

UNIVERSIDADE FEDERAL DE VIÇOSA

**Bactérias láticas autóctones nacionais como alternativa para produção de
fermentos lácteos: uma abordagem da eficiência da desidratação por spray
drying**

Jaqueline Aparecida Honorato
Magister Scientiae

**VIÇOSA - MINAS GERAIS
2025**

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Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, para obtenção do título de *Magister Scientiae*.

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**Ficha catalográfica elaborada pela Biblioteca Central da Universidade
Federal de Viçosa - Campus Viçosa**

T

H774b
2025
Honorato, Jaqueline Aparecida, 1998-
Bactérias lácticas autóctones nacionais como alternativa para
produção de fermentos lácteos: uma abordagem da eficiência da
desidratação por spray drying / Jaqueline Aparecida Honorato. –
Viçosa, MG, 2025.

1 dissertação eletrônica (71 f.): il. (algumas color.).

Texto em português e inglês.

Inclui apêndices.

Orientador: Antônio Fernandes de Carvalho.

Dissertação (mestrado) - Universidade Federal de Viçosa,
Departamento de Tecnologia de Alimentos, 2025.

Inclui bibliografia.

DOI: <https://doi.org/10.47328/ufvbbt.2025.415>

Modo de acesso: World Wide Web.

1. Queijo - Microbiologia. 2. Queijo - Secagem.
3. Queijo-de-minas. 4. Fermentação. 5. Bactérias - Efeito do frio.
I. Carvalho, Antônio Fernandes de, 1964-. II. Universidade
Federal de Viçosa. Departamento de Tecnologia de Alimentos.
Programa de Pós-Graduação em Ciência e Tecnologia de
Alimentos. III. Título.

CDD 22. ed. 664.024

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APROVADA: 14 de abril de 2025.

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Jaqueline Aparecida Honorato
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À minha família.

AGRADECIMENTOS

Diferente de todas as outras páginas, esta talvez seja a que mais me falta palavras. O caminho até aqui nem sempre foi fácil; na verdade, os momentos fáceis podem ser contados nos dedos. Início agradecendo a Deus por me guiar e amparar em todos os momentos principalmente nos momentos que tudo pareceu impossível.

Agradeço aos meus pais, Carminha e João, pelo amor incondicional, por entenderem minhas ausências e sempre me incentivarem a ser e a dar o meu melhor. Por me moldarem a ser quem sou hoje, pelas inúmeras orações. A vocês, meu eterno amor, gratidão e respeito.

Agradeço aos meus irmãos, Janice, Clarisse e Mateus, por serem meus melhores amigos e por todo o amor. Aos meus sobrinhos, por serem o bem mais precioso da minha vida. Aos meus amigos por todo o amor, suporte e acolhimento ao longo de toda a minha vida.

Agradeço à minha avó do coração, Isaulina, por ser o meu refúgio e meu amor.

Agradeço as amigadas que fiz ao longo do mestrado, em especial à Fabíola, por não me desamparar, por tornar a vida mais cheia de alegria e luz, por ser um exemplo para mim e por dividir essa fase comigo. Obrigada por tudo.

Agradeço à minha estagiária, Gabi, por todo apoio e companheirismo. Tenho certeza de que boa parte disso tudo não teria acontecido sem a sua ajuda. Muito obrigada.

Agradeço à Andressa, minha amiga e coorientadora por tudo que és e representa na minha vida. Obrigada por tudo, em todos os momentos da minha vida.

Agradeço ao meu namorado, Artur, por escolher dividir esta vida comigo e por ser meu abrigo em todos os momentos, pela paciência e por todo amor.

Agradeço ao meu orientador, Antônio Fernandes, por ser, além de meu professor, um exemplo da pessoa que desejo ser, por sua alegria e por nunca me desamparar.

Agradeço à Sol, à Rafa e à Raiane por toda a ajuda ao longo desta caminhada.

Agradeço ao Inovaleite por ser minha segunda casa por tantos anos.

À Universidade Federal de Viçosa, pela oportunidade de realizar a pós-graduação e ao DTA desde 2017.

E, por último, agradeço aos ausentes, que sei que, do céu, vibram junto comigo a cada conquista. Tudo aqui é por vocês.

Este trabalho foi realizado com o apoio das seguintes agências de

pesquisa brasileiras: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001, Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

“Não fui eu que lhe ordenei? Seja forte e corajoso! Não se apavore, nem se desanime, pois o Senhor, o seu Deus, estará com você por onde você andar”

Josué 1.9

RESUMO

HONORATO, Jaqueline Aparecida, M.Sc., Universidade Federal de Viçosa, abril de 2025. **Bactérias lácticas autóctones nacionais como alternativa para produção de fermentos lácteos: uma abordagem da eficiência da desidratação por spray drying.** Orientador: Antonio Fernandes de Carvalho. Coorientadores: Solimar Goncalves Machado e Andressa Fusieger.

Diversos microrganismos são utilizados em processos industriais e podem atuar como ferramentas chave nos processos de fermentação, conservação e nos benefícios probióticos na preparação de produtos alimentícios, farmacêuticos, biocombustíveis e bioquímicos. No contexto de culturas iniciadoras, também conhecidas como culturas starter, as bactérias lácticas (BAL) autóctones apresentam papel importante na produção de alimentos lácteos. A microbiota láctica autóctone está naturalmente presente no leite cru e no ambiente lácteo, e são responsáveis pela fermentação do açúcar do leite (lactose), produzindo ácido láctico como subproduto. Além de contribuírem para a formação do sabor e textura dos alimentos, as BAL têm propriedades benéficas para a saúde, como a produção de vitaminas e a melhoria da digestibilidade dos alimentos. Este estudo avaliou o potencial tecnológico de linhagens de BAL isoladas de queijo Minas artesanal e investigou os efeitos de estresses térmicos subletais na viabilidade de *Lactocaseibacillus paracasei* durante a secagem por atomização e armazenamento. Vinte linhagens de BAL foram inicialmente estudadas quanto à atividade proteolítica, capacidade de acidificação, produção de diacetil e perfil de lactofermentação. Com base nessas características, dez linhagens foram selecionadas para avaliação posterior do comportamento de crescimento bacteriano e tolerância ao estresse térmico subletal. A linhagem 120 de *L. paracasei* demonstrou o melhor desempenho e foi submetida à secagem por atomização, com ou sem adaptação térmica prévia. A viabilidade celular foi avaliada imediatamente após a secagem e durante o armazenamento a 4°C e 25°C por 90 dias. As análises estatísticas revelaram que o tempo de armazenamento afetou significativamente a sobrevivência celular, enquanto a pré-adaptação ao estresse subletal não levou a um aumento estatisticamente significativo na viabilidade pós-secagem. Essas descobertas indicam que a secagem por atomização é uma técnica viável para preservação de BAL, fornecendo uma alternativa econômica à liofilização. Mais estudos são necessários para otimizar as condições de secagem e melhorar a sobrevivência do BAL para aplicações industriais.

Palavras-chave: Viabilidade celular; *Lactocaseibacillus paracasei*; Adaptação celular ; estresse subletal; pré-adaptação térmica; spray drying

ABSTRACT

HONORATO, Jaqueline Aparecida, M.Sc., Universidade Federal de Viçosa, April, 2025. **National autochthonous lactic acid bacteria as an alternative for the production of lactic ferments:**

A new approach to the efficiency of dehydration by spray drying

. Adviser: Antonio Fernandes de Carvalho. Co-advisers: Solimar Goncalves Machado and Andressa Fusieger.

Several microorganisms are used in industrial processes and can act as key tools in fermentation, preservation and probiotic benefits in the preparation of food, pharmaceutical, biofuel and biochemical products. In the context of starter cultures, also known as lactic acid bacteria (LAB), native lactic acid bacteria (LAB) play an important role in the production of dairy foods. The native lactic acid microbiota is naturally present in raw milk and in the dairy environment, and is responsible for the fermentation of milk sugar (lactose), producing lactic acid as a byproduct. In addition to contributing to the formation of food flavor and texture, LAB have beneficial properties for health, such as the production of vitamins and the improvement of food digestibility. This study evaluated the technological potential of LAB strains isolated from artisanal Minas cheese and investigated the effects of sublethal thermal stresses on the viability of *Lacticaseibacillus paracasei* during spray drying and storage. Twenty LAB strains were initially studied for proteolytic activity, acidification capacity, diacetyl production, and lactofermentation profile. Based on these characteristics, ten strains were selected for further evaluation of bacterial growth behavior and tolerance to sublethal heat stress. *L. paracasei* strain 120 demonstrated the best performance and was subjected to spray drying, with or without prior thermal adaptation. Cell viability was assessed immediately after drying and during storage at 4°C and 25°C for 90 days. Statistical analyses revealed that storage time significantly affected cell survival, while pre-adaptation to sublethal stress did not lead to a statistically significant increase in post-drying viability. These findings indicate that spray drying is a viable technique for LAB preservation, providing a cost-effective alternative to freeze-drying. Further studies are needed to optimize drying conditions and improve LAB survival for industrial applications.

Keywords: Cell Viability; *Lacticaseibacillus paracasei*; Stress adaptation; Storage stability

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1. INTRODUÇÃO GERAL

Diversos microrganismos são utilizados em processos industriais e podem atuar como ferramentas chave nos processos de fermentação, conservação e nos benefícios probióticos na preparação de produtos alimentícios, farmacêuticos, biocombustíveis e bioquímicos (Alonso, 2016). No contexto de culturas iniciadoras, também conhecidas como culturas *starter*, as bactérias lácticas (BAL) autóctones apresentam papel importante na produção de alimentos lácteos. A microbiota láctica autóctone está naturalmente presente no leite cru e no ambiente lácteo, e são responsáveis pela fermentação do açúcar do leite (lactose), produzindo ácido lático como subproduto. Além de contribuírem para a formação do sabor e textura dos alimentos, as BAL têm propriedades benéficas para a saúde, como a produção de vitaminas e a melhoria da digestibilidade dos alimentos (Fox et al., 2017d). Nos últimos anos, a viabilidade e produção de fermentos autóctones funcionais bem definidos para a produção de queijos tradicionais em condições controladas, usando a tecnologia tradicional padronizada, tem se tornado um diferencial (Munekata et al., 2023; Terzić-Vidojević et al., 2015, 2020).

As BAL autóctones são frequentemente associadas na produção de queijos e iogurtes tradicionais, que têm sabores e características únicas, de acordo com a região em que são produzidos. No entanto, a sua utilização na indústria de laticínios pode apresentar desafios, como a necessidade de controlar a microbiota para garantir a qualidade e segurança dos alimentos produzidos. Além disso, a utilização de culturas *starter* comerciais pode ser mais eficiente em termos de produção e padronização de sabor, o que pode levar à inviabilização do uso de BAL autóctones na indústria de lácteos (Fox et al., 2017d; Tamang et al., 2016). Contudo, o isolamento da microbiota autóctone, caracterização microbiológica e tecnológica, bem como a secagem, pode ser um processo padrão e viável para a inserção de BAL autóctones na realidade industrial, obtendo-se assim fermentos autóctones.

As culturas *starter* são frequentemente fornecidas para as indústrias de alimentos como um concentrado em pó, obtido por liofilização ou por spray-dryer. Adicionalmente, essas culturas também podem ser fornecidas congeladas. No caso das culturas em pó, a alta concentração celular permite que as culturas sejam inoculadas diretamente no tanque de fermentação, sendo denominadas culturas DVS (*Direct Vat Set*) (Fox et al., 2017d). As culturas DVS se caracterizam pela alta viabilidade celular e elevada uniformidade entre diferentes lotes de produção. Além disso, a necessidade de inóculo é muito pequena, por exemplo, 1 g dessa cultura pode ser suficiente para inocular 500 litros de leite (Carminati et al., 2010; Reale et al.,

2019). As culturas DVS podem ser acondicionadas em filmes laminados que permanecem estáveis por anos quando armazenados a 5 °C na ausência de luz e, diversos produtos como iogurtes, leites fermentados e queijos podem ser feitos a partir dessas culturas concentradas (Carminati et al., 2010; Reale et al., 2019; L. Wang et al., 2023).

