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Phytate Reduction in Bran-Enriched Bread by Phytase-Producing Bifidobacteria

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Bread fermented with the selected *Bifidobacterium* strains had similar technological and sensorial quality as the controls, resulting in breads with significantly lower ($p < 0.05$) levels of InsP_6 with residual amounts of *myo*-inositol triphosphates (InsP_3). The fact that the phytate-degrading enzymes are produced by strains of bifidobacteria, which are GRAS/QPS (generally regarded as safe/qualified presumption of safety) microorganisms makes this strategy particularly suitable to reduce the content of InsP_6 in rich fiber products for human consumption.

KEYWORDS: Phytate-degrading enzyme; phytic acid; whole-wheat bread; enriched-fiber bread; *Bifidobacterium*

INTRODUCTION

Nowadays, society is aware of nutrition−health interactions, and therefore, the demand for healthier, more nutritious and safer foods is increasing. High fiber products, as whole-grain or bran-enriched meal, are a good example of foods that positively influence human health. Epidemiological studies support the protective role of whole-grain foods against diseases associated with metabolic syndrome. Metabolic syndrome includes disorders in the metabolism of glucose, lipoproteins, insulin actions, arterial hypertension and obesity, which constitute a high risk of developing cardiovascular diseases and type-2 diabetes (*1*, *2*). Consumption of low amounts of fiber has been associated with atherosclerosis, coronary heart disease and colon cancer, while increasing the amount of fiber in the human diet has been associated with decrease in the incidence of these diseases (*3*). As a consequence, consumption of whole-grain bread or fiber-enriched bread has increased in recent years (*2*). However, whole-grain foods are also thought to impair mineral absorption. The phytate or phytic acid [*myo*-inositol (1,2,3,4,5,6)-hexakisphosphate, InsP_6] present in these products is considered to be the major factor causing negative effects on mineral uptake in humans and animals (*4*). Phytate behaves in a broad pH region as a highly negatively charged ion and has a tremendous affinity for food components with positive charge(s), such as minerals, trace elements and proteins. Minerals and trace elements of concern in this regard include zinc, iron, calcium, magnesium, manganese, and copper (*4*−*6*). The formation of insoluble mineral−phytate complexes at physiological pH values is regarded as the major reason for reduced mineral bioavailability, because these complexes are nonabsorbable in the human gastrointestinal tract. Furthermore, the human small intestine has only a very limited capability to hydrolyze phytate (*7*) due to the lack of endogenous phytate-degrading enzymes. Many investigations have demonstrated that a diet rich in phytate may cause deficiencies in minerals (*5*, *8*−*10*). The risk of mineral deficiency is important mainly in vulnerable population groups including babies, child-bearing women, strict vegetarians, the elderly and inhabitants of developing countries (*11*−*13*). However, both *in vivo* and *in vitro* studies have indicated that hydrolysis of phytate to partially phosphorylated *myo*-inositol phosphate esters is a way to overcome the negative effect of phytate on mineral absorption (*14*, *15*).

Phytate hydrolysis has a double benefit: first it eliminates the antinutrient compound phytate, resulting in a better absorption of minerals by the human gut, and second the partially lower phosphorylated *myo*-inositol phosphates generated may positively affect human health. Individual *myo*-inositol phosphate esters have been proposed to be metabolically active. The InsP_3 is a second messenger, bringing about a range of cellular functions including cell proliferation via intracellular Ca^{2+} mobilization and preventive effects on diabetes complications and treatment of chronic inflammations and cardiovascular diseases (*16*, *17*).

