

2 **Nutritional and functional performance of high β -glucan barley flours in breadmaking: mixed breads**
3 **vs wheat breads**

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25 **Keywords** Nutritional quality · Barley · Wheat · Functional properties · Bread

2 **Abstract** **The ability of high-β-glucan barley (HBGB) flour vs regular commercial barley (CB) and**
3 **common wheat flour (WT) to make highly nutritious wheat (WT) blended breads** meeting functional and
4 sensory standards has been investigated. Mixed breads obtained by 40% replacement of WT flour by
5 **HBGB flours are more nutritious than those replaced by CB flours, and much more than regular WT**
6 **flour breads** in terms of elevated levels of dietary fibre fractions (soluble, insoluble, resistant starch and β-
7 glucans), slowly digestible starch subfraction and bioaccessible polyphenols providing higher antiradical
8 activity. WT/CB and WT/HBGB breads can be respectively labelled as source of fibre ([3 g DF/100 g food)
9 and high-fibre breads ([6 g DF/100 g food), according to Nutritional Claims for dietary fibre foods The
10 consumption of 100 g of WT/HBGB can meet up to almost 50% the required dietary fibre, providing a β-
11 glucan intake high enough to meet the requirements of the EFSA health claim (3 g/day), contributing a
12 reduced blood cholesterol level. **The techno-functional performance of fresh blended breads and the**
13 **sensory appreciation were in general preserved, or even improved.**

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2 Introduction

3 Consumers increasingly demanding healthier and tastier foods are shifting away from getting
4 nutrients via fortified foods and turning toward products that are naturally high in components with health
5 promoting effects. This trend has boosted that traditionally neglected cereals for human nutrition such as
6 barley, but rich in health-related components, are currently being reconsidered a part of a healthy human
7 diet (Manach, et al., 2004). Barley is increasingly incorporated in already established as well as in new food
8 products -pasta and bread- either as a whole grain or as a food ingredient (Holtekjølen & Knutsen, 2011),
9 mainly due to the presence of β -glucan and phenolic compounds which have the potential to lower
10 cholesterol and blood glucose levels (Cavallero et al., 2002), and proteins which have been recognized as a
11 rich source of some essential amino acids (Newman et al., 1978).

12 The nutritional value of food supplemented with barley depends on both the level of supplementation and on
13 the type of barley used (hull-less or hulled). Hull-less cultivars have better nutritional value than hulled ones
14 as they contain more proteins, lipids and soluble dietary fibres (Soares et al., 2007), mainly β -glucan
15 (Izydorczyk et al., 2000), which content generally underlies a natural fluctuation depending on the variety
16 and conditions before and after harvesting (Ehrenbergerová et al., 2008). In breadmaking applications,
17 replacement of wheat flour by significant amounts of non-gluten forming flours such as barley, can seriously
18 constrain dough viscoelasticity and gas retention capability of blended dough matrices that have a
19 weakened mixed protein network (diluted gluten/non gluten proteins). Diluted gluten matrices often lead to
20 impaired physico-chemical and sensory quality of fermented goods after baking in terms of volume, texture,
21 color, and taste. In addition, the baking process itself may induce depolymerization of the fiber constituents
22 (Andersson et al., 2004). In general, wheat flour substitution levels ranging from 15–20% of barley flour are
23 the most usual in practice (Alu'datt et al., 2012), although there are reports of successful incorporation of 20
24 and 26% of hull-less barley (Swanson & Penfield, 1988), and even higher –from 40 to 100%- (Trogh et al.,

2 2004; Rieder et al., 2012, Kinner et al., 2011), with variable chemical, physico-chemical, nutritional and
3 biological properties achieved in final breads.

4 **The paper is aimed at exploring comparatively the ability of high-β-glucan barley flours of superior**
5 **nutritional value vs regular commercial barley to be included in substantial amounts in blended**
6 **matrices with common wheat, to make highly nutritious bread meeting functional and sensory**
7 **standards.** Dough machinability, bread nutritional and functional profiles ~~and keeping-behaviour~~ were
8 assessed in blended barley-wheat matrices, and compared with the wheat flour counterparts. For common
9 wheat flour replacement purposes, refined high grade wheat flour (70% extraction rate) was used to keep,
10 as much as possible, viscoelasticity and gas retention ability of the basic wheat dough matrix.

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12 **Materials and methods**

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14 *Materials*

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16 Commercial flours from refined common wheat (WT), and common whole grain barley (CB), were
17 purchased from the Spanish market. **Refined wheat flour (70% extraction rate) of 356 x 10⁻⁴ J energy of**
18 **deformation W, 0.64 curve configuration ratio P/L, 95% Gluten Index, 62% water absorption in**
19 **Brabender Farinograph, was used.** High β-glucan barley (HBGB) produced by ConAgra (USA) under the
20 branded name of Sustagrain® (whole barley flour prepared in the grinding and bolting of varieties of
21 cleaned waxy, hulless barley) was furnished by Ingredion Germany GmbH. Supra Vital wheat gluten [GL]
22 and vegetable fat were acquired from Indespan (Spain); Ireks Vollsauer sour dough was from Ireks (Spain);
23 Aquasorb A-500 carboxymethylcellulose from Ashland-Aqualon (USA) was provided by Ricardo Molina
24 (Spain).

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2 *Methods*

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4 *Chemical and nutritional composition of wheat and barley flours*

5 Moisture, protein, ash and fat contents of commercial flours WT, CB and HBGB were determined following
6 the ICC methods 110/1, 105/2, 104/1, and 136, respectively (ICC, 1976-1996). Total, soluble and insoluble
7 dietary fibre contents were determined according to the AOAC method 991.43 (AOAC, 1991). Two replicates
8 were made for each flour analysis. Digestible carbohydrates were calculated by difference (FAO, 2003).
9 Amylose/ amylopectin ratio (Megazyme kit K-AMYL 07/11) was estimated by using a modification of a Con
10 A method developed by Yun and Matheson (1990) that uses an ethanol pre-treatment step to remove lipids
11 prior to analysis (modified from Morrison and Laignelet, 1983). Resistant starch determination was
12 performed according to AOAC Official Method 2002.02 (AOAC, 2000) by using Megazyme kit K-RSTAR
13 08/11. β -glucan content (Megazyme kit K-BGLU 07/11) was determined following the ICC Standard Method
14 No. 166. Total polyphenol content was determined according to the Folin-Ciocalteu procedure (Singleton et
15 al., 1999) in enzyme extracts as described previously (Angioloni and Collar, 2011a). Anti-radical activity
16 determined by using the radical scavenging capacity assay according to the DPPH method (Brand-
17 Williams et al., 1995), modified by Sánchez-Moreno et al. (1998) and applied earlier (Angioloni and Collar,
18 2011a). β -D-glucanase was assessed by using the azo-barley glucan method (Megazyme kit KMBGL
19 04/01). ~~Extraction of wheat (WT) and barley (CB, HBGB) flour proteins was carried out according to a
20 modified procedure of Kwon et al., (1996) and Osborne fractionation scheme (globulin, glutelin-1, glutelin-2
21 and prolamin fractions) as compiled by Alu'datt et al. (2012). A sequential solvent extraction procedure
22 including 0.5 M NaCl, 0.1 M NaOH, 50% glacial acetic acid and 70% ethanol was used to extract proteins,
23 at a flour to solvent ratio of 1:10 (w/v) for 2 h at room temperature. The fractionated proteins extracts were
24 lyophilized and designated as globulin, glutelin-1, glutelin-2 and prolamin fractions, respectively. The yield of~~

