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Hull-less barley pearling fractions: Nutritional properties and their effect on the functional and technological quality in bread-making

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23 **Title: Hull-less barley pearling fractions: nutritional properties**
24 **and their effect on the functional and technological quality in**
25 **bread-making.**

26

27

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41

42 **Keywords:** barley, pearling, bioactive compounds, β -glucan.

43

44 **Abbreviations:** DDT, dough development time; DF, dietary fibre; DON, deoxynivalenol;
45 dw, dry weight; HLB, hull-less barley; TPC, total phenolic compounds; TAA, total
46 antioxidant activity; TE, trolox equivalents; TPA, Texture Profile Analysis.

47

48

49 **Abstract**

50 Breads enriched with hull-less barley (HLB) have been characterized. An HLB cultivar was
51 sequentially pearled, and the fractions were analyzed for their bioactive components. Ash,
52 proteins, dietary fibre (DF) and total antioxidant activity (TAA) decreased from the external
53 to the internal layers, while β -glucans showed an inverse trend.

54 Two functional ingredients were selected: an external fraction (5-15% w/w) and a
55 debranned inner fraction (25-100%). Five mixtures of refined commercial flour, with
56 increasing replacement with pearled HLB fractions, were used for each ingredient to
57 prepare bread.

58 The addition of the external layers led to a higher enrichment in ash, proteins, DF and
59 TAA, and showed significant changes in the rheological parameters, with detrimental
60 effects on the bread volume and texture. At a 10%-substitution level, the technological
61 properties were acceptable and similar to those shown by the control, while the nutritional
62 value was significantly improved. Conversely, the addition of the inner kernel fraction was
63 also successfully employed at high replacement levels, with only a few physical and
64 rheological changes. This ingredient led to a lowering of the improvement in the
65 antioxidant compounds, but it clearly enhanced the DF and β -glucan contents in the
66 bakery products.

67

68

69 **1. Introduction**

70 Consumer awareness about high-fibre diets and food naturally rich in components with
71 health-promoting effects is increasing (Siró et al., 2008). Thus, there is a great interest in
72 improving the nutritional profile of white wheat baked goods through supplementation with
73 flour or bran of different origins. In a multigrain approach, the use of other cereals is a
74 recent trend in the baking industry to obtain multiple functional benefits in bakery products
75 (Bartłomiej et al., 2012). Among the different cereals, barley has been studied in particular
76 as a source of dietary fibre (DF), especially because of its high natural β -glucan content,
77 non-starch unbranched polysaccharides, composed of (1→4) and (1→3) linked β -D-
78 glucopyranosyl units. In addition, barley is an important source of other bioactive
79 compounds, that show marked antioxidant activity (Liu and Yao, 2007).

80 Of the various barley cultivars, hull-less barley (HLB) has recently been receiving
81 considerable research attention concerning the development of functional food, as it is an
82 excellent source of both soluble and insoluble fibre. Hull-less (or "naked") barley (*Hordeum*
83 *vulgare* L. var. *nudum* Hook. f.) is a form of domesticated barley, in which, unlike hulled
84 barleys but similarly to wheat, the lemma and palea (hull) are non-adherent to the
85 caryopsis. The total β -glucan content of HLB is higher than that of hulled barley genotypes,
86 whereas the insoluble DF content is lower (Xue et al., 1997).

87 The development of functional bakery products could offer an excellent opportunity to
88 introduce several new uses of barley. Furthermore, it is crucial to obtain ingredients that
89 can be incorporated into regular food at physiologically effective levels, without
90 compromising the technological quality of the bakery products (Poutanen et al., 2014). The
91 addition of DF to baking products, through cereal bran or other by-products, generally
92 leads to a reduction in loaf volume, changes in crust color and a denser crumb texture,

93 and therefore represents a technological drawback. These effects are particularly evident
94 when barley grain flour or barley fractions are used (Ragaei et al., 2011), and represent a
95 drawback to the increasing consumption of whole-grain products, since consumers
96 generally prefer white bread.

97 Cereal grain fractionation technologies have been proposed as a way of obtaining new
98 ingredients from raw grain materials, with technologically optimized functional and
99 nutritional attributes (Liu et al., 2009). Among the different fractionation processes,
100 sequential pearling, which involves an abrasive scouring process that gradually removes
101 kernel layers, has provided interesting results for the development of new products.

102 Hulled barley is generally pearled in order to discard the hull and bran fractions and to
103 obtain pot and pearled barley, which represents $\approx 60-70\%$ of the total grain weight (Jadhav
104 et al., 1998). Because of its anatomical structure, the application of pearling to HLB is not
105 necessary; however, this process could be an interesting way of providing grain fractions
106 with unique compositions, which could be useful for the development of consumer-friendly
107 bakery products. In fact, the pearling degree could be modulated to separate different
108 grain fractions, with different health benefits or detrimental effects on the technological
109 quality and on safety (Sovrani et al., 2012).

110 The aims of this study were: i) to quantitatively fingerprint the bioactive compounds of HLB
111 kernel fractions, while evaluating the detrimental factors, through a sequential pearling
112 process, in order to design new functional ingredients; ii) to evaluate the nutritional
113 enhancement and the technological impact connected to the incorporation (at several
114 replacement levels) of differently pearled HLB fractions into bread.

115

116 **2. Materials and methods**

117 *2.1. Sequential barley grain pearling*

118 A grain lot of HLB (cultivar Mona: a two-row, spring, regular starch variety) was purchased
119 from Società Italiana Sementi (San Lazzaro di Savena, BO, Italy).

120 Six fractions of kernels were obtained through incremental pearling, according to the
121 approach described in Sovrani et al. (2012). The pearling consisted of consecutive
122 passages of barley grain in an abrasive-type grain testing mill (TM-05C model, Satake,
123 Tokyo, Japan). Starting from unprocessed grain, the kernels were initially pearled to
124 remove 5% of the original grain weight, and this resulted in a first fraction (0-5% w/w). The
125 remaining kernels were then pearled to remove a second fraction of 5% (5-10% w/w). The
126 pearling process was continued until other 3 fractions (designed 10-15%, 15-20%, 20-
127 25%, respectively) plus a residual 75% of the kernel (25-100%), were collected.

