

UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA NUTRIÇÃO

KARINA FELIX DIAS FERNANDES

**APLICAÇÃO DE REVESTIMENTO FORMULADO COM ALGINATO
DE SÓDIO E CULTURA MISTA DE BACTÉRIAS LÁTICAS PARA
CONTROLE DE ANTRACNOSE E PRESERVAÇÃO DA QUALIDADE
PÓS-COLHEITA EM GOIABA E MANGA**

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GOIABA E MANGA**

Tese apresentada ao Programa de Pós-Graduação em Ciências da Nutrição do Centro de Ciências da Saúde, Universidade Federal da Paraíba, como requisito obrigatório para obtenção de Título de Doutora em Ciências da Nutrição.

Orientador: Prof. Dr. Evandro Leite de Souza

Coorientadora: Profa. Dra. Kataryne Árabe Rimá de Oliveira

João Pessoa

2026

Catálogo na publicação
Seção de Catalogação e Classificação

F363a Fernandes, Karina Felix Dias.

Aplicação de revestimento formulado com alginato de sódio e cultura mista de bactérias lácticas para controle de antracnose e preservação da qualidade pós-colheita em goiaba e manga / Karina Felix Dias Fernandes. - João Pessoa, 2026.

203 f. : il.

Orientação: Evandro Leite de Souza.

Coorientação: Kataryne Árabe Rimá de Oliveira.

Tese (Doutorado) - UFPB/CCS.

1. Revestimentos comestíveis. 2. Controle biológico.
3. Microrganismos antagonistas. 4. Colletotrichum spp.
5. Frutas tropicais. I. Souza, Evandro Leite de. II.
Oliveira, Kataryne Árabe Rimá de. III. Título.

UFPB/BC

CDU 664.8(043)

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GOIABA E MANGA**

Tese APROVADA em 18 de Junho de 2026.

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Aos meus Pais, Irmão e Noivo,
Dedico.

AGRADECIMENTOS

A Deus, por sua infinita misericórdia, por ter me concedido o dom da vida, por me dar fé, coragem e ser meu sustento em todos os momentos.

Aos meus amados pais, Claudinete Felix Dias Fernandes e Genival Fernandes da Silva, por todo carinho, amor, incentivo, dedicação e ensinamentos, por confiarem que a educação é o melhor caminho, por sempre acreditarem nos meus sonhos e me apoiarem a realizá-los. Amo vocês e serei eternamente grata.

A Kaio Felix Dias Fernandes, meu irmão, por todo apoio, incentivo, amor, companheirismo diário e por ser fundamental na minha formação pessoal.

A Miguel Veríssimo da Silva Neto, meu noivo, por todo amor, companheirismo e incentivo diário, seu apoio foi imprescindível para a realização do doutorado, ao seu lado minha vida tem mais sentido.

A toda minha família, em especial a minha tia Arleide Felix Dias, por todo apoio em João Pessoa-PB, por me oferecer sua casa, tornado possível à realização dos meus estudos desde a graduação até o doutorado.

Ao Prof. Dr. Evandro Leite de Souza, por aceitar ser orientador desta tese. Agradeço a confiança, incentivo, disponibilidade, dedicação, profissionalismo e ensinamentos constantes.

A Profa. Dra. Kataryne Árabe Rimá de Oliveira, por aceitar ser Coorientadora desta tese. Agradeço por seus ensinamentos, confiança, disponibilidade, dedicação e incentivos diários.

Aos membros da banca examinadora, Profa. Dra. Estefânia Fernandes Garcia, Prof. Dr. Fillipe de Oliveira Pereira, Profa. Dra. Elma Regina Silva de Andrade Wartha e Prof. Dr. Marcos Antônio Barbosa de Lima, pela disponibilidade e valiosas contribuições a esta tese.

A todos que fazem parte do Laboratório de Microbiologia e Bioquímica dos Alimentos (LMBA/DN/ CCS/ UFPB), pela convivência, companheirismo e todos os ensinamentos prestados, em especial a Profa. Dra. Maria Lúcia da Conceição, e as técnicas Dra. Heloísa Maria Almeida do Nascimento e Dra. Sônia Paula Alexandrino de Oliveira.

Aos amigos e parceiros do LMBA, Dra. Maiara da Costa Lima, MsC. Jaielison Yandro Pereira da Silva, MsC. Talita Silveira Queiroga e Dra. Viviane Priscila Barros de Medeiros, e ao aluno de Iniciação Científica Ítallo Fernandes Cirilo, agradeço o companheirismo durante a realização dos experimentos.

Ao Laboratório de Bromatologia (DN/ CCS/ UFPB), em especial a Profa. Dra. Maria Elieidy Gomes de Oliveira, exemplo de profissionalismo e generosidade.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela concessão de bolsa de estudos durante a realização de todo doutorado.

A Universidade Federal da Paraíba, em especial ao Departamento de Nutrição e aos Professores do Programa de Pós-graduação em Ciências da Nutrição, por todos os ensinamentos e oportunidade de realização do doutorado.

Ao corpo editorial dos periódicos *Comprehensive Reviews in Food Science and Food Safety* (doi:10.1111/1541-4337.13427) e *Food Control* (doi:10.1016/j.foodcont.2025.111341) pelo reconhecimento do mérito e pela publicação dos manuscritos derivados desta tese.

A todos que de alguma forma contribuíram para a realização desta tese, meu muito obrigada!

RESUMO

Goiaba e manga são frutas tropicais amplamente comercializadas, mas por serem climatéricas, apresentam alterações fisiológicas, que as tornam mais suscetíveis à ação de patógenos. A antracnose, causada por fungos do gênero *Colletotrichum*, é uma das principais doenças pós-colheita dessas frutas. Embora o controle convencional da doença seja a utilização de fungicidas químicos, os impactos deletérios associados ao uso contínuo desses compostos têm impulsionado a busca por alternativas sustentáveis. Nesse contexto, o uso de bactérias lácticas (BAL) como agentes de biocontrole, associado a revestimentos comestíveis, como alginato de sódio (AS), destaca-se como uma estratégia inovadora e eficaz para a inibição de fitopatógenos e biopreservação de frutas. Esta tese teve como objetivo desenvolver, caracterizar e aplicar um novo revestimento à base de AS e cultura mista de BAL para controlar a antracnose e preservar a qualidade pós-colheita de goiaba (cv.) Paluma e manga (cv.) Palmer. O efeito de uma cultura mista composta por três cepas de diferentes espécies de BAL (*Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129 e *Limosilactobacillus fermentum* 263) foi avaliado *in vitro* contra dez isolados de *Colletotrichum*. Em seguida, foi desenvolvida uma formulação contendo AS e cultura mista de BAL liofilizada (AS+BAL), a qual foi caracterizada quanto às propriedades térmicas, morfológicas, físico-químicas e de biodegradabilidade. A sobrevivência, bem como as respostas fisiológicas e metabólicas das BAL incorporadas ao revestimento e ao filme, também foi avaliada sob diferentes condições de armazenamento (15 dias a 25 °C e 4 °C). O efeito dos revestimentos no controle do desenvolvimento de lesões de antracnose em goiaba e manga, bem como na manutenção de parâmetros indicadores de qualidade pós-colheita, foi avaliado durante 15 dias de armazenamento a 25 ± 0,5 °C. A cultura mista inibiu o crescimento micelial de todos os isolados testados, alcançando até 100% de eficácia no método de difusão em poços. A caracterização dos filmes demonstrou que a incorporação das BAL promoveu modificações na matriz polimérica, resultando em maior estabilidade térmica, superfície mais homogênea e sem poros. O filme AS+BAL apresentou menor rugosidade, espessura, teor de umidade, solubilidade em água, higroscopicidade e atividade da água, sem alteração nos parâmetros de cor, quando comparado ao filme sem BAL. Além disso, houve aumento na capacidade antioxidante após a incorporação de BAL nos filmes. Ambos os filmes demonstraram alta biodegradabilidade em solo, água doce e água do mar, com degradação quase completa em 20 dias. As amostras armazenadas sob refrigeração apresentaram contagens de células viáveis >7,5 log UFC/g, e 31,73 e 55,85% de subpopulações de células com membrana não permeabilizada e com atividades enzimáticas (células viáveis), respectivamente, ao final do armazenamento. A aplicação do revestimento AS+BAL em goiaba e manga retardou o desenvolvimento e reduziu o diâmetro das lesões de antracnose em até 78,94% em goiaba e 83,44% em manga após 15 dias de armazenamento (25 ± 0,5 °C). Nas frutas revestidas, observou-se preservação da qualidade pós-colheita, evidenciada pela menor perda de peso e pela maior manutenção da cor, bem como dos teores de ácido ascórbico e carotenoides ao final do armazenamento. Adicionalmente, as frutas tratadas com AS+BAL apresentaram melhor preservação das características sensoriais, especialmente cor, aroma e textura, além de menor percepção de maturação ao longo do período de armazenamento. Os resultados destacam que o revestimento AS+BAL pode ser eficaz para o biocontrole pós-colheita da antracnose em goiaba e manga. Além disso, este revestimento pode ser uma alternativa sustentável e biodegradável para a preservação da qualidade das frutas durante o período pós-colheita.

Palavras-chave: revestimentos; controle biológico; microrganismos antagonistas; *Colletotrichum* spp.; frutas tropicais.

ABSTRACT

Guava and mango are widely marketed tropical fruits; however, as climacteric fruits, they undergo physiological changes that make them more susceptible to pathogens. Anthracnose, caused by fungi of the genus *Colletotrichum*, is one of the main post-harvest diseases of these fruits. Although conventional disease control involves the use of chemical fungicides, the deleterious impacts associated with the continuous use of these compounds have driven the search for sustainable alternatives. In this context, the use of lactic acid bacteria (LAB) as biocontrol agents, combined with edible coatings such as sodium alginate (SA), stands out as an innovative and effective strategy for inhibiting phytopathogens and biopreserving fruits. This thesis aimed to develop, characterize, and apply a new SA-based coating and LAB mixed culture to control anthracnose and preserve the post-harvest quality of guava (cv.) Paluma and mango (cv.) Palmer. The effect of a mixed culture composed of three strains of different LAB species (*Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129, and *Limosilactobacillus fermentum* 263) was evaluated *in vitro* against ten *Colletotrichum* isolates. Subsequently, a formulation containing AS and a mixed culture of lyophilized LAB (AS+LAB) was developed, which was characterized in terms of thermal, morphological, physicochemical, and biodegradability properties. The survival, as well as the physiological and metabolic responses of the LAB incorporated into the coating and film, were also evaluated under different storage conditions (15 days at 25 °C and 4 °C). The effect of coatings on controlling the development of anthracnose lesions in guava and mango, as well as on maintaining post-harvest quality indicator parameters, was evaluated during 15 days of storage at 25 °C. The mixed culture inhibited mycelial growth of all isolates tested, achieving up to 100% effectiveness in the well diffusion method. Film characterization demonstrated that the incorporation of LAB promoted modifications in the polymeric matrix, resulting in greater thermal stability, a more homogeneous surface, and no pores. The AS+LAB film showed lower roughness, thickness, moisture content, water solubility, hygroscopicity, and water activity, without alteration in color parameters, when compared to the film without LAB. Furthermore, the ABTS^{•+}, DPPH[•], and FRAP assays indicated an increase in antioxidant capacity after the incorporation of LAB into the films. Both films demonstrated high biodegradability in soil, freshwater, and seawater, with almost complete degradation in 20 days. Samples stored under refrigeration showed viable cell counts >7.5 log CFU/g, and 31.73% and 55.85% of cell subpopulations with non-permeabilized membranes and enzymatic activities (viable cells), respectively, at the end of storage. The application of the AS+LAB coating to guava and mango delayed the development and reduced the diameter of anthracnose lesions by up to 78.94% in guava and 83.44% in mango after 15 days of storage (15 days; 25 ± 0.5 °C). In the coated fruits, preservation of post-harvest quality was observed, evidenced by less weight loss and greater maintenance of color, as well as ascorbic acid and carotenoid content at the end of storage. Additionally, fruits treated with AS+LAB showed better preservation of sensory characteristics, especially color, aroma, and texture, as well as less perceived ripening throughout the storage period. The results highlight that the AS+LAB coating can be effective for post-harvest biocontrol of anthracnose in guava and mango. Furthermore, this coating may be a sustainable alternative for preserving fruit quality during the post-harvest period.

Keywords: coatings; biological control; antagonistic microorganisms; *Colletotrichum* spp.; tropical fruits.

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1 INTRODUÇÃO

A fruticultura brasileira destaca-se mundialmente como um dos setores mais importantes na agroindústria, consolidando-se devido a sua relevância econômica, sobretudo na região Nordeste (Kist *et al.*, 2025). Dentre as frutas mais produzidas nesta região, destacam-se goiaba (*Psidium guajava* L.) e manga (*Mangifera indica* L.), devido ao sabor agradável, fácil acesso comercial e presença de diversos nutrientes em sua composição, como açúcares, minerais, fibras, ácido ascórbico, carotenoides e compostos fenólicos (Bilal; Hashmi, 2023; Semwal *et al.*, 2024). Em 2023, o Brasil produziu 2,39 milhões de toneladas dessas frutas, ocupando a quinta posição entre os maiores produtores mundiais (FAO, 2026). No mesmo período, o valor conjunto da produção ultrapassou 4,5 bilhões de reais (Kist *et al.*, 2025).

No entanto, por serem frutas climatéricas goiabas e mangas apresentam elevada suscetibilidade à deterioração fisiológica e ao desenvolvimento de patógenos durante o armazenamento, reduzindo sua vida útil e favorecendo perdas pós-colheita (Mahapatra *et al.*, 2024; Nguyen *et al.*, 2026). Dentre as perdas pós-colheita de frutas causadas por infecções de microrganismos, destacam-se os danos provocados pelo desenvolvimento de fungos fitopatogênicos, as quais podem causar perdas de até 25% da produção total, especialmente quando as condições ambientais favorecem o desenvolvimento das doenças (Bano *et al.*, 2023).

A antracnose causada por fungos do gênero *Colletotrichum* é considerada uma das mais importantes doenças pós-colheita que acometem goiabas e mangas em todo o mundo devido à severidade dos sintomas desenvolvidos e das significativas perdas econômicas causadas pelo estabelecimento da infecção (Chen *et al.*, 2024; Li *et al.*, 2024). Para o controle de antracnose, tradicionalmente são usados fungicidas químicos como tiofanato-metílico (Wu *et al.*, 2022) e difenoconazole (Carraro *et al.*, 2024), entretanto, devido aos efeitos deletérios a saúde humana associados ao uso contínuo desses produtos, tem-se intensificado a busca por estratégias alternativas com potencial fungitóxico e menor impacto ambiental (Wu *et al.*, 2023).

Nesse contexto, tecnologias pós-colheita seguras, economicamente viáveis e ambientalmente sustentáveis vêm sendo desenvolvidas, destacando-se o uso de microrganismos antagonistas (leveduras e bactérias) como agentes de biopreservação (Evangelista-Martínez *et al.* 2022; Zhou *et al.*, 2022). As bactérias lácticas (BAL) são reconhecidas como microrganismos com potencial para exercer efeitos antagonistas contra contaminantes alimentares, incluindo fungos fitopatogênicos (Hirozawa *et al.*, 2023). Essa ação é atribuída à sua capacidade de sintetizar metabólitos secundários com atividade antimicrobiana, ativar respostas de defesa em

plantas hospedeiras e competir eficientemente por nutrientes e espaço (Barrios-Salgado *et al.*, 2022; Mpeluza *et al.*, 2023). O efeito antagonista das BAL contra fitopatógenos em frutas tem sido investigado em diferentes estudos (Bambace *et al.*, 2024; de Simone *et al.*, 2024; Li *et al.*, 2024; Melo *et al.*, 2021). A aplicação de microrganismos antagonistas pode ainda ser combinada com outros tratamentos, visando potencializar seus efeitos, como o uso em revestimentos comestíveis. Essa abordagem pode melhorar sua viabilidade e consequentemente sua atividade antimicrobiana (Wang *et al.*, 2023). Além disso, os revestimentos comestíveis podem retardar o processo de respiração, amadurecimento e deterioração microbiana das frutas, repercutindo em ampliação do seu período de armazenamento (Azarakhsh *et al.*, 2024; Boubakeur *et al.*, 2026; Silva *et al.*, 2023).

O alginato de sódio é um polissacarídeo comumente utilizado para formular revestimentos comestíveis, principalmente, devido às suas propriedades de geleificação e espessamento, além das características de biodegradabilidade, baixa toxicidade e baixo custo (Wang *et al.*, 2025). Os revestimentos à base de alginato de sódio têm sido comumente utilizados como carreadores de microrganismos antagonistas para aplicação em frutas, sendo relatados resultados promissores em relação à conservação destes produtos (Bambace *et al.*, 2024; Boubakeur *et al.*, 2026; Li *et al.*, 2024; Wei *et al.*, 2025). Além disso, revestimentos de alginato de sódio têm mostrado vantagens tecnológicas em relação a outros revestimentos formulados com polissacarídeos, não afetando negativamente a sobrevivência dos agentes antagonistas durante o armazenamento (Speranza *et al.*, 2018).

Apesar dos avanços na aplicação de BAL e de revestimentos comestíveis para a conservação pós-colheita de frutas, permanecem limitados os estudos que exploram revestimentos de alginato de sódio incorporados com culturas mistas de BAL, particularmente para o controle da antracnose causada por diferentes espécies de *Colletotrichum* bem como para a manutenção da qualidade pós-colheita de goiabas e mangas. Nesse contexto, esta tese teve como objetivo desenvolver um revestimento à base de alginato de sódio incorporado com uma cultura mista de cepas de BAL e caracterizá-lo quanto às propriedades térmicas, morfológicas, físico-químicas e de biodegradabilidade. Adicionalmente, avaliou-se o efeito antagonista dessa cultura mista de BAL contra diferentes espécies de *Colletotrichum in vitro*. Em seguida, investigaram-se os efeitos da aplicação do revestimento no controle da antracnose causada por diferentes isolados de *Colletotrichum* em goiaba (cv. Paluma) e manga (cv. Palmer). Na etapa final, foram avaliados os efeitos do revestimento sobre parâmetros físicos, físico-químicos e sensoriais relacionados à qualidade pós-colheita dessas frutas durante o armazenamento.

2 REFERENCIAL TEÓRICO

2.1 PRODUÇÃO E COMERCIALIZAÇÃO DE GOIABA E MANGA

O total de frutas destinadas ao mercado abrange várias espécies, contudo algumas têm se destacado por suas características nutricionais e pelo volume de produção, a exemplo de goiaba e manga (FAO, 2026). De acordo com o Anuário brasileiro de fruticultura (2025), o Brasil produziu em 2023, 582 mil toneladas de goiaba e 1,75 milhão de toneladas de manga, com destaque para os estados de Pernambuco e Bahia como os maiores produtores, responsáveis por cerca de 80% do total produzido no país.

No ano de 2023, o valor econômico dessas frutas em conjunto ultrapassou a marca de 4 bilhões de reais (Kist *et al.*, 2025). Em 2024, manga foi a fruta mais exportada pelo Brasil, com volume de 258 mil toneladas, e os principais destinos foram os países da Europa e os Estados Unidos (FAO, 2026). Contudo, apesar do expressivo volume destinado à exportação, a maior parte da produção dessas frutas é consumida no mercado interno, principalmente na forma *in natura*, além de ser utilizada na elaboração de diversos produtos alimentícios, incluindo sucos, polpas e doces (Kist *et al.*, 2025; Santos *et al.*, 2023; Bilal; Hashmi, 2023).

A goiaba é uma fruta doce e aromática da família *Myrtaceae* nativa da América Central. Valorizada por seu sabor delicioso e composição, em termos de nutrientes, com destaque para minerais como cálcio, ferro e fósforo (Hasan *et al.*, 2022). A polpa apresenta alto teor de vitaminas A, B1 e C (Formiga *et al.*, 2022). Além disso, a goiaba vermelha também é uma importante fonte de licopeno, pigmento com reconhecida atividade antioxidante (Mathiazhagan *et al.*, 2024).

Por essas características, a goiaba é uma fruta consumida em diversos países, sendo de maior importância comercial naqueles de zonas tropicais como os localizados na América Latina, especialmente México e Brasil, bem como em alguns países da África e Ásia (Chen *et al.*, 2024; Semwal *et al.*, 2024). Em destaque, Índia, Brasil, Filipinas, México e Colômbia são os principais produtores desta fruta. A maioria das variedades vendidas na Europa é importada da África do Sul e do Brasil, sendo comercialmente classificados como brancas ou vermelhas, dependendo da cor da polpa (Angulo-López *et al.*, 2021).

No Brasil, a goiaba encontra-se naturalmente distribuída por todo o território nacional, o que a torna um importante recurso para a produção agrícola local (Santos *et al.*, 2023). O cultivar Paluma (Figura 1) está entre os mais produzidos no Brasil devido sua maior aceitação para consumo *in natura* e para o processamento industrial (Kist *et al.*, 2025). A goiaba *in natura*

apresenta elevada demanda nos mercados nacional e internacional (FAO, 2026). Em 2024, o volume de goiaba exportado pelo Brasil totalizou 589 toneladas (Kist *et al.*, 2025). No entanto, grande parte da produção é destinada à indústria alimentícia, sendo utilizada na fabricação de conservas, bebidas, polpas, néctares, geleias e xaropes. Esses produtos processados de goiaba, especialmente as geleias e polpas, possuem grande aceitação no mercado, contribuindo significativamente para a diversificação da oferta e agregação de valor (Santos *et al.*, 2023).

Figura 1 - Frutas de goiaba (*Psidium guajava* L.) cv. Paluma.



Fonte: CEAGESP, 2023.

Em relação ao processo de maturação, devido a suas características de respiração, a goiaba é caracterizada como uma fruta altamente perecível, que sofre rápida perda de firmeza, cor verde e brilho, completando seu processo de amadurecimento em poucos dias após a colheita (Hasan *et al.*, 2022; Shu *et al.*, 2024). Quando colhida no estágio verde-maduro, a vida útil da goiaba pode chegar a até seis dias em temperatura ambiente. Por isso, os produtores comumente colhem a fruta em um estágio imaturo (verde) para aumentar a disponibilidade comercial da fruta, estendendo a vida útil para oito a dez dias (Formiga *et al.*, 2022). Além disso, as frutas são sensíveis a danos por frio quando armazenadas a uma temperatura abaixo de 13 °C (Hsiao *et al.*, 2026). Tudo isso limita significativamente a produção e a comercialidade da goiaba, com perdas pós-colheita importantes (Shu *et al.*, 2024).

A manga é uma fruta tropical, amplamente consumida no mundo, principalmente devido ao sabor e composição nutricional (Qasim *et al.*, 2025). Dentre seus constituintes, a manga é rica em carboidratos, ácidos graxos, vitaminas e minerais, ácido ascórbico, ácidos orgânicos, fibras alimentares, polifenóis, carotenoides, flavonoides e mangiferina (Yahia *et al.*, 2023). As propriedades nutricionais da manga, especialmente os antioxidantes, são essenciais para a saúde

humana, pois são conhecidos por estimular o sistema imunológico, prevenir doenças cardiovasculares, catarata e alguns tipos de câncer (Bambalele *et al.*, 2021; Yahia *et al.*, 2023). Além disso, as qualidades sensoriais da manga merecem destaque por se tratar de uma fruta de sabor doce e agradável (Bilal; Hashmi, 2023; Li *et al.*, 2024). A manga está entre as principais frutas tropicais cultivadas no mundo, classificada como a segunda mais importante, depois da banana. Apresenta uma predominância do consumo *in natura*, contudo parte da produção também é destinada a indústria de processamento de alimentos, como sucos, néctares e geleias (Bilal; Hashmi, 2023; Oliver-Simancas *et al.*, 2021; Sousa *et al.*, 2021).

No Brasil, existe uma grande diversidade de cultivares de manga, os quais variam de acordo com a região de cultivo, contudo, de forma geral, há predomínio da comercialização dos cultivares Tommy Atkins, Palmer, Rosa, Espada, Keitt e Haden. Dentre esses, os cultivares de manga preferidos para exportação do Brasil são Tommy Atkins e Palmer, tendo como principais compradores países da União Europeia e Estados Unidos (Kist *et al.*, 2025). O cultivo de mangueiras cultivar Palmer (Figura 2) tem crescido significativamente nos últimos anos devido às suas melhores características sensoriais, com destaque para menor teor de fibras e maior teor de sólidos solúveis (Sousa *et al.*, 2021).

Em geral, a manga Palmer termina o amadurecimento oito dias após a colheita, período quando começa a perder a qualidade visual (Sousa *et al.*, 2021). Após esse tempo, os pigmentos presentes nas frutas passam por transformações bioquímicas específicas e direcionadas ao processo de amadurecimento até a senescência (Almeida *et al.*, 2024). Além disso, doenças pós-colheita, e lesões por frio também são responsáveis por diminuir a qualidade dessas frutas (Bambalele *et al.*, 2021, Li *et al.*, 2024).

Figura 2 - Frutas de manga (*Mangifera indica* L.) cv. Palmer



Fonte: CEAGESP, 2023.

No geral, por serem frutas tropicais, goiaba e manga são comumente consumidas *in natura*, e, portanto, cada alteração em suas características e atributos de qualidade as torna menos atraentes para os consumidores o que dificulta sua comercialização (González-Cuello *et al.*, 2024; Wang *et al.*, 2023). As condições individuais ou combinadas que resultam em perdas pós-colheita, impactam na diminuição de receita para os produtores e todos os envolvidos na cadeia de abastecimento. Uma proporção significativa de perdas dessas frutas ocorre durante o armazenamento e transporte como resultado de manuseio e instalações inadequadas (Bambalele *et al.*, 2021).

2.2 PRINCIPAIS CAUSAS DE PERDAS PÓS-COLHEITA EM FRUTAS

As perdas pós-colheita de frutas ao longo da cadeia de abastecimento comprometem a disponibilidade e o acesso a esses alimentos (Silué *et al.*, 2024). Essas perdas correspondem às reduções quantitativas e qualitativas dos produtos que ocorrem desde a colheita até sua chegada ao consumidor (Bano *et al.*, 2023). As principais causas dessas perdas incluem práticas inadequadas de manuseio e armazenamento, transporte deficiente e limitações na infraestrutura (Bambalele *et al.*, 2021). O metabolismo intrínseco dessas frutas também influencia significativamente nas perdas pós-colheita, uma vez que estas frutas sofrem efeitos do hormônio etileno, que quando produzido em grandes concentrações acelera muitas atividades enzimáticas e consequentemente a sua senescência (Alonso-Salinas *et al.*, 2024).

O etileno está envolvido na maioria das etapas do desenvolvimento da planta, incluindo germinação de sementes, crescimento de raízes, desenvolvimento de flores, amadurecimento de frutas e senescência (Alonso-Salinas *et al.*, 2024). Com base no perfil respiratório e na produção de etileno durante o amadurecimento, as frutas podem ser divididas em duas classes: climatéricas e não climatéricas (Bianchetti *et al.*, 2024). Dentre as frutas climatéricas estão incluídas goiaba e manga que apresentam a característica de um aumento rápido e significativo da respiração durante o amadurecimento. O processo de amadurecimento das frutas envolve alterações fisiológicas e bioquímicas como acúmulo de pigmentos, amolecimento, formação de aroma e sabor, além de mudanças na cor, causada pela degradação da clorofila e ao acúmulo de pigmentos como carotenoides e antocianinas (Yadav *et al.*, 2022).

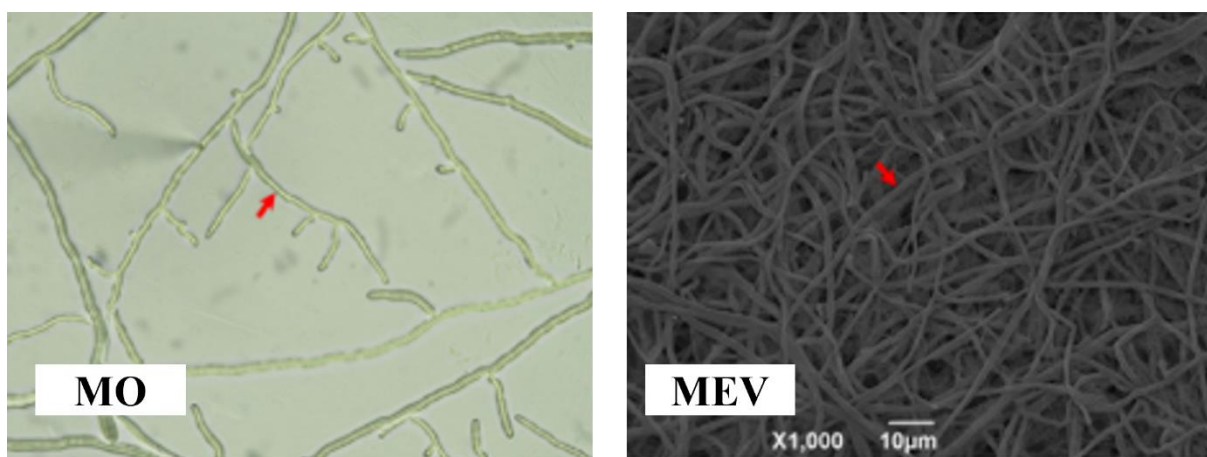
Além da maturação fisiológica, quando expostas a condições de alta temperatura e umidade, goiaba e manga são suscetíveis à invasão e infecção por vários fungos patogênicos, resultando em declínio na qualidade da fruta e perda de valor de mercado (Tovar *et al.*, 2019;

Wu *et al.*, 2022). As principais doenças provocadas por fungos associadas à manga e goiaba são: antracnose (*Colletotrichum* spp.), podridão da extremidade do caule (*Botryodiplodia theobromae*, *Lasiodiplodia theobromae*, *Phomopsis mangifera* ou *Dithiorella dominicana*), podridão negra (*Aspergillus niger*, *Aspergillus variegatus* e *Aspergillus saubreyi*) e podridão alternaria (*Alternaria alternata*) (Gwiazdowski *et al.*, 2024; Zakaria 2021). Essas doenças são limitadoras para produção, estando presente em várias regiões onde as frutas são cultivadas (Soares *et al.*, 2024).

2.2.1 Antracnose em frutas

O gênero *Colletotrichum*, formado por fungos filamentosos caracterizados pela produção de hifas septadas, conídios hialinos e acérvulo (Figura 3), é um importante fitopatógeno em todo o mundo, sendo responsável por causar a antracnose em vegetais nos trópicos, subtropicos e zonas temperadas (Soares *et al.*, 2024). A taxonomia deste gênero é constantemente atualizada, e a mais recente reconhece 340 espécies pertencentes ao gênero *Colletotrichum* (Talhinhas; Baroncelli, 2023). Os agentes causadores da antracnose da goiaba e manga têm sido tradicionalmente identificados como *C. gloeosporioides* (Nguyen *et al.*, 2026) e *C. acutatum* (Ishii *et al.*, 2022). Entretanto, outras espécies de *Colletotrichum* têm sido reportadas como causadoras de antracnose nessas frutas, a citar: *C. asianum*, *C. brevisporum*, *C. dianesei*, *C. fructicola*, *C. musae*, *C. siamense*, *C. tropicale* e *C. karstii* (Lima *et al.*, 2013; Fernandes *et al.*, 2024; Soares *et al.*, 2024; Vitale *et al.*, 2020).

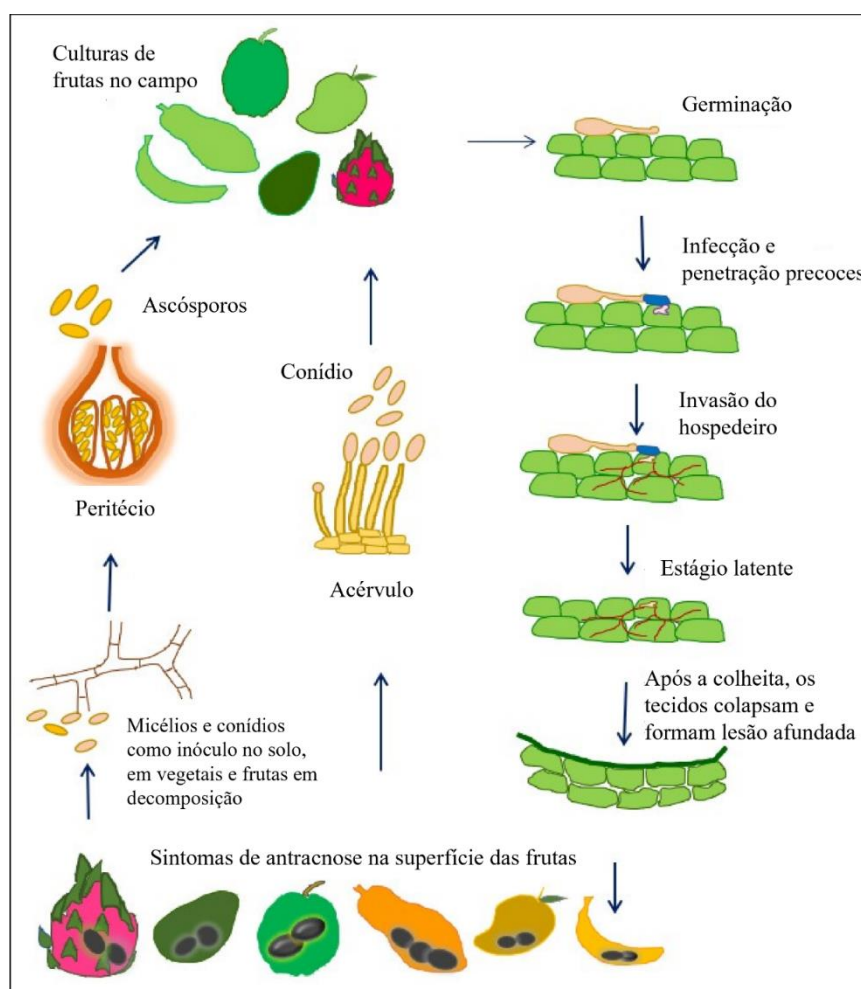
Figura 3 - Morfologia de *C. gloeosporioides* por microscopia óptica (MO) e microscopia eletrônica de varredura (MEV).



Fonte: Adaptado de Wang *et al.*, 2023.

A Figura 4 resume o ciclo da doença da antracnose em frutas tropicais, com a infecção ocorrendo comumente nos campos durante os estágios de floração e frutificação. Essa infecção geralmente começa com a germinação dos conídios e é seguida pela formação de apressórios e pinos de penetração, que pode ocorrer através de feridas ou aberturas naturais (Mpeluza *et al.*, 2023). Após a infecção, muitos patógenos da doença adotam quiescência ou latência, permanecendo dormentes dentro dos tecidos do hospedeiro até que as condições ambientais (incluindo umidade, temperatura) e a fisiologia sejam propícias para sua reativação, que ocorre, principalmente quando as frutas amadurecem (de Oliveira *et al.*, 2023; Osondu *et al.*, 2022). Assim, os sintomas da antracnose tendem a se desenvolver principalmente após a colheita, durante o armazenamento, transporte e comercialização (Nguyen *et al.*, 2026; Ranjith *et al.*, 2022).

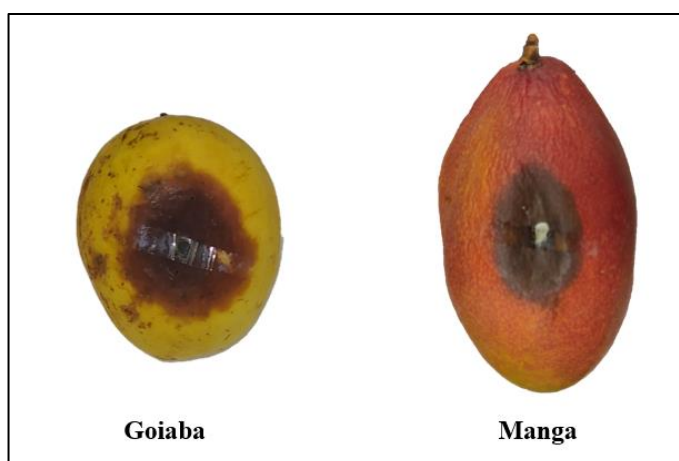
Figura 4 – Ciclo da doença antracnose em frutas tropicais.



Fonte: Adaptado de Zakaria, 2021.

Os sintomas da antracnose variam muito dependendo do tecido da planta, nos caules ou folhas variam de pequenas manchas afundadas marrom-acinzentadas a manchas mais escuras nos galhos; já as flores infectadas parecem secas e podres, enquanto nas frutas a antracnose produz lesões marrons ou pretas, circulares, afundadas, podendo apresentar massa viscosa de esporos, com linha nítida de demarcação entre as áreas infectadas e não infectadas, podem evoluir causando necrose do tecido vegetal (Ciofini *et al.*, 2022). Sintomas característicos da antracnose em goiaba e manga são ilustrados na Figura 5. Essas lesões aceleram o processo de deterioração de frutas, afetando sua qualidade geral e provocando perdas econômicas em toda cadeia de produção até a aceitação do consumidor (Liu *et al.*, 2025; Tovar *et al.*, 2019).

Figura 5 - Sintomas de antracnose causada por *Colletotrichum* em goiaba cv. Paluma e manga cv. Palmer.



Fonte: própria, 2026.

As infecções em frutas causadas por *Colletotrichum* possuem grande impacto econômico, afetando vários setores da cadeia de abastecimento. Cerca de 30% a 60% da perda de rendimento de frutas a cada ano é atribuída a este patógeno (Tovar *et al.*, 2019; Wu *et al.*, 2022). O prolongamento da vida útil pós-colheita dessas frutas beneficiaria o mercado, permitindo expansão e flexibilidade de comercialização, com aumento da qualidade do produto e redução de perdas (Sousa *et al.*, 2021).

Diferentes técnicas são atualmente empregadas para manter a qualidade pós-colheita de frutas, como físicas, químicas e biológicas. Principalmente na fase pós-colheita, o controle da antracnose é dependente do uso de fungicidas químicos tradicionais, que permitem a conservação prolongada das frutas (Cuéllar-Torres *et al.*, 2022). Nas últimas duas décadas,

fungicidas químicos sintéticos, como clorotalonil, propiconazol, metilcarbamato e tiofanato-metil, foram os agentes mais populares usados para a preservação de frutas frescas (Li *et al.*, 2024; Ranjith *et al.*, 2022).

O uso frequente e em altas doses desses fungicidas pode favorecer o surgimento de microrganismos resistentes, além de causar contaminação ambiental, especialmente do solo e da água, com potencial impacto na redução da biodiversidade de organismos não-alvo (Kim *et al.*, 2022). Já os efeitos na saúde de pessoas expostas a fungicidas dependem do tipo de agrotóxico, da duração e da via de exposição, bem como do estado de saúde. Um problema importante é a possibilidade de bioacumulação dessas substâncias no tecido adiposo, embora também possam ser metabolizadas e excretadas do corpo (Gwiazdowski *et al.*, 2024).

Os efeitos negativos à saúde associados aos fungicidas químicos tradicionais incluem manifestações dermatológicas, gastrointestinais, neurológicas, respiratórias, reprodutivas, hormonais e potencial carcinogênico (Mpeluza *et al.*, 2023). Diante dessas preocupações, tem-se intensificado o debate regulatório e científico sobre o uso dessas substâncias. Nos últimos anos, a União Europeia tem adotado regulamentações mais rigorosas para o uso e a importação de determinados fungicidas químicos, em razão de suspeitas relacionadas às suas propriedades de desregulação endócrina. Como consequência, tem havido maior restrição à entrada de produtos importados tratados com essas substâncias (Ishii *et al.*, 2022).

Estratégias alternativas mais seguras para controlar/ reduzir a gravidade e a incidência de lesões de antracnose em frutas tropicais têm sido descritas na literatura, incluindo barreiras físicas, antimicrobianos naturais e controle biológico com microrganismos antagonistas (Fernandes *et al.*, 2024). O uso de microrganismos antagônicos e seus metabólitos destacam-se entre os métodos usados para preservar a qualidade de alimentos em geral e aumentar sua segurança, sendo essa abordagem conhecida como biopreservação ou biocontrole (Huang *et al.*, 2024).

Uma descrição detalhada sobre esta temática é apresentada no artigo intitulado “*Interventions based on alternative and sustainable strategies for postharvest control of anthracnose and maintain quality in tropical fruits*” publicado na revista *Comprehensive Reviews in Food Science and Food Safety*, em 2024 (APÊNDICE B), como um dos produtos derivados desta tese. O artigo de revisão reúne informações atuais e relevantes sobre as principais intervenções inovadoras relativas à aplicação de estratégias alternativas sustentáveis no controle pós-colheita de espécies patogênicas de *Colletotrichum* em frutas tropicais.

2.3 BIOCONTROLE E BACTÉRIAS LÁTICAS COMO AGENTES DE BIOPRESERVAÇÃO DE FRUTAS

O controle biológico é considerado uma alternativa promissora para a aplicação em frutas, pois oferece soluções sustentáveis e eficazes (Huang *et al.*, 2024). Esse método consiste na utilização de organismos vivos para reduzir ou mitigar os danos causados por pragas ou patógenos, sendo esses denominados como agentes de controle biológico, que podem incluir microrganismos como fungos, bactérias ou vírus (Hirozawa *et al.*, 2023). O uso de microrganismos no controle de fitopatógenos é relevante para oferecer uma alternativa aos fungicidas sintéticos artificiais, viabilizando a certificação de produtos agrícolas como orgânicos (Gwiazdowski *et al.*, 2024).

Atualmente, o uso de técnicas de biopreservação para controlar as doenças pós-colheita de frutas também está se tornando mais popular devido à alta demanda dos consumidores por frutas livres de produtos químicos sintéticos (Ranjith *et al.*, 2022). Neste contexto, as bactérias podem ser utilizadas por apresentarem diversos mecanismos antagônicos aos fungos fitopatogênicos, como a competição por nutrientes, produção de enzimas hidrolíticas, indução de resistência na planta, síntese de compostos voláteis e formação de biofilmes (Hirozawa *et al.*, 2023). O uso de bactérias como agentes de biocontrole tem sido muito importante no manejo integrado de cultivos e produção orgânica, onde se destaca seu valor como controle pós-colheita de doenças fúngicas, sendo considerada eficiente a curto, médio e longo prazo, e não representando risco ao meio ambiente nem causadora de danos a humanos e animais (Gwiazdowski *et al.*, 2024).

As BAL são amplamente distribuídas na natureza, e abrangem mais de 60 gêneros, no entanto os mais frequentemente usados em aplicações industriais são *Lactobacillus* spp., *Lactococcus* spp., *Enterococcus* spp., *Leuconostoc* spp., *Oenococcus* spp., *Pediococcus* spp., *Streptococcus* spp. e *Weissella* spp. (Hirozawa *et al.*, 2023). Como agentes de biocontrole, estas bactérias são capazes de se adaptar ao ambiente onde o patógeno se encontra, o que é fundamental para um potencial antagonista pós-colheita (de Simone *et al.*, 2024).

O uso de BAL e seus compostos antimicrobianos para a preservação de frutas tem sido aceito como uma técnica para aumentar a vida de prateleira, qualidade e propriedades sensoriais de frutas (Agriopoulou *et al.*, 2020). Alguns estudos investigaram o efeito antagonista de cepas de microrganismos probióticos frente a fungos patogênicos em frutas. de Simone *et al.* (2021) avaliaram a eficácia de inibição de cepas de BAL potencialmente probióticas frente a *Botrytis cinerea* em kiwis minimamente processados, apresentando uma capacidade promissora de

retardar o crescimento do fungo. As propriedades antifúngicas de cepas de BAL também foram testadas contra *Aspergillus niger*, *Fusarium* sp. e *Rhizopus stolonifer* em tomates cereja e foi constatado o poder inibitório das cepas sobre os fungos avaliados (Melo *et al.*, 2021).

Dentre as BAL, Lactobacilos, e alguns dos gêneros recentemente reclassificados em sua taxonomia (Zheng *et al.*, 2020) tem se destacado na aplicação como agentes antagonistas em frutas (Hirozawa *et al.*, 2023; Huang *et al.*, 2024; Pan *et al.*, 2025). BAL são consideradas GRAS (*Generally Recognized as Safe*) pela *Food and Drug Administration* (FDA) e de QPS (*Qualified Presumption of Safe*) pela *European Food Safety Authority* (EFSA), características que reforçam sua segurança e potencial para aplicação em controle biológico de alimentos (Gajendran; Rajamani, 2025; Hirozawa *et al.*, 2023; Liu *et al.*, 2024; Yap *et al.*, 2022).

Os efeitos inibitórios das BAL podem ser atribuídos à produção de metabólitos que são capazes de conferir atividades antimicrobianas, incluindo ácidos orgânicos, aldeídos, cetonas, ácidos graxos e bacteriocinas, como também pela indução de defesas nos hospedeiros (Wong; Li, 2023). A produção de compostos antimicrobianos promove a proteção direta contra organismos indesejados, enquanto a indução de respostas de defesa nas plantas confere proteção indireta, aumentando a resistência aos microrganismos fitopatogênicos (Li *et al.*, 2024). Como mecanismos de ação, as bacteriocinas e ácidos orgânicos exercem atividade antifúngica ao penetrarem nas membranas celulares em sua forma não dissociada, promovendo a acidificação do pH intracelular e interrompendo as atividades metabólicas fúngicas (Bangar *et al.*, 2022; Yap *et al.*, 2022).

Lactiplantibacillus plantarum C10 foi testado frente à *Trichothecium roseum*, causador do apodrecimento em melão, sendo reportado efeito inibitório sobre o crescimento fúngico (Lv *et al.*, 2018). A atividade antagônica de *Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129 e *Limosilactobacillus fermentum* 263 foi testada isoladamente frente a diferentes cepas de *Colletotrichum* em goiaba e manga, e os resultados constataram que as cepas testadas inibiram o crescimento fúngico, bem como reduziram a severidade das lesões de antracnose nas frutas (Fernandes; De Oliveira; De Souza, 2021).

Apesar dos efeitos promissores obtidos, a aplicação de BAL isoladamente em frutas pode ocasionar impactos sensoriais indesejáveis, podendo também diminuir a sobrevivência das cepas durante o armazenamento. A incorporação de culturas de BAL em revestimentos comestíveis tem sido proposta como uma alternativa viável para melhorar a estabilidade dos microrganismos, sendo aplicada pela primeira vez por Tapia *et al.* (2007), em um revestimento composto com alginato e gelatina carregado com *Bifidobacterium lactis* Bb-12 para chips de maçã e mamão.

Desde então, outros estudos foram realizados para avaliar a eficácia antimicrobiana de probióticos em revestimentos para frutas, sendo observado um aumento do potencial de inibição do crescimento de patógenos vegetais. A aplicação de revestimentos a base de alginato de sódio adicionado de *L. rhamnosus*, *L. paracasei* e *L. plantarum* foi capaz de inibir o crescimento de bactérias e fungos em morangos durante armazenamento refrigerado, além de melhorar a qualidade físico-química das frutas (Li *et al.*, 2024).

2.4 APLICAÇÃO DE REVESTIMENTOS COMESTÍVEIS EM FRUTAS

Revestimentos comestíveis são aplicados em frutas como tratamentos pós-colheita biodegradáveis. Eles formam uma fina camada sobre a superfície da fruta, criando uma barreira protetora ao oxigênio e umidade (Kohli *et al.*, 2024). A incorporação de revestimentos comestíveis na preservação de frutas oferece vários benefícios tecnológicos, incluindo aparência melhorada, propriedades antimicrobianas, intensidade respiratória reduzida e inibição da produção de etileno (Li *et al.*, 2024).

Nos últimos anos, os materiais formadores de revestimento aplicados em frutas mais estudados incluem: celulose (Nkede *et al.*, 2024), gelatina (Azarakhsh *et al.*, 2024); goma arábica (López-Ortiz *et al.*, 2025; Lundgren *et al.*, 2021), carboximetilcelulose (Zhang *et al.*, 2024; Nguyen *et al.*, 2026), quitosana (Osondu *et al.*, 2022; Zhou *et al.*, 2024; Parente *et al.*, 2025) e alginato de sódio (Alvarez *et al.*, 2021; Hasan *et al.*, 2022; Yang *et al.*, 2022; Dias *et al.*, 2023; Wong; Li, 2023; Bambace *et al.*, 2024; Martínez-Molina *et al.*, 2024; Zhang *et al.*, 2024; Pan *et al.*, 2025).

Classificado como um polímero natural, extraído de algas marinhas, o alginato de sódio é amplamente aplicado na produção de vários revestimentos comestíveis devido à sua sustentabilidade, biodegradabilidade, biocompatibilidade, e suas excelentes propriedades de formação de filme (Wang *et al.*, 2025). Estruturalmente, o alginato de sódio é composto por frações de α -L-guluronato ligadas em (1→4) e β -D-manuronato, também ligadas em (1→4). A proporção e a distribuição desses blocos em sua estrutura influenciam diretamente suas propriedades funcionais, como a formação de gel, a solubilidade e a viscosidade (Abka-Khajouei *et al.*, 2022). Além disso, uma das propriedades mais úteis dos alginatos é sua capacidade de reagir com cátions, especialmente cátions multivalentes, como os íons cálcio, formando géis fortes e estáveis (Metha *et al.*, 2024).

Os revestimentos formados por alginato de sódio são transparentes, uniformes e solúveis em água (Hasan *et al.*, 2022). Tais revestimentos têm menor permeabilidade para oxigênio, o

que pode retardar a oxidação lipídica e a perda de umidade de vários vegetais e frutas, auxiliando na redução da perda de peso e do crescimento microbiano (Wang *et al.*, 2025). A utilização de polímeros biodegradáveis em embalagens de alimentos oferece uma solução eficaz e ecologicamente correta, podendo ser uma alternativa viável aos materiais tradicionais (Metha *et al.*, 2024).

Os revestimentos comestíveis também podem atuar como carreadores de substâncias ativas como antimicrobianos e antioxidantes, para estender sua eficiência na preservação e controlar o crescimento microbiano na superfície dos alimentos (Wong, Li, 2024; Zhang *et al.*, 2024). Os revestimentos à base de alginato de sódio têm sido comumente utilizados como carreadores de microrganismos probióticos para aplicação em frutas, sendo relatados resultados promissores em relação à conservação destes produtos (Tabela 1). Além disso, os revestimentos à base de alginato se destacam por suas vantagens tecnológicas em relação a outros revestimentos de polissacarídeos aplicados em frutas, principalmente por promoverem maior proteção aos microrganismos incorporados, favorecendo a manutenção de sua viabilidade e atividade funcional durante o período de armazenamento (Martínez-Molina *et al.*, 2024).

Tabela 1 – Estudos de produção e aplicação de revestimentos comestíveis à base de alginato de sódio adicionados de bactérias láticas em frutas (*in natura* ou minimamente processadas).

Composição do revestimento	Fruta	Principais resultados	Referência
Alginato de sódio, inulina, oligofrutose, <i>L. rhamnosus</i> e <i>B. lactis</i>	Maçã	As contagens de <i>L. rhamnosus</i> e <i>B. lactis</i> foram mantidas nos revestimentos até o 8º dia de armazenamento. A aplicação dos revestimentos foi eficaz contra <i>L. innocua</i> e <i>E. coli</i> . As amostras revestidas com <i>B. lactis</i> permaneceram sensorialmente aceitáveis durante o armazenamento.	Alvarez <i>et al.</i> , 2021
Alginato de sódio, <i>L. brevis</i> , <i>L. pentosus</i> e <i>L. fermentum</i>	Goiaba e manga	A aplicação dos revestimentos controlou a antracnose, reduzindo seu desenvolvimento e severidade nas frutas durante o armazenamento. Esses revestimentos impactaram positivamente alguns parâmetros físico-químicos indicativos da qualidade pós-colheita de goiaba e manga.	Fernandes; de Oliveira; de Souza, 2021
Alginato de sódio e <i>Lactocaseibacillus casei</i>	Manga e melão	Os revestimentos foram capazes de manter a qualidade de manga e melão, retardando o aumento dos sólidos solúveis totais, pH e açúcares (glicose e frutose), assim como reduziu a perda de peso e preservou a firmeza e perfil sensorial. As contagens de <i>L. casei</i> foram mantidas no revestimento.	Dias <i>et al.</i> , 2023
Alginato de sódio e <i>Lactiplantibacillus plantarum</i>	Uva	A viabilidade de <i>L. plantarum</i> adicionada ao revestimento foi mantida por 14 dias de armazenamento refrigerado. Os revestimentos	de Simone <i>et al.</i> , 2024

		foram capazes de inibir o desenvolvimento da deterioração de uva de mesa causada por <i>Aspergillus niger</i> .	
Alginato, inulina, oligofrutose e <i>Lacticaseibacillus casei</i>	Maça	O revestimento foi capaz de manter a viabilidade probiótica durante o período de armazenamento e inibiu o crescimento do patógeno <i>E. coli</i> . Por outro lado, a qualidade sensorial das maçãs foi afetada negativamente pela adição de <i>L. casei</i> .	Bambace <i>et al.</i> , 2024
Alginato de sódio e <i>Limosilactobacillus fermentum</i>	Melão	A viabilidade de <i>L. fermentum</i> foi mantida no revestimento aplicado em melão. O revestimento foi capaz de preservar as características físico-químicas e atributos sensoriais da fruta.	Martínez-Molina <i>et al.</i> , 2024
Alginato de sódio e <i>Lactobacillus rhamnosus</i> , <i>L. plantarum</i> e <i>L. paracasei</i>	Morango	Os revestimentos adicionados de BAL apresentaram atividade antibacteriana frente a <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> e <i>Rhizopus stolonifer</i> .	Li <i>et al.</i> , 2024
Alginato de sódio e <i>Lactiplantibacillus plantarum</i>	Maracujá	O revestimento preservou a aparência, a textura e as principais qualidades nutricionais (açúcares, ácidos e vitamina C) da fruta.	Pan <i>et al.</i> , 2025

Fonte: própria, 2026.

Considerando as vantagens potenciais do uso de revestimentos como tecnologias alternativas para a preservação de frutas, a aplicação de um revestimento formulado com alginato de sódio adicionado de cultura mista de cepas de BAL potencialmente probióticas, isoladas de subprodutos do processamento de frutas, para a inibição de diversas espécies patogênicas de *Colletotrichum*, pode se apresentar como uma estratégia viável e eficiente para a preservação da qualidade pós-colheita de goiaba e manga. A aplicação dessa tecnologia poderá proporcionar vantagens para a comercialização de goiaba e manga, considerando as dificuldades que os produtores enfrentam para o controle da antracnose nestas frutas e as consideráveis perdas econômicas para a sua cadeia produtiva decorrentes do progresso da doença.

3 MATERIAL E MÉTODOS

O presente estudo foi desenvolvido em diferentes etapas que estão apresentadas no esquema da Figura 6. Inicialmente, foram realizados os testes *in vitro*, nos quais foi avaliada a atividade antagonista da cultura mista de BAL frente a diferentes isolados de *Colletotrichum* spp. utilizados como microrganismo alvo. A avaliação da sobrevivência e do estado fisiológico da cultura mista de BAL em revestimentos de alginato de sódio foi realizada em diferentes condições de armazenamento. Posteriormente, a formulação de revestimento de alginato de sódio adicionado da cultura mista de BAL foi testada *in situ* quanto à sua eficácia na diminuição do progresso e severidade da antracnose em manga e goiaba durante o armazenamento sob temperatura ambiente. Também foram realizadas análises das características físico-químicas e estruturais do revestimento, assim como a avaliação da preservação de características físico-químicas e sensoriais indicadoras da qualidade pós-colheita de goiaba e manga após a aplicação dos diferentes revestimentos ao longo do armazenamento.

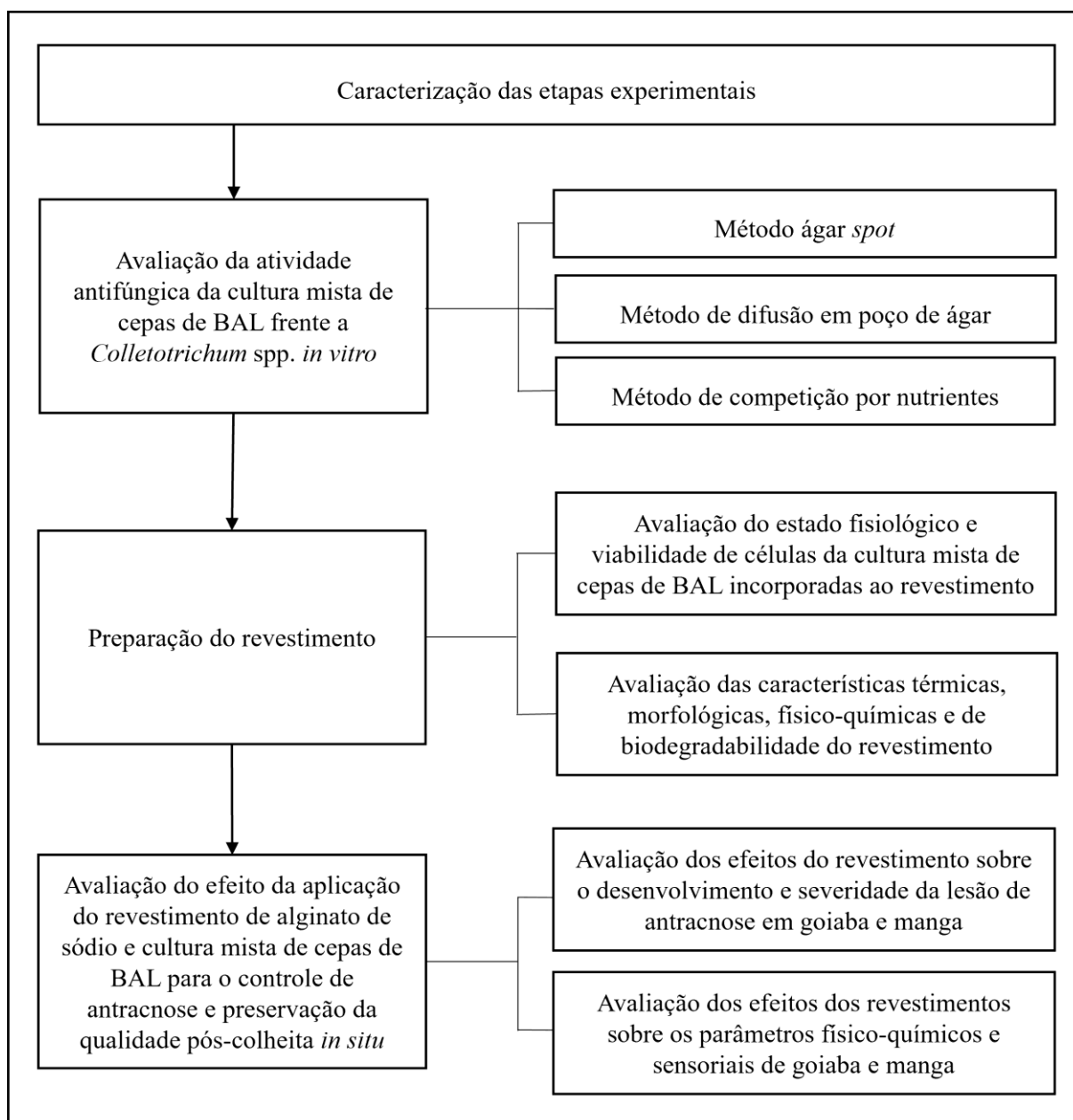
Os experimentos foram realizados no Laboratório de Microbiologia e Bioquímica de Alimentos (LMBA); Laboratório de Bromatologia, ambos pertencentes ao Departamento de Nutrição/Centro de Ciências da Saúde/Universidade Federal da Paraíba – Campus I; e Laboratório de Materiais e Biosistemas (LAMAB), pertencente ao Departamento de Engenharia de Materiais/ Centro de Tecnologia/ Universidade Federal da Paraíba – Campus I.