Para a obtenção dessas culturas, a técnica de liofilização é uma das mais usadas comercialmente. Contudo, apresenta alto custo de energia na produção, tornando interessante aplicar outras tecnologias alternativas que permitam produção em larga escala a um custo menor (Santivarangkna et al., 2007). A secagem por spray-dryer emergiu como uma tecnologia alternativa para a secagem de culturas lácticas de interesse industrial. Dentre as vantagens, pode-se citar a produção em larga escala que, consequentemente, reduz os custos de produção, menor consumo de energia (Moreira et al., 2021; Schuck et al., 2016). Ademais, estudos apontaram que é possível armazenar os fermentos obtidos por spray-dryer a temperatura de refrigeração (4 °C) e a temperatura ambiente (25 °C) (Farahmandi et al., 2021; Ghandi et al., 2013).

Durante a secagem por spray-dryer, as células são expostas a diferentes estresses, como estresse osmótico, térmico e oxidativo, e os mecanismos de adaptação celular envolvem a acumulação de compostos intracelulares, como substâncias osmoprotetoras. Para garantir a sobrevivência das células durante esses processos e evitar a perda de viabilidade das células durante o armazenamento no estado desidratado, essas substâncias osmoprotetoras podem ser adicionadas ao meio de cultivo. Dessa forma, essas substâncias auxiliam na proteção das células lácticas, permitindo sua preservação efetiva ao longo do armazenamento (Moreira et al., 2021; Peighambardoust et al., 2011; Yang et al., 2021). Adicionalmente, a aplicação potencial de estresses subletais para aumentar a resistência microbiana ao processo de desidratação tem sido empregada, uma vez que esses tipos de estresses induzem a síntese transitória de proteínas específicas e levam a alterações fisiológicas que, geralmente, aumentam a capacidade de resistência do microrganismo a condições adversas (Ghandi et al., 2012, 2013; Jin et al., 2023; Y. Wang et al., 2020; Yang et al., 2021, 2023).

Neste contexto, a produção de um fermento a partir de BAL autóctones pelo processo de desidratação em spray-dryer pode ser uma opção promissora para a indústria de alimentos, oferecendo vantagens em termos de adaptação ao ambiente local, sustentabilidade, facilidade de armazenamento e transporte, e redução de custos da cadeia produtiva. A fim de se estudar a secagem por atomização associada a condições que mantenham a viabilidade celular e o desempenho tecnológico de culturas iniciadoras nacionais, esse projeto teve como finalidade o estudo da aplicação de estresse subletal em *Lactocaseibacillus paracasei* para aumentar a

resistência térmica da mesma durante a desidratação por atomização. Na sequência, os pós produzidos foram analisados e os tratamentos de pré-adaptação comparados.

2. IMPORTÂNCIA DO ESTUDO

Esse projeto é a continuidade das linhas de pesquisa do laboratório InovaLeite que unem conhecimentos científicos para estruturar a produção de fermentos lácticos para a fabricação de queijos. O InovaLeite possui uma ampla coleção de culturas e destacam-se as BAL que foram recentemente isoladas de queijos artesanais da Região Entre Serras da Piedade ao Caraça/MG. Em um projeto paralelo, esses isolados estão sendo identificados e caracterizados quanto ao seu potencial tecnológico e, posteriormente, serão utilizados nesse projeto para a produção de fermento.

A utilização de BAL autóctones para a produção de fermento é uma abordagem promissora para a indústria de lácteos, uma vez que essas bactérias estão naturalmente presentes no ambiente em que serão utilizadas e, portanto, são adaptadas às condições específicas do local. Isso pode levar a uma produção de fermento mais eficiente e com melhor desempenho, além de reduzir a dependência por fermentos importados. Dentre as técnicas empregadas para a produção de fermentos, a liofilização está bem estabelecida comercialmente e a desidratação por spray-drying apresenta características promissoras. Dentre as vantagens da técnica por spray-drying destacam-se o menor custo energético de produção, alta eficiência de secagem e produção em grande escala, partículas de tamanho uniforme, estabilidade do produto a temperatura ambiente, ganhos na cadeia logística e, conseqüentemente, um fermento mais barato para a indústria de lácteos.

Entretanto, a viabilidade das BAL selecionadas será importante para a produção do fermento lácteo, bem como a aplicação progressiva de estresses subletais que pode levar a um fermento mais eficiente, com melhor desempenho e maior estabilidade, resultando em um produto final de alta qualidade. A aplicação desses novos fermentos em queijos permitirá a valorização da biodiversidade nacional e dos diferentes *terroirs* produtores de queijo artesanal, e também benefícios significativos para a indústria de laticínios brasileira.

3. OBJETIVOS

Avaliar e comparar a viabilidade celular e o desempenho tecnológico de pós de *Lb. Paracasei* produzidos pela técnica de desidratação por atomização submetidos a pré-adaptação térmica e sem pré-adaptação (controle).

3.1 Objetivos específicos

- Submeter as cepas (n=20) para aplicação de testes tecnológicos e avaliar o potencial das mesmas.
- Submeter as cepas (n=10) ao estresse térmico subletal e selecionar uma cepa de acordo com a viabilidade celular após a aplicação do estresse térmico.
- Submeter as cepas a secagem por atomização.
- Avaliar o efeito da aplicação de estresse subletal na viabilidade celular.
- Avaliar o efeito da secagem por atomização na viabilidade celular das cepas de BAL ao longo de noventa dias e em diferentes temperaturas de estocagem.

4. REFERENCIAL TEORICO

4.1. Bactérias lácticas e autóctones

As culturas selvagens ou autóctones são aquelas presentes em alimentos *in natura*, nos equipamentos de processo ou no ambiente, e apresentam características interessantes, uma vez que sobrevivem às condições adversas do ambiente e predominam nesse ecossistema. Para que sobrevivam ao ambiente que habitam, necessitam resistir à competição com outros microorganismos, geralmente pela produção de substâncias antimicrobianas tais como os ácidos orgânicos, etanol, CO₂, H₂O₂ ou bacteriocinas (Grujović et al., 2022; Munekata et al., 2023).

É importante ressaltar que uma cultura iniciadora autóctone consiste em uma única ou um grupo de linhagens microbianas bem adaptadas ao processo tecnológico, isoladas e selecionadas a partir do produto original. Sua implementação implica na dominância dos microorganismos da cultura iniciadora sobre a microbiota do leite cru ou pasteurizado, garantindo um microambiente favorável ao processo tecnológico e otimizando o processo fermentativo. Isso pode permitir o desenvolvimento das propriedades organolépticas típicas e

específicas do produto autóctone e/ou exaltar algumas características sensoriais ou funcionais (Bassi et al., 2015; Chourasia et al., 2021; Munekata et al., 2023).

A microbiota autóctone em queijo é uma característica que tem sido preservada há séculos. O uso de leite cru é um componente essencial para muitas variedades tradicionais de queijo e desempenha um papel importante tanto na produção quanto na maturação do queijo (Munekata et al., 2023). Devido ao seu sabor único, os queijos artesanais produzidos por fermentação espontânea de leite cru recebem grande atenção dos consumidores em todo o mundo. Esses produtos lácteos são conhecidos por sua grande variedade e determinadas regiões geográficas representam um tesouro nacional e patrimônio cultural (Terzić-Vidojević et al., 2015). As culturas autóctones desempenham um papel importante na obtenção de Denominação de Origem Protegida (DOP), que se aplica a alimentos produzidos, processados e preparados em uma determinada área geográfica usando métodos e tecnologias reconhecidas. No Brasil, elas também contribuem para a Indicação Geográfica (IG) e para a qualidade dos produtos artesanais (Kamimura et al., 2019).

As bactérias lácticas (BAL; em inglês: Lactic Acid Bacteria) desempenham um papel importante na produção de queijo e sua presença contribui para as grandes diferenças nas características organolépticas e bioquímicas (Fox et al., 2017d). BAL compõem naturalmente a microbiota autóctone de diversos alimentos, incluindo o leite cru e seus derivados e compartilham diversas características morfológicas, metabólicas e fisiológicas. Possuem forma de bacilos, cocos, Gram positivas, catalase negativas, não formadoras de esporos, anaeróbicas facultativas e produzem ácido láctico como produto principal da fermentação dos açúcares (Holzapfel & Wood, 2014; Lahtinen et al., 2011).

Atualmente, gêneros que compõem este são, por exemplo, *Aerococcus*, *Atopobium*, *Bifidobacterium*, *Brochotrix*, *Carnobacterium*, *Enterococcus*, *Tetragenococcus*, *Vagococcus*, *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Oenococcus*, *Weissella*, *Lactococcus* e *Streptococcus* (Liu et al., 2014). A principal característica que agrupa esses gêneros é a produção de ácido láctico a partir da fermentação de carboidratos e algumas características distintivas podem ser observadas na Tabela 1 (Fox et al., 2017d; Lahtinen et al., 2011).

BAL são responsáveis por uma série de processos fermentativos essenciais na produção de queijos. Embora possuam como principal função a produção de ácido láctico, também apresentam uma atuação muito importante na maturação do queijo, visto que suas enzimas estão envolvidas de modo direto nos processos de proteólise, lipólise e conversão de aminoácidos em compostos aromáticos (Fox et al., 2017b, 2017d; Wilkinson & LaPointe, 2020). Além disso,

algumas cepas podem produzir substâncias antimicrobianas, principalmente bacteriocinas, sendo esses importantes na modulação do crescimento de microrganismos patogênicos, podendo inibir ou inativar os mesmos (Macori & Cotter, 2018; Pineda et al., 2021)

Tabela 1. Algumas características distintivas das BAL encontradas em culturas iniciadoras comerciais e naturais de acordo com Fox et al. (2017b).

Nome	Tipo ^a	Forma	% de ácido láctico em leite ^b	Metabolismo do citrato	Crescimento				Crescimento em 4% de NaCl ^d	Fermentação do açúcar	Isômero de lactato
					10 °C	15 °C	40 °C	45 °C			
Culturas comerciais											
<i>Streptococcus thermophilus</i>	T	Cocos	0,6	-	-		+	+	-	Homo	L
<i>Lactobacillus helveticus</i>	T	Bastão	2,5	-	-	-	+	+	-	Homo	DL
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	T	Bastão	1,8	-	-	-	+	+	-	Homo	D
<i>Lactobacillus delbrueckii</i> subsp. <i>Lactis</i>	T	Bastão	1,8	-	-	-	+	+	-	Homo	D
<i>Lactococcus lactis</i> subsp. <i>Cremoris</i>	M	Cocos	0,8	-	+	+	-	-	-	Homo	L
<i>Lactococcus lactis</i> subsp. <i>Lactis</i>	M	Cocos	0,8	±	+	+	+	-	+	Homo	L
<i>Leuconostoc lactis</i>	M	Cocos	<0,5	+	+	+	-	-		Hetero	D
<i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i>	M	Cocos	0,2	+	+	+	-	-		Hetero	D
Culturas naturais											
<i>Lacticaseibacillus casei</i> subsp. <i>Casei</i>		Bastão	1,4	±		+		-		Homo	L
<i>Lacticaseibacillus paracasei</i> subsp. <i>paracasei</i>		Bastão		±	+	+	+	±		Homo	L
<i>Lacticaseibacillus paracasei</i> subsp. <i>tolerans</i>		Bastão	0,3 – 1,2			+		-		Homo	L
<i>Lacticaseibacillus rhamnosus</i>		Bastão		±		+	+	+		Homo	L
<i>Lactiplantibacillus plantarum</i>		Bastão				+		-		Homo	DL
<i>Latilactobacillus curvatus</i>		Bastão				+		-		Homo	DL
<i>Limosilactobacillus fermentum</i>		Bastão				+	+	+		Hetero	DL
<i>Enterococcus faecalis</i>		Cocos		+	+	+	+	+	+	Homo	L
<i>Enterococcus faecium</i>		Cocos			+	+	+	+	+	Homo	L

^a T = termofílico; M = mesofílico. ^b Valores aproximados, cepas individuais variam. ^c Ativado pela frutose-1,6-fosfato e nos lactobacilos também pelo Mn²⁺. ^d Todos crescem em meio contendo 2% de NaCl; *E. faecalis* e *E. faecium* cresce em meio contendo 6,5% de NaCl

Existem muitos estudos que descrevem a presença de BAL na microbiota autóctone de diferentes queijos artesanais elaborados com leite cru, bem como estudos que têm demonstrado que essas BAL autóctones podem melhorar as propriedades tecnológicas, sensoriais e a vida de prateleira dos queijos (Castellanos-Rozo et al., 2020; Christian et al., 2020; Cosentino et al., 2018; Dal Bello et al., 2010; Dolci et al., 2020; Quijada et al., 2020). No contexto atual, uma possível perda de populações microbianas nativas presentes em queijos artesanais levanta uma preocupação crescente. É importante o isolamento e estudo das características de segurança e tecnológicas das culturas autóctones pois permitirá a conservação cultural e identificação de culturas com características específicas. Ademais, o uso de fermentos de culturas iniciadoras autóctones no processo de fabricação de queijos artesanais pode superar os problemas de uniformidade e padronização (Araújo-Rodrigues et al., 2021; Hosken et al., 2023).

