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ORIGINAL PAPER

# Nutritional and functional performance of high β-glucan barley flours in breadmaking: mixed breads versus wheat breads

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

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

## Introduction

Consumers increasingly demanding healthier and tastier foods are shifting away from getting nutrients via fortified foods and turning towards products that are naturally high in components with health-promoting effects. This trend has boosted that traditionally neglected cereals for human nutrition such as barley, but rich in health-related components, are currently being reconsidered a part of a healthy human diet [38]. Barley is increasingly incorporated in already established as well as in new food products—pasta and bread—either as a whole grain or as a food ingredient [31], mainly due to the presence of β-glucan and phenolic compounds which have the potential to lower cholesterol and blood glucose levels [12] and proteins which have been recognised as a rich source of some essential amino acids [43].

The nutritional value of food supplemented with barley depends on both the level of supplementation and on the type of barley used (hull-less or hulled). Hull-less cultivars have better nutritional value than hulled ones as they contain more proteins, lipids and soluble dietary fibres [54], mainly β-glucan [34], which content generally underlies a natural fluctuation depending on the variety and conditions before and after harvesting [20]. In breadmaking applications, replacement of wheat flour by significant amounts of non-gluten forming flours such as barley can seriously constrain dough viscoelasticity and gas retention capability of blended dough matrices that have a weakened mixed protein network (diluted gluten/non-gluten proteins). Diluted gluten matrices often lead to impaired physico-chemical and sensory quality of fermented goods after baking in terms of volume, texture, colour and taste. In addition, the baking process itself may induce depolymerisation of the fibre constituents [4]. In general, wheat flour substitution levels ranging from 15 to 20 % of barley flour are the most usual in practice [3], although there are reports of successful incorporation of 20 and 26 % of hull-less barley [55] and even higher—from 40 to 100 %—[35, 48, 58] with

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variable chemical, physico-chemical, nutritional and biological properties achieved in final breads.

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

## Materials and methods

### Materials

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

### Methods

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

Moisture, protein, ash and fat contents of commercial flours WT, CB and HBGB were determined following the ICC methods 110/1, 105/2, 104/1 and 136, respectively [33]. Total, soluble and insoluble dietary fibre contents were determined according to the AOAC method 991.43 [7]. Two replicates were made for each flour analysis. Digestible carbohydrates were calculated by difference [23]. Amylose/amylopectin ratio (Megazyme kit K-AMYL 07/11) was estimated by using a modification of a Con A method developed by Yun and Matheson [62] that uses an ethanol pre-treatment step to remove lipids prior to analysis (modified from [40]). Resistant starch determination was performed according to AOAC Official Method 2002.02 [8] by using Megazyme kit K-RSTAR 08/11. β-glucan content (Megazyme kit K-BGLU 07/11) was determined following the ICC Standard Method No. 166. Total polyphenol content was determined according to the Folin–Ciocalteu procedure [51] in enzyme extracts as described previously [5]. Anti-radical activity determined by using the radical scavenging capacity assay according to the DPPH method [11], modified by Sánchez-Moreno et al. [49] and applied earlier [5]. β-D-Glucanase was assessed by using the azo-barley glucan method (Megazyme kit KMBGL 04/01).

#### *Functional properties of wheat–barley blended flours*

Blended wheat–barley flours were prepared by substituting 40 % of either CB or HBGB from the total per cent of WT flour, and functional characteristics of WT and blended flours were assessed as it follows. solvent retention capacity (SRC) was determined according the AACC method 56–11 [1] to quantify potential contributions to water-holding capacity by other flour components having water-uptake capabilities [57]. The solvents used were sucrose (50 % w/v), sodium bicarbonate (5 % w/v) and lactic acid (5 % v/v); 25 mL of prepared solvent were added to 5 g of flour in 30-mL centrifuge bottles. Centrifugation at 1,239×*g* (3,000 rpm) was performed for 15 min. After decanting, a gel remained. Gels were weighed and the SRC value (%) calculated as % SRC = [[(gel wt/flour wt) × (86/(100 − % flour moisture)) − 1)] × 100] for each solvent. The water-holding capacity (WHC) was determined using methods modified from Heywood et al. [30] and Lin and Zayas [37] as described by [57].

