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TRABALHO DE CONCLUSÃO DE CURSO

POTENCIAL TECNOLÓGICO E BIOATIVO DE FERMENTOS NATURAIS INOCULADOS COM CEPAS AUTÓCTONES DA PARAÍBA

TECHNOLOGICAL AND BIOACTIVE POTENTIAL OF SOURDOUGH STARTERS INOCULATED WITH AUTOCHTHONOUS STRAINS FROM PARAÍBA

Gabriel Victor Pinheiro Barbosa

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2025

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RESUMO

Este trabalho teve como objetivo desenvolver um fermento natural dominante a partir de cepas autóctones de bactérias ácido-láticas (BAL) isoladas de diferentes regiões climáticas do Estado da Paraíba, com potencial tecnológico e bioativo para aplicação na panificação. Foram selecionadas as cepas *Lactiplantibacillus plantarum* 47 (Lp47) e *Levilactobacillus brevis* 83 (Lb83) com base em testes de desempenho tecnológico, tolerância e adaptabilidade realizados em estudos anteriores. Os fermentos foram elaborados com adição de culturas puras às massas de farinha e água e submetidos a análises físico-químicas, microbiológicas, bioativas, reológicas e de textura ao longo de 24 horas de fermentação. Os resultados demonstraram que ambas as cepas promoveram rápida acidificação e crescimento celular, com estabilização das BAL em 8,49 log UFC/g para Lp47 e 8,72 log UFC/g para Lb83. A atividade metabólica resultou na diminuição dos açúcares disponíveis e produção de ácidos orgânicos como lático, acético, fórmico e cítrico. A fermentação resultou em aumento significativo ($p<0,05$) dos compostos fenólicos totais e da atividade antioxidante. As análises reológicas revelaram que, após 24 horas, os fermentos apresentaram comportamento semelhante ao de gel, com o módulo de armazenamento (G') superior ao de perda (G"), indicando um comportamento prioritariamente elástico e valores de módulo complexo (G*) inferiores a 15 kPa, favoráveis ao aumento do volume dos pães. As amostras fermentadas com Lp47 e Lb83 também apresentaram redução significativa na rigidez e adesividade, características desejáveis para massas panificáveis mais maleáveis e com melhor incorporação de ingredientes. Os dados obtidos reforçam a viabilidade tecnológica das cepas autóctones selecionadas, que demonstraram estabilidade microbiológica, melhora do perfil funcional e propriedades reológicas adequadas para aplicação industrial. Conclui-se que o uso dessas culturas iniciadoras permite conduzir a fermentação de forma controlada, rápida e eficiente, promovendo a produção de fermentos naturais mais padronizados, funcionais e adaptados às condições regionais da panificação brasileira.

Palavras-chave: Bactéria ácido-láctica, fermentação natural, compostos bioativos, cepas autóctones, sourdough, *Lactobacillus spp*.

ABSTRACT

This study aimed to develop a dominant sourdough starter using autochthonous strains of lactic acid bacteria (LAB) isolated from different climatic regions of the state of Paraíba, with technological and bioactive potential for application in baking. The strains *Lactiplantibacillus plantarum* 47 (Lp47) and *Levilactobacillus brevis* 83 (Lb83) were selected based on technological performance, tolerance, and adaptability tests conducted in previous studies. The sourdoughs were prepared by adding pure cultures to flour and water doughs and were subjected to physicochemical, microbiological, bioactive, rheological, and texture analyses over a 24-hour fermentation period. The results showed that both strains promoted rapid acidification and cell growth, with LAB stabilizing at 8.49 log CFU/g for Lp47 and 8.72 log CFU/g for Lb83. Metabolic activity led to a decrease in available sugars and the production of organic acids such as lactic, acetic, formic, and citric acids. Fermentation resulted in a significant increase ($p<0.05$) in total phenolic compounds and antioxidant activity. Rheological analyses revealed that, after 24 hours, the sourdoughs exhibited gel-like behavior, with storage modulus (G') higher than loss modulus (G"), indicating predominantly elastic behavior, and complex modulus (G*) values below 15 kPa, which favor bread volume increase. Doughs fermented with Lp47 and Lb83 also showed a significant reduction in stiffness and adhesiveness—desirable characteristics for more malleable doughs with improved ingredient incorporation. The data obtained reinforces the technological viability of the selected autochthonous strains, which demonstrated microbiological stability, improved functional profile, and suitable rheological properties for industrial application. It is concluded that the use of these starter cultures enables controlled, rapid, and efficient fermentation, promoting the production of more standardized, functional sourdoughs adapted to the regional conditions of Brazilian baking.

Keywords: Lactic acid bactéria, natural fermentation, bioactive compounds, autochthonous strains, sourdough, *Lactobacillus spp*

1. INTRODUÇÃO

A fermentação é uma das técnicas mais antigas no processamento de alimentos sendo amplamente reconhecida por suas propriedades funcionais, capacidade de modificar o sabor e aprimoramento da biodisponibilidade dos nutrientes (Fan *et al*., 2024). Na panificação, a fermentação atua principalmente na mudança da estrutura da massa, tornando-a menos densa e mais aerada, resultado da produção de CO_2, gerado pelo metabolismo dos microrganismos envolvidos no processo fermentativo (De Vuyst *et al*., 2017).

Na fabricação de pães, dois tipos de agentes de fermentação são comumente utilizados: o fermento biológico instantâneo — preparações comerciais de *Saccharomyces cerevisiae* — e o fermento natural, formado por comunidades microbianas complexas, compostas predominantemente por leveduras e bactérias ácido-láticas (BAL). Nos últimos anos, produtos obtidos a partir da fermentação com fermento natural — como pães, biscoitos, pizzas, tortas, torradas e bolos — passaram a integrar o portfólio de grandes indústrias de panificação, como Bauducco (Brasil), Marilan (Brasil) e Knead Love (EUA) (Lima *et al.*, 2023). Essa expansão reflete não apenas a valorização do sabor e da textura dos produtos de fermentação natural, mas também a crescente busca dos consumidores por alimentos mais saudáveis e sustentáveis.

Estudos recentes (Graça *et al.,* 2022; Paucean *et al.,* 2024; Fang *et al.,* 2023) têm evidenciado que o fermento natural pode ser uma fonte rica de compostos bioativos. Estes compostos não apenas contribuem para o sabor, aroma e textura dos produtos fabricados com fermento natural, mas também conferem benefícios à saúde quando consumidos (Calinoiu *et al.,* 2018; Fekri *et al.,* 2024). A capacidade metabólica de BAL de produzir uma ampla gama de compostos bioativos são influenciadas por diversos fatores, incluindo pH, tempo e temperatura da fermentação (Pérez-Alvarado et al, 2022). Neste contexto, explorar os potenciais bioativos do fermento natural não só amplia nosso entendimento sobre os processos fermentativos, mas também abre novas perspectivas para o desenvolvimento de produtos com propriedades funcionais e benéficas a saudade (Papadimitriou et al, 2019).

O fermento natural é definido como um consórcio microbiano tradicionalmente formado por uma mistura fermentada de farinha de trigo ou centeio e água, na qual predominam leveduras e BAL, que podem se desenvolver espontaneamente ou ser inoculadas com culturas iniciadoras selecionadas (De Vuyst *et al.*, 2014; Pétel *et al.*, 2017).

A fermentação natural é um sistema dinâmico marcado por mudanças contínuas na disponibilidade de nutrientes ao longo do processamento. Esse sistema está sujeito à liberação simultânea de carboidratos fermentáveis — como glicose, maltose e frutose — por ação de

enzimas endógenas ou exógenas, com esses açúcares sendo rapidamente absorvidos e metabolizados por leveduras e BAL (Guerzoni, 2015).

Do ponto de vista microbiológico, o fermento natural é um ecossistema específico e estressante, caracterizado por um pH baixo, alta concentração de carboidratos, baixa disponibilidade de oxigênio e contagens celulares mais elevadas de BAL [$\geq 10^8$ unidades formadoras de colônias (UFC)/g] em comparação com as leveduras ($\leq 10^7$ UFC/g) (De Vuyst *et al*., 2014).

As vantagens da utilização do fermento natural e seus efeitos nos pães têm sido apresentadas por vários estudos (Siepmann *et al*., 2018; Lima *et al*., 2023; De Vero *et al*., 2021). No entanto, sua aplicação em escala industrial ainda enfrenta desafios significativos, principalmente dependendo do tipo de fermento natural empregado (Lima *et al*., 2023).

Atualmente, existem quatro métodos que podem ser utilizados para se iniciar uma massa de fermentação natural: fermentação espontânea; aplicação de uma cultura iniciadora em altas concentrações; uso de fermento seco, líquido ou liofilizado; e adição de uma cultura iniciadora seguido de propagação. Embora todas essas abordagens possam resultar em bons fermentos, algumas demandam tempo e mão de obra especializada para garantir a qualidade e a manutenção do fermento (Reale *et al*., 2020). Com isso, o uso de culturas iniciadoras tem se mostrado uma alternativa promissora, pois permite conduzir o processo fermentativo de forma mais rápida e controlada (Siepmann *et al*., 2019).

Quanto às cepas iniciadoras do fermento, para garantir uma fermentação eficiente, é essencial utilizar cepas que possuam boa adaptação às condições da panificação e que sejam resistentes à microbiota já presente no ambiente, utensílios e ingredientes utilizados.

As BAL destacam-se pela produção de ácidos orgânicos, responsáveis pela acidificação da massa, enquanto as leveduras atuam como principal agente fermentador (Catzeddu, 2019; Fekri *et al*., 2024). A significativa redução do pH durante a fermentação ativa diversas enzimas presentes nas farinhas, como proteases e amilases, que contribuem para a melhora da qualidade do fermento natural. As proteases, por exemplo, liberam aminoácidos como precursores de aroma e sabor (Catzeddu, 2019) e favorecem a maciez e a extensibilidade da massa (Siepmann *et al*., 2018). Como resultado, os fermentos inoculados com BAL apresentam uma estrutura mais adequada para aplicação em massas panificáveis, pois são menos adesivos, mais elásticos e possuem maior fluência a altas deformações.

Do ponto de vista social, a valorização de microrganismos autóctones em processos fermentativos apresenta grande relevância. Esses microrganismos podem ser adaptados a condições específicas de cada região, promovendo a valorização da biodiversidade local,

fortalecendo a produção artesanal e gerando alternativas de renda para comunidades. Nesse contexto, a fermentação natural relaciona-se diretamente aos Objetivos de Desenvolvimento Sustentável (ODS), como o ODS 3 (Saúde e Bem-Estar), ao contribuir com a oferta de alimentos funcionais e mais saudáveis; e o ODS 9 (Indústria, Inovação e Infraestrutura), ao estimular processos biotecnológicos inovadores aplicados ao setor de panificação.

Diante dos efeitos positivos associados ao uso de culturas iniciadoras em processos fermentativos e da necessidade de avaliar, em especial, as cepas autóctones devido à sua maior capacidade de adaptação a condições ambientais estressantes, este estudo teve como objetivo desenvolver um fermento natural a partir de cepas de BAL isoladas de diferentes regiões climáticas da Paraíba, explorando seu potencial tecnológico e bioativo.

2. OBJETIVOS

2.1 Objetivo Geral:

Caracterizar e avaliar o potencial tecnológico e bioativo de fermentos naturais inoculados com cepas de bactérias ácido-láticas (BAL) autóctones do estado da Paraíba.

2.2 Objetivos Específicos:

- Desenvolver um fermento natural a partir de cepas de bactérias ácido-láticas (BAL) isoladas das diferentes regiões climáticas da Paraíba;
- Avaliar características microbiológicas dos fermentos inoculados;
- Avaliar características físico-químicas dos fermentos inoculados;
- Avaliar características tecnológicas dos fermentos inoculados;
- Avaliar características reológicas dos fermentos inoculados;
- Avaliar potencial bioativo dos fermentos inoculados.

3. METODOLOGIA

3.1 Locais de Execução

A elaboração dos fermentos naturais, manutenção das cepas e avaliação da viabilidade de bactérias ácido láticas foram realizadas no Laboratório de Microbiologia de Alimentos do Centro de Tecnologia e Desenvolvimento Regional (CTDR) da Universidade Federal da Paraíba (UFPB). As análises físico-químicas e de bioatividade foram realizadas no Laboratório de Análises Físico-químicas/CTDR/UFPB. A quantificação de compostos fenólicos e a determinação de açúcares e ácidos orgânicos foi realizada no Laboratório de Análise de Águas e Bebidas, do Departamento de Tecnologia de Alimentos (DTA) do Instituto Federal do Sertão de Pernambuco (IFSertãoPE). As análises de textura e reologia foram realizadas no Laboratório Tecnológico em Segurança Alimentar/ CTDR/UFPB.

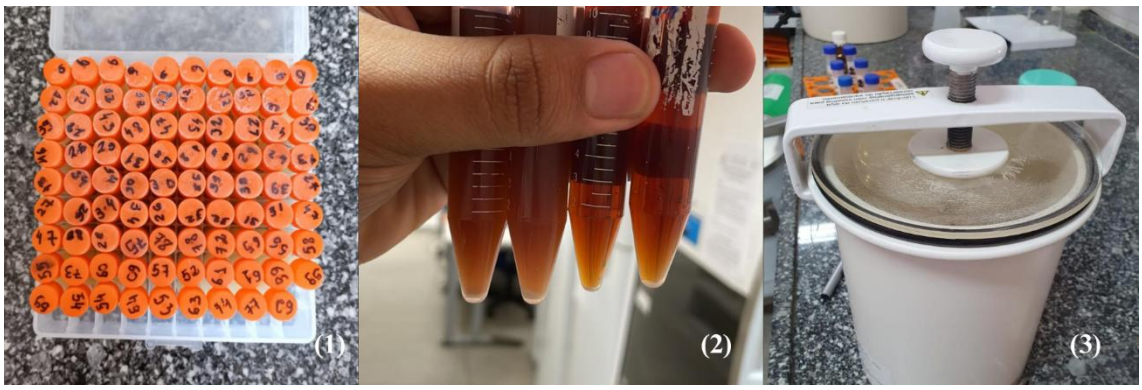
3.2 Materiais

Neste trabalho, foram utilizados dois tipos de fermentos naturais, o Lp47 (Fermento natural inoculado com *Lactiplantibacillus plantarum* 47) e o Lb83 (Fermento natural inoculado com *Levilactobacillus brevis* 83). Os dois fermentos foram elaborados com 50 ml de água potável, 100g de farinha de trigo tipo 1 (Finna. Lote:102023) e 10 ml das culturas isoladas de *Lactiplantibacillus plantarum* 47 e *Levilactobacillus brevis* 83 (Medeiros *et al.*, 2025), pertencentes ao banco de cepas do Laboratório de Microbiologia de Alimentos do Centro de Tecnologia e Desenvolvimento Regional (CTDR) da UFPB. Essas cepas foram selecionadas devido ao seu bom desempenho em testes tecnológicos, de seleção e tolerância, características importantes para produtos de panificação.