128 The whole grain samples and the residual 75% of the unprocessed kernels were milled
129 using a laboratory centrifugal mill (ZM-100; Retsch, Haan, Germany) with a 1 mm opening.
130 Then, both the milled and pearled samples were ground to pass through a 0.5 mm screen
131 and stored at -25°C until the chemical analyses.

132

133 *2.2. Substitution of flour with barley pearled fractions in the bread making* 134 *procedure*

135 On the basis of the results obtained through the sequential pearling of the HLB kernels,
136 two different fractions (external layer and debranned inner kernel) were prepared and used
137 to obtain functional flour for bread-making. Starting from unprocessed grain, the barley
138 kernels were initially pearled to remove 5% of the original grain weight, and this most

139 external fraction was discarded. The remaining kernels were then pearled to remove a
140 second 10% fraction of the original grain weight (5-15%), and this fraction was used as a
141 first “functional ingredient” (external layer). The remaining kernels were further pearled
142 until 25% of the original grain weight was removed (this fraction was discarded); the
143 residual pearled grain (25-100%) was ground to pass through a 0.5 mm screen, and used
144 as a second “functional ingredient” (debranned inner kernel).

145 The two selected fractions were used to replace conventional refined wheat flour for
146 bread-making, at different percentages. The particle size of the selected pearling fractions
147 was similar to that of refined commercial flour; in both cases, more than 80% of the
148 particles fell within the < 200 µm size range.

149 Five mixtures of refined bread-making commercial flour with increasing pearled barley
150 fraction replacement rates (5%, 10%, 15%, 20%, 25%) were made from the two pearling
151 fractions selected as functional ingredient, used for bread making, and compared with a
152 control with no wheat flour replacement. The refined flour and the selected pearled barley
153 fractions were accurately mixed using a rotary laboratory blender (Beccaria S.r.l., Cuneo,
154 Italy). The Chopin® alveograph parameters of the used commercial refined flour were:
155 deformation energy (W) $325 \text{ J } 10^{-4}$ and curve configuration ratio (P/L) 0.52.

156 The bread was prepared according to the method previously described in Blandino et al.
157 (2013). Three composite loaves were prepared for each replacement level and used as
158 replicates for chemical and technological analyses.

159

160 *2.3. Chemicals*

161 The total Dietary Fibre (DF) and Mixed-Linkage β-Glucan kits for the enzymatic
162 determination were supplied by Megazyme (Megazyme International Ireland Ltd, Wicklow,

163 Ireland). The solvents (HPLC) and formic acid (50%, LC–MS grade) were purchased from
164 Sigma–Aldrich (Milan, Italy). The water was obtained from Milli-Q Instruments (Millipore
165 Corp., Bedford, MA, USA). The antibody-based immunoaffinity columns were supplied by
166 VICAM (Waters Corporation, Watertown, MA, USA). The analytical standards (purity \geq
167 95%) and all the other chemicals (reagent-grade level) were purchased from Sigma–
168 Aldrich (Milan, Italy).

169

170 *2.4. Chemical analyses on the pearling fractions and breads*

171 **2.4.1. Sample preparation**

172 The flours and barley pearled fractions were analysed without any pre-treatment.
173 Bread samples were ground in a laboratory mill (ZM-100; Retsch, Haan, Germany), and in
174 the case of DF, total phenolic content (TPC) and total antioxidant activity (TAA)
175 determinations, they were also freeze-dried (Heto Drywinner 8, Copenhagen, Denmark).
176 The lyophilized samples were ground in an oscillatory mill (Mixer Mill MM440, Retsch
177 GmbH, Hann, Germany). The barley pearled fractions, whole flour and freeze-dried ground
178 bread were sieved (particles size $<250\ \mu\text{m}$) prior to the TAA analyses.

179

180 **2.4.2. Proximate composition**

181 All the samples were characterized for their moisture, total protein, ash, dietary fibre (total
182 and insoluble) and β -glucan contents. The adopted methods have already been described
183 for the characterization of wheat pearling fractions and derived bread (Blandino et al.,
184 2013; Sovrani et al. 2012). The conversion factors employed to calculate the total protein
185 content were 5.83 and 5.70 for barley and bread, respectively.

186

187 **2.4.3. Total phenolic content (TPC)**

188 The phenolic extracts were obtained as previously reported in Blandino et al. (2013), then
189 opportune volumes (from 30 to 100 μL , according to the expected concentration) were
190 made to react with 100 μL of Folin-Ciocalteu reagent and 350 μL of sodium carbonate
191 (5%), and distilled water was added to a total volume of 2900 μL . After 1 h of incubation,
192 the absorbance was measured at 760 nm (Evolution 60S spectrophotometer, Thermo
193 Scientific, Milan, Italy). TPC was expressed as ferulic acid equivalents through a
194 calibration curve.

195

196 **2.4.4. Total antioxidant activity (TAA)**

197 TAA was determined employing DPPH \bullet and ABTS $\bullet+$ methods (direct measurement on
198 solid samples), as previously described (Blandino et al., 2013; Sovrani et al., 2012;).
199 DPPH \bullet antiradical activity was determined in both methanolic (DPPH MeOH) and
200 hydroalcoholic (DPPH H $_2$ O) solutions.

201 As far as the DPPH MeOH method is concerned, the samples were opportunely weighted
202 (0.5 – 20 mg, in order to obtain final inhibition percentage values <70%), then 700 μL of
203 methanol and 700 μL of a 100 μM DPPH \bullet methanolic solution were added. The reaction
204 was carried out in the dark under stirring for 25 min, then the samples were promptly
205 centrifuged for 1 min at 14000 rpm (Microcentrifuge 5417 R, Eppendorf Italia, Milan, Italy),
206 and absorbance was measured at 515 nm after exactly 30 min of reaction (Evolution 60S
207 spectrophotometer, Thermo Scientific, Milan, Italy). A control solution (without sample)
208 was tested under the same conditions, in order to calculate the DPPH \bullet inhibition
209 percentage.

210 The DPPH H $_2$ O method was performed in the same way as described for the DPPH in the

211 methanolic solution, but adding 1400 μL of water and 1400 μL of DPPH• methanolic
212 solution to the solid samples.

