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Brans from hull-less barley, emmer and pigmented wheat varieties: from by-products to bread nutritional improvers using selected lactic acid bacteria and xylanase

Erica Pontonio[1], Cinzia Dingeo[1], Raffaella Di Cagno[2*], Massimo Blandino[3], Marco Gobbetti[2], Carlo Giuseppe Rizzello[1]

[1]*Department of Soil, Plant and Food Science, University of Bari Aldo Moro, 70126 Bari, Italy*

[2]*Faculty of Science and Technology, Free University of Bozen-Bolzano, 39100 Bolzano, Italy*

[3]*Department of Agriculture Selvi. Ter., University of Turin, 10095 Grugliasco, Italy*

*****C**orresponding author** Email address: raffaella.dicagno@unibz.it

Abstract

Aiming at meeting the recommendations of the World Health Organization regarding the total fiber daily intake, an integrate biotechnological approach, combining xylanase treatment and lactic acid bacteria fermentation of milling by-products from pigmented wheat varieties, hull-less barley and emmer was proposed as suitable strategy to include bran in breadmaking. The effects on the biochemical and nutritional features were investigated. Enhanced radical scavenging activity, increased concentrations of free amino acids and peptides and optimal *in vitro* protein digestibility value as well as relevant phytic acid degradation were achieved during bran fermentation. The main nutritional features of each matrix were enhanced and distinguished. Fortified breads contained higher total dietary fibers and protein contents as compared to a wheat bread and showed improved nutritional and sensory profiles according to the fermented bran used.

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Keywords: milling by-products, lactic acid bacteria, sourdough fermentation, nutritional profile, high fiber content

Abbreviations

B1, bran obtained from red-grained wheat variety (cv Aubusson); B2, bran obtained from blue-grained wheat variety (cv Skorpion); B3, bran obtained from yellow-grained wheat variety (cv Bona Vita); B4, bran obtained from spring hull-less barley (var. Rondo); B5, bran obtained from emmer (var. Giovanni Paolo); FB1, fermented bran obtained from red-grained wheat variety (cv Aubusson); FB2, fermented bran obtained from blue-grained wheat variety (cv Skorpion); FB3, fermented bran obtained from yellow-grained wheat variety (cv Bona Vita); FB4, fermented bran obtained from spring hull-less barley (var. Rondo); FB5, fermented bran obtained from emmer (var. Giovanni Paolo); FB1-B, bread containing 30% (wt/wt) of FB1; FB2-B, bread containing 30% (wt/wt) of FB2; FB3-B, bread containing 30% (wt/wt) of FB3; FB4-B, bread containing 30% (wt/wt) of FB4; FB5-B, bread containing 30% (wt/wt) of FB5; WB, wheat flour bread; WSE, Water/salt-soluble extract; ME, methanol extract; TFAA, Total Free Amino Acids; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TTA, Total titratable acidity; MCPA (2-methyl-4-chlorophenoxyacetic acid); QF; quotient of fermentation; OPA, o-phtaldialdehyde; BHT, butylated hydroxytoluene; IVPD, *in vitro* protein digestibility; HI, hydrolysis index; *pGI*, predicted glycemic index; DF, dietary fibers.

1. Introduction

Epidemiological and clinical studies show as the consumption of dietary fibers (DF) is crucial for decreasing the risks of obesity, type 2 diabetes, cancer, and cardiovascular diseases (CVD) (Kuznesof et al., 2012; Lattimer and Haub, 2010). The World Health Organization recommends a total fiber daily intake, which varies from 20 to 45 g depending on countries dietary habit (Stephen et al., 2017). The regular consumption of DF particularly that from cereal sources, may improve the health status through multiple mechanisms: reduction in lipid levels, weight regulation, improved glucose metabolism, blood pressure control, and reduction in chronic inflammation (Satija and Hu, 2012). Nevertheless, the average daily intake of fiber in many populations is still lower than that recommended (King et al., 2012; Stephen et al., 2017). Recent studies described the perception of high-fiber foods as unpalatable and relatively higher expensive as compared to their refined counterparts (Baixauli et al., 2008). However, consumers are aware of the beneficial influence that DF and whole meal products have on their health status (Mialon et al., 2002). Bran and germ fractions are the main sources of fibers in whole grains (Katina et al., 2007, Messia et al., 2016). Besides fibers such as cellulose, hemicellulose and lignin (Šramková et al., 2009), bran also contains proteins and bioactive compounds (e.g., phenols, anthocyanins and carotenoids), which related to the antioxidant activity (Adom and Liu, 2002). Although the concentration of such phytochemicals is limited in conventional and widely diffused wheat varieties (Carson and Edwards, 2009), it increases in the so-called pigmented wheat varieties. According to the most recent consumer expectations and to the food industry trend of introducing non-wheat cereals to get bakery products with multiple functional benefits, the use of barley, einkorn, emmer, spelt and pigmented wheat cultivars is increasing globally (Bartlomiej et al., 2012; Pasqualone et al., 2015; Zanoletti et al., 2017).

Barley has a high natural content of β-glucan, a polysaccharide comprising glucose residues made of 1,3-beta-d-lucopyranose (30% of linkages) and 1,4-beta-d-glucopyranose (70% of linkages). Moreover, barley is an important source of bioactive compounds with antioxidant activity (Liu

and Yao, 2007). Among barley cultivars, hull-less barley (HLB) has recently received considerable attention for the manufacture of functional foods as an excellent source of both soluble and insoluble DF (Blandino et al., 2015). Hulled wheat-related species (i.e., einkorn, emmer and spelt) are among the most ancient cereal crops of the Mediterranean area (Piergiovanni et al., 1996). These cereals were popular for centuries, being progressively replaced by the modern wheat cultivars. In the late 90's they regained popularity due to the high commercial potential. In particular, the appreciation of emmer is for the elevated content of DF, resistant starch and antioxidant compounds (Galterio et al., 2003). The sourdough fermentation seems the most suitable option to manage with the techno-functionality of fiber-rich cereal ingredients (Gobbetti et al., 2014). Inspired by the sourdough biotechnology, selected lactic acid bacteria starters were successfully used to ferment wheat and rye bran (Coda et al., 2015; Katina et al., 2007) and germ (Rizzello et al., 2010a) aiming at improving the technological, nutritional, and sensory properties, and at degrading the anti-nutritional factors such as phytic acid (Gobbetti et al., 2014). Moreover, the combination of lactic acid bacteria and cell-wall-degrading enzymes were successfully used to improve nutritional profile and technological properties of wheat bran (Arte et al., 2015).

Based on the above knowledge, xylanase treatment and fermentation with selected sourdough lactic acid bacteria were used to produce an ingredient for breadmaking from pigmented wheat, hull-less barley and emmer brans. The main functional, nutritional, technological and sensory properties of the fortified wheat bread were highlighted.

2. Materials and methods

2.1 Grain cultivation

Spring hull-less barley (*Hordeum vulgare* L. var. Rondo), emmer (*Triticum turgidum* subsp. *dicoccum* var. Giovanni Paolo), blue- and yellow-grained wheat (*T. aestivum* L.) varieties (cv Skorpion and cv Bona Vita, respectively) and one conventional red-grained wheat variety (cv Aubusson) were used.