2 ~~protein (%) is expressed using the following equation: (Weight of Freeze Dried Extract x Protein Content x~~
3 ~~100%)/ (% Protein in Meal x Weight of Meal).~~

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5 *Functional properties of wheat - barley blended flours*

6 Blended wheat - barley flours were prepared by substituting 40% of either CB or HBGB from the total
7 percent of WT flour, and functional characteristics of WT and blended flours were assessed as it follows.

8 Solvent-Retention Capacity (SRC) was determined according the AACC method 56-11 (AACC, 2005) to
9 quantify potential contributions to water holding capacity by other flour components having water-uptake

10 capabilities (Traynham et al., 2007). The solvents used were sucrose (50% w/v), sodium bicarbonate (5%
11 w/v), and lactic acid (5% v/v). 25 mL of prepared solvent were added to 5 g of flour in 30 mL centrifuge

12 bottles. Centrifugation at 1,239•g (3,000 rpm) was performed for 15 min. After decanting, a gel remained.

13 Gels were weighed and the SRC value (%) calculated as % SRC = $[(\text{gel wt/flour wt}) \cdot (86/(100 - \% \text{ flour}$
14 $\text{moisture})) - 1] \cdot 100]$ for each solvent. The Water-Holding Capacity (WHC) was determined using methods

15 modified from Heywood et al. (2002) and Lin and Zayas (1987) as described by Traynham et al., 2007.

16 15 g of total flour was dispersed in 285 mL of distilled water in a 500 mL centrifuge bottle. Bottles were
17 shaken for 10 min, then centrifuged at either 1,592•g or 4,424•g (3,000 and 5,000 rpm, respectively) for 30

18 min. After decanting the supernatant, each bottle was weighed and WHC (g of water/g flour) was calculated

19 as: $\text{WHC} = ((\text{weight of bottle after decanting} - \text{weight of dry bottle}) - \text{total flour weight (g)})/\text{total flour weight}$
20 (g) . Swelling was determined as the volume occupied by a known weight of flour (Nelson, 2001), and was

21 evaluated by mixing 5 g (± 0.1 mg) of flour with 100 mL of distilled water and hydrating overnight. Fat

22 adsorption capacity (FAC) was determined according to Ahn et al. (2005). 1 g of flour sample was weighed
23 into a 50-mL pre-weighed centrifuge tube and thoroughly mixed with 10 mL of sunflower vegetable oil. The

24 protein-oil mixture was centrifuged (2000 × g for 5 min). Immediately after centrifugation, the supernatant

25 was carefully removed, and the tube was weighed. FAC (g of oil/g of sample) was calculated as $\text{FAC} = (F_2 -$

2 $F_1)/F_0$, where F_0 is the weight of the dry sample (g), F_1 is the weight of the tube plus the dry sample (g), and
3 F_2 is the weight of the tube plus the sediment (g). Foam capacity (FC) and Foam stability (FS) were
4 determined as described by Narayana and Narasinga Rao (1982) and modified by Alu'datt et al (2012). 2 g
5 of flour sample was mixed with 40 mL distilled water at 30 C in a 100 mL measuring cylinder. The
6 suspension was stirred and shaken for 5 min at 1600 rpm to produce foam and the foam stability was
7 expressed as the volume of foam over a time period from 0 to 60 min. The volume of foam was measured
8 after 0 min (V_T , FC) and the volume of foam after 60 min (V_1) was recorded. Foaming stability was
9 expressed as $\% = (V_1/V_T)100$.

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11 *Bread making of wheat and wheat – barley blended flours*

12 Doughs and breads were prepared for control (WT) and wheat - barley blended flours (WT/CB, WT/HBGB,
13 60/40, w/w). Flour (100 g), water (63% -WT-, 80% -WT/CB-, 80% -WT/HBGB-, flour basis), commercial
14 compressed yeast (4% flour basis), salt (1.5% flour basis), vegetable fat (4% flour basis), sugar (1% flour
15 basis), commercial sour dough (3% flour basis), gluten (2% flour basis), carboxymethylcellulose (1% flour
16 basis), and calcium propionate (0.5%) were mixed in a 10 kg mixer at 60 revolutions min^{-1} for 10 min up to
17 optimum dough development. Fermented doughs were obtained after bulk fermentation (10 min), dividing
18 (700 g), rounding, molding, and proofing up to maximum volume increment (30 min), and were baked at 220
19 °C for 30 min to make WT, WT/CB, and WT/HBGB breads, respectively. ~~Breads were sliced (1 cm) and
20 stored in polypropylene bags for 1, 3, 6, 8 and 10 days at 26°C until analysis.~~

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22 *Dough rheological measurements*

23 ~~Dough machinability of dough samples was assessed by either texture profile analysis (TPA) or stickiness
24 measurements (Hosoney and Chen cell) in a TA-Xtplus texture analyser (Stable Micro Systems,
25 Godalming, UK). A 5 cm diameter probe, a 75 s waiting period and 60% compression (Collar et al., 1999)~~

2 ~~for TPA and a Hosoney and Chen cell (Armero and Collar, 1997) for stickiness were used. All~~
3 ~~measurements were made in triplicate.~~