3.3 Manutenção das Cepas

As cepas selecionadas, descrita no item 3.2, foram mantidas em caldo de Man, Rogosa e Sharpe (MRS) suplementado com 200 μL de solução de glicerol 80% sob congelamento (-18 °C). A cada necessidade de uso uma alíquota de cada cepa foi tomada dos respectivos criotubos, posteriormente sendo inoculadas em caldo MRS e incubadas em jarra de Permution® (modelo JA0400, Curitiba, Brasil) com gerador de atmosfera específico Anaerobac® (Probac do Brasil, São Paulo, Brasil) à 37 °C por 48h até formação de biomassa visível.

Figura 01 – Manutenção das cepas: (1) banco de cepas; (2) ressuspensão das cepas; (3) incubação em anaerobiose.



Fonte: Dados da pesquisa

3.4 Elaboração dos Fermentos Naturais

A elaboração do fermento foi realizada seguindo protocolo descrito por Medeiros *et al*., (2025). Para o preparo dos inóculos as cepas selecionadas de BAL foram inoculadas em caldo MRS e incubadas a 37± 5ºC até a fase exponencial. Posteriormente as células foram centrifugadas e ressuspendidas em solução salina (0,1%) estéril. Imediatamente após o preparo dos fermentos (Tempo 0), as amostras foram incubadas em B.O.D. (30± 5ºC) até o horário das análises subsequentes.

3.5 Estudo das Características Físico-Químicas, Microbiológicas, Bioativas e Tecnológicas dos Fermentos Naturais Inoculados com *L. Plantarum* 47 e *L. Brevis* 83

Após a elaboração dos fermentos naturais inoculados foram realizadas as análises físico-químicas das amostras. As análises de pH e acidez titulável total foram realizadas nos tempos 0, 2, 4, 6, 8 e 24h, para observar o comportamento do fermento ao longo do tempo. As análises de quantificação de compostos fenólicos e atividade antioxidante foram realizadas nos tempos 0, 8 e 24h.

3.5.1 pH e Acidez Titulável Total

O pH foi medido utilizando potenciômetro acoplado a um eletrodo de vidro em triplicata (Modelo Q400AS, Quimis, Diadema, São Paulo, Brasil). A acidez titulável total (g/100 g) foi determinada por titulação com NaOH 0,1 N e solução de fenolftaleína como indicador (AOAC, 2016), em intervalos de 0, 2, 4, 6, 8 e 24 horas.

3.5.2 Quantificação e Teor de Compostos Fenólicos

Foi preparado um extrato etanólico (5g/50mL) de massa fermentada para as análises de quantificação e teor de compostos fenólicos totais. A metodologia foi descrita por Fang *et al*. (2023), com adaptações.

Para determinação do teor de fenólicos totais, 0,5mL do extrato foi colocado em um balão volumétrico de 10mL. Em seguida, foram adicionados 1mL de Folin-Ciocalteu (agente cromogênico para fenóis), 2mL de solução de Na_2CO_3 a 15% e o volume foi completado com água destilada até atingir 10mL. A mistura reagiu em temperatura ambiente por 2 horas. As leituras das amostras aconteceram nos tempos 0, 8 e 24 horas de fermentação e os valores de absorbância foram determinados na OD_{765nm}. Foi construída uma curva padrão com solução de ácido gálico, e uma mistura de água e reagente foi utilizada como branco. Os resultados foram expressos em: Equivalente de Ácido Gálico (GAE) por mg de amostra.

A quantificação de compostos fenólicos foi determinada por cromatografia líquida de alta eficiência (HPLC), seguindo a metodologia descrita por Lima *et al*. (2024). Utilizou-se um cromatógrafo líquido Agilent 1260 (Santa Clara, EUA), equipado com bomba de solvente quaternário com desgaseificação em linha (modelo G1311C), compartimento termostatizado para colunas (modelo G1316A), amostrador automático (modelo G1329B) e detector de arranjo de diodos (DAD, modelo G1315D). A coleta e o processamento dos dados foram realizados por meio do software OpenLAB CDS ChemStation Edition (Agilent Technologies, Santa Clara, EUA). Para a análise cromatográfica, foi empregada uma coluna ultrarrápida Eclipse Plus RRHT RP-C18 (50 × 4,6 mm, 1,8 μm; Zorbax, SC, EUA). A fase móvel foi composta por solução de ácido fosfórico a 0,5% v/v (solvente A) e metanol acidificado com 0,5% v/v de ácido fosfórico (solvente B). Os compostos foram identificados e quantificados por meio da comparação com padrões externos, utilizando tempo de retenção, curvas de calibração e similaridade espectral.

3.5.3. Atividade Antioxidante

Foi preparado um extrato etanólico de (5 g/50 mL) de massa fermentada para as análises de atividade antioxidante. A metodologia foi descrita por Fang *et al.* (2023), com adaptações, seguindo método ABTS. 0,1mL do extrato foi misturado com 3,9mL de solução de trabalho ABTS+, a mistura ficou sem o contato com a luz por 10 minutos, em seguida foi determinada a absorbância um espectrofotômetro (Eppenddorf BioSpectrometer basic) com leitura da densidade óptica em OD_{734nm}. As leituras das amostras aconteceram nos tempos 0, 8 e 24h. Uma mistura de etanol a 15% e reagente foi utilizado como branco. Foi construída a curva

padrão com solução padrão Trolox. Os resultados foram expressos em: Equivalente de Trolox por g de amostra.

Figura 02 – Atividade antioxidante: (1) solução de trabalho ABTS+; (2) preparação da curva padrão; (3) leitura das amostras.

Fonte: Dados da pesquisa

3.5.4. Viabilidade das BAL

Foram realizadas avaliações das amostras de fermento para determinar a contagem de células viáveis de BAL em intervalos de 0, 2, 4, 6, 8 e 24 horas.

Figura 03 – Viabilidade das BAL: (1) diluição das amostras; (2) diluição seriada; (3) inoculação por microgota; (4) contagem das células viáveis.

Fonte: Dados da pesquisa

Para cada análise, 3g da amostra foram diluídas em 27mL de solução de água peptonada 0,1% (p/v) e diluídas serialmente (1:9, v/v) no mesmo diluente (10^{-2} – 10^{-6}). Alíquotas de 20µL de cada diluição seriada foram inoculadas (usando o método de microgota) (Liu *et al*., 2018) em placas contendo ágar MRS e incubadas a 37± 5 °C por 48 h. Após o período de incubação, as células viáveis de BAL foram enumeradas e os resultados foram expressos como log UFC/g.

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3.5.5. Perfil de Ácidos Orgânicos e Açúcares

Maltose, sacarose, glicose, frutose, etanol, ácido láctico e acético, glicerol e manitol foram determinados por cromatografia líquida de alta eficiência (HPLC) composto por um

detector de índice de refração. As análises foram descritas por Paramithiotis *et al.*, (2006) e Gunduz *et al.*, (2022), com adaptações.

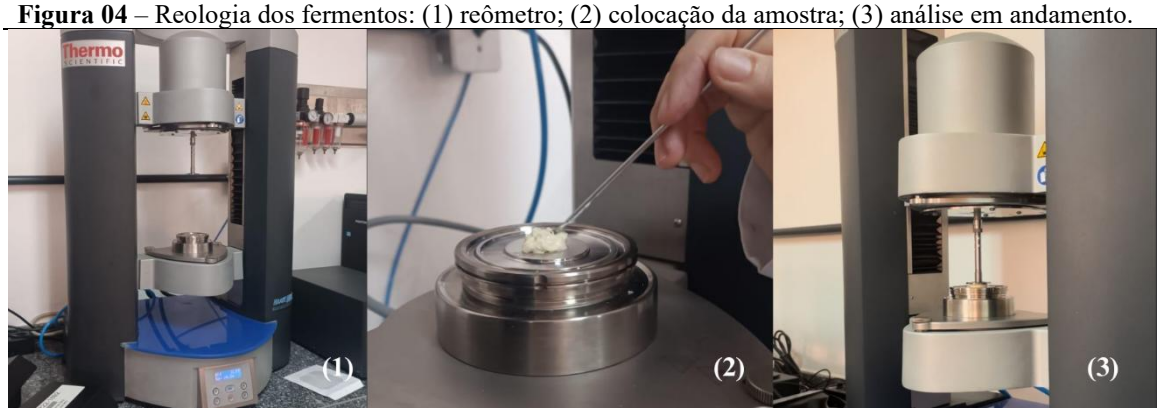
Uma alíquota de 10g da amostra foi homogeneizada em 90mL de tampão fosfato (25 mM e pH 5,6) e centrifugada por 10 minutos a 12.000 rpm, a uma temperatura de 4±5 °C. Em seguida, 1mL do sobrenadante foi misturado com 50µL de ácido perclórico (70% v/v, a 30±5 °C) e mantido sob refrigeração (4°C) por 24 horas. Os aglomerados de proteínas foram removidos por centrifugação (5.000 rpm, 60 min, 4±5 °C) e o sobrenadante obtido foi filtrado através de um filtro de seringa de 0,45µm. Posteriormente, 20µL do extrato filtrado foram injetados em uma coluna Aminex, HPX-87H, acoplada a um detector de índice de refração. A eluição foi realizada a 35±5 °C utilizando H_2SO_4 5mM como fase móvel, com vazão de 0.5 mL/min.

Os dados obtidos foram processados utilizando o software OpenLAB CDS ChemStation Edition™ (Agilent Technologies). Curvas padrão e análises quantitativas foram realizadas para cada composto.

3.5.6. Reologia dos Fermentos Inoculados com *L. plantarum* 47 e *L. brevis* 83

Para a realização das análises, foi utilizado um reômetro MARS III Haake (Karlsruhe, Alemanha), contendo um sistema de placas paralelas lisas de aço inox com diâmetro de 3,5 cm (T35TIL) e um gap de 1 mm, em temperatura de 30 ºC. Os experimentos foram realizados em triplicata após 8h e 24h de fermentação seguindo a metodologia descrita por Amaral *et al.* (2022). Para determinar a região viscoelástica linear, foi realizado um escaneamento de tensão (1 – 100 Pa) na frequência de 1 Hz. Em seguida, foram obtidos espectros mecânicos por meio de varreduras de frequência entre 0,1 e 10 Hz, mantendo-se a tensão fixa dentro da faixa de viscoelasticidade linear.

Figura 04 – Reologia dos fermentos: (1) reômetro; (2) colocação da amostra; (3) análise em andamento.



Fonte: Dados da pesquisa

O módulo complexo (G*) e a tangente de delta, foram calculados utilizando as equações (1) e (2), respectivamente.

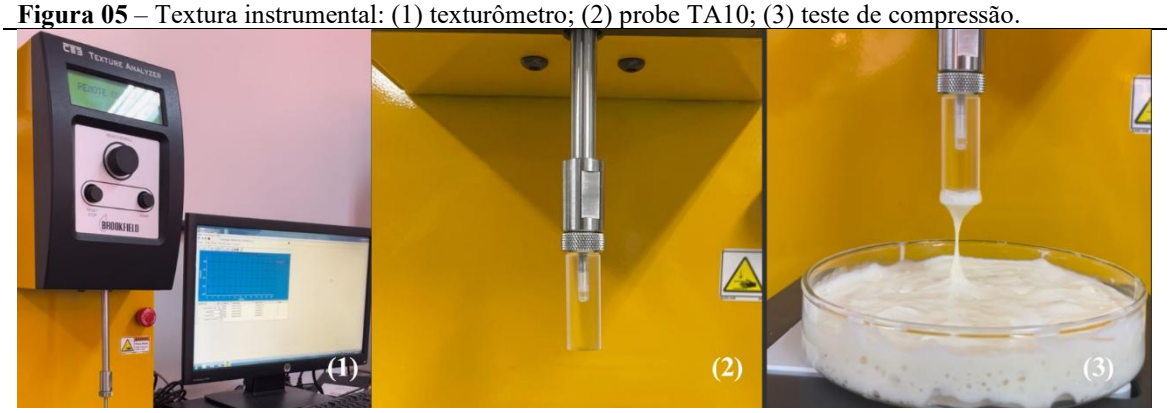
$$(1)\ G^* = \sqrt[2]{(G')^2 + (G")^2} \qquad (2)\ Tan(\delta) = \frac{G"(\omega)}{G'(\omega)}$$

A média e o desvio padrão dos valores dos módulos elástico e viscoso foram graficados, enquanto a média e o desvio dos valores do módulo complexo e tangente de delta na frequência de 1 Hz foram tabulados e analisados estatisticamente.

3.5.7. Textura Instrumental

Os testes de compressão foram realizados após 8 e 24h de fermentação, em triplicata, seguindo a metodologia descrita por Amaral *et al.* (2022). Foram analisados os parâmetros dureza e adesividade. Para tal, foi utilizado um texturômetro (Texture Analyzer CT3 - Brookfield Engineering Labs, Middleboro, EUA), equipado com uma geometria cilíndrica acrílica (TA10) com 12,5 mm de diâmetro. Os resultados foram obtidos pelo programa próprio do equipamento (Texture Expert for Windows, versão 1.19).

Figura 05 – Textura instrumental: (1) texturômetro; (2) probe TA10; (3) teste de compressão.



Fonte: Dados da pesquisa

3.5.8. Análises Estatísticas

Os ensaios foram realizados em triplicata apresentando média e desvio padrão. A análise estatística foi realizada para determinar diferenças significativas ($p < 0,05$) utilizando a Análise de Variância (ANOVA) seguido pelo teste Tukey. Para o cálculo dos dados, foi utilizado o software Jamovi versão 2.3.28.

4. RESULTADOS E DISCUSSÃO

4.1. CARACTERIZAÇÃO FÍSICO-QUÍMICA DO FERMENTO

Foram avaliados os parâmetros físico-químicos dos fermentos inoculados com Lp47 e Lb83 durante a fermentação em diferentes tempos. Os valores de pH e ATT estão dispostos na Tabela 01. Além disso, o conteúdo de açúcares e ácidos orgânicos estão presentes na Tabela 02.