Cereals were grown side by side on the same experimental field located in Carmagnola Italy (Piedmont; 44° 50’ N, 7° 40’ E; altitude 245 m) during the growing season 2016/2017. The plot size for each cultivar was 5 X 100 m (500 m^2). The soil of the experimental site had loam texture. Sowing was carried out in 12 cm wide rows at a seeding rate of 450 seeds/m^2. Before planting, fertilization plan included 60 kg/ha of P_2O_5 and K_2O. A total of 130 kg N/ha was also used as fertilizer for wheat and emmer according to the following design: 50 kg N/ha at wheat tillering; and 80 kg N/ha at stem elongation. Moreover, 80 kg N/ha were used as ammonium nitrate to hull-less barley at stem elongation. Fluroxypyr and MCPA (2-methyl-4-chlorophenoxyacetic acid) were used for weeding control at the beginning of stem elongation. No fungicide was applied to control foliar and head disease in any of the cultivar. The mechanical harvesting of all cultivars was carried out on 14 July 2017, by means of a Walter Wintersteiger cereal plot combine-harvester. Red-, yellow- and blue-grained wheat, emmer and barley were provided by Limagrain Italia SpA (Italy), Osivo a. s. (Slovakia), the Agricultural Research Institute Kromeriz, Ltd. (the Czech Republic), Apsovsementi s.p.a (Italy) and Società Italiana Sementi s.p.a (Italy), respectively.

Five kg grain sample for each cereal cultivar were roller-milled to obtain their bran fraction. Five kg grain sample for each cereal cultivar were roller-milled to obtain their bran fraction. After tempering, performed according to the moisture content and hardness of each grain variety, roller-milling was carried out using a laboratory-scale mill (Labormill 4RB, Bona, Italy). The mill was cleaned thoroughly by aspiration to avoid equipment contamination and washed with alcohol to minimize microbial contamination.

2.2 Gross chemical composition of brans

Moisture was determined using a Sartorius MA30 thermo-balance (Sartorius AG, Goettingen, Germany). The total protein (conversion factor: 5.70) and fat contents were determined according to the Kjeldahl (Kjeltec system I, Foss Tecator AB, Höganäs, Sweden) and Soxhlet (AOAC 2003-05, 2006) methods, respectively. After enzymatic treatment with amyloglucosidase, carbohydrates were

quantified through the Glucose GOD-PAP kit (Roche Diagnostics GmbH, Nonnenwald, Germany) following the manufacturer's instructions. Insoluble and soluble DF contents were determined through gravimetric determination after enzymatic digestion according to the AOAC 991.42 and 993.19 procedures, respectively. Ash content was determined in a muffle furnace according to the AOAC 923.03 procedure.

2.3 Microorganisms and growth conditions

Ten strains of lactic acid bacteria, belonging to the species *Lactobacillus plantarum* (T6B10, STF28 and Lin 22), *Lactobacillus rossiae* (T0A16), *Weissella confusa* (BAN8 and KAS3) and *Pediococcus pentosaceus* (BAR 4, BAN1, BAN2 and NEJ1) were preliminary selected among 70 strains according to their pro-technological and functional properties (Supplementary Table S1 and Supplementary Figure S1) and used in this study. Proteolysis by means of total free amino acids (TFFA), and phytase and radical scavenging (in the methanolic extract) activities were the functional features considered. The kinetics of growth and acidification were considered as the pro-technological traits (Supplementary Figure S1). All the strains belong to the Culture Collection of the Department of Soil, Plant and Food Science (University of Bari Aldo Moro, Italy). Strains were routinely cultivated on modified De Man, Rogosa and Sharpe (mMRS) (Oxoid, Basingstoke, Hampshire, UK) agar medium (maltose and fresh yeast extract were added at 1% and 5%, respectively, and the final pH was 5.6) until the late exponential phase of growth was reached (*ca*. 8 h) (Nionelli et al., 2014; Pontonio et al., 2015; Rizzello et al., 2016).

2.4 Bran fermentation

Aiming at evaluating the performances in bran matrix, the ten lactic acid bacteria were singly inoculated in 50 g of wheat bran doughs. Dough yield (DY, dough weight × 100/flour weight) was 300. In detail, 16.66 g of wheat bran (cv. Aubusson) and 33.33 g of tap water containing the cell suspension (final cell density in the dough of *ca.* 7.0 Log cfu/g) were incubated at 30°C for 24 h. Cell

147 suspensions were prepared as described by Rizzello et al. (2010a). Non-inoculated bran doughs prior
148 (CT_0) and after (CT_{24}) incubation were used as the controls. Based on these results, the two best
149 performing strains (*L. plantarum* T6B10 and *W. confusa* BAN8) were selected and used as a mixed
150 starter for sourdough fermentation of brans from wheat (Aubusson, FB1, Skorpion, FB2; Bonavita,
151 FB3), barley (var. Rondo, FB4) and emmer (var. Giovanni Paolo, FB5). A xylanase, (Depol 761,
152 Biocatalysts Limited, Chicago, USA) at 1% (wt/wt) based on weight of bran, was used to increase
153 the release of soluble fiber (Arte et al., 2015). Fermentations were carried out in triplicate. Bran
154 doughs prior fermentation (B1, B2, B3, B4 and B5) were used as the controls.
155
156 **2.5 Microbiological, biochemical and nutritional characterization of bran doughs**
157 For microbiological analysis, ten grams of bran doughs were suspended in 90 ml of sterile sodium
158 chloride (0.9%, wt/vol) solution and homogenized in a Bag Mixer 400P (Interscience, St Nom,
159 France) at room temperature. Presumptive lactic acid bacteria were determined on mMRS (Oxoid)
160 supplemented with cycloheximide (0.1 g/l), at 30°C for 48 h under anaerobiosis. Total *Enterobacteria*
161 were determined on Violet Red Bile Glucose Agar (VRBGA, Oxoid) at 37°C for 24 h and total
162 mesophilic bacteria were determined on Plate Count Agar (PCA, Oxoid) at 30°C for 48 h. Molds
163 were enumerated on Potato Dextrose Agar (PDA, Oxoid) at 32-35°C for 48 h. Cell density of yeasts
164 was estimated on Sabouraud Dextrose Agar (SDA, Oxoid), supplemented with chloramphenicol (0.1
165 g/l) at 30°C for 48 h.
166 The values of pH were determined by a pH-meter (Model 507, Crison, Milan, Italy) with a food
167 penetration probe. Total titratable acidity (TTA) was determined on 10 g of dough homogenized with
168 90 ml of distilled water and expressed as the amount (ml) of 0.1 M NaOH to reach pH of 8.3.
169 Water/salt-soluble extracts (WSE) from doughs were prepared according to the method originally
170 described by Osborne (1907) and modified by Weiss et al. (1993). Briefly, 9 g of samples were
171 suspended in 12 ml of 50 mM Tris–HCl (pH 8.8), incubated at 4°C for 1 h under stirring conditions

(*ca*. 150 rpm), and centrifuged at 12000 *x* g for 20 min. The supernatant was used for the determination of organic acids, TFAA and peptides.

Organic acids were determined by High Performance Liquid Chromatography (HPLC), using an ÄKTA Purifier system (GE Healthcare, Buckinghmshire, UK) equipped with an Aminex HPX-87H column (ion exclusion, Biorad, Richmond, CA), and an UV detector operating at 210 nm. Elution was at 60°C, with a flow rate of 0.6 ml/min, using H_2SO_4 10 mM as mobile phase (Rizzello et al., 2010a). The quotient of fermentation (QF) was determined as the molar ratio between lactic and acetic acids. TFAA were analyzed by a Biochrom 30 series Amino Acid Analyzer (Biochrom Ltd., Cambridge Science Park, England) with a Na-cation-exchange column (20 by 0.46 cm internal diameter), as described by Rizzello et al. (2010a). For the peptides analysis, WSE were treated with trifluoroacetic acid (0.05% wt/vol) and subject to dialysis (cut-off 500 Da) to remove proteins and FAA, respectively. Then peptides concentration was determined by the *o*-phtaldialdehyde (OPA) method as described by Church et al. (1983).

