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GIOVANNA ALENCAR LUNDGREN

DESENVOLVIMENTO E APLICAÇÃO DE REVESTIMENTOS COM GOMA ARÁBICA E ÓLEO ESSENCIAL DE *Conyza bonariensis* (L.) Cronquist PARA CONTROLE DE ANTRACNOSE EM BANANA

JOÃO PESSOA
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Tese apresentada ao programa de Pós-graduação em Ciências da Nutrição, Centro de Ciência da Saúde, Universidade Federal da Paraíba, em cumprimento aos requisitos para obtenção do título de Doutora em Ciências da Nutrição.

Orientador: Prof. Dr. Evandro Leite de Souza

JOÃO PESSOA

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Universidade Federal da Paraíba

Centro de Ciências da Saúde

Programa de Pós-graduação em Ciências da Nutrição

Ata da 35ª (trigésima quinta) Sessão Pública de Defesa de Tese para obtenção do grau de Doutor em Ciências da Nutrição, da discente Giovanna de Alencar Lundgren.

Aos vinte dias do mês de agosto de dois mil e vinte e um (20/08/2021) às 08:30 h, reuniram-se em cerimônia pública on line por meio da ferramenta Google meet, **meet.google.com/ota-dbkb-bau**, os membros da Banca Examinadora e a doutoranda Giovanna de Alencar Lundgren, candidata ao grau de Doutor em Ciências da Nutrição. A Banca Examinadora foi constituída pelos seguintes Professores:Dr. Evandro Leite de Souza (Presidente/Orientador), Dr. Fillipe de Oliveira Pereira (Examinador Interno), Drª Estefânia Fernandes Garcia (Examinadora Interna), Drª Maria Elieidy Gomes de Oliveira (Examinadora Interna Suplente), Drª Ingrid Conceição Dantas Gonçalves (Examinadora Externa), Dr. Marcos Paz Saraiva Câmara (Examinador Externo) e Dr. Willie Anderson Vieira (Examinador Externo Suplente). Dando início a sessão pública, o Prof. Evandro Leite de Souza, convidou a mim, Carlos Fernando da Silva para secretariá-los comunicando o fim específico da reunião. A seguir, foi cedida a palavra à examinada, para que, no tempo previsto no Regimento do PPGCN/CCS/UFPB, fizesse sua exposição de motivos e objetivos da sua tese, metodologia adotada, resultados encontrados, discussão e conclusão, trabalho esse sob o título "DESENVOLVIMENTO E APLICAÇÃO DE REVESTIMENTOS COM GOMA ARÁBICA E ÓLEO ESSENCIAL DE *Conyza bonariensis* (L.) Cronquist PARA REDUÇÃO DO DESENVOLVIMENTO DE ANTRACNOSE EM BANANA". Concluída a exposição, a candidata, em seguida, foi arguida sucessivamente por cada membro da banca examinadora. Dando continuidade, os membros examinadores foram inquiridos pelo Sr. Presidente se estavam aptos a proferir o julgamento e, recebendo respostas afirmativas, procederam com a deliberação da avaliação. Após certo espaço de tempo, o Sr Presidente proclamou como "APROVADA" a tese de doutorado intitulada "DESENVOLVIMENTO E APLICAÇÃO DE REVESTIMENTOS COM GOMA ARÁBICA E ÓLEO ESSENCIAL DE *Conyza bonariensis* (L.) Cronquist PARA REDUÇÃO DO DESENVOLVIMENTO DE ANTRACNOSE EM BANANA" A
seguir, preenchendo devidamente os mapas concernentes aos valores e conceitos, declarou que sua autora estava em condições de receber o grau de Doutor em Ciências da Nutrição, devendo a Universidade Federal da Paraíba, providenciar na forma da lei a expedição do respectivo Diploma. Nada mais havendo a ser tratado, eu, Carlos Fernando da Silva Secretário do Programa de Pós- Graduação em Ciências da Nutrição, lavrei a presente Ata, que vai datada e assinada pelos membros da Banca Examinadora. João Pessoa, 20 de agosto de 2021.

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RESUMO

A banana é popular devido ao sabor e textura agradáveis e facilidade de consumo. No entanto, entra rapidamente em senescência devido a alterações bioquímicas e ocorrência de deteriorações microbianas, com destaque para a antracnose, caracterizada como doença pós-colheita de elevada severidade causada pelo fungo *Colletotrichum musae*. A aplicação de compostos naturais com atividade antifúngica, a exemplo de óleos essenciais, tem sido estudada como alternativa para o controle de doenças pós-colheita, principalmente na formulação de revestimentos comestíveis. *Conyza bonariensis* é uma planta encontrada em todo território brasileiro e seu óleo essencial (OE) tem demonstrado atividade antimicrobiana. Esse estudo teve como resultado dois produtos, um teórico, com desenvolvimento de um artigo de revisão com o objetivo de auxiliar a pesquisa em revestimentos comestíveis elaborados com polissacarídeos e OEs e auxiliar na sua aplicação otimizada como tratamento pós-colheita de frutas. Os resultados deste trabalho teórico indicaram que a aplicação de revestimentos elaborados com polissacarídeos e OEs reduzem as perdas pós-colheita e mantêm as características físico-químicas e sensoriais em frutas durante o armazenamento. Um segundo produto de natureza experimental teve como objetivo avaliar desenvolver e aplicar revestimentos com goma arábica (GA) e óleo essencial de *C. bonariensis* (OECB) para o retardo do desenvolvimento de antracnose em bananas cv. 'Prata'. Foram realizadas análises de identificação dos constituintes do OECB, dos efeitos antifúngicos *in vitro* da GA e OECB sobre o crescimento micelial radial de seis isolados de *C. musae*, dos efeitos do OECB sobre a permeabilidade de membrana e atividade enzimática dos conídios de *C. musae*, dos efeitos de revestimentos formulados com GA e OECB sobre o desenvolvimento de antracnose em bananas artificialmente contaminadas com os isolados de *C. musae* testados durante o armazenamento sob temperatura ambiente (25 ± 0.5 ºC), bem como sobre aspectos físico-químicos (pH, sólidos solúveis, acidez titulável, cor e perda de peso) indicadores de qualidade pós-colheita destas frutas. Os compostos majoritários no OECB foram sesquicineol (48,43%), sesquisabineno (10,87%), limoneno (9,63%) e timol (6,15%). A GA (0,1 g/mL) não foi eficaz de inibir o crescimento micelial de *C. musae*, enquanto o OECB (0,1; 0,2; 0,4; 0,6; 0,8 e 1 µL/mL) foi eficaz em inibir o crescimento micelial fúngico. O OECB (0,6 µL/mL) causou alteração da permeabilidade de membrana (28,8 – 43,2%) e atividade enzimática (59,5 – 84,6%) dos conídios de *C. musae*. A aplicação de revestimentos com GA (0,1 g/mL) e OECB (0,4; 0,6; 0,8; 1 µL/mL) causou redução do desenvolvimento das lesões de antracnose em bananas, com valores de redução do diâmetro da lesão de até 45,8% no 5º dia de armazenamento, os quais foram, em sua maioria, similares aos efeitos causados pelo fungicida comercial utilizado como controle positivo. As bananas não-revestidas demonstraram maiores índices de severidade da doença. O revestimento com GA + OECB foi capaz de retardar as alterações em parâmetros físico-químicos indicadores de qualidade pós-colheita em bananas, particularmente da perda de massa e da mudança de cor. Estes resultados indicam que a aplicação de revestimentos formulados com GA e OECB pode ser considerada uma possível estratégia para retardar o desenvolvimento da antracnose e prolongar o período de armazenamento de bananas.

Palavras-chave: *Musa acuminata*, *Colletotrichum*, efeito antifúngico, doenças pós-colheita, revestimento comestível.

