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Article

Screening Sourdough Starter Cultures from Yeast and Lactic Acid Bacteria Isolated from Mexican Cocoa Mucilage and Coffee Pulp for Bread Quality Improvement

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Abstract

This study aimed to identify and evaluate yeasts and lactic acid bacteria (LAB) isolated from Mexican cocoa mucilage (*Theobroma cacao*) and coffee pulp (*Coffea arabica*) for their potential use as sourdough starter co-cultures to improve bread quality. Functional screens included assessments of amylolytic, proteolytic, and phytase activities, CO₂ production, acidification capacity, and exopolysaccharide (EPS) synthesis. *Saccharomyces cerevisiae* YCTA13 exhibited the highest fermentative performance, surpassing commercial baker's yeast by 52.24%. *Leuconostoc mesenteroides* LABCTA3 showed a high acidification capacity and EPS production, while *Lactiplantibacillus plantarum* 20B3HB had the highest phytase activity. Six yeast–LAB combinations were formulated as mixed starter co-cultures and evaluated in sourdough breadmaking. The B3Y14 co-culture (LABCTA3 + YCTA14) significantly improved the bread volume and height by 35.61% and 17.18%, respectively, compared to the commercial sourdough starter, and reduced crumb firmness by 59.66%. Image analysis of the bread crumb revealed that B3Y14 enhanced the crumb structure, resulting in greater alveolar uniformity and a balanced gas cell geometry. Specifically, B3Y14 showed low alveolar regularity (1.16 ± 0.03) and circularity (0.40 ± 0.01), indicating a fine and homogeneous crumb structure. These findings highlight the synergistic potential of selected allochthonous yeast and LAB strains in optimizing sourdough performance, positively impacting bread texture, structure, and quality.

Keywords: sourdough; bread; yeast; lactic acid bacteria; coffee pulp; cocoa mucilage



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1. Introduction

Sourdough is a leavening agent used in the production of bakery products. It is obtained through the spontaneous fermentation of flour and water. Lactic acid bacteria (LAB) and yeasts are the main microorganisms involved in this fermentation process. Through their metabolic activity, these microorganisms acidify and leaven the dough while producing antifungal and aromatic compounds. The latter compounds contribute to the

characteristic flavor of sourdough. During spontaneous fermentation, LAB and yeasts, autochthonous microorganisms from flour, dough ingredients, and the environment, add a unique flavor profile to the sourdough [1–3].

The metabolic activity of microorganisms during fermentation triggers biochemical changes in the main components of the dough—carbohydrates, proteins, and lipids—through enzymatic activities and metabolite production. These transformations impact bread quality, improving crumb firmness, shelf life, and aromatic and sensory profiles. Moreover, using starter co-cultures in sourdough fermentation with allochthonous microorganisms enables the development of specific bread attributes that enhance quality. Therefore, starter co-culture microorganisms must be selected based on criteria such as adaptation to the fermentation environment and metabolite production, enabling better fermentation control and desired product characteristics [2–5].

Yeast selection criteria include high CO₂ production, tolerance to low pH, and the ability to grow in the presence of lactic and acetic acids. For LAB, the criteria include acidification capacity and exopolysaccharide (EPS) production. Additionally, enzymatic activities such as amylolytic, proteolytic, and phytasic activities are advantageous over the other selection criteria for yeast and LAB. Ogunsakin et al. [6] evaluated and screened LAB and yeasts from sorghum as starter cultures for sourdough fermentation, selecting strains with functional attributes. For LAB, the selection was based on EPS production and acidification properties, while for yeast, the selection criteria included gas production and low pH and acetic acid tolerance. Similarly, Shen et al. [7] isolated yeast and LAB strains from Chinese sourdoughs and screened them for their ability to enhance the sensorial quality of whole wheat steamed bread. Their results demonstrated that *Saccharomyces cerevisiae* and *Lactobacillus johnsonii* exhibited high CO₂ and acid production compared to the other sourdough strains used. These microorganisms were combined with the acetic acid bacteria *Acetobacter pasteurianum* to formulate a mixed starter, leading to increased bread volume, enhanced volatile organic compound production, and improved breadcrumb softness.

Additionally, several studies have demonstrated that microorganisms present in different food sources, including kefir, yogurt, and plant-derived fermented foods rich in sugars, such as coffee, cocoa, pears, oranges, and grapes, can be used to start sourdough fermentation [8–15]. In addition, fermented coffee pulp and cocoa mucilage can be used as sources of diverse genera of LAB (*Lactobacillus* and *Leuconostoc*) and yeasts (*Saccharomyces*, *Pichia*, *Rhodotorula*, and *Kazachstania*), which have also been identified in sourdough fermentations [16–29]. Therefore, using the microbiota in fermented coffee pulp and cocoa mucilage is of interest for developing sourdough starter cultures. This study aims to select LAB and yeast strains from fermented coffee pulp and cocoa mucilage for their potential application as starter cultures in sourdough, contributing to bread production enhancement.

2. Materials and Methods

2.1. Chemicals, Enzymes, and Broths

Culture media and the main components were obtained from the following suppliers: yeast extract, MRS broth, and nutrient agar were purchased from DIBICO (Mexico City, Mexico); agar (CAS 9002-18-0), casein peptone (CAS 91079-40-2), dextrose (D-glucose, CAS 50-99-7), and potato dextrose agar (PDA) were supplied by MCD LAB (Mexico City, Mexico); and wheat flour was provided by Fábrica de Harina Elizondo (Mexico City, Mexico). Sugars and salts included D-glucose (CAS 50-99-7), sucrose (CAS 57-50-1) (CTR Scientific, Monterrey, Mexico), calcium chloride (CaCl₂, CAS 10043-52-4), potassium chloride (KCl, CAS 7447-40-7) (Reasol, Mexico City, Mexico), magnesium sulfate heptahydrate (MgSO₄·7H₂O, CAS 10034-99-8), manganese sulfate monohydrate (MnSO₄·H₂O, CAS 10034-96-5), and iron sulfate heptahydrate (FeSO₄·7H₂O, CAS 7782-63-0) (Golden Bell,

Mexico City, Mexico). Additional reagents included starch (CAS 9005-25-8) and Lugol's iodine solution (iodine, CAS 7553-56-2; potassium iodide, CAS 7681-11-0) (HYCEL), as well as cobalt chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, CAS 7791-13-1), ammonium nitrate (NH_4NO_3 , CAS 6484-52-2), and hydrochloric acid (HCl , CAS 7647-01-0), all purchased from J.T. Baker through El Crisol S.A. de C.V. (San Luis Potosí, Mexico). Sodium phytate (Na -phytate, CAS 14306-25-3) was obtained from Makesy (Haltom City, TX, USA), and sodium hydroxide (NaOH , CAS 1310-73-2) was acquired from Química Suastes (Mexico City, Mexico). The commercial cultures used were the San Francisco Style Sourdough Starter Culture (Cultures for Health, Wake Forest, NC, USA) and *Saccharomyces cerevisiae* Saf-instant (Safmex, Toluca, Mexico). Sigma-Aldrich reagents, including α -amylase from porcine pancreas (EC 3.2.1.1; CAS 9000-90-2), ammonium vanadate (NH_4VO_3 , CAS 7803-55-6), iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, CAS 10025-77-1), lactic acid (CAS 50-21-5), phytase from wheat (*Triticum aestivum*, EC 3.1.3.26; CAS 9001-89-2), *Aspergillus melleus* proteinase (EC 3.4.21.63; CAS 9001-92-7), and skim milk powder (CAS 999999-99-4) were obtained from Tesla (Mexico City, Mexico). DNA extraction reagents were supplied by Qiagen Mexico (Mexico City, Mexico) in the Blood and Cell Culture DNA Kit.

2.2. Microorganisms and Growth Conditions

The strains used in this study were obtained from the microorganism collection of the Grupo de Tecnología de Alimentos at the Unidad de Investigación y Desarrollo de Alimentos (UNIDA), Instituto Tecnológico de Veracruz, Veracruz, Mexico. These strains were previously isolated from cocoa mucilage [30,31], and in the present work, they were identified and evaluated for their selection as sourdough starter cultures. The collection included four LAB strains identified as *Lactiplantibacillus pentosus* LABCTA1, *Leuconostoc mesenteroides* LABCTA3, *Lactiplantibacillus plantarum* LABCTA6 and LABCTA8, along with eight yeast strains identified as *Saccharomyces cerevisiae*: YCTA5, YCTA9, YCTA10, YCTA12, YCTA13, YCTA14, YCTA15, and YCTA16. Additionally, from coffee pulp, one LAB strain, *Lpb. plantarum* 20B3H, and two yeast strains, *Wickerhamomyces anomalus* YX1SR15 and *W. anomalus* YX1SR23, were previously identified and selected as candidate strains for sourdough starter cultures [32,33]. Yeast strains *W. anomalus* YX1SR15 and YX1SR23 isolated from coffee pulp were selected for their tolerance to a pH between 3.0 and 5.0 and for their high amylolytic, proteolytic and phytasic activities; furthermore, these strains were negative for hemolytic activity [32]. Lactic acid bacterial strain *Lpb. plantarum* 20B3H from coffee pulp was selected for its high amylolytic activity, which would allow the strain to grow in sourdough fermentation, as it also presents proteolytic and phytasic activities; furthermore, this strain was identified as EPS-producer, which is a key screening criterion [33]. *Lpb. plantarum* 20B3H was negative for hemolytic activity. Yeast strains were grown in YPD broth at 30 °C for 24 h, and LAB strains were cultured in MRS broth at 37 °C for 24 h. Commercial *Saccharomyces cerevisiae* was obtained from Saf-instant® brand (Toluca, Mexico). San Francisco Style Sourdough Starter Culture (Cultures for Health) was obtained from Cultures for Health (Wake Forest, NC, USA).

2.3. Molecular Identification of Yeast and LAB Strains from Cocoa Fermentation

Isolated yeast and LAB strains were incubated on YPD and MRS agar at 30 ± 1 °C for 24 h for identification. DNA extraction was performed using the Blood and Cell Culture DNA kit. PCR amplification and sequencing of the extracted DNA were performed by Macrogen (Seoul, Republic of Korea). According to Macrogen's standard protocol, yeast identification was performed by amplifying the internal transcribed spacer (ITS) regions ITS1-5.8S-ITS2 using the ITS4/ITS5 primers and LAB identification targeted the V1–V3 region of the 16S rRNA gene using the 785F/907R primers. PCR-specific primer bands

and reagents were determined and provided by MacroGen. The sequences were analyzed using BLASTN version 2.13.0+ (GenBank, NCBI, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 5 May 2023), performed by MacroGen (Seoul, Republic of Korea).

2.4. Enzymatic Activities of Yeast and LAB Isolates

2.4.1. Phytase Activity

A phytate-selective medium (PSM) was used following the methodology of Jorquera et al. [34] PSM medium contained 10 g/L D glucose, 4 g/L $C_6H_6O_{24}P_{6\bullet}12Na$ (sodium phytate), 2 g/L $CaCl_2$, 5 g/L NH_4NO_3 , 0.5 g/L KCl, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $FeSO_4 (7H_2O)$, 0.01 g/L $MnSO_4 \cdot H_2O$, and 15 g/L agar. A colony of each yeast and LAB strain was cultured in 10 mL of YPD and MRS broth, respectively, at 30 °C for 16 h. After this, a spot of 10 µL of yeast and BAL culture broth was inoculated into Petri dish. Degradation halos around colonies after eight days of incubation indicate phytase activity. To eliminate false positives, Petri dishes were flooded with an aqueous solution of 2% (*w/v*) cobalt chloride. After 5 min at room temperature, plates were then flooded with an equal-volume solution of ammonium molybdate (6.25% *w/v*) and ammonium vanadate (0.42% *w/v*) that was freshly prepared. After an additional 5 min of incubation, the solution was removed, and clear zones were examined. As a positive control, 10 µL of a 1% (*w/v*) phytase solution from wheat was used, while 10 µL of sterile water functioned as the negative control.

2.4.2. Proteolytic Activity

For yeasts, the screening medium consisted of PDA supplemented with 20 g/L of skim milk, while for LAB, the screening medium was composed of agar with 20 g/L of skim milk [6]. Two colonies of each yeast and LAB strain were cultured in 20 mL of YPD and MRS broth, respectively, and incubated at 30 °C for 16 h. Then, 10 µL of culture broth was inoculated onto Petri dishes. After 5 days of incubation, a 1 N HCl solution was added to cover the agar surface and incubated for 30 min. The acid precipitated non-hydrolyzed proteins, leaving clear zones where protease activity had degraded casein. Finally, the HCl solution was removed, and plates were rinsed with distilled water. The presence of degradation halos around colonies indicated proteolytic activity. As a positive control, 10 µL of a 1% (*w/v*) *Aspergillus melleus* proteinase solution was inoculated into the Petri dishes, while 10 µL of sterile water was used as the negative control.

2.4.3. Amylolytic Activity

For yeast and LAB, amylolytic activity screening was performed using nutrient agar supplemented with 0.2% (*w/v*) starch [35,36]. Two colonies of yeast and LAB strains were cultured in 20 mL of YPD and MRS broth, respectively, and incubated at 30 °C for 16 h. After 5 days of incubation, the plates were flooded with 1% Lugol solution. The presence of degradation halos around colonies indicates amylolytic activity. As a positive control, starch hydrolysis was tested using 10 µL of a 1% (*w/v*) α -amylase solution from porcine pancreas, while 10 µL of sterile water was used as the negative control.

2.5. Gas Production by Yeast

CO₂ production by yeast, which determines its fermentation capacity in bread dough, was measured using the drainage method [37,38]. A mixture of 20 g of flour, 15 mL of distilled water, and 0.5 g of yeast pellet (obtained by centrifugation of an overnight cell culture at 2795 g for 15 min at 4 °C) was placed in an Erlenmeyer flask sealed with a perforated rubber stopper, coupled to a drainage glass tube leading to a 100 mL water-filled graduated burette. The displacement of water in the burette was recorded every hour for 4 h. Commercial *S. cerevisiae* was cultured and used as a control.

2.6. Lactic Acid Production Capacity

LAB strains were screened based on their acid production capacity. Two colonies of each LAB strain were inoculated into 20 mL of MRS broth and incubated at 30 °C for 16 h. The activated LAB was inoculated into 50 mL of MRS broth with 1% (*v/v*) inoculum and incubated at 30 °C. After 16 h of incubation, culture broth samples were collected to determine pH, titratable acidity (TTA), and the lactic acid concentration. TTA was measured using 0.1 N NaOH until pH 8.5, while the lactic acid concentration was determined by a spectrophotometric method.

Determination of the Lactic Acid Concentration

The lactic acid concentration was determined using the method described by Borshchevskaya et al. [39], with modifications. The method is based on the spectrophotometric quantification of the colored product formed by the reaction between lactate ions with iron (III) chloride at 390 nm. A calibration curve was constructed within a range of 0–120 mM of lactic acid, with a correlation coefficient of 0.978. A 0.2% (*w/v*) iron (III) chloride solution was prepared. Then, 50 µL of lactic acid at the corresponding concentration was added to 2 mL of the iron (III) chloride solution. The reference solution contained 2 mL of the iron (III) chloride solution. For the determination of the lactic acid concentration in the culture supernatant, two colonies of each LAB strain were inoculated into 20 mL of MRS broth and incubated at 30 °C for 16 h. After the incubation, the supernatant was obtained by centrifugation (2795 g for 15 min) and diluted 20-fold with deionized water. Finally, the lactic acid concentration was determined using the calibration curve.

