ISBN: 978-628-96613-3-0. ISSN: 2414-6390. Digital Object Identifier: https://dx.doi.org/10.18687/LACCEI2026.1.1.1532

Peptide-Rich Fermentates from Surplus Bread: Screening for ACE/α-Glucosidase Inhibition, Antioxidant Capacity and Antimicrobial Activity

Benjamín Vázquez-Rodríguez[1]; David A. Perez-Perez[2], Erick Heredia-Olea[3]
[1] Institute for Obesity Research, Tec de Monterrey, bv@tec.mx
[2,3] School of Engineering and Sciences, Tec de Monterrey, davidantonio.perez@tec.mx, erickho@tec.mx

Abstract*– This study evaluates the valorization of surplus bread as a substrate for microbial production of bioactive peptides with antidiabetic, antioxidant, and antimicrobial properties. Surplus bread was enzymatically hydrolyzed (alcalase, α-amylase, amyloglucosidase) and fermented with a collection of 35 isolates (20 yeasts, 15 lactic acid bacteria) obtained from sourdough, lyophilized sourdough, kefir and commercial probiotics. Fermentates were characterized for peptide content (α-amino nitrogen), enzyme inhibition (ACE, α-glucosidase), antioxidant capacity (DPPH, FRAP) and bacteriostatic activity (agar well diffusion against S. aureus and E. coli). ANOVA indicated a significant effect of strain on peptide release (R^2 = 87.7%, adj. R^2 = 76.2%), and strain and peptide concentration significantly influenced antioxidant assays (DPPH $R^2 \approx 97.6\%$, FRAP $R^2 \approx 92.6\%$). Fermentation increased peptide levels 1.5–4.8-fold relative to hydrolysates. Several LAB (L_1, L_2, L_{11}, L_{12}) consistently produced 60–70% ACE and α-glucosidase inhibition, while several yeast isolates (Y_{11}, Y_{19}, Y_{20}) achieved the highest ACE inhibition (up to >90%). Antioxidant capacity reached ~40–48% in top-performing strains. Antimicrobial assays revealed clear inhibition halos against S. aureus for specific LAB (L_1, L_3, L_{11}, L_{13}) but no activity against E. coli, indicating selective Gram-positive antagonism. These results demonstrate that surplus bread can be converted into multifunctional peptide-rich fermentates with potent antidiabetic and antioxidant effects and targeted antimicrobial activity. The work establishes a strain repository and supports further proteomic and structural characterization to optimize peptide yields and bioactivity. Valorization of surplus bread aligns with circular bioeconomy and SDG objectives by reducing food waste while generating value-added functional ingredients with translational potential

Keywords -- Surplus bread; bioactive peptides; fermentation; ACE inhibition; antioxidant capacity

I. Introduction

Food-surplus valorization has become a critical global priority: nearly 1.3 billion tonnes of food are wasted annually, and surplus bread represents a protein-rich residue that remains underexploited despite its potential as a substrate for biotechnological innovation. Recent work has shown that food residues such as brewer's spent grain (BSG), maize and bread can be transformed by fermentation into value-added products (biofuels, polymers, organic acids, pigments) that align with the United Nations Sustainable Development Goals for responsible consumption, health and climate action [1]. However, the potential of surplus bread specifically as a feedstock for producing multifunctional bioactive peptides (BPs) has been insufficiently explored, creating a clear knowledge gap in substrate suitability, strain performance and comparative bioactivity profiles

Bioactive peptides are short, sequence-defined amino-acid fragments released from parent proteins primarily by enzymatic hydrolysis or microbial proteolysis during fermentation. Lactic acid bacteria (LAB) and yeasts secrete proteases that hydrolyse dietary proteins into smaller fragments with diverse biological functions [2]. BPs have attracted attention for applications spanning antihypertensive and antidiabetic therapies to antimicrobial and antioxidant agents; their mechanisms include inhibition of metabolic enzymes [3], modulation of oxidative stress [4] and disruption of microbial membranes [5], [6]. Structurally, many BPs are 2–20 residues long and often contain hydrophobic and basic residues that confer activity and, in some cases, resistance to digestive peptidases.

Beyond sustainability, producing BPs from surplus bread carries important public-health relevance. Fermentation-derived antimicrobial peptides act via membrane disruption and, in some cases, interference with nucleic acid or protein synthesis [7], offering alternative modes of action against multidrug-resistant pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [8]. Concurrently, BPs generated during fermentation can inhibit clinically relevant enzymes (ACE, DPP-IV, α-amylase, α-glucosidase), providing biochemical routes to modulate blood pressure and glycaemic control when formulated as functional ingredients or nutraceuticals [9]. These dual antimicrobial and metabolic activities, together with food-grade provenance, biodegradability and compatibility with food matrices, make surplus-bread fermentates attractive for preventive and adjunctive applications within a circular-economy framework.