ABSTRACT

Banana is a popular fruit because of its pleasant taste and texture and ease of consumption. However, bananas rapidly enter into senescence due to biochemical alterations and the occurrence of microbial deterioration, especially anthracnose, which is characterized as a highly severe postharvest disease caused by the fungus Colletotrichum musae. The application of natural compounds with antifungal activity, such as essential oils, has been studied as an alternative for the control of postharvest diseases in fruits, mainly in the formulation of edible coatings. *Conyza bonariensis* is a plant found throughout Brazil and its essential oil has shown antimicrobial activity. This study resulted in two products, one theoretical, with the development of a review article with the aim of assisting research on edible coatings made with polysaccharides and essential oils and assisting in their optimized application as a post-harvest treatment of fruits. The results of this theoretical work indicated that the application of coatings made with polysaccharides and essential oils reduce post-harvest losses and maintain the physicochemical and sensory characteristics of fruits during storage. A second experimental product aimed to evaluate the development and application of coatings with gum arabic (GA) and essential oil of *C. bonariensis* (OECB) to delay the development of anthracnose in bananas cv. 'Silver'. Identification analyzes of OECB constituents, in vitro antifungal effects of GA and OECB on radial mycelial growth of six C. musae isolates, OECB effects on membrane permeability and enzymatic activity of C. musae conidia were carried out. , of the effects of coatings formulated with GA and OECB on the development of anthracnose in bananas artificially contaminated with *C. musae* isolates tested during storage at room temperature (25 ± 0.5 ºC), as well as on physical-chemical aspects (pH, soluble solids, titratable acidity, color and weight loss) indicators of postharvest quality of these fruits. The major compounds in the OECB were sesquicineol (48.43%), sesquisabinene (10.87%), limonene (9.63%) and thymol (6.15%). GA (0.1 g/mL) was not effective in inhibiting the mycelial growth of *C. musae*, while OECB (0.1; 0.2; 0.4; 0.6; 0.8 and 1 µL/ mL) was effective in inhibiting fungal mycelial growth. OECB (0.6 µL/mL) caused changes in membrane permeability (28.8 – 43.2%) and enzymatic activity (59.5 – 84.6%) of *C. musae* conidia. The application of coatings with GA (0.1 g/mL) and OECB (0.4; 0.6; 0.8; 1 µL/mL) caused a reduction in the development of anthracnose lesions in bananas, with reduced values of lesion diameter of up to 45.8% on the 5th day of storage, which were mostly similar to the effects caused by the commercial fungicide used as a positive control. Uncoated bananas showed higher rates of disease severity. The coating with GA + OECB was able to delay changes in physicochemical parameters indicative of postharvest quality in bananas, particularly of mass loss and color change. These results indicate that the application of coatings formulated with GA and CBEO can be considered a possible strategy to delay the development of anthracnose and prolong the storage period of bananas.

Keywords: *Musa acuminata*, *Colletotrichum*, antimicrobial, antifungal, postharvest diseases, edible coating

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1. INTRODUÇÃO

A banana é uma fruta tropical largamente cultivada, e, devido ao seu sabor, textura, valor nutricional e presença de diferentes compostos bioativos benéficos à saúde, tem sido uma das frutas mais consumidas no mundo. Caracteriza-se, como fonte de compostos fenólicos, carboidratos, vitaminas e minerais (SINGH et al., 2016). No entanto, a banana é uma fruta climatérica com vida de prateleira relativamente curta em decorrência de transtornos fisiológicos, doenças pós-colheita e rápida senescência (AHMED; PALTA, 2016; SINGH et al., 2016) caracterizada por escurecimento da casca, amaciamento da polpa e alterações de sabor e aroma (CAMPELO et al., 2020).

A antracnose pós-colheita, causada pelo fungo *Colletotrichum musae* (Berk. E M. Curtis) Arx, é a doença mais importante em bananas, causando até aproximadamente 30% de perdas de frutas comercializáveis (RANASINGHE; JAYAWARDENA; ABEYWICKRAMA, 2003; SILVA et al., 2016). A antracnose é causada por uma infecção latente, onde os esporos de *C. musae* infectam a banana ainda imatura no campo, embora os sintomas só apareçam durante o estágio de amadurecimento. A antracnose em banana inclui uma variedade de sintomas, como manchas na casca, lesões marrons à negras deprimidas na polpa, e, em condições de alta umidade, podem apresentar frutificações rosadas do fungo após o amadurecimento (SIVAKUMAR; BAUTISTA-BAÑOS, 2014).

Os produtores de frutas de importância comercial têm feito amplo uso de fungicidas convencionais para o controle de doenças decorrentes de infecções fúngicas (VIEIRA et al., 2017). A imersão das frutas em soluções de fungicidas à base de tiabendazol, tiofanato metílico, prochloraz e imazalil tem sido a principal forma de controle da antracnose em diversas frutas (BRAGA et al., 2019a). No entanto, preocupações crescentes continuam a existir em relação aos riscos para a saúde humana impostos pelo uso destes fungicidas, como, por exemplo, o risco de causar câncer nos manipuladores e consumidores em decorrência da exposição contínua, bem como a preocupação com a poluição ambiental (RIVERO et al., 2015). Além disso, o uso continuado destes compostos sintéticos tem induzido o surgimento de biótipos resistentes de patógenos fúngicos (FISHER et al., 2018).

Portanto, a fim de minimizar o uso de fungicidas comerciais na agroindústria de bananas, qualquer medida de controle alternativo sustentável que tenha o potencial de retardar efetivamente o surgimento dos sintomas da infecção por antracnose pode representar papel importante na extensão da vida de prateleira destas frutas e, consequentemente, na expansão

das possibilidades de comercialização (THAKUR et al., 2019). Nesta perspectiva, o uso de revestimentos comestíveis à base de polímeros adicionados de óleos essenciais tem sido investigado como estratégia de diminuir do uso de fungicidas comerciais na pós-colheita de frutas (ANDRADE et al., 2017; OLIVEIRA et al., 2018; BRAGA et al., 2019a).

A goma arábica é um biopolímero obtido a partir de caules e ramos da espécie vegetal *Acacia senegal* (Linne), Leguminoseae, com toxicidade oral aguda baixa e ausência de genotoxicidade, sendo reconhecido como polissacarídeo biodegradável de alto potencial biotecnológico para formulação de filmes ou revestimentos em decorrência das suas propriedades emulsificantes (MAQBOOL et al., 2011; ANDRADE et al., 2017; EFSA, 2017). Contudo, a atividade antimicrobiana da goma arábica tem sido reportada como fraca ou inexistente frente a fungos fitopatógenos (MAQBOOL et al., 2011; ANDRADE et al., 2017), necessitando da sua combinação com compostos ou substâncias possuidoras de propriedades antimicrobianas para aplicações com o propósito de controle do crescimento fúngico.

Os óleos essenciais são os compostos mais importantes entre a ampla gama de substâncias naturais com propriedades antifúngicas extraídas de plantas, sendo formados por uma combinação de metabólitos secundários voláteis que apresentam atividade direta contra fitopatógenos (GUERRA et al., 2016). *Conyza bonariensis* (L.) Cronq. é uma planta perene e bem difundida em todo o mundo, com crescimento espontâneo e considerada altamente prolífica (BLAINSKI et al., 2015; THABIT et al., 2015), o que facilita a obtenção de material para a produção de óleo essencial. Além disso, estudos têm demonstrado que extratos de *C. bonariensis* apresentam baixa toxicidade pré-clínica, embora apresentem atividade antibacteriana e antifúngica (SHAH et al., 2013; DE PAULA et al., 2018). Entretanto, ainda existem poucos estudos na literatura sobre as propriedades antimicrobianas do óleo essencial de *C. bonariensis* (ARAUJO et al., 2013; LOMBARDO et al., 2016).

A combinação de goma arábica e óleos essenciais tem sido explorada como uma possível estratégia de potenciação e extensão das suas propriedades antifúngicas quando utilizados na formulação de recobrimentos possíveis de uso em frutas de interesse comercial (MAQBOOL et al., 2011; ANDRADE et al., 2017). No entanto, estudos verificando a eficácia da incorporação do óleo essencial de *C. bonariensis* em revestimentos a base de goma arábica para controlar doenças fúngicas em banana são escassos ou inexistentes.