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5 *Bread measurements*

6 *Physico-chemical and sensory determinations*

7 Colour determinations were carried out on bread crumb and crust using a Minolta colorimeter (Minolta CR-
8 400, Konica Minolta Sensing, Inc., Osaka, Japan), and results were expressed in accordance with the
9 Hunter Lab colour space. Parameters determined were L (L = 0 [black] and L = 100 [white]), a (-a =
10 greenness and +a = redness), b (-b = blueness and +b = yellowness), ΔE -total colour difference- (Francis
11 and Clydesdale, 1975), BI -browning index- (Ramirez-Jimenez et al., 2001) and WI -whiteness index- (Hsu
12 et al., 2003). All measurements were made in triplicate. Crumb grain characteristics were assessed in bread
13 slices using a digital image analysis system. Images were previously acquired with a ScanJet II cx flatbed
14 scanner (Hewlett-Packard, Palo Alto, CA, USA) supported by a Deskscan II software. The analysis was
15 performed on 40 mm × 40 mm squares taken from the centre of the images. Data were processed using
16 SigmaScan Pro 5 (Jandel Corporation, San Rafael, CA, USA). The crumb grain features evaluated were
17 mean cell area, cells/cm², cell/total area ratio, wall/total area ratio and crumb area/total cell ratio (Collar et
18 al., 2005). Sensory analysis of fresh breads was performed with a panel of eight trained judges (four males
19 and four females aged 24-55) using semi structured scales, scored 0-10 (lowest:0; highest:10) for each
20 sensory attribute. Evaluated attributes were external appearance, crumb texture, aroma intensity, taste
21 intensity, and overall acceptability.

22 Bread primary and secondary mechanical characteristics (TPA in a double compression cycle) of fresh and
23 stored breads were recorded in a TA-XTplus texture analyser (Stable Micro Systems) using a 10 mm
24 diameter probe, a 5 kg load cell, 50% penetration depth and a 30 s gap between compressions on slices of
25 25 mm width (Armero and Collar, 1998). For textural measurements, three slices of two freshly made

2 breads were used for each sample. at different storage periods (0 to 10 days). The obtained firming
3 curves were modelled using the Avrami equation, and model factors were estimated by fitting experimental

4
$$\theta = \frac{T_{\infty} - T_t}{T_{\infty} - T_0} = e^{-kt^n}$$
 — where θ is the fraction of the
5 recrystallisation still to occur; T_0 , T_{∞} and T_t are crumb firmness at time zero, ∞ and time t, respectively, k is
6 a rate constant, and n is the Avrami exponent (Armero and Collar, 1998).

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8 *Enzymatic/biochemical determinations*

9 Starch hydrolysis kinetics and relevant starch fractions in WT and wheat - barley blended (WT/CB,
10 WT/HBGB) breads was determined following the AACC (2000) method 32-40, adapted as previously
11 described (Angioloni & Collar, 2011b). Each bread sample (100 mg) was incubated with pancreatic α -
12 amylase (10 mg) and amyloglucosidase (12 U) in 4 mL of 0.1 mol/L sodium maleate buffer (pH 6.0) in a
13 shaking water bath (200 strokes/min) at 37 °C for 0, 0.5, 1, 1.5, 2, 3, 4, and 16 h). After incubation, samples
14 were heated at 100 °C for 5 min, and ethanol:water (95:5, v:v) was added for enzyme inactivation, prior to
15 centrifugation at 720 g for 10 min. The glucose content of the supernatant was measured using a glucose
16 oxidase/peroxidase (GOPOD) kit. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were
17 measured after incubation for 30 min and 120 min respectively (Englyst et al., 2003). Total digestible starch
18 (DS) was determined in the supernatant after 16 h of incubation while resistant starch (RS) was determined
19 in the pellet as the starch remaining after 16 h incubation. The digestion kinetics and expected glycaemic
20 index (eGI) of bread were calculated in accordance with the procedure followed by Chung et al. (2008)
21 based on the method established by Goñi et al.(1997). A first order kinetic equation [$C = C_{\infty} (1 - e^{-kt})$] was
22 applied to describe the kinetics of starch hydrolysis, where C , C_{∞} and k were the hydrolysis degree at each
23 time, the maximum hydrolysis extent and the kinetic constant, respectively. The hydrolysis index (HI) was
24 calculated as the relation between the area under the hydrolysis curve (0-16 h) of blended bread samples

2 and the area of standard material from white bread (control) (Chung et al., 2008). The expected glycaemic
3 index (eGI) was calculated using the equation proposed by Granfeldt, et al. (1992): $eGI = 8.198 + 0.862HI$.
4 Bioaccessible phenol determination was conducted in bread samples by conducting an “in vitro” digestive
5 enzymatic mild extraction that mimics the conditions in the gastrointestinal tract according to the procedure
6 of Glahn et al. (1998) as recently used by Vitali et al. (2009) for biscuits and Angioloni and Collar (2011a) for
7 breads. This method skips out the colonic fermentation in the large intestine. Briefly, 10 mL of distilled water
8 and 0.5 mL of pepsin (20 g L^{-1} in 0.1 mol L^{-1} HCl) were added to 500 mg of ground sample, pH was
9 adjusted to 2 using 5 mol L^{-1} HCl and the sample was incubated at $37 \text{ }^{\circ}\text{C}$ in a shaking water bath for 1 h.
10 Simulation of gastric digestion was stopped by addition of 1 M NaHCO_3 (to adjust pH to 7.2). Then, 2.5 mL
11 of bile (cholic and deoxycholic sodium salts)/pancreatin solution (2 g L^{-1} of pancreatin and 12 g L^{-1} of bile
12 salt in 0.1 M NaHCO_3) and 2.5 mL of NaCl/KCl (120 mmol L^{-1} NaCl and 5 mmol L^{-1} KCl) were added to the
13 sample, and simulation of intestinal digestion was conducted for the following 2.5 h. Samples were
14 centrifuged at 3500 g for 10 min and the supernatants were used for determination of bioaccessible phenols
15 after removing proteins from digestive extracts by addition of trichloroacetic acid (20%, w/w) by the Folin-
16 Cioalteau method, as described for flours.

