C&e Focus Issue

UNLOCKING THE POTENTIAL OF CEREAL GRAINS

Solutions Through Science-Based Innovation

Study of the Chemical Changes and Evolution of Microbiota During Sourdoughlike Fermentation of Wheat Bran

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ABSTRACT

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Several studies have emphasized the possibility of enhancing nutritional properties of cereal by-products through biotechnological processes. Bran fermentation positively affects the bioavailability of several functional compounds. Moreover, bran fermentation could increase water-extractable arabinoxylans (WEAX), compounds with positive effects on glucose metabolism and prebiotic properties. This study was aimed at increasing the amount of bran bioactive compounds through a sourdoughlike fermentation process. Wheat bran fermentations were conducted through continuous propagation by back-slopping of fermented bran (10% inoculum) until a stable microbiota was established, reaching high counts of lactic acid bacteria and yeasts (10^9 and 10^7 CFU/g, respectively). At each refreshment step, bacterial strains were isolated, clustered, molecularly analyzed by randomly amplified polymorphic DNA, and identified at the species level by 16S rRNA gene sequencing. *Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus sakei, Lactobacillus plantarum, Pediococcus pentosaceus,* and *Pichia fermentans* dominated the stable sourdough ecosystem. After fermentation, levels of soluble fiber increased (+30%), and WEAX and free ferulic acid were respectively fourfold and tenfold higher than in raw bran, results probably related to microbial xylan-degrading activity, whereas phytic acid was completely degraded. These preliminary data suggest that fermented bran could be considered an interesting functional ingredient for nutritional enhancement.

Several studies indicate that diets high in whole grain work as protective factors against chronic diseases (Gil et al 2011; Qing Ye et al 2012; McKeown et al 2013). These effects are likely related, at least in part, to their high content of fiber and bioactive compounds, with antioxidants and anticarcinogenic properties, mainly present in bran and germ of cereal grains (Fardet 2010; Okarter and Liu 2010). Removal of these fractions during milling to improve the shelf life of flour results in severe depletion of fiber and bioactive compounds. The loss of about 58% of fiber, 83% of magnesium, 61% of folate, and 79% of vitamin E has been shown in comparing the contents of important nutrients in wholemeal flour and white flour (Truswell 2002). The aleurone layer (the outermost layer of the endosperm) has been shown to contain many of these functional compounds (Brouns et al 2012), but it is partially eliminated in wheat flour milling and is a by-product mostly used for animal feed. The increasing demand for functional foods and the possibility of taking advantage of agro-industrial by-products have attracted great interest in using bran-enriched products. Doing so should lead to a greater value for wheat industries, reducing their environmental impact and getting an economic return. The main reasons behind the low utilization rate of wheat bran in the baking industry are the gritty texture, bitter and pungent flavor, and coarse mouthfeel of bread caused by bran. However, the fermentation of cereal bran, such as wheat and rye, has shown to be an interesting pretreatment to improve technological, sensorial, and nutritional properties of bran-enriched products (Katina et al 2007, 2012; Poutanen et al 2009; Coda et al 2014) as well as to degrade antinutritive factors such as phytic acid to increase mineral bioavailability (Lopez et al 2000). Bioprocessing of wheat bran with enzymes and microbes has been used to improve loaf volume, crumb structure, and shelf life of bread supplemented with fermented bran (Salmenkallio-Marttila et al 2001; Coda et al 2014). Moreover, Katina et al (2012) showed that yeast fermentation of wheat bran from peeled kernels increased the level of folates (+40%), free phenolic acids (+500%), and soluble arabinoxylans (AX) (+60%). Many of the observed changes during fermentation (e.g., dietary fiber solubilization) can be explained by the contribution of endogenous or microbial enzymes, especially xylanases (Katina et al 2007; Poutanen et al 2009). From a nutritional point of view, AX and compounds resulting from their hydrolysis, such as AX oligosaccharides (AXOS) and xylooligosaccharides, deserve particular attention. AX represent the major component of the dietary fiber fraction of cereal grains and are mainly found in the outer tissues of the wheat caryopsis as a water-unextractable fraction (WUAX), whereas most water-extractable AX (WEAX) are distributed in the endosperm. AX consist of a linear backbone of xylose residues, unsubstituted or mono- or disubstituted with residues of arabinose. AX also contain large amounts of ferulic acid and other phenolic compounds covalently linked to them (Saeed et al 2011). These compounds have different physicochemical and functional properties depending on their solubility and structural characteristics. Because of their ability to induce viscosity, WEAX are able to delay the rate

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of digestion and absorption of carbohydrates, leading to positive effects on the postprandial glycemic and insulinemic responses (Lu et al 2000, 2004; Möhlig et al 2005). A European Food Safety Authority panel reported a cause-and-effect relationship between the consumption of AX produced from wheat endosperm and a reduction in postprandial glycemic response (EFSA 2011). Moreover, the soluble oligosaccharides (AXOS and xylooligosaccharides) resulting from hydrolysis of bran WUAX have a potential prebiotic effect and anticarcinogenic, antioxidant, and hypocholesterolemic properties (Broekaert et al 2011; Francois et al 2012).