Com isso, o objetivo do presente estudo foi avaliar o efeito inibitório do óleo essencial de *C. bonariensis* sobre diferentes isolados de *C. musae* e a sua incorporação em revestimentos à base de goma arábica empregados paro o retardo do desenvolvimento de antracnose em banana

cv. Prata ao longo do armazenamento, bem como a avaliação de alguns parâmetros físicos e químicos indicadores de qualidade pós-colheita.

2. REFERENCIAL TEÓRICO

2.1 BANANAS: ASPECTOS MERCADOLÓGICOS E DE QUALIDADE

Acredita-se, que a banana foi domesticada há mais de dez mil anos nas regiões tropicais do sul da Ásia e, em seguida, se disseminou pelos trópicos (DENHAM, 2003; ZHANG et al., 2005). As bananeiras que produzem frutos comestíveis pertencem à classe das Monocotyledoneae e família Musaceae, incluindo vários híbridos do gênero *Musa* (CORDEIRO, 2000). Dentro do gênero, as bananas podem ser divididas em grupos genômicos diploides (AA), triploides (AAA, AAB, ABB) e tetraploides (AAAA, AAAB, AABB, ABBB), de modo que esses genomas são denominados A e B, referindo-se as espécies *M. acuminata* Colla e *M. balbisiana* Colla, respectivamente. Além dos grupos genômicos, também foram estabelecidos subgrupos para denominar um complexo de cultivares originários de diversas mutações de uma única cultivar original, como no caso do grupo AAA, subgrupo *Cavendish*, e grupo AAB, subgrupos Prata e Terra (ALVES, 1999; GASPAROTTO et al., 2006). Estas duas espécies são as mais conhecidas comercialmente, sendo o subgrupo *Cavendish* o ponto de partida de todas as bananeiras de frutas comestíveis (BORGES, 2006).

As principais cultivares de banana difundidas no Brasil são Prata, Pacovan, Prata Anã, Maçã, Mysore, Terra, D'Angola, Nanica, Nanicão e *Grand Nine*. Dentre estas, as cultivares Nanica, Nanicão e *Grand Nine* são destinadas, principalmente, para exportação (BORGES, 2006).

A banana é uma das frutas mais presentes na dieta dos consumidores brasileiros, sendo considerada importante fonte de calorias (QAMAR; SHAIKH, 2018). A banana possui composição com aproximadamente 22% de carboidratos, tendo baixa quantidade de proteínas e lipídeos e presença de vitaminas A, B_1, B_2, B_3, C, D e E, sendo as vitaminas D e E encontradas nos mais baixos teores. Ainda, possui em sua composição a presença dos minerais potássio, fosforo, cálcio, magnésio e ferro. A textura macia da polpa, sabor doce e baixa acidez são aspectos importantes relacionados com a ampla aceitação da banana por consumidores de diferentes faixas etárias (MATSUURA, 2001; FORSTER et al., 2003; FASOLIN et al., 2007; AURORE; PARFAIT; FAHRASMANE, 2009; SALOMÃO; SIQUEIRA, 2015). Na Tabela 1 são apresentadas informações relacionadas a composição nutricional de uma porção de 100 g de banana (cv. Prata), segundo a Tabela Brasileira de Composição de Alimentos (TACO, 2011).

A polpa de banana contém diferentes compostos bioativos, como flavonoides, carotenoides e aminas biogênicas, repercutindo em elevada propriedade antioxidante (SINGH et al., 2016). Quercetina, miricetina, kaempferol e cianidina fazem parte dos flavonóis, os quais se caracterizam como a principal classe de flavonoides encontrado em bananas (KEVERS et al., 2007; BORGES et al., 2014). Estes flavonoides podem atuar como protetores da ação de radicais livres derivados do oxigênio e das espécies reativas de oxigênio (ROS), embora sejam ainda escassos os estudos sobre os efeitos sinérgicos dos diferentes compostos fenólicos presentes na banana (SINGH et al., 2016).

Tabela 1 - Composição nutricional em 100 g de banana cv. Prata

Componente	**Quantidade**
Água	71,9 g
Energia	98 kcal
Proteína	1,3 g
Lipídio total	0,1 g
Carboidrato	26,0 g
Fibra	2,0 g
Cinzas	0,8 g
Cálcio	8 mg
Ferro	0,4 mg
Magnésio	26 mg
Fósforo	22 mg
Potássio	358 mg
Sódio	1 mg
Manganês	0,42 mg
Cobre	0,05 mg
Riboflavina	0,02 mg
Piridoxina	0,10 mg
Zinco	0,1 mg
Vitamina C	21,6 mg

Fonte: TACO, 2011

Os carotenoides proporcionam benefícios à saúde devidos às suas funções fisiológicas como provitaminas e atividade antioxidante, principalmente, na eliminação do oxigênio singlete (SIDHU; ZAFAR, 2018). O conteúdo de carotenoides em bananas consiste, principalmente, em carotenoides provitamina A e podem auxiliar populações que apresentem deficiência desse nutriente (FUNGO; PILLAY, 2013; BORGES et al., 2014). Serotonina, dopamina e norepinefrina são algumas das aminas biogênicas presentes na casca e polpa da banana (GONZÁLEZ-MONTELONGO; GLORIA LOBO; GONZÁLEZ, 2010). Estas aminas biogênicas desempenham funções importantes no corpo e cérebro humano, impactando no

humor, estabilidade emocional e capacidade de concentração (SINGH et al., 2016; SIDHU; ZAFAR, 2018).

A banana é uma fruta climatérica, com vida de prateleira curta entre seis e oito dias após madura quando armazenada sob temperatura ambiente (HUANG et al., 2014). Ainda, apresenta altas taxas respiratórias que desencadeiam intensas modificações bioquímicas após a colheita (VIVIANI; LEAL, 2007). As elevadas taxas respiratórias conjuntamente com a produção de etileno aceleram o processos de deterioração da banana (PRILL et al., 2012). As mudanças que ocorrem durante o amadurecimento e senescência da banana envolvem várias vias bioquímicas, como a degradação do amido em açúcares simples, modificações na textura, cor da casca e da polpa, bem como na concentração de compostos voláteis e ácidos orgânicos, repercutindo em redução da adstringência (LOBO; ROJAS, 2020).

O amido é convertido em açúcares simples causando aumento no teor de sólidos solúveis ao longo do processo de amadurecimento da banana (MADUWANTHI; MARAPANA, 2017). A alteração na cor da casca ocorre devido à quebra da clorofila, a qual torna-se ausente em frutas maduras, e da diminuição dos carotenoides durante a mudança de cor da casca, os quais, em seguida, voltam a se elevar até alcançar conteúdo similar àquele encontrado quando da fruta verde (LOBO; ROJAS, 2020). O pH da polpa e acidez total titulável são atributos de qualidade pós-colheita importantes na avaliação do amadurecimento de frutas e conforme o amadurecimento avança ocorre diminuição do pH e elevação da acidez (HAILU; WORKNEH; BELEW, 2013).

A produção mundial de bananas tem aumentado de forma constante nos últimos 20 anos, saindo de aproximadamente 58 milhões de toneladas em 1996 para alcançar aproximadamente 114 milhões de toneladas em 2017 (FAOSTAT, 2019). O aumento desta produção pode estar relacionado com a maior demanda pelo produto devido ao crescimento populacional, bem como ao emprego de tecnologias que implicam em aumento da produtividade mesmo ocorrendo decréscimo da área cultivada entre 2012 e 2016 (FAOSTAT, 2019). No ano de 2017, a produção mundial de bananas foi de 113,9 milhões de toneladas, sendo a Índia o maior produtor com produção anual de 30,4 milhões de toneladas, seguida por China, Indonésia e Brasil com produção anual de 11,1; 7,1 e 6,6 milhões de toneladas, respectivamente (FAO/WHO, 2019).