213 ABTS•+ reagent was diluted in an ethanol:water mixture (50:50, v/v) to obtain an
214 absorbance of 0.700 ± 0.020 at 734 nm. Samples were tested at a 0.5 - 10 mg per 6 mL
215 of ABTS•+ solution ratio (inhibition percentage values <70%). The reaction solutions were
216 stirred for 25 min and then centrifuged at 14000 rpm for 1 min (Microcentrifuge 5417 R,
217 Eppendorf Italia, Milan, Italy). The absorbance of both the samples and the control
218 solutions was measured after exactly 30 min, and then the inhibition percentage values
219 were calculated.

220 For all the methods, the final results were expressed as mmol of trolox equivalents (TE)
221 per kg of sample (dw) through a calibration curve.

222

223 **2.4.5. Deoxynivalenol (DON) contamination**

224 The DON content was analysed on the HLB fractions obtained after a sequential pearling
225 using a high performance liquid chromatography (HPLC-MS-MS) method as previously
226 described in Sovrani et al. (2012). The limit of detection (LOD) and the limit of
227 quantification (LOQ) were $5 \mu\text{g kg}^{-1}$ and $16 \mu\text{g kg}^{-1}$, respectively.

228

229 *2.5. Bread-making technological quality analyses*

230 **2.5.1. Rheological properties of the flour**

231 The mixing and pasting behaviour of the control and of the different replaced flours was
232 studied using a Mixolab[®] analyser (Chopin Technologies, Paris, France), according to the
233 ICC Standard Method 173 (ICC, 2010). This instrument allows specific information to be
234 obtained about the behaviour (of dough constituents (starch, protein, water) by

235 continuously measuring the torque (Nm) produced by the passage of the dough between
236 two mixing blades, subject to the dual stress of mixing and temperature changes. The key
237 parameters derived from the Mixolab[®] curve are: water absorption capacity (WA, %);
238 Dough Development Time (DDT, min); dough stability (min); amplitude (Nm), which
239 represents the width of the curve to C1 and refers to dough elasticity; C2 (Nm), which
240 measures the protein strength after a decrease in dough consistency and provides an
241 indication of the protein quality; C3 (Nm), which measures starch gelatinization; C4 (Nm),
242 which measures the stability of the hot gel connected to the amylase activity; C5 (Nm),
243 which measures the starch retrogradation during the cooling phase.

244

245 **2.5.2. Bread crust and crumb color**

246 The color of the bread crust and crumb were determined using a Minolta Chroma Meter
247 reflectance spectrophotometer (Model CR-400, Minolta Co., Osaka, Japan). Standard
248 illuminant C was used as the reference. The analysis was performed in triplicate at 3
249 different points for each loaf in bread crust and crumb. The color values of L*, a*, and b*
250 were determined directly by the instrument, in accordance with the Commission
251 Internationale de L'Eclairage (1986) methods.

252

253 **2.5.3. Combined acoustic-mechanical analysis of the bread crust**

254 A penetration test was carried out to assess the mechanical and acoustic properties of the
255 bread crust, using a TA-XT Plus Texture Analyzer (SMS-Stable Micro Systems, Surrey,
256 UK), combined with an AED Acoustic Envelope Detector supplied by the same
257 manufacturer. Force and acoustic emission acquisitions of the crust were made
258 simultaneously using the Texture Exponent software (Stable Micro Systems), with a data

259 rate of 500 points per second during a compression/penetration test. Each loaf was
260 penetrated by a P/6 6-mm steel cylindrical probe, a deformation of 20 mm was applied
261 with a test speed of 1 mm s^{-1} , and an instrumental trigger of 0.05 N was used. The
262 microphone was placed at a fixed distance of 10 mm from the sample for the acoustic
263 measurements. In order to minimize the noise, the acoustic measurements were filtered
264 through an integrated 1-kHz high pass filter, and a 24 dB instrumental gain was applied.
265 The analysis was performed in triplicate at 3 different points for each loaf. The following
266 mechanical and acoustic parameters were determined from the force-distance and
267 acoustic spectra: total energy (mJ), maximum acoustic emission (dB (SPL)), and number
268 of acoustic emission peaks, using 15 dB (SPL) as the peak threshold value (Blandino et al,
269 2013).

270

271 **2.5.4. Bread volume**

272 Loaf volume was determined 1 h after baking, by means of the rapeseed displacement
273 method, AACC Standard 10-05.01 (AACC, 2008).

274

275 **2.5.5. Breadcrumb texture profile analysis**

276 Texture measurements were performed on two slices (20 mm thick), cut out from the
277 central part of the three replicated loaves for each mixture of refined flour and pearled
278 fractions, 4 h after baking. On average, six measurements per slice were made. The bread
279 slices were compressed in the central area using an SMS P/35 flat probe (Stable Micro
280 Systems) for a 50% deformation of the slice, with a waiting time between two bites of 30
281 seconds, using 1 mm s^{-1} as the speed test (Blandino et al., 2013). An instrumental trigger
282 of 0.05 N was applied. The typical texture profile analysis parameters were determined

283 from the force-distance curves, and were calculated by the software: hardness (N),
284 cohesiveness (adimensional), gumminess (N), springiness (mm), chewiness (mJ), and
285 resilience (adimensional).

286

287 *2.6. Statistical analysis*

288 All the analyses were performed at least in triplicate; the results for the bread samples are
289 reported as the means of the three loaf replicates. Significant differences were estimated
290 by means of analysis of variance (ANOVA). The residual normal distribution was verified
291 using the Kolmogorov-Smirnov test, while variance homogeneity was verified using the
292 Levene test. Multiple comparison tests were performed according to the REGW-F test on
293 treatment means. The SPSS for Windows, Version 20.0, statistical package (SPSS Inc.,
294 Chicago), was used for all the statistical analysis.

295

296

297 **3. Results and discussion**

298

299 *3.1. Bioactive compounds and DON in the barley pearled fractions*

300 The ash, protein, DF and β -glucan contents of the barley wholegrain were 1.7%, 11.6%,
301 14.6%, 4.3%, respectively. The TAA analyzed through the DPPH in methanol, DPPH in
302 water and ABTS methods was 12.7, 28.7 and 38.6 mmol TE kg⁻¹ d.w., respectively. The
303 DON contamination in the wholegrain was under the LOQ.