Fifteen grams of total flour was dispersed in 285 mL of distilled water in a 500-mL centrifuge bottle. Bottles were shaked for 10 min, then centrifuged at either 1,592×*g* or 4,424*g* (3,000 and 5,000 rpm, respectively) for 30 min. After decanting the supernatant, each bottle was weighed and WHC (g of water/g flour) was calculated as: WHC = ((weight of bottle after decanting − weight of dry bottle) − total flour weight (g))/total flour weight (g). Swelling was determined as the volume occupied by a known weight of flour [42] and was evaluated by mixing 5 g (±0.1 mg) of flour with 100 mL of distilled water and hydrating overnight. Fat adsorption capacity (FAC) was determined according to Ahn et al. [2]. One gram of flour sample was weighed into a 50-mL pre-weighed centrifuge tube and thoroughly mixed with 10 mL of sunflower vegetable oil. The protein–oil mixture was centrifuged (2,000×*g* for 5 min). Immediately after centrifugation, the supernatant was carefully removed, and the tube was weighed. FAC (g of oil/g of sample) was calculated as FAC = $(F_2–F_1)/F_0$, 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 $F_2$ is the weight of the tube plus the sediment (g). Foam capacity (FC) and foam stability (FS) were determined as described by Narayana and Narasinga Rao [41] and modified by Alu'datt et al. [3]. Two grams of flour sample was mixed with 40 mL distilled water at 30 °C in a 100-mL measuring cylinder. The suspension was stirred and shaken for 5 min at 1,600 rpm to produce foam, and the foam stability was expressed as the volume of foam over a time period from 0 to 60 min. The volume of foam was measured after 0 min ($V_T$, FC), and the volume of foam after 60 min ($V_1$) was recorded. Foaming stability was expressed as $\% = (V_1/V_T)100$.

*Breadmaking of wheat and wheat–barley blended flours*

Doughs and breads were prepared for control (WT) and wheat–barley blended flours (WT/CB, WT/HBGB, 60/40, w/w). Flour (100 g), water (63 % -WT-, 80 % -WT/CB-, 80 % -WT/HBGB-, flour basis), commercial compressed yeast (4 % flour basis), salt (1.5 % flour basis), vegetable fat (4 % flour basis), sugar (1 % flour basis), commercial sour dough (3 % flour basis), gluten (2 % flour basis), carboxymethylcellulose (1 % flour basis) and calcium propionate (0.5 %) were mixed in a 10 kg mixer at 60 revolutions min-1 for 10 min up to optimum dough development. Fermented doughs were obtained after bulk fermentation (10 min), dividing (700 g), rounding, moulding and proofing up to maximum volume increment (30 min) and were baked at 220 °C for 30 min to make WT, WT/CB and WT/HBGB breads, respectively.

*Bread measurements*

*Physico-chemical and sensory determinations* Colour determinations were carried out on bread crumb and crust using a Minolta colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan), and results were expressed in accordance with the Hunter Lab colour space. Parameters determined were $L$ ($L = 0$ [black] and $L = 100$ [white]), a ($-a$ = greenness and $+a$ = redness), $b$ ($-b$ = blueness and $+b$ = yellowness), ΔE—total colour difference—[24], BI—browning index—[47] and WI—whiteness index—[32]. All measurements were made in triplicate. Crumb grain characteristics were assessed in bread slices using a digital image analysis system. Images were previously acquired with a ScanJet II cx flatbed scanner (Hewlett-Packard, Palo Alto, CA, USA) supported by a Deskscan II software. The analysis was performed on 40 × 40 mm squares taken from the centre of the images. Data were processed using SigmaScan Pro 5 (Jandel Corporation, San Rafael, CA, USA). The crumb grain features evaluated were mean cell area, cells/cm$^2$, cell/total area ratio, wall/total area ratio and crumb area/total cell ratio [16]. Sensory analysis of fresh breads was performed with a panel of eight trained judges (four males and four females aged 24–55 years) using semi structured scales, scored 0–10 (lowest:0; highest:10) for each sensory attribute. Evaluated attributes were external appearance, crumb texture, aroma intensity, taste intensity and overall acceptability.