Tabela 01. Avaliação do pH e acidez titulável total de fermentos inoculados com *Lactiplantibacillus plantarum* 47 e *Levilactobacillus brevis* 83 durante a fermentação (0, 2, 4, 6, 8 e 24 horas).

			pH			
	0h	**2h**	**4h**	**6h**	**8h**	**24h**
Fp47	5,48±0,01^{Aa}	5,43±0,05^{Aa}	4,24±0,03^{Ab}	3,87±0,03^{Abc}	3,79±0,03^{Ac}	3,47±0,02^{Ac}
Fb83	5,52±0,03^{Aa}	5,54±0,01^{Aa}	5,09±0,01^{Bb}	4,55±0,19^{Bc}	4,11±0,02^{Bd}	3,57±0,02^{Ae}
			Acidez Titulável Total (g/100g)			
	0h	**2h**	**4h**	**6h**	**8h**	**24h**
Fp47	0,47±0,06^{Aa}	0,63±0,06^{Aa}	1,37±0,15^{Ab}	2,00±0,17^{Ac}	2,53±0,15^{Ad}	3,30±0,10^{Ae}
Fb83	0,40±0,00^{Aa}	0,60±0,00^{Aab}	0,83±0,06^{Bb}	1,43±0,06^{Bc}	1,90±0,10^{Bd}	3,13±0,06^{Ae}

A-C Letras maiúsculas iguais na mesma coluna não diferem estatisticamente (p>0,05) entre si.
a-e Letras minúsculas iguais na mesma linha não diferem estatisticamente (p>0,05) entre si.
Fp47: Fermento inoculado com *Lactiplantibacillus plantarum* 47; Fb83: Fermento inoculado com *Levilactobacillus brevis* 83.
Fonte: Dados da pesquisa.

Durante o experimento o pH das amostras variou de 3,47±0,02 a 5,54±0,01, sem diferenças significativas entre as amostras no início da inoculação (0h). Entretanto, após 4h de fermentação, o pH de Fb47 caiu significativamente para 4,24±0,03, enquanto o pH de Fb83 permaneceu em 5,09±0,01. Após 6 horas, o pH de Fp47 atingiu 3,87±0,02 e manteve-se estável até as 24 horas. Os valores finais obtidos foram semelhantes aos estudos de Gunduz *et al*. (2020) e Ventimiglia *et al*. (2015), que também observaram rápida acidificação, essencial para uma fermentação eficiente (Arora *et al.*, 2021).

A acidez titulável total (ATT), que reflete a acidez da massa, mostrou comportamento inversamente proporcional ao pH, aumentando ao longo do tempo de fermentação, com valores variando entre 0,40±0,00 e 3,30±0,10. Isso ocorre porque os ácidos orgânicos, produtos do metabolismo das BAL, se acumulam na massa durante a fermentação, conforme descrito por De Vuyst *et al*. (2017).

Tabela 02. Conteúdo de açúcares e ácidos orgânicos em fermento natural inoculado com *Lactiplantibacillus plantarum* 47 e *Levilactobacillus brevis* 83 em diferentes tempos de fermentação.

	Tempo	Açúcares (g/kg)			Ácidos Orgânicos (g/kg)		
		Maltose	Glicose	Frutose	Cítrico	Láctico	Fórmico
Fp47	0h	ND	0,08	0,57	ND	ND	0,22
	8h	0,09	0,08	0,42	ND	0,98	0,30
	24h	2,50	0,09	0,53	ND	1,21	0,22
Fb83	0h	1,72	0,37	0,81	ND	9,09	0,74
	8h	1,48	0,13	0,57	ND	ND	0,17
	24h	3,23	0,21	0,58	17,22	ND	0,50

ND – Não detectado. Fp47: Fermento inoculado com *Lactiplantibacillus plantarum* 47; Fb83: Fermento inoculado com *Levilactobacillus brevis* 83.
Fonte: Dados da pesquisa.

Foram identificados por HPLC os açúcares: maltose, glicose e frutose. A alta atividade metabólica das BAL esgotou rapidamente as fontes de carboidratos, como evidenciado pelos baixos níveis de açúcares e os altos valores de ATT. Nas culturas heterofermentativas, como Lb83, a glicose é metabolizada pela via da fosfocetolase para produzir ácido láctico e etanol, enquanto a frutose é utilizada como aceptor de elétrons alternativo para a produção de ácido acético e manitol (Ganzle, 2015). Não foi detectada maltose em Fp47 após a inoculação, no entanto, durante a fermentação foi observado um aumento progressivo desse açúcar devido à atividade das amilases, enzimas responsáveis pela quebra do amido em açúcares simples, como a maltose (Fu *et al.*, 2024).

Apenas a cepa Lb83 produziu ácido cítrico (17,22 g/L após 24 horas de fermentação), o qual pode ser convertido em diversos compostos, como succinato, ácido lático, acético ou etanol. O ácido láctico foi detectado nos dois fermentos, no entanto, em Fb83 foi encontrado em maiores concentrações logo após a inoculação (0h), estando ausente nos tempos subsequentes, enquanto Fp47 acumulou ácido láctico progressivamente ao longo do tempo de fermentação. Este comportamento sugere diferentes rotas metabólicas entre as cepas.

O único composto presente em ambas as cepas independente do tempo de fermentação foi o ácido fórmico, com valores variando de 0,17 a 0,74. Esse composto possui importante ação antimicrobiana, atuando sinergicamente com outros ácidos como o láctico e o acético (De Vuyst *et al.*, 2017) podendo ser produto da degradação do ácido láctico ou de rotas alternativas do metabolismo do ácido cítrico (Ganzle, 2015).

4.4.7. CARACTERIZAÇÃO BIOATIVA DO FERMENTO

Na Tabela 03 estão dispostos os valores da atividade antioxidante, além da quantificação e teores totais dos compostos fenólicos dos fermentos naturais.

Tabela 03. Compostos fenólicos, teor fenólico total e atividade antioxidante dos fermentos inoculados com *Lactiplantibacillus plantarum* 47 e *Levilactobacillus brevis* 83 durante a fermentação (0, 8 e 24 horas)

Compostos Fenólicos Quantificados por HPLC* (µg / g)						
	Ácido Fumárico			**Epigalocatequina Galato**		
	0h	**8h**	**24h**	**0h**	**8h**	**24h**
Fp47	2,68	ND	ND	2,74	2,66	3,07
Fb83	ND	ND	ND	ND	3,64	4,03
Teor Fenólico Total (mg GAE/100g)**						
	0h		**8h**		**24h**	
Fp47	29,85±0,31^{Aa}		30,90±0,37^{Aa}		36,17±1,40^{Ab}	
Fb83	29,38±0,62^{Aa}		31,75±0,58^{Ab}		35,16±1,07^{Ac}	
ABTS (µmol Tx/100g)						
	0h		**8h**		**24h**	
Fp47	8,57±0,85^{Aa}		9,33±0,60^{Aa}		11,37±0,76^{Ab}	
Fb83	8,47±1,05^{Aa}		9,87±1,26^{Aa}		11,77±0,06^{Ab}	

A-B Letras maiúsculas iguais na mesma coluna não diferem estatisticamente (p>0,05) entre si.
a-c Letras minúsculas iguais na mesma linha não diferem estatisticamente (p>0,05) entre si.
ND – Não detectado. Fp47: Fermento inoculado com *Lactiplantibacillus plantarum* 47; Fb83: Fermento inoculado com *Levilactobacillus brevis* 83.
* Análise feita por HPLC.
** Análise feita por espectrofotometria.
Fonte: Dados da pesquisa.

A fermentação teve impacto positivo na produção dos compostos fenólicos totais, com os valores variando entre 29,38 e 36,17g a cada 100g de fermento. Observou-se um aumento significativo ao longo da fermentação ($p<0,05$), com as maiores concentrações sendo registradas após 24 horas. Esses compostos são benéficos devido as suas propriedades antioxidantes e anti-inflamatórias, com a capacidade das bactérias ácido-láticas de liberar ácidos fenólicos ligados à farinha, especialmente em farinhas integrais (Calinoiu *et al.*, 2018).

Em estudo realizado por Graça *et al*. (2022), o teor de compostos fenólicos em fermento natural de trigo após 24h foi de 8,6%, com a adição de farinha integral impulsionando o crescimento para 38%. O fermento Fp47 apresentou um crescimento de 21,2% em relação ao tempo inicial (0h), enquanto Fb83 registrou um aumento de 19,8%.

Esses resultados indicam que a fermentação natural demonstrou impacto significativo na disponibilidade dos compostos fenólicos, resultando em um aumento considerável destes compostos. Esse processo não apenas potencializa as propriedades bioativas dos fermentos, mas também contribui para um perfil nutricional mais enriquecido dos produtos finais.

A análise de eliminação do radical ABTS•+ foi usada para avaliar as propriedades antioxidantes dos fermentos inoculados. Os fermentos apresentaram atividade antioxidante de 39% para Fb83 e 32% para Fp47 em relação ao início da fermentação, demonstrando que a fermentação também amplificou a atividade antioxidante. Os valores variaram entre 8,47±1,05 e 11,77±0,06, mostrando diferenças significativas após 24h de fermentação. As cepas

comportaram-se de maneira semelhante, não mostrando diferença significativa entre si ($p>0,05$).

Estudos anteriores com diferentes tipos de farinhas (Fang *et al*., 2023; Li *et al*., 2024; Liu *et al*., 2024) evidenciaram uma tendência de aumento da atividade antioxidante em pães e fermentos naturais inoculados com bactérias ácido lácticas, o que respalda os resultados obtidos neste trabalho. Isso pode acontecer devido a acidificação decorrente da fermentação que pode beneficiar a formação de peptídeos com potencial antioxidante (Fang *et al*., 2023; Pejcz *et al*., 2024), além de aumentar a disponibilidade dos compostos fenólicos, como já observado.

A epigalocatequina galato (EGCG), um flavonoide do grupo das catequinas e amplamente reconhecida por suas propriedades antioxidantes (Silva Júnior *et al.,* 2021), foi detectada logo após a inoculação em Fp47, aumentando após 24 horas de fermentação. Esse flavonoide tem sido associado à prevenção de diversas condições de saúde, como doenças cardiovasculares, neurodegenerativas, síndromes metabólicas, envelhecimento e câncer (Thanikachalam *et al*., 2020).

Comportamento semelhante foi observado para Fb83, embora, nesse caso, a detecção da EGCG tenha ocorrido apenas a partir das 8 horas de fermentação. Além disso, o ácido fumárico foi encontrado em Fp47 no tempo 0h, mas foi degradado ao longo da fermentação. Cepas de *L. plantarum* e *L. brevis* são conhecidas por aumentar as concentrações de catequina durante a fermentação (Gaur e Ganzle, 2023).

Acredita-se que as enzimas esterases sejam uma das responsáveis pela conversão de EGCG durante a fermentação. No entanto, esses dados se baseiam na quantificação da redução dos compostos originais e no aumento subsequente dos metabólitos esperados (Gaur e Ganzle, 2023), indicando a necessidade de estudos mais aprofundados sobre as vias metabólicas envolvidas. Segundo os autores, revisões recentes sobre o metabolismo de compostos fenólicos durante a fermentação de alimentos por lactobacilos ainda são limitadas, o que demonstra a escassez de literatura disponível sobre o tema.

A contagem de células viáveis de BAL nos fermentos inoculados foi consistente com os valores de pH e ATT, estabilizando-se ao atingir um pH médio de 4,4. A estabilização pode ser observada após 4 horas para Fp47 (8,49±0,09 log UFC/g) e 6 horas para Fb83 (8,72±0,06 log UFC/g). Os valores da contagem de células viáveis de bactérias ácido láticas estão dispostos na Tabela 04.

Tabela 04. Contagem de células viáveis em fermentos inoculados com *Lactiplantibacillus plantarum* 47 e *Levilactobacillus brevis* 83 durante a fermentação (0, 2, 4, 6, 8 e 24 horas).

	BAL (UFC/g)					
	0h	**2h**	**4h**	**6h**	**8h**	**24h**
Fp47	7.63 ± 0.21^{Aa}	7.98 ± 0.03^{Aa}	8.49 ± 0.09^{Ab}	8.85 ± 0.00^{Ab}	8.85 ± 0.00^{Ab}	8.86 ± 0.01^{Ab}
Fb83	7.57 ± 0.04^{Aac}	6.85 ± 0.21^{Ba}	8.01 ± 0.19^{Bc}	8.72 ± 0.06^{Bbd}	8.68 ± 0.004^{Ad}	8.71 ± 0.13^{Ad}

A-C Letras maiúsculas iguais na mesma coluna não diferem estatisticamente (p>0,05) entre si.
a-e Letras minúsculas iguais na mesma linha não diferem estatisticamente (p>0,05) entre si.
Fp47: Fermento inoculado com *Lactiplantibacillus plantarum* 47; Fb83: Fermento inoculado com *Levilactobacillus brevis* 83.
Fonte: Dados da pesquisa.

Esse comportamento é consistente com os resultados de Ventimiglia *et al*. (2015) e Gunduz *et al*. (2022), que também observaram rápida adaptação das BAL isoladas de fermento natural. Além disso, a rápida adaptação da cepa Lp47 demonstra sua eficiência e potencial de aplicação industrial. Em estudo semelhante, Fang *et al*. (2023) inocularam cepas isoladas de diferentes matrizes alimentares em fermentos naturais; no entanto, somente após 12 horas *L. plantarum* conseguiu se estabilizar (8,5 log UFC/g). No estudo de Santos *et al*. (2024), as contagens de *L. pentosus* e *L. fermentum* isoladas de frutas foram, em média, de 6,66 log UFC/g após 24 horas de fermentação, com um aumento de um ciclo logarítmico nesse período.

O rápido crescimento e a intensa acidificação observados para as cepas *L. plantarum* 47 e *L. brevis* 83 podem estar diretamente relacionados à sua origem autóctone e à sua capacidade de adaptação ao meio estressor do fermento natural. Essas características fazem com que as cepas autóctones se destaquem pelo seu desempenho tecnológico em processos fermentativos, assegurando uma fermentação eficiente, direcionada e mais estável.

Esses resultados indicam que a fermentação natural com as cepas Lp47 e Lb83 contribui não apenas melhorando a disponibilidade de compostos bioativos, mas também aumenta a atividade antioxidante dos fermentos, com destaque para a cepa Lp47, que demostrou uma rápida adaptação e estabilização no ambiente fermentativo.

