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Application of *Bifidobacterium* strains to the breadmaking process

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Abstract

The possible use of bifidobacterial strains from different origin (adult and infant humans, and chicken) as novel starter cultures for breadmaking was evaluated. Fermentative parameters of doughs (pH, volume, total titrable acidity [TTA], lactic and acetic acids production and rheofermentative parameters) and technological parameters of breads (specific volume, bread shape and crumb hardness) were analyzed. Human bifidobacterial strains could replace *Lactobacillus* strains, commercialized for breadmaking, as they yielded breads with similar characteristics but with the advantage of having softer crumbs. Important differences between the behavior of chicken bifidobacterial strains and human bifidobacterial strains were found when comparing bread TTA, bread shape and bread volume. Breads made with chicken strains showed significantly lower ($p < 0.05$) specific bread volume than those made with human strains, while showing similar values of TTA. The effects observed when using bifidobacterial strains from different origin as novel starter cultures for breadmaking seemed to depend on the strain and its origin.

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1. Introduction

Breadmaking is one of the most complex process that involves fermentation. Associations between lactic acid bacteria (LAB) and yeasts are widely used in the production of different types of breads [1]. Artisan bread production, which often employs sourdough processes or the use of pre-ferments and starters, provides a wide regional variety of breads and specialty products [2–4]. The most typical sourdough LAB belongs to the genus *Lactobacillus* and includes obligately (e.g. *L. brevis*) and facultatively heterofermentative species (e.g. *L. plantarum*) as well as obligately homofermentative species. Yeast and homo- and heterofermentative lactobacilli affect the characteristics of sourdoughs in different ways. Yeasts greatly contribute to the leavening, while lactobacilli play an important role in the acidification and structure of the grains and crumbs [5]. Yeasts and lactobacilli generally coexist and could establish a symbiotic relationship [6]. The metabolic activities of the dominating fermentation microbiota will influence all aspects of bread quality [7].

Lactobacilli that are developed in the dough may originate from naturally selected strains initially present in the cereals or flours or by addition of defined starter cultures containing one or more known strains [4]. This bacterial group contributes to technological, nutritional and sensory properties, and shelf-life [8–11]. Particularly, the capacities of lactobacilli to ferment a large range of wheat flour carbohydrates (i.e. maltose, sucrose, glucose and fructose) partly determines their overall competitiveness and have important technological repercussions during sourdough fermentations resulting from the acidification process and generation of compounds involved in flavor and preservation.

Bifidobacterium strains are natural inhabitants of the intestine of humans and animals. These strains are considered to be integrants of lactic acid bacteria genera, although they are phylogenetically unrelated. Bifidobacteria have a unique hexose metabolism that occurs via the phosphoketolase pathway [12]. Bifidobacteria produce the vitamins B-1, B-2, B-6, B-12, nicotinic acid and folic acid [13]. Enzymatic hydrolysis with participation of bifidobacteria increases bioaccessibility of lipids and proteins [14]. This type of bacteria can digest lactose and in consequence symptoms of lactose nontolerance diminish; because of that they are used in fermented dairy products [15]. Several *Bifidobacterium* species have been incorporated in yogurts as they are considered to

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Table 1
Microbial counts in wheat dough before and after the fermentation process[a]

