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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[a], Cinzia Dingeo[a], Raffaella Di Cagno[b,*], Massimo Blandino[c], Marco Gobbetti[b], Carlo Giuseppe Rizzello[a]

[a] Department of Soil, Plant and Food Science, University of Bari Aldo Moro, 70126 Bari, Italy
[b] Faculty of Science and Technology, Free University of Bozen-Bolzano, 39100 Bolzano, Italy
[c] Department of Agricultural, Forest and Food Sciences, University of Turin, 10095 Grugliasco, Italy

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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. The effects on the biochemical and nutritional features were investigated. Enhanced radical scavenging activity, increased concentrations of free amino acids (up to three times) and peptides and optimal in vitro protein digestibility (up to ca. 87%) 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 were characterized by a concentration in total dietary fibers and protein of ca. 7 and 13% of dry matter, respectively. Compared to wheat bread the addition of pre-fermented brans caused a significant increase in protein digestibility (up to 79%), and a relevant decrease of the predicted glycemic index (ca. 8%) of the fortified bread. According to the results, this study demonstrates the potential of xylanase treatment and lactic acid bacteria fermentation to be used as suitable strategy to include bran in breadmaking, meeting both nutritional and sensory requests of modern consumers.

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

Abbreviations: LAB, lactic acid bacteria; 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); FB, fermented bran; 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

* Corresponding author.
E-mail address: raffaella.dicagno@unibz.it (R. Di Cagno).

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on their health status (Mialon et al., 2002). Bran fraction is rich in fibers (e.g., cellulose, hemicellulose and lignin) (Katina et al., 2007; Messia et al., 2016; Šramková et al., 2009), proteins and antioxidant compounds (e.g., phenols, anthocyanins and carotenoids) (Adom and Liu, 2002). The concentration of such phytochemicals increases in the so-called pigmented wheat varieties as compared to conventional and widely diffused wheat varieties (Carson and Edwards, 2009). 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 (Bartłomiej 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 had been 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 to be 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 (LAB) 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 LAB 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 LAB were used to produce an ingredient for bread-making 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* subsp. *aestivum*) 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 × 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 kilogram 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). Mill was cleaned thoroughly by aspiration to avoid equipment contamination and washed with alcohol to minimize microbial contamination.

2.2. Gross chemical and microbiological 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.

Ten grams of each bran dough (see below) were suspended in 90 ml of sterile sodium chloride (0.9%, wt/vol) solution and homogenized in a Bag Mixer 400P (Interscience, St Nom, France) at room temperature to enumerate the microbial cell number. Serial 10-fold dilutions were then plated into modified De Man, Rogosa and Sharpe (maltose and fresh yeast extract were added at 1 and 5%, respectively, and the final pH was 5.6) (mMRS, Oxoid, Basingstoke, Hampshire, UK) supplemented with cycloheximide (0.1 g/l), Plate Count Agar (PCA, Oxoid) supplemented with cycloheximide (0.1 g/l) and Sabouraud Dextrose Agar (SDA, Oxoid), supplemented with chloramphenicol (0.1 g/l) were incubated at 30 °C for 48 h, and used to enumerate total mesophilic bacteria, presumptive LAB and yeasts, respectively. Total *Enterobacteria* were determined on Violet Red Bile Glucose Agar (VRBGA, Oxoid) at 37 °C for 24 h and molds were enumerated on Potato Dextrose Agar (PDA, Oxoid) at 32–35 °C for 48 h.

2.3. Microorganisms

2.3.1. Cultivation

Aiming at investigating a wide microbial diversity, seventy strains of LAB (Supplementary Table S1) belonging to the Culture Collection of the Department of Soil, Plant and Food Science (University of Bari Aldo Moro, Italy) were used in this study. Strains were routinely cultivated on mMRS (Oxoid) medium until the late exponential phase of growth was reached (approximatively 12 h).

2.3.2. Screening

Aiming at selecting strains to be used as mixed starter for bran fermentation, the pro-technological and functional features of LAB were evaluated when singly inoculated in their own isolation matrix (wheat, quinoa, hemp and hop flours and wheat germ) (Nionelli et al., 2014, 2018a, 2018b; Pontonio et al., 2015; Rizzello et al., 2010a, 2016). Cells were harvested by centrifugation (10,000 × *g*, 10 min, 4 °C), washed twice in 50 mM phosphate buffer, pH 7.0, and re-suspended in tap water. The DY (dough yield, dough weight × 100/flour weight) was 200 and the initial cell density of each LAB was ca. 7.0 Log cfu/g. Doughs were prepared in sterile beakers (diameter, 100 mm; height 90 mm), mixed manually for 5 min and incubated at 30 °C for 24 h.

Fermentation was carried out in triplicate. After fermentation, samples were stored at 4 °C and analyzed within 2 h. Non-inoculated doughs were used as controls. Proteolytic by means of total free amino acids (TFAA), phytase and radical scavenging (in the methanolic extract) activities were considered as functional features. Kinetics of growth and acidification were also considered as pro-technological criteria of selection.