Para a seleção de culturas iniciadoras e aplicação em queijos, as linhagens microbianas devem ser Geralmente Reconhecidas Como Seguras (GRAS; em inglês: Generally Recognized as Safe), possuir características tecnológicas, se adaptarem às condições tecnológicas usadas durante o processo de fabricação de queijo, colaborar no procedimento fermentativo e de maturação (Araújo-Rodrigues et al., 2021; Carafa et al., 2019; De Pasquale et al., 2019; Fox et al., 2017d; Gaglio et al., 2021). Entretanto, cada tipo de queijo requer culturas iniciadoras específicas, conforme a Tabela 2.

Além disso, a produção e comercialização dessas culturas deve ser regulamentada pelos órgão vigentes. No Brasil, a Instrução Normativa (IN) nº 211 de 01 de março de 2023 da Agência Nacional de Vigilância Sanitária (ANVISA) estabelece as funções tecnológicas, os limites máximos e as condições de uso para os aditivos alimentares e os coadjuvantes de tecnologia autorizados para uso em alimentos. O Anexo II da IN nº 211 especifica as funções tecnológicas dos coadjuvantes de tecnologia e apresenta como item os fermentos biológicos, sendo definidos como “levedura e outros microrganismos utilizados em processos de tecnologia alimentar que envolvem fermentação” (Brasil, 2023). Contudo, os limites máximos e condições de uso não estão especificados.

Tabela 2. Culturas iniciadoras utilizadas na fabricação de queijos.

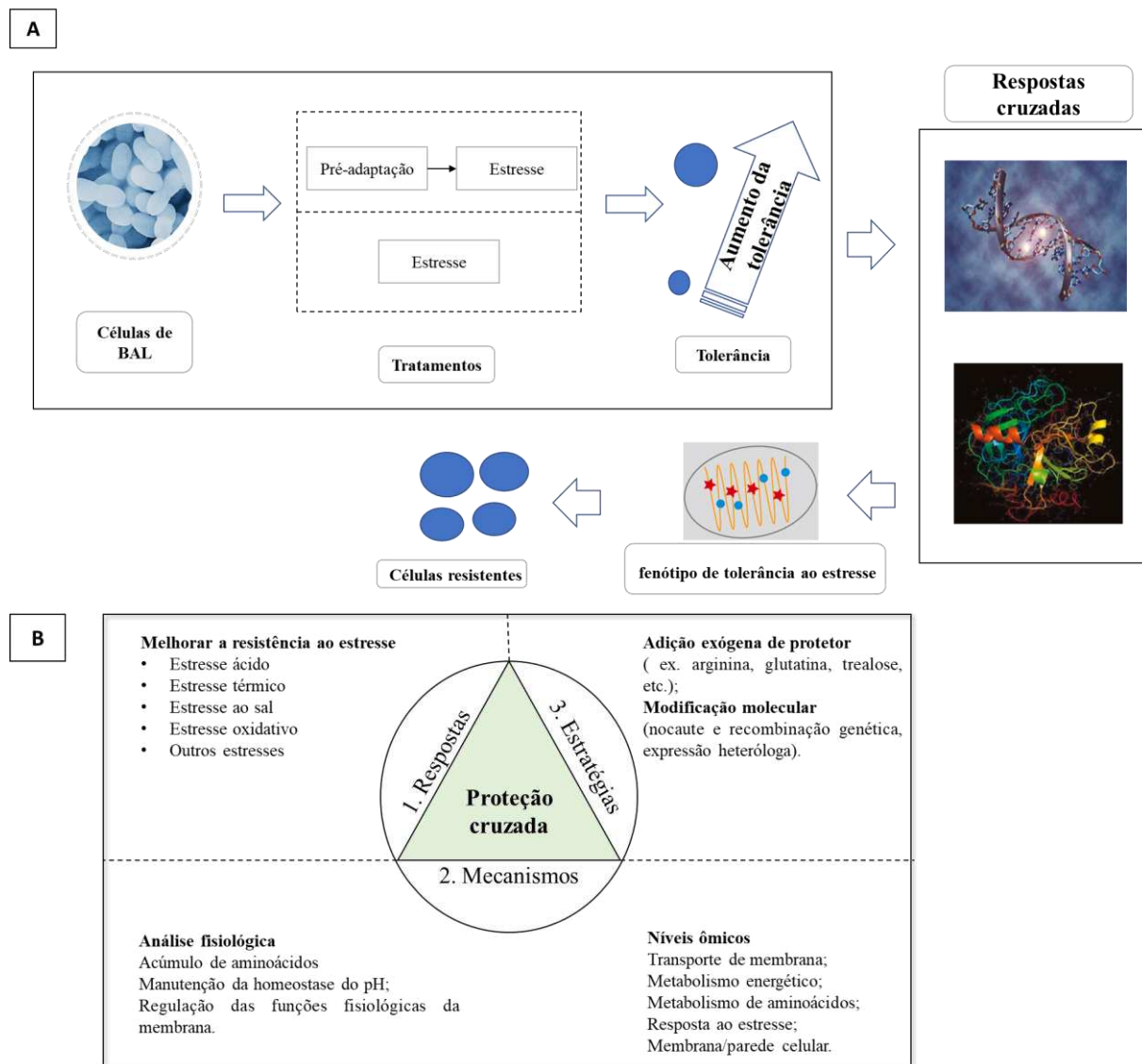
Queijo	Culturas iniciadoras	Outras culturas	Produtos importantes além do ácido láctico
Emmental	<i>Streptococcus thermophilus</i> e <i>Lactobacillus helveticus</i> (<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i> galactose-positivo também pode ser usado)	<i>Propionibacterium freudenreichii</i>	CO ₂ , propionato e acetato
Mussarela e outros queijos italianos	<i>Streptococcus thermophilus</i> e <i>Lactobacillus helveticus</i> ou culturas naturais de soro de leite		
Cheddar	<i>Lactococcus lactis</i> subsp. <i>cremoris</i> e <i>Lactococcus lactis</i> subsp. <i>lactis</i> ou culturas mistas mesófilas O, L ou DL (às vezes, culturas termofílicas são incluídas)		
Edam e Gouda	Principalmente culturas mistas mesófilas DL		CO ₂ e acetato
Camembert e Brie	Culturas mistas mesófilas O, L ou DL	<i>Penicillium camemberti</i> , <i>Geotrichum candidum</i>	
Tilsit, Limburger e Munster	Culturas mistas mesófilas O, L ou DL	<i>Brevibacterium linens</i> , <i>Geotrichum candidum</i>	Compostos de enxofre, por exemplo, metional
Fresco e Quark	Principalmente culturas mistas mesófilas DL		Diacetil e acetato

Fonte: (Fox et al., 2017d).

4.2. Estresses subletais em BAL

Na aplicação tecnológica, as BAL encontram diversos tipos de estresses abióticos e bióticos (como estresses ácidos, térmicos, osmóticos, oxidativos e outros), que afetam as atividades metabólicas e a eficiência da fermentação e produção de alimentos. Em resposta as várias condições de estresse, as BAL possuem diferentes mecanismos para resistir aos danos causados por ambientes estressantes (Papadimitriou et al., 2016). Nesse contexto, destaca-se o mecanismo de proteção cruzada (Figura 1). Esse mecanismo pode ser definido como a adaptação das células transitoriamente a uma condição branda/subletal que, posteriormente, são desafiadas com um estresse letal, o que pode aumentar significativamente a tolerância das células durante diversas condições de estresse (Barbosa et al., 2015; R. Huang et al., 2016; Papadimitriou et al., 2016; Yang et al., 2021).

Figura 1. O princípio da resposta ao estresse e o potencial mecanismo de proteção cruzada em BAL. A: uma breve introdução à proteção cruzada. B: a resposta ao estresse e os mecanismos subjacentes de proteção cruzada e estratégias para obter células adaptadas.



Fonte: adaptado de Yang et al. (2021).

Cepas de BAL resistem a vários estresses por meio da exposição prévia a uma condição de pré-adaptação branda, o que indica que uma condição subletal pode induzir a proteção cruzada efetiva contra estresse letal (Yang et al., 2021). O estresse subletal é um fenômeno que ocorre quando as bactérias são expostas a condições adversas que não resultam em sua morte imediata, mas afetam seu crescimento e capacidade de sobrevivência. No entanto, as células bacterianas possuem uma variedade de mecanismos de defesa inatos para lidar com essas

condições desafiadoras, conhecidos como mecanismos de resposta ao estresse (Kulkarni et al., 2018; Mbye et al., 2020; Papadimitriou et al., 2016).

Esses mecanismos incluem as chaperonas, que auxiliam no correto dobramento de proteínas malformadas; proteases, que degradam proteínas danificadas irreversivelmente; sistemas de transporte, que ajudam a manter a osmolaridade celular adequada; catalases e superóxido dismutases, que combatem espécies reativas de oxigênio; além de bombas de prótons, descarboxilases e transportadores, que atuam para manter o pH intracelular adequado (Chen & Lu, 2018; Papadimitriou et al., 2016; Yang et al., 2021). A resposta ao estresse mais bem estudada em bactérias é a resposta ao choque térmico, que se caracteriza pela indução transitória da síntese de proteínas específicas e de alterações fisiológicas que, geralmente, aumentam a capacidade de resistência do microrganismo a condições adversas, como aquelas existentes nos processos de desidratação e estocagem no estado desidratado (Papadimitriou et al., 2016; Sugimoto et al., 2008; Varmanen & Savijoki, 2011; Yang et al., 2021).

Os estresses térmico, osmótico, oxidativo e ácido são geralmente considerados os principais mecanismos que causam a inativação de bactérias durante e após a secagem por pulverização (S. Huang et al., 2017). A submissão progressiva a esses tipos de estresses se torna interessante para a indústria de lácteos, uma vez que a síntese de chaperonas é um mecanismo de resposta geral equivalente ao estresse térmico (Lim et al., 2000). Estudos recentes têm sugerido que a exposição de BAL a algum estresse subletal é benéfica para a manutenção da viabilidade celular após a secagem por pulverização, visto que ocorre a produção de proteínas de choque térmico e outros metabólitos que aumentam a resistência das bactérias (Barbosa et al., 2015; Cnossen et al., 2020; Dijkstra et al., 2014; Ghandi et al., 2012; Hao et al., 2021; Y. Wang et al., 2020; Yang et al., 2023).

Em estudo realizado por Cnossen et al. (2020) foi avaliada a exposição de células de *Lactococcus lactis* ao H₂O₂ antes da secagem por desidratação em spray-dryer. Os resultados demonstraram um aumento da sua resistência celular ao calor por meio da aquisição de uma resposta cruzada. Resultados semelhantes foram descritos por Dijkstra et al. (2014), onde cepas de *L. lactis* apresentaram tolerância tanto em condições de estresse oxidativo quanto ao térmico, indicando uma correlação entre as respostas adaptativas a essas condições de estresse.

O estresse ácido é um tipo de força autoimposta para as BAL devido às suas propriedades e funções metabólicas. O ácido láctico é um metabólito primário das BAL, que se acumula em grande quantidade durante a fermentação de açúcar, o que resulta em condições de estresse ácido (Guan & Liu, 2020; Wu et al., 2012). Além disso, outros metabólitos (como ácido

fórmico, ácido acético e outros) produzidos pelas BAL também podem causar desafios ácidos (He et al., 2016). O estresse ácido provoca várias condições adversas, afetando o sistema homeostático, interrompendo o sistema de transporte de substratos e inibindo vias metabólicas (Beales, 2004). Para restabelecer a conformação funcional de proteínas e enzimas essenciais ao metabolismo, o mecanismo das chaperonas também é importante para reparar danos macromoleculares (Papadimitriou et al., 2016). Essa resposta adaptativa ao estresse ácido é semelhante à encontrada para o estresse térmico, logo, as células adaptadas a condições ácidas são reconhecidas por apresentar maior tolerância a tratamentos térmicos (Lim et al., 2000).