Genus/strain	Species	Origin	Yeast counts (Log CFU/g)		LAB counts (Log CFU/g)	
			NFD[b]	FD[c]	NFD[b]	FD[c]
Control		Commercial	7.5 ± 0.1	7.6 ± 0.1	<4.5 ± 0.0	<4.5 ± 0.1
Bifidobacterium						
BIF326	*B. longum*	Human adult faeces	7.7 ± 0.1	7.7 ± 0.0	8.5 ± 0.0	8.6 ± 0.4
BIF349	*B. longum*		8.2 ± 0.6	8.3 ± 0.6	8.5 ± 0.4	8.9 ± 0.1
BIF307	*B. longum*		7.7 ± 0.2	7.7 ± 0.1	8.8 ± 0.9	8.8 ± 0.9
BIF31S	*B. catenulatum*		7.5 ± 0.0	7.6 ± 0.1	8.6 ± 0.1	8.5 ± 0.1
BIF324	*B. catenulatum*		7.6 ± 0.1	7.6 ± 0.1	8.8 ± 0.1	8.6 ± 0.2
BIF12R	*B. longum*		8.1 ± 0.8	8.3 ± 0.7	8.2 ± 0.1	8.4 ± 0.1
BIF22	*B. longum*	Human infant faeces	7.7 ± 0.1	7.7 ± 0.0	7.7 ± 0.1	7.7 ± 0.0
BIF112	*B. longum*		7.6 ± 0.1	7.7 ± 0.1	7.9 ± 0.1	7.8 ± 0.1
BIF113	*B. breve*		7.8 ± 0.0	7.9 ± 0.0	7.5 ± 0.1	7.9 ± 0.2
BIF211	*B. breve*		7.7 ± 0.1	7.9 ± 0.1	7.8 ± 0.0	7.9 ± 0.0
BIF-ID21	*B. longum*	Chicken small intestine	7.6 ± 0.1	7.7 ± 0.1	8.0 ± 0.7	8.2 ± 0.7
BIF-ID23	*B. longum*		7.8 ± 0.1	7.7 ± 0.1	7.8 ± 0.1	7.9 ± 0.1
BIF-IG21	*B. longum*	Chicken large intestine	7.7 ± 0.2	7.7 ± 0.0	8.1 ± 0.4	8.0 ± 0.3
BIF-IG24	*B.longum*		7.7 ± 0.0	7.7 ± 0.1	7.7 ± 0.1	7.8 ± 0.1
Lactobacillus						
L-62	*L. brevis*	FloraPan™ (commercial)	7.6 ± 0.1	7.7 ± 0.1	8.1 ± 0.2	8.4 ± 0.3
L-73	*L. plantarum*	FloraPan™ (commercial)	7.5 ± 0.0	7.8 ± 0.1	7.5 ± 0.1	7.8 ± 0.1

[a] Results are expressed as means of three independent experiments. Means ± S.D. ($n = 3$).
[b] Not fermented dough.
[c] Fermented dough.

have beneficial healthy effects. This bacterial group has been also successfully used as starter cultures in cereal fermentations [16], their potential use in breadmaking process has been scarcely explored.

In the present study, *Bifidobacterium* strains from different origins have been applied as novel starter cultures for bread production. The participation of bifidobacteria in leavening, acidification, and technological properties of wheat breads has been determined in comparison with that of facultatively or obligately heterofermentative lactobacilli currently commercialized.

2. Materials and methods

2.1. Microbial strains and growth conditions

The *Bifidobacterium* species and strains included in this study are listed in Tables 1 and 2. The strains BIF12R, BIF31S, BIF324, BIF307, BIF326 and BIF349 were isolated from human adult faeces [17]; strains BIF22, BIF211, BIF112, and BIF113 were isolated from human infant faeces; strains BIF-ID21 and BIF-ID23 were isolated from chicken small intestine; and strains BIF-IG21 and BIF-IG24 were isolated from chicken large intestine [18]. These bifidobacteria were identified by genus and species-specific PCR as described elsewhere [19,20].

The strains of the *L. brevis* L-62 CHCC2097 (obligate heterofermentative) and *L. plantarum* L-73 CHCC2102 (facultative heterofermentative) commercialized as starter cultures for dough fermentations were also tested (FloraPan™, Lallemand, Saint-Simon, France). Compressed baker's yeast (Levamax, Spain) was also included for bread making.