17
18 *Chromatographic determinations*
19 Analysis of the polyphenol composition was achieved by using Reversed Phase-High Performance Liquid
20 Chromatography (RP-HPLC). HPLC-analysis was performed on an Agilent model1200, equipped with
21 quaternary pump, automatic injector, autosampler and Diode Array Detector. The instrument was controlled
22 and the chromatographic data were analysed using a personal computer loaded with the HP ChemStation
23 software. Reversed phase separations were conducted on a Zorbax Eclipse XDB-C18 column with
24 dimensions $150 \times 4.6 \text{ mm}$ and $5 \text{ }\mu\text{m}$ particle size. The mobile phase consisted of 0.1% formic acid in water
25 as eluent A and 0.1% formic acid in acetonitrile as eluent B. The solvent gradient program was set as

2 follows: initial conditions 100% A; 0-60min, 0-25% B; 60-70 min, 25-70% B; 70-85 min, 70-100% B. Column
3 temperature was set at 24 °C, flow rate was 1 ml min⁻¹ and the injection volume was 20 µL. Samples were
4 deproteinised and desaccharified prior to injection by precipitation with methanol. For identification
5 purposes, a spectral library was constructed comprising the retention times and spectra of the standards
6 under the chromatographic conditions specified above. Calibration curves using an external calibration
7 method were also prepared and used for quantitative analysis, and the results expressed as mg/kg of
8 sample, as is. Phenolic acids and flavonoids were detected at a wavelength of 320 nm.

9

10 Statistical analysis

11 Univariate (ANOVA) and multivariate (non linear multiple regression) analysis of data was performed by
12 using Statgraphics V.7.1 program (Bitstream, Cambridge, MN).

13

14 Results and discussion

15

16 Bread is a complex viscoelastic porous matrix, composed mainly of gluten, starch, lipids and water, whose
17 sensory, technological and nutritional final quality is multifactor dependent. Basic ingredients, additives and
18 technological and/or processing aids, and breadmaking process influence, in variable degree, the overall
19 quality of fresh and stored breads. Physicochemical, biochemical, nutritional and techno-functional patterns
20 of single (WT, CB and HBGB) and blended (WT/CB, WT/HBGB) flours are investigated (Tables 1-2), prior
21 to depict comparatively the sensory (Table 3), technological (Table 4), and nutritional profiles (Table 5) of
22 blended barley/wheat vs wheat bread matrices.

23

24 *Physico-chemical, nutritional and functional performance of single (WT, CB and HBGB) and blended*
25 *(WT/CB, WT/HBGB) flours.*

2 Single WT, CB and HBGB flours exhibited different chemical, biochemical and nutritional profiles (Table 1).
3 Comparatively (HBGB vs CB, WT, per 100g flour basis, d.b), high- β -glucan barley flour, accounted for
4 much higher protein (19.35% vs 12.92%, 14.12%), slightly higher fat (5.87% vs 1.94%, 1.34%), and ash
5 (2.00% vs 1.74%, 0.63%), and much higher total dietary fibre (35.01% vs 17.43%, 2.22%), resistant starch
6 (8.33% vs 4.84, 2.05%), β -glucan (13.30% vs 5.16%, 0.23%), antiradical activity (65%, 62% vs 12%), and
7 total polyphenol (2197mg vs 1003mg, 713 mg) contents than both CB and WT flours, in good accordance
8 with a superior nutritional profile for hullless barley samples (Soares et al., 2007). It has been reported that
9 hullless barley contains much higher (1/3,1/4)- β -D-glucan levels than either wheat or hulled barley (Bhatty,
10 1999). The high content of β -glucan in barley (2.5 -11.3%) compared to wheat (0.4 -1.4%) has made barley
11 increasingly interesting for bread production (Lazaridou et al., 2007). High-amylose and waxy hullless barley
12 have been described to contain approximately 7 or 8% β -glucans, whereas regular hullless barley comprises
13 significantly less (4.6%) (Gao et al., 2009; Tiwari and Cummins, 2008), in good accordance with data
14 obtained for CB (4.50%, flour m. b.), and significantly lower than amounts obtained for HBGB (12.20%, m.
15 b.). Contribution of β -glucan degrading enzymes from the commercial wheat flour is believed to be limited
16 since wheat flour has been shown to have low activity of β -glucanases (Wang et al., 1998), and values
17 retrieved for β -glucanase activity in CB and HBGB in this work were significantly much lower (<46U/kg).
18 Hullless barley is a good source of dietary fiber providing soluble and insoluble dietary fiber fractions (Bhatty,
19 1999; Izydorczyk et al., 2000). Mixed-linkage (1 / 3), (1 / 4)- β -D-glucans (hereafter termed as β -glucan) are
20 a major part of the soluble dietary fiber in barley. A study by Xue et al., 1997 showed that the total β -glucan
21 content is higher, whereas the insoluble dietary fiber content is significantly lower in naked barley compared
22 to hulled barley genotypes. This was not the case of the hullless barley used in this study (HBGB) that
23 exhibits 18.5% of insoluble dietary fibre vs 10.05% and 1.09% for CB and WT flour, respectively.
24 Differences could be attributed to a natural fluctuation depending on the variety and conditions before and
25 after harvesting, as reported earlier (Ehrenbergerová et al., 2008). Hullless barley, normally used as a non-

2 wheat source of dietary fibre, significantly promotes total as well as soluble dietary fibre contents of the
3 resulting bread products, when it replaces 15% of wheat flour (Gill et al., 2002). Skendi et al. (2010)
4 reported a positive effect of barley β -glucan addition on wheat bread specific volume, which was more
5 pronounced for high MW β -glucan and wheat flours with weak gluten quality.

6 On the contrary, lower amounts for digestible starch (37.8% vs 65.9%, 81.5%) and β -glucanase activity (22
7 U/kg vs 53, 985 U/kg) were observed in barley flours especially for HBGB vs CB and WT flours (Table 1) ,
8 in good agreement with current dietary guidelines for starch fractions (Miller-Jones, 2009), and with suitable
9 integrity of the molecular weight of β -glucans to keep hypocholesterolemic effects (Newman et al., 1989),
10 respectively.