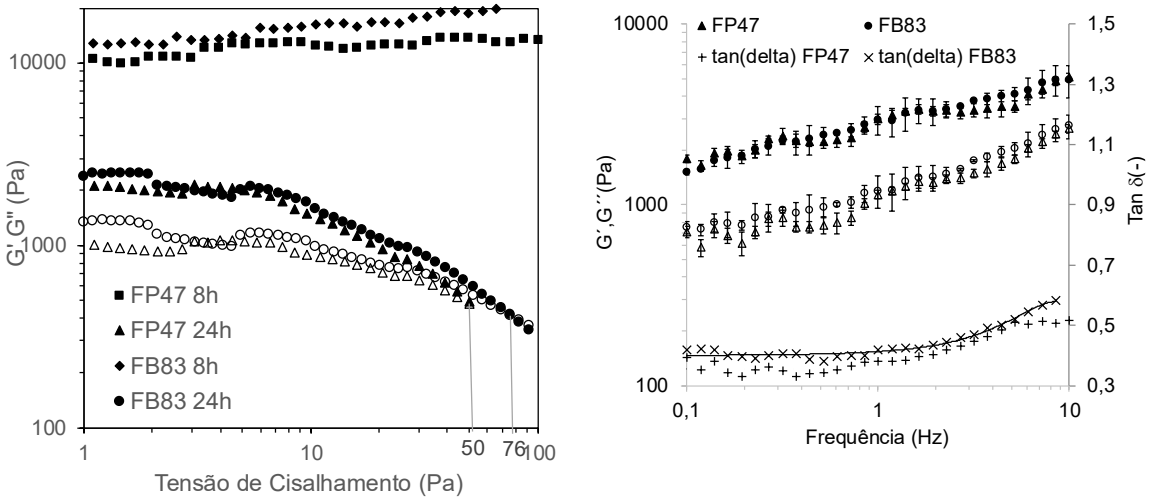
4.4.8. CARACTERIZAÇÃO TECNOLÓGICA DO FERMENTO

As características tecnológicas dos fermentos inoculados com *L. plantarum* 47 e *L. brevis* 83 foram analisadas em diferentes tempos de fermentação.

A Figura 2 (A) mostra o comportamento dos módulos elástico (G') e viscoso (G"), em função da tensão, para os fermentos inoculados com Lp47 e Lb83 após 8 h e 24h de fermentação. Em 24h, a região viscoelástica linear das amostras foi inferior a 2 Pa de tensão. Khatkar e Schofield (2002a) observaram um decréscimo contínuo de G' para massas de trigo em tensões entre 20-100 Pa, caracterizando ausência de região linear. O cruzamento das curvas dos módulos elástico e viscoso em alta tensão indica o início do escoamento do material. Nas

amostras com 24 horas de fermentação, observou-se que o fermento Fp47 iniciou o escoamento a 50 Pa, enquanto o LB83 teve início a 75 Pa, indicando uma estrutura mais resistente e coesa.

Figura 06 – Variação dos módulos de elasticidade e de perda em função da tensão de cisalhamento (A) e da frequência (B) de fermentos inoculados com *Lactiplantibacillus plantarum* 47 e *Levilactobacillus brevis* 83, isoladamente, após 8h e 24h de fermentação.



Símbolo fechado (G') e símbolo aberto (G").
Fp47: Fermento inoculado com *Lactiplantibacillus plantarum* 47; Fb83: Fermento inoculado com *Levilactobacillus brevis* 83.
Fonte: Dados da pesquisa.

Em 8h de fermentação, G' manteve-se linear até 100 Pa, comportamento semelhante ao relatado por Khatkar e Schofield (2002a), para massas de glúten puro. Gerez *et al.* (2008) e Yildirim-Mavis (2019) relatam que as características reológicas das massas podem ser modificadas por alterações físico-químicas, como a quebra de grandes agregados proteicos em frações menores, resultante da ação proteolítica das BAL e o enfraquecimento das ligações dissulfeto, que desestabilizam as estruturas proteicas (Tomic *et al*., 2023). Além disso, o módulo de armazenamento foi superior ao módulo de perda em todos os tempos de fermentação, indicando que os fermentos apresentaram comportamento predominantemente elástico e estruturas semelhantes a gel.

Os baixos níveis de pH e o aumento da ATT durante a fermentação ativam várias enzimas presentes nas farinhas, como amilases e hemicelulases (Yildirim-Mavis, 2019). A acidez elevada também modifica a estrutura do glúten, resultando em repulsão eletrostática intramolecular e exposição de grupos hidrofóbicos (Aprodu *et al*., 2019). Essas alterações estruturais explicam as mudanças reológicas observadas nos fermentos Fp47 e Fb83 após 24h de fermentação, quando atingiram pH 3,47 e 3,57 e ATT de 3,30 e 3,13, respectivamente.

Os resultados indicam que, após 8h, a rede de glúten permanece preservada, com os grânulos de amido estando ligados à matriz proteica, o que confere maior rigidez à amostra em comparação com as de 24 horas — sendo esta aproximadamente 10 vezes menor (Figura 2 A).

Embora o aumento da concentração de amido nas massas de glúten esteja associado a maior elasticidade em tensões inferiores a 20 Pa, como observado por Khatkar e Schofield (2002a), não houve diferença na concentração de amido dos fermentos ao longo da fermentação. Portanto, a maior rigidez observada em 8h pode ser atribuída à maior capacidade de retenção de água do glúten (Li *et al*., 2020).

Após 24 horas, com a estrutura proteica alterada pela ação enzimática e microbiana, a reologia passa a ser governada por interações amido-amido e amido-proteína, em vez de proteína-proteína, reduzindo o intervalo de viscoelasticidade linear (Khatkar e Schofield, 2002). Características que favorecem um processo de mistura mais homogêneo com os demais ingredientes da massa, além de demandar menor gasto energético.

A Figura 2(B) mostra o espectro mecânico dos fermentos em 24 horas, onde G' foi maior que G", indicando um comportamento principalmente elástico, semelhante a gel (Galle *et al*., 2011). Winter e Chambon (1986) definem o ponto de gel como a condição em que os módulos dinâmicos seguem uma lei da potência ao longo da frequência, mantendo o valor de tan δ constante. Esse comportamento foi observado para as amostras com 24 horas de fermentação, até 2 Hz para Fb83 e 1 Hz para Fp47. Em frequências superiores a 2 Hz, tan δ aumentou, comportamento similar ao observado em massas de farinha e água por Dongdong *et al*. (2023). Os valores de G* e tan δ, obtidos na região do intervalo linear a 1 Hz, estão apresentados na Tabela 05.

Tabela 05. Propriedades reológicas sob cisalhamento (a 1Hz) e compressão de fermentos inoculados com *Lactiplantibacillus plantarum* 47 e *Levilactobacillus brevis* 83, isoladamente, após 8h e 24h de fermentação.

Cepas	**Medidas sob Cisalhamento**			
	Módulo Complexo – G* (kPa)		**Tan δ (−)**	
	8h	**24h**	**8h**	**24h**
Fp47	$155,3 \pm 31,9^{Aa}$	$3,2 \pm 0,2^{Ab}$	$0,34 \pm 0,07^{Aa}$	$0,38 \pm 0,00^{Aa}$
Fb83	$180,8 \pm 45,8^{Aa}$	$3,2 \pm 0,6^{Ab}$	$0,38 \pm 0,05^{Aa}$	$0,40 \pm 0,00^{Aa}$
Cepas	**Medidas sob Compressão**			
	Dureza (N)		**Adesividade (mJ)**	
	8h	**24h**	**8h**	**24h**
Fp47	$0,48 \pm 0,04^{Aa}$	$0,60 \pm 0,08^{Aa}$	$2,87 \pm 0,50^{Aa}$	$1,43 \pm 0,15^{Ab}$
Fb83	$0,42 \pm 0,06^{Aa}$	$0,40 \pm 0,04^{Aa}$	$4,37 \pm 0,61^{Ba}$	$1,07 \pm 0,20^{Ab}$

[A] Letras maiúsculas iguais na mesma coluna não diferem estatisticamente (p>0,05) entre si.
[a-b] Letras minúsculas iguais na mesma linha não diferem estatisticamente (p>0,05) entre si.
Fp47: Fermento inoculado com *Lactiplantibacillus plantarum* 47; Fb83: Fermento inoculado com *Levilactobacillus brevis* 83.
Fonte: Dados da pesquisa.

O fator de perda (tan δ) reflete o comportamento viscoelástico do material, sendo a razão entre G" e G'. Quando a deformação é rápida, o fermento tende a se comportar mais como líquido (Yu *et al.*, 2019). Géis físicos, por sua vez, podem quebrar-se e fluir conforme a tensão e a taxa de deformação. No ponto de gel, a estrutura molecular é frágil e com certo grau de mobilidade, uma vez que a conexão dos largos agregados moleculares ainda estão se formando. Dessa forma, é provável que o fermento com 24 horas apresente pequenos agregados de glúten hidrolisado ligados ao amido, capazes de conectar e romper suas ligações físicas.

Não houve diferença significativa no valor de tan δ a 1 Hz entre as amostras, com valores entre 0,34 - 0,4. Valores semelhantes de G" foram relatados por Khatkar e Schofield (2002b), variando entre 0,33 – 0,58 para diversas farinhas de trigo analisadas. Valores de tan δ inferiores a 1 indicam um comportamento mais sólido/elástico do material (Li *et al.*, 2022). Observou-se uma redução significativa no modulo complexo (G*) após 24 horas de fermentação, passando de aproximadamente 180 kPa para 3,2 kPa, sem diferença significativa em relação ao tipo de cepa inoculada.

Van Bockstaele *et al*. (2008) identificaram uma relação inversa entre G* e o volume de pães, observando que valores de G* inferiores a 15 kPa favorecem o aumento do volume dos pães, chegando a 650 cm^3/100g de farinha com G* de 7 kPa. Assim, após 24 horas de inoculação, os fermentos Fp47 e Fb83 apresentaram valores de G* adequados para a produção de pães com maior volume, evidenciando seu potencial tecnológico para aplicação na panificação.

Foram analisadas a dureza e a adesividade dos fermentos após 8h e 24h. A dureza, que corresponde a força (N) máxima aplicada durante a deformação, não apresentou diferença significativa entre os fermentos, tendo variado entre 0,4–0,6 N. A adesividade, definida como a energia necessária para separar superfícies aderidas (Amaral *et al.*, 2022), variou de 1,0 a 4,3 mJ. Após 8 horas de fermentação, Fb83 foi significativamente mais adesivo que Fp47; no entanto, essa diferença desapareceu após 24h, quando a adesividade de Fp47 foi reduzida pela metade e a de Fb83 diminuiu cerca de quatro vezes.

Os amidos tendem a apresentar comportamento mais adesivos após a gelatinização, o que não ocorreu durante a fermentação. De acordo com Yildiz *et al*. (2012), a maior concentração de glúten aumenta a adesividade da massa. Portanto, a menor adesividade observada entre as amostras Fp47 e Fb83 ao longo do tempo de fermentação pode ser atribuída à hidrólise parcial da rede de glúten.

5. CONCLUSÃO

Este trabalho teve como objetivo desenvolver um fermento natural utilizando cepas de bactérias ácido-láticas isoladas de fermentos naturais das diferentes regiões climáticas da Paraíba e explorar seu potencial tecnológico e bioativo. De maneira geral, os resultados indicam que as cepas Lp47 e Lb83 são alternativas promissoras para uso como culturas iniciadoras em fermentações naturais, conferindo aos fermentos características físico-químicas, bioativas e tecnológicas satisfatórias. Os fermentos apresentaram rápida acidificação e crescimento expressivo das células viáveis, assegurando a estabilidade e a viabilidade, com potencial de replicação em escala industrial. As culturas iniciadoras promoveram aumento no teor de compostos fenólicos totais e na atividade antioxidante ao longo da fermentação. As análises reológicas e de compressão revelaram alterações estruturais significativas no fermento, com redução de aproximadamente dez vezes na rigidez entre 8 e 24 horas de fermentação. Diante do exposto os resultados reforçam que o uso de cepas autóctones no preparo de fermentos naturais oferecem um bom desenvolvimento tecnológico, sendo recomendado para iniciar o processo de fermentação, proporcionando um processo rápido, controlado e seguro.

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APÊNDICE A – Artigo

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Autochthonous strains of *Lactiplantibacillus plantarum and Levilactobacillus brevis* with technological and bioactive potential as starter cultures for sourdough production

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Starter culture

ABSTRACT

This study investigated sourdoughs from different climatic regions in northeastern Brazil to identify autochthonous strains of lactic acid bacteria (LAB) and evaluate their technological potential. Sourdough samples were collected from humid, sub-humid, and semi-arid regions, yielding 129 bacterial isolates. Of these, 24 strains exhibited typical LAB characteristics and were identified via MALDI-TOF mass spectrometry as *Lactiplantibacillus plantarum*, *Companilactobacillus paralimentarius*, and *Levilactobacillus brevis*. *C. paralimentarius* dominated in humid regions, while *L. plantarum* prevailed in other climates. From principal component analysis, seventeen strains with good technological potential were selected based on acidification, proteolytic activity, and exopolysaccharide production. These strains then underwent tolerance tests for acid, salt, sucrose, and ethanol stress. *L. plantarum* 47 (Lp47) exhibited superior acid and salt tolerance. *L. brevis* 83 (Lb83) presented tolerance to high ethanol concentrations. These strains were then used as starter cultures to evaluate fermentative performance. Lp47 and Lb83 demonstrated rapid growth and acidification under fermentation stress, respectively acidifying the medium at 4 and 6 h. Both strains progressively enhanced antioxidant activity and phenolic compound production during fermentation, with no significant differences ($p > 0.05$). Rheological tests revealed that sourdoughs with Lp47 and Lb83 were less sticky, more elastic, and exhibited greater deformation resistance after 24 h. These findings underscore the potential of Lp47 and Lb83 as effective starter cultures for sourdough production, ensuring faster, controlled fermentation while enhancing textural and bioactive properties, particularly for large-scale applications. Exploring the biotechnological potential of sourdough fermentation processes expands our understanding and opens new possibilities in gastronomy and food science for developing functional, healthy and high value food products. Our study highlights the importance of autochthonous strains in enhancing fermentation. These strains accelerated production, improved efficiency, and enhanced bread quality, demonstrating their potential to significantly impact the food industry.

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1. Introduction

Fermentation is one of the oldest food processing techniques. It is renowned for its ability to enhance flavor, improve nutrient bioavailability, and offer functional benefits (Fan et al., 2024). In baking, fermentation transforms dense dough into a leavened product, contributing to texture and flavor enhancement (De Vuyst et al., 2017).

The biochemical changes that occur in naturally fermented bread can be perceived in the texture and flavor of baked goods, as well as in its increased volume, shelf life, and improved nutritional value (Fang et al., 2023; Sanmartín et al., 2024). Among the benefits provided by the action of LAB is the production of exopolysaccharides (EPS) by specific species, such as *L. plantarum* and *L. brevis*, which, in addition to influencing the texture and rheology of dough, contribute to consumer health by stimulating short-chain fatty acid (SCFA) production, which can prevent inflammation and intestinal infections (Gill et al., 2018; Pérez-Alvarado et al., 2022).