O estresse osmótico permite que o microrganismo crie estratégias para evitar a desidratação rápida e excessiva por meio do acúmulo de solutos compatíveis. Ele é causado devido ao aumento da pressão osmótica e, como consequência, é uma das principais barreiras para as BAL durante a fabricação de alimentos fermentados salgados (Huang et al., 2016; Le Marrec, 2011; Papadimitriou et al., 2016). *Lactobacillus acidophilus*, por exemplo, pode adquirir maior tolerância (aumento de 1,6 a 3000 vezes na sobrevivência) a altas concentrações de NaCl ou bile através de pré-adaptação em concentrações subletais de NaCl (2%) ou bile (0,05%) por um determinado período de tempo (Kim et al., 2001; Kulkarni et al., 2018).

Yang et al. (2023) avaliou a pré-adaptação ao calor para combater as tensões físicas encontradas durante o processo de liofilização e secagem por pulverização e desenvolver um pó de *Tetragenococcus halophilus* de alta atividade. Os resultados revelaram que as células de *T. halophilus* mantiveram uma viabilidade superior no pó seco quando submetidas à pré-adaptação ao calor antes do processo de secagem. Além disso, a temperatura de transição vítrea do pó seco aumentou quando as células foram pré-adaptadas, evidenciando uma maior estabilidade ao longo da vida útil. A pré-adaptação também exibiu um desempenho fermentativo superior das células secas, indicando que essa pode ser uma estratégia promissora na produção de pós bacterianos por liofilização ou secagem por pulverização.

4.3. Desidratação por atomização em spray-dryer

A secagem é um processo amplamente utilizado para conservação de alimentos, garantindo uma vida útil estável e prolongada, reduzindo custos de transporte e facilitando a comercialização. A liofilização continua sendo a técnica mais utilizada para preservar as bactérias, mas é um processo demorado e de alto custo. Na indústria de laticínios, a técnica de secagem por atomização/spray drying é uma das mais predominantes para produção de produtos lácteos em pó (Schuck et al., 2016). Quando comparada à liofilização, a secagem por

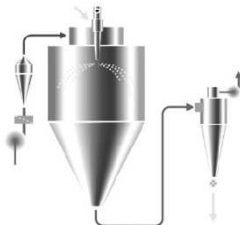
atomização representa um menor custo de energia e maior produtividade. Dessa forma, permanecem os desafios associados ao uso de secagem por pulverização para produzir culturas viáveis e em altas concentrações celulares (Moreira et al., 2021).

A técnica desidratação por atomização em spray-dryer envolve a dispersão do fluido em pequenas gotas em uma corrente de ar quente para permitir a rápida evaporação da água presente nas gotas. O princípio subjacente a essa técnica é baseado na lei de Fourier, que estabelece que quanto maior a área de troca, mais eficiente é a transferência de energia na forma de calor, resultando em uma taxa de secagem mais elevada (Bimbenet, 1978; Santivarangkna et al., 2007). O uso da secagem por atomização na produção de fermentos lácteos tem se mostrado promissor para garantir a viabilidade e estabilidade das BAL utilizadas na produção de produtos lácteos fermentados devido à vantagem de poder ser realizada em larga escala, reduzindo assim os custos de produção, consumo de energia mais baixo, além de alguns estudos apontarem a possibilidade de serem armazenadas em temperatura ambiente (Moreira et al., 2021; Peighambardoust et al., 2011).

No entanto, algumas linhagens são sensíveis à secagem por spray devido à exposição a condições de estresse térmico, oxidativo e osmótico durante o processo de desidratação (R. Huang et al., 2016; Moreira et al., 2021; Peighambardoust et al., 2011; Schuck et al., 2016; Yang et al., 2023). Contudo, existem estratégias que podem ser aplicadas para otimizar o processo e torná-lo interessante (S. Huang et al., 2017). Os fatores mais influentes e estresses adversos estão apresentados na Figura 2.

Hao et al. (2021) avaliou a termotolerância, sobrevivência e estabilidade de *L. lactis* subsp. *cremoris*, *Lactobacillus rhamnosus* e *L. acidophilus* após secagem por pulverização de acordo com o aumento da temperatura de crescimento (3-5 °C acima das condições padrão). A sobrevivência de *L. lactis* subsp. *cremoris* e *L. acidophilus* adaptados ao calor após a secagem por pulverização aumentou em 21,0% e 13,7%, respectivamente, enquanto o aumento mostrado pelo *L. rhamnosus* foi relativamente insignificante (9,9%). Os pós secos por pulverização contendo células adaptadas ao calor mostraram uma redução substancial da viabilidade na primeira semana de armazenamento, atingindo 1,03–1,23 log, em comparação com 0,87–0,90 log de redução mostrada pelos controles.

Figura 2. Fatores mais influentes e estresses adversos durante as etapas de crescimento celular, secagem por pulverização, armazenamento e aplicação.

	CRESCIMENTO BACTERIANO	SPRAY DRYING	ESTOCAGEM E APLICAÇÃO
			
FATORES DE INFLUÊNCIA	Cepa bacteriana Meio de cultura Temperatura pH Fase do crescimento Concentração celular inicial Método da concentração celular	Temperatura Matriz de secagem Atomização Tempo de retenção Umidade do pó	Injúria celular Temperatura Matriz de secagem Umidade do pó Atividade de água Material de armazenamento Condição de reidratação
ESTRESSE ADVERSO	Estresse ácido Estresse metabólico Estresse nutricional Estratégia: realizar estresse subletal para induzir a tolerância ao estresse	Estresse térmico Estresse osmótico Estresse oxidativo Estresse mecânico	Estresse oxidativo Estresse mecânico Estresse nutricional

Fonte: adaptado de Huang et al. (2017).

É importante ressaltar que a temperatura do ar de entrada influencia a taxa de secagem das gotas, além de influenciar a temperatura das partículas no final do processo. Quanto mais alta a temperatura do ar de entrada, mais rápida será a desidratação celular e mais intenso será o tratamento térmico sofrido (Fu & Chen, 2011; Moreira et al., 2021; Peighambardoust et al., 2011; J. Wang et al., 2016). Por outro lado, o tipo e a concentração do material carreador determinam o tamanho das gotas que serão secas. Quando fluidos viscosos são atomizados, tendem a se formar gotas maiores, dificultando a evaporação da água (Lian et al., 2002; Peighambardoust et al., 2011; Santivarangkna et al., 2007). Isso leva a um aumento no tempo de residência na câmara de secagem, resultando em maior dano térmico. Em contrapartida, a formação de gotas menores resulta em pós finos e maior consumo de energia. Portanto, é necessário encontrar um intervalo ideal de teor de sólidos que, geralmente, situa-se entre 20% e 30% (m/v) (S. Huang et al., 2017).

A condução de calor dentro da gota a ser seca depende das propriedades térmicas do material carreador. Materiais com baixa condutividade térmica tendem a limitar a condução de calor e, conseqüentemente, proteger as células contra danos térmicos. Desse modo, a escolha

do material carreador e sua concentração são importantes para limitar os danos celulares durante a secagem (De Castro-Cislaghi et al., 2012; S. Huang et al., 2017; Lian et al., 2002; Perdana et al., 2014). Dentre os meios carreadores mais utilizados na secagem por atomização de BAL destacam-se o manitol, o leite desnatado reconstituído, maltodextrina, goma arábica, gelatina e amido solúvel.

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CAPÍTULO I

Technological potential and viability of lactic acid bacteria subjected to sublethal thermal stress and spray drying
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Manuscript prepared and submitted to *Food Research International*

Technological potential and viability of lactic acid bacteria subjected to sublethal thermal stress and spray drying

Potencial tecnológico e viabilidade de bactérias lácticas submetidas a estresse térmico subletal e secagem por atomização

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ABSTRACT

Lactic acid bacteria (LAB) play a fundamental role in the food industry, particularly in the fermentation and maturation of dairy products. However, ensuring their viability in industrial applications remains a challenge, especially during drying processes. This study evaluates the technological potential of LAB strains isolated from Minas artisanal cheese and investigates the effects of sublethal thermal stresses on the viability of *Lacticaseibacillus paracasei* during spray drying and storage. Twenty LAB strains were initially screened for proteolytic activity, acidification capacity, diacetyl production, and lactofermentation profile. Based on these characteristics, ten strains were selected for further evaluation of bacterial growth behavior and tolerance to sublethal heat stress. The *L. paracasei* strain 120 demonstrated the best performance and was subjected to spray drying, with or without prior thermal adaptation. Cell viability was assessed immediately after drying and during storage at 4°C and 25°C for 90 days. Statistical analyses revealed that storage time significantly affected cell survival, whereas pre-adaptation to sublethal stress did not lead to a statistically significant increase in viability post-drying. These findings indicate that spray drying is a viable technique for LAB preservation, providing a cost-effective alternative to lyophilization. Further studies are needed to optimize drying conditions and improve LAB survival for industrial applications.

Keywords: Cell Viability. *Lacticaseibacillus paracasei*. Stress adaptation. Storage stability.

1. INTRODUCTION

In cheese production, lactic acid bacteria (LAB) play different roles, participating either in the fermentation or maturation process. In the former case, they are responsible for initiating lactic acid production, being referred to as starter lactic acid bacteria (SLAB) (Settanni and Moschetti, 2010). The production of lactic acid from lactose lowers the pH of the milk (Fox et al., 2016), and rapid acidification extends the shelf life of the product by preventing the growth of undesirable microorganisms, while being essential for the aroma and texture of the final product (Vuyst, 2000; König et al., 2017).

During cheese maturation, non-starter lactic acid bacteria (NSLAB) become predominant, contributing to the catabolism of amino acids, particularly aromatic amino acids (phenylalanine, tyrosine, tryptophan), branched-chain amino acids (leucine, isoleucine, and valine), and methionine, which are responsible for the specific texture and flavor of matured cheeses (Fox et al., 2016). The combined metabolic activity of LAB not only ensures microbial stability but also enhances the sensory attributes of cheese, making them key players in the dairy industry.

Given the extensive use of LAB in fermented foods, most of these bacteria are classified as GRAS (Generally Recognized As Safe) by the U.S. Food and Drug Administration (FDA), which deems genera such as *Lactobacillus* and *Bifidobacterium* safe for use (Cui et al., 2018; Grujović et al., 2019; Yadav and Shukla, 2017). To enable the use of these cultures in industries, lyophilization is one of the most commonly techniques. However, it involves high energy costs, making it interesting to explore alternative technologies that allow for large-scale production at a lower cost (Santivarangkna et al., 2007).

Spray drying has emerged as an alternative technology for drying lactic acid cultures of industrial interest. Among its advantages are large-scale production, which reduces production costs, and lower energy consumption (Moreira et al., 2021; Schuck et al., 2016). Furthermore, studies have shown that the cultures obtained by spray drying can be stored at refrigeration temperatures (4 °C) or room temperature (25 °C) (Farahmandi et al., 2021; Ghandi et al., 2013).

During spray drying, the cells are exposed to different stresses, such as osmotic, thermal, and oxidative stress, and the cellular adaptation mechanisms involve the osmoprotectant accumulation (Gaucher et al., 2020). To ensure cell survival during these processes and prevent cell viability loss during storage in the dehydrated state, these osmoprotective substances can be added to the culture medium. These compounds contribute to the protection of LAB cells, enhancing their stability and viability throughout the storage period (Moreira et al., 2021;

Peighambardoust et al., 2011; Yang et al., 2021). Additionally, the potential application of sublethal stresses to increase microbial resistance to the dehydration process has been employed, as these types of stresses induce the transient synthesis of specific proteins and lead to physiological changes that generally increase the microorganism's resistance to various conditions (Ghandi et al., 2013; Jin et al., 2023; Wang et al., 2020; Yang et al., 2023, 2021).

In this context, the development of starter cultures from indigenous LAB through spray-dryer technology presents a promising option for the food industry. This approach offers advantages in terms of adaptation to the local environment, sustainability, ease of storage and transport, and cost reduction in the production chain. However, the success of this strategy depends on enhancing microbial survival during the drying process and subsequent storage. Given that sublethal thermal stress has been reported to enhance bacterial resistance to environmental challenges, further investigation is needed to determine its effectiveness in improving LAB viability under industrial drying conditions. Therefore, this study aimed to evaluate the technological potential of LAB strains isolated from Minas artisanal cheese and assess the effects of sublethal thermal stress on the viability of *Lacticaseibacillus paracasei* during spray drying and storage. The findings of this research may contribute to the development of more resilient starter cultures, enhancing their application in the dairy industry and supporting the advancement of cost-effective biotechnological solutions.