During the breadmaking process phytate is sequentially hydrolyzed by the action of cereal phytate-degrading enzymes (phytase) to a mixture of *myo*-inositol pentakis-, tetrakis-, tri-, di-, and monophosphates (InsP_5, InsP_4, InsP_3, InsP_2, InsP_1, respectively) and orthophosphate. However, breads with high fiber content or whole-grain breads still contain high phytate levels due a slow enzymatic dephosphorylation (*18*, *19*). Enzymatic phytate degradation in doughs depends on many factors, including fermentation time, temperature, pH, water content of dough, flour extraction rate, starter culture, mineral content, leavening agent and the bread-making process (*20*). The strategies to reduce or eliminate the phytate from food include the addition of exogenous phytate-degrading enzymes; changes in breeding; agronomic conditions; genetic engineering or changes in food processes such as prolonged process time or change in pH of the product (*4*, *21*). In general, the addition of sourdough into bread formulation has

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been shown to improve degradation of InsP_6 (*4*). The addition of exogenous phytate-degrading enzymes, mainly from fungal origin (phosphatases and phytase), is a practical alternative that causes a decrease in phytate content prepared with whole-flour or fiber-enriched breads (*18*, *19*, *22*). The increase in phytase activity appears to be the best strategy to decrease the phytate content of cereal products. The addition of phytase-producing microorganisms and enzyme preparations would be one way to design foods with better nutritional characteristics by reduction of InsP_6 content. Phytase activity has been detected in *Bifidobacterium* for the first time (*23*, *24*). It was also suggested that phytase-producing bifidobacteria could be useful for producing fermented cereal based products because they could perfectly replace *Lactobacillus* strains, usually employed as commercial starter cultures, and at the same time lead to a significant InsP_6 degradation, without the necessity of prolonging the fermentation time. *Bifidobacterium* phytase-producer strains could also generate a specific *myo*-inositol phosphate isomer profile during food fermentation, improving nutritional value and health benefits of the product. It has been suggested that strains of the genus *Bifidobacterium* could be used in the production of bakery products (*25*, *26*) and contribute to the prevention of deteriorating fungi (*27*). Palacios et al. (*28*) investigated the use of bifidobacterial strains as starter during long fermentation process of whole-wheat dough, which showed a good adaptation to the dough ecosystem and contributed to different acidification degrees promoting the degradation of phytate. *Bifidobacterium longum* strain was investigated as starter culture in the whole-wheat breadmaking process, which resulted in bread with similar technological quality as the control (in the absence of bifidobacteria) with lower levels of InsP_6 (*29*).

This investigation is aimed at developing breads under standard conditions with direct method of bread-making process, in two formulations (100% and 50% of whole-wheat flour) by using the combination of *Bifidobacterium pseudocatenulatum* ATCC 27919 and *Bifidobacterium infantis* ATCC15697 with high phytate-degrading activity as starter cultures. The nutritional, technological and sensorial quality of final products has been evaluated in comparison with control (in the absence of bifidobacteria) and samples supplemented with commercial fungal phytase.

MATERIALS AND METHODS

Materials. Commercial Spanish wheat flour and whole-wheat flour were purchased from the local market. The characteristics of the commercial wheat flours used were (g kg^{-1} in dry matter): moisture 152.8 ± 0.1 and 149.6 ± 0.1, protein ($N \times 5.7$) 116.9 ± 0.6 and 125.9. ± 0.7, lipids 11.1 ± 0.1 and 16.6 ± 0.1, ash 5.3 ± 0.9 and 12.9 ± 0.2, and endogenous phytase activity was 2.8 ± 0.4 and 5.0 ± 0.7 U kg^{-1}, for wheat flour and whole-wheat flour, respectively.

Compressed yeast (*Saccharomyces cerevisiae*, Levamax, Spain) was used as a starter for the bread making process. Commercial phytase (EC 3.1.3.8) from *Aspergillus niger* (11.4 U mL^{-1}, Ronozyme Phytase Novo from Novozymes, Bioindustrial, Madrid, Spain) was added as positive control in dough formulation. One unit of phytase activity was defined as 1.0 mg of Pi liberated per minute at pH 5.0 and 30 °C.

The strains of the *Bifidobacterium* genus used in this study, which have phytate-degrading enzymes (*23*, *24*), were *B. infantis* ATCC 15697 and *B. pseudocatenulatum* ATCC 27919 originally isolated from feces of infants, and obtained from the American Type Culture Collection (ATCC).