3.1 FRUTAS E COMPONENTES DO REVESTIMENTO

Goiabas (*Psidium guajava* L.) cv. Paluma, caracterizadas por estarem no segundo estágio de maturação, ou seja, com até 80% de casca verde e °Brix aproximadamente 8 (Gull *et al.*, 2026), e mangas (*Mangifera indica* L.) cv. Palmer, caracterizadas por estarem em estágio de maturação média com °Brix de aproximadamente 6 (Almeida *et al.*, 2024; Silué *et al.*, 2022), foram adquiridas na EMPASA (Empresa Paraibana de Abastecimento e Serviços Agrícolas, João Pessoa, Paraíba, Brasil). As frutas selecionadas estavam livres de lesões físicas e sinais visíveis de infecção, sendo padronizadas com base no tamanho, aparência, cor e forma. Antes do uso nos experimentos, as frutas foram higienizadas com detergente neutro e água corrente potável, desinfetadas superficialmente por imersão em hipoclorito de sódio (150 ppm, pH 7,2 ajustado com NaOH 1 M) por 15 minutos, enxaguadas com água estéril e secas por duas horas em temperatura ambiente ($25 \pm 0,5$ °C) em cabine de segurança (Fernandes; de Oliveira; de Souza, 2021).

O alginato de sódio (Dinâmica Química Contemporânea Ltda., Indaiatuba, São Paulo, Brasil), glicerol (Química Moderna, Barueri, São Paulo, Brasil), óleo de girassol (Cargill Agrícola SA, Recife, PE, Brasil) e tween 80 (Sigma - Aldrich, St. Louis, MO, EUA) foram utilizados para formular o revestimento. O cloreto de cálcio (PA) (Synth, Diadema, São Paulo, Brasil) foi aplicado nas frutas revestidas para formar um gel por meio de reticulação com alginato de sódio.

Figura 6 – Etapas do desenho experimental do estudo.

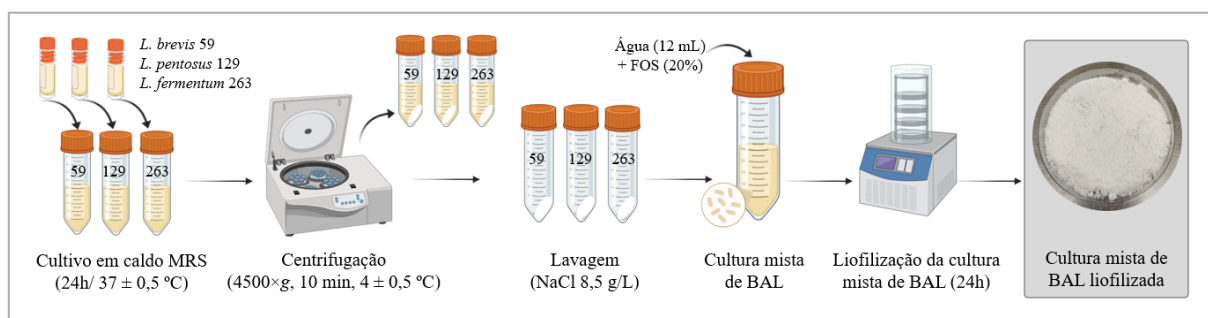


Fonte: Própria, 2026.

3.2 MICRORGANISMOS TESTE

Três diferentes cepas de BAL, a citar *Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129 e *Limosilactobacillus fermentum* 263 foram selecionadas para uso na formulação do revestimento com base em resultados anteriores que demonstraram sua capacidade antifúngica frente à *Colletotrichum* spp. (Fernandes; de Oliveira; de Souza, 2021). As cepas de BAL fazem parte da Coleção do Laboratório de Microbiologia e Bioquímica dos Alimentos (LMBA), e foram previamente isoladas de subprodutos do processamento de polpas de frutas de acerola (*Malpighia glabra* L.), manga (*Mangifera indica* L.) e abacaxi (*Ananas comosus*), respectivamente. Essas cepas foram caracterizadas como detentoras de propriedades probióticas, destacando-se a atividade antimicrobiana frente a microrganismos contaminantes de alimentos (Garcia *et al.*, 2016). Os estoques foram armazenados ($-20 \pm 0,5$ °C) em caldo de Man, Rogosa e Sharpe (MRS) (HiMedia, Mumbai, Índia) com glicerol (Sigma Aldrich; 20 mL/100 mL). As culturas de trabalho foram mantidas em ágar MRS (HiMedia) sob refrigeração ($4 \pm 0,5$ °C) e transferidas para um novo meio de manutenção mensalmente.

Antes do uso nos ensaios, cada cepa de BAL testada foi cultivada em caldo de MRS a $37 \pm 0,5$ °C por 20 a 24 h sob anaerobiose (Sistema Anaeróbico Anaerogen, Oxoid, Hampshire, Reino Unido) (Figura 7). As células foram coletadas por centrifugação ($4500 \times g$, 10 min, $4 \pm 0,5$ °C), lavadas em solução salina esterilizada (NaCl 8,5 g/L) e ressuspensas em água destilada esterilizada com fruto-oligossacarídeos (FOS, crioprotetor) (20% p/v) para obter uma suspensão de células com contagens de células viáveis entre 9 - 10 log UFC/mL. A suspensão celular mista foi preparada com a mistura das suspensões de cada cepa (proporção 1:1:1) (Freire *et al.*, 2021). Para a liofilização, a suspensão da cultura mista de BAL adicionada de FOS foi transferida para placas de vidro com tampas (2 mL de suspensão em cada placa) e submetidas ao congelamento (-80 ± 2 °C, 24 h) (Di *et al.*, 2023). O processo de liofilização foi realizado em temperatura de -55 ± 2 °C, com pressão de vácuo de <138 µHG e um taxa de liofilização de 1 mm/h, por aproximadamente 20 horas em liofilizador de bancada (modelo L-101, Liotop, São Carlos, São Paulo, Brasil) (Di *et al.*, 2023). A cultura mista liofilizada foi armazenada sob congelamento (-18 ± 2 °C) até a realização dos ensaios. Para aplicação nos testes *in vitro*, a cultura mista liofilizada foi ressuspensa em água destilada estéril (6%), atingindo contagens de células viáveis entre 9 - 10 log UFC/mL.

Figura 7 - Esquema do cultivo e liofilização da cultura mista de cepas de BAL.

Fonte: própria, 2026.

Dez isolados de *Colletotrichum*, nomeadamente *C. asianum* CMM 4057, *C. fructicola* CMM 4069, *C. tropicale* CMM 4071, *C. siamense* CMM 4077, *C. karstii* CMM 4101, *C. gloeosporioides* CMM 320, *C. fructicola* LM 680, *C. siamense* LM 681; *C. tropicale* LM 679 e *C. chrysophilum* LM 685, procedentes da Coleção de Cultura de Fungos Fitopatogênicos “Prof. Maria Menezes” (UFRPE, Recife, PE, Brasil) e da Coleção do Laboratório de Micologia (Universidade Federal Rural de Pernambuco, Recife, Brasil) foram utilizados como microrganismos alvo. Esses fungos foram previamente isolados de goiabas e mangas (Tabela 2). Para os ensaios de atividade antifúngica, foram utilizados discos de ágar (5 mm de diâmetro) contendo estruturas do isolado de *Colletotrichum* alvo obtidas da margem de uma colônia cultivada em ágar batata dextrose (ABD) por sete dias a $25 \pm 0,5$ °C (Fernandes; de Oliveira; de Souza, 2021).

Tabela 2 – Isolados de *Colletotrichum* utilizados como microrganismos alvo

Isolados de <i>Colletotrichum</i>	Fruta hospedeira	Referência
<i>C. tropicale</i> LM 679	Goiaba	Soares <i>et al.</i> , 2024
<i>C. fructicola</i> LM 680	Goiaba	Soares <i>et al.</i> , 2024
<i>C. siamense</i> LM 681	Goiaba	Soares <i>et al.</i> , 2024
<i>C. chrysophilum</i> LM 685	Goiaba	Soares <i>et al.</i> , 2024
<i>C. asianum</i> CMM 4057	Manga	Lima <i>et al.</i> , 2013
<i>C. fructicola</i> CMM 4069	Manga	Lima <i>et al.</i> , 2013
<i>C. tropicale</i> CMM 4071	Manga	Lima <i>et al.</i> , 2013
<i>C. siamense</i> CMM 4077	Manga	Lima <i>et al.</i> , 2013
<i>C. karstii</i> CMM 4101	Manga	Lima <i>et al.</i> , 2013
<i>C. gloeosporioides</i> CMM 320	Manga	Lima <i>et al.</i> , 2013

LM: Coleção Laboratório de Micologia (Universidade Federal Rural de Pernambuco, Recife, Brasil).

CMM: Coleção de Cultura de Fungos Fitopatogênicos “Prof. Maria Menezes” (UFRPE, Recife, PE, Brasil).

Fonte: própria, 2026.

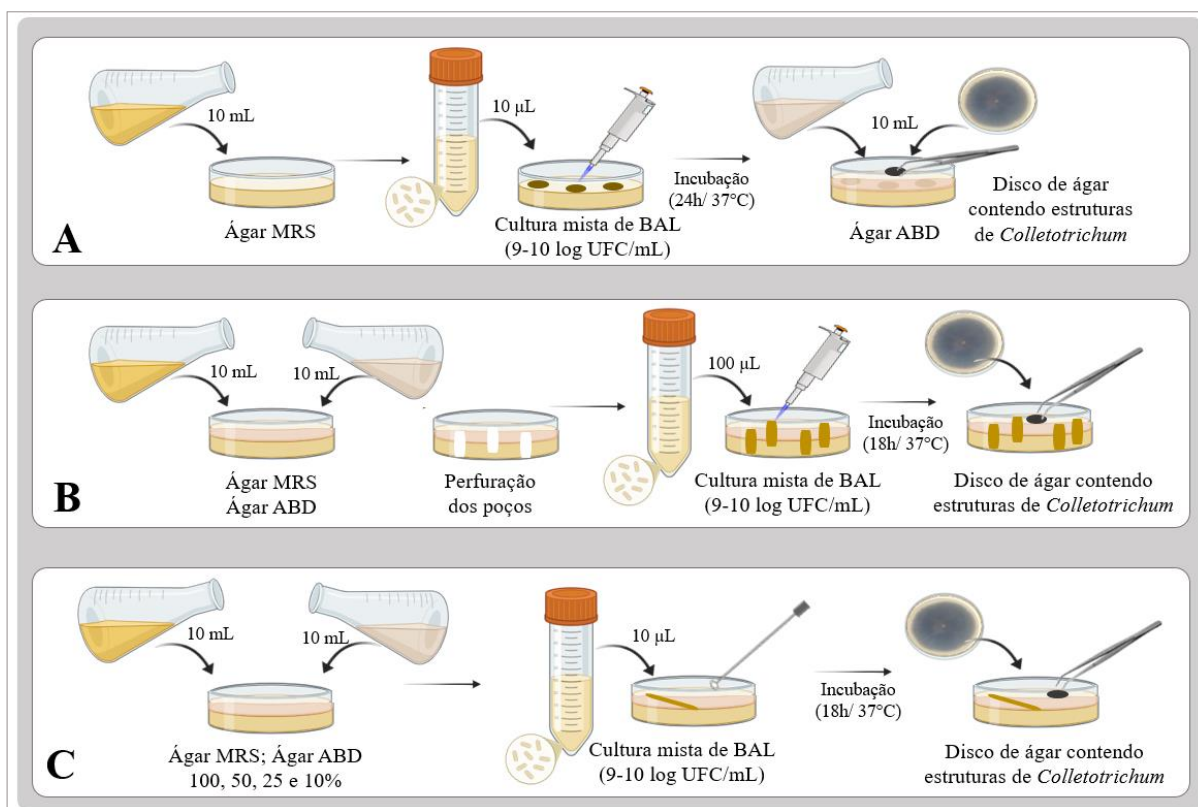
3.3 AVALIAÇÃO DA ATIVIDADE ANTIFÚNGICA *in vitro* DA CULTURA MISTA DE CEPAS DE BAL SOBRE ISOLADOS DE *Colletotrichum*

A atividade antifúngica da cultura mista de cepas de BAL frente aos diferentes isolados alvo de *Colletotrichum* (Figura 8) foi realizada utilizando os métodos ágar *spot* (Afzali *et al.*, 2020), difusão em poços de ágar (El Oirdi *et al.*, 2021) e competição por nutrientes (Mpeluza *et al.*, 2023). Os efeitos antifúngicos da cultura mista de BAL testada foram medidos com base na redução do crescimento micelial do fungo com a equação:

$$\text{ICM}\% = [(CT - TT) / CT] \times 100 \quad (\text{Equação 1})$$

onde ICM% é o percentual de inibição do crescimento micelial; CT é o crescimento total de fungos no ensaio de controle e TT é o crescimento total de fungos no ensaio com a cultura mista de BAL (Lundgren *et al.*, 2021).

Figura 8 - Esquema dos métodos de avaliação da atividade fúngica *in vitro*.



Fonte: adaptado de Fernandes (2021). A: método de ágar spot; B: método de difusão em poços de ágar; C: método de competição por nutrientes.

3.3.1 Método ágar *spot*

Para a realização do método ágar spot (Figura 8A) (Afzali *et al.*, 2020), foram preparadas placas de Petri (90 mm de diâmetro) contendo 10 mL de ágar MRS. Sobre a superfície do meio de cultura, foram adicionadas, em posições equidistantes, quatro alíquotas de 10 µL da cultura mista de cepas de BAL testada (9-10 log UFC/mL), previamente liofilizada e reidratada (6%) em água destilada estéril. Após a absorção do inóculo pelo meio de cultura, as placas foram invertidas e incubadas sob anaerobiose (Anaerogen) durante 24 horas a $37 \pm 0,5$ °C. No final do período de incubação, as placas com ágar MRS previamente inoculadas foram cobertas com 10 mL de ágar ABD semissólido, e em seguida inoculadas (no centro da superfície da camada de ABD) com um disco de ágar (5 mm de diâmetro) contendo estruturas do isolado de *Colletotrichum* alvo (previamente cultivada por sete dias a $25 \pm 0,5$ °C em ABD), e incubadas por cinco dias ($25 \pm 0,5$ °C). Em paralelo, uma placa contendo ágar MRS não inoculado com a cultura mista de cepas de BAL foi utilizada como controle negativo. Os efeitos antifúngicos da cultura mista de cepas de BAL foram avaliados com base na redução do crescimento micelial do fungo, utilizando a Equação 1 (seção 3.3).

3.3.2 Método difusão em poços de ágar

Para o método de difusão em poço de ágar (Figura 8B) (El Oirdi *et al.*, 2021), foram utilizadas placas de Petri (90 mm de diâmetro) contendo uma camada dupla de ágar MRS (10 mL) e ABD (10 mL). Após a solidificação dos meios de cultura, quatro poços de 6 mm de diâmetro foram perfurados na dupla camada de ágar em uma distância de dois cm do ponto central da placa. Em seguida, uma alíquota de 100 µL da cultura mista de cepas de BAL testada (9-10 log UFC/mL) liofilizada e reidratada (6%) em água destilada foi dispensada em cada poço. Para permitir a difusão no ágar, as placas foram pré-incubadas durante 18 horas ($37 \pm 0,5$ °C) sob anaerobiose (Anaerogen). Água destilada estéril em substituição a suspensão da cultura mista de cepas de BAL foi usada como controle negativo. Após o período de incubação, um disco de ágar (5 mm de diâmetro) contendo estruturas do isolado alvo de *Colletotrichum* (previamente cultivado por sete dias a $25 \pm 0,5$ °C em ABD), foi transferido para o centro da placa com ágar MRS sobreposto com ABD, seguido por incubação ($25 \pm 0,5$ °C) por cinco dias. Os resultados foram expressos como ICM%, que foi calculada conforme equação 1 (seção 3.3).

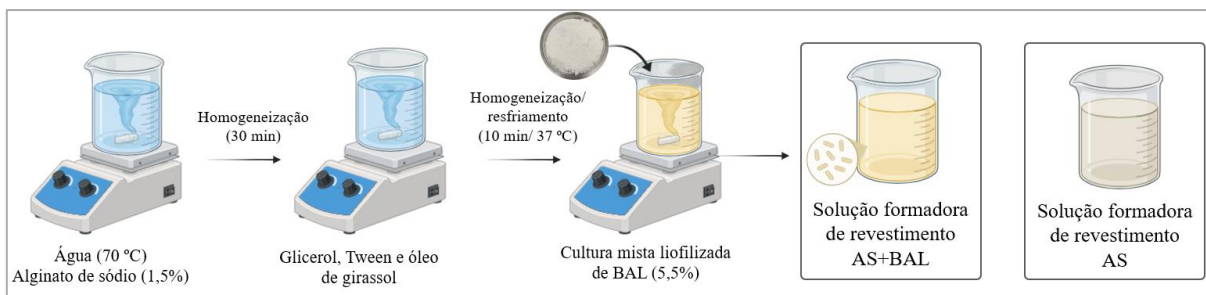
3.3.3 Método de competição por nutrientes

A abordagem de cultura dupla foi usada para avaliar a capacidade da cultura mista de BAL liofilizadas de competir por nutrientes contra *Colletotrichum* em concentrações variadas de nutrientes (Figura 8C) (Mpeluza *et al.*, 2023). Os meios ABD e MRS foram preparados em quatro concentrações diferentes: concentração total (100%), metade da concentração de nutrientes (50%), um quarto da concentração total de nutrientes (25%) e um décimo da concentração normal de nutrientes (10%). Inicialmente, o meio ABD (10 mL) foi recoberto com ágar MRS (10 mL). Em seguida, a cultura mista de cepas de BAL liofilizadas e reidratadas em água destilada (6%), com concentração de 9–10 log UFC/mL, foi semeada em uma das extremidades da placa utilizando uma alça bacteriológica de 10 µL. As placas foram então pré-incubadas por 18–20 h a $37 \pm 0,5$ °C, sob condições de anaerobiose (Anaerogen). Após esse período, um disco de 5 mm contendo estruturas do isolado alvo de *Colletotrichum* foi colocado na extremidade oposta da placa, seguido de incubação a $25 \pm 0,5$ °C por cinco dias. Os resultados foram expressos como ICM%, calculado conforme a equação 1 (seção 3.3).

3.4 PREPARAÇÃO DO REVESTIMENTO

O revestimento foi produzido conforme apresentado na Figura 9. Inicialmente, o alginato de sódio (1,5 g/100 mL) foi dissolvido em água destilada esterilizada (70 ± 1 °C) sob agitação (240 rpm) por 30 min até dissolução completa. Em seguida, foram incorporados glicerol (0,75 g/100g) para aumentar a flexibilidade do revestimento; tween 80 (0,05 g/100g) para melhorar a aderência à fruta e óleo de girassol (0,04 g/100g), como fonte lipídica para aumentar as características de barreira à água do revestimento. Todos os ingredientes foram agitados por 10 min ($25 \pm 0,5$ °C) até dissolução completa (Fernandes; de Oliveira; de Souza, 2021). Após resfriamento da dispersão preparada, a cultura mista de cepas de BAL liofilizada (5,5%, p/v) foi ressuspensa na dispersão (AS+BAL) (Figura 7), de forma a alcançar uma concentração celular final de aproximadamente 9 log UFC/mL. Um revestimento contendo alginato de sódio sem adição de BAL (AS), bem como a aplicação de água destilada esterilizada adicionada de glicerol (2,5 mL/100mL) foram testados como controles negativos. Como controle positivo, foram aplicados nas frutas fungicidas químicos comerciais, Thiophanate-methyl (10 µg a.i./mL) e Difenconazole (0.5 µg a.i./mL).

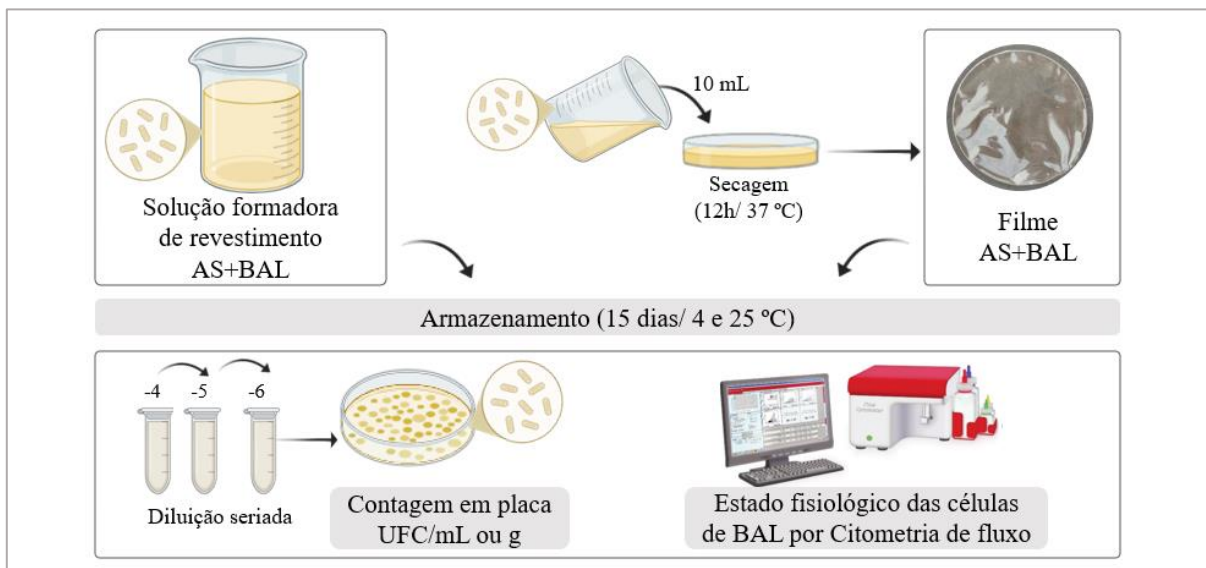
Figura 9 - Esquema da produção do revestimento de alginato de sódio adicionado da cultura mista de BAL.



Fonte: adaptado de Fernandes (2021).

Os filmes de AS e AS+BAL (Figura 10) foram moldados vertendo-se assepticamente 10 mL de cada solução formadora de revestimento em placas de Petri estéreis (90 mm de diâmetro), sendo posteriormente submetidos à secagem por aproximadamente 12 h ($25 \pm 0,5$ °C) em cabine de biossegurança (Iñiguez-Moreno *et al.*, 2021). Após a secagem, os filmes foram armazenados sob diferentes condições de temperatura (4 e 25 ± 1 °C para a realização das análises de viabilidade celular das BAL (Díaz-Díaz *et al.*, 2023; Mahapatra *et al.*, 2024).

Figura 10 – Esquema das análises de sobrevivência e avaliação do estado fisiológico das células de BAL.



Fonte: própria, 2026.

3.5 SOBREVIVÊNCIA E AVALIAÇÃO DO ESTADO FISIOLÓGICO DA CULTURA MISTA DE BAL EM REVESTIMENTOS DE ALGINATO DE SÓDIO

As contagens de células viáveis da cultura mista de BAL incorporada à solução formadora de revestimento e ao filme AS+BAL foi realizada em diferentes intervalos de tempo de armazenamento (dias 0, 1, 6, 9, 12 e 15) sob temperatura ambiente ($25 \pm 0,5$ °C) e refrigerado ($4 \pm 0,5$ °C). A solução formadora de revestimento (1 mL/100 mL) e o filme (1 g/100 mL) foram diluídos em água peptonada estéril (0,1 g/100 mL), seguido por homogeneização em agitador mecânico estéril (180 rpm) durante 10 min. Diluições seriadas (1:9) foram preparadas no mesmo diluente e semeadas em ágar MRS. As placas foram incubadas ($37 \pm 0,5$ °C) por 48 h sob condições anaeróbicas (Anaerogen) e os resultados foram expressos como UFC/mL ou UFC/g (de Simone *et al.*, 2024).

Simultaneamente, as cepas da cultura mista de BAL recuperadas da solução formadora do revestimento foram lavadas com solução salina tamponada com fosfato estéril (PBS; 8 g/L NaCl, 0,2 g/L KCl, 1,44 g/L Na_2HPO_4 , 0,24 g/L KH_2PO_4 , pH 7,4) por centrifugação ($4500 \times g$, 10 min, 4 °C) e submetidas à coloração com iodeto de propídio (PI, Sigma-Aldrich, 10 µg/mL) e diacetato de carboxifluoresceína (cFDA, Sigma-Aldrich, 2,5 µg/mL) por 15 min no escuro. Após os procedimentos de coloração, as suspensões celulares foram lavadas com um volume igual de PBS por centrifugação ($4500 \times g$, 10 min, 4 °C) para remover o excesso de corante. A coloração com os fluorocromos PI e cFDA foi utilizada para avaliar a integridade da membrana citoplasmática e a atividade enzimática nas células, respectivamente (Rodrigues *et al.*, 2022).

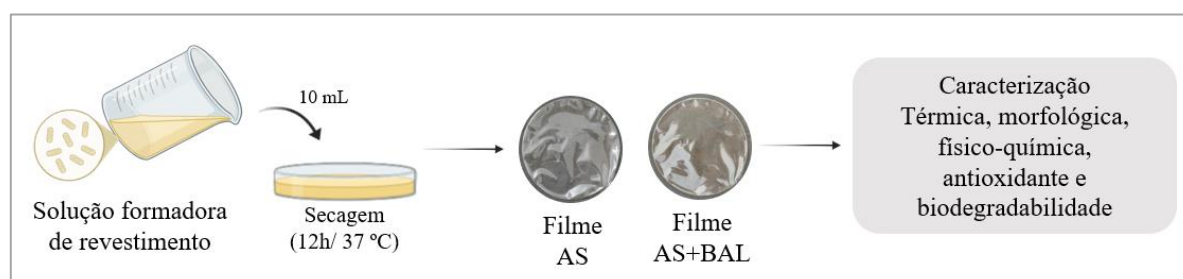
Os procedimentos de aquisição e análise dos dados das células coradas foram realizados em um citômetro de fluxo com emissão de laser de argônio em 488 nm e 640 nm (BD Accuri C6, Becton Dickinson, Franklin Lakes, NJ, EUA), contendo dois detectores de dispersão de luz (FSC e SSC) e quatro detectores de fluorescência. O canal FL1, que detecta luz com comprimento de onda de $533 \text{ nm} \pm 30 \text{ nm}$, coletou a fluorescência verde para verificar a marcação com cFDA, enquanto o canal FL3, que detecta luz com comprimentos de onda $> 670 \text{ nm}$, coletou a fluorescência vermelha para verificar a marcação com PI. Foram lidos 10.000 eventos por amostra com compensação automática dos dados obtidos. A população bacteriana foi identificada a partir de um gráfico de densidade FSC versus SSC, e os resultados da coloração foram plotados em uma região específica do gráfico FL1 versus FL3. Os quadrantes foram usados para identificar a subpopulação celular de acordo com as leituras de fluorescência baseadas no corante utilizado. Na dupla coloração com PI e cFDA, as subpopulações celulares foram caracterizadas como PI-cFDA+, correspondendo a células não permeabilizadas com

atividade enzimática (células viáveis), e PI+cFDA–, correspondendo a células permeabilizadas sem atividade enzimática (células mortas) (do Nascimento *et al.*, 2025; Rodrigues *et al.*, 2022). A análise dos dados foi realizada com o *software* BD Accuri C6 (Becton Dickinson). Os resultados foram expressos como o tamanho (%) das subpopulações celulares com diferentes estados fisiológicos.

3.6 AVALIAÇÃO DAS CARACTERÍSTICAS FÍSICO-QUÍMICAS E ESTRUTURAIS DOS FILMES

As características físico-químicas dos filmes formulados com alginato de sódio adicionado da cultura mista de cepas de BAL (AS+BAL) e do filme de alginato de sódio (AS) sem adição de BAL, produzidos conforme descrito na seção 3.4, foram avaliadas por meio de análises de espectroscopia na região do infravermelho (FTIR), calorimetria diferencial exploratória (DSC), microscopia eletrônica de varredura (MEV), rugosidade da superfície, umidade, solubilidade em água, higroscopicidade, atividade de água, cor, capacidade antioxidante, e biodegradabilidade (Figura 11).

Figura 3 - Esquema da produção e análises de caracterização dos filmes alginato de sódio (AS) e alginato de sódio adicionado da cultura mista de BAL (AS + BAL).



Fonte: própria, 2026.

3.6.1 Espectroscopia na região do infravermelho

Os grupos funcionais presentes nos filmes foram determinados por espectroscopia no infravermelho por transformada de Fourier (FTIR). Os espectros foram obtidos na faixa de comprimento de onda de 400 a 4000 cm^{-1} , com resoluções de 4 e 64 varreduras, utilizando o equipamento FTIR da Shimadzu (Modelo Prestige-21 IR Affinity-1 FTIR 8400S, Kyoto, Japão) (Wu *et al.*, 2024). As medições foram realizadas no modo de absorbância (Díaz-Díaz *et al.*, 2023), e os espectros foram analisados para identificar os grupos funcionais característicos na

estrutura do filme, seguindo metodologias estabelecidas para a caracterização de polissacarídeos (Mollah *et al.*, 2023).

3.6.2 Calorimetria diferencial exploratória

As propriedades térmicas dos filmes foram avaliadas por calorimetria diferencial exploratória (DSC) utilizando um calorímetro TA-60WS (Shimadzu, Kyoto, Japão). As amostras foram submetidas a uma taxa de aquecimento e resfriamento de 10 °C/min sob um fluxo de gás nitrogênio seco (50 mL/min). As análises foram realizadas em duas rampas de aquecimento, partindo da temperatura ambiente até 300 °C, seguidas por uma rampa de resfriamento (Bao *et al.*, 2023).

3.6.3 Microscopia eletrônica de varredura

A microscopia eletrônica de varredura (MEV) foi realizada em um microscópio TESCAN VEGA de 4ª geração (TESCAN, República Tcheca). As amostras dos filmes foram montadas usando fita de carbono condutora e revestidas por pulverização catódica com uma camada de ouro de 10 nm de espessura por revestidor iônico modelo G20 (Ion Sputter Coater, Schaefer-Tec, Itália). As amostras foram examinadas utilizando uma tensão de aceleração de 10 kV para as superfícies dos filmes (Medeiros *et al.*, 2022).

3.6.4 Rugosidade da superfície

A análise da rugosidade da superfície dos filmes foi realizada com perfilômetro óptico 3D sem contato (Talysurf CCI MP, Leicester, Reino Unido) conectado a uma unidade computadorizada. Foram realizadas cinco medidas para cada amostra, sendo uma na área central e duas de acordo com o movimento da lente (2 mm para a direita, esquerda, abaixo e acima), seguindo um desenho cruzado. A rugosidade final dos padrões Ra e Sz (µm), indicando rugosidade média e altura, respectivamente, foi obtida pela média de cinco pontos das amostras dos filmes (Braga *et al.*, 2020).

3.6.5 Teor de umidade

O teor de umidade foi determinado conforme descrito por Lopes *et al.* (2025). Inicialmente, os filmes foram pesados e, em seguida, acondicionados em placas de Petri de vidro previamente secas e pesadas, sendo mantidos em estufa a 105 °C por 24 h, até atingirem peso constante. O teor de umidade, expresso como a porcentagem de perda de peso após a secagem, foi determinado em base seca, conforme a equação:

$$\text{Umidade (\%)} = (M1 - (M3 - M2)) \times 100 \quad (\text{Equação 2})$$

onde M1 é a massa inicial do filme (antes da secagem em estufa), M2 é a massa da placa de Petri e M3 é a massa da placa de Petri com o filme após a secagem.

3.6.6 Solubilidade em água

A solubilidade em água foi determinada por gravimetria (Santos *et al.*, 2021). Os filmes foram colocados em uma cápsula de alumínio, secos a 105 °C em estufa para determinação do peso inicial do filme. Para a determinação da solubilidade em água, os filmes secos inicialmente foram submersos em 50 mL de água destilada e agitados a 150 rpm por 24 h em agitador orbital (Tecnal, TE-424, Brasil). Os materiais insolúveis foram secos a 105 °C por 24 h e o peso final foi determinado. A solubilidade em água foi calculada, seguindo a equação:

$$\text{Solubilidade (\%)} = [(M4 - M5) / M4] \times 100 \quad (\text{Equação 3})$$

onde M4 é a massa inicial dos filmes e M5 é massa do material insolúvel remanescente após a imersão em água.

3.6.7 Higroscopicidade

Uma alíquota (1 g) de cada filme formulado foi pesada em placas de Petri, as quais foram colocadas em dessecador contendo solução saturada de NaCl, com umidade relativa de aproximadamente 75% e temperatura de 25 ± 0,5 °C, sendo mantidas em câmara com temperatura controlada (BOD, 25 ± 0,5 °C) por sete dias e pesadas diariamente até atingirem o equilíbrio higroscópico. A higroscopicidade foi expressa em g de água adsorvida por 100 g de massa seca (do Nascimento *et al.*, 2025).

3.6.8 Atividade de água

A atividade de água (a_w) de cada filme foi determinada utilizando higrômetro digital (Aqualab 3TE, Decagon, Pullman, WA, EUA), previamente calibrado, com as medições realizadas a $25 \pm 0,5$ °C (da Silva *et al.*, 2024).

3.6.9 Parâmetros de cor

Os parâmetros de cor de cada amostra de filme foram medidos com a utilização de um colorímetro Konica Minolta (modelo CR-400, Minolta Corp., Ramsey, NJ, EUA). O instrumento foi calibrado com uma placa branca padrão. Os parâmetros medidos e registrados conforme o sistema CIELAB (CIE Lab*), incluindo L^* (luminosidade), a^* (coordenada vermelho/verde), b^* (coordenada amarelo/azul), bem como croma (C^*) e ângulo de matiz (h°) (Kalkan *et al.*, 2025).

3.6.10 Capacidade antioxidante

Para determinar a atividade antioxidante dos filmes, foram obtidos extratos metanólicos. Para isso, 1 g de cada filme foi homogeneizado em 20 mL de metanol a 80% (v/v) e, após agitação, as amostras foram armazenadas por 24 h. Todos os extratos foram centrifugados a $1696 \times g$ (4.000 rpm) por 15 minutos, $4 \pm 0,5$ °C. O sobrenadante foi filtrado, e os extratos foram mantidos sob refrigeração ($4 \pm 0,5$ °C) até a realização das análises. A atividade antioxidante dos extratos foi determinada pelos métodos ABTS $\bullet^+$ (ensaio com o radical cátion 2,2'-azino-bis(3-etilbenzotiazolina-6-sulfonato)), DPPH $\bullet$ (ensaio com o radical 2,2-difenil-1-picril-hidrazil) e FRAP (Ferric Reducing Antioxidant Power).

O radical ABTS $\bullet^+$ foi gerado pela reação de ABTS $\bullet^+$ com persulfato de potássio (2,45 mM final), sendo mantido no escuro por 12–16 h a $25 \pm 0,5$ °C. Em seguida, a solução foi diluída até atingir absorbância de $0,700 \pm 0,02$ a 734 nm. Aliquotas dos extratos foram adicionadas à solução de ABTS $\bullet^+$ e incubadas por 30 min à temperatura ambiente, utilizando-se o solvente de extração como controle. A absorbância foi medida a 734 nm em espectrofotômetro de microplacas (EON, BioTek Instruments Inc., Highland Park, EUA). A solução estoque de DPPH $\bullet$ foi preparada dissolvendo 24 mg de 2,2-difenil-1-picrilhidrazil em 100 mL de etanol absoluto. A partir desta, foi obtida uma solução de trabalho por diluição 1:10 (v/v). Aliquotas dos extratos foram misturadas à solução de DPPH $\bullet$ e incubadas por 30 min no

escuro. A absorbância foi determinada a 517 nm em espectrofotômetro de microplacas. A atividade antioxidante dos ensaios ABTS^{•+} e DPPH[•] foi quantificada por curva padrão de Trolox (50–2000 µM) e expressa em equivalentes de Trolox (µmol/g de amostra) (Gull *et al.*, 2026).

A capacidade redutora do ferro foi determinada pelo método FRAP conforme do Nascimento *et al.* (2025). O ensaio baseia-se na redução do complexo férrico a ferroso pelos compostos antioxidantes presentes nos extratos, sendo utilizada curva padrão de sulfato ferroso. A absorbância foi medida a 595 nm em espectrofotômetro de microplacas. Para o ensaio FRAP, os resultados foram expressos como µmol de FeSO₄ equivalente por grama de amostra.

3.6.11 Determinação da taxa de biodegradação dos filmes

A taxa de biodegradação dos filmes formulados com alginato de sódio adicionado da cultura mista de cepas de BAL e sem adição de BAL foi determinada em condições laboratoriais durante o armazenamento (Filipini *et al.*, 2020). Após a obtenção e secagem (Figura 10), as amostras dos filmes foram enterradas a 1 cm de profundidade em bandejas plásticas (500 mL de capacidade) contendo 300 g de composto orgânico. Para o teste de biodegradação em água do mar e água doce, os filmes foram submersos em 300 mL e mantidos em bandejas plásticas (500 mL de capacidade).

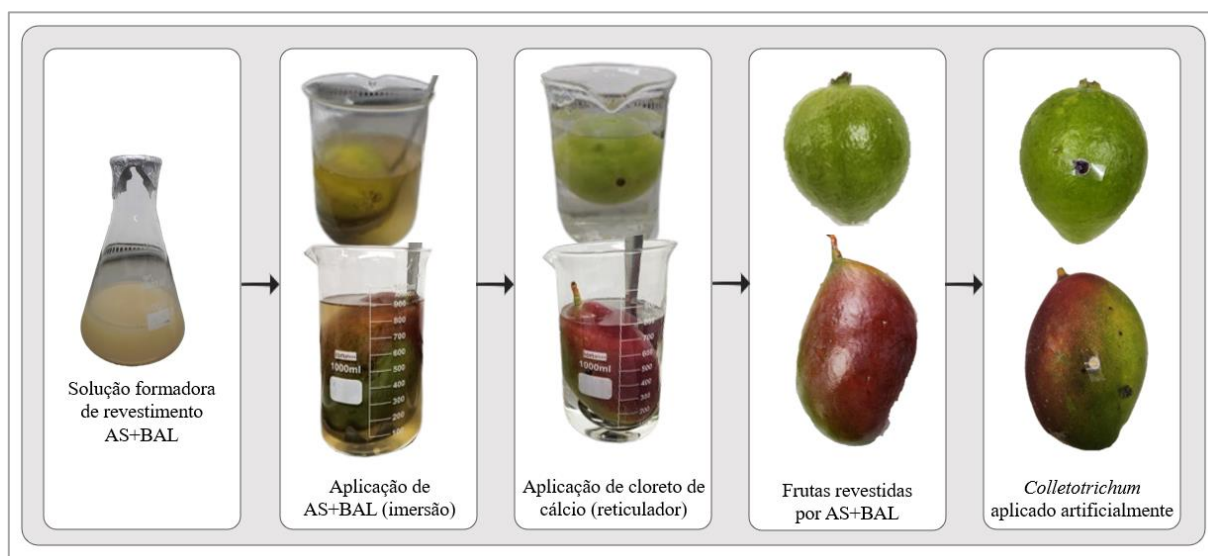
Todas as amostras foram mantidas em temperatura ambiente ($25 \pm 0,5$ °C) e condições aeróbicas. As amostras dos filmes foram recuperadas nos dias 0, 6, 12, 15 e 20. O processo de biodegradação foi monitorado por inspeção visual dos filmes, e as amostras foram coletadas e fotografadas periodicamente até que não fosse mais possível identificar os fragmentos dos corpos de prova (López-Díaz *et al.*, 2023).

3.7 AVALIAÇÃO DOS EFEITOS DO REVESTIMENTO SOBRE O DESENVOLVIMENTO E SEVERIDADE DE ANTRACNOSE EM GOIABA E MANGA

A epiderme de cada fruta (goiaba e manga) foi ferida (cinco feridas por fruta) com o uso de agulhas esterilizadas (3 mm de profundidade e 2 mm de largura) na região central do mesmo lado da fruta. Posteriormente, cada fruta foi imersa em 400 mL da dispersão formadora do revestimento de alginato de sódio (1,5 g/100 mL) adicionado da cultura mista de cepas de BAL liofilizadas (6 g/100 mL) por 2 min, e, em seguida, imersa em solução de cloreto de cálcio (2 g/100 mL) por 1 min como forma de promover a reticulação do revestimento (Oliveira *et al.*,

2021) (Figura 12). As frutas foram imersas em um revestimento de alginato de sódio sem adição de BAL e em água destilada esterilizada adicionada de glicerol (2,5 mL/100 mL) como controles negativos. Fungicidas químicos comerciais foram aplicados como controles positivos (tiofanato-metílico (10 µg a.i./mL) e difenoconazole (0.5 µg a.i./mL), sob as mesmas condições do revestimento.

Figura 4 – Etapas de aplicação do revestimento de alginato de sódio adicionado da cultura mista de BAL (AS + BAL) em goiaba e manga.



Fonte: Própria, 2026.

Após a secagem (2 horas), um disco de ágar (5 mm de diâmetro) contendo estruturas do isolado de *Colletotrichum* testado (obtidas da margem de uma colônia cultivado em ABD por sete dias a $25 \pm 0,5$ °C) foi inoculado no ponto de punção da fruta (Figura 12) (Fernandes; de Oliveira; de Souza, 2021). Em goiabas, foram inoculadas quatro espécies isoladas dessa fruta: *C. fruticola* LM 680, *C. siamense* LM 681, *C. tropicale* LM 679 e *C. chrysophilum* LM 685. Em mangas, foram inoculadas seis espécies isoladas dessa fruta: *C. asianum* CMM 4057, *C. fruticola* CMM 4069, *C. tropicale* CMM 4071, *C. siamense* CMM 4077, *C. karstii* CMM 4101, *C. gloeosporioides* CMM 320.

As frutas foram acondicionadas em incubadora tipo BOD ($25 \pm 0,5$ °C e 55% de umidade relativa) e avaliadas quanto ao progresso dos sintomas visíveis característicos da antracnose, assim como pela medição do diâmetro das lesões nos dias 6, 9, 12 e 15 de armazenamento (Braga *et al.*, 2019). As frutas foram avaliadas entre o 6º e o 15º dia de armazenamento, período no qual se observaram os primeiros sinais visíveis de antracnose. As

lesões foram detectadas a partir do 6º dia em goiabas, tanto revestidas quanto não revestidas, e em mangas, independentemente do isolado de *Colletotrichum* utilizado na inoculação.

Os resultados foram expressos como a porcentagem de redução do diâmetro da lesão de antracnose (%RDLA) medida com base na diferença do diâmetro da lesão em frutas revestidas com dispersão da AS + BAL quando comparado ao diâmetro da lesão em frutas não tratadas (controle negativo) e calculados com a equação (Braga *et al.*, 2019):

$$\%RDLA = [(N - F / N) \times 100 \quad (\text{Equação 4})$$

onde N é o diâmetro da lesão de antracnose em frutas não tratadas (controle negativo) e F é o diâmetro da lesão de antracnose em frutas revestidas.

Adicionalmente, foi determinado o índice de severidade (%) das lesões de antracnose em goiaba e manga, com base na relação entre a área da superfície da fruta e a área da lesão. A área da superfície da fruta foi estimada com base na forma geométrica mais próxima, assumida como elipse, utilizando os raios de comprimento (r1) e largura (r2), conforme a equação: Área = $r1 \times r2 \times \pi$. O índice de severidade (%) foi calculado de acordo com a equação proposta por Macedo *et al.* (2020):

$$\% \text{Índice de severidade} = [(AF - LA) / AF] \times 100 \quad (\text{Equação 5})$$

onde AF é a área total da superfície da fruta e LA é a área da lesão de antracnose na fruta avaliada.

3.8 AVALIAÇÃO DOS EFEITOS DO REVESTIMENTO SOBRE PARÂMETROS FÍSICOS, FÍSICO-QUÍMICOS E SENSORIAIS DE GOIABA E MANGA

A determinação dos parâmetros físicos, físico-químicos, fitoquímicos, antioxidantes e sensoriais das frutas submetidas aos diferentes tratamentos (AS+BAL, AS e controle), sem inoculação com isolados de *Colletotrichum*, foi realizada imediatamente após a aplicação dos revestimentos e aos 6, 9, 12 e 15 dias de armazenamento a $25 \pm 0,5$ °C. As avaliações físico-químicas incluíram a determinação de sólidos solúveis totais, acidez titulável, pH e teor de ácido ascórbico. Os parâmetros físicos analisados compreenderam perda de peso, cor e firmeza das frutas. O conteúdo fitoquímico foi avaliado por meio da quantificação de compostos fenólicos totais, flavonoides e β -caroteno. A atividade antioxidante foi determinada pelos métodos ABTS^{•+}, DPPH[•] e FRAP. Adicionalmente, foi realizada análise sensorial das amostras ao longo do período de armazenamento.

3.8.1 Sólidos Solúveis Totais

Para a determinação dos sólidos solúveis totais (SST), cada fruta foi cortada longitudinalmente; no caso da manga, o corte foi realizado ao longo do eixo longitudinal, contornando o caroço, de modo a obter duas porções laterais e duas metades. A casca e a semente (na manga) foram retiradas e a polpa esmagada em processador de frutas doméstico. Duas gotas foram extraídas e depositadas no prisma de um refratômetro digital (Brix/RI Chek Model, Reichert Analytical Instruments, Depew, NY, EUA) para leitura (AOAC 2016, método 932,12). Os resultados dos SST foram corrigidos para $20 \pm 0,5$ °C e expressos em °Brix.

3.8.2 Acidez Titulável

A acidez titulável (AT) foi determinada com 10 mL de polpa, homogeneizada com 100 mL de água destilada, usando o método de titulação com solução padronizada de NaOH 0,1 M na presença de fenolftaleína como indicador. Os resultados de AT são expressos em gramas de ácido cítrico por 100 g de fruta (AOAC, 2016, método 920,15).

3.8.3 pH

Os valores de pH foram determinados com 10 mL de polpa, homogeneizada com 100 mL de água destilada, utilizando potenciômetro com eletrodo de vidro combinado (Modelo Q400AS, Quimis, São Paulo, Brasil) calibrado previamente com solução tampão com pH 7 e 4 (AOAC, 2016; Método 981,12).

3.8.4 Ácido ascórbico

O conteúdo de ácido ascórbico foi determinado pelo método de titulação de neutralização com solução de 2,6-diclorofenol indofenol (Pan *et al.*, 2025). Para isso, 10 g de polpa triturada foram homogeneizadas e diluídas em ácido oxálico 0,5%. Uma alíquota de 10 mL foi removida da solução final para titulação com 2,6-diclorofenol indofenol até aparecimento de tonalidade rosa claro. O volume de titulação foi usado na seguinte equação:

$$\text{Ácido ascórbico (mg/100g)} = (V \times F / A) \times 100 \quad (\text{Equação 6})$$

onde V é o volume da solução de 2,6-diclorofenol indofenol gasto na titulação; F é o fator da solução de 2,6-diclorofenol indofenol e A é a quantidade em mL da amostra utilizada. Os resultados foram expressos em mg de ácido ascórbico por 100 g de fruta (Pan *et al.*, 2025).

3.8.5 Perda de peso

A avaliação da perda de peso das frutas foi determinada com base no peso inicial (dia zero) e no peso final da fruta em cada período de armazenamento. Os resultados foram expressos como porcentagem da perda de peso inicial (Sousa *et al.*, 2021).

3.8.6 Parâmetros de cor

A cor da superfície da casca e da polpa foi medida em cada fruta em dois locais opostos (Shu *et al.*, 2024), usando um colorímetro Konica Minolta (modelo CR-400, Minolta Corp., Ramsey, NJ, EUA), conforme descrito na seção 3.6.9. Adicionalmente, a diferença total de cor (ΔE) foi calculada de acordo com Saikaew *et al.* (2025), com base nos valores L^* , a^* e b^* , utilizando a seguinte equação:

$$\Delta E = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2} \quad (\text{Equação 7})$$

onde L_0 , a_0 e b_0 representam os valores de cor iniciais. A magnitude de ΔE foi interpretada da seguinte forma: imperceptível ($0 < \Delta E \leq 0,5$), pouco perceptível ($0,5 < \Delta E \leq 1,5$), perceptível ($1,5 < \Delta E \leq 3,0$), bem visível ($3,0 < \Delta E \leq 6,0$), muito visível ($6,0 < \Delta E \leq 12,0$) e extremamente visível ($\Delta E > 12,0$) (Razali *et al.*, 2021).

3.8.7 Firmeza

Para a avaliação da firmeza, cada fruta foi analisada em dois pontos opostos na região equatorial, utilizando um analisador de textura TA-XTplus (Stable Micro Systems Ltd., Reino Unido), equipado com sondas cilíndricas de diferentes diâmetros: 6 mm para manga (Parente *et al.*, 2025) e 2 mm para goiaba (Chen *et al.*, 2022). As sondas penetraram aproximadamente 10 mm no tecido da fruta, sendo registrada a força máxima de penetração. Os resultados foram expressos em Newton (N).

3.8.8 Teor de fenólicos, flavonoides e atividade antioxidante

Para a determinação do teor de fenólicos e flavonoides totais, e da atividade antioxidante das frutas ao longo do período de armazenamento, foram obtidos extratos metanólicos. Para isso, 5 g de polpa triturada foram homogeneizados e diluídos em 100 mL de metanol a 80% (80:20, v/v) e, após agitação, as amostras foram armazenadas por 24 h. Em seguida, todos os extratos foram centrifugados a $1696 \times g$ (4.000 rpm) por 15 min a $4 \pm 0,5$ °C. O sobrenadante foi filtrado e os extratos mantidos sob refrigeração ($4 \pm 0,5$ °C) até a realização das análises. As análises realizadas incluíram a determinação de compostos fenólicos totais, flavonoides totais, carotenoides totais e atividade antioxidante pelos métodos ABTS $\bullet^+$, DPPH $\bullet$ e FRAP.

3.8.8.1 Compostos fenólicos totais

O teor de fenólicos totais foi determinado pelo ensaio de Folin-Ciocalteu, utilizando ácido gálico como padrão. Aliquotas de 30 μ L de cada amostra foram transferidas para uma microplaca de 96 poços. Em seguida, 150 μ L de solução de Folin-Ciocalteu (10%) foram adicionados e a placa foi mantida por 6 min em temperatura ambiente ($25 \pm 0,5$ °C). Posteriormente, 120 μ L da solução de carbonato de sódio (7,5%) foram adicionados aos poços e deixados por 5 min em banho-maria ($50 \pm 0,5$ °C). Por fim, a absorbância foi lida em um comprimento de onda de 765 nm utilizando um espectrofotômetro de microplacas. Uma curva padrão de ácido gálico determinou o teor total de compostos fenólicos. Somente a solução extratora foi utilizada para analisar o branco da curva padrão e as amostras. Os resultados foram expressos em mg de equivalentes de ácido gálico (EAG) por 100 gramas de amostra (mg EAG/100 g) (Ouahabi *et al.*, 2024).

3.8.8.2 Flavonoides totais

O teor total de flavonoides foi determinado por um método previamente descrito por Guevara-Figueroa *et al.* (2010). Uma alíquota de 0,5 mL do extrato metanólico de cada fruta foi adicionada a 2 mL de água destilada, seguida da adição de 150 μ L de nitrito de sódio a 5%. Após 5 min, foram adicionados 150 μ L de cloreto de alumínio a 10%. Em seguida, após 6 min, foram incorporados 1 mL de hidróxido de sódio 1 M e 1,2 mL de água destilada. A absorbância foi medida a 510 nm em espectrofotômetro de microplacas, utilizando como branco a solução na ausência dos extratos. O teor total de flavonoides foi quantificado por meio de curva padrão

de catequina (Sigma-Aldrich), e os resultados foram expressos em mg de equivalentes de catequina (EC) por 100 g de amostra (mg EC/100 g) (Zitha *et al.*, 2022).

3.8.8.3 Carotenoides totais

Os extratos das frutas foram lidos imediatamente após a filtração em um espectrofotômetro de microplacas em um comprimento de onda de 450 nm. Uma curva de calibração foi obtida usando β -caroteno como padrão. Os resultados foram expressos em mg β -caroteno/100 g de amostra (Hasan *et al.*, 2022).

3.8.8.4 Determinação atividade antioxidante

A atividade antioxidante dos extratos das frutas foi determinada pelos métodos ABTS $\bullet^+$, DPPH $\bullet$ e FRAP, conforme descrito na seção 3.6.10.

3.8.9 Sensorial

Os protocolos das análises sensoriais foram avaliados pelo Comitê de Ética em Pesquisa com Seres Humanos do Centro de Ciências da Saúde/UFPB, e somente após a sua aprovação os experimentos foram realizados (CAAE: 92207925.0.0000.5188). As amostras foram avaliadas por meio de métodos padrão, visando à verificação da conformidade com os parâmetros microbiológicos estabelecidos pela legislação brasileira vigente (Legislação Brasileira, 2019). A metodologia de *Preferred Elicitation Attribute* (PAE) foi realizada de acordo com Grygorczyk *et al.* (2013), com o objetivo de realizar uma análise exploratória. Foram recrutados 16 julgadores, de 23 a 40 anos, de ambos os sexos, através de contato pessoal. Como critérios de exclusão, foram considerados casos de intolerância, alergia ou seletividade alimentar relacionada ao contato ou consumo das frutas utilizadas no estudo. Adicionalmente, indivíduos que apresentaram problemas respiratórios nos dias das sessões foram excluídos, a fim de não comprometer a acurácia da avaliação.

Os testes sensoriais foram conduzidos em três dias distintos, com uma sessão dedicada a cada fruta, correspondente ao período de armazenamento (dias 0, 6 e 15). As avaliações sensoriais foram realizadas individualmente pelos provadores, com duração aproximada de 120 min em cada sessão. As análises foram realizadas sob condições controladas de temperatura e iluminação em cabines individuais. Cada avaliador recebeu uma fruta inteira e uma porção da

polpa das frutas revestidas com AS+BAL, AS e não revestidas (controle), apresentadas em pratos plásticos brancos, identificadas com números aleatórios de três dígitos, seguindo uma sequência cega e aleatória, imediatamente após a remoção do armazenamento ($25 \pm 0,5$ °C).

Todas as formulações foram apresentadas simultaneamente e as avaliações sensoriais realizadas individualmente. Os julgadores foram solicitados a avaliar as frutas íntegras e porções de polpa das frutas revestidas (aproximadamente 20 gramas) e listar quais atributos sensoriais são importantes para caracterizá-las. Os atributos elicitados foram descritos em um quadro branco e agrupados em aparência geral, cor, aroma e textura por meio de uma discussão com todos do grupo. Atributos que não seriam facilmente avaliados ou que tivessem o mesmo significado foram excluídos por consenso do grupo. Os provadores, então, definiram as âncoras dos termos descritores e, após uma pausa de 15 min, receberam as amostras de frutas íntegras e porções de frutas (de forma monádica), juntamente com a folha de avaliação. Em seguida, foi solicitado a cada provador avaliar a intensidade dos atributos para cada amostra usando uma escala hedônica de 9 pontos (1 = pouco/baixo e 9 = muito/alto) (Grygorczyk *et al.*, 2013).

3.10 ANÁLISES ESTATÍSTICAS

A análise dos efeitos sobre o crescimento micelial fúngico foram realizadas em triplicata em três experimentos independentes. A análise do progresso da lesão de antracnose e dos parâmetros físico-químicos das frutas foram realizadas considerando um delineamento experimental inteiramente casualizado com três repetições para cada grupo experimental (tipo de tratamento) e seis frutas por repetição em três experimentos independentes. Os resultados foram expressos como média $\pm$ desvio padrão. O teste de normalidade de Kolmogorov–Smirnov foi executado para avaliar se os dados obtidos tinham distribuição normal. O teste de Levene foi executado para avaliar se os dados satisfaziam a suposição de homogeneidade (variância igual). A análise de variância (ANOVA), seguida pelo teste post hoc de Tukey ou Duncan, foi executada para determinar diferenças ($p < 0,05$) entre os resultados. A análise estatística foi realizada com o *software* computacional Sigma Stat 3.5 (Jandel Scientific Software, San Jose, CA, EUA). A análise dos dados obtidos nos experimentos de FTIR e DSC foi realizada utilizando o *software* Origin 9.1 (OriginLab Corporation, Northampton, MA, EUA). Os dados de *Preferred Elicitation Attribute* (PAE) foram avaliados utilizando a Análise de Procrustes Generalizada (APG). Essa análise foi realizada utilizando o *software* computacional GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, EUA).

4 RESULTADOS

Conforme as normas estipuladas pelo colegiado do Programa de Pós-graduação em Ciências da Nutrição/CCS/UFPB, os resultados desta tese estão apresentados na forma de dois artigos originais apresentados nos apêndices C e D.

O manuscrito original intitulado “*A novel coating formulated with sodium alginate loaded with a mixed lactic acid bacteria culture to control anthracnose in guava and mango*” (apêndice C), publicado no periódico *Food Control* (doi:10.1016/j.foodcont.2025.111341) aborda a eficácia de um revestimento formulado com alginato de sódio e adicionado da cultura mista de cepas de bactérias lácticas (BAL), para controlar o progresso e severidade da lesão da antracnose, causada por diferentes isolados de fungos do gênero *Colletotrichum* em goiaba cv. Paluma e manga cv. Palmer durante o armazenamento. Neste estudo, uma cultura mista de três diferentes espécies de BAL nomeadamente *Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129 e *Limosilactobacillus fermentum* 263, foi testada quanto a sua capacidade antagonista frente a dez isolados de *Colletotrichum*. Em testes *in vitro*, a cultura mista foi capaz de inibir o crescimento micelial de todos os isolados fúngicos testados. Os revestimentos de alginato de sódio apresentaram >31% de subpopulações de células BAL com membrana não permeabilizada (células viáveis, PI-CFDA+) no final do armazenamento refrigerado e contagens viáveis >7 log UFC/g. A aplicação de revestimentos com alginato de sódio com a cultura mista de BAL atrasou o desenvolvimento e diminuiu a gravidade das lesões de antracnose em goiaba e manga contaminadas artificialmente com qualquer um dos isolados de *Colletotrichum* testados. O revestimento formulado pode ser uma estratégia inovadora de biocontrole para proteção de goiaba e manga contra o desenvolvimento de doenças pós-colheita, especialmente antracnose.

