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Use of *Lactobacillus* from Pulque in Sourdough

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Abstract

The purpose of this study is to determine whether there are significant differences in the use of lactic acid bacteria isolated from pulque dough *Lactobacillus plantarum* and *Lactobacillus paracasei* ssp. *paracasei* alone and combined with baker's yeast in the properties of the dough and the finished product. The best bacteria were selected using growth kinetics and statistical analysis with the Sigma Plot 11 program. Physical-chemical tests pH, % acidity of the dough. Physical tests, texture profile analysis, image analysis of the crumb structure and sensory analysis were performed on the finished product. The results show that the most suitable LAB to ferment dough in 5 hours is *Lactobacillus paracasei* ssp. *paracasei* which reduce pH and increase acidity more quickly. The combination of *Lactobacillus paracasei* ssp. *paracasei* plus baker's yeast presents better quality attributes in terms of texture, flavor and appearance for the final consumer.

Keywords

Lactic Acid Bacteria, Bread, Texture Profile Analysis, Pulque Bread, Dough Pulque

1. Introduction

The sourdough in wheat bread production clearly improves dough properties, bread texture and flavor, delays the staling process and prevents bread from mould and bacterial spoilage [1] [2]. These benefits result from a common trend of sourdough fermentations are the unique symbiosis of certain hetero- and homo-fermentative

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lactic acid bacteria whit certain yeasts. *Lactobacillus sanfranciscensis*, *L. brevis* and *L. plantarum* are the most frequently isolated lactic acid bacteria, while *Saccharomyces cerevisiae* is the yeast most frequently present [1]-[4]. In spontaneous sourdough fermentations, it is likely that apart from the dominant lactic acid bacteria and yeast species that are isolated in numbers suggest a significant contribution to the fermentation, a secondary microbiota exists atm lower numbers. This secondary microbiota includes lactic acid bacteria species such as *L. alimentarius*, *L. acidophilus*, *L. fructivorans*, *L. fermentum*, *L. reuteri* and *L. pontis* [3] [4] and yeast species such as *S. exiguous*, *Candida krusei* and *C. milleri* [5].

Within Mexican gastronomy, the breadmaking industry is not only a source of employment but part of artisanal, business and cultural development for Mexican people. There are many natural products that can serve as raw material for breadmaking, among them pulque, a traditional Mexican drink dating back to the pre-Hispanic era. Pulque is rich in carbohydrates and microorganisms such as lactic acid bacteria, acetic acids and yeasts. These bacteria ferment the sugars forming acetic acid, ethanol, lactic acid and CO_2 depending on the species, and contribute to food biopreservation, improve sensory attributes such as taste, aroma and texture, and increase nutritional value. Yeasts help the formation of gas by fermenting sugar to ethanol and CO_2 [6]. Pulque bread can contain alcohol and lactic acid produced by the inoculum of pulque which may inhibit undesirable bacteria. The bread is made with sourdough [6] which is a mixed culture of lactic acid bacteria and yeast. The introduction of new methods to improve bread creates new challenges for obtaining a good quality product given the wide selection of additives, such as enzymes, emulsifiers, antioxidants and preservatives, which unquestionably improve, above all, the flavor, texture and shelf life of bread. Nevertheless, the majority of these are made with chemical products, going against the current consumer trend to prefer natural foods without chemical additives. Using microorganisms from natural products may be effective in improving the characteristics of bread. In this work we determined the physical-chemical characteristics of bread made with lactic acid bacteria of the genus-*Lactobacillus* isolated from sourdough pulque.

2. Materials and Methods

2.1. Materials

Lactic acid bacteria isolated from the pulque's doughs. Flour bulk silver leaf (Elizondo), fat (La Gloria), standard sugar (Golden Hills), egg (Bachoco), yeast (tradipan), milk powder (Nido), salt (Fina), pulque (obtained from Singuilucan region in the state of Hidalgo, Mexico) and distilled water and sterile saline.

Lactic Acid Bacteria. Lactic acid bacteria studied were isolated and identified by the technique API 50 CHL, colonial morphology (color, size, type, edge, elevation, reflected light, transmitted light) and cellular (Gram staining and catalase) sourdough with pulque in the Microbiology Laboratory Sciences Research Center and Food Technology of ICAp-UAEH, which they were encoded as LB1 to LB10 and they kept in MRS agar plus 10% glycerol according of method of Sanchez [7].