Phytic acid concentration were measured using K-PHYT 05/07 kit assay (Megazyme Intl., Ireland), following the manufacturer's instructions. Total phenols were determined on the methanolic extract (ME) of bran doughs. Five grams of each sample were mixed with 50 ml of 80% methanol to get ME. The mixture was purged with nitrogen stream for 30 min, under stirring condition, and centrifuged at 4600 x g for 20 min. MEs were transferred into test tubes, purged with nitrogen stream and stored at *ca*. 4°C before analysis. The concentration was determined as described by Slinkard and Singleton (1997) and expressed as gallic acid equivalent. The radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was used for determining the free radical scavenging activity (Rizzello et al., 2010a) in both the WSE and ME. The synthetic antioxidant butylated hydroxytoluene (BHT) was included in the analysis as the reference (75 ppm). The *in vitro* protein digestibility (IVPD) was determined by the method proposed by Akeson and Stahmann (1964) with some modifications (Rizzello et al., 2014). Samples were subjected to a sequential enzyme treatment mimicking the *in vivo* digestion in the gastro intestinal tract and IVPD was expressed as the percentage of the total protein which was solubilized

after enzyme hydrolysis. The concentration of protein of digested and non-digested fractions was determined by the Bradford method (Bradford, 1976).

2.6 Breadmaking

Breads (DY of 180) containing fermented bran from wheat cultivars (Aubusson, FB1-B, Skorpion, FB2-B; Bona Vita, FB3-B), barley (FB4-B) and emmer (FB5-B) were manufactured at the pilot plant of the Department of Soil, Plant and Food Science (University of Bari, Italy). Breads were produced according to the two-stage protocol commonly used for typical Italian sourdough breadmaking. The protocol was adapted to bran, including fermentation for 24 h at 30°C (step I), and subsequent mixing with wheat flour, water, and baker's yeast (2 h at 30°C, step II). The bread formula was as follows: 97.2 g of white flour, 77.8 g of water, 75 g of fermented brans (30%, wt/wt) and salt (1%, wt/wt). A baker's yeast wheat bread (WB) was manufactured without the addition of bran (DY, 180) and used as the control. Baker's yeast was added at the percentage of 2% (wt/wt), corresponding to a final cell density of *ca.* 9 Log cfu/g in all breads. Doughs were mixed at 60 × g for 5 min with an IM 5-8 high-speed mixer (Mecnosud, Flumeri, Italy) and fermentation was at 30°C for 2h. All breads were baked at 220°C for 50 min (Combo 3, Zucchelli, Verona, Italy). Wheat flour use for breadmaking had the following chemical composition: moisture, 14.2%; protein, 11.4% of dry matter (d.m.); fat, 1.1% of d.m.; carbohydrates, 86.8% of d.m. of which fiber (3.1% of d.m.) and ash, 0.6% of d.m. The Alveograph properties were W value between 200 and 250 and a P/L in the range of 0.6 – 0.7.

The Texture Profile Analysis (TPA) of bread was carried out with a Universal Testing machine (model 3344, Instron, Norwood, MA, USA), equipped with 3.6 cm diameter cylindrical probe, 1000 N load cell. The chromaticity co-ordinates of the bread crust L, a, and b (determined by a Minolta CR-10 camera) were also reported in the form of a color difference, dE*ab, as follows:

$$dE^*_{ab} = \sqrt{(dL)^2 + (da)^2 + (db)^2}$$

where dL, da, and db are the differences for L, a, and b values between sample and reference (a white ceramic plate having L = 67.04, a = 2.44, and b = 18.28).

The values of pH and TTA, concentration of organic acids, TFAA, total phenols and phytic acid, and radical scavenging activity were determined as reported above. Water activity (a_w) was determined at 25ºC by the Aqualab Dew Point 4TE water activity meter (Decagon Devices Inc., USA). Breadmaking was carried out in triplicate and each bread was analyzed twice.

2.7 Nutritional characterization of breads

The starch hydrolysis was analyzed using a procedure that mimicked the *in vivo* digestion (De Angelis et al., 2009). Aliquots of breads, containing 1 g of starch, were undergo to enzymatic process and the released glucose content was measured with D-Fructose/D-Glucose Assay Kit (Megazyme). The degree of starch digestion was expressed as the percentage of potentially available starch hydrolyzed after 180 min. Wheat flour bread (WB) leavened with baker's yeast was used as the control to estimate the hydrolysis index (HI = 100). The predicted glycemic index (*pGI*) was calculated using the equation: GI = 0.549 x HI + 39.71 (Capriles and Areas, 2013). IVPD of breads was determined as reported above.

2.8 Sensory analysis

Sensory analysis of breads was carried out by ten panellists (five male and five females, mean age: 35 years, range: 18-54 years). After a roundtable discussion about the attributes, 7 were selected as the most frequently recognized by all the members of the panel. These were included in a score sheet for the quantitative evaluation with a scale from 0 to 10, with 10 the highest score. Salty taste, previously described as another wheat sourdough bread attribute, was also included (Rizzello, et al., 2010b).

2.9 Statistical analysis

Fermentations were carried out in triplicate and each analysis was repeated twice. Data were subjected to one-way ANOVA; pair-comparison of treatment means was achieved by Tukey's procedure at $P<0.05$, using the statistical software, Statistica 12.5 (TIBCO Software Inc., Palo Alto, USA) for Windows. Principal Components analysis was performed through Xlstat 2014 (Addinsoft, New York, USA).

3. Results

3.1 Gross chemical composition, biochemical and microbiological characterization of brans

The gross chemical composition of brans used in this study are reported in Table 1. No significant ($p>0.05$) differences were found in term of carbohydrates. The content of the DF strictly depended on the bran. The values ranged from 10.0 ± 0.3% (B5) to 26.3 ± 0.4% (B2) (Table 1). The protein, fat and ash contents also significantly ($p<0.05$) differed. Bran of wheat cultivars (B1, B2 and B3) contained the lowest and highest concentrations of protein (15.9 ± 0.5% – 17.7 ± 0.4%) and fat and ash (4.1 ± 0.4% – 4.5 ± 0.6% and 3.2 ± 0.5% – 3.5 ± 0.3%), respectively (Table1). Contrarily, the highest concentration of protein (18.8 ± 0.5% - 18.9 ± 0.4%) and the lowest of fat (3.3 ± 0.3% - 3.9 ± 0.3%) and ash (2.3 ± 0.3% - 2.6 ± 0.5%) characterized bran of barley (B4) and emmer (B5) varieties. Table 2 summarizes the microbiological and biochemical characterization of bran doughs prior the fermentation. Total mesophilic bacteria and presumptive lactic acid bacteria ranged from 5.7 ± 0.2 to 5.8 ± 0.3 Log cfu/g and from 3.5 ± 0.1 to 5.8 ± 0.3 Log cfu/g, respectively. Molds and yeasts were from 1.2 ± 0.3 to 3.7 ± 0.2 Log cfu/g and from 2.3 ± 0.2 to 3.4 ± 0.3 Log cfu/g, respectively. Cell density of *Enterobacteria* was in the range 3.7 ± 0. 3 - 4.8 ± 0.2 Log cfu/g (Table 2).

Values of pH and TTA were 5.70 ± 0.01 – 6.60 ± 0.02 and 1.4 ± 0.1 – 13.8 ± 0.3 (ml NaOH 0.1 M), respectively (Table 2). The concentrations of TFFA and phytic acid varied from 675 ± 15 (B1) – 1653 ± 31 (B2) mg/kg to 330 ± 15 (B5) - 900 ± 21 mg/100g (B3). The concentration of total phenols and the radical scavenging activity varied from 1.22 ± 0.02 to 1.93 ± 0.04 mmol/kg to 34.4 ± 0.6 to 59.3

± 0.8% (Table 2). WSE had concentrations of peptides ranging between 13.0 ± 0.6 (B4) and 19.4 ± 0.4 (B2) mg/g (Table 2). No radical scavenging activity was detected in any of bran doughs.