Lactobacilli and bifidobacteria were routinely grown in MRS broth (Scharlau Chemie, Barcelona, Spain) supplemented with 4 mM cysteine in the case of bifidobacteria (MRSC; Sigma, St. Louis, MO, USA). This medium was inoculated at 1% (v/v) with 24 h old cultures, previously propagated once in the same conditions. Cultures were incubated at 37 °C anaerobically (AnaeroGen™, Oxoid, England). Preliminary studies showed that 24 h of incubation gave the counts usually encountered in sourdough (around 10^8 CFU/g) [21]. Therefore, after 24 h of incubation, microbial cells were harvested by centrifugation (10,000 × *g*, 15 min, 4 °C, Sorvall RC-5B, DuPont Instruments), washed twice in 0.085% NaCl solution. The obtained cells were use to inoculate the dough. Lactobacilli and bifidobacteria counts were determined during the whole breadmaking process (after kneading, before and after fermentation period) in MRS and MRSC agar, respectively. To confirm the selective recovery of lactobacilli or bifidobacteria representative colonies of the highest dilution plates were isolated and tested for their sensibility to mupirocin disks (Oxoid, Hampshire, UK). Isolates resistant to mupirocin were considered to be bifidobacteria while sensitive isolates were considered to be lactobacilli [22]. 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 triplicate.

2.2. Determination of the proofing behavior of doughs

The characteristics of the commercial wheat flour used were: moisture 13.95%, protein ($N \times 5.7$) 11.34% dry matter (d.m.), ash 0.30% d.m. and Falling number 428 s.

Dough properties throughout the fermentation were continuously registered by the rheofermentometer (Chopin Rheofermentometer F3, Groupe Tripette et Renaud, Villeneuve-La-Garenne Cedex, France), which gives information about dough development, gas production and gas retention [23].The flour (250 g) was mixed with 1% yeast, 1%-24 h incubation LAB (prepared as mentioned previously) and 125 mL of tap water in the bowl of the Alveograph (Chopin Alveograph M82, Groupe Tripette et Renaud, Villeneuve-La-Garenne, Cedex, France) during 1 min, then 2% sodium chloride salt was added. Kneading lasted 8 min, and after that period, 315 g dough were placed in the rheofermentometer vessel, pressed with a 2 kg cylindrical weight and the chamber was hermetically closed. Fermentation was carried out at 37 °C for 3 h.

Table 2
Characteristics of fermented dough in the presence of different lactic acid bacteria[a]

Genus/strain	V (cm^3)	pH	TTA[b] (mL 0.1N NaOH)	Lactic acid (mmol/g d.m.)	Acetic acid (mmol/g d.m.)
Control	128 ± 7	5.5 ± 0.2	2.81 ± 1.01	0.021 ± 0.005	0.021 ± 0.005
Bifidobacterium					
BIF326	140 ± 7	4.9 ± 0.1	4.42 ± 1.38	0.234 ± 0.024	0.017 ± 0.005
BIF349	140 ± 7	5.0 ± 0.0	3.69 ± 0.37	0.225 ± 0.005	0.020 ± 0.005
BIF307	135 ± 0	5.5 ± 0.0	2.66 ± 0.30	0.073 ± 0.003	0.023 ± 0.004
BIF31S	130 ± 7	5.3 ± 0.0	3.93 ± 0.35	0.085 ± 0.005	0.019 ± 0.003
BIF324	132 ± 17	5.2 ± 0.0	4.40 ± 0.49	0.092 ± 0.003	0.022 ± 0.001
BIF12R	135 ± 10	5.5 ± 0.1	4.13 ± 0.01	0.139 ± 0.012	0.024 ± 0.005
BIF22	130 ± 3	5.6 ± 0.0	3.13 ± 0.42	0.026 ± 0.004	0.032 ± 0.004
BIF112	130 ± 3	5.5 ± 0.0	3.39 ± 0.55	0.071 ± 0.002	0.030 ± 0.006
BIF113	120 ± 7	5.4 ± 0.0	3.09 ± 0.83	0.061 ± 0.001	0.020 ± 0.006
BIF211	127 ± 17	5.5 ± 0.1	3.09 ± 0.93	0.046 ± 0.007	0.011 ± 0.005
BIF-ID21	132 ± 3	5.2 ± 0.3	4.69 ± 0.62	0.035 ± 0.010	0.024 ± 0.003
BIF-ID23	125 ± 7	5.2 ± 0.0	4.70 ± 0.42	0.135 ± 0.022	0.021 ± 0.004
BIF-IG21	127 ± 17	5.3 ± 0.2	4.40 ± 0.68	0.035 ± 0.006	0.028 ± 0.004
BIF-IG24	120 ± 10	5.4 ± 0.0	3.50 ± 0.28	0.048 ± 0.005	0.019 ± 0.002
Lactobacillus					
L-62	140 ± 7	5.3 ± 0.1	4.18 ± 0.63	0.093 ± 0.004	0.033 ± 0.005
L-73	128 ± 10	5.5 ± 0.1	4.00 ± 0.88	0.142 ± 0.016	0.027 ± 0.003

[a] Results are expressed as means of three independent experiments. Means ± S.D. ($n = 3$).
[b] Total titrable acids; V: dough volume (cm^3).