304 Specific health related constituents and compounds with antioxidant properties in the
305 kernel fractions obtained from the sequential pearling of the HLB is reported in Table 1.
306 ANOVA showed highly significant differences ($P < 0.001$) between the kernel pearling
307 fractions for all the considered parameters.

308 A progressive decrease in the percentage of ash was observed, thus confirming that the
309 mineral components were mainly distributed in the outer layers of the kernel (Yeung and
310 Vasanthan, 2001). The ash content was on average 3.6 times higher in the 0-5% fractions
311 than in the whole kernels. The residual ash content in the inner kernel fraction (25-100%)
312 was only 39% of the total content for a 25% mass removal. Since useful elements and
313 heavy metals, such as cadmium and lead, are included in the ash, it could be useful to
314 preserve the nutritionally interesting minerals of cereals, by removing the more
315 contaminated fractions. In fact, the bread supplemented through the addition of HLB flour
316 presented an increase in cadmium, lead and arsenic (Škrbíc et al., 2009). As previously
317 shown, heavy metals were only found in the most external fractions after wheat sequential
318 pearling (0-5%) (Sovrani et al., 2012).

319 The protein content was higher in the outermost layers (0-5% and 5-10%), while it then
320 reduced slightly until the 20-25% fraction, in agreement with Liu et al. (2009) and Yeung

321 and Vasanthan (2001). The external layers corresponding to the 25% of the kernel weight
322 contributed by 42% to the total protein content. HLB proteins could play an important
323 nutritional role, since they have a higher concentration of limiting amino acids (lysine and
324 threonine) than wheat or hulled barley (Boros et al., 1996). Moreover, these essential
325 amino acids are located more in the external kernel layers (Sumner et al., 1985).

326 Sequential pearling has shown that both the total and insoluble fibre decreased
327 progressively from the external to the internal layers, while the β -glucan content increased
328 going towards the inner kernel layers, in agreement with the results of several authors
329 (Sumner et al., 1985; Yeung and Vasanthan, 2001; Zheng et al., 2000). No significant
330 differences were observed between the first two pearling fractions (0-5% and 5-10%) for
331 the total and insoluble DF, which resulted 2.5 and 3.4 times higher than the whole grain,
332 respectively. β -glucans showed the lowest concentration in the most external layer (0-5%),
333 while the highest β -glucan content was in the 20-25% fraction. The pearled inner core (25-
334 100%) constituted 79% of the total kernel content of this soluble fibre. Liu et al. (2009)
335 reported a peak of the β -glucan content at a 60% pearling removal level for HLB, but their
336 concentration then decreased slightly. A different distribution of the β -glucan content in
337 kernels was shown by Zheng et al. (2000): in normal starch and with a medium-low β -
338 glucan content, these compounds were more concentrated in the subaleurone and in the
339 endosperm adjacent to the subaleurone (30-40% pearled fractions); while in high β -glucan
340 genotypes, including both waxy and high amylose varieties, the β -glucans were more
341 uniformly distributed in the endosperm.

342 The TAA decreased progressively going towards the kernel core. As far as the DPPH
343 methods are concerned, no significant differences were observed between the 0-5% and
344 5-10% pearled fractions. The contribution of the 10% external kernel layer to the TAA of
345 the kernel was on average 46%. Madhujith et al. (2006) reported that, in HLB, as in hulled

346 barley, the recovery of antioxidant compounds, in particular TPC, declined gradually going
347 from the outermost layer towards the kernel centre.

348 The DON contamination in the barley fractions is reported in Table 2. The content of this
349 mycotoxin was under the LOQ for all the considered pearled fractions, with the exception
350 of the outermost layer (0-5%), where this mycotoxin was found in traces.

351 Although the DON contamination of the considered barley lot was extremely low, these
352 findings confirm that, even for HLB, the risk of mycotoxin contamination and other
353 contaminants is higher in the external kernel layers (Sovrani et al., 2012).

354

355 *3.2. Chemical characterization of the wheat flour and selected functional* 356 *ingredients*

357 On the basis of the collected data, two functional ingredients were chosen among the
358 different pearling fractions of HLB: an external fraction (5-15% w/w) and a debranned inner
359 fraction (25-100%). The outermost layer (0-5%), although rich in functional components,
360 was discarded, thus reducing the risk of the presence of contaminants. The former
361 ingredient was selected mainly as a source of total DF (28%), but also of ash, proteins and
362 compounds with antioxidant activity, such as TPC (Table 2). This pearled barley fraction
363 had 9, 2.4, 14, 25 and 85 times higher ash, protein, DF, β -glucan and TPC contents,
364 respectively, than the refined wheat commercial flour. Considering the different methods,
365 the TAA was found to be from 61 to 138 times higher than in the refined wheat flour.

366 Conversely, the other barley ingredient was particularly rich in β -glucans (4.3%), but it
367 also showed higher ash (+2 times), protein (+1.4 times), DF (+3.8 times), TPC (+40
368 times) and TAA (+5 times) contents, compared to the refined wheat white flour.

369

370 *3.3. Rheological parameters of the replaced flours*

371 Five mixtures of refined flour for bread-making, with increasing replacements with selected
372 pearled barley fraction (5%, 10%, 15%, 20%, and 25%) were obtained for each barely
373 fraction and characterized for their rheological properties using a Mixolab[®] analyser; the
374 refined flour (no replacement) was analyzed as the control.

375 The progressive replacement of flour with the external fraction led to a significant increase
376 in the water required for the flour hydration process at each level (Table 3). Conversely,
377 only at the 25%-replacement level, with the debranned inner kernel, was the water
378 absorption higher than the refined control.

379 Compared to the control, the addition of the external barley fraction significantly reduced
380 the DDT at a 10% replacement level (-17%) and the dough stability at a 5% replacement
381 level (-14%). As the addition of this fraction was increased, the dough stability also
382 increased, and this parameter was significantly higher at 20% and 25% of substitution than
383 in the control. Substituting the white flour with the inner kernel, instead, did not lead to any
384 significant differences in dough stability, while a lower DDT than that of the control was
385 only observed at a 20% replacement level. However, the addition of this inner fraction
386 significantly influenced the C2 point (protein strength), which was already increased at a
387 5% level (+10%). No differences were observed for the C2 point when the external fraction
388 was used.