Key challenges remain (proteolytic instability, scalable production and purification, and the need for rigorous in vivo and clinical validation) but advances in strain selection, controlled fermentation, encapsulation and downstream processing can mitigate these barriers [9]. Addressing the identified gap, this study aims to valorize surplus bread via enzymatic hydrolysis followed by controlled fermentation with selected yeasts and LAB, systematically screening peptide yield and functional endpoints (ACE and α-glucosidase inhibition, DPPH and FRAP antioxidant capacity, and antimicrobial

activity) to identify high-performing fermentates and inform downstream optimization for translational development.

II. MATERIALS AND METHODS

A. Biological material

For the development of this project, surplus bread was supplied by the company BIMBO, collected from batches approaching the end of their shelf life. The material was manually inspected to remove impurities and discard pieces showing visible damage or contamination. Afterwards, the bread was ground and stored in sterile bags at –20 °C until its use in enzymatic hydrolysis and fermentation experiments.

B. Isolation of Probiotic Microorganisms

Samples were collected from kefir grains, sourdough starters, and commercial probiotic concentrates. Serial dilutions were prepared in sterile distilled water and plated on selective media: MRS agar for LAB, YM agar for yeasts, and PDA for molds. Plates were incubated at 37°C for bacteria and 25–30°C for fungi. Colonies were selected based on morphology, purified by repeated streaking, and preserved in glycerol stocks at –80°C. Negative controls (sterile media) and positive controls (reference strains) were included to validate isolation procedures.

C. Enzymatic Hydrolysis of Surplus Bread

Prior to fermenting the surplus bread with probiotic organisms, the material was ground and subjected to enzymatic pretreatment using alcalase (100 µL g^{-1}, 60 min, 60 °C, pH 8.0), α-amylase (17.5 µL g^{-1}, 20 min, 95 °C, pH 6.0), and amyloglucosidase (3.03 µL g^{-1}, 30 min, 60 °C, pH 4.5). After enzymatic hydrolysis, the pH was adjusted to 6.5 using 1 M NaOH, followed by a thermal treatment for enzyme inactivation. Hydrolysates were incubated for 35 min at 90 °C and subsequently cooled for 30 min in an ice bath. The hydrolysates were then centrifuged at 10,000 rpm for 10 min, transferred into sterile flasks, and refrigerated for subsequent fermentation. Sterile aliquots of 3 mL were taken to determine total protein content using the BCA method and peptide concentration through α-amino nitrogen quantification.

D. Fermentation Process

Hydrolysates were inoculated with a cell concentration of 6–7 Log CFU g^{-1}. Fermentation with LAB was carried out at 37 °C for 24 h at 150 rpm, while yeast fermentations were conducted at 25 °C for 48 h at 150 rpm.

E. Enzyme Inhibition Assays: α-Glucosidase and ACE

The α-glucosidase inhibition assay was performed using different extract concentrations (200, 400, 600, 800, 1,000, and 1,200 µg/mL). Rat intestinal acetone powder (RIAP) was suspended in distilled water (10 mg/mL) and centrifuged for 10 min at 10,000 rpm. The assay buffer was HEPES 100 mM at pH 6.8, and the substrate was 4-nitrophenyl-α-D-glucopyranoside (2 mM). The assay components were added to 96-well microplates in the following order: 100 µL of enzyme solution (10 mg RIAP per 1 mL distilled water), 50 µL of acarbose (positive control), seaweed extract, or buffer, and finally 50 µL of substrate. Samples were incubated at 25 °C for 2 h. Absorbance was recorded at 405 nm at the beginning and end of incubation, α-glucucosidase inhibitory activity was calculated as inhibition percentage using Eq. 1

$$\%\, I_{\alpha-\text{Glucosidase}} = \frac{(A_{(BLK)}) - (A_{sample} - A_{(-CTRL)})}{(A_{(BLK)})} x\ 100 \quad (1)$$

Where: $A_{(BLNK)}$: the absorbance of the control blank sample, $A_{(sample)}$: the absorbance of the sample with enzyme and $A_{(-CTRL)}$: the absorbances of the sample without enzyme

For the angiotensin-converting enzyme (ACE) inhibition assay, 50 µL of sample (10 µg/mg) was added to a 2 mL Eppendorf tube, followed by 40 µL of hippuryl-His-Leu (HHL, Sigma-Aldrich, St. Louis, MO, USA). The mixture was pre-incubated at 37 °C for 5 min. Subsequently, 10 µL of ACE enzyme (rabbit lung, Sigma-Aldrich) was added and incubated for 30 min at 37 °C. After incubation, 120 µL of hydrochloric acid (HCl) was added, followed by 1 mL of ethyl acetate. Samples were centrifuged at 3,000 × g for 5 min, and 800 µL of the ethyl acetate phase was recovered, transferred to a new tube, and evaporated using a Genevac EZ-2 Plus evaporator (SP Scientific, PA, USA) for 30 min. The residue was resuspended in 200 µL of ultrapure water, and hippuric acid release was quantified at 228 nm, using a microplate reader, ACE inhibitory activity was calculated as inhibition percentage using Eq. 2.

$$\%\, I_{\text{ACE}} = \frac{(A_{(+CTRL)} - A_{(BLK)}) - (A_{sample} - A_{(-CTRL)})}{(A_{(+CTRL)} - A_{(BLK)})} \times 100 \quad (2)$$

Where: $A_{(+CTRL)}$: the absorbance of the positive control, $A_{(BLNK)}$: the absorbance of the blank sample, $A_{(sample)}$: the absorbance of the sample and $A_{(+CTRL)}$: the absorbances of the negative control.