11 ~~Since protein plays an important role in dough structural development, an increase in the protein content~~
12 ~~would suggest a priori that increased HBGB inclusion levels in the formulations may produce doughs and~~
13 ~~breads of a promising quality than those with lower inclusion levels, from the point of view of both functional~~
14 ~~and nutritional performances. Protein content and yield of protein extracts from WT, CB and HBGB flours~~
15 ~~(Table 2) revealed significant quantitative differences for all sequential protein extracts (concentration) and~~
16 ~~protein distribution (yield). Higher protein concentration and yield were both obtained for **glutelin-1 and**~~
17 ~~**prolamin fractions, followed by globulin and glutelin-2.** WT showed the highest protein concentration~~
18 ~~for glutelin-1 (50.09%), followed by CB (42.99%) and HBGB (33.02%), **whereas solids of prolamin and**~~
19 ~~**globulin extracts were similar in protein content (30% and 10%, respectively), and glutelin-2 extracts**~~
20 ~~**contained low protein regardless the flour (<3%).** Glutelin-1 and **prolamin** extracted together from **63%**~~
21 ~~(HBGB) to **80%** (WT) of total flour protein. Obtained results suggested different protein profiles and hence~~
22 ~~different functional properties, particularly for HBGB.~~

23 Hydration properties (WHC, swelling and SRC), FAC, FC and FS were determined in WF and blended
24 wheat - barley flours prepared by substituting 40% of either CB or HBGB from the total % of WT flour (Table
25 **2**). WHC reports the ability of a protein matrix to absorb and retain bound, hydrodynamic, capillary, and

2 physically entrapped water against gravity (Damodaran and Paraf, 1997). In general, WHC values for all the
3 flour blends were lower at 5,000 rpm than 3,000 rpm, as would be expected and reported before (Traynham
4 et al., 2007), because of the greater centrifugal force being applied to samples. WHC of WT and WT/CB
5 blends were not statistically different but significantly lower than values for WT/HBGB blend, in good
6 accordance with the trend shown for swelling (Table 2). This persistent difference might be ascribed to the
7 significantly higher protein content of the HBGB flour (Table 1), and probably to the formation of large
8 clusters of protein molecules or protein aggregates bound by hydrogen bonds and other non-covalent
9 forces. SRC testing used to establish a practical functionality profile of flour (Heywood et al., 2002), takes
10 into account several flour constituents influencing water-retention potential, including pentosans, damaged
11 starch, and glutenin, using sucrose, sodium carbonate, and lactic acid solutions, respectively. **SRC is the**
12 **weight of solvent held by flour after centrifugation, and it is expressed as percent of flour weight, on**
13 **a 14% moisture basis.** For flour typically used to produce bread by the sponge–dough method, optimal
14 SRC profile values would be $\geq 100\%$ glutenin, $\leq 96\%$ pentosans, $\leq 72\%$ damaged starch (Heywood et al.,
15 2002). In this work, a straight dough breadmaking system was used instead, and some mean values for
16 water retention components of WT, and blended wheat - barley flours (WT/CB and WT/HBGB) were
17 outside the typical range for a sponge–dough bread system, especially for WT/HBGB and water retention of
18 pentosans (174%) and damaged starch (134%). FAC values indicate that when barley was added to the
19 wheat flour samples, their FAC values increased only with HBGB up to 1.34 g fat/g flour. Lin and Zayas
20 (1987) suggested that the ability of protein to bind fat depends on nonpolar side chains that bind
21 hydrocarbon chains, thereby contributing to increased oil absorption (Ahn et al., 2005). The results imply
22 that the increased FAC can be partly attributed to a marked decrease in bulk density because fat absorption
23 depends on the physical entrapment of oil (Siu et al., 2002). Both FC (14 mL) and FS (64%) of WT flour did
24 not significantly change with 40% replacement of WT by CB, but with HBGB replacement (Table 2). The
25 presence of HBGB in wheat blends gave a decrease in either FC or FS by 70 % and 22 %, respectively.

2

3 *Techno-functional, sensory and nutritional profiles of blended barley/wheat vs wheat matrices.*

4 Bread dough is a viscoelastic material that exhibits an intermediate rheological behaviour between a
5 viscous liquid and an elastic solid. Bread crumb is a porous solid matrix with cellular structure composed
6 mainly of gluten, starch, and water, and minor constituents such as lipids and non-starch polysaccharides in
7 presence of other ingredients, additives and technological aids representing a typical viscoelastic
8 biopolymer foam system. Major breadmaking steps significantly change the viscoelasticity of wheat doughs.
9 During mixing, distribution of materials, hydration and energy input for stretching and alignment of protein
10 molecules take place involving shear and extensional deformation. During fermentation, the expansion of
11 the air bubbles previously incorporated during mixing provides the characteristic aerated structure of bread,
12 which is relevant to its appeal. During proof and baking the growth of gas bubbles determines the expansion
13 of the dough and therefore the ultimate volume and texture of the baked product (Collar, 2013). It is known
14 that the addition of barley or barley fractions in foods will influence techno-functional and sensory bread
15 product quality in terms of color, taste, and texture through changes induced in dough viscoelastic
16 behaviour. The polysaccharides in barley affect the quality of baking products (Holtekjølen and Knutsen,
17 2011), and the baking process itself may alter the fiber constituents (depolymerization) (Andersson et al.,
18 2004). Rheological measurements that are increasingly being used as sensitive indicators of polymers
19 molecular structure and predictors of end-use performance, have been successfully applied to bread
20 doughs as indicators of the gluten and starch biopolymers molecular structure and predictors of its
21 functional behaviour in breadmaking. In this study, 40% of WT replacement by either CB or HBGB was
22 established in preliminary trials as the maximum level of substitution that did not compromise significantly
23 dough machinability (data not shown) in terms of stickiness (<1 N), dough hardness (<80 N), cohesiveness
24 (>0.5), adhesiveness (<100 N.s) and springiness (>0.8). The addition of barley flour either CB or HBGB
25 significantly decreased the bread volume (-22%), and the crumb cohesiveness (-14%) but increased crumb

2 hardness (90%) with respect to refined WT flour bread types, particularly for WT/HBGB blends (Table 3).
3 Resulting blended breads are visibly different from control WT breads ($\Delta E \geq 3$) in both crust and crumb
4 colour, particularly for crust colour in WT/HBGB breads ($\Delta E \geq 3$) and for the crumb colour in WT/CB breads
5 ($\Delta E \geq 16$). Crumb pore uniformity and crumb grain structure were not significantly affected, though in the
6 barley supplemented breads the crumb quality decreased in terms of lower cell size and thicker cell walls.
7 The refined control bread WT was scored significantly lower in both taste intensity and overall acceptability.
8 The wheat-barley blended breads deserved similar ratings than WT control breads concerning external
9 appearance, aroma intensity and crumb firmness (Table 3). The incorporation of barley flour diminishes
10 bread quality, particularly loaf volume, of wheat composite breads (Gill et al., 2002) due to both the dilution
11 of wheat gluten and the mechanical interference with gluten network formation by insoluble dietary fibre
12 (Salmenkallio-Marttila et al., 2001) causing additional rupture of gas cells (Courtin and Delcour, 2002), as
13 previously observed for high-fibre supplemented wheat breads (Collar, 2008). Besides, both soluble and
14 insoluble fibres, tightly bind high amounts of water, which may make it less available for the development of
15 the gluten network and may further result in less volume production after baking (Gill et al., 2002). Lower
16 volume in barley blended matrices is in good accordance with the higher total dietary fibre content of barley
17 flours (Table 1) that resulted in particularly prominent total dietary fibre level (g/100 g bread, as is) in breads
18 thereof (4% WT/CB, 12% WT/HBGB), compared to the WT counterparts (Table 1 and Table 5). Other
19 authors have stated the positive effect on dough rheology and bread quality of barley β -glucan isolates to
20 wheat flour of a poor bread making cultivar made with optimised water addition (Skendi et al., 2010),
21 probably ascribed to the higher viscosity of the water phase of the dough and thereby stabilising gas cells in
22 the way reported for water-extractable arabinoxylans in wheat dough (Courtin and Delcour, 2002). In fact, β -
23 glucan content (Table 1) of barley flours with high water binding capacity (Table 2) gave blended breads
24 with a significantly high β -glucan content (1.51% CB, 3.23% HBGB) compared to WT counterparts (0.11%).