Kinetics of growth and acidification were determined and modelled in agreement with the Gompertz equation, as modified by Zwietering et al. (1990): $y = k + A \exp.\{- \exp.[(\mu max \text{ or } Vmax\ e/A)(\lambda\text{-}t) + 1]\}$; where y is the growth expressed as Log cfu/g/h or the acidification rate expressed as dpH/dt (units of pH/h) at the time t; k is the initial level of the dependent variable to be modelled (Log cfu/g or pH units); A is the cell density or pH (units) variation (between inoculation and the stationary phase); μmax or Vmax is the maximum growth rate expressed as Δ Log cfu/g/h or the maximum acidification rate expressed as dpH/h, respectively; λ is the length of the lag phase measured in hours. Experimental data were modelled by the non-linear regression procedure of the Statistica 12.0 software (Statsoft, Tulsa, USA). The values of pH of doughs were determined by a M.507 pHmeter (Crison, Milan, Italy) equipped with a food penetration probe.

Water/salt-soluble extracts (WSE) from doughs were prepared according to the method originally described by Osborne (1907) and modified by Weiss et al. (1993). Briefly, sample containing 1 g of flour was suspended in 4 ml of 50 mM Tris–HCl (pH 8.8), incubated at 4 °C for 1 h under stirring conditions (150 rpm) and centrifuged at 12,000 *xg* for 20 min. The supernatant was used for the determination of TFAA concentration and phytase activity. 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). Phytase activity was determined by monitoring the rate of hydrolysis of *p*-nitrophenyl phosphate (*p*-NPP) (Sigma, 104–0). The assay mixture contained 200 μl of 1.5 mM *p*-NPP (final concentration) in 0.2 M Na-acetate, pH 5.2, and 400 μl of WSE. The mixture was incubated at 45 °C and the reaction was stopped by adding 600 μl of 0.1 M NaOH. The *p*-nitrophenol released was determined by measuring the absorbance at 405 nm (Rizzello et al., 2010a). One unit (U) of activity was defined as the amount of enzyme required to liberate 1 μmol/min of *p*-nitrophenol under the assay conditions. The radical scavenging activity was determined on the ME methanolic extract (ME) of doughs. Three grams of each sample were mixed with 30 ml of methanol (80%, vol/vol) to get ME. The mixture was purged with nitrogen stream for 30 min, under stirring condition, and centrifuged at 4600 × *g* for 20 min. The supernatants (MEs) were transferred into test tubes, purged with nitrogen stream and stored at ca. 4 °C before analysis. The radical DPPH· was used for determining the free radical scavenging activity (Rizzello et al., 2010a). The synthetic antioxidant butylated hydroxytoluene (BHT) was included in the analysis as the reference (75 ppm). The reaction was monitored by reading the absorbance at 517 nm.

Based on the results collected under the above conditions, the ten best performing strains were selected and further characterized.

2.3.3. Starter selection for bran fermentation

Aiming at evaluating the performances in bran matrix, the ten best performing LAB were singly inoculated in 50 g of wheat bran doughs (DY 300). Doughs and cell suspensions were prepared as described above. Non-inoculated bran doughs prior (CT_0) and after (CT_{24}) incubation were used as the controls.

Singly fermented doughs were characterized according to the pro-technological (growth and acidification) and metabolic (e.g., proteolysis, release of antioxidant compounds and phytic acid degradation) traits affecting the nutritional properties of the dough.

Cell density of LAB, value of pH, concentration of organic acids and TFAA and radical scavenging activity in the ME were determined as reported above. Total titratable acidity (TTA) was determined on 10 g of dough homogenized with 90 ml of distilled water and expressed as the amount (ml) of 0.1 M NaOH to reach pH of 8.3. Phytic acid concentration was measured using K-PHYT 05/07 kit assay (Megazyme Intl., Ireland), following the manufacturer's instructions. Total phenols were determined on the ME of bran doughs as described by Slinkard and Singleton (1977) and expressed as gallic acid equivalent. The two best performing strains were selected and used for wheat, barley and emmer brans fermentation.

2.4. Bran fermentation and characterization

Lactobacillus plantarum T6B10 and *Weissella confusa* BAN8 were used as a mixed starter (1:1) for sourdough fermentation of brans from wheat (Aubusson, FB1, Skorpion, FB2; Bonavita, FB3), barley (var. Rondo, FB4) and emmer (var. Giovanni Paolo, FB5). A xylanase, (Depol 761, Biocatalysts Limited, Chicago, USA) at 1% (wt/wt) based on weight of bran, was used to increase the release of soluble fiber (Arte et al., 2015). Doughs (DY 300) and cell suspensions were prepared as described above (paragraph 2.3.3). Fermentations were carried out in triplicate. Bran doughs prior fermentation (B1, B2, B3, B4 and B5) were used as the controls. Microbiological, biochemical and nutritional analyses were carried out as reported above. To determine the presence and the eventual dominance of the single strains after fermentation, at least 15 colonies of presumptive LAB were randomly selected from mMRS plates containing the two highest sample dilutions, isolated and subjected to genotypic characterization by Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis (Nionelli et al., 2018a). The RAPD-profiles of the isolated strains were compared to those of the starters used for the fermentation.