F. Antioxidant capacity assays: DPPH and FRAP

The radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, MA, USA) was adapted from the method reported by Singh and Vij (2018). A volume of 50 µL of standardized sample, prepared across a concentration range of 100 to 1,000 ppm BSA, from each replicate of LAB and yeast fermentates, was mixed with 50 µL of 60 µM DPPH solution (methanol as solvent). The mixture was dispensed in triplicate into a 96-well microplate, with distilled water serving as the blank. Plates were incubated for 1 h at 37 °C in the absence of light. Absorbance was measured at 515 nm using a microplate reader (Synergy HT KC4, BioTek, VT, USA), radical scavenging capacity was calculated as DPPH inhibition percentage using Eq. 3

$$\%DPPH_{Inhibition} = \frac{A_{(BLK)} - A_{sample}}{A_{(BLK)}} x\ 100 \quad (3)$$

Where: $A_{(BLNK)}$: the absorbance of the blank sample, $A_{(sample)}$: the absorbance of the sample.

In a 96-well microplate, 25 µL of sample was mixed with 100 µL of aqueous $FeSO_4$ (75 µM) per well and incubated for 10 min at room temperature. Subsequently, 100 µL of aqueous ferrozine (500 µM) was added. Absorbance was measured at 560 nm using MQ water as blank and radical scavenging capacity was calculated as FRAP inhibition percentage using Eq. 4

$$\%FRAP_{Inhibition} = \frac{A_{(BLK)} - A_{sample}}{A_{(BLK)}} x\ 100 \quad (4)$$

Where: $A_{(BLNK)}$: the absorbance of the blank sample, $A_{(sample)}$: the absorbance of the sample.

G. Inhibition Halos in Pathogenic Bacteria

Growth inhibition assays against pathogenic bacteria were performed using *Escherichia coli* and *Staphylococcus aureus* as primary candidates. The bacteria were inoculated in Muller-Hinton medium, and each Petri dish was divided into four sectors. In each sector, a sterile punch was used to remove a disc of approximately 50 mm in diameter, creating wells where 50 µL of fermentate and a control (kanamycin) were added. The Petri dishes were incubated for 24 h, after which bacterial growth and/or inhibition of growth were evaluated.

H. Statistical analysis

DPPH and FRAP data were analyzed using multifactorial ANOVA in a two-way factorial design (factor A: Strain; factor B: peptide concentration) to assess main effects and interaction, Peptide synthesis was evaluated by one-way ANOVA for strain effects The significance threshold was set at $p \leq 0.05$. All statistical analysis were performed using Minitab 22.4.

III. RESULTS AND DISCUSSION

A. Isolation of Probiotic Microorganisms

Table 1 summarizes the obtained isolated microorganisms, a total of 20 yeast and 15 LAB strains were isolated from sourdough, lyophilized sourdough, dairy kefir, and commercial probiotics. Yeast isolates consistently formed small, round, cream-colored colonies, with oval to round cell morphologies observed under microscopy. LAB predominantly exhibited medium-sized, cream-colored colonies with bacillary morphology, except for one coccoid variant (L_{12}) isolated from lyophilized sourdough. The phenotypic consistency across sources suggests the dominance of well-adapted microbial populations with similar morphological traits, supporting their potential for reproducible fermentation performance.

TABLE 1
ISOLATION SOURCES OF YEAST AND LAB STRAINS

LAB Strain	Isolation source	Yeast Strain	Isolation source
L_1	Probiotic	Y_1	Sourdough
L_2	Probiotic	Y_2	Lyophilized sourdough
L_3	Sourdough	Y_3	Lactic kefir
L_4	Sourdough	Y_4	Lactic kefir
L_5	Lactic kefir	Y_5	Lactic kefir
L_6	Sourdough	Y_6	Sourdough
L_7	Lyophilized sourdough	Y_7	Sourdough
L_8	Lactic kefir	Y_8	Lyophilized sourdough
L_9	Sourdough	Y_9	Lactic kefir
L_{10}	Lyophilized sourdough	Y_{10}	Lactic kefir
L_{11}	Probiotic	Y_{11}	Lyophilized sourdough
L_{12}	Lyophilized sourdough	Y_{12}	Lyophilized sourdough
L_{13}	Lactic kefir	Y_{13}	Lyophilized sourdough
L_{14}	Lactic kefir	Y_{14}	Lyophilized sourdough
L_{15}	Lactic kefir	Y_{15}	Lyophilized sourdough
-	-	Y_{16}	Lyophilized sourdough
-	-	Y_{17}	Sourdough
-	-	Y_{18}	Sourdough
-	-	Y_{19}	Sourdough
-	-	Y_{20}	Lactic kefir