3.2 Selection of mixed starter for lactic acid fermentation

Preliminarily, the ten lactic acid bacteria strains were singly used to ferment (30°C for 24 h) wheat bran (cv. Aubusson), which was chosen as the common matrix for the screening (Table 3). After 24 h of fermentation, all lactic acid bacteria increased of *ca.* 2.5 Log cfu/g. *L. plantarum* T6B10 and *W. confusa* BAN8 reached the highest values (Table 3). A cell density of 6.8 ± 0.2 Log cfu/g was found in CT_{24}. No *Enterobacteriaceae* were detectable in 10 g of sample. Because of the lactic acid fermentation, the values of pH were lower than 4, being the lowest when doughs were fermented with *L. plantarum* T6B10 and *W. confusa* BAN8. TTA increased to values higher than 10 ml NaOH 0.1M only in fermented samples (Table 3). The concentration of lactic acid was higher than 41.5 ± 0.4 mmol/kg and reached the highest value when *L. plantarum* T6B10 was used. Similarly, the highest concentration of acetic acid was found in the dough fermented with *W. confusa* BAN8 (Table 3). However, acetic acid was found only in doughs fermented with obligately heterofermentative strains (*W. confusa* and *L. rossiae*). The concentration of lactic acid of started doughs was *ca.* 20% higher than that found in CT_{24}. The QF of fermented doughs was *ca*. 7 (Table 3). Compared to CT_{24}, the concentration of TFAA was *ca*. 4 times higher. A similar trend was observed for the concentration of total phenols and radical scavenging activity, which were up to 77% higher than those found in CT_{24}. On the contrary, decreases of 12 - 25% were found for phytic acid concentration as compared to CT_{24} (Table 3). Values of TFAA concentration and radical scavenging activity of doughs fermented with *L. plantarum* T6B10 and *W. confusa* BAN8 were significantly ($p<0.05$) higher than the median values. Similarly, when *W. confusa* BAN8 was used as starter, the lowest value of phytic acid concentration was achieved. Based on the above results, *L. plantarum* T6B10 and *W. confusa* BAN8 were chosen to be used as mixed starter to ferment wheat, barley and emmer brans.

300 **3.3 Bran fermentation with selected mixed starter**

301 Table 4 shows the biochemical and nutritional properties of the brans fermented with the mixed
302 starter. After 24 h of fermentation, the cell number of lactic acid bacteria increased by *ca.* 2 Log cfu/g,
303 regardless the type of bran. The values of pH decreased during the fermentation, being in the range
304 of 3.9 – 4.1, without significant ($p>0.05$) differences among doughs. On the contrary, TTA significant
305 ($p<0.05$) differed, with the highest and lowest values for FB1 (cv. Aubusson) and FB5 (var. Rondo),
306 respectively. Overall, the use of the mixed starter led to an increase of *ca.* 4 - 30% of the lactic acid
307 concentration in fermented brans, as compared to single strains. While, higher concentrations of
308 acetic acids were found in brans fermented with mixed starter as compared to the single strains (Table
309 3 and Table 4). Compared to bran doughs prior the fermentation (Table 2), the concentration of TFAA
310 increased up to three times. FB1 and FB4 showed the highest and lowest increases, respectively
311 (Tables 2 and 4). The fermentation also promoted an overall increase of the peptide concentration up
312 to 40%.

313 The fermentation with the mixed starter led also to an improvement of the nutritional features (Table
314 4). As compared to the un-fermented doughs (Table 2), fermented brans had lower concentrations of
315 phytic acid (Table 4). The lowest decrease was found when the B5 was fermented, indeed the
316 concentration of phytic acid was 24% lower in FB5 as compared to B5. The highest decrease (60%)
317 was found when B3 was fermented, although FB3 still contained the highest concentration (370 ± 21
318 mg/100g). According to the type of bran, the concentrations of phenols increased from 10 to 60%
319 during fermentation. The radical scavenging activity of the ME increased from 10% (FB5) to 70%
320 (FB1), reaching the highest values in FB2, FB3 and FB4, which agreed with the total phenol
321 concentrations. A similar trend was found for the radical scavenging activity of the WSE, which
322 reached values ranging from 30.7 ± 0.4% (FB1) to 44.7 ± 0.3% (FB5) (Table 4).

323 The IVPD values of fermented bran doughs ranged between 80.1 ± 0.4% and 87.1 ± 0.5%, being the
324 highest and lowest for FB2 and FB5, respectively.

325

3.4 Characterization of the breads fortified with fermented bran

The physical-chemical, biochemical and nutritional characteristics of the breads are summarized in Table 5. The inclusion of FB in the bread formula caused a marked water retention during baking, which was confirmed by the higher values of moisture and a_w of the fortified breads with respect to WB. Before baking, the pH of the dough fermented with baker's yeast alone was significantly ($p<0.05$) higher than those of the doughs containing 30% (wt/wt) of FB, regardless the type of bran. According to the type of FB used, the values of TTA were significantly ($p<0.05$) higher (up to three times) than that of WB. The use of FB in the bread formula, led to higher concentrations of lactic and acetic acids with respect to WB. Values of 25.31 ± 0.6 – 45.77 ± 0.6 mmol/kg and 4.86 ± 0.5 – 6.69 ± 0.5 mmol/kg were found for lactic and acetic acids, respectively (Table 5). Compared to WB, the fortified breads had also higher concentrations of TFAA (up to 4 times) and total phenols (up to 40%). The comparison also showed higher values of radical scavenging activities for both WSE (up to *ca.* 28%) and ME (up to *ca.* 70%). Fortified breads had lower contents of phytic acid (up to 10 times) as compared to WB.

Compared to WB, the use of FB as an ingredient caused significant ($p<0.05$) increases of DF (up to 6 times) and proteins (up 2 times) (Table 5). Compared to WB, a significant decrease (*ca.* 20%) of the HI was observed. The lowest decrease was found for FB3-B (*ca.* 80%), corresponding to a *pGI* of 65.1 ± 0.2. Significant ($p<0.05$) increases of IVPD were observed, which varied depending on the type of bran (Table 5).

3.5 Textural properties and sensory profile of the bread fortified with fermented bran

Compared to WB, the specific volumes of breads fortified with FB3, FB4 and FB5 increased (Table 6). On the contrary, decreases of resilience and cohesiveness (up to *ca.* 30%) and increases of hardness (up to *ca.* 2 times), gumminess (up to *ca.* 4 times) and chewiness (up to *ca.* 4 times) were found when FB were added to the bread formula. The magnitude of changes strictly depended on the bran used (Table 6). Among breads fortified with fermented brans, FB5-B had the highest values of

all textural properties (Table 6). However, the highest hardness value was found in FB3-B. Contrarily, lowest values of gumminess and chewiness were found when FB1 was used in breadmaking. FB4-B had the lowest value of hardness. No significant ($p>0.05$) differences were found in term of resilience. The addition of FB in bread formula, significantly ($p<0.05$) influenced the color of the crust, leading to a decrease of lightness (*L*) and to an increase of the *a* values (Table 6). The *b* value did not significantly ($p>0.05$) differ among breads. However, *dE* (calculated based on the chromaticity co-ordinates) significantly differ from WB when FB were added in the bread formula (Table 6). FB5-B had the lowest and higher values of *L* and *dE*, respectively. FB4-B showed the lowest *a* value (Table 5).

Overall, the elasticity of the fortified breads was not significantly ($p>0.05$) influenced by the type of bran used. The use of FB in the bread formula led to an increase of the crust and crumb color as well as the acidic aroma and taste as compared to the WB (Figure 1). The PCA analysis, explaining *ca*. the 95% of the total variance of the data, scattered the breads containing wheat (FB1-B, FB2-B and FB3-B) and barley (FB4-B) and emmer (FB5-B) brans in two different zones of the plane. FB1-B, FB2-B and FB3-B shared similar profiles. Breads FB4-B and FB5-B were separated due to low scores of acidic aroma and taste.