2.7. Screening LAB Exopolysaccharide (EPS) Production

EPS production was tested with the method described by Menezes et al. [40] using MRS agar supplemented with 4% (*w/v*) sucrose. A single colony of activated bacteria was streaked onto the Petri dish, and the plates were incubated at 30 °C for 48 h. The presence of slimy or ropy colonies indicated EPS production.

2.8. Sourdough Elaboration and Breadmaking

To produce *de novo* sourdough with the selected microorganisms, six compound starter co-cultures with three yeast strains of *S. cerevisiae* (YCTA5, YCTA13, and YCTA14) and two LAB strains of *Lpb. plantarum* 20B3H and *Leu. mesenteroides* LABCTA3 were formulated to observe the individual effect of each yeast strain with LAB. The multistage sourdough feeding protocol (Figure 1) was adapted from an online sourdough course by Ramon-Simon [41]. To formulate the six starter co-cultures, a targeted combinatorial strategy was applied based on the functional screening results. Yeast and LAB strains were pre-selected due to their performance in functional assays, including CO₂ production, acidification rate, proteolytic and phytase activities, and EPS-producing ability. These strains were paired to reflect different technological profiles and potential synergistic interactions as combinations of high EPS-producing LAB with high CO₂-producing yeasts. The resulting six co-cultures were thus not exhaustive of all possible combinations but were rationally selected to represent functional diversity and to evaluate the contributions of the selected microorganisms to sourdough bread quality.

To prepare the starter culture, cells from an overnight culture were harvested and washed twice with sterile water. Then, 1 mL of the starter culture inoculum, previously adjusted to 10⁷ CFUs/g for yeast and 10⁸ CFUs/g for LAB, was added to a mix of flour (50 g) and water (50 mL) to start sourdough production. Sourdough fermentation was performed at 30 °C with seven backslipping steps using different flour, water and dough proportions. The first three backslipping steps followed a flour/water/sourdough ratio (*w/w/w*) of

1:1:1 (24 h, 24 h, 12 h), while the last three were carried out using a 2:2:1 proportion (12 h, 6 h and 6 h) followed by a 4 h fermentation period to obtain the final sourdough (Figure 1). A commercial sourdough starter, San Francisco Style Sourdough Starter Culture, was used as control (Cultures for Health, Akron, OH, USA). To ensure experimental comparability, the entire content of the starter packet (~7 g) was directly inoculated into 50 g of wheat flour and 50 mL of sterile water, following the same backslopping conditions applied to mixed starters (Figure 1).

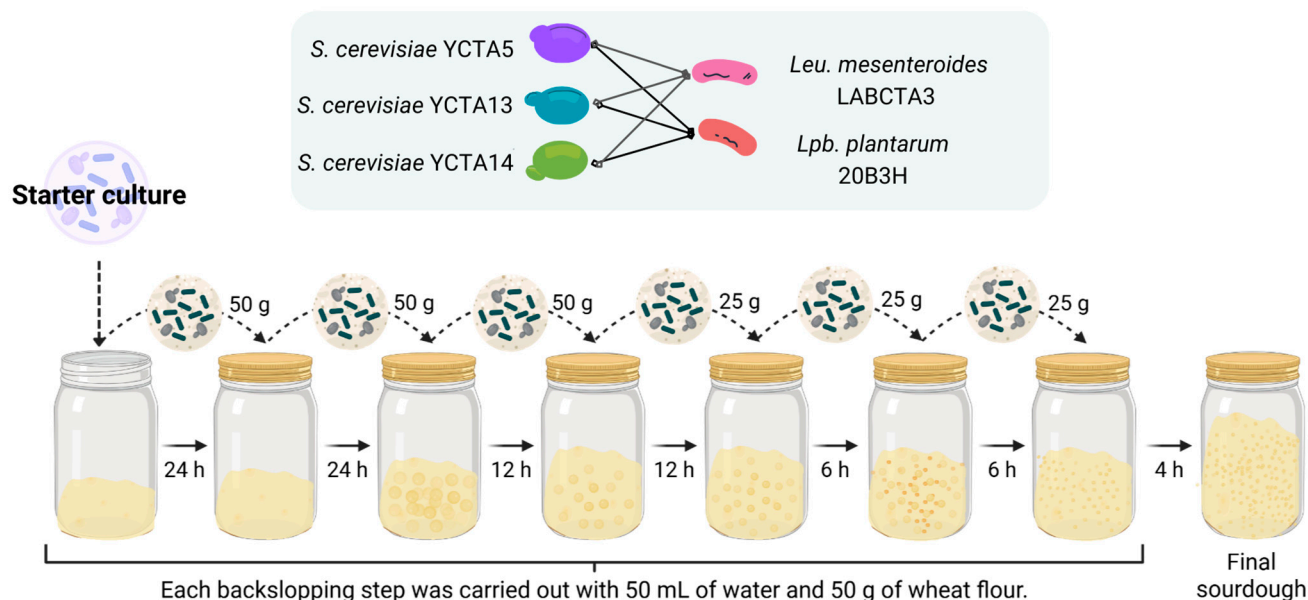


Figure 1. Sourdough preparation with the formulated mixed starter cultures.

Sourdough bread formulation was carried out using the AACC 10-10.03 method, with modifications. A formulation with 100 g of flour, 70 mL of water, 20 g of active sourdough, 1.5 g of salt, and 6 g of sugar was mixed using a KitchenAid mixer (KitchenAid, model KP26M9XCCU, Benton Harbor, MI, USA). The bread dough was placed in plastic bowls and covered with disposable food storage stretch lids and proofed at 30 °C for 6 h in an incubator (Gallenkamp, Harry Mazal S.A., Mexico City, Mexico). Then, the bread dough was manually shaped and molded in rectangular mini-loaf mold made of heavy-gauge steel with a non-stick coating with dimensions of 6.3 cm (W) × 16.0 cm (L) × 4.5 cm (H) (Cuisinart, Stamford, CT, USA); the mold was covered with the stretch lids and proofed for an additional 60 min at 30 °C. Finally, the bread dough was fermented overnight at 4 °C and the next day left, it rested at room temperature for 60 min before being baked in a convection oven (Gourmia, model GTF7660, Brooklyn, NY, USA) at 200 °C for 35 min with steam by adding water in the oven in a baking mold. The loaves were unmolded 15 min after baking, cooled for 120 min on a metallic rack, and then packed in polyethylene bags for further analysis.

2.9. Bread Quality

To determine which starter co-culture produced the best overall bread characteristics, bread quality was analyzed based on loaf volume (AACC Method 10-05.01), height (measured using digital Vernier calipers adapted as a height gauge), weight, crumb firmness (AACC Method 74-10.02), and crumb structure by image analysis. Loaf volume, height, and weight were evaluated 120 min after baking. For crumb firmness and structure, bread samples were kept in polyethylene hermetic bags until analyses were performed 24 h after baking.

2.9.1. Crumb Firmness Analysis

For crumb firmness, a compression test was carried out using a Texture Analyzer TA.XT Plus (Stable Micro Systems, Godalming, UK). From each loaf, 15-mm thick slices were obtained using a food slicer (Gourmia, model GFS-700, Brooklyn, NY, USA), discarding the central and ends slices. Two slices were compressed with a cylindrical P/25 probe (25 mm diameter) until a deformation of 3.0 mm was reached. The force required for compression was recorded as the firmness value.

2.9.2. Crumb Structure by Image Analysis

Crumb structure was assessed using digital image analysis in ImageJ software, version 1.54 (National Institutes of Health, Bethesda, MD, USA; <https://imagej.nih.gov/ij/>, accessed on 15 April 2025), based on standardized image processing steps. The crumb image analysis was conducted using high-resolution photographs captured with a SONY DSC-HX400V camera (zoom 2.5×, ISO 1000, F8.0, 1/30 s) (SONY, Tokyo, Japan). Images were saved as bitmap files, with a 300 DPI resolution and in real-color format (RGB, 256 million colors). All bread photos were scaled and standardized using a ruler. Images were cropped (ImageJ v1.54) to a field of view of 40 mm × 40 mm region from the crumb center to ensure comparability. The cropped color images were duplicated, and one was converted into an 8-bit grayscale image. Image contrast was enhanced by functions, normalized, and equalized. The grayscale images were automatically binarized/thresholded with the Otsu algorithm without manual adjustment [42–44]. This standardization ensures the objective, reproducible quantification of alveoli size distribution. Alveoli characteristics, including the area, circularity of pores (shape factor), and Feret's diameter, were extracted from the binary images using the “Analyze Particles” function, excluding objects touching the edges. The circularity index parameter is defined as $CI = \frac{4\pi A}{P^2}$, where CI is the circularity index, A is the cell/alveoli area, and P is the cell perimeter; a perfect circle has a shape factor of 1.0, whereas a line has a shape factor approaching zero [45]. Alveoli were classified into five size categories: class 1 (0.05–0.49 mm²), class 2 (0.50–0.99 mm²), class 3 (1.00–4.99 mm²), class 4 (5.00–49.99 mm²), and class 5 (>50 mm²) [46]. Porosity was calculated as the ratio of the total alveolar area to the crumb area analyzed. Uniformity was assessed by calculating the coefficient of variation (CV) of Feret's diameter, and circularity was reported as the average shape factor of all alveoli retained for analysis.

2.10. Statistical Analysis

All the experiments were performed in triplicate, and results are expressed as means with standard deviations. Statistical analysis was performed using the JMP Pro software (version 14.0, SAS Institute Inc., Cary, NC, USA) with one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Non-parametric data (e.g., alveolar distribution classes) were analyzed using the Kruskal–Wallis test.

3. Results and Discussion

3.1. Microorganisms Identified in Cocoa Mucilage

In this study, eight yeast and four LAB strains from cocoa mucilage were identified to screen their potential in formulating starter co-cultures (Table 1). Among yeasts, all strains were identified as *S. cerevisiae*, which was the dominant species of isolates from cocoa mucilage. *S. cerevisiae* has been widely reported as predominant in sourdough fermentation, mainly due to the favorable environmental conditions [47–52]. This yeast species is widely found in sourdough fermentation ecosystems due to its essential role as a leavening agent, its high fermentation efficiency, production of volatile organic compounds, and synergistic interactions with LAB, such as cross-feeding of metabolites, gas retention, and

mutual tolerance of inhibitory metabolites, which allows us to enhance the overall quality functional properties of bread. Together, these contributions are critical for developing the characteristic sensory properties of sourdough-based baked products [28,47,53–57].

Table 1. Identification of microorganisms in cocoa mucilage based on ITS and 16S rRNA.

Identification Method	Microorganism	Length (bp)	Match/Total (bp)	Identity (%)
ITS region	<i>Saccharomyces cerevisiae</i> YCTA5	850	841/842	99.9
ITS region	<i>Saccharomyces cerevisiae</i> YCTA9	832	832/832	100
ITS region	<i>Saccharomyces cerevisiae</i> YCTA10	833	832/832	100
ITS region	<i>Saccharomyces cerevisiae</i> YCTA12	828	823/823	100
ITS region	<i>Saccharomyces cerevisiae</i> YCTA13	834	833/833	100
ITS region	<i>Saccharomyces cerevisiae</i> YCTA14	849	833/835	99.8
ITS region	<i>Saccharomyces cerevisiae</i> YCTA15	848	834/834	100
ITS region	<i>Saccharomyces cerevisiae</i> YCTA16	844	827/827	100
16S rDNA	<i>Lactiplantibacillus pentosus</i> LABCTA1	1246	691/695	99.4
16S rDNA	<i>Leuconostoc mesenteroides</i> LABCTA3	912	886/891	99.4
16S rDNA	<i>Lactiplantibacillus plantarum</i> LABCTA6	1427	680/683	99.6
16S rDNA	<i>Lactiplantibacillus plantarum</i> LABCTA8	1593	673/679	99.1

Regarding LAB species, one strain of *Lpb. pentosus* (LABCTA1), one strain of *Leu. mesenteroides* (LABCTA3), and two strain of *Lpb. plantarum* (LABCTA6 and LABCTA8) were identified in cocoa mucilage (Table 1). *Lpb. pentosus* is not commonly a dominant LAB species in spontaneous sourdoughs but is often used as a starter in laboratory sourdough. Nevertheless, it has also been isolated from artisan sourdoughs in Italy and cereal-based substrates [48,58]. *Lpb. plantarum* has been widely reported to be isolated in sourdough fermentation as it is predominantly found in sourdough elaborated in commercial bakeries rather than in the laboratory [59–63]. *Leu. mesenteroides* species are not common in sourdough fermentation; however, similar to *Lpb. pentosus*, it has been used as a starter culture in sourdough [64,65]. Despite their varying prevalence in sourdough, all three strains—*Lpb. pentosus*, *Lpb. plantarum*, and *Leu. Mesenteroides*—have been used as starter cultures in sourdough fermentation. Adding these LAB species to sourdough has been associated with improved bread sensory profiles, enhanced loaf volume, and reduced staling rates [27,66,67].

3.2. Screening and Selection of Yeast and LAB Strains

3.2.1. Quantitative Halo-Based Enzymatic Screening of Strains from Cocoa Mucilage

The isolated strains were assessed for enzyme production using qualitative halo-based assays on agar plates to evaluate amylase, protease, and phytase activities, and the diameter of the hydrolysis zones (in mm) was used as indicator of enzymatic potential. The results of the degradation halos for these enzymatic activities are presented in Table 2. Notably, all strains exhibited low levels of amylase activity, showing halo diameter values between 0.20 and 1.43 mm. During sourdough fermentation, yeasts and LAB with amylolytic activity interact synergistically to metabolize starch. Acidic conditions during sourdough fermentation can induce structural and chemical modifications in both native and damaged starch granules, depending on pH, exposure time, and the type and concentration of organic acids present [68]. Prolonged exposure to the fermentative environment leads to starch surface damage (roughening, fissures, and localized erosion) and partial hydrolysis of α -1,4-glycosidic linkages [69–71]. These changes occur mostly in the amorphous regions, enhancing the accessibility of starch to amylolytic enzymes and enabling yeast to efficiently metabolize starch [71,72]. This reduces the fermentation time by increasing fermentable sugar contents in the dough, which enhance gas formation during proofing and baking,

leading to an improved bread volume [73–75]. Furthermore, amylase reduces retrogradation by breaking down starch into smaller molecules, i.e., glucose, maltose, and dextrins. During bread staling, starch recrystallizes, which firms up the crumb. However, amylase disrupts this process by inhibiting the formation of double helices in amylose and in the linear regions of amylopectin, thereby reducing crystallinity, slowing retrogradation, and retarding the staling rate [76–78]. The screen of amylolytic activity showed that yeast strains had moderate amylolytic halos ($1\text{ mm} < \text{halo} \leq 4\text{ mm}$). Although commercial *S. cerevisiae* typically lacks significant amylolytic activity and requires exogenous enzymes during industrial breadmaking, wild *S. cerevisiae* strains isolated from spontaneous fermentations have been reported to have amylolytic activity, possibly due to environment adaptation or horizontal gene transfer [79–81]. *S. cerevisiae* YCTA9 presented the largest amylolytic halo diameter, and *S. cerevisiae* YCTA10 showed a significantly smaller halo diameter compared to the former. In contrast, there were no significant differences in halo diameter among YCTA5, YCTA9, and YCTA12–YCTA16, as they all belonged to the same statistical group (Table 2). Amylolytic activity in yeasts was significantly higher than in LAB, as reported by Palla et al. [81] and Damayanti et al. [82]. Among LAB, amylolytic activity was generally low ($<1\text{ mm}$), with *Lpb. plantarum* LABCTA6 showing the smallest halo diameter, and *Leu. mesenteroides* LABCTA3 the largest. However, the differences observed between LABCTA1, LABCTA6, and LABCTA8, and between LABCTA3 and LABCTA8 were not significant (Table 2). The amylolytic activity of LABCTA3 was significantly higher than LABCTA6, with halo diameters of, respectively, 0.90 mm and 0.81 mm. These findings align with previous research studies, such as Palla et al. [79], who analyzed 98 yeast strains isolated from PDO Tuscan sourdough and identified three *S. cerevisiae* strains as positive for amylase activity, showing hydrolysis halos of variable size up to 4 mm in diameter. Similarly, Akamine et al. [80] reported that one strain of *S. cerevisiae* isolated from laboratory-made sourdough showed a large amylase hydrolysis halo diameter ($\geq 2\text{ cm}$). Regarding LAB, strains of *Lpb. plantarum* and *Leu. mesenteroides* have shown variable amylolytic activities, with halo diameters ranging from non-detectable (i.e., absence of a visible halo on starch-iodine agar plates) to values from 9 to 48 mm [83–85]. The amylase-producing capacity of both LAB and yeast strains depends on their source of isolation. Microorganisms obtained from starch- or amylose-rich environments tend to produce higher amounts of amylase than non-starch rich sources [86]. Consistently, Amapu et al. [84] reported 14 amylolytic LAB strains isolated from various substrates, including wet-milled cereals (sorghum, millet, corn, and acha), cassava flour, and tomato. Although isolates from corn flour exhibited the highest amylase activity (up to 1.10 U/mL) and hydrolysis halos (48 mm), the isolate from tomato (*L. brevis*) showed a large hydrolysis halo (42 mm) but the lowest reducing sugar concentration (0.30 mg/mL), suggesting limited enzymatic activity despite visual starch degradation. In contrast, the strain from corn (*L. plantarum* AMZ5) not only had the largest halo but also the highest concentration of reducing sugars (0.55 mg/mL), reinforcing the idea that starchy flours such as corn may serve as more effective inducers of amylase production. A similar pattern was observed for the strains isolated from cocoa mucilage in this study, which exhibited relatively low amylolytic activity, most likely due to the non-starchy nature of their isolation source.