For the peptides analysis, WSE were treated with trifluoroacetic acid (0.05% wt/vol) and centrifuged (10,000 × *g* for 10 min) to remove proteins. Then, samples were transferred into dialysis tubes (cut-off 500 Da, Fisher Scientific, Rodano, Italy) and dialyzed against water (1 l per 5 ml of sample) at 4 °C for 48 h to remove FAA. Retentates were freeze-dried and then resuspended in 50 mM Tris–HCl (pH 8.8). Then peptide concentration was determined by the *o*-phtaldialdehyde (OPA) method as described by Church et al. (1983). 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).

2.5. 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 (Zeus IBA S.R.L., Florence, Italy) (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. 7 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 2 h. All breads were baked at 220 °C for 50 min (Combo 3, Zucchelli, Verona, Italy). Wheat flour used for breadmaking had the following

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 (%)	24.7 ± 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^{c}	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}

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. Giovanni Paolo. Data are expressed on dry matter.
$^{a-d}$Values in the same row with different superscript letters differ significantly ($p < .05$).

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 25 °C 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.

2.6. 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.

2.7. Sensory analysis

Sensory analysis of breads was carried out by 10 trained panellists (5 male and 5 females, mean age: 35 years, range: 18–54 years), according to the method described by Haglund et al. (1998). 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. Elasticity, gumminess, acidic aroma and taste, color crumb and crust and salty taste were chosen as attributes to characterize the bread. A quarter of each piece of bread (including crust and crumb) was presented (in randomized order) on a plastic plate encoded with a three-digit number. Mineral water was available to clear the palate between samples. According to the IFST Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods, assessors gave informed consent to tests and could withdraw from the panel at any time, without penalty or having to give a reason.

2.8. 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 $p < .05$, 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 ($p > .05$) 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 ($p < .05$) differed. B1 contained the lowest and the highest concentrations of protein (15.9 ± 0.5%) and fat (4.5 ± 0.6%), respectively. B5 was characterized by the lowest contents of both fat and ash (3.3 ± 0.4 and 2.3 ± 0.4%, respectively). The highest concentration of protein (18.9 ± 0.4%) was found in bran of barley variety (B4).

Table 2 summarizes the microbiological and biochemical characterization of bran doughs prior the fermentation. Total mesophilic bacteria and presumptive LAB ranged from 5.7 ± 0.2 to 5.8 ± 0.3 Log cfu/g and from 3.5 ± 0.1 to 3.8 ± 0.3 Log cfu/g, respectively. Molds and yeasts were from 1.2 ± 0.2 to 3.7 ± 0.2 Log cfu/g and from 2.3 ± 0.1 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 concentration of TFFA varied from 675 ± 15 (B1) to 1653 ± 31 (B2) mg/kg. Total phenols concentration and radical scavenging activity in the ME were in the range 1.22 ± 0.03 (B4) - 1.93 ± 0.04 (B5) mmol/kg and 34.4 ± 0.6 (B1) to 59.3 ± 0.8% (B4), respectively (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 found in any of bran doughs. Phytic acid was found in the range 330 ± 15 (B5) - 900 ± 21 mg/100 g (B3). According to the TFAA concentrations, highest and lowest IVPD were found in B1 and B2, respectively (Table 2).

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 0.1 M)	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/100 g)	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.
IVPD (%)	25.4 ± 0.6^{a}	35.1 ± 0.2^{c}	31.2 ± 0.7^{b}	29.4 ± 0.4^{a}	32.4 ± 0.8^{b}

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 from; B4, dough made with barley (var. Rondo) bran; B5, dough made with emmer (var. Giovanni Paolo) bran.
LAB, Lactic acid bacteria.
n.d. not detectable.
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 < .05).

3.2. Lactic acid bacteria

3.2.1. Screening

LAB strains were singly used to ferment wheat, quinoa, hemp and hop flours and wheat germ at 30 °C for 24 h. To allow the comparison between results from different food matrices, the increase (%) of TFAA concentration, phytase and radical scavenging activities, as compared to the corresponding non-inoculated doughs, were considered (Fig. 1). Increases of TFAA were in the range 13–84%, being the highest for L. *plantarum* T6B10 and the lowest for *Lc. lactis* LVS26. Similarly, highest and lowest values of phytase activity were reached when L. *plantarum* T6B10 (81.7%) and *Lc. lactis* LVS 26 (3.8%) were used, respectively. Moreover, strains of *Leuc. citreum* STF28, *W. confusa* KAS3 and BAN8 and L. *plantarum* T0A16 fell in the 75% percentile of the phytase activity. Increases of the radical scavenging activity were also found after the fermentation with LAB. Highest increases were found when L. *plantarum* LIN 2 and T6B10 and L. *rossiae* T0A16 (ca. 44%) were used as starter (Fig. 1). However, strains of *W. confusa* BAN1 and BAN2 and *P. pentosaceous* BAR4 fell in 75% percentile. According to the pro-technological features, *W. confusa* BAN8 showed the highest cell density increase (A_G, 2.4 log10 cfu/g) followed by *W. confusa* NEY (A_G, 2.2 log10 cfu/g) and *P. pentosaceous* NEJ1 (2.1 log10 cfu/g). Moreover, both L. *plantarum* T6B10 and *W. confusa* BAN8 fell into the 75% and 25% percentile of the A_A and λ_A and λ_G, respectively (Fig. 1).