The parameters registered included: H_m, height under constraint of dough at maximum development time (mm); h, height of dough at the end of the test (mm); $(H_m - h)/H_m$ that is inversely related to dough stability; H'_m, maximum height of CO_2 production (mm); $T_1{}'$, time of the maximum gas formation (min); V_T, total volume of CO_2 (mL) produced during 3 h of fermentation (mm); V_r, total volume of the CO_2 (mL) retained by the dough; R_C, the CO_2 retention coefficient V_r/V_T, which measures the amount of CO_2 liberated from the dough and therefore is related to the porosity of the dough; T_X, the time (min) when the porosity of the dough develops. Each experiment was carried out in duplicate.

2.3. Breadmaking process

Three-hundred grams of wheat flour, 150 mL of tap water, 3 g baker yeast (1% flour basis), 6 g sodium chloride salt (2% flour basis), and the starter cells (bifidobacterial or lactobacilli) were used to produce 450 g of dough in a Brabender Farinograph. In control dough only baker's yeast were used to produce the dough.

After kneading for 5 min and dividing into pieces of 50 g, doughs were individually placed into aluminum pans. Fermentation was carried out at 37 °C and 80% relative humidity for 75 min. Fermentation was monitored by measuring pH, temperature and volume increase of the dough. A graduated cylinder was used for following dough volume increase during proofing. After the fermentation step, doughs were baked in an electric oven at 170 °C for 18 min and cooled at room temperature for 120 min. The experiments were done in triplicate.

2.4. Determination of pH, TTA, and lactic and acetic acids

pH and total titrable acids (TTA) of dough and bread were measured following the method of Arbeitsgemeinschaft Getreideforschung [24]. Ten grams dough or bread, blended with 100 mL acetone/water (5/95, v/v) under constant agitation, were titrated against 0.1N NaOH until a final pH of 8.5. TTA was calculated before and after fermentation period, as well as after breadmaking process. The results were expressed as the amount (mL) of NaOH 0.1N used for titrating 10 g of dough or bread [25].

Concentration of lactic acid and acetic acid in dough and bread were analyzed using the specific enzymatic kit (Boehringer, Mannheim, Germany) by UV method. The results were expressed as mmoles of lactic or acetic acid per gram of dough or bread of dry matter. Four replicates were run for each value.

2.5. Technological evaluation of the baked bread

The bread quality was evaluated by assessing the most characteristics technological parameters: loaf volume (rapeseed displacement), weight, specific volume, width/height ratio [26], moisture content [27], and bread crumb hardness [28]. Texture of fresh bread was measured after maintaining bread at room temperature for 2 h. Crumb hardness was measured in a texturometer TA-XT2i (Stable Microsystems, Surrey, UK). Briefly, a 2 cm thick slice was compressed with a 10 mm probe up to 50% at 1 mm/min speed [28]. Each technological evaluation was carried out in triplicate, with the exception of the crumb hardness that was made in quadruplicate.

2.6. Statistical analysis

Data parameters measured during the breadmaking process are the mean of values obtained in three independent experiments. In each experiment, parameters were determined at least in duplicate. Multiple sample comparison of the means was statistically analyzed with the program Statgraphics Plus 7.1. Fisher's least significant differences (LSD) test was used to define differences between means at the 5% significance level ($p < 0.05$).