2. MATERIALS AND METHODS

2.1 LAB strains

LAB isolated from Minas artisanal cheese produced in the Entre Serras da Piedade do Caraça region (Minas Gerais, Brazil) were selected as the target strains for this study. 16S rRNA partial gene sequencing showed the highest abundance of *Lacticaseibacillus*, *Pediococcus*, and *Lactiplantibacillus* species (Rodrigues et al., 2024). Based on these findings, five strains from four species were selected, including *Lacticaseibacillus paracasei*, *Lacticaseibacillus rhamnosus*, *Lactiplantibacillus plantarum* and *Pediococcus pentosaceus*, resulting in a total of twenty strains (n = 20). These strains were stored at -80 °C in de Man, Rogosa and Sharpe broth (MRS; Kasvi, São José dos Pinhais, PR, Brazil) with 20% (v/v) glycerol; before use, strains were transferred into MRS broth and incubated at 37 °C overnight, and checked for purity in MRS agar and Plate Count Agar (PCA; Kasvi, Paraná, Brazil) after incubation at 37 °C.

2.2 Technological potential

LAB strains (n = 20) were subjected to technological assays, such as proteolytic activity, acidifying capacity, lactofermentation profile and diacetyl production. Before each assay, aliquots of 1% (v/v) of the refreshed culture were cultured in MRS broth at 37 °C for 24 hours. All analyses were conducted in triplicate, with two independent repetitions.

Proteolytic activity was evaluated using skim milk agar diffusion assay. 20 µL of the refreshed culture of each strain were placed onto the plates surface containing PCA enriched with skimmed UHT milk (10% w/v; Nestlé, São Paulo, SP, Brazil) and incubated at 37 °C for 4 days (Franciosi et al., 2009). The presence of proteolytic activity was indicated by a clear zone around the colonies. *Pseudomonas fluorescens* 07A was used as a positive control (Alves et al., 2016).

Acidification capacity was performed by the inoculation of 1% (v/v) of the cultured strains into skimmed UHT milk (Nestlé) and incubation at 37 °C. pH values and lactic acid production were measured at the time of inoculation (T0), and after 6 (T6), 12 (T12), and 24 hours (T24). The pH value was obtained by direct measuring with digital pHmeter (Hanna Instruments, São Paulo, SP, Brazil) and titratable acidity by titration with 0.1N NaOH, being expressed as percentage of lactic acid (Dal Bello et al., 2012).

For lactofermentation profile and diacetyl production, aliquots (1%, v/v) of each cultured strain were inoculated into skimmed UHT milk (Nestlé) and incubated at 37 °C for 24 h. Based on the characteristics of the formed curd, lactofermentation patterns were empirically analyzed and classified as follows: uniform, uniform with whey presence, uniform and fragile (in appearance), broken with whey presence, and absence of curd (Fusieger et al., 2020). Subsequently, 1 mL of the activated cultures in milk were transferred to tubes containing 0.5 mL of α -naphthol (1% w/v) and 0.5 mL of KOH (16% w/v) and incubated at 37 °C for 10 min. Diacetyl production was indicated by the formation of a red ring at the top of the tubes (Franciosi et al., 2009). *Lactococcus lactis* subsp. *lactis* bv. *diacetylactis* ATCC 13675 was used as positive control. Based on the results of the technological potential assessment, ten LAB strains (n = 10) were selected to evaluate bacterial growth behavior and tolerance to sublethal stress.

2.3 Bacteria growth behavior

Aliquots of 1% (v/v) of the stock cultures of the selected LAB strains (n = 10) were cultured in MRS broth at 37 °C for 24 hours. Then, the strains were cultured once again and LAB concentration was determined at times 0, 4, 8, 12, 16, 18, 20, 22, and 24 hours by plate counting through the microdrop method (Morton, 2001). In detail, at each time point, aliquots of 100 µL were collected and added to 900 µL of saline solution 0.85% (w/v), homogenized, and 10-fold diluted in saline solution 0.85% (w/v). 20 µL of selected dilutions were deposited on the surface of MRS agar and incubated at 37 °C for 48 hours. The enumeration of colony-forming units was performed in triplicate and conducted in droplets containing between 8 and 80 colonies. Viable cell results were expressed as log CFU/mL.

2.4 Application of thermal sublethal stress

To increase the tolerance of the selected LAB strains (n = 10) to dehydration conditions, the strains were subjected to thermal stress conditions according to Zhang et al. (2016), with some modifications. Aliquots of 1% (v/v) of the selected strains were cultured in MRS broth at 37 °C until 1.5×10^8 CFU/mL was reached. Then, the selected strains were subjected to 12 thermal treatments, combining three temperatures (45 °C, 50 °C, 55 °C) and four different exposure times (0, 30, 60, 90 minutes). Bacterial survival was assessed using microdrop plating method, as previously described, and the viability was expressed as log CFU/mL. In this study, only one strain was considered for the subsequent steps, the one that presented the best technological potential (acidification capacity, diacetyl production, lactofermentation pattern and proteolytic activity) combined with the ability to pre-adapt to thermal stress. Thus, the strain *L. paracasei* 120 was selected for the subsequent step of the research, which is the spray drying process.

2.5 LAB spray drying

2.5.1 Preparation of dehydration medium

Drying medium was prepared according to Martins et al. (2019), with modification. Briefly, 220 g of skimmed milk powder (Nestlé) was dispersed in 660 g of sterile distilled water to obtain reconstituted and concentrated skimmed milk with a final solid content of 30-35 °Brix, as measured by a refractometer (Biobrix, São Paulo, SP, Brazil). The dehydration medium was heated to 85 °C for 30 minutes, and then cooled to 4 °C.

2.5.2 Preparation of cell concentrate and powder production

The selected strain *L. paracasei* 120 was activated (1%, v/v) in MRS broth at 37 °C for the time required to reach approximately 1.5×10^9 CFU/mL; this time was determined based on the growth curve obtained in item 2.3. Subsequently, a thermal pre-adaptation procedure was performed, with two groups: with thermal treatment (CST) and SST no thermal treatment (SST). After incubation, the inoculum was centrifuged at $3,260 \times g$ for 10 min at 4 °C (Heraeus Megafuge 8R; Thermo Fisher Scientific, Waltham, MA, USA), and resuspended in the dehydration medium to achieve a cell concentration of approximately 1.5×10^{10} CFU/g.

The cell concentrates (cells resuspended in drying medium) were collected for cell enumeration before spray drying. Aliquots of 1 mL of the mixtures were dispersed into 9 mL of sterile saline solution (0.85% w/v) and serial dilutions were made in the same diluent. Cell enumeration was performed by the microdrop plating technique on MRS agar plates, and the results were expressed in log CFU/mL.

Subsequently, cell concentrates (CST and SST) were subjected to the spray drying. The cell concentrates were dried in a spray-dryer, model MSD 1.0 (Labmaq do Brasil, São Paulo, SP, Brazil), using previously determined drying parameters: inlet air temperature = 170 °C; outlet air temperature = 85 °C; and suspension flow rate = 0.6 to 0.8 kg/h. The spray drying experiment for each treatment was completed in approximately 1 hour. Samples of the obtained powders, each consisting of 1 g, were aseptically divided and placed into sterile Falcon tubes, sealed with aluminum foil, and then vacuum packed. The dried bacterial powder samples were stored at 4 ± 1 °C and 25 ± 1 °C for 90 days. In total, four treatments were evaluated (120CST_4°C, 120SST_4°C, 120CST_25°C, and 120SST_25°C) and three independent repetitions of spray drying were undertaken.

2.5.3 Evaluation of cell viability of the powders

Cell viability was assessed in the powders immediately after spray drying and during the storage period at 4 and 25 °C at times 1, 15, 30, 60, and 90 days. During storage, 1 g of powder was resuspended in 9 mL of 0.85% saline solution (w/v), and serial dilutions were made in the same diluent. Enumeration of viable cells was carried out using the microdrop plating technique on MRS agar plates. The survival rate (%) of *L. paracasei* 120 in the powders was expressed as live cells at any time during the storage period $\times 100$ / live cells before drying (Yang et al., 2023).

2.5.4 Evaluation of water activity and moisture of the powders

Water activity (A_w) of the powders was determined at 25 °C using the AquaLab meter (3TE; Decagon Devices Inc., Pullman, WA, USA). The sample cup was filled to half its depth, placed in the sample chamber, and the A_w measured using the standard procedure for the instrument. Moisture content of the powders was determined in a moisture analyzer (ID-200; Marte, São Paulo, SP, Brazil) using infrared balance (Almada-Érix et al., 2021). All measurements were performed in triplicate.

2.6 Statistical analyses

Statistical analyses and data visualization were performed using R software version 4.3.3 and RStudio 2023.03.0+386 (R Core Team, 2023; RStudio Team, 2020). Growth curves parameters were calculated using the ‘ipolygrowth’ package (Wang & Barger, 2023), while graphical representations were generated with ‘ggplot2’ package (Wickham, 2016). For the analysis of bacterial response to stress and drying conditions, the ‘ExpDes.pt’ and ‘ggplot2’ packages were employed (Ferreira et al., 2013; R Core Team, 2023; Wickham, 2016).

The bacterial response to sublethal stress, expressed as $\log_{10}$ CFU/mL, was initially assessed by applying the Shapiro-Wilk test to verify the normality of model residuals. Subsequently, an analysis of variance (ANOVA) was performed using a split-plot design within a completely randomized layout, where temperature was defined as the main plot factor and exposure time as the subplot factor. Tukey’s test was used for post-hoc comparisons.

The drying experiment was conducted using a $2 \times 2 \times 5$ factorial design, involving two treatments (CST: with thermal stress; SST: without thermal stress), two storage temperatures (4 °C and 25 °C), and five evaluation times (1, 15, 30, 60, and 90 days). The normality of the residuals was verified using the Shapiro-Wilk test, followed by ANOVA. For multiple comparisons between means, Tukey’s test was applied as a post-hoc procedure, considering a statistical significance level of $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Technological Potential

The results of the technological potential are shown in **Table 1**. All twenty LAB strains exhibited extracellular proteolytic activity. This enzymatic activity in cheese lead to the generation of aroma and flavor, as well as contributing to the release of bioactive peptides in

milk that are beneficial to consumer health (Teixeira et al., 2021). For acidification capacity, most strains showed a decrease in pH after 24 hours of incubation, as well as lactic acid production. Strains 10, 304, 581, and 651 (*Pediococcus pentosaceus*) reduced the pH by 1.0 unit and had low lactic acid production during 24 hours. Strains from the *Lacticaseibacillus* and *Lactiplantibacillus* genera exhibiting different levels of acidification over 24 hours. The decrease in pH during cheese production helps to ensure the appropriate texture of the cheese and inhibits undesirable microorganisms (Bansal and Veena, 2024). The consistency of titratable acidity values in most strains suggests a reliable acidification process, which is essential for maintaining cheese quality and characteristics (Teixeira et al., 2021).

According to Delgado et al. (2002), strains that reduce the pH of milk to 5.3 within 6 hours can be considered good acid producers. Similarly, strains that develop acidity ≥ 0.25 g of lactic acid/100 mL after 6 hours of incubation can be used as starter cultures in cheese production (Herreros et al., 2005). In this context, only strains 10, 304, 581, and 651 did not showed adequate titratable acidity after 6 hours of incubation, while the others showed variations from 0.25 to 0.29 (**Table 1**). Margalho et al. (2020) observed higher acidification rates in *L. paracasei* and *L. brevis* compared to other Lactobacilli isolated from artisanal cheeses in Minas Gerais.

Other important characteristics of LAB is their ability to synthesize diacetyl (2,3-butanedione), a volatile compound produced as a final product when citrate is converted to pyruvate (Tian et al., 2023). It provides a buttery flavor to fermented dairy products and is a common characteristic associated with *L. lactis* subsp. *lactis* bv. *diacetylactis* (Fusieger et al., 2020). In this study, eleven strains exhibited positive results for diacetyl production, being one *Lactiplantibacillus plantarum* (strain 694), three *Lacticaseibacillus paracasei* (strains 23, 28, 31), and five *Lacticaseibacillus rhamnosus* (strains 59, 68, 262, 280, and 613) (**Table 1**). The strain 20 (*Lacticaseibacillus paracasei*) showed the most intense red color (data not shown). Strains 1, 2, 549, 683 (*Lacticaseibacillus rhamnosus*) and 10, 304, 581, 632, 651 (*Pediococcus pentosaceus*) did not show diacetyl production (**Table 1**). In the study performed by Margalho et al. (2020), diacetyl production was also evaluated in the genera *Lacticaseibacillus*, *Lactiplantibacillus*, *Pediococcus*, and *Levilactobacillus*, and similar results were found, with most isolates from *Lb. rhamnosus* (80%), *Lb. brevis* (72.2%), and *Lb. paracasei* (65%) producing diacetyl. *Lacticaseibacillus rhamnosus* was defined as moderate to high in diacetyl production.