O segundo manuscrito original, intitulado “*Characterization and preservation performance of a novel sodium alginate coating incorporating a mixed lactic acid bacteria culture on guava and mango*” (Apêndice D), encontra-se submetido ao periódico *Food Chemistry* e apresenta os resultados obtidos a partir da caracterização de um revestimento formulado à base de alginato de sódio incorporado com cultura mista de bactérias ácido lácticas, bem como a avaliação de seus efeitos na manutenção da qualidade pós-colheita de goiaba cv. Paluma e manga cv. Palmer durante o armazenamento. A cultura mista de BAL utilizada na formulação do revestimento foi composta pelas cepas *Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129 e *Limosilactobacillus fermentum* 263. As análises de caracterização indicaram que a incorporação das BAL promoveu maior estabilidade estrutural

ao filme, conferindo propriedades térmicas, físico-químicas, morfológicas e de biodegradabilidade adequadas para aplicação como revestimento comestível em frutas. Além disso, as análises de biodegradabilidade, conduzidas em diferentes meios, demonstraram degradação significativa em curto período (20 dias), indicando que a incorporação das BAL não comprometeu o desempenho ambiental da matriz polimérica. Adicionalmente, aplicação dos revestimentos (AS + BAL) em goiaba e manga contribuiu para a preservação das características pós-colheita das frutas, retardando a perda de massa e a degradação do ácido ascórbico, além de auxiliar na manutenção dos parâmetros de cor durante 15 dias de armazenamento. Além disso, os atributos sensoriais corroboraram os resultados físico-químicos, evidenciando a manutenção da cor, aroma e textura das frutas revestidas ao longo do período de armazenamento. De modo geral, o revestimento formulado apresentou potencial promissor na conservação pós-colheita de goiabas e mangas, contribuindo para a manutenção da qualidade e ampliação da vida útil das frutas.

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APÊNDICES

APÊNDICE A – Depósito de Patente

20/06/2023 870230052813
15:08

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Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2023 012287 6

Dados do Depositante (71)

Depositante 1 de 1

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Tipo de Pessoa: Pessoa Jurídica

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Esta solicitação foi enviada pelo sistema Petição Eletrônica em 20/06/2023 às 15:08, Petição 870230052813

Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): REVESTIMENTO DE ALGINATO DE SÓDIO ADICIONADOS DE CULTURA MISTA DE BACTÉRIAS LÁTICAS, MÉTODO DE PRODUÇÃO E APLICAÇÃO COMO TECNOLOGIA PÓS-COLHEITA EM FRUTAS

Resumo: A presente invenção está inserida na área de biotecnologia de preservação de produtos hortifrutícolas e se refere ao desenvolvimento de um revestimento composto da combinação do polímero alginato de sódio e bactérias lácticas para aplicação como tecnologia pós-colheita em frutas. A aplicação do revestimento objetiva promover o controle do desenvolvimento de fungos fitopatógenos e manter as características de qualidade (físico-químicas e sensoriais) destes produtos ao longo do armazenamento. Além disso, apresenta-se como alternativa biológica e viável frente ao uso de fungicidas sintéticos em frutas no período pós-colheita. O produto apresenta ação comprovada frente a fungos causadores de infecções pós-colheita em frutas, com manutenção das características de qualidade dos produtos revestidos, e surge como nova tecnologia de preservação e conservação de frutas, com destacável valor agregado e potencial de atender as demandas correntes do mercado e consumidores.

**PETICIONAMENTO
ELETRÔNICO**

Esta solicitação foi enviada pelo sistema Petição Eletrônica em 20/06/2023 às 15:08, Petição 870230052813

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Documentos anexados

Tipo Anexo	Nome
Comprovante de pagamento de GRU 200	00_Comprovante_01-03-2023.pdf
Relatório Descritivo	01_Relatorio_descritivo-1-5.pdf
Reivindicação	02_Reivindicacoes-6-7.pdf
Resumo	03_Resumo-8.pdf

Acesso ao Patrimônio Genético

- ☒ Declaração Negativa de Acesso - Declaro que o objeto do presente pedido de patente de invenção não foi obtido em decorrência de acesso à amostra de componente do Patrimônio Genético Brasileiro, o acesso foi realizado antes de 30 de junho de 2000, ou não se aplica.

Declaração de veracidade

- ☒ Declaro, sob as penas da lei, que todas as informações acima prestadas são completas e verdadeiras.

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NOME FANTASIA:

INSTITUTO NACIONAL DA PROPRIEDADE I

CNPJ: 42.521.088/0001-37

PAGADOR:

UNIVERSIDADE FEDERAL DA PARAIBA

CNPJ: 24.098.477/0001-10

NR. DOCUMENTO 30.101

NOSSO NUMERO 29409162300921835

CONVENIO 02940916

DATA DE VENCIMENTO 29/03/2023

DATA DO PAGAMENTO 01/03/2023

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“REVESTIMENTO DE ALGINATO DE SÓDIO ADICIONADOS DE CULTURA MISTA DE BACTÉRIAS LÁTICAS, MÉTODO DE PRODUÇÃO E APLICAÇÃO COMO TECNOLOGIA PÓS-COLHEITA EM FRUTAS”

[001] A presente invenção está inserida na área de biotecnologia de preservação de produtos hortifrutícolas e refere-se ao desenvolvimento de revestimentos compostos do polímero alginato de sódio adicionado de bactérias lácticas. A aplicação dos revestimentos no período pós-colheita de frutas objetiva promover o controle do desenvolvimento de fungos fitopatogênicos e manter as características de qualidade (físico-químicas e sensoriais) dos produtos revestidos ao longo do armazenamento. Além disso, esses revestimentos apresentam-se como alternativa biológica e viável frente ao uso de fungicidas sintéticos no período pós-colheita de frutas.

[002] A procura e o consumo de frutas estão em constante aumento. No entanto, existem barreiras que dificultam a comercialização desses produtos. Frutas são alimentos com elevado perecibilidade, sendo facilmente alterados durante seu desenvolvimento e maturação, especialmente na fase de pós-colheita em consequência da manipulação excessiva, utilização de embalagens inadequadas, armazenamento e transporte em temperaturas elevadas, que são fatores facilitadores do desenvolvimento de patógenos causadores de podridão, com destaque para a ação de fungos fitopatogênicos. Em decorrência das doenças ocasionadas por esses agentes, têm-se a deterioração mais acelerada com comprometimento da aparência, qualidade e aceitação das frutas pelos consumidores, resultando em perdas econômicas elevadas.

[003] Atualmente, a principal estratégia utilizada para o controle das doenças pós-colheita em frutas é a aplicação de agentes fungicidas sintéticos no campo e no processamento pós-colheita. Porém, a crescente

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preocupação dos consumidores com relação aos impactos negativos causados para o meio ambiente e para a saúde humana como consequência do uso generalizado e contínuo dos fungicidas químicos, aliados a capacidade dessas substâncias de induzir o surgimento de estirpes de fungos fitopatógenos resistentes, têm estimulado a busca por métodos alternativos efetivos para a prevenção do desenvolvimento de infecções pós-colheitas em frutas.

[004] Nessa perspectiva, bactérias láticas com capacidade de exercer efeitos antagonísticos frente a microrganismos contaminantes de alimentos, incluindo fungos fitopatógenos, são consideradas ferramentas biotecnológicas promissoras para o uso na formulação de novos materiais e tecnologias para o biocontrole de doenças pós-colheita em frutas, com ênfase nas espécies de *Lactobacillus* e gêneros relacionados, que possuem amplo histórico de uso seguro em alimentos. Além disso, as bactérias láticas estão entre os microrganismos mais prevalentes na microbiota autóctone de frutas, com adaptação natural as condições encontradas nesses alimentos.

[005] A incorporação de bactérias láticas em matrizes adequadas caracteriza-se como aspecto importante para garantir a propagação e sobrevivência destes microrganismos quando aplicados em estratégias de tecnologias de preservação de alimentos, a exemplo da incorporação em revestimentos comestíveis. Esses materiais também podem ser utilizados como estratégias inovadoras para o controle da podridão fúngica e para a manutenção de parâmetros indicadores de qualidade pós-colheita em frutas, visto que retardam o processo de respiração, amadurecimento e deterioração destes produtos, repercutindo em ampliação do seu período de armazenamento, transporte e distribuição.

[006] Dentre os compostos utilizados na produção de revestimentos comestíveis, o alginato de sódio, um polímero extraído de algas marinhas, tem se destacado devido as suas propriedades de geleificação e

espessamento e de sua estrutura química, formada por frações de α -L-guluronato ligadas por (1 $\rightarrow$ 4) e manuronato β -D-ligadas (1 $\rightarrow$ 4). Apresenta-se como um produto de baixo custo, pouco sabor residual, biodegradável e de baixa toxicidade, permitindo assim seu uso na indústria alimentícia, farmacêutica e biotecnológica.

[007] Os revestimentos de alginato de sódio têm sido utilizados como carreadores de microrganismos probióticos para aplicação em frutas, sendo relatados resultados promissores em relação à conservação destes produtos. Além disso, revestimentos de alginato de sódio mostraram vantagens tecnológicas em relação a outros revestimentos de polissacarídeos aplicados em frutas, não afetando a sobrevivência de cepas de bactérias lácticas com propriedades probióticas e tecnológicas durante o armazenamento. Dentre os microrganismos adicionados a revestimentos de alginato de sódio aplicados em frutas, destacam-se *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus* e *Lactocaseibacillus rhamnosus*.

[008] A aplicação de revestimento formulado com alginato de sódio adicionado de bactérias lácticas tem efeitos relacionados com melhorias das características pós-colheita e também com inibição de fungos fitopatogênicos, melhorando os aspectos de qualidade e reduzindo perdas econômicas para a cadeia produtiva em decorrência as doenças que acometem as frutas.

[009] Algumas patentes já foram descritas na literatura com a produção de revestimentos de alginato de sódio, assim como sua utilização em alimentos. A invenção WO 2021 21456 1 A1, intitulada "Alginate composition for food products, method for its production and process for producing coated food products" refere-se a uma composição de alginato para revestimento de produtos alimentícios, em particular produtos alimentícios frescos e sem pele, como salsichas, e um método para produzir a composição de alginato. Tal invenção faz referência

apenas a produtos alimentícios específicos revestidos com a composição de alginato.

[010] A invenção US 2016 032417 3 A1, intitulada “Edible coating for preserving fruit pieces, manufacturing and application method thereof” refere-se a um revestimento comestível composto de alginato de sódio e reticulado com ascorbato de cálcio ou lactato de cálcio para ser aplicado em frutas minimamente processadas. Na composição também pode conter ácido cítrico como agente antioxidante, e ácido málico como agente antimicrobiano, mas sem referir especificações para o biocontrole de fitopatógenos.

[011] A invenção WO 2006 10369 8 A1, intitulada “Edible films and coatenfgs based on fructooligosaccharides with probiotic properties” refere-se a filmes e revestimentos comestíveis à base de frutooligossacarídeos com propriedades probióticas, contudo sem aplicação antimicrobiana como protetor pós-colheita.

[012] Na literatura não foi encontrada nenhuma patente descrevendo a aplicação de revestimentos de alginato de sódio adicionados de bactérias lácticas para o controle de doenças fúngicas pós-colheita em frutas, bem como para a manutenção das características de qualidade dos produtos durante o armazenamento. Assim, esta invenção é pioneira e promissora em trazer a combinação de alginato de sódio e bactérias lácticas na elaboração de revestimentos com capacidade de inibição de fungos fitopatógenos, tais como *Colletotrichum asianum*, *Colletotrichum fructicola*, *Colletotrichum tropicale*, *Colletotrichum siamense*, *Colletotrichum karstii*, e *Colletotrichum gloeosporioides*. Na prática, o produto pode ser utilizado mesmo sob temperatura ambiente em determinadas condições de estágio de maturação das frutas destinadas ao mercado interno ou externo.

[013] A produção do revestimento composto de alginato de sódio e bactérias lácticas foi realizada seguindo etapas sequenciais: **(1)** Dissolução

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do alginato de sódio (1,5 g/100 mL) em água destilada esterilizada aquecida (70°C) sob agitação magnética (120 rpm) por 30 minutos até a dissolução completa. **(2)** Incorporação de glicerol (0,75 g/100 g) na dissolução para aumentar a flexibilidade dos revestimentos, surfactante tween 80 (0,05 g/100 g) para melhorar a aderência às frutas e óleo de girassol (0,04 g/100g) como fonte de lipídios para aumentar as características de barreira à água, seguido de agitação por 10 minutos sob temperatura ambiente ($25 \pm 0,5^\circ\text{C}$) até a dissolução completa. **(3)** Incorporação da cultura mista de células de bactérias lácticas (proporção 1:1:1), (*Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129 e *Limosilactobacillus fermentum* 263) na dispersão de alginato de sódio, colhidas após centrifugação (4500 x g, 4°C, 15 minutos), lavagem (NaCl, 0,85%, p/v) e liofilização (com frutooligossacarídeo como crioprotetor, 20% p/v); para obtenção de concentração celular final 9,5 – 10,5 log UFC/mL, preferencialmente de 9 log UFC/mL. **(4)** Imersão das frutas, previamente higienizadas, na dispersão de alginato de sódio e bactérias lácticas por 1 a 5 minutos, preferencialmente por 2 minutos. **(5)** Preparação da solução reticuladora do revestimento com cloreto de cálcio (2 g/100 mL) diluído em água destilada esterilizada aplicada após imersão no revestimento para induzir o mecanismo de geleificação e reação de reticulação do alginato de sódio. **(6)** Drenagem e secagem sob temperatura ambiente ($25 \pm 0,5^\circ\text{C}$) por 60 minutos.

REIVINDICAÇÕES

1. “REVESTIMENTO DE ALGINATO DE SÓDIO ADICIONADOS DE CULTURA MISTA DE BACTÉRIAS LÁTICAS, MÉTODO DE PRODUÇÃO E APLICAÇÃO COMO TECNOLOGIA PÓS-COLHEITA EM FRUTAS” apropriados para proteção de frutas no período pós-colheita em decorrência da ação inibitória contra fungos fitopatogênicos, com manutenção das características de qualidade (físico-químicas e sensoriais) e extensão da sua vida de prateleira, constituído pela obtenção de uma dispersão composto por (1) alginato de sódio e (2) bactérias lácticas (BAL), caracterizado por compreender as seguintes etapas: **(1)** dissolução do alginato de sódio (1,5 g/100 mL) em água destilada esterilizada aquecida (70°C) sob agitação magnética (120 rpm) por 30 minutos até a dissolução completa; **(2)** incorporação de glicerol (0,75 g/100 g) na dissolução para aumentar a flexibilidade dos revestimentos, surfactante tween 80 (0,05 g/100 g) para melhorar a aderência às frutas, e óleo de girassol (0,04 g/100 g) como fonte de lipídios para aumentar as características de barreira à água, seguido de agitação por 10 minutos sob temperatura ambiente ($25 \pm 0,5^\circ\text{C}$ até a dissolução completa; **(3)** incorporação da cultura mista de células de bactérias lácticas (proporção 1:1:1), (*Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129 e *Limosilactobacillus fermentum* 263) na dispersão de alginato de sódio, colhidas após centrifugação (4500 x g/ 4°C, 15 minutos), lavagem (NaCl, 0,85%, p/v) e liofilização (com frutooligossacarídeo como crioprotetor, 20% p/v) e para obtenção de concentração celular final 9,5 – 10,5 log UFC/mL, preferencialmente de 9 log UFC/mL; **(4)** imersão das frutas, previamente higienizadas, na dispersão de alginato de sódio e bactérias lácticas por 1 a 5 minutos, preferencialmente por 2 minutos; **(5)** preparação da solução reticuladora do revestimento com cloreto de cálcio (2 g/100mL) diluído em água

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destilada esterilizada aplicada após imersão no revestimento para induzir o mecanismo de geleificação e reação de reticulação do alginato de sódio; **(6)** drenagem e secagem sob temperatura ambiente ($25 \pm 0,5^{\circ}\text{C}$) por 60 minutos.

2. “REVESTIMENTO DE ALGINATO DE SÓDIO ADICIONADOS DE CULTURA MISTA DE BACTÉRIAS LÁTICAS, MÉTODO DE PRODUÇÃO E APLICAÇÃO COMO TECNOLOGIA PÓS-COLHEITA EM FRUTAS”, de acordo com a reivindicação 1, constituído de etapas sequenciais para obtenção do revestimento de alginato de sódio caracterizado pela incorporação de cultura mista bactérias lácticas liofilizadas e imersão de frutas na dispersão, reticulação e secagem.

3. “REVESTIMENTO DE ALGINATO DE SÓDIO ADICIONADOS DE CULTURA MISTA DE BACTÉRIAS LÁTICAS, MÉTODO DE PRODUÇÃO E APLICAÇÃO COMO TECNOLOGIA PÓS-COLHEITA EM FRUTAS”, de acordo com a reivindicação 2, caracterizado pelo fato da dispersão de alginato de sódio e cultura mista de bactérias lácticas liofilizadas formarem um revestimento quando aplicado na superfície de frutas com capacidade de proteção frente à doenças pós-colheita, causadas por fungos fitopatogênicos, mantendo a qualidade dos produtos revestidos por tempo prolongado, vindo ao encontro das exigências do mercado sem, entretanto, proporcionar prejuízos ao consumidor.

RESUMO**“REVESTIMENTO DE ALGINATO DE SÓDIO ADICIONADOS DE CULTURA MISTA DE BACTÉRIAS LÁTICAS, MÉTODO DE PRODUÇÃO E APLICAÇÃO COMO TECNOLOGIA PÓS-COLHEITA EM FRUTAS”**

A presente invenção está inserida na área de biotecnologia de preservação de produtos hortifrutícolas e se refere ao desenvolvimento de um revestimento composto da combinação do polímero alginato de sódio e bactérias lácticas para aplicação como tecnologia pós-colheita em frutas. A aplicação do revestimento objetiva promover o controle do desenvolvimento de fungos fitopatógenos e manter as características de qualidade (físico-químicas e sensoriais) destes produtos ao longo do armazenamento. Além disso, apresenta-se como alternativa biológica e viável frente ao uso de fungicidas sintéticos em frutas no período pós-colheita. O produto apresenta ação comprovada frente a fungos causadores de infecções pós-colheita em frutas, com manutenção das características de qualidade dos produtos revestidos, e surge como nova tecnologia de preservação e conservação de frutas, com destacável valor agregado e potencial de atender as demandas correntes do mercado e consumidores.

APÊNDICE B – Artigo de Revisão



Received: 13 April 2024 | Revised: 16 July 2024 | Accepted: 21 July 2024

DOI: 10.1111/1541-4337.13427

COMPREHENSIVE REVIEW



Interventions based on alternative and sustainable strategies for postharvest control of anthracnose and maintain quality in tropical fruits

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Funding information

Coordenação de Aperfeiçoamento de
Pessoal de Nível Superior; Fundação de
Apoio à Pesquisa do Estado da Paraíba

Abstract

Colletotrichum spp. is a phytopathogen causing anthracnose in a variety of tropical fruits. Strategies used to control postharvest diseases in tropical fruits typically rely on the use of synthetic fungicides, which have stimulated the emergence of resistant pathogens. Safer alternative strategies to control anthracnose in tropical fruits have been described in the literature. This review presents and discusses the main innovative interventions concerning the application of sustainable alternative strategies in the postharvest control of pathogenic *Colletotrichum* species in tropical fruits, with a particular emphasis on the studies published in the last 5 years. The available studies have shown the use of various methods, including physical barriers, natural antimicrobials, and biological control with antagonistic microorganisms, to reduce anthracnose lesion severity and incidence in tropical fruits. The available literature showed high inhibitory activity in vitro, reduced anthracnose incidence and lesion diameter, and total disease inhibition in tropical fruits. Most studies focused on the inhibition of *Colletotrichum gloeosporioides* on avocado, papaya, and mango, as well as of *Colletotrichum musae* on banana; however, the inhibition of other *Colletotrichum* species was also demonstrated. The application of emerging sustainable alternative methods, including natural antimicrobial substances, also stimulated the induction of defense systems in tropical fruits, including enzymatic activity, such as polyphenol oxidase, peroxidase, and phenylalanine ammonia-lyase. The retrieved data helped to understand the current state of the research field and reveal new perspectives on developing efficient and sustainable intervention strategies to control pathogenic *Colletotrichum* species and anthracnose development in tropical fruits.

KEYWORDS

anthracnose, natural antimicrobial, physical barriers, postharvest preservation, tropical fruits

1 | INTRODUCTION

Tropical fruits are cultivated predominantly in hot climate zones and include a diversity of cultivars that are increasingly consumed worldwide due to their high contents of nutrients and bioactive compounds and good sensory acceptance by consumers (Irfan et al., 2023). Most tropical fruits are very perishable in nature, which causes significant losses due to physiological disorders, mechanical injuries, and especially the action of fungal phytopathogens causing fruit rot (Bilal & Hashmi, 2023; Sharma et al., 2021; Uclaray et al., 2022). Because tropical fruits are commonly consumed fresh, every alteration in their characteristics and quality attributes makes them less attractive to consumers and difficult to commercialize (Anjum et al., 2020).

Anthrachnose caused by *Colletotrichum* spp. is considered one of the most important postharvest diseases affecting tropical fruits worldwide due to the severity of the developed symptoms, which lead to high postharvest losses (Shah et al., 2021; Wu et al., 2023; Zhou et al., 2022). Anthracnose is characterized by black spot lesions on the fruit skin that eventually penetrate the pulp and accelerate natural ripening (Garcia et al., 2022), reducing fruit quality and market value during export and retailing (Mokgalapa et al., 2022).

Fungal infections, especially anthracnose, contribute to economic losses of about 30%–50% in the tropical fruit supply chain (Bordoh et al., 2020). For example, avocado losses due to anthracnose are estimated at about 5%–25% of the production in developed countries and 20%–50% in developing countries, with a greater prevalence of *Colletotrichum gloeosporioides* (Cuéllar-Torres et al., 2022; Mokgalapa et al., 2022). *Colletotrichum musae* is the most prevalent pathogenic species causing anthracnose in banana, resulting in postharvest losses of about 30%–40% in fruit commercial value (Mangoba & Alvindia, 2023; Qiao et al., 2022; Yang et al., 2022). Still, anthracnose caused by *Colletotrichum* spp. is the most serious disease during the growth and postharvest of papaya, guava, and mango, causing about 30%–60% loss in the supply chain of these fruits (Bilal & Hashmi, 2023; Landi et al., 2021; Wang et al., 2022, 2023; Wu et al., 2023). *C. gloeosporioides* is considered the main causative agent of anthracnose in papaya, guava, and mango; however, other different *Colletotrichum* species, such as *C. asianum*, *C. fruticola*, *C. tropicale*, *C. siamense*, *C. karstii*, *C. musae*, *C. brevisporum*, and *C. acutatum*, have also been associated with anthracnose lesions in these fruits (Danh et al., 2021; Fernandes et al., 2021; Mangoba & Alvindia, 2023; Wang et al., 2023).

In conventional agriculture, fruits are consistently sprayed with chemical fungicides (e.g., dithiocarbamate, benomyl, thiabendazole, imazalil, and Prochloraz) in the

preharvest period from after flowering up to harvest (Alvindia & Mangoba, 2020; Shah et al., 2021). In the postharvest phase, the control of anthracnose also greatly relies on using chemical fungicides that allow prolonged fruit conservation (Cuéllar-Torres et al., 2022). However, the use of these chemical fungicides for long periods imposes several challenges, including increased resistance to fungicides, risk to human and animal health, and concerns regarding environmental sustainability (Wang et al., 2023). These aspects highlight the importance of seeking feasible options for anthracnose control in tropical fruits that fit the concept of sustainable agriculture, aiming to meet the global demand for qualitative and quantitative agricultural production, allowing more intelligent management of production factors, and helping to mitigate environmental problems associated with chemical fungicides (Evangelista-Martínez et al., 2022).

Some alternative and more sustainable postharvest treatments have been investigated to control anthracnose in tropical fruits, including chemical (essential oils [EOs], organic acids, and chitosan [CH]) (Alvindia & Mangoba, 2020; Qiao et al., 2022; Wang et al., 2023), physical (coatings, hot water, and cold plasma) (Cuéllar-Torres et al., 2022; Wu et al., 2023), and biological methods (lactic acid bacteria [LAB] and yeasts) used separately (Aguirre-Gutiérrez et al., 2021; Konsue et al., 2020) or in combination (e.g., polymers with antagonistic bacteria and CH with EO or organic acid) (Fernandes et al., 2021; Iniguez-Moreno et al., 2020; Landi et al., 2021; Osondu et al., 2022; Peralta-Ruiz et al., 2020; Reyna et al., 2022).

This review presents and discusses the main emerging innovative interventions concerning the application of sustainable alternative strategies in the postharvest control of pathogenic *Colletotrichum* species in tropical fruits using the literature review framework with particular emphasis on the studies published in the last 5 years.

2 | POSTHARVEST QUALITY LOSSES OF TROPICAL FRUITS

2.1 | Commercial importance and nutritional aspects of tropical fruits

The world trade volume of major tropical fruits in 2023 reached a new peak of USD 11.2 billion, marking an increase of approximately 12% compared to 2022, prominently featured in the international trade for pineapple, avocado, papaya, and the commodity cluster composed of mango, mangosteen, and guava (Food and Agriculture Organization [FAO], 2024). In terms of global exports, these fruits represented approximately 8.9 million tons in 2023, with pineapple as the most predominant fruit in

commercialized production volume. However, regarding value, avocado represents over 60% of the global trade among the main tropical fruits (FAO, 2024).

Tropical fruits cultivated in distinct warm climate zones comprise various cultivars with differences regarding structure, physiology, and sensory characteristics (color, taste, and flavor) (Irfan et al., 2023). Furthermore, tropical fruits are widely accepted by consumers due to their high contents of nutrients and bioactive compounds, such as vitamins, minerals, carotenoids, and dietary fibers, commonly linked to a reduced risk of chronic noncommunicable diseases (López-Zazueta et al., 2023).

The trade and export of tropical fruits depend on compliance with strict phytosanitary standards mostly imposed by developed countries, which impact agricultural trade in developing countries as the major tropical fruit producers (Zakaria, 2023). In addition, the expenditure on chemical fertilizers and pesticides significantly affects the production cost of tropical fruits due to their high use frequency (FAO, 2024).

2.2 | Factors causing postharvest loss of tropical fruits

The high perishability of tropical fruits in nature results in significant losses during production, processing, storage, transportation, and market (Irfan et al., 2023). Approximately 14% of the world's food production is lost after harvesting and before reaching retail. In some cases, this loss can reach up to 50% of total tropical fruit production (FAO, 2024; Irfan et al., 2023; Zakaria, 2023).

Tropical fruits are significantly affected by several factors during production, including abiotic and biotic stresses, and these impacts can increase immediately after harvest, especially in climacteric fruits, such as avocado, banana, guava, mango, and papaya. These fruits undergo a climacteric peak after harvest and before ripening due to cellular respiration, ethylene gas production, and enzymatic activity (e.g., pectinase and polygalacturonase) (García et al., 2022). Postharvest losses in tropical fruits are a serious concern in the agro-food sector in different regions (Bilal & Hashmi, 2023). However, the postharvest losses in tropical fruits vary with the cultivar, soil, climatic conditions, and production and handling techniques (Gonçalves et al., 2021). Short postharvest life, susceptibility to mechanical injury, high respiration rate, water loss, chilling injury, microbial diseases, and insect pests are among the major factors causing postharvest losses in various tropical fruits (Vinod et al., 2023).

Because fluctuations in temperature, humidity, and oxygen concentration occur during storage and trans-

portation, the ripening process induces changes in fruit metabolism (transpiration and respiration), resulting in aging and decay (Bilal & Hashmi, 2023; Israfi et al., 2022). In addition to the losses resulting from physiological changes, the growth of microorganisms, mainly phytopathogenic fungi, becomes the main factor accounting for fruit postharvest losses. Postharvest decay caused by latent fungal infections sharply affects the fruit quality during transport to the market destination and storage (Ramírez-Gil et al., 2021). Fungal infection affects fruit ripening, reactive oxygen species (ROS) generation, and the production of genes and enzymes linked to disease resistance and cell wall degradation (Wu et al., 2023).

Furthermore, the microbial contamination of healthy tropical fruits is favored by specific environmental factors in tropical regions. Overall, the high temperature (around 27°C) and moisture (around 80%) highly affect the growth of phytopathogenic fungi in tropical fruits. Still, prolonged rain periods and high temperatures in tropical regions favor the progression of fungal disease in fruits (Nguyen et al., 2020; Sharma et al., 2021; Uclaray et al., 2022; Zakaria, 2023). Tropical fruits have intrinsic characteristics that provide a more favorable environment in which fungi can thrive, such as high moisture content (70%–95%) and high polysaccharide concentration (Israfi et al., 2022). The characteristics of the fruit maturation phase, such as the occurrence of disaggregated cell wall components, ethylene accumulation, and decrease of acidity, organic compounds, antifungal substances (e.g., phenolic compounds and phytoalexins), and plant-defense mechanisms, can accelerate fungal disease progress (Ciofini et al., 2022).

Anthracoze caused by *Colletotrichum* sp., stem-end rot caused by a fungal complex consisting of *Lasiodiplodia theobromae*, *Phomopsis mangifera*, *Fusarium solani*, and/or *Alternaria alternata*, black rot caused by *Aspergillus* sp., gray mold caused by *Botrytis cinerea*, and *Rhizopus* rot caused by *Rhizopus stolonifer* are among the most common postharvest diseases attacking tropical fruits. Some of these fungal infections occur at flowering and colonize endophytically with epidermis protection. Therefore, quiescent infections are very difficult to detect and control in the early life cycle stages (Ranjith et al., 2021; Vinod et al., 2023). For example, pathogenic *Colletotrichum* species commonly infect young fruits and remain undetected until the fruit becomes ripe, making disease control even more difficult.

Colletotrichum sp. infection in tropical fruits overall occurs before harvest; however, symptoms only appear when the fruit is exposed to favorable conditions for fungal growth (López-Zazueta et al., 2023). The infection can occur by direct cuticle penetration or entry through stomata, lenticels, wounds, or scar tissue

abscission (Gonçalves et al., 2021). Most *Colletotrichum* species initially establish the infection through a short biotrophic phase with large intracellular primary hyphae (Shi et al., 2021). Then, the pathogen switches to a necrotrophic phase, leading to dark and rounded spots on the fruit peel. The center of the lesion may contain a mass of acervulus and orange to pink conidia with a velvety appearance to better fix to the host, which can evolve to cause tissue necrosis (Gonçalves et al., 2021; Wu et al., 2023).

Anthraxnose is especially severe for climacteric fruits, such as banana, guava, avocado, mango, and papaya, because their ripening stage includes physiological and biochemical changes, providing adequate conditions for fungi growth. The sharp decline in tropical fruit quality requires adopting effective strategies to decrease these impacts, considering the important role of the tropical fruit agro-industry sector in developing countries.

3 | ALTERNATIVE SUSTAINABLE METHODS FOR CONTROLLING ANTHRACNOSE IN TROPICAL FRUITS

Refrigeration is the most traditional method to slow down ripening and postharvest fungal disease development in tropical fruits. However, despite its advantages, refrigeration has limited efficacy because the cold chain can be interrupted at retail locations and households. Furthermore, refrigeration is commonly used in combination with chemical fungicides, resulting in high costs, health risks, and environmental damage (Garcia et al., 2022).

Excessive and continuous use of synthetic fungicides has been a concern because of their resistance-inducing effects on phytopathogenic fungi (Chit-aree et al., 2021; Reyna et al., 2022; Uclaray et al., 2022; Wang et al., 2023). The Fungicide Resistance Action Committee (FRAC) reported that *Colletotrichum* species develop resistance against methyl benzimidazole carbamates, quinone external inhibitors, and demethylation inhibitor classes of fungicides (Cortaga et al., 2023). In addition, consumers are increasingly concerned about exposure to fungicide residues and their effects on public health, leading to the necessity of adopting strict phytosanitary control policies by importing countries to restrict the use of synthetic fungicides (Gonçalves et al., 2021).

In this context, research has focused on developing sustainable alternative strategies to reduce the harmful effects induced by anthracnose in tropical fruits (Figure 1). Table 1 summarizes the main results of retrieved studies evaluating the effects of sustainable alternative strategies on the postharvest control of pathogenic *Colletotrichum* species in tropical fruits.

3.1 | Alternative sustainable chemical strategies to control anthracnose in tropical fruits

EOs obtained from aromatic plants have been widely investigated due to their broad antimicrobial activity and reduced induction of resistance in phytopathogenic fungi (Bilal & Hashmi, 2023). EO and plant extracts have an overall negligible effect on the environment and present little risk to human health compared to chemical fungicides (Khalik et al., 2019). Because of these characteristics, EOs have also gained popularity and consumer acceptability (Khalik et al., 2019).

EOs are secondary metabolites produced by aromatic plants, and their antimicrobial properties are linked to the presence of several terpenes, aldehydes, and alcohols in their composition (Reyna et al., 2022). In addition, EOs are approved as “Generally Recognized as Safe” (GRAS) by the Food and Drug Administration (FDA, USA) and recognized as an alternative for chemical fungicides to protect foods due to their well-reported antimicrobial and antioxidative effects (Bilal & Hashmi, 2023; Danh et al., 2021; FDA, 2023; Landi et al., 2021; Reyna et al., 2022).

The available literature has shown that EOs have antifungal effects and can control anthracnose development and reduce deterioration, besides improving fruit quality without affecting its natural ripening (Bilal & Hashmi, 2023). EO showed efficacy in inhibiting *Colletotrichum* sp. with a reduction of anthracnose lesions like Prochloraz, a commercial chemical fungicide widely used to control the anthracnose in fruits in the postharvest phase (Chit-aree et al., 2021). However, these antifungal effects have varied with the tested EO, concentration, and target fungal species (Braga et al., 2019; Chit-aree et al., 2021; Lundgren et al., 2022; Mangoba & Alvindia, 2023; Shah et al., 2021). The antifungal effects of EO have been linked to their capability to disturb cell membrane structure and enzymatic/metabolic activities vital for fungal growth and fungal spore germination (Bilal & Hashmi, 2023; Lundgren et al., 2022; Reyna et al., 2022).

Aiming to improve the antimicrobial properties of EO, some combinations of EO with organic or inorganic salts, also recognized as GRAS, have been investigated. Organic or inorganic salts, such as carbonate, sorbate, benzoate, and silicate, demonstrate advantages for potential commercial use. These substances are readily available and economical and have excellent water solubility, where the use of potassium sorbate stands out because it offers several benefits, including improvements in the texture and appearance of the fruits, slowing down breathing, and increasing antioxidant activity (Bilal & Hashmi, 2023).

CH is another widely investigated natural chemical agent, which is a long-chain polymer of 2-acetamido

TABLE 1 Studies assessing the effects of sustainable alternative strategies to postharvest control of pathogenic *Colletotrichum* species in tropical fruits and main reported effects.

Chemical and physical methods Treatment and tested concentration	Target microorganism	Fruit	Main effects	Reference
<i>Cymbopogon winterianus</i> (CWE) (0.05%–2.5%)	<i>C. musae</i>	Banana	CWE at 1.5%–2.5% controlled the development of <i>C. musae</i> in vitro. CWE caused mycelial blast, cytoplasmic discharge, and spore edema. Minimum effective concentration (MEC) of CWE was 1.50% in the in situ assay (7 days at 22° C). No visible phytotoxicity or changes in aroma were observed on banana fruit treated with CWE, even at the highest concentration (2.5%). CWE had strong fungicidal effects against <i>C. musae</i>	Mangoba and Alvindia (2023)
Rosemary essential oil (RMO) (1%–2%) and potassium sorbate (PS) (2%)	<i>C. gloeosporioides</i>	Mango	RMO (1%) and potassium sorbate (2%) (PS) completely inhibited the growth of <i>C. gloeosporioides</i> in vitro. The combination of PS and rosemary oil (RMO) as a coating (RMO + PS) delayed ripening, regulated decay incidence, and preserved the sensory attributes of mango, as well as increased antioxidant activity and phenolic metabolism compared to control and synthetic fungicide (9 days at 20° C). The treatment upregulated the activity of defense-related enzymes, such as peroxidase and phenylalanine ammonia-lyase, besides being effective against <i>C. gloeosporioides</i> and reduced decay when applied as a coating on mango	Bilal and Hashmi (2023)
Kojic acid (KA) (0.28%–0.71%)	<i>C. brevisporium</i>	Papaya	KA decreased the lesion length in papaya after storage (7 days at 22° C). KA enhanced antioxidant activity in fruit, including increased enzymatic activity (superoxide dismutase—SOD and catalase—CAT)	Wang et al. (2023)

(Continues)

Table 1 - Studies assessing the effects of sustainable alternative strategies to postharvest control of pathogenic *Colletotrichum* species in tropical fruits and main reported effects.

TABLE 1 (Continued)

Chemical and physical methods Treatment and tested concentration	Target microorganism	Fruit	Main effects	Reference
Plasma-activated water (PAW) prepared with dielectric barrier discharge (DBD) plasma	<i>C. asianum</i>	Mango	Treatment showed inhibitory effects against pathogenic fungi enzymes (cell wall degrading enzymes and laccase) and downregulated pathogenic genes. The combination of plasma-activated water and modified atmosphere packaging effectively controlled the development of mango anthracnose, improved fruit quality, and extended storability (8 days at 25°C)	Wu et al. (2023)
High degree of polymerization agave fructans (HDPAF) (5%–20%)	<i>C. gloeosporioides</i>	Avocado	HDPAF, at different concentrations, effectively controlled anthracnose (reduction in disease severity and incidence) (9 days at 21°C). Polyphenoloxidase, peroxidase, and phenylalanine ammonia-lyase activity were increased after treatment, indicating the induction of defense mechanisms. Furthermore, the fruit quality parameters were positively affected	Cuellar-Torres et al. (2022)
Cold plasma (DBD) (1–9 min)	<i>C. asianum</i>	Mango	DBD plasma showed an antifungal effect, decreasing spore germination rate and viability. Subcellular structures were damaged, leading to spore death. Treatment controlled mango anthracnose (disease incidence and lesion diameter) and maintained mango quality (10 days at 25°C)	Wu et al. (2022)
Chitosan (CH) (low and high molecular weight) (0.5%–1.5%)	<i>C. gloeosporioides</i>	Avocado	Low molecular weight CH (1.5%) reduced the incidence (%) of anthracnose in inoculated and naturally infected fruits compared with Prochloraz fungicide, besides improving fruit defense mechanisms (5 days at 25°C)	Mokgalapa et al. (2022)

(Continues)

TABLE 1 (Continued)

Chemical and physical methods		Target microorganism	Fruit	Main effects	Reference
Treatment and tested concentration	Wild oregano phenolic extract (WOPE)-loaded yeast microcapsules (0.001%–0.1%)	<i>C. gloeosporioides</i>	Papaya	Applying postharvest treatment as a spray-type on papaya caused mycelial inhibition toward <i>C. gloeosporioides</i> and reduced anthracnose symptoms in fruits naturally infected and artificially inoculated with the pathogen (14 days at 27°C). Treatment delayed ripening extending the fruit storability	Uclaray et al. (2022)
	Poly(lactic acid)/polybutylene adipate-co-terephthalat, pine essential oil (10%–20%), and chitosan (1%)	<i>C. gloeosporioides</i>	Avocado	Polymeric nets showed fungicidal effects, mainly on <i>C. gloeosporioides</i> in vitro, and the spore germination phase was the most affected. The incidence of anthracnose was remarkably reduced (~80%); however, the ripening of avocado was not affected by using the tested polymeric net (9 days at 25°C)	Correa-Pacheco et al. (2022)
	Hinokitiol (0.5%–2%)	<i>C. musae</i>	Banana	Hinokitiol exhibited promising antifungal activities both in vitro and in situ (16 days at 25°C). The ability to destroy the cell membrane integrity could be the main action mode against <i>C. musae</i> . In addition, the treatment effectively delayed banana ripening by regulating the physicochemical index. The activities of defense-related enzymes, including superoxide dismutase, peroxidase, and ascorbate peroxidase, were increased by hinokitiol	Qiao et al. (2022)
Chitosan (CH) hydrochloride loaded (1.5%) with vanillic acid (0.07%) or caffeic acid (0.07%)		<i>C. gloeosporioides</i>	Avocado	Complexes of phenolic acids and CH reduced anthracnose decay (28 days at 6.5°C) and induced upregulation of fruit defense response genes; CH-vanillic acid coating showed a greater impact on fungi than CH-caffeic acid. Fourier-transform infrared spectroscopic analysis data showed the chemical interaction of phytochemicals with CH	Osundu et al. (2022)

(Continues)

TABLE 1 (Continued)

Chemical and physical methods Treatment and tested concentration	Target microorganism	Fruit	Main effects	Reference
Sodium alginate (2%) and tree essential oil nanoemulsion (TEON) (0.02%–0.1%) bilayer films incorporated with TiO ₂ nanoparticles (0.02%–0.1%)	<i>C. musae</i>	Banana	The addition of TEON improved the antifungal and antioxidant properties of the bilayer film. TiO ₂ improved the TEON retention and UV blocking properties, as well as the oxygen and water vapor barrier properties of the developed film. The treatment reduced oxidative damage, electrolyte leakage loss, water loss, delayed softening, and controlled anthracnose in banana (28 days at 25°C)	Yang et al. (2022)
CH (1%) and mint essential oil (0.2%)	<i>C. gloeosporioides</i>	Papaya	Treatments exhibited antifungal activity in vitro, inhibiting mycelial growth and conidial germination. Lower incidence and severity of anthracnose were obtained in fruits treated with CH (20 days at 12°C), which was also observed by microscopic analysis. The addition of MEO did not provide an additional effect. CH treatment maintained postharvest papaya quality	Reyna et al. (2022)
Ethanol extracts of <i>Dipteris punctata</i> (40%–50%)	<i>C. musae</i>	Banana	The extracts showed high antifungal activity against <i>C. musae</i> , highlighting the extracts from branches, residues, and the coumarin pattern. The preventive application of the extracts, at the highest concentrations, was more effective in reducing anthracnose lesions in banana (8 days at 25°C)	Sousa et al. (2022)
CH (1%) and thyme essential oil (TEO) (0.4%)	<i>C. gloeosporioides</i>	Mango	Thyme oil completely inhibited mycelial growth of <i>C. gloeosporioides</i> in vitro. However, the combined application of CH + TEO was more effective than thyme oil alone in controlling the disease in artificially inoculated mango (9 days at 25°C). The retention of physicochemical attributes and high acceptability scores confirmed the efficacy of CH + TEO in extending the shelf life of mango	Shah et al. (2021)

(Continues)

TABLE 1 (Continued)

Chemical and physical methods Treatment and tested concentration	Target microorganism	Fruit	Main effects	Reference
Cinnamon, basil, and lemongrass EO (0.4%)	<i>C. acutatum</i>	Mango	Cinnamon, basil, and lemongrass EO effectively inhibited the growth of <i>C. acutatum</i> . However, these EOs (except basil EO) severely damaged fruit peels. The antifungal activity was closely related to the main EO compounds. Basil EO effectively controlled anthracnose development in mango artificially infected with <i>C. acutatum</i> (7 days at 25°C)	Danh et al. (2021)
Unripe kaffir essential oil (UKLO) (0.15%) and ripe kaffir essential oil (RKLO) (0.15%)	<i>C. gloeosporioides</i>	Mango	UKLO (0.15%–5%) showed the highest inhibition against <i>C. gloeosporioides</i> mycelial growth in vitro. UKLO and RKLO (0.15%) suppressed the disease development of <i>C. gloeosporioides</i> in vivo (9 days at 25°C). Mango coated with UKLO (0.15%) had a reduced disease severity index. Physicochemical indicators and consumer acceptability of coated fruits did not differ compared to control groups during storage	Chit-aree et al. (2021)
<i>Conyza bonariensis</i> (L.) Cronquist essential oil (CBEO) (0.4%–1%) and gum arabic (GA) (0.1%)	<i>C. musae</i>	Banana	CBEO had inhibitory effects on various <i>C. musae</i> isolates in vitro, whereas GA was not capable of inhibiting them. The application of coatings with GA combined with different concentrations of CBEO was effective in reducing the anthracnose lesion development in banana contaminated with <i>C. musae</i> during storage (5 days at 25°C). Additionally, the coatings delayed the alterations in physicochemical parameters indicative of postharvest quality and more prolonged storability of banana	Lundgren et al. (2021)

(Continues)

TABLE 1 (Continued)

Chemical and physical methods Treatment and tested concentration	Target microorganism	Fruit	Main effects	Reference
Chitosan (0.5%) and/or <i>Ruta graveolens</i> L. essential oil (REO) (1%)	<i>C. gloeosporioides</i>	Papaya	Anthraxnose incidence and disease severity were reduced with CH, REO, and CH-REO emulsions (9 days at 25°C). Incorporation of REO into the CHI-coating improved the control of postharvest decay of papaya compared to the use of these treatments individually. It suggested that CH stabilized the volatile and/or hydrophobic substances of highly reactive EO. The additive effects of CH and REO reduced postharvest decay and affected gene expression linked to plant-defense mechanisms in papaya	Landi et al. (2021)
Chitosan (0.5%) and <i>Ruta graveolens</i> L. essential oil (REO) (1%)	<i>C. gloeosporioides</i>	Papaya	CH emulsions added with REO inhibited <i>C. gloeosporioides</i> growth in vitro and in situ on papaya. CHI-REO emulsions affected physicochemical parameters in papaya, delaying ripening without affecting the sensory characteristics	Peralta-Ruiz et al. (2020)
Chitosan (0.5%–2%) and hot water (HW)	<i>C. fructicola</i>	Papaya	The combined treatment of hot water dip and CH coating (2%) (HW–CH20) not only improved the efficacy obtained with hot water dips alone on papaya (28 days at 10°C) but also that of treatment with synthetic fungicide. This combined treatment reduced maturity signs throughout storage, increased the shelf-life period, and did not negatively affect the sensory characteristics in contrast to chemical treatments and hot water dips applied individually	Vilaplana et al. (2020)

(Continues)

TABLE 1 (Continued)

Chemical and physical methods		Target microorganism	Fruit	Main effects	Reference
Treatment and tested concentration					
Gelatinized tapioca starch (5%) and synthesized copper nanoparticles (CuNPs) (0.02%–0.0245%)		<i>C. gloeosporioides</i>	Mango	CuNPs showed dose-dependent antifungal activity against <i>C. gloeosporioides</i> with 100% growth inhibition at 0.02% copper. <i>C. gloeosporioides</i> did not grow on the surface of nutrient agar in contact with the films containing 0.0245% CuNPs, whereas the fungus grew normally on films without CuNPs. For the antifungal tests on mango, both the control (polymer) and the CuNPs-containing coatings equally inhibited fungal growth, possibly due to the low oxygen permeability of the protein and starch components in the films (4 days at 25°C)	Nguyen et al. (2020)
<i>Allium longicuspis</i> extracts (ALE) (0.75%–2.5%)		<i>C. gloeosporioides</i>	Mango	ALE (0.75%–2.5%) completely inhibited the growth of <i>C. gloeosporioides</i> in vitro (mycelial growth and conidial germination). ALE in a minimum effective concentration (MEC) of 0.75% can be applied as a protective, curative, and simultaneous treatment in mango to inhibit the anthracnose infection. Based on protective, curative, and simultaneous treatments (7 days at 25°C), the efficacy of ALE was relatively superior to that of synthetic fungicides	Alvindia and Mangoba (2020)
Chitosan (2%) and <i>Ruta graveolens</i> L essential oil (REO) (0.5%–1.5%)		<i>C. gloeosporioides</i>	Guava	The coatings reduced the growth of <i>C. gloeosporioides</i> in guava, increasing their stability (12 days at 25°C). CH and REO at 1.5% showed excellent stability concerning the quality indicator attributes and microbiological decay compared to the uncoated guava and showed a high percentage of inhibition in the development of anthracnose lesions	Tovar et al. (2019)

(Continues)

TABLE 1 (Continued)

Chemical and physical methods Treatment and tested concentration	Target microorganism	Fruit	Main effects	Reference
Chitosan (0.25%–1%), <i>Mentha piperita</i> L. (MPEO) (0.03%–0.125%), and <i>M. x villosa</i> Huds essential oil (MVEO) (0.03%–0.125%)	<i>C. gloeosporioides</i> and <i>C. brevisporum</i>	Papaya	CH (0.25%–1%), MPEO, and MVEO (0.15–1.25 µL/mL) alone effectively inhibited the growth of <i>C. gloeosporioides</i> and <i>C. brevisporum</i> isolates in vitro. Combinations of CH (5 and 7.5 mg/mL) and MPEO or MVEO (0.15–1.25 mL/mL) inhibited the growth of <i>Colletotrichum</i> isolates and mostly presented additive or synergistic interactions. Development of anthracnose lesions was reduced by coatings formed by CH (0.5%) and MPEO or MVEO (0.03%–0.125%) combinations during storage (10 days, 25 ± 0.5°C). Efficacy of coating with CH (0.5%) and MPEO or MVEO (0.06% and 0.125%) was similar or higher than that caused by a commercial fungicide formulation	Braga et al. (2019)
<i>Aloe vera</i> (AV) gel and/garlic essential oil (GO) (0.05%–0.1%)	<i>C. musae</i>	Banana	The highest antifungal activity was observed in the AV + GO 0.1% treatment, which significantly inhibited mycelial growth and spore germination by 87.7% and 91.2%, respectively, compared to the control. An in vivo study indicated that AV gel combined with GO 0.1% effectively reduced anthracnose disease incidence (92.5%) and severity (81.0%) in banana (15 days at 20°C). AV gel coating enriched with GO improved the postharvest quality attributes of banana	Khaliq et al. (2019)

(Continues)

TABLE 1 (Continued)

Chemical and physical methods			
Treatment and tested concentration	Target microorganism	Fruit	Reference
Chitosan/vanillin and ethylene adsorbents	<i>Colletotrichum</i> spp.	Mango	Jaimun and Sangsuwan (2019)
Main effects			
Coating layers composed of CH, vanillin, and zeolite on wrapping paper effectively inhibited anthracnose during storage (10 days at 25°C). The treatment removed ethylene, which delayed the ripening process of mango with reduction of changes in physicochemical quality parameters and the highest sensory acceptance score (30 days at 13°C)			
<i>Aloe vera</i> extract; fresh <i>aloe vera</i> ; and food grade (15%–50%)	<i>C. gloeosporioides</i>	Papaya	Mendy et al. (2019)
Main effects			
Filtered fresh <i>Aloe vera</i> caused higher inhibition of mycelial growth of fungi compared to food grade <i>Aloe vera</i> amended with PDA media in a dose-response in vitro study. Both treatments prevented wound infection on papaya inoculated with <i>C. gloeosporioides</i> (3 days at 28°C)			
Biological methods			
Treatment and tested concentration	Target microorganism	Fruit	Reference
<i>Streptomyces</i> sp. HSL-9B (1–8 × EC 50)	<i>C. gloeosporioides</i>	Mango	Zhou et al. (2022)
Main effects			
Strain HSL-9B extracts inhibited the mycelial growth and spore germination of <i>C. gloeosporioides</i> , decreasing the decay of mango during storage (10 days at 28°C). Based on the sequencing genome of strain HSL-9B, biosynthesis gene clusters of secondary metabolites involved in antagonism were identified. Chromatography-mass spectrometry detected 15 compounds in strain HSL-9B extracts			

(Continues)

TABLE 1 (Continued)

Biological methods Treatment and tested concentration	Target microorganism	Fruit	Main effects	Reference
<i>Streptomyces</i> sp. AGS-58 (0.1%–0.5%)	<i>C. stamense</i>	Mango	<i>Streptomyces</i> sp. AGS-58 exhibited the highest inhibitory activity against <i>C. stamense</i> CT1. According to morphological evidence, extracellular exudates of AGS-58 colonies inhibited conidial germ tube formation and hyphal extension. AGS-58 droplets decreased anthracnose to a similar extent as the commercial fungicide benomyl. Spraying mango with the extracellular exudate affected the germination of <i>C. stamense</i> conidia and prevented anthracnose progression (10 days at 28° C)	Evangelista-Martínez et al. (2022)
Yeast <i>Meyerozyma caribbica</i> by electrospraying process (7 log CFU/mL)	<i>C. gloeosporioides</i>	Mango and papaya	The encapsulation using electrospraying of the biocontrol agent <i>Meyerozyma caribbica</i> was a feasible process, and the encapsulated yeast exhibited high initial viable cell counts, which remained stable during storage at 25 and 4° C. After 180 days of storage, the capsules inhibited the growth of <i>C. gloeosporioides</i> in vitro and in situ (15 days at 25 and 13° C) like yeast fresh cells	Aguirre-Gutiérrez et al. (2021)
<i>Trichoderma harzianum</i> , <i>Bacillus subtilis</i> and <i>Pseudomonas anomala</i> (8.1 log CFU/mL)	<i>C. gloeosporioides</i>	Mango	Dip treatment of mango in a spore suspension of <i>T. harzianum</i> for 5 min was the effective to provided anthracnose control (81.67%) during storage (28 days at 25° C). In addition, treatment with <i>T. harzianum</i> + <i>Pichia anomala</i> was effective with 93.39% anthracnose disease control. Under field conditions, three consecutive sprays of <i>T. harzianum</i> , starting with the initiation of disease on leaves, followed by other two sprays at an interval of 15 days, were the most effective for the management of mango anthracnose both on leaves and fruits	Sharma et al. (2021)

(Continues)

TABLE 1 (Continued)

Biological methods		Target microorganism	Fruit	Main effects	Reference
Treatment and tested concentration					
<i>Bacillus mycoides</i> A1 and A2 and <i>Bacillus tequilensis</i> A3 (7 log CFU/mL)		<i>C. gloeosporioides</i>	Avocado	Three bacterial isolates (A1–A3) reduced the mycelial growth of the <i>C. gloeosporioides</i> by 75%, 70%, and 60%, respectively. Two of these isolates were identified by 16S rRNA as <i>B. mycoides</i> (A1 and A2) and the third was identified as <i>B. tequilensis</i> (A3). <i>B. mycoides</i> cell-free supernatant reduced the mycelial growth of a member of the <i>C. gloeosporioides</i> species complex isolated from avocado by 65%, whereas <i>B. tequilensis</i> A3 supernatant did so by 25% (7 days at 28° C). Preventive treatment using a cell-free supernatant of <i>B. mycoides</i> A1 diminished the severity of anthracnose disease (41.9%) on avocado fruit	Guerrero-Barajas et al. (2020)
<i>Torulaspora indica</i> and <i>Pseudozyma hubeiensis</i> (8 log CFU/mL)		<i>C. gloeosporioides</i>	Mango	Only <i>Papiliotrema asperensis</i> DMKU-SP67 inhibited the growth of <i>C. gloeosporioides</i> and reduced anthracnose severity by 94.1% (7 days at 25° C), which was comparable to that of using fungicide benomyl (93.9%). The antifungal volatile organic compounds produced by yeast strain reduced the disease severity on mango but at lower rates than using yeast cells	Konsue et al. (2020)
Sodium alginate (SA) (1.5%)/lactic acid bacteria (LAB) (9–10 log CFU/mL)		<i>Colletotrichum</i> spp.	Guava and mango	Eight LAB strains caused strong inhibition of the mycelial growth of distinct pathogenic <i>Colletotrichum</i> species in vitro. Application of SA coatings loaded with <i>Levilactobacillus brevis</i> 59, <i>Lactiplantibacillus pentosus</i> 129, and <i>Limosilactobacillus fermentum</i> 263 (strains that showed better inhibitory effects) controlled the development and reduced the severity of anthracnose in guava and mango (15 days at 25° C). These coatings positively impacted some physicochemical parameters indicative of postharvest quality and more prolonged storability of guava and mango	Fernandes et al. (2021)

(Continues)

TABLE 1 (Continued)

Biological methods		Target microorganism	Fruit	Main effects	Reference
Treatment and tested concentration					
<i>Meyeromyces caribbica</i> (9 log CFU/mL) and sodium alginate (SA) (1.5%)		<i>C. gloeosporioides</i>	Avocado	Films with <i>M. caribbica</i> inhibited <i>C. gloeosporioides</i> growth in vitro. Application of SA coatings contributed to maintaining <i>M. caribbica</i> viability and improved their efficacy in controlling anthracnose in avocado. Furthermore, in vivo, preventive treatments were more effective than curative treatments in disease control; for 12 days at 25°C, the severity was halved, whereas for 10 days at 6°C, the reduction reached 100%. Additionally, weight loss was reduced in avocado treated with bioactive coatings	Iniguez-Moreno et al. (2020)
Yeast <i>Meyeromyces caribbica</i> (7 log CFU/mL)		<i>C. gloeosporioides</i>	Mango	<i>M. caribbica</i> powder formulation stored for 6 months at 4°C was effective in the postharvest control of <i>C. gloeosporioides</i> in mango (15 days at 25°C). The formulation reduced the incidence and severity of the disease in mango to 53.4% and 23.9%, respectively. The yeast application (fresh and powder formulation) did not affect mango quality parameters during storage	Aguirre-Gutiérrez et al. (2019)
<i>Stenotrophomonas rhizophila</i> (8 log CFU/mL)		<i>C. gloeosporioides</i>	Mango	The marine bacterium <i>Stenotrophomonas rhizophila</i> was effective in inhibiting <i>C. gloeosporioides</i> in vitro. The results showed that inhibition of the colony diameter and spore germination of the phytopathogen was due to the production of hydrolytic enzymes, volatile organic compounds, and siderophores competing for space and nutrients. When the antagonist bacterium was applied to the fruit, disease incidence was reduced by 95%, and the lesion diameter of anthracnose decreased by 85%; this treatment was more efficient against anthracnose than the application of synthetic fungicides (7 days at 28°C)	Reyes-Perez (2019)

(Continues)

TABLE 1 (Continued)

Biological methods Treatment and tested concentration	Target microorganism	Fruit	Main effects	Reference
<i>Metschnikowia pulcherrima</i> (8 log CFU/mL), salicylic acid (SLA) (0.05%), and calcium chloride (CaCl ₂) (0.1%)	<i>C. gloeosporioides</i>	Mango	<i>M. pulcherrima</i> showed an additive effect with SLA and CaCl ₂ , increasing the efficacy in controlling anthracnose disease (lesion expansion) (24 days at 25°C). These treatments delayed changes in quality characteristics and enhanced the activities of defensive enzymes in mango. Furthermore, the combination of SA, CaCl ₂ , and <i>M. pulcherrima</i> yeast was more effective than each treatment alone	Shao et al. (2019)

Abbreviations: ALE, *Allium longicaule* extracts; CaCl₂, calcium chloride; CBEO, *Coryza bonariensis* (L.) Cronquist essential oil; CuNPs, copper nanoparticles; DBD, dielectric barrier discharge plasma; EO, essential oil; Go, garlic essential oil; HDPAF, high degree of polymerization agave fructans; HW, hot water; MEC, minimum Effective Concentration; MEO, mint essential oil; MPEO, *Mentha piperita* L.; MVEO, *M. x villosa* Huds essential oil; PAN; plasma-activated water; REO, *Ruta graveolens* L. essential oil; RMO, Rosemary oil; SLA, salicylic acid; TEON, tree essential oil nanoemulsion; TSS, total soluble solids; UKLO, unripe kaffir essential oil; WOPE, wild oregano phenolic extract.