3. Results and discussion

3.1. Characterization of doughs fermented by different bacterial strains

3.1.1. Microbial evolution during dough fermentation

Bifidobacterial strains isolated from different origins were evaluated as possible starter cultures for dough fermentation, using similar amount of microbial counts as found in bread sourdough. Bacterial starters showed initial levels of 3.1×10^7 to 6.3×10^8 CFU/g, whereas yeast initial population ranged from 3.1×10^7 to 1.6×10^8 CFU/g (Table 1). Both populations were in the range of the microbial counts found in bread sourdough [21]. Fermentation was carried out at 37 °C for 75 min. During the fermentation step both populations did not

show significant ($p < 0.05$) variations, likely due to the duration of the fermentation time, and remained almost at constant levels in all doughs (Table 1). These data indicated that the inoculated bifidobacterial strains could be adapted to the dough environment at least maintaining their viability. In control doughs initial levels of lactobacilli were about 10^4 to 10^5 CFU/g, which is in the range found in white flours [25].

3.1.2. Dough volume

The dough volume reached after the fermentation stage did not differ significantly ($p < 0.05$) in all inoculated samples when compared to the control (128 cm^3). Interestingly, the highest volume increase (140 cm^3) achieved in doughs fermented by the heterofermentative commercial starter *L. brevis* (L-62) were also reached in doughs fermented by the adult bifidobacterial strains *B. longum* BIF326 and *B. longum* BIF349. The lowest dough volume was obtained in the presence of BIF113 or BIF-IG24. Considering the microbial counts obtained in fermented doughs, no relationship could be established between the microbial counts and the fermented dough volume. Overall, the dough fermented by adult bifidobacterial showed higher volume than those fermented by either infant or chicken bifidobacterial strains (Table 1).

3.1.3. pH, organic acid production and TTA values in fermented doughs

The pH value of control dough (with commercial baker's yeast) decreased from 5.9 to 5.5 after the fermentation stage (Table 2). Some differences were found regarding the pH values of dough fermented by bifidobacterial strains of different origin or lactobacilli. *L. brevis* and *L. plantarum* strains reduced the dough pH values to 5.3 and 5.5, respectively. However, some bifidobacterial strains reduced the pH to lower values (≤pH 5.2). Compared to the control, these reductions in pH were significantly different ($p < 0.05$) in doughs inoculated with the adult bifidobacterial strains *B. longum* BIF326 and *B. longum* BIF349, reaching values of 4.9 and 5.0, respectively. The doughs fermented by infant bifidobacterial strains reached similar pH values (between 5.4 and 5.6) to the control dough. Concerning the amount of lactic and acetic acid produced in fermented doughs, the production of lactic acid was predominant while the concentration of acetic acid was low and similar to control values (Table 2). No significant differences in the acetic acid amount were found between samples containing lactic acid bacteria (LAB) and the control dough in the absence of them. However, significant differences were found within the dough containing different lactic acid bacterial strains. The concentration of lactic acid showed great differences among bacterial strains. *B. longum* BIF326 and *B. longum* BIF349 were the strains that resulted in doughs with the highest lactic acid values (0.234 and 0.225 mmol/g d.m., respectively), followed by *L. plantarum*, *B. longum* BIF12R, and *B. longum* BIF-ID23. The remaining strains showed far lower values than the strains mentioned above. In general, doughs fermented by adult bifidobacterial strains showed significantly higher ($p < 0.05$) lactic acid content that those fermented by infant strains, with the exception of BIF112. Again, considering the microbial counts obtained in fermented doughs, no relationship could be established between the short fatty acids production and the microbial counts, thus the observed differences might be attributed to the bacterial metabolism. Corsetti et al. [29] stated that sourdoughs produced with homofermentative or facultative heterofermentative LAB had the lowest pH, while Gianotti et al. [5] reported that the pH variation depends on both lactic acid and acetic production and, to a lesser extent, on CO_2 dissolution in the water phase. For sourdough bread production, a molar ratio of lactic to acetic acid from 2.0 to 2.7 is considered an optimal value for its sensory quality [30,31]. In this study, the highest molar ratio of lactic to acetic acid was obtained with the strains *B. longum* BIF326 and *B. longum* BIF349 (13.48 and 11.04, respectively). Molar ratio between 2.0 and 2.7 were obtained when doughs were fermented by the infant strain *B. longum* BIF112 (2.4) and the large intestine chicken strain *B. longum* BIF-IG24 (2.6). The samples made with *L. brevis* showed a nearby value (2.8).