The proteolysis process during sourdough fermentation is primarily influenced by the interaction between LAB and yeasts. However, studies have reported that the proteolytic activity of yeast strains isolated from sourdough is negligible compared to that of LAB [87–89]. Proteolysis contributes to enhancing both the technological and sensory qualities of bread by releasing peptides with antimicrobial properties and generating precursors for flavor and color compounds. These metabolites are key to the development and enhancement of the sensory profile of sourdough bread [90–93]. Moreover, the partial degradation of

gluten proteins modifies the viscoelastic properties of the dough, which can improve the bread volume and a soften crumb [94]. In addition, proteolysis delays the staling process by modifying the crumb structure and increasing moisture retention through changes in the water-binding capacity of the dough caused by protein breakdown [95].

Table 2. Enzymatic activities of yeasts and LAB from cocoa mucilage and coffee pulp.

Strain	Halo Diameter (mm)			Reference
	Amylolytic Activity	Proteolytic Activity	Phytasic Activity	
Yeast				
<i>S. cerevisiae</i> YCTA5	1.36 ± 0.04 ^{ab}	34.15 ± 1.16 ^{bc}	16.05 ± 1.19 ^a	This study
<i>S. cerevisiae</i> YCTA9	1.43 ± 0.03 ^a	38.31 ± 1.15 ^a	15.85 ± 0.41 ^a	This study
<i>S. cerevisiae</i> YCTA10	1.28 ± 0.03 ^b	36.51 ± 1.83 ^{ab}	14.94 ± 1.04 ^a	This study
<i>S. cerevisiae</i> YCTA12	1.37 ± 0.02 ^{ab}	37.78 ± 0.33 ^{ab}	14.68 ± 0.24 ^a	This study
<i>S. cerevisiae</i> YCTA13	1.39 ± 0.05 ^{ab}	31.98 ± 1.16 ^c	14.33 ± 0.21 ^a	This study
<i>S. cerevisiae</i> YCTA14	1.37 ± 0.05 ^{ab}	39.86 ± 0.35 ^b	14.21 ± 0.66 ^a	This study
<i>S. cerevisiae</i> YCTA15	1.37 ± 0.06 ^{ab}	15.97 ± 0.07 ^d	15.48 ± 0.38 ^a	This study
<i>S. cerevisiae</i> YCTA16	1.40 ± 0.01 ^{ab}	36.26 ± 0.41 ^{ab}	13.73 ± 0.31 ^a	This study
<i>W. anomalus</i> YX1SR15	24.92 ± 0.02 ^{ab}	25.08 ± 0.02 ^{ab}	17.51 ± 0.11 ^{ab}	[32] *
<i>W. anomalus</i> YX1SR23	20.04 ± 0.02 ^{ab}	27.40 ± 0.04 ^{ab}	15.82 ± 0.06 ^{ab}	[32] *
LAB				
<i>Lpb. pentosus</i> LABCTA1	0.80 ± 0.01 ^B	27.80 ± 0.67 ^B	36.89 ± 2.14 ^B	This study
<i>Leu. mesenteroides</i> LABCTA3	0.90 ± 0.01 ^A	40.00 ± 0.00 ^A	32.75 ± 0.04 ^B	This study
<i>Lpb. plantarum</i> LABCTA6	0.81 ± 0.03 ^B	15.78 ± 1.09 ^C	48.48 ± 0.61 ^A	This study
<i>Lpb. plantarum</i> LABCTA8	0.86 ± 0.01 ^{AB}	24.15 ± 1.31 ^B	46.00 ± 0.08 ^A	This study
<i>Lpb. plantarum</i> LAB20B3HB	0.20 ± 0.01 ^{ab}	29.47 ± 0.31 ^{ab}	22.00 ± 0.08 ^{ab}	[33] **

Amylolytic activity = low ≤ 1 mm; moderate 1 mm < halo ≤ 4 mm; high > 4 mm. Proteolytic activity = low ≤ 1 mm; moderate 1 mm < halo ≤ 5 mm; high > 5 mm. Phytasic activity = low ≤ 1 mm; moderate 1 mm < halo ≤ 15 mm; high > 15 mm. Different letters in the same column and the same group of microorganisms indicate significant differences (Tukey's HSD test, $p < 0.05$). * Data from Romero-Isaza [32]. ** Data from Vázquez-Vázquez [33].

The screen for proteolytic activity showed that the degradation halo varied among strains (Table 2). Although *S. cerevisiae* YCTA14 and YCTA9 exhibited the largest proteolytic halos, there were no significant differences between YCTA10, YCTA12, YCTA14, and YCTA16. In contrast, YCTA13 and particularly YCTA15 showed significantly lower proteolytic activity than YCTA14 and YCTA9. Among *Lactiplantibacillus* strains, *Lpb. pentosus* LABCTA1 and *Lpb. plantarum* LABCTA8 were not statistically different and showed intermediate degradation halos. Regarding the bacteria strains, *Leu. mesenteroides* LABCTA3 displayed the highest proteolytic activity, while *Lpb. plantarum* LABCTA6 exhibited the lowest value among bacterial strains. Contrary to the values commonly reported, recent studies have shown that strains of the species *S. cerevisiae* and LAB as *Lpb. plantarum* and *Limosilactobacillus fermentum* isolated from traditional sourdoughs have shown the ability to hydrolyze protein with a variable range from negligible to large halo diameters (>25 mm) [81,96,97]. In this study, LAB and yeast strains were isolated from coffee and cocoa fermentation, exhibiting varying proteolytic activities among yeast and LAB strains that ranged from approximately 15 to 40 mm; this reflects that the proteolytic activity is strain and source specific. Similar results have been reported for yeast and bacterial strains isolated from cocoa and coffee postharvest processes exhibiting proteolytic activity. Sukmawati et al. [98] screened 231 yeast isolates from fermented dried cocoa beans and identified three isolates producing proteolytic enzymes. Rodarten et al. [99] isolated 144 microorganisms from coffee fruit and found that 52.5% of bacteria and 2.6% of yeasts were capable of secreting proteases. Additionally, Muzaifa et al. [100] identified proteolytic

yeast strains isolated from Civet coffee, finding two strains of *Wickerhamomyces anomalus* and *Trichosporon asahii* species displaying protease activity. These findings highlight the importance of the strain isolation source and the potential application of allochthonous strains in starter co-culture formulation.

Finally, phytase-producing microorganisms play a key role in sourdough fermentation, as this enzyme is essential for breaking down phytic acid, an antinutritional factor that chelates essential minerals like iron, calcium, and zinc, reducing their bioavailability. Studies have demonstrated that sourdough fermentation activates both microbial and endogenous phytases, enhancing the bioaccessibility of minerals in whole-grain products [40,101]. The screening of phytase activity revealed notable differences among yeast and bacterial strains. Among yeasts, even though *S. cerevisiae* YCTA5 exhibited the highest phytase activity and *S. cerevisiae* YCTA16 displayed the lowest activity, the results obtained for YCTA5 to YCTA16 yeasts were not statistically different. Regarding bacterial strains, *Lpb. plantarum* LABCTA6 recorded the highest phytase activity, followed by *Lpb. plantarum* LABCTA8. *Lpb. plantarum* LABCTA6 and *Lpb. plantarum* LABCTA8 exhibited comparable phytase activity, with no statistically significant differences in halo diameters. Meanwhile, *Lpb. pentosus* LABCTA1 and *Leu. mesenteroides* LABCTA3 showed the lowest activity levels, but no statistical difference was observed between them, and both strains exhibited significantly lower activity than *Lpb. plantarum* LABCTA6 and LABCTA8. These results suggest that the isolated microorganisms may be efficient at phytate breakdown. Similarly, Palla et al. found that certain yeast strains display measurable phytase activity, indicating that yeasts may also contribute to phytic acid degradation in sourdough fermentation, although their enzymatic efficiency tends to be lower compared to LAB [102]. Additionally, Fekri et al. [103] isolated and identified phytate-degrading probiotic LAB and yeast strains from traditional sourdough, confirming that their enzymatic activity significantly improves whole wheat bread quality. Supporting this, Rizzello et al. [104] demonstrated that sourdough fermentation with selected LAB strains significantly increases phytase activity, leading to enhanced mineral bioavailability. These studies align with Gobetti et al. [105], who emphasized that the selection of phytase-producing strains improves mineral bioavailability and reduces anti-nutritional factors in the final product.

To drive the formulation of starter co-cultures, yeasts were assessed based on their CO₂ production, while LAB were evaluated according to their acidification capacity and EPS production. The integration of these results and the final selection of starter co-cultures are presented in the following sections.

3.2.2. Dough Fermentability by Yeast Isolated from Cocoa Mucilage and Coffee Pulp

The determination of leavening activity is directly related to the fermentability of bread dough by yeast strains, making it one of the most important selection criteria for a sourdough starter culture. The fermentability of dough by the evaluated yeast strains is shown in Figure 2.

Compared to commercial *S. cerevisiae*, which exhibited a leavening activity of 19.17 mL·h⁻¹, the dough fermented with strain *S. cerevisiae* YCTA13 displayed the highest gas production at 29.17 mL·h⁻¹, followed by *S. cerevisiae* YCTA14 (22.33 mL·h⁻¹) and *S. cerevisiae* YCTA5 (20.83 mL·h⁻¹). These results highlight the superior fermentative capacity of *S. cerevisiae* YCTA13. However, the *S. cerevisiae* YCTA14 and *S. cerevisiae* YCTA5 strains also demonstrated higher leavening activity than the commercial strain. In contrast, the *W. anomalus* YX1SR15 and YX1SR23 yeast strains, isolated from coffee fermentation, exhibited negligible leavening activity under the experimental conditions, despite their high enzymatic activity values, showing limited dough fermentability support through low CO₂ production. Similar results were reported by Sergeeva et al. [106] who evalu-

ated the breadmaking potential of *W. anomalus* CBSS605T and *T. delbrueckii* YIT3 strains, finding that both strains showed lower leavening ability in wheat dough compared to control *S. cerevisiae*, and that these strains needed more time to form the required structure of dough and bread crumb. This may be because *W. anomalus* has been described as a Crabtree-negative yeast, which leads to the low production of ethanol and CO₂ [107–109]. Thus, due to their limited leavening capacities, *W. anomalus* strains were discarded from the starter co-culture formulation.

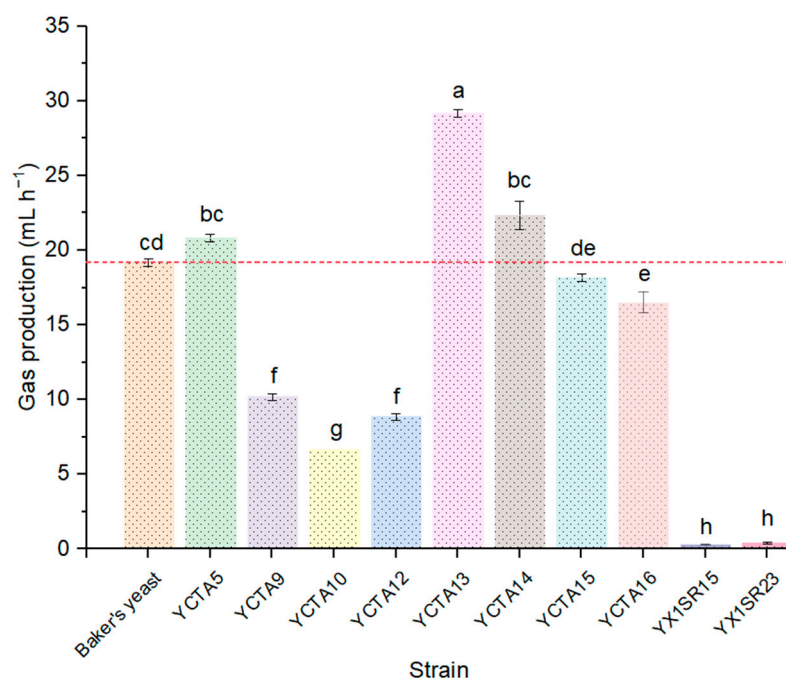


Figure 2. Gas production rate of yeast strains isolated from cocoa mucilage and coffee pulp (mL·h⁻¹), measured after 4 h of incubation at 30 °C. Baker's yeast (Commercial *S. cerevisiae*); YCTA5–YCTA16, *S. cerevisiae* YCTA5–YCTA16; YX1SR15, *W. anomalus* YX1SR15; YX1SR23, *W. anomalus* YX1SR23. The dotted red line represents the gas production rate of the commercial baker's yeast (*Saccharomyces cerevisiae*), which was used as a reference control for comparison. Different letters above the bars indicate significant differences (Tukey's HSD test, $p < 0.05$).