The aim of the current study was to improve the amount of bioactive compounds of wheat bran through a sourdoughlike fermentation process to use the fermented bran as a functional ingredient. The outer layers of grain are rich in dietary fiber, phytochemicals, vitamins, minerals, and endogenous enzymes. Therefore, bran fermentation could be an interesting tool to enhance the bioavailability of these functional compounds and, thus, improve the nutritional quality of the wheat bran. In particular, this study was performed to determine the effect of fermentation on fiber solubilization, mainly on the AX fraction, and on the amount of other bioactive compounds, such as ferulic acid and phytic acid. Moreover, the microbes involved in bran fermentation were isolated, clustered, and identified for future study regarding their potential use as starter cultures to increase the amount of bran bioactive compounds.

MATERIALS AND METHODS

Fermentation Process and Sampling. Spontaneous fermentations (without microbial starters) were developed from commercial native wheat bran (raw, untreated) (mean particle size 475–633 µm, Molino Quaglia, Vighizzolo D'Este, Italy) by mixing 28% bran and 72% water in a large beaker (2,000 mL) and covering it with aluminum foil. The fermentation process was performed as a traditional type I sourdough, characterized by low incubation temperatures and daily refreshments to keep the microorganisms in an active state (Meroth et al 2003; De Vuyst and Neysens 2005). Fermentation batches were produced in triplicate, at 18°C, through continuous propagation by back-slopping of the fermented bran until a stable microbiota was established (13 days). At every refreshment step (once a day), the fermented bran was used as a 10% inoculum for the subsequent fermentation cycle. Fresh samples were taken from unfermented and fermented bran for microbiological analyses. In addition, samples were frozen for later measurements of pH, total titratable acidity (TTA), and lactic acid and for the quantification of bioactive compounds (dietary fiber, WEAX, free ferulic acid [FFA], and phytic acid).

Microbial Quantification and Isolation. Lactic acid bacteria (LAB), yeasts, and contaminant bacteria were quantified. A sample of 10 g was homogenized for 10 min with 90 mL of sterile saline–tryptone diluent (containing, per liter, 8.5 g of NaCl and 1.0 g of tryptone, pH 6.0) in a BagMixer 400 stomacher (Interscience, Saint-Nom-la-Bretèche, France), serially diluted 1:10 with quarter-strength Ringer's solution and plated on different media. LAB were determined on MRS5 agar (all ingredients were provided by Oxoid, Basingstoke, U.K.) containing 0.001% cycloheximide (Oxoid) to prevent fungal overgrowth. Plates were incubated anaerobically at 30°C for 72 h. Yeasts and molds were determined on Rose Bengal Chloramphenicol (RBC) agar (Biolife, Milan, Italy). Plates were incubated aerobically at 25°C for five days. Contaminant bacteria were determined on casein-peptone soymeal-peptone agar (Merck KGaA, Darmstadt, Germany), and plates were incubated aerobically at 30°C for two days. At each refreshment step of one batch of sourdoughlike fermentation, between 10 and 15 colonies of all morphologies were picked from MRS5 and RBC plates and streaked out several times on their respective agar plates to ensure their purity. After microscopic and morphological examination, among a total of 165 isolates, 98 LAB and 13 yeasts were obtained and further characterized.

Molecular Characterization of LAB Strains by Randomly Amplified Polymorphic DNA–Polymerase Chain Reaction (RAPD-PCR) Analysis. RAPD-PCR profiles were used to perform a first strain differentiation and to explore the genetic diversity of LAB isolated from the fermented bran. Total genomic DNA from the strains was extracted with a microlysis kit (Labogen, Rho, Italy) following the manufacturer's instructions. RAPD-PCR reactions were performed with primer M13 (5′-GAGGGT GGCGGTTCT-3′, Huey and Hall 1989). Amplification conditions, as well as electrophoresis and analysis of the amplification products, were as previously described by Andrighetto et al (2002) and Morandi et al (2006). Grouping of the RAPD-PCR profiles was obtained with the BioNumeric 5.0 software package (Applied Maths, Kortrijk, Belgium) following the unweighted pair-group method with arithmetic averages cluster analysis. The value for the repeatability of the RAPD-PCR assay, DNA extraction, and running conditions, evaluated by analysis of repeated DNA extracts of the type strains, was 95%.