2.2. Methods

Flour analysis. Moisture and total solids determination was using 44-19 method [8], ash by the method 14-006 [9] and protein by the method 20 - 57 [9].

Pulque's doughs preparation. Determination of the growth kinetics of *Lactobacillus* strains: from BAL strains preserved, were activated in MRS agar petri dishes (DIFCO) cross-streak, were subsequently incubated at 37˚C for 48 hours [10]. The selection of BAL for the dough fermented was based on the production of CO_2, BAL growth: once the growth of each of the strains selected on the basis of the results obtained (BL2, BL6 and BL7), will take a colony and inoculated into Erlenmeyer flask with 99 mL of MRS broth which was incubated at 37˚C for 48 hours (performed in triplicate), which was used to incubate with 1 mL for each of the 33 test tubes, (3 tubes 3 each control and fermentation time) containing 9 mL of MRS broth which corresponded to the following times (0, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 minutes). These tubes were incubated at 37˚C, after, each time decimal dilutions from 10^{-1} to 10^{-6}. In MRS agar petri dishes were placed 50 µl of the last three dilutions using the scanning technique in plate. Preparation of inoculum: culturing the BAL (BL2, BL6 and BL7) were inoculated at 1% V/V in Erlenmeyer flasks containing 99 mL of MRS broth, and incubated for 24 h at 37˚C. Biomass was obtained by centrifugation (5000 rpm, 4˚C for 15 min). Biomass was resuspended in 50 mL sterile saline (0.9%), cell concentration containing 108 CFU/mL. It was added directly to the dough [11].

Fermentation of pulque's dough. 110 g dough were prepared with each of the selected bacteria (LB2, LB6 and LB7), 11 samples were obtained 10 g of dough portions each corresponding to each fermentation time (0, 30, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min), then incubated at 37˚C. In a sterile bag (Nasco Whirl-Pak) insertion of a portion of dough 10 g plus 100 mL of sterile distilled water, the mixture was ground in a Stomacher (40 recirculator, Sward) at 250 rpm for 90 s. Subsequent dilutions were made from 10^{-1} to 10^{-7}. Then plated on MRS agar petri dishes, 50 µl of the last three dilutions using the plate scanning technique for 24 h. Analytical determination of pH was using 10 g dough and 100 mL of distilled water were ground with a food processor (Brand Taurus) in a beaker for 3 min at speed 1 processor. After potentiometer brand electrode (Oakton SN) was submerged and the values were recorded. It made in triplicate [9]. Analytical determination of acidity: according 10 g of sample put up and dissolved in 50 mL of distilled water. It was added 2 mL of a solution of 1% phenolphthalein. The solution was titrated with 0.1 N sodium hydroxide until it formed a transparent turns from pink. It made in triplicate.

Texture Profile Analysis with Peltier Control Unit for the doughs. 25 g samples of 3 × 3 cm in diameter and height were compressed on a texture analyzer TAHDi (Texture Technologies, New York, USA/Stable Microsystems, Surrey, UK) and a control unit Peltier with a load cell of 50 kg and a cylindrical probe Delrin half inch at a speed of 1 mm/s by compressing the dough in two cycles to 50% of its original height with a waiting period of 5 s between each. Compression is carried out every 30 m for 5 h. To obtain the following parameters after APT: hardness, elasticity, adhesiveness and cohesiveness, the analysis made in triplicate.