The results showed that *S. cerevisiae* YCTA5, YCTA13, and YCTA14 exhibited the highest CO₂ production among the evaluated strains. The results from YCTA5 and YCTA14 are not significantly different. This may be linked to their efficient metabolism of maltose and other fermentable sugars, as reported by Sánchez-Adriá et al. [110], who described the enhanced leavening activity and CO₂ production in yeast species as *S. cerevisiae*, *Kazachstania humilis* and *Torulaspora delbrueckii* when compared to commercial yeast. Similarly, Woo et al. [111] observed that a high gas-producing strain (*S. cerevisiae* BW1) improved gas retention and dough aeration in gluten-free sourdoughs. Ferraz et al. [112] demonstrated that higher yeast concentrations are related to increased gas production, which improve dough rise and bread texture. Selecting yeast strains with superior CO₂ production ensures a more efficient fermentation process and improved final product quality. Ogunsakin et al. [6] highlighted that yeast strains with CO₂ production and tolerance to acidic stress (pH 3.0–4.0) are ideal candidates for sourdough applications. Yeast isolates from cocoa fermentation have been reported to be acid-tolerant, as they can grow in acidic environments with lactic and acetic acid concentrations ranging from 0.5 to 2% (v/v) [113–115]. The cocoa-isolated yeast strains evaluated in this study were previously screened for acid stress tolerance. When comparing the specific growth rates (μ) of *S. cerevisiae* YCTA5, YCTA13, and YCTA14 in YPD broth as control (0.72, 1.68 and 0.65 h⁻¹, respectively) with their performance under stress

conditions, pH 3.0 (0.58, 0.92, and 1.03 h⁻¹), pH 4.0 (0.60, 0.60, and 0.65 h⁻¹), and 2% *v/v* lactic acid (0.39, 0.56, and 0.48 h⁻¹), it can be concluded that these strains are able to grow under the acidic conditions (pH 3.8–4.0) during sourdough fermentation [3]. This factor is expected to influence yeast performance positively in sourdough fermentation. In addition, Shen et al. [7] reported that yeast strains with high fermentability and enzymatic activity enhanced bread sensory properties. Based on gas production, enzymatic activity, and acid stress tolerance, the yeast strains *S. cerevisiae* YCTA5, YCTA13, and YCTA14 were selected for further analysis in co-culture with the chosen LAB strains, as co-culturing *S. cerevisiae* with LAB as *Lpb. plantarum* has been reported to improve bread quality characteristics such as leavening, volume, and texture [116].

3.2.3. Determination of Lactic Acid Production by LAB from Cocoa Mucilage and Coffee Pulp

The acid-producing capacity of LAB strains isolated from cocoa mucilage and coffee pulp was assessed by measuring pH, titratable acidity (TTA), and lactic acid concentrations in broth cultures after 16 h of fermentation at 30 °C (Figure 3). As shown in Figure 3a, *Leu. mesenteroides* LABCTA3 exhibited the lowest pH value at 3.82, followed by *Lpb. plantarum* 20B3HB at 3.91 and LABCTA8 at 4.07. Although these last two values were not significantly different from those of *Lpb. plantarum* LABCTA1 (4.28) and LABCTA6 (4.29), they suggest a strain-dependent acidification capacity. The pH results were supported by TTA values between 2.71 and 3.92 mL of NaOH (Figure 3b), with strains *Leu. mesenteroides* LABCTA3 showing the highest TTA value (4.33 mL of NaOH), which was significantly higher than *Lpb. plantarum* LABCTA6 (2.71 mL of NaOH). In terms of lactic acid production (Figure 3c), *Leu. mesenteroides* LABCTA3 also exhibited the highest concentration (8.84 g/L), followed by *Lpb. plantarum* 20B3HB (8.74 g/L), both of which were significantly higher than *Lpb. plantarum* LABCTA1 (7.28 g/L) and LABCTA8 (7.24 g/L). These results confirm a strong correlation between pH, TTA, and lactic acid production. The variability observed among strains in our results may be attributed to differences in metabolic pathways, fermentation rates, and stress adaptation mechanisms [7]. The correlation between pH, TTA, and lactic acid concentration features the efficiency of these strains in acid production, which is crucial for their potential application as sourdough starter culture strains.

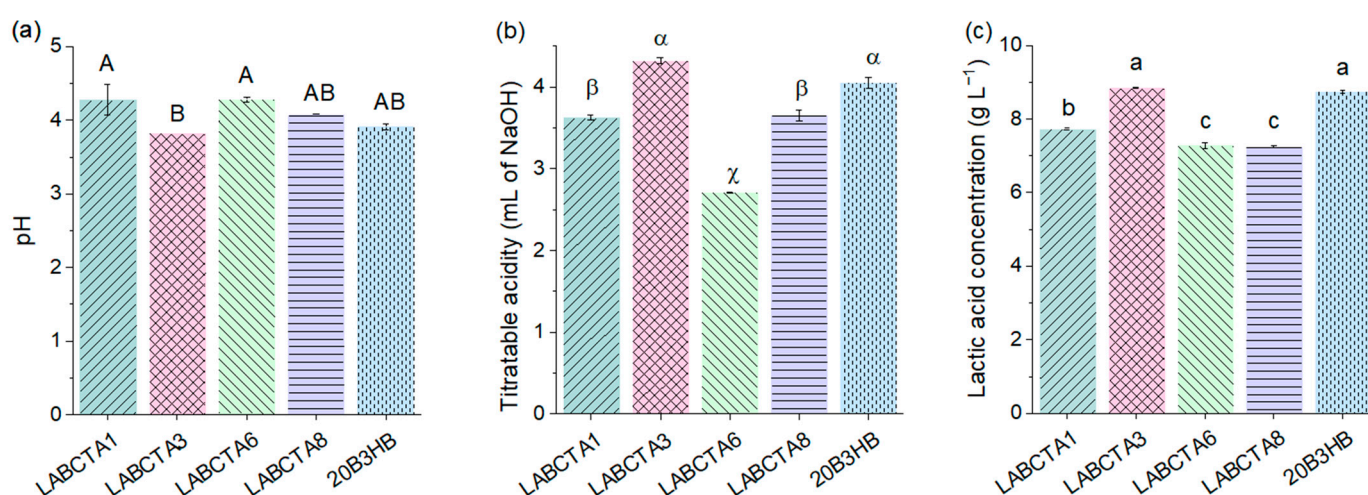


Figure 3. Results of the analysis of fermented broths after 16 h at 30 °C in MRS broth: (a) pH values, (b) titratable acidity values (mL of NaOH), (c) lactic acid production of LAB (g/L). LABCTA1, *Lpb. pentosus* LABCTA1; LABCTA3, *Leu. mesenteroides* LABCTA3; LABCTA6, *Lpb. plantarum* LABCTA6; LABCTA8, *Lpb. plantarum* LABCTA8; 20B3HB, *Lpb. plantarum* 20B3HB. Different letters above the bars indicate significant differences (Tukey's HSD test, $p < 0.05$).

The acidification capacity of LAB influences dough pH, microbial stability, texture, shelf-life, and overall bread quality. A decrease in pH is essential for inhibiting undesirable microorganisms, extending shelf life, and enhancing the development of volatile compounds that contribute to the sensory profile of sourdough bread [4,5,117–119]. Additionally, the production of organic acids, such as lactic and acetic acids, has been associated with an improved crumb structure and gluten network stabilization. Wu et al. [120] reported that LAB with a high acidification capacity induced a lower dough pH, which enables gluten hydrolysis and depolymerization; these changes promote gluten network development and viscoelasticity. On the other hand, acetic acid, which is often produced by heterofermentative LAB, contributes to an improved crumb softness and bread shelf life due to its antifungal properties [121]. During sourdough fermentation, organic acids such as lactic and acetic acids contribute to delaying bread staling by modifying starch retrogradation and protein interactions. These acids reduce the rearrangement of linear amylose chains through acid hydrolysis, leading to chain shortening and decreased crystallization [122,123]. Similarly, in amylopectin, an acidic environment increases the proportion of shorter branches and disrupts the formation of double helices which drives amylopectin retrogradation [69,124,125]. Furthermore, carboxyl groups from organic acids can react with hydroxyl groups in starch to form ester bonds, resulting in chemically modified starches with a lower retrogradation capacity [123,125]. Regarding gluten, the acids reduce the dough pH below the isoelectric point, altering the gluten protein's surface charge and denaturing its structure; this promotes the formation of disulfide bond formation between gliadin and glutenin, enhancing dough cohesivity and extensibility [126,127]. The resulting protein network reduces water mobility and limits molecular rearrangements, thereby slowing bread retrogradation during storage [128]. With regard to our results, similar acidification patterns have been reported in studies evaluating the performance of *Lpb. plantarum* and *Leu. mesenteroides* in sourdough fermentation. For example, *Lpb. plantarum* strains can decrease the pH to values between 3.7 and 4.0 after 16–24 h of fermentation, which aligns with the results obtained in this study [129]. Likewise, *Leu. mesenteroides* has been reported to exhibit high lactic and acetic acid production due to its heterofermentative metabolism, contributing to the characteristic sourness and aromatic profile of sourdough. Additionally, *Leu. mesenteroides* has been associated with EPS production, which improves moisture retention in the crumb and enhances bread texture [8]. This introduces the next LAB selection criteria to be discussed, which will be crucial in determining the most suitable bacteria strains.

3.2.4. Exopolysaccharide (EPS) Production by LAB from Cocoa Mucilage and Coffee Pulp

When selecting the LAB strains, it is important to consider EPS production, as it contributes to bread structure stabilization and has been widely reported in sourdough fermentation studies [130–134]. The results of the LAB screen are presented in Table 3, and only strain *Leu. mesenteroides* B4 exhibited EPS production among the strains isolated from cocoa fermentation.

EPSs interact with gluten and starch, enhancing viscoelasticity and preventing crumb staling [135]. They act as natural hydrocolloids, reducing the need for synthetic additives and improving overall bread quality [8]. The results obtained in this study align with those reported in the literature, as several LAB strains have been reported to produce EPSs in sourdough fermentation [134–136]. It is important to highlight that *Lpb. plantarum* 20B3HB, a previously screened sourdough starter culture candidate, exhibits EPS production as well [33]. Xu et al. [137] found that *Lpb. plantarum* and *Leuconostoc* species isolated from fermented foods exhibited high EPS production, positively influencing bread texture and shelf-life. Similarly, Menezes et al. [40] reported that sourdough fermented

with EPS-producing LAB improved crumb softness and delayed retrogradation. Among EPS-producing LAB species, *Leu. mesenteroides* is known for its production of heteropolysaccharides, primarily dextran, which enhances dough hydration, elasticity, and the sensory attributes of sourdough bread [134,137]. In contrast, *Lpb. plantarum* produces homopolysaccharides, which act as structural stabilizers in the dough matrix, improving gas retention and the crumb structure [40,134]. Variability in EPS production among LAB strains can be attributed to genetic differences in polysaccharide biosynthesis pathways, fermentation conditions, and carbohydrate utilization preferences [111]. Considering their EPS production, along with acidification capacity and enzymatic activity, the *Leu. mesenteroides* LABCTA3 and *Lpb. plantarum* 20B3HB strains were selected as candidates for inclusion in co-culture with the selected yeast strains for sourdough fermentation.

Table 3. EPS production screen of lactic acid bacteria strains after 48 h at 30 °C in MRS agar supplemented with 4% (*w/v*) sucrose.

Bacteria Strain	EPS Production	Reference	<i>Leu. mesenteroides</i> LABCTA3
<i>Lpb. pentosus</i> LABCTA1	-	This work	
<i>Leu. mesenteroides</i> LABCTA3	+	This work	
<i>Lpb. plantarum</i> LABCTA6	-	This work	
<i>Lpb. plantarum</i> LABCTA8	-	This work	
<i>Lpb. plantarum</i> 20B3HB	+	Vazquez-Vazquez [33] **	

(+) Growth/production; (-) growth/absence of production. Representative image of EPS produced by *Leu. mesenteroides* LABCTA3. ** Data from Vazquez-Vazquez [33].

3.3. Sourdough Bread Quality

Six different mixed starter co-cultures were formulated based on the selection of the highest CO₂-producing yeasts (*S. cerevisiae* YCTA5, YCTA13 and YCTA14 from cocoa mucilage) and two LAB strains with a highly acidification capacity and EPS production (*L. mesenteroides* LABCTA3 from cocoa mucilage and *Lpb. plantarum* 20B3HB from coffee pulp). Co-culture formulations are shown in Table 4. These co-cultures were designed to combine the metabolic properties of each strain in sourdough fermentation and to assess their impacts on the final bread parameters, such as the volume, height, and crumb firmness, which are linked to the microbial synergy during sourdough fermentation [138]. Microbial synergy refers to the cooperative interactions between yeasts and LAB that enhance fermentation and result in improved bread quality parameters [120,139].

Table 4. Starter co-culture formulations of selected LAB and yeast strains.

Starter Co-Culture	LAB Strain	Yeast Strain
B3Y5	<i>Leu. mesenteroides</i> LABCTA3	<i>S. cerevisiae</i> YCTA5
B3Y13	<i>Leu. mesenteroides</i> LABCTA3	<i>S. cerevisiae</i> YCTA13
B3Y14	<i>Leu. mesenteroides</i> LABCTA3	<i>S. cerevisiae</i> YCTA14
B20Y5	<i>Lpb. plantarum</i> 20B3HB	<i>S. cerevisiae</i> YCTA5
B20Y13	<i>Lpb. plantarum</i> 20B3HB	<i>S. cerevisiae</i> YCTA13
B20Y14	<i>Lpb. plantarum</i> 20B3HB	<i>S. cerevisiae</i> YCTA14

In this study, the analysis of bread quality parameters revealed statistically significant differences among the treatments (Figure 4). The B3Y14 co-culture showed the significantly highest specific volume (4.31 cm³/g) and loaf height (9.40 cm), and softest crumb (2.23 N). Starter co-culture B20Y5 also showed greater performance with a high specific volume (3.99 cm³/g) and loaf height (8.88 cm³/g), though statistically lower than B3Y14

(4.31 cm³/g) but higher than the commercial control (3.57 cm³/g). In contrast, co-cultures B3Y5 and B20Y14 exhibited the lowest specific volume (3.21 cm³/g and 3.23 cm³/g, respectively) and height (8.04 cm and 7.98 cm, respectively), with no significant difference between them, and both were significantly lower than the control (3.57 cm³/g, 8.63 cm), while B20Y14 had the lowest loaf height (7.98 cm). Regarding crumb firmness, B3Y14 yielded the softest crumb (2.23 N), which was significantly different from all other treatments, while B20Y13 (4.55 N), B3Y13 (4.25 N), and B3Y5 (4.15 N) resulted in the firmest crumbs, with no significant differences among them, but significantly firmer than the control (3.88 N) and B3Y14. The results for bread made with the B3Y14 and B20Y5 starters are explained by the microbial synergy between the highly acid-tolerant, CO₂-producing yeasts and LAB strains with EPS production and enzymatic activity, leading to optimal gas retention, dough expansion, and crumb softness. In contrast, the B3Y5 and B20Y14 starter co-cultures showed a weak synergistic effect, likely due to competition for substrates, the production of inhibitory metabolites, or the absence of effective metabolic cross-feeding, as described by Ponomarova et al. [140] and Canon et al. [141]. These metabolic interactions may limit nutrient availability and metabolic cooperation between yeast and LAB, resulting in restricted dough rise and firmer crumbs, as described by Sun et al. [142]. The starter co-cultures B3Y13 and B20Y13 showed intermediate results, indicating partial functional compatibility that does not improve bread quality, which may be due to the low acid tolerance of the yeast strain. This observation is consistent with Carbonetto et al. [143], who found that in most sourdough yeast–LAB combinations, the presence of LAB reduced yeast populations, while LAB was rarely affected by yeasts. They emphasized that negative or neutral interactions seem to be dominant, often resulting from direct competition for substrates or the production of inhibitory metabolites. Furthermore, effective metabolic cross-feeding is not always established, and interactions are frequently competitive rather than cooperative, which affects fermentation and bread quality. The starter co-cultures B3Y13 and B20Y13 showed intermediate results, indicating partial functional compatibility that does not improve bread quality. The improvement in breadcrumb softness with the B3Y14 starter co-culture is consistent with Graça et al. [14], who used the incorporation of yogurt as a fermentation starter and thus obtained softer crumb structures and reduced staling. Yu et al. [10] demonstrated that spontaneous sourdoughs containing *Lpb. plantarum* and *Leuconostoc* species significantly improved bread porosity and overall structural quality. This result agrees with Woo et al. [111] and Elhariry et al. [116], who emphasized the cooperative fermentation effects of LAB and yeasts on stabilizing gas cells and enhancing crumb elasticity.