Microbial Growth Conditions. Bifidobacteria were grown in Garche broth in which inorganic phosphate (K_2HPO_4 and NaH_2PO_4) was replaced by 0.74 g/L phytic acid dipotassium salt (Sigma-Aldrich, St. Louis, MO) and 0.1 M 3-[*N*-morpholino]propanesulfonic acid buffer (MOPS, Sigma-Aldrich, St. Louis, MO) (*24*). The medium was inoculated at 5% (v/v) with 18 h old cultures, previously propagated under the same conditions. Cultures were incubated at 37 °C and in anaerobic conditions (AnaeroGen, Oxoid, England) until the beginning of the stationary phase of growth was reached (~14−18 h). Bacterial growth was monitored by measuring optical density at 600 nm. Bacterial cells were harvested by centrifugation (10000*g*, 15 min, 4 °C, Sorvall RC-5B, DuPont Instruments), washed twice and suspended in 0.085% NaCl solution. The obtained cell suspensions were used to inoculate the dough (*26*).

Lactobacilli and bifidobacteria counts were determined after the dough fermentation period in MRS cysteine−lactose agar using the double layer technique after anaerobic incubation at 37 °C, for 48 h. Yeast counts were determined in Rose Bengal Agar (Scharlau Chemie, Barcelona, Spain) after aerobic incubation at 30 °C, for 72 h. Determinations were carried out in duplicate.

Breadmaking Procedure. Two flour formulations were used for making bread dough: flour A, 100% whole-wheat flour (WWF); and flour B, 50% whole-wheat flour + 50% wheat flour. The bread dough formula consisted of flour A or B (1000 g), compressed yeast (2.5% flour basis), sodium salt (2.0% flour basis), tap water (up to optimum absorption, 500 Brabender units, 65.0% or 60.1% for A and B flours, respectively) and ascorbic acid (0.01%). The ingredients were mixed for 6.5 or 4.5 min (A or B flours, respectively), rested for 10 min, divided (100 g), kneaded and then rested (15 min), doughs were manually sheeted and rolled, proofed (up to optimum volume increase, at 28 °C, 85% relative humidity) and baked (165 °C, 30 min for 100% of WWF or 170 °C, 27 min for 50% of WWF) according to Haros et al. (*19*).

Commercial phytase was added as positive control to dough formulations prepared in paralleled at a concentration equivalent to 10 times the activity in the flour (A or B), which was measured under the same conditions.

Cell suspensions of bifidobacteria (*B. infantis* + *B. pseudocatenulatum*) were added to the dough formulations. *Bifidobacterium*-fermented samples were compared with control samples (containing yeast and/or added fungal phytase).

Fermentation was monitored by measuring pH, temperature and volume increase of the dough. The pH values were registered at regular period times with a pH meter (Crison instruments, Barcelona, Spain). After the fermentation step, doughs were baked in an electric oven and cooled at room temperature for 75 min (*19*). The experiments were done in duplicate.

Bread Quality. The technological parameters analyzed were loaf specific volume (cm^3/g), width/height ratio of the central slice (cm/cm), moisture content (%) and the crumb texture, determined by a texture profile analysis using Texture Analyzer TA-XT2i (*30*). Digital image analysis was used to measure bread crumb structure. Images were previously squared at 240 pixels per cm with a flatbed scanner (HP ScanJet 4400C, Hewlett-Packard, USA) supporting by HP PrecisianScan Pro 3.1 Software. A single 10 mm × 10 mm square field of view of two central slices (10 mm thick) of each of two loaves was used, thereby yielding 4 digital images per treatment. Data was processed using Sigma Scan Pro Image Analysis Software (version 5.0.0, SPSS Inc., USA). The crumb grain features chosen were cell area/total area, cm^2/cm^2; wall area/total area, cm^2/cm^2; number of cells per cm^2; and mean cell area, μm^2.

The tristimulus color parameters L^* (lightness), a^* (redness to greenness), b^* (yellowness to blueness) of the baked loaves (crumb and crust) were determined using a digital colorimeter (Chromameter CR-400, Konika Minolta Sensing, Japan). Each sample was measured 12 times in different points to minimize the heterogeneity produced by the bran.

Initial InsP_6 concentration in flour, the remaining concentration of InsP_6 in bread and the lower *myo*-inositol phosphates generated were measured by high pressure liquid chromatographic method described by Türk and Sandberg (*20*), later modified by Sanz-Penella et al. (*31*).

