1 a	30.6 a	178 a
Trasimeno (two-row)	2.8 a	9.3 b	20 b	14 b	4.4 a	10.4 a	26.7 a	28.3 b	<LOQ b
<i>P</i> (F)	<0.001	0.003	<0.001	<0.001	<0.001	0.076	0.123	<0.001	<0.001
sem ^a	0.1	0.7	1.2	1.7	0.3	1.5	1.9	2.6	1.1

The results are expressed on d.w. basis.

Table 2.Ash, protein, DF and β -glucan contents, DON contamination and TAA in pearled barley fractions.

Barley cultivar	Kernel pearling fractions	Ash (%)	Proteins (%)	DF		β -glucans (%)	TAA			DON ($\mu\text{g kg}^{-1}$)
				Total (%)	Insoluble (%)		DPPH MeOH	DPPH H ₂ O (mmol TE kg ⁻¹)	ABTS	
Ketos six-row	0-5%	8.5 a	6.1 g	79 a	79 a	0.2 f	21 de	34 E	33 f	1074 a
	5-10%	7.0 b	9.4 f	68 b	66 b	1.2 e	45 b	77 C	114 b	532 b
	10-15%	6.4 c	18.3 bc	46 c	41 c	2.2 d	55 a	142 A	154 a	268 c
	15-20%	6.1 c	19.3 a	37 d	31 d	2.6 d	47 b	128 A	143 a	148 d
	20-25%	5.4 d	18.9 ab	23 e	20 e	3.5 c	31 c	98 B	95 c	110 e
	25-30%	4.3 e	17.7 c	21 e	14 f	4.0 b	25 d	59 D	66 d	73 f
	30-35%	3.3 f	16.2 d	19 e	9 g	4.5 ab	19 e	48 De	54 de	59 f
	35-40%	2.6 g	15.3 e	10 f	1 h	4.7 a	17 e	41 E	41 ef	52 f
	Residue 40-100%	0.9 h	9.1 f	11 f	3 h	4.9 a	3 f	8 F	9 g	< LOQ g
<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
sem ^a	0.7	0.9	6.5	3.4	0.9	5.6	18.0	19.5	20	
Trasimeno two-row	0-5%	9.4 a	5.3 f	72 a	71 a	0.8 g	20 e	45 De	30 f	< LOQ
	5-10%	7.3 b	9.6 d	65 b	64 b	1.2 g	31 d	66 C	61 cd	< LOQ
	10-15%	6.8 c	16.7 a	49 c	43 c	2.2 f	66 a	132 A	139 a	< LOQ
	15-20%	5.7 d	17.2 a	36 d	31 d	3.1 e	49 b	126 A	129 a	< LOQ
	20-25%	4.6 e	15.7 b	27 e	21 e	3.9 d	36 c	86 B	110 b	< LOQ
	25-30%	3.7 f	14.5 c	26 e	16 ef	4.6 cd	21 e	53 Cd	66 c	< LOQ
	30-35%	3.0 g	13.9 c	19 f	12 fg	5.1 bc	16 f	47 De	50 de	< LOQ
	35-40%	2.6 h	14.1 c	22 ef	9 g	5.5 ab	14 f	40 E	40 e	< LOQ
	Residue 40-100%	1.1 i	7.3 e	9 g	2 h	6.0 a	3 g	7 F	14 g	< LOQ
<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
sem ^a	0.3	0.9	5.4	4.2	1.3	4.9	13.4	13.4		

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

^a sem: standard error of mean**Table 3.**

Ash, protein, DF, β -glucan and TPC content and TAA in refined wheat flour and an enriched fractions obtained through barley pearling.

Product	Ash (%)	Proteins (%)	DF		β -glucans (%)	TPC (mg kg ⁻¹)	TAA		
			Total (%)	Insoluble (%)			DPPH MeOH	DPPH H2O (mmol TE kg ⁻¹)	ABTS
White flour	0.5 b	12.2 b	2.2 b	0.5 b	0.2 b	649 b	0.4 b	0.8 b	11.5 b
Barley pearled fraction	5.1 a	18.1 a	24.7 a	23.1 a	3.3 a	5239 a	25.7 a	55.8 a	114.5 a
<i>P</i> (F)	< 0.001	< 0.001	0.002	0.004	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
sem ^a	0.1	0.3	0.7	1.2	0.09	75	1.1	2.9	5.9

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

^a sem: standard error of mean

Table 4.

Mixolab rheological parameters^a of mixture of refined wheat flour and an enriched fraction obtained through barley kernel pearling.

Replacement level	Water absorption (%)	DDT (min)	Stability (min)	Amplitude (Nm)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
0	55.0 f	3.5 a	8.7 a	0.09 b	0.42 a	1.82 a	1.61 a	2.33 a
5	57.2 e	3.6 a	6.4 bc	0.09 b	0.34 b	1.74 b	1.51 b	2.22 a
10	57.9 d	3.6 a	5.2 d	0.09 ab	0.29 de	1.72 b	1.49 b	2.22 a
15	58.5 c	3.3 a	5.6 d	0.09 ab	0.29 e	1.65 c	1.42 b	2.18 a
20	59.5 b	3.1 a	5.9 cd	0.10 ab	0.30 cd	1.66 c	1.46 b	2.28 a
25	60.5 a	3.1 a	6.9 b	0.11 a	0.31 c	1.63 c	1.43 b	2.25 a
<i>P</i> (F)	<0.001	0.096	<0.001	0.029	<0.001	<0.001	0.001	0.103
sem ^b	0.30	0.51	0.62	0.02	0.01	0.04	0.08	0.12

^a Mixolab parameters: water absorption = the amount of water required in dough development; DDT = Dough Development Time; stability = time of dough stability at constant temperature; amplitude = dough elasticity; C2 = protein weakness; C3 = starch gelatinization; C4 = hot gel stability; C5 = starch retrogradation in the cooling phase. C2, C3, C4 and C5: end points of the corresponding mixing phases.