Bread Analysis. A series of bread analyses was performed with three loaves at day 0, after 2 h of cooling, before packaging. The crust and crumb, were determined put on a whole loaf, remove the crust and weigh it, the result is subtracted from the initial weight of the bread crumbs give the amount that should be related to 100. The coefficient of elevation was determined the width/height were measured with a vernier, at the center slice of bread [12]. Loaf weight and volume (rapeseed displacement method) were calculated. Images of the bread were obtained three slices of bread for each formulation of 1 cm thick on a scanner HP Officejet J5700 (tiff image format), with a resolution of 600 pp and analyzed by ImageJ 1.45 program (National Institutes of Health, Bethesda, MD, USA). Texture profile analysis (TPA) of bread: Loaves of 25 g of 3 × 3 cm in diameter and height were compressed on a TAHDi texture analyzer (Texture Technologies, New York, USA/Stable Microsystems, Surrey, UK), with a load cell of 50 kg and a probe P25/L perspex cylinder 25 mm at a speed of 1 mm/s by compressing the doughs in two cycles to 50% of its original height with a waiting period of 5 s between each. Obtain the following parameters APT: hardness, elasticity, resilience and cohesiveness, the analysis was performed in triplicate. The color was analyzed according Anzaldúa [13]. The sensory analysis was performed to 35 consumers, according to Anzaldúa [13].

Statistical Analysis. The statistical analysis was a completely randomized design with a statistical package Sigma Plot 11. An analysis of variance (ANOVA) and mean comparisons by the Tukey method ($P \leq 0.05$). The **Table 1** shows the formulations used for the manufacture of bread's pulque whit the yeast + BAL 2, 6 and 7.

Table 1. Formulations used for the manufacture of breads and doughs with pulque, and yeast inoculum.

Ingredients (%)	Pulque's dough	LAB 2, 6, 7 + dough	LAB 2, 6, 7 + yeast
Flour	100	100	100
Sugar	15.6	15.6	15.6
Salt	1.2	1.2	1.2
Fat	25.0	25.0	25.0
Milkpowder	5.0	5.0	5.0
Egg	20.5	20.5	20.5
Water	20.0	20.0	20.0
Pulque	15.0	0.0	0.0
Yeast	0.0	0.0	0.2
LAB 2, 6, 7	0.0	15.0	15.0

The percentage is based on a function of the flour. LB7 = *Lactobacillus paracasei* ssp. *paracasei*; LB6 = *Lactobacillus paracasei* ssp. *paracasei*; LB2 = *Lactobacillus paracasei* ssp. *paracasei*.

3. Results and Discussion

3.1. Proximate Analysis of Wheat Flour

According to the results obtained from this analysis the flour was found to be Grade I for bread making, maximum moisture 14.0%, minimum protein 9.5% and maximum ash 0.55% giving an excellent baking quality due to the protein content [14].

3.2. LAB Growth Kinetics

From the microscopic observations made using Gram stain, catalase testing, API testing and colonial morphology, the bacteria isolated from dough fermented with pulque were identified as reported in **Table 2**, where 50% were *Lactobacillus paracasei* ssp. *paracasei* and the remaining 50% were *Lactobacillus plantarum*, from which we selected LB2, LB6 and LB7 (*Lactobacillus paracasei* ssp. *paracasei*) since they reported the highest growth rate and significant differences between them ($\alpha = 0.05$).

It can be seen from the behavior of the LAB in the dough that due to their rapid growth and adaptability to the medium, the results are favorable.

3.3. The Acidification Properties during Pulque's Dough Fermentation

In the **Figure 1**, it showed the effect of the LAB starter on pH values was monitored over 300 min pulque's dough fermentation and was compared with an uniculated *Saccharomyces cereviceae* dough prepared under the same conditions. As expected the pH value nearly constant (5.2 to 4.7). During the same period the acidity increase of 0.33% to 0.49% v/v lactic acid during the fermentation of 5 h. Start yeast dough with pH 6.32 and only get 6.22 of 5 h, the acidity was to 0.27% - 0.32% v/v lactic acid. Doughs were inoculated only with Lactobacillus a final pH of 4.67, for LB2, 4.91 y 4.51 to LB6 and LB7 respectively, the acidity was between 0.43% to 0.53%, 0.37% to 0.52%, 0.32% to 0.44% v/v lactic acid. The acidity of the doughs with *Lactobacillus* sp, showed to the time 6 h a pH of 5.7 and acidity was 0.18% v/v lactic acid, in this investigation the 3 strains of *Lactobacillus paracasei* ssp. *paracasei* 2, 6 and 7 decreased the pH and the acidity was higher than previously reported [15]. The pH differed depending on the fermentation temperature and LAB concentration, whereas the acidity was greatly affected by the fermentation temperature [16]. The fermentation temperature and time affected the volume of the dough, although there was no significant difference. The volume of the sourdough rapidly increased early and maintained a constant volume until the end of the fermentation process.