B. Enzymatic Hydrolysis of Surplus Bread and Probiotic fermentation

ANOVA analysis results indicated LAB strain has a significant effect on peptide concentration after fermentation ($p < 0.001$). The model had an R^2 of 87.71% and an adjusted R^2 of 75.24%. Fig 1 shows the concentration of peptides present in the bread hydrolysate and after fermentation. Probiotic organisms demonstrated the ability to increase the peptide content in the medium by a factor of 2 to 4.

Previous researches have quantified peptide release in soy yogurt using the OPA method, reporting values that increased from approximately 0.033–0.046 after two weeks to 0.054–0.067 by day 28, equivalent to a 60–70% increment in proteolysis during storage [10]. Their study employed commercial probiotic strains such as *Lactobacillus acidophilus* LAFTI® L10, *Bifidobacterium lactis* LAFTI® B94, and *Lactobacillus paracasei* LAFTI® L26, highlighting moderate peptide liberation in a soy milk substrate supplemented with lactose. In another study peptide concentrations where reported in surplus bread hydrolysates and fermented hydrolysates prior to purification, with values in the range of 0.8–1.2 mg/g in hydrolysates and increasing to approximately 2.0–2.8 mg/g

after fermentation with *Lactobacillus brevis* AM7, representing increments of about 150–200% [11].

In this study, peptide release with LAB (L_{11}, L_4, L_{10}, L_{12}) increased from 0.39–0.72 mg g^{-1} in hydrolysates to 1.5–1.85 mg g^{-1} after fermentation (1.5 – 3.71-fold increments), while yeast strains (Y_{12}–Y_{16}) increased from 0.37–0.71 mg g^{-1} to 0.7–1.8 mg g^{-1} (1.5–4.8-fold increments). These differences reflect the proteolytic potential from food source derived probiotic microorganisms for producing high value bioactive molecules derived from surplus food.

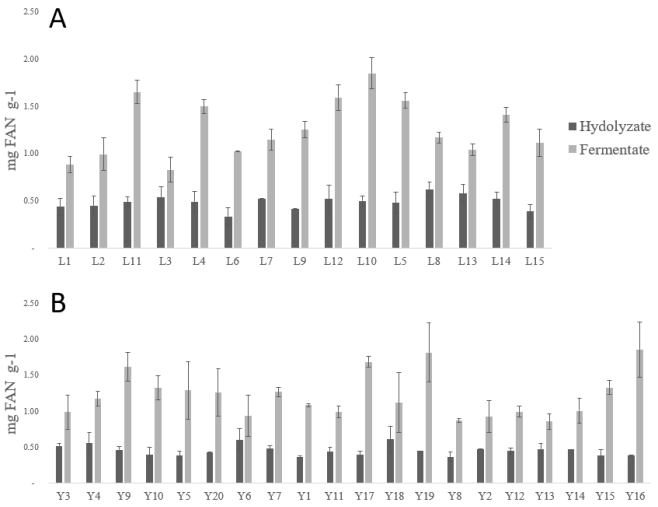

Fig 1. Free amino nitrogen (FAN) concentration (mg FAN g^{-1}) in bread hydrolysates and after fermentation with LAB (A) and yeast strains (B).

B. Enzyme Inhibition Assays: α-Glucosidase and ACE

The inhibition of enzymes involved with antidiabetic activities are shown on **Fig 2**. Fermentation significantly enhanced enzymatic inhibition compared to hydrolysates alone. Among LAB, strains L_1, L_2, L_{14}, and L_{10}, isolated from probiotic and lyophilized sourdough—showed the strongest dual inhibition, exceeding 70% against both ACE and α-glucosidase. Yeast strains Y_7, Y_{11}, Y_{20}, and Y_{19}, isolated from kefir and sourdough, also demonstrated potent activity, with inhibition levels above 60% for both enzymes, even close to 100% inhibition for ACE, for Y_{11} isolated from sourdough. These results suggest that selected strains can generate bioactive peptides with potential antihypertensive and antidiabetic effects