Sensory analysis of fresh breads was performed with a panel of trained judges using semistructured scales, scored from 1 to 10, in which extremes were described. The visual, textural and organoleptic characteristics evaluated were crumb structure, crumb softness, crumb elasticity, aroma (quality and intensity), and flavor (quality and intensity).

Statistical Analysis. Data parameters measured during the breadmaking process are the mean of values obtained in two independent experiments. In each experiment, parameters were determined at least in duplicate. Multiple sample comparison of the means and Fisher's least significant differences (LSD) were applied to establish statistical significant differences between treatments. All statistical analyses were carried out with the software Statgraphics Plus 7.1 (Bitstream, Cambridge, MN), and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSION

The selection of *Bifidobacterium* strains for this study was based on previous studies, which showed their high phytase activity in comparison with other *Bifidobacterium* strains. *B. pseudocatenulatum* ATCC27919 and *B. infantis* ATCC15697 were selected from a total of 100 strains isolated from different ecosystems (*23*, *24*, *28*, *29*).

Prior to the development of cereal-based products by using the selected *Bifidobacterium* strains as starter cultures, a preliminary study of the role of the bakery yeast on phytate hydrolysis during the bread-making process was carried out. The phytate content in the final product without the yeast addition showed similar values as those of products made with yeast, under the same processing conditions ($p < 0.05$). Increasing the concentration of yeast (from 1 to 3%) in the formulation did not produce significant differences in the phytate reduction. These results were consistent with previous reports which showed that yeast was not involved in the phytate hydrolysis (*32*, *33*).

Characteristics of Dough during Fermentation. Dough volume of bran-enriched bread showed a progressive increase along the fermentation period due to the production of carbon dioxide, reaching a maximum volume after approximately 1 h at 30 °C. Prolonged incubation periods resulted in decreases in the dough volume due to the dough permeability. The presence of the bifidobacterial strains in the bread formulation did not promote significant changes in developing the optimum dough volume. The addition of the bifidobacterial strains to formulation significantly increased the lactic acid bacteria (LAB) counts, from 10^3-10^4 CFU/g present in the control dough to 10^7 CFU/g found in the *Bifidobacterium* inoculated samples, whereas the yeast counts remained almost constant, around 10^8 CFU/g independently of the condition studied. Both population counts present in the control samples were in the range of those found in bread sourdough by other authors (*26*, *28*).

The dough pH remained unchanged at values of 5.0−5.1 in all the analyzed conditions in both whole-wheat dough and bran-enriched dough.

Degradation of Phytate and Generation of Derived *myo*-Inositol Phosphates. During the bread-making process, endogenous phytate-degrading enzymes from cereal and microbial sources could be active (*18*, *19*). In order to determine whether the inoculation of the selected *Bifidobacterium* strains conferred additional benefits to bread derived from their phytate-degrading activity, the InsP_6 and lower *myo*-inositol phosphate contents were analyzed (**Table 1**). The results were compared with bread formulated under the same conditions with the addition of yeast (as negative control, C) and with yeast + fungal phytase (as positive control, Phy).

The inclusion of commercial fungal phytase in bread formulation significantly reduced the amount of phytate after one hour fermentation (**Table 1**) as was previously observed by other researchers (*18*, *19*, *22*). It also hydrolyzed efficiently the lower *myo*-inositol phosphates, which are considered to exert positive biological functions, particularly the InsP_3 (*16*, *17*). The breads inoculated with *Bifidobacterium* strains showed significant reductions of InsP_6 levels compared with control ones (**Table 1**). In whole-wheat bread the InsP_6 reduction was approximately 44%, whereas in bran-enriched bread it was about 67%. In the last case, the InsP_6 degradation was similar to that of the sample supplemented with fungal phytase (Phy), whereas the lower *myo*-inositol phosphates remained significantly at higher levels (InsP_{5-3}). A similar observation was recorded in the whole-wheat bread, where the lower *myo*-inositol phosphates remained at high levels in breads inoculated with bifidobacteria (**Table 1**). Though the lower inositol phosphate levels did not present significant differences between control and *Bifidobacterium* inoculated samples, preliminary studies using high performance ion chromatography indicated that the stereoisomers formed as a result of InsP_6 degradation presented slight differences (results not shown). In general, depending on phytase type, the predominant attack of the phosphoester bond of the inositol ring could be in position D3, D4, D5 or D6, which produce different isomers of the lower inositol phosphates. *Bifidobacterium* strains used in this study produce a particular profile of *myo*-inositol phosphates (*34*).