4. Discussion

Throughout Europe, the recommended DF intake is *ca*. 25–32 g/d and 30–35 g/d for adult women and men, respectively. Less for children and elderly, depending on age (Stephen et al., 2017). Nevertheless, observational studies indicate that the averaged intake of DF is far below the recommendations (Stephen et al., 2017). Nutrition guidelines from United States (U.S. Department of Health and Human Services) and Europe (European Food Safety Authority, EFSA) exhort consumers to meet their daily DF intake through the consumption of a variety of fruits, vegetables and whole grains. Bread is a good and suitable vehicle for health promotion because of the low cost and worldwide consumption (Dziki et al., 2014). Traditionally, wheat bread is made from refined

flour, with milling process removing outer layers (bran) and germ, those fractions that are the richest of DF and other bioactive compounds (Benítez et al., 2018). Besides the functionality, other desirable food attributes are freshness, minimal processing and a clean label (Nielsen Company, 2015). Bread fortified with DF is an example of minimally processed food, which combines healthy benefits. Nevertheless, the fiber as an ingredient in the bread formula may lead to worsening of the technological and sensory properties (Ciccoritti et al., 2017). Based on the traditional use of sourdough, fermentation by lactic acid bacteria is the most efficient tool for the manufacture of baked goods with high concentration of fiber, improving the technological aptitude of whole meal flours, and promoting optimal rheology, nutritional and sensory properties (Coda et al., 2014; Manini et al., 2014; Pontonio et al., 2017).

In this scenario, bran from hull-less barley, emmer and pigmented wheat cultivars were fermented by selected lactic acid bacteria and used in breadmaking. Based on a selection process among 70 strains of lactic acid bacteria according to pro-technological and functional features (Supplementary Figure 1) later (Table 3), *Lactobacillus plantarum* T6B10 and *Weissella confusa* BAN8 were chosen (Pontonio et al., 2015; Rizzello et al., 2016) and used as mixed starter for bran fermentation. Metabolic traits associated with improvements of the functional and nutritional features in bran. Kinetics of growth and acidification, proteolysis, and liberation of phenolic compounds were the main criteria used to screen.

Aiming at enhancing the solubilization of protein from bran (Arte et al., 2015), the use of cell-wall-degrading enzymes was also investigated in combination with microbial fermentation. Besides providing DF, bran is a source of protein, being a valuable substitute for other protein-rich sources in the food industry. Nonetheless, several factors affect protein bioavailability, including bran's layered structure.

The fermentation of brans from hull-less cereals allowed optimal lactic acid bacteria growth and acidification. Bran is rich in essential amino acids (lysine and tryptophan), vitamins (e.g., thiamin and niacin), antioxidants (e.g., ferulic acid and alkylresorcinols), and minerals (phosphorus and iron)

(Arte et al., 2015; Rizzello et al., 2010a, 2010b). Nevertheless, the bioavailability of most of these nutrients is often questioned. Bran and, especially, the aleuronic layer contain considerable levels of phytic acid, which strongly chelates minerals, thus reducing the bioavailability. Because of the pH-activation of endogenous phytases (Kumar et al., 2010), the concentration of phytic acid markedly decreased (230 ± 14 mg/100g) during fermentation. Proteolysis via the combined activity of endogenous proteases (also activated by acidification) and lactic acid bacteria peptidases led to an increase of TFAA (up to 3899 ± 41 mg/Kg) (Ganzle, 2014). Amino acids and short-chain peptides affect the taste of fermented foods and are important precursors for volatile flavor compounds, which generate during baking (Ganzle et al., 2008). Overall, lactic acidification also improves the level of extractable phenolic compounds, whose profile is further modified by the activity of lactic acid bacteria enzymes (e.g., feruloyl-esterase and β-glucosidase) (Filannino et al., 2015). The increase of the concentration of total phenols found in the fermented bran reflected on the antioxidant activity. Indeed, such activity increased up to *ca*. 65% as compared to non-fermented bran. The amino acid composition, their bioavailability and protein digestibility are basic indexes to determine the quality of a protein source (Sarwar Gilani et al., 2012) and the nutritional profile of a food (Bilgiçli et al., 2007). The addition of bran may decrease the IVPD (Bilgiçli et al., 2007; Rizzello et al., 2012) because of the possible formation of complexes between fiber components and proteins. The fermentation by lactic acid bacteria flanked by the use of xylanase led to values of IVPD of *ca*. 87%, much higher than those commonly found for wheat bran (Arte et al., 2015; Bilgicli et al., 2006).

Data from fermented brans were elaborated through Principal Component Analysis (PCA) (Figure 2). The two PCs explained *ca.* 85% of the total variance of the data. Fermented brans showed peculiar profiles and fell into different zones of the plane. Factor 1 clearly separated fermented wheat (FB1, FB2 and FB3) from fermented barley (FB4) and emmer (FB5) brans. Factor 2 differentiated conventional wheat (FB1) and pigmented wheat cultivars (FB2 and FB3). The use of the same process conditions (e.g., starter cultures, temperature and time of fermentation) enhanced the feature of each bran and allowed the discrimination among them. Indeed, FB2 contained the highest concentrations

of peptides and had the highest value of IVPD. FB3 had the highest concentration of TFAA. Both these fermented brans shared a high concentration of total phenols and radical scavenging activity. The highest radical scavenging activity was found for FB4 and FB1 had the lowest content of phytic acid.

Food quality is a multivariate notion: foods carry an image of tasting good being good for health. Taste and health need to be improved in parallel. Consequently, fermented brans were used to fortify wheat breads. The results mirrored those found in fermented brans. All fortified breads showed increased concentrations of TFAA (597 ± 11 - 888 ± 19 mg/Kg) and phenolic compounds (2.55 ± 0.03 – 4.23 ± 0.05 mmol/Kg), enhanced radical scavenging activity (up to 60%) and reduced phytic acid concentration. FB5-B was characterized by the highest concentrations of both TFAA and total phenols, while the lowest content of phytic acid was found in FB4-B. Compared to a baker's yeast wheat bread (control), breads fortified with fermented brans exhibited also a more balanced sensory profile, mainly due to the acidic taste and aroma. The use of fermented bran in the formula led to breads having HI and *pGI* values markedly lower (20% and 12%, respectively) than those of the control. Beside the well-known effect related to the considerable supply of DF, a strong contribution is provided by the biological acidification, which is one of the main factors that decreases starch hydrolysis rate (Pontonio et al., 2017). Compared to the control, fortified breads had high levels of DF (up to 7% of d.m.) and proteins (up to 13% of d.m.). Despite the bran fortification, the protein digestibility of fortified breads was *ca*. 40% higher than the control, thus hypothesizing a key role of the lactic acid bacteria proteolysis (Rizzello et al., 2019). According to EC Regulation (Regulation EC No. 1924/2006) on nutrition and health claims on food products, experimental fortified breads can be labelled as "source of fiber", since containing at least 3 g of fiber per 100 g of bread.

5. Conclusion

This study combines the use of selected lactic acid bacteria and cell-wall-degrading enzymes to enhance the nutritional profile of bran. Treatment with exogenous xylanase solubilizes proteins

entrapped within bran layers, making them available for microbial/endogenous proteolysis, which improves protein digestibility. Fermentation with selected lactic acid bacteria improves the nutritional and functional features of fermented brans. Each fermented bran has peculiar features, offering choices to fortify breads, which depend on specific nutritional aims. This study supplies a realistic option that combines waste recycle and consumer expectations for healthy foods.

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Author Contributions Statement

EP, carried out the selection of lactic acid bacteria, elaborated the results and wrote the draft of the manuscript; CD carried out the selection of lactic acid bacteria, the microbiological, bio-chemical and nutritional analysis and the baking tests; RDC coordinated the scientific units and was responsible for the research funding; MB was responsible for cereal cultivation and gross chemical composition of brans and breads; MG critically revised the manuscript; CGR was the scientific advisor and designed the experimental work. All authors read and approved the final manuscript.