Bread primary and secondary mechanical characteristics (TPA in a double compression cycle) of fresh and stored breads were recorded in a TA-XTplus texture analyser (Stable Micro Systems) using a 10 mm diameter probe, a 5 kg load cell, 50 % penetration depth and a 30-s gap between compressions on slices of 25 mm width [9]. For textural measurements, three slices of two freshly made breads were used for each sample.

*Enzymatic/biochemical determinations* Starch hydrolysis kinetics and relevant starch fractions in WT and wheat–barley blended (WT/CB, WT/HBGB) breads were determined following the AACC (2000) method 32–40, adapted as previously described [6]. Each bread sample (100 mg) was incubated with pancreatic α-amylase (10 mg) and amyloglucosidase (12 U) in 4 mL of 0.1 mol/L sodium maleate buffer (pH 6.0) in a 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 were heated at 100 °C for 5 min, and ethanol/water (95:5, v:v) was added for enzyme inactivation, prior to centrifugation at 720*g* for 10 min. The glucose content of the supernatant was measured using a glucose oxidase/peroxidase (GOPOD) kit. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured after incubation for 30 and 120 min, respectively [21]. Total digestible starch (DS) was determined in the supernatant after 16 h of incubation, while resistant starch (RS) was determined in the pellet as the starch remaining after 16-h incubation. The digestion kinetics and expected glycaemic index (eGI) of bread were calculated in accordance with the procedure followed by Chung et al. [13] based on the method established by Goñi et al. [28]. A first-order kinetic equation [$C = C_\infty (1-e^{kt})$] was applied to describe the kinetics of starch hydrolysis, where $C$, $C_\infty$ and $k$ were the hydrolysis degree at each time, the maximum hydrolysis extent and the kinetic constant, respectively. The hydrolysis index (HI) was calculated as the relation between the area under the hydrolysis curve (0–16 h) of blended bread samples and the area of standard material from white bread (control) [13]. The eGI was calculated using the equation proposed by Granfeldt et al. [29]: $\text{eGI} = 8.198 + 0.862\ \text{HI}$.

Bioaccessible phenol determination was conducted in bread samples by conducting an "in vitro" digestive enzymatic mild extraction that mimics the conditions in the gastrointestinal tract according to the procedure of Glahn et al. [27] as recently used by Vitali et al. [59] for biscuits and Angioloni and Collar [5] for breads. This method skips

**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) | 14.12 ± 0.28b | 12.92 ± 0.34a | 19.95 ± 0.23c |
| Fat (g/100 g flour) | 1.56 ± 0.11a | 1.94 ± 0.11b | 5.87 ± 0.09c |
| Ash (g/100 g flour) | 0.63 ± 0.04a | 1.74 ± 0.07b | 2.00 ± 0.08c |
| Digestible starch (g/100 g flour) | 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) | 2.2 ± 0.2a | 17.4 ± 1.5b | 35.0 ± 2.6c |
| Soluble fibre (g/100 g flour) | 0.95 ± 0.11a | 5.91 ± 0.28b | 14.95 ± 0.33c |
| Insoluble fibre (g/100 g flour) | 1.27 ± 0.28a | 11.53 ± 1.09b | 20.17 ± 1.44c |
| Resistant starch (g/100 g flour) | 2.05 ± 0.26a | 4.84 ± 1.22b | 8.33 ± 1.42c |
| β-glucans (g/100 g flour | 0.23 ± 0.11a | 5.16 ± 0.17b | 13.30 ± 0.71c |
| Total polyphenols (mg/100 g, as is) | 713 ± 37a | 1.003 ± 50b | 2.197 ± 107c |
| Antiradical activity, % | 12 ± 2a | 65 ± 4b | 62 ± 4c |
| β-glucanase activity (U/kg) | 984 ± 70c | 53 ± 6b | 22 ± 10a |

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

out the colonic fermentation in the large intestine. Briefly, 10 mL of distilled water 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 adjusted to 2 using 5 mol $L^{-1}$ HCl, and the sample was incubated at 37 °C in a shaking water bath for 1 h.