-D-glucose and 2-amino-D-glucose units crosslinked by β -1,4 glycosidic bonds, obtained from the exoskeleton of crustaceans, insects, and fungi (Shah et al., 2021). CH is one of the most used polymers in coating formulations due to its antimicrobial, film-forming, and barrier properties, which help to prevent fresh fruit postharvest decay. CH is considered a natural biocompatible polysaccharide and an effective eco-friendly alternative to synthetic fungicides (Landi et al., 2021; Mokgalapa et al., 2022; Reyna et al., 2022).

The antifungal activity of CH has been linked to its ability to damage the fungal membrane due to the interaction of CH amino groups with fungal membrane phospholipids, resulting in increased plasma membrane permeability (Osondu et al., 2022; Reyna et al., 2022). In addition, it affects the absorption of minerals and nutrients by the fungal cell and delays fungal spore germination (Landi et al., 2021; Reyna et al., 2022). Still, CH causes morphological alterations in the fungal mycelium and delays its growth by affecting several stages of mycelium development (Reyna et al., 2022). In this context, the application of CH would have a direct action to control phytopathogenic fungi, highlighting its role as a chemical strategy, going beyond the function of creating a physical barrier that alters only the fruit maturation process (Mokgalapa et al., 2022).

The temperature and exposure time used to cause the CH dispersion needed to prepare coatings and films can affect the CH intrinsic properties and the antifungal effects. Furthermore, the deacetylation degree, concentration, and molecular weight of CH affect its antifungal efficacy, as well as the development of ionic and/or covalent interactions with the coated food matrix (Mokgalapa et al., 2022; Shah et al., 2021; Vilaplana et al., 2020). Low molecular weight CH coatings reduced the incidence of anthracnose in avocado artificially inoculated with *C. gloeosporioides* and naturally infected with *Colletotrichum* spp. Furthermore, the percentage of anthracnose incidence in fruits treated with CH was lower compared to those caused by Prochloraz. The delay in anthracnose development was linked to the ability of CH to penetrate the *C. gloeosporioides* cell wall, causing genetic material perforation, affecting cellular homeostasis, and leading to fungal cell death (Mokgalapa et al., 2022).

In addition to EO and CH, other natural substances have been investigated as alternatives for anthracnose control in tropical fruits. Extracts from *Cymbopogon winterianus* caused rapid inhibition of *C. musae* in banana, with effects associated with the synergistic action of their components (e.g., γ -sitosterol and α -terpineol). The results also showed that *C. winterianus* extracts were more effective than synthetic fungicide (Mancozeb) in suppressing anthracnose after 7 days (Mangoba & Alvindia, 2023).

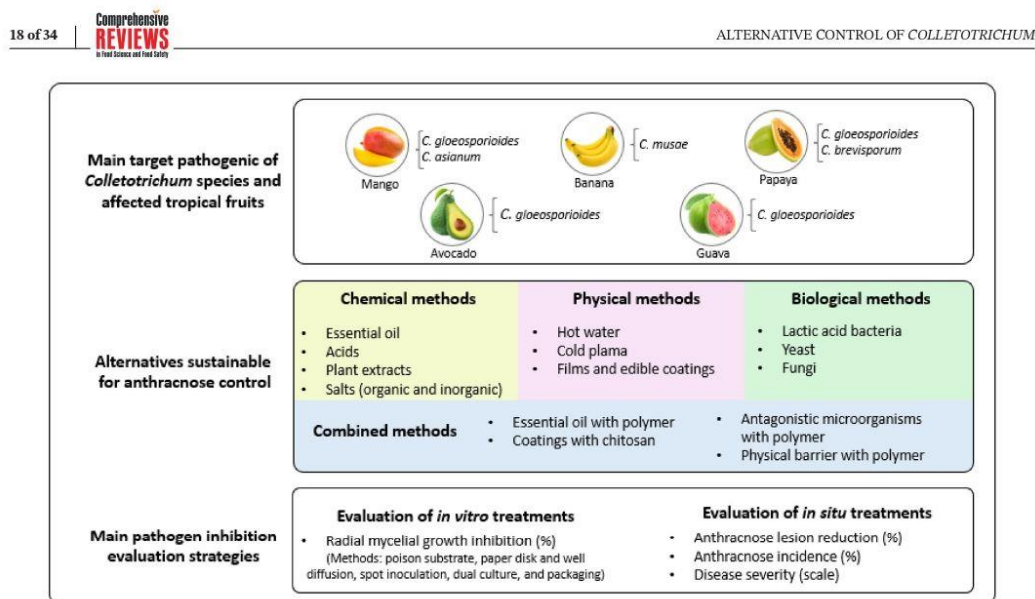


FIGURE 1 Sustainable strategies for controlling pathogenic *Colletotrichum* species in tropical fruits.

Hinokitiol, extracted by steam distillation from the woody tissues of *Chamaecyparis taiwanensis*, was effective in controlling anthracnose caused by *C. musae* in banana (Quiao et al., 2022), affecting the normal growth of the mycelium, and destroying the integrity of the cell membrane, leading to the leakage of proteins, nucleic acids, and soluble carbohydrates from fungal mycelium (Quiao et al., 2022).

Ethanol extracts from different parts of *Dipteryx punctata* were effective in controlling anthracnose caused by *C. musae* in banana. The antifungal effects were associated with the interaction between the *D. punctata* extract constituents and the compounds naturally occurring in banana, as well as with increased plant resistance to pathogenic fungi due to the induction of more efficient structural defense mechanisms and production of secondary metabolites, such as precursors of phytoalexins and chitinase compounds, account for fungal cell membrane rupture (Sousa et al., 2022).

Due to the complexity in composition and possible multi-target action mechanisms, the application of plant extracts (and EO) has demonstrated a low possibility of inducing resistance in phytopathogenic fungi, which is an advantage compared to the action of synthetic fungicides formulated mainly with a single-active principle (Shah et al., 2021). This could also reduce the concentrations of plant extracts (and EO) used to inhibit anthracnose infection development. *Allium longicuspis* extracts exhibited similar, or even higher toxic effects on *C. gloeosporioides* even at a low concentration compared with a synthetic fungicide (Mancozeb) (Alvindia & Mangoba, 2020).

Applying kojic acid (KA) in papaya effectively induced resistance against *C. brevisporum*, increasing the activity of antioxidant enzymes. KA is a secondary metabolite produced by various microorganisms, especially *Aspergillus* spp., and is considered safe for humans. KA has several applications for food quality management as a natural antioxidant and food storage extension (Wang et al., 2023).

3.2 | Alternative sustainable physical methods to control anthracnose in tropical fruits

Physical methods involving thermal and nonthermal technologies have been investigated as alternative strategies to control anthracnose in fruits. Postharvest heat treatments, such as hot water immersion, have been applied to tropical fruits to reduce the load of target pathogens on the fruit surface or in the cell layer close to the fruit skin. Immersion in hot water is a treatment commonly applied to control anthracnose as it has low operational costs, is waste-free, and causes low environmental impact. The efficacy of hot water immersion to control *Colletotrichum* spp. in tropical fruits has been linked to the growth inhibition of the germinal tube of pathogenic fungi infecting fruit surface (Vilaplana et al., 2020).

Figure 0-1 - Sustainable strategies for controlling pathogenic *Colletotrichum* species in tropical fruits.

Cold plasma is a nonchemical processing technology generated by dielectric barrier, microwave, and radio frequency discharges, with the advantages of simple operation, low-temperature rise, and wide applications. The interaction between microorganisms and cold plasma is intricate, and cold plasma sterilization is mainly attributed to biological oxidation and physical damage in target microorganisms. Compared with other nonthermal microbial decontamination technologies, cold plasma has the advantages of being a short-time treatment, having no residue, being simple equipment, and having fewer adverse effects on food characteristics and the environment. Cold plasma is used to extend the shelf life of tropical fruits, and studies have exploited the inactivation effects of cold plasma on *Colletotrichum* spp. to prevent and control anthracnose in mango (Wu et al., 2022, 2023).

Biopolymeric coatings and edible films have been investigated to extend the storability of tropical fruits. Biopolymeric coatings and films are formulated with different biological ingredients, such as proteins, lipids, and mainly polysaccharides, capable of creating a physical barrier to keep internal carbon dioxide in and external oxygen out of coated fruit, besides reducing moisture loss to the environment (Garcia et al., 2022). Gum arabic (GA) is a natural polysaccharide extracted from *Acacia* species and used in foods as an emulsifier, flavor intensifier, and film-forming agent. However, GA alone is commonly ineffective in inhibiting postharvest fungal pathogens, directing the need to combine it with other effective antimicrobial substances (e.g., EO), to inhibit pathogenic *Colletotrichum* species and anthracnose development in tropical fruits. The application of coatings with GA combined with different concentrations of *Conyza bonariensis* EO was effective in reducing the anthracnose lesion development in banana contaminated with *C. musae*. Overall, the disease severity indexes in coated banana did not differ from those in banana treated with a mixture of commercial fungicides (trifloxystrobin + tebuconazole) (Lundgren et al., 2022).

Gelatin-tapioca starch films and coatings containing copper nanoparticles showed strong antifungal effects against *C. gloeosporioides*. Tapioca starch creates a physical barrier preventing fungal entry and reducing moisture, whereas copper nanoparticles exert antifungal effects disturbing fungal cell wall integrity. The antifungal effects of copper nanoparticles have been linked to their small size and high surface-to-volume ratio, improving the interactions with a wide spectrum of target fungi (Nguyen et al., 2020). The application of a high polymerization degree agave fructans controlled anthracnose development in avocado in a dose-dependent manner, and these effects were attributed to the activation of plant defense-related enzymes, such as polyphenol oxidase, peroxidase,

and phenylalanine ammonia-lyase, enhancing the fruit resistance to pathogenic fungi (Cuéllar-Torres et al., 2022).

Sodium alginate (SA) is a polysaccharide abundant in nature as a structural component in marine brown algae (*Phaeophyceae*, mainly *Laminaria*). SA is considered GRAS and used as a texturizer, emulsifier, stabilizer, thickener, and gelling agent in foods. Additionally, despite not showing antifungal activity, SA can trap and carry volatile compounds, such as EO (Yang et al., 2022), and antagonistic microorganisms, including LAB (Fernandes et al., 2021) and yeasts (Iñiguez-Moreno et al., 2020), to promote antifungal effects against pathogenic *Colletotrichum* species.

Aloe vera (AV) gel is a biodegradable natural preservative used to reduce microbial growth and maintain papaya quality during storage (Mendy et al., 2019). AV gel has favorable features for food application, such as nontoxicity, high stability, hydrophilicity, and gel-forming properties. The antifungal activity of AV gel coating enriched with garlic EO was attributed to the presence of bioactive compounds, such as aloin or barbaloin, that inhibited the mycelial growth and spore germination of *C. musae* (Khaliq et al., 2019).

Active-coated papers have been studied as a potential packaging material for commercial application in wrapping mango to control anthracnose and delay fruit ripening. Coating layers composed of CH, vanillin, and zeolite on wrapping paper inhibited anthracnose development in mango during storage, which was linked to direct contact of fungi with antimicrobial compounds on wrapping paper (Jaimun & Sangsuwan, 2019).

3.3 | Alternative sustainable biological methods to control anthracnose in tropical fruits

The application of antagonistic microorganisms for the biological control of anthracnose in tropical fruits is an environment-friendly strategy to replace chemical fungicide use. Antagonistic microorganisms, especially bacteria and yeasts, indirectly regulate and decrease the adverse effects of different biotic stress factors by enhancing systemic plant resistance due to the production of bacteriocins, metabolites, peptides, iron-chelating siderophores, and lytic enzymes (Aguirre-Güitrón et al., 2021; Sharma et al., 2021).

Among potential antagonistic microorganisms considered for use in tropical fruits, yeasts deserve particular attention because most yeasts are not pathogenic, have a simple nutritional requirement, and do not produce allergenic spores, mycotoxins, and secondary metabolites. Furthermore, yeasts grow rapidly and colonize a broad

range of substrates for a prolonged period under various conditions (Konsue et al., 2020).

The inhibition of anthracnose in tropical fruits with antagonistic yeasts, such as *Meyerozyma caribbica*, *Metschnikowia pulcherrima*, and *Pseudozyma hubeiensis*, may be achieved through a number of mechanisms, including competition between the antagonistic yeast and the target pathogen for nutrients and space in the fruit, biofilm production, and adhesion to the fruit as well as to the pathogen to stunt further growth or production of hydrolytic enzymes (e.g., chitinase, phenylalanine ammonia-lyase, and β -1,3-glucanase) and volatile organic compounds (Aguirre-Güitrón et al., 2019, 2021; Iñiguez-Moreno et al., 2020; Konsue et al., 2020; Shao et al., 2019).

The action mechanism of volatile organic compounds produced by yeasts, such as alcohols and esters, involves damage to the plasma membrane and rapid protein denaturation, causing impairments in cell metabolism and respiration and leading to cell lysis. Volatile organic compounds could have some advantages as antimicrobials because the contact between the biocontrol agent and the pathogen is not required to perform the antifungal activity. Furthermore, the biocontrol efficacy of yeasts producing volatile organic compounds may result from a synergistic effect between the volatile organic compounds and carbon dioxide in the environment (Iñiguez-Moreno et al., 2020).

The yeast *M. caribbica* showed high inhibition against *C. gloeosporioides* in vitro and caused a reduction in anthracnose lesions in avocado, mango, and papaya (Aguirre-Güitrón et al., 2019, 2021; Iñiguez-Moreno et al., 2020). Yeast *P. hubeiensis* has a high potential for controlling anthracnose caused by *C. gloeosporioides* in mango. The antifungal effects of antagonistic yeasts have been associated with the competition for space and nutrients, the production of volatile organic compounds and hydrolytic enzymes, parasitism, and biofilm formation (Aguirre-Güitrón et al., 2019, 2021; Iñiguez-Moreno et al., 2020; Konsue et al., 2020).

In addition, fungi, such as *Trichoderma harzianum* and *Streptomyces* sp., are considered potential agents to control anthracnose development in tropical fruits because of their high efficiency in producing functional metabolites, as well as competition for nutrients, mycoparasitism, and antibiosis (Evangelista-Martinez et al., 2022; Sharma et al., 2021; Zhou et al., 2022). *Streptomyces* sp. HSL-9B extracts inhibited the mycelial growth and spore germination of *C. gloeosporioides*, decreasing the mango decay during storage (Zhou et al., 2022).

The marine bacterium *Stenotrophomonas rhizophila* inhibited *C. gloeosporioides* growth in vitro and on mango through the production of volatile organic compounds and extracellular enzymes, namely, β -1,3-glucanase and chitinase, linked to fungal cell wall hydrolysis (Reyes-Perez

et al., 2019). The inhibitory effects of *Bacillus mycoides* and *Bacillus tequilensis* against *C. gloeosporioides* were related to the production of enzymes and metabolites involved in fungal growth inhibition (Guerrero-Barajas et al., 2020). The inhibitory effects of LAB against pathogenic *Colletotrichum* species have been associated with the reduction in environmental pH caused by the production of organic acids and different antimicrobial peptides and bacteriocins. The competition for nutrients between antagonistic LAB and target microorganisms coexisting in the same environment could be another factor in controlling fruit pathogenic fungi (Fernandes et al., 2021).

Additionally, different methods have been combined to improve their efficacy in controlling anthracnose in tropical fruits. Coatings with CH loaded with vanillic acid or caffeic acid controlled anthracnose development in avocado artificially infected with *C. gloeosporioides*. The inhibitory effects of these coatings were linked to the activation of defense mechanisms in the fruit and enhanced interaction of phenolic compounds with target fungi, causing cellular damage (Osondu et al., 2022). Combining CH and thyme EO resulted in a synergistic effect on reducing anthracnose severity in mango, which was linked to increased persistence of antifungal compounds on the fruit surface due to reduced volatility. The CH and thyme EO combination was more effective than a synthetic fungicide (Prochloraz) (Shah et al., 2021).

The combination of different concentrations of CH and *Mentha piperita* L. or *Mentha* $\times$ *villosa* Huds EO exerted additive or synergistic interactions against *C. gloeosporioides* and *C. brevisporum* and caused partial or total inhibition of anthracnose lesion development in papaya during storage. These results indicate the possibility of reducing the active doses of the antifungal compounds when applied in combination to inhibit *C. gloeosporioides* and *C. brevisporum* (Braga et al., 2019).

The combination of plasma-activated water and modified atmosphere packaging controlled mango anthracnose, improved fruit quality, and prolonged storability (Wu et al., 2023). The combined application of *M. pulcherrima* with salicylic acid and calcium chloride showed an additive interaction, increasing the efficacy in controlling anthracnose in mango, with better results than when applied alone (Shao et al., 2019).

4 | QUANTITATIVE ANALYSES OF SUSTAINABLE STRATEGIES TO CONTROL ANTHRACNOSE IN TROPICAL FRUITS

Studies from available literature have assessed the efficacy of several alternative sustainable strategies, including physical barriers, natural substances, and antagonistic

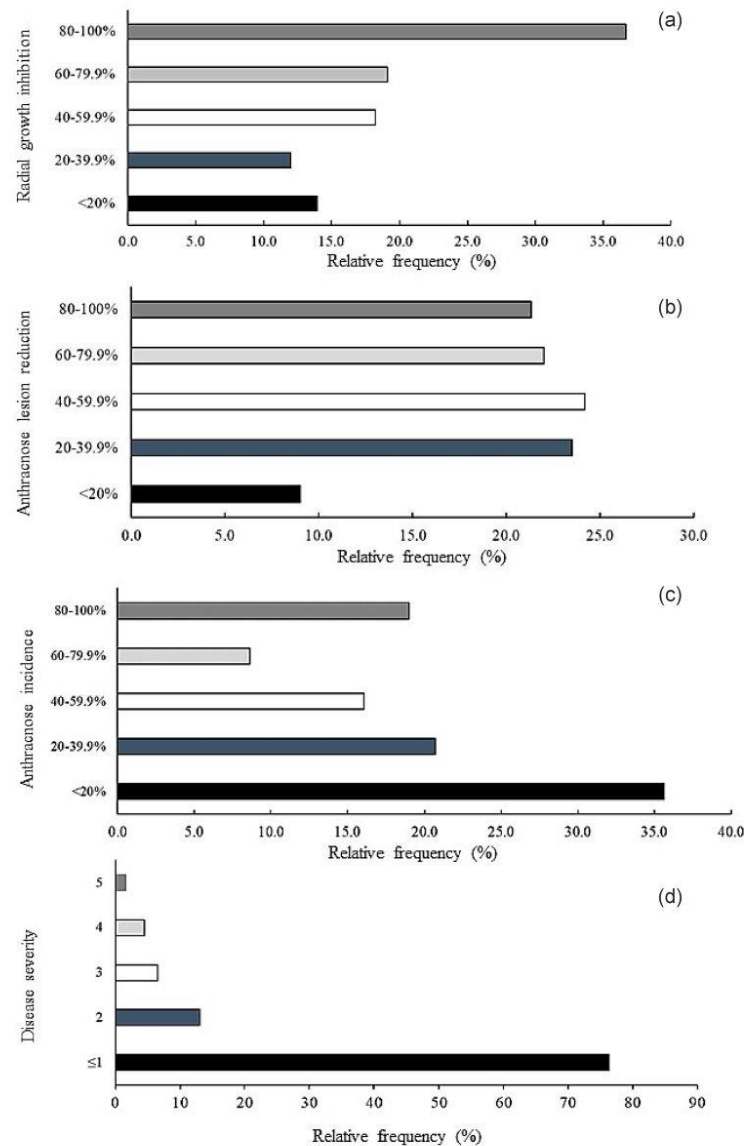


FIGURE 2 Relative frequency distributions of radial growth inhibition (a), anthracnose lesion reduction (b), anthracnose incidence (c), and disease severity (d) caused by different sustainable strategies to control pathogenic *Colletotrichum* species.

microorganisms, to reduce anthracnose lesion severity or incidence and maintain postharvest quality parameters in different tropical fruits (Table 1). To better understand this information from the robust perspective of published literature, a quantitative analysis of data from available studies

was performed, and detailed results are shown in Figures 2 and 3.

The variables collected from experiments to assess the efficacy of tested strategies to control anthracnose in vitro were the radial mycelial growth inhibition (%), whereas

Figure 2 - Relative frequency distributions of radial growth inhibition (a), anthracnose lesion reduction (b), anthracnose incidence (c), and disease severity (d) caused by different sustainable strategies to control pathogenic *Colletotrichum* species.

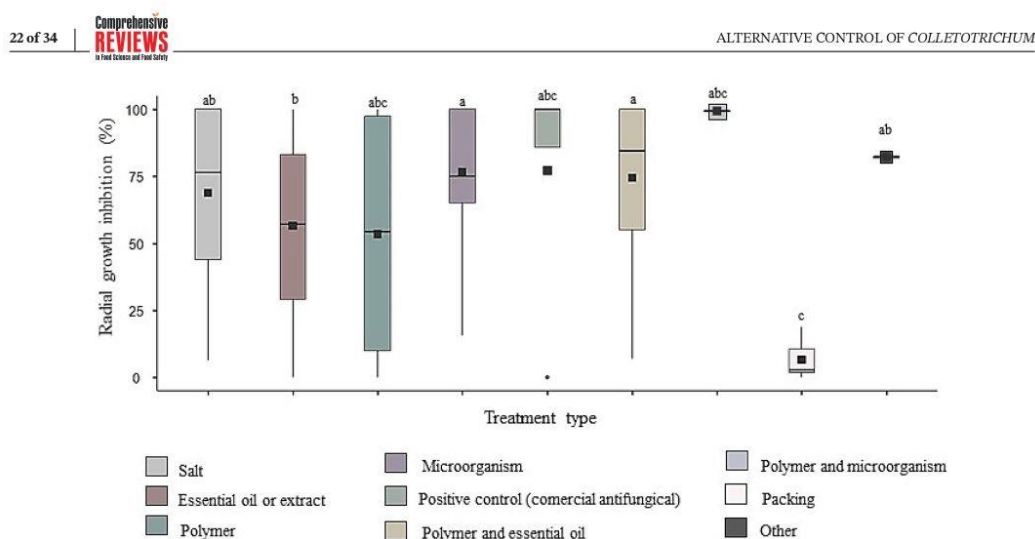


FIGURE 3 Assumed variation of radial mycelial growth inhibition (%) caused by salt, essential oil or extract, polymer, antagonistic microorganisms, commercial fungicide, polymer and essential oil, polymer and microorganism, packing, and others. Different letters between the boxplots are significantly different ($p > .05$), based on Kruskal–Wallis's test.

from in situ experiments were (i) reduction of anthracnose lesion diameter (%), including preventive and curative methods, (ii) disease incidence (%), and (iii) disease severity (scale). These data were collected from each analysis performed in the retrieved studies reported in this review, as cited in Table 1. Information concerning potential factors affecting the anthracnose inhibition was collected, that is, target fungi (*Colletotrichum* species), tested fruit, treatment type, application method in vitro and in situ (in fruit), antifungal substance concentration, and antagonistic microorganisms tested.

Analysis of this quantitative evaluation included only data concerning applying sustainable strategies against *Colletotrichum* species in vitro or on fresh tropical fruits during the postharvest period. Data on microbial inhibition were recovered from tables or graphics of retrieved studies, whether necessary. Relative frequency distributions were measured and classified in a range of different microbial reduction levels (Figure 2). In addition, data variations (boxplot) of radial mycelial growth inhibition (%) were created to show the data distribution regarding the type of the tested strategies (Figure 3).

The Kolmogorov–Smirnov test checked the normal data distribution, and the variables collected from studies to assess the efficacy of the tested strategies in controlling anthracnose were compared to data of chemical fungicide (positive control) or a reference value representing no efficacy in anthracnose inhibition, using the Mann–Whitney U and Wilcoxon signed-rank tests, respectively. Differences among the results of the different tested strate-

gies were assessed using Kruskal–Wallis's test, which considered a p -value of .05 for a significant difference.

A total of 47 studies, with 1345 observations, were acquired. From retrieved studies, 450 (33.5%) experiments measured in vitro the radial mycelial growth inhibition, and 895 (66.5%) experiments measured in situ the preventive (54.6%) and/or curative treatment of anthracnose lesion (14.0%), disease incidence (12.9%), and/or disease severity (18.0%). From recovered in situ studies, 100% of the observations measured the growth reduction of an artificial inoculum in different fresh tropical fruits, including papaya (252, 28.1%), mango (235, 26.3%), banana (207, 23.1%), guava (117, 13.1%), and avocado (84, 9.4%).

Target fungi included several *Colletotrichum* species. The most frequently tested species were *C. gloeosporioides* (637; 47.4%) > *C. musae* (315; 23.4%) > *C. brevisporum* (134; 10.0%) > *C. fruticola* (49; 3.6%) > *C. asianum* (45; 3.3%) > *C. siamense* (42; 3.1%) > *C. karstii* (37; 2.8%)/*C. tropicale* (37; 2.8%) > *C. karstii* (37; 2.8%) > *Colletotrichum* spp. (30; 2.2%) > *C. acutatum* (16; 1.2%) > *C. fragariae* (1; 0.1%)/*C. lagenarium* (1; 0.1%)/*C. higginsianum* Sacc (1; 0.1%). Investigations have shown *C. gloeosporioides* as the major species causing anthracnose in tropical fruits (Shi et al., 2021; Zakaria, 2023).

The strategies tested to control anthracnose in these studies were classified as polymer combined with EO (574, 32.1%) > polymer combined with antagonistic microorganisms (282, 15.8%) > EO or plant extract alone (214, 12.0%) > polymer alone (188, 10.5%) > antagonistic microorganism alone (94; 5.3%) > packing

Figure 3 - Assumed variation of radial mycelial growth inhibition (%) caused by salt, essential oil or extract, polymer, antagonistic microorganisms, commercial fungicide, polymer and essential oil, polymer and microorganism, packing, and others. Different letters between the boxplots are significantly different ($p > 0.05$), based on Kruskal–Wallis's test.

(60, 3.4%) > physical barrier combined with polymer (16, 0.9%) > salt combined with EO (14, 0.8%) > physical barrier alone (14, 0.8%) > and salt alone (5, 0.3%). Other types of treatments, which might not be classified as cited before, comprised four observations (0.2%).

To estimate the overall efficacy of the tested strategies for controlling pathogenic *Colletotrichum* species in vitro and in situ, the data of radial mycelial growth inhibition (%) and anthracnose lesion reduction (%) produced by the tested strategies were compared to zero, whereas the data of anthracnose incidence (%) and disease severity were compared to 100% and 5 (scale), respectively. These values were chosen for comparison because they represent no efficacy in anthracnose inhibition.

The Wilcoxon signed-rank tests (data presenting non-normal distribution according to the Shapiro–Wilk test, $p < .05$) showed that radial mycelial growth inhibition (%) and anthracnose lesion reduction (%) were significantly different ($p < .001$) from zero. Anthracnose incidence (%) and disease severity differed from 100% and 5 (scale), respectively.

In addition, results of tested sustainable strategies and chemical fungicide (positive control), including data from different variables, that is, radial mycelial growth inhibition (%), anthracnose lesion reduction (%), anthracnose incidence (%), and disease severity (scale), were compared using the Mann–Whitney test (data presenting nonnormal distribution according to the Shapiro–Wilk test, $p < .05$). Results showed no significant difference ($p > .05$) between the tested strategies and chemical fungicides, indicating that alternative sustainable interventions could have effects like chemical fungicides against *Colletotrichum* species in vitro and in situ; however, with a reduced potential for having negative environmental impact (irresponsible disposal) (Aguirre-Güitrón et al., 2021; Sharma et al., 2021) and decreased fungal resistance concerns (Wang et al., 2023) compared to traditional strategies. In these analyses, other factors (e.g., tested fruit, tested treatment, application method, antifungal substance concentration, and target fungi) were not measured.

The overall evaluation of these data indicates that investigated sustainable strategies effectively controlled pathogenic *Colletotrichum* species in vitro and in situ. Figure 2 shows the relative frequency distributions, that is, the percentage of times that a value range was observed relative to a total number of observations regarding radial mycelial growth inhibition (Figure 2A), anthracnose lesion reduction (Figure 2B), disease incidence (Figure 2C), and disease severity (Figure 2D).

Data variation regarding the radial mycelial growth inhibition (%) (Figure 3) indicates significant differences among the tested interventions ($p < .05$). Antagonist microorganisms had a stronger inhibitory effect (average:

76.7%) than EO or extracts (mean: 56.6%) and packing (average: 6.7%). Biocontrol has been consistently reported as effective and environmentally harmless alternative to sustainable agriculture (Evangelista-Martínez et al., 2022; Jaiswal et al., 2022; Sharma et al., 2021). In addition, EO or extracts have a stronger inhibitory effect in combination with a polymer (average: 74.4%), indicating that this combination could produce synergic effects, which agree with the results from previous studies (Braga et al., 2019; Ludgren et al., 2022; Yang et al., 2022). Packing was the less effective strategy. The analysis also showed no significant differences ($p > .05$) in the inhibitory effects when the tested sustainable interventions were compared to the chemical fungicides. These findings regarding the in vitro data help better infer the in situ results and contribute to direct more effective choices in testing the fruits.

Quantitative analysis by summarization of individual studies from available literature shows overall that applying alternative sustainable strategies could represent a perspective to improving postharvest tropical fruit quality.

5 | EFFECTS OF ALTERNATIVE SUSTAINABLE STRATEGIES TO CONTROL ANTHRACNOSE ON QUALITY PARAMETERS OF TROPICAL FRUITS

Due to transpiration and respiration processes, fresh tropical fruits have limited postharvest life (Wu et al., 2023). In general, different quality parameters, such as losses in weight and firmness, color alteration, and decrease in soluble solids content, acidity, color, and phenolic compounds, are changed in tropical fruits during storage. Applying alternative strategies to preventing and controlling postharvest diseases caused by phytopathogenic fungi reduces these changes and delays fruit senescence and decay (Cuéllar-Torres et al., 2022; Iñiguez-Moreno et al., 2020; Reyna et al., 2022; Wu et al., 2023; Yang et al., 2022; Zhou et al., 2022). The impacts of chemical, physical, and biological treatments on the quality attributes of tropical fruits have also been investigated, and the main results are summarized in Table 2.

5.1 | Physical quality parameters

Weight loss was the main physical parameter evaluated in the retrieved studies, with a typical increase in tropical fruit weight loss during storage. However, edible coatings containing active compounds effectively reduced the progression of fruit weight loss. Coatings exert a moisture barrier to delay metabolic activities and fruit dehydration (Aguirre-Güitrón et al., 2019; Correa-Pacheco et al., 2022;

TABLE 2 Main effects of alternative sustainable strategies on postharvest control of pathogenic *Colletotrichum* species on physicochemical and sensory quality parameters of tropical fruits.

Chemical and physical methods				Main effects	Reference
Treatment	Fruit	Storage conditions	Quality parameters		
Plasma-activated water (PAW) prepared with dielectric barrier discharge (DBD) cold plasma	Mango	25°C, 90% RH (relative humidity) for 8 days	Weight loss, firmness and nutritional quality (total soluble solids—TSS, and malonaldehyde—MDA)	Treatment maintained the quality and extended the shelf life of mango, maintaining firmness and delaying MDA and TSS decrease. In addition, mango treated with PAW showed better color, appearing brighter, reddish, and yellowish, and had no noticeable fragrance effect as determined by sensory analysis	Wu et al. (2023)
High degree of polymerization agave fructans (HDPAF)	Avocado	22°C, RH 55%–60% for 9 days	Weight loss, firmness, TSS, titratable acidity, and pH	HDPAF (mainly at 20%) retrieved the acceleration of both firmness loss and physiological weight loss. The other quality parameters (color, pH, TSS, and titratable acidity) were not negatively affected by HDPAF treatment	Cuellar-Torres et al. (2022)
Dielectric barrier discharge (DBD) cold plasma	Mango	25°C and 90% RH for 10 days	Weight loss, firmness, color, and TSS	DBD plasma maintained mango quality parameters by delaying the deterioration process with increased hardness and reduced TSS. There was no difference for the parameters of color (L^* , a^* , and b^*) and weight loss of fruit among treatments	Wu et al. (2022)
Poly(lactic acid)/polybutylene adipate-co-terephthalate + pine essential oil + chitosan	Avocado	25°C for 9 days	Weight loss, firmness, TSS, dry matter, color, and CO ₂ production	The ripening process of avocado was not affected by using any of the tested polymeric nets, with slightly firmer fruit being observed. The treatments did not influence the production of CO ₂ , nor the other quality parameters evaluated	Correa-Pacheco et al. (2022)
Hinokitiol	Banana	13°C for 21 days and at 20°C C for 7 days	Weight loss, color, firmness, TSS, titratable acidity, ethylene release, and respiration rate	Treatment effectively delayed the ripening of banana, regulating the physicochemical index postharvest, reducing the weight loss rate, and maintaining high values of firmness, low values of TSS, MDA, besides reducing ethylene release and respiration rate	Qiao et al. (2022)
Sodium alginate and tea tree essential oil nanoemulsion (TEON) bilayer films incorporated with TiO ₂ nanoparticles	Banana	25°C and 75% RH for 16 days	Weight loss, firmness, TSS, and starch content	Postharvest fruit quality was improved by treatments (mainly SA enriched with TEON and TiO ₂), showing excellent physical appearance, reduced mass, and electrolyte loss, lower TSS content, satisfactory starch content, and higher firmness	Yang et al. (2022)

(Continues)

Table 2 - Main effects of alternative sustainable strategies on postharvest control of pathogenic *Colletotrichum* species on physicochemical and sensory quality parameters of tropical.

TABLE 2 (Continued)

Chemical and physical methods				Main effects	Reference
Treatment	Fruit	Storage conditions	Quality parameters		
Chitosan and mint essential oil (MEO)—alone and combined	Papaya	12°C for 20 days	Weight loss, firmness, TSS, and ascorbic acid	Treatments maintained the postharvest quality of fruits, with greater firmness, decreased TSS content, and delayed loss of ascorbic acid (vitamin C), but the combination with MEO did not improve these results	Reyna et al. (2022)
Curcumin-loaded zein nanoparticles (CZNP)	Mango	32°C for 9 days	Weight loss, color, and firmness	Coating prolonged the postharvest quality of Nam Dokmai mango, delaying color change and loss of weight and firmness during storage	Chuacharoen and Sabtirov (2022)
Chitosan-thyme EO coating	Mango	12°C, 80%–85% RH for 28 days and 25°C for 5 days (total 33 days)	Weight loss, firmness, TSS, and titratable acidity	Application of treatments was effective in the retention (minor change) of physicochemical properties of fruit, such as titratable acidity, total soluble solids, firmness, weight loss, changes in color parameters, and high sensory acceptability	Shah et al. (2021)
Kafir lime EO	Mango	5°C for 9 days	Weight loss, firmness, TSS, and titratable acidity	There was no significant difference between treatments for weight loss, firmness, soluble solids, and titratable acidity of mango between treatments. The results were related to the short preservation time (10 days) and the low applied EO concentration. However, unripe kaffir EO tended to protect more against weight loss and TSS alteration	Chit-aree et al. (2021)
Conyza bonariensis (L.) Cronquist essential oil/gum arabic	Banana	25°C for 9 days	Weight loss, TSS, titratable acidity, color, and pH	Coatings reduced the alterations in physicochemical parameters and more prolonged storability of banana. Specifically, coated banana had lower pH and higher TA and TSS, besides causing smaller weight loss and delayed alternation in color	Lundgren et al. (2021)
Chitosan (CH) and Ruta graveolens L. essential oil	Papaya	20°C, 88% RH for 12 days	Weight loss, firmness, TSS, titratable acidity, pH, color, water activity, and sensory characteristics	CH-RGEO emulsions delayed the ripening according to maturation index, as well as firmness loss and weight loss, and did not affect the sensory characteristics of papaya. Control and CH-treated fruits had the major reduction in the brightness	Peralta-Ruiz et al. (2020)
Chitosan/hot water (HW)	Papaya	10°C at 28 days plus 20°C at 7 days (35 days)	Weight loss, surface color, firmness, TSS, titratable acidity, pH, and sensory characteristics	Treatments, especially HW-CH ₂ O, maintained the physicochemical parameters and had no negative sensory effects during storage, showing decreased loss of mass and firmness, better color parameters (keeping the green color of fruits), and lower TSS	Vilaplana et al. (2020)

(Continues)

TABLE 2 (Continued)

Chemical and physical methods				
Treatment	Fruit	Storage conditions	Quality parameters	Main effects
Chitosan and <i>Ruta graveolens</i> L essential oil	Guava	24° C for 12 days	Weight loss, firmness, TSS, titratable acidity, pH, CO ₂ respiration rate, maturation index, color, water activity and sensory	Guava coated with CH + RGEO at 1.5% showed stability concerning decay index, weight loss, maturation index, respiration rate, color, firmness, and water activity as compared to the uncoated guava
<i>Aloe vera</i> gel/garlic essential oil (GEO)	Banana	20° C for 15 days	Weight loss, firmness, TSS, titratable acidity, and total phenolic contents	AV gel in combination with GEO improved the postharvest quality attributes of banana, delaying changes in weight loss, TSS, firmness, and titratable acidity. These coatings enhanced total phenolic contents and antioxidant activities
Chitosan/vanillin and ethylene adsorbents	Mango	13° C for 30 days	Weight loss, firmness, TSS, titratable acidity, and color	Mango wrapped with chitosan, vanillin, and zeolite paper showed delayed changes in physicochemical parameters (weight loss, firmness, titratable acidity, total soluble solids, and color changes), resulting in shelf-life extension, and higher sensory acceptance score
Biological methods				
Treatment	Fruit	Storage conditions	Measured quality parameters	Main effects
<i>Streptomyces</i> sp. HSL-9B	Mango	28° C and 85% RH for 10 days	Total carotenoid content, and total soluble sugar	Treatment did not influence mango quality, with no changes for total carotenoid content and soluble solids content. However, the total soluble sugar content in the fruits treated with strain HSL-9B extracts was higher than that in the control group
Yeast <i>Meyerozyma caribbica</i> by electrospaying process	Mango and papaya	25° C for 15 days or 13° C for 4 days then 25° C for 11 days	Weight loss, skin color, and firmness	After application of the antagonist yeast, no significant changes in weight loss, color, and firmness were detected; however, fruits stored at 13° C lost less weight and delayed color changes than fruits stored at 25° C
Sodium alginate/LAB	Guava and mango	25° C for 15 days	Weight loss, TSS, titratable acidity, pH, and ascorbic acid	Treatments positively impacted some physicochemical parameters in coated fruits, which had lower TSS and titratable acidity, increased pH and retention of ascorbic acid, and decreased weight loss
<i>M. caribbica</i> /sodium alginate	Avocado	25° C for 15 days or 6° C for 10 days and matured at 25° C for 5 days	Weight loss	Fruit treated with bioactive coatings had reduced weight loss, with increased production of volatile organic compounds. Furthermore, storage at 6° C allowed a reduced weight loss compared to storage at 25° C

(Continues)

TABLE 2 (Continued)

Biological methods				
Treatment	Fruit	Storage conditions	Measured quality parameters	Main effects
<i>M. pulcherrima</i> , salicylic acid, and calcium chloride	Mango	25°C for 12 days	Skin color, firmness, TSS, and ascorbic acid	Treatments delayed changes in mango's quality characteristics (a^* value, firmness, TSS content, and ascorbic acid) while enhancing the activities of anti-disease enzymes. Furthermore, the combination of SA solution, CaCl_2 solution, and <i>M. pulcherrima</i> suspension had an additive effect, increasing the efficacy of maintaining mango quality during storage
Yeast <i>Meyerozyma caribbica</i>	Mango	25°C, 80% RH for 15 days or 13°C (80% RH) for 4 days and then for 11 days at 25°C	Weight loss, color, and firmness	The yeast application (fresh and powder formulation) did not affect the quality parameters of mango, and no significant difference was observed in weight loss, color, and firmness parameters for the different storage times evaluated. Nevertheless, fruits stored at 13°C had less weight loss than those stored at 25°C

Abbreviations: CaCl_2 , calcium chloride; CZNP, curcumin-loaded zein nanoparticles; DBD, dielectric barrier discharge; EO, essential oil; HDPAF, high degree of polymerization agave fructans; HW, hot water; LAB, lactic acid bacteria; MDA, malondialdehyde; MEO, mint essential oil; PAW, plasma-activated water; RGEQ, *Rutta graveolens* L. essential oil; RH, relative humidity; SA, sodium alginate; TA, titratable acidity; TEON, tea tree essential oil nanoemulsion; TSS, total soluble solids.

Fernandes et al., 2021; Vilaplana et al., 2020; Wu et al., 2023). This effect is associated with the hygroscopic water pressure between the fruit and the environment and is influenced by cuticle composition and thickness that differ according to the fruit cultivar and maturity stage (Iniguez-Moreno et al., 2020; Khaliq et al., 2019). Additionally, storage conditions, such as low temperature, high relative humidity, and low air movement, reduce fruit weight loss (Jaimun & Sangsuwan, 2019).

Change in water activity is another parameter evaluated in tropical fruits, allowing us to analyze the behavior of free and bound water during fruit ripening. The higher maturity index is commonly associated with lower water activity in fruits. The decrease in fruit water activity is caused by the transformation of free water into bound water, which is responsible for the transpiration and hydrolysis reactions during fruit ripening. The application of CH-based coatings has been shown to be effective in slowing down water activity reduction in guava and papaya during storage (Peralta-Ruiz et al., 2020; Tovar et al., 2019). This activity could be due to the combination of CH with emulsions forming a hydrophobic complex that reinforces the coating matrix, leading to enhanced mechanical performance and barrier capacity and preventing water loss (Peralta-Ruiz et al., 2020; Tovar et al., 2019).

Several studies indicated the gradual decline of fruit firmness during storage. The cell wall collapse negatively affects the texture and firmness of mango and banana (Bilal & Hashmi, 2023; Yang et al., 2022). This process involves pectin hydrolysis and the transformation of cell wall components by hydrolytic enzymes (Reyna et al., 2022; Vilaplana et al., 2020; Wu et al., 2023). Physical treatments using cold plasma (Wu et al., 2022) and biological powder formulation of *M. caribbica* (Aguirre-Güitrón et al., 2021) preserved the firmness of mango and guava during storage. These effects were linked to high antifungal activity and inhibitory effects on the activity of cell wall degradation enzymes under low-temperature storage, such as polygalacturonase, pectin methylesterase, galactosidases, and β -1,4-glucanases (Aguirre-Güitrón et al., 2019; Iniguez-Moreno et al., 2020).

Furthermore, the formation of lesions in fungal-infected tropical fruits induces ethylene production that increases enzymatic activities and contributes to fruit softening (Jaimun & Sangsuwan, 2019). Applying different preservative strategies can reduce the ripening process (peak ethylene production) in mango, leading to shelf-life extension and firmness loss reduction (Jaimun & Sangsuwan, 2019; Qiao et al., 2022).

The natural ripening process causes color changes in tropical fruits (Aguirre-Güitrón et al., 2021). The instrumental color parameters L^* (luminosity), a^* (red), b^*

(yellow/blue), and °Hue were measured in different tropical fruits (Aguirre-Güitrón et al., 2019, 2021; Chuacharoen & Sabliov, 2022; Correa-Pacheco et al., 2022; Cuéllar-Torres et al., 2022; Jaimun & Sangsuwan, 2019; Lundgren et al., 2021; Peralta-Ruiz et al., 2020; Qiao et al., 2022; Shah et al., 2021; Shao et al., 2019; Tovar et al., 2019; Vilaplana et al., 2020; Wu et al., 2022, 2023). °Hue represents the hue angle on a color wheel (Cuéllar-Torres et al., 2022). Luminosity and saturation typically increase in papaya during the postharvest period (Vilaplana et al., 2020). The color changes during the maturation and senescence are attributed to the progressive chlorophyll degradation and development of pigments, such as carotenoids, xanthophylls, and anthocyanins (Aguirre-Güitrón et al., 2019; Shah et al., 2021). The blackening on the fruit surface is associated with the initial harvest index, normal ripening process, and damage caused by fungal infection (Correa-Pacheco et al., 2022). Different treatments delay these changes, reducing respiration rates, ethylene production, chlorophyll degradation rate, and/or reducing the activity of enzymes related to browning, such as polyphenol oxidase and peroxidase (Chuacharoen & Sabliov, 2022; Jaimun & Sangsuwan, 2019; Peralta-Ruiz et al., 2020).

The changes in respiratory rate, measured by oxygen reduction and increased CO₂ levels during storage, have also been evaluated in guava and banana (Quiao et al., 2022; Tovar et al., 2019) and associated with water loss, increased total soluble solids (TSS), decreased TA, malondialdehyde (MDA) accumulation, and increased sensitivity to ethylene (Quiao et al., 2022). Coatings formulated with hydrophobic compounds reduce respiratory rate because they form a semipermeable film surface that modifies the internal fruit atmosphere and reduces senescence and fruit metabolic activity during storage (Chuacharoen & Sabliov, 2022; Lundgren et al., 2021; Tovar et al., 2019; Wu et al., 2023).

In addition, changes in respiration rate could reduce the continuous conversion of complex sugars to simple sugars and organic acids to simple sugars, which are effects reported in mango and banana coated with GA and EO (Chit-aree et al., 2021; Lundgren et al., 2021). Sugars maintain cell osmotic potential, provide energy for stress defense, and act as membrane protectants and ROS scavengers in fruits. Changes in fruit carbohydrates are an important acclimatory response to stress affecting product quality and appearance (Oliveira et al., 2023). Reduced maturation progression in fruits resulting from the application of the tested alternative strategies would extend the shelf-life and reduce fruit losses, improving the economic viability and consumers' interest in treated tropical fruits.

5.2 | Biochemical quality parameters

As an indicator of fruit quality, TSS is directly linked to soluble sugar content and metabolic and maturation processes in fruits (Chit-aree et al., 2021). The increase in TSS may be associated with the conversion of pectic substances and enzymatic carbohydrate hydrolysis (Shah et al., 2021). Application of some alternative strategies slowed down TSS content decrease in tropical fruits during storage (Fernandes et al., 2021; Shao et al., 2019; Yang et al., 2022), which was linked to the reduced occurrence of fungal diseases and respiratory activity in fruits (Wu et al., 2022).

Coatings formulated with polymers, such as CH and SA, create a surface barrier to gas exchange and limit oxygen diffusion and water evaporation in fruits. These coatings could inhibit fruit ripening, reducing TSS (because of reducing the conversion of starch into soluble sugar rate), slowing respiration rate, and, consequently, oxidative reactions, a major cause of ascorbic acid decay in fruit during storage (Khaliq et al., 2019; Shah et al., 2021; Yang et al., 2022). Guava, mango, and papaya coated with SA loaded with *Lactobacillus* strains and with combined CHI and EO had greater retention of ascorbic acid during storage (Fernandes et al., 2021; Reyna et al., 2022). These results could be linked to the capability of the coatings to reduce oxygen diffusion and slow respiration rates in fruits (Fernandes et al., 2021; Reyna et al., 2022).

TA is an important parameter indicative of fruit quality during storage. Major organic acids (e.g., malic and citric acids) and ascorbic acid are commonly used to determine TA in tropical fruits. Reducing TA indicates changes in the composition and fruit storability (Jaimun & Sangsuwan, 2019; Khaliq et al., 2019). Tropical fruits treated with alternative sustainable postharvest methods have shown higher TA value than untreated fruits (Jaimun & Sangsuwan, 2019; Khaliq et al., 2019). This effect may be linked to the ability of these treatments (mainly coatings) to reduce the fruit respiration rate and the conversion of organic acids into sugars or volatile compounds through several biochemical reactions (Fernandes et al., 2021; Peralta-Ruiz et al., 2020; Shah et al., 2021). However, in some tropical fruits (e.g., avocado), TA increases can be associated with high contents of free fatty acids due to triglyceride lipolysis, possibly caused by fruit decay during storage (Cuéllar-Torres et al., 2022).

The pH is a parameter associated with TA in fruits, and increases in pH values are linked to the consumption of undissociated organic acids and sugars as a substrate for respiratory metabolism (Cuéllar-Torres et al., 2022; Tovar et al., 2019). Most alternative treatments have regulated the progress of pH increase in tropical fruits, especially when these fruits are treated with antagonistic LAB that

acts reducing fruit pH due to the production of organic acids and different antimicrobial molecules during their metabolism (Fernandes et al., 2021).

Some studies evaluated enzymatic and antioxidant activities, MDA contents, and total phenolic compound contents in tropical fruits. The browning process impacts the marketing and quality of these fruits and may be related to their phenolic compound contents, influencing antioxidant and antifungal properties (Oliveira et al., 2023). As reported for fruits treated with AV-based coating, the variation in total phenolic contents during storage affects pathogen physiology and stimulates host plant resistance against anthracnose (Khaliq et al., 2019).

The increasing content of MDA can indicate peroxidation degree and senescence in mango, as well as cytotoxicity. MDA accumulation occurs because of membrane lipid peroxidation and causes irreversible damage to the integrity of fruit cell membranes and organelles (Wu et al., 2022). Enzymes related to disease processes play a critical role in protecting cell membranes, preventing fruit decay (pathogen infections), maintaining fruit color, and enhancing the resistance to ROS in stressful situations (Quiao et al., 2022; Shao et al., 2019; Vilaplana et al., 2020; Wu et al., 2023).

The activity of some major defense-related enzymes typically varies during storage. Hinokitiol application maintained the integrity of cell membranes and induced defense mechanism by enhancing the activity of superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase in banana (Quiao et al., 2022). These mechanisms are related to the ability of superoxide dismutase to rapidly catalyze the dismutation of superoxide free radicals into hydrogen peroxide, which is dissolved in H₂O and O₂ by catalase, peroxidase, and ascorbate peroxidase, protecting the cell membrane integrity (Quiao et al., 2022). Similarly, the application of thyme EO to mango enhanced the activity of peroxidase, phenylalanine ammonia-lyase, and polyphenol oxidase, increasing resistance to pathogens. Phenylalanine ammonia-lyase may have stimulated the biosynthesis of phenylpropanoids, protecting the plant against various biotic and abiotic stresses (Shah et al., 2021).

Some studies have evaluated parameters indicative of the quality and sensory acceptability in tropical fruits treated with alternative sustainable postharvest technologies (Jaimum & Sangsuwan, 2019; Martinez-Blay et al., 2020; Peralta-Ruiz et al., 2020; Shah et al., 2021; Tovar et al., 2019; Vilaplana et al., 2020; Wu et al., 2023). The combined treatment with CH and hot water (Vilaplana et al., 2020), vanillin (Jaimum & Sangsuwan, 2019), *Ruta graveolens* L. EO (Peralta-Ruiz et al., 2020; Tovar et al., 2019), and thyme EO (Shah et al., 2021) did not affect the flavor and odor of tropical fruits, indicating the barrier effect caused by CH,

as well as the importance of reducing the treatment dose to below the threshold limit for strange flavor perception (e.g., bitter herbal flavors). In general, flavor alterations related to sensory characteristics are one of the main disadvantages of the postharvest application of EO on mango (Shah et al., 2021). On the other hand, plasma-activated water had no perceptible effect on mango flavor because it is a colorless and odorless liquid (Wu et al., 2023). However, it is important to highlight that only one available study tested fruit consumer acceptance. A deeper investigation of consumer acceptance is necessary for future research to better understand the possible limitations of the revised postharvest strategies.

6 | LIMITATIONS OF USE AND POTENTIAL PRACTICAL APPLICATION

The efficacy of alternative and sustainable strategies applied to control anthracnose in tropical fruits could be directly affected by several factors. The fruit cultivation conditions, compound concentration, application methods, and storage temperature are influential factors to the observed results (Martinez-Blay et al., 2020; Mokgalapa et al., 2022; Osondu et al., 2022). Fruits stored under refrigeration have shown better results for disease control compared to fruits stored under room temperature, with the effects being influenced by the delay of maturation progress (Chit-aree et al., 2021; Iníguez-Moreno et al., 2020). Fruits with high respiration rates, such as banana, mango, and avocado, had an increased maturation process due to the release of large amounts of ethylene, which may reduce the efficacy of applied treatments (García et al., 2022; Jaimun & Sangsuwan, 2019; Lundgren et al., 2021). Therefore, it is important to align the selected treatment with the specific fruit matrix to obtain more reliable techniques and better anthracnose control.

EOs have high volatility during storage, which may contribute to decreasing their efficacy in controlling anthracnose over time (Lundgren et al., 2021). The application of higher EO concentrations to compensate for this volatility could reduce the fruit sensory acceptance; however, the incorporation of food-grade oils into edible films is an alternative to reduce EO volatile losses and increase the treatment efficacy (Shah et al., 2021). In the case of edible coatings/films, the temperature and exposure time used to dissolve and apply polymers in fruits could affect their mechanical and physical properties, altering the barrier effects (Braga et al., 2019). The polarity can increase the reactivity of the polymers with water, causing dissolution or swelling of the coatings, a characteristic that could be improved with the inclusion of hydrophobic substances, such as EO (Braga et al., 2020). Additionally,

studies with plant extracts to determine toxicological features and potential negative environmental impact are still scarce (Sousa et al., 2022). The target pathogenic fungi species must also be considered, as different pathogens may have varying susceptibilities to treatments, requiring specific approaches for each case (Aguirre-Güitrón et al., 2021; Braga et al., 2019).

Despite all the potential benefits of applying antagonistic microorganisms to control anthracnose in tropical fruits, the antimicrobial efficacy of bacteria and yeast can also be compromised by several factors. The concentration of cells can undergo large variations from the initial point of incorporation into the food product and during storage (Aguirre-Güitrón et al., 2019; Fernandes et al., 2021; Iñiguez-Moreno et al., 2020). The intrinsic characteristics of antagonistic microorganisms, commercial viability, applied dose, and complex action mechanisms also need to be carefully evaluated, because these factors would directly interfere with the antifungal effects (Evangelista-Martínez et al., 2022; Sharma et al., 2021; Zhou et al., 2022). Moreover, there is still a lack of evidence from the perspective of management and consumption safety of these antagonistic microorganisms, which limits the carrying out of specific tests (including acceptance and sensory tests) and the application to fruits on an industrial scale. It is important to emphasize that there is no available study evaluating the application of these alternative and sustainable strategies under field conditions. A single article applied biocontrol agents in the fruit growing area, but with limited observations to assess disease progress in leaves and fruits (Sharma et al., 2021).

7 | CONCLUSION

This review considered studies that tested alternative sustainable interventions to the use of chemical fungicides to control pathogenic species of *Colletotrichum* causing anthracnose in tropical fruits. The available studies have shown the use of various methods, including physical barriers (biopolymeric coatings) and natural antimicrobials (EO or plant extract alone), to reduce anthracnose lesion severity or incidence in tropical fruits. In general, the anti-*Colletotrichum* efficacy of tested interventions varied with several factors, such as the ability of the polymer matrix to produce and release antimicrobial compounds, activation of defense enzymes, antioxidant activity, kind and concentration of natural extracts applied, the gas diffusion, and the fruit respiration rate during storage. However, the characterization of the bioactive compounds responsible for the antifungal effects, action mechanism,

and effects of combinations/interactions among different strategies/technologies remain little explored and could direct future research, particularly to guarantee or improve the stability and optimize their practical application. Investigations with biological antagonists, such as yeast, fungi, and bacteria, showed their efficacy in inhibiting *Colletotrichum* growth in vitro and in tropical fruits and could be used as bioformulations to replace chemical fungicides. Most studies focused on the inhibition of *C. gloeosporioides* on avocado, papaya, and mango and *C. musae* on banana; however, the inhibition of other *Colletotrichum* species was also demonstrated. The application of emerging sustainable alternative methods also improved some postharvest quality parameters and stimulated the induction of defense systems in tropical fruits, including enzymatic activity. However, the studies gathered in this review provide little data on the effects of the examined strategies on the sensory parameters of tropical fruits, and these characteristics could be evaluated in future studies. In addition to these factors, it is important to conduct future investigations on the isolation, identification, characterization, and control of specific *Colletotrichum* species recovered from anthracnose lesions in different tropical fruits.

In addition to the advantages related to the application of sustainable strategies, features related to the viability of their use in agriculture need to be clarified to allow their introduction into integrated pest and disease management protocols against *Colletotrichum* spp. Some of the investigated strategies, such as biopolymeric coatings/films (dispersion and application), cold plasma, and antagonist microorganisms, could have limitations (in terms of structure and management) for their practical application, making it necessary to improve techniques to use on an agro-industrial scale. Though some of the natural compounds investigated are widely available on the market (e.g., EO), their applicability to fruits may not present major challenges using infrastructure already available in the processing industries. As a result, producers could potentially introduce alternatives to control anthracnose in tropical fruits (enabling a reduction of chemical fungicides use) if the knowledge would gradually transfer to the agricultural community.

AUTHOR CONTRIBUTIONS

Karina Felix Dias Fernandes: Conceptualization; data curation; formal analysis; visualization; writing—original draft; investigation; writing—review and editing. **Talita Silveira Queiroga:** Formal analysis; visualization; investigation; writing—original draft. **Maiara da Costa Lima:** Formal analysis; visualization; writing—original draft;

investigation; data curation. **Kataryne Áraabe Rimá de Oliveira:** Conceptualization; data curation; formal analysis; visualization; writing—original draft; investigation; supervision; writing—review and editing. **Evandro Leite de Souza:** Conceptualization; data curation; formal analysis; visualization; writing—original draft; investigation; supervision; project administration; writing—review and editing.

ACKNOWLEDGMENTS

The authors thank the Paraíba State Research Foundation (FAPESQ) for a postdoctoral scholarship awarded to M. C. Lima (Funding number: 001) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) for a PhD scholarship awarded to K. F. D. Fernandes and an MSc scholarship awarded to T. S. Queiroga (Finance code 001).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data used to perform the quantitative analysis presented in this manuscript may be available upon reasonable request.

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How to cite this article: Fernandes, K. F. D., Queiroga, T. S., Lima, M. C., de Oliveira, K. Á. R., & de Souza, E. L. (2024). Interventions based on alternative and sustainable strategies for postharvest control of anthracnose and maintain quality in tropical fruits. *Comprehensive Reviews in Food Science and Food Safety*, 23, e13427. <https://doi.org/10.1111/1541-4337.13427>

APÊNDICE C - Artigo original I

Food Control 175 (2025) 111341



Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

A novel coating formulated with sodium alginate loaded with a mixed lactic acid bacteria culture to control anthracnose in guava and mango

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ARTICLE INFO

Keywords:

Alternative control
Colletotrichum spp.
Coatings
Antagonistic microorganisms
Psidium guajava L.
Mangifera indica L.