Another study [12] demonstrated that *Lactobacillus* spp. isolated from kefir grains collected in Bogor, Bandung, Jakarta, and Yogyakarta exhibited variable α-glucosidase inhibitory activity. The highest values were observed in *Lactobacillus rhamnosus* BD2 (73.6%) and *Lactobacillus kefiri* YK4 (64.3%) when cultured in MRS broth. However, when these isolates were transferred to reconstituted skim milk, inhibition decreased markedly to 25.7% and 36.2%, respectively, underscoring the influence of substrate composition on bioactivity. In comparison, bacterial isolates obtained from surplus bread hydrolysates, including strains L_1, L_3, L_4, L_{10}, L_{11}, and L_{12}, maintained consistently high inhibitory activities ranging from 65% to 80%. These isolates originated from diverse ecological sources such as sourdough (fresh stock, lyophilized), kefir, and commercial probiotic products. The results indicate that surplus bread fermentates provided a more favorable environment for sustained peptide release and enzymatic inhibition

In contrast, yeast strains in surplus bread fermentates demonstrated α-glucosidase inhibitory activities between 55% and 75% (Y_1, Y_3, Y_7, Y_{11}, and Y_{13}). The highest values were observed in strains Y_{11} and Y_{13}, which surpassed the inhibition levels reported for the kefir-derived bacterial isolates. These yeast strains originated from kefir and sourdough fermentates, reflecting the ecological diversity and importance of this microorganism in their respective complex communities. The inclusion of yeasts revealed an additional dimension of bioactivity absent in the kefir-based study, emphasizing their contribution to peptide liberation and metabolic modulation.

ACE-inhibitory activity has been observed in soy yogurt fermented with Lactobacillus acidophilus LAFTI® L10, Bifidobacterium lactis LAFTI® B94, *Lactobacillus paracasei* LAFTI® L26, together with yogurt starters *Lactobacillus delbrueckii* ssp. and *Streptococcus thermophilus* [10]. The inhibition values observed in this system ranged between 30–50%, reflecting moderate bioactivity under dairy fermentation conditions. In comparison, surplus bread hydrolysates fermented with LAB and yeast isolates demonstrated markedly higher inhibition levels. LAB strains such as L_1, L_2, L_{11}, and L_{12} consistently achieved 60–70% inhibition, while yeast strains Y_{12}–Y_{16} reached values up to 75%, representing the strongest inhibition among the tested fermentates. The contrast indicates that commercial probiotic strains in a soy milk substrate produced limited ACE-inhibitory activity, whereas diverse isolates from sourdough, kefir, and surplus food matrices generated substantially greater inhibition. Differences in microbial diversity and substrate composition appear to account for the higher peptide release and stronger inhibitory effects observed in the surplus bread fermentates compared to the soy yogurt system.

A distinctive outcome of this study is the remarkably high ACE inhibitory activity observed in yeast isolates. Strain Y_{11} exceeded 90% inhibition, while Y_{19} and Y_{20} reached approximately 75% (Figure 2), resulting in the most effective ACE inhibitors within the surplus bread fermentation experiments. These values are substantially greater than those reported in leterature [3], previously there was found maximum ACE inhibition of about 78.52–72.85% in bovine colostrum fermentation using *Candida lipolytica* MIUG D99 and *C. lipolytica* MIUG D6 in conjunction with kefir grains. The

higher ACE inhibition activity of yeast fermentates can be explained by previously highlighted mechanisms [13], where peptides generated during fermentation might interact directly with critical residues of the ACE active site, including His353, Ala354, Tyr523, and Val380, through hydrogen bonding, hydrophobic contacts, and π-stacking interactions. The study demonstrated that the potency of inhibition is strongly influenced by the number and stability of hydrogen bonds. By analogy, the elevated inhibition observed in this research yeast fermentates might be explained by the previously discussed isolated yeast proteolytic activity, resulting in peptide synthesis capable of forming multiple stabilizing interactions within the C-domain of ACE, resulting in inhibition levels that surpass those previously documented in conventional dairy and colostrum fermentations.

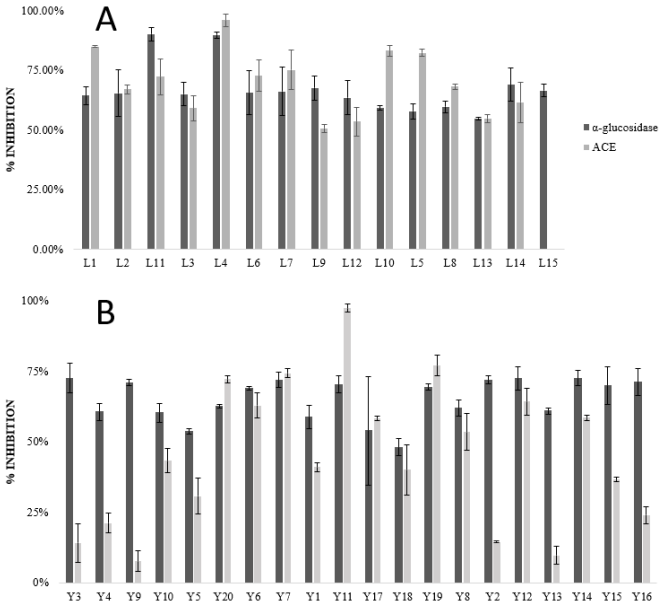

Fig. 2. Enzymatic inhibition activity of fermented bread samples was evaluated against angiotensin-converting enzyme (ACE) and α-glucosidase. The graph displays inhibition percentages for LAB (A) and yeast strains (B).