The results of the crumb alveolar analysis regarding alveolar porosity (AP), regularity/uniformity (AR), and alveolar circularity index (CI) exhibited differences between treatments (Figure 5). Porosity in bread crumb is an indicator of the retained gas during fermentation and baking. Although the highest AP was observed using co-culture B20Y5, no significant differences in alveolar porosity were detected between treatments. Porosity values are related to the gas cell structure, as alveoli are directly shaped by fermentation and gluten network stability. Low porosity values (<0.20) are associated with dense crumbs, while moderate porosity (0.20–0.35) indicates efficient gas retention and a stable crumb structure; in contrast, high porosity (>0.35) may result in large void formation or crumb collapse [144,145]. The results obtained in this study, ranging from 0.20 to 0.30, fit within the moderate range, which is desirable in sourdough bread, as it reflects a balanced interaction between microbial fermentation and dough structure, avoiding excessive expansion and crumb collapse.

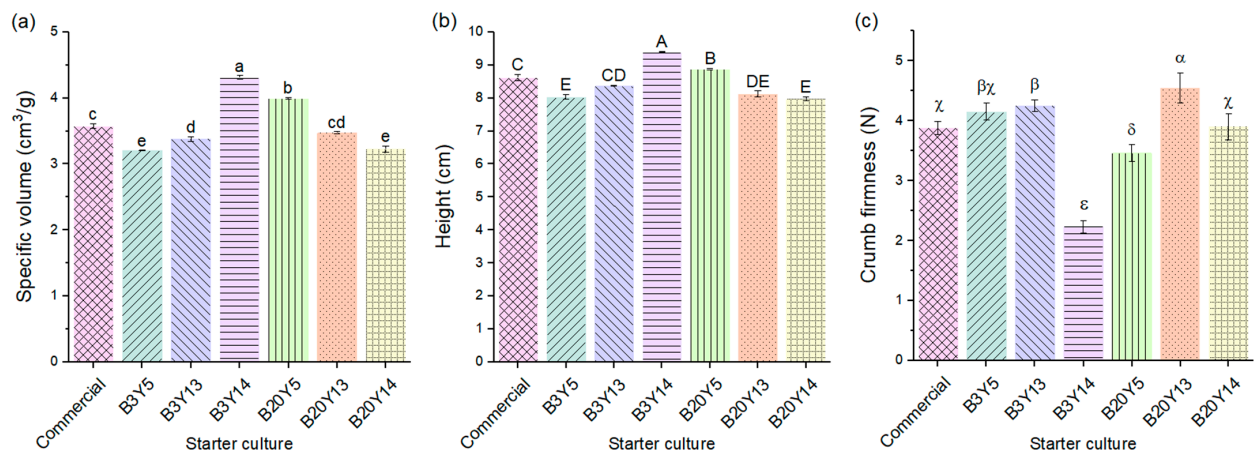


Figure 4. Effect of different starter co-cultures on sourdough bread properties. (a) Specific volume (cm³/g), (b) height (cm), and (c) crumb firmness (N). Different letters above the bars indicate significant differences (Tukey's HSD test, $p < 0.05$). For the starter co-culture nomenclature, see Table 4.

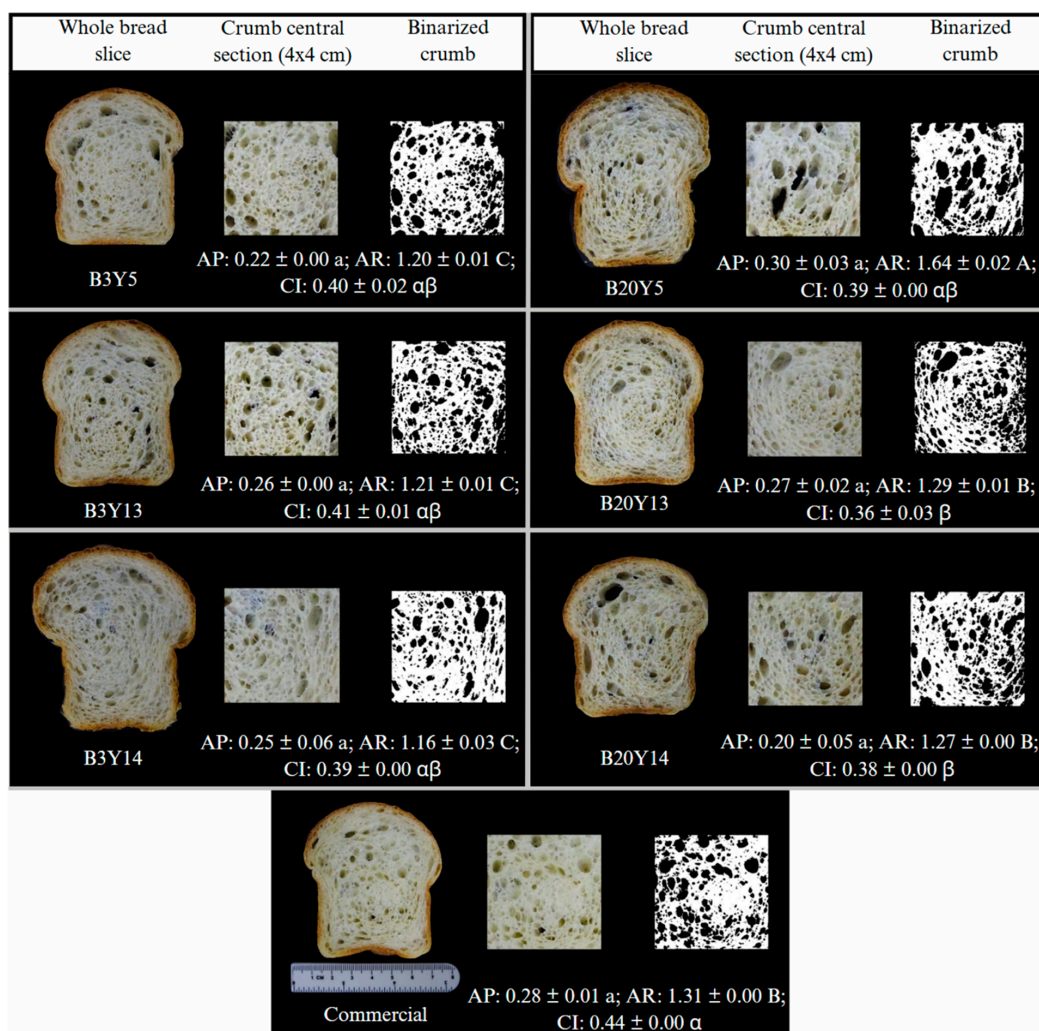


Figure 5. Crumb structure analysis of sourdough breads prepared with different starter co-cultures. From left to right: whole bread slice, central crumb section, and binarized crumb. Alveoli porosity (AP), regularity (AR), and circularity index (CI). For the starter co-culture nomenclature, see Table 4. Values sharing the same letter or symbol within each class are not significantly different (Tukey's HSD test, $p > 0.05$). Images were scaled using a reference ruler during acquisition, and standardization was ensured across all treatments. The ruler in the slides was omitted from the final figure for clarity.

Regarding crumb alveolar regularity (AR), this parameter is associated with sensory perception and structural consistency, as it reflects crumb uniformity. Regularity was assessed through the coefficient of variation of Feret's diameter, calculated using Fiji/ImageJ. Significant differences were found among treatments ($p < 0.05$). The B20Y5 co-culture exhibited the highest alveolar regularity value (1.64), indicating the most significant variation in alveolar diameter size and more heterogeneous crumb structure. These results align with Gonzales-Barron and Butler [42], who reported that higher gas cell variability is associated with crumb uniformity. In contrast, the use of co-culture B3Y14 showed the lowest alveolar regularity, which was significantly lower than B20Y5 and the commercial control (1.31), suggesting a uniform and consistent crumb. Similarly, B3Y5 and B3Y13 showed uniform crumb structures that were statistically comparable to B3Y14. The commercial starter presented an intermediate regularity that was not significantly different from B20Y13 and B20Y14. These results indicate that B3Y14 favored the formation of a homogeneous alveolar structure, which is desirable in sourdough bread, as a uniform alveolar distribution is linked to a better crumb structure and mouthfeel properties [145]. This uniformity may be attributed to the balanced metabolic activity of its microbial components, which favor even gas production and prevent the coalescence of alveoli, especially under controlled fermentation conditions [146].

Alveolar shape, quantified through the circularity index (CI), reflects the geometric regularity of gas cells within the bread crumb. Circularity values close to 1.0 indicate perfectly round alveoli [147]. Lower circularity values (<0.50) have been consistently reported in sourdough breads compared to those leavened with commercial yeast (>0.80) due to differences in fermentation kinetics, dough acidification, and gluten network development [147–149]. In our study, the commercial sourdough starter yielded the highest circularity, with only statistically significant differences ($p < 0.05$) relative to B20Y13 and B20Y14. Intermediate values were observed in B3Y5, B3Y13, B3Y14, and B20Y5 (0.39–0.41), which did not differ significantly from the commercial reference. While breads made with baker's yeast alone typically present circularity values between 0.80 and 0.90, sourdough breads commonly show lower values, ranging from 0.45 to 0.70 depending on microbial diversity, dough rheology, and fermentation time [46,148]. The circularity values observed in this study (<0.45) align with the structural behavior of sourdough bread, as mentioned before. These lower circularity values may result from metabolic interactions involving acidification, CO₂ production, proteolysis, and EPS-producing strains. Such interactions influence gas cell expansion and stabilize or disrupt gluten matrices, as previously observed in sourdoughs fermented with *Leu. mesenteroides* and *S. cerevisiae* [95,140,142,150].

The alveolar area distribution was classified into five size classes (Figure 6). Class 1 alveoli (0.05–0.49 mm²), associated with a fine and homogeneous crumb, predominated in all treatments, accounting for more than 70% of the crumb structure in all cases. Intermediate-size alveoli (classes 2 and 3) ranged from 8 to 15%, contributing to a moderately open crumb structure [148], while large alveoli (classes 4 and 5), often considered structural defects [144,145], represented $\leq 6\%$ of the total alveolar area. Statistical analysis using the Kruskal–Wallis test revealed no significant differences ($p > 0.05$) among treatments within any of the alveolar size classes. Consequently, it was not possible to associate specific microbial consortia with distinct alveolar class distributions. These results suggest that although microbial combinations may influence the crumb microstructure in other ways (e.g., alveolar regularity and circularity), the overall proportion of each alveolar size remains relatively stable across treatments.

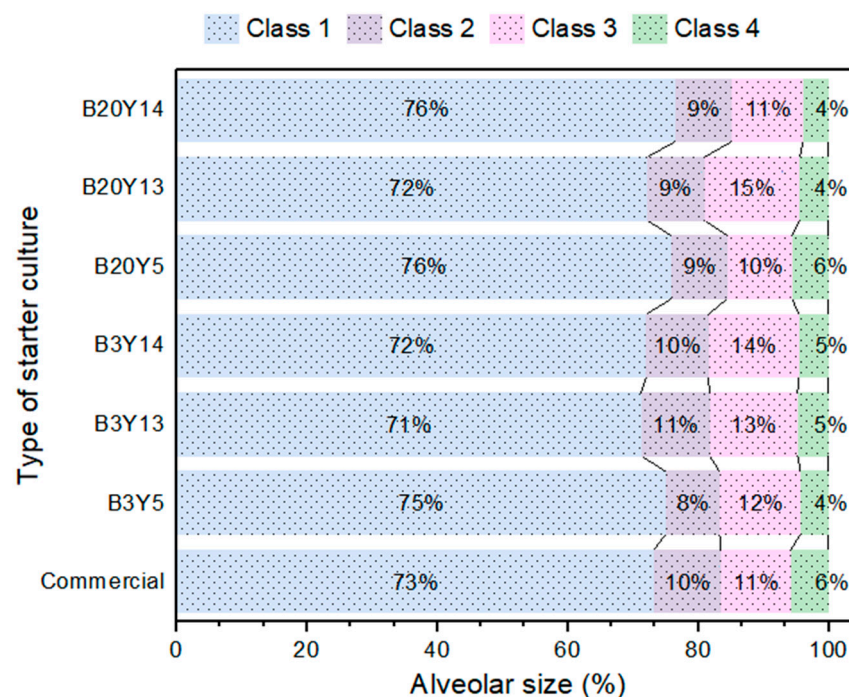


Figure 6. Percentage distribution of alveolar size in bread crumb produced with different starter co-cultures. Alveoli were classified by individual area into five classes: class 1 (0.05–0.49 mm²), class 2 (0.50–0.99 mm²), class 3 (1.00–4.99 mm²), class 4 (5.00–49.99 mm²), and class 5 (>50 mm²). For the starter co-culture nomenclature, see Table 4. No statistically significant differences were found among treatments within any alveolar class (Kruskal–Wallis test, $p > 0.05$).

In conclusion, the image analysis of the crumb structure provided insights into the impact of different starter co-cultures on the alveolar structure in sourdough breads. While porosity values remained within a desirable moderate range, differences in alveolar regularity and the circularity index revealed notable variations in crumb uniformity and gas cell morphology. In particular, the use of the B3Y14 co-culture, composed of *Leu. mesenteroides* LABCTA3 and *S. cerevisiae* YCTA14, promoted a homogeneous alveolar distribution and balanced gas cell geometry.

4. Conclusions

This study demonstrates that sourdough starter cultures formulated with yeast and LAB strains isolated from cocoa and coffee fermentations can enhance the technological and sensory qualities of sourdough bread. The selected *S. cerevisiae* YCTA13 strain showed superior leavening activity with 52.24% increase in CO₂ production when compared with commercial *S. cerevisiae*, improving dough aeration. *Leuconostoc mesenteroides* LABCTA3 displayed promising EPS production and acidification ability by reaching the lowest pH value (3.82), the highest titratable acidity (4.33 mL NaOH), and the highest lactic acid concentration (8.84 g/L), highlighting its potential as a functional LAB component in sourdough fermentation.

The B3Y14 co-culture (LABCTA3 + YCTA14) significantly improved the bread volume and height by 35.53 and 17.18%, respectively, compared to the commercial sourdough starter, and reduced crumb firmness by 59.66%. Additionally, B3YCTA14 reduced crumb firmness by 42.5%, resulting in a significantly softer texture. This co-culture also enhanced the crumb structure by increasing alveolar porosity by 3.8%, reducing regularity by 11.5%, and lowering the circularity index by 9.1% compared to the commercial control, reflecting a more uniform and typical sourdough morphology.

These findings highlight the importance of screening strains for selection based on functional properties, particularly CO₂ production, acidification and EPS production, to optimize sourdough performance through starter culture application. Furthermore, using microorganisms from sources other than sourdough opens new possibilities to find functional starter cultures. Future research should focus on deepening the understanding of microbial interactions and their impacts on bread quality, the aroma profile, and shelf-life.