The production of different acids as a result of carbohydrate fermentation was reflected in the TTA increase from 1.5–1.7 to 2.6–4.4 mL. Similar increase was found by Vernocchi et al. [32] in the fermentation of wheat dough with *L. plantarum* strains. Significant ($p < 0.05$) increase in the TTA was induced in the presence of *B. longum* BIF326 and *B. catenulatum* BIF324 (adult bifidobacterial strains), and also with chicken strains (with the exception of *B. longum* BIF-IG24). The lowest total titrable acidity was obtained in the presence of BIF307. No relationship could be established between the TTA and the microbial counts present in the doughs, thus, results might be ascribed to the bacterial metabolism more than to the amount of microbial counts.

3.1.4. Dough development during proofing in rheofermentometer

Three strains from different origins were randomly chosen (*L. plantarum*, *B. longum* BIF326 from human faeces and *B. longum* BIF-IG24 from chicken faeces) in order to determine their behavior during wheat dough fermentation. The lactic acid bacteria strains were combined with yeast for determining their rheofermentative parameters (Table 3). The rheofermentograms showed similar shape with slight differences in several

Table 3
Fermentation parameters obtained using the rheofermentometer

	Control	BIF326	BIF-IG24	*L. plantarum*
Dough development				
H_m (mm)	48.7 ± 0.5	51.4 ± 0.4	49.2 ± 0.5	49.6 ± 0.9
h (mm)	21.3 ± 0.2	35.7 ± 0.9	18.9 ± 0.6	34.2 ± 0.4
$(H_m - h)/H_m$ (%)	56.3 ± 1.0	30.5 ± 1.2	61.6 ± 1.3	31.0 ± 0.9
T_1 (min)	126 ± 7	126 ± 9	127 ± 6	133 ± 9
T_2 (min)	145 ± 9	150 ± 11	144 ± 7	159 ± 9
Gas behavior				
H'_m (mm)	65.2 ± 0.8	63.7 ± 1.0	68.4 ± 0.6	69.6 ± 0.7
T_X (min)	90 ± 7	76 ± 5	87 ± 6	88 ± 5
V_T (mL)	1427 ± 25	1403 ± 17	1466 ± 21	1451 ± 29
V_r (mL)	1234 ± 17	1210 ± 27	1257 ± 15	1263 ± 21
R_C	86.5 ± 0.8	86.3 ± 1.0	85.8 ± 1.3	87.0 ± 1.7
T'_1 (min)	111 ± 9	85 ± 7	114 ± 9	112 ± 5

Parameters definition is described in materials and methods section.

parameters among strains (data not shown). The inoculation of *L. plantarum* produced dough similar to that fermented by the yeast alone (control), in spite of the higher cell concentration obtained in the dough containing *L. plantarum*. The unique difference of *L. plantarum* compared to the control dough was the dough height at the end of the experiment (h), which was 12.9 mm higher than that of the control sample. The strain *B. longum* BIF326 also showed higher dough height at the end than that of the control; whereas the presence of BIF-IG24 resulted in a decrease of that value. The sample inoculated with the strain BIF326 showed an early dough development, although no differences were detected on the time to reach the maximum dough height (T_1). The maximum dough height in the presence of BIF326 was slightly higher (51.4 mm) than that observed in the control sample (48.7 mm). The overall result is an increase of dough stability in the presence of BIF326 or *L. plantarum*, and the opposite effect when dough contained BIF-IG24. In addition, the time needed to reach the maximum gas formation (T'_1) was 85 min, whereas the control sample required 111 min. This result could be attributed to the higher cell concentration observed in the BIF326 compared to the other lactic acid bacteria tested. The pH decrease caused by the bifidobacterial strain was probably responsible for an initial activation of yeast metabolism. No further increase in the total CO_2 was observed when yeast was associated to *B. longum* BIF326 strain, neither with the other LAB strains. As was stated by Rosell et al. [33], the relation between gas production and retention is related to the dough ability to be stretched in thin membranes, and in turn, it is associated to quality of the protein network. No differences in the retention coefficients were observed in the presence of the LAB strains tested, despite the dough containing BIF326 induced an early appearance of dough porosity (Tx). This strain could have specific proteolytic activity, which may weaken the gluten network without affecting the dough porosity.