Resistant starch is also formed, leading to reductions in the glycemic index (Arora et al., 2021). The allergenic potential of wheat and rye flours is also reduced due to proteolytic activity and the breakdown of the gluten network and its fractions (Păcularu-Burada et al., 2020; Pérez-Alvarado et al., 2022). The antifungal activity of LAB allows for the production of bread without the addition of chemical preservatives (Papadimitriou et al., 2019; Pérez-Alvarado et al., 2022). LAB also produce a wide range of bioactive compounds. (Pérez-Alvarado et al., 2022).

The demand for large-scale sourdough production has been rising due to growing consumer interest in healthy, natural products (Paucean et al., 2024; Albagli et al., 2021). In 2020, the global sourdough market was valued at US$2.19 billion, with an anticipated annual growth rate of 8 % through 2030 (Lima et al., 2023). Major baking companies have integrated sourdough products into their portfolios to meet consumer demand (Lima et al., 2023).

Sourdough can be categorized into four main types. Type I sourdough is produced through spontaneous fermentation involving lactic acid bacteria (LAB) and yeasts, and requires daily refreshment via backslopping—a technique involving re-inoculating previous dough (mother dough) with fresh flour and water until LAB stabilization is achieved. This process demands both time and skilled handling, presenting challenges for standardization and cost efficiency (Reale et al., 2020).

To address these limitations, starter cultures can be employed. These cultures are tailored to withstand baking conditions and microbial competition from ingredients, the environment, and tools. The deliberate use of specific strains facilitates targeted fermentation, imparting desired sensory, technological, functional, and nutritional traits to the final product (Montemurro et al., 2020). Types II, III, and IV sourdoughs exemplify such applications: Type II involves high-concentration starter cultures without backslopping, Type III undergoes drying, and Type IV combines starter inoculation with backslopping (Pérez-Alvarado et al., 2022; Catzeddu, 2019; Papadimitriou et al., 2019).

Geographic location has been shown to influence the microbial composition of sourdough (Suo et al., 2020). Successful starter cultures must adapt to the sourdough's ecological conditions to dominate fermentation (De Vuyst et al., 2014). Autochthonous strains, which evolve in the challenging environment of spontaneous fermentations, are highly resilient and efficient in fermentation.

Despite its distinct climatic regions, there is a lack of studies in northeastern Brazil which evaluate LAB diversity or which identify predominant fermentation species. Different climates can influence the microbial composition of natural leavens (Landis et al., 2021; Pérez-Alvarado et al., 2022). Isolating, identifying, and studying these compositions allows identification of strains adapted to specific regions that bring unique characteristics to bakery products.

Focusing on technological functionality and bioactive potential, this study sought to develop sourdough using LAB strains isolated from sourdough samples collected across the varying climate regions of northeastern Brazil. We sought to evaluate the performance of autochthonous strains when used to initiate sourdough fermentation.

2. Materials and methods

2.1. Sample collection

Sourdough samples were collected from three different climatic regions in the northeastern Brazil: two samples from the humid region (João Pessoa/PB - 7.1193° S, 34.8754° W), two samples from the subhumid region (Solânea/PB, Brazil - 6.7572° S, 35.6577° W), and two samples from the semi-arid region (Cajazeiras/PB, Brazil - 6.8917° S, 38.5613° W). Sourdough fermentation requires time and specialized professionals, which implies higher costs, (Catzeddu, 2019). For this reason, establishments which produce sourdoughs are still scarce in the region. A 100g portion of active sourdough was collected (in duplicate) from each establishment at approximately 8 h after the last propagation. The samples were placed in insulated containers with ice and transported to the Food Microbiology Laboratory at the Center for Technology and Regional Development (CTDR) of the Federal University of Paraíba (UFPB).

2.2. Isolation, pre-identification, and culture conditions

The isolation of LAB followed the method proposed by Garcia et al. (2016). Using the spread plate technique, 25g aliquots of sourdough were diluted in 225 mL of peptone water, followed by serial dilution (10^{-6}), and inoculation in Petri dishes containing De Man, Rogosa, and Sharpe (MRS) agar (HiMedia, Mumbai, India). The plates were incubated at 37 ± 0.5 °C for 48 h in a bacteriological incubator (ACB 80 Labor, Americana, Brazil).

After incubation, colonies displaying distinct morphologies were selected, with five colonies from each morphology isolated for further testing. These colonies were incubated again at 37 ± 0.5 °C for 48 h and subsequently subjected to phenotypic tests for pre-identification. Isolates classified as Gram-positive and catalase-negative, exhibiting cocci, coccobacilli, or bacilli morphology, were selected for further study. The selected isolates were stored at −18 ± 0.5 °C in cryotubes containing MRS broth (HiMedia, Mumbai, India) supplemented with glycerol (Sigma Aldrich, St. Louis, USA; 20 mL/100 mL).

For the assays described in items 2.3.1, 2.3.2, 2.3.3, and 2.3.4, strains were prepared by collecting 100 μL aliquots from each cryotube which had been inoculated in 10 mL of MRS broth and incubated at 37 ± 0.5 °C for 48 h. 20 μL of these broth cultures were subsequently plated onto MRS agar and incubated again at 37 ± 0.5 °C for 48 h. Identification was performed using Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry, as described by Freiwald and Sauer (2009). Biomass from isolated colonies was transferred to a stainless steel MALDI-TOF plate using a sterile toothpick. Each sample was treated with 1 μL of formic acid solution and then (after drying) with 1 μL of a solution containing alpha-cyano-4-hydroxycinnamic acid in an acetonitrile:water mixture (1:1:1) at 10 mg/mL (Bruker Daltonics, Bremen, Germany).

The samples were analyzed using a Bruker Biotyper 4.1 MALDI-TOF mass spectrometer, calibrated with the Bruker Bacterial Test Standard (BTS). The mass spectra generated were processed using the MALDI Biotyper 4.1 software (Bruker Daltonics, Bremen, Germany), and results were expressed as BioTyper log scores, indicating similarity between the unknown isolates and reference database entries. Isolates with log scores above 2.0, indicating a high probability of species identification, and classified under Generally Recognized as Safe (GRAS) genera, were selected for further study. The identification spectra were also compared with entries in the Bruker Biotyper reference library.

2.3. Technological Potential Tests for Strain Selection

All strains identified as LAB were subjected to the selection tests described below.

2.3.1. Acidification and growth

The methodology described by Reale et al. (2020) was employed to assess acidification and growth. pH values were recorded using a pH meter (Model Q400AS, Quimis, Diadema, São Paulo, Brazil). Optical density (OD_{595}nm) was measured using an Eppendorf BioSpectrometer Basic (Eppendorf AG, Germany). Measurements were taken at 0, 2, 4, 6, and 18 h. Results were expressed as changes in OD_{595nm} at each time point relative to the initial reading (0 h).

2.3.2. Exopolysaccharide (EPS) production

EPS production was quantified following the method outlined by Rodrigues et al. (2021). Precipitates were collected through centrifugation, washed with sterile distilled water, and dried at 55 ± 0.5 °C until reaching a constant weight. The results for EPS were determined by measuring dry weight (mg/L).

2.3.3. Proteolytic activity

Proteolytic activity was evaluated in accordance with Domingos-Lopes et al. (2017). A 10 μL aliquot of each isolate was inoculated onto MRS agar plates containing lactose-free skim milk (Camponesa, Lagoa da Prata, Minas Gerais, Brazil) using micro drops and incubated at 37 ± 0.5 °C for 48 h. Following incubation, plates were rinsed with 1N HCl (P.A. Proquímios©, Rio de Janeiro, RJ, Brasil). Positive proteolytic activity was indicated by the formation of a clear zone around colonies.

2.3.4. Salt, sucrose, acid, and ethanol tolerance

Tolerance testing followed the protocol described by Reale et al. (2020). Isolates were cultured to an OD_{595nm} of 0.6, and inoculated at 10 % (v/v) into MRS broths (1.8 mL) modified to simulate stress conditions, including: acidic solutions (pH 3.5, 4.5, and 6.5), sodium chloride solutions (2 %, 4 %, and 6 % NaCl), sucrose solutions (20 % and 30 % sucrose), and ethanol solutions (2 %, 4 %, and 6 % ethanol). Samples were incubated for 24 h at 35 ± 0.5 °C under anaerobic conditions (Permution®, Model JA0400, Curitiba, PR, Brazil) using an Anaerobac® anaerobic atmosphere generator. Optical density (OD_{595nm}) was measured by spectrophotometry at 0h, 6 h, and 24 h. Results were expressed as the difference in OD between 6 h or 24 h and the initial measurement at 0h.

Based on the results obtained for the analyzed LAB strains, and following the methods described in item 2.3, a PCA assay was performed to identify the strains with the best technological performance, considering the characteristics preferred for sourdough inoculation (described in item 2.6).

2.4. Preparation of sourdough

Sourdough was prepared following the protocol of Minervini et al. (2010) using 100 g of type 1 wheat flour (Finna, Fortaleza, Brazil), 50 mL of potable water, and 10 mL of selected cultures (100 μL aliquots from *Lactiplantibacillus plantarum* 47 and *Levilactobacillus brevis* 83 cryotube were inoculated in 5 mL of MRS broth and incubated at 37 ± 0.5 °C/48 h, followed by inoculum adjustment until OD_{595nm} = 2, equivalent to 8.00 log. CFU/mL). The samples were incubated in a Biochemical Oxygen Demand (B.O.D) incubator at 30 ± 0.1 °C. Immediately after preparation (t_0), samples were analyzed according to the procedures described in section 2.5.

2.5. Physicochemical, microbiological, bioactive, and technological characterization of sourdough inoculated with Lactiplantibacillus plantarum 47 and Levilactobacillus brevis 83

2.5.1. pH and total titratable acidity

Using a pH meter with a glass electrode, pH was measured in 10 g of sourdough (triplicate). Total titratable acidity (g/100 g) was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator (Association of Official Analytical Chemists International - AOAC, 2016) at 0, 2, 4, 6, 8, and 24 h.

2.5.2. Profile and quantification of phenolic compounds

An ethanolic extract (5g/50 mL) of fermented dough was prepared for quantification and profiling phenolic compounds according to Fang et al. (2023). For total phenolic content by spectrophotometric determination using Folin-Ciocalteu reagent, absorbance was measured at 765 nm, with readings at 0, 8, and 24 h. Gallic acid was used to prepare a standard curve, and results were expressed as Gallic Acid Equivalents (GAE) per gram of sample. The individual phenolic compounds were analyzed by high-performance liquid chromatography (HPLC) using an Agilent 1260 liquid chromatography system with diode array detection (DAD), as described by Lima et al. (2024). Supplementary Table A8 presents the external standards used for quantification and the method validation parameters. Chromatograms of the sample at 0 and 24 h of fermentation are presented in the supplementary data (Figure A.2).

2.5.3. Antioxidant activity

Antioxidant activity was evaluated using an ethanolic extract (100 g/kg) of fermented dough to assess ABTS•+ radical scavenging activity, based on Fang et al. (2023). Absorbance at OD_{734nm} was measured at 0, 8, and 24 h. A 15 % ethanol mixture was used as the blank, and a standard curve was constructed using Trolox solution. Results were expressed as Trolox Equivalents per gram of sample.

2.5.4. Viability of LAB

LAB viability was determined at 0, 2, 4, 6, 8, and 24 h of fermentation.

Aliquots (20 μL) from serial dilutions were inoculated onto MRS agar plates using the micro drop method (Liu et al., 2018) and incubated at 37 ± 0.5 °C for 48 h. Viable cell counts were expressed as log CFU/g.

2.5.5. Sugars and organic acid

Maltose, sucrose, glucose, fructose, ethanol, lactic acid, acetic acid, glycerol, and mannitol concentrations were analyzed using HPLC, according to the methods of Paramithiotis et al. (2006) and Gunduz et al. (2022). Supernatants were filtered through a 0.45 μm syringe filter, and 10 μL was injected into an Agilent Hi-Plex H column (7.7 × 300 mm, 8 μm, Santa Clara, USA) with a DAD and refractive index detector (RID). Elution was performed at 35 ± 0.5 °C using 5 mM H_2SO_4 at a flow rate of 0.5 mL/min. The data were processed using the OpenLAB CDS ChemStation EditionTM software (Agilent Technologies). The HPLC sample peaks were identified by comparing their retention times with those of sugar (Sigma-Aldrich, St. Louis, USA), and organic acid (Vetec Química Fina, Rio de Janeiro, Brazil) standards.

2.5.6. Rheological properties of sourdough inoculated with Lactiplantibacillus plantarum 47 and Levilactobacillus brevis 83

Rheological analysis was conducted using a Haake MARS III rheometer (Karlsruhe, Germany) equipped with a parallel plate system (diameter: 3.5 cm, gap: 1 mm) at 30 ± 0.5 °C. Experiments were performed after 8 and 24 h of fermentation, in accordance with Amaral et al. (2022). To determine the linear viscoelastic region, a strain sweep (1–100 Pa) at 1 Hz was conducted, followed by mechanical spectra through frequency sweeps from 0.1 to 10 Hz, with a fixed strain within the linear viscoelastic range.

The complex modulus (G) and the loss tangent (tan δ) were calculated

using Equations (1) and (2) below.

$$G^{*} = \sqrt[2]{(G')^{2}+(G'')^{2}} \quad (1)$$

$$Tan(\delta) = \frac{G''(\omega)}{G(\omega)} \quad (2)$$

2.5.7. Texture profile analysis

Texture analysis was performed at 8 and 24 h of fermentation following the methodology of Amaral et al. (2022). Hardness and adhesiveness were assessed under compression conditions using a Texture Analyzer CT3 (Brookfield Engineering Labs, Middleboro, USA) equipped with a cylindrical acrylic probe (TA10, 12.5 mm diameter). Measurements were carried out in two compression cycles, with a deformation of 50 % and a test speed of 1 mm/s. Data acquisition and analysis were conducted using the Texture Expert for Windows software (version 1.19).

2.6. Statistical analysis

All assays were performed in triplicate, with results expressed as mean values ± standard deviation. Statistical significance ($p < 0.05$) was determined using Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. Data calculations were performed using Jamovi software (version 2.3.28). Principal Component Analysis (PCA) was applied to evaluate and categorize the datasets derived from the selection and tolerance tests (2.3 Technological Potential Tests for Strain Selection). The resulting groups were divided into quartiles.