C. Antioxidant capacity assays: DPPH and FRAP

ANOVA analysis results indicated that both LAB strain and peptide concentration and their interaction significantly affected DPPH antioxidant capacity results ($p < 0.001$). The model had an R^2 of 97.56% and an adjusted R^2 of 95.15%. In DPPH assays, strains L_1, L_2, L_3, and L_9 exhibited the highest radical scavenging activity, reaching 40% at the highest concentration tested. These strains originated from probiotic and lyophilized sourdough sources, indicating strong antioxidant potential. Antioxidant activity assessed by DPPH inhibition varied notably between studies. Yusuf et al. (2021) reported values of 44.3% and 41.6% for *Lactobacillus kefiri* JK5 and JK17, respectively, isolated from kefir grains and evaluated in MRS broth [12]. Bacterial strains applied to the fermentation of surplus bread hydrolysates demonstrated inhibition values of similar magnitude, comparable to those reported for the best kefir-derived strains.

Yeast fermentates, evaluated through DPPH inhibition as a function of peptide concentration, revealed antioxidant activities ranging from 30% to nearly 40% at the highest peptide levels (1000 ppm). Strains Y_3, Y_5, Y_{11}, and Y_{14} showed the strongest responses, approaching or matching the activity observed in the top-performing bacterial strains. These yeasts were sourced from sourdough and kefir fermentates, and their performance highlights the relevance of non-bacterial isolates in antioxidant peptide generation.

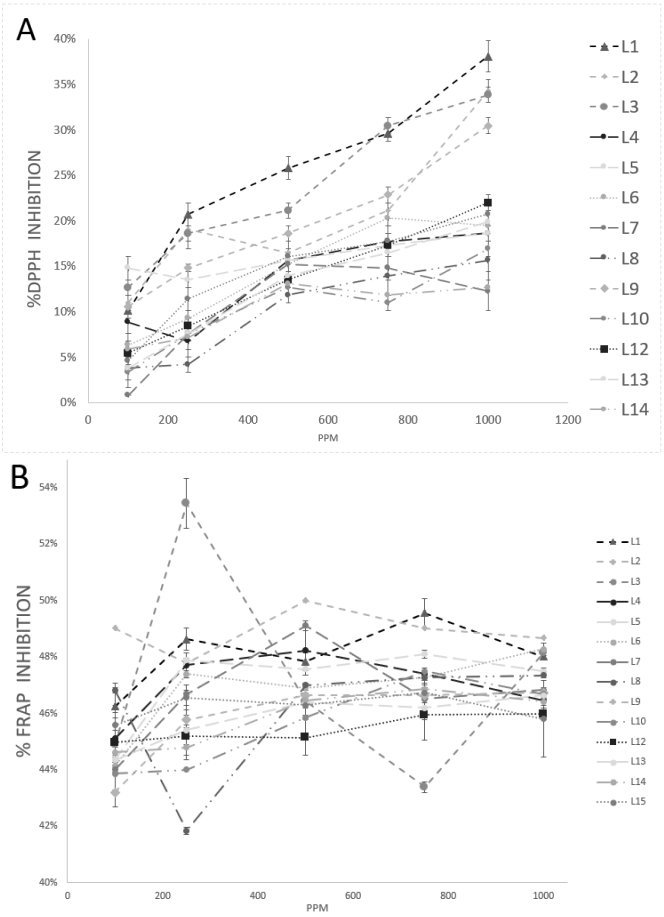

Fig. 3. Antioxidant capacity of LAB strains was assessed using DPPH (A) and FRAP (B) assays across increasing concentrations (PPM).

ANOVA analysis results indicated that both LAB strain and peptide concentration and their interaction significantly affected FRAP antioxidant capacity results ($p < 0.001$). The model had an R^2 of 92.6% and an adjusted R^2 of 85.31%. In the FRAP assay, %. L_1, L_2, L_3, and L_5 exhibited the highest radical scavenging activity, reaching at the highest concentration tested., ranging between 46 - 48% across all concentrations. The remaining strains showed moderate activity, with DPPH

values ranging from 20–35% and FRAP values between 45–46%, these differences between FRAP and DPPH results between the isolated strains and different concentrations tested might be explained by the different proteases produced by the LAB strains that hydrolyze different peptide bonds, generating a different antioxidant capacity profile for he given surplus substrate [2].

These results highlight the potential antioxidant capacity of selected strains under both assay conditions, supporting their potential application in oxidative stress modulation and functional food development.