Furthermore, lactofermentation profile varied among the strains, seventeen strains (85%) exhibited a uniform pattern with whey, one strain (5%) showed a uniform pattern with whey, one strain (5%) showed a uniform but fragile pattern, and one strain (5%) had no coagulum (**Table 1**). Milk coagulation is the first step in cheese production, determined by the destabilization of casein micelles, leading to curd formation. This phase can occur due to the activity of coagulating enzymes and/or due to the activity of bacterial strains that will release lactic acid. Lactic acid production reduces pH and increases milk coagulation through whey expulsion, while also inhibiting the growth of undesirable microorganisms (Bansal and Veena, 2024). The results obtained indicate the potential use of the isolates as coagulant agents during cheese production.

After evaluating the technological potential of the twenty LAB strains, ten strains (n = 10) with promising characteristics for application in cheese manufacturing processes were selected, including high acidification capacity, diacetyl production, proteolytic activity, and efficiency in lactic fermentation. These strains were then subjected to analyze their growth behavior and exposure to sublethal thermal stress.

3.2 Bacteria Growth Behavior

Microbial growth is commonly described by a sigmoidal curve, consisting of three phases. **Figure 1** shows the growth curves of the ten LAB strains analyzed over time. These curves allow the identification of different growth phases, such as the adaptation phase to the culture medium, the exponential phase where cells multiply rapidly, the stationary phase where the number of cells in multiplication and apoptosis are equal, and finally the death phase. The specific growth rate reaches its maximum value during the exponential phase of the growth curve, at which point the metabolism of microorganisms occurs in a more intense and efficient manner.

The generation time, in turn, allows us to estimate how long a cell takes to divide. **Table 2** presents detailed data on bacterial population in CFU/mL over the respective incubation times. It was observed that strain 68 showed the lowest maximum growth rate, while strain 651 exhibited the highest rate. Regarding the time required to reach the growth peak, strain 549 was the only one that took longer, while strain 304 reached the peak more quickly. The other isolates showed growth peak times ranging from 4 to 7 hours. In terms of doubling time, five strains had times of less than 2 hours, while the other five had times longer than this interval. The

maximum population was reached at different incubation times: strains 304 and 120 at 16 hours; strains 28, 683, and 549 at 18 hours; strains 68, 280, and 196 at 20 hours; and finally, strains 613 and 651 reached the maximum population after 24 hours of incubation (**Table 2**).

3.3 Application of Thermal Sublethal Stress

Knowing the growth behavior of each strain, the subsequent stage was the application of thermal sublethal stress, as shown in **Figure 2**. The results show that bacterial survival varied among the strains in response to the combinations of temperature and time assessed. The analysis of variance for eight strains (304, 613, 651, 683, 68, 120, 196, and 549) revealed that temperature and time had significant effects on the response to sublethal stress ($p \leq 0.05$). Moreover, the interaction between temperature and time was also significant ($p \leq 0.05$), indicating that the effect of time on the response depends on the applied temperature. In general, a progressive reduction in bacterial count (Log_{10} CFU/mL) was observed with increasing exposure time, especially at higher temperatures (50 °C and 55 °C). The significant interaction between temperature and time highlights that the effect of time depends on the applied temperature, with more pronounced differences at 55 °C and prolonged times (60 and 90 minutes). Additionally, variations among the strains suggest different levels of resistance to thermal stress, with some showing greater viability under more severe conditions.

On the other hand, strain 280 showed that temperature had no significant effect on the response ($p = 0.187$), while time had a significant effect ($p = 0.027$). With the interaction being non-significant, the effects of time were evaluated separately. Tukey's test revealed that the initial time ($t = 0$) had the highest mean (8.51), statistically different from the 60-minute time (4.75), which had the lowest mean. These results indicate that, regardless of the applied temperature, there was a reduction in the number of bacterial cells over time for this strain.

For strain 28, the analysis of variance indicated that neither temperature nor time, nor their interaction, had significant effects on the response to sublethal stress ($p > 0.05$). These could be attributed to the high variability observed in the subplot (time) error, which may have reduced the statistical power of the analysis, demonstrating the limitation in detecting significant differences with the available data.

Several mechanisms have been proposed to explain the effects of heat adaptation on bacterial viability. One hypothesis suggests that heat adaptation could promote an increase in the saturation and length of fatty acids in cellular membranes, which would help maintain

intrinsic protein activity and membrane fluidity (Russell and Fukunaga, 1990). Another hypothesis suggests that the induction of heat shock proteins would be one of the key factors responsible for the beneficial effects of heat adaptation, as these proteins are involved in the proper folding of nascent polypeptides, assembly of protein complexes, and in the degradation and translocation of proteins (Bukau and Horwich, 1998; De Angelis and Gobbetti, 2004). Furthermore, experimental studies have shown that cell survival during heat adaptation can be influenced by various factors, such as the bacterial growth medium, culture age, and type of shock medium used. In particular, thermal adaptation in complex media may confer greater thermal tolerance to bacteria than in simple buffering systems (Telxelra et al., 1994).

In the study by Zhang et al. (2016), *Lactobacillus salivarius* strains were subjected to a thermal pre-adaptation process, which involved incubation in drying medium at different temperatures (45 °C, 50 °C, 52 °C, and 55 °C) and exposure times (5, 10, and 15 minutes), aiming to increase their thermal resistance for later use in the spray-drying process. The results indicated that for all drying regimes tested, there was a sharp reduction in viable cell counts of *L. salivarius* when samples were heated to 52 °C or 55 °C for 5 minutes. In contrast, when bacteria were exposed to 45 °C or 50 °C for up to 15 minutes, no significant changes ($P > 0.05$) in viable cell counts were observed. These findings are consistent with our results and the literature, which suggests that higher temperatures, such as 50 °C and 55 °C, can be detrimental to bacterial viability, possibly due to destabilization of the cell membranes and inactivation of essential proteins (Bukau and Horwich, 1998; Russell and Fukunaga, 1990).

Similarly, the preliminary studies by Bommasamudram et al. (2022) indicated that no growth of *L. casei* was observed when cells were exposed to 55 °C and 60 °C. In contrast, when the cells were subjected to 45 °C and 50 °C, growth was observed, with these temperatures being classified as sublethal thermal stress for the bacteria. These results reinforce the idea that adaptation to lower temperatures may be more effective in preserving cell viability in processes such as spray drying. This thermal pre-treatment was shown to increase cell viability when later challenged by higher temperatures, as indicated by Gouesbet et al. (2002), who reported that exposure to 50 °C increased the resistance of *Lactobacillus delbrueckii* subsp. *bulgaricus* cells to lethal temperatures.

Other studies also support the idea that thermal pre-adaptation can improve cell resistance to spray drying. According to Desmond et al. (2002), the preliminary adaptation of *L. paracasei* NFBC338 cultures to thermal stress increased the bacterium's survival capacity, mainly by enhancing its cell resistance during the drying process. This effect can be explained

by the reduction of harmful chemical reactions to microorganisms, which are minimized at lower temperatures, resulting in better preservation of cell viability (Wang et al., 2015).

Recently, cross-protection strategies have been explored to improve the resistance of lactic acid bacteria (LAB) cells under stress conditions. These strategies, involving pre-adaptation of cells to different types of stress, have been successful in increasing LAB cell resistance to adverse conditions, such as high temperatures and drying. Research by R. Huang et al. (2016), Lenz et al. (2010), and Sánchez et al. (2007) has shown that pre-adaptation can activate various defense systems in cells, such as energy metabolism, which ensures ATP production for cells, amino acid metabolism, which maintains cellular microenvironment homeostasis, and peptidoglycan synthesis, which preserves cell membrane integrity.

Considering the technological characteristics (**Table 1**) combined with its excellent ability to pre-adapt to thermal stress (**Figure 2**), strain *L. paracasei* 120 was selected for the subsequent step of the research, being the spray drying process.

3.4 Cell Survival Rate After Spray Drying

Spray drying is recognized as a viable strategy for the production of dairy powders and dry probiotics (Fu et al., 2018). By dehydrating LAB cells, it is expected to inhibit their metabolic activities, thereby extending the shelf life of cell viability (Fu and Chen, 2011). However, the spray drying process has been associated with decreased cell viability when applied to bacterial strains (Hao et al., 2021). Different genera, species, and strains may exhibit varying degrees of response due to differences in gene expression, stress protein levels, and metabolic pathways (Iaconelli et al., 2015; Salar-Behzadi et al., 2013). A common criterion for assessing LAB cell survival after spray drying is to measure cell viability, which refers to the ability of the cells to multiply in a rich medium (Ali et al., 2019). Since bacterial cells can activate stress response systems under stressful conditions, a feasible approach to increase LAB survival after spray drying is to enhance the intrinsic tolerance of the cells to stress (Desmond et al., 2002; Gaucher et al., 2020; S. Huang et al., 2016).

The results of *L. paracasei* 120 survival rate after spray drying are shown in **Figure 3**. The analysis of variance indicated that only the storage time factor had a significant effect on bacterial survival ($p \leq 0.001$), while the factors treatment and temperature, as well as their interactions, were not significant ($p > 0.05$). Tukey's test showed that the 15-day and 30-day storage times had the highest survival means, while the 1-day storage time had the lowest mean,

significantly different from the other time points (**Figure 3**). These results suggest that bacterial survival is strongly influenced by storage time, regardless of the treatment (CST and SST) or temperature (4 °C and 25 °C) used.

Due to the biological properties of LAB as living microorganisms, the microencapsulation of LAB cells through spray drying demonstrates several unique characteristics that differ from the processing of other bioactive substances. In this context, the response of LAB cells to environmental and protective stresses needs to be considered (Hao et al., 2021). In our research, both heat-pre-adapted cells and control cells were spray-dried with the same drying medium, composed of skimmed milk powder and water, at a final soluble solids concentration of approximately 35 °Brix. This composition may possibly explain the survival of non-heat-pre-adapted cells, as milk proteins play a significant role in thermal protection properties, which is widely recognized in the literature (Burgain et al., 2015; Maciel et al., 2014; Yang et al., 2023; Yudiastuti et al., 2022).

Moreover, according to Hao et al. (2021), high temperature can promote the rate of enzymatic reactions, as is commonly observed for most chemical reactions. The higher rate of enzymatic reactions may compensate for the low number of viable cells, resulting in similar metabolic activity between heat-adapted cultures and controls. In their study, similar results were observed, where the difference in viability between *L. rhamnosus* control cultures and heat-adapted cultures, after the spray-drying process, remained statistically insignificant ($p = 0.06$, $n = 3$), even after triplicate experiments.

Although we did not observe statistical differences between pre-adapted and control cells, the increased survival of heat-adapted *L. paracasei* 120 cells after spray drying was an expected trend. Heat stress, in addition to dehydration stress, is considered a major factor responsible for the inactivation of LAB cells during spray drying (Ghandi et al., 2012; Perdana et al., 2013). Another factor contributing to these results is that during spray drying, the particle temperature typically varies between the exit air temperature and the wet bulb temperature of the exit air, being approximately 10-20 °C lower than the exit air temperature (Silva et al., 2017). In our study, the temperature of the bacterial powder post-spray drying ranged between 36 °C to 40 °C, which likely contributed to the survival of control cells, considering that the studied strain is thermophilic. Furthermore, Gardiner et al. (2000) showed a relationship between viable counts of *Lacticaseibacillus paracasei* NFBC338 and *Ligilactobacillus salivarius* UCC18 with an increase in exit air temperature, reaching optimal viability at 80-85

°C, aligning with the results obtained in our research, as the studied exit air temperature was 85 °C.

Additionally, the specific cellular response mechanism that varies among species and strains involves conserved chaperones, such as the GroES, GroEL, DnaK, and DnaJ families, which are frequently identified in LAB cells after heat shock (De Angelis and Gobbetti, 2004; Sanders et al., 1999). At elevated ambient temperatures, not only does the expression of stress proteins increase in LAB cells, but the ability of chaperones to bind to unfolded polypeptides also improves substantially (Sherman and Goldberg, 1993).