3. Results and discussion

3.1. Isolation, pre-identification, and identification of isolates using mass spectrometry (MALDI-TOF)

Approximately 129 isolates were obtained from sourdough samples collected in northeastern Brazil. Of these, 24 were characterized as gram-positive, catalase-negative bacteria, displaying cocci, bacilli, and coccobacilli morphologies. These characteristics, which are typical of LAB (Gopal, 2020; De Vuyst et al., 2021; Sevgili et al., 2023), were used as criteria for the selection of isolates for subsequent stages of the study. The LAB isolates (24) were identified using mass spectrometry, yielding Log BioTyper scores greater than 2.0, which is an indicative high-confidence identification. Of these strains, 12 originated from the humid region (João Pessoa city), six from the sub-humid region (Solânea city), and six from the semi-arid region (Cajazeiras city), this is summarized in Table 1.

The identification of the isolates revealed three distinct microbial groups, with *Companilactobacillus paralimentarius* predominating in the humid region, and *Lactiplantibacillus plantarum* being dominant in both the sub-humid and semi-arid regions. Previous studies by De Vuyst et al. (2017) and Comasio et al. (2020) have indicated that restricted microbial compositions in individual sourdoughs are common, particularly in mature sourdoughs, due to intense competition between bacterial strains. The presence of these LAB species highlights both their potential to adapt to the specific sourdough fermentation ecosystem, and the diversity within the same species (De Vuyst et al., 2017).

Table 01
Identification of LAB from Different Climatic Regions of northeastern Brazil by MALDI-TOF Mass Spectrometry.

Sourdough	Total Number of Isolates	Identification	Number of Identified Isolates
JP-H	12	*Lactiplantibacillus plantarum*	5
		Companilactobacillus paralimentarius	7
SL-SU	6	*Lactiplantibacillus plantarum*	4
		Levilactobacillus brevis	2
CJ-SA	6	*Lactiplantibacillus plantarum*	3
		Companilactobacillus paralimentarius	1
		Levilactobacillus brevis	2

JP-H: João Pessoa (Humid); SL – SU: Solânea (sub-humid); CJ-SA: Cajazeiras (semi-arid).

The sourdough microbiota represents a complex and dynamic ecosystem, and both its composition and functionality are significantly influenced by exogenous factors (Gunduz et al., 2022; Pérez-Alvarado et al., 2022) such as temperature. Using different temperatures, Menezes et al. (2020) propagated a single sourdough and found microbiota variations. As an example, *Lactiplantibacillus plantarum* is not typically found at temperatures below 30 °C (De Vuyst et al., 2017).

Landis et al. (2021) investigated 500 sourdough samples from four continents and identified *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* as the most frequently coexisting species. Reale et al. (2020) found that among 73 isolates obtained from Italian sourdoughs, *Lactiplantibacillus plantarum* was the most prevalent. These findings confirm the species identified within the studied sourdoughs as characteristic of this type of fermentation product. According to De Vuyst et al. (2017), species such as *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* are present because they are tolerant to acidic conditions, which are common in sourdoughs.

Given the complexity involved in sourdoughs, their composition of microorganisms and their maintenance, it is important to know the strains that best adapt to each region studied, in order to develop sourdough with desirable and predictable characteristics. Considering industrial-scale production and standardization of the final product, this preliminary guidance is essential.

3.2. Strain Selection - Technological Potential Tests

Tests were conducted to evaluate the acidification capacity and growth of each strain at different incubation times, as well as both exopolysaccharide (EPS) production and proteolytic activity (Table A.2).

In the acidification capacity test, the pH values ranged from 7.94 to 4.00. Of the 24 strains analyzed, 13 maintained a pH below 4.5 at 18 h of incubation. Acidification is a key characteristic of LAB and represents an important technological attribute of sourdough (Reale et al., 2020; Arora et al., 2021; Gunduz et al., 2022). In mature sourdoughs, pH typically ranges between 3.8 and 4.5, influenced by multiple factors (Catzeddu, 2019).

As to growth, *Levilactobacillus brevis* 83 exhibited an OD_{595nm} of 3.74 after 18 h, thereby demonstrating superior performance in comparison to the other strains. Seventeen strains exhibited an OD_{595nm} greater than 0.6 within 8 h of incubation, indicating robust multiplication, a desirable for starter cultures used in sourdough fermentation (Reale et al., 2020). Rapid LAB growth and concomitant acidification are important technological features, particularly at an industrial scale, since they accelerate fermentation and impart desirable artisanal qualities to products in a shorter time frame (Arora et al., 2021; Gunduz et al., 2022).

All analyzed strains produced EPS in amounts ranging from 0.1 to 5.7 mg/L. Aktepe et al. (2024) reported that *L. brevis* produced between 5.5 and 17.14 g/L, while *L. plantarum* ranged from 4.99 to 17.17 g/L. In the same study, it was found that *C. crustorum, C. paralimentarius*, and *Ll. curvatus* did not produce EPS. Manini et al. (2016) reported similar results for *L. brevis, L. sakei*, and *Ln. mesenteroides.* In baking, EPS improves rheological, textural, and sensory properties (Reale et al., 2020). EPS also improves the shelf life of baked goods (Pérez-Alvarado et al., 2022), provides health benefits, stimulates SCFA production, and reduces intestinal inflammation and infections (Gill et al., 2018; Pérez-Alvarado et al., 2022).

All of the isolates demonstrated proteolytic activity, which can

enhance the extensibility and volume of breads, especially those made with strong flours (Santos et al., 2024).

Based on the technological test results, principal component analysis (PCA) was performed to screen the most promising strains for further selection. The selected strains presented superior performance in the evaluated tests, and are shown clustering near the variable arrows on the PCA plot in Fig. 1. The strains selected for further testing were 32, 34, 39, 41, 47, 53, 66, 79, 80, 81, 82, 83, 84, 113, 114, 121, and 123.

3.3. Tolerance to salt, sucrose, acid, and ethanol

After principal component analysis (PCA), the selected strains were subjected to tests assessing their adaptation to acidic environments, as well as their tolerance to salt, sucrose, and ethanol - conditions commonly encountered in sourdough fermentation of both sweet and savory breads. The tests aimed to identify versatile strains capable of ensuring consistent and high-quality fermentation across diverse conditions.

3.3.1. NaCl tolerance test (OD_{595} nm) at different concentrations after 24 hours of incubation of selected LAB strains

The sodium tolerance test, minimum and maximum OD_{595nm} values at 6 h ranged from 0.00 to 2.75 (Table A.3). The performance and identification of the 17 strains after 24 h are presented in Fig. 2A. The strain clustering at different NaCl concentrations can be observed, revealing the strain associated with the best adaptation. Strains incubated at the highest sodium chloride concentrations exhibited significant growth, suggesting an adaptive response during the initial hours of exposure. All LAB presented high tolerance to salt ($OD_{595nm} > 3.00$), with *Lactiplantibacillus plantarum* 47 demonstrating the highest growth at 6 % NaCl.

Reale et al. (2020) has reported that *Fructilactobacillus sanfranciscensis*, *Lactobacillus brevis*, and *Furfuralibacterium rossiae* exhibit tolerance to 6 % NaCl. However, Gänzle et al. (1998) observed growth inhibition of *F. sanfranciscensis* in a medium containing 4 % NaCl, highlighting the strain's sensitivity to osmotic stress, and underscoring the importance of selecting robust strains capable of maintaining efficacy as starter cultures for fermented foods.

3.3.2. Sucrose tolerance test (OD_{595} nm) at different concentrations after 24 hours of incubation of selected LAB strains

Sucrose, widely used in the production of sweet breads, fermented cakes, and French pastries (Reale et al., 2020), is metabolized by some hetero-fermentative LAB (De Vuyst et al., 2017). However, high sucrose concentrations induce osmotic stress, potentially inhibiting growth during fermentation (Ge et al., 2011). The OD_{595nm} values for strains incubated in sucrose solutions varied between 0.04 and 12.01 (Table A.4). Fig. 2B presents the performance of the 17 strains at 24 h. In the figure, the clustering of strains at different sucrose concentrations can be observed, revealing the strain associated with the best adaptation. The LAB exhibited growth ($OD_{595nm} > 4.00$) and resistance to high sugar concentrations, with *L. plantarum* strains 81, 80, 53, and 47 reaching $OD_{595nm} > 7.00$.

In related experiments, Reale et al. (2020) found a significant reduction in LAB growth in MRS broth with 30 % sucrose ($OD_{595nm} \cong 0.3$), and moderate growth at 20 % sucrose ($OD_{595nm} \cong 0.6$). *L. pseudomesenteroides* was identified as the most tolerant species, while *Companilactobacillus paralimentarius* and *Lactiplantibacillus paraplantarum* were among the most sensitive. Vilanova et al. (2015) observed that *Leuconostoc mesenteroides* benefited from sugar addition, underscoring the diverse behaviors exhibited by different LAB species and strains. These data highlight the importance of using strains acclimated to the intended fermentation environment.

3.3.3. pH tolerance test (OD_{595} nm) of selected LAB strains at different pH values after 24 hours of incubation

The acid stress test, OD_{595nm} values ranged from 0.07 to 9.87 (Table A.5). At 24 h at pH 3.5, strains *L. plantarum* 47 and 53 demonstrated resistance, with OD_{595nm} values of 5.73 and 1.29, respectively, while the remaining strains presented an $OD_{595nm} < 0.6$. At pH 4.5, all strains exhibited robust growth ($OD_{595nm} > 2.00$). Fig. 2C illustrates the identification and performance of the 17 LAB after 24 h of incubation. In the figure, one may observe the clustering of strains at different pH concentrations, the strain associated with the best adaptation is emphasized.

Gunduz et al. (2022) reported that *Lactiplantibacillus plantarum* remains viable at pH 3.5, whereas *Fructilactobacillus sanfranciscensis* does not. Selecting LAB strains tolerant to low pH is critical to ensuring the viability and efficacy of starter cultures in fermentation processes. *L. plantarum* strains 47 and 53 demonstrated tolerance to low pH,

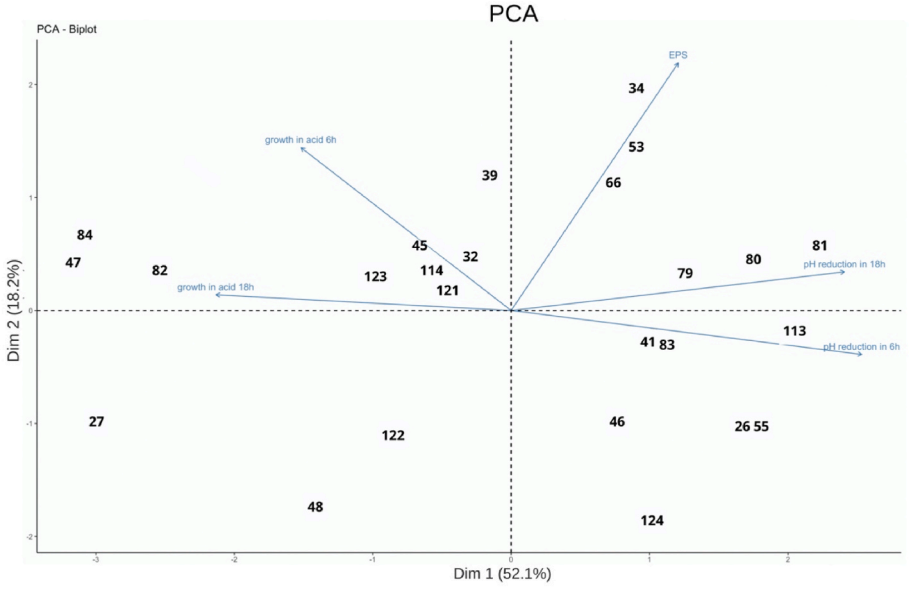

Fig. 1. Principal component analysis relating to technological tests.

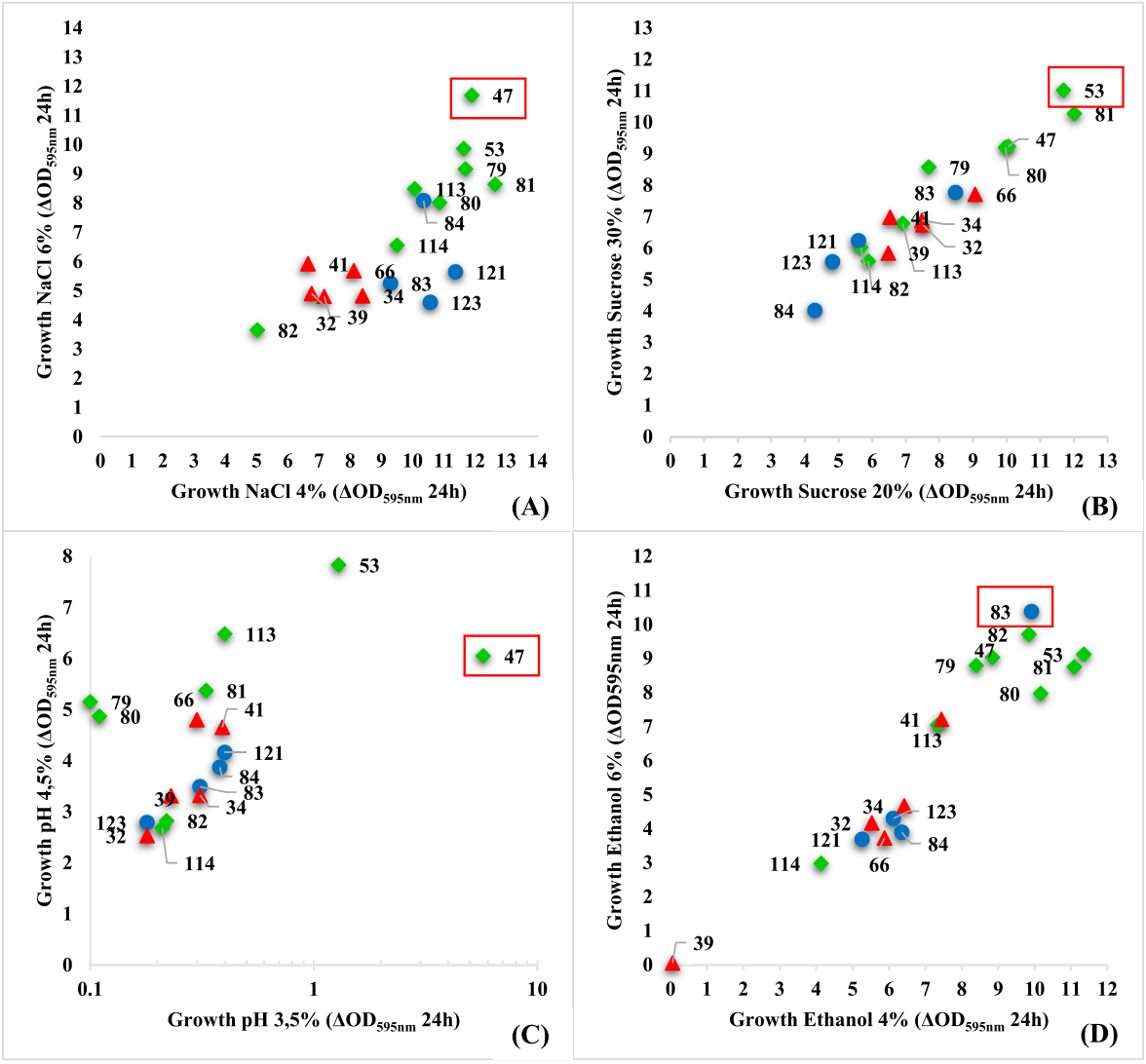

Fig. 2. Scatter plot showing the distribution of the 17 LAB strains based on growth capacity (OD_{595nm}) in MRS supplemented with: A: NaCl at 4 % (w/v) and 6 % (w/v) after 24 h of incubation. B: Sucrose at 20 % (w/v) and 30 % (w/v) after 24 h of incubation. C: Acidified pH at 3.5 and 4.5 after 24 h of incubation. D: Ethanol at 4 % (w/v) and 6 % (w/v) after 24 h of incubation. *Lactiplantibacillus plantarum* (◆); *Companilactobacillus paralimentarius* (▲); *Levilactobacillus brevis* (●).

making them effective under rapid acidification and promoting reproducibility and stability in industrial applications (Gunduz et al., 2022).