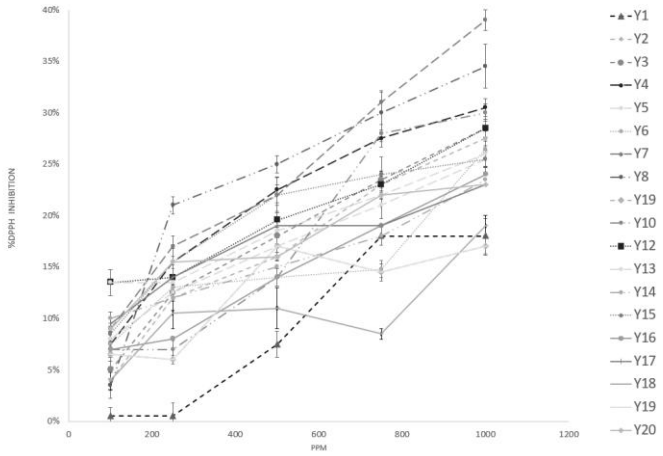

Fig. 4. Antioxidant capacity of yeast strains was assessed using DPPH

The ANOVA results showed that yeast strain, peptide concentration, and their interaction had a significant effect on DPPH inhibition ($p < 0.001$ for all factors). The model demonstrated an excellent fit, with an R^2 of 97.97% and an adjusted R^2 of 95.97%, confirming its reliability for evaluating antioxidant activity across treatments Most isolated yeast strains exhibited a dose-dependent increase in activity. Strains Y_4, Y_7, Y_4, Y_{12}, and Y_{18} showed the highest antioxidant capacity, surpassing 40% at the highest concentration tested. These fermentates exhibited more pronounced response curves compared to the rest, indicating superior radical scavenging potential. The remaining strains demonstrated moderate activity, with final values ranging between 20–35%. This variability reflects differences in yeast metabolic performance and peptide release during fermentation.

D. Inhibition Halos in Pathogenic Bacteria

LAB strains L_1, L_3, L_{11} and L_{13}, exhibited the most pronounced inhibition, forming clear halos indicative of strong antagonistic activity (Fig 5). These strains originated from probiotic, sourdough and lactic kefir sources, suggesting enhanced metabolite production with antimicrobial potential.

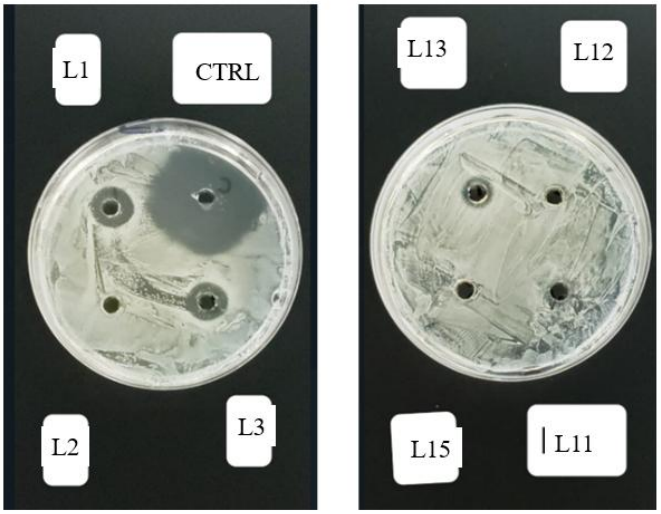

Fig. 5. Antimicrobial capacity of LAB strains against *Staphylococcus aureus*

In contrast, fermentates tested against *Escherichia* coli showed no inhibition zones, indicating a possible strain-specific activity limited to Gram-positive pathogens. These results support the selective antimicrobial ability of certain LAB, particularly against *Staphylococcus aureus*; regarding the bacteriostatic activity of bioactive peptides produced by lactic acid bacteria, previous studies have reported comparable results. Antibiotic peptides synthesized by *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, and their mixed cultures exhibited inhibitory effects not only against Gram-positive bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes*, but also against Gram-negative pathogens including Escherichia coli, *Pseudomonas aeruginosa*, and *Salmonella enterica* [14].

IV. CONCLUSIONS

The fermentation of a surplus food product nearing the end of its shelf life, such as packaged bread, was confirmed as a promising technology for the development of products with antidiabetic, antioxidant and antimicrobial bioactivity. This exploratory study, designed as a proof of concept, establishes the foundation for future research aimed at identifying the isolated strains and characterizing the peptic compound profiles synthetized by the probiotic cultures. A significant achievement of these experiments was the establishment of a strain collection comprising 35 probiotic microorganisms. An additional outcome of this study highlights the importance of employing probiotic organisms isolated from foods and commercial probiotic products. Their use not only ensures biological relevance and functional diversity but also strengthens the translational potential of the findings. Moreover, the valorization of surplus food materials, such as packaged bread nearing the end of its shelf life, directly connects this research to sustainability strategies by reducing waste and generating

value-added products. Notably, the enzymatic inhibition levels obtained in these assays were considerably high, underscoring the potential of these fermentates to contribute to the development of functional formulations with antidiabetic and antimicrobial applications.