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Abbreviations

The following abbreviations are used in this manuscript:

LAB	Lactic acid bacteria
EPS	Exopolysaccharide
AP	Alveolar porosity
AR	Alveolar regularity
CI	Circularity index

References

1. Suo, B.; Chen, X.; Wang, Y. Recent research advances of lactic acid bacteria in sourdough: Origin, diversity, and function. *Curr. Opin. Food Sci.* **2021**, *37*, 66–75. [[CrossRef](#)]
2. Arora, K.; Ameer, H.; Polo, A.; Di Cagno, R.; Rizzello, C.G.; Gobbetti, M. Thirty years of knowledge on sourdough fermentation: A systematic review. *Trends Food Sci. Technol.* **2021**, *108*, 71–83. [[CrossRef](#)]
3. Hernández-Parada, N.; González-Ríos, O.; Suárez-Quiroz, M.L.; Hernández-Estrada, Z.J.; Figueroa-Hernández, C.Y.; Figueroa-Cárdenas, J.D.D.; Rayas-Duarte, P.; Figueroa-Espinoza, M.C. Exploiting the native microorganisms from different food matrices to formulate starter cultures for sourdough bread production. *Microorganisms* **2023**, *11*, 109. [[CrossRef](#)]
4. Calvert, M.D.; Madden, A.A.; Nichols, L.M.; Haddad, N.M.; Lahne, J.; Dunn, R.R.; McKenney, E.A. A review of sourdough starters: Ecology, practices, and sensory quality with applications for baking and recommendations for future research. *PeerJ* **2021**, *9*, e10972. [[CrossRef](#)] [[PubMed](#)]
5. Corsetti, A.; Gänzle, M. Technology of sourdough fermentation and sourdough applications. In *Handbook on Sourdough Biotechnology*; Springer: Boston, MA, USA, 2013; pp. 1–298. [[CrossRef](#)]
6. Ogunakin, A.O.; Vanajakshi, V.; Anu-Appaiah, K.A.; Vijayendra, S.V.N.; Walde, S.G.; Banwo, K.; Sanni, A.I.; Prabhasankar, P. Evaluation of functionally important lactic acid bacteria and yeasts from Nigerian sorghum as starter cultures for gluten-free sourdough preparation. *LWT* **2017**, *82*, 326–334. [[CrossRef](#)]

7. Shen, J.; Shi, K.; Dong, H.; Yang, K.; Lu, Z.; Lu, F.; Wang, P. Screening of sourdough starter strains and improvements in the quality of whole wheat steamed bread. *Molecules* **2022**, *27*, 3510. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Lim, S.B.; Tingirikari, J.M.R.; Seo, J.S.; Li, L.; Shim, S.; Seo, J.H.; Han, N.S. Isolation of lactic acid bacteria starters from Jeung-Pyun for sourdough fermentation. *Food Sci. Biotechnol.* **2018**, *27*, 73–78. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Mohd Roby, B.H.; Muhialdin, B.J.; Abadl, M.M.T.; Mat Nor, N.A.; Marzlan, A.A.; Lim, S.A.H.; Mustapha, N.A.; Meor Hussin, A.S. Physical properties, storage stability, and consumer acceptability for sourdough bread produced using encapsulated Kombucha sourdough starter culture. *J. Food Sci.* **2020**, *85*, 2286–2295. [\[CrossRef\]](#)
10. Yu, Y.; Wang, L.; Qian, H.; Zhang, H.; Qi, X. Contribution of spontaneously-fermented sourdoughs with pear and navel orange for the bread-making. *LWT* **2018**, *89*, 336–343. [\[CrossRef\]](#)
11. Nionelli, L.; Montemurro, M.; Pontonio, E.; Verni, M.; Gobetti, M.; Rizzello, C.G. Pro-technological and functional characterization of lactic acid bacteria to be used as starters for hemp (*Cannabis sativa* L.) sourdough fermentation and wheat bread fortification. *Int. J. Food Microbiol.* **2018**, *279*, 14–25. [\[CrossRef\]](#)
12. Păcularu-Burada, B.; Ceoromila (Cantaragiu), A.-M.; Vasile, M.A.; Bahrim, G.-E. Novel insights into different kefir grains usefulness as valuable multiple starter cultures to achieve bioactive gluten-free sourdoughs. *LWT* **2022**, *165*, 113670. [\[CrossRef\]](#)
13. Limbad, M.; Maddox, N.G.; Hamid, N.; Kantono, K. Sensory and physicochemical characterization of sourdough bread prepared with a coconut water kefir starter. *Foods* **2020**, *9*, 1165. [\[CrossRef\]](#)
14. Graça, C.; Edelmann, M.; Raymundo, A.; Sousa, I.; Coda, R.; Sontag-Strohm, T.; Huang, X. Yoghurt as a starter in sourdough fermentation to improve the technological and functional properties of sourdough-wheat bread. *J. Funct. Foods* **2022**, *88*, 104877. [\[CrossRef\]](#)
15. Ripari, V.; Gänzle, M.G.; Berardi, E. Evolution of sourdough microbiota in spontaneous sourdoughs started with different plant materials. *Int. J. Food Microbiol.* **2016**, *232*, 35–42. [\[CrossRef\]](#)
16. Schwan, R.; Silva, C.; Batista, L. Coffee fermentation. In *Handbook of Plant-Based Fermented Food and Beverage Technology*, 2nd ed.; Taylor & Francis: Boca Raton, FL, USA, 2012; pp. 677–690. [\[CrossRef\]](#)
17. Nigam, P.S.; Singh, A. Cocoa and coffee fermentations. In *Encyclopedia of Food Microbiology*, 2nd ed.; Elsevier Inc.: Oxford, UK, 2014; pp. 485–492. ISBN 9780123847331.
18. Haile, M.; Kang, W.H. The role of microbes in coffee fermentation and their impact on coffee quality. *J. Food Qual.* **2019**, *2019*, 4836709. [\[CrossRef\]](#)
19. Bae, H.M.; Haile, M.; Kang, W.H. Evaluation of antioxidant, organic acid, and volatile compounds in coffee pulp wine fermented with native yeasts isolated from coffee cherries. *Food Sci. Technol. Int.* **2022**, *28*, 716–727. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Roussos, S.; de los Angeles Aquihuatl, M.; del Refugio Trejo-Hernández, M.; Gaime-Perraud, I.; Favela, E.; Raimbault, G.; Viniegra-González, G. Biotechnological management of coffee pulp—Isolation, screening, characterization, selection of caffeine-degrading fungi and natural microflora present in coffee pulp and husk. *Appl. Microbiol. Biotechnol.* **1995**, *42*, 756–762. [\[CrossRef\]](#)
21. Arellano-González, M.A.; Ramírez-Coronel, M.A.; Torres-Mancera, M.T.; Pérez-Morales, G.G.; Saucedo-Castañeda, G. Antioxidant activity of fermented and nonfermented coffee (*Coffea arabica*) pulp extracts. *Food Technol. Biotechnol.* **2011**, *49*, 374–378.
22. Machuca-Guevara, J.I.; Suárez-Peña, E.A.; Darricau, E.M.; Mialhe-Matonnier, E.L. Molecular characterization of microorganisms during cocoa-bean fermentation process (*Theobroma cacao*). *Rev. Peru. Biol.* **2019**, *26*, 535–542. [\[CrossRef\]](#)
23. Sarbu, I.; Csutak, O. The microbiology of cocoa fermentation. In *Caffeinated and Cocoa Based Beverages, Volume 8: The Science of Beverages*; Preedy, V.R., Ed.; Elsevier: London, UK, 2019; pp. 423–446. ISBN 9780128158647. [\[CrossRef\]](#)
24. Cempaka, L.; Aliwarga, L.; Purwo, S.; Kresnowati, M.T.A.P. Dynamics of cocoa bean pulp degradation during cocoa bean fermentation: Effects of yeast starter culture addition. *J. Math. Fund. Sci.* **2014**, *46*, 14–25. [\[CrossRef\]](#)
25. Lau, S.W.; Chong, A.Q.; Chin, N.L.; Talib, R.A.; Basha, R.K. Sourdough microbiome comparison and benefits. *Microorganisms* **2021**, *9*, 1355. [\[CrossRef\]](#)
26. De Vuyst, L.; Neysens, P. The sourdough microflora: Biodiversity and metabolic interactions. *Trends Food Sci. Technol.* **2005**, *16*, 43–56. [\[CrossRef\]](#)
27. Landis, E.A.; Oliverio, A.M.; McKenney, E.A.; Nichols, L.M.; Kfoury, N.; Biango-Daniels, M.; Shell, L.K.; Madden, A.A.; Shapiro, L.; Sakunala, S.; et al. The diversity and function of sourdough starter microbiomes. *eLife* **2021**, *10*, e61644. [\[CrossRef\]](#)
28. Lhomme, E.; Urien, C.; Legrand, J.; Dousset, X.; Onno, B.; Sicard, D. Sourdough microbial community dynamics: An analysis during French organic bread-making processes. *Food Microbiol.* **2016**, *53*, 41–50. [\[CrossRef\]](#)
29. Von Gastrow, L.; Amelot, R.; Segond, D.; Guézennec, S.; Valence, F.; Sicard, D. Microbial community dispersal in sourdough. *bioRxiv* **2021**. [\[CrossRef\]](#)
30. Cervantes-Ilizaliturri, A.-A. Aislamiento y Caracterización de Bacterias Ácido-Lácticas Aisladas del Cacao con Capacidad Antifúngica. Master's Thesis, Instituto Tecnológico de Veracruz, Veracruz, México, 2020.
31. Hernández-Parada, N. Selección de Levaduras y Bacterias Autóctonas de la Fermentación de Cacao Mexicano Para la Formulación de Cultivos Iniciadores. Master's Thesis, Instituto Tecnológico de Veracruz, Veracruz, México, 2021.

32. Romero-Isaza, S.P. Aislamiento y Caracterización de Levaduras Autóctonas del Café Para la Formulación de Cultivos Iniciadores de Masa Madre. Master's Thesis, Instituto Tecnológico de Veracruz, Veracruz, México, 2023.
33. Vázquez-Vázquez, M. Selección y Caracterización Fisiológica y Metabólica de Bacterias Ácido-Lácticas (BAL) Aisladas de la Fermentación de Café Para la Formulación de Cultivos Iniciadores. Master's Thesis, Instituto Tecnológico de Veracruz, Veracruz, México, 2024.
34. Jorquera, M.A.; Hernández, M.T.; Rengel, Z.; Marschner, P.; De La Luz Mora, M. Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. *Biol. Fertil. Soils* **2008**, *44*, 1025–1034. [\[CrossRef\]](#)
35. Hasan, M.M.; Marzan, L.W.; Hosna, A.; Hakim, A.; Azad, A.K. Optimization of some fermentation conditions for the production of extracellular amylases by using *Chryseobacterium* and *Bacillus* isolates from organic kitchen wastes. *J. Genet. Eng. Biotechnol.* **2017**, *15*, 59–68. [\[CrossRef\]](#)
36. Kwon, Y.M.; Choi, H.S.; Lim, J.Y.; Jang, H.S.; Chung, D. Characterization of amylolytic activity by a marine-derived yeast *Sporidiobolus pararoseus* PH-Gra1. *Mycobiology* **2020**, *48*, 195–203. [\[CrossRef\]](#)
37. Ahi, M.; Hatamipour, M.S.; Goodarzi, A. Optimization of leavening activity of baker's yeast during the spray-drying process. *Drying Technol.* **2010**, *28*, 490–494. [\[CrossRef\]](#)
38. Borzani, W. Measurement of the gassing power of bakers' yeast: Correlation between the dough volume and the incubation time. *Braz. Arch. Biol. Technol.* **2004**, *47*, 213–217. [\[CrossRef\]](#)
39. Borshchevskaya, L.N.; Gordeeva, T.L.; Kalinina, A.N.; Sineokii, S.P. Spectrophotometric determination of lactic acid. *J. Anal. Chem.* **2016**, *71*, 755–758. [\[CrossRef\]](#)
40. Menezes, L.A.A.; De Marco, I.; Oliveira dos Santos, N.N.; Costa Nunes, C.; Leite Cartabiano, C.E.; Molognoni, L.; Pereira, G.V.M.; Daguer, H.; De Dea Lindner, J. Reducing FODMAPs and improving bread quality using type II sourdough with selected starter cultures. *Int. J. Food Sci. Nutr.* **2021**, *72*, 912–922. [\[CrossRef\]](#)
41. Ramon-Simon, X. Técnicas de Panadería con Masa Madre, Online course. Available online: <https://www.scoolinary.com/es/courses/tecnicas-panaderia-masa-madre> (accessed on 19 December 2022).
42. Gonzales-Barron, U.; Butler, F. A comparison of seven thresholding techniques with the K-means clustering algorithm for measurement of bread-crumbs features by digital image analysis. *J. Food Eng.* **2006**, *74*, 268–278. [\[CrossRef\]](#)
43. Scheuer, P.M.; Ferreira, J.A.S.; Mattioni, B.; de Miranda, M.Z.; de Francisco, A. Optimization of image analysis techniques for quality assessment of whole-wheat breads made with fat replacer. *Food Sci. Technol.* **2015**, *35*, 133–142. [\[CrossRef\]](#)
44. Rosales-Juárez, M.; González-Mendoza, B.; López-Guel, E.C.; Lozano-Bautista, F.; Chanona-Pérez, J.; Gutiérrez-López, G.; Farrera-Rebollo, R.; Calderón-Domínguez, G. Changes on dough rheological characteristics and bread quality as a result of the addition of germinated and non-germinated soybean flour. *Food Bioproc. Technol.* **2008**, *1*, 152–160. [\[CrossRef\]](#)
45. López-Guel, E.C.; Lozano-Bautista, F.; Mora-Escobedo, R.; Farrera-Rebollo, R.R.; Chanona-Pérez, J.; Gutiérrez-López, G.F.; Calderón-Domínguez, G. Effect of soybean 7S protein fractions, obtained from germinated and nongerminated seeds, on dough rheological properties and bread quality. *Food Bioproc. Technol.* **2012**, *5*, 226–234. [\[CrossRef\]](#)
46. Hayta, M.; Hendek Ertop, M. Evaluation of microtextural properties of sourdough wheat bread obtained from optimized formulation using scanning electron microscopy and image analysis during shelf life. *J. Food Sci. Technol.* **2018**, *55*, 3024–3031. [\[CrossRef\]](#)
47. Aydın, F.; Günen, T.U.; Kahve, H.İ.; Güler, E.; Özer, G.; Aktepe, Y.; Çakır, İ. Molecular and technological characterization of *Saccharomyces cerevisiae* from sourdough. *Fermentation* **2023**, *9*, 329. [\[CrossRef\]](#)
48. Martorana, A.; Giuffrè, A.M.; Capocasale, M.; Zappia, C.; Sidari, R. Sourdoughs as a source of lactic acid bacteria and yeasts with technological characteristics useful for improved bakery products. *Eur. Food Res. Technol.* **2018**, *244*, 1873–1885. [\[CrossRef\]](#)
49. Guerzoni, M.E.; Serrazanetti, D.I.; Vernocchi, P.; Gianotti, A. Physiology and biochemistry of sourdough yeasts. In *Handbook on Sourdough Biotechnology*; Gobbetti, M., Gänzle, M., Eds.; Springer: Boston, MA, USA, 2013; pp. 155–181. ISBN 9781461454250. [\[CrossRef\]](#)
50. Koçak, N.; Ardiç, M. Isolation, molecular characterization, and population analysis of *Saccharomyces cerevisiae* from sourdough collected from different provinces. *Gıda* **2024**, *49*, 179–192. [\[CrossRef\]](#)
51. Lahue, C.; Madden, A.A.; Dunn, R.R.; Smukowski Heil, C. History and domestication of *Saccharomyces cerevisiae* in bread baking. *Front. Genet.* **2020**, *11*, 584718. [\[CrossRef\]](#)
52. Leo, V.V.; Viswanath, V.; Deka, P.; Zothanpuia, Ramji, D.R.; Pachuau, L.; Carrie, W.; Malvi, Y.; Singh, G.; Singh, B.P. *Saccharomyces* and their potential applications in food and food processing industries. In *Industrially Important Fungi for Sustainable Development*; Springer: Cham, Switzerland, 2021; Volume 1, pp. 393–427. [\[CrossRef\]](#)
53. Zhang, G.; Sun, Y.; Sadiq, F.A.; Sakandar, H.A.; He, G. Evaluation of the effect of *Saccharomyces cerevisiae* on fermentation characteristics and volatile compounds of sourdough. *J. Food Sci. Technol.* **2018**, *55*, 2079–2086. [\[CrossRef\]](#)