Molecular Identification of the LAB and Yeast Strains. For the LAB, a fragment of ≈800 bp of the 16S rRNA gene was amplified by PCR with the primers p8FPL (AGTTTGATCCTGGCT CAG) and p806R (GGACTACCAGGGTATCTAAT) (Hosseini et al 2009). For the isolated yeasts, a fragment of ≈500–1,300 bp of the D1/D2 domain of the 26S rDNA gene was amplified by PCR with the primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG) and NL-4 (5′-GGTCCGTGTTTCAAGACGG) (Kurtzman and Robnett 1997). PCR reaction was performed in a 25 µL total volume containing 2 units of Taq DNA polymerase (Finnzymes, Espoo, Finland), 0.5 µM of each primer, 200 µM of each dNTP, 1.5mM $MgCl_2$, and 50–100 ng of genomic DNA. PCR amplifications were performed with a Mastercycler thermal cycler (Eppendorf, Hamburg, Germany). The PCR parameters were as follows: initial denaturation at 94°C for 5 min; 30 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 7 min. The amplified PCR products were visualized by 1% agarose gel electrophoresis stained with SYBR Safe DNA gel stain (Thermo Fisher Scientific, Waltham, MA, U.S.A.). The gels were photographed under ultraviolet light with a UV transilluminator. Amplicons were sent for sequencing to Macrogene Europe (Amsterdam, The Netherlands).

Sequence alignment was carried out with ClustalW software. The BLAST algorithm was used to determine the most related sequence relatives in the National Center for Biotechnology Information nucleotide sequence database (www.ncbi.nlm.nih.gov/BLAST).

pH, TTA, and Lactic Acid. pH and TTA were determined on 10 g of fermented bran suspended in 100 mL of distilled water. For the determination of TTA, this suspension was titrated with 0.1M NaOH to a final pH of 8.5, detected by a pH meter (PHM 250, Radiometer, Copenhagen, Denmark); TTA was expressed as milliliters of 0.1M NaOH needed to achieve the final pH of 8.5. All samples were analyzed in duplicate. Lactic acid was determined by HPLC with refractive index detection as described by Lefebvre et al (2002).

Chemical Analysis. Analysis of moisture, ash, lipids, and proteins was carried out following AACC International Approved Methods 44-15.02, 08-01.01, 30-10.01, and 46-12.01, respectively. Sugars were assessed by high-performance anion-exchange liquid chromatography with pulsed amperometric detection (HPAEC-PAD) (Rocklin and Pohl 1983). Briefly, 1 g of the sample was extracted with 200 mL of distilled water at 60°C for 60 min (Zygmunt et al 1982); the extract solution was analyzed with an HPAEC-PAD equipped with a CarboPac PA1 (4 × 250 mm) column plus a CarboPac PA1 (4 × 50 mm) guard column (Dionex, Sunnyvale, CA, U.S.A.) and an ED50 pulsed amperometric detector (Dionex). Starch content was calculated by difference (100 – amount of all the other chemical components). Soluble and insoluble dietary fiber was evaluated by the enzymatic–gravimetric procedure

(AOAC International Method 991.43, which corresponds to AACCI Approved Method 32-07.01). Total AX and WEAX in native bran and fermented bran were determined by HPAEC-PAD as described by Saulnier and Quemener (2009) with some modification, after hydrolysis with trifluoroacetic acid (TFA) (Courtin et al 2000; Gebruers et al 2008). For total AX levels, native bran or fermented bran samples (150 mg) were hydrolyzed in 5 mL of 2M TFA for 60 min at 110°C. For WEAX evaluation, extracts were prepared by suspending the samples (2 g) in 10 mL of deionized water, shaking for 60 min at 7°C, and centrifuging (10,000 × *g*, 10 min, 4°C). To the aqueous extracts, 2.5 mL of 4M TFA was added, and the solution was heated for 60 min at 110°C. The hydrolyzed samples were analyzed by an HPAEC system equipped with CarboPac PA1 (4 × 250 mm) column plus a CarboPac PA1 guard column (4 × 50 mm) (Dionex), a ternary pump (SP8800, Spectra Physics, Santa Clara, CA, U.S.A.), and an ED50 pulsed amperometric detector (Dionex). A gradient elution with a flow rate of 1 mL/min was used: 0 min (96% H_2O, 4% 250mM NaOH), 4 min (100% H_2O, 0% 250mM NaOH), 22 min (20% H_2O, 80% 250mM NaOH), 32 min (96% H_2O, 4% 250mM NaOH), and hold up to 41 min. Moreover, a 300mM NaOH postcolumn with a flow rate of 0.6 mL/min was added. Pulsed amperometric detection was carried out with the following pulse potentials and durations: E_{OX} = +0.1 V (t_{OX} = 0.3 s), E_{DET} = +0.6 V (t_{DET} = 0.1 s), and E_{RED} = –0.8 V (t_{RED} = 0.3 s). AX content was then defined as 0.88 times the sum of the monosaccharide xylose and arabinose concentrations.