ABSTRACT

Guava and mango are tropical fruits widely consumed due to their pleasant flavor and nutritional composition. Anthracnose caused by *Colletotrichum* spp. is considered a primary post-harvest disease affecting these fruits. Applying biocontrol agents, such as lactic acid bacteria (LAB), combined with coating materials is an innovative fruit biopreservation method. This study investigated the effects of a novel sodium alginate coating loaded with a mixed culture of LAB in controlling anthracnose caused by different pathogenic *Colletotrichum* isolates in guava (cv.) Paluma and mango (cv.) Palmer during 15 days of room temperature storage (25 ± 0.5 °C). A mixed culture formed by three different LAB species strains (*Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129, and *Limosilactobacillus fermentum* 263) was tested against ten target *Colletotrichum* isolates. The mixed culture inhibited the mycelial growth of all target *Colletotrichum* isolates *in vitro*, with up to 100 % inhibition. In the nutrient competition method, the mixed LAB culture survived and caused mycelial growth inhibition even at low nutrient concentrations. The coatings showed sizes of LAB cell subpopulations with non-permeabilized membrane (viable cells, PI-CFDA+) of >31 % at the end of refrigerated storage and viable cell counts of >7 log CFU/g. Applying coatings with sodium alginate loaded with mixed LAB culture delayed the development and decreased the severity of anthracnose lesions in guava and mango artificially contaminated with either of the tested *Colletotrichum* isolates. The coating formulated with sodium alginate and mixed LAB culture can be effective for postharvest anthracnose biocontrol in guava and mango.

1. Introduction

Guava (*Psidium guajava* L.) and mango (*Mangifera indica* L.) are economically significant crops grown in tropical and subtropical regions due to their pleasant flavor and nutritional richness (Shu et al., 2024; Wang et al., 2023). The Food and Agriculture Organization (FAO) classifies guava, mangosteen, and apple as the same commodity. India was the most prominent world producer of these fruits in 2023, reaching a volume of 26 million tons. In the same period, Brazil produced 2.1 million tons of these fruits, of which 11 % was destined for export, making Brazil the fourth largest world exporter (FAO, 2024).

However, guava and mango are climacteric fruits with high respiration rates, undergoing rapid ripening and senescence, resulting in a short storage period. In addition, these fruits are sensitive to infection by

pathogens, especially anthracnose, caused mainly by fungi belonging to the *Colletotrichum* genus, leading to considerable postharvest losses (Formiga et al., 2022; Li, Bi, et al., 2024). Anthracnose is a latent infection on the fruit skin surface that remains predominantly dormant during its growth stage but appears as sunken black or brown spots after the fruit is harvested (Ranjith et al., 2021). These physiological and fungal-induced changes, associated with inadequate handling, account for significant post-harvest losses. In developing countries, these losses are estimated to reach 30–60 % of total mango production (Silué et al., 2022) and 20–40 % of total guava production (Yadav et al., 2022).

Postharvest chemical treatments are still the most common method used to control postharvest spoilage in fruits compared to other methods, such as low temperature, heat treatment, irradiation, and modified atmosphere (Ranjith et al., 2021; Zakaria, 2021). In the last

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<https://doi.org/10.1016/j.foodcont.2025.111341>

Received 14 February 2025; Received in revised form 31 March 2025; Accepted 5 April 2025

Available online 6 April 2025

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two decades, synthetic chemical fungicides, such as benomyl, prochloraz, thiabendazole, chlorothalonil, propiconazole, methylcarbamate, and thiophanate-methyl, have been the most popular agents for preserving fresh fruits (Li, Cheng, et al., 2024; Ranjith et al., 2021; Vega-Alvarez et al., 2020). However, the adverse health effects associated with chemical fungicides, including dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and hormonal dysfunction, along with the negative environmental impacts, have raised the demand for effective alternatives (Gwiazdowski et al., 2023).

The use of antagonistic microorganisms and their metabolites is a known approach for the biopreservation or biocontrol of phytopathogens in fruits (Ranjith et al., 2021; Alvarez et al., 2021). The application of lactic acid bacteria (LAB) or its antifungal compounds has assumed relevance as an eco-friendly alternative to chemical interventions, aiming for a sustainable improvement of the shelf life, safety, quality, and sensory properties of the fruits (Agriopoulou et al., 2020; de Simone et al., 2024). Among the mechanisms underlying the LAB antifungal effects, the production of organic acids stands out, leading to the alteration in the pH of the environment and creating adverse conditions for pathogen development (Barrios-Salgado et al., 2022).

However, there is a limitation to applying LAB in fruit preservation due to the low stability and adhesion of these microorganisms on the fruit surface. Therefore, different approaches to incorporating LAB into edible coating components have been investigated, aiming to prolong their stability and survival in the fruit (Bambace et al., 2024; de Simone et al., 2024; Dias et al., 2023; Seyedzadeh-Hashemi et al., 2023). Sodium alginate, a natural anionic linear polysaccharide derived from marine brown algae, is commonly used to formulate these coatings, mainly due to its gelling and thickening properties (Nair et al., 2020; Shigematsu et al., 2018). In addition, it exhibits beneficial properties, including excellent film-forming characteristics, low permeability to O₂ and water vapor, and safety in its application in food (Kontominas, 2020). Sodium alginate-based coatings have been used as LAB carriers for application in fruits, with promising results concerning the preservation of these products (Bambace et al., 2024; Xu et al., 2020; Alvarez et al., 2021).

The efficacy of LAB strains, namely *Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129, and *Limosilactobacillus fermentum* 263, against different pathogenic *Colletotrichum* isolates was previously tested when applied alone and loading a sodium alginate coating (Fernandes, de Oliveira, & de Souza, 2021). However, a mixed LAB culture could present advantages related to its multiple action mechanisms, reducing the adaptation capacity of the target pathogens (Ibrahim et al., 2021). Thus, to enhance the previous results, this study aimed to develop and apply a sodium alginate coating loaded with a mixed culture of *L. brevis* 59, *L. pentosus* 129, and *L. fermentum* 263 to control anthracnose caused by different pathogenic *Colletotrichum* species in guava (cv.) Paluma and mango (cv.) Palmer during storage.

2. Materials and methods

2.1. Raw materials

Guavas cv. Paluma, characterized in the second maturation stage with up to 80 % green peel and °Brix of ≥10, harvested about 120–150 days after flowering and pollination (de Oliveira et al., 2020; Yadav et al., 2022), and mangos cv. Palmer, characterized in the medium maturation stage with 50 % of yellow or red peel and °Brix of ≥12, harvested about 90–100 days after flowering (Ranjith et al., 2021; Sousa et al., 2021), were obtained from EMPASA (Supplies and Services Company of Paraíba, João Pessoa, PB, Brazil). Fruits without physical injury and visual infections were selected and standardized based on size, appearance, color, and shape. Fruits were washed with neutral detergent and potable running water, surface disinfected (sodium hypochlorite, 150 ppm, pH 7.2 adjusted with 1 M NaOH) for 15 min, rinsed with potable water, and dried for 2 h under room temperature (25 ±

0.5 °C) in a safety cabinet (Lundgren et al., 2021). Sodium alginate (Dinâmica Química Contemporânea Ltda., Indaiatuba, SP, Brazil), glycerol (Química Moderna, Barueri, SP, Brazil), sunflower oil (Cargill Agrícola S.A, Recife, PE, Brazil), and Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) were used to formulate the coatings. Calcium chloride (Synth, Diadema, SP, Brazil) was used to form a gel by crosslinking with sodium alginate.

2.2. Mixed lactic acid bacteria culture and growth conditions

Three different lactic acid bacteria strains, namely *Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129, and *Limosilactobacillus fermentum* 263, were selected for use in the coating formulation based on previous results that demonstrated their antifungal capacity against *Colletotrichum* spp. (Fernandes, de Oliveira, de Souza, 2021). These strains were isolated from fruit pulp processing by-products [Acerola (*Malpighia emarginata* D.C.), mango (*Mangifera indica* L.), and pineapple (*Ananas comosus* L.), respectively] and characterized as probiotic candidates, with antimicrobial activity against food-contaminating microorganisms (Garcia et al., 2016). Stocks were maintained (−20 ± 0.5 °C) in de Man, Rogosa, and Sharpe (MRS) broth (HiMedia, Mumbai, India) with glycerol (Sigma-Aldrich; 20 mL/100 mL). Working cultures were kept on MRS agar (HiMedia) under refrigeration (4 ± 0.5 °C) and transferred to a new media monthly. Before use in the assays, each LAB strain tested was cultured in MRS broth at 37 ± 0.5 °C for 20–24 h under anaerobiosis (Anaerobic System Anaerogen, Oxoid, Hampshire, UK). Cells were collected by centrifugation (4500×g, 10 min, 4 ± 0.5 °C), washed in sterile saline (NaCl, 0.85 g/100 mL), and resuspended in sterile distilled water containing fructooligosaccharides [FOS (20 % w/v); cryoprotectant; F8052, SigmaAldrich, Saint Louis, MA, EUA]] to obtain a cell suspension with viable cell counts between 9 and 10 CFU/mL. The mixed cell suspension was prepared by mixing the suspensions of each strain (ratio 1:1:1 v/v) (Freire et al., 2021).

For lyophilization, the LAB mixed culture suspension added with FOS was transferred to glass vials with lids (2 mL of suspension in each vial) and subjected to freezing (−80 °C, 24 h) (Di et al., 2023). The lyophilization was carried out at a temperature of −55 ± 2 °C, with a vacuum pressure of <138 µHg, a lyophilization rate of 1 mm/h, for approximately 10 h, using a benchtop lyophilizer (model L-101, Liotop, São Carlos, SP, Brazil).

2.3. *Colletotrichum* strains and growth conditions

Ten different *Colletotrichum* strains from distinct species, kindly provided by the Prof. Maria Menezes Plant Pathogenic Fungi Culture Collection (Federal Rural University of Pernambuco, Recife, PE, Brazil) and the Mycology Laboratory Collection (Federal Rural University of Pernambuco, Recife, PE, Brazil), were used as target organisms. The groups were separated according to the origin fruit source: *C. asianum* CMM 4057, *C. fructicola* CMM 4069, *C. tropicale* CMM 4071, *C. siamense* CMM 4077, *C. karstii* CMM 4101, and *C. gloeosporioides* CMM 320 isolated from mango; and *C. fructicola* LM 680, *C. siamense* LM 681, *C. tropicale* LM 679, and *C. chrysophilum* LM 685 isolated from guava (Table 1). These strains were identified with phylogenetic inference (Lima et al., 2013; Soares et al., 2024) and were capable of inducing anthracnose development in guava and mango (Lima et al., 2013, 2015). Stock cultures were maintained in the dark in potato dextrose agar (PDA; HiMedia) under refrigeration (4 ± 0.5 °C). Before use in the experiments, working cultures were grown on PDA for 7 days (25 ± 0.5 °C). Each strain was inoculated separately in guava and mango, and after developing lesions with characteristic anthracnose symptoms, the strains were recovered and cultured on PDA (25 ± 0.5 °C, 7 days). All *Colletotrichum* strains induced anthracnose characteristic symptoms in fruits, i.e., irregular brown spots, depressed, and, in some cases, soaked areas for guava (Oliveira, de Oliveira, Vieira, Câmara, & de Souza, 2018); and sunken, prominent, and generally rounded dark brown to

Table 1
Colletotrichum isolates used in the tests.

<i>Colletotrichum</i> isolates	Host fruit	Reference
<i>C. tropicale</i> LM 679	Guava	Soares et al. (2024)
<i>C. fructicola</i> LM 680	Guava	Soares et al. (2024)
<i>C. siamense</i> LM 681	Guava	Soares et al. (2024)
<i>C. chrysophium</i> LM 685	Guava	Soares et al. (2024)
<i>C. asianum</i> CMM 4057	Mango	Lima et al. (2013)
<i>C. fructicola</i> CMM 4069	Mango	Lima et al. (2013)
<i>C. tropicale</i> CMM 4071	Mango	Lima et al. (2013)
<i>C. siamense</i> CMM 4077	Mango	Lima et al. (2013)
<i>C. karstii</i> CMM 4101	Mango	Lima et al. (2013)
<i>C. gloeosporioides</i> CMM 320	Mango	Lima et al. (2013)

LM: Mycology Laboratory Collection (Federal Rural University of Pernambuco, Recife, Brazil).

CMM: "Prof. Maria Menezes" Phytopathogenic Fungi Culture Collection (Federal Rural University of Pernambuco, Recife, PE, Brazil).

black spots for mango (de Oliveira et al., 2017). Each strain was tested separately in the experiments.

2.4. Evaluation of the antifungal activity of mixed LAB culture against *Colletotrichum* spp. *in vitro*

2.4.1. Agar spot method

The antifungal activity of LAB strains in mixed culture against the different target *Colletotrichum* strains was initially evaluated with an agar spot method (Afzali et al., 2020). Ten μ L aliquots from the mixed LAB culture ($9\text{--}10 \log \text{CFU/mL}$) lyophilized and rehydrated in sterile distilled water were dispensed as spots on the MRS agar surface (HiMedia) and incubated anaerobically for 24 h ($37 \pm 0.5^\circ\text{C}$). At the end of the incubation period, the plates were overlaid with 10 mL of semi-solid PDA, inoculated (on the center of the PDA layer surface) with an agar plug (5 mm diameter) containing structures of the target *Colletotrichum* isolate (previously grown for 7 days at $25 \pm 0.5^\circ\text{C}$ in PDA) and incubated aerobically for 5 days ($25 \pm 0.5^\circ\text{C}$). Agar non-inoculated with the mixed LAB culture was used as a negative control. The antifungal effects of the mixed culture LAB were measured based on the fungal mycelial growth reduction with the equation (Mpeluza et al., 2023):

$$\text{MGI\%} = \frac{[(\text{CT} - \text{TT}) / \text{CT}] \times 100}{(\text{Eq. 1})}$$

where MGI% is the percentage of inhibition of mycelial growth, CT is the total mycelial growth of fungi in the control assay and TT is the total mycelial growth of fungi in tested media with mixed culture of LAB.

2.4.2. Agar-well diffusion method

Six mm-diameter wells were punched in a dual-agar plate 2 cm away from a central point. A 100- μ L aliquot of a suspension of the mixed LAB culture ($9\text{--}10 \log \text{CFU/mL}$) lyophilized and rehydrated in sterile distilled water was dispensed in the wells (El Oirdi et al., 2021). The plates were pre-incubated overnight ($37 \pm 0.5^\circ\text{C}$) under anaerobiosis to allow the diffusion into the agar. Distilled water was used as a control for each application. An agar plug (5 mm diameter) containing structures of the tested *Colletotrichum* strain was transferred to the center of the MRS plate overlaid with PDA and incubated aerobically ($30 \pm 0.5^\circ\text{C}$) for 5 days. The percentage of the mycelial growth inhibition (MGI%) was calculated as described in Eq. (1).

2.4.3. Competition for nutrients

The dual-culture method evaluated the ability of the lyophilized mixed LAB culture to compete for nutrients with *Colletotrichum* strains at varied nutrient concentrations (Mpeluza et al., 2023). PDA and MRS media were prepared at four different concentrations: full strength concentration (100 %), half nutrient concentration (50 %), one-fourth of the full nutrient concentration (25 %), and one-tenth of the full nutrient

concentration (10 %). PDA was overlaid with MRS agar, and the mixed LAB culture ($9\text{--}10 \log \text{CFU/mL}$) was streaked on the side of the medium (with bacteriological loop, $10\text{--}\mu\text{L}$). At the same time, a 5 mm plug of *Colletotrichum* mycelia was placed on the other side of the plate and incubated aerobically ($30 \pm 0.5^\circ\text{C}$) for 5 days. The percentage of the mycelial growth inhibition (MGI%) was calculated as described in Eq. (1).

2.5. Preparation of coatings with sodium alginate loaded with mixed LAB culture

Sodium alginate (1.5 g/100 mL) was dissolved in distilled sterile heated water (70°C) under stirring (240 rpm) for 30 min until its complete dissolution (Iniguez-Moreno et al., 2021). Glycerol (0.75 g/100 g) was incorporated into the sodium alginate dispersion to increase the flexibility of the formulated coatings. The surfactant Tween 80 (0.05 g/100 g) was incorporated into the coatings to improve their adherence to the fruit. Sunflower oil (0.04 g/100 g) was incorporated into the coatings as a source of lipids to increase the water barrier characteristics. All ingredients were stirred for 10 min at room temperature until their complete dissolution (Shigematsu et al., 2018). After cooling the prepared dispersion ($30 \pm 1^\circ\text{C}$), the lyophilized mixed LAB culture was resuspended in the dispersion (Khodaei and Hamidi-Esfahani, 2019) to achieve a final cell concentration between 9.5 and $10.5 \log \text{CFU/mL}$ (Speranza et al., 2018). A sodium alginate coating with glycerol (0.75 g/100 g) without LAB was also applied. Calcium chloride (2 g/100 g) dispersed in sterile distilled water was applied to the fruit after coating to induce a gelling mechanism and crosslinking reaction (Oliveira et al., 2021).

Sterile distilled water containing glycerol (0.75 g/100 g) was used as a negative control, and commercial formulations of the fungicides thiophanate-methyl ($10 \mu\text{g i.a./mL}$) and difenoconazole ($0.5 \mu\text{g i.a./mL}$) were used as a positive control.

2.6. Enumeration of viable cell counts of the mixed LAB culture on sodium alginate coatings

The viable cell counts of the mixed LAB culture incorporated into the sodium alginate coatings were carried out after formulation and partial coating dehydration. A 10-mL aliquot of each coating formulation was aseptically transferred to sterile Petri dishes and placed for drying for approximately 24 h ($37 \pm 0.5^\circ\text{C}$) in a biosecurity cabinet (Iniguez-Moreno et al., 2021). On different room temperature ($25 \pm 0.5^\circ\text{C}$) and refrigerated ($4 \pm 0.5^\circ\text{C}$) storage time intervals (days 1, 6, 9, 12, and 15), the coating forming solution (1 mL) and dry coating (1g) were diluted in 9 mL of sterile peptone water (0.1 g/100 mL) and homogenized with a sterile mechanical shaker (180 rpm) during 10 min. Serial dilutions (1:9 v/v) were prepared in the same diluent and plated on MRS agar. Plates were incubated ($37 \pm 0.5^\circ\text{C}$) for 48 h under anaerobic conditions, and the results were expressed as CFU/g or CFU/mL (de Simone et al., 2024).

2.7. Staining procedure

The strains forming the mixed LAB culture were simultaneously recovered from the coating, washed with phosphate-buffered saline (PBS) by centrifugation ($4500 \times g$, 10 min, 4°C), and subjected to a propidium iodide staining process (PI, Sigma-Aldrich, $10 \mu\text{g/mL}$) and carboxyfluorescein diacetate (cFDA, Sigma-Aldrich, $2.5 \mu\text{g/mL}$) for 15 min in the dark to assess the cytoplasmic membrane integrity and cell membrane potential, respectively. After staining, cell suspensions were washed with PBS (1:1 v/v) by centrifugation ($4500 \times g$, 10 min, 4°C) to remove excess dye. Staining, data acquisition, and analysis with a flow cytometer were performed as previously described (BD Accuri C6, Becton Dickinson, Franklin Lakes, NJ, USA) (de Sousa Guedes and de Souza, 2018; Rodrigues et al., 2022). Cell subpopulations characterized

Table 1. *Colletotrichum* isolates used in the tests.

as PI-cFDA+ were considered non-permeabilized cells with enzymatic activity (live cells), and cell subpopulations characterized as PI + cFDA- were considered permeabilized cells without enzymatic activity (dead cells).

2.8. Evaluation of the effects of coatings on the development and severity of anthracnose in guava and mango

Four coating formulations were evaluated for each fruit: negative control (fruit coated with sterile distilled water containing glycerol (0.75 g/100 g), sodium alginate (fruit coated with sodium alginate), sodium alginate + LAB (fruit coated with sodium alginate and mixed culture of LAB), and positive controls [thiophanate-methyl (10 µg a.i./mL) and difenoconazole (0.5 µg a.i./mL)]. The surface (epidermis) of each fruit (guava or mango) was wounded (five wounds per fruit) with a sterilized needle (3 mm deep and 2 mm wide) in the middle region on the same fruit side. Each fruit was carefully immersed in 400 mL of the coating-forming solution for 2 min and in calcium chloride solution (2 g/100 mL) for 1 min to allow crosslinking of the polymer in the sample. This interaction enhances the coating stability, making it firmer and facilitating adhesion to the fruit surface (Frent et al., 2022).

Fruit was air-dried on absorbent filter papers and maintained in a biosafety cabinet for 2 h for complete drying. An agar plug (5 mm diameter) containing cellular structures of the *Colletotrichum* strains, obtained from the margin of a 7-day-old colony on PDA (25 ± 0.5 °C, 7 days), was inoculated on the puncture point of an individual fruit. The agar plug was fixed with tape on the fruit surface (Braga et al., 2019). The fruits were distributed individually in polyethylene containers lined with paper towels previously moistened with sterile distilled water to form a humid chamber and covered with a commercially sterile plastic bag to keep a high relative humidity (approximately 85 %). Containers were maintained at room temperature (25 ± 0.5 °C, mimicking fruit during retail commercialization), and humidity chambers were removed after 48 h (de Oliveira et al., 2017). On different storage time intervals (days 6, 9, 12, and 15), the fruits were examined for visible characteristic anthracnose symptoms and anthracnose lesion diameter measurements (Braga et al., 2019). The fruits were evaluated from day 6–15 of storage because the first visible signs of anthracnose lesions arose from day 6 of storage onward in coated and uncoated guava and mango regardless of the *Colletotrichum* isolate used to contaminate them.

The percentage of anthracnose lesion diameter reduction (ALDR%) was determined considering the difference of the lesion diameter in fruit coated with sodium alginate + LAB mixed dispersion when compared to the lesion diameter in uncoated fruit (negative control) and calculated with the equation:

$$\%ALDR = [(N - F) / N] \times 100 \quad (\text{Eq. 2})$$

where N is the anthracnose lesion diameter in uncoated fruit (negative control), and F is the anthracnose lesion diameter in coated fruit.

The severity index (%) of anthracnose lesions in guava and mango was determined considering the fruit surface area and the anthracnose lesion area. The fruit surface area was determined based on the more proximate fruit geometric form (ellipse) using measures of length (r1) and width (r2) radius according to the following equation:

$$\text{Area} = r1 \times r2 \times \pi.$$

The severity index (%) was calculated with the equation (Macedo, Lundgren, Braga, de Souza, & Câmara, 2020):

$$\% \text{Severity index} = [(FA - LA) / FA] \times 100 \quad (\text{Eq. 3})$$

where FA is the total surface area of the fruit, and LA is the area of the anthracnose lesion on the evaluated fruit.

2.9. Statistical analysis

The effects on the fungal mycelial growth were analyzed in triplicate in three independent experiments. The analysis of the anthracnose lesion development in fruit was done considering a completely randomized experimental design with three replicates for each experimental group (treatments) and eight fruits per replicate in three independent experiments. The results were expressed as average ± standard deviation. Kolmogorov-Smirnov normality test assessed whether the obtained data had a normal distribution. Levene's test determines if the data satisfied the homogeneity assumption (equal variance). Analysis of variance (ANOVA) followed by post hoc Tukey or Duncan test determined differences ($p < 0.05$) among the results. The Sigma Stat 3.5 software (Jandel Scientific Software, San Jose, CA, USA) was used for the statistical analysis.

3. Results and discussion

3.1. Antifungal effects of the mixed LAB culture on *Colletotrichum* spp. in vitro

Initially, the agar spot method evaluated the antifungal activity of the lyophilized mixed LAB culture against ten different *Colletotrichum* isolates. The mixed LAB culture strongly inhibited the mycelial growth of all *Colletotrichum* isolates, with %MGI values ranging from 77 to 100 % (total inhibition) (Table 2). *C. fructicola* LM 680, *C. siamense* CMM 4077, and *C. gloeosporioides* CMM 320 were the target isolates most sensitive to the mixed LAB culture ($p < 0.05$), with a total growth inhibition (%MGI 100 %).

In the well-diffusion method, the mycelial growth inhibition achieved >90 % for three *Colletotrichum* isolates and 100 % for the other seven isolates tested (Table 2). In this method, the *Colletotrichum* isolates were more sensitive to the mixed LAB culture than the spot agar method ($p < 0.05$).

Different *in vitro* methods allowed evaluation of the effects of the antagonistic activity of the mixed LAB culture against ten different *Colletotrichum* isolates from species causing anthracnose in guava and mango. Combining various methods should be more efficient for selecting bacteria as antagonists in fungal biocontrol strategies.

The antagonistic effects of the LAB comprising the mixed LAB culture used in this study have already been evaluated against different *Colletotrichum* isolates. However, the tests were carried out with each LAB strain solely using *in vitro* methodologies and when applied to fruits (Fernandes, de Oliveira, de Souza, 2021). When tested solely, *L. brevis*

Table 2
Mycelial growth inhibition (MGI%, average ± standard deviation) caused by mixed culture of lactic acid bacteria.

<i>Colletotrichum</i> isolates/Methods	MGI %	
	Spot	Well diffusion
<i>C. tropicale</i> LM 679	92.23 (±2.83) ^{Pa}	91.24 (±1.58) ^{Pa}
<i>C. fructicola</i> LM 680	100.00 (±0.00) ^{Aa}	100.00 (±0.00) ^{Aa}
<i>C. siamense</i> LM 681	85.26 (±2.40) ^{Cb}	92.53 (±2.21) ^{Pa}
<i>C. chrysophyllum</i> LM 685	78.53 (±2.98) ^{Cb}	91.96 (±1.71) ^{Pa}
<i>C. asianum</i> CMM 4057	80.74 (±1.68) ^{Cb}	100.00 (±0.00) ^{Aa}
<i>C. fructicola</i> CMM 4069	81.201 (±2.28) ^{Cb}	100.00 (±0.00) ^{Aa}
<i>C. tropicale</i> CMM 4071	79.45 (±1.43) ^{Cb}	100.00 (±0.00) ^{Aa}
<i>C. siamense</i> CMM 4077	100.00 (±0.00) ^{Aa}	100.00 (±0.00) ^{Aa}
<i>C. karstii</i> CMM 4101	77.54 (±1.98) ^{Cb}	100.00 (±0.00) ^{Aa}
<i>C. gloeosporioides</i> CMM 320	100.00 (±0.00) ^{Aa}	100.00 (±0.00) ^{Aa}

Results are expressed as percent inhibition rates of radial mycelial growth compared with control treatment (without adding LAB).

^{A–D}: Average values in the same column with different uppercase letters are significantly different ($p < 0.05$), based on Tukey's test.

^{a–c}: Average values in the same row with different lowercase letters are significantly different ($p < 0.05$), based on Duncan's test.

Table 2. Mycelial growth inhibition (MGI%, average ± standard deviation) caused by mixed culture of lactic acid bacteria.

59, *L. pentosus* 129, and *L. fermentum* 263 inhibited the mycelial growth of the target *Colletotrichum* isolates, with inhibition percentage ranging from 55 to 100 % in the agar spot method and 100 % in the agar-well diffusion method (Fernandes, de Oliveira, de Souza, 2021). In the agar well diffusion method, more mixed LAB culture is added to each well (100 µl) than the agar spot method (10 µL of mixed LAB inoculum in each spot). This difference may intensify the antagonistic effects, i.e., the production of antifungal metabolites and competition for space, of LAB against *Colletotrichum* isolates (El Oirdi et al., 2021; Mpeluza et al., 2023).

A previous study reported that LAB strains inhibited the mycelial growth of *C. gloeosporioides* isolated from mango (Konsue et al., 2020). The LAB strains could produce antifungal metabolites inhibitory to *C. gloeosporioides* by acidifying the growing medium. *Bacillus atrophaeus* DM6120 also suppressed the mycelial growth of *Colletotrichum nymphaeae* (54.92 %) when measured with the dual culture method (Alijani et al., 2022).

In addition to producing organic acids, LAB may present other antagonistic action mechanisms, such as the production of hydrolytic enzymes, competition for space, and parasitism (Hirozawa et al., 2023). LAB (*L. plantarum*, *L. paracasei*, and *L. pentosus*) isolated from fermented beverages showed inhibitory activity near 100 % against *C. gloeosporioides*. These effects were linked to producing a wide range of antifungal compounds, such as bacteriocins, hydrogen peroxide, carbon dioxide, and natamycin, which increase their antifungal capacity (Barrios-Salgado et al., 2022).

L. fermentum and *L. plantarum* were effective in inhibiting *C. gloeosporioides* *in vitro*, and the mechanisms underlying these effects could be linked to the production of some metabolites, including organic acids, fatty acids, and antifungal peptides. Organic acids directly contribute to this inhibition as they penetrate the cytoplasm, reducing intracellular pH and causing a decrease in the activity of target cells. Antifungal peptides, in turn, are responsible for creating small channels in the lipid layer of the cell membrane, facilitating interactions with target microorganisms that interrupt their metabolism and pathogenicity (Ranjith et al., 2021).

3.2. Effects of the mixed LAB culture on nutrient competition and *Colletotrichum* mycelial growth

Mixed LAB culture and *Colletotrichum* isolates were used to determine the competition for nutrients using PDA and MRS agar at four different concentrations (100 %, full nutrient concentration; 50 %, half-nutrient concentration; 25 %, one-fourth nutrient concentration; 10 %, one-tenth nutrient concentration). The results showed that the mixed LAB culture was more efficient against most of the target *Colletotrichum* isolates when the nutrient concentrations were higher (100 and 50 % total nutrient concentration). At reduced nutrient concentrations (25 and 10 %), the mixed LAB culture efficacy was lower ($p > 0.005$) against all *Colletotrichum* isolates tested (Table 3).

The nutrient competition test was performed to indicate possible action mechanisms of the LAB strains forming the mixed culture. The results showed that the demand for nutrients in the mixed LAB culture may be more significant in ensuring the production of antifungal metabolites than in the single cultures. Possibly, when cultivated in low nutrient concentrations (10 and 25 %), *L. plantarum* 21 and *L. plantarum* 56 completely inhibited (100 %) the *C. gloeosporioides* mycelial growth. However, when the PDA concentration was 100 %, the inhibition ranged from 55 to 60 % (Mpeluza et al., 2023).

The mixed LAB culture showed remarkable inhibitory effects against the target *Colletotrichum* isolates *in vitro*. However, in addition to the antagonistic effects, the ability of the mixed culture to maintain viable cell counts in the polymeric coatings is a crucial factor to be considered for its application in fruits (Bambace et al., 2024). This is a relevant technological characteristic since the concentration of the inoculated LAB cells and their survival over time should directly influence the

Table 3
Mycelial growth inhibition (MGI%, average $\pm$ standard deviation) caused by mixed culture of lactic acid bacteria grown in different concentrations of PDA and MRS agar.

<i>Colletotrichum</i> isolates	MGI %			
	Comp. nutrients (100 %)	Comp. nutrients (50 %)	Comp. nutrients (25 %)	Comp. nutrients (10 %)
<i>C. tropicale</i>	61.03	47.10	39.94	24.18
IM 679	(± 1.75) ^{Aa}	(± 1.66) ^{Ab}	(± 1.96) ^{Ac}	(± 1.27) ^{ABd}
<i>C. fructicola</i>	32.59	33.17	20.31	10.63
IM 680	(± 2.04) ^{Ba}	(± 1.84) ^{Ca}	(± 2.07) ^{Bb}	(± 2.77) ^{Bc}
<i>C. siamense</i>	55.37	46.30	30.80	17.36
IM 681	(± 1.81) ^{Ba}	(± 1.05) ^{Ab}	(± 2.05) ^{Bc}	(± 2.17) ^{CDd}
<i>C. chrysophilum</i>	42.37	33.77	12.57	8.19
IM 685	(± 1.21) ^{Ca}	(± 1.54) ^{Cb}	(± 1.71) ^{Bc}	(± 1.30) ^{Bc}
<i>C. asiarum</i>	27.08	24.56	24.15	19.24
CMM 4057	(± 2.08) ^{Ba}	(± 1.60) ^{Dab}	(± 2.57) ^{BCDab}	(± 1.82) ^{BCb}
<i>C. fructicola</i>	33.02	26.05	22.47	11.31
CMM 4069	(± 1.42) ^{Ba}	(± 1.53) ^{Bb}	(± 2.25) ^{CDb}	(± 1.89) ^{Bc}
<i>C. tropicale</i>	22.87	39.94	28.00	26.51
CMM 4071	(± 1.24) ^{Bc}	(± 2.23) ^{Ba}	(± 1.60) ^{BCb}	(± 1.64) ^{Ab}
<i>C. siamense</i>	28.13	24.72	30.06	11.05
CMM 4077	(± 2.12) ^{Dab}	(± 1.27) ^{Bb}	(± 1.25) ^{Bca}	(± 1.86) ^{Bc}
<i>C. karstii</i>	41.33	48.75	44.09	12.12
CMM 4101	(± 1.53) ^{Cb}	(± 2.53) ^{Aa}	(± 3.82) ^{Aab}	(± 1.74) ^{Dc}
<i>C. gloeosporioides</i>	38.89	29.56	21.67	12.59
CMM 320	(± 1.85) ^{Ca}	(± 2.37) ^{CDb}	(± 2.08) ^{CDc}	(± 2.12) ^{DEd}

Results are expressed as percent inhibition rates of radial mycelial growth compared with control treatment (without adding LAB). 100 % - full strength; 50 % - half-strength; 25 % - one-fourth concentration; 10 % - one-tenth concentration.

A–E; Average values in the same column with different uppercase letters are significantly different ($p < 0.05$), based on Tukey's test.

a–d; Average values in the same row with different lowercase letters are significantly different ($p < 0.05$), based on Tukey's test.

inhibitory effects against the target microorganisms, impacting the success of the control process (Zhou et al., 2022).

3.3. Survival of lactic bacteria in coating-forming solutions and dried coating

Fig. 1 shows the viable cell counts of the LAB strains composing the mixed culture in the coating-forming solutions and dry coating stored under different conditions (25 ± 0.5 °C and 4 ± 0.5 °C for 15 days). On the first day of storage, there was a drop ($p < 0.05$) in the viable cell counts of the mixed LAB culture in the coating-forming solutions and dry coating, regardless of the storage temperature. From day 6 onwards,

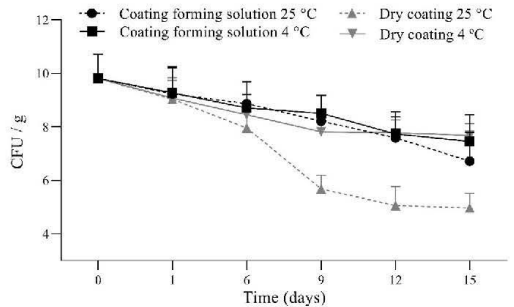


Fig. 1. Viable cell counts (CFU/g; average $\pm$ standard deviation) of mixed culture of lactic acid bacteria loaded into sodium alginate coating forming solution and dry coating during 15 days of room temperature storage (25 ± 0.5 °C and 4 ± 0.5 °C).

Table 3. Mycelial growth inhibition (MGI%, average $\pm$ standard deviation) caused by mixed culture of lactic acid bacteria grown in different concentrations of PDA and MRS agar.

Figure 1 - Viable cell counts (CFU/g; average $\pm$ standard deviation) of mixed culture of lactic acid bacteria loaded into sodium alginate coating forming solution and dry coating during 15 days of room temperature storage (25 ± 0.5 °C and 4 ± 0.5 °C).

there was a gradual decrease in the viable cell counts, which was more pronounced in the dry coating stored at 25 °C. At the end of the evaluated period (15 days), coating-forming solutions and dry coating stored at 4 °C had higher viable cell counts ($>7.5 \log \text{CFU/g}$) than those stored at 25 °C ($p < 0.05$). During refrigeration storage, the coating-forming solution and dry coating maintained high viable cell counts ($>7 \log \text{CFU/g}$).

Lyophilization is the most used drying method for LAB preservation, as it could lead to higher bacterial survival rates than most other drying techniques (Wang et al., 2024) despite being an energy-, time-, and cost-consuming method (Gagnet et al., 2024). Furthermore, dried LAB powders are convenient for the food industry and consumer markets due to their easy transportation and stable storability (Han et al., 2021). A cryoprotectant is essential during drying to maintain high viable bacterial cell counts. FOS are among the most used cryoprotectants to protect bacteria during drying (Han et al., 2021) and application of LAB to films (Oliveira-Alcantara et al., 2020). In a process involving dehydration, such as the production of films and coatings, the protective effects of prebiotics could be related to the glass transition phenomenon, as well as to their interaction with proteins and phospholipids from the membrane, acting as stabilizers during dehydration (Oliveira-Alcantara et al., 2020).

These materials were indicated as suitable carriers, providing a favorable environment for the survival of the tested LAB strains, even when stored at a higher temperature (25 °C). Temperature, water activity, pH, and oxygen influence bacterial survival (Seyedzadeh-Hashemi et al., 2023; Silva et al., 2021). However, polymeric matrices (e.g., sodium alginate) can reduce the impact of these factors by increasing the resistance of cells to environmental conditions through interaction with the matrix (Shahrampour et al., 2020; Wang et al., 2022). At the end of the storage period, the highest LAB viable cell counts were detected in coating and dry films stored under refrigeration (4 °C). Probiotic survival was also more significant under low temperatures in carboxymethylcellulose-based symbiotic films (Seyedzadeh-Hashemi et al., 2023).

The growth capacity of the mixed LAB culture on all films showed a decreasing trend during storage. This viable cell count reduction can be attributed to increased osmotic pressure and decreased nutrient availability during storage (Yang et al., 2023). The viable cell counts of *Lactobacillus acidophilus* and *L. plantarum* added to edible films

containing protein (whey protein isolate) and carbohydrate (carrageenan) decreased after the drying period. This result may be attributed to the drying temperature (25 °C), leading to the decline in bacterial cell counts (Sogut et al., 2022).

3.4. Physiological status of mixed LAB culture in coating-forming solutions and dried coating

Table 4 shows the cell subpopulation sizes of the mixed LAB culture loading the coating-forming solution and the dry coating double-stained with PI and cFDA during 15 days of room and refrigeration temperature storage.

The PI-cFDA + cell subpopulation from mixed culture LAB showed sizes between 22.58 and 31.73 % in coating-forming solution and 38.52–56.08 % in dry coating after 15 days of storage at room and refrigeration temperature. Under these conditions, the PI + cFDA-cell subpopulations were 4.71–10.37 % in the coating-forming solution and 17.71–3.63 % in the dried coating. The cell subpopulations with non-permeabilized membrane (PI-cFDA+) showed the highest sizes in the dry coating, mainly on days 9 or 12 of refrigerated storage (58.38–60.67 %). During this same storage period, the coating-forming solution under refrigerated storage kept high live cell subpopulations (46.96–50.11 %). In both conditions, viable cell counts were high (around $7.7 \log \text{CFU/mL}$). The results indicate a possible adaptation of LAB cells in the mixed culture, with increased viable cell counts under some storage conditions, especially in the dry coating under refrigerated storage.

The percentage of viable and metabolically active cells of the mixed LAB culture maintained in the coating solution and the dry coating during storage may be linked to the ability of some LAB to resist stressors. When challenged with different types of stress, LAB can develop various protective/adaptive strategies, allowing it to withstand adverse conditions and rapid environmental changes (e.g., temperature, pH, water activity). This tolerance is facilitated by synthesizing specialized biomolecules, such as chaperones, enzymes, antioxidants, and exopolysaccharides (Derumets et al., 2024). Furthermore, the entrapment of LAB cells in the edible coatings and films may be an alternative to overcome the detrimental influence of physicochemical stresses associated with food processing and storage conditions (Sionek et al., 2024). Coating components, such as sodium alginate and FOS, can serve as a substrate source for the maintenance and adaptation of

Table 4

Sizes (%; average $\pm$ standard deviation; n: 3) of subpopulations with different physiological characteristics of mixed culture of lactic acid bacteria in coating-forming solutions and dried coating during storage (25 and 4 ± 0.5 °C).

Coatings	Storage temperature	Size of cell subpopulation (%)	Day of storage					
			0	1	6	9	12	15
Coating-forming solution	25 (±0.5 °C)	PI-cFDA +	10.02 (±0.34) ^F	19.64 (±0.32) ^D	12.47 (±0.47) ^E	32.82 (±0.17) ^A	24.42 (±0.50) ^B	22.58 (±0.37) ^C
		PI + cFDA -	10.29 (±0.54) ^A	3.46 (±0.47) ^E	8.62 (±0.33) ^B	4.60 (±0.41) ^D	6.55 (±0.43) ^C	4.71 (±0.26) ^D
		PI-cFDA +	4.86 (±0.12) ^F	6.51 (±0.43) ^E	17.95 (±0.22) ^D	46.96 (±0.30) ^B	50.11 (±0.30) ^A	31.73 (±0.23) ^C
		PI + cFDA -	52.40 (±0.35) ^A	26.58 (±0.51) ^A	19.50 (±0.43) ^C	17.68 (±0.50) ^D	8.90 (±0.26) ^F	10.37 (±0.32) ^E
	4 (±0.5 °C)	PI-cFDA +	Nd	28.69 (±0.27) ^D	48.37 (±0.55) ^A	41.76 (±0.21) ^B	41.49 (±0.44) ^B	38.52 (±0.42) ^C
		PI + cFDA -	Nd	10.34 (±0.36) ^E	24.32 (±0.28) ^C	48.43 (±0.50) ^B	49.64 (±0.31) ^A	17.71 (±0.25) ^D
Dried coating	25 (±0.5 °C)	PI-cFDA +	Nd	35.17 (±0.38) ^E	52.78 (±0.48) ^D	58.38 (±0.54) ^B	60.67 (±0.35) ^A	56.08 (±0.28) ^C
		PI + cFDA -	Nd	14.45 (±0.47) ^D	31.00 (±0.22) ^A	25.46 (±0.40) ^C	27.07 (±0.32) ^B	3.63 (±0.32) ^E
		PI-cFDA +	Nd	28.69 (±0.27) ^D	48.37 (±0.55) ^A	41.76 (±0.21) ^B	41.49 (±0.44) ^B	38.52 (±0.42) ^C
		PI + cFDA -	Nd	10.34 (±0.36) ^E	24.32 (±0.28) ^C	48.43 (±0.50) ^B	49.64 (±0.31) ^A	17.71 (±0.25) ^D
	4 (±0.5 °C)	PI-cFDA +	Nd	35.17 (±0.38) ^E	52.78 (±0.48) ^D	58.38 (±0.54) ^B	60.67 (±0.35) ^A	56.08 (±0.28) ^C
		PI + cFDA -	Nd	14.45 (±0.47) ^D	31.00 (±0.22) ^A	25.46 (±0.40) ^C	27.07 (±0.32) ^B	3.63 (±0.32) ^E

Nd: not detected.

^{A–E}: Average values in a same row with different uppercase letters are significantly different ($p < 0.05$), based on Tukey's test.

Table 4. Sizes (%; average $\pm$ standard deviation; n: 3) of subpopulations with different physiological characteristics of mixed culture of lactic acid bacteria in coating-forming solutions and dried coating during storage (25 and 4 ± 0.5 °C).

metabolic activities by LAB (de Simone et al., 2024; Di et al., 2023).

3.5. Effects of sodium alginate coatings loaded with mixed LAB culture on anthracnose lesion development and severity in guava and mango during storage

The effects of coatings with sodium alginate loaded with mixed culture of *L. brevis* 59, *L. pentosus* 129, and *L. fermentum* 263 (sodium alginate + LAB), sodium alginate alone, and chemical fungicides (thiophanate-methyl and difenoconazole) on the development of anthracnose lesions on guava and mango artificially contaminated with different pathogenic *Colletotrichum* isolates were evaluated during 15 days of room temperature storage (Tables 5 and 6; Supplementary material data, S1 and S2). Sodium alginate coating loaded with mixed LAB culture effectively reduced the development of anthracnose lesions on guava and mango contaminated with any of the target *Colletotrichum* strains during storage. However, the ALDR% values were variable among the tested coating formulations and target *Colletotrichum* strains.

Sodium alginate coating loaded with the mixed LAB culture caused ALDR% values in the range of 37.04–78.94 % in guava contaminated with *Colletotrichum* isolates during the evaluated storage period. The highest ALDR% values ($p < 0.05$) were detected on the 15th day of storage in guava regardless of the *Colletotrichum* isolate tested (ALDR% > 70 %). Generally, the lowest ALDR% values ($p < 0.05$) were detected in guava contaminated with *C. tropicale* LM 679 and *C. siamense* LM 681, especially on day 6 of storage. Guava coated with sodium alginate reached ALDR% values ($p < 0.05$) ranging from 35.06 to 48.96 % on day 15 of storage.

Sodium alginate coating loaded with the mixed LAB culture caused ALDR% values ($p < 0.05$) in the range of 43.64–86.43 % in mango contaminated with the tested *Colletotrichum* isolates during the evaluated storage period. The highest ALDR% values ($p < 0.05$) were achieved on day 15 of storage for most of the tested isolates, except in mango contaminated with *C. gloeosporioides* CMM 320, which obtained the highest value on day 9 of storage (ALDR%: 86.43 %). The lowest ALDR% ($p < 0.05$) was generally detected in mango contaminated with *C. karstii* CMM 4101 (ALDR%: 43.64–70.47 %). Mango coated with sodium alginate reached ALDR% values ($p < 0.05$) ranging from 37.40 to 46.27 % on day 15 of storage.

Coatings with sodium alginate alone caused lower ALDR% values (p

< 0.05) in guava and mango compared to coatings with sodium alginate + LAB (ALDR%: 20.37–53.76 % in guava and ALDR%: 13.33–58.19 % in mango) regardless of the tested *Colletotrichum* isolates and evaluated storage. Treatments with traditional chemical fungicides showed the lowest ALDR% values ($p < 0.05$) when compared to sodium alginate + LAB in guava (ALDR% thiophanate-methyl: 4.27–38.41 %; difenoconazole 12.0–51.77 %) and mango (ALDR% thiophanate-methyl: 5.99–35.87; difenoconazole 7.76–34.42 %) during the evaluated storage period.

The analysis of the area under the disease progress curve showed that coatings with sodium alginate + LAB led to the lowest disease severity indices (measured by the area of the damaged fruit; $p < 0.05$) in guava and mango. Uncoated fruit (negative control) or treated with chemical fungicides (thiophanate-methyl and difenoconazole) showed the highest disease severity rates ($p < 0.05$) (Figs. 2 and 3).

C. fructicola LM 680 and *C. chrysophyllum* LM 685 caused the highest disease severity rates in guava, and *C. siamense* CMM 4077 and *C. gloeosporioides* CMM 320 caused the highest disease severity rates in mango.

The application of sodium alginate coatings loaded with mixed LAB culture delayed the development and decreased the severity of anthracnose in guava and mango during 15 days of room temperature storage. The mixed culture of lyophilized LAB impacted the results, with ALDR% higher than the values obtained with the application of isolated LAB strains (Fernandes, de Oliveira, de Souza, 2021). These results indicate that combining LAB strains can generate a synergistic effect, increasing its efficacy in controlling *Colletotrichum* (Simões et al., 2023).

LAB produce organic acids, mainly lactic acid, during their metabolism. The production of organic acids decreases the pH of the environment, making it less favorable for the growth of spoilage microorganisms, particularly fungi (Bangar et al., 2022). In addition, LAB produce other antimicrobial compounds, such as bacteriocins, with detrimental effects on specific pathogens. This selective antimicrobial activity can further aid in the preservation of foods by targeting specific spoilage organisms without affecting beneficial microflora (Yap et al., 2022). Specifically, *L. plantarum* KB2 LAB 03 demonstrated inhibitory effects against *C. coccoides* in artificially contaminated potatoes, with action mechanisms related to producing organic acids (lactic and acetic acid). Protein antifungal compounds, release of reuterin, and hydroxy fatty acids were also associated with the antimicrobial efficacy of LAB

Table 5

Percentage (average $\pm$ standard deviation) of anthracnose lesion diameter reduction in guava cv. Paluma coated with sodium alginate (SA) or loaded with mixed culture of lactic acid bacteria (LAB) strains and inoculated with distinct pathogenic *Colletotrichum* species during 15 days of room temperature storage (25 ± 0.5 °C).

Colletotrichum isolates	Treatments	Day of storage			
		6	9	12	15
<i>C. tropicale</i> LM 679	SA + LAB	37.04 (± 1.70) ^{Ad}	50.93 (± 2.57) ^{Ac}	65.61 (± 1.84) ^{Ab}	74.94 (± 2.47) ^{Aa}
	SA	20.37 (± 1.70) ^{Cc}	38.40 (± 2.88) ^{Bb}	50.00 (± 2.27) ^{Ba}	48.93 (± 1.01) ^{Ba}
	Thiophanate-methyl (10 mg a.i./mL)	26.88 (± 1.88) ^{Bb}	38.41 (± 2.40) ^{Ba}	17.50 (± 1.15) ^{Cc}	12.78 (± 1.36) ^{Dd}
	Difenoconazole (5 mg a.i./mL)	20.87 (± 1.75) ^{Cc}	32.63 (± 1.58) ^{Ba}	21.21 (± 1.39) ^{Cc}	29.07 (± 1.01) ^{Cb}
<i>C. fructicola</i> LM 680	SA + LAB	62.13 (± 1.32) ^{Ac}	70.92 (± 1.39) ^{Ab}	73.07 (± 1.99) ^{Aab}	77.55 (± 1.02) ^{Aa}
	SA	45.38 (± 2.12) ^{Bd}	46.32 (± 1.39) ^{Bc}	48.36 (± 1.79) ^{Bb}	41.36 (± 1.78) ^{Ba}
	Thiophanate-methyl (10 mg a.i./mL)	5.36 (± 1.79) ^{Dc}	21.85 (± 1.34) ^{Da}	11.15 (± 1.88) ^{Db}	4.27 (± 1.69) ^{Dc}
	Difenoconazole (5 mg a.i./mL)	23.81 (± 2.18) ^{Cb}	31.24 (± 1.90) ^{Ca}	34.05 (± 1.43) ^{Ca}	25.00 (± 1.19) ^{Cb}
<i>C. siamense</i> LM 681	SA + LAB	53.33 (± 2.80) ^{Ad}	70.23 (± 2.46) ^{Ac}	74.46 (± 1.53) ^{Ab}	78.94 (± 1.47) ^{Aa}
	SA	26.38 (± 2.19) ^{Cd}	33.02 (± 2.13) ^{Bc}	38.31 (± 1.79) ^{Bb}	46.10 (± 2.35) ^{Ba}
	Thiophanate-methyl (10 mg a.i./mL)	38.33 (± 1.53) ^{Ba}	14.17 (± 2.21) ^{Dc}	9.52 (± 2.18) ^{Dc}	23.56 (± 1.50) ^{Cb}
	Difenoconazole (5 mg a.i./mL)	35.63 (± 2.25) ^{Ba}	21.91 (± 1.15) ^{Cb}	18.00 (± 1.10) ^{Cbc}	12.50 (± 3.01) ^{Dc}
<i>C. chrysophyllum</i> LM 685	SA + LAB	61.79 (± 1.23) ^{Ac}	71.43 (± 1.64) ^{Ab}	70.41 (± 1.45) ^{Ab}	77.53 (± 1.12) ^{Aa}
	SA	51.55 (± 1.97) ^{Ba}	53.76 (± 1.91) ^{Ba}	43.38 (± 1.58) ^{Bb}	35.06 (± 1.78) ^{Bc}
	Thiophanate-methyl (10 mg a.i./mL)	12.34 (± 2.25) ^{Cab}	15.12 (± 1.53) ^{Da}	11.33 (± 1.26) ^{Dab}	7.22 (± 1.42) ^{Db}
	Difenoconazole (5 mg a.i./mL)	51.77 (± 2.01) ^{Ba}	40.80 (± 2.28) ^{Cb}	32.08 (± 1.91) ^{Cc}	12.00 (± 1.87) ^{Dd}

Results are expressed as percentage reduction rates of anthracnose lesion diameter in coated guava compared with the uncoated fruit.

^{A–D}: Average values in a same column, for a same *Colletotrichum* strain. with different uppercase letters are significantly different ($p < 0.05$), based on Tukey's test.

^{a–d}: Average values in a same row with different lowercase letters are significantly different ($p < 0.05$), based on Tukey's test.

Table 5. Percentage (average $\pm$ standard deviation) of anthracnose lesion diameter reduction in guava cv. Paluma coated with sodium alginate (SA) or loaded with mixed culture of lactic acid bacteria (LAB) strains and inoculated with distinct pathogenic *Colletotrichum* species during 15 days of room temperature storage (25 ± 0.5 °C).

Table 6

Percentage (average $\pm$ standard deviation) of anthracnose lesion diameter reduction in mango cv. Palmer coated with sodium alginate (SA) or loaded with mixed culture of lactic acid bacteria (LAB) strains and inoculated with distinct pathogenic *Colletotrichum* species during 15 days of room temperature storage (25 ± 0.5 °C).

Colletotrichum isolates	Treatments	Days of storage			
		6	9	12	15
<i>C. asianum</i> CMM 4057	SA + LAB	65.79 (± 1.53) ^{Ad}	70.04 (± 2.00) ^{Ac}	76.61 (± 1.75) ^{Ab}	83.44 (± 1.71) ^{Aa}
	SA	34.34 (± 2.48) ^{Bb}	46.22 (± 2.04) ^{Ba}	36.79 (± 2.74) ^{Bb}	45.63 (± 1.25) ^{Ba}
	Thiophanate-methyl (10 mg a.i./mL)	34.09 (± 1.90) ^{Ba}	26.94 (± 2.85) ^{Cb}	26.49 (± 2.25) ^{Cb}	10.38 (± 3.33) ^{Cc}
	Difenoconazole (5 mg a.i./mL)	22.39 (± 1.94) ^{Ca}	11.78 (± 1.98) ^{Cc}	10.42 (± 2.25) ^{Cc}	16.78 (± 1.67) ^{Cb}
<i>C. fructicola</i> CMM 4069	SA + LAB	43.64 (± 1.82) ^{Ad}	51.14 (± 2.59) ^{Ac}	72.34 (± 2.33) ^{Ab}	77.80 (± 1.92) ^{Aa}
	SA	27.27 (± 1.82) ^{Bc}	38.72 (± 2.00) ^{Bb}	46.86 (± 2.77) ^{Ba}	42.05 (± 1.14) ^{Bb}
	Thiophanate-methyl (10 mg a.i./mL)	23.81 (± 2.18) ^{Bca}	13.46 (± 2.51) ^{Cb}	9.95 (± 2.28) ^{Cd}	7.20 (± 1.74) ^{Cd}
	Difenoconazole (5 mg a.i./mL)	22.39 (± 1.94) ^{Ca}	18.42 (± 2.59) ^{Ca}	27.56 (± 2.03) ^{Cc}	25.76 (± 2.74) ^{Cc}
<i>C. tropicale</i> CMM 4071	SA + LAB	62.04 (± 2.34) ^{Ab}	74.06 (± 2.22) ^{Aa}	74.28 (± 1.37) ^{Aa}	79.47 (± 1.35) ^{Aa}
	SA	26.20 (± 1.46) ^{Bc}	49.26 (± 1.93) ^{Ba}	50.30 (± 2.46) ^{Ba}	37.40 (± 2.15) ^{Bb}
	Thiophanate-methyl (10 mg a.i./mL)	19.65 (± 2.08) ^{Cb}	19.63 (± 3.08) ^{Cb}	27.88 (± 2.28) ^{Ca}	7.37 (± 2.21) ^{Cc}
	Difenoconazole (5 mg a.i./mL)	13.68 (± 2.19) ^{Cc}	34.42 (± 2.26) ^{Ca}	28.15 (± 1.22) ^{Cb}	32.52 (± 2.15) ^{Ca}
<i>C. siamense</i> CMM 4077	SA + LAB	69.91 (± 1.69) ^{Ac}	79.30 (± 1.61) ^{Ab}	81.41 (± 1.34) ^{Aab}	83.42 (± 1.60) ^{Aa}
	SA	56.68 (± 3.05) ^{Ba}	57.75 (± 1.27) ^{Ba}	52.60 (± 2.39) ^{Bab}	46.27 (± 2.66) ^{Bb}
	Thiophanate-methyl (10 mg a.i./mL)	13.86 (± 1.87) ^{Cc}	39.65 (± 2.19) ^{Ca}	24.22 (± 2.07) ^{Cb}	25.88 (± 2.01) ^{Cb}
	Difenoconazole (5 mg a.i./mL)	19.48 (± 1.87) ^{Ca}	10.74 (± 1.46) ^{Cbc}	7.76 (± 1.49) ^{Cc}	15.00 (± 1.99) ^{Cab}
<i>C. karstii</i> CMM 4101	SA + LAB	45.08 (± 2.40) ^{Ac}	46.89 (± 1.02) ^{Abc}	52.41 (± 2.25) ^{Ab}	70.47 (± 2.39) ^{Aa}
	SA	20.95 (± 2.52) ^{Cb}	13.33 (± 1.33) ^{Cc}	17.96 (± 1.40) ^{Cbc}	39.11 (± 2.27) ^{Ba}
	Thiophanate-methyl (10 mg a.i./mL)	35.87 (± 2.40) ^{Ba}	28.00 (± 2.40) ^{Bb}	22.78 (± 2.22) ^{BCc}	25.71 (± 2.08) ^{Cbc}
	Difenoconazole (5 mg a.i./mL)	15.49 (± 1.48) ^{Cb}	20.00 (± 3.33) ^{Cab}	23.38 (± 2.02) ^{Ba}	19.05 (± 1.59) ^{Cab}
<i>C. gloeosporioides</i> CMM 320	SA + LAB	81.71 (± 1.51) ^{Ab}	86.43 (± 0.88) ^{Aa}	77.14 (± 2.58) ^{Ac}	75.33 (± 1.30) ^{Ac}
	SA	51.43 (± 2.86) ^{Bb}	58.19 (± 1.83) ^{Ba}	49.00 (± 1.17) ^{Bb}	41.99 (± 2.70) ^{Bc}
	Thiophanate-methyl (10 mg a.i./mL)	7.95 (± 1.18) ^{Cb}	24.07 (± 2.50) ^{Ca}	9.54 (± 1.63) ^{Cb}	5.99 (± 1.72) ^{Cc}
	Difenoconazole (5 mg a.i./mL)	9.74 (± 1.94) ^{Cb}	9.59 (± 2.46) ^{Cb}	10.77 (± 3.08) ^{Cab}	16.48 (± 2.34) ^{Ca}

Results are expressed as percentage reduction rates of anthracnose lesion diameter in coated guava compared with the uncoated fruit.

^{A–D}: Average values in a same column for a same *Colletotrichum* isolate with different uppercase letters are different ($p < 0.05$), based on Tukey's test.

^{a–d}: Average values in a same row with different lowercase letters are different ($p < 0.05$), based on Tukey's test.

(Stęglińska et al., 2022).

