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

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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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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.

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

1. Introduction

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

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,

93 and therefore represents a technological drawback. These effects are particularly evident
94 when barley grain flour or barley fractions are used (Ragaee 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 ≈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

2. Materials and methods

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

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.

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.l., Cuneo, Italy). 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.

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).

2.4. Chemical analyses on the pearling fractions and breads

2.4.1. Sample preparation

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.

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.

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 µL, according to the expected concentration) were made to react with 100 µL of Folin-Ciocalteu reagent and 350 µL of sodium carbonate (5%), and distilled water was added to a total volume of 2900 µL. 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.

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

The DPPH H_2O method was performed in the same way as described for the DPPH in the

methanolic solution, but adding 1400 µL of water and 1400 µL 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.

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 µg kg^{-1} and 16 µg kg^{-1}, respectively.

2.5. Bread-making technological quality analyses

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

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.

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 penetrated by a P/6 6-mm steel cylindrical probe, a deformation of 20 mm was applied with a test speed of 1 mm s^{-1} , and an instrumental trigger of 0.05 N was used. The microphone was placed at a fixed distance of 10 mm from the sample for the acoustic measurements. In order to minimize the noise, the acoustic measurements were filtered through an integrated 1-kHz high pass filter, and a 24 dB instrumental gain was applied.

The analysis was performed in triplicate at 3 different points for each loaf. The following mechanical and acoustic parameters were determined from the force-distance and acoustic spectra: total energy (mJ), maximum acoustic emission (dB (SPL)), and number of acoustic emission peaks, using 15 dB (SPL) as the peak threshold value (Blandino et al, 2013).

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).

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

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

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 progressively from the external to the internal layers, while the β-glucan content increased going towards the inner kernel layers, in agreement with the results of several authors (Sumner et al., 1985; Yeung and Vasanthan, 2001; Zheng et al., 2000). No significant 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, respectively. β-glucans showed the lowest concentration in the most external layer (0-5%), while the highest β-glucan content was in the 20-25% fraction. The pearled inner core (25-100%) constituted 79% of the total kernel content of this soluble fibre. Liu et al. (2009) reported a peak of the β-glucan content at a 60% pearling removal level for HLB, but their concentration then decreased slightly. A different distribution of the β-glucan content in kernels was shown by Zheng et al. (2000): in normal starch and with a medium-low β-glucan content, these compounds were more concentrated in the subaleurone and in the endosperm adjacent to the subaleurone (30-40% pearled fractions); while in high β-glucan genotypes, including both waxy and high amylose varieties, the β-glucans were more uniformly distributed in the endosperm.

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

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

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

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

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 (Ragaee 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 Škrbíc 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% (Ragaee 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% (Škrbíc 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 at 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 (Ragaee 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

4. Conclusion

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

In short,, these findings have shown that the selective pearling process of HLB could be an 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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Tables

Table 1.

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
			Total	Insoluble		DPPH MeOH	DPPH H20	ABTS	
	(%)	(%)	(%)		(%)	(mmol TE kg^{-1})			(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
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
P (F)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 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

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

Table 2.

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

Product	Moisture	Ash	Proteins	DF	β-glucans	TPC	TAA		
							DPPH MeOH	DPPH H20	ABTS
	(%)	(%)	(%)	(%)	(%)	(mg kg^{-1})	(mmol 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

Results are expressed on a d.w. basis.

[a] sem: standard error of mean

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	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
	P (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
	P (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

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

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

[b] sem: standard error of mean.

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
	P (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 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
	P (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

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

694 [a] sem: standard error of mean.

Table 5.

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

Barley fraction	Replacement level	Bread	Bread crumbs						Crumb color		
		Volume (ml)	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
	P (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 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
	P (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

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

[a] sem: standard error of mean.

Table 6.

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	TAA		
							DPPH MeOH	DPPH H_2O	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
	P (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
	P (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

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.