The FFA content was determined as described by Bartolomé and Gómez-Cordovés (1999), with some modifications. After addition of internal standard (d3-hydroxycinnamic acid), samples were extracted for 10 min with 80% ethanol (v/v) in an ultrasonic bath. After centrifugation (20,000 × *g*, 15 min), the supernatant was collected, evaporated to dryness, acidified with 1M HCl, and extracted two times with ethyl acetate. The organic solutions were combined and dried under N_2. Sample derivatization was conducted for 1 h at 70°C with *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (1% BSFTA-TMCS, Supelco, Bellafonte, PA, U.S.A.). The analytical quantification of FFA was performed by isotope-dilution gas chromatography–mass spectrometry (GC-MS), by means of a GC (GC-17A, Shimadzu, Tokyo, Japan) interfaced with a single-quadrupole MS (MS-QP5050, Shimadzu). GC separation was performed on a DB-5-MS capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Folsom, CA, U.S.A.). The analysis of phytic acid was performed by HPLC with spectrophotometric detection as described by Oberleas and Harland (2007). All analyses were performed in triplicate.

Statistical Analysis. Results are expressed on a dry weight basis as mean ± standard deviation. One-way ANOVA was used to test the statistical differences in WEAX content between the different refreshment steps. When the difference among the samples in ANOVA was statistically significant, pairwise comparisons of these samples were analyzed with Tukey's test. A paired Student's *t* test was used to compare values of FFA and phytic acid levels before and after sourdoughlike fermentation. Data were processed by GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego CA, U.S.A., www.graphpad.com).

RESULTS AND DISCUSSION

Microbial Counts. At the start of the sourdoughlike fermentation process, low counts (<10^6 CFU/g) were found for both LAB and yeast populations. However, LAB rapidly increased after the first day of bran fermentation, reaching levels of 10^9 CFU/g. Yeast population developed more slowly than LAB and fluctuated during the first four days of fermentation. Their counts stabilized at a level of 10^7 CFU/g after eight refreshments. The contaminants disappeared after five days of fermentation (Table I).

Molecular Characterization of Microbial Strains. For strain identification, we used a polyphasic approach. First, RAPD-PCR was performed on all strains to explore the genetic diversity, and the resulting fingerprints were compared with a user-generated BioNumerics database for a preliminary identification. This identification was then confirmed by DNA sequence analysis.

Figure 1 shows the different banding patterns of the 73 cocci. The strains fell into two main clusters corresponding to *Pediococcus* and *Leuconostoc* genera. Among the *Leuconostoc* cluster, there were *Leuconostoc mesenteroides* and *Leuconostoc citreum* strains. Intraspecific comparison accomplished by RAPD-PCR profiles revealed a high biodiversity among the strains. Figure 2 shows the RAPD-PCR banding patterns of 25 rod-shaped strains. Almost all the strains were grouped according to species except for two *Lactobacillus plantarum* strains that did not fall in the cluster of *L. plantarum.* A quite high degree of DNA polymorphism was detected in *Lactobacillus brevis*, for which the similarity levels reached only 50% for some of the strains.

Growth rate and yield of microorganisms are governed by a multitude of ecological factors such as temperature, ionic strength, dough yield, and microbial products (lactate, acetate, CO_2, and ethanol), as well as factors resulting from substrates present in the cereal fraction and from enzymatic reactions (Gänzle et al 1998; Meroth et al 2003). Table II shows the endogenous LAB and yeast development during several refreshments of the sourdoughlike fermentation of wheat bran. *Ln. mesenteroides*, *Lactobacillus curvatus*, and *P. pentosaceus* were found from the first step of the process as bran endogenous bacteria and dominated until the end of the fermentation. *Ln. citreum* could be de-

TABLE I
Microbial Counts, pH, Total Titratable Acidity (TTA), Lactic Acid, and WEAX in the Different Refreshment Steps of Sourdoughlike Fermentation of Wheat Bran[z]

Refreshment Step (days)	Contaminants	Yeast	LAB	pH	TTA	Lactic Acid	WEAX
0	5.8	4.8	4.8	6.6 ± 0.1	2.9 ± 0.1	0.02 ± 0.02	0.5 ± 0.0a
1	7.4	5.4	8.2	6.5 ± 0.1	4.6 ± 0.2	ND	1.7 ± 0.2b
2	7.2	4.0	9.5	4.3 ± 0.1	14.9 ± 1.1	ND	1.8 ± 0.1b
3	6.5	2.4	9.7	4.2 ± 0.1	17.5 ± 0.3	ND	2.7 ± 0.1ce
4	3.5	3.4	9.7	4.1 ± 0.1	18.0 ± 0.9	ND	3.0 ± 0.2cd
5	<2.0	3.6	9.6	4.2 ± 0.1	17.3 ± 0.2	ND	3.0 ± 0.3cd
6	<2.0	4.6	9.7	4.1 ± 0.1	18.3 ± 0.4	ND	2.9 ± 0.1cde
7	<2.0	5.2	9.7	4.1 ± 0.1	18.5 ± 0.7	ND	2.9 ± 0.1cde
8	<2.0	5.7	9.6	4.1 ± 0.1	18.8 ± 0.8	ND	2.8 ± 0.0cde
9	<2.0	6.9	9.6	4.1 ± 0.1	18.8 ± 0.8	ND	3.0 ± 0.1cde
10	<2.0	7.1	9.5	4.1 ± 0.1	18.0 ± 0.7	ND	2.6 ± 0.2ce
11	<2.0	7.0	9.6	4.1 ± 0.1	18.6 ± 0.5	ND	3.2 ± 0.4d
12	<2.0	7.1	9.6	4.1 ± 0.1	18.8 ± 0.4	ND	3.0 ± 0.3cde
13	<2.0	7.1	9.6	4.1 ± 0.1	18.9 ± 0.7	5.8 ± 0.4	2.6 ± 0.4e