3.2.2. Selection

Based on the above results, *L. plantarum* (T6B10, STF28 and Lin 22), *L. rossiae* (T0A16), *W. confusa* (BAN8 and KAS3) and *P. pentosaceus* (BAR 4, BAN1, BAN2 and NEJ1) were selected and used for further analyses.

The ten LAB 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 LAB strains 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 *Enterobacteria* 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 0.1 M 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 (9.2 ± 0.8 mmol/kg) (Table 3). However, acetic acid (8.8 ± 0.7–9.2 ± 0.8 mmol/kg) 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 < .05) higher than the median values. Similarly, when *W. confusa* BAN8 was used as starter, the lowest value of phytic acid concentration (391 ± 11 mg/100 g) 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.

3.3. Bran fermentation with selected mixed starter

Table 4 shows the biochemical and nutritional properties of the fermented brans (FB) with the mixed starter. After 24 h of fermentation, the cell number of LAB increased by ca. 2 Log cfu/g, regardless the type of bran. LAB isolated from fermented brans were bio-typed. Regardless the bran used, both L. *plantarum* T6B10 and *W. confusa* BAN8 reached the same cell density (ca. 9 Log cfu/g) at the end of fermentation. However, slight differences were found in terms of relative abundance (Supplementary Fig. S1). Indeed, *L. plantarum* T6B10 seems to dominate on *W. confusa* BAN8 in all FB, with the only exception of FB4 (Supplementary Fig. S1).

The values of pH decreased during the fermentation, being in the range of 3.9–4.1, without significant (p > .05) differences among