3.2. Characteristics of breads

Bread loaves obtained in the presence of different strain groups had several differences (Table 4). The control bread showed neutral pH, whereas the bread made with bifidobacterial strains or lactic acid bacteria showed significantly lower pH, with the exception of *B. longum* BIF326, BIF349, BIF307, BIF22 and BIF112. In breads done with chicken bifidobacterial starters, the pH values decreased till 5.7–5.5; whereas in those made with the adult strains *B. catenulatum* BIF31S and BIF324, pH values were 5.3 and 5.2, respectively. Similar values to the ones obtained in breads made from lactobacilli starters [9,34]. The same trend was observed in the cases of breads inoculated with commercial lactobacilli, especially with *L. brevis* (Table 3). When comparing TTA of fermented dough, a moderate decrease of TTA (from 26.8% to 49.0%) occurred due to the effect of baking on volatile short acids (Table 4). Similar results were obtained by Corsetti et al. [29], who found a reduction up to 42.3%. No significant differences were found between specific volume of breads made with the different bacterial strains (values between 3.45 and 3.80 cm^3/g), with the exception of those breads made with chicken bifidobacterial strains, where the specific volume was significantly lower (2.75–2.95 cm^3/g) than that of the control. The small differences observed in the presence of the different bacterial strains can be explained by the fact that gas production mainly relies on yeast activity, and not on bacterial metabolism.

Related to bread hardness no significant ($p < 0.05$) differences were found between strains of different origins, with the exception of breads made with the strain *B. longum*

Table 4
Technological parameters of bread[a]

Starter strain	Humidity (%)	Specific volume (cm^3/g)	Bread shape[b] (cm/cm)	pH	TTA[c] (mL 0.1N NaOH)	Hardness (N)
Baker's yeast	48.66 ± 1.76	3.63 ± 0.51	1.15 ± 0.02	6.9 ± 0.7	1.79 ± 0.37	2.17 ± 0.29
BIF326	48.94 ± 0.44	3.80 ± 0.70	1.09 ± 0.04	6.3 ± 0.1	2.69 ± 0.22	1.84 ± 0.14
BIF349	48.96 ± 2.65	3.60 ± 0.42	1.21 ± 0.11	6.5 ± 0.1	2.40 ± 0.04	1.94 ± 0.07
BIF307	48.07 ± 1.27	3.45 ± 0.35	1.18 ± 0.02	6.7 ± 0.2	1.95 ± 0.07	2.16 ± 0.12
BIF31S	46.77 ± 1.23	3.45 ± 0.07	1.25 ± 0.01	5.3 ± 0.5	2.62 ± 0.30	2.10 ± 0.08
BIF324	48.64 ± 2.54	3.55 ± 0.49	1.22 ± 0.12	5.2 ± 0.1	3.20 ± 0.14	2.16 ± 0.14
BIF12R	47.93 ± 1.52	3.80 ± 0.14	1.18 ± 0.06	5.9 ± 0.2	2.61 ± 0.02	2.16 ± 0.27
BIF22	49.25 ± 1.43	3.55 ± 0.21	1.13 ± 0.00	6.9 ± 0.1	1.86 ± 0.06	2.15 ± 0.11
BIF112	48.95 ± 2.05	3.53 ± 0.49	1.21 ± 0.00	6.7 ± 0.2	1.73 ± 0.25	1.96 ± 0.09
BIF113	48.75 ± 0.07	3.45 ± 0.07	1.24 ± 0.10	5.7 ± 0.0	2.17 ± 0.66	2.51 ± 0.19
BIF211	48.68 ± 0.37	3.45 ± 0.35	1.17 ± 0.10	5.7 ± 0.2	2.25 ± 0.78	2.51 ± 0.23
BIF-ID21	47.91 ± 2.08	2.95 ± 0.07	1.23 ± 0.06	5.6 ± 0.3	3.04 ± 0.79	2.68 ± 0.10
BIF-ID23	46.48 ± 1.67	2.75 ± 0.21	1.26 ± 0.13	5.7 ± 0.1	3.15 ± 0.42	2.13 ± 0.50
BIF-IG21	46.07 ± 0.46	2.85 ± 0.07	1.26 ± 0.13	5.5 ± 0.4	2.92 ± 0.76	2.53 ± 0.01
BIF-IG24	46.17 ± 1.78	2.95 ± 0.07	1.25 ± 0.10	5.7 ± 0.0	2.34 ± 0.23	2.79 ± 0.14
L-62	45.53 ± 0.07	3.70 ± 0.03	1.16 ± 0.06	5.5 ± 0.0	3.03 ± 0.00	2.56 ± 0.20
L73	46.42 ± 1.43	3.60 ± 0.14	1.23 ± 0.00	5.9 ± 0.1	2.45 ± 0.07	2.89 ± 0.32