O Brasil produziu no ano de 2019 (até o mês agosto) aproximadamente 6,9 milhões de toneladas de bananas, sendo a região Nordeste a segunda maior produtora e responsável por 35,53% da produção nacional, seguindo a região Sudeste como a primeira produtora (IBGE, 2019). Porém, o Brasil tem pequena participação no comércio internacional de bananas,

estimado em torno de 3% da produção, visto que a principal via de escoamento da produção ainda é o mercado interno (COLTRO; KARASKI, 2019). Esta pequena participação no mercado externo também ocorre devido, principalmente, aos altos índices de perdas da fruta durante a produção e comercialização (COLTRO; KARASKI, 2019).

Apesar da alta produtividade brasileira de produtos agrícolas, considerável parte da produção é perdida por causa de problemas que ocorrem na fase de produção e pós-colheita. Tem sido estimado que as perdas de produtos agrícolas nos diferentes seguimentos das cadeias agroalimentares na América Latina e Caribe são de, aproximadamente, 28% na produção, 22% no manejo e armazenamento, 17% no mercado e distribuição e 28% pelos consumidores, sendo tais perdas decorrentes de diversos fatores físicos, fisiológicos e biológicos (BORGES, 2006; SPAGNOL et al., 2018).

2.1. DOENÇAS PÓS-COLHEITA EM BANANAS

A baixa qualidade de bananas produzidas no Brasil tem sido relacionada, principalmente, aos problemas fitossanitários. De modo geral, fungos, bactérias, nematoides, vírus e insetos têm contribuído de forma expressiva na redução da produção e nas perdas pós-colheita de bananas (BORGES, 2006; GASPAROTTO, 2010; SILVA et al., 2013). As principais doenças pós-colheita que afetam a banana são a antracnose (Figura 1) e “podridão da coroa” (Figura 2), que se manifestam, principalmente, na fruta madura (BORGES, 2006).

Figura 1- Antracnose causada por *Colletotrichum musae* em banana

Fonte: Agrolink.

Figura 2- Podridão da coroa: podridão e necrose de tecidos em bananas

Fonte: Don Edwards, UC Davis, 2016

A podridão-da-coroa, também chamada de podridão-da-almofada, é uma doença causada comumente pelos fungos *Fusarium roseum*, *Verticillum theobromae* e *Colletotrichum musae*, Lasiodiplodia spp. podendo encontrar-se associados a bactérias oportunistas, durante a prática de despencamento das frutas para comercialização (SIRIWARDANA et al., 2017). A antracnose da banana é uma doença causada por *C. musae*, sendo as lesões desenvolvidas nas frutas consideradas o mais grave problema pós-colheita em bananas (BORGES, 2006).

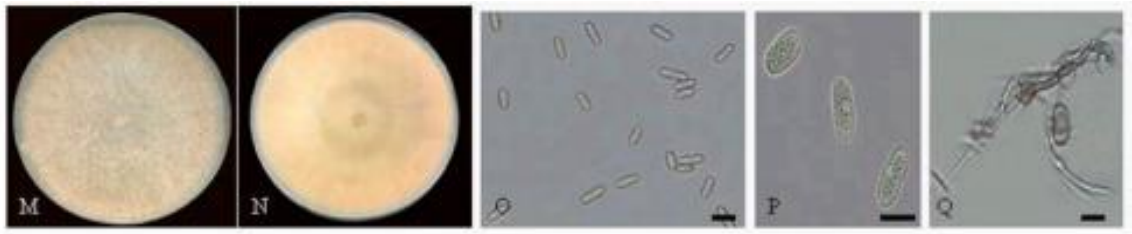
2.1.1. *Colletotrichum musae* e desenvolvimento de antracnose em bananas

Cerca de 2.200 espécies de plantas são reportadas em associação com o gênero *Colletotrichum* (FARR & ROSSMAN, 2015), sendo um dos um dos mais importantes agentes etiológicos de doenças de plantas em todo o mundo (DEAN et al., 2012), este gênero possui tanto espécies patogênicas como não-patogênicas (VIEIRA et al., 2014).

A primeira classificação da espécie *C. musae* foi como *Myxoporium musae* Berk. & M.A. Curtis (BERKELEY, 1874), sendo, em seguida, transferida para o gênero *Gloesporium* Desm. & Montag., passando a ser nomeada *Gloesporium musarum* Cooke & Massee (COOKE, 1887; BAXTER; VAN DER WESTHUIZEN; EICKER, 1985), e, por último, reclassificada no gênero *Colletorichum*. A classificação taxonômica da espécie *C. musae* ocorreu com base no teleomorfo, que está inserido no filo Ascomycota, classe Sordariomycetes, subclasse Sordariomycetidae, ordem Incertae sedis e família Glomerellaceae (INDEX FUNGORUM, 2019).

Frequentemente, a espécie *C. musae* apresenta colônias com micélios aéreos abundantes e de coloração branca, os quais com o passar do tempo tornam-se acinzentados com considerável massa de conídios, envoltos em uma mucilagem. Não há relatos na literaturad a presença de escleródios e setas. Os conídios são, geralmente, hialinos, retos, unicelulares, cilíndricos, obtusos nos ápices, possuindo entre 10 e 18 µm de comprimento e entre 3 e 6,5 µm de largura (ARX, 1957a, 1957b; SUTTON, 1980; SU et al., 2011). Os apressórios se apresentam de forma irregular, médios e com coloração castanho-escura (SUTTON, 1980; SU et al., 2011).

Figura 1- Características morfológicas de *Colletotrichum musae* isolado de banana: frente da placa de Petri (M) e verso da placa de Petri (N) em meio de cultivo ágar batata dextrose, conídios (O-P) e apressório (Q) (barras = 10 µm)



Fonte: Sakinah, Suzianti e Latiffah, 2014.

A temperatura ótima para o crescimento, esporulação e germinação dos conídios ocorre entre 27 a 30 °C. A taxa de germinação se torna um pouco mais baixa se no escuro ou na presença de luz, sendo mais favorável a alternância da luminosidade. O valor de pH ótimo para o seu crescimento é 7 (THANGAMANI et al., 2011).

A antracnose é uma doença de natureza fúngica também denominada como podridão negra ou podridão das frutas maduras, tendo *C. musae* como seu agente etiológico em bananas. A infecção pode ocorrer de duas formas: i) latente, a qual acorre ainda no campo, permanecendo dormente até o início do amadurecimento; e ii) não latente, a qual ocorre pela invasão de fitopatógenos em ferimentos nas frutas ainda verdes (CORDEIRO, 2000). O amadurecimento das frutas atacadas por *C. musae* acontece de forma mais rápida quando comparado com frutas sadias, representando risco adicional para a cadeia de produção e comercialização de bananas. De forma geral, a antracnose não afeta a polpa da banana, embora esse dano possa ocorrer em condições de altas temperaturas ou em estádio de maturação avançado (CORDEIRO, 2000; PLOETZ, 2003; CORDEIRO; MATOS, 2005).

A antracnose em bananas pode causar perdas de até 40% da produção, estando o fungo difundido em todas as regiões brasileiras. A infecção se inicia na lavoura nos frutas verdes, mas

o desenvolvimento da doença só ocorre durante o amadurecimento, formando de pequenas a grandes lesões necróticas e deprimidas (OLIVEIRA, 2006; NEGREIROS et al., 2013; VIEIRA et al., 2017). Os sintomas só aparecem em frutas verdes quando ocorre intensa injúria (PLOETZ, 2003).

Em condições de alta umidade, as frutas ficam cobertas de frutificações do patógeno com coloração variando de rosa a salmão e presença de acérvulos acizentados (CORDEIRO; MATOS, 2005). A produção de conídios ocorre em restos culturais sob condições favoráveis de alta umidade e podem ser dispersos pela chuva, vento e insetos (PLOETZ, 2003). A germinação ocorre entre 4 e 24 horas com formação de apressório em presença de água livre. Entre 24 e 48 horas, ocorre a penetração na fruta com acúmulo de fitoalexinas devido à reação de hipersensibilidade nas células adjacentes da epiderme, tornando a infecção latente até a maturação da fruta. Entretanto, a infecção também pode ocorrer por meio de ferimentos nas frutas (GOWEN, 1995).