Table 1. *myo*-Inositol Phosphate Concentration in Final Product[a]

% whole-wheat flour	treatment	μmol/g bread (d.m.)[b] InsP_6	InsP_5	InsP_4	InsP_3	InsP_6+ InsP_5
100	C	1.383 a	0.797 a	2.255 a	1.472 a	2.180 a
	Phy	0.150 c	0.024 b	0.009 b	0.056 b	0.173 c
	Bif	0.770 b	0.712 a	2.384 a	1.594 a	1.482 b
50	C	0.607 a	0.260 a	0.845 a	1.269 a	0.867 a
	Phy	0.203 b	0.040 c	0.023 b	0.050 b	0.242 b
	Bif	0.199 b	0.145 b	0.850 a	1.310 a	0.345 b

[a] Control (C), fungal phytase addition (Phy) and *Bifidobacterium* addition (Bif). Mean, $n = 3$. Values followed by the same letter in the same column are not statistically different at the 95% confidence level ($p < 0.05$). The statistical analysis of the different flours was made separately. [b] InsP_3 to InsP_6: *myo*-inositol phosphate containing 3−6 phosphates per inositol residue.

The activity of the endogenous cereal phytase during the bread-making (mixing, resting, proofing and the beginning of baking) could be increased by pH changes (*4*, *29*, *35*, *36*). However, in the current investigation the dough pH remained constant from the beginning of mixing to the end of fermentation, which suggests that the additional InsP_6 hydrolysis was due to the phytate-degrading activity of the inoculated bifidobacteria. Palacios et al. (*28*) investigated the effect of the *Bifidobacterium* strains as starters in whole-wheat dough along the fermentation. During the first 2 h of fermentation the samples with bifidobacteria did not hydrolyze a higher amount of InsP_6 than the control ones, only the doughs with significantly lower pH reduced the content of InsP_6 significantly (*28*).

The InsP_6 forms the more stable complexes with minerals, its degradation being effective for improving the iron bioavailability at least until the InsP_3 (*37*). The literature explains as the numbers of phosphate groups are progressively removed from the InsP_6, the mineral binding strength decreases and solubility increases, although InsP_5 still has adverse effects on mineral absorption (*5*, *37*). In the current investigation InsP_6 + InsP_5 in whole-wheat bread and bran-enriched bread were reduced ($p < 0.05$) in *Bifidobacterium* inoculated samples by 32% and 60% in relation to the control levels, respectively. Palacios et al. (*29*) used a strain *B. longum* as starter in whole-wheat bread fermented for one hour at 37 °C, leading to lower InsP_6 + InsP_5 reductions (22%) with respect to the control sample, showing that the strain combination used in the present study is more effective.

The use of commercial fungal phytase could improve the mineral bioaccessibility of cereal-based products by removing phytate, which is a common practice in animal nutrition. In general, commercial phytases are produced employing filamentous fungi, usually from *Aspergillus* and *Trichoderma* strains, at the moment only used in animal feeding. The phytate-degrading *Bifidobacterium* strains may be suitable organisms for the production of food-grade phytase and for their direct use in food production.