54. Yang, H.; Sadiq, F.A.; Liu, T.; Zhang, G.; He, G. Use of physiological and transcriptome analysis to infer the interactions between *Saccharomyces cerevisiae* and *Lactobacillus sanfranciscensis* isolated from Chinese traditional sourdoughs. *LWT* **2020**, *126*, 109268. [\[CrossRef\]](#)
55. Sieuwerts, S.; Bron, P.A.; Smid, E.J. Mutually stimulating interactions between lactic acid bacteria and *Saccharomyces cerevisiae* in sourdough fermentation. *LWT* **2018**, *90*, 201–206. [\[CrossRef\]](#)
56. Korcari, D.; Ricci, G.; Capusoni, C.; Fortina, M.G. Physiological performance of *Kazachstania unispora* in sourdough environments. *World J. Microbiol. Biotechnol.* **2021**, *37*, 114. [\[CrossRef\]](#)
57. De Vuyst, L.; Van Kerrebroeck, S.; Harth, H.; Huys, G.; Daniel, H.M.; Weckx, S. Microbial ecology of sourdough fermentations: Diverse or uniform? *Food Microbiol.* **2014**, *37*, 11–29. [\[CrossRef\]](#)
58. Lancetti, R.; Sciarini, L.; Pérez, G.T.; Salvucci, E. Technological performance and selection of lactic acid bacteria isolated from Argentinian grains as starters for wheat sourdough. *Curr. Microbiol.* **2021**, *78*, 255–264. [\[CrossRef\]](#)
59. Liu, T.; Li, Y.; Zhao, M.; Mo, Q.; Feng, F. Weight-reducing effect of *Lactobacillus plantarum* ZJUFT17 isolated from sourdough ecosystem. *Nutrients* **2020**, *12*, 977. [\[CrossRef\]](#)
60. Tolu, V.; Fraumene, C.; Carboni, A.; Loddo, A.; Sanna, M.; Fois, S.; Roggio, T.; Catzeddu, P. Dynamics of microbiota in three backslotted liquid sourdoughs that were triggered with the same starter strains. *Fermentation* **2022**, *8*, 571. [\[CrossRef\]](#)
61. Minervini, F.; Lattanzi, A.; De Angelis, M.; Di Cagno, R.; Gobbetti, M. Influence of artisan bakery- or laboratory-propagated sourdoughs on the diversity of lactic acid bacterium and yeast microbiotas. *Appl. Environ. Microbiol.* **2012**, *78*, 5328–5340. [\[CrossRef\]](#)
62. Scheirlinck, I.; Van Der Meulen, R.; Van Schoor, A.; Vancanneyt, M.; De Vuyst, L.; Vandamme, P.; Huys, G. Influence of geographical origin and flour type on diversity of lactic acid bacteria in traditional Belgian sourdoughs. *Appl. Environ. Microbiol.* **2007**, *73*, 6262–6269. [\[CrossRef\]](#)
63. Scheirlinck, I.; Van Der Meulen, R.; De Vuyst, L.; Vandamme, P.; Huys, G. Molecular source tracking of predominant lactic acid bacteria in traditional Belgian sourdoughs and their production environments. *J. Appl. Microbiol.* **2009**, *106*, 1081–1092. [\[CrossRef\]](#)
64. Manini, F.; Casiraghi, M.C.; Poutanen, K.; Brasca, M.; Erba, D.; Plumed-Ferrer, C. Characterization of lactic acid bacteria isolated from wheat bran sourdough. *LWT* **2016**, *66*, 275–283. [\[CrossRef\]](#)
65. Zotta, T.; Piraino, P.; Parente, E.; Salzano, G.; Ricciardi, A. Characterization of lactic acid bacteria isolated from sourdoughs for Cornetto, a traditional bread produced in Basilicata (southern Italy). *World J. Microbiol. Biotechnol.* **2008**, *24*, 1785–1795. [\[CrossRef\]](#)
66. Bi, Q.; Hong, T.; Mei, X.; Xu, X.; Xu, D. Effect of high-molecular weight dextran-enriched sourdough fermented using *Leuconostoc mesenteroides* ATCC 8293 on bread quality and gluten. *Food Biosci.* **2023**, *53*, 102777. [\[CrossRef\]](#)
67. Gumustop, I.; Ortakci, F. Analyzing the genetic diversity and biotechnological potential of *Leuconostoc pseudomesenteroides* by comparative genomics. *Front. Microbiol.* **2023**, *13*, 1074366. [\[CrossRef\]](#)
68. Deckardt, K.; Khiaosa-Ard, R.; Grausgruber, H.; Zebeli, Q. Evaluation of various chemical and thermal feed processing methods for their potential to enhance resistant starch content in barley grain. *Starch/Stärke* **2014**, *66*, 558–565. [\[CrossRef\]](#)
69. Majzoobi, M.; Beparva, P. Effects of acetic acid and lactic acid on physicochemical characteristics of native and cross-linked wheat starches. *Food Chem.* **2014**, *147*, 312–317. [\[CrossRef\]](#)
70. Majzoobi, M.; Radi, M.; Farahnaky, A.; Tongdang, T. Effects of L-ascorbic acid on physicochemical characteristics of wheat starch. *J. Food Sci.* **2012**, *77*, C258–C264. [\[CrossRef\]](#)
71. Wang, Z.; Wang, L. Impact of sourdough fermentation on nutrient transformations in cereal-based foods: Mechanisms, practical applications, and health implications. *Grain Oil Sci. Technol.* **2024**, *7*, 124–132. [\[CrossRef\]](#)
72. Sun, L.; Sun, X.; Du, Y.; Fang, Y.; Yang, W.; Hu, Q.; Pei, F. Effect of the starch structure fermented by *Lactobacillus plantarum* LB-1 and yeast on rheological and thermomechanical characteristics of dough. *Food Chem.* **2022**, *369*, 130877. [\[CrossRef\]](#)
73. David, I.; Mișcă, C.; Bujancă, G.; Rinovetz, A.; Danci, M. The monitoring of enzyme activity of glucoamylase and endoxylanase on the bread dough with consitographic method. *J. Agroaliment. Processes Technol.* **2023**, *19*, 338–343.
74. David, I.; Danci, M.; Chiș-Junior, S. The consistographic determinations of different types of amylase on the bread dough. *J. Hortic. For. Biotechnol.* **2014**, *18*, 203–207.
75. Lee, J.Y.; Im, Y.K.; Chin, J.E.; Bai, S. Construction of amylolytic yeasts secreting xylanase and phytase to improve bread quality. *Korean J. Microbiol.* **2020**, *56*, 170–176. [\[CrossRef\]](#)
76. Chiravi, K.; Khan, M.A.; Wadikar, D.D.; Mahesh, C.; Semwal, A.D. Starch retrogradation: Role of food ingredients and extrinsic factors. *Food Sci. Eng.* **2023**, *4*, 204–215. [\[CrossRef\]](#)
77. Palacios, H.R.; Schwarz, P.B.; D’Appolonia, B.L. Effect of α -amylases from different sources on the retrogradation and recrystallization of concentrated wheat starch gels: Relationship to bread staling. *J. Agric. Food Chem.* **2004**, *52*, 5978–5986. [\[CrossRef\]](#)
78. Wang, S.; Li, C.; Copeland, L.; Niu, Q.; Wang, S. Starch retrogradation: A comprehensive review. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 568–585. [\[CrossRef\]](#)
79. Lalue, C.; Bertolini, M.C.; Ernandes, J.R.; Martini, A.V.; Martini, A. New amylolytic yeast strains for starch and dextrin fermentation. *Appl. Environ. Microbiol.* **1988**, *54*, 2447–2451. [\[CrossRef\]](#)

80. Akamine, I.T.; Mansoldo, F.R.P.; Cardoso, V.S.; de Souza Dias, E.P.; Vermelho, A.B. Hydrolase activities of sourdough microorganisms. *Fermentation* **2023**, *9*, 703. [\[CrossRef\]](#)
81. Palla, M.; Cristani, C.; Giovannetti, M.; Agnolucci, M. Identification and characterization of lactic acid bacteria and yeasts of PDO Tuscan bread sourdough by culture dependent and independent methods. *Int. J. Food Microbiol.* **2017**, *250*, 19–26. [\[CrossRef\]](#)
82. Damayanti, E.; Ichsyani, M.; Istiqomah, L.; Anggraeni, A.S.; Kurniadi, M. Fermentation of amylolytic yeast and lactic acid bacteria to improve the quality of modified cassava. *IOP Conf. Ser. Mater. Sci. Eng.* **2021**, *1011*, 012004. [\[CrossRef\]](#)
83. Díaz-Ruiz, G.; Guyot, J.P.; Ruiz-Teran, F.; Morlon-Guyot, J.; Wachter, C. Microbial and physiological characterization of weakly amylolytic but fast-growing lactic acid bacteria: A functional role in supporting microbial diversity in pozol, a Mexican fermented maize beverage. *Appl. Environ. Microbiol.* **2003**, *69*, 4367–4374. [\[CrossRef\]](#)
84. Amapu, T.; Ameh, J.; Ado, S.; Abdullahi, I.; Dapiya, H. Amylolytic potential of lactic acid bacteria isolated from wet milled cereals, cassava flour and fruits. *Br. Microbiol. Res. J.* **2016**, *13*, 1–8. [\[CrossRef\]](#)
85. Elida, M.; Agustina, A.; Ermiami, E.; Desminarti, S. Isolate characterization and amylolytic properties of lactic acid bacteria from traditional fermented dadih. *IOP Conf. Ser. Earth Environ. Sci.* **2022**, *1097*, 012025. [\[CrossRef\]](#)
86. Gopinath, S.C.B.; Anbu, P.; Arshad, M.K.M.; Lakshmipriya, T.; Voon, C.H.; Hashim, U.; Chinni, S.V. Biotechnological processes in microbial amylase production. *Biomed. Res. Int.* **2017**, *2017*, 1272193. [\[CrossRef\]](#)
87. Huang, X.; Schuppan, D.; Tovar, L.E.R.; Zevallos, V.F.; Loonen, J.; Gänzle, M. Sourdough fermentation degrades wheat alpha-amylase/trypsin inhibitor (ATI) and reduces pro-inflammatory activity. *Foods* **2020**, *9*, 943. [\[CrossRef\]](#)
88. Zotta, T.; Piraino, P.; Ricciardi, A.; McSweeney, P.L.H.; Parente, E. Proteolysis in model sourdough fermentations. *J. Agric. Food Chem.* **2006**, *54*, 2567–2574. [\[CrossRef\]](#)
89. Rizzello, C.G.; De Angelis, M.; Coda, R.; Gobbetti, M. Use of selected sourdough lactic acid bacteria to hydrolyze wheat and rye proteins responsible for cereal allergy. *Eur. Food Res. Technol.* **2006**, *223*, 405–411. [\[CrossRef\]](#)
90. Pérez-Alvarado, O.; Zepeda-Hernández, A.; García-Amezquita, L.E.; Requena, T.; Vinderola, G.; García-Cayuela, T. Role of lactic acid bacteria and yeasts in sourdough fermentation during breadmaking: Evaluation of postbiotic-like components and health benefits. *Front. Microbiol.* **2022**, *13*, 969460. [\[CrossRef\]](#) [\[PubMed\]](#)
91. Nutter, J.; Saiz, A.I.; Iurlina, M.O. Microstructural and conformational changes of gluten proteins in wheat-rye sourdough. *J. Cereal Sci.* **2019**, *87*, 91–97. [\[CrossRef\]](#)
92. Nouska, C.; Hatzikamari, M.; Matsakidou, A.; Biliaderis, C.G.; Lazaridou, A. Enhancement of textural and sensory characteristics of wheat bread using a chickpea sourdough fermented with a selected autochthonous microorganism. *Foods* **2023**, *12*, 3112. [\[CrossRef\]](#)
93. Ebrahimi, M.; Sadeghi, A.; Mortazavi, S.A. The use of cyclic dipeptide producing LAB with potent anti-aflatoxigenic capability to improve techno-functional properties of clean-label bread. *Ann. Microbiol.* **2020**, *70*, 24. [\[CrossRef\]](#)
94. Muskovics, G.; Farkas, A.; Bugyi, Z.; Tömösközi, S. Changes of gluten protein composition during sourdough fermentation in rye flour. *Cereal Chem.* **2024**, *101*, e10837. [\[CrossRef\]](#)
95. Hernández-Figueroa, R.H.; Mani-López, E.; Palou, E.; López-Malo, A. Sourdoughs as natural enhancers of bread quality and shelf life: A review. *Fermentation* **2024**, *10*, 7. [\[CrossRef\]](#)
96. Syrokou, M.K.; Tziompra, S.; Psychogiou, E.E.; Mpisti, S.D.; Paramithiotis, S.; Bosnea, L.; Mataragas, M.; Skandamis, P.N.; Drosinos, E.H. Technological and safety attributes of lactic acid bacteria and yeasts isolated from spontaneously fermented Greek wheat sourdoughs. *Microorganisms* **2021**, *9*, 671. [\[CrossRef\]](#)
97. Prasev, I.; Denkova-Kostova, R.; Koleva, A.; Denkova, Z.; Goranov, B.; Dobrev, I.; Kostov, G. Examination of some technological properties of lactic acid bacteria of the genera *Lactiplantibacillus* and *Levilactobacillus* isolated from spontaneously fermented sourdough. Part 1: Enzymatic profile. *BIO Web Conf.* **2025**, *70*, 02003. [\[CrossRef\]](#)
98. Sukmawati, D.; Al Husna, S.N.; Saszieta, D.; Priskaningrum, A.M.; Mutiani, A.M.; Sari, A.B.T.; El-Enshasy, H.A. Screening and isolation of indigenous cellulolytic and proteolytic yeasts from fermented dried cocoa beans. *AIP Conf. Proc.* **2024**, *2896*, 050019. [\[CrossRef\]](#)
99. Rodarte, M.P.; Dias, D.R.; Vilela, D.M.; Schwan, R.F. Atividade proteolítica de bactérias, leveduras e fungos filamentosos presentes em grãos de café (*Coffea arabica* L.). *Acta Sci. Agron.* **2011**, *33*, 457–464. [\[CrossRef\]](#)
100. Muzaifa, M.; Nilda, C.; Rahmi, F. Identification of proteolytic and pectinolytic yeasts from civet (*Paradoxorus hermaphroditus*) and evaluation of their potential to modify coffee bean quality. *J. Agric. Sci. Technol.* **2023**, *13*, 85–95. [\[CrossRef\]](#)
101. Mohammadi-Kouchesfahani, M.; Hamidi-Esfahani, Z.; Azizi, M.H. Isolation and identification of lactic acid bacteria with phytase activity from sourdough. *Food Sci. Nutr.* **2019**, *7*, 3700–3708. [\[CrossRef\]](#)
102. Palla, M.; Blandino, M.; Grassi, A.; Giordano, D.; Sgherri, C.; Quartacci, M.F.; Reyneri, A.; Agnolucci, M.; Giovannetti, M. Characterization and selection of functional yeast strains during sourdough fermentation of different cereal wholegrain flours. *Sci. Rep.* **2020**, *10*, 14629. [\[CrossRef\]](#)
103. Fekri, A.; Torbati, M.; Yari Khosrowshahi, A.; Bagherpour Shamloo, H.; Azadmard-Damirchi, S. Functional effects of phytate-degrading, probiotic lactic acid bacteria and yeast strains isolated from Iranian traditional sourdough on the technological and nutritional properties of whole wheat bread. *Food Chem.* **2020**, *306*, 125620. [\[CrossRef\]](#)