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613

614 **Legend to figures**

615 **Figure 1.** Principal component analysis (PCA) based on sensory analysis of breads (DY, 180) FB1-
616 B, bread containing 30% (wt/wt) of fermented bran doughs obtained from red-grained wheat variety
617 (cv Aubusson) (FB1); FB2-B, bread containing 30% (wt/wt) of fermented bran obtained from blue-
618 grained wheat variety (cv Skorpion) (FB2); FB3-B, bread containing 30% (wt/wt) of fermented bran
619 obtained from yellow-grained wheat variety (cv Bona Vita) (FB3); FB4-B, bread containing 30%
620 (wt/wt) of fermented bran obtained from spring hull-less barley (var. Rondo) (FB4); FB5-B, bread
621 containing 30% (wt/wt) of fermented bran obtained from emmer (var. Schrank) (FB5); WB, white
622 wheat bread.

623 **Figure 2.** Principal component analysis (PCA) based on biochemical and nutritional characteristics
624 of wheat, emmer and barley bran doughs (DY 300) fermented with *Lactobacillus plantarum* T6B10
625 and *Weissella confusa* BAN8 at 30°C for 24 h. The ingredients and technological parameters for the
626 preparation of fermented bran doughs (FB1, FB2, FB3, FB4 and FB5) are reported in the Materials
627 and methods section.

628 **Table 1**. Gross chemical composition of wheat, barley and emmer brans

	B1	**B2**	**B3**	**B4**	**B5**
Carbohydrates (%)	71.6 ± 0.6^{a}	71.1 ± 0.5^{a}	71.8 ± 0.5^{a}	72.8 ± 0.7^{b}	74.6 ± 0.6^{c}
Total dietary fiber (%)	25.5 ± 0.5^{c}	26.3 ± 0.4^{cd}	25.3 ± 0.7^{c}	21.6 ± 0.5^{b}	10.0 ± 0.3^{a}
***Insoluble fiber* (%)**	24.0 ± 0.6^{c}	24.7 ± 0.5^{c}	24.1 ± 0.4^{c}	19.1 ± 0.3^{b}	8.6 ± 0.5^{a}
***Soluble fiber* (%)**	1.5 ± 0.2^{ab}	1.6 ± 0.1^{b}	1.2 ± 0.3^{a}	2.5 ± 0.3^{c}	1.4 ± 0.2^{a}
Protein (%)	15.9 ± 0.5^{a}	17.7 ± 0.4^{b}	17.6 ± 0.3^{b}	18.9 ± 0.4^{c}	18.8 ± 0.5^{c}
Fat (%)	4.5 ± 0.6^{cd}	4.3 ± 0.5^{bc}	4.1 ± 0.4^{b}	3.9 ± 0.3^{ab}	3.3 ± 0.4^{a}
Ash (%)	3.4 ± 0.3^{b}	3.2 ± 0.5^{b}	3.5 ± 0.3^{b}	2.6 ± 0.5^{a}	2.3 ± 0.4^{a}

629 B1, wheat bran cv. Aubusson; B2, wheat bran cv. Skorpion; B3, wheat bran cv. Bona Vita; B4, hull-less barley var. Rondo, B5; emmer bran var.

630 Giovanni Paolo.

631 Data are expressed on dry matter.

632 $^{a\text{-}e}$ Values in the same row with different superscript letters differ significantly ($p<0.05$)

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637 **Table 2**. Microbiological, biochemical and nutritional characterization of wheat, barley and emmer bran doughs (DY 300) prior the fermentation.

	B1	**B2**	**B3**	**B4**	**B5**
Microbiological characterization					
Total mesophilic bacteria (Log cfu/g)	5.7 ± 0.2^{a}	5.7 ± 0.3^{a}	5.7 ± 0.4^{a}	5.8 ± 0.3^{a}	5.8 ± 0.2^{a}
LAB (Log cfu/g)	3.5 ± 0.1^{a}	3.5 ± 0.3^{a}	3.5 ± 0.2^{a}	3.6 ± 0.2^{b}	3.8 ± 0.3^{c}
Yeast (Log cfu/g)	2.3 ± 0.2^{a}	2.3 ± 0.2^{a}	2.3 ± 0.1^{a}	3.4 ± 0.1^{b}	3.4 ± 0.3^{b}
Molds (Log cfu/g)	1.2 ± 0.2^{a}	1.3 ± 0.1^{b}	1.2 ± 0.3^{a}	3.7 ± 0.2^{c}	3.2 ± 0.2^{c}
Enterobacteriaceae (Log cfu/g)	4.6 ± 0.1^{b}	4.7 ± 0.1^{c}	4.7 ± 0.2^{c}	3.7 ± 0.3^{a}	4.8 ± 0.2^{d}
Biochemical characterization					
pH	6.6 ± 0.2^{b}	6.3 ± 0.3^{b}	6.2 ± 0.2^{b}	5.7 ± 0.1^{a}	6.4 ± 0.3^{b}
TTA (ml NaOH 0.1M)	1.4 ± 0.1^{a}	11.6 ± 0.4^{c}	7.2 ± 0.3^{b}	13.8 ± 0.3^{d}	11.0 ± 0.5^{c}
Lactic acid (mmol/Kg)	n.d.	n.d.	n.d.	n.d.	n.d.
Acetic acid (mmol/Kg)	n.d.	n.d.	n.d.	n.d.	n.d.
TFAA (mg/Kg)	675 ± 15^{a}	1653 ± 31^{e}	1455 ± 24^{d}	1000 ± 22^{b}	1290 ± 33^{c}
Peptide concentration (mg/g)	13.4 ± 0.3^{a}	19.4 ± 0.4^{c}	15.2 ± 0.7^{b}	13.0 ± 0.6^{a}	14.7 ± 0.4^{b}
Nutritional features					
Phytic acid (mg/100g)	620 ± 17^{c}	670 ± 22^{c}	900 ± 21^{d}	500 ± 17^{b}	330 ± 15^{a}

Total phenols (mmol/Kg)	1.39 ± 0.02^{c}	1.27 ± 0.03^{b}	1.27 ± 0.02^{b}	1.22 ± 0.03^{a}	1.93 ± 0.04^{d}
Radical scavenging activity/ME (%)	34.4 ± 0.6^{a}	55.3 ± 0.8^{b}	57.0 ± 0.7^{c}	59.3 ± 0.8^{c}	35.3 ± 0.5^{a}
Radical scavenging activity/WSE (%)	n.d.	n.d.	n.d.	n.d.	n.d.

638 B1, dough made with wheat (cv. Aubusson) bran; B2, dough made with wheat (cv. Skorpion) bran; B3, dough made with wheat (cv. Bona Vita) bran

639 from; B4, dough made with barley (var. Rondo) bran; B5, dough made with emmer (var. Giovanni Paolo) bran.

640 LAB, Lactic acid bacteria.

641 n.d. not detectable.

642 The data are the means of three independent experiments ± standard deviations (n = 3).

643 $^{a\text{-}e}$ Values in the same row with different superscript letters differ significantly ($p<0.05$)

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Table 3. Cell density of lactic acid bacteria (LAB), pH, TTA, concentration of lactic and acetic acids, total free amino acids (TFAA), phytic acid and phenols concentrations, quotient of fermentation (QF) and radical scavenging activity of fermented wheat bran (cv. Aubusson) started with single selected lactic acid bacteria strains (initial cell density of *ca.* 7 Log cfu/g) fermented at 30°C for 24 h. The minimum (m) and maximum (M) refer to whole number of isolates. Values for individual *Lactobacillus plantarum* T6B10 and *Weissella confusa* BAN8, which were further selected and used as a mixed starter for bran fermentation, are also included.