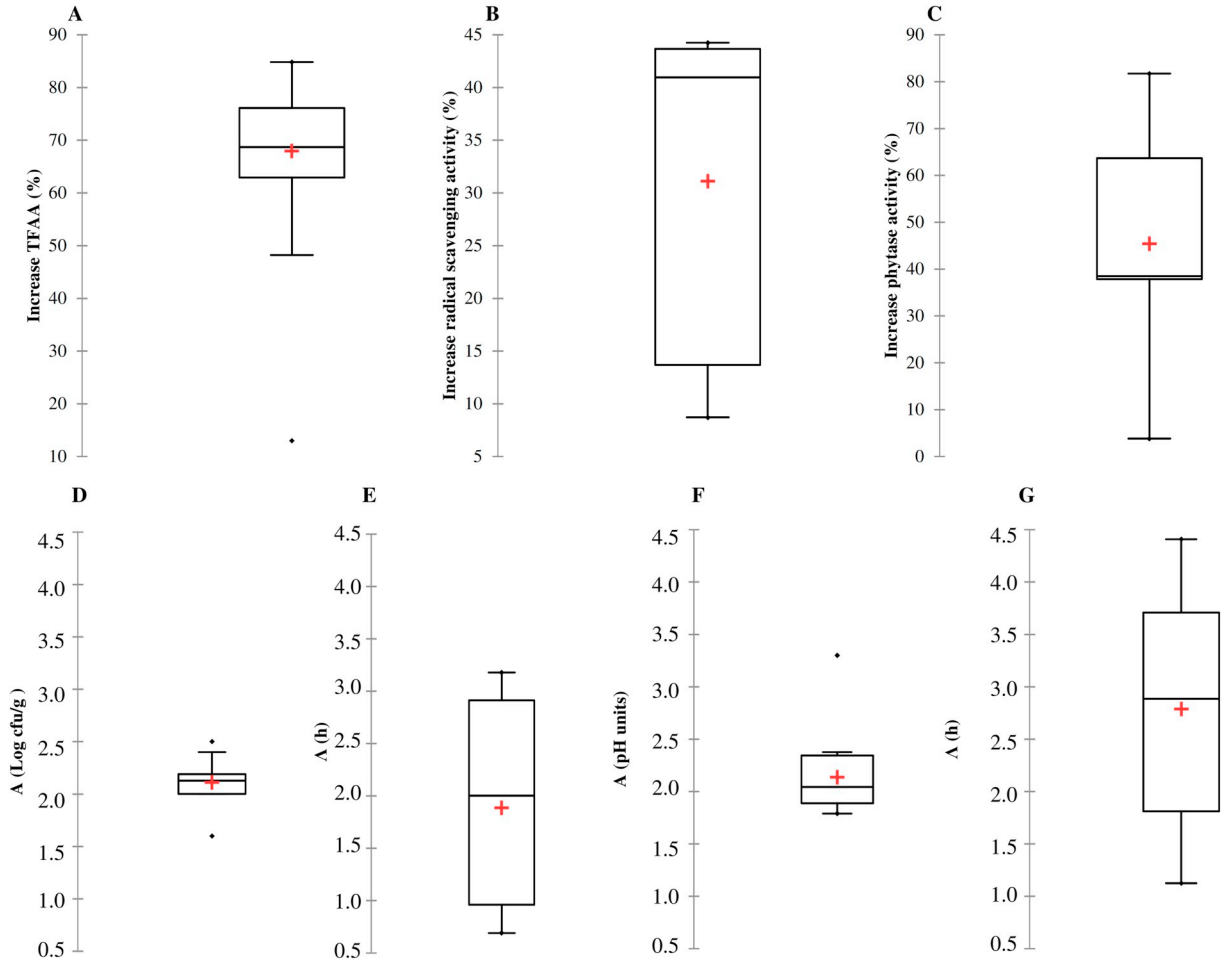

Fig. 1. Boxplot showing the functional (panels A–C) and pro-technological (panels D–G) characterization of 70 strains of lactic acid bacteria belonging to the species *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus rossiae*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Weissella cibaria*, *Weissella confusa*, *Leuconostoc citreum* and *Leuconostoc mesenteroides* of the Culture Collection of the Department of Soil, Plant and Food Science of the University of Bari A. Moro, Italy and isolated from raw or spontaneously fermented wheat, hemp, hop, quinoa, wheat germ and bran. The increase (%) of TFAA concentration (panel A), radical scavenging activity (B) and phytase activity (C) in wheat, hemp, hop, quinoa, wheat germ and bran (DY 200) singly inoculated with the strains and fermented for 24 h at 30 °C, compared to a not inoculated dough incubated in the same conditions. Panels D, E and F, G represent the boxplot of the acidification and growth kinetics parameters of the strains in the above-mentioned conditions, respectively. Median (black line inside the box), mean (+), minimum and maximum (*) values are represented. The top and the bottom of the box represent the 75th and 25th percentile of the data, respectively. The top and the bottom of the bars represent the 5th and the 95th percentile of the data, respectively.

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^{a}	n.d.	9.2 ± 0.8^{a}
QF	n.d.	n.d.	6.4^{a}	6.9^{a}	n.d.	6.6^{a}
TFAA (mg/kg)	675 ± 15^{a}	690 ± 21^{a}	1980 ± 26^{b}	2625 ± 39^{d}	2043 ± 36^{b}	2478 ± 38^{c}
Phytic acid (mg/100 g)	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 ($p < .05$).

Table 4
Biochemical and nutritional characteristics of the wheat, barley and emmer bran fermented with *Lactobacillus plantarum* T6B10 and *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 0.1 M)	52.0 ± 0.5^{d}	49.2 ± 0.4^{c}	51.2 ± 0.6^{d}	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^{b}	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^{b}
QF	8.0^{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^{c}	19.9 ± 0.2^{a}	20.4 ± 0.3^{ab}	21.2 ± 0.4^{b}
Nutritional characteristics					
Phytic acid (mg/100 g)	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^{c}	65.5 ± 0.5^{d}	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^{d}
IVPD (%)	82.5 ± 0.6^{b}	87.1 ± 0.5^{c}	81.0 ± 0.7^{a}	83.6 ± 0.5^{b}	80.1 ± 0.4^{a}

FB1, fermented dough made with wheat (cv. Aubusson) bran; FB2, fermented dough made with wheat (cv. Skorpion) bran; FB3, fermented dough made with wheat (cv. Bona Vita) bran; FB4, fermented dough made with barley (var. Rondo) bran; FB5, 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 ($p < .05$).

doughs. On the contrary, TTA significantly ($p < .05$) differed, with the highest and lowest values for FB1 (cv Aubusson) and FB5 (var. Rondo), respectively. Overall, the use of the mixed starter led to increases up to 30 and 14% of the lactic and acetic acids concentrations, respectively in FB (Table 4) as compared to maximum achieved with single strains (Table 3). Compared to bran doughs prior the fermentation (Table 2), the concentration of TFAA increased up to three times (Table 4). FB1 and FB2 showed the highest and lowest increases, respectively (Tables 2 and 4). The fermentation also promoted an overall increase of the peptide concentration up to 40%.

The fermentation with the mixed starter led also to an improvement of the nutritional features (Table 4). As compared to the un-fermented doughs (Table 2), fermented brans had lower concentrations of phytic acid (Table 4). The lowest decrease was found when the B5 was fermented, indeed the concentration of phytic acid was 24% lower in FB5 as compared to B5. The highest decrease (60%) was found when B3 was fermented, although FB3 still contained the highest concentration (370 ± 21 mg/100 g). According to the type of bran, the concentrations of phenols increased from 10 to 60% during fermentation. The radical scavenging activity of the ME increased from 10 (FB5) to 70% (FB1), where the highest values were reached in FB2, FB3 and FB4. These results corroborated the data of total phenol concentrations. Although values of the radical scavenging activity of the WSE were undetectable in un-fermented doughs (Table 2) values ranging from 30.7 ± 0.4% (FB1) to 44.7 ± 0.3% (FB5) were achieved through fermentation (Table 4).