389 Spiking wheat flour with barley flour generally leads to an increase in water absorption,
390 and a reduction in dough stability (Rieder et al., 2012), because of a weaker gluten
391 network and lower elastic dough (Trogh et al., 2004). According to Rosell et al. (2010),
392 these effects are mainly related to the fact that the added barley fibres compete for water
393 with the flour proteins and starch, as well as to the physical negative effect of fibre on the

394 formation of the gluten network. Moreover, the increase in water absorption of the dough is
395 not only related to the insoluble fibre, but also to the non-starch polysaccharide content, in
396 particular β -glucans (Holtekjølen et al., 2008b). Izydorczyk et al. (2001) reported that the
397 presence of β -glucan in barley seems to override the negative effects associated with the
398 dilution of wheat gluten upon mixing with fibre and starch, and leads to a strengthening of
399 the dough. The increase in dough strength, due to β -glucan addition, also depends on the
400 quality of the wheat flour that is used, with a greater effect for poor bread-making flour than
401 for good bread-making flour (Skendi et al., 2010).

402 As far as the starch components of the Mixolab curve is concerned, the addition of the
403 barley fractions led to different dough behaviour: the external kernel layer clearly reduced
404 starch gelatinization (C3 point) and retrogradation (C5), mainly as a consequence of the
405 greater dilution of wheat flour starch with barley DF (Ragaei et al., 2011). Conversely, the
406 replacement with the debranned inner kernel fraction significantly increased the starch
407 gelatinization (C3) and the amylase activity (C4), while all the composited dough mixtures
408 resulted in a greater starch retrogradation (C5) than the refined control. These effects
409 could be related to the addition of starch from the barley endosperm, which is rich in β -
410 glucans, and which could affect both the hot and cold starch pasting properties (Sumner et
411 al., 1985).

412

413 *3.4. Bread technology properties*

414 The control (no replacement) and the 5%-, 10%-, 15%-, 20%-, 25%-substituted types of
415 bread were produced for both two barley fractions and analyzed for their technological
416 properties: crust color, bread crunchiness, volume and TPA test.

417 ANOVA showed significant differences in L^* and a^* for the crusts of bread made with

418 different replacement levels of both the external and internal fractions of the barley (Table
419 4). The b^* component did not change significantly: since this component represents the
420 yellowness of the bread crust, no difference was induced in this colour component by the
421 substitutions, for any of the percentages considered.

422 As has also been observed for the use of barley flour (Holtekjølen et al., 2008a), the
423 addition of an external barley kernel layer significantly reduced crust lightness (L^*) and
424 slightly increased the redness values (a^*). In the bread made at replacement levels of 10%
425 using the external HLB fraction, the lightness of the crust was reduced by 14%. In a
426 previous study based on the addition of pearled wheat fractions (Blandino et al., 2013), the
427 reduction in crust lightness at the same replacement level was 12% lower than the control.
428 The substitution with the inner pearled fraction, instead, led to a significant increase in L^*
429 and a reduction in a^* . At a 20%-replacement level, the L^* value was significantly higher
430 (8%) than the refined control, while a^* was reduced by 27%.

431 The differences between the two substitution ingredients can be confirmed from an
432 analysis of the bread crumb color (Table 5). The external HLB layer replacements can be
433 categorized perfectly on the basis of the a^* component (red-green), while the debranned
434 kernel substitution does not modify this parameter significantly in any of the percentages
435 considered until 25%.

436 An increase in the yellow b^* component was observed for the external layer fraction
437 substitutions from 5% to 25% levels, while a slight decrease of L^* was noted in all the
438 samples compared to the control, but it was only significantly different for the 5, 10 and
439 15% substitutions. A darker crumb color was also observed by Trogh et al. (2004) for the
440 addition of HLB flour. Yeung and Vasanthan (2001) reported that the pearling of HLB is
441 required, at least to a 32% level, to ensure a bright color in barley-based foods.

442 Sumner et al. (1985) reported that the removal of the outer kernel layers by pearling

443 resulted in an increase in lightness (L^*) of pearled barley grain and a reduction in the red
444 (a^*) and yellow (b^*) values. Similarly, Zheng et al. (2000) observed that the L^* value
445 increased progressively for 10 to 30% pearling fractions, while a similar whiteness was
446 observed in the inner kernel layers.

447 The results of the mechanical and acoustical properties of the composite bread crust are
448 reported in Table 4. The total break energy parameter was used to evaluate the easiness
449 of crust breaking; the penetration into the crumb was continued until 20 mm of the total
450 compression. This parameter was significantly reduced (-19%) at the 10% replacement
451 level of white flour with the external barley fraction, and a descending trend was observed
452 as the flour replacement level was increased. A decrease was also evidenced for both of
453 the evaluated acoustic parameters [maximum acoustic emission and average peak
454 number, using a threshold value of 15 dB (SPL)] as the replacement percentage was
455 increased. The maximum acoustic emission detected during compression was found to be
456 lower at each substitution, with significant differences from the 10% replacement level with
457 respect to the control. A steep decrease in the average acoustic peak number was found
458 between the control and the replaced samples from a 5% replacement level, thus
459 highlighting the loss of crust crunchiness in the substituted samples.

460 No significant differences in the total break energy were observed for the addition of the
461 inner pearled kernel, while its incorporation only significantly reduced the maximum
462 acoustic emission and the average peak number at 20% and 25% replacement levels: this
463 highlighted a loss of perceivable crunchiness (lower acoustic emission peaks) even for this
464 kind of substitution, while a non-significant change in the achieved energy values was
465 observed.