Simulation of gastric digestion was stopped by addition of 1 M NaHCO3 (to adjust pH to 7.2). Then, 2.5 mL of bile (cholic and deoxycholic sodium salts)/pancreatin solution (2 g $L^{-1}$ of pancreatin and 12 g $L^{-1}$ of bile 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 sample, and simulation of intestinal digestion was conducted for the following 2.5 h. Samples were centrifuged at 3,500*g* for 10 min, and the supernatants were used for determination of bioaccessible phenols after removing proteins from digestive extracts by addition of trichloroacetic acid (20 %, w/w) by the Folin–Ciocalteu method, as described for flours.

*Chromatographic determinations* Analysis of the polyphenol composition was achieved by using reversed-phase high-performance liquid chromatography (RP-HPLC). HPLC analysis was performed on an Agilent model 1200, equipped with quaternary pump, automatic injector, autosampler and diode array detector. The instrument was controlled, and the chromatographic data were analysed using a personal computer loaded with the HP ChemStation software. Reversed phase separations were conducted on a Zorbax Eclipse XDB-C18 column with dimensions 150 × 4.6 mm and 5 μm particle size. The mobile phase consisted of 0.1 % formic acid in water as eluent A and 0.1 % formic acid in acetonitrile as eluent B. The solvent gradient programme was set as follows: initial conditions 100 % A; 0–60 min, 0–25 % B; 60–70 min, 25–70 % B; 70–85 min, 70–100 % B. Column temperature was set at 24 °C, flow rate was 1 ml $min^{-1}$, and the injection volume was 20 μL. Samples were deproteinised and desaccharified prior to injection by precipitation with methanol. For identification purposes, a spectral library was constructed comprising the retention times and spectra of the standards under the chromatographic conditions specified above. Calibration curves using an external calibration method were also prepared and used for quantitative analysis, and the results expressed as mg/kg of sample, as is. Phenolic acids and flavonoids were detected at a wavelength of 320 nm.

### Statistical analysis

Univariate (ANOVA) and multivariate (nonlinear multiple regression) analysis of data was performed by using Statgraphics version 7.1 program (Bitstream, Cambridge, MN).

## Results and discussion

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

**Table 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 3,000 rpm, g water/g flour | 0.83 ± 0.10a | 0.85 ± 0.08a | 1.05 ± 0.07b |
| Water-holding capacity 5,000 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 |

Within row, 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

**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) | 1,890 ± 75c | 1,480 ± 45b | 1,360 ± 50a |
| 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

**Table 4** Kinetic parameters for starch hydrolysis of wheat and wheat/barley (60:40, w:w) blended breads

| Parameter | WT | WT/CB | WT/HBGB |
|---|---|---|---|
| $C_\infty$ | 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 | 0.99 | 0.99 | 0.98 |
| eGI | 94 ± 1b | 91 ± 2b | 85 ± 1a |

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

C∞: equilibrium concentration; k: kinetic constant; H90: total starch hydrolysis at 90 min; HI: hydrolysis index; $r^2$ adjusted squared coefficient for the fitting model; eGI: expected glycaemic index. WT: wheat flour; CB: commercial barley flour; HBGB: high β-glucan barley flour

(Table 5) of blended barley/wheat versus wheat bread matrices.

## Physico-chemical, nutritional and functional performance of single (WT, CB and HBGB) and blended (WT/CB, WT/HBGB) flours

Single WT, CB and HBGB flours exhibited different chemical, biochemical and nutritional profiles (Table 1). Comparatively (HBGB vs. CB, WT, per 100 g flour basis, d.b), high β-glucan barley flour accounted for much higher protein (19.35 vs. 12.92 %, 14.12 %), higher fat (5.87 vs. 1.94 %, 1.34 %), ash (2.00 vs. 1.74 %, 0.63 %), much higher total dietary fibre (35.01 vs. 17.43 %, 2.22 %), resistant starch (8.33 vs. 4.84 %, 2.05 %), β-glucan (13.30 vs. 5.16 %, 0.23 %) and total polyphenol (2,197 vs. 1,003 mg, 713 mg)