Lactobacillus doughs inoculated more with commercial yeast resulted for LB2 + yeast a final pH of 5.8 and acidity of 0.32% v/v lactic acid, for LB5 and LB7 + yeast the pH was of 6.22 and 6.24 respectively, and the acidity of 0.32% v/v lactic acid for the 2. The dough acid fermentation whit *Lb. sanfranciscencis* and *S. cerevisiae* reached within 24 h pH of 3.96 and acidity of 0.85%, the fermentation temperature of 25˚C, yeast growth was most active, but as the temperature decreased, the number of yeast cells decreased slowly, the causing fermentation activity to also decrease [16]. According to the results obtained and consulted bibliography the best

Table 2. Identification of the *lactobacillus* bacterias isolated from dough fermented with pulque.

Bacteria code	Bacteria identified
LB1	*Lactobacillus paracasei* ssp. *paracasei*
LB2	*Lactobacillus paracasei* ssp. *paracasei*
LB3	*Lactobacillus paracasei* ssp. *paracasei*
LB4	*Lactobacillus plantarum*
LB5	*Lactobacillus plantarum*
LB6	*Lactobacillus paracasei* ssp. *paracasei*
LB7	*Lactobacillus paracasei* ssp. *paracasei*
LB8	*Lactobacillus plantarum*
LB9	*Lactobacillus plantarum*
LB10	*Lactobacillus plantarum*