Means followed by different letters are significantly different (the level of significance is shown in table).

^b sem: standard error of mean.

Table 5.

Crust color, texture and acoustic emission tests of composite bread with refined flour and an enriched fraction obtained through barley kernel pearling.

Replacement level	Crust color			Crust crunchiness		
	L*	a*	b*	Total break energy (mJ)	Maximum acoustic emission (dB (SPL))	Number of acoustic emission peaks (threshold 15 dB (SPL))
	(C)	(C)	(C)			
0	64.2 a	11.6 a	35.3 a	134 a	64.7 a	55 A
5	59.6 b	10.7 a	30.4 bc	90 b	62.7 ab	50 A
10	56.6 c	10.8 a	29.4 c	89 b	60.0 ab	23 B
15	50.0 e	11.0 a	29.5 c	84 bc	56.5 b	25 B
20	52.7 d	12.0 a	31.8 b	83 bc	55.1 b	18 B
25	54.2 d	11.1 a	31.9 b	73 c	45.2 c	12 B
<i>P</i> (F)	< 0.001	0.403	< 0.001	<0.001	<0.001	<0.001
sem ^a	2.1	1.6	1.7	11.1	6.9	16.0

Means followed by different letters are significantly different (the level of significance is shown in table).

^a sem: standard error of mean.

Table 6.

Crumb Texture Profile Analysis of composite bread with refined flour and an enriched fraction obtained through barley kernel pearling.

Replacement level	Bread crumbs						Crumb color			
	Hardness (N)	Cohesiveness (-)	Springiness (mm)	Gumminess (N)	Chewiness (mJ)	Resilience (-)	L* (C)	a* (C)	b* (C)	
0	a	3.0 e	0.88 a	9.6 a	2.6 e	25.2 f	0.5 a	47.7 ab	0.6 e	12.8 c
5	b	4.6 de	0.85 ab	9.6 a	3.9 de	36.9 ef	0.5 a	43.0 cd	1.0 e	12.5 c
10	c	6.6 d	0.83 bc	9.3 ab	5.5 d	51.2 d	0.5 a	40.3 d	1.6 d	13.2 c
15	d	10.1 c	0.83 bc	9.2 ab	8.4 c	77.6 c	0.5 a	45.2 bc	2.8 c	17.4 b
20	d	16.3 b	0.81 cd	9.1 b	13.1 b	118.6 b	0.5 a	50.3 a	3.6 b	19.3 a
25	e	20.8 a	0.80 d	9.0 b	16.6 a	149.4 a	0.5 a	48.8 ab	4.1 a	20.2 a
<i>P</i> (F)	<0.001	<0.001	<0.001	<0.001	<0.001	0.253	<0.001	<0.001	<0.001	<0.001
sem ^a	2.8	0.03	0.4	2.0	17.3	0.04	3.32	0.5	1.6	

Means followed by different letters are significantly different (the level of significance is shown in table).

^a sem: standard error of mean.

Table 7.

Moisture, ash, protein, DF, β -glucan, TPC, AR content and TAA of composite bread with refined flour and an enriched fraction obtained through barley kernel pearling.

Replacement level	Moisture (%)	Ash (%)	Proteins (%)	DF		β -glucans (%)	TPC (mg kg ⁻¹)	TAA		
				Total (%)	Insoluble (%)			DPPH MeOH	DPPH H ₂ O (mmol TE kg ⁻¹)	ABTS
0	25.8 e	2.7 e	12.57 c	2.9 d	1.1 e	0.15 f	501 e	0.17 e	1.47 e	9.4 e
5	26.7 d	2.9 d	12.93 b	3.8 d	2.3 d	0.33 e	1156 d	0.40 d	3.67 d	14.2 d
10	27.7 cd	3.2 c	13.23 a	5.9 c	4.2 c	0.52 d	1277 d	0.66 c	5.48 c	16.2 d
15	27.5 bc	3.5 b	12.52 c	7.2 b	4.7 bc	0.75 c	1939 c	0.57 c	6.02 c	14.6 c
20	28.4 bc	3.6 a	12.52 c	6.9 b	5.7 b	0.91 b	2211 b	1.07 b	9.84 b	19.8 b
25	30.0 a	3.6 a	12.66 c	11.6 a	7.6 a	1.07 a	2525 a	1.33 a	12.04 a	21.7 a
<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001					
sem ^a	0.707	0.069	0.142	0.801	0.950	0.029	192	0.088	0.780	1.21

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

^a sem: standard error of mean

Figure 1. Volume of composite bread with refined flour and an enriched fraction obtained through barley kernel pearling.