[a] Results are expressed as means of two independent experiments. Means ± S.D. ($n = 3$).
[b] Width/height rate.
[c] Total titrable acids.

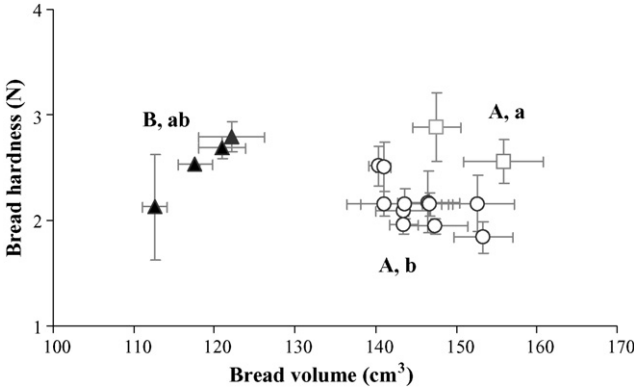

Fig. 1. Bread hardness related to bread volume. Human bifidobacterial strains (○); chicken bifidobacterial strains (▲); *Lactobacillus* strains (□). Uppercase and lowercase letters indicate significance between bacterial groups at $p < 0.05$ in bread volume and hardness, respectively. Control volume: 46.6 cm^3 (AB) and control hardness: 2.17 N (ab). Different letters indicate significant difference. Errors bars are the mean of at least three replicates ± S.D.

BIF-IG24 and *L. plantarum* L73 that had significantly higher values (2.79 and 2.88 N, respectively). Breads made with chicken bifidobacterial strains showed a linear increase ($R^2 = 0.987$) of the crumb hardness with the increase of bread volume (Fig. 1). Conversely, commercial lactobacilli strains and human strains showed an inverse relationship between both parameters.

Regarding the bread shape (width/height ratio of central slice), no significant differences were observed between the control and the samples with different starters, but significant ($p < 0.05$) differences were observed within the doughs containing different lactic acid bacterial strains. Only the samples made with the human strain *B. longum* BIF326 (1.09 cm/cm) induced a reduction of the bread shape, thus, improved the shape of the loaf slices. In opposition, the samples made with the chicken strains *B. longum* BIF-ID23 and BIF-IG21 resulted in a significant increase of the width/height ratio, indicating lower oven rise during baking.

The bread obtained with the bifidobacterial strains *B. longum* BIF326 or *B. longum* BIF349, which showed the lowest pH and the highest lactic acid content in dough (Table 2), had the lowest crumb hardness (1.84 and 1.94 N, respectively), and together with commercial lactobacilli strains the highest bread volume (Fig. 1). Similar results were obtained by Gül et al. [1], who reported that bread produced with dough of low pH and high ratio of lactic and acetic acids had the highest volume and moreover the lowest rate of staling during storage.

Bifidobacterial strains from different origin can be used as starter cultures for breadmaking. Important differences between the behavior of chicken bifidobacterial strains and human bifidobacterial strains were found when comparing bread TTA, bread shape, moisture and bread volume relationship. The breads made with chicken strains showed lower bread specific volume than those made with human strains.

Human bifidobacterial strains can be used as starters for breadmaking leading to breads with similar characteristics than the ones obtained from *Lactobacillus* strains.

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