466 ANOVA showed a significant decrease in bread volume, related to the increasing
467 percentage replacement of refined flour for both of the considered barley fractions (Table

468 5). In both cases, this effect had already begun at a 5% replacement level, and resulted in
469 reductions of 17% and 12% for the external and the inner barley fractions, respectively.
470 Škrbić et al. (2009) found a volume reduction of 23% for bread supplemented with HLB
471 flour at a 15% level. In the present study, the reduction in bread volume for the external
472 and debranned inner fractions was 27% and 14%, respectively, for the same substitution
473 percentage. At a 20%-replacement level, the reduction in the bread volume was 36% and
474 24% for the previously reported barley fractions, respectively. The inclusion of 20% of
475 wholegrain barley flour has been reported to reduce loaf volume by 28% (Ragae et al.,
476 2011). In another trial, conducted at the same level of replacement, but using an
477 intermediate pearled fraction obtained from wheat, the volume was reduced by 8%
478 compared to the control (Blandino et al., 2013).

479 The TPA for breadcrumbs (Table 5) was used to evaluate the mechanical parameters
480 related to biting actions. An increase in crumb hardness was found in the high-substituted
481 breads; a positive increment was observed in the hardness of the external HLB layer-
482 substituted breads, with significant differences for the 15% substitution. A significant
483 variation was also observed for the inner kernel substituted breads for the 20% value. The
484 cohesiveness parameter made it possible to understand more clearly how the product
485 reacted to the second deformation; decreasing values were observed when the
486 substitution percentage was increased. The two parameters together pointed out an
487 increase in the gumminess and chewiness parameters, which meant a more difficult
488 breadcrumb to chew for higher substitutions. However, the external layer substitutions had
489 a greater impact on these parameters than the debranned kernel substitutions.

490 The lower loaf volume and firmer crumb hardness values are mainly related to the addition
491 of DF, which leads to a gluten–starch matrix dilution and, consequently, to a lower capacity
492 to enclose the gas cells during fermentation and baking (Gill et al., 2002). Moreover, the

493 same values are related to the effect of the non-starch polysaccharide content (β -glucans),
494 which bind part of the water in the dough, and reduce the development of the gluten
495 network (Holtekjolen et al., 2008b).

496

497 *3.5. Chemical characterization of the bread and nutritional considerations*

498 The ash, DF, β -glucan and TPC contents and the TAA of the bread increased linearly as
499 the refined flour was replaced with both of the selected pearled barley fractions, although
500 the nutritional impact of the compared ingredients was different, depending on their
501 composition (Table 6). Compared to the control (0% replacement), the 20%-substituted
502 bread obtained with the addition of the external barley fraction significantly increased the
503 ash, DF, β -glucan and TPC contents, by 1.3, 2.8, 1.4 and 9.3 times, respectively.
504 Conversely, the inclusion of the debranned inner grain at a 20% level increased the total
505 DF, β -glucans and TPC, by 1.6, 9.8, 1.7 and 1.6, respectively; while it only slightly
506 increased the ash content. The addition of the external barley fraction led to a higher TAA
507 than the inner fraction: at a 20% of replacement level, the TAA was on average 8.7 and
508 1.7 times higher than the refined control.

509 As far as the DF nutritional claim is concerned (ECC, 2006), both the considered HLB
510 fractions allowed bread to be obtained that could be classified as “Source of Fiber” bread
511 (DF > 3%) at a 5% replacement level. Furthermore, only the inclusion of the external
512 barley fraction over a 15%-replacement level led to a product that could be classified as a
513 “Good Source of Fiber” (DF > 6%). Increases of 25% (Gill et al., 2002) and 41% (Škrbić et
514 al., 2009) of the total DF were obtained for a 15%-replacement level of white wheat flour
515 with HLB whole flour. The DF contents in the 15%-substituted bread of the present study
516 were 2.3 and 1.5 times higher than the refined control, for the external and the inner

517 fractions, respectively.

518 Considering a bread intake of 300g per day (Kinner et al., 2011), the EFSA requirements
519 pertaining to β -glucans for maintenance of normal blood cholesterol concentrations
520 (3g/day; EFSA, 2009) could only be achieved with a 25% composite bread made using the
521 debranned inner kernel. A 300-gram daily portion of bread made with a 15%-replacement
522 level of the external or the inner fraction could satisfy up to 62% and 75% of the
523 recommended daily doses , respectively. These percentages are similar to those reported
524 by Škrbíc et al. (2009) for the substitution of white flour with HLB milling flour. Kinner et al.
525 (2011) developed bread made of 100% HLB, which was able to meet the suggested β -
526 glucan requirement for a bread intake of 200g per day. A mixed bread, with 40% of a high
527 β -glucan HLB flour, also provides a high enough intake of this component to satisfy the
528 suggested EFSA health claim (Collar and Angioloni, 2014).

529 Moreover, the inclusion of the external pearled fraction has been shown to lead to a clearly
530 higher increase in TPC and TAA, compared to wholegrain barley flour, even when hulled
531 genotypes are considered: TPC and TAA were increased twofold in the bread enriched
532 with 30g/100g of wholegrain barley flour, compared to a control recipe without enrichment
533 (Ragaei et al., 2011). A similar TAA enhancement was observed by Holtekjølen et al.
534 (2008a) when 40% of wheat flour was replaced with barley flour, while in our/the present
535 study this enhancement was already reached for a 5% replacement level with the external
536 HLB fraction.

537

538 **4. Conclusion**

539 These results highlight the potential of using fractions obtained from the sequential
540 pearling of barley in bakery products as functional ingredients. HLB has been confirmed to
541 be a good source of insoluble and soluble DF and other bioactive compounds.
542 Furthermore, since the distribution of various components is not homogeneous throughout
543 the kernel, sequential pearling may be an efficient way of obtaining ingredients enriched in
544 specific bioactive nutrients. The impact on the functional enhancement or on changes in
545 the technological properties of bakery products could be extremely different: the addition
546 of external kernel layers leads to great enrichment of the ash, protein, DF, TPC and other
547 antioxidant compounds, but has shown a detrimental impact on bread volume and texture,
548 as well as changes in the rheological parameters. When deciding on the amount of
549 incorporation of this barley fraction in the formulation of bakery products, it will be
550 necessary to consider the sensory acceptability of consumers and also the possible
551 application strategies to mitigate these undesirable effects. At a 10%-substitution level,
552 the technological properties were similar to the control, while the nutritional value of the
553 composite bread was clearly improved, particularly as far as DF and TAA are concerned.
554 Conversely, it has been shown that the inner core of the HLB barley kernel, after the
555 removal of the external fraction through pearling, can be successfully incorporated into
556 bread formulations, even at high replacement levels, with few physical and rheological
557 changes in the composite bread, compared to a refined control. This ingredient has led to
558 less improvement in the antioxidant compounds than the previous one, but it clearly
559 enhances the DF and β -glucan contents in bakery products.