The IVPD values of fermented bran doughs ranged between 80.1 ± 0.4% and 87.1 ± 0.5%, being the highest and lowest for FB2 and FB5, respectively. Increases up to two times (Table 4) were found as compared to un-fermented bran doughs (Table 2).

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 slight water retention during baking, which was confirmed by the higher values of 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 ($p < .05$) 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 ($p < .05$) 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 70%). The comparison also showed higher values of radical scavenging activities for both WSE (up to ca. 40%) and ME (up to 3 times). The former varied from 30.2 ± 0.5% (FB1-B) to 39.2 ± 0.4% (FB2-B) and the latter was in the range 27.8 ± 0.4% (FB5-B) – 62.0 ± 0.4 (FB2-B). 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 ($p < .05$) increases of DF (up to 6 times) and proteins (up 2 times) (Table 5). Compared to WB, a significant decrease (ca. 8%) of the *pGI* was observed. The lowest value was found for FB3-B (65.1 ± 0.2). Significant ($p < .05$) increases of IVPD were observed, which varied depending on the type of bran (Table 5). Lowest (65 ± 2%) and highest (79 ± 2%) values were found in FB2-B and FB4-B, respectively.

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

Compared to WB (2.31 ± 0.01 cm^3/g), the specific volumes of breads fortified with FB3, FB4 and FB5 increased (2.65 ± 0.07–2.88 ± 0.04 cm^3/g, in FB3-B and FB5-B, respectively) (Table 6). On the contrary, decreases (up to ca. 40%) of resilience (0.80 ± 0.03–0.82 ± 0.07 for FB3-B and FB5-B, respectively) and cohesiveness (0.40 ± 0.05–0.62 ± 0.06 for FB2-B and FB5-B, respectively) were found when FB were added to the bread formula (Table 6).

Hardness (3040 ± 28–5270 ± 39 in FB4-B and FB3-B, respectively), gumminess (15.8 ± 0.5–31.3 ± 0.9 for FB1-B and FB5-B, respectively) and chewiness (1290 ± 24–2594 ± 29 for FB1-B and FB5-B, respectively) increased up to ca. 2 and 4 times, respectively when FB were used in breadmaking (Table 6). 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 ($p > .05$) differences were found in term of resilience.

The addition of FB in bread formula, significantly ($p < .05$)

Table 5
Physical-chemical, biochemical and nutritional characteristics of experimental breads (DY, 180) containing 30% (wt/wt) of wheat, barley and emmer bran doughs and fermented with *Lactobacillus plantarum* T6B10 and *Weissella confusa* BAN8 (initial cell density of ca. 7 Log cfu/g) at 30 °C for 24 h.

	FB1-B	FB2-B	FB3-B	FB4-B	FB5-B	WB
Physical-chemical characteristics						
Moisture (%)	27.3 ± 0.4^{b}	27.2 ± 0.7^{b}	26.4 ± 0.2^{a}	26.8 ± 0.4^{ab}	27.2 ± 0.6^{b}	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^{b}	0.92 ± 0.02^{a}
Biochemical characteristics						
pH	4.1 ± 0.5^{b}	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^{d}	25.4 ± 0.3^{e}	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^{e}	36.38 ± 0.4^{d}	37.24 ± 0.5^{d}	25.31 ± 0.6^{b}	28.65 ± 0.4^{c}	3.3 ± 0.5^{a}
Acetic acid (mmol/Kg)	6.69 ± 0.5^{c}	6.61 ± 0.7^{c}	5.29 ± 0.6^{b}	4.86 ± 0.4^{b}	5.32 ± 0.6^{b}	1.27 ± 0.3^{a}
FQ	6.8	5.5	7.0	5.2	5.4	2.6
TFAA (mg/Kg)	654 ± 13^{c}	597 ± 11^{b}	858 ± 14^{d}	632 ± 16^{c}	888 ± 19^{e}	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^{b}	12.7 ± 0.5^{b}	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^{b}	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^{d}	0.87 ± 0.05^{b}	0.89 ± 0.04^{b}	0.27 ± 0.02^{a}
IVPD (%)	74 ± 1^{c}	65 ± 2^{b}	79 ± 1^{d}	79 ± 2^{d}	68 ± 2^{b}	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/100 g)	141 ± 13^{c}	304 ± 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^{e}	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}

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. Bonavita) 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; WB, bread made with baker's yeast.
Data of protein, fat, carbohydrates, fiber, and ash are expressed on dry weight basis.
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 ($p < .05$).