3.5 Powder Moisture and Aw During Storage

As shown in **Figure 4**, the water content in both types of *L. paracasei* 120 dried powders remained below 5% throughout the storage period, which are consistent with the results reported by Zayed and Roos (2004). This suggests that the high survival of the dry bacterial powder during storage was attributed to maintaining the water content within a range of 2.8–5.6%. The statistical analysis revealed that only the time factor had a significant effect on moisture ($p = 0.0035$), while the treatment and temperature factors, as well as their interactions, were not significant ($p > 0.05$). Tukey's test showed that the moisture content was higher at 30, 60, and 90 days, with statistically similar averages. In contrast, the initial time (1 day) had the lowest moisture content, significantly differing from the others, but was statistically similar to the moisture content at 15 days. These results suggest that moisture increases initially and remained stable after 30 days, regardless of the treatment or temperature used.

The **Figure 5** presents the A_w over time for the analysed treatments. The analysis of variance revealed that only the time factor had a significant effect on A_w ($p = 0.0137$), while the treatment and temperature factors, as well as their interactions, were not significant ($p > 0.05$). Tukey's test showed that the highest mean value of A_w was observed at 90 days, while the lowest value occurred at the initial time (1 day). The intermediate times (15, 30, and 60 days) had statistically similar average values ($p > 0.05$). These results indicate that A_w remained stable throughout most of the storage period, with a significant increase observed only at 90 days, regardless of the treatment or temperature used.

At low A_w values, the mechanism of cell inactivation may be related to lipid oxidation, a process that promotes the release of free radicals, which are harmful to cells (França et al., 2007). Lipid oxidation can affect the cell membrane, causing irreversible damage to its

structure, and consequently leading to a loss of cell viability (Ezraty et al., 2017). On the other hand, at higher A_w values, the inactivation of cells in the powders may be attributed to the higher thermal conductivity of water molecules, which transfer heat more efficiently to the cells during the drying process. According to Higl et al. (2007), at higher A_w , cells may enter a partially active state, in which some enzymatic systems remain active while others do not. This imbalance can lead to the accumulation of intermediate metabolites inside the cells, causing harmful effects to the microorganisms. These results are consistent with the data obtained in this study, as illustrated in **Figure 5**, which shows that the survival rate of the strain was lowest at 1 and 90 days, when the A_w values were low and high, respectively.

4. CONCLUSION

In this study, thermal pre-adaptation was applied with the aim of increasing the microbial resistance of the *L. paracasei* strain, isolated from artisanal Minas cheese from the Entre Serras de Minas region, to preserve an initial culture with commercial potential, based on its technological potential demonstrated throughout this research. For this purpose, two treatments were applied to the cell concentrate: one with thermal pre-adaptation and one without, with the goal of producing dry cultures via spray drying. The experiment was conducted using laboratory-scale equipment, considering the survival rate of cells after drying and during storage at different temperatures (refrigeration at 4 °C and ambient at 25 °C), with the latter directly associated with transportation and logistics costs. Both treatments demonstrated advantages in these aspects. However, a better understanding of the cellular response of LAB under stress conditions is still necessary to improve the production of active LAB powders via spray drying. Despite the fact that the application of this drying technique is still limited in the context of dairy fermentation, the results obtained in this study support the benefits of this approach for the preservation of viable and active starter cultures.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors have no competing interests to declare.

Data availability

Data will be made available on request.

Acknowledgments

The authors are thankful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, DF, Brazil, Finance code 001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, DF, Brazil) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Belo Horizonte, MG, Brazil).

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6. CONCLUSÃO GERAL

Neste estudo, ambos os tratamentos mostraram benefícios, indicando que, mesmo sem a aplicação da pré-adaptação térmica, a cepa de *Lb paracasei* manteve sua viabilidade durante e após o processo de secagem. No entanto, um entendimento mais aprofundado sobre a resposta celular das bactérias ácido-láticas (BAL) a condições de estresse é essencial para otimizar a produção de culturas em pó ativas via secagem por atomização.

Embora a liofilização ainda seja a técnica mais amplamente empregada para a preservação de bactérias devido à sua alta eficácia na manutenção da viabilidade celular, trata-se de um processo oneroso e demorado. Na indústria de laticínios, a secagem por atomização, ou spray drying, destaca-se como uma alternativa viável, amplamente utilizada na produção de derivados lácteos em pó. Em comparação com a liofilização, a secagem por atomização apresenta vantagens econômicas significativas, como menor consumo energético e maior produtividade.

Dessa forma, embora os resultados deste estudo reforcem o potencial da secagem por atomização para a preservação de culturas starter viáveis e ativas, ainda são necessários estudos adicionais para superar os desafios inerentes à aplicação desta técnica na produção de culturas microbianas com alta concentração celular e viabilidade prolongada. O aprofundamento das investigações sobre os mecanismos de resistência celular e a otimização das condições de processamento poderá contribuir significativamente para a viabilização desta abordagem na indústria de fermentação láctea.

APÊNDICE A – TABLE 1

Table 1: Technological Potential

LAB Strain	Lactofermentation Profile	Proteolitic Activity	pH				Lactic acid production				Diacetyl production
			T0	T6	T12	T24	T0	T6	T12	T24	
1	Uniform with whey	+	6.66 ± 0.03	6.17 ± 0.09	6.17 ± 0.09	4.75 ± 0.07	0.17	0.27	0.35	0.62	-
2	Uniform with whey	+	6.66 ± 0.03	6.13 ± 0.09	5.59 ± 0.00	4.78 ± 0.02	0.17	0.28	0.36	0.65	-
549	Uniform with whey	+	6.66 ± 0.03	6.14 ± 0.09	5.59 ± 0.03	4.65 ± 0.15	0.17	0.28	0.36	0.71	-
683	Uniform with whey	+	6.66 ± 0.03	6.13 ± 0.07	5.56 ± 0.06	4.71 ± 0.02	0.17	0.29	0.37	0.64	-
694	Uniform with whey	+	6.66 ± 0.03	6.14 ± 0.06	5.64 ± 0.05	4.71 ± 0.03	0.17	0.28	0.37	0.64	+
23	Uniform with whey	+	6.66 ± 0.03	6.11 ± 0.09	5.50 ± 0.03	4.84 ± 0.06	0.17	0.25	0.41	0.68	+
28	Uniform	+	6.66 ± 0.03	6.22 ± 0.06	5.52 ± 0.02	4.83 ± 0.03	0.17	0.26	0.41	0.64	+
31	Uniform with whey	+	6.66 ± 0.03	6.17 ± 0.06	5.50 ± 0.00	4.87 ± 0.05	0.17	0.26	0.39	0.67	+
120	Uniform with whey	+	6.66 ± 0.03	6.16 ± 0.03	5.43 ± 0.07	4.54 ± 0.09	0.17	0.29	0.45	0.75	+
196	Uniform with whey	+	6.66 ± 0.03	6.13 ± 0.08	5.44 ± 0.04	4.65 ± 0.03	0.17	0.26	0.4	0.75	+
59	Uniform with whey	+	6.66 ± 0.03	6.23 ± 0.14	5.49 ± 0.06	4.90 ± 0.12	0.17	0.26	0.41	0.62	+
68	Uniform but fragile	+	6.66 ± 0.03	6.28 ± 0.04	5.72 ± 0.06	4.84 ± 0.10	0.17	0.25	0.37	0.62	+
262	Uniform with whey	+	6.66 ± 0.03	6.28 ± 0.05	5.95 ± 0.11	5.11 ± 0.39	0.17	0.25	0.29	0.58	+
280	Uniform with whey	+	6.66 ± 0.03	6.21 ± 0.04	5.86 ± 0.07	4.94 ± 0.11	0.17	0.25	0.32	0.65	+
613	Uniform with whey	+	6.66 ± 0.03	6.19 ± 0.08	5.49 ± 0.03	4.84 ± 0.08	0.17	0.28	0.42	0.65	+
10	No coagulum	+	6.66 ± 0.03	6.36 ± 0.08	6.17 ± 0.05	5.96 ± 0.10	0.17	0.24	0.26	0.31	-
304	Uniform with whey	+	6.66 ± 0.03	6.30 ± 0.08	6.17 ± 0.05	5.95 ± 0.08	0.17	0.23	0.27	0.31	-
581	Uniform with whey	+	6.66 ± 0.03	6.31 ± 0.06	6.11 ± 0.05	5.46 ± 0.41	0.17	0.24	0.27	0.42	-
632	Uniform with whey	+	6.66 ± 0.03	6.20 ± 0.05	5.71 ± 0.04	4.84 ± 0.08	0.17	0.27	0.35	0.60	-
651	Uniform with whey	+	6.66 ± 0.03	6.32 ± 0.09	6.19 ± 0.05	5.94 ± 0.06	0.17	0.23	0.27	0.31	-

APÊNDICE B – FIGURE 1

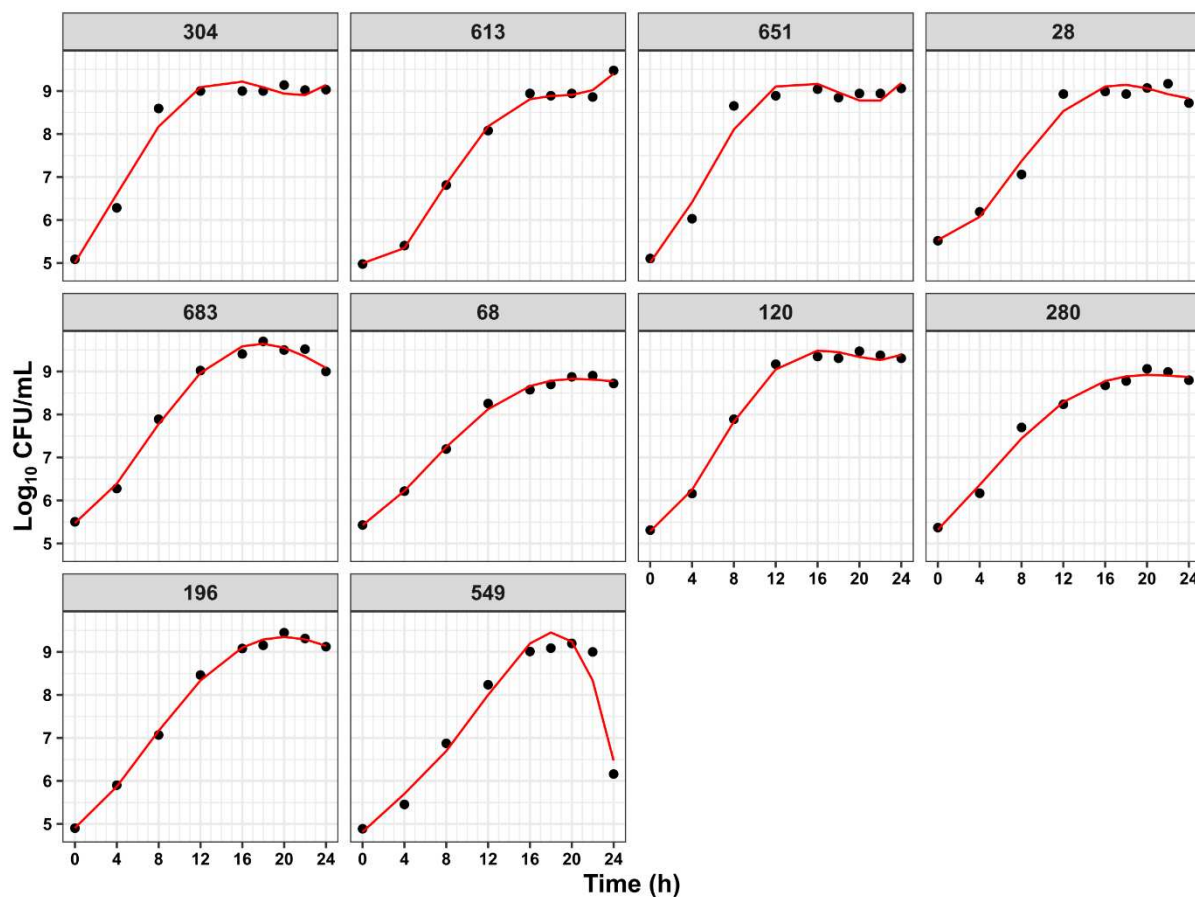


Figure 1. Bacterial growth curves of the ten LAB strains, with observed data and fitting lines.

In each individual panel, representing a specific isolate, the points correspond to the results of $\log_{10}$ CFU/mL over 24 hours. The adjusted red line represents the estimated growth model for each isolate, indicating the expected trajectory based on the collected data.