560 In short,, these findings have shown that the selective pearling process of HLB could be an
561 easy applicable strategy to obtain different functional ingredients that could be an

562 interesting concentrated sources of fibre and natural antioxidants, and which could be
563 suggested for the manufacturing of fibre-rich bakery products with acceptable sensory
564 characteristics.

565

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576

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669

670 **Tables**

671

672 **Table 1.**673 Ash, protein, DF and β -glucan contents, TAA and DON contamination in pearled HLB fractions.

Kernel pearling fractions	Ash (%)	Proteins (%)	DF		β -glucans (%)	TAA			DON ($\mu\text{g kg}^{-1}$)
			Total (%)	Insoluble (%)		DPPH MeOH	DPPH H2O (mmol TE kg^{-1})	ABTS	
0-5%	6.2 a	21.8 a	39.7 a	36 a	1.9 e	75 a	149 A	237 a	30 a
5-10%	5.0 b	20.9 a	35.8 ab	33 ab	3.3 d	69 a	133 A	189 b	< LOQ b
10-15%	4.8 b	19.8 b	33.0 b	27 bc	4.6 c	49 b	107 B	143 c	< LOQ b
15-20%	4.2 c	19.0 bc	26.4 c	23 c	4.9 bc	39 c	83 C	106 d	< LOQ b
20-25%	3.3 d	18.2 c	22.3 d	17 d	6.7 a	22 d	51 D	67 e	< LOQ b
Residue 25-100%	1.0 e	8.9 d	9.8 e	4 e	5.3 b	4 e	8 E	10 f	< LOQ b
<i>P</i> (F)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001				
sem ^a	0.3	0.8	5.8	6.7	0.9	6.9	18.6	24.7	9

674

675 The results are expressed on a d.w. basis.

676

677 **Table 2.**

678 Moisture, ash, protein, DF, β -glucan and TPC contents and TAA in refined wheat flour and the different fractions obtained
679 through HLB pearling.

Product	Moisture (%)	Ash (%)	Proteins (%)	DF (%)	β -glucans (%)	TPC (mg kg ⁻¹)	TAA		
							DPPH MeOH (mmol TE kg ⁻¹)	DPPH H2O	ABTS
white wheat flour	15.5	0.6	12.0	2.0	0.11	45	0.3	1.0	1.8
barley pearled fraction (external layer 5-15%)	10.5	5.4	28.6	28.0	2.74	3890	45.6	124.6	111.6
barley pearled fraction (debranned inner kernel 25-100%)	11.9	1.1	17.5	7.8	4.24	249	1.7	7.4	5.3

680

681 Results are expressed on a d.w. basis.

682 ^a sem: standard error of mean

683

Table 3. Mixolab rheological parameters^a of flours for bread-making enriched with different levels of HLB pearled fractions.

Barley fraction	Replacement level	Water absorption (%)	DDT (min)	Stability (min)	Amplitude (Nm)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
external layer 5-15%	0	54.2 f	5.2 a	9.0 c	0.09 a	0.47 a	2.10 a	1.65 ab	3.11 a
	5	57.3 e	4.5 ab	7.7 e	0.08 a	0.45 a	1.97 b	1.43 b	2.82 b
	10	58.3 d	4.3 b	8.4 d	0.08 a	0.43 a	1.94 bc	1.75 a	2.83 b
	15	59.3 c	4.0 b	9.0 c	0.09 a	0.43 a	1.93 bc	1.85 a	2.69 c
	20	61.1 b	4.2 b	9.9 b	0.09 a	0.44 a	1.90 c	1.79 a	2.60 c
	25	62.4 a	5.1 a	10.6 a	0.10 a	0.46 a	1.90 c	1.79 a	2.62 c
	<i>P</i> (F)	<0.001	0.003	<0.001	0.325	0.086	<0.001	0.009	<0.001
sem ^a	0.30	0.65	0.39	0.02	0.03	0.05	0.23	0.07	
debranned kernel 25-100%	0	52.6 bc	5.1 a	9.7 a	0.09 a	0.49 c	2.15 d	1.64 c	3.01 b
	5	52.6 bc	5.1 a	9.9 a	0.08 a	0.53 b	2.26 c	1.74 b	3.17 a
	10	52.4 c	4.5 ab	9.8 a	0.09 a	0.54 ab	2.29 bc	1.78 ab	3.22 a
	15	52.7 bc	4.7 ab	9.7 a	0.08 a	0.56 ab	2.33 ab	1.80 ab	3.20 a
	20	53.3 ab	4.1 b	9.6 a	0.08 a	0.56 ab	2.34 ab	1.81 ab	3.17 a
	25	53.7 a	4.3 ab	9.7 a	0.08 a	0.58 a	2.37 a	1.84 a	3.18 a
	<i>P</i> (F)	<0.001	0.027	0.682	0.661	0.001	<0.001	<0.001	<0.001
sem ^a	0.51	0.74	0.35	0.02	0.04	0.05	0.07	0.07	

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

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

689 ^b sem: standard error of mean.

691 **Table 4.**

692 Crust color, texture and acoustic emission tests of composite breads enriched with different levels of HLB pearling fractions.