2 This fact can partially counteract the deleterious effect of barley flour addition in the volume of wheat-barley
3 mixed breads.

4 ~~During storage, fresh breads aged in variable extent following different staling kinetics (Table 5). The wheat-~~
5 ~~barley blended breads followed slower (WT/CB) or quicker (WT/HBGB) staling kinetics along storage~~
6 ~~compared to the control WT breads, as it can be concluded from the Avrami exponent n values: 0.690 (WT)~~
7 ~~vs 0.417 (WT/CB) and 0.808 (WT/HBGB). Besides, final crumb hardness T_{∞} was lower for blended breads~~
8 ~~(29 N) than for control WT breads (75 N), supporting a good keeping behaviour of supplemented barley~~
9 ~~breads with respect to control WT breads.~~

10 Nutritional intrinsic characteristics of barley flours (i.e. good source of dietary fibre –resistant starch and β -
11 glucan-, and high protein contents and dough processing (i.e. hydration, other ingredients, breadmaking
12 test) play relevant roles both in the nutritional features and in the enzyme accessibility to natural
13 biopolymers—protein and starch—present in mixed breads. Nutritional information on wheat-barley breads
14 (Table 5) showed most appealing nutritional quality than WT breads, especially for HBGB breads in terms of
15 lower digestible starch, high soluble and insoluble dietary fibre, β -glucan, resistant starch and bioaccessible
16 polyphenol contents of sensorially accepted samples. A significant reduction of starch content in bread with
17 wheat flour substitution by barley flour, which regulates the total amount of accessible macronutrients was
18 observed. Starch nutritional fractions determined in wheat and wheat-barley breads by “in vitro” starch
19 digestion included rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS)
20 (Table 6). RDS, SDS and RS contents ranged from 34.7 (WT/HBGB) to 58.5% (WT), from 3.4 (WT/CB) to
21 9.3% (WT/HBGB) and from 1.80 (WT) to 7.0% (WT/HBGB), respectively. The lowest RDS content and
22 highest SDS and RS contents, which are considered suitable nutritional trends for dietary starch fractions
23 (Englyst et al., 2003), were observed for the blend sample WT/HBGB, which showed a rather low extent of
24 starch hydrolysis (Table 4) with the lowest values for C_{∞} , k and H_{90} , and expected Glycaemic Index (85).
25 The incorporation of barley flour into wheat bread formulation seems to reduce starch hydrolysis, probably

2 because of their lower starch and higher fibre and protein contents, especially for HBGB flour (Table 1). The
3 reduced rate and overall reduced starch digestibility of barley mixed breads may be affected by high content
4 of viscous soluble dietary fibre components like in legume matrices (Angioloni and Collar, 2012) supported
5 by the high amount of β -glucan determined in wheat-barley breads (Table 5). In addition, high protein
6 content of barley flours (Table 1) can promote starch–protein interactions restricting enzyme attack as
7 pointed out for lentils (Chung et al., 2008). Barley bread samples contain about four (WT/CB) to twelve
8 times (WT/HBGB) the fibre of the regular white bread, so that breads can be respectively labelled as source
9 of fibre ([3 g DF/100 g food) and high-fibre breads ([6 g DF/100 g food), according to Nutritional Claims for
10 DF foods (Off J Eur Comm, 2006).

11 The shift from digestible to non-digestible carbohydrates and moisture variations (35.6 g/100 g bread as is –
12 WT-, 38.6%-WT/CB-, 40.5% -WT/HBGB-) were responsible for the observed differences in the energy
13 extent of high barley breads (198 kcal –WT/CB- , 166 kcal –WT/HBGB-) vs WT breads (219 kcal). 100 g of
14 control wheat breads made with refined wheat flour account for 7.9 g of protein, 1.15 g of total dietary fibre,
15 0.56 g of fat and 45 g of digestible carbohydrates (Table 5). According to the Recommended Dietary
16 Allowances (RDA), and Adequate Intakes (AI) for macronutrients (Otten et al., 2006), a daily intake of 100 g
17 of high barley breads WT/CB and WT/HBGB provide 30 and 19% of digestible carbohydrate, 13-16% and
18 15-18% of protein, and 11-16% and 31-48% of the dietary fibre recommended for adults (male-female),
19 respectively. Daily consumption of 100 g of wheat bread delivers 35% of digestible carbohydrates, from 14
20 (male) to 17% (female) of proteins, and from 3 (male) to 4.6% (female) of dietary fibre. Compared to wheat,
21 high-barley breads allow the ingestion of higher protein and almost 50% the required dietary fibre
22 (WT/HBGB), and lower digestible carbohydrates, in good agreement with the dietary guidelines for health
23 (FAO/WHO,1997). In addition, 100-200 g (WT/HBGB-WT/CB) of a daily serving of high-barley breads
24 provide a β -glucan intake high enough to meet the requirements of the EFSA health claim (3 g/day),
25 contributing a reduced blood cholesterol level (EFSA, 2011). Taking into account the health benefits and the