APÊNDICE C – FIGURE 2

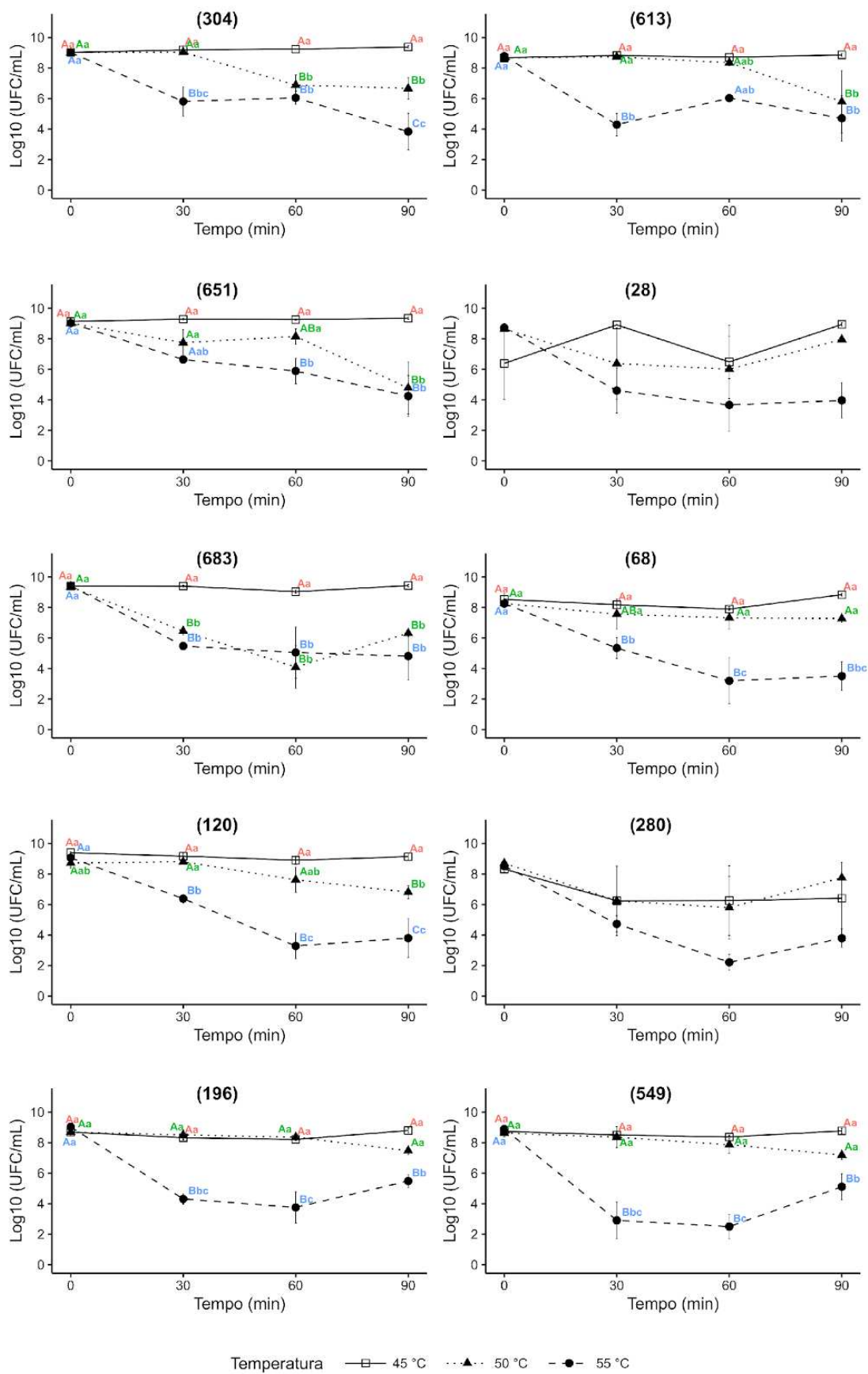


Figure 2. Bacterial survival of ten lactic acid bacteria isolates in response to sublethal stress at three temperatures (45 °C, 50 °C, and 55 °C) and four exposure times (0, 30, 60, and 90 minutes). The lines and dots represent the means and respective standard errors of Log₁₀ of CFU/mL for each temperature. Distinct capital letters indicate statistically significant difference between temperatures within each time, while distinct lowercase letters indicate significant difference between times within each temperature (Tukey test, $p < 0.05$). Red letters correspond to the statistical results for a temperature of 45 °C, green letters for 50 °C, and blue letters for 55 °C.

APÊNDICE D – FIGURE 3

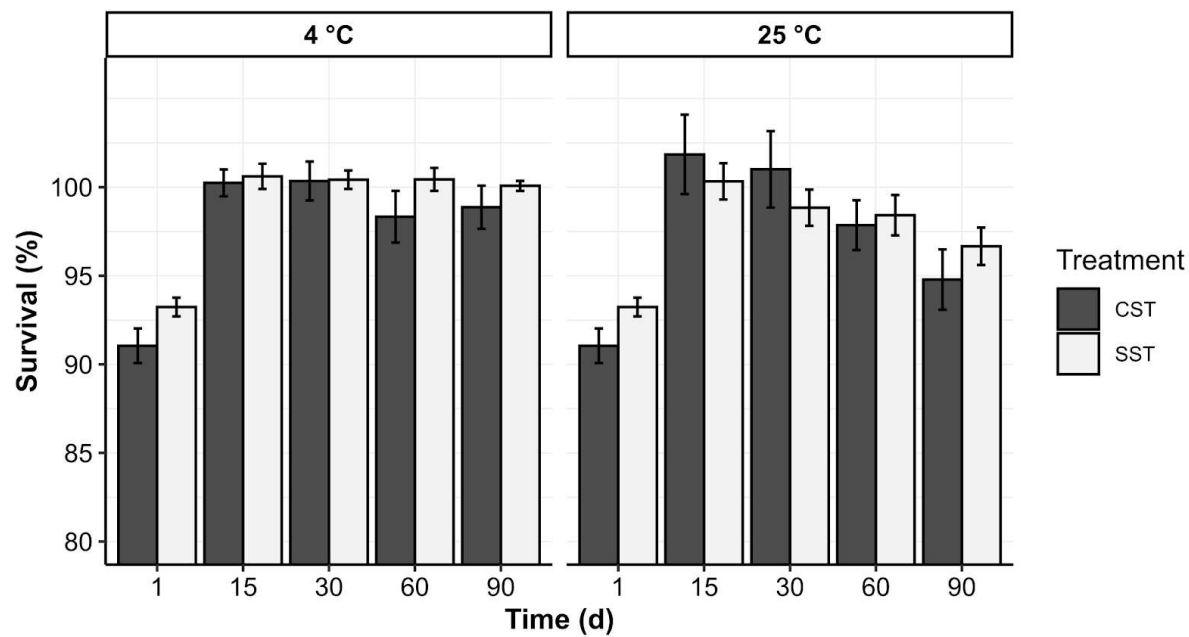


Figure 3. Percentage of survival (%) over time (days) for the CST (with heat stress) and SST (without heat stress) treatments, with two different storage temperatures (4 and 25 °C). Error bars correspond to the standard error of the mean.

APÊNDICE E – FIGURE 4

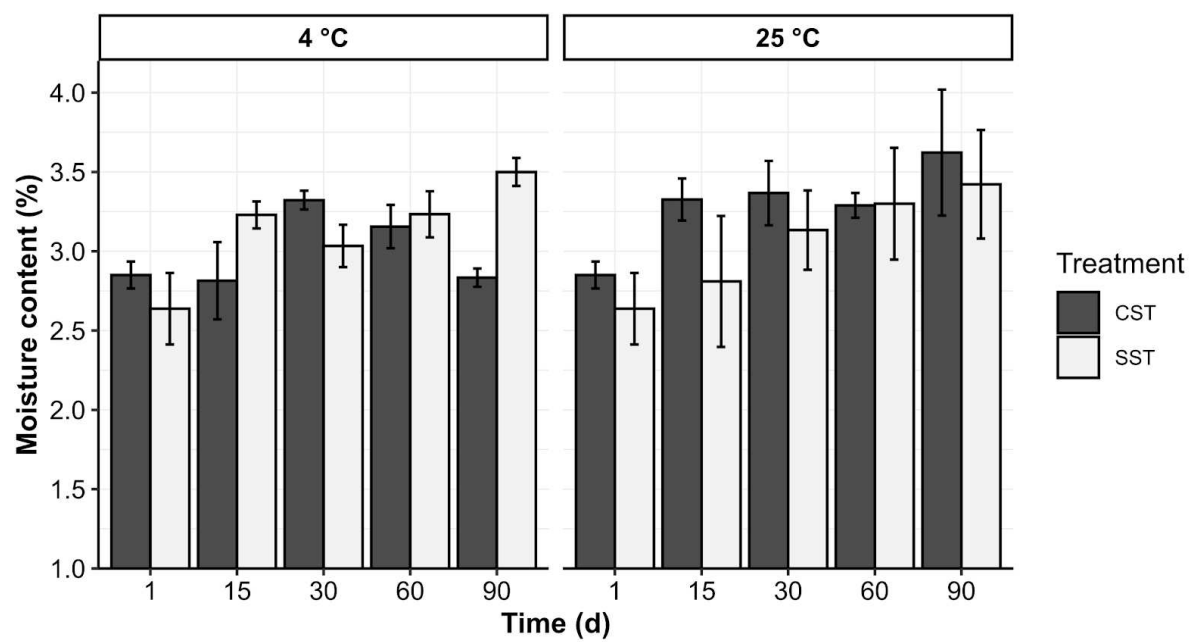


Figure 4. Moisture (%) over time (days) for the CST (with heat stress) and SST (without heat stress) treatments, with two different storage temperatures (4 and 25 °C). Error bars correspond to the standard error of the mean.

APÊNDICE F – FIGURE 5

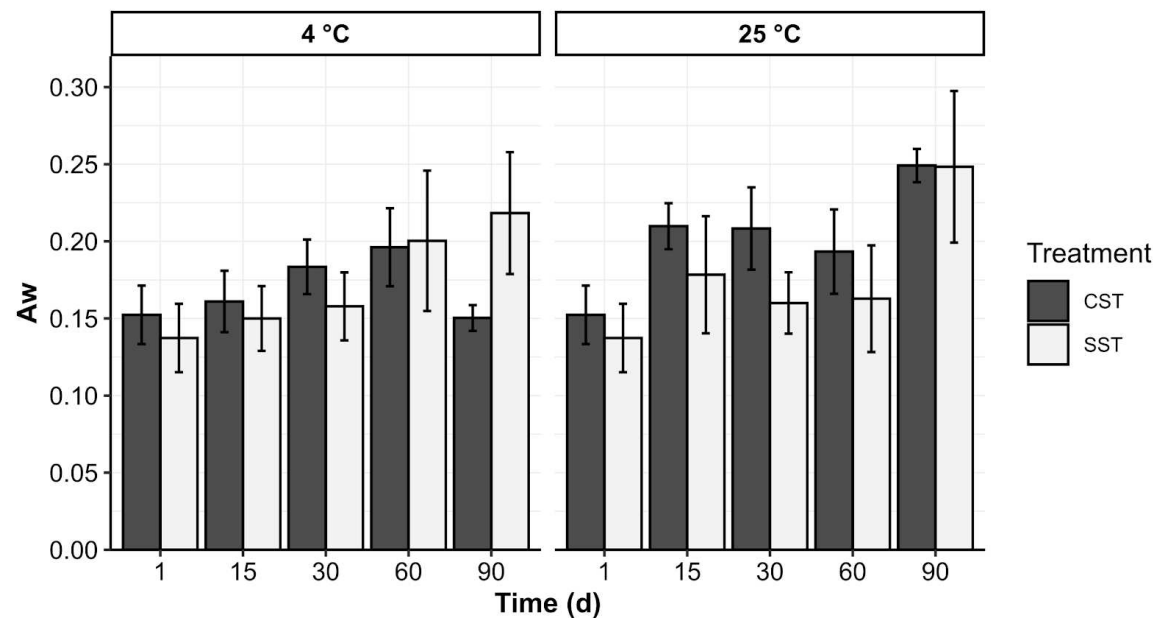


Figure 5. Aw over time (days) for CST (with heat stress) and SST (without heat stress) treatments, with two different storage temperatures (4 and 25 °C). Error bars correspond to the standard error of the mean.