	CT_0	**CT_{24}**	***Minimum***	***Maximum***	***L. plantarum* T6B10**	***W. confusa* BAN8**
LAB (Log cfu/g)	3.5 ± 0.1^{a}	6.8 ± 0.2^{b}	9.5 ± 0.1^{c}	10.0 ± 0.2^{d}	9.9 ± 0.1^{d}	10.0 ± 0.2^{d}
pH	6.5 ± 0.2^{c}	5.9 ± 0.1^{b}	3.8 ± 0.3^{a}	3.9 ± 0.4^{a}	3.8 ± 0.3^{a}	3.9 ± 0.2^{a}
TTA (ml NaOH 0.1 M)	1.4 ± 0.1^{a}	4.1 ± 0.3^{b}	12.6 ± 0.2^{c}	15.8 ± 0.3^{d}	15.3 ± 0.2^{d}	12.6 ± 0.2^{c}
Lactic acid (mmol/Kg)	n.d.	30.5 ± 0.2^{a}	41.5 ± 0.4^{b}	67.3 ± 0.8^{d}	67.3 ± 0.8^{d}	60.7 ± 0.6^{c}
Acetic acid (mmol/Kg)	n.d.	n.d.	8.8 ± 0.7^{a}	9.2 ± 0.8^{ab}	n.d.	9.2 ± 0.8^{ab}
QF	n.d.	n.d.	6.4^{a}	6.9^{a}	n.d.	6.6^{a}
TFAA (mg/kg)	675 ± 15^{a}	690 ± 21^{ab}	1980 ± 26^{c}	2625 ± 39^{f}	2043 ± 36^{cd}	2478 ± 38^{e}
Phytic acid (mg/100g)	519 ± 5^{d}	487 ± 8^{c}	391 ± 11^{a}	457 ± 19^{b}	421 ± 9^{ab}	391 ± 11^{a}
Total phenols (mmol/Kg)	1.44 ± 0.02^{a}	3.32 ± 0.03^{b}	3.92 ± 0.04^{c}	5.89 ± 0.05^{f}	5.55 ± 0.06^{e}	4.62 ± 0.03^{d}
Radical scavenging activity/ME (%)	32.7 ± 0.3^{a}	42.5 ± 0.6^{b}	56.3 ± 0.6^{c}	75.6 ± 0.7^{e}	66.6 ± 0.5^{d}	66.3 ± 0.6^{d}

Aubusson (B1) bran was used as common matrix for bran fermentation.

The data are the means of three independent experiments ± standard deviations (n = 3).

[a-f] Values in the same row with different superscript letters differ significantly ($p<0.05$)

673 **Table 4**. Biochemical and nutritional characteristics of the wheat, barley and emmer bran fermented with *Lactobacillus plantarum* T6B10 and

674 *Weissella confusa* BAN8 (initial cell density of *ca.* 7 Log cfu/g) at 30°C for 24 h.

	FB1	**FB2**	**FB3**	**FB4**	**FB5**
Biochemical characteristics					
pH	4.1 ± 0.2^{a}	4.0 ± 0.1^{a}	4.1 ± 0.4^{a}	3.9 ± 0.2^{a}	3.9 ± 0.3^{a}
TTA (ml NaOH 0.1M)	52.0 ± 0.5^{cd}	49.2 ± 0.4^{c}	51.2 ± 0.6^{c}	41.2 ± 0.5^{b}	37.4 ± 0.4^{a}
Lactic acid (mmol/Kg)	82.0 ± 0.8^{c}	82.5 ± 0.5^{c}	86.8 ± 0.7^{d}	70.8 ± 0.9^{ab}	69.65 ± 0.6^{a}
Acetic acid (mmol/Kg)	10.2 ± 0.3^{c}	8.1 ± 0.2^{a}	10.5 ± 0.4^{c}	9.8 ± 0.3^{b}	8.8 ± 0.4^{ab}
QF	8.01^{b}	10.3^{d}	8.3^{c}	7.2^{a}	7.93^{b}
TFAA (mg/Kg)	2401 ± 24^{a}	2844 ± 33^{c}	3899 ± 41^{e}	3088 ± 47^{d}	2601 ± 12^{b}
Peptide concentration (mg/g)	20.9 ± 0.3^{b}	33.8 ± 0.4^{d}	19.9 ± 0.2^{a}	20.4 ± 0.3^{ab}	21.2 ± 0.4^{bc}
Nutritional characteristics					
Phytic acid (mg/100g)	230 ± 14^{a}	340 ± 11^{d}	370 ± 21^{e}	280 ± 12^{c}	250 ± 10^{b}
Total phenols (mmol/Kg)	2.52 ± 0.01^{b}	2.90 ± 0.02^{cd}	3.28 ± 0.03^{e}	2.83 ± 0.02^{c}	2.11 ± 0.01^{a}
Radical scavenging activity/ME (%)	59.8 ± 0.2^{b}	63.4 ± 0.7^{c}	64.1 ± 0.5^{cd}	65.5 ± 0.5^{e}	55.4 ± 0.6^{a}
Radical scavenging activity/WSE (%)	30.7 ± 0.4^{a}	42.3 ± 0.5^{d}	34.9 ± 0.3^{b}	38.5 ± 0.4^{c}	44.7 ± 0.3^{de}
IVPD (%)	$82.5 \pm 0.6b^{c}$	87.1 ± 0.5^{e}	81.0 ± 0.7^{b}	83.6 ± 0.5^{cd}	80.1 ± 0.4^{a}

675 FB1, fermented dough made with wheat (cv. Aubusson) bran; FB2, fermented dough made with wheat (cv. Skorpion) bran; FB3, fermented dough
676 made with wheat (cv. Bona Vita) bran; FB4, fermented dough made with barley (var. Rondo) bran; FB5, fermented dough made with emmer (var.
677 Giovanni Paolo) bran.
678 The data are the means of three independent experiments ± standard deviations (n = 3).
679 [a-e] Values in the same row with different superscript letters differ significantly ($p<0.05$)
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690 **Table 5**. Physical-chemical, biochemical and nutritional characteristics of experimental breads (DY, 180) containing 30% (wt/wt) of wheat, barley
691 and emmer bran doughs and fermented with *Lactobacillus plantarum* T6B10 and *Weissella confusa* BAN8 (initial cell density of *ca.* 7 Log cfu/g) at
692 30°C for 24 h.

	FB1-B	**FB2-B**	**FB3-B**	**FB4-B**	**FB5-B**	**WB**
Physical-chemical characteristics						
Moisture (%)	27.3 ± 0.4^{ab}	27.2 ± 0.7^{ab}	26.4 ± 0.2^{a}	26.8 ± 0.4^{ab}	27.2 ± 0.6^{ab}	31.0 ± 0.2^{c}
a_w	0.97 ± 0.04^{ab}	0.99 ± 0.06^{abc}	0.98 ± 0.05^{ab}	0.97 ± 0.04^{ab}	0.98 ± 0.01^{ab}	0.92 ± 0.02^{a}
Biochemical characteristics						
pH	4.1 ± 0.5^{ab}	4.0 ± 0.4^{a}	3.9 ± 0.3^{a}	3.9 ± 0.5^{a}	3.9 ± 0.4^{a}	5.3 ± 0.3^{c}
TTA	23.6 ± 0.4^{e}	25.4 ± 0.3^{f}	19.4 ± 0.5^{b}	22.6 ± 0.3^{c}	19.8 ± 0.4^{b}	9.1 ± 0.3^{a}
Lactic acid (mmol/Kg)	45.77 ± 0.6^{f}	36.38 ± 0.4^{d}	37.24 ± 0.5^{de}	25.31 ± 0.6^{b}	28.65 ± 0.4^{c}	3.3 ± 0.5^{a}
Acetic acid (mmol/Kg)	6.69 ± 0.5^{de}	6.61 ± 0.7^{d}	5.29 ± 0.6^{bc}	4.86 ± 0.4^{b}	5.32 ± 0.6^{c}	1.27 ± 0.3^{a}
FQ	6.8^{c}	5.5^{b}	7.0^{c}	5.2^{b}	5.4^{b}	2.6^{a}
TFAA (mg/Kg)	654 ± 13^{cd}	597 ± 11^{b}	858 ± 14^{e}	632 ± 16^{c}	888 ± 19^{f}	264 ± 10^{a}
Nutritional characteristics						
Protein (%)	12.2 ± 0.3^{b}	12.5 ± 0.4^{b}	12.5 ± 0.5^{b}	12.7 ± 0.4^{bc}	12.7 ± 0.5^{bc}	6.3 ± 0.1^{a}
Fat (%)	1.65 ± 0.01^{c}	1.73 ± 0.02^{d}	1.67 ± 0.01^{c}	1.49 ± 0.01^{b}	1.64 ± 0.02^{c}	0.61 ± 0.04^{a}