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 ($p > .05$) differ among breads. However, *dE* (calculated based on the chromaticity co-ordinates) significantly differ from WB (33.1 ± 0.5) when FB (38.5 ± 0.6–50.4 ± 0.7 in FB4-B and FB5-B, respectively) 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 ($p > .05$) 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 (Fig. 2). 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

Table 6
Textural characteristics of experimental breads (DY, 180) containing 30% (wt/wt) of wheat, barley and emmer bran doughs and fermented with *Lactobacillus plantarum* T6B10 and *Weissella confusa* BAN8 (initial cell density of ca. 7 Log cfu/g) at 30 °C for 24 h.

	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}	2.71 ± 0.05^{cd}	2.88 ± 0.04^{d}	2.31 ± 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^{a}	0.56 ± 0.04^{b}	0.62 ± 0.06^{bc}	0.70 ± 0.07^{c}
Gumminess	15.8 ± 0.5^{b}	19.0 ± 0.9^{d}	22.4 ± 0.8^{e}	16.9 ± 0.4^{c}	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^{d}	2.5 ± 0.1^{a}
b	23.0 ± 0.3^{ab}	23.0 ± 0.2^{a}	23.7 ± 0.4^{b}	23.3 ± 0.2^{ab}	23.3 ± 0.4^{ab}	23.4 ± 0.3^{ab}
dE	45.2 ± 0.6^{c}	45.7 ± 0.4^{c}	41.2 ± 0.5^{b}	38.5 ± 0.6^{b}	50.4 ± 0.7^{d}	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; WB, bread made with baker's yeast.
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 ($p < .05$).

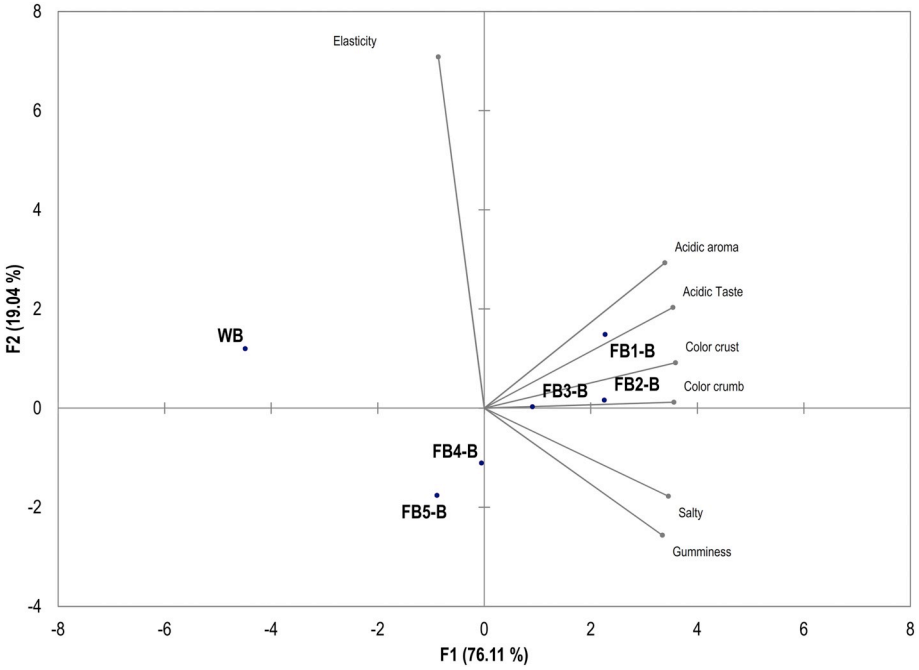

Fig. 2. Principal component analysis (PCA) based on sensory analysis of breads (DY, 180) FB1-B, bread containing 30% (wt/wt) of fermented bran doughs obtained from red-grained wheat variety (cv Aubusson) (FB1); FB2-B, bread containing 30% (wt/wt) of fermented bran obtained from blue-grained wheat variety (cv Skorpion) (FB2); FB3-B, bread containing 30% (wt/wt) of fermented bran obtained from yellow-grained wheat variety (cv Bona Vita) (FB3); FB4-B, bread containing 30% (wt/wt) of fermented bran obtained from spring hull-less barley (var. Rondo) (FB4); FB5-B, bread containing 30% (wt/wt) of fermented bran obtained from emmer (var. Schrank) (FB5); WB, white wheat bread. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