Bread Quality. Effect of *Bifidobacterium* Addition. The effect of the use of *Bifidobacterium* strains, with phytate-degrading activity, on technological quality parameters of bread loaves was

Table 2. Effect of the Treatment on Different Technological Parameters in Bread[a]

		bread technological parameters					
% whole-wheat flour	treatment	moisture,[b] %	loaf volume,[c] mL	loaf weight,[c] g	specific vol,[c] cm^3/g	width/height ratio,[c] cm/cm	firmness,[c] N
100	C	37.5 a	220.0 a	83.1 a	2.58 a	1.60 a	5.00 a
	Phy	35.4 a	218.8 a	82.4 a	2.66 a	1.62 a	4.64 a
	Bif	35.9 a	231.3 a	82.8 a	2.79 a	1.83 a	4.42 a
50	C	34.4 a	260.8 a	82.5 a	3.12 a	1.68 a	2.28 a
	Phy	33.1 a	218.8 b	82.8 a	2.74 b	1.64 a	2.48 a
	Bif	34.8 a	268.8 a	82.7 a	3.26 a	1.67 a	2.94 b

[a] Control (C), fungal phytase addition (Phy) and *Bifidobacterium* addition (Bif). Values followed by the same letter in the same column are not statistically different at the 95% confidence level ($p < 0.05$). The statistical analysis of the different flours was made separately. [b] Mean, $n = 2$. [c] Mean, $n = 4$.

Table 3. Effect of Treatment on Bread Color[a]

		color parameters					
		crust			crumb		
% whole-wheat flour	treatment	L^*	a^*	b^*	L^*	a^*	b^*
100	C	53.33 a	13.31 a	31.54 a	56.39ab	5.27 a	21.18 a
	Phy	50.68 b	14.32 a	31.62 a	55.16 a	4.95 ab	19.86 b
	Bif	51.88ab	13.79 a	31.08 a	57.25 b	4.85 b	20.99 a
50	C	59.46 a	11.83 a	34.95 a	61.03 a	2.06 ab	18.56 a
	Ph	63.03 b	10.41 b	35.49 a	63.52 b	1.66 a	17.85 b
	Bif	60.19 a	10.58 ab	35.35 a	64.87 b	2.29 b	19.77 c

[a] Control (C), fungal phytase addition (Phy) and *Bifidobacterium* addition (Bif). Mean, $n = 12$. Values followed by the same letter in the same column are not statistically different at the 95% confidence level ($p < 0.05$). The statistical analysis of the different flours was made separately.

Table 4. Effect of the Treatment on the Crumb Structure[a]

% whole-wheat flour	treatment	cell area/ total area, cm^2/cm^2	wall area/ total area, cm^2/cm^2	no. of cells/cm^2	mean cell area, μm^2
100	C	0.296 a	0.704 a	175 ab	18 a
	Phy	0.303 a	0.697 a	199 a	16 a
	Bif	0.263 a	0.737 a	162 b	16 a
50	C	0.370 a	0.630 a	182 a	23 ab
	Phy	0.433 b	0.567 b	158 b	26 a
	Bif	0.317 a	0.683 a	179 a	18 b

[a] Codes: Control (C). Fungal phytase addition (Phy) and *Bifidobacterium* addition (Bif). Mean, $n = 8$. Values followed by the same letter in the same column are not statistically different at the 95% confidence level ($p < 0.05$). The statistical analysis of the different flours was made separately.

analyzed (**Tables 2**−**4**). **Table 2** shows some technological parameters of the final product. In general, the parameters did not show significant differences between treatments. The loaf weight and volume have positive effects on bread at the retail end. The consumers often get attracted by the bread loaf with higher weight and volume, the moisture and the carbon dioxide diffused out of the loaf during baking being some of the reasons that are reflected in these parameters. The loaf humidity ranged between 35.4 and 37.5% for whole-wheat breads, whereas bran-enriched breads significantly showed lower loaf humidity, between 33.1 and 34.8%. Loaf volume, weight and specific volume ranged from 218.8 to 268.3 mL, 82.4 to 83.1 g, and 2.58 to 3.26 mL/g, respectively. The loaf volume and, hence, the specific volume were significantly modified by the whole-wheat flour content in the formulation, whereas the loaf weight remained practically constant (**Table 2**). The only exception was the treatment with fungal phytase in formulation B, which showed significantly lower loaf volume and specific volume than the control one. Rosell et al. (*38*) found the same behavior when fungal phytase was added, whereas an opposite tendency was published by Haros et al. (*19*), who hypothesized that the advanced or complete hydrolysis of phytate could activate the endogenous alpha-amylase by calcium liberation, which acts as cofactor of the enzyme increasing the volume during fermentation. The parameter which describes the loaf shape did not show significant differences with the treatment (**Table 2** and **Figure 1**).