**Table 5** 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 | | 2.356 ± 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)[23]

(2)Bureau of Nutritional Sciences, Food Directorate, Health Products and Food Branch. 2010. Proposed Policy: Definition and Energy Value for Dietary Fibre

contents than both CB and WT flours, in good accordance with a superior nutritional profile for hulless barley samples [54]. It has been reported that hulless barley contains much higher (1/3,1/4)-β-D-glucan levels than either wheat or hulled barley [10]. The high content of β-glucan in barley (2.5–11.3 %) compared to wheat (0.4–1.4 %) has made barley increasingly interesting for bread production [36]. High-amylose and waxy hulless barley have been described to contain approximately 7 or 8 % β-glucans, whereas regular hulless barley comprises significantly less (4.6 %) [25, 56], in good accordance with data obtained for CB (4.50 %, flour m. b.), and significantly lower than amounts obtained for HBGB (12.20 %, m. b.). Contribution of β-glucan degrading enzymes from the commercial wheat flour is believed to be limited since wheat flour has been shown to have low activity of β-glucanases [60], and values retrieved for β-glucanase activity in CB and HBGB in this work were significantly much lower (<46U/kg). Hulless barley is a good source of dietary fibre providing soluble and insoluble dietary fibre fractions [10, 34]. Mixed-linkage (1/3), (1/4)-β-D-glucans (hereafter termed as β-glucan) are a major part of the soluble dietary fibre in barley. A study by [61] showed that the total β-glucan content is higher, whereas the insoluble dietary fibre content is significantly lower in naked barley compared to hulled barley genotypes. This was not the case of the hulless barley used in this study (HBGB) that exhibits 18.5 % of insoluble dietary fibre versus 10.05 and 1.09 % for CB and WT flour, respectively. Differences could be attributed to a natural fluctuation depending on the variety and conditions before and after harvesting, as reported earlier [20]. Hulless barley, normally used as a non-wheat source of dietary fibre, significantly promotes total as well as soluble dietary fibre contents of the resulting bread products, when it replaces

15 % of wheat flour [26]. Skendi et al. [53] reported a positive effect of barley β-glucan addition on wheat-bread-specific volume, which was more pronounced for high MW β-glucan and wheat flours with weak gluten quality.

On the contrary, lower amounts for digestible starch (37.8 vs. 65.9 %, 81.5 %) and β-glucanase activity (22 U/kg vs. 53, 985 U/kg) were observed in barley flours especially for HBGB versus CB and WT flours (Table 1), in good agreement with current dietary guidelines for starch fractions [39], and with suitable integrity of the molecular weight of β-glucans to keep hypocholesterolemic effects [44], respectively.

Hydration properties (WHC, swelling and SRC), FAC, FC and FS were determined in WF and blended wheat–barley flours prepared by substituting 40 % of either CB or HBGB from the total % of WT flour (Table 2). WHC reports the ability of a protein matrix to absorb and retain bound, hydrodynamic, capillary and physically entrapped water against gravity [18]. In general, WHC values for all the flour blends were lower at 5,000 rpm than 3,000 rpm, as would be expected and reported before [57], because of the greater centrifugal force being applied to samples. WHC of WT and WT/CB blends were not statistically different but significantly lower than values for WT/HBGB blend, in good accordance with the trend shown for swelling (Table 2). This persistent difference might be ascribed to the significantly higher protein content of the HBGB flour (Table 1) and probably to the formation of large clusters of protein molecules or protein aggregates bound by hydrogen bounds and other non-covalent forces. SRC testing used to establish a practical functionality profile of flour [30] takes into account several flour constituents influencing water-retention potential, including pentosans, damaged starch and glutenin, using sucrose, sodium carbonate and lactic acid solutions, respectively. SRC is the weight of solvent held by flour after centrifugation, and it is expressed as per cent of flour weight, on a 14 % moisture basis. For flour typically used to produce bread by the sponge–dough method, optimal SRC profile values would be $\geq$100 % glutenin, $\leq$96 % pentosans, $\leq$72 % damaged starch [30]. In this work, a straight dough breadmaking system was used instead, and some mean values for water-retention components of WT and blended wheat–barley flours (WT/CB and WT/HBGB) were outside the typical range for a sponge–dough bread system, especially for WT/HBGB and water retention of pentosans (174 %) and damaged starch (134 %). FAC values indicate that when barley was added to the wheat flour samples, their FAC values increased only with HBGB up to 1.34 g fat/g flour. Lin and Zayas [37] suggested that the ability of protein to bind fat depends on nonpolar side chains that bind hydrocarbon chains, thereby contributing to increased oil absorption [2]. The results imply that the increased FAC can be partly attributed to a marked decrease in bulk density because fat absorption depends on the physical entrapment of oil [52]. Both FC (14 mL) and FS (64 %) of WT flour did not significantly change with 40 % replacement of WT by CB, but with HBGB replacement (Table 2). The presence of HBGB in wheat blends gave a decrease in either FC or FS by 70 and 22 %, respectively.