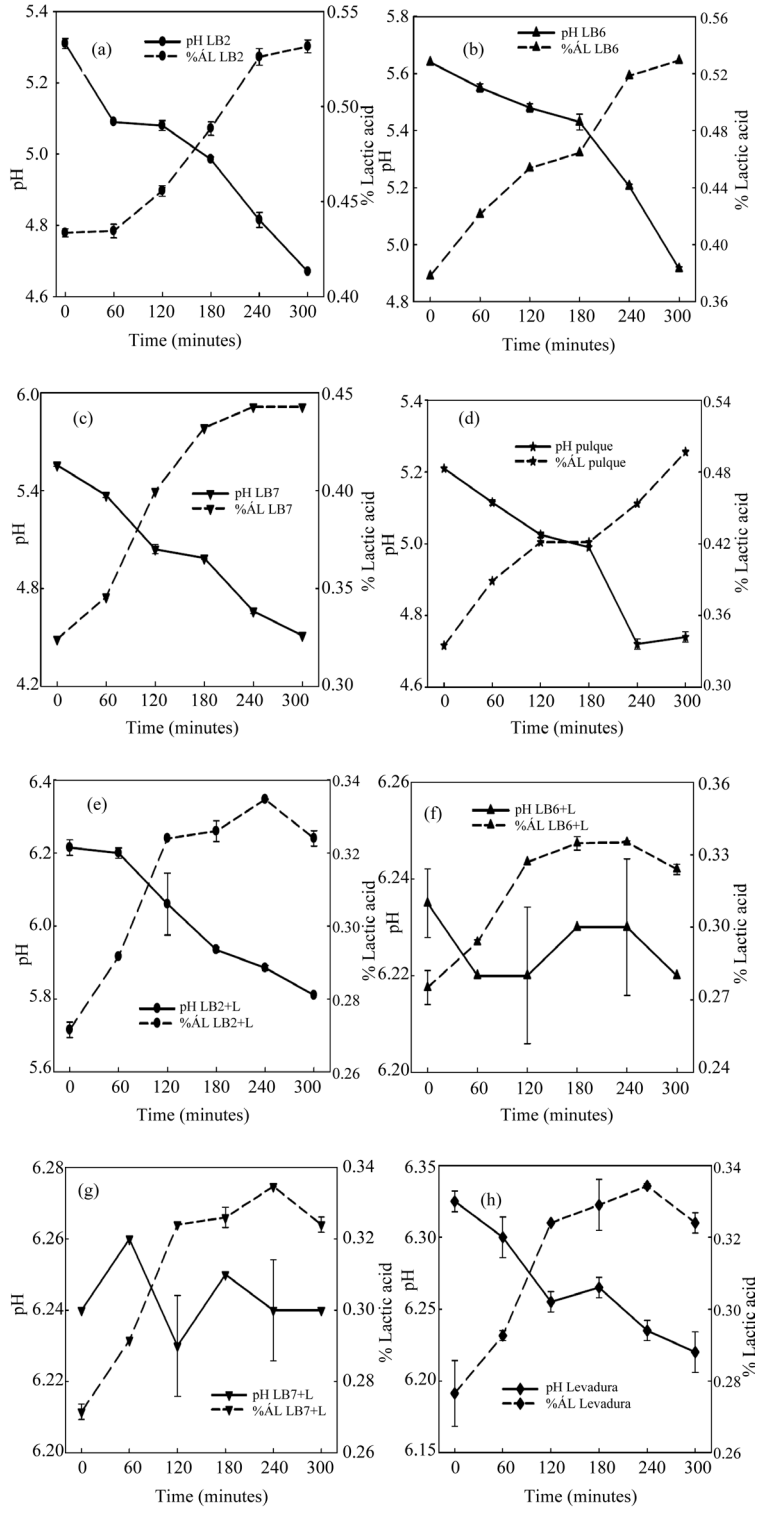

Figure 1. (a) pH and % lactic acid of dough with LB2; (b) pH and % lactic acid of dough with LB6; (c) pH and % lactic acid of dough with LB7; (d) pH and % lactic acid of dough with pulque; (e) pH and % lactic acid of dough with LB2 + L; (f) pH and % lactic acid of dough with LB6 + L; (g) pH and % lactic acid of dough with LB7 + L; and (h) pH and % lactic acid of dough with yeast.

results from the fermentation of the doughs was the LB7 + yeast mixture.

3.4. Behavior of LAB in Dough

Figure 1 showed that the LB2 remained constant with a count of 8.86 Log UFC/mL until minute 150 when it presented a decrease in growth. LB6 and LB7 showed a growth decrease from minute 60 reaching the end of fermentation with a count of 8.65 and 8.35 Log UFC/mL respectively.

The fermentation of dough has beneficial effects since it prolongs shelf life, increases volume, delays hardening, and improves the flavor and nutritional value of the bread [17] [18].

In determining the lift coefficient (**Table 3**) its importance can be said to lie in the quality of the bread and it being well-made since there are no proteolytic bacteria that can impair the retention of gases that are essential for the rising (volume) of the dough [14]. The breads made solely with *Lactobacillus* presented a higher lift coefficient indicating the presence of proteolytic bacteria which inhibited the increase in volume of the bread. In contrast, the breads made with baker's yeast and *Lactobacillus* did present a good lift coefficient; here the leavening agents were more active achieving a better "volume" of bread. The importance of the volume is that it is directly related to the volume of the crumb pores, and it also indicates that the bread is well made and of good quality. As can be seen, LB7 was of the poorest quality with an index of 0.52, followed by all the breads inoculated solely with lactobacilli and pulque which had an index of 0.45 - 0.46 and were also of poor quality in terms of volume. The breads inoculated with lactobacilli and baker's yeast had an apparent density index of 0.21 - 0.22, indicating a good quality bread since it varies between (0.20 - 0.22). In the percentages of crust and crumb we find that the breads with lactobacilli have 81.09% - 83.03% crumb and 16.97% - 18.91% crust which tells us there is a slight alteration since well-made bread varies from 70% - 80% in crumb and 20% - 30% in crust; this may be caused by a poor bread making technique and an inadequate oven temperature whereby the starch did not produce a sufficient amount of dextrin [19].

The obtaining of the maximum volume may be due to the release of exopolysaccharides which strengthen the gluten network and the starch, influencing both the size and retrogradation of the product, hence the bread with yeast and lactobacilli had good volume [14].

The plastic and mechanical qualities of the dough are of great importance in breadmaking since they all allow the formation of smaller cells under the pressure of the gas retained by the fine gluten network [14]. If this does not happen, diverse alterations or faults appear as seen in the images of the breads with *Lactobacillus*. Thus, if the dough is too solid, the gas is not distributed evenly throughout, causing deep or less deep cracks to form in the crust or crumb which are ports of entry for microorganisms, such as fungi [20]. A frequent fault is also the formation of moist striations under the crust due to the lack of sponginess of the dough. Sometimes during breadmaking there is a partial separation between the crust and crumb, as observed when the dough, rich in gassy bubbles, is placed in the hot oven too soon. This causes the solid crust to form before the gas is released, and the pressure of the gas separates the crust from the crumb. On the other hand, when the gas is released before the

Table 3. The physical tests and texture profile analysis of the bread with *Lactobacillus*, pulque and yeast.