The textural parameters did not show significant changes between treatments. The textural profile of the crumb from the sample supplemented with commercial phytase showed no significant difference compared with control sample. The firmness is related to the force required to compress the food between the molars, which is a quality characteristic for bakery products, because it is strongly correlated with the perception of freshness by the consumers (*39*). In whole-wheat bread, the firmness in the sample added with bifidobacteria did not show significant differences comparing to the control ones (**Table 2**). The rest of texture profile analysis parameters showed the same tendency. The gumminess and chewiness showed values from 3.86 to 4.17 N and from 4.41 to 4.54 N, respectively. The springiness, cohesiveness and resilience also showed no significant differences between treatments, with values between 1.156 and 1.076, 0.775 and 0.780, and 0.428 and 0.429, respectively. The bran-enriched bread inoculated with *Bifidobacterium* showed significantly harder crumb than control ones (**Table 2**). The parameters that depend on firmness (gumminess and chewiness) also showed the same tendency. The rest of the parameters of different treated samples did not show any significant differences comparing to the control samples. Palacios et al. (*29*) studied the addition of *B. longum* in whole-wheat bread and observed that the firmness did not change significantly compared to the control one.

The CIEL*a*b* parameters of whole-wheat breads (samples A) were significantly different from those of the samples elaborated with 50% of whole-wheat flour (samples B). As expected, the samples with higher bran amount showed more darkness (lower L^*), higher redness and lower yellowness (**Table 3**). Comparing between treatments, it was observed that the samples inoculated with bifidobacteria did not shown significant changes in the crust color parameters, whereas the samples added with fungal phytase showed slight differences, but they were not perceptible under simple visual observation by consumers ($n = 50$). Regarding the crumb color, the samples inoculated with bifidobacteria or fungal phytase presented slight differences in comparison with the control samples, but these were not perceptible under simple visual observation by consumers (**Figure 1**).

The bran normally impedes the normal formation and development of the gas cell structure, restricting and forcing gas cells to expand in a particular dimension, because it incorporates into the