Torna-se ideal, que a tomada de medidas de controle da antracnose tenham início de aplicação ainda no campo de produção, conforme as recomendações sugeridas para o manejo de doenças (CORDEIRO, 2000), como eliminação de folhas velhas, brácteas e restos florais, cobertura dos cachos com saco polietileno perfurado, limpeza e desinfestação dos tanques de despencamento e renovação periódica da água do tanque de lavagem das frutas. Durante a fase de colheita e pós-colheita é importante a tomada de cuidados a fim de evitar ferimentos nas frutas, os quais facilitam a penetração de fitopatógenos (AMORIM; BERGAMIN FILHO; REZENDE, 2018). O último passo para o controle da doença vem a ser a utilização de fungicidas, o que pode ser feito por imersão ou atomização (CORDEIRO, 2000; VENTURA; HINZ, 2002; CORDEIRO; MATOS, 2005). Atualmente, somente três produtos são registrados no Brasil para uso no controle da antracnose em bananas, dos quais os ingredientes ativos são imazalil, tiofonato metílico e tiabendazol (MAPA, 2019), com concentrações de aplicação entre 200 e 400 ppm (AMORIM; BERGAMIN FILHO; REZENDE, 2018).

Existem diversas restrições relacionadas ao uso de fungicidas na pós-colheita de frutas, como fitotoxicidade dos produtos aplicados, efeitos residuais, tendência dos patógenos para desenvolver resistência devido ao sítio específico de ação, toxicidade e carcinogenicidade (VIEIRA et al., 2017; HAWKINS; FRAAIJE, 2018). Além disso, tem ocorrido uma tendência de mercado por busca de alternativas de controle de perdas pós-colheita de frutas sem a utilização de produtos químicos (TAJKARIMI; IBRAHIM; CLIVER, 2010; DE COSTA; GUNAWARDHANA, 2012).

A adoção de medidas de controle de doenças pós-colheita torna-se indispensável como estratégia de manutenção da qualidade e aumento de tempo de prateleira de frutas, especialmente quando o período entre a colheita e o consumo for espaçado (LINS et al., 2011; YAHIA; CARRILLO-LÓPEZ, 2019). Diante disso, pode-se inferir que o potencial de conservação das frutas está diretamente relacionado, não só ao manejo adequado, mas também ao seu ponto de colheita e aos tratamentos fitossanitários de campo e pós-colheita, os quais podem interferir na velocidade da sua deterioração (NABI et al., 2017).

2.2. APLICAÇÃO DE REVESTIMENTOS COMESTÍVEIS EM FRUTAS

A tecnologia de aplicação de revestimentos comestíveis tem ganhado destaque como ferramenta para ampliar o tempo de preservação de frutas (OLIVEIRA et al., 2018; BRAGA et al., 2019a; MACEDO et al., 2020). O principal objetivo de aplicação de revestimentos comestíveis é exercer papel funcional e coadjuvante, contribuindo para a preservação da textura e valor nutricional da fruta revestida, reduzindo as trocas gasosas superficiais e a perda ou ganho excessivo de água e não substituindo o uso de embalagens ou emprego do frio em definitivo (JONGEN, 2002; TURHAN, 2010).

Os revestimentos devem ser formulados com materiais considerados como GRAS (geralmente reconhecidos como seguros), ou seja, serem atóxicos e seguros para o uso em alimentos. Essa premissa é de importância, visto que serão aplicadas ou formados diretamente sobre a superfície das frutas, sendo, muitas vezes, imperceptíveis ao olho nu e com características estruturais que dependem da formulação da solução filmogência precursora (FDA, 2011).

Uma das vantagens mais importantes do uso de revestimentos comestíveis é a possibilidade de incorporação de aditivos que podem aumentar a sua eficácia, como antioxidantes, antimicrobianos e flavorizantes. A incorporação destes agentes pode repercutir em vida de prateleira mais prolongada do produto revestido devido a inibição do crescimento de microrganismos e retardo de alterações bioquímicas, repercutindo em menores perdas nutricionais (HASSAN et al., 2018).

Polissacarídeos, lipídios e proteínas são as matérias-primas mais empregadas na formulação de revestimentos comestíveis, de modo que a escolha da formulação depende fundamentalmente das características do produto a ser revestido e do objetivo da aplicação do revestimento. Atualmente, existe a tendência de classificar os materiais empregados na

formulação de revestimentos em hidrofóbicos e hidrofílicos (ZARITZKY, 2010; HASSAN et al., 2018).

Os materiais hidrofóbicos contêm moléculas que não configuram regiões polares definidas, incluindo grupos aromáticos e alquilas (CH_3, CH_2-CH_3). Quando na presença de água, estes materiais se aglomeram e excluem as moléculas polares ao seu redor. Proteínas hidrofóbicas, óleos e ácidos graxos são alguns dos materiais inseridos na categoria de materiais hidrofóbicos utilizados na formulação de revestimentos (ASSIS; BRITTO, 2014).

Os materiais hidrofílicos possuem estruturas com predominância de grupos hidroxila, carboxila ou amino (OH, COO–NH_3). Em função das características destes grupos, a cadeia carbônica apresenta sítios parcialmente carregados positivamente e outros carregados negativamente. Esta característica da estrutura química favorece o acúmulo e o rearranjo de moléculas polares e, principalmente, da água em torno destes sítios. Alguns exemplos de materiais hidrofílicos são os polissacarídeos, como celulose, quitina, goma xantana, goma guar, goma arábica, pectina e amido, além dos polissacarídeos polieletrólitos, como carboximetilcelulose, quitosana e alginato. Os materiais hidrofílicos favorecem a dispersão do soluto e formação mais homogênea do filme. Dependendo da estrutura química, podem formar géis ou até mesmo requerer alterações químicas para se alcançar completa solubilização (ASSIS; BRITTO, 2014).

A camada formada pelo revestimento nas frutas causa preenchimento parcial dos estômatos e lenticelas das células vegetais, e, com isso, diminuem a transferência de umidade e as trocas gasosas (transpiração e respiração, respectivamente) (MANNOZZI et al., 2017). A degradação da textura e a perda de peso ocorrem devido os produtos frescos serem muito sensíveis a perda de água, de forma que os revestimentos comestíveis formam uma barreira entre a casca e o ambiente externo, evitando a perda excessiva de água (NAWAB; ALAM; HASNAIN, 2017; ARNON-RIPS; POVERENOV, 2018).

Esta modificação na atmosfera também é capaz de manter a firmeza e retardar a senescência das frutas, visto que o processo de amadurecimento está diretamente relacionado ao aumento da produção de etileno. O etileno necessita do oxigênio para ser produzido, portanto, a permeação do oxigênio para o interior da fruta, causada pelo revestimento, reduz a produção de etileno dificultando o amadurecimento (ZHANG et al., 2019). O revestimento também pode melhorar a aparência da fruta armazenada, visto que confere brilho, o que é um aspecto atraente para o consumidor. Ademais, o atraso no amadurecimento também causa retardo da quebra da clorofila e síntese de carotenoides (ULLAH et al., 2017).

As duas formas mais conhecidas de aplicação de revestimento em vegetais são: i) por meio de imersão rápida do vegetal na solução filmogênica, de modo que, após a retirada da imersão, o vegetal é colocado em repouso até que a película seja formada por meio da evaporação da água contida na solução; e ii) por meio de aspersão, onde a solução dos ingredientes utilizados na formulação do revestimento é aspergida sobre o vegetal. Outras formas de aplicação podem ser a escovação, leito fluidizado, aplicação eletrostática e eletrospray (SILVA-VERA et al., 2018).