## Techno-functional, sensory and nutritional profiles of blended barley/wheat versus wheat matrices

Bread dough is a viscoelastic material that exhibits an intermediate rheological behaviour between a viscous liquid and an elastic solid. Bread crumb is a porous solid matrix with cellular structure composed mainly of gluten, starch and water, and minor constituents such as lipids and non-starch polysaccharides in presence of other ingredients, additives and technological aids representing a typical viscoelastic biopolymer foam system. Major breadmaking steps significantly change the viscoelasticity of wheat doughs. During mixing, distribution of materials, hydration and energy input for stretching and alignment of protein molecules takes place involving shear and extensional deformation. During fermentation, the expansion of the air bubbles previously incorporated during mixing provides the characteristic aerated structure of bread, which is relevant to its appeal. During proof and baking, the growth of gas bubbles determines the expansion of the dough and therefore the ultimate volume and texture of the baked product [15]. It is known that the addition of barley or barley fractions in foods will influence techno-functional and sensory bread product quality in terms of colour, taste and texture through changes induced in dough viscoelastic behaviour. The polysaccharides in barley affect the quality of baking products [31] and the baking process itself may alter the fibre constituents (depolymerisation) [4]. Rheological measurements that are increasingly being used as sensitive indicators of polymers molecular structure and predictors of end-use performance have been successfully applied to bread doughs as indicators of the gluten and starch biopolymers molecular structure and predictors of its functional behaviour in breadmaking. In this study, 40 % of WT replacement by either CB or HBGB was established in preliminary trials as the maximum level of substitution that did not compromise significantly dough machinability (data not shown) in terms of stickiness (<1 N), dough hardness (<80 N), cohesiveness (>0.5), adhesiveness (<100 N.s) and springiness (>0.8). The addition of barley flour either CB or HBGB significantly decreased the bread volume (−22 %) and the crumb cohesiveness (−14 %) but increased crumb hardness (90 %) with respect to refined WT flour bread types, particularly for WT/HBGB blends (Table 3). Resulting blended breads are visibly different from control WT

breads ($\Delta E \geq 3$) in both crust and crumb colour, particularly for crust colour in WT/HBGB breads ($\Delta E \geq 3$) and for the crumb colour in WT/CB breads ($\Delta E \geq 16$). Crumb pore uniformity and crumb grain structure were not significantly affected, though in the barley supplemented breads the crumb quality decreased in terms of lower cell size and thicker cell walls. The refined control bread WT was scored significantly lower in both taste intensity and overall acceptability. The wheat–barley blended breads deserved similar ratings than WT control breads concerning external appearance, aroma intensity and crumb firmness (Table 3). The incorporation of barley flour diminishes bread quality, particularly loaf volume, of wheat composite breads [26] due to both the dilution of wheat gluten and the mechanical interference with gluten network formation by insoluble dietary fibre (Salmenkallio-Marttila et al. 2001) causing additional rupture of gas cells [17], as previously observed for high-fibre supplemented wheat breads [14]. Besides, both soluble and insoluble fibres, tightly bind high amounts of water, which may make it less available for the development of the gluten network and may further result in less volume production after baking [26]. Lower volume in barley blended matrices is in good accordance with the higher total dietary fibre content of barley flours (Table 1) that resulted in particularly prominent total dietary fibre level (g/100 g bread, as is) in breads thereof (4 % WT/CB, 12 % WT/HBGB), compared to the WT counterparts (Tables 1, 5). Other authors have stated the positive effect on dough rheology and bread quality of barley β-glucan isolates to wheat flour of a poor breadmaking cultivar made with optimised water addition [53], probably ascribed to the higher viscosity of the water phase of the dough and thereby stabilising gas cells in the way reported for water-extractable arabinoxylans in wheat dough [17]. In fact, β-glucan content (Table 1) of barley flours with high water binding capacity (Table 2) gave blended breads with a significantly high β-glucan content (1.51 % CB, 3.23 % HBGB) compared to WT counterparts (0.11 %). This fact can partially counteract the deleterious effect of barley flour addition in the volume of wheat–barley mixed breads.