2 nutritional added value derived from barley flour incorporation, especially high β -glucan (HBGB) into wheat
3 bread formulation, and considering that blended matrices were sensorially scored higher than wheat
4 breads, quantitative and qualitative phenol composition, and antiradical activity were determined.
5 Polyphenols released from the food matrix during the simulated digestive process (bioaccessible
6 polyphenols) are potentially bioavailable and/or susceptible to absorption through the gut barrier, and the
7 degree to which they produce an antioxidant effect depends on their rate of absorption. There are few data
8 in the literature on polyphenol bioaccessibility, and no references were found in high barley breads.
9 Bioaccessible polyphenol content of both the WT and WT/CB blended breads (Table 5) did not differ
10 significantly (598 mg/100 g bread, as is), but were lower ($p < 0.99$) than bioaccessible polyphenols
11 determined in WT/HBGB breads (857 mg/100 g bread, as is), in good agreement with the trend observed
12 for the level of these bioactive components in flours (Table 1). Dietary fibre and other compounds of proven
13 resistance to the action of digestive enzymes, such as resistant starch, resistant protein, Maillard
14 compounds and other associated compounds, may reduce the bread phenol bioaccessibility (Saura-Calixto
15 et al., 2000). The lowest phenol bioaccessibility, expressed as the percentage of total phenol content in
16 flours, obtained for WT/HBGB breads, 42%, could be associated with their high fibre content. Additionally,
17 the high amount of β -glucans able to produce viscous films that entrap nutrients, phytochemicals included,
18 could explain its lower expected phenol bioaccessibility. Results were in line with the antiradical activity of
19 flours and breads (Table 1, Table 5), since several polyphenols (phenolic acids and flavonoids) released
20 from the food matrix exhibit antiradical activity, a health-protecting factor. P-coumaric acid and cinnamic
21 acid (hydroxycinnamic acids) were determined in all breads, and particularly in higher amounts in
22 WT/HBGB samples (61 and 2356 mg/ 100 g bread, as is). Moreover, caffeic, gallic and syringic acids were
23 respectively in WT (0.5mg), WT/CB (48.5mg) and WT/HBGB (996) breads, and catequin (71mg) only in
24 WT/HBGB breads.

25

2 Conclusions

3 **Mixed breads obtained by 40% replacement of WT flour by HBGB flours are much more** nutritious in terms
4 of elevated intake of important nutrients such as dietary fibre fractions (soluble, insoluble, resistant starch
5 and β -glucans), slowly digestible starch subfraction and bioaccessible polyphenols providing higher
6 antiradical activity with health-promoting effects, **compared to their WT/CB and wheat flour counterparts.**
7 WT/CB and WT/HBGB breads can be respectively labelled as source of fibre ([3 g DF/100 g food) and high-
8 fibre breads ([6 g DF/100 g food), according to Nutritional Claims for dietary fibre foods The consumption of
9 100 g of WT/HBGB can meet up to almost 50% the required dietary fibre, and lower digestible
10 carbohydrates, providing a β -glucan intake high enough to meet the requirements of the EFSA health claim
11 (3 g/day), contributing a reduced blood cholesterol level. Despite the incorporation of barley flours
12 diminishes bread loaf volume, blended breads deserved higher scores in both taste intensity and overall
13 acceptability, and similar ratings than WT control breads concerning external appearance, aroma intensity
14 and crumb firmness. In addition, ~~keeping behaviour during storage was good, and~~ crumb pore uniformity
15 and crumb grain structure were not significantly affected, though in the barley supplemented breads the
16 crumb quality decreased in terms of lower cell size and thicker cell walls.
17 Addition of **high β -glucan hulless flour** with enhanced hydration properties, to common wheat flour in mixed
18 matrices at 40% of wheat flour replacement allowed to obtain **highly** enhanced-value grain-based breads in
19 terms of higher nutritional value and health-promoting impact, preserving **in general** the techno-functional
20 performance and the sensory appreciation of breads thereof.

21
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Table 1.- Chemical, biochemical and nutritional composition of flours (per 100 g flour, dry basis (d.b.)).

Parameter	Flours, d. b.		
	Wheat	Commercial barley	High β -glucan barley
Moisture, (g/ 100 g flour, as is)	14.3±0.1c	12.8±0.1b	8.3±0.1a
Protein, (g/ 100 g flour, as is)	14.12±0.28b	12.92±0.34a	19.95±0.23c
Fat, (g/ 100 g flour, as is)	1.56±0.11a	1.94±0.11b	5.87±0.09c
Ash, (g/ 100 g flour, as is)	0.63±0.04a	1.74±0.07b	2.00±0.08c
Digestible starch, (g/ 100 g flour, as is)	81.5±1.9c	65.9±1.5b	37.8±1.0a
Amylose/amylopectin ratio	23/77b	29/71c	14/86a
Total Dietary Fibre, (g/ 100 g flour, as is)	2.2±0.2a	17.4±1.5b	35.0±2.6c
Soluble Fibre, (g/ 100 g flour, as is)	0.95±0.11a	5.91±0.28b	14.95±0.33c
Insoluble Fibre, (g/ 100 g flour, as is)	1.27±0.28a	11.53±1.09b	20.17±1.44c
Resistant Starch, (g/ 100 g flour, as is)	2.05±0.26a	4.84±1.22b	8.33±1.42c
β -glucans, (g/ 100 g flour, as is)	0.23±0.11a	5.16±0.17b	13.30±0.71c
Total polyphenols, (mg/100 g, as is)	713±37a	1003±50b	2197±107c
Antiradical activity, %	12±2a	65±4b	62±4c
β -glucanase activity, (U/kg)	984±70c	53±6b	22±10a

2 Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other (p> 0.05).

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Protein content and yield of protein extracts from wheat,
commercial barley and high β -glucan barley flours

Protein extracts	wheat	commercial barley	high β -glucan barley
-	protein content (% N x 6,25)		-
globulin	10.41	9.06	10.25
glutelin-1	50.09	42.99	33.02
glutelin-2	1.41	2.47	3.19
prolamin	0.18	1.20	1.11
-	yield (% protein basis)		-
globulin	19.96	19.56	23.41
glutelin-1	56.15	55.95	38.08
glutelin-2	1.70	2.88	2.73
prolamin	0.00	0.02	0.18

4 Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).

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Table 3-2.- Functional properties of wheat flour, and wheat/barley flour blends (60/40, w/w)

Property	Wheat	Wheat:CB 60:40	Wheat:HBGB 60:40
Water Holding Capacity 3000 rpm, g water/g flour	0.83±0.10a	0.85±0.08a	1.05±0.07b
Water Holding Capacity 5000 rpm, g water/g flour	0.71±0.05a	0.80±0.09a	1.05±0.06b
Swelling, mL/g	2.32±0.10a	2.68±0.28a	3.74±0.48b
Solvent Retention Capacity, %			
Water	62±5a	76±7b	101±6c
Sucrose	116±18a	124±13a	174±15b
Sodium carbonate	104±8b	84±6a	134±9c
Lactic acid	132±12b	96±9a	136±13b
Fat Adsorption Capacity, g/g	1.13±0.09a	1.22±0.09a	1.34±0.16a
Foam Capacity, mL	14±2b	12±1b	4±1a
Foam Stability, %	64±3b	67±3b	50±4a

CB commercial barley flour; HBGB high β-glucan barley flour
 Within row, values (mean of three replicates) with the same following letter do not differ significantly from each other (p> 0.05).