Bread	Lift Coefficient	Specific Volume (cm^3/g)	% Crust	% Crumb	Hardness (g)	Adhesiveness (g * s) (−)	Elasticity (mm)	Cohesiveness (g * s)
LB7	2.611 ± 0.02^{a}	0.52 ± 0.00^{a}	18.49 ± 0.84^{b}	81.51 ± 0.84^{a}	256.70 ± 0.06^{d}	0.00 ± 0.00^{a}	0.742 ± 0.01^{ab}	0.283 ± 0.13^{a}
LB6	2.571 ± 0.03^{a}	0.45 ± 0.00^{b}	18.00 ± 1.12^{b}	82.00 ± 1.12^{a}	412.58 ± 0.21^{b}	0.00 ± 0.00^{a}	0.785 ± 0.04^{ab}	0.289 ± 0.01^{a}
LB2	2.314 ± 0.34^{a}	0.46 ± 0.01^{b}	18.91 ± 0.66^{b}	81.09 ± 0.66^{a}	175.46 ± 0.35^{e}	0.00 ± 0.00^{a}	0.665 ± 0.12^{ab}	0.266 ± 0.01^{a}
Pulque	1.972 ± 0.00^{b}	0.45 ± 0.01^{b}	16.97 ± 0.87^{b}	83.03 ± 0.87^{a}	422.43 ± 0.38^{a}	0.26 ± 0.11^{a}	0.776 ± 0.00^{ab}	0.337 ± 0.03^{a}
LB2Y	1.503 ± 0.01^{c}	0.21 ± 0.00^{c}	30.15 ± 1.04^{a}	69.85 ± 1.04^{b}	46.40 ± 0.39^{f}	170.97 ± 0.80^{c}	0.517 ± 0.01^{b}	0.220 ± 0.08^{a}
LB6Y	1.501 ± 0.01^{c}	0.22 ± 0.02^{c}	29.33 ± 0.07^{a}	70.67 ± 0.07^{b}	46.40 ± 0.40^{f}	0.10 ± 0.12^{a}	0.615 ± 0.16^{ab}	0.202 ± 0.07^{a}
LB7Y	1.488 ± 0.01^{c}	0.22 ± 0.02^{c}	29.07 ± 0.86^{a}	70.93 ± 0.86^{b}	47.18 ± 0.58^{f}	13.94 ± 0.03^{b}	0.858 ± 0.03^{a}	0.264 ± 0.04^{a}
Baker's Yeast	1.487 ± 0.00^{c}	0.21 ± 0.01^{c}	30.65 ± 0.68^{a}	69.35 ± 0.68^{b}	373.95 ± 0.57^{c}	0.61 ± 0.45^{a}	0.575 ± 0.33^{ab}	0.285 ± 0.08^{a}

[a]Means in the column with a different letter are significantly different according to the Tukey test ($P < 0.05$). LB7 = *Lactobacillus paracasei* ssp. *paracasei*; LB6 = *Lactobacillus paracasei* ssp. *paracasei*; LB2 = *Lactobacillus paracasei* ssp. *paracasei*; LB7Y = *Lactobacillus paracasei* ssp. *paracasei* + yeast; LB6Y = *Lactobacillus paracasei* ssp. *paracasei* + yeast; LB2Y = *Lactobacillus paracasei* ssp. *paracasei* + yeast.

crust is formed by not being placed in the very hot oven, a flat bread is produced [21] [22], reported that the bread samples with *Lactobacillus* was the highest for the control (303.9 cm^3) this it is not coincide whit this investigation, the results obtained were smaller (210.0 cm^3). The sample LB7 was of 520 cm^3, value more high that the reported for this author. The pulque's bread too was high (540 cm^3). The differences can be must to the process and formulations different.

