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

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

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

49 **Abstract**

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

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

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

67

69 **1. Introduction**

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

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

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

and therefore represents a technological drawback. These effects are particularly evident
 when barley grain flour or barley fractions are used (Ragaee et al., 2011), and represent a
 drawback to the increasing consumption of whole-grain products, since consumers
 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 obtain pot and pearled barley, which represents ≈60-70% of the total grain weight (Jadhav 103 et al., 1998). Because of its anatomical structure, the application of pearling to HLB is not 104 necessary; however, this process could be an interesting way of providing grain fractions 105 with unique compositions, which could be useful for the development of consumer-friendly 106 bakery products. In fact, the pearling degree could be modulated to separate different 107 grain fractions, with different health benefits or detrimental effects on the technological 108 quality and on safety (Sovrani et al., 2012). 109

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

115

116 2. Materials and methods

117 2.1. Sequential barley grain pearling

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

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

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

132

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

On the basis of the results obtained through the sequential pearling of the HLB kernels, two different fractions (external layer and debranned inner kernel) were prepared and used to obtain functional flour for bread-making. Starting from unprocessed grain, the barley kernels were initially pearled to remove 5% of the original grain weight, and this most external fraction was discarded. The remaining kernels were then pearled to remove a second 10% fraction of the original grain weight (5-15%), and this fraction was used as a first "functional ingredient" (external layer). The remaining kernels were further pearled until 25% of the original grain weight was removed (this fraction was discarded); the residual pearled grain (25-100%) was ground to pass through a 0.5 mm screen, and used as a second "functional ingredient" (debranned inner kernel).

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

Five mixtures of refined bread-making commercial flour with increasing pearled barley fraction replacement rates (5%, 10%, 15%, 20%, 25%) were made from the two pearling fractions selected as functional ingredient, used for bread making, and compared with a control with no wheat flour replacement. The refined flour and the selected pearled barley fractions were accurately mixed using a rotary laboratory blender (Beccaria S.r.I., Cuneo, ltaly). The Chopin[®] alveograph parameters of the used commercial refined flour were: deformation energy (W) 325 J 10⁻⁴ and curve configuration ratio (P/L) 0.52.

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

159

160 **2.3.** Chemicals

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

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

Bread samples were ground in a laboratory mill (ZM-100; Retsch, Haan, Germany), and in the case of DF, total phenolic content (TPC) and total antioxidant activity (TAA) determinations, they were also freeze-dried (Heto Drywinner 8, Copenhagen, Denmark). The lyophilized samples were ground in an oscillatory mill (Mixer Mill MM440, Retsch GmbH, Hann, Germany). The barley pearled fractions, whole flour and freeze-dried ground bread were sieved (particles size <250 µm) prior to the TAA analyses.</p>

179

180 **2.4.2. Proximate composition**

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

186

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

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

195

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

TAA was determined employing DPPH• and ABTS•+ methods (direct measurement on solid samples), as previously described (Blandino et al., 2013; Sovrani et al., 2012;). DPPH• antiradical activity was determined in both methanolic (DPPH MeOH) and hydroalcoholic (DPPH H_2O) solutions.

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

210 The DPPH H₂O method was performed in the same way as described for the DPPH in the

methanolic solution, but adding 1400 μ L of water and 1400 μ L of DPPH• methanolic solution to the solid samples.

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

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

222

223 2.4.5. Deoxynivalenol (DON) contamination

The DON content was analysed on the HLB fractions obtained after a sequential pearling using a high performance liquid chromatography (HPLC-MS-MS) method as previously described in Sovrani et al. (2012). The limit of detection (LOD) and the limit of quantification (LOQ) were 5 μ g kg⁻¹ and 16 μ g kg⁻¹, respectively.

228

229 **2.5.** Bread-making technological quality analyses

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

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

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

244

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

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

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

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

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

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**

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

274

275 **2.5.5. Breadcrumb texture profile analysis**

Texture measurements were performed on two slices (20 mm thick), cut out from the central part of the three replicated loaves for each mixture of refined flour and pearled fractions, 4 h after baking. On average, six measurements per slice were made. The bread slices were compressed in the central area using an SMS P/35 flat probe (Stable Micro Systems) for a 50% deformation of the slice, with a waiting time between two bites of 30 seconds, using 1 mm s⁻¹ as the speed test (Blandino et al., 2013). An instrumental trigger of 0.05 N was applied. The typical texture profile analysis parameters were determined from the force-distance curves, and were calculated by the software: hardness (N), cohesiveness (adimensional), gumminess (N), springiness (mm), chewiness (mJ), and resilience (adimensional).

286

287 2.6. Statistical analysis

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

295

3. Results and discussion

298

3.1. Bioactive compounds and DON in the barley pearled fractions

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

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

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

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

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

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

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

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

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

Although the DON contamination of the considered barley lot was extremely low, these findings confirm that, even for HLB, the risk of mycotoxin contamination and other 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

On the basis of the collected data, two functional ingredients were chosen among the 357 different pearling fractions of HLB: an external fraction (5-15% w/w) and a debranned inner 358 fraction (25-100%). The outermost layer (0-5%), although rich in functional components, 359 was discarded, thus reducing the risk of the presence of contaminants. The former 360 ingredient was selected mainly as a source of total DF (28%), but also of ash, proteins and 361 compounds with antioxidant activity, such as TPC (Table 2). This pearled barley fraction 362 had 9, 2.4, 14, 25 and 85 times higher ash, protein, DF, β-glucan and TPC contents, 363 respectively, than the refined wheat commercial flour. Considering the different methods, 364 the TAA was found to be from 61 to 138 times higher than in the refined wheat flour. 365 Conversely, the other barley ingredient was particularly rich in β -glucans (4.3%), but it 366 also showed higher ash (+2 times), protein (+1.4 times), DF (+3.8 times), TPC (+40 367 368 times) and TAA (+5 times) contents, compared to the refined wheat white flour.