Barley fraction	Replacement level	Crust color			Crust crunchiness		
		L* (C)	a* (C)	b* (C)	Total break energy mJ	Maximum acoustic emission dB (SPL)	Number of acoustic emission peaks [threshold 15 dB (SPL)]
external layer 5-15%	0	67.0 a	9.1 b	31.9 a	137 a	81 a	438 a
	5	61.1 ab	9.9 ab	32.7 a	136 a	71 ab	75 b
	10	57.9 bc	11.7 ab	33.5 a	111 b	63 bc	35 bc
	15	53.2 c	13.5 a	32.3 a	89 c	57 cd	25 bc
	20	54.3 bc	12.7 ab	32.4 a	77 c	49 de	9 c
	25	53.0 c	13.5 a	32.2 a	71 c	43 e	4 c
	<i>P</i> (F) sem ^a	< 0.001 5.5	0.024 3.2	0.627 2.3	<0.001 18.7	<0.001 9.7	<0.001 50.0
debranned kernel 25-100%	0	68.4 b	6.7 ab	29.4 a	128 a	79 a	387 a
	5	70.9 ab	5.8 ab	27.3 a	113 a	81 a	428 a
	10	71.0 ab	6.2 ab	28.2 a	107 a	81 a	498 a
	15	69.0 b	7.1 a	28.3 a	104 a	80 a	443 a
	20	73.8 a	4.9 b	27.7 a	112 a	73 b	110 b
	25	73.4 a	4.6 b	26.5 a	101 a	74 b	175 b
	<i>P</i> (F) sem ^a	< 0.001 2.4	0.020 1.7	0.108 2.2	0.072 20.3	0.003 3.2	<0.001 98.9

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

694 ^a sem: standard error of mean.

695 **Table 5.**

696 Volume and crumb Texture Profile Analysis of composite breads enriched with different levels of HLB pearling fractions.

Barley fraction	Replacement level	Bread Volume (ml)	Bread crumbs						Crumb color		
			Hardness N	Cohesiveness (-)	Springiness mm	Gumminess N	Chewiness mJ	Resilience (-)	L* (C)	a* (C)	b* (C)
external layer 5-15%	0	2213 a	2.3 c	0.9 a	9.5 a	2.0 c	19.0 c	0.5 ab	59.5 a	-0.2 f	12.5 d
	5	1835 b	3.7 c	0.9 b	9.7 a	3.2 c	30.5 c	0.5 a	47.2 cd	0.4 e	11.1 e
	10	1713 bc	4.2 c	0.9 ab	9.5 a	3.6 c	33.9 c	0.5 a	45.4 d	1.0 d	12.9 d
	15	1623 c	8.0 b	0.8 c	9.4 a	6.7 b	63.1 b	0.5 abc	48.4 bcd	2.0 c	16.7 c
	20	1407 d	13.9 a	0.8 d	9.1 b	11.3 a	102.1 a	0.5 bc	55.3 ab	2.7 b	19.1 b
	25	1333 d	14.4 a	0.8 d	9.1 b	11.6 a	105.4 a	0.5 c	53.6 abc	3.4 a	20.5 a
	<i>P</i> (F)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
sem ^a	134	3.0	0.01	0.2	2.4	20.5	0.03	5.8	0.3	1.0	
debranned kernel 25-100%	0	2278 a	1.6 b	0.9 a	9.7 a	1.4 b	14.1 b	0.6 a	63.6 a	-0.3 a	12.9 b
	5	1994 b	2.0 b	0.9 abc	9.5 b	1.7 b	16.2 b	0.5 b	65.8 a	-0.1 a	15.0 ab
	10	1973 b	1.8 b	0.9 ab	9.6 ab	1.6 b	15.0 b	0.5 b	66.5 a	-0.3 a	14.0 ab
	15	1964 b	2.4 b	0.9 bc	9.5 b	2.1 b	19.9 b	0.5 ab	69.1 a	-0.3 a	14.5 ab
	20	1718 c	5.1 a	0.9 bc	9.5 b	4.4 a	41.4 a	0.5 ab	69.4 a	0.0 a	16.3 a
	25	1685 c	4.9 a	0.8 c	9.5 b	4.2 a	39.9 a	0.5 b	68.2 a	0.0 a	15.7 ab
	<i>P</i> (F)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.097	0.144
sem ^a	62	0.8	0.01	0.1	0.7	6.5	0.02	4.9	0.4	2.2	

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

698 ^a sem: standard error of mean.

699 **Table 6.**

700 Ash, protein, DF, β -glucan, TPC content and TAA of composite breads enriched with different levels of HLB pearling fractions.

Barley fraction	Replacement level	Ash (%)	Proteins (%)	DF (%)	β -glucans (%)	TPC (mg kg ⁻¹)	TAA		
							DPPH MeOH	DPPH H ₂ O (mmol TE kg ⁻¹)	ABTS
external layer 5-15%	0	2.7 f	12.0 e	2.6 d	0.18 f	177 d	0.20 e	1.2 e	7.1 e
	5	2.8 e	12.4 de	3.4 c	0.35 e	413 cd	0.77 d	6.3 d	12.5 d
	10	3.0 d	12.6 cd	4.5 b	0.49 d	832 c	1.30 cd	10.3 c	17.4 c
	15	3.3 c	13.0 bc	6.1 ab	0.62 c	763 c	1.49 abc	14.7 b	21.0 bc
	20	3.6 b	13.4 b	7.5 a	0.75 b	1180 b	1.86 ab	16.2 ab	24.8 ab
	25	3.8 a	14.1 a	7.9 a	0.89 a	1804 a	2.22 a	18.8 a	25.9 a
	<i>P</i> (F) sem ^a	< 0.001 0.10	< 0.001 0.4	< 0.001 1.6	< 0.001 0.03	< 0.001 391	< 0.001 0.7	< 0.001 2.8	< 0.001 3.9
debranned kernel 25-100%	0	2.7 c	11.3 b	2.7 d	0.09 f	175 a	0.25 d	1.1 f	5.0 d
	5	2.7 bc	11.1 b	3.2 cd	0.38 e	172 a	0.32 c	1.3 e	5.6 c
	10	2.7 abc	10.8 b	3.7 bc	0.52 d	181 a	0.35 bc	1.6 d	6.1 c
	15	2.8 abc	10.4 ab	4.1 b	0.75 c	198 a	0.39 b	2.0 c	6.6 b
	20	2.8 ab	10.7 a	4.3 ab	0.93 b	271 a	0.41 ab	2.2 b	6.9 b
	25	2.9 a	10.4 a	5.1 a	1.13 a	295 a	0.47 a	2.5 a	7.5 a
	<i>P</i> (F) sem ^a	0.005 0.10	< 0.001 0.38	< 0.001 0.69	< 0.001 0.09	0.308 124	< 0.001 0.06	< 0.001 0.15	< 0.001 0.42

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

702 ^a sem: standard error of mean.

703