The three tested LAB strains added separately to the coating have proven also antimicrobial activity. The antifungal activity of *L. brevis* has been attributed to the production of organic acids, such as lactic acid, acetic acid, and phenylacetic acid. These organic acids exert antifungal activity due to their penetration into cell membranes in an undissociated form, decreasing intracellular pH and interrupting fungal metabolic activities (Silva et al., 2023). *L. fermentum* demonstrated antimicrobial activity linked to producing bacteriocins with high hydrophobic residues and a net negative charge, increasing membrane disruption in pathogens and the electrostatic binding between peptides and the cell membrane (Wai et al., 2022). The potent inhibitory activity of *L. pentosus* against fungi was attributed to the production of lactic acid and acetic acid. The lipophilic nature and protonated form of these organic acids at low pH could facilitate their diffusion through the fungal membrane and cause damage to fungal cells due to accumulation in a dissociated form in the cytoplasm (Simões et al., 2023). The synergistic or additive effect of the multiple compounds produced may account for the antifungal efficacy of the tested strains when applied in mixed inclusions (Simões et al., 2023).

Additionally, some LAB strains can influence host plant defense responses by stimulating signaling pathways related to the expression and activity of phenylalanine ammonium lyase (PAL), phenol peroxidase (POX), and polyphenol oxidase (PPO) enzymes and phenolic compounds accumulation (Pruthviraj et al., 2024), increasing the resistance against phytopathogens, such as *Colletotrichum*. Compounds synthesized by LAB can also increase cell wall stability and enhance the antioxidant capacity of plant cells through signaling pathways that modulate plant defense responses, including salicylic acid (Jaffar et al., 2023).

It is also worth noting that the efficacy of sodium alginate coatings loaded with the mixed LAB culture to reduce the development of anthracnose lesions remained high at the end of the storage period.

These results may be associated with the increased concentration of compounds with antifungal properties produced by LAB, which may be interconnected in the food matrix. Incorporating live LAB cells into edible coatings favors the maintenance of adequate concentrations of antimicrobial compounds on food surfaces, improving their antimicrobial effects (Li, Cheng, et al., 2024). It is also worth mentioning that the differences in lesion diameter values observed in fruits can be attributed to modifications in the metabolic activity of LAB in coated matrices, given the availability of nutrients to produce antimicrobial compounds (de Simone et al., 2024).

Variations in storage temperature could also modify the antifungal potential of mixed LAB cultures applied in the sodium alginate coatings. Low temperatures can slow the fruit ripening process, reducing the availability of essential nutrients for fungal development (García et al., 2022). Lower temperatures can also reduce the metabolic activity of fungi, making them less efficient in the fruit infection process, which would enhance the effectiveness of the coating (Martínez-Blay et al., 2020). In addition, in the feasibility tests of this study, it was observed that mixed LAB cultures added to sodium alginate coatings remained physiologically active at low temperatures, which could be favorable in combining treatment with refrigeration. The combination of different technologies positively impacts expanding fruit storage, emphasizing the application of low temperatures during the supply chain (Silués et al., 2022; Álvarez et al., 2021).

Another significant result was the greater efficacy of sodium alginate coatings loaded with mixed LAB culture to inhibit anthracnose development in guava and mango compared to commercial synthetic fungicides commonly applied to control this disease. The control of diseases caused by *Colletotrichum* still depends mainly on using fungicides. Consequently, *Colletotrichum* species can develop field resistance to fungicides (Ishii et al., 2022). In guava, the isolate that showed the most significant resistance to treatments with chemical fungicides was

Table 6. Percentage (average $\pm$ standard deviation) of anthracnose lesion diameter reduction in mango cv. Palmer coated with sodium alginate (SA) or loaded with mixed culture of lactic acid bacteria (LAB) strains and inoculated with distinct pathogenic *Colletotrichum* species during 15 days of room temperature storage (25 ± 0.5 °C).

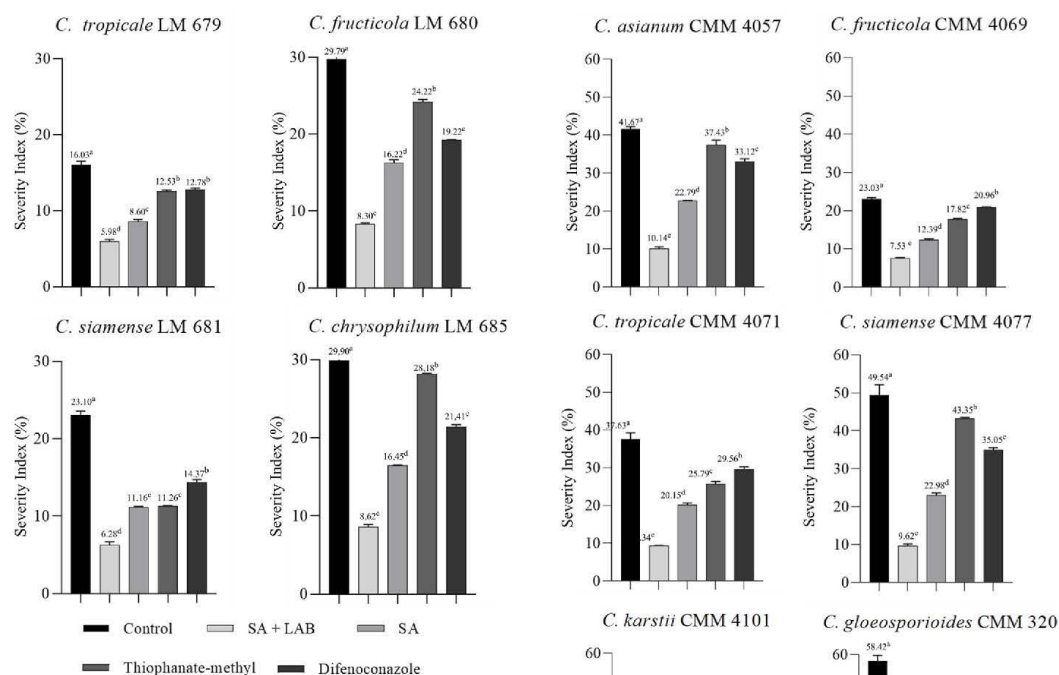


Fig. 2. Severity index (%) of anthracnose lesion in guava cv. Paluma coated with sodium alginate (SA) alone or loaded with mixed culture of lactic acid bacteria (LAB), thiophanate-methyl (10 mg a.i./mL) or difenoconazole (5 mg a.i./mL) and inoculate with different pathogenic *Colletotrichum* species after 15 days of room temperature storage (25 ± 0.5 °C). a-e Average values followed by different letters for the same fungal isolate are significantly different ($p < 0.05$), based on Tukey's test.

C. chrysophilum, which caused an anthracnose lesion severity index close to that obtained in control fruits. In mango, *C. asianum* showed the most significant resistance to chemical fungicides. The resistance of different *Colletotrichum* isolates to thiophanate-methyl has been linked to mutation points in the β -tubulin gene (Wu et al., 2022), while the resistance to difenoconazole has been linked to mutations in the cytochrome *b* gene (Carraro et al., 2024).

In previous studies, sodium alginate coating treatments also effectively reduced fruit postharvest spoilage. Sodium alginate coatings produce a barrier effect on the fruit surface, which inhibits the gas exchange of oxygen and carbon dioxide due to perspiration and respiration, delaying fruit senescence and pathogen infection (Hasan et al., 2022; Zhang et al., 2024).

4. Conclusion

The mixed culture of *L. brevis* 59, *L. pentosus* 129, and *L. fermentum* 263 inhibited the mycelial growth of ten pathogenic *Colletotrichum* isolates. Incorporating the mixed LAB culture in a sodium alginate-based coating maintained high viable cell counts and large subpopulation sizes of physiologically active cells after 15 days of storage, mainly under refrigeration. Applying the sodium alginate coating incorporated with the mixed LAB culture effectively controlled the development and reduced the anthracnose severity in guava and mango artificially contaminated with different *Colletotrichum* isolates during 15 days of storage. Therefore, using sodium alginate coatings and mixed LAB

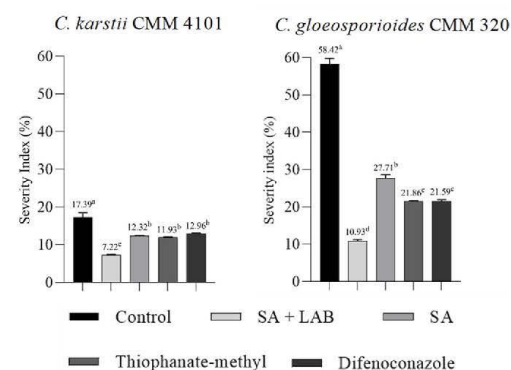


Fig. 3. Severity index (%) of anthracnose lesion in mango cv. Palmer coated with sodium alginate (SA) alone or loaded with mixed culture of lactic acid bacteria (LAB), thiophanate-methyl (10 mg a.i./mL) or difenoconazole (5 mg a.i./mL) and inoculate with different pathogenic *Colletotrichum* species after 15 days of room temperature storage (25 ± 0.5 °C). a-e Average values followed by different letters for the same fungal isolate are significantly different ($p < 0.05$), based on Tukey's test.

culture is a potentially effective alternative to mitigate the pathogen *Colletotrichum* and minimize post-harvest fruit losses. It is also worth noting that the methodologies of this study were designed so that they can be applied in the fruit processing industry, considering the commonly available infrastructure, so that there are no significant changes in the application method used, allowing the replacement of chemical fungicides and waxes traditionally applied by the sodium alginate coating added to the mixed LAB culture. However, although the initial investment in infrastructure, research, and development may be significant, advances in technology, production techniques, and safety can reduce costs over time, making large-scale applications more viable.

To complement the action of sodium alginate coatings containing the

Figure 2 - Severity index (%) of anthracnose lesion in guava cv. Paluma coated with sodium alginate (SA) alone or loaded with mixed culture of lactic acid bacteria (LAB), thiophanate-methyl (10 mg a.i./mL) or difenoconazole (5 mg a.i./mL) and inoculate with different pathogenic *Colletotrichum* species after 15 days of room temperature storage (25 ± 0.5 °C).

Figure 3 - Severity index (%) of anthracnose lesion in mango cv. Palmer coated with sodium alginate (SA) alone or loaded with mixed culture of lactic acid bacteria (LAB), thiophanate-methyl (10 mg a.i./mL) or difenoconazole (5 mg a.i./mL) and inoculate with different pathogenic *Colletotrichum* species after 15 days of room temperature storage (25 ± 0.5 °C).

mixed LAB culture, studies are warranted to determine the physico-chemical, structural, and biodegradation characteristics of the coating and its influence on physicochemical and sensory parameters indicative of the post-harvest quality of guava cv. Paluma and mango cv. Palmer during storage.

CRedit authorship contribution statement

Karina Felix Dias Fernandes: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kataryne Árabe Rimá de Oliveira:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ítalo Fernandes Cirilo:** Methodology, Investigation, Formal analysis, Data curation. **Maiara da Costa Lima:** Methodology, Investigation, Formal analysis, Data curation. **Marcos Paz Saraiva Câmara:** Methodology, Investigation, Conceptualization. **Evandro Leite de Souza:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Funding

The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil, Funding code 001) awarded scholarships to Karina Felix Dias Fernandes (PhD scholarship), Ítalo Cirilo Fernandes (undergraduate scholarship), and Maiara da Costa Lima (Post-doctoral scholarship).

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.foodcont.2025.111341>.

Data availability

Data will be made available on request.

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Supplementary 1. Anthracnose lesion inhibition in guava cv. Paluma coated with sodium alginate (SA) alone or loaded with mixed culture of lactic acid bacteria (SA + LAB) and inoculated with different *Colletotrichum* species after 15 days of room temperature storage (25 ± 0.5 °C).

Fungi	Fruit				
	A	AA	AAA	AAAA	AAAAA
<i>C. tropicale</i> LM 679					
<i>C. fructicola</i> LM 680					
<i>C. siamense</i> LM 681					
<i>C. chrysophilum</i> LM 685					

(A) negative control - lesion severity (uncoated fruit) in guava cv. Paluma; (AA) guava cv. Paluma coated with (SA) and mixed culture of lactic acid bacteria strains; (AAA) guava cv. Paluma coated with SA alone; (AAAA) guava cv. Paluma coated with thiophanate-methyl (10 mg a.i./mL); (AAAAA) guava cv. Paluma coated with difenoconazole (5 mg a.i./mL).

Supplementary 2. Anthracnose lesion inhibition in mango cv. Palmer coated with sodium alginate (SA) alone or loaded with mixed culture of lactic acid bacteria (SA + LAB) and inoculated with different *Colletotrichum* species after 15 days of room temperature storage (25 ± 0.5 °C).

Fungi	Fruit				
	A	AA	AAA	AAAA	AAAAA
<i>C. asianum</i> CMM 4057					
<i>C. fructicola</i> CMM 4069					
<i>C. tropicale</i> CMM 4071					
<i>C. siamense</i> CMM 4077					
<i>C. karstii</i> CMM 4101					
<i>C. gloeosporioides</i> CM 320					

(A) negative control - lesion severity (uncoated fruit) in mango cv. Palmer; (AA) mango cv. Palmer coated with (SA) and mixed culture of lactic acid bacteria strains; (AAA) mango cv. Palmer coated with SA alone; (AAAA) mango cv. Palmer coated with thiophanate-methyl (10 mg a.i./mL); (AAAAA) mango cv. Palmer coated with difenoconazole (5 mg a.i./mL).

APÊNDICE D - Artigo original II

Characterization and preservation performance of a novel sodium alginate coating incorporating a mixed lactic acid bacteria culture on guava and mango

Abstract

This study characterized a sodium alginate (SA)-based coating incorporating a mixed culture of lactic acid bacteria (LAB) (*Lactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129, and *Limosilactobacillus fermentum* 263) and evaluated its effectiveness in maintaining postharvest quality of guava cv. Paluma and mango cv. Palmer during 15 days of room-temperature storage. LAB incorporation modified the polymeric matrix, increasing thermal stability and producing more homogeneous, smooth, pore-free films with lower roughness, thickness, moisture content, water solubility, hygroscopicity, and water activity, while maintaining color and increasing antioxidant capacity. Both coatings exhibited high biodegradability in soil, freshwater, and seawater, with nearly complete degradation within 20 days. Coated fruits showed reduced mass loss and greater retention of color, ascorbic acid, and carotenoids. SA-LAB-coated fruits also maintained better sensory quality, particularly color, aroma, and texture, with delayed apparent ripening. These findings demonstrate the potential of SA-LAB coatings as sustainable materials for preserving fruit quality during postharvest storage.

Keywords: Edible coating; lactic acid bacteria; film characterization; antioxidant activity; tropical fruits; postharvest preservation.

1 Introduction

Guava (*Psidium guajava* L.) and mango (*Mangifera indica* L.) are fruits widely valued for their pleasant taste and high content of vitamins, phenolic compounds, flavonoids, and other antioxidants, characteristics that make them important from both nutritional and economic perspectives (Misra et al., 2025; Qasim et al., 2025). However, due to their climacteric nature, characterized by high respiration rates and intense ethylene production, these fruits exhibit accelerated postharvest metabolism, resulting in rapid ripening, subsequent deterioration, and, consequently, significant losses, thereby reducing storage and marketing periods (Mahapatra et al., 2024; Nguyen et al., 2026).

Several strategies have been employed to reduce commercial losses in fruit production and distribution, including refrigerated storage, controlled atmosphere, and chemical treatments (Gull et al., 2026); however, these approaches have significant limitations. Refrigeration can induce physiological cold disorders in tropical fruits such as guava and mango, thereby restricting their long-distance transport and export (Ali et al., 2022; Chen et al., 2022). Similarly, controlled or modified-atmosphere storage is costly and can lead to heterogeneous ripening (Liu et al., 2025). Additionally, the use of conventional chemical treatments, often based on synthetic agrochemicals, poses risks to human health, including gastrointestinal, neurological, and respiratory effects, as well as environmental impacts, including soil and water pollution and the development of resistant pathogens over time (Gwiazdowski et al., 2023).

Given the limitations of conventional approaches, alternative strategies for preserving the postharvest quality of fruits have been widely explored. In this context, the use of lactic acid bacteria (LAB) has emerged as a promising approach, supported by their Generally Recognized as Safe (GRAS) status granted by the United States Food and Drug Administration (FDA) and Qualified Presumption of Safety (QPS) status from the

European Food Safety Authority (EFSA), particularly for controlling ripening and pathogens (Gajendran; Rajamani, 2025). LAB are widely used as bioprotective agents in foods due to their ability to produce antimicrobial metabolites, including organic acids (e.g., lactic, formic, and phenyllactic acids), antimicrobial peptides (e.g., bacteriocins), hydrogen peroxide, phenolic compounds, and enzymes such as proteases and lipases, as well as their capacity to modulate bioactive compounds and compete with pathogens for space and nutrients (Gajendran; Rajamani, 2025; Huang et al., 2024; Kalkan et al., 2025).

In recent years, different LAB species have been investigated for application in fruits, including *Lactiplantibacillus plantarum* (De Simone et al., 2024; Huang et al., 2024), *Lactobacillus rhamnosus* (Azarakhsh et al., 2024; Kalkan et al., 2025; Li et al., 2024), *Lacticaseibacillus casei* (Dias et al., 2023), and *Lactococcus lactis* (Yang et al., 2023). However, LAB are susceptible to adverse physicochemical and biological conditions on the fruit surface, which can compromise their viability and survival, and, consequently, reduce their efficacy as biopreservative agents on fruits (De Simone et al., 2024; Huang et al., 2024).

Edible coatings can act as delivery systems for active components, including LAB (De Simone et al., 2024). Furthermore, the application of these coatings has proven effective in reducing fruit ripening and spoilage, as they form thin films that act as semi-permeable barriers, reducing water loss, gas exchange, and microbial contamination (Gull et al., 2026). In this context, sodium alginate (SA) stands out as a promising material for carrying LAB. SA is a natural polymer extracted from seaweed and is widely used in the food industry as a thickener, stabilizer, and gelling agent (Wei et al., 2025). Additionally, its biodegradable and non-toxic properties, combined with its ability to form suitable matrices for incorporating probiotic microorganisms, make SA a particularly attractive material for edible coating applications (Kalkan et al., 2025; Pandey et al., 2025).

It is important to note that, to be effective as functional edible coatings, these materials must possess adequate mechanical, thermal, and structural properties that ensure integrity, stability, and performance as barrier systems (Kalkan et al., 2025; Wei et al., 2025). These properties are directly related to their ability to form continuous, uniform coatings, regulate mass transfer, and maintain functional performance during storage and application (Bao et al., 2023). Although a previous study demonstrated the antifungal potential of a novel coating formulated with SA and a mixed culture of LAB comprising *Levilactobacillus brevis*, *Lactiplantibacillus pentosus*, and *Limosilactobacillus fermentum* (Fernandes et al., 2025), its functional characteristics and efficacy in preserving postharvest quality parameters of fruits remain insufficiently explored. This study aimed to evaluate the thermal, physicochemical, morphological, and biodegradability properties of this novel SA-based edible coating incorporating a mixed LAB culture, as well as its performance in preserving the postharvest quality of guava and mango during room-temperature storage.

2 Material and methods

2.1 Raw materials

Guavas cv. Paluma, characterized in the second maturation stage with up to 80% green peel and °Brix of approximately 8 (Gull et al., 2026), and mangos cv. Palmer, characterized in the second stage of maturation, with light green peel and °Brix of approximately 6 (Almeida et al., 2023; Silué et al., 2022), were obtained from EMPASA (Supplies and Services Company of Paraíba, João Pessoa, PB, Brazil). Fruits without physical injury and visual infections were selected and standardized based on size, appearance, color, and shape. Fruits were washed with neutral detergent and potable running water, disinfected surface (sodium hypochlorite, 150 ppm, pH 7.2 adjusted with

1 M NaOH) for 15 min, rinsed with potable water, and dried for 2 h under room temperature (25 ± 0.5 °C) in a safety cabinet (Fernandes et al., 2025). Sodium alginate (Dinâmica Química Contemporânea Ltda., Indaiatuba, SP, Brazil), glycerol (Química Moderna, Barueri, SP, Brazil), sunflower oil (Cargill Agrícola S.A, Recife, PE, Brazil), and Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) were used to formulate the coatings. Calcium chloride (Synth, Diadema, SP, Brazil) was used to form a gel by crosslinking with sodium alginate.

2.2 Mixed lactic acid bacteria culture and growth conditions

Three LAB strains, namely *Levilactobacillus brevis* 59, *Lactiplantibacillus pentosus* 129, and *Limosilactobacillus fermentum* 263, were used for the coating formulation based on results from previous studies demonstrating their antifungal activity against *Colletotrichum* spp. (Fernandes et al., 2021). These strains were isolated from fruit pulp-processing by-products [acerola (*Malpighia emarginata* D.C.), mango (*Mangifera indica* L.), and pineapple (*Ananas comosus* L.)] and were characterized as probiotic candidates with antimicrobial activity against food-contaminating microorganisms (Garcia et al., 2016). Stocks were maintained (-20 ± 0.5 °C) in de Man, Rogosa, and Sharpe (MRS) broth (HiMedia, Mumbai, India) with glycerol (Sigma-Aldrich; 20 mL/100 mL). Working cultures were kept on MRS agar (HiMedia) under refrigeration (4 ± 0.5 °C) and transferred to a new medium monthly. Before use in the assays, each LAB strain tested was cultured in MRS broth at 37 ± 0.5 °C for 20–24 h under anaerobiosis (Anaerobic System Anaerogen, Oxoid, Hampshire, UK). Cells were collected by centrifugation ($4500 \times g$, 10 min, 4 ± 0.5 °C), washed in sterile saline (NaCl, 0.85 g/100 mL), and resuspended in sterile distilled water containing fructooligosaccharides [FOS (20% w/v); cryoprotectant; F8052, Sigma-Aldrich, Saint

Louis, MA, EUA)] to obtain a cell suspension with viable cell counts between 9 – 10 CFU/mL. The mixed cell suspension was prepared by combining suspensions of each strain at a 1:1:1 (v/v) ratio (Fernandes et al., 2025). For lyophilization, the LAB mixed culture suspension added with FOS was transferred to glass vials with lids (2 mL of suspension in each vial) and subjected to freezing (-80 °C, 24 h) (Di et al., 2023). The lyophilization was carried out at -55 ± 2 °C, under a vacuum of <138 µHg, at a lyophilization rate of 1 mm/h for approximately 10 h, using a benchtop lyophilizer (model L-101, Liotop, SP, Brazil).

2.3 Preparation of the coating with SA loaded with mixed LAB culture

The SA (1.5 g/100 mL) was dissolved in sterile distilled water, heated to 70 °C, and stirred at 240 rpm for 30 min until complete solubilization (Mahapatra et al., 2024). Glycerol (0.75 g/100 g), Tween 80 (0.05 g/100 g), and sunflower oil (0.04 g/100 g) were added to the alginate dispersion to improve coating flexibility, fruit adhesion, and water barrier properties, respectively. The mixture was stirred for 10 min at room temperature until complete homogenization. After cooling to 30 ± 1 °C, the lyophilized mixed LAB culture was resuspended in the formulation at 5.5% (w/v) (SA-LAB), yielding a final cell concentration of 9.5-10.5 log CFU/mL (Khodaei & Hamidi-Esfahani, 2019; Speranza et al. 2018). A formulation containing sodium alginate without LAB (SA) was also evaluated.

The films were molded by pouring 10 mL of each coating emulsion into a sterile polystyrene Petri dish (90 mm diameter). The Petri dishes were kept in an incubator to dry the emulsion at room temperature (25 ± 0.5 °C) for approximately 12 h. After drying, the films were stored under refrigeration (4 ± 1 °C) for subsequent analyses (Díaz-Díaz et al., 2023; Mahapatra et al., 2024).

2.4 Films characterization

2.4.1 Fourier transform infrared spectroscopy (FTIR)

Functional groups present in the films were determined in spectra obtained by FTIR spectroscopy. Spectra were obtained over the wavelength range of 400-4000 cm^{-1} , with resolutions of 4 and 64 scans, using Shimadzu FTIR equipment (Model Prestige-21 IR Affinity-1 FTIR 8400S, Kyoto, Japan) (Wu et al., 2024). The measurements were performed in absorbance mode (Díaz-Díaz et al., 2023), and the spectra were analyzed to identify the characteristic functional groups in the film structure, following established methodologies for polysaccharide characterization (Mollah et al., 2023).

2.4.2 Differential Scanning Calorimetry (DSC)

The thermal properties of the films were evaluated by DSC using a TA-60WS calorimeter (Shimadzu, Kyoto, Japan). The samples were subjected to a heating and cooling rate of 10 $^{\circ}\text{C}/\text{min}$ under a flow of dry nitrogen gas (50 mL/min). The analyses were performed on two heating ramps, starting from room temperature up to 300 $^{\circ}\text{C}$, followed by a cooling ramp (Bao et al., 2023).

2.4.3 Scanning electron microscopy (SEM)

The morphology of the samples was evaluated by SEM using a TESCAN VEGA 4th-generation scanning electron microscope (TESCAN, Czech Republic). Before the SEM observations, film samples of similar size were sputter-coated with a conductive gold layer using a G20 ion sputter coater (Ion Sputter Coater, Schaefer-Tec, Italy). The samples were examined using an accelerating voltage of 10 kV (Kalkan et al., 2025). Representative images were subsequently selected to illustrate the characteristic surface morphology of each sample.

2.4.4 Surface roughness analysis

Surface roughness analysis of films was performed using a non-contact 3D optical profiler (Taylor Hobson, Talysurf CCI MP, Leicester, UK), connected to a computerized unit. An 8 μm film cut-off was used with a 50 $\times$ lens (numerical aperture 0.4) and a 1 $\times$ scanning speed in XY mode (0.3 $\mu\text{m} \times 0.3 \mu\text{m}$). Five measurements were taken for each sample: one in the central area and two along the lens's movement (2 mm to the right, left, down, and up), following a cross design. The final roughness of Ra and Sa standards (μm), indicating the average roughness and average height of the film surfaces, respectively, was obtained by averaging five points from film samples (Braga et al., 2020).

2.4.5 Moisture, water solubility, hygroscopicity, and water activity

Moisture content was determined according to Lopes et al. (2025). The film samples were weighed, placed in previously dried Petri dishes, and dried in an oven at 105 $^{\circ}\text{C}$ for 24 h until constant weight. Moisture was calculated using the equation:

$$\text{Moisture (\%)} = [(M1 - (M3 - M2)) / M1] \times 100 \quad (\text{Equation 1})$$

where M1 is the initial mass of the sample, M2 is the mass of the Petri dish, and M3 is the mass of the dish with the sample after drying.

The solubility of the films in water was determined by gravimetry (Santos et al., 2021). The films were placed in Petri dishes and dried in an oven at 105 $^{\circ}\text{C}$ to determine their initial weight. To determine water solubility, the initially dry films were submerged in 50 mL of distilled water and agitated at 150 rpm for 24 h using an orbital shaker (Tecnal, TE-424, SP, Brazil). The insoluble materials were dried at 105 $^{\circ}\text{C}$ for 24 h, and the final weight was determined. Water solubility was calculated using the equation:

201 Solubility (%) = $[(M4 - M5) / M4] \times 100$ (Equation 2)

202 where M4 is the initial mass of the dry film, and M5 is the mass of the remaining insoluble
203 material after immersion in water.

204 For hygroscopicity analysis, an aliquot (one g) of each film was weighed onto
205 Petri dishes and placed in a desiccator containing a saturated NaCl solution (relative
206 humidity $\approx 75\%$) at 25 ± 0.5 °C. The samples were kept in a temperature-controlled
207 chamber (BOD, 25 ± 0.5 °C) for 7 days and weighed daily until they reached hygroscopic
208 equilibrium. Hygroscopicity was expressed as grams of water adsorbed per 100 g of dry
209 mass (Nascimento et al., 2025).

210 The water activity (a_w) of each film was determined using a previously calibrated
211 digital hygrometer (Aqualab 3TE, Decagon, WA, USA), with measurements taken at 25
212 ± 0.5 °C (Silva et al., 2024).

213

214 2.4.6 Color

215 The color parameters of each film sample were measured on a standard white
216 surface using a previously calibrated colorimeter (CR-400, Konica Minolta, Ramsey, NJ,
217 USA). Color measurements were expressed in the CIELAB system (CIE Lab*), including
218 L* (lightness), a* (red/green coordinate), b* (yellow/blue coordinate), as well as chroma
219 (C*) and hue angle (h°) (Kalkan et al., 2025).

220

221 2.4.7 Antioxidant capacity

222 To determine the antioxidant activity of the films, methanol extracts were
223 obtained. For this, 1 g of each film was homogenized in 20 mL of 80% methanol (v/v).
224 After agitation, the samples were left to stand for 24 h for extraction. Then, the extracts
225 were centrifuged at $1696 \times g$ for 15 min at 4 °C. The supernatant was collected, filtered

(125 mm filter paper), and stored under refrigeration (4 °C) until analysis. Antioxidant capacity was subsequently evaluated using the ABTS^{•+}, DPPH[•], and FRAP assays.

In the ABTS^{•+} assay, ABTS^{•+} was generated by reacting ABTS with potassium persulfate (2.45 mM final), then kept in the dark for 12–16 h at 25 ± 0.5 °C, and subsequently diluted to an absorbance of 0.700 ± 0.02 at 734 nm. Aliquots of the sample extract were mixed with the ABTS^{•+} solution, incubated in the dark for 30 min, and the absorbance was measured at 734 nm. Water was used as the control, and the extraction solution as the blank. The antioxidant capacity was calculated from a Trolox standard curve (10–2000 µM) and expressed as µmol/g (Trolox equivalents) (Nascimento et al., 2025). For the DPPH[•] assay, a stock solution of DPPH[•] (24 mg/100 mL of absolute ethanol) was prepared and diluted (1:10, v/v). Aliquots of the extracts were mixed with the working solution and incubated for 30 min in the dark. Absorbance was measured at 517 nm, using distilled water as the control and the extraction solvent as the blank. Antioxidant capacity was calculated using a Trolox standard curve (10–2000 µM) and expressed as µmol g⁻¹ (Trolox equivalents) (Gull et al., 2026). For the FRAP assay, aliquots of the extract were mixed with distilled water and FRAP reagent, then incubated for 30 min at 37 ± 0.5 °C. Absorbance was measured at 595 nm, using the FRAP reagent as the blank. The standard curve was prepared using ferrous sulfate solutions (50–2000 µM), and the results were expressed as µmol/g (FeSO₄ equivalents) (Nascimento et al., 2025).

2.4.8 Biodegradability in soil and water

The biodegradation rate of sodium alginate-based films, with and without the addition of a mixed culture of lactic acid bacteria (LAB), was evaluated under laboratory storage conditions, as described by Filipini et al. (2020). After drying, the samples were

buried 1 cm deep in plastic trays containing organic compost. For aqueous degradation assays (freshwater and seawater), the films were immersed in 300 mL of each solution contained in plastic trays. All samples were kept under aerobic conditions at room temperature (25 ± 0.5 °C). Samples were collected at 0, 6, 12, 15, and 20 days. The biodegradation process was monitored by visual inspection, and samples were collected and photographed periodically until it became impossible to identify film fragments.

2.5 Evaluation of the physical and physicochemical quality of coated guava and mango fruits

For coating application, three formulations were used: SA-LAB (sodium alginate with a mixed LAB culture), SA (sodium alginate), and a control (sterile distilled water containing 2.5 mL/100 mL glycerol). Fruits were individually immersed in 400 mL of each coating formulation for 2 min, then in a calcium chloride solution (2 g/100 mL) for 1 min to induce gelation and crosslinking of the coating. Subsequently, fruits were air-dried on absorbent paper and kept in a biological safety cabinet for 2 h to ensure complete drying.

The evaluation of physical and physicochemical parameters was performed immediately after coating application and at 6, 9, 12, and 15 days of storage (25 ± 0.5 °C). The analyses included total soluble solids, titratable acidity, pH, ascorbic acid content, weight loss, firmness, color, phytochemical contents (total phenolics, flavonoids, and β -carotene), and antioxidant capacity.

2.5.1 Total soluble solids

For the determination of total soluble solids (TSS), the fruits were cut longitudinally. In the case of mango, the cut was made along the longitudinal axis,

contouring the pit, followed by the removal of the peel. The resulting pulp was ground in a domestic food processor (Mixer Daily RI2532, Philips Walita, MG, Brazil) until homogeneous. Then, two drops of each pulp were extracted and applied to the prism of a digital refractometer (Brix/RI Chek Model, Reichert Analytical Instruments, NY, USA). Readings were taken according to AOAC (2016), corrected to 20 ± 0.5 °C, and the results expressed in °Brix.

2.5.2 Titratable acidity

Titrate acidity (TA) was determined in 10 mL of homogenized pulp by titration with 0.1 M NaOH solution, using phenolphthalein as an indicator. Analyses were performed according to AOAC (2016), and results are expressed in grams of citric acid per 100 g of fresh fruit.

2.5.3 pH

The pH values were determined in 10 mL of homogenized pulp using a potentiometer with a combined glass electrode (Model Q400AS, Quimis, SP, Brazil) calibrated with buffer solutions at pH 7 and 4 (AOAC, 2016).

2.5.4 Ascorbic acid content

The ascorbic acid content was determined by titration with 2,6-dichlorophenolindophenol. Ten grams of homogenized pulp were diluted in a solution of oxalic acid (0.5%). Then, a 10 mL aliquot of the solution was titrated with 2,6-dichlorophenolindophenol until a persistent light pink color appeared. The ascorbic acid content was calculated using the equation:

$$\text{Ascorbic acid (mg/100g)} = (V \times F/A) \times 100 \quad (\text{Equation 3})$$

where V is the volume of 2,6-dichlorophenol indophenol solution spent on the titration, F is the factor of 2,6-dichlorophenolindophenol solution, and A is the quantity (mL) of the sample used (Pan et al, 2025). Results were expressed as mg of ascorbic acid per 100 g of fruit.

2.5.5 Weight loss

Fruit weight loss was determined by the difference between the initial weight (day 0) and the weight at each storage period. The results were expressed as a percentage of the initial weight loss (Gull et al., 2026).

2.5.6 Firmness

To assess firmness, each fruit was analyzed at two opposite points in the equatorial region. Measurements were performed using a TA-Xtplus texture analyzer (Stable Micro Systems Ltd., United Kingdom), equipped with cylindrical probes of different diameters: 6 mm for mango (Parente et al., 2026) and 2 mm for guav (Chen et al., 2022). The probes penetrated approximately 10 mm into the fruit tissue, and the maximum penetration force was recorded. The results were expressed in Newtons (N).

2.5.7 Color

The peel and pulp colors of the fruits were measured at two opposite points on each fruit using a colorimeter previously calibrated with a standard white plate (Ali et al., 2022). The color parameters were recorded in the CIELAB system, as described in section 2.5.8 Additionally, the total color difference (ΔE) was calculated according to Saikaew et al. (2025), based on the L^* , a^* , and b^* values, using the following equation:

$$\Delta E = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2} \quad (\text{Equation 4})$$

where L_0 , a_0 , and b_0 represent the initial color values. The magnitude of ΔE was interpreted as follows: not noticeable ($0 < \Delta E < 0.5$), slightly noticeable ($0.5 < \Delta E < 1.5$), noticeable ($1.5 < \Delta E < 3.0$), well visible ($3.0 < \Delta E < 6.0$), and greatly visible ($6.0 < \Delta E < 12.0$) (Razali et al., 2021).

2.5.8 Phytochemical contents and antioxidant capacity

To determine the bioactive compounds and antioxidant activity of the fruits during storage, methanolic extracts were obtained. For this purpose, 5 g of crushed pulp were homogenized with 100 mL of 80% methanol (80:20, v/v). Determinations of antioxidant activity using the ABTS^{•+}, DPPH[•], and FRAP methods were performed as described in section 2.4.7.

2.5.8.1 Total phenolic compounds

The total phenolic content was determined by the Folin-Ciocalteu method, using gallic acid as a standard. Aliquots of the extract were mixed with Folin–Ciocalteu reagent (10%) and sodium carbonate solution (7.5%), and absorbance was measured at 765 nm using a microplate spectrophotometer (BioTek Eon, Winooski, VT, USA). The results were obtained using a gallic acid standard curve and expressed in mg of gallic acid equivalents (GAE) per 100 g of sample (Traore et al., 2024).

2.5.8.2 Total flavonoids compounds

The total flavonoid content was determined by sequentially reacting the methanolic extract with sodium nitrite (5%), aluminum chloride (10%), and sodium hydroxide (1 M). Absorbance was measured at 510 nm using a microplate

spectrophotometer. Results were obtained using a catechin standard curve and expressed in mg of catechin equivalents (CE) per 100 g of sample (Zitha et al., 2022).

2.5.8.3 Total carotenoids compounds

Total carotenoids were determined by absorbance at 450 nm with a microplate spectrophotometer, with β -carotene as the standard for the calibration curve. Results were obtained using a β -carotene standard curve and expressed as μ g of β -carotene equivalents per 100 g of sample (Hasan et al., 2022).

2.6 Sensory analysis

This study was approved by a Research Ethics Committee with Human Beings of the Health Sciences Center/UFPB (CAAE: 61451522.0.0000.5188). Samples were evaluated for microbiological conformity using standard methods, in accordance with current Brazilian legislation (Brasil, 2019). Fruit consumers were invited to participate in the Preference Attribute Elicitation (PAE) session ($n = 16$; 9 women and 7 men; aged 23–40 years), conducted as described by Grygorczyk et al. (2013) with the aim of conducting an exploratory analysis. Initially, participants received a whole fruit and a portion of pulp (~20 g) from each formulation (coated with SA-LAB, SA, or an uncoated control). The samples were presented on white plastic plates, coded with random three-digit numbers, in random order and blinded, immediately after removal from storage (25 ± 0.5 °C). Participants were asked to indicate the attributes relevant to the characterization and acceptance of the product. The generated terms were recorded and, by consensus, grouped into the appearance, color, aroma, and texture categories. Redundant, ambiguous, or difficult to evaluate attributes were excluded. Thereafter, the main descriptors and their respective intensity extremes were defined. After a 15-minute interval, the samples were

represented monadically and in randomized order. Participants evaluated the intensity of the attributes using structured nine-point scales, based on the previously defined descriptors. Sensory tests were conducted on three different days, with one session dedicated to each fruit, corresponding to the storage period (days 0, 6, and 15). Each PAE session lasted approximately 120 minutes.

2.7 Statistical analysis

The characterization analyses of the coatings were conducted using a completely randomized design, with three replicates per sample. The evaluation of physical and physicochemical parameters and bioactive compounds in the fruits was performed using a completely randomized design, with three replicates per treatment, each replicate consisting of eight fruits, across three independent experiments. The results were expressed as average $\pm$ standard deviation. The normality of the data was assessed using the Kolmogorov–Smirnov test, and the homogeneity of variances using Levene's test. When the assumptions were met, the data were subjected to analysis of variance (ANOVA), followed by Tukey's or Duncan's post-hoc tests, considering a significant difference at $p < 0.05$. Statistical analyses were performed using SigmaStat 3.5 software (Jandel Scientific Software, San Jose, CA, USA). PAE data were evaluated with Generalized Procrustes Analysis (GPA) using GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, CA, USA).

3 Results and discussion

3.1 Determination of the physical and structural properties of films

3.1.1 FTIR spectroscopy

FTIR spectroscopy can be used to measure molecular interactions by monitoring shifts in absorption peaks of bonds in polysaccharide molecules (Wu et al., 2024). The spectra obtained from the SA and SA-LAB films are shown in Figure 1A. Both spectra exhibited characteristic SA bands, indicating the preservation of the polymer's fundamental chemical structure in both systems evaluated. However, the incorporation of LAB resulted in subtle spectral changes, suggesting physicochemical interactions between the polymer matrix components and the bacteria. The band observed around 3300 cm^{-1} is attributed to the O–H stretching vibration of hydroxyl groups, moisture, and the hydrophilic character of alginate (Mollah et al., 2023). The reduction in the intensity of this band in the SA-LAB film may indicate changes in water retention (Pan et al, 2025), possibly due to interactions between the polymer and bacterial cells. Furthermore, the bands located in the $1400\text{--}1600\text{ cm}^{-1}$ region correspond to vibrations of the alginate carboxylate groups (--COO^-), while the band near 1730 cm^{-1} can be attributed to the C=O stretching of protonated carboxylic groups (--COOH) (Mollah et al., 2023; Thombare et al., 2023). The changes observed in this region between the SA and SA-LAB films suggest possible interactions between the alginate functional groups and the constituents of the LAB cells, resulting in modifications to the polymer network organization (Sharma et al., 2023). This interpretation is consistent with the changes observed in the characteristic alginate bands and may explain the greater structural stability observed in the films containing LAB (Mollah et al., 2023; Thombare et al., 2023). Additionally, the spectral region between 1000 and 1200 cm^{-1} corresponds to C–O and C–C bond vibrations within the polysaccharide structure (Thombare et al., 2023). The presence of multiple peaks in this range is characteristic of polysaccharides such as sodium alginate (Mollah et al., 2023), and the differences observed between SA and SA-LAB films may

reflect changes in polymer chain arrangement and/or interactions with compounds associated with LAB incorporation (Sharma et al., 2023).

3.1.2 Differential Scanning Calorimetry (DSC)

The DSC is a thermoanalytical technique that measures the difference in heat flow between a sample and a reference as a function of temperature, enabling the identification of thermal transitions and phase changes of the material (Wai Chun et al., 2021). Biopolymers undergo temperature-dependent structural changes during application, which can directly affect their properties (Bao et al., 2023). In this context, the thermal behavior of SA and SA-LAB films was evaluated and compared to understand potential structural modifications resulting from the incorporation of LAB into the polymeric matrix (Figure 1B). In SA-LAB films, the initial endothermic peaks were less pronounced, indicating less loss of bound water. However, the SA film showed a more intense endothermic peak between 50 °C and 100 °C, suggesting greater evaporation of adsorbed water, possibly due to the greater availability of hydrophilic groups in the matrix (Araújo et al., 2024). Exothermic peaks associated with matrix decomposition occurred between 200 °C and 250 °C in SA-LAB films, at a later stage, indicating greater structural preservation and thermal stability (Braga et al., 2020). In SA films, these events began at 150 °C, evidencing earlier degradation and greater matrix fragility. These results indicate that incorporating LAB promotes structural reinforcement, thereby improving thermal stability and material integrity (Wai Chun et al., 2021).

Taken together, the FTIR and DSC results suggest that the incorporation of LAB induced changes in the interactions within the sodium alginate matrix without compromising its fundamental chemical structure. The alterations observed in the FTIR bands, particularly in the hydroxyl region and characteristic polysaccharide vibrations,

are consistent with modifications in network organization and water-associated interactions (Mollah et al., 2023; Pan et al., 2025; Sharma et al., 2023). These structural changes may contribute to the thermal behavior observed by DSC, including reduced loss of bound water and enhanced thermal stability of SA-LAB films, as stronger intermolecular interactions can restrict chain mobility and influence thermal transitions within the alginate matrix (Bao et al., 2023; Braga et al., 2020; Wai Chun et al., 2021).

3.1.3 Scanning electron microscopy (SEM)

The morphological structure of the developed films, as observed by SEM, is shown in Figure 2. The SA sample exhibited a structured polymeric network. In the SA-LAB film, rod-shaped bacterial cells were well incorporated into the coating matrix, resulting in a smooth and pore-free surface. This feature indicates enhanced protection of bacterial cells within the film, effective interfacial adhesion, and their interaction with the polymeric network (Akman et al., 2021). In agreement with the present study, Kalkan et al. (2025) observed a smooth surface in SA films containing *Lacticaseibacillus rhamnosus*, in which the uniform distribution and relatively smaller size of the bacterial cells contributed to a more homogeneous film matrix without pore formation.

3.1.4 Surface roughness

The highest Ra (average roughness) and thickness values were observed in the SA film (Table 1), indicating a more irregular surface characterized by greater variations between peaks and valleys across the film (Figure 3). This topography increases the effective surface area, reflecting higher surface roughness (Boubakeur et al., 2026). In contrast, the SA-LAB film exhibited a more uniform, homogeneous surface than SA alone. This behavior suggests that the incorporation of LAB contributed to the reduction

of surface irregularities, promoting a more organized and stable structure. The formation of a more homogeneous matrix can positively influence the mechanical properties of SA-based films (Janik et al., 2023). In this regard, thickness is a critical parameter for determining mechanical and barrier properties and is influenced by the film preparation method, the smoothness of the Petri dish surface, and the drying process conditions (Engin et al., 2022). Additionally, the reduced thickness of SA-LAB films may contribute to more efficient release of functional agents by shortening the diffusion path to the target surface (Almeida et al., 2023). The reduction in roughness may be related to interactions between bacterial cells and the alginate matrix during film formation, favoring the reorganization of the polymer network. This interpretation is supported by FTIR results, which indicated changes in intermolecular interactions (Mollah et al., 2023; Pan et al., 2025; Sharma et al., 2023), and by DSC results, which demonstrated greater thermal stability in films containing LAB (Bao et al., 2023; Braga et al., 2020; Wai Chun et al., 2021). Taken together, these findings suggest that the incorporation of LAB promoted a promoted the formation of a more cohesive polymer matrix, which was reflected in lower surface roughness (Janik et al., 2023).

3.1.5 Moisture, water solubility, hygroscopicity, and water activity

Moisture content is a critical parameter in the characterization of films, as it reflects their affinity for water and is associated with the hydrophilic/hydrophobic balance of the matrix (Lopes et al., 2025). Furthermore, it is directly related to the viability and stability of probiotic microorganisms during storage (Kalkan et al., 2025). The SA film exhibited a moisture content of 23.49 ± 0.74 %, whereas the incorporation of the mixed LAB culture reduced this value to $13.15 \pm 2.47\%$ ($p < 0.05$) (Table 1). Similar results were reported by Boubakeur et al. (2026) in SA-based films containing inulin and

Lactiplantibacillus plantarum. Although this reduction may suggest lower water availability, the more compact and homogeneous structure of SA-LAB films (as evidenced by SEM and FTIR analyses) likely limits free water content without compromising bacterial stability, indicating a more densely structured polymeric matrix. In addition, moisture content in edible films is influenced by factors such as polymer concentration, the type and concentration of plasticizers, and the water-binding capacity of the polymeric matrix (Abedinia et al., 2021).

Water solubility is an essential parameter in the characterization of films, as it is directly related to water resistance and material integrity (Lopes et al., 2025). The solubility of the films is presented in Table 1. The SA film showed a solubility of $36.82 \pm 1.20\%$, whereas the SA-LAB film exhibited a lower value of $28.60 \pm 3.68\%$ ($p < 0.05$). The reduction in solubility may suggest that the presence of bacterial cells promotes greater structural integrity of the SA matrix, limiting its dissolution in aqueous media (Kalkan et al., 2025). In general, films with lower solubility are more suitable for use in high-moisture foods, such as fruits, as they help maintain product quality and stability (Santos et al., 2021).

The hygroscopicity of the film (Table 1) was reduced by incorporating the mixed LAB culture, decreasing from $61.03 \pm 3.62\%$ in the SA film to $44.89 \pm 2.57\%$ in the SA-LAB film ($p < 0.05$). SA-based films are naturally hygroscopic, which can result in sticky surfaces when stored under ambient relative humidity conditions (Metha et al., 2024). In this sense, Botalo et al. (2024) observed high hygroscopicity in SA films, associated with surface adhesion of the material. However, incorporating compounds such as whey protein isolate or curcumin reduced hygroscopicity, maintaining a dry, non-sticky surface (Botalo et al., 2024). In general, hygroscopic films exhibit poor moisture barrier properties. Thus, incorporating additional components into the polymer matrix is an

important strategy for reducing water uptake and controlling moisture transfer, thereby improving film performance (Bhatia et al., 2023).

The water activity (a_w) of the SA film was 0.46 ± 0.03 , whereas the SA-LAB film showed a lower value of 0.38 ± 0.03 ($p < 0.05$). The reduction in water activity in the SA-LAB sample agrees with the moisture content and hygroscopicity results, suggesting that the presence of LAB induces changes in the polymeric matrix, possibly associated with increased intermolecular interactions within the film structure (Kalkan et al., 2025).

3.1.6 Color

The color parameters of the films are presented in Table 1. The incorporation of the LAB mixed culture did not significantly affect ($p > 0.05$) any of the evaluated parameters, thereby maintaining the SA-LAB film's visual characteristics, as in SA. The color of packaging materials can influence consumer perception and acceptance of food products (Bao et al., 2023); therefore, maintaining the optical properties and transparency of edible coatings/films is also relevant to their potential applications (Engin et al., 2022). From a technological perspective, the absence of significant changes in color parameters suggests that the incorporation of LAB did not disrupt the polymer matrix's structural organization to the extent that would alter light absorption or scattering (Huang et al., 2024). In agreement with these results, *Lactiplantibacillus plantarum* did not affect luminosity (L) values in films ($p > 0.05$), as reported by Kalkan et al. (2025).

3.1.7 Antioxidant activity

The antioxidant activity of the films is presented in Table 1. The SA-LAB film showed significantly higher values compared to the SA film ($p < 0.05$). In the ABTS^{•+} assay, the SA film exhibited 0.12 ± 0.02 $\mu\text{mol Trolox TEAC/g}$, while the SA-LAB film

showed 0.19 ± 0.03 $\mu\text{mol Trolox TEAC/g}$. Similar results were observed with the DPPH $\cdot$ method, in which the SA film showed 0.42 ± 0.07 $\mu\text{mol Trolox TEAC/g}$, whereas the SA-LAB film showed a value approximately twofold higher, at 0.90 ± 0.07 $\mu\text{mol Trolox TEAC/g}$. In the FRAP assay, the SA film showed 1.70 ± 0.12 $\mu\text{mol FeSO}_4/\text{g}$, while the SA-LAB film reached 3.31 ± 0.11 $\mu\text{mol FeSO}_4/\text{g}$, confirming the increase in antioxidant capacity after LAB incorporation. This increase can be attributed to the presence of bioactive metabolites produced by LAB, such as peptides and exopolysaccharides, which exhibit reducing capacity and free-radical-scavenging activity, thereby enhancing the antioxidant functionality of the films (Silva et al., 2023). In addition to the contribution of LAB metabolites, SA films themselves may also play an important role in oxidative stability. Although alginate-based films exhibit lower intrinsic antioxidant activity, their antioxidant potential, combined with their semipermeable barrier properties, may contribute to modulating the generation of reactive oxygen species (ROS) in food matrices, particularly in fruits, thereby helping to preserve oxidative stability and postharvest quality (Nguyen et al., 2026; Pandey et al., 2025).

3.1.8 Biodegradability in soil and water

The use of biodegradable polymers as edible coatings/films has received increasing attention due to the environmental impacts associated with the indiscriminate use of non-degradable plastic materials (Aziz et al., 2022). In this context, the biodegradability of SA and SA-LAB films was evaluated in soil, freshwater, and seawater (Figure 4) to determine degradation times and confirm their potential as alternatives to synthetic polymers, which are characterized by long environmental persistence.

During soil storage, the films exhibited progressive biodegradation, beginning after 12 days and reaching almost complete degradation by 20 days. These results indicate

that the SA-based films exhibit satisfactory biodegradability under natural conditions and that the incorporation of LAB does not affect the degradation time in soil. The rate of biodegradation in soil is influenced by film composition, the profile and concentration of microorganisms present, and environmental conditions such as temperature, humidity, and climate (Shaikh et al., 2021). In this context, moisture, temperature, and microbial enzymatic activity promote polymer chain degradation, leading to the formation of fragmented structures (López-Díaz et al., 2023). Furthermore, a material is considered biodegradable when 90% of its mass is degraded by biological activity within 6 months (Filipini et al., 2020).

When immersed in freshwater, the films had more accelerated degradation compared to the other evaluated media. From day 12 onwards, significant dissolution was observed, and by the end of the experiment, the films were completely solubilized. This behavior may be related to the hydrophilic nature of SA, which favors rapid water absorption (Kumar et al., 2023). Although the presence of bacterial cells contributed to a more compact polymeric structure, it did not affect the overall dissolution time under these conditions, suggesting that, under high hydration, the hydrophilic nature of SA predominates over structural differences in the matrix. In seawater tests, the films began to show signs of biodegradation on day 12 and were almost completely solubilized by day 20.

The presence of calcium ions (Ca^{2+}) in this medium may have promoted slight cross-linking within the polymer matrix, possibly delaying initial solubilization due to the formation of stable interactions between polymer chains (Gubitosa et al., 2023; Frent et al., 2022). Furthermore, environmental factors such as pH and water temperature can influence medium dynamics and, consequently, the degradation rate of the films (Ayari et al., 2025). Notably, the incorporation of LAB did not affect the overall biodegradation

behavior of the films, which exhibited high biodegradation capacity across the evaluated media, confirming their potential as sustainable materials for application on fruits.

3.2 Effects of coating application on the physical and physicochemical quality of guava and mango fruits

Guava and mango are climacteric fruits and present significant perishability challenges due to their high respiration rates and ethylene production during ripening (Qasim et al., 2025; Yadav et al., 2022). In this context, the effects of SA-LAB and SA coatings on the physical and physicochemical parameters of guava and mango during 15 days of storage (25 ± 0.5 °C) are presented in Figures 5 and 6.

3.2.1 Total soluble solids

Total soluble solids (TSS) content is widely used as an indicator of fruit ripening stage and sensory quality and is directly related to sugar accumulation (Wei et al., 2024). In general, TSS increased in guava and mango throughout the storage period, a behavior expected during climacteric ripening. This increase is primarily attributable to starch hydrolysis (Kohli et al., 2024). The highest TSS values were recorded in control fruits (without coating) on day 15 of storage, reaching 13.47 ± 0.29 for guava and 18.03 ± 0.15 for mango (Figures 5 and 6). In contrast, fruits subjected to coatings showed lower TSS values ($p < 0.05$) at the same storage time. Guava coated with SA and SA-LAB showed TSS values of 12.47 ± 0.43 and 12.00 ± 0.10 , respectively. For mango coated with SA and SA-LAB, these values were 16.97 ± 0.15 and 15.97 ± 0.59 , respectively. The lower increase in TSS in coated fruits, particularly in the SA-LAB coating treatment, suggests that the coating modulated respiratory metabolism and delayed ripening (Qasim et al., 2025). This effect may be associated with the formation of a semipermeable barrier,

which reduces gas exchange and consequently slows biochemical processes related to senescence (Sg et al., 2026; Kohli et al., 2024).

3.2.2 Titratable acidity

Acidity is an important parameter for determining fruit ripeness and flavor (López-Ortiz et al., 2025). Throughout storage, a reduction in titratable acidity (TA) was observed in all treatments for both guava and mango (Figures 5 and 6). On day 15 of storage, fruits coated with SA-LAB showed the highest acidity levels, reaching 1.15 ± 0.05 g/100 g for guava and 1.09 ± 0.04 g/100 g for mango. In contrast, lower TA values ($p < 0.05$) were observed in control fruits, with 0.76 ± 0.03 g/100 g and 0.71 ± 0.07 g/100 g for guava and mango, respectively. These results may be related to reduced degradation of organic acids, resulting from lower oxygen availability provided by the coatings (González-Cuello et al., 2024). A similar trend in acidity preservation was reported by Li et al. (2024), with higher TA levels in strawberries coated with alginate containing LAB compared to uncoated fruits, reinforcing the role of active coatings in maintaining postharvest quality.

3.2.3 pH

pH is an important indicator of changes in the organic acid content of fruits during storage (Li et al., 2024). During ripening, these acids are consumed in respiratory metabolism, resulting in reduced acidity and, consequently, increased pH (Silva et al., 2024). An increase in pH values was observed in all samples throughout storage, with the highest values recorded on day 15 in control (uncoated) fruits (Figures 5 and 6). For guava, pH values were 4.35 ± 0.07 in the control and 4.10 ± 0.01 in the SA-LAB coating treatment. For mango, the observed values were 5.92 ± 0.03 in the control and 4.89 ± 0.02

in the SA-LAB coating treatment. The lower pH values observed in fruits coated with SA-LAB indicate greater retention of organic acids, reinforcing the efficacy of this coating in delaying ripening (Huang et al., 2024). Similar results have been reported in the literature, in which coatings containing LAB delayed the increase in pH and stabilized pH fluctuations, thereby extending fruit shelf life (Dias et al., 2023; Li et al., 2024).

3.2.4 Ascorbic acid content

Ascorbic acid (AA) acts as an antioxidant, neutralizing free radicals formed in fruits and protecting them against degradation, which usually begins during ripening due to oxidative processes (Majeed et al., 2024). Guava and mango fruits coated with SA and SA-LAB showed higher ascorbic acid content compared to control (uncoated) fruits from day 6 of storage, with the highest values observed in fruits treated with SA-LAB ($p < 0.05$) (Figures 5 and 6). At the end of the storage period, AA content in guava was 122.80 ± 4.96 mg/100 g in the SA-LAB coating treatment, 80.90 ± 2.85 mg/100 g in the SA coating treatment, and 50.57 ± 4.75 mg/100 g in the control. For mango, the observed values were 61.67 ± 2.52 mg/100 g for the SA-LAB coating treatment, 38.53 ± 2.24 mg/100 g for the SA coating treatment, and 25.75 ± 1.70 mg/100 g for the control. AA is susceptible to oxidation and gradually degrades during storage; however, fruits with higher AA content generally exhibit greater storage stability, extended shelf life, and superior postharvest quality (Li et al., 2024).

The application of bioactive coatings may reduce oxidative losses, likely by limiting respiration rate and metabolic stress in fruits (Gull et al., 2026). A coating formulated with alginate and supplemented with *L. rhamnosus* substantially reduced ascorbic acid loss and extended the shelf life of strawberries (Li et al., 2024). This

behavior may be attributed to the antioxidant metabolic activity of LAB and their role in modulating oxidative processes within the fruit surface environment (Gull et al., 2026).

3.2.5 Weight loss

Weight loss in fruits during storage occurs mainly due to moisture loss, which compromises appearance and freshness, and can cause wrinkling (Qasim et al., 2025). This process is associated with water loss through respiration and transpiration in fruits (Pan et al, 2025). Weight loss progressively increased during storage in all treatments; however, control (uncoated) fruits consistently showed the highest values from day 6 onwards (Figure 5 and 6). The application of SA and SA-LAB significantly reduced this loss in guavas and mangos until day 15. At the end of storage, control guavas showed a weight loss of $40.70 \pm 2.49\%$, while fruits coated with SA-LAB showed a lower value ($p < 0.05$), $20.27 \pm 2.04\%$. A similar trend was observed in mangos, with losses of $20.14 \pm 1.42\%$ in control fruits and $10.11 \pm 0.72\%$ in those coated with SA-LAB.

Consistent results in reducing weight loss have been observed with the application of sodium alginate to guava (Mahapatra et al., 2024) and mango (Zhang et al., 2024). This effect can be attributed to SA -based coatings acting as semipermeable barriers that regulate gas exchange, reduce moisture loss, and decrease the fruit respiration rate (Pandey et al., 2025).