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Table 54.- Kinetic parameters for **crumb firming and** starch hydrolysis of wheat and wheat/barley (60:40, w:w) blended breads.

Parameter	WT	WT/CB	WT/HBGB
<i>Crumb firming kinetics</i>			
T_{∞} (N)	75±2b	29±1a	28±1a
k	0.046±0.002a	0.135±0.006b	0.248±0.003c
n	0.690±0.002b	0.417±0.003a	0.808±0.001c
T_0 (N)	4±1a	3±2a	8±1b
r^2	0.95	0.96	0.92
<i>Starch hydrolysis kinetics</i>			
C_{∞}	81±1b	79±1b	75±2a
k	0.072±0.002b	0.094±0.003c	0.052±0.001a
H_{90}	81±1b	79±2b	74±1a
HI (%)	100±1c	96±2b	89±3a
r^2	0.99	0.99	0.98
eGI	94±1b	91±2b	85±1a

5 Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$). T_0 : initial crumb
6 firmness; T_{∞} : final crumb firmness; k : rate constant; n : Avrami exponent; C_{∞} : equilibrium concentration; k : kinetic constant; H_{90} : total starch
7 hydrolysis at 90 min; HI: hydrolysis index; r^2 : adjusted squared coefficient for the fitting model; eGI: expected Glycaemic Index. WT: wheat flour ;
8 CB: commercial barley flour; HBGB: high β -glucan barley flour .

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Table 65.- Nutritional information (per 100 g bread, as is) of wheat and wheat/barley (60:40, w:w) blended breads.

Nutritional information	WT		WT/CB		WT/HBGB	
	Nutrient (per 100 g)	Energy, kcal (%)	Nutrient (per 100 g)	Energy, kcal (%)	Nutrient (per 100 g)	Energy, kcal (%)
Moisture (g)	35,6±0,2a		38,6±0,1b		40,5±0,3c	
Fat (g)	0,56±0,01a	5 (2)	0,56±0,03a	5 (3)	1,11±0,04b	10 (6)
Protein (g)	7,89±0,41b	32 (14)	7,31±0,09a	29 (15)	8,10±0,09b	32 (19)
Ash (g)	0,35±0,02a		0,56±0,01b		0,96±0,02c	
Total dietary fibre (g)	1.15±0,03a	2 (1)	4.01±0,04b	8 (4)	11.91±0,07c	24 (14)
Soluble dietary fibre (g)	0,36±0,02a		1,24±0,04b		3,69±0,07c	
Insoluble dietary fibre (g)	0,79±0,05a		2,77±0,04b		8,22±0,11c	
β-glucans	0,11±0,01a		1,51±0,03b		3,23±0,07c	
Resistant starch (g)	1.8±0.1a		4.4±0.2b		7.0±0.3c	
Digestible carbohydrates* (g)	45c	180 (82)	39b	156 (79)	25a	100 (60)
Rapidly Digestible Starch (g)	58.5±0.3c		53.1±0.4b		34.7±0.6a	
Slowly Digestible Starch (g)	7.5±0.2b		3.4±0.3a		9.3±0.1c	
Expected Glycaemic Index	94±1b		91±2b		85±1a	
∑ Energy (kcal)		219 (100)		198 (100)		166 (100)
Bioaccessible phenols (mg)	598±23a		597±29a		857±33b	
Antiradical activity (%)	40±5		58±8		75±8	
p-coumaric acid (mg)	18±0.3		29±2.2		61±5.5	

Cinnamic acid (mg)	252±13.2	571±13.2	2356±17.6
Caffeic acid (mg)	0.5±0.1	-	-
Gallic acid (mg)	-	48.5±3.3	-
Syringic acid (mg)	-	-	996±16.5
Catequin (mg)	-	-	71±8.7

(*) Indirect determination: Digestible CHO = 100 - [Moisture + Protein + Fat + Ash + Dietary Fibre] (1)
 Energy conversion factors: CHO = 4 kcal/g, Protein = 4 kcal/g, Fat = 9 kcal/g, and Dietary Fibre = 2 kcal/g (2).
 Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p>0.05$). CB: commercial barley flour; HBGB: high β -glucan barley flour.
 (1)FAO/WHO (2003) Food Energy - Methods of Analysis and Conversion Factors. FAO Food and Nutrition Paper 77, Rome.
 (2)Bureau of Nutritional Sciences, Food Directorate, Health Products and Food Branch. 2010. Proposed Policy: Definition and Energy Value for Dietary Fibre.

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Table 3.- Physico-chemical and sensory characteristics of wheat and wheat/barley (60:40, w:w) blended breads.

Characteristic	WT	WT/CB	WT/HBGB
Volume (mL)	1890±75	1480±45	1360±50
Specific volume (mL/g)	3.2±0.3b	2.5±0.2a	2.3±0.3a
ΔE crumb	-	16.28±1.11b	10.58±1.03a
ΔE crust	-	1.26±0.02a	3.44±0.05b
Hardness (N)	3.9±0.4a	3.3±0.3a	7.5±0.3b
Cohesiveness	0.84±0.02c	0.72±0.01a	0.77±0.03b
Mean cell área (mm ²)	2,0±0,2b	1,4±0,1a	1,4±0,2a
Cells/cm ²	50,76±0,32b	52,88±0,11c	47,36±0,44a
Cell/total area ratio (%)	10,15±1,15b	7,40±1,01a	6,63±1,23a
Wall/total área ratio (%)	89,85±1,34a	92,60±1,45ab	93,37±1,36b
External appearance (0-10)	7±1a	6±0.5a	7±0.5a
Aroma intensity (0-10)	6±1a	7±1a	7±1a
Taste intensity (0-10)	4±1a	6±1b	6.5±1b
Firmness (0-10)	3±0.5a	3±1a	4±1a
Overall acceptability (0-10)	6.5±1a	7.5±0.5a	7±0.5a

Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p>0.05$). WT: wheat flour; CB: commercial barley flour; HBGB: high β -glucan barley flour.

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