[z] LAB = lactic acid bacteria; WEAX = water-extractable arabinoxylan. Microbial counts are measured in log CFU/g. TTA is measured in milliliters of 0.1M NaOH per 10 g. Lactic acid and WEAX are reported as % db. ND = not determined. Data not sharing the same letters are significantly different ($P < 0.05$).

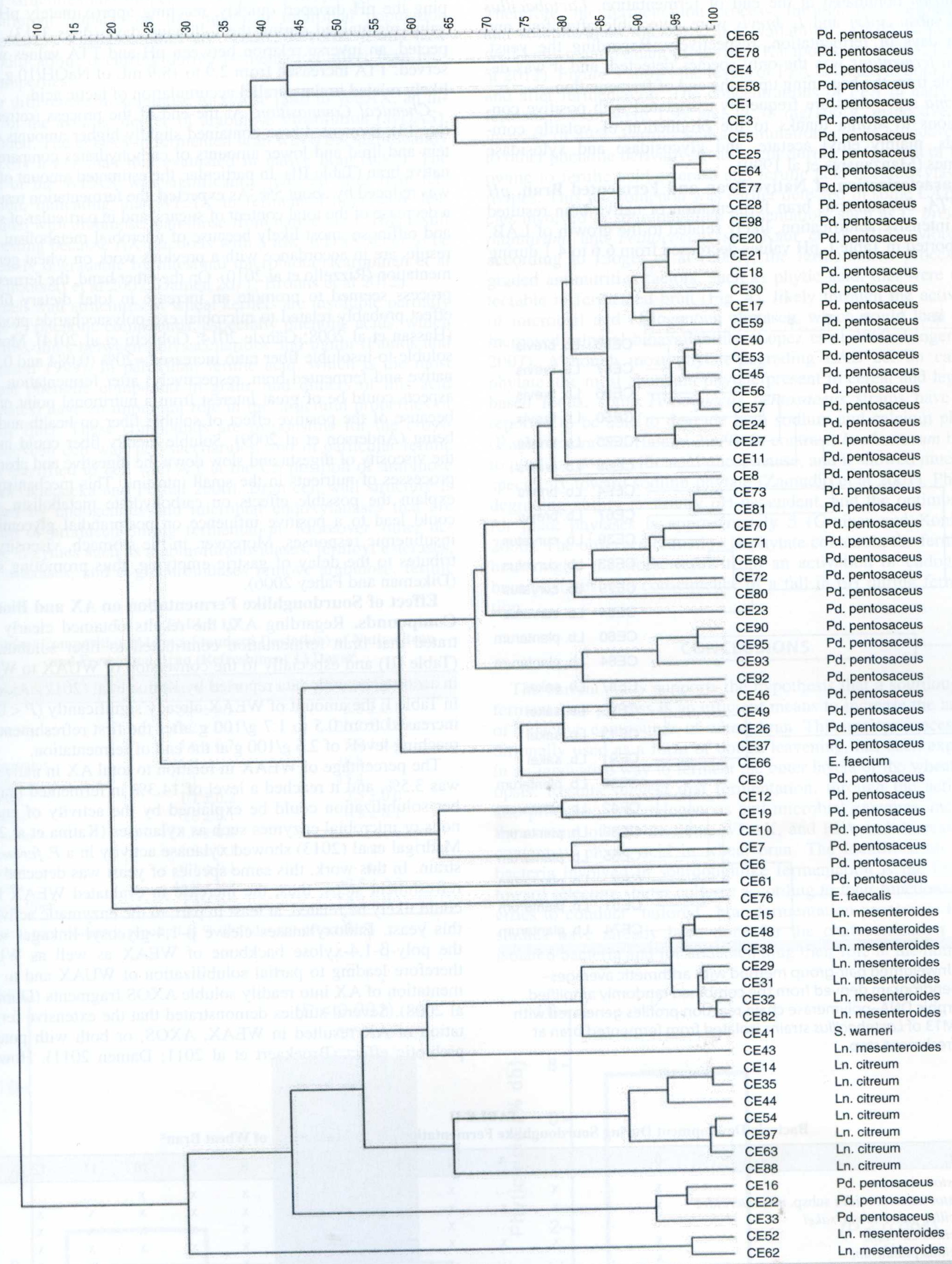

Fig. 1. Unweighted pair-group method with arithmetic averages–based dendrogram derived from the combined randomly amplified polymorphic DNA–polymerase chain reaction profiles generated with primer M13 of cocci strains isolated from fermented bran at each refreshment step.

tected as an endogenous species until the 10th refreshment, but it disappeared at the end of fermentation. On the other hand, *L. plantarum* dominated at the end of fermentation. *Lactobacillus sakei* subsp. *sakei* and *L. brevis* were detectable after four and seven days of fermentation, respectively. Regarding the yeast, *Pichia fermentans* was the only species detected, and it was detectable from the beginning up to the end of fermentation.