104. Rizzello, C.G.; Coda, R.; Gobbetti, M. Use of sourdough fermentation and nonwheat flours for enhancing nutritional and healthy properties of wheat-based foods. In *Flour and Breads and Their Fortification in Health and Disease Prevention*, 2nd ed.; Elsevier Inc.: London, UK, 2017; pp. 219–228. ISBN 9780128023099. [\[CrossRef\]](#)
105. Gobbetti, M.; De Angelis, M.; Di Cagno, R.; Calasso, M.; Archetti, G.; Rizzello, C.G. Novel insights on the functional/nutritional features of the sourdough fermentation. *Int. J. Food Microbiol.* **2019**, *302*, 103–113. [\[CrossRef\]](#)
106. Sergeeva, A.; Novichenko, A.; Ivanova, V.; Gurev, S.; Korovyansky, V. Evaluation of breadmaking potential of the yeasts *Wickerhamomyces anomalus* (CBS S605T) and *Torulaspora delbrueckii* (YIT3). *E3S Web of Conf.* **2024**, *539*, 02033. [\[CrossRef\]](#)
107. Walker, G.M. *Pichia anomala*: Cell physiology and biotechnology relative to other yeasts. *Antonie Van Leeuwenhoek* **2011**, *99*, 25–34. [\[CrossRef\]](#)
108. Schwentke, J.; Sabel, A.; Petri, A.; König, H.; Claus, H. The yeast *Wickerhamomyces anomalus* AS1 secretes a multifunctional exo- β -1,3-glucanase with implications for winemaking. *Yeast* **2014**, *31*, 349–359. [\[CrossRef\]](#)
109. López-Enríquez, L.; Vila-Crespo, J.; Rodríguez-Nogales, J.M.; Fernández-Fernández, E.; Ruipérez, V. Modulation of the aromatic profile of Verdejo wine through sequential inoculation of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae*. *Fermentation* **2023**, *9*, 977. [\[CrossRef\]](#)
110. Sánchez-Adriá, I.E.; Sanmartín, G.; Prieto, J.A.; Estruch, F.; Fortis, E.; Randez-Gil, F. Technological and acid stress performance of yeast isolates from industrial sourdough. *LWT* **2023**, *184*, 114957. [\[CrossRef\]](#)
111. Woo, S.H.; Park, J.; Sung, J.M.; Choi, E.J.; Choi, Y.S.; Park, J.D. Characterization of lactic acid bacteria and yeast from grains as starter cultures for gluten-free sourdough. *Foods* **2023**, *12*, 4367. [\[CrossRef\]](#)
112. Ferraz, R.; Flores, S.H.; Frazzon, J.; Thys, R.C.S. The effect of co-fermentation on sourdough breadmaking using different viable cell concentrations of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* as starter cultures. *J. Culin. Sci. Technol.* **2021**, *19*, 1–17. [\[CrossRef\]](#)
113. Romanens, E.; Freimüller Leischfeld, S.; Volland, A.; Stevens, M.; Krähenmann, U.; Isele, D.; Fischer, B.; Meile, L.; Miescher Schwenninger, S. Screening of lactic acid bacteria and yeast strains to select adapted anti-fungal co-cultures for cocoa bean fermentation. *Int. J. Food Microbiol.* **2019**, *290*, 262–272. [\[CrossRef\]](#)
114. Samagaci, L.; Ouattara, H.G.; Goualié, B.G.; Niamke, S.L. Growth capacity of yeasts potential starter strains under cocoa fermentation stress conditions in Ivory Coast. *Emir. J. Food Agric.* **2014**, *26*, 861–870. [\[CrossRef\]](#)
115. Pereira, G.V.M.; Miguel, M.G.C.P.; Ramos, C.L.; Schwan, R.F. Microbiological and physicochemical characterization of small-scale cocoa fermentations and screening of yeast and bacterial strains to develop a defined starter culture. *Appl. Environ. Microbiol.* **2012**, *78*, 5395–5405. [\[CrossRef\]](#)
116. Elhariry, H.M.; Mahmoud, R.M.; Hassan, A.A.; Aly, M.A. Development of co-culture sourdough systems for improving bread quality and delaying staling. *Food Biotechnol.* **2011**, *25*, 252–272. [\[CrossRef\]](#)
117. Coda, R.; Di Cagno, R.; Gobbetti, M.; Rizzello, C.G. Sourdough lactic acid bacteria: Exploration of non-wheat cereal-based fermentation. *Food Microbiol.* **2014**, *37*, 51–58. [\[CrossRef\]](#)
118. De Angelis, M.; Gobbetti, M. Environmental stress responses in *Lactobacillus*: A review. *Proteomics* **2004**, *4*, 106–122. [\[CrossRef\]](#)
119. Gänzle, M.; Gobbetti, M. Physiology and biochemistry of lactic acid bacteria. In *Handbook on Sourdough Biotechnology*; Springer: Boston, MA, USA, 2013; pp. 183–216. ISBN 9781461454250. [\[CrossRef\]](#)
120. Wu, Y.; Yu, M.; Li, Y.; Zhu, S.; Huang, D. The synergistic effect of lactic acid bacteria fermentation combined with gluten on the starch digestive properties of highland barley. *Food Biosci.* **2024**, *58*, 103822. [\[CrossRef\]](#)
121. Settanni, L.; Ventimiglia, G.; Alfonzo, A.; Corona, O.; Miceli, A.; Moschetti, G. An integrated technological approach to the selection of lactic acid bacteria of flour origin for sourdough production. *Food Res. Int.* **2013**, *54*, 1569–1578. [\[CrossRef\]](#)
122. Wang, S.; Copeland, L. Effect of acid hydrolysis on starch structure and functionality: A review. *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 1081–1097. [\[CrossRef\]](#)
123. Zhang, M.; Jia, H.; Wang, B.; Ma, C.; He, F.; Fan, Q.; Liu, W. A prospective review on the research progress of citric acid modified starch. *Foods* **2023**, *12*, 458. [\[CrossRef\]](#)
124. Ulbrich, M.; Natan, C.; Flöter, E. Acid modification of wheat, potato, and pea starch applying gentle conditions—Impacts on starch properties. *Starch/Stärke* **2014**, *66*, 903–913. [\[CrossRef\]](#)
125. Shao, D.; Zhang, J.; Shao, T.; Li, Y.; He, H.; Wang, Y.; Ma, J.; Cao, R.; Li, A.; Du, X. Modification of structure, pasting, and in vitro digestion properties of glutinous rice starch by different lactic acid bacteria fermentation. *Foods* **2025**, *14*, 367. [\[CrossRef\]](#)
126. Mou, T.; Xu, R.; Li, Q.; Li, J.; Liu, S.; Ao, X.; Chen, S.; Liu, A. Screening of antifungal lactic acid bacteria and their impact on the quality and shelf life of rye bran sourdough bread. *Foods* **2025**, *14*, 1253. [\[CrossRef\]](#)
127. Li, M.; Zhang, H.; Lyu, L.; Deng, Q.; Fan, H.; Xu, X.; Xu, D. Effects of sourdough on bread staling rate: From the perspective of starch retrogradation and gluten depolymerization. *Food Biosci.* **2024**, *59*, 103877. [\[CrossRef\]](#)
128. Zhang, T.; Hong, S.; Zhang, J.R.; Liu, P.H.; Li, S.; Wen, Z.; Xiao, J.; Zhang, G.; Habimana, O.; Shah, N.P.; et al. The effect of lactic acid bacteria fermentation on physicochemical properties of starch from fermented proso millet flour. *Food Chem.* **2024**, *437*, 137764. [\[CrossRef\]](#)

129. Ventimiglia, G.; Alfonzo, A.; Galluzzo, P.; Corona, O.; Francesca, N.; Caracappa, S.; Moschetti, G.; Settanni, L. Codominance of *Lactobacillus plantarum* and obligate heterofermentative lactic acid bacteria during sourdough fermentation. *Food Microbiol.* **2015**, *51*, 57–68. [\[CrossRef\]](#)
130. Di Monaco, R.; Torrieri, E.; Pepe, O.; Masi, P.; Cavella, S. Effect of sourdough with exopolysaccharide (EPS)-producing lactic acid bacteria (LAB) on sensory quality of bread during shelf life. *Food Bioprocess Technol.* **2015**, *8*, 691–701. [\[CrossRef\]](#)
131. Galle, S.; Arendt, E.K. Exopolysaccharides from sourdough lactic acid bacteria. *Crit. Rev. Food Sci. Nutr.* **2014**, *54*, 891–901. [\[CrossRef\]](#)
132. Milanovic, V.; Osimani, A.; Garofalo, C.; Belleggia, L.; Maoloni, A.; Cardinali, F.; Mozzon, M.; Foligni, R.; Aquilanti, L.; Clementi, F. Selection of cereal-sourced lactic acid bacteria as candidate starters for the baking industry. *PLoS ONE* **2020**, *15*, e0236190. [\[CrossRef\]](#)
133. Tieking, M.; Korakli, M.; Ehrmann, M.A.; Gänzle, M.G.; Vogel, R.F. In situ production of exopolysaccharides during sourdough fermentation by cereal and intestinal isolates of lactic acid bacteria. *Appl. Environ. Microbiol.* **2003**, *69*, 945–952. [\[CrossRef\]](#)
134. Valerio, F.; Bavaro, A.R.; Di Biase, M.; Lonigro, S.L.; Logrieco, A.F.; Lavermicocca, P. Effect of amaranth and quinoa flours on exopolysaccharide production and protein profile of liquid sourdough fermented by *Weissella cibaria* and *Lactobacillus plantarum*. *Front. Microbiol.* **2020**, *11*, 967. [\[CrossRef\]](#)
135. Sha, H.Y.; Wang, Q.Q.; Li, Z.J. Comparison of the effect of exopolysaccharide-producing lactic acid bacteria from sourdough on dough characteristics and steamed bread quality. *Int. J. Food Sci. Technol.* **2023**, *58*, 378–386. [\[CrossRef\]](#)
136. Dertli, E.; Mercan, E.; Arici, M.; Yilmaz, M.T.; Sağdıç, O. Characterisation of lactic acid bacteria from Turkish sourdough and determination of their exopolysaccharide (EPS) production characteristics. *LWT* **2016**, *71*, 116–124. [\[CrossRef\]](#)
137. Xu, D.; Hu, Y.; Wu, F.; Jin, Y.; Xu, X.; Gänzle, M.G. Comparison of the functionality of exopolysaccharides produced by sourdough lactic acid bacteria in bread and steamed bread. *J. Agric. Food Chem.* **2020**, *68*, 8907–8914. [\[CrossRef\]](#)
138. Agbo, B.E.; Edeghor, U.; Lennox, J.; Aminadokiari, D. Bread fermentation using synergistic activity between lactic acid bacteria (*Lactobacillus bulgaricus*) and baker's yeast (*Saccharomyces cerevisiae*). *Int. J. Curr. Microbiol. App. Sci.* **2016**, *5*, 26–34.
139. Wang, Z.; Yan, J.; Ma, S.; Tian, X.; Sun, B.; Huang, J.; Li, L.; Wang, X.; Bao, Q. Effect of wheat bran dietary fiber on structural properties of wheat starch after synergistic fermentation of *Lactobacillus plantarum* and *Saccharomyces cerevisiae*. *Int. J. Biol. Macromol.* **2021**, *190*, 86–92. [\[CrossRef\]](#)
140. Ponomarova, O.; Gabrielli, N.; Sévin, D.C.; Müllender, M.; Zirngibl, K.; Bulyha, K.; Andrejev, S.; Kafkia, E.; Typas, A.; Sauer, U.; et al. Yeast creates a niche for symbiotic lactic acid bacteria through nitrogen overflow. *Cell Syst.* **2017**, *5*, 345–357.e6. [\[CrossRef\]](#)
141. Canon, F.; Nidelet, T.; Guédon, E.; Thierry, A.; Gagnaire, V. Understanding the mechanisms of positive microbial interactions that benefit lactic acid bacteria co-cultures. *Front. Microbiol.* **2020**, *11*, 575580. [\[CrossRef\]](#)
142. Sun, X.; Wu, S.; Li, W.; Koksel, F.; Du, Y.; Sun, L.; Fang, Y.; Hu, Q.; Pei, F. The effects of cooperative fermentation by yeast and lactic acid bacteria on the dough rheology, retention and stabilization of gas cells in a whole wheat flour dough system—A review. *Food Hydrocoll.* **2023**, *135*, 108155. [\[CrossRef\]](#)
143. Carbonetto, B.; Nidelet, T.; Guezenc, S.; Perez, M.; Segond, D.; Sicard, D. Interactions between *Kazachstania humilis* yeast species and lactic acid bacteria in sourdough. *Microorganisms* **2020**, *8*, 240. [\[CrossRef\]](#)
144. Zghal, M.C.; Scanlon, M.G.; Sapirstein, H.D. Prediction of bread crumb density by digital image analysis. *Cereal Chem.* **1999**, *76*, 734–742. [\[CrossRef\]](#)
145. Shibata, M.; Tsuta, M.; Sugiyama, J.; Fujita, K.; Kokawa, M.; Araki, T.; Nabetani, H. Image analysis of bread crumb structure in relation to mechanical properties. *Int. J. Food Eng.* **2013**, *9*, 115–120. [\[CrossRef\]](#)
146. Onipe, O.O.; Beswa, D.; Jideani, A.I.O. Confocal laser scanning microscopy and image analysis for elucidating crumb and crust microstructure of bran-enriched South African fried dough and batter. *Foods* **2020**, *9*, 605. [\[CrossRef\]](#)
147. Peñaloza-Espinosa, J.; De, G.J.; Rosa-Angulo, L.; Mora-Escobedo, R.; Chanona-Pérez, J.; Farrera-Rebollo, R.; Calderón-Domínguez, G. Sourdough and bread properties as affected by soybean protein addition. In *Soybean—Applications and Technology*; Ng, T.-B., Ed.; IntechOpen: London, UK, 2011. [\[CrossRef\]](#)
148. Dos Santos, J.G.; de Ávila, P.M.; Borges Gomes, R.A.; de Souza Monteiro, R.; Vieira, S.M.; Pimenta Pereira, P.A. Use of natural yogurt, pineapple juice, and beer wort as starter cultures in sourdough combined with white and whole wheat flours to improve the technological characteristics of milk breads. *MOJ Food Process. Technol.* **2023**, *11*, 9–14. [\[CrossRef\]](#)
149. Scheuer, P.M.; Southgate, A.N.N.; Martelli, M.F.; Dias, C.; da Silva, M.E.; Coelho, A.A.; de Francisco, A. Quality properties of a bread made with levain and cocoa waste. *J. Culin. Sci. Technol.* **2022**, *20*, 409–420. [\[CrossRef\]](#)
150. Hu, Y.; Zhang, J.; Wang, S.; Liu, Y.; Li, L.; Gao, M. Lactic acid bacteria synergistic fermentation affects the flavor and texture of bread. *J. Food Sci.* **2022**, *87*, 1823–1836. [\[CrossRef\]](#)

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