3.3.4. *Ethanol tolerance test (OD_{595} nm) after 24 hours of incubation of selected LAB strains*

Hetero-fermentative LAB metabolize carbohydrates to produce various byproducts, including ethanol (Gopal, 2020; Sevgili et al., 2023). Ethanol is also generated in yeast metabolism (De Vuyst et al., 2017; Fu et al., 2024) and typically accumulates to high levels during sourdough fermentation, potentially limiting LAB metabolic activity (Pradal et al., 2024). In the ethanol stress test, OD_{595nm} values ranged from 0.01 to 11.35 (Table A.6), and except for *Companilactobacillus paralimentarius* 39, all strains demonstrated high viability (OD_{595nm} > 2.00) at 24 h of incubation and at varying ethanol concentrations. Fig. 2D illustrates the performance of the strains after 24 h. In the figure, it is possible to observe the clustering of strains at different ethanol concentrations, the strain associated with the best adaptation is emphasized. At 6 % ethanol, *Levilactobacillus brevis* 83 presented the highest tolerance, while *L. plantarum* 53 exhibited the highest tolerance at 4 % ethanol.

Pradal et al. (2024) observed that ethanol accumulation during fermentation, averaging 3 g/kg at 48 h, can significantly affect LAB metabolism. Reale et al. (2020) identified *Furl. rossiae*, *L. plantarum*, and *F. sanfranciscensis* as the most ethanol-tolerant species. At 4 % ethanol, LAB demonstrated average growth ($OD_{595nm} \cong 0.9$), and reduced growth at 6 % ethanol concentrations ($OD_{595nm} < 0.8$), indicating the negative impact of ethanol.

The selection of LAB strains with capacity to tolerate ethanol is of crucial importance for control of the fermentation process. In this study, *L. plantarum* 47 exhibited high growth ($OD_{595nm} > 1.00$) within the first 6 h at 6 % ethanol, indicating its suitability for industrial-scale fermentation (due to rapid adaptation). *L. brevis* 83 exhibited the highest overall ethanol tolerance at 24 h.

3.3.5. *Selection of strains tolerant to various stress conditions*

The LAB strains underwent another PCA to identify the two best-performing strains in the tolerance tests,. Growth was assessed under various conditions, including pH 3.5, 6 % NaCl, 30 % sucrose, and 6 % ethanol. *L. plantarum* 47 demonstrated the highest tolerance to acidic and saline environments and was among the top five strains in other conditions. At high sucrose concentrations, *L. plantarum* 53 exhibited the greatest resistance and performed well across the other parameters. *L. brevis* 83 demonstrated superior performance at the highest ethanol concentration and achieved effective results in the other tests, and

marking the only instance where *L. plantarum* was outperformed.

Based on the PCA results (Figure A.1), *Lactiplantibacillus plantarum* 47 and *Levilactobacillus brevis* 83 were selected, as they clustered in the upper and lower right quadrants, respectively, where desirable traits are strongly represented. Further, *L. plantarum* 53 was temporarily excluded to enable the performance evaluation of different LAB species during fermentation.

3.4. Physicochemical sourdough characterization

The physicochemical parameters of sourdoughs inoculated with *Lactiplantibacillus plantarum* 47 and *Levilactobacillus brevis* 83 were evaluated (Table 02).

The pH of the samples ranged from 3.47 to 5.54. No significant difference ($p > 0.05$) was observed immediately after inoculation (0 h). However, after 6 h, the pH of *Lactiplantibacillus plantarum* 47 (Lp47) decreased to 3.87 and remained stable until 24 h. The final values were respectively 3.47 ± 0.02 and 3.57 ± 0.02 for Lp47 and Lb83. In a study conducted by Robert et al. (2006), two *L. plantarum* strains only reached pH < 4 after 15 h of fermentation. This was also observed by Üçok and Sert (2020). Similar results were reported by Gunduz et al. (2022) and Ventimiglia et al. (2015) in starters inoculated with *L. plantarum* strains, reaching pH < 4 after 12, and 21 h of fermentation.

Generally, in sourdough production, fermentation time can vary from 8 to more than 14 h (Gänzle, 2014). However, this time period is determined by the acidification of the dough. Thus, the sooner the dough acidifies, the faster its fermentation (Arora et al., 2021).

The rapid acidification of dough by starter cultures is particularly pronounced when autochthonous cultures are employed (Gunduz et al., 2022). Total titratable acidity (TTA) values ranged from 0.40 to 3.30 mg/100g, revealing an inverse relationship with pH. This is consistent with the accumulation of organic acids in the dough during fermentation (De Vuyst et al., 2017). In a study conducted by Manini et al. (2016), *L. brevis* and *L. plantarum* strains presented variations respectively ranging from 1.9 to 4.4, and from 1.8 to 10.2, by 8 h of fermentation.

3.5. Bioactive characterization

The phenolic compound, total phenolic content, and antioxidant activity (bioactive characteristics) of the sourdoughs inoculated with *Lactiplantibacillus plantarum* 47 and *Levilactobacillus brevis* 83 were evaluated during fermentation at the different times (Table 3).

As to the quantified phenolic compounds, of the 41 phenolic compounds analyzed, only two were present in the sample. Fumaric acid was detected in Lp47 at inoculation (0 h), but was subsequently degraded during fermentation. This has been observed by Verni et al. (2024). Epigallocatechin gallate (EGCG) appeared in Lp47 at inoculation, and increased over time. In Lb83, EGCG was first detected at 8 h and continued to accumulate until 24 h. EGCG is a catechin-class flavonoid known for its antioxidant properties (Silva Júnior et al., 2021), and for its potential protective effects against various diseases (Thanikachalam et al., 2020). Both L. *plantarum* and *L. brevis* strains have demonstrated ability to enhance catechin concentrations during fermentation (Gaur and Gänzle, 2023).

The conversion of EGCG during fermentation is likely facilitated by esterases, as indicated by changes in compound concentrations and the emergence of expected metabolites (Gaur and Gänzle, 2023). However, detailed studies on these metabolic pathways are still needed. According to recent reviews, there remains a scarcity of comprehensive research on the metabolism of phenolic compounds by lactobacilli during food fermentation, it remains a gap in the current literature.

Fermentation resulted in a notable elevation in the synthesis of phenolic compounds, with concentrations reaching 29.38 and 36.17 g/100g. A statistically significant progressive increase was observed ($p < 0.05$) throughout the study period. *Lactiplantibacillus plantarum* 47 (Lp47) demonstrated a 21.2 % increase relative to the initial time (0 h), while *Levilactobacillus brevis* 83 (Lb83) exhibited a 19.8 % increase. Graça et al. (2022) reported that phenolic compound content in wheat sourdough increased by 8.6 % after 24 h. Whole wheat flour inclusion further enhanced this increase to 38 %. The production of phenolic compounds offers multiple functional benefits, including antioxidant (Călinoiu and Vodnar, 2018) and anti-inflammatory effects (Fekri et al., 2024). Certain LAB species can release phenolic acids, and bound to the flour (Rizzello et al., 2019; Fekri et al., 2024), these can be converted into flavor precursors (Pérez-Alvarado et al., 2022).

To evaluate antioxidant properties, the ABTS•+ radical scavenging assay was performed on the inoculated sourdoughs, yielding respective minimum and maximum values of 8.47 and 11.77. Lb83 and Lp47 exhibited antioxidant activity increases of 39 % and 32 % relative to the initial time (0 h). The strains presented no statistically significant difference ($p > 0.05$) between each other. Fermentation not only enhanced the available phenolic content but also increased the antioxidant activity of the sourdoughs.

Table 02
Evaluation of pH and Total Titratable Acidity of sourdoughs inoculated with *Lactiplantibacillus plantarum* 47 and *Levilactobacillus brevis* 83, individually, during fermentation (0, 2, 4, 6, 8, and 24 h).

pH						
	0h	2h	4h	6h	8h	24h
Lp47	5.48 ± 0.01^{Aa}	5.43 ± 0.05^{Aa}	4.24 ± 0.03^{Ab}	3.87 ± 0.03^{Abc}	3.79 ± 0.03^{Ac}	3.47 ± 0.02^{Ac}
Lb83	5.52 ± 0.03^{Aa}	5.54 ± 0.01^{Aa}	5.09 ± 0.01^{Bb}	4.55 ± 0.19^{Bc}	4.11 ± 0.02^{Bd}	3.57 ± 0.02^{Ae}
Total Titratable Acidity (mg/100g)						
	0h	2h	4h	6h	8h	24h
Lp47	0.47 ± 0.06^{Aa}	0.63 ± 0.06^{Aa}	1.37 ± 0.15^{Ab}	2.00 ± 0.17^{Ac}	2.53 ± 0.15^{Ad}	3.30 ± 0.10^{Ae}
Lb83	0.40 ± 0.00^{Aa}	0.60 ± 0.00^{Aab}	0.83 ± 0.06^{Bb}	1.43 ± 0.06^{Bc}	1.90 ± 0.10^{Bd}	3.13 ± 0.06^{Ae}

$^{A–C}$ Identical uppercase letters in the same column do not differ statistically ($p > 0.05$) from each other.
$^{a-e}$ Identical lowercase letters in the same row do not differ statistically ($p > 0.05$) from each other. Lp47: *Lactiplantibacillus plantarum* 47; Lb83: *Levilactobacillus brevis* 83.

Table 03
Phenolic compounds, total phenolic content and antioxidant activity of sourdoughs inoculated with *Lactiplantibacillus plantarum* 47 and *Levilactobacillus brevis* 83, and fermented individually for 0, 8 and 24 h.

Phenolic Compounds quantified by HPLC[a] (µg/g)						
	Fumaric Acid			Epigallocatechin Gallate		
	0h	8h	24h	0h	8h	24h
Lp47	2.68	N/D	N/D	2.74	2.66	3.07
Lb83	N/D	N/D	N/D	N/D	3.64	4.03
Total Phenolic Content[b] (mg GAE/100g)						
	0h		8h		24h	
Lp47	29.85 ± 0.31^{Aa}		30.90 ± 0.37^{Aa}		36.17 ± 1.40^{Ab}	
Lb83	29.38 ± 0.62^{Aa}		31.75 ± 0.58^{Ab}		35.16 ± 1.07^{Ac}	
ABTS (µmol TE/100g)						
	0h		8h		24h	
Lp47	8.57 ± 0.85^{Aa}		9.33 ± 0.60^{Aa}		11.37 ± 0.76^{Ab}	
Lb83	8.47 ± 1.05^{Aa}		9.87 ± 1.26^{Aa}		11.77 ± 0.06^{Ab}	

$^{A–B}$ Identical uppercase letters in the same column do not differ statistically ($p > 0.05$) from each other.
$^{a-c}$ Identical lowercase letters in the same row do not differ statistically ($p > 0.05$) from each other.
N/D – Not detected. Lp47: *Lactiplantibacillus plantarum* 47; Lb83: *Levilactobacillus brevis* 83.
[a] Analysis performed by HPLC.
[b] Analysis performed by spectrophotometry.

Previous studies on various types of flour (Fang et al., 2023; Li et al., 2024; Liu et al., 2024) have similarly demonstrated increased antioxidant activity in sourdough breads inoculated with LAB. This supports the findings of our research. The results suggest that the studied strains effectively enhance the bioactive properties of sourdoughs. The viable cell count of LAB, expressed as log CFU/g (Table 4), in sourdoughs inoculated with Lp47 and Lb83 corresponded with the observed pH and total titratable acidity (TTA) values. Cell counts stabilized after 4 h for Lp47 and after 6 h for Lb83. This illustrates the rapid adaptive capacity of *L. plantarum* 47 in a short fermentation time. No significant difference ($p > 0.05$) in LAB viability was observed between the sourdoughs after 8 h of fermentation. Similar viability behavior for LAB strains isolated from sourdough has been reported in studies by Ventimiglia et al. (2015) and Gunduz et al. (2022).

In a related study, Fang et al. (2023) observed that strains isolated from different food matrices and inoculated into sourdough, including *Lactiplantibacillus plantarum*, stabilized at 8.5 log CFU/g only after 12 h. Similarly, Santos et al. (2024) reported that *Lactiplantibacillus pentosus* and *Lactobacillus fermentum* isolated from fruit reached an average count of 6.66 log CFU/g after 24 h of fermentation, showing a single logarithmic cycle increase over this period. The rapid growth and acidification of Lp47 and Lb83 in our study may be attributed to their autochthonous origin and their ability to adapt to the stress conditions typical of sourdough fermentation.

Autochthonous strains are noted for their high technological potential in fermentation processes. Through exposure to adverse conditions, these strains have developed traits that enable rapid growth and pH stabilization, ensuring efficient, targeted, and stable fermentations (Gunduz et al., 2022).

The content of sugars and organic acids at 0, 8, and 24 h of fermentation are summarized in Table 5. Maltose, glucose, and fructose were identified through HPLC analysis. Following inoculation, maltose was not detected in Lp47; however, its concentration progressively increased due to amylase activation during fermentation (Fu et al., 2024). The observed reduction in sugar levels is indicative of elevated LAB metabolic activity, as they deplete carbohydrate sources to produce organic acids (De Vuyst et al., 2017). This finding corroborates the TTA data.