Nutritional intrinsic characteristics of barley flours (i.e. good source of dietary fibre—resistant starch and β-glucan) and high protein contents and dough processing (i.e. hydration, other ingredients, breadmaking test) play relevant roles both in the nutritional features and in the enzyme accessibility to natural biopolymers—protein and starch—present in mixed breads. Nutritional information on wheat–barley breads (Table 5) showed most appealing nutritional quality than WT breads, especially for HBGB breads in terms of lower digestible starch, high soluble and insoluble dietary fibre, β-glucan, resistant starch and bioaccessible polyphenol contents of sensorially accepted samples. A significant reduction in starch content in bread with wheat flour substitution by barley flour, which regulates the total amount of accessible macronutrients was observed. Starch nutritional fractions determined in wheat and wheat–barley breads by "in vitro" starch digestion included RDS, SDS and resistant starch (RS) (Table 5). RDS, SDS and RS contents ranged from 34.7 (WT/HBGB) to 58.5 % (WT), from 3.4 (WT/CB) to 9.3 % (WT/HBGB) and from 1.80 (WT) to 7.0 % (WT/HBGB), respectively. The lowest RDS content and highest SDS and RS contents, which are considered suitable nutritional trends for dietary starch fractions [21], were observed for the blend sample WT/HBGB, which showed a rather low extent of starch hydrolysis (Table 4) with the lowest values for $C_{\infty}$, k and $H_{90}$, and eGI (85). The incorporation of barley flour into wheat bread formulation seems to reduce starch hydrolysis, probably because of their lower starch and higher fibre and protein contents, especially for HBGB flour (Table 1). The reduced rate and overall reduced starch digestibility of barley mixed breads may be affected by high content of viscous soluble dietary fibre components like in legume matrices (Angioloni and Collar, 2012) supported by the high amount of β-glucan determined in wheat–barley breads (Table 5). In addition, high protein content of barley flours (Table 1) can promote starch–protein interactions restricting enzyme attack as pointed out for lentils [13]. Barley bread samples contain about four (WT/CB) to twelve times (WT/HBGB) the fibre of the regular white bread, so that breads can be, respectively, labelled as source of fibre (3 g DF/100 g food) and high-fibre breads (6 g DF/100 g food), according to Nutritional Claims for DF foods [45].

The shift from digestible to non-digestible carbohydrates and moisture variations (35.6 g/100 g bread as is –WT-, 38.6 %-WT/CB-, 40.5 %-WT/HBGB-) were responsible for the observed differences in the energy extent of high-barley breads (198 kcal –WT/CB- -, 166 kcal –WT/HBGB-) versus WT breads (219 kcal). Hundred grams of control wheat breads made with refined wheat flour account for 7.9 g of protein, 1.15 g of total dietary fibre, 0.56 g of fat and 45 g of digestible carbohydrates (Table 5). According to the Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for macronutrients [46], a daily intake of 100 g of high-barley breads WT/CB and WT/HBGB provides 30 and 19 % of digestible carbohydrate, 13–16 % and 15–18 % of protein, and 11–16 % and 31–48 % of the dietary fibre recommended for adults (male–female), respectively. Daily consumption of 100 g of wheat bread delivers 35 % of digestible carbohydrates, from 14 (male) to 17 % (female) of proteins, and from 3 (male) to 4.6 % (female) of dietary fibre. Compared to wheat, high-barley breads allow the ingestion of higher protein and almost 50 % the required dietary fibre (WT/HBGB) and lower digestible carbohydrates, in good agreement with the dietary guidelines for health [22]. In