Carbohydrates (%)	86.7 ± 0.6^{b}	86.6 ± 0.8^{b}	86.7 ± 0.5^{b}	87.2 ± 0.5^{bc}	86.9 ± 0.8^{b}	79.4 ± 0.9^{a}
Total dietary fiber (%)	7.02 ± 0.02^{d}	7.15 ± 0.01^{e}	6.88 ± 0.02^{d}	4.20 ± 0.03^{b}	6.31 ± 0.04^{c}	1.87 ± 0.02^{a}
Insoluble fiber (%)	6.71 ± 0.03^{d}	6.82 ± 0.04^{e}	6.67 ± 0.04^{d}	4.02 ± 0.05^{b}	6.23 ± 0.02^{c}	1.73 ± 0.03^{a}
Soluble fiber (%)	0.31 ± 0.02^{c}	0.33 ± 0.02^{c}	0.21 ± 0.02^{b}	0.19 ± 0.02^{ab}	0.39 ± 0.02^{d}	0.14 ± 0.02^{a}
Ash (%)	1.11 ± 0.02^{d}	1.05 ± 0.02^{c}	1.14 ± 0.03^{de}	0.87 ± 0.05^{b}	0.89 ± 0.04^{b}	0.27 ± 0.02^{a}
IVPD (%)	74 ± 1^{d}	65 ± 2^{b}	79 ± 1^{e}	79 ± 2^{e}	68 ± 2^{bc}	46 ± 1^{a}
pGI	65.4 ± 0.3^{a}	65.2 ± 0.4^{a}	65.1 ± 0.2^{a}	66.8 ± 0.2^{b}	68.0 ± 0.5^{b}	71.2 ± 0.4^{c}
Phytic acid (mg/100g)	141 ± 13^{bc}	$304 \pm 15d^{e}$	252 ± 17^{d}	30 ± 8^{a}	104 ± 14^{b}	352 ± 14^{f}
Total phenols (mmol/Kg)	3.74 ± 0.04^{e}	3.62 ± 0.03^{d}	2.55 ± 0.03^{b}	3.37 ± 0.03^{c}	4.23 ± 0.05^{f}	2.39 ± 0.03^{a}
Peptide concentration (mg/g)	372 ± 5^{a}	471 ± 3^{c}	648 ± 5^{f}	511 ± 4^{e}	425 ± 5^{b}	486 ± 5^{d}
Radical scavenging/ME (%)	36.8 ± 0.4^{d}	62.0 ± 0.4^{ef}	33.5 ± 0.5^{c}	61.5 ± 0.4^{e}	27.8 ± 0.4^{b}	20.3 ± 0.3^{a}
Radical scavenging/WSE (%)	30.2 ± 0.5^{b}	39.2 ± 0.4^{e}	33.4 ± 0.5^{c}	33.2 ± 0.6^{c}	35.9 ± 0.5^{d}	28.2 ± 0.3^{a}

693 FB1-B, bread containing fermented dough made with wheat (cv. Aubusson) bran; FB2-B, bread containing fermented dough made with wheat (cv.
694 Skorpion) bran; FB3-B, bread containing fermented dough made with wheat (cv. Bonavita) bran from; FB4-B, bread containing fermented dough
695 made with barley (var. Rondo) bran; FB5-B, bread containing fermented dough made with emmer (var. Giovanni Paolo) bran.
696 Data of protein, fat, carbohydrates, fiber, and ash are expressed on dry weight basis.
697 The data are the means of three independent experiments ± standard deviations (n = 3).

[a-f] Values in the same row with different superscript letters differ significantly ($p<0.05$)

713 **Table 6**. Textural characteristics of experimental breads (DY, 180): FB1-B, bread containing 30% (wt/wt) of FB1; FB2-B, bread containing 30%
714 (wt/wt) of FB2; FB3-B, bread containing 30% (wt/wt) of FB3; FB4-B, bread containing 30% (wt/wt) of FB4; FB5-B, bread containing 30% (wt/wt)
715 of FB5; WB, white wheat bread.

	FB1-B	**FB2-B**	**FB3-B**	**FB4-B**	**FB5-B**	**WB**
Specific volume (cm^3/g)	2.28 ± 0.05^{ab}	2.17 ± 0.06^{a}	2.65 ± 0.07^{c}	2.7 ± 0.05^{cd}	2.88 ± 0.04^{d}	2.3 ± 0.02^{b}
Resilience	0.81 ± 0.06^{ab}	0.82 ± 0.07^{bc}	0.80 ± 0.03^{a}	0.82 ± 0.04^{bc}	0.82 ± 0.07^{bc}	0.85 ± 0.04^{c}
Cohesiveness	0.43 ± 0.04^{a}	0.40 ± 0.05^{a}	0.42 ± 0.09^{ab}	0.56 ± 0.04^{bc}	0.62 ± 0.06^{cd}	0.70 ± 0.07^{de}
Gumminess	15.8 ± 0.5^{b}	19.0 ± 0.9^{d}	22.4 ± 0.8^{e}	16.9 ± 0.4^{bc}	31.3 ± 0.9^{f}	7.3 ± 0.2^{a}
Chewiness (g)	1290 ± 24^{b}	1560 ± 35^{d}	1799 ± 27^{e}	1397 ± 31^{c}	2594 ± 29^{f}	625 ± 13^{a}
Hardness (g)	3710 ± 32^{c}	4700 ± 42^{d}	5270 ± 39^{f}	3040 ± 28^{b}	5000 ± 42^{e}	2590 ± 22^{a}
Crust color						
L	53.9 ± 0.4^{b}	53.8 ± 0.3^{b}	58.7 ± 0.8^{c}	61.0 ± 0.4^{d}	52.0 ± 0.5^{a}	68.1 ± 0.7^{e}
a	4.4 ± 0.2^{c}	5.2 ± 0.3^{cd}	3.5 ± 0.1^{b}	3.2 ± 0.2^{b}	7.9 ± 0.4^{e}	2.5 ± 0.1^{a}
b	23.0 ± 0.3^{a}	23.0 ± 0.2^{a}	23.7 ± 0.4^{bc}	23.3 ± 0.2^{b}	23.3 ± 0.4^{b}	23.4 ± 0.3^{b}
dE	45.2 ± 0.6^{d}	45.7 ± 0.4^{d}	41.2 ± 0.5^{bc}	38.5 ± 0.6^{b}	50.4 ± 0.7^{e}	33.1 ± 0.5^{a}

FB1-B, bread containing fermented dough made with wheat (cv. Aubusson) bran; FB2-B, bread containing fermented dough made with wheat (cv. Skorpion) bran; FB3-B, bread containing fermented dough made with wheat (cv. Bona Vita) bran from; FB4-B, bread containing fermented dough made with barley (var. Rondo) bran; FB5-B, bread containing fermented dough made with emmer (var. Giovanni Paolo) bran.

The data are the means of three independent experiments ± standard deviations (n = 3).

[a-e] Values in the same row with different superscript letters differ significantly ($p<0.05$).