In obligate and facultative hetero-fermentative LAB cultures, glucose is metabolized via the phosphoketolase pathway to produce lactic acid and ethanol, while fructose serves as an alternative electron acceptor, yielding acetic acid (Galle et al., 2011; Gänzle, 2015) and mannitol (Paramithiotis et al., 2006; Galle et al., 2011). The adaptive capacity of *Lactiplantibacillus plantarum* is attributed to its extensive metabolic versatility, facilitated by numerous sugar transport systems (Corsetti et al., 2016) as well as the production of stress proteins, which confer protection under adverse conditions (De Vuyst et al., 2017).

Organic acids play a pivotal role in natural fermentation systems, significantly affecting product structure, sensory characteristics, and shelf-life (Fang et al., 2023). The formation of organic acids is directly associated with the characteristic flavor and aroma of sourdough breads (Pérez-Alvarado et al., 2022), resulting in notes that can range from lactic, fruity, to toasted (Vilanova et al., 2015). Breads with more pronounced flavors have been gaining increasing popularity (Fekri et al., 2024), and are preferred by consumers (Zhao et al., 2024).

Some LAB species produce citric acid, which can be converted to succinate for regenerating reduced cofactors, or to lactic, acetic, or ethanolic acids (Gänzle, 2015).

Of the tested strains, only *Levilactobacillus brevis* 83 (Lb83) produced citrate, at a concentration of 17.22 g/L after 24 h.

Lactic acid, a primary product of LAB metabolism, follows various pathways depending on the bacterial species and process conditions. Lb83 exhibited the highest lactic acid concentration at inoculation (0 h), which then diminished over time. In contrast, *L. plantarum* 47 (Lp47) initially presented no lactic acid but accumulated it progressively during fermentation. As noted by Gänzle (2015) and Zhang et al. (2010), lactic acid oxidation (to acetic acid), accompanied by adenosine triphosphate (ATP) production, supports bacterial growth. This transformation is mediated by lactate Dehydrogenase, and depending on the strain, can lead to the formation of additional end products such as ethanol, formic acid, and CO_2 (De Angelis and Gobbetti, 2011).

Formic acid was present in both strains consistently, regardless of fermentation time, and in concentrations ranging from 0.17 to 0.74 g/kg. Formic acid is recognized for its antimicrobial properties (De Vuyst et al., 2017) and can originate from lactic acid degradation (De Angelis and Gobbetti, 2011) or from alternative citric acid metabolic pathways (Gänzle, 2015).

Table 04
Viable cell count of LAB (log CFU/g) in sourdoughs inoculated with *Lactiplantibacillus plantarum* 47 and *Levilactobacillus brevis* 83, individually, during fermentation at 0, 2, 4, 6, 8, and 24 h.

Sample	LAB (CFU/g)					
	0h	2h	4h	6h	8h	24h
Lp47	7.63 ± 0.21^{Aa}	7.98 ± 0.03^{Aa}	8.49 ± 0.09^{Ab}	8.85 ± 0.00^{Ab}	8.85 ± 0.00^{Ab}	8.86 ± 0.01^{Ab}
Lb83	7.57 ± 0.04^{Aac}	6.85 ± 0.21^{Ba}	8.01 ± 0.19^{Bc}	8.72 ± 0.06^{Bbd}	8.68 ± 0.004^{Ad}	8.71 ± 0.13^{Ad}

$^{A-C}$ Identical uppercase letters in the same column do not differ statistically ($p > 0.05$) from each other.
$^{a-d}$ Identical lowercase letters in the same row do not differ statistically ($p > 0.05$) from each other.
Lp47: *Lactiplantibacillus plantarum* 47; Lb83: *Levilactobacillus brevis* 83.

Table 05
Content of sugars and organic acids in sourdough inoculated with *Lactiplantibacillus plantarum* 47 and *Levilactobacillus brevis* 83 at different fermentation times.

Time	Strains	Sugars (g/kg)			Organic Acids (g/kg)		
		Maltose	Glicose	Frutose	Citric	Lactic	Formic
0h	**Lp47**	N/D	0.08	0.57	N/D	N/D	0.22
	Lb83	1.72	0.37	0.81	N/D	9.09	0.74
8h	**Lp47**	0.09	0.08	0.42	N/D	0.98	0.30
	Lb83	1.48	0.13	0.57	N/D	N/D	0.17
24h	**Lp47**	2.50	0.09	0.53	N/D	1.21	0.22
	Lb83	3.23	0.21	0.58	17.22	N/D	0.50

N/D – Not detected. Lp47: *Lactiplantibacillus plantarum* 47; Lb83: *Levilactobacillus brevis* 83.

3.6. Rheological and textural properties

At 24 h, the linear viscoelastic region of the samples was below 2 Pa of stress. The crossing of the elastic (G′) and viscous (G″) modulus curves at high stress indicates the onset of material flow, with Lp47 beginning to flow at 50 Pa and Lb83 showing greater structural integrity, and starting to flow at 75 Pa (Fig. 3A).

At 8 h, a linear G′ behavior was observed up to 100 Pa (Fig. 3A), consistent with the findings of Khatkar and Schofield (2002) for dough made with pure gluten. The stiffness of the dough at 8 h was approximately ten times greater than that at 24 h (Fig. 3A). These results suggest that the gluten network remains intact in the early stage of sourdough fermentation.

Certain factors, such as large protein aggregate breakdown into smaller fractions, explain this change during fermentation. Breakdown occurs due to the proteolytic capacity of LAB (Di Cagno et al., 2002), and the weakening of disulfide bonds, which destabilize the protein structures (Tomic et al., 2023). It is likely these changes that occurred in the dough inoculated with *Lp47* and *Lb83* during fermentation, knowing that after 24 h they reached respective pH values of 3.47 and 3.57, and TTA values of 3.30 and 3.13, (Table 02).

The mechanical spectrum of sourdoughs at 24 h is depicted in

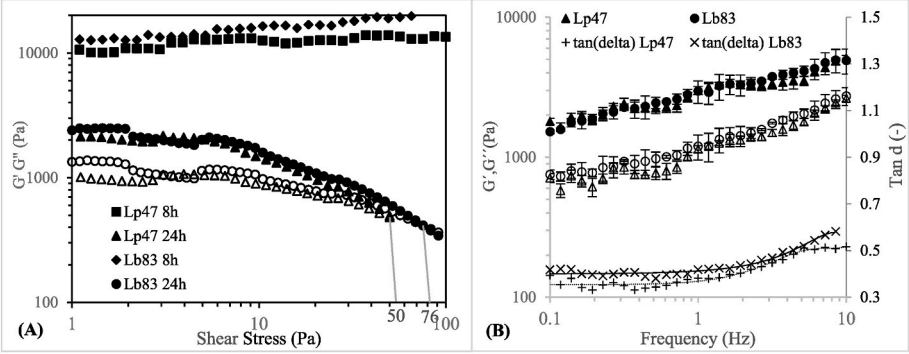

Fig. 3. Variation of Elastic and Loss Moduli as a function of shear stress (A) and frequency (B) for sourdoughs inoculated with *Lactiplantibacillus plantarum* 47 (Lp47) and *Levilactobacillus brevis* 83 (Lb83), individually, after 8 h and 24 h of fermentation. Closed symbol (G′) and open symbol (G″).

Fig. 3B. For both strains, the storage modulus (G′) was consistently higher than the loss modulus (G″), indicating the gel-like structure and predominantly elastic behavior in the samples (Galle et al., 2011; Abedfar et al., 2020; Li et al., 2022). Winter and Chambon (1986) define the gel point as the moment when tan δ becomes constant across frequency. This behavior was observed for the samples at 24 h of fermentation (Fig. 3B), up to 2 Hz for Lb83 and up to 1 Hz for Lp47.

Dough behaves more like a liquid when deformation occurs rapidly (Yu et al., 2019). Physical gels present a fragile molecular structure and some mobility at the gel point, and can break and flow (depending on the stress and deformation rate) due to the formation of large molecular aggregates. Most of the constituents in dough are small molecular size aggregates (Winter, 2016). Thus, after 24 h of fermentation, dough likely contains small aggregates of hydrolyzed gluten bound to starch granules. These can connect and break their physical bonds.

The complex modulus (G*) and loss factor (tan δ) values obtained in the linear region at 1 Hz are summarized in Table 6. No significant differences were observed in the tan δ values between samples, which ranged from 0.34 to 0.4. Khatkar and Schofield (2002) reported comparable loss modulus values of between 0.33 and 0.58 for various wheat flours.

A tan δ value of less than 1 indicates that the samples exhibit more solid/elastic behavior than viscous behavior (Li et al., 2022; Vela et al., 2023). Over the fermentation period, a significant decrease in G* was noted at 24 h, dropping from approximately 180 kPa–3.2 kPa. There were no significant differences ($p > 0.05$) based on the inoculated strain type.

Table 06
Rheological properties under shear (at 1Hz) and compression of sourdoughs inoculated with *Lactiplantibacillus plantarum* 47 and *Levilactobacillus brevis* 83, individually, after 8h and 24h of fermentation.

Strains	Shear Measurements			
	Complex Modulus – G* (kPa)		Tan δ (–)	
	8h	24h	8h	24h
Lp47	155.3 ± 31.9^{Aa}	3.2 ± 0.2^{Ab}	0.34 ± 0.07^{Aa}	0.38 ± 0.00^{Aa}
L83	180.8 ± 45.8^{Aa}	3.2 ± 0.6^{Ab}	0.38 ± 0.05^{Aa}	0.40 ± 0.00^{Aa}
Cepas	Compression measurements			
	Hardness (N)		Adhesiveness (mJ)	
	8h	24h	8h	24h
Lp47	0.48 ± 0.04^{Aa}	0.60 ± 0.08^{Aa}	2.87 ± 0.50^{Aa}	1.43 ± 0.15^{Ab}
Lb83	0.42 ± 0.06^{Aa}	0.40 ± 0.04^{Aa}	4.37 ± 0.61^{Ba}	1.07 ± 0.20^{Ab}

[A] Identical uppercase letters in the same column do not differ statistically ($p > 0.05$) from each other.
[a-b] Identical lowercase letters in the same row do not differ statistically ($p > 0.05$) from each other.
Lp47: *Lactiplantibacillus plantarum* 47; Lb83: *Levilactobacillus brevis* 83.

Van Bockstaele et al. (2008) established an inverse relationship between the complex modulus (G*) and bread volume, indicating that when G* < 15 kPa, higher bread volumes are achieved. Thus, the sourdoughs inoculated with Lp47 and Lb83, and exhibited G* values within this range at 24 h, were well-suited for producing breads with increased volume.

Compression testing was conducted to evaluate the hardness and adhesiveness of sourdoughs at 8 and 24 h (Table 6). Hardness is defined as the maximum force (N) applied during deformation (Ndjang et al., 2024), and presented no significant difference ($p > 0.05$) between the Lp47 and Lb83 sourdoughs at the analyzed time points. Adhesiveness represents a material's tendency to adhere to a surface upon contact, and is quantified as the energy required to separate the adhered surfaces (Amaral et al., 2022).

The sourdough inoculated with Lb83 exhibited significantly higher adhesiveness ($p < 0.05$) than Lp47 after 8 h of fermentation. At 24 h, the adhesiveness of Lp47 had decreased by half, while that of Lb83 was reduced fourfold; though at this stage, no significant difference was observed between the two formulations. Üçok and Sert (2020) observed opposing results during fermentation, reporting an increase in the adhesiveness of the ferments inoculated with *L. plantarum*.

According to Yildiz et al. (2012), increased gluten concentration contributes to greater adhesiveness in flour dough. Thus, the reduction in adhesiveness observed over the fermentation period for both the Lp47 and Lb83 samples may be attributable to partial hydrolysis of the gluten network. Adhesiveness directly affects dough handling and in large-scale production, can lead to machine contamination, waste, and financial losses due to production interruptions (Üçok and Sert, 2020).

The rheological results, under both compression and shear conditions whether at high or low deformation, indicate that sourdoughs exhibit better properties for use in bread after 24 h of fermentation. Specifically, they display less adhesiveness, enhanced elasticity, and greater flow under high deformation, facilitating a more homogeneous mixing process with other dough ingredients and lowering energy costs during preparation.

4. Conclusion

LAB identification revealed that the climatic characteristics of the sourdough's origin, influence microbial composition, leading to distinct microbial groupings. *Lactiplantibacillus plantarum* was the only species present across all three regions. Overall, the results demonstrated the feasibility of using Lp47 or Lb83 as starter cultures for sourdough production. Inoculation with these strains resulted in favorable physicochemical, bioactive, and technological properties. The sourdoughs exhibited rapid acidification and growth of viable cells, providing stability and scalability for industrial applications. The use of starter cultures enhanced the content of phenolic compounds and antioxidant activity during fermentation. These findings highlight the effective

technological performance of the tested autochthonous sourdough strains, recommending their safe use for initiating fermentation in a rapid and controlled manner.

CRediT authorship contribution statement

Ana Regina Simplício de Medeiros: Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis. **Noádia Priscila Araújo Rodrigues:** Writing – review & editing, Methodology, Data curation. **Marcus Vinicius de Souza Couto:** Investigation, Formal analysis. **Gabriel Victor Pinheiro Barbosa:** Methodology, Investigation, Formal analysis. **Tatiana Zanella Rodrigues:** Methodology, Formal analysis. **Ingrid Conceição Dantas Gonçalves:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Eloiza Helena Campana:** Methodology, Formal analysis, Data curation. **Ana Luiza Mattos Braga:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis. **Marcos dos Santos Lima:** Supervision, Methodology, Formal analysis. **Maristela Alves Alcântara:** Validation, Supervision, Methodology, Formal analysis. **Angela Maria Tribuzy de Magalhães Cordeiro:** Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Estefânia Fernandes Garcia:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Implications for gastronomy

Sourdough has gained considerable prominence in the baking market over the past few decades, establishing itself as a significant niche within the gastronomic sector. This is due to its unique ability to blend the ancient tradition of fermentation with the growing consumer demand for natural, nutritious, sustainable foods that offer robust and appealing flavors. Lactic acid bacteria (LAB) represent a diverse group of microorganisms that play a critical role in the fermentation of foods. Their use in food products is considered safe, and they are recognized as GRAS (Generally Recognized as Safe). The biochemical changes occurring in sourdough during fermentation, driven by enzymatic activity, directly influence the quality of the bread. These changes are perceptible in the sensory characteristics of texture and flavor, as well as in the improved shelf life, volume, and nutritional value of the final product. In this regard, exploring the biotechnological potential of sourdough enhances our understanding of fermentation processes and opens new avenues for the development of products with functional and health-promoting attributes. As consumers increasingly seek to understand the origins and production processes of their food, studies such as this contribute to expanding knowledge, enabling chefs, bakers, and researchers to optimize sourdough fermentation processes, improving both sourdough efficiency and overall product quality.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijgfs.2025.101221.

Data availability

No data was used for the research described in the article.

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