2.2.1 Goma Arábica

O exsudato de troncos e ramos da *Acacia senegal* (Linne), após dessecado de forma espontânea, gera um produto conhecido como goma arábica ou goma acácia (Figura 4). A goma arábica é um polissacarídeo ácido de estrutura ramificada, cuja cadeia principal é formada por unidades de D-galactopiranose unidas por ligações glicosídicas β-D-(1→3). A esta cadeia

Figura 2: Exsudato de tronco da *Acacia senegal*

Fonte: Google images.

principal, por meio de ligações β(1→6), estão ligadas as cadeias laterais com diferentes estruturas químicas formadas de D-galactopiranose, L-ramnose, L-arabinofuranose (SHIRWAIKAR et al., 2008; PATEL; GOYAL, 2015). A goma arábica contém 12 a 15% de água e várias enzimas ocluídas (oxidases, peroxidases e pectinases), e, em base seca, possui de 1 a 2% de diferentes de proteínas. Além disso, pode apresentar substâncias associadas como polifenois e minerais (magnésio, potássio, cálcio e sódio) em cerca de 3 a 4%. Embora tenha elevado peso molecular, a goma arábica apresenta comportamento reológico newtoniano em

meio aquoso (10% na formulação), sendo consequência da sua estrutura molecular compactada e altamente ramificada (MATHIAS et al., 2013).

Para se obter a goma arábica, as árvores são sujeitas às condições de seca ou são feridas. No entanto, a produção do exsudato é condicionada, fazendo-se uma incisão transversal no córtex e descascando-o acima e abaixo do corte, expondo uma área de trocas. Entre duas a oito semanas, as lágrimas formadas na superfície de exposição são colhidas, sendo o intervalo de tempo dependente das condições climáticas (PATEL; GOYAL, 2015).

A goma arábica é amplamente utilizada pela indústria devido a sua capacidade de emulsificação, formação de filme, sabor suave e propriedades de encapsulamento. Ainda, possui excelente solubilidade em água, propriedades tensoativas e produz soluções com baixa viscosidade em altas concentrações de sólidos. O seu uso tem ocorrido em alimentos (doces, bolos, biscoitos, bebidas e produtos secos embalados), medicamentos (como transportador em cápsulas e suplementos de alto teor de fibra solúvel), produtos cosméticos (cremes e loções) e tintas litográficas (BALDWIN, 2016).

Os três grandes campos de aplicações da goma arábica são na elaboração de produtos de confeitaria, emulsão de aromas em bebidas e encapsulamento de aromas. Destaca-se, a sua aplicação na indústria de confeitos, sendo utilizada como ingrediente para formulação de uma variedade de produtos, tais como gomas, pastilhas, *marshmallows* e caramelos (*toffees*). A goma arábica é estável em condições ácidas, sendo extensamente utilizada como emulsificante na produção de óleos aromatizantes concentrados de cola e cítricos. A goma é capaz de inibir a floculação e a coalescência de gotas de óleo durante longos períodos. Além disso, as suas emulsões permanecem estáveis por até um ano quando diluídas em até aproximadamente 500 vezes. A goma arábica tem sido empregada em microencapsulamento para transformar os compostos responsáveis pelo aroma em alimentos da forma de líquidos voláteis para pós com o objetivo de facilitar a incorporação em formulações de produtos alimentícios (YE et al., 2012).

Além disso, a goma arábica tem sido estudada para aplicação como revestimento em algumas frutas com o intuito de aumentar a vida de prateleira e manter a qualidade pós-colheita. A aplicação de goma arábica (12%, p/v) em pimentões verdes demonstrou efeitos positivos sobre a manutenção da qualidade pós-colheita do produto ao longo do armazenamento (ULLAH et al., 2017). Mangas revestidas com dispersão de goma arábica enriquecida com cloreto de cálcio tiveram redução do dano oxidativo e apresentaram melhores indicadores de qualidade pós-colheita (KHALIQ et al., 2016).

Mirtilos revestidos com dispersões de goma arábica enriquecidas com extratos de rosela vermelha e branca apresentaram menor crescimento de leveduras e bactérias, reduzindo a sua deterioração (YANG et al., 2019). Ameixas revestidas com dispersões de gomara arábica e óleos essenciais de *Origanum vulgare* L. e *Rosmarinus officinalis* L. apresentaram retardo na ocorrência de podridão mole causada por *Rhizopus stolonifer*, bem como maior realce de cor e sabor, manutenção da firmeza e diminuição da perda de peso ao longo do armazenamento (ANDRADE et al., 2017).

Devido a goma arábica já existir em uso comercial facilita o seu uso como revestimento, porém não foi comprovado até o momento que ela apresenta atividade antimicrobiana por este motivo seria interessante atribuir aditivos ao seu uso como revestimento comestível.

2.3. ÓLEOS ESSENCIAIS

Os óleos essenciais são compostos de metabólitos vegetais secundários lipofílicos e altamente voláteis, que podem ser fisicamente separados de outros componentes vegetais ou tecidos membranosos (TUREK; STINTZING, 2013; BAŞER; BUCHBAUER, 2020). São definidos pelas normas ISO 4720:2009 (ISO, 2018) e ISO 9235:2013 (ISO, 2013) como o "produto obtido por destilação a vapor de partes de plantas (folhas, flores e ramos), por prensagem a frio de epicarpos (casca) de citrinos ou por destilação a seco, após separação da fase aquosa (se existir) por processos físicos". O principal processo de separação usado atualmente pela indústria de óleos essenciais é a destilação fracionada à vácuo (SILVESTRE et al., 2019).

Os constituintes dos óleos essenciais podem variar na quantidade e na diversidade e esse fenômeno pode ocorrer devido a diversos fatores, como variabilidade genética, clima, temperatura, altitude, sazonalidade, fotoperiodismo, localização geográfica, horário da colheita, uso de agroquímicos e tipo de procedimento de extração. Estes fatores podem também influenciar no rendimento do óleo essencial (SIMÕES; SCHENKEL; MELLO, 2017; SILVESTRE et al., 2019).

Além de seu uso difundido como material aromatizante (TUREK; STINTZING, 2013), os óleos essenciais representam uma alternativa "natural" para uso pela indústria alimentícia, farmacêutica e agrícola devido às suas propriedades antimicrobianas, antivirais, nematicidas, antifúngicas, inseticidas e antioxidantes (POURGHANBARI et al., 2016; PAVELA; SEDLÁK, 2018; TAHIR et al., 2018; BARROS et al., 2019; BRAGA et al., 2019a; BARBOSA et al.,

2020) ou mesmo atividades que alterem o sistema nervoso central (LÓPEZ et al., 2017; SMERIGLIO et al., 2018). Estas características compiladas resultam em amplo espectro de aplicações como conservantes em alimentos (PATRA; KIM; BAEK, 2015; ALMEIDA et al., 2019) ou mesmo quando incorporados em embalagens ou revestimentos aplicados em alimentos (DE OLIVEIRA et al., 2017; BRAGA et al., 2019a; MACEDO et al., 2020).

A variedade de possíveis aplicações de óleos essenciais resulta da grande diversidade de componentes comumente encontrados na sua composição (TUREK; STINTZING, 2013). Os principais constituintes dos óleos essenciais podem ser categorizados em duas famílias estruturais com relação ao seu esqueleto de hidrocarbonetos, ou seja: Terpenóides, que são produzidos pela combinação de duas, três ou quatro unidades de isopreno (monoterpenos, sesquiterpenos ou diterpenos, respectivamente); e fenilpropanóides. Estas duas famílias têm precursores metabólitos primários distintos e são biossintetizados por diferentes vias (JUGREET et al., 2020).

Acredita-se, que a atividade antifúngica dos componentes de óleos essenciais se estabeleça devido à alta hidrofobicidade dos seus componentes, causando danos na membrana celular dos fungos (YU; ZHANG; WANG, 2021). Porém, outros mecanismos de ação têm sido relatados para explicar a atividade antifúngica de óleos essenciais, tais como: desestruturação da mitocôndria fúngica, inibição do transporte de elétrons e inibição da atividade de ATPase mitocondrial (TIAN et al., 2012; LAGROUH; DAKKA; BAKRI, 2017; YU; ZHANG; WANG, 2021).