3.2.6 Firmness

Firmness is an essential parameter for evaluating fruit ripeness, shelf life, and commercial quality, and is also one of the main criteria consumers consider when purchasing (Schlogl et al., 2025). Fruit firmness gradually decreased during storage in all samples (Figures 5 and 6); however, SA and SA-LAB coatings delayed this reduction.

Sodium alginate-based coatings exhibit properties that help maintain fruit firmness and mitigate texture changes associated with ripening and senescence (Pandey et al., 2025). On day 9 in guava and on day 6 in mango, control (uncoated) fruits showed lower firmness than coated fruits. At the end of storage, SA-LAB-coated fruits exhibited firmness values of 15.84 ± 1.46 N in guava and 31.54 ± 1.62 N in mango, while control fruits showed significantly lower values ($p < 0.05$) of 4.70 ± 1.54 N and 16.43 ± 2.34 N, respectively. The reduction in firmness in guava and mango can be attributed to the activity of cell wall-degrading enzymes, such as polygalacturonase, cellulase, hemicellulase, pectin methylesterase, and β -galactosidase, which increase during storage and promote pectin solubilization, starch breakdown, cell wall disintegration, and reduced turgor (Qasim et al., 2025; Nguyen et al., 2026; Schlogl et al., 2025).

In this context, the higher firmness observed in SA-LAB-coated fruits suggests modulation of these enzymatic processes, likely due to the formation of a more compact, semipermeable barrier that limits oxygen availability and reduces metabolic activity associated with cell wall degradation (Nguyen et al., 2026; Pandey et al., 2025). A similar effect was reported for coatings formulated with gelatin, *L. rhamnosus*, and inulin, which reduced enzymatic activity, delayed polymer breakdown, and helped maintain strawberry firmness during storage (Azarakhsh et al., 2024).

Taken together, the application of SA-LAB coating effectively preserved the physicochemical quality of guava and mango fruits during storage by delaying ripening-associated changes. The maintenance of higher titratable acidity, lower pH increase, reduced weight loss, and slower changes in soluble solids indicated a reduction in metabolic activity and senescence progression (Pandey et al., 2025; Qasim et al., 2025; Sg et al., 2026; Kohli et al., 2024). Additionally, the higher retention of ascorbic acid and firmness in SA-LAB-coated fruits demonstrated the protective effect of the active coating

on oxidative stability and cell wall integrity (Gull et al., 2026; Li et al., 2024; Schlogl et al., 2025). These combined effects suggest that the incorporation of LAB enhanced the functionality of the alginate matrix, providing a more effective semipermeable barrier against physiological deterioration and extending the commercial quality period of climacteric fruits (Nguyen et al., 2026; Pandey et al., 2025).

3.2.7 Phytochemical contents and antioxidant activity

The phytochemical content, including total phenolic compounds (TPC), total flavonoids (TFC), β -carotene, and antioxidant activity of guava and mango coated with SA-LAB, SA, and the control, are presented in Figures 5 and 6, respectively. Phenolic compounds are important secondary metabolites, mainly classified as phenolic acids and polyphenols, and play a relevant role in disease prevention and the promotion of human health (Lebaka et al., 2021). TPC decreased during storage in all samples, with a more pronounced reduction in control (uncoated) fruits. Fruits coated with SA-LAB showed greater preservation of phenolic compounds, with significantly higher values ($p < 0.05$) at the end of storage, reaching 82.26 ± 2.05 mg GAE/100 g in guava and 38.43 ± 0.22 mg GAE/100 g in mango. In contrast, SA and control treatments exhibited lower TPC values, indicating reduced retention of these compounds.

The decline in TPC during storage is associated with ripening, which promotes the degradation of phenolic compounds (Traore et al., 2024). Conversely, the higher TPC retention observed, particularly in SA-LAB-treated fruits, may be related to reduced activity of phenol-degrading enzymes (Hasan et al., 2022). Additionally, as TPC is closely linked to antioxidant capacity and metabolic stability, its preservation suggests a delay in oxidative processes. In this context, the application of edible coatings likely helps maintain these compounds by limiting oxygen availability and reducing enzymatic

oxidation (Gull et al., 2026). Supporting this, Nguyen et al. (2026) reported higher TPC levels in mangos coated with sodium alginate containing *L. plantarum* compared to uncoated fruits.

Flavonoids (TFC) constitute an important class of antioxidants of plant origin (Gull et al., 2026). Overall, TFC followed a trend like TPC, with a reduction from the 6th day of storage, more pronounced in control (uncoated) fruits. In guavas, at the end of storage (day 15), the SA-LAB-coating treatment showed the highest TFC content (24.72 ± 1.07 mg CE/100 g), followed by the SA-coating treatment (18.42 ± 1.43 mg CE/100 g), both higher ($p < 0.05$) than the control (13.91 ± 2.58 mg CE/100 g). In mangos, SA-LAB-coated fruits also showed greater flavonoid preservation (8.57 ± 0.72 mg CE/100 g). SA-coated fruits exhibited intermediate values (1.90 ± 0.72 mg CE/100 g) that remained higher ($p < 0.05$) than the control, in which TFC was only detectable up to the 12th day, indicating a more pronounced degradation of these compounds. TFC content is affected by postharvest handling and storage conditions (Saikaew et al., 2025). The reduction observed during storage may be due to its conversion to other phenolic compounds during ripening and to increased enzymatic activity in oxidative metabolism (Hasan et al., 2022). Conversely, the higher levels of TPC and TFC observed in SA-LAB-coated fruits may be related to the mitigation of oxidative stress, possibly due to the preservation of antioxidant defense systems, including enzymes such as superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase, thereby contributing to the maintenance of the fruit's nutritional and sensory quality (Gull et al., 2026).

Carotenoids are fat-soluble pigments responsible for the yellow, orange, and red colors of fruits (Lebaka et al., 2021). In addition to their role in appearance, they act as precursors of vitamin A and exhibit important antioxidant activity (Lebaka et al., 2021; Mathiazhagan et al., 2024). Guava and mango stand out for their high levels of these

pigments, primarily β -carotene (Hasan et al., 2022; Saikaew et al., 2025). The carotenoid content, expressed as β -carotene, gradually increased throughout storage in all samples. The highest values were observed in control (uncoated) fruits at the end of storage, reaching $62.69 \pm 1.79 \mu\text{g BE}/100 \text{ g}$ in guava and $401.64 \pm 3.09 \mu\text{g BE}/100 \text{ g}$ in mango. The SA and SA-LAB coatings reduced this progressive increase ($p < 0.05$). In guavas, at the end of storage, fruits coated with SA showed $44.26 \pm 1.93 \mu\text{g BE}/100 \text{ g}$, while those treated with SA-LAB showed a significantly lower value ($p < 0.05$) of $34.52 \pm 1.20 \mu\text{g BE}/100 \text{ g}$. For mangos, on day 15, SA-coated fruits showed a carotenoid content of $375.90 \pm 4.80 \mu\text{g BE}/100 \text{ g}$, whereas those coated with SA-LAB also showed a significantly lower value ($p < 0.05$) of $123.12 \pm 3.01 \mu\text{g BE}/100 \text{ g}$. These results indicate that the SA-LAB coating was the most effective in delaying ripening and preserving fruit quality during storage. This effect may be attributed to reduced chlorophyll degradation and lower ethylene activity, limiting the activation of enzymes involved in chlorophyll breakdown and carotenoid biosynthesis (Qasim et al., 2025). Furthermore, the lower accumulation of carotenoids may be associated with reduced oxidative metabolism and slowed senescence-related processes (Ali et al., 2022; Hasan et al., 2022).

Total antioxidant capacity represents the overall potential of fruits to mitigate oxidative stress by neutralizing free radicals, which can damage cellular components and contribute to the development of various chronic diseases (Khedr & Khedr, 2023; Gull et al., 2026). The effects of SA and SA-LAB coatings on the antioxidant capacity of guava and mango were evaluated using ABTS^{•+}, DPPH[•], and FRAP assays during storage (Figures 5 and 6). In general, all evaluated antioxidant activities decreased over time in both control (uncoated) and treated fruits. However, the SA-LAB coating retained more antioxidant activity from the 6th day of storage in both guava and mango than the control

($p < 0.05$). An exception was observed in the ABTS^{•+} assay for mango, in which differences were detected only on the 15th day.

At the end of storage, ABTS^{•+} values in guava were higher in the SA–LAB-coated fruits, reaching 5.76 ± 0.28 $\mu\text{mol Trolox TEAC/g}$, followed by SA-coated fruits with 4.49 ± 0.13 $\mu\text{mol Trolox TEAC/g}$ and the control with 2.05 ± 0.13 $\mu\text{mol Trolox TEAC/g}$. A similar trend was observed in mango, albeit with lower magnitude, where SA–LAB-coated fruits (2.21 ± 0.04 $\mu\text{mol Trolox TEAC/g}$) had higher values ($p < 0.05$) than SA-coated fruits (2.10 ± 0.04 $\mu\text{mol Trolox TEAC/g}$) and the control (2.05 ± 0.03) $\mu\text{mol Trolox TEAC/g}$. For the DPPH[•] assay, at the end of storage, SA-LAB-coated guava showed values of 4.45 ± 0.14 $\mu\text{mol Trolox TEAC/g}$ in the SA–LAB-coated guava, followed by 3.94 ± 0.17 $\mu\text{mol Trolox TEAC/g}$ in SA-coated guava and 3.50 ± 0.15 $\mu\text{mol Trolox TEAC/g}$ in the control. In mango, the values of 2.18 ± 0.09 $\mu\text{mol Trolox TEAC/g}$ were observed in SA–LAB-coated fruit, 1.77 ± 0.06 $\mu\text{mol Trolox TEAC/g}$ in SA-coated fruit, and 1.50 ± 0.06 $\mu\text{mol Trolox TEAC/g}$ in the control, demonstrating a consistent effect of the coating containing LAB in preserving antioxidant activity. At the end of storage, the FRAP assay showed the highest values among the methods evaluated for both fruits. In guava, values were 20.30 ± 1.07 $\mu\text{mol FeSO}_4/\text{g}$ in SA–LAB-coated fruit, 17.01 ± 0.97 $\mu\text{mol FeSO}_4/\text{g}$ in SA-coated fruit, and 14.42 ± 0.47 $\mu\text{mol FeSO}_4/\text{g}$ in the control, with significant differences. In mango, values of 7.62 ± 0.32 $\mu\text{mol FeSO}_4/\text{g}$ were observed in SA-LAB-coated fruit, 6.43 ± 0.39 $\mu\text{mol FeSO}_4/\text{g}$ in SA-coated fruit, and 5.23 ± 0.31 $\mu\text{mol FeSO}_4/\text{g}$ in the control, confirming the superiority of the SA-LAB treatment in maintaining reducing potential throughout storage.

The decline in antioxidant activity during storage, particularly in control fruit, is associated with the degradation of bioactive compounds during ripening and senescence (Siva et al., 2025). These processes involve increased activity of oxidative enzymes,

structural changes in tissues, and higher respiration rates, which accelerate the depletion of phenolics, flavonoids, carotenoids, and ascorbic acid (Kamboj et al., 2025; Siva et al., 2025). In contrast, edible coatings helped preserve antioxidant activity by acting as semipermeable barriers, reducing oxygen diffusion and the activity of enzymes involved in phenolic degradation, thereby promoting the stability of bioactive compounds during storage (Majeed et al., 2024; Sg et al., 2026).

In particular, the barrier formed by sodium alginate can restrict respiratory processes in fruits, attenuating metabolic activity and further contributing to the preservation of antioxidant compounds (Li et al., 2024). The superior performance of the SA-LAB treatment suggests an additional effect of LAB, which, beyond the physical barrier provided by sodium alginate, may have contributed to the reduction of oxidative reactions, resulting in greater retention of antioxidant activity and improved preservation of bioactive compounds throughout storage (Azarakhsh et al., 2024; Nguyen et al., 2026). The incorporation of *L. rhamnosus* and inulin into the gelatin coating increased the antioxidant activity of strawberries during storage, owing to cell stability and the probiotic's functional characteristics (Azarakhsh et al., 2024). In addition, the higher values obtained in the FRAP assay compared to ABTS^{•+} and DPPH[•] may be related to its higher sensitivity to reducing compounds present in fruit matrices, as it primarily reflects the overall reducing power of antioxidants, including no phenolic compounds such as ascorbic acid and carotenoids, which may contribute more markedly to this method (Hasan et al., 2022; Zeng et al., 2023).

3.2.8 Color

The evaluation of color in climacteric fruits is an important parameter, as it reflects the progression of postharvest ripening and influences consumer perception of

quality (González-Cuello et al., 2024; Schlogl et al., 2025; Silva et al., 2024). In general, the parameters Luminosity (L^*), a^* (red/green), b^* (yellow/blue), Chroma (C), and Hue angle (h°), presented in Tables 2 and 3, indicated progressive changes in the color of the peel and pulp of guavas and mangos during storage, with greater intensity of changes in the control fruits (without coating).

The L^* values of fruit peel increased throughout storage in all samples. However, from day 6 onwards, control guavas (without coating) showed significantly higher values ($p < 0.05$) compared to SA and SA-LAB treatments, indicating a more pronounced color transition from green to yellow (Supplementary material S2). At the end of storage, SA-LAB-coated guava showed the lowest L value (62.45 ± 2.11), followed by SA (70.80 ± 1.45), while control fruits exhibited the highest value (80.06 ± 0.84). A similar trend was observed in mangos, with a more marked increase in L^* in control fruits from day 6 onwards (Supplementary material S3). At the end of storage, SA-LAB-coated fruits showed the lowest L^* value (51.46 ± 1.80), followed by SA (56.77 ± 0.92), whereas control fruits showed the highest value (62.73 ± 1.28).

The a^* parameter represents the green–red axis, with negative values indicating a predominance of green. In guavas, from day 6 onwards, SA and SA-LAB-coated fruits differed ($p < 0.05$) from the control, maintaining negative values throughout storage. At the end of storage, SA and SA-LAB-coated fruits reached -4.20 ± 0.83 and -11.20 ± 0.79 , respectively, whereas control fruits showed higher a^* values (8.90 ± 1.27), indicating more advanced ripening. A similar pattern was observed in mangos from day 9 onwards, with SA-LAB maintaining stable negative values throughout storage (-11.89 ± 1.01 to -10.13 ± 0.95 , $p > 0.05$), while control fruits showed higher values at the end of storage (13.88 ± 3.58). The b^* parameter represents the blue–yellow axis, with positive values indicating increased yellowness. On day 15, control guava peels showed the

highest b^* value (59.10 ± 1.70), whereas SA and SA-LAB coating treatments exhibited lower values ($p < 0.05$): 40.95 ± 1.53 and 40.53 ± 0.51), respectively. A similar pattern was observed in mango peel, where control fruits reached higher b^* values (43.18 ± 1.84), while these values in SA and SA-LAB-coated fruits remained lower at 29.48 ± 1.58 and 25.43 ± 1.54), respectively. Overall, these results indicate a progressive increase in yellow coloration during storage, associated with fruit ripening (Schlogl et al., 2025).

Chroma (C) values increased during storage in all treatments; however, the increase was more pronounced in control (uncoated) fruits, for both guava and mango, with significant differences ($p < 0.05$) from coated samples from day 6 onwards. On day 15, guavas showed the greatest differences among treatments, with SA-LAB presenting the lowest C value (42.74 ± 1.75), followed by SA (51.27 ± 2.73), while control fruits showed the highest value (59.18 ± 0.41). In mangos, SA-LAB (25.23 ± 1.59) and SA (26.17 ± 1.21) did not differ significantly ($p > 0.05$), but both were lower than the control (41.45 ± 1.67). The Hue angle (h°) confirmed progressive color changes associated with ripening. In guavas, control fruits showed a marked decrease from 117.89 ± 2.09 to $78.34 \pm 2.00 h^\circ$, while coated fruits maintained significantly higher values ($p < 0.05$), with SA-LAB showing the smallest variation ($105.95 \pm 2.32 h^\circ$). A similar trend was observed in mangos at the end of storage, with SA-LAB maintaining the highest h° value (111.57 ± 0.92), followed by SA (85.45 ± 2.94), whereas control fruits had lower values (78.67 ± 1.70), indicating more advanced ripening.

The ΔE value represents the overall change in fruit color during storage in relation to the initial condition (Saikaew et al., 2025). At the end of storage, coated fruits showed lower color variation ($p < 0.05$), particularly those treated with SA-LAB. In guavas, SA-LAB exhibited the lowest ΔE (11.63 ± 2.01), followed by SA (21.81 ± 0.57), whereas control fruits showed the highest variation (42.89 ± 1.12). A similar trend was observed

in mangos, with ΔE values of 9.92 ± 1.66 for SA-LAB, 21.83 ± 1.98 for SA, and 40.82 ± 1.29 for the control. These findings indicate more pronounced visual changes in uncoated fruits (Razali et al., 2021), likely associated with intensified oxidative processes and greater degradation of chlorophylls and carotenoids during storage (González-Cuello et al., 2024).

Guavas and mangos exhibit a characteristic dark green color at harvest, which progressively changes during ripening (Ali et al., 2022; Schlogl et al., 2025). This transition toward yellowish tones is mainly driven by chlorophyll degradation and carotenoid accumulation (González-Cuello et al., 2024). In parallel, chlorophyll catabolism, particularly relevant in climacteric fruits, is a well-documented hallmark of postharvest ripening and senescence (Ali et al., 2022; Majeed et al., 2024), mediated by the increased activity of enzymes involved in pigment breakdown, such as chlorophyll-degrading enzymes and related oxidative systems (Qasim et al., 2025). In this context, the coatings likely attenuated these color changes by acting as semipermeable barriers that reduce oxygen diffusion and respiration rates, thereby slowing down metabolic pathways associated with chlorophyll degradation and carotenoid biosynthesis (Majeed et al., 2024). Additionally, the more compact matrix observed in coated fruits may have further limited internal oxidative processes, thereby delaying the progression of visual ripening compared to uncoated fruits (Azarakhsh et al., 2024; Nguyen et al., 2026).

The pulp color results for guava and mango are presented in Figures 5 and 6. The L^* parameter decreased throughout storage in all samples; however, SA-LAB-coated fruits maintained significantly higher values ($p < 0.05$) compared to the other treatments. On day 15, guavas coated with SA-LAB showed an L of 68.98 ± 1.55 , followed by SA (59.12 ± 1.35) and control (51.97 ± 1.86). A similar pattern was observed in mangos, with values of 71.94 ± 1.53 for SA-LAB, 64.17 ± 2.05 for SA-coating, and 52.03 ± 1.76 for

control, indicating greater luminosity retention in coated fruits. The a^* parameter increased during storage in all samples. In guavas, at the end of storage, SA-LAB-coated fruits showed the lowest increase (23.84 ± 1.46), followed by SA (31.34 ± 0.67), while control fruits reached the highest value (34.27 ± 1.91), indicating more intense pink coloration. A similar trend was observed in mangos at the end of storage, with values of 4.90 ± 0.73 for SA-LAB-coated fruits, 9.42 ± 0.38 for SA-coated fruits, and 15.75 ± 0.41 for control ($p < 0.05$). The b parameter also increased during storage. In guavas, no differences ($p > 0.05$) were observed among treatments at the end of storage. In mangos, control fruits showed the highest b value (64.01 ± 1.84), differing ($p < 0.05$) from SA-LAB (58.83 ± 1.10) and SA-coated fruits (59.51 ± 1.38), indicating more pronounced yellowing in uncoated fruits.

Chroma (C) increased throughout storage, indicating intensification of pulp color. In guavas, control fruits showed the highest value (42.81 ± 1.96), followed by SA (37.11 ± 1.70) and SA-LAB-coated fruits (31.00 ± 1.04). In mangos, control fruits also showed higher values (68.47 ± 1.07), differing significantly ($p < 0.05$) from SA-LAB (63.49 ± 1.64) and SA (65.10 ± 0.95). In contrast, hue angle (h°) decreased during storage. In guavas, SA-LAB-coated fruits showed the highest value at the end of storage (37.49 ± 1.20), followed by SA (33.67 ± 0.82), while control fruits showed the lowest value (28.25 ± 1.29) ($p < 0.05$). A similar response was observed in mangos, with higher h° values in SA-LAB (83.90 ± 0.92) and SA-coated fruits (80.67 ± 1.01), and lower values in control fruits (73.82 ± 1.86), indicating more advanced ripening.

The ΔE values in guava pulp at the end of storage were lower in the SA-LAB (14.64 ± 1.17), followed by the SA-coated fruits (25.50 ± 3.15), while the fruits in the control showed higher values (34.05 ± 4.03). Similarly, in mango pulp, the lowest ΔE values were also observed in the SA-LAB (15.44 ± 0.39), followed by SA-coated fruits

(24.19 ± 3.07) and the control (37.20 ± 2.98). The greater variations in ΔE observed in the control indicate a higher intensity of color changes during storage (Saikaew et al., 2025).

Color parameters are key indicators of fruit quality, as they reflect biochemical changes associated with ripening, particularly chlorophyll degradation and carotenoid biosynthesis in guava (Silva et al., 2024) and mango (Lebaka et al., 2021). As ripening progresses, these transformations become more evident, leading to reddish coloration in guava pulp (González-Cuello et al., 2024) and a shift from whitish to yellow–orange tones in mango pulp (Yahia et al., 2023). In this context, the attenuation of color changes observed in coated fruits may be associated with the semipermeable nature of alginate-based coatings, which limit oxygen diffusion and reduce respiration rates, thereby slowing pigment degradation and synthesis pathways (Wang et al., 2025). Previous studies support this effect, showing that alginate-based coatings containing thyme essential oil delayed color changes in guava (Mahapatra et al., 2024), while chitosan-based active coatings helped preserve the color of mango pulp during storage (Parente et al., 2026).

3.3 Sensory properties

Among rapid sensory evaluation methods for food, the panel of preferred attributes (PAE) stands out as a quick, lower-cost alternative. This method allows consumers to collaboratively identify, quantify, categorize, and prioritize key attributes, as well as provide hedonic information (Da Silva et al., 2025). In this context, the PAE of guava and mango coated with SA-LAB, SA, and the control was performed at the beginning of storage and after 6 and 15 days (Figure 7), using a nine-point hedonic scale

970 to quantify attribute intensity, where 1 indicated the lowest perception and 9 the highest
 971 intensity of each evaluated descriptor (Siva et al., 2025).
 972 For guava, at the beginning of storage (Figure 7A), the control (uncoated fruit) was
 973 mainly described as having green peel (7.81 ± 1.47), white pulp (4.00 ± 1.46), and intact
 974 structure (8.25 ± 0.78). The SA-LAB coating was associated with sweet (5.69 ± 1.66),
 975 characteristic (7.38 ± 1.89), and fermented (4.81 ± 1.60) aromas. In contrast, the SA
 976 coating was primarily linked to a visible coating layer (7.06 ± 1.95) and reddish pulp
 977 (4.63 ± 1.20). A shiny appearance was associated with both SA-LAB- (7.88 ± 0.96) and
 978 SA-coated (8.06 ± 1.44) samples. These findings indicate that, even at the start of storage,
 979 edible coatings mainly influenced guava appearance and aroma. By day 6 of storage
 980 (Figure 7B), the control was associated with sweet (6.59 ± 2.79), fermented (6.06 ± 2.16),
 981 and characteristic (8.35 ± 1.22) aromas, yellow peel (8.41 ± 0.71), red pulp (8.35 ± 0.77),
 982 a more advanced ripening stage (8.24 ± 0.56), and a moist appearance (7.59 ± 1.66). The
 983 SA-LAB treatment preserved white pulp color (6.47 ± 1.28) and brightness (7.88 ± 0.93).
 984 The SA treatment was characterized by fruit integrity (8.18 ± 0.81), green peel ($8.04 \pm$
 985 0.99), firm texture (8.65 ± 0.61), and a visible coating (5.82 ± 1.70). Overall, particularly
 986 given the color descriptors, these results suggest that the coatings delayed guava ripening
 987 by preserving traits like those observed at the beginning of storage, whereas the control
 988 fruit reached a more advanced stage of ripening. These observations are consistent with
 989 the peel and pulp color data shown in Table 2. By day 15 of storage (Figure 7C), the
 990 control showed a profile like that observed on day 6. The SA-LAB coating maintained
 991 the same general pattern, preserving white pulp color (4.75 ± 0.93) and brightness (6.38
 992 ± 1.03), although the visible coating (4.75 ± 1.18) became more noticeable. The SA
 993 coating also retained most of the characteristics observed at the previous time point,
 994 except that the coating became less visible on the fruit surface.

Sensory analysis results indicated that edible coatings delayed guava ripening and helped maintain desirable sensory attributes throughout storage. SA-LAB coating treatment stood out for preserving visual characteristics, such as brightness, over time, though with increased surface perception of the coating. In contrast, the control showed faster ripening and deterioration-associated attributes.

For mango, at the beginning of storage (Figure 7D), the control (uncoated) was mainly characterized by smooth texture (7.10 ± 1.27). The SA-coating treatment exhibited green (6.50 ± 1.41) and purple (6.43 ± 1.86) peel colors as its main attributes. The SA-LAB coating treatment, in turn, was associated with acidic (4.56 ± 0.96), sweet (4.25 ± 1.18), characteristic (7.11 ± 1.60), and fermented (4.06 ± 1.00) aromas, as well as yellow pulp color (5.71 ± 1.65) and a bright appearance (7.69 ± 1.08). The attributes of apparent coating and firm texture were associated with both the SA and SA-LAB coating treatments. On the 6th day of storage (Figure 7E), the control was characterized by a red peel color (5.65 ± 1.37), a moist texture (7.24 ± 1.15), greater ripeness (8.12 ± 0.93), and a characteristic aroma (7.94 ± 0.97). The SA-LAB treatment maintained its association with acidic (5.06 ± 1.03), sweet (5.53 ± 1.01), and fermented (4.00 ± 1.28) aromas, as well as smooth texture (7.12 ± 1.11) and bright appearance (7.32 ± 1.41). The SA coating treatment exhibited attributes such as green (6.97 ± 1.30) and purple (4.88 ± 1.05) peel coloration, greater fruit integrity (8.18 ± 1.24), and a visible coating (8.65 ± 0.61). Yellow pulp color was associated with both the control (7.35 ± 1.41) and the SA-coating treatment (6.06 ± 1.03). On the 15th day of storage (Figure 7F), the control showed yellow pulp (8.75 ± 0.45) and red peel (5.00 ± 0.82), along with characteristic and sweet aromas (7.44 ± 0.81), indicating a more advanced ripening stage (8.88 ± 0.34). The SA-LAB coating treatment was associated with purple peel (4.00 ± 0.82) coloration, firm texture (6.00 ± 0.89), and acidic (6.25 ± 1.34) and fermented (5.63 ± 0.89) aromas. In

turn, SA was characterized by bright appearance (7.38 ± 1.03), greater integrity (7.25 ± 0.93), green peel coloration (6.94 ± 1.00), and smooth texture (5.60 ± 0.89). The visible coating attribute showed a similar association between SA (8.88 ± 0.50) and the SA-LAB coating treatment (8.94 ± 0.25).

Overall, these results indicate that edible coatings influenced the evolution of mango sensory characteristics during storage. SA coating contributed mainly to maintaining attributes associated with fruit integrity and less-advanced ripening stages, while SA-LAB coating was more closely associated with aroma and appearance attributes, preserving brightness but also exhibiting fermentative notes over time. In contrast, the control showed a more pronounced progression of ripening, with intensification of characteristics typical of fully ripe fruits.

In the present study, the preservation of a bright appearance in SA- and SA-LAB-coated fruits may be associated with the formation of a continuous, homogeneous polymeric film on the fruit surface, which reduces surface dehydration and light scattering, thereby enhancing visual gloss and overall appearance (Silva et al., 2023). This physical effect is commonly linked to the barrier properties of sodium alginate-based matrices, which help maintain surface integrity during storage (Metha et al., 2024; Wang et al., 2025). On the other hand, the fermented aroma detected in SA-LAB-coated fruits may be related to the metabolic activity of LAB, including the production of volatile organic compounds such as organic acids, esters, and alcohols, which can accumulate at the fruit surface under storage conditions and contribute to the sensory profile over time (Bangar et al., 2022; Oliveira et al., 2025).

The application of coatings containing *L. plantarum* to strawberries resulted in acceptable sensory attributes of color, flavor, taste, and texture over 10 days of storage, without significant influence of the different probiotic concentrations on fruit sensory

characteristics (Khodaei & Hamidi-Esfahani, 2019). Similarly, Dias et al. (2023) reported that edible coatings based on sodium alginate, incorporated with *Lactocaseibacillus casei*, improved the sensory quality of minimally processed mangos and melons during storage, primarily by maintaining a desirable appearance and delaying the perception of ripening-related attributes.

4 Conclusion

The incorporation of a mixed culture of LAB into the SA-based coating was successful, as evidenced by functional characterization analyses that indicated a well-structured, coherent film morphology. The SA-LAB films also exhibited satisfactory physicochemical and surface properties, including controlled roughness, moisture content, and solubility, thereby supporting their structural stability and suitability for use as edible coatings. Overall, both SA and SA-LAB coatings also showed high biodegradation capacity across the different media evaluated, with significant degradation over a short period, demonstrating the efficiency of the polymeric matrix under the tested environmental conditions and that the incorporation of LAB does not compromise its environmental performance. In addition, the SA-LAB coating effectively maintained the postharvest quality of Paluma guavas and Palmer mangos during storage at room temperature. A reduction in mass loss, delayed degradation of ascorbic acid and carotenoids, as well as better preservation of color, were observed in coated fruits. Sensory attributes corroborated the physicochemical results, indicating maintenance of color, aroma, and texture throughout storage. The SA-LAB coating examined shows potential as a sustainable alternative for fruit-coating applications, reducing environmental impact and extending the marketing period.

Funding

The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil, Funding code 001) awarded scholarship to Karina Felix Dias Fernandes.

Declaration of Competing Interest

The authors declare no competing interests.

Data availability

All data are available in the present manuscript.

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FIGURES

Figure 1. Fourier Transform Infrared Spectroscopy (FTIR) spectra (A) and Differential Scanning Calorimetry (DSC) curves (B) of coatings formulated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB) and sodium alginate alone (SA).

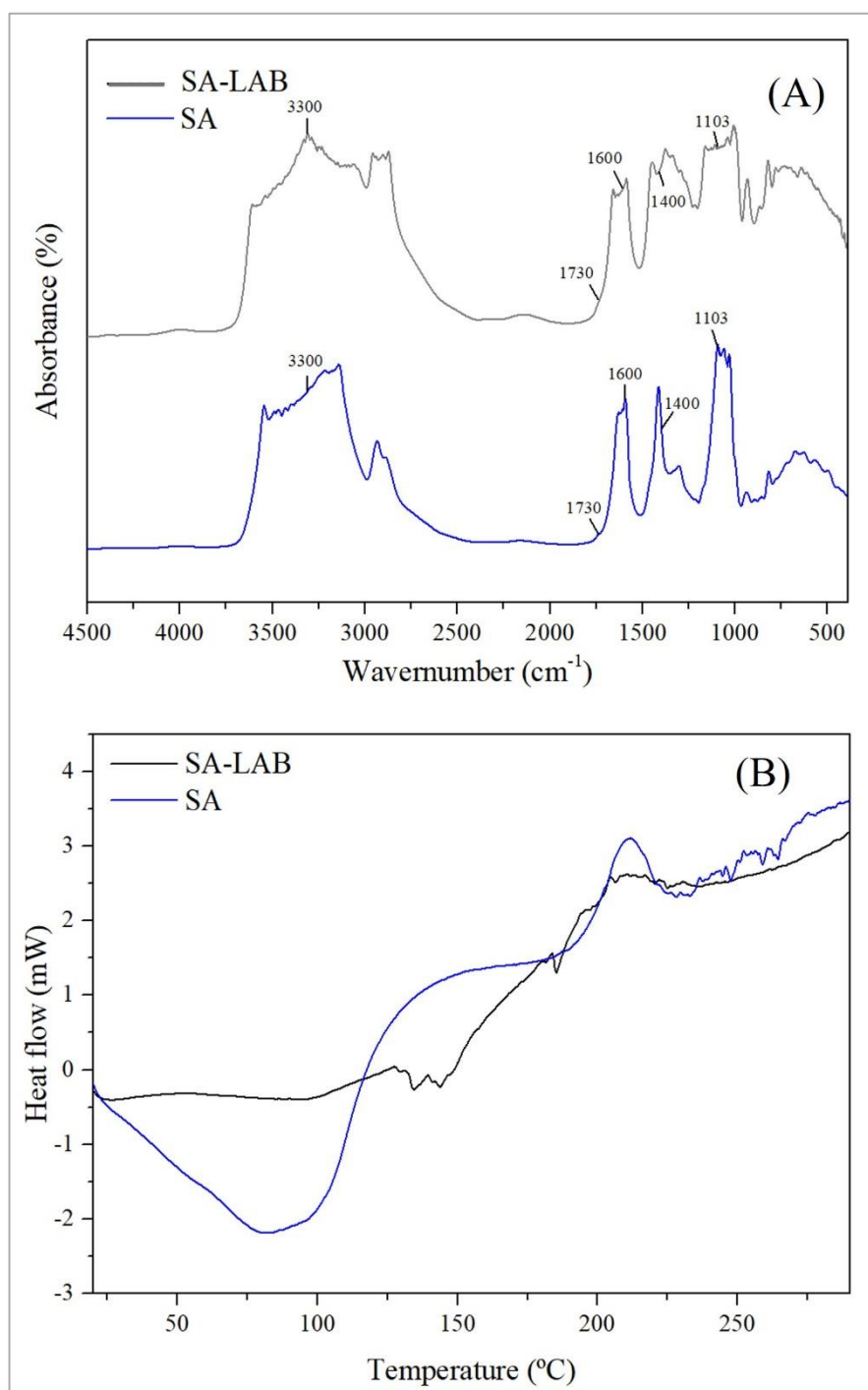
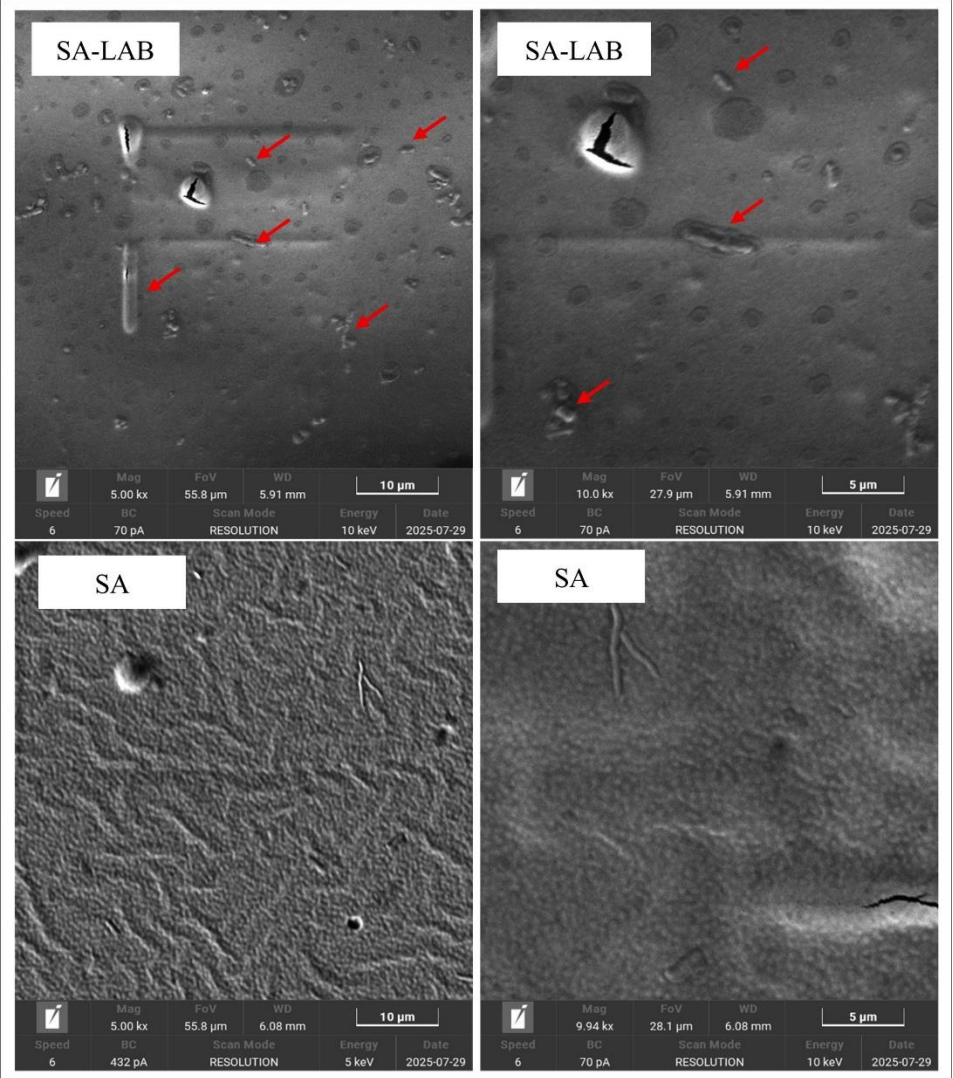


Figure 2. Scanning electron microscopy (SEM) images of coating samples formulated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB) and sodium alginate alone (SA).



Legend: Red arrows indicate rod-shaped structures compatible with lactic acid bacterial cells embedded in the alginate matrix.

Figure 3. Images captured with surface roughness analysis of coatings formulated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB) and sodium alginate alone (SA).

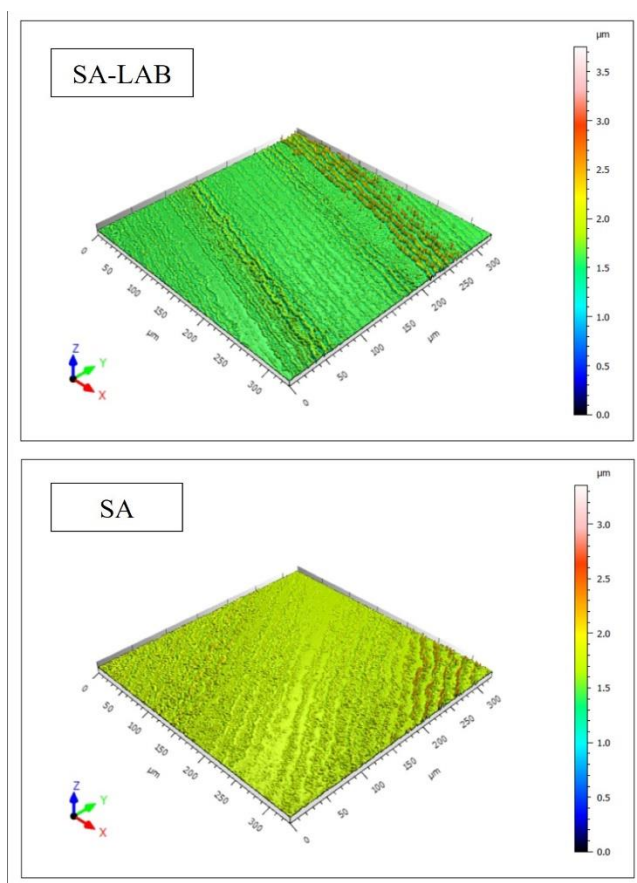
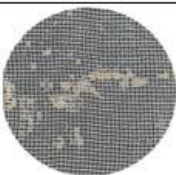
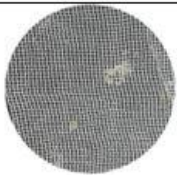
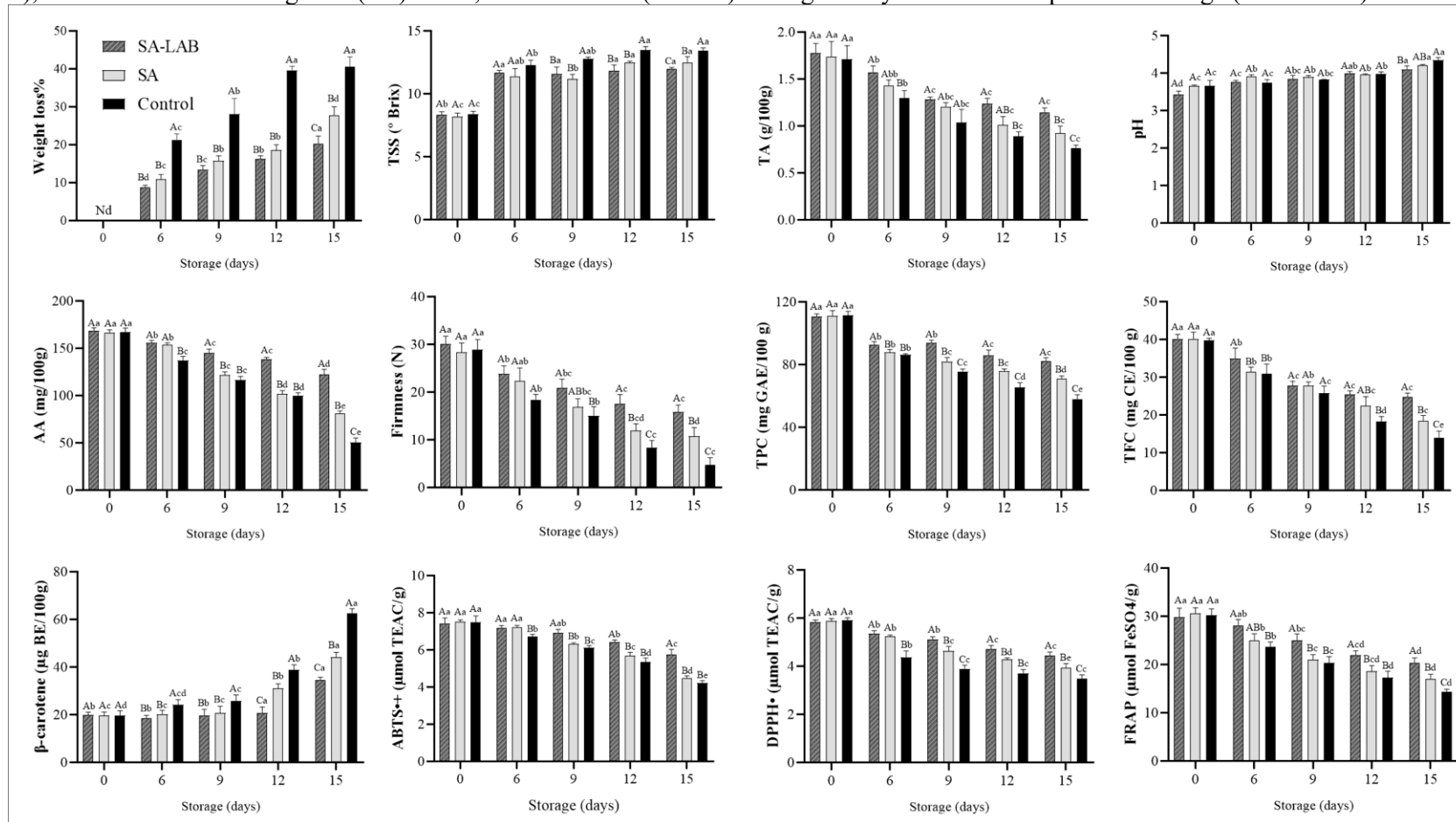


Figure 4. Biodegradability in soil, water, and seawater of coatings formulated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB) and sodium alginate alone (SA).

Coatings	Storage days				
	0	6	12	15	20
Soil					
SA-LAB					
SA					
Water					
SA-LAB					Nd
SA					Nd
Seawater					
SA-LAB					
SA					

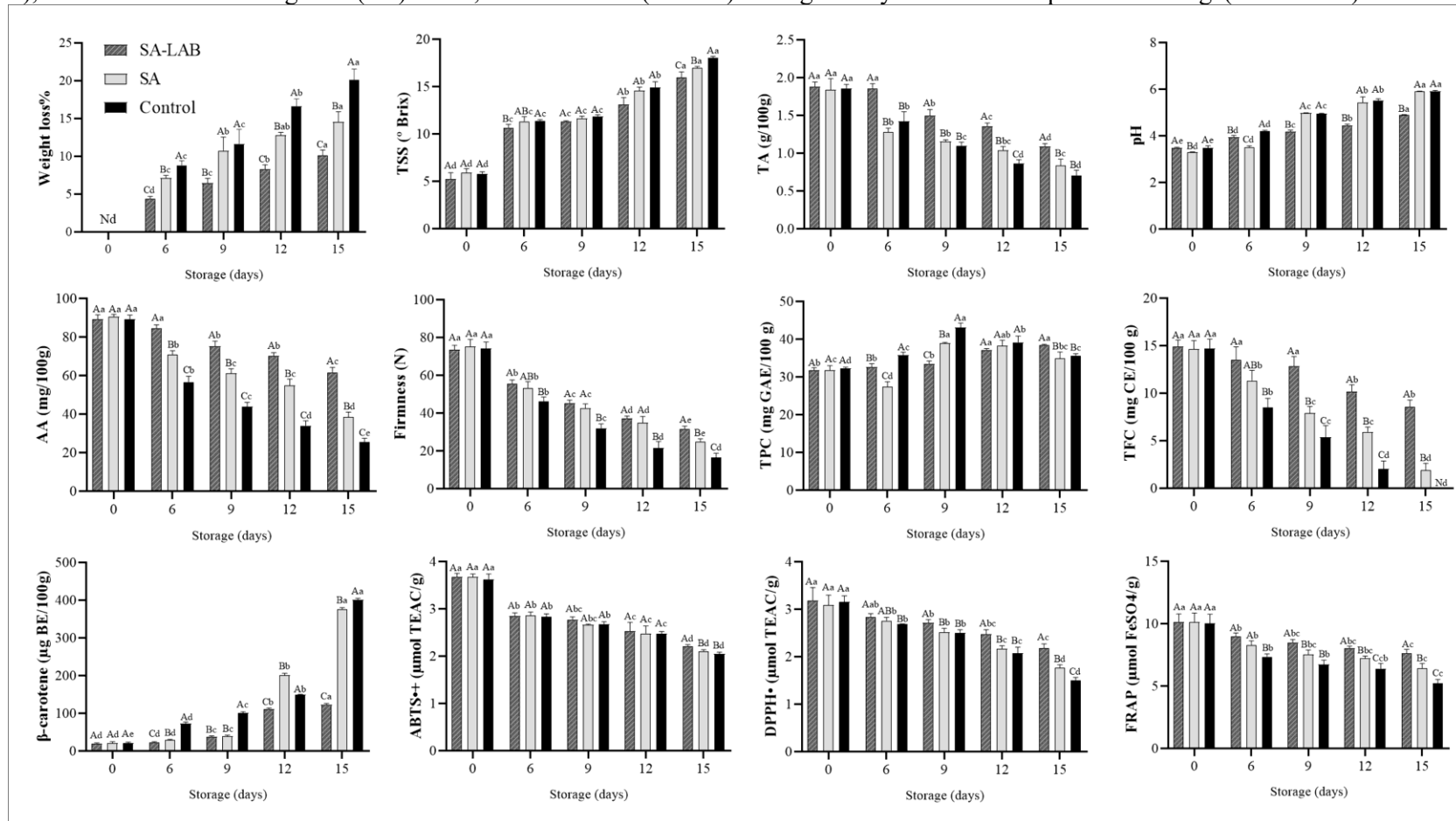
Legend: Nd: Not detected.

Figure 5. Physicochemical parameters (average $\pm$ standard deviation) of guava cv. Paluma coated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB), coated with sodium alginate (SA) alone, and uncoated (control) during 15 days of room temperature storage ($25 \pm 0.5^\circ\text{C}$).



Legend: Control: uncoated guava (sterile distilled water + glycerol 2.5 mL/100 mL). TSS: Total Soluble Solids. TA: Titratable Acidity. AA: Ascorbic acid. TPC: Total phenolic content. TFC: Total flavonoid content. GAE: Gallic acid equivalent. CE: Catechin equivalent. BE: β -carotene equivalent. DPPH: Radical scavenging activity. FeSO₄: Ferrous Sulfate. FRAP: Ferric-reducing power. TEAC: Trolox Equivalent Antioxidant Capacity. Nd: Not determined. A–C: different letters for treatments at the same storage time indicate significant differences ($p < 0.05$) based on Tukey's test. a–e: different letters for the same treatment indicate significant differences across storage times ($p < 0.05$) based on Tukey's test.

Figure 6. Physicochemical parameters (average $\pm$ standard deviation) of mango cv. Palmer coated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB), coated with sodium alginate (SA) alone, and uncoated (control) during 15 days of room temperature storage ($25 \pm 0.5^\circ\text{C}$).



Legend: Control: uncoated guava (sterile distilled water + glycerol 2.5 mL/100 mL). TSS: Total Soluble Solids. TA: Titratable Acidity. AA: Ascorbic acid. TPC: Total phenolic content. TFC: Total flavonoid content. GAE: Gallic acid equivalent. CE: Catechin equivalent. BE: β -carotene equivalent. DPPH: Radical scavenging activity. FeSO₄: Ferrous Sulfate. FRAP: Ferric-reducing power. TEAC: Trolox Equivalent Antioxidant Capacity. Nd: Not determined. A–C: different letters for treatments at the same storage time indicate significant differences ($p < 0.05$) based on Tukey's test. a–e: different letters for the same treatment indicate significant differences across storage times ($p < 0.05$) based on Tukey's test.

Figure 7. Generalized Procrustes Analysis (GPA) biplots of sensory characteristics in coated guava (A–C: 0, 6, and 15 days of room temperature storage) and mango (D–F: 0, 6, and 15 days of room temperature storage).

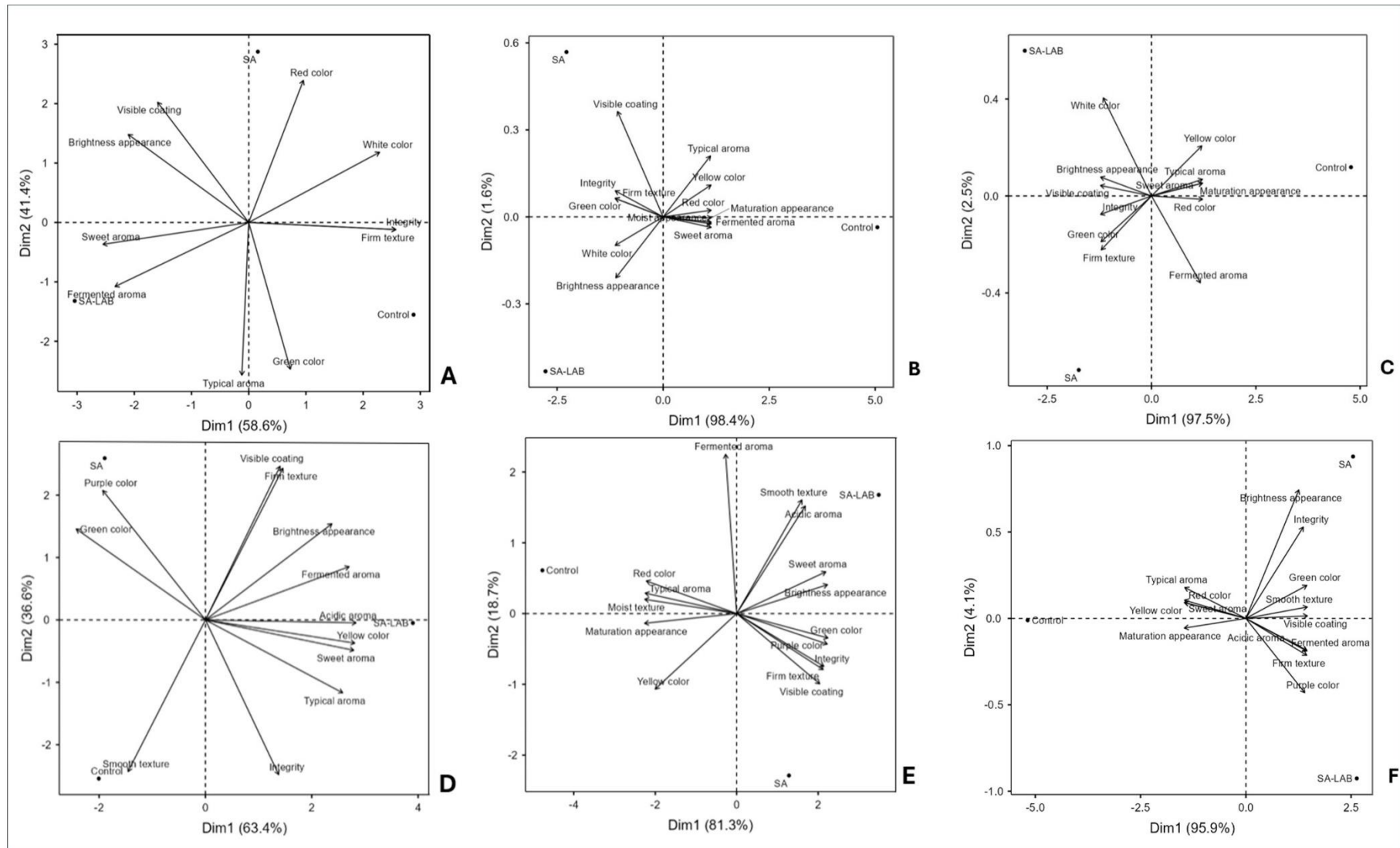
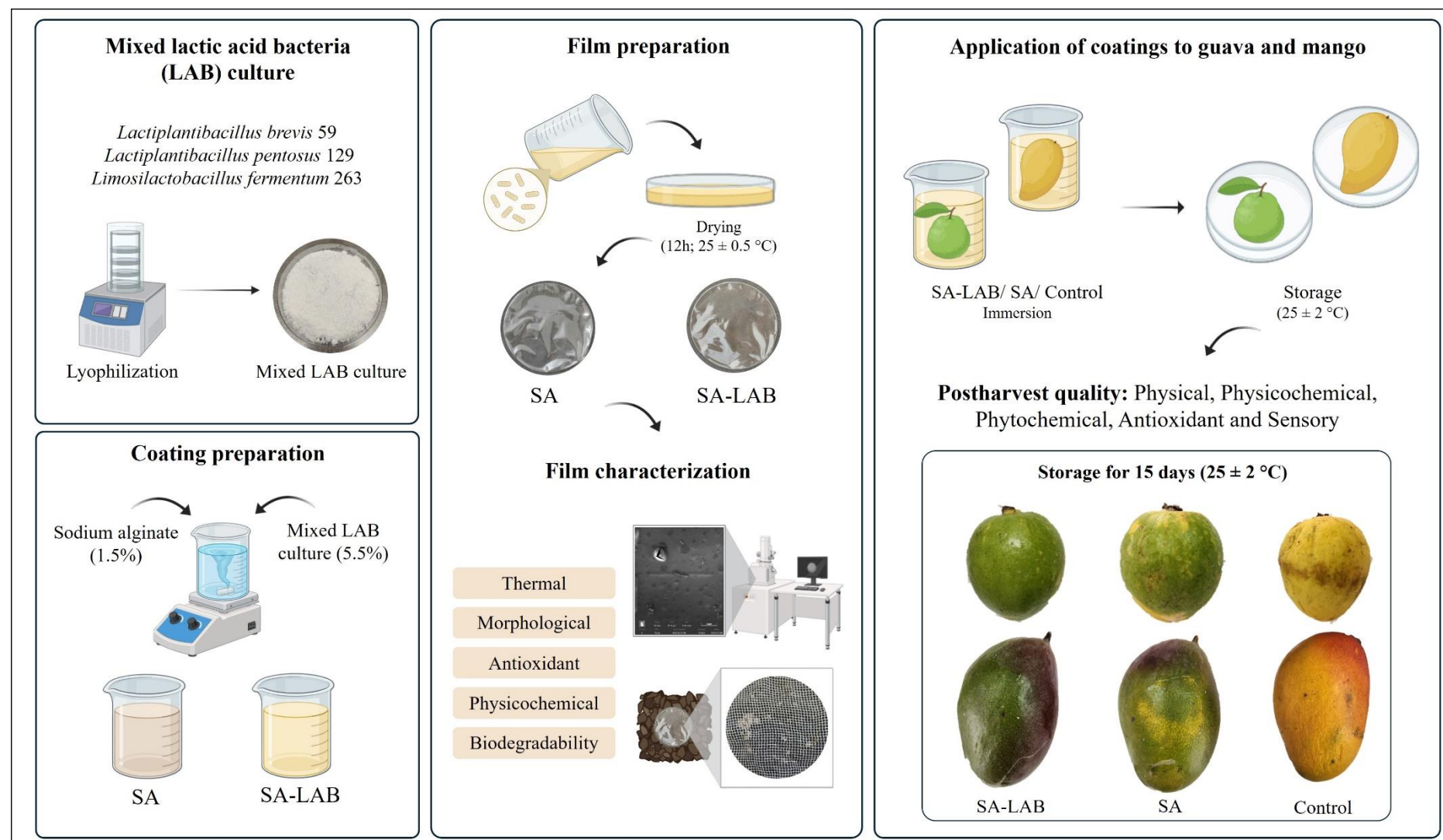


Figure 8. Abstract Graphical.



TABLES

Table 1. Physical, physicochemical, and colorimetric properties of coatings formulated with sodium alginate and a mixed culture of lactic acid bacteria (SA-LAB) and sodium alginate alone (SA).

Parameter/ Coatings	SA-LAB	SA
Roughness [Ra] (nm)	80.04 ± 6.30 ^b	109.80 ± 8.40 ^a
Thickness (µm)	83.60 ± 1.90 ^b	111.80 ± 10.20 ^a
Moisture (%)	13.15 ± 2.47 ^b	23.49 ± 0.74 ^a
Water solubility (%)	28.60 ± 3.68 ^b	36.82 ± 1.20 ^a
Hygroscopicity (%)	44.89 ± 2.57 ^b	61.03 ± 3.62 ^a
Water activity (Aw)	0.38 ± 0.03 ^b	0.46 ± 0.03 ^a
Color parameters		
L	97.85 ± 0.23 ^a	98.16 ± 0.72 ^a
a*	-4.30 ± 0.23 ^a	-4.65 ± 0.21 ^a
b*	-4.53 ± 0.27 ^a	-4.71 ± 0.23 ^a
Choma	6.36 ± 0.29 ^a	6.54 ± 0.43 ^a
Hue	225.96 ± 1.38 ^a	227.46 ± 1.93 ^a
Antioxidant capacity		
ABTS•+ (µmol Trolox TEAC/g)	0.19 ± 0.03 ^a	0.12 ± 0.02 ^b
DPPH• (µmol Trolox TEAC/g)	0.90 ± 0.07 ^a	0.42 ± 0.07 ^b
FRAP (µmol FeSO ₄ /g)	3.31 ± 0.11 ^a	1.70 ± 0.12 ^b

DPPH: Radical scavenging activity. FeSO₄: Ferrous sulphate. FRAP: Ferric-reducing power. TEAC: Trolox Equivalent Antioxidant Capacity.

a-b: Average values in the same column, for the same measured parameter, with different uppercase letters are significantly different (p < 0.05), based on Duncan's test.