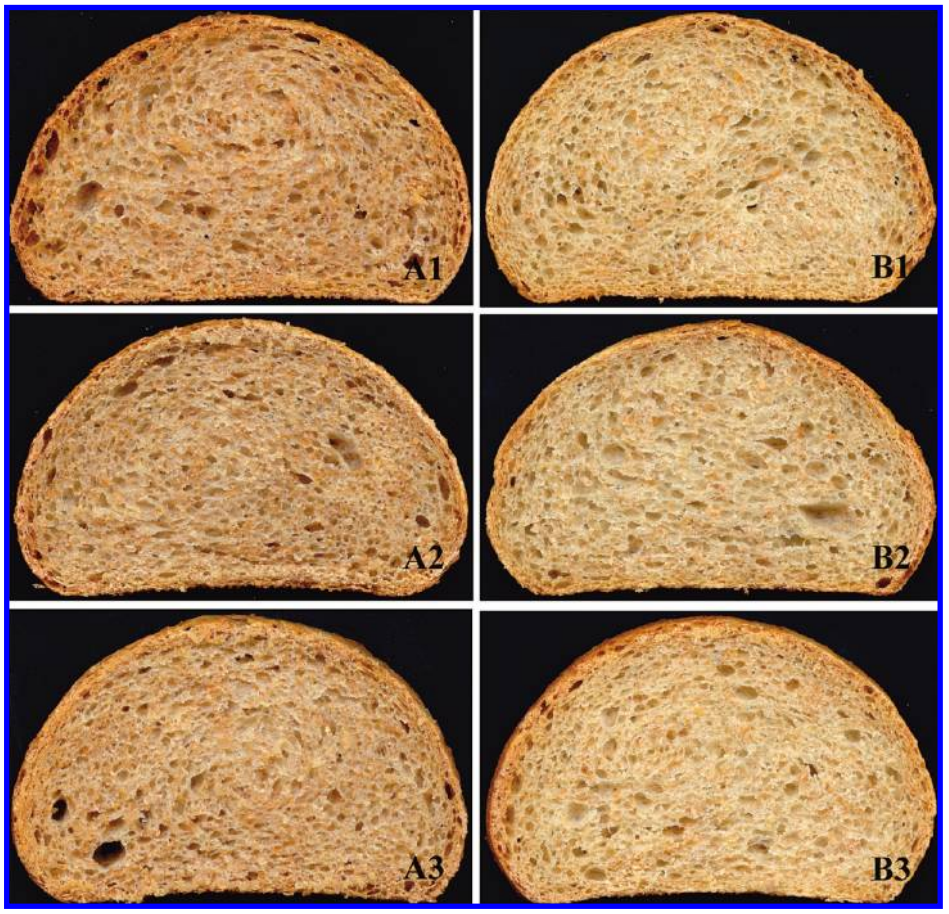

Figure 1. Effect of the treatment on crumb structure of bread. (**A**) Whole-wheat bread. (**B**) Bran-enriched bread. (**1**) Control. (**2**) Added with fungal phytase. (**3**) Added with bifidobacteria.

Table 5. Sensory Evaluation[a]

		sensorial parameters[b]							
% whole-wheat flour	treatment	crumb structure	crumb softness	crumb elasticity	crust crunchiness	aroma quality	aroma intensity	flavor quality	flavor intensity
100	C	6.0 a	5.7 a	5.4 a	6.0 a	7.5 a	6.3 a	7.0 a	6.4 a
	Phy	5.4 a	5.3 a	5.2 a	5.3 a	7.0 a	6.6 a	6.3 a	6.5 a
	Bif	5.8 a	5.6 a	5.3 a	5.3 a	6.6 a	6.2 a	6.2 a	6.2 a
50	C	6.4 a	7.1 a	7.7 a	7.1 a	7.9 a	6.8 a	7.5 a	6.6 a
	Phy	6.6 a	7.1 a	7.7 a	7.1 a	7.4 a	6.6 a	7.1 a	6.4 a
	Bif	6.0 a	7.0 a	7.2 a	7.0 a	7.2 a	6.3 a	6.8 a	6.0 a

[a] Control (C), fungal phytase addition (Phy), *Bifidobacterium* addition (Bif). Mean, $n = 10$. Values followed by the same letter in the same column are not statistically different at the 95% confidence level ($p < 0.05$). The statistical analysis of the different flours was made separately. [b] Parameters were evaluated on a scale from 0 to 10 (from lowest acceptation to highest acceptation).

cell walls of dough (*40*). That may be the explanation for the fact that bran-enriched bread showed better crumb structure than the whole-wheat bread (**Table 4** and **Figure 1**), where the distortion of the cell structure contributed to changes in the size, shape and cell distributions (*40*). In this sense, the specific volume and firmness corroborated this tendency (**Table 2**). On the other hand, the parameters which described the crumb structure did not present significant differences between the control and *Bifidobacterium* inoculated breads. The inclusion of fungal phytase in the bran-enriched bread increased the cell area, whereas the wall area and number of cells/cm^2 decreased (**Table 4**).

Sensory analysis confirmed the results obtained by instrumental methods, about crumb structure, softness and elasticity, crust crunchiness, flavor and aroma, which did not show significant differences between treatments (**Table 5**).

It has been shown that strains of the genus *Bifidobacterium* have phytase activity (*23*, *24*), suggesting their possible use in the production of bakery products with high level of bran. The fact that the phytase is produced by strains of bifidobacteria, which are GRAS/QPS (generally regarded as safe/qualified presumption of safety) microorganisms, makes this strategy particularly suitable to reduce the content of InsP_6 in rich-fiber products for human consumption. Bran-enriched wheat breads in the presence of the selected human *Bifidobacterium* strains had technological and sensorial quality similar to the control ones. Moreover, the inoculation of *Bifidobacterium* resulted in products with significantly lower ($p < 0.05$) levels of InsP_6 keeping residual amount of InsP_3. The commercial phytases, which are used as feed additives, are not added to foods meant for human consumption. Therefore, *Bifidobacterium* strains or the enzyme preparations would be

the best approach to reduce the content of InsP_6 in fiber-rich products for human consumption.

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