Os óleos essenciais, quando incorporados em revestimentos comestíveis, são continuamente liberados na superfície das frutas, mantendo uma concentração adequada dos componentes antimicrobianos ao longo do tempo e, consequentemente, estendendo o período de armazenamento (SOUZA et al., 2019). Estudos com a aplicação de revestimentos incorporados com óleos essenciais têm se mostrado eficazes no controle de espécies de *Colletotrichum*, a exemplo de revestimentos formulados com quitosana e óleo essencial de *Cymbopogon citratus*, os quais apresentaram capacidade de controlar o crescimento micelial de cinco espécies patogênicas de *Colletotrichum* in vitro e quando aplicados em goiaba, mamão e manga (OLIVEIRA et al., 2018); cera de abelha enriquecida com óleo essencial de manjericão, a qual reduziu o desenvolvimento de antracnose em mangas (KARUNANAYAKE et al., 2020); e revestimentos formulados com goma arábica e extratos de frutos secos de *Garcinia atroviridis*, os quais causaram inibição do crescimento micelial de *Colletotrichum gloeosporioides* (MUSTAFA; BORDOH; AHMAD, 2018).

2.3.1 Óleo essencial de *Conyza bonariensis* (L.) Cronquist

A espécie vegetal *C. bonariensis* (L.) Cronquist (Asteraceae) (Figura 5) é uma erva daninha dicotiledônea de folha larga (LOURA et al., 2020), com origem na América do Sul, embora atualmente seja amplamente distribuída em todo o mundo. No Brasil, é conhecida como buva, voadeira, margaridinha-do-campo, rabo-de-foguete, arnicão, lagarteira, rabo-de-raposa, capiçoba, acatóia, entre outras denominações (MAIA et al., 2002; LORENZI, 2014). É uma planta nativa que ocorre em todas as regiões brasileiras e pode ser encontrada em todos os biomas (ZAPPI et al., 2015).

Figura 3: Parte aérea de *Conyza bonariensis* (L.) Cronquist: A) Caule e folhas, B) Folhas e flores.

Fonte: (A) Mangolin, Oliveira Júnior e Machado, 2014; (B) European Enviroment Agency

As folhas de *C. bonariensis* são utilizadas em preparações culinárias, podendo ser consumida refogada, crua ou como especiaria devido a sua característica picante, sendo considerada uma planta alimentícia não convencional (PANC) (KINUPP; LORENZI, 2014; DA SILVA et al., 2018). Caracteriza-se como planta rica em compostos espasmogênicos e espasmolíticos, o que se relacionada ao seu amplo uso medicinal na Ásia para tratamento de diarreia e obstipação (BUKHARI et al., 2013). Extratos de *C. bonariensis* administrados por via oral foram considerados seguros em doses de até 5000 mg/kg em modelos animais (DE PAULA et al., 2018) e seu óleo essencial tem sido considerado possuidor de baixa toxicidade (DELORENZI et al., 2017).

O primeiro estudo que analisou o óleo essencial de diferentes partes de *C. bonariensis* foi publicado em 2005, sendo relatado rendimento entre 0,04 e 0,32% de acordo com a parte da

planta utilizada para extração. Foram detectados 31 diferentes componentes no óleo essencial, dos quais 17 foram identificados, embora apenas um (Matricaria metil-éster) tenha sido identificado no material extraído de todas as partes da planta, sendo majoritário no óleo essencial extraído das raízes. Os demais componentes mais prevalentes no óleo essencial extraído do caule, folhas e inflorescências foram manool, limoneno e carvona, respectivamente (BARBOSA et al., 2005).

Outro estudo determinou os constituintes do óleo essencial de amostras de *C. bonariensis* coletadas em cinco diferentes localidades no Brasil, sendo identificados (E)-β-farneceno e/ou limoneno como constituintes majoritários, com concentrações variadas de acordo com a região de origem (MAIA et al., 2002). Outro estudo encontrou elevadas concentrações de limoneno e/ou óxido de cariofileno no óleo essencial obtido das folhas de *C. bonariensis*, as quais variaram de acordo com a estação do ano da coleta do material vegetal utilizado para extração do óleo essencial (MABROUK et al., 2011). Estes dois compostos são utilizados pela planta como defesa, sendo relatada ação inseticida para o limoneno e ação antifúngica para o óxido de cariofileno (THABIT et al., 2014; DA SILVA et al., 2018).

Foi relatado efeito antibacteriano do extrato metanólico de *C. bonariensis* e suas frações de solvente subsequentes contra *Escherichia coli* e *Pseudomonas aureginosa* e efeito antifúngico contra *Cladosporium cucumerinum* e *Candida albicans* (SHAH et al., 2013). Cepas de diferentes bactérias causadoras de doenças transmitidas por alimentos (*Shigella dysenteriae*, *Escherichia coli*, *Salmonella typhimurium*, *Streptococcus pyogenes* e *Staphylococcus aureus*) foram utilizadas para avaliar o efeito antibacteriano do extrato etanólico de *C. bonariensis*, sendo reportado forte efeito inibitório das cepas testadas (THABIT et al., 2015). Estudo anterior também demonstrou que o extrato metanólico de *C. bonariensis* exerceu atividade antimicrobiana de moderada a fraca frente cepas de diferentes bactérias patogênicas (*Enterococcus faecalis*, *Enterobacer aerogene*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Shigella flexneri*, *Staphylococcus aureus* e *Streptococcus pyogenes*) e fungos patogênicos (*C. albicans*, *Candida grablata*, *Candida kruzei* e *Candida parapsilosis*) (DE PAULA et al., 2018). Extratos etanólicos de *C. bonariensis* em concentrações abaixo de 10% também demonstraram atividade antimicrobiana contra cepas de bactérias (*S. aureus*) e fungos patogênicos (*Malassezia* spp. e *Candida* spp.) (MUSSIN et al., 2017). Alguns poucos estudos têm demonstrado que o óleo essencial de *C. bonariensis* possui efeito inibitório frente bactérias (*Bacillus cereus* e *Staphylococcus epidermidis*) e fungos (*Candida albicans* e *Phyllosticta citricarpa*) (ARAUJO et al., 2013; LOMBARDO et al., 2016).

Entretanto, ainda existem poucas informações disponíveis na literatura sobre os efeitos antimicrobianos do óleo essencial de *C. bonariensis*, sendo necessário avançar em estudos sobre sua ação antifúngica, particularmente frente fungos fitopatógenos e somente dois gêneros de fungos fitopatogênicos foram avaliados (*Cladosporium* e *Phyllosticta*).

Portanto, considerando os aspectos anteriormente citados, que caracterizam o potencial biológico do óleo essencial de *C. bonariensis* e a sua possível incorporação em revestimentos formulados com goma arábica, a aplicação combinada destes compostos pode representar uma alternativa de melhoria na tecnologia de preservação pós-colheita de bananas destinadas ao consumo interno e/ou exportação.

3. MATERIAL E MÉTODOS

3.1. OBTENÇÃO DE DADOS PARA ELABORAÇÃO DE ARTIGO DE REVISÃO DE LITERATURA

A seleção dos artigos se iniciou a partir da busca nas bases de dados Pubmed e Scopus de artigos publicados entre os anos de 2010 e 2019 no idioma inglês. Os termos descritores utilizados de forma isolada ou em combinação foram:

"coating," "edible films," "composition," "essential oil," "phytoconstituents," "antimicrobial activity," "antifungal," "antibacterial," "postharvest diseases," "microbiological safety," "effects," "effectiveness," "application," "fruit," "vegetable," "quality standards," "sensory aspects," and "nutritional aspects".

Os artigos selecionados para uso na elaboração da revisão foram avaliados de forma independente por cinco avaliadores e após a leitura do título e resumo foram selecionados 30 artigos.

A revisão limitou-se a pesquisar artigos originais que avaliaram a eficácia de revestimentos comestíveis formados por polissacarídeos e óleos essenciais com o intuito de preservar frutas frescas. Os artigos que utilizaram revestimentos comestíveis formados por polissacarídeos e constituintes individuais presentes nos óleos essenciais também foram avaliados, pois estes compostos normalmente apresentam propriedades antimicrobianas semelhantes às dos óleos essenciais.