3.5. Texture Profile Analysis of the Bread

Table 3 shows the hardness results where all the breads are significantly different. This results match with the reported for Eliasson and Larsson [23]. The pulque bread required the most force to compress. While there was no adhesiveness in the breads inoculated with lactobacilli, there was in those inoculated with added yeast, LB2 plus yeast needing more effort to remove the probe which is equivalent to the human palate. The elasticity of the bread with LB2 and LB6 is different while in the bread with LB7 and pulqueit is the same. The cohesiveness of the 4 samples is different in the bread with LB6 and LB7 but not so in the bread with pulque and LB2. The breadcrumb should recover quickly after being pressed and not be too spongy or resume its shape too slowly. The degree of elasticity in the crumb is important in determining the ease with which butter can be spread over it, above all when hard [24]. In this study the hardness was highest that the reported for Gul *et al*. [22] it could be for the differents formulations and process of elaboration for the bread. Researchers reported that LAB increased shelf life of bread and delayed staling. The hardness in this study decreased with differents samples. A great improvement of textural properties of bread added with commercial and microorganism derived emulsifiers. The emulsifiers addition enhanced bread specific volume and improved bread appearance, textural properties of breads [25]. In the **Table 4**, it showed the bread's color, the yellow coloration decreases with respect to the kind of LAB that is added.

3.6. Bread Structure Analysis

The breads made with lactobacilli plus yeast had a finer crumb, predominantly with a cell size less than 1 mm^2 (**Table 4**) in higher proportion to the breads made only with lactobacilli; in these latter breads large holes were found that were considered defects. The consumers chose in the bread preference for porous appearance and floury odor, this parameter is better in LB6 + L, LB2 + L and LB7 + L in comparison whit the Yeast [23]. The cell density results for different kind of commercial sweet breads evaluated by the two different there holding methods and classified by different cell areas [26]. The differences among bread crumb structures were also detected, showing that pound cake bread had the largest number of cells per unit area as compared to the other samples. This difference may be a consequence of the bread making process and the ingredients used, such as fat, sugar, additives or others. The three kinds of sweet commercial breads presented different cells areas depending on the range of objects used to evaluate them. When all cells were considered, there were not significant differences in the mean cell areas of Danish and yeast sweet bread samples, while in all other cases, there were significant variations.

Table 4. Color and crumb structure analysis of bread with *Lactobacillus*, pulque and yeast.

Bread	L	a	b	Cells (No.)	Cells/total area	Cell/cm^2	Cell size (mm^2)	Uniformity (mm^2)
LB6 + Y	757 ± 6.81^a	0.177 ± 0.01^e	10.33 ± 3.06^a	757 ± 6.81^a	0.177 ± 0.01^e	10.33 ± 3.06^a	3.9 ± 0.00^a	98.19 ±1.32^a
LB2 + Y	491 ± 6.66^b	0.241 ± 0.00^d	8.00 ± 4.58^a	491 ± 6.66^b	0.241 ± 0.00^d	8.00 ± 4.58^a	4.2 ± 0.00^a	96.77 ± 4.44^a
LB7 + Y	385 ± 3.00^c	0.314 ± 0.02^c	7.33 ± 2.31^a	385 ± 3.00^c	0.314 ± 0.02^c	7.33 ± 2.31^a	5.6 ± 0.03^a	90.40 ± 7.06^b
Pulque	295 ± 3.00^d	0.200 ± 0.01^e	10.67 ± 9.02^a	295 ± 3.00^d	0.200 ± 0.01^e	10.67 ± 9.02^a	4.4 ± 0.02^a	74.30 ± 6.97^c
Yeast	327 ± 1.51^d	0.328 ± 0.64^c	11.3 ± 1.02^a	327 ± 1.51^d	0.328 ± 0.64^c	11.3 ± 1.02^a	4.4 ± 0.02^a	59.91 ± 0.07^d
LB7	177 ± 2.65^e	0.324 ± 0.01^c	5.33 ± 1.15^a	177 ± 2.65^e	0.324 ± 0.01^c	5.33 ± 1.15^a	4.7 ± 0.01^a	32.65 ± 2.63^d
LB2	113 ± 2.08^f	0.451 ± 0.04^b	5.33 ± 0.58^a	113 ± 2.08^f	0.451 ± 0.04^b	5.33 ± 0.58^a	5.0 ± 0.01^a	26.28 ± 5.72^d
LB6	104 ± 2.52^g	0.543 ± 0.02^a	5.67 ± 2.08^a	104 ± 2.52^g	0.543 ±0.02^a	5.67 ± 2.08^a	6.9 ± 0.03^a	17.51 ± 1.21^e