Pichia ssp. yeasts are frequently associated with positive contributions to aroma thanks to the production of volatile compounds, mainly ethyl acetate, and glycosidase and xylosidase enzymes (Manzanares et al 1999).

Characterization of Native Bran and Fermented Bran. *pH and TTA.* Spontaneous bran fermentation of native bran resulted in an intensive acidification, likely related to the growth of LAB. As reported in Table I, pH values decreased from 6.6 to 4.1 during bran fermentation. In particular, pH did not change significantly during the first 24 h of fermentation, but after the first back-slopping the pH dropped quickly, reaching approximately pH 4.1, a value maintained during the following days (days 3–13). As expected, an inverse relation between pH and TTA values was observed: TTA increased from 2.9 to 18.9 mL of NaOH/10 g, values likely related to the parallel accumulation of lactic acid.

Chemical Composition. At the end of the process (refreshment day 13), fermented bran contained slightly higher amounts of protein and lipid and lower amounts of carbohydrates compared with native bran (Table III). In particular, the estimated amount of starch was reduced by about 5%. As expected, the fermentation resulted in a decrease of the total content of sugars, and in particular of sucrose and raffinose, most likely because of microbial metabolism. These results are in accordance with a previous work on wheat germ fermentation (Rizzello et al 2010). On the other hand, the fermentation process seemed to promote an increase in total dietary fiber, an effect probably related to microbial exo-polysaccharide production (Hassan et al 2008; Gänzle 2014; Gobbetti et al 2014). Moreover, soluble-to-insoluble fiber ratio increased ≈20% (0.084 and 0.103 in native and fermented bran, respectively) after fermentation. These aspects could be of great interest from a nutritional point of view, because of the positive effect of soluble fiber on health and well-being (Anderson et al 2009). Soluble dietary fiber could increase the viscosity of digesta and slow down the digestive and absorptive processes of nutrients in the small intestine. This mechanism may explain the possible effects on carbohydrate metabolism, which could lead to a positive influence on postprandial glycemic and insulinemic responses. Moreover, in the stomach, viscosity contributes to the delay of gastric emptying, thus promoting satiety (Dikeman and Fahey 2006).

Effect of Sourdoughlike Fermentation on AX and Bioactive Compounds. Regarding AX, the results obtained clearly illustrated that bran fermentation contributed to fiber solubilization (Table III) and especially to the conversion of WUAX to WEAX, in accordance with data reported by Katina et al (2012). As shown in Table I, the amount of WEAX already significantly ($P < 0.001$) increased from 0.5 to 1.7 g/100 g after the first refreshment step, reaching levels of 2.6 g/100 g at the end of fermentation.

The percentage of WEAX in relation to total AX in native bran was 3.5%, and it reached a level of 14.3% in fermented bran. Fiber solubilization could be explained by the activity of endogenous or microbial enzymes such as xylanases (Katina et al 2006). Madrigal et al (2013) showed xylanase activity in a *P. fermentans* strain. In this work, this same species of yeast was detected in all refreshment steps; thus, the increase in evaluated WEAX levels could likely be related, at least in part, to the enzymatic activity of this yeast. Endoxylanases cleave β-1,4-glycosyl linkages within the poly-β-1,4-xylose backbone of WEAX as well as WUAX, therefore leading to partial solubilization of WUAX and to fragmentation of AX into readily soluble AXOS fragments (Dornez et al 2008). Several studies demonstrated that the extensive fermentation of AX resulted in WEAX, AXOS, or both with potential prebiotic effect (Broekaert et al 2011; Damen 2011). However,