ACKNOWLEDGMENT

The authors gratefully acknowledge the contribution, technical support and funding provided by the Centro de Biotecnología FEMSA, whose backing was instrumental for the experimental execution and logistical development of this study. This work is dedicated to the memory of Dr. Sergio O. Serna-Saldivar in recognition of his guidance, mentorship and seminal contributions to baking science, bioenergetics and cereal science; he also contributed to the conception of the project and served as an advisor, and his scientific leadership, rigorous scholarship and enduring mentorship continue to inform and inspire this research.

REFERENCES

[1] B. P. Singh, R. E. Aluko, S. Hati, and D. Solanki, "Bioactive peptides in the management of lifestyle-related diseases: Current trends and future perspectives," 2021, *Bellwether Publishing, Ltd.* doi: 10.1080/10408398.2021.1877109.

[2] S. Kayacan Çakmakoğlu *et al.*, "Production of bioactive peptides during yogurt fermentation, their extraction and functional characterization," *Food Biosci.*, vol. 61, Oct. 2024, doi: 10.1016/j.fbio.2024.104805.

[3] A. Gaspar-Pintiliescu *et al.*, "Angiotensin-converting enzyme inhibition, antioxidant activity and cytotoxicity of bioactive peptides from fermented bovine colostrum," *Int. J. Dairy Technol.*, vol. 73, no. 1, pp. 108–116, Feb. 2020, doi: 10.1111/1471-0307.12659.

[4] T. Bin Zou, T. P. He, H. Bin Li, H. W. Tang, and E. Q. Xia, "The structure-activity relationship of the antioxidant peptides from natural proteins," Jan. 01, 2016, *MDPI AG*. doi: 10.3390/molecules21010072.

[5] S. Nebbia *et al.*, "Antimicrobial potential of food lactic acid bacteria: Bioactive peptide decrypting from caseins and bacteriocin production," *Microorganisms*, vol. 9, no. 1, pp. 1–19, Jan. 2021, doi: 10.3390/microorganisms9010065.

[6] L. I. Vorob'eva, E. Y. Khodzhaev, E. A. Rogozhin, T. A. Cherdyntseva, and A. I. Netrusov, "Characterization of extracellular yeast peptide factors and their stress-protective effect on probiotic lactic acid bacteria," *Microbiology (Russian Federation)*, vol. 85, no. 4, pp. 411–419, Jul. 2016, doi: 10.1134/S0026261716040160.

[7] R. D. Joerger, "Alternatives to Antibiotics: Bacteriocins, Antimicrobial Peptides and Bacteriophages," *Poult. Sci.*, vol. 82, no. 4, pp. 640–647.

[8] C. Zhang and M. Yang, "Antimicrobial Peptides: From Design to Clinical Application," Mar. 01, 2022, *MDPI*. doi: 10.3390/antibiotics11030349.

[9] S. Sharma, R. Singh, and S. Rana, "Bioactive peptides: A review," 2011, *Institute of Biophysics and Biomedical Engineering*. doi: 10.1093/fqs/fyx006.

[10] O. N. Donkor, A. Henriksson, T. Vasiljevic, and N. P. Shah, "Probiotic Strains as Starter Cultures Improve Angiotensin-converting Enzyme Inhibitory Activity in Soy Yogurt," *Jorunal of Food Science* , vol. 70, no. 8, pp. 375–381, 2005, [Online]. Available: www.ift.org

[11] L. Nionelli *et al.*, "Antifungal effect of bioprocessed surplus bread as ingredient for bread-making: Identification of active compounds and impact on shelf-life," *Food Control*, vol. 118, Dec. 2020, doi: 10.1016/j.foodcont.2020.107437.

[12] D. Yusuf, L. Nuraida, R. Dewanti-Hariyadi, and D. Hunaef, "In vitro Antioxidant and α-Glucosidase Inhibitory Activities of Lactobacillus spp. Isolated from Indonesian Kefir Grains," *Applied Food Biotechnology*, vol. 8, no. 1, pp. 39–46, 2021, doi: 10.22037/afb.v8i1.30367.

[13] C. Innocente-Alves *et al.*, "Characterization of Novel Angiotensin-Converting Enzyme Inhibitory Peptides," *ACS Med. Chem. Lett.*, vol. 16, no. 12, pp. 2486–2491, Dec. 2025, doi: 10.1021/acsmedchemlett.5c00569.

[14] S. Amiri, R. Rezaei Mokarram, M. Sowti Khiabani, M. Rezazadeh Bari, and M. Alizadeh Khaledabad, "Characterization of antimicrobial peptides produced by Lactobacillus acidophilus LA-5 and Bifidobacterium lactis BB-12 and their inhibitory effect against foodborne pathogens," *LWT*, vol. 153, Jan. 2022, doi: 10.1016/j.lwt.2021.112449.