370 **3.3.** Rheological parameters of the replaced flours

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

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

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

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

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

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

412

413 3.4. Bread technology properties

The control (no replacement) and the 5%-, 10%-, 15%-, 20%-, 25%-substituted types of bread were produced for both two barley fractions and analyzed for their technological 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

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

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

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

An increase in the yellow b* component was observed for the external layer fraction substitutions from 5% to 25% levels, while a slight decrease of L* was noted in all the samples compared to the control, but it was only significantly different for the 5, 10 and 15% substitutions. A darker crumb color was also observed by Trogh et al. (2004) for the addition of HLB flour. Yeung and Vasanthan (2001) reported that the pearling of HLB is 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

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

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

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

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

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

The lower loaf volume and firmer crumb hardness values are mainly related to the addition of DF, which leads to a gluten–starch matrix dilution and, consequently, to a lower capacity to enclose the gas cells during fermentation and baking (Gill et al., 2002). Moreover, the same values are related to the effect of the non-starch polysaccharide content (β-glucans),
which bind part of the water in the dough, and reduce the development of the gluten
network (Holtekjolen et al., 2008b).

496

497 3.5. Chemical characterization of the bread and nutritional considerations

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

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

517 fractions, respectively.

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

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

537

538 **4. Conclusion**

559

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

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

enhances the DF and β -glucan contents in bakery products.

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

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Tables

Table 1.

$Ash, protein, DF and \beta$ -glucan contents, TAA and DON contamination in pearled HLB fractions.

Kernel pearling	Ash	Proteins	DF				DON		
fractions	(%)	(%)	Total (%	Insoluble %)	<mark>β-glucans</mark> (%)	DPPH MeOH	DPPH H20 (mmol TE kg ⁻¹)	ABTS	(µg kg⁻¹)
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<="" td=""></loq>
10-15%	4.8 b	19.8 b	33.0 b	27 bc	4.6 c	49 b	107 B	143 c	<loq b<="" td=""></loq>
15-20%	4.2 c	19.0 bc	26.4 c	23 c	4.9 bc	39 c	83 C	106 d	<loq b<="" td=""></loq>
20-25%	3.3 d	18.2 c	22.3 d	17 d	6.7 a	22 d	51 D	67 e	<loq b<="" td=""></loq>
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<="" td=""></loq>
P(F) sem ^a	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001 សូទ	< 0.001 18 6	< 0.001 24 7	< 0.001 ໑

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

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.

	Moisture	Ash	Proteins	DF	β-glucans	TPC		ΤΑΑ	
Product							DPPH MeOH	DPPH H20	ABTS
	(%)	(%)	(%)	(%)	(%)	(mg kg⁻¹)	(mmo	ol TE kg ⁻¹)	
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.

^a sem: standard error of mean

Barley fraction	Replacement level	Water absorption (%)	DDT (min)	Stability (min)	Amplitude (Nm)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
external laver	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
5-15%	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	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
25-100%	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

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

^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.

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.**

Barley	Replacement		Crust cold	or	Crust crunchiness					
fraction		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 laver	0	67.0 a	9.1 b	31.9 a	137 a	81 a	438 a			
5-15%	5	61.1 ab	9.9 ab	32.7 a	136 a	71 ab	75 b			
0 10 / 0	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)	< 0.001	0.024	0.627	<0.001	<0.001	<0.001			
	sem ^a	5.5	3.2	2.3	18.7	9.7	50.0			
debranned kernel	0	68.4 b	6.7 ab	29.4 a	128 a	79 a	387 a			
25-100%	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)	< 0.001	0.020	0.108	0.072	0.003	<0.001			
	sem ^a	2.4	1.7	2.2	20.3	3.2	98.9			

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

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

^a sem: standard error of mean.

Barley	Replacement	Bread			Bread ci	rumbs			Crumb color				
fraction	level	Volume (ml)	Hardness N	Cohesiveness (-)	Springiness mm	Gumminess N	Chewiness mJ	Resilience (-)	L* (C)	a* (C)	b * (C)		
external	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		
layer	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		
5-15%	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	0.002	<0.001	<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	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		
kernel	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		
25-100%	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	0.004	0.097	0.144	0.031		
	sem ^a	62	0.8	0.01	0.1	0.7	6.5	0.02	4.9	0.4	2.2		

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

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

⁶⁹⁸ ^a sem: standard error of mean.

695 **Table 5.**

699 **Table 6.**

Barley	Replacement	Ash	Proteins	DF	β-glucans	TPC		TAA	
fraction	level						DPPH MeOH	DPPH H₂O	ABTS
		(%)	(%)	(%)	(%)	(mg kg⁻¹)		(mmol TE kg ⁻¹)	
external layer	0	2.7 f	12.0 e	2.6 d	0.18 f	177 d	0.20 e	1.2 e	7.1 e
5-15%	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)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	sem ^a	0.10	0.4	1.6	0.03	391	0.7	2.8	3.9
debranned kernel	0	2.7 c	11.3 b	2.7 d	0.09 f	175 a	0.25 d	1.1 f	5.0 d
25-100%	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)	0.005	< 0.001	< 0.001	< 0.001	0.308	< 0.001	< 0.001	< 0.001
	sem ^a	0.10	0.38	0.69	0.09	124	0.06	0.15	0.42

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

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).

^a sem: standard error of mean.