below the recommendations (Stephen et al., 2017). Nutrition guidelines from United States (U.S. Department of Health and Human Services, 2015) and Europe (European Food Safety Authority) (EFSA, 2010) 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 LAB 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 LAB and used in bread-making. Based on a selection process among 70 strains of LAB according to pro-technological and functional features (Fig. 1 and Table 3), *L. plantarum* T6B10 and *W. 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 LAB growth and acidification. Both starters outcompete the resident microbiota with a slightly higher dominance of L. *plantarum* T6B10 on *W. confusa* BAN8 in four out of five fermented brans. Data from fermented brans were elaborated through Principal Component Analysis (PCA) (Fig. 3). 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. Overall, the used of a mixed starter led to better nutritional profiles of the brans as compared to the single strains. 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. Overall, 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 during fermentation reaching the lowest value (230 ± 14 mg/100 g) in FB1 (Table 4). Proteolysis via the combined activity of endogenous proteases (also activated by acidification) and LAB peptidases led to an increase of TFAA with FB3 containing the highest concentration (up to 3899 ± 41 mg/Kg) (Gänzle, 2014). The use of a mixed starter allows the combination of peptidases with different substrate specificity thus influencing the composition and the concentration of amino acids in doughs (Gänzle et al., 2008). Amino acids and short-chain peptides affect the taste of fermented foods and are important precursors for volatile flavor compounds, which generate during baking (Gänzle et al., 2008). 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. However, the fermentation by LAB flanked by the use of xylanase led to values of IVPD of ca. 87% (FB2), much higher than those commonly found for wheat bran (Arte et al., 2015; Bilgiçli et al., 2006). Overall, lactic acidification also improves the level of extractable

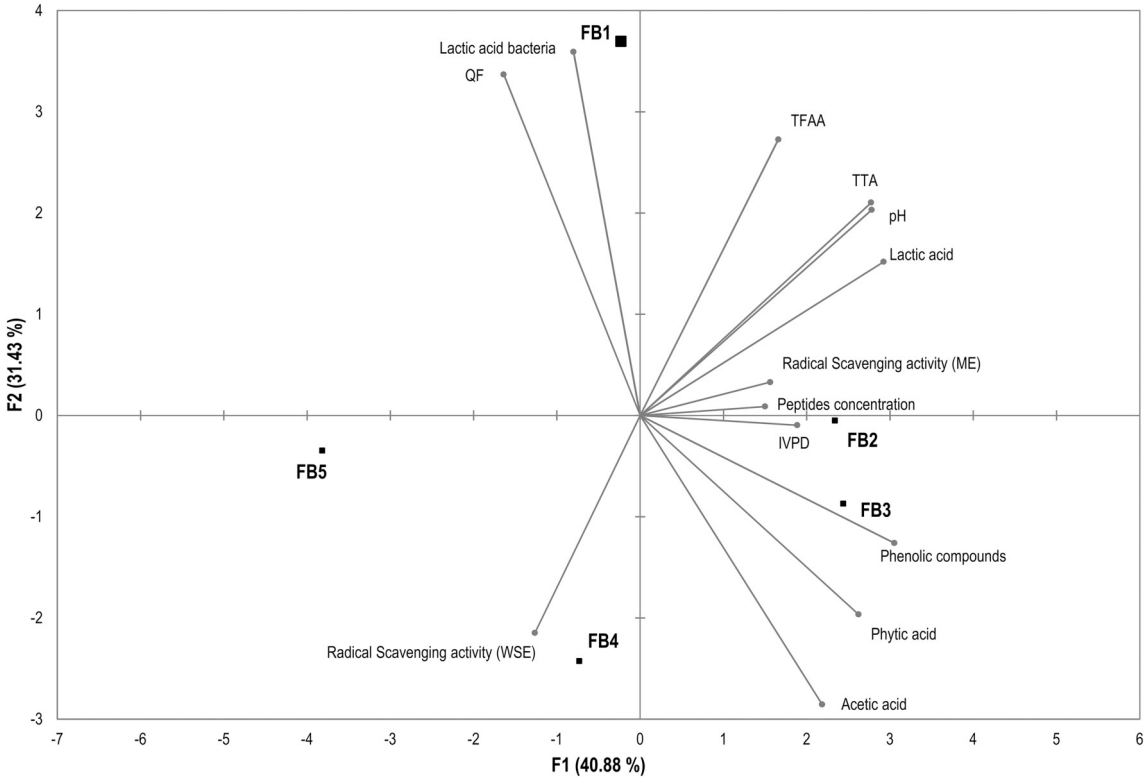

Fig. 3. Principal component analysis (PCA) based on biochemical and nutritional characteristics of wheat, emmer and barley bran doughs (DY 300) fermented with *Lactobacillus plantarum* T6B10 and *Weissella confusa* BAN8 at 30 °C for 24 h. The ingredients and technological parameters for the preparation of fermented bran doughs (FB1, FB2, FB3, FB4 and FB5) are reported in the Materials and methods section.

phenolic compounds having antioxidant activity and whose profile is further modified by the activity of LAB enzymes (e.g., feruloyl-esterase and β-glucosidase) (Filannino et al., 2015). Indeed, higher values of radical scavenging activity were found in fermented brans with FB1 and FB4 characterized by the highest increase (ca. 73%) and value (65.5 ± 0.5%), respectively (Table 4). Overall, high values of TTA were reached at the end of fermentation probably due to the significant content of the phenolic acid (Laddomada et al., 2015) as well as the good buffering capacity (Salmenkallio-Marttila et al., 2001) of bran fractions.

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 improved nutritional value with each sample having its own peculiar profile. Overall, 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 were found. 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 *pGI* values lower (8%) 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 ca. 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 LAB proteolysis (Rizzello et al., Unpublished results). 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 LAB 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 LAB 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.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.108384.

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