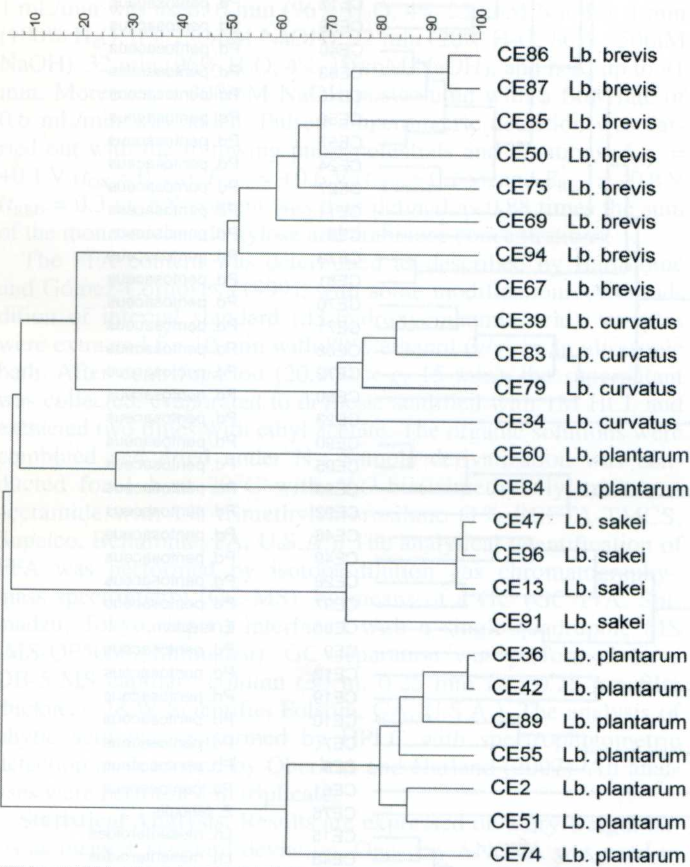

Fig. 2. Unweighted pair-group method with arithmetic averages–based dendrogram derived from the combined randomly amplified polymorphic DNA–polymerase chain reaction profiles generated with primer M13 of *Lactobacillus* strains isolated from fermented bran at each refreshment step.

TABLE II
Bacteria Development During Sourdoughlike Fermentation (13 Refreshments) of Wheat Bran[z]

Bacteria	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Leuconostoc citreum	x	…	x	…	x	x	x	x	x	x	x	…	…	…
Leuconostoc mesenteroides subsp. *mesenteroides*	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Lactobacillus sakei subsp. *sakei*	…	…	…	…	x	x	x	x	x	x	x	x	x	x
Lactobacillus curvatus	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Lactobacillus plantarum	x	…	…	…	…	…	…	…	x	…	…	x	x	x
Lactobacillus brevis	…	…	…	…	…	…	…	x	x	x	x	x	x	x
Pediococcus pentosaceus	…	x	x	x	x	x	x	x	x	x	x	x	x	x
Pichia fermentans	x	x	x	x	x	x	x	x	x	x	x	x	x	x

[z] Detected bacteria are marked with an x.

the physiological impact of AX consumption strongly depended on their structures and properties, because different types of AX have different impacts on the intestinal microbial population (Damen 2011). Further studies are needed to verify if and how AX modifications occurring during fermentation affect bran prebiotic properties.

Clear differences in arabinose-to-xylose ratio in WEAX, an indicator of the average degree of arabinose substitution (avDAS), were found. The avDAS in fermented bran was 0.65, significantly ($P < 0.01$) lower than that evaluated in native bran (0.88). The avDAS of the WEAX was significantly reduced after bran fermentation, indicating that WEAX in fermented bran were less substituted with arabinose than those from native bran. This result is interesting because AX fractions with a low avDAS seem to be more easily degradable by intestinal microbiota (Karppinen et al 2001; Grootaert et al 2009; Damen 2011; Brouns et al 2012).

The cell wall structures of wheat kernels are insoluble partially because of phenolic compounds, especially phenolic acids, which form cross-links between polysaccharides and lignin (Faulds and Williamson 1999). In particular, ferulic acid, which is the most abundant phenolic compound in grain, and dimers of this acid (diferulates) have an important role in the structural properties of aleurone fiber. These compounds are responsible for the cross-links between cell wall polysaccharides, and in particular ferulic acid is esterified to cell wall AX at the C5 position of arabinose residues (Klepacka and Fornal 2006). Bran cell wall structure is degraded by endogenous and microbial endoxylanases that are activated or produced during fermentation. Moreover, other degrading enzymes such as arabinofuranosidases, feruloyl esterases, acetyl esterases, and α-glucuronidases remove arabinose, ferulic acid, acetic acid, and (4-*O*-methyl)glucuronic acid side chains from the xylan backbone, respectively (Grootaert et al 2007). Therefore, the fermentation process, through fiber degradation and solubilization, increased the availability of FFA, which has well-known antioxidant properties (Katapodis et al 2003; Fang et al 2012). Figure 3 shows the levels of FFA detected in bran before and after fermentation. The concentrations of FFA in native and fermented bran were 1.99 and 11.38 mg/100 g, respectively, an increase of 82%. Ferulic acid accumulation and its bioconversion to other phenolic derivatives can occur during the growth of LAB, owing to ferulic acid esterase and ferulic acid decarboxylase activities. This phenomenon was earlier detected in some LAB species involved in sourdoughlike fermentation, such as *L. brevis*, *L. plantarum*, and *Pediococcus* sp. (Kaur et al 2013). Moreover, according to Lioger et al (2007), the fermentation process degraded antinutritive factors, such as phytic acid, that were undetectable in fermented bran (Fig. 4), likely through the activation of microbial and endogenous phytases, which could lead to an increased mineral bioavailability (Lopez et al 2000; Lioger et al 2007). Although most phytate-degrading LAB act on calcium phytate, the most abundant phytate present in cereal and legume-based foods, some *Pediococcus pentosaceus* strains have been reported to be able to degrade both sodium and calcium phytate (Raghavendra and Halami 2009). In contrast, *L. plantarum* is able to produce nonspecific acid phosphatase, and it showed much less specificity toward sodium phytate (Zamudio et al 2001). Phytate-degrading ability is strictly pH-dependent, and the optimum pH for plant phytases is approximately 5 (Greiner and Konietzny 2006). The observed reduction in phytate content in the fermented bran might, therefore, result from an activation of endogenous bran phytases as a consequence of a fall in pH during fermentation.