Table 2. Colorimetric parameters (average $\pm$ standard deviation) of guava cv. Paluma coated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB), sodium alginate alone (SA), or uncoated (negative control) over 15 days of storage at room temperature (25 ± 0.5 °C).

Parameters/ treatments	Storage days				
	0	6	9	12	15
Fruit peel					
L* (Luminosity)					
SA-LAB	53.98 $\pm$ 1.16 ^{Ac}	56.00 $\pm$ 1.25 ^{Bbc}	59.75 $\pm$ 2.14 ^{Bab}	60.75 $\pm$ 1.50 ^{Ba}	62.45 $\pm$ 2.11 ^{Ca}
SA	54.46 $\pm$ 1.56 ^{Ac}	57.38 $\pm$ 1.92 ^{Bc}	60.33 $\pm$ 2.35 ^{Bbc}	65.06 $\pm$ 2.27 ^{Bab}	70.80 $\pm$ 1.45 ^{Ba}
Negative control	54.51 $\pm$ 1.47 ^{Ad}	70.03 $\pm$ 2.32 ^{Ac}	75.42 $\pm$ 1.37 ^{Ab}	78.57 $\pm$ 1.60 ^{Aab}	80.06 $\pm$ 0.84 ^{Aa}
a* (red/green)					
SA-LAB	-18.81 $\pm$ 1.04 ^{Ac}	-17.10 $\pm$ 0.58 ^{Bbc}	-16.52 $\pm$ 0.89 ^{Bb}	-15.79 $\pm$ 1.10 ^{Cb}	-11.20 $\pm$ 0.79 ^{Ca}
SA	-18.39 $\pm$ 0.92 ^{Ad}	-18.20 $\pm$ 0.25 ^{Bd}	-14.53 $\pm$ 1.24 ^{Bc}	-11.90 $\pm$ 0.86 ^{Bb}	-4.20 $\pm$ 0.83 ^{Ba}
Negative control	-17.20 $\pm$ 1.51 ^{Ac}	-11.76 $\pm$ 1.75 ^{Ad}	-6.06 $\pm$ 1.25 ^{Ac}	4.23 $\pm$ 0.64 ^{Ab}	8.90 $\pm$ 1.27 ^{Aa}
b* (yellow/blue)					
SA-LAB	39.49 $\pm$ 0.61 ^{Aa}	39.31 $\pm$ 1.20 ^{Ba}	39.26 $\pm$ 1.21 ^{Ba}	40.81 $\pm$ 0.23 ^{Ba}	40.53 $\pm$ 0.51 ^{Ba}
SA	38.69 $\pm$ 1.72 ^{Aa}	38.57 $\pm$ 1.12 ^{Ba}	37.43 $\pm$ 1.81 ^{Ba}	38.52 $\pm$ 0.90 ^{Ba}	40.95 $\pm$ 1.53 ^{Ba}
Negative control	37.73 $\pm$ 2.28 ^{Ad}	43.48 $\pm$ 2.56 ^{Ac}	46.06 $\pm$ 1.81 ^{Abc}	50.76 $\pm$ 1.43 ^{Ab}	59.10 $\pm$ 1.70 ^{Aa}
Chroma					
SA-LAB	37.56 $\pm$ 0.72 ^{Ab}	38.80 $\pm$ 0.23 ^{Bb}	41.33 $\pm$ 0.68 ^{Bab}	42.65 $\pm$ 1.18 ^{Ba}	42.74 $\pm$ 1.75 ^{Ca}
SA	38.36 $\pm$ 0.82 ^{Ac}	41.47 $\pm$ 1.98 ^{Bbc}	41.03 $\pm$ 1.21 ^{Bbc}	45.33 $\pm$ 0.83 ^{Bb}	51.27 $\pm$ 2.73 ^{Ba}
Negative control	37.73 $\pm$ 1.59 ^{Ac}	52.29 $\pm$ 0.93 ^{Ab}	55.42 $\pm$ 1.51 ^{Aab}	55.40 $\pm$ 2.28 ^{Aab}	59.18 $\pm$ 0.41 ^{Aa}
Hue (h°)					
SA-LAB	118.43 $\pm$ 1.07 ^{Aa}	114.99 $\pm$ 1.64 ^{Aab}	111.01 $\pm$ 1.74 ^{Abc}	109.46 $\pm$ 0.83 ^{Ac}	105.95 $\pm$ 2.32 ^{Ac}
SA	119.40 $\pm$ 2.24 ^{Aa}	115.67 $\pm$ 1.32 ^{Aa}	107.61 $\pm$ 2.93 ^{Ab}	97.53 $\pm$ 2.27 ^{Bc}	88.19 $\pm$ 2.20 ^{Bd}
Negative control	117.89 $\pm$ 2.09 ^{Aa}	112.64 $\pm$ 1.76 ^{Ab}	95.29 $\pm$ 1.21 ^{Bc}	91.64 $\pm$ 1.68 ^{Cc}	78.34 $\pm$ 2.00 ^{Cd}
ΔE					
SA-LAB	0.00 $\pm$ 0.00 ^{Ad}	3.02 $\pm$ 1.18 ^{Bc}	6.41 $\pm$ 2.13 ^{Bb}	7.69 $\pm$ 1.15 ^{Ab}	11.63 $\pm$ 2.01 ^{Ca}
SA	0.00 $\pm$ 0.00 ^{Ac}	3.11 $\pm$ 1.17 ^{Bc}	7.80 $\pm$ 3.47 ^{Bb}	12.51 $\pm$ 3.53 ^{Ab}	21.81 $\pm$ 0.57 ^{Ba}
Negative control	0.00 $\pm$ 0.00 ^{Ac}	18.16 $\pm$ 4.58 ^{Ad}	25.69 $\pm$ 3.57 ^{Ac}	35.41 $\pm$ 1.44 ^{Bb}	42.89 $\pm$ 1.12 ^{Aa}
Fruit pulp					
L* (Luminosity)					
SA-LAB	81.58 $\pm$ 2.00 ^{Aa}	78.85 $\pm$ 2.29 ^{Aab}	75.40 $\pm$ 0.68 ^{Ab}	74.72 $\pm$ 1.67 ^{Ab}	68.98 $\pm$ 1.55 ^{Ac}
SA	80.37 $\pm$ 2.01 ^{Aa}	71.62 $\pm$ 2.03 ^{Bb}	65.75 $\pm$ 1.51 ^{Bbc}	61.72 $\pm$ 1.93 ^{Bcd}	59.12 $\pm$ 1.35 ^{Bd}
Negative control	80.73 $\pm$ 1.97 ^{Aa}	66.21 $\pm$ 2.26 ^{Cb}	60.32 $\pm$ 1.46 ^{Bc}	54.58 $\pm$ 1.45 ^{Cd}	51.97 $\pm$ 1.86 ^{Cd}
a* (red/green)					
SA-LAB	18.21 $\pm$ 1.22 ^{Ab}	18.37 $\pm$ 1.39 ^{Ab}	21.29 $\pm$ 1.46 ^{Bab}	21.41 $\pm$ 1.30 ^{Bab}	23.84 $\pm$ 1.46 ^{Ba}
SA	17.69 $\pm$ 1.72 ^{Ad}	21.56 $\pm$ 1.28 ^{Ac}	26.70 $\pm$ 0.71 ^{Ab}	28.34 $\pm$ 0.24 ^{Ab}	31.34 $\pm$ 0.67 ^{Aa}
Negative control	17.06 $\pm$ 1.93 ^{Ad}	21.89 $\pm$ 2.77 ^{Ac}	26.41 $\pm$ 1.49 ^{Abc}	30.90 $\pm$ 1.27 ^{Aab}	34.27 $\pm$ 1.91 ^{Aa}
b* (yellow/blue)					
SA-LAB	18.65 $\pm$ 1.23 ^{Ab}	21.62 $\pm$ 0.89 ^{Aab}	23.30 $\pm$ 1.36 ^{Aa}	22.05 $\pm$ 1.48 ^{Aa}	22.51 $\pm$ 0.79 ^{Aa}
SA	18.71 $\pm$ 0.71 ^{Ab}	19.50 $\pm$ 0.47 ^{Bab}	21.34 $\pm$ 1.41 ^{Aab}	21.29 $\pm$ 1.12 ^{Aab}	21.60 $\pm$ 0.97 ^{Aa}
Negative control	17.92 $\pm$ 0.85 ^{Ab}	19.89 $\pm$ 0.72 ^{Bb}	21.95 $\pm$ 0.90 ^{Aab}	23.07 $\pm$ 1.58 ^{Aa}	23.48 $\pm$ 1.01 ^{Aa}
Chroma					
SA-LAB	25.80 $\pm$ 1.06 ^{Ab}	25.62 $\pm$ 0.86 ^{Bb}	25.78 $\pm$ 3.09 ^{Bb}	27.59 $\pm$ 1.15 ^{Bab}	31.00 $\pm$ 1.04 ^{Ca}
SA	25.17 $\pm$ 1.63 ^{Ac}	31.32 $\pm$ 1.25 ^{Ab}	33.57 $\pm$ 1.31 ^{Aab}	34.92 $\pm$ 1.74 ^{Aab}	37.11 $\pm$ 1.70 ^{Ba}
Negative control	25.52 $\pm$ 1.63 ^{Ac}	31.51 $\pm$ 2.23 ^{Ab}	35.72 $\pm$ 2.20 ^{Ab}	38.06 $\pm$ 1.45 ^{Aab}	42.81 $\pm$ 1.96 ^{Aa}
Hue					
SA-LAB	49.29 $\pm$ 0.85 ^{Aa}	48.64 $\pm$ 1.58 ^{Aa}	41.46 $\pm$ 1.23 ^{Ab}	39.17 $\pm$ 0.81 ^{Abc}	37.49 $\pm$ 1.20 ^{Ac}
SA	49.21 $\pm$ 1.42 ^{Aa}	45.06 $\pm$ 1.25 ^{Ab}	40.74 $\pm$ 0.49 ^{Ac}	35.52 $\pm$ 0.66 ^{Bd}	33.67 $\pm$ 0.82 ^{Bd}
Negative control	48.22 $\pm$ 1.63 ^{Aa}	40.47 $\pm$ 1.95 ^{Bb}	34.46 $\pm$ 1.63 ^{Bc}	31.60 $\pm$ 1.17 ^{Ccd}	28.25 $\pm$ 1.29 ^{Cd}
ΔE					
SA-LAB	0.00 $\pm$ 0.00 ^{Ad}	5.00 $\pm$ 1.60 ^{Bc}	8.48 $\pm$ 1.33 ^{Bb}	8.86 $\pm$ 1.89 ^{Cb}	14.64 $\pm$ 1.17 ^{Ba}
SA	0.00 $\pm$ 0.00 ^{Ad}	8.86 $\pm$ 0.72 ^{Bc}	17.51 $\pm$ 1.81 ^{Ab}	21.73 $\pm$ 2.49 ^{Ba}	25.50 $\pm$ 3.15 ^{Aa}

Negative control	0.00 ± 0.00^{Ad}	15.88 ± 1.78^{Ac}	22.97 ± 2.24^{Ab}	30.08 ± 2.99^{Aa}	34.05 ± 4.03^{Aa}
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Negative control: uncoated guava (sterile distilled water + glycerol 2.5 mL/100 mL).

A-C: Average values in the same column, for a same measured parameter, with different uppercase letters are significantly different ($p < 0.05$), based on Tukey's test.

a-c: Average values in the same row with different lowercase letters are significantly different ($p < 0.05$). based on Tukey's test.

Table 3. Colorimetric parameters (average $\pm$ standard deviation) of mango cv. Palmer coated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB), sodium alginate alone (SA), or uncoated (negative control) over 15 days of storage at room temperature (25 ± 0.5 °C).

Parameters/ treatments	Storage days				
	0	6	9	12	15
Fruit peel					
L* (Luminosity)					
SA-LAB	45.56 $\pm$ 2.79 ^{Ab}	45.87 $\pm$ 0.98 ^{Bb}	45.72 $\pm$ 1.08 ^{Cb}	47.73 $\pm$ 1.40 ^{Cab}	51.46 $\pm$ 1.80 ^{Ca}
SA	45.30 $\pm$ 1.54 ^{Ad}	45.91 $\pm$ 1.69 ^{Bcd}	49.70 $\pm$ 1.82 ^{Bbc}	52.45 $\pm$ 1.45 ^{Bb}	56.77 $\pm$ 0.92 ^{Ba}
Negative control	45.60 $\pm$ 1.69 ^{Ac}	50.62 $\pm$ 1.38 ^{Ad}	54.46 $\pm$ 0.62 ^{Ac}	58.90 $\pm$ 1.39 ^{Ab}	62.73 $\pm$ 1.28 ^{Aa}
a* (red/green)					
SA-LAB	-11.89 $\pm$ 1.01 ^{Aa}	-11.05 $\pm$ 0.60 ^{Aa}	-10.38 $\pm$ 0.70 ^{Ba}	-11.70 $\pm$ 0.45 ^{Ca}	-10.13 $\pm$ 0.95 ^{Ca}
SA	-11.40 $\pm$ 2.39 ^{Ac}	-9.31 $\pm$ 1.97 ^{Ac}	-8.17 $\pm$ 0.93 ^{Ac}	-2.76 $\pm$ 0.48 ^{Bb}	2.64 $\pm$ 0.75 ^{Ba}
Negative control	-13.22 $\pm$ 1.59 ^{Ad}	-10.45 $\pm$ 1.10 ^{Acd}	-5.82 $\pm$ 1.23 ^{Ac}	4.39 $\pm$ 0.98 ^{Ab}	13.88 $\pm$ 3.58 ^{Aa}
b* (yellow/blue)					
SA-LAB	18.41 $\pm$ 0.83 ^{Ac}	21.17 $\pm$ 1.25 ^{Bbc}	23.26 $\pm$ 2.24 ^{Bab}	23.50 $\pm$ 0.92 ^{Bab}	25.43 $\pm$ 1.54 ^{Ba}
SA	17.13 $\pm$ 0.97 ^{Ad}	19.93 $\pm$ 0.71 ^{Bcd}	21.55 $\pm$ 1.46 ^{Bc}	25.10 $\pm$ 1.55 ^{Bb}	29.48 $\pm$ 1.58 ^{Ba}
Negative control	18.06 $\pm$ 0.83 ^{Ad}	24.35 $\pm$ 1.24 ^{Ac}	28.90 $\pm$ 1.48 ^{Ac}	32.28 $\pm$ 1.88 ^{Ab}	43.18 $\pm$ 1.84 ^{Aa}
Chroma					
SA-LAB	17.43 $\pm$ 2.61 ^{Ac}	20.21 $\pm$ 0.71 ^{Bbc}	21.69 $\pm$ 1.89 ^{Bab}	23.14 $\pm$ 1.59 ^{Ba}	25.23 $\pm$ 1.59 ^{Ba}
SA	16.71 $\pm$ 1.67 ^{Ac}	19.14 $\pm$ 1.87 ^{Bbc}	21.37 $\pm$ 0.82 ^{Bb}	22.84 $\pm$ 1.41 ^{Bab}	26.17 $\pm$ 1.21 ^{Ba}
Negative control	16.95 $\pm$ 2.25 ^{Ad}	25.45 $\pm$ 1.81 ^{Ac}	32.67 $\pm$ 0.76 ^{Ab}	37.55 $\pm$ 1.23 ^{Aa}	41.45 $\pm$ 1.67 ^{Aa}
Hue					
SA-LAB	119.04 $\pm$ 2.17 ^{Aa}	115.86 $\pm$ 1.73 ^{Aab}	113.62 $\pm$ 1.92 ^{Ab}	114.35 $\pm$ 1.32 ^{Ab}	111.57 $\pm$ 0.92 ^{Ab}
SA	121.03 $\pm$ 1.63 ^{Aa}	117.40 $\pm$ 2.52 ^{Aa}	104.53 $\pm$ 2.83 ^{Bb}	95.26 $\pm$ 1.24 ^{Bc}	85.45 $\pm$ 2.94 ^{Bd}
Negative control	120.19 $\pm$ 1.41 ^{Aa}	105.86 $\pm$ 1.05 ^{Bb}	96.62 $\pm$ 2.04 ^{Cc}	85.59 $\pm$ 3.11 ^{Cd}	78.67 $\pm$ 1.70 ^{Cc}
ΔE					
SA-LAB	0.00 $\pm$ 0.00 ^{Ac}	4.08 $\pm$ 1.06 ^{Bb}	6.21 $\pm$ 0.86 ^{Bb}	5.80 $\pm$ 0.94 ^{Cb}	9.92 $\pm$ 1.66 ^{Ca}
SA	0.00 $\pm$ 0.00 ^{Ad}	4.35 $\pm$ 2.23 ^{Bc}	7.25 $\pm$ 2.53 ^{Bc}	13.74 $\pm$ 2.65 ^{Bb}	21.83 $\pm$ 1.98 ^{Ba}
Negative control	0.00 $\pm$ 0.00 ^{Ac}	8.99 $\pm$ 1.30 ^{Ad}	16.07 $\pm$ 0.56 ^{Ac}	26.36 $\pm$ 1.44 ^{Ab}	40.82 $\pm$ 1.29 ^{Aa}
Fruit pulp					
L* (Luminosity)					
SA-LAB	81.83 $\pm$ 1.29 ^{Aa}	79.68 $\pm$ 0.74 ^{Aa}	75.55 $\pm$ 0.84 ^{Ab}	74.81 $\pm$ 1.56 ^{Abc}	71.94 $\pm$ 1.53 ^{Ac}
SA	82.29 $\pm$ 1.20 ^{Aa}	78.36 $\pm$ 1.41 ^{ABb}	74.04 $\pm$ 1.07 ^{Ac}	68.39 $\pm$ 0.59 ^{Bd}	64.17 $\pm$ 2.05 ^{Be}
Negative control	81.08 $\pm$ 1.45 ^{Aa}	76.48 $\pm$ 1.42 ^{Bb}	66.53 $\pm$ 2.15 ^{Bc}	59.54 $\pm$ 1.42 ^{Cd}	52.03 $\pm$ 1.76 ^{Cc}
a* (red/green)					
SA-LAB	-4.72 $\pm$ 0.36 ^{Ad}	-3.97 $\pm$ 0.13 ^{Cd}	-2.21 $\pm$ 0.17 ^{Cc}	1.58 $\pm$ 0.62 ^{Cb}	4.90 $\pm$ 0.73 ^{Ca}
SA	-4.40 $\pm$ 0.53 ^{Ac}	-2.54 $\pm$ 0.47 ^{Bd}	4.74 $\pm$ 0.28 ^{Bc}	6.15 $\pm$ 0.43 ^{Bb}	9.42 $\pm$ 0.38 ^{Ba}
Negative control	-4.25 $\pm$ 0.37 ^{Ad}	5.38 $\pm$ 0.69 ^{Ac}	11.38 $\pm$ 0.25 ^{Ab}	12.37 $\pm$ 0.31 ^{Ab}	15.75 $\pm$ 0.41 ^{Aa}
b* (yellow/blue)					
SA-LAB	51.94 $\pm$ 0.83 ^{Ab}	52.93 $\pm$ 1.50 ^{Ab}	54.61 $\pm$ 0.78 ^{ABb}	57.53 $\pm$ 0.86 ^{Aa}	58.83 $\pm$ 1.10 ^{Ba}
SA	51.51 $\pm$ 0.49 ^{Acd}	49.64 $\pm$ 0.56 ^{Ad}	53.26 $\pm$ 0.54 ^{Bc}	57.03 $\pm$ 0.63 ^{Ab}	59.51 $\pm$ 1.38 ^{Ba}
Negative control	52.23 $\pm$ 0.71 ^{Ac}	52.18 $\pm$ 1.92 ^{Ac}	56.90 $\pm$ 1.65 ^{Abc}	60.38 $\pm$ 2.34 ^{Aab}	64.01 $\pm$ 1.84 ^{Aa}
Chroma					
SA-LAB	57.31 $\pm$ 2.31 ^{Ab}	61.01 $\pm$ 0.96 ^{Aab}	63.34 $\pm$ 1.26 ^{Aa}	62.99 $\pm$ 1.32 ^{Aa}	63.49 $\pm$ 1.64 ^{Ba}
SA	56.83 $\pm$ 1.83 ^{Ab}	62.12 $\pm$ 1.87 ^{Aa}	63.31 $\pm$ 0.82 ^{Aa}	64.22 $\pm$ 0.67 ^{Aa}	65.10 $\pm$ 0.95 ^{Ba}
Negative control	57.66 $\pm$ 0.93 ^{Ad}	60.63 $\pm$ 1.52 ^{Acd}	62.59 $\pm$ 1.43 ^{Abc}	64.15 $\pm$ 1.25 ^{Ab}	68.47 $\pm$ 1.07 ^{Aa}
Hue					
SA-LAB	92.53 $\pm$ 1.91 ^{Aa}	94.25 $\pm$ 1.86 ^{Aa}	93.62 $\pm$ 1.50 ^{Aa}	93.10 $\pm$ 1.53 ^{Aa}	83.90 $\pm$ 0.92 ^{Ab}
SA	93.96 $\pm$ 1.45 ^{Aa}	88.80 $\pm$ 1.36 ^{Bb}	86.73 $\pm$ 0.78 ^{Bbc}	85.72 $\pm$ 0.73 ^{Bc}	80.67 $\pm$ 1.01 ^{Ad}
Negative control	93.85 $\pm$ 1.37 ^{Aa}	84.47 $\pm$ 1.03 ^{Cb}	79.86 $\pm$ 0.67 ^{Cc}	78.47 $\pm$ 1.18 ^{Cc}	73.82 $\pm$ 1.86 ^{Bd}
ΔE					
SA-LAB	0.00 $\pm$ 0.00 ^{Ac}	3.26 $\pm$ 0.86 ^{Ad}	7.40 $\pm$ 1.32 ^{Cc}	11.20 $\pm$ 1.47 ^{Cb}	15.44 $\pm$ 0.39 ^{Ca}
SA	0.00 $\pm$ 0.00 ^{Ac}	4.74 $\pm$ 2.71 ^{Ad}	12.47 $\pm$ 0.94 ^{Bc}	18.31 $\pm$ 0.86 ^{Bb}	24.19 $\pm$ 3.07 ^{Ba}

Negative control	0.00 ± 0.00^{Ac}	10.82 ± 1.84^{Ad}	21.95 ± 2.40^{Ac}	28.46 ± 3.05^{Ab}	37.20 ± 2.98^{Aa}
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Negative control: uncoated guava (sterile distilled water + glycerol 2.5 mL/100 mL).

A-C: Average values in the same column, for a same measured parameter, with different uppercase letters are significantly different ($p < 0.05$), based on Tukey's test.

a-c: Average values in the same row with different lowercase letters are significantly different ($p < 0.05$), based on Tukey's test

Supplementary 1. Sensory attributes (average $\pm$ standard deviation) guava cv. Paluma coated with sodium alginate and a mixed culture of lactic acid bacteria (SA-LAB), sodium alginate alone (SA), and uncoated (negative control) during 15 days of room temperature storage (25 ± 0.5 °C).

Attributes/ Days of storage	SA-LAB	SA	Control
0 day			
Brightness appearance	7.88 ± 0.96^a	8.06 ± 1.44^a	6.56 ± 1.86^b
Visible coating	6.56 ± 1.75^b	7.06 ± 1.95^a	1.00 ± 0.00^c
Integrity	8.00 ± 0.63^a	8.00 ± 0.82^a	8.25 ± 0.78^a
Green color	7.31 ± 1.20^a	7.69 ± 1.08^a	7.81 ± 1.47^a
Red color	4.56 ± 1.50^a	4.63 ± 1.20^a	4.19 ± 1.64^a
White color	3.63 ± 1.46^a	3.50 ± 1.90^a	4.00 ± 1.46^a
Sweet aroma	5.69 ± 1.66^a	5.50 ± 2.07^a	5.63 ± 1.78^a
Typical aroma	7.38 ± 1.89^a	7.25 ± 1.88^a	7.31 ± 1.35^a
Fermented aroma	4.81 ± 1.60^a	1.81 ± 0.83^b	1.44 ± 0.73^b
Firm texture	8.38 ± 0.81^a	8.56 ± 0.96^a	8.38 ± 1.09^a
6 days			
Maturation appearance	3.18 ± 1.51^b	3.06 ± 1.35^b	8.24 ± 0.56^a
Brightness appearance	7.88 ± 0.93^a	7.24 ± 1.30^a	4.77 ± 1.52^a
Visible coating	5.00 ± 1.50^a	5.82 ± 1.70^a	1.06 ± 0.24^b
Integrity	7.94 ± 1.09^a	8.18 ± 0.81^a	6.41 ± 1.66^a
Moist appearance	2.77 ± 0.97^b	3.06 ± 0.90^b	7.59 ± 1.66^a
Green color	7.94 ± 0.97^a	8.04 ± 0.99^a	1.47 ± 1.46^b
Yellow color	1.88 ± 1.22^b	1.77 ± 1.35^b	8.41 ± 0.71^a
Red color	1.88 ± 1.17^c	2.94 ± 1.30^b	8.35 ± 0.77^a
White color	6.47 ± 1.28^a	6.06 ± 1.48^b	1.06 ± 0.24^c
Sweet aroma	2.82 ± 1.70^b	2.77 ± 1.44^b	6.59 ± 2.79^a
Typical aroma	4.41 ± 1.78^b	5.47 ± 2.29^b	8.35 ± 1.22^a
Fermented aroma	2.18 ± 1.47^b	2.41 ± 1.54^b	6.06 ± 2.16^a
Firm texture	8.59 ± 0.71^a	8.65 ± 0.61^a	4.94 ± 0.90^b
15 days			
Maturation appearance	4.94 ± 1.53^b	5.38 ± 1.36^b	9.00 ± 0.00^a
Brightness appearance	6.38 ± 1.03^a	5.31 ± 0.87^b	1.81 ± 0.98^c
Visible coating	4.75 ± 1.18^a	3.81 ± 1.80^a	1.00 ± 0.00^b
Integrity	7.56 ± 1.26^a	7.00 ± 0.97^a	2.63 ± 1.03^b
Green color	8.06 ± 0.93^a	8.00 ± 0.97^a	1.06 ± 0.25^b
Yellow color	2.69 ± 1.58^b	2.81 ± 1.47^b	8.63 ± 0.62^a
Red color	3.38 ± 1.46^c	4.38 ± 1.63^b	8.56 ± 0.73^a
White color	4.75 ± 0.93^a	3.00 ± 1.27^b	1.06 ± 0.25^c
Sweet aroma	3.69 ± 1.08^b	4.06 ± 1.29^b	7.13 ± 1.41^a
Typical aroma	2.94 ± 1.44^b	3.56 ± 1.21^b	6.38 ± 1.03^a
Fermented aroma	5.53 ± 1.70^b	6.53 ± 1.07^{ab}	7.06 ± 1.35^a
Firm texture	7.59 ± 1.50^a	7.88 ± 1.05^a	4.71 ± 1.36^b

^{a-c}: Average values in the same row with different lowercase letters are significantly different ($p < 0.05$), based on Tukey's test.

Supplementary 2. Sensory attributes (average $\pm$ standard deviation) mango cv. Palmer coated with sodium alginate and a mixed culture of lactic acid bacteria (SA-LAB), sodium alginate alone (SA), and uncoated (negative control) during 15 days of room temperature storage (25 ± 0.5 °C).

Attributes/ Days of Storage	SA-LAB	SA	Control
0 day			
Brightness appearance	7.69 ± 1.08^a	7.31 ± 1.45^a	4.94 ± 1.61^b
Visible coating	8.75 ± 0.58^a	9.00 ± 0.00^a	1.13 ± 0.50^b
Integrity	8.13 ± 1.02^a	7.63 ± 1.63^a	8.13 ± 0.81^a
Green color	6.37 ± 1.25^a	6.50 ± 1.41^a	6.25 ± 1.57^a
Yellow color	5.71 ± 1.65^a	4.31 ± 1.92^a	4.65 ± 1.46^a
Purple color	5.94 ± 0.86^a	6.43 ± 1.86^a	5.87 ± 1.40^a
Sweet aroma	4.25 ± 1.18^a	3.81 ± 1.42^a	3.75 ± 1.13^a
Typical aroma	7.11 ± 1.60^a	5.84 ± 1.64^b	6.26 ± 1.88^{ab}
Fermented aroma	4.06 ± 1.00^a	2.56 ± 1.55^b	1.00 ± 0.00^c
Acidic aroma	4.56 ± 0.96^a	4.44 ± 1.71^a	4.38 ± 1.89^a
Firm texture	8.38 ± 0.96^a	8.44 ± 0.73^a	7.94 ± 0.85^{ab}
Smooth texture	6.74 ± 2.21^a	6.69 ± 1.30^a	7.10 ± 1.27^a
6 days			
Maturation appearance	5.00 ± 1.06^c	5.94 ± 1.03^b	8.12 ± 0.93^a
Brightness appearance	7.32 ± 1.41^a	7.00 ± 1.23^a	5.00 ± 0.87^b
Visible coating	7.06 ± 0.97^b	8.65 ± 0.61^a	1.06 ± 0.24^c
Integrity	8.06 ± 0.90^a	8.18 ± 1.24^a	7.12 ± 1.41^b
Green color	6.71 ± 1.05^a	6.97 ± 1.30^a	5.24 ± 1.35^b
Yellow color	4.00 ± 0.94^c	6.06 ± 1.03^b	7.35 ± 1.41^a
Purple color	4.71 ± 0.99^a	4.88 ± 1.05^a	3.71 ± 0.92^b
Red color	4.06 ± 0.97^b	4.00 ± 1.06^b	5.65 ± 1.37^a
Sweet aroma	5.53 ± 1.01^a	5.32 ± 1.23^a	5.29 ± 1.45^a
Typical aroma	7.18 ± 1.07^a	7.12 ± 1.41^a	7.94 ± 0.97^a
Fermented aroma	4.00 ± 1.28^a	2.65 ± 0.86^b	3.65 ± 1.46^a
Acidic aroma	5.06 ± 1.03^a	3.47 ± 0.80^b	3.65 ± 0.93^b
Firme texture	7.71 ± 0.92^a	7.59 ± 1.18^a	5.94 ± 2.11^b
Smooth texture	7.12 ± 1.11^a	6.53 ± 0.94^a	6.53 ± 1.18^a
Moist texture	5.88 ± 1.32^b	6.94 ± 1.35^a	7.24 ± 1.15^a
15 days			
Maturation appearance	6.38 ± 1.03^b	5.94 ± 1.34^b	8.88 ± 0.34^a
Brightness appearance	5.88 ± 0.89^b	7.38 ± 1.03^a	4.19 ± 0.91^c
Visible coating	8.94 ± 0.25^a	8.88 ± 0.50^a	1.00 ± 0.00^b
Integrity	6.81 ± 1.11^a	7.25 ± 0.93^a	6.00 ± 0.73^b
Green color	6.56 ± 0.96^a	6.94 ± 1.00^a	3.00 ± 0.97^b
Yellow color	6.63 ± 1.20^b	6.63 ± 0.81^b	8.75 ± 0.45^a
Purple color	4.00 ± 0.82^a	3.19 ± 0.83^b	1.06 ± 0.25^c
Red color	3.06 ± 1.00^b	3.07 ± 0.93^b	5.00 ± 0.82^a
Sweet aroma	5.00 ± 0.52^b	5.56 ± 1.09^b	7.44 ± 0.81^a
Typical aroma	5.94 ± 0.68^b	6.19 ± 0.98^b	8.25 ± 1.13^a
Fermented aroma	5.63 ± 0.89^a	5.50 ± 1.16^a	3.81 ± 0.83^b
Acidic aroma	6.25 ± 1.34^a	6.06 ± 0.77^a	2.63 ± 0.72^b
Firm texture	6.00 ± 0.89^a	5.55 ± 1.07^a	3.56 ± 0.89^b
Smooth texture	5.44 ± 0.73^a	5.60 ± 0.89^a	3.06 ± 0.93^b

^{a-c}: Average values in the same row with different lowercase letters are significantly different ($p < 0.05$), based on Tukey's test.

Supplementary 3. General aspects of guava cv. Paluma coated with sodium alginate and a mixed culture of lactic acid bacteria (SA-LAB), guava coated only with sodium alginate (SA), and uncoated (negative control) during 15 days of room temperature storage ($25 \pm 0.5\text{ }^{\circ}\text{C}$).

Treatments	Days of storage				
	0	6	9	12	15
SA-LAB					
AS					
Negative control					
Negative control: uncoated guava (sterile distilled water + glycerol 2.5 mL/100 mL).					

Supplementary 4. General aspects of mango cv. Palmer coated with sodium alginate and a mixed culture of lactic acid bacteria (SA-LAB), mango coated only with sodium alginate (SA), and uncoated (negative control) during 15 days of room temperature storage ($25 \pm 0.5\text{ }^{\circ}\text{C}$).

Treatments	Days of storage				
	0	6	9	12	15
SA-LAB					
SA					
Negative control					
Negative control: uncoated mango (sterile distilled water + glycerol 2.5 mL/100 mL).					

Supplementary 5. Physicochemical parameters (average $\pm$ standard deviation) of guava cv. Paluma coated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB), coated with sodium alginate (SA) alone, and uncoated (negative control) during 15 days of room temperature storage (25 ± 0.5 °C).

Parameters/ Treatments	Days of storage				
	Zero	6	9	12	15
TSS (° Brix)					
SA-LAB	8.33 (± 0.25) ^{Ab}	11.67 (± 0.20) ^{Aa}	11.60 (± 0.52) ^{Ba}	11.84 (± 0.45) ^{Ba}	12.00 (± 0.10) ^{Ca}
SA	8.17 (± 0.31) ^{Ac}	11.41 (± 0.61) ^{Aab}	11.17 (± 0.38) ^{Bb}	12.50 (± 0.10) ^{Ba}	12.47 (± 0.43) ^{Ba}
Negative control	8.40 (± 0.20) ^{Ac}	12.27 (± 0.40) ^{Ab}	12.77 (± 0.15) ^{Aab}	13.47 (± 0.29) ^{Aa}	13.43 (± 0.20) ^{Aa}
TA (g/100g)					
SA-LAB	1.78 (± 0.10) ^{Aa}	1.57 (± 0.07) ^{Ab}	1.28 (± 0.03) ^{Ac}	1.24 (± 0.06) ^{Ac}	1.15 (± 0.05) ^{Ac}
SA	1.74 (± 0.16) ^{Aa}	1.43 (± 0.06) ^{ABb}	1.21 (± 0.04) ^{Abc}	1.01 (± 0.09) ^{ABc}	0.92 (± 0.08) ^{Bc}
Negative control	1.71 (± 0.14) ^{Aa}	1.30 (± 0.08) ^{Bb}	1.04 (± 0.14) ^{Abc}	0.89 (± 0.05) ^{Bc}	0.76 (± 0.03) ^{Cc}
pH					
SA-LAB	3.43 (± 0.09) ^{Ad}	3.76 (± 0.05) ^{Ac}	3.85 (± 0.09) ^{Abc}	3.99 (± 0.05) ^{Aab}	4.10 (± 0.01) ^{Ba}
SA	3.67 (± 0.01) ^{Ac}	3.81 (± 0.05) ^{Ab}	3.90 (± 0.04) ^{Ab}	3.96 (± 0.02) ^{Ab}	4.21 (± 0.02) ^{ABa}
Negative control	3.66 (± 0.15) ^{Ac}	3.75 (± 0.08) ^{Ac}	3.83 (± 0.02) ^{Abc}	3.99 (± 0.05) ^{Ab}	4.35 (± 0.07) ^{Aa}
AA (mg/100g)					
SA-LAB	168.63 (± 2.92) ^{Aa}	155.83 (± 2.36) ^{Ab}	145.00 (± 4.00) ^{Ac}	138.33 (± 2.08) ^{Ac}	122.80 (± 4.96) ^{Ad}
SA	166.30 (± 3.25) ^{Aa}	153.63 (± 2.12) ^{Ab}	121.68 (± 3.35) ^{Bc}	101.67 (± 3.60) ^{Bd}	80.90 (± 2.85) ^{Be}
Negative control	167.43 (± 3.88) ^{Aa}	137.23 (± 4.13) ^{Bb}	116.6 (± 3.61) ^{Bc}	100.12 (± 2.94) ^{Bd}	50.57 (± 4.75) ^{Ce}
Firmness (N)					
SA-LAB	30.15 (± 1.58) ^{Aa}	23.86 (± 1.66) ^{Ab}	20.89 (± 1.82) ^{Abc}	17.58 (± 1.90) ^{Ac}	15.84 (± 1.46) ^{Ac}
SA	28.37 (± 1.90) ^{Aa}	22.41 (± 2.63) ^{Aab}	16.89 (± 1.74) ^{ABbc}	11.98 (± 1.37) ^{Bcd}	10.77 (± 1.80) ^{Bd}
Negative control	28.87 (± 2.15) ^{Aa}	18.40 (± 1.11) ^{Ab}	15.06 (± 1.88) ^{Bb}	8.42 (± 1.44) ^{Cc}	4.70 (± 1.54) ^{Cc}
Weight loss (%)					
SA-LAB	Nd	8.83 (± 0.60) ^{Bd}	13.53 (± 0.98) ^{Bc}	16.22 (± 0.91) ^{Bb}	20.27 (± 2.04) ^{Ca}
SA	Nd	10.91 (± 1.24) ^{Bc}	15.81 (± 1.29) ^{Bb}	18.64 (± 1.32) ^{Bb}	27.76 (± 2.27) ^{Ba}
Negative control	Nd	21.35 (± 1.55) ^{Ac}	28.17 (± 4.04) ^{Ab}	39.69 (± 1.01) ^{Aa}	40.70 (± 2.49) ^{Aa}
TPC (mg GAE/100 g)					
SA-LAB	110.53 (± 1.87) ^{Aa}	92.86 (± 1.76) ^{Ab}	93.74 (± 1.79) ^{Ab}	86.04 (± 3.25) ^{Ac}	82.26 (± 2.05) ^{Ac}
SA	110.98 (± 3.44) ^{Aa}	87.99 (± 1.71) ^{Bb}	81.97 (± 2.56) ^{Bc}	76.02 (± 1.20) ^{Bc}	71.23 (± 1.50) ^{Bd}
Negative control	111.58 (± 2.41) ^{Aa}	86.36 (± 0.59) ^{Bb}	75.66 (± 1.47) ^{Cc}	65.42 (± 3.06) ^{Cd}	58.06 (± 2.64) ^{Ce}
TFC (mg CE/100 g)					
SA-LAB	40.10 (± 1.26) ^{Aa}	34.97 (± 2.70) ^{Ab}	27.77 (± 1.15) ^{Ac}	25.47 (± 0.92) ^{Ac}	24.72 (± 1.07) ^{Ac}
SA	40.07 (± 1.89) ^{Aa}	31.45 (± 1.25) ^{Bb}	27.86 (± 0.92) ^{Ab}	22.42 (± 2.43) ^{ABc}	18.42 (± 1.43) ^{Bc}
Negative control	39.84 (± 0.50) ^{Aa}	31.04 (± 2.45) ^{Bb}	25.87 (± 1.79) ^{Ac}	18.36 (± 1.24) ^{Bd}	13.91 (± 2.58) ^{Ce}
β -carotene (μ g BE/100g)					
SA-LAB	19.94 (± 1.13) ^{Ab}	18.67 (± 1.00) ^{Bb}	19.63 (± 2.70) ^{Bb}	20.85 (± 2.35) ^{Cb}	34.52 (± 1.20) ^{Ca}
SA	19.53 (± 1.64) ^{Ac}	20.25 (± 1.59) ^{Bc}	20.70 (± 2.90) ^{Bc}	31.05 (± 1.94) ^{Bb}	44.26 (± 1.93) ^{Ba}
Negative control	19.63 (± 2.01) ^{Ad}	24.22 (± 2.10) ^{AcD}	25.93 (± 2.37) ^{Ac}	38.97 (± 1.99) ^{Ab}	62.69 (± 1.79) ^{Aa}
Antioxidant capacity					
ABTS•+ (μ mol TEAC/g)					
SA-LAB	7.42 (± 0.30) ^{Aa}	7.21 (± 0.10) ^{Aa}	6.93 (± 0.17) ^{Aab}	6.41 (± 0.13) ^{Ab}	5.76 (± 0.28) ^{Ac}
SA	7.55 (± 0.11) ^{Aa}	7.21 (± 0.11) ^{Aa}	6.34 (± 0.05) ^{Bb}	5.68 (± 0.19) ^{Bc}	4.49 (± 0.13) ^{Bd}
Negative control	7.49 (± 0.34) ^{Aa}	6.71 (± 0.13) ^{Bb}	6.13 (± 0.11) ^{Bc}	5.36 (± 0.20) ^{Bd}	4.23 (± 0.13) ^{Be}
DPPH• (μ mol TEAC/g)					
SA-LAB	5.83 (± 0.10) ^{Aa}	5.36 (± 0.12) ^{Ab}	5.11 (± 0.11) ^{Ab}	4.70 (± 0.16) ^{Ac}	4.45 (± 0.14) ^{Ac}
SA	5.88 (± 0.11) ^{Aa}	5.24 (± 0.06) ^{Ab}	4.63 (± 0.19) ^{Bc}	4.28 (± 0.06) ^{Bd}	3.94 (± 0.17) ^{Be}
Negative control	5.92 (± 0.10) ^{Aa}	4.37 (± 0.27) ^{Bb}	3.89 (± 0.15) ^{Cc}	3.71 (± 0.16) ^{Bc}	3.50 (± 0.15) ^{Cc}
FRAP (μ mol FeSO4/g)					
SA-LAB	29.85 (± 1.84) ^{Aa}	28.06 (± 1.28) ^{Aab}	25.01 (± 1.33) ^{Abc}	21.91 (± 0.94) ^{AcD}	20.30 (± 1.07) ^{Ad}
SA	30.62 (± 1.16) ^{Aa}	25.09 (± 1.30) ^{ABb}	21.09 (± 1.01) ^{Bc}	18.69 (± 1.09) ^{Bcd}	17.01 (± 0.97) ^{Bd}
Negative control	30.22 (± 1.33) ^{Aa}	23.68 (± 0.99) ^{Bb}	20.36 (± 1.28) ^{Bc}	17.31 (± 1.30) ^{Bd}	14.42 (± 0.47) ^{Cd}

TSS: Total Soluble Solids. TA: Titratable Acidity. AA: Ascorbic acid. Negative control: uncoated guava (sterile distilled water + glycerol 2.5 mL/100 mL). GAE: Gallic acid equivalent. CE: Catechin equivalent. BE: β -carotene equivalent. DPPH: Radical scavenging activity. FeSO4: Ferrous sulphate. FRAP: Ferric-reducing power. TEAC: Trolox Equivalent Antioxidant Capacity. Nd: Not determined. A-C: Average values in the same column, for a same measured parameter, with different uppercase letters are significantly different ($p < 0.05$), based on Tukey's test. a-e: Average values in the same row with different lowercase letters are significantly different ($p < 0.05$), based on Tukey's test.

Supplementary 6. Physicochemical parameters (average $\pm$ standard deviation) of mango cv. Palmer coated with sodium alginate and mixed culture of lactic acid bacteria (SA-LAB), coated with sodium alginate (SA) alone, and uncoated (negative control) during 15 days of room temperature storage (25 ± 0.5 °C).

Parameters/ Treatments	Storage days				
	0	6	9	12	15
TSS (°Brix)					
SA-LAB	5.23 (± 0.68) ^{Ad}	10.63 (± 0.38) ^{Bc}	11.30 (± 0.10) ^{Ac}	13.13 (± 0.71) ^{Ab}	15.97 (± 0.59) ^{Ca}
SA	5.93 (± 0.40) ^{Ad}	11.33 (± 0.49) ^{ABc}	11.67 (± 0.21) ^{Ac}	14.57 (± 0.38) ^{Ab}	16.97 (± 0.15) ^{Ba}
Negative control	5.81 (± 0.20) ^{Ad}	11.40 (± 0.10) ^{Ac}	11.87 (± 0.15) ^{Ac}	14.90 (± 0.61) ^{Ab}	18.03 (± 0.15) ^{Aa}
TA (g/100g)					
SA-LAB	1.88 (± 0.06) ^{Aa}	1.86 (± 0.06) ^{Aa}	1.50 (± 0.08) ^{Ab}	1.36 (± 0.06) ^{Ac}	1.09 (± 0.04) ^{Ad}
SA	1.84 (± 0.15) ^{Aa}	1.28 (± 0.05) ^{Bb}	1.16 (± 0.02) ^{Bb}	1.04 (± 0.05) ^{Bbc}	0.84 (± 0.09) ^{Bc}
Negative control	1.86 (± 0.05) ^{Aa}	1.42 (± 0.13) ^{Bb}	1.10 (± 0.05) ^{Bc}	0.87 (± 0.05) ^{Cd}	0.71 (± 0.07) ^{Bd}
pH					
SA-LAB	3.49 (± 0.02) ^{Ac}	3.94 (± 0.07) ^{Bd}	4.18 (± 0.07) ^{Bc}	4.45 (± 0.05) ^{Bb}	4.89 (± 0.02) ^{Ba}
SA	3.31 (± 0.01) ^{Bd}	3.52 (± 0.05) ^{Cd}	4.98 (± 0.01) ^{Ac}	5.43 (± 0.24) ^{Ab}	5.91 (± 0.01) ^{Aa}
Negative control	3.48 (± 0.10) ^{Ac}	4.19 (± 0.04) ^{Ad}	4.96 (± 0.01) ^{Ac}	5.51 (± 0.07) ^{Ab}	5.92 (± 0.03) ^{Aa}
AA (mg/100g)					
SA-LAB	89.17 (± 2.36) ^{Aa}	86.67 (± 1.61) ^{Aa}	75.27 (± 2.58) ^{Ab}	70.27 (± 1.58) ^{Ab}	61.67 (± 2.52) ^{Ac}
SA	90.50 (± 1.32) ^{Aa}	70.83 (± 2.02) ^{Bb}	61.17 (± 2.36) ^{Bc}	55.07 (± 3.10) ^{Bc}	38.53 (± 2.24) ^{Bd}
Negative control	89.33 (± 2.01) ^{Aa}	56.73 (± 2.86) ^{Cb}	43.90 (± 2.15) ^{Cc}	34.00 (± 2.55) ^{Cd}	25.75 (± 1.70) ^{Ce}
Firmness (N)					
SA-LAB	73.55 (± 2.34) ^{Aa}	55.54 (± 2.00) ^{Ab}	45.37 (± 1.51) ^{Ac}	37.15 (± 1.33) ^{Ad}	31.54 (± 1.62) ^{Ac}
SA	75.31 (± 3.62) ^{Aa}	53.15 (± 3.49) ^{ABb}	42.72 (± 2.12) ^{Ac}	34.95 (± 2.28) ^{Ad}	24.76 (± 1.68) ^{Bc}
Negative control	74.18 (± 3.46) ^{Aa}	46.38 (± 2.19) ^{Bb}	32.00 (± 2.32) ^{Bc}	21.71 (± 3.29) ^{Bd}	16.43 (± 2.34) ^{Cd}
Weight loss (%)					
SA-LAB	Nd	4.37 (± 0.34) ^{Cd}	6.49 (± 0.61) ^{Bc}	8.32 (± 0.58) ^{Cb}	10.11 (± 0.72) ^{Ca}
SA	Nd	7.15 (± 0.35) ^{Bc}	10.76 (± 1.78) ^{Ab}	12.82 (± 0.35) ^{Bab}	14.58 (± 1.33) ^{Ba}
Negative control	Nd	8.84 (± 0.59) ^{Ac}	11.69 (± 1.89) ^{Ac}	16.63 (± 1.00) ^{Ab}	20.14 (± 1.42) ^{Aa}
TPC (mg GAE/100 g)					
SA-LAB	31.83 (± 0.59) ^{Ab}	32.65 (± 0.88) ^{Bb}	33.44 (± 0.79) ^{Cb}	37.17 (± 0.37) ^{Aa}	38.43 (± 0.22) ^{Aa}
SA	31.86 (± 1.12) ^{Ac}	27.45 (± 1.27) ^{Cd}	38.96 (± 0.18) ^{Ba}	38.24 (± 1.49) ^{Aab}	34.93 (± 1.67) ^{Bbc}
Negative control	32.28 (± 0.32) ^{Ad}	35.81 (± 0.76) ^{Ac}	43.15 (± 1.12) ^{Aa}	39.20 (± 1.61) ^{Ab}	35.71 (± 0.42) ^{Bc}
TFC (mg CE/100 g)					
SA-LAB	14.94 (± 0.66) ^{Aa}	13.48 (± 1.39) ^{Aa}	12.85 (± 1.00) ^{Aa}	10.18 (± 0.71) ^{Ab}	8.57 (± 0.72) ^{Ab}
SA	14.65 (± 0.90) ^{Aa}	11.35 (± 1.06) ^{ABb}	7.88 (± 0.72) ^{Bc}	5.93 (± 0.51) ^{Bc}	1.90 (± 0.72) ^{Bd}
Negative control	14.73 (± 0.96) ^{Aa}	8.50 (± 0.95) ^{Bb}	5.39 (± 1.20) ^{Cc}	2.03 (± 0.84) ^{Cd}	Nd
β-carotene (μg BE/100g)					
SA-LAB	20.03 (± 1.31) ^{Ad}	22.34 (± 2.45) ^{Cd}	37.55 (± 2.74) ^{Bc}	110.87 (± 2.95) ^{Cb}	123.12 (± 3.01) ^{Ca}
SA	21.09 (± 3.91) ^{Ad}	29.33 (± 1.72) ^{Bd}	39.29 (± 2.50) ^{Bc}	201.72 (± 4.23) ^{Bb}	375.90 (± 4.80) ^{Ba}
Negative control	20.90 (± 2.48) ^{Ac}	73.17 (± 3.64) ^{Ad}	100.94 (± 3.34) ^{Ac}	148.78 (± 1.94) ^{Ab}	401.64 (± 3.09) ^{Aa}
Antioxidant capacity					
ABTS•+ (μmol TEAC/g)					
SA-LAB	3.67 (± 0.08) ^{Aa}	2.85 (± 0.06) ^{Ab}	2.77 (± 0.06) ^{ABc}	2.54 (± 0.18) ^{Ac}	2.21 (± 0.04) ^{Ad}
SA	3.68 (± 0.06) ^{Aa}	2.86 (± 0.07) ^{Ab}	2.66 (± 0.02) ^{ABc}	2.48 (± 0.16) ^{Ac}	2.10 (± 0.04) ^{Bd}
Negative control	3.63 (± 0.11) ^{Aa}	2.83 (± 0.06) ^{Ab}	2.68 (± 0.05) ^{Ab}	2.48 (± 0.04) ^{Ac}	2.05 (± 0.03) ^{Bd}
DPPH• (μmol TEAC/g)					
SA-LAB	3.19 (± 0.27) ^{Aa}	2.84 (± 0.07) ^{Aab}	2.72 (± 0.06) ^{Ab}	2.48 (± 0.09) ^{ABc}	2.18 (± 0.09) ^{Ac}
SA	3.09 (± 0.21) ^{Aa}	2.76 (± 0.07) ^{ABb}	2.51 (± 0.09) ^{Bb}	2.16 (± 0.07) ^{Bc}	1.77 (± 0.06) ^{Bd}
Negative control	3.15 (± 0.13) ^{Aa}	2.68 (± 0.01) ^{Bb}	2.50 (± 0.07) ^{Bb}	2.08 (± 0.12) ^{Bc}	1.50 (± 0.06) ^{Cd}
FRAP (μmol FeSO4/g)					
SA-LAB	10.14 (± 0.64) ^{Aa}	8.97 (± 0.28) ^{Ab}	8.48 (± 0.25) ^{ABc}	8.05 (± 0.14) ^{ABc}	7.62 (± 0.32) ^{Ac}
SA	10.12 (± 0.74) ^{Aa}	8.31 (± 0.33) ^{Ab}	7.52 (± 0.38) ^{Bbc}	7.23 (± 0.17) ^{Bbc}	6.43 (± 0.39) ^{Bc}
Negative control	10.02 (± 0.75) ^{Aa}	7.36 (± 0.22) ^{Bb}	6.74 (± 0.34) ^{Bb}	6.41 (± 0.41) ^{Cbc}	5.23 (± 0.31) ^{Cc}

TSS: Total Soluble Solids. TA: Titratable Acidity. AA: Ascorbic acid. Negative control: uncoated mango (sterile distilled water + glycerol 2.5 mL/100 mL). GAE: Gallic acid equivalent. CE: Catechin equivalent. BE: β -carotene equivalent. DPPH: Radical scavenging activity. FeSO4: Ferrous sulphate. FRAP: Ferric-reducing power. TEAC: Trolox Equivalent Antioxidant Capacity. Nd: Not determined. A-C: Average values in the same column, for a same measured parameter, with different uppercase letters are significantly different ($p < 0.05$), based on Tukey's test. a-c: Average values in the same row with different lowercase letters are significantly different ($p < 0.05$), based on Tukey's test.

ANEXOS

ANEXO A - Parecer Consubstanciado do Comitê de Ética em Pesquisa

CENTRO DE CIÊNCIAS DA
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**PARECER CONSUBSTANCIADO DO CEP****DADOS DO PROJETO DE PESQUISA**

Título da Pesquisa: APLICAÇÃO DE REVESTIMENTO FORMULADO COM ALGINATO DE SÓDIO E CULTURA MISTA DE BACTÉRIAS LÁTICAS PARA PRESERVAÇÃO DA QUALIDADE PÓS-COLHEITA EM FRUTAS.

Pesquisador: KARINA FELIX DIAS FERNANDES

Área Temática:

Versão: 1

CAAE: 92207925.0.0000.5188

Instituição Proponente: Centro De Ciências da Saúde

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 7.899.849

Apresentação do Projeto:

Adequado. Trata-se de um projeto de tese vinculado ao programa de Pós-graduação em Ciência da Nutrição do Centro de Ciências da Saúde da Universidade Federal da Paraíba que tem a orientação o professor Dr. Evandro Leite de Souza e discente pesquisadora Karina Félix Dias Fernandes. A proposta da pesquisa tem como título "APLICAÇÃO DE REVESTIMENTO FORMULADO COM ALGINATO DE SÓDIO E CULTURA MISTA DE BACTÉRIAS LÁTICAS PARA PRESERVAÇÃO DA QUALIDADE PÓS-COLHEITA EM FRUTAS, e

Objetivo da Pesquisa:

Adequado. A proposta da pesquisa tem como objetivo principal de desenvolver e aplicar um revestimento formulado com alginato de sódio adicionado de cultura mista de cepas de BAL para a preservação da qualidade pós-colheita de goiaba (cv. Paluma) e manga (cv. Palmer) durante o armazenamento.

Avaliação dos Riscos e Benefícios:

Adequado. Como riscos a pesquisadora apresenta que a pesquisa pode envolver resistência ou insegurança aos participantes ao analisar produtos com características sensoriais fora do seu

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hábito alimentar, e como benefícios descreve a contribuição para o avanço científico na área de conservação pós-colheita de frutas tropicais, com possíveis impactos positivos na qualidade e segurança dos alimentos ofertados à população.

Comentários e Considerações sobre a Pesquisa:

A proposta da pesquisa está muito bem escrita e delineada, e, a pesquisadora aponta que este estudo através da tecnologia alternativa aplicada, seja capaz de conservar a qualidade e a segurança microbiológica das frutas, contribuindo para o prolongamento da vida útil, a redução das perdas pós-colheita, a diminuição do desperdício e o incentivo a práticas mais sustentáveis de manejo e conservação.

Considerações sobre os Termos de apresentação obrigatória:

Adequado. Todos os documentos obrigatórios foram anexados na plataforma do CEP, como: Projeto completo, Carta de Anuência do Laboratório de Microbiologia e Bioquímica dos Alimentos e do Laboratório de Técnica Dietética, Certidão de Aprovação da pesquisa emitida pelo Programa de Pós-graduação em Ciências da Nutrição, Orçamento, Cronograma de execução, TCLE e folha de rosto.

Recomendações:

Recomenda-se que caso ocorra quaisquer alteração no projeto, a pesquisadora responsável deverá submeter a emenda, informando as alterações e anexando os documentos necessários. Também recomenda-se que ao término da pesquisa, a pesquisadora encaminhe ao CEP do CCS da UFPB, o relatório final e documento devolutivo, comprovando que os dados foram divulgados.

Conclusões ou Pendências e Lista de Inadequações:

Tendo em vista a não observância de nenhum impedimento ético no projeto de pesquisa, a relatora atribui o parecer favorável a execução do projeto como este se apresenta.

Considerações Finais a critério do CEP:

Certifico que o Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba e CEP/CCS aprovou a execução do referido projeto de pesquisa. Outrossim, informo que a autorização para posterior publicação fica condicionada à submissão do Relatório Final na Plataforma Brasil, via Notificação, para fins de apreciação e aprovação por este egrégio Comitê.

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Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2621475.pdf	23/09/2025 11:45:20		Aceito
Outros	TERMO_DE_COMPROMISSO_DE_DIVULGACAO_DOS_RESULTADOS_Karina assinado.pdf	23/09/2025 11:39:59	KARINA FELIX DIAS FERNANDES	Aceito
Declaração de Pesquisadores	TERMO_DE_COMPROMISSO_DO29_PESQUISADOR29 assinado.pdf	23/09/2025 11:38:58	KARINA FELIX DIAS FERNANDES	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE assinado.pdf	23/09/2025 11:38:31	KARINA FELIX DIAS FERNANDES	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_CEP_Completo.docx	23/09/2025 11:35:52	KARINA FELIX DIAS FERNANDES	Aceito
Declaração de Instituição e Infraestrutura	CARTA_DE_ANUENCIA_LATED_Karina assinado.pdf	23/09/2025 11:34:54	KARINA FELIX DIAS FERNANDES	Aceito
Declaração de Instituição e Infraestrutura	CARTA_ANUENCIA_LMBA assinado.pdf	23/09/2025 11:34:41	KARINA FELIX DIAS FERNANDES	Aceito
Outros	Certidao_PPGCN.pdf	23/09/2025 11:30:32	KARINA FELIX DIAS FERNANDES	Aceito
Orçamento	ORCAMENTO.docx	23/09/2025 11:25:20	KARINA FELIX DIAS FERNANDES	Aceito
Outros	INSTRUMENTO_DE_COLETA.docx	23/09/2025 11:24:47	KARINA FELIX DIAS FERNANDES	Aceito
Cronograma	CRONOGRAMA.docx	23/09/2025 11:23:12	KARINA FELIX DIAS FERNANDES	Aceito
Folha de Rosto	Folha_de_rosto_Karina.pdf	23/09/2025 11:15:31	KARINA FELIX DIAS FERNANDES	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

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JOAO PESSOA, 13 de Outubro de 2025

Assinado por:
Eliane Marques Duarte de Sousa
(Coordenador(a))

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