^{a}Means in the column with a different letter are significantly different according to the Tukey test ($P < 0.05$). LB7 = *Lactobacillus paracasei* ssp. *paracasei*; LB6 = *Lactobacillus paracasei* ssp. *paracasei*; LB2 = *Lactobacillus paracasei* ssp. *paracasei*; LB7 + Y = *Lactobacillus paracasei* ssp. *paracasei* + yeast; LB6 + Y = *Lactobacillus paracasei* ssp. *paracasei* + yeast; LB2 + Y = *Lactobacillus paracasei* ssp. *paracasei* + yeast.

Table 5. Sensory analysis of the different breads with *Lactobacillus*, pulque and yeast.

BREAD	Sponginess	Aroma	Flavor	Color	Acceptability
LB2 + Y	4.5 ± 0.51[a]	4.06 ± 0.87[b]	4.52 ± 0.79[b]	4.00 ± 0.83[a]	4.020 ± 0.68[b]
LB6 + Y	4.44 ± 0.70[a]	4.44 ± 0.73[a]	4.64 ± 0.97[b]	3.22 ± 0.99[b]	4.14 ± 0.65[b]
LB7 + Y	4.38 ± 0.60[a]	4.36± 0.83[ab]	4.66 ± 1.02[b]	3.26 ± 1.24[b]	4.16 ± 0.76[b]
Yeast	4.35 ± 0.18[a]	4.32 ± 0.65[ab]	4.57 ± 0.78[b]	3.32 ± 0.87[b]	4.11 ± 0.63[b]
Pulque	1.76 ± 0.23[b]	4.32 ± 0.32[ab]	4.87 ± 0.65[a]	3.12 ± 0.87[b]	4.98 ± 0.56[a]
LB7	1.84 ± 0.68[b]	4.22 ± 0.86[ab]	4.84 ± 0.70[a]	3.24 ± 1.20[b]	4.86 ± 0.75[a]
LB2	1.82 ± 0.77[b]	4.48 ± 0.68[a]	4.96 ± 1.05[a]	2.36 ± 0.87[c]	4.96 ± 1.18[a]
LB6	1.58 ± 0.50[b]	3.68 ± 0.94[c]	4.90 ± 1.01[a]	3.16 ± 1.13[b]	4.90 ± 0.65[a]

[a]Means in the column with a different letter are significantly different according to the Tukey test ($P < 0.05$). LB7 = *Lactobacillus paracasei* ssp. *paracasei*; LB6 = *Lactobacillus paracasei* ssp. *paracasei*; LB2 = *Lactobacillus paracasei* ssp. *paracasei*; LB7Y = *Lactobacillus paracasei* ssp. *paracasei* + yeast; LB6Y = *Lactobacillus paracasei* ssp. *paracasei* + yeast; LB2Y = *Lactobacillus paracasei* ssp. *paracasei* + yeast.

3.7. Sensory Analysis of the Bread

Table 5 shows the results of the sensory evaluation of the breads with *Lactobacillus* compared to those inoculated with *Lactobacillus* plus yeast. All the breads inoculated only with *Lactobacillus* were equal in terms of sponginess having the minimum scale values; however, when the baker's yeast was added their sponginess was of the highest values. Bread with *Lactobacillus* was the best accepted among the judges in spite of its sponginess not being statistically the best. The organoleptic attributes of the bread the best in terms organoleptic properties was with *Lb. sake* and *S. cereviciae* [22]. In this study the best formulation was with LB7, LB2 and LB6 for that results it is not coincide [22] [27]. But these results are similar whit Martinez [1] reported that *Lb. plantarum* and *Lb. brevis* affected positively the sensory properties of sourdoughs breads.

4. Conclusion

According to the results obtained and consulted from the bibliography, the best result from the fermentation of the doughs was the LB7 + yeast mixture and this was similar in breadmaking.

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