TABLE III
Chemical Composition (Mean ± Standard Deviation) of Native Bran and Fermented Bran (Refreshment 13 Days)[z]

Components	Native Bran	Fermented Bran
Ash	5.3 ± 0.1	5.6 ± 0.1
Proteins	19.2 ± 0.1	20.7 ± 0.1
Lipids	5.6 ± 0.7	7.0 ± 0.4
Carbohydrates	26.0	18.8
Starch	21.1	15.4
Glucose	1.1 ± 0.2	2.5 ± 0.0
Fructose	0.8 ± 0.1	0.8 ± 0.1
Raffinose	1.0 ± 0.0	nd
Sucrose	1.8 ± 0.1	nd
Maltose	0.1 ± 0.1	nd
Total fiber	43.9 ± 0.3	47.5 ± 0.3
Soluble fiber	3.4 ± 0.2	4.4 ± 0.1

[z] Chemical composition is reported as % db; nd = not detectable.

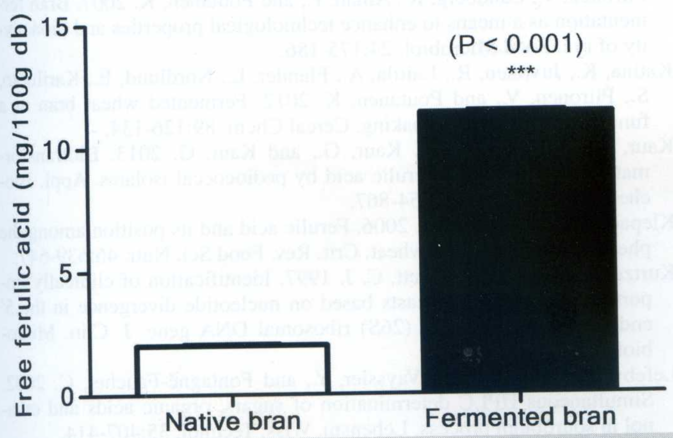

Fig. 3. Free ferulic acid content (mg/100 g db) in native and fermented bran.

CONCLUSIONS

The current study supports the hypothesis that a sourdoughlike fermentation process is an efficient means to increase the amount of bioactive compounds of wheat bran. This ancient process, traditionally used as a form of dough leavening, has been exploited in an innovative way to ferment the outer layers of the wheat caryopsis. Results suggest that fermentation, through the activation and production of endogenous and microbial enzymes, increases the amount of soluble fiber, WEAX, and FFA and decreases the content of phytic acid in wheat bran. The identification of the bacteria involved in sourdoughlike fermentation is the first step toward selecting starter cultures according to their functional properties to conduct "tailored" bran fermentation processes. Further studies have already been done for the characterization of the isolated bacteria and for understanding their role in the nutritional

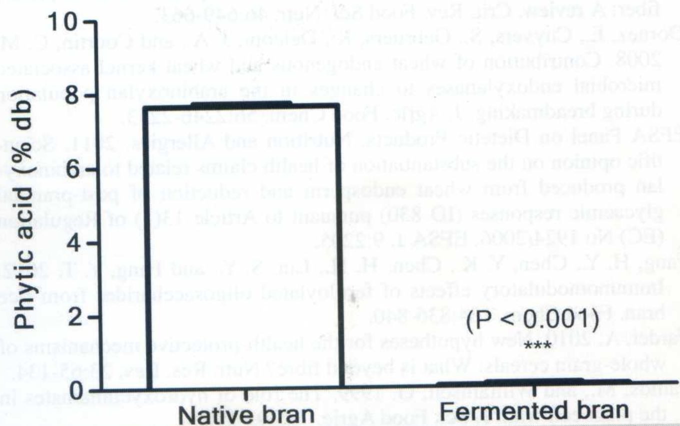

Fig. 4. Phytic acid content (% db) in native and fermented bran.

enhancement of fermented bran. These studies provide additional information for the future purpose of adding fermented bran as a functional ingredient for bran-enriched products.

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