addition, 100–200 g (WT/HBGB-WT/CB) of a daily serving of high-barley breads provides a β-glucan intake high enough to meet the requirements of the EFSA health claim (3 g/day), contributing a reduced blood cholesterol level [19]. Taking into account the health benefits and the nutritional added value derived from barley flour incorporation, especially high β-glucan (HBGB) into wheat bread formulation, and considering that blended matrices were sensorially scored higher than wheat breads, quantitative and qualitative phenol composition and antiradical activity were determined. Polyphenols released from the food matrix during the simulated digestive process (bioaccessible polyphenols) are potentially bioavailable and/or susceptible to absorption through the gut barrier, and the degree to which they produce an antioxidant effect depends on their rate of absorption. There are few data in the literature on polyphenol bioaccessibility, and no references were found in high barley breads. Bioaccessible polyphenol content of both the WT and WT/CB blended breads (Table 5) did not differed significantly (598 mg/100 g bread, as is), but were lower ($p < 0.99$) than bioaccessible polyphenols determined in WT/HBGB breads (857 mg/100 g bread, as is), in good agreement with the trend observed for the level of these bioactive components in flours (Table 1). Dietary fibre and other compounds of proven resistance to the action of digestive enzymes, such as resistant starch, resistant protein, Maillard compounds and other associated compounds, may reduce the bread phenol bioaccessibility [50]. The lowest phenol bioaccessibility, expressed as the percentage of total phenol content in flours, obtained for WT/HBGB breads, 42 %, could be associated with their high fibre content. Additionally, the high amount of β-glucans able to produce viscous films that entrap nutrients, phytochemicals included, could explain its lower expected phenol bioaccessibility. Results were in line with the antiradical activity of flours and breads (Tables 1, 5), since several polyphenols (phenolic acids and flavonoids) released from the food matrix exhibit antiradical activity, a health-protecting factor. P-coumaric acid and cinnamic acid (hydroxycinnamic acids) were determined in all breads and particularly in higher amounts in WT/HBGB samples (61 and 2,356 mg/100 g bread, as is). Moreover, caffeic, gallic and syringic acids were, respectively, in WT (0.5 mg), WT/CB (48.5 mg) and WT/HBGB (996) breads and catequin (71 mg) only in WT/HBGB breads.

## Conclusions

Mixed breads obtained by 40 % replacement of WT flour by HBGB flours are much more nutritious in terms of elevated intake of important nutrients such as dietary fibre fractions (soluble, insoluble, resistant starch and β-glucans), SDS subfraction and bioaccessible polyphenols providing higher antiradical activity with health-promoting effects, compared to their WT/CB and wheat flour counterparts. WT/CB and WT/HBGB breads can be, respectively, labelled as source of fibre (3 g DF/100 g food) and high-fibre breads (6 g DF/100 g food), according to Nutritional Claims for dietary fibre foods The consumption of 100 g of WT/HBGB can meet up to almost 50 % the required dietary fibre and lower digestible carbohydrates, providing a β-glucan intake high enough to meet the requirements of the EFSA health claim (3 g/day), contributing a reduced blood cholesterol level. Despite the incorporation of barley flours diminishes bread loaf volume, blended breads deserved higher scores in both taste intensity and overall acceptability and similar ratings than WT control breads concerning external appearance, aroma intensity and crumb firmness. In addition, crumb pore uniformity and crumb grain structure were not significantly affected, though in the barley supplemented breads, the crumb quality decreased in terms of lower cell size and thicker cell walls.

Addition of high β-glucan hulless flour with enhanced hydration properties to common wheat flour in mixed matrices at 40 % of wheat flour replacement allowed to obtain highly enhanced-value grain-based breads in terms of higher nutritional value and health-promoting impact, preserving in general the techno-functional performance and the sensory appreciation of breads thereof.

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