Os estudos incluídos abordaram: (i) a atividade antimicrobiana e os efeitos dos revestimentos comestíveis na infecção e deterioração quando aplicados em frutas; e (ii) a influência dos revestimentos comestíveis sobre parâmetros físico-químicos e sensoriais de frutas revestidas mesmo que não tenham sido avaliados os efeitos antimicrobianos. Artigos originais utilizando apenas ensaios *in vitro* frente microrganismos fitopatogênicos e deteriorantes não foram utilizados. Estudos que utilizaram proteínas (por exemplo, gelatina, caseína, zeína e proteína de soja) e lipídios (por exemplo, cera de carnaúba e cera de abelha) como materiais de base para a produção de revestimentos comestíveis também foram excluídos.

Foram descritos aspectos metodológicos relativos à produção de revestimentos formados por polissacarídeos, óleos essenciais ou constituintes individuais, como: (i) tipo e característica do polissacarídeo, óleo essencial ou constituinte individual; e (ii) concentrações testadas dessas substâncias (ou compostos) e seu uso combinado para formular os revestimentos. Também foram apresentados e discutidos aspectos metodológicos empregados para a produção e aplicação de coberturas comestíveis em frutas.

Considerando a aplicação de revestimentos comestíveis em frutas, foram avaliados os microrganismos patógenos utilizados nos ensaios antimicrobianos *in situ*, os principais efeitos contra esses microrganismos e a influência dos revestimentos comestíveis nos parâmetros gerais de qualidade das frutas revestidas, como: pH, acidez titulável, sólidos solúveis totais, cor, firmeza, perda de peso, taxa respiratória, compostos fenólicos, ácido ascórbico, atividade enzimática e características sensoriais.

Diferentes tabelas foram montadas organizando as informações e considerando: (i) os materiais, como o tipo de material de revestimento e frutas; (ii) a concentração de materiais de revestimento, o microrganismo alvo, os tipos de ensaios realizados e os principais efeitos antimicrobianos exercidos pelos materiais usados para preparar os revestimentos (ensaios *in vitro*) e/ou por revestimentos formulados quando aplicados em frutas (em ensaios *situ*); e (iii) os efeitos dos revestimentos formulados nos parâmetros físico-químicos e sensoriais relacionados à qualidade geral das frutas revestidas.

3.2. LOCAL DE EXECUÇÃO DOS EXPERIMENTOS

Os experimentos de avaliação dos efeitos da solução de GA e emulsão de OECB sob os isolados fúngicos, a análise de citometria de fluxo, a avaliação dos revestimentos sob o desenvolvimento da lesão de antracnose em bananas e os parâmetros físico-químicos foram realizados no Laboratório de Bioquímica e Microbiologia de Alimentos (LMBA) pertencente ao Departamento de Nutrição/Centro de Ciências da Saúde, enquanto as análises de caracterização do óleo essencial foram realizadas no Laboratório Multiusuário de Caracterização e Análises pertencente ao Instituto de Pesquisa em Fármacos e Medicamentos (IPeFArM), ambos da Universidade Federal da Paraíba e situados no campus I (João Pessoa – PB).

3.3. MATERIAL

3.3.1. Obtenção do óleo essencial de *C. bonariensis* e da goma arábica

O óleo essencial de *C. bonariensis* (OECB) foi extraído no IPeFArM/UFPB. As folhas de *C. bonariensis* foram coletadas pela manhã (antes das 8:00 horas) no horto de Plantas Medicinais do IPeFArM/UFPB. A espécie foi identificada e depositada no Herbário Lauro Pires Xavier - JPB (UFPB) sob o número JPB 26391 e registrado no SISGEN com código ABB39C8.

Para a extração do óleo essencial utilizou-se o método de hidrodestilação por aparelho de Clevenger. O período de extração durou aproximadamente 8 horas, e, posteriormente, o óleo essencial foi filtrado com sulfato de sódio anidro e armazenado em recipientes âmbar com vedação sob temperatura de refrigeração (4 ± 0,5°C) (GUERRA et al., 2016).

A goma arábica (GA) (CAS 9000-01-5, densidade 0,424 g/cm^3, pureza de 99,98%, brilho > 250 °C) foi obtida da Dinâmica Química Contemporânea Ltda. (Diadema, São Paulo, Brasil).

3.3.2. Isolados fúngicos e preparação do inóculo

Seis isolados de *Colletotrichum musae* (RP3, RP4, RP10, MM5, GM20 e LN2) foram utilizados como micro-organismos teste, os quais foram cedidos pela Coleção de Culturas de Fungos Fitopatogênicos "Profa. Maria Menezes" (Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brasil). Estes isolados foram recuperados de bananas acometidas de antracnose e identificados com o uso de inferência filogenética, os isolados com as siglas iniciando em RP são resistentes ao fungicida comercial e os demais são susceptíveis ao fungicida comercial (VIEIRA et al., 2017).

Os isolados estoques foram mantidos em discos de 5 mm de batata dextrose ágar – BDA (HiMedia, Mumbai, Índia) em microtubos contendo 1,5 mL de água destilada esterilizada, sendo mantidos a 5 ± 0,5 °C no escuro. Para os ensaios de atividade antifúngica, foram utilizadas as culturas fúngicas crescidas em BDA (25 ± 0,5 °C) durante 7 dias. Antes dos ensaios, cada isolado foi inoculado em uma banana, e, após o desenvolvimento de lesões características de antracnose, foram re-isolados e cultivados em BDA (25 ± 0,5 °C) durante sete dias. As lesões induzidas foram semelhantes para todos os isolados testados, sendo caracterizadas como manchas marrom-escuro/pretas, geralmente, arredondadas e levemente deprimidas (VIEIRA et al., 2017).

3.3.3. Obtenção das frutas

As bananas (*Musa* sp., cv. Prata) foram adquiridas de uma fazenda agrícola localizada no município de João Pessoa (7 ° 08′29 ″ S, 34 ° 50′48 ″ W, Paraíba, Brasil), no estádio de maturação comercial (casca amarela com pontas verdes e índice de maturidade cinco) (BHUIYAN et al., 2020) e selecionadas considerando uniformidade de tamanho, forma, aparência e ausência de danos mecânicos ou infecções fúngicas visíveis.

Antes da realização dos ensaios, as frutas foram lavadas com detergente neutro e água corrente, sendo, em seguida, imersas em solução de hipoclorito de sódio para desinfecção da superfície (1 mL/100 mL, pH 7,2 ajustado com NaOH a 1 M) durante 5 minutos, lavadas com água destilada esterilizada e secas em temperatura ambiente (25 ± 0,5 °C).

3.3.4. Obtenção dos fungicidas

A formulação comercial de fungicidas composta por trifloxistrobina (100 g/L) + tebuconazol (200 g/L) (Bayer S.A., Rio de Janeiro, Brasil), a qual tem sido aplicada para controle de antracnose em diversas frutas no Brasil (BRAGA et al., 2019b; MACEDO et al., 2020), foi utilizada como fungicida padrão nos experimentos *in situ*. A formulação foi diluída em água destilada esterilizada (1:1000, v/v) para atingir a concentração de final de 0,1 g/L de trifloroxistrobina e 0,2 g/L de tebuconazol, de acordo com as instruções do fabricante.

3.4. MÉTODOS

3.4.1. Identificação dos constituintes do óleo essencial de *C. bonariensis*

Os constituintes do OECB foram identificados por cromatografia gasosa utilizando cromatógrafo gasoso acoplado a espectrômetro de massas (CGMS-QP2010 Ultra Shimadzu, Kyoto, Japão), utilizando as seguintes condições analíticas: coluna capilar RTX-5MS (proporções de 30 m x 0,25 mm x 0,25 µm); temperatura do programa: 60 a 240 ºC (3 ºC/min); temperatura do injetor: 250 ºC; temperatura do detector: 220 ºC; gás de transporte: hélio, com taxa de fluxo de 0,99 mL/min; impacto de elétrons: 70 eV; e faixa de massa (m/z): 40 a 500. A identificação de cada constituinte foi realizada comparando seus espectros de massa com as bibliotecas NIST/EPA/NIH Mass Spectral Database (Instituto Nacional de Padrões Tecnológicos, Norwalk, CT) e FFNSC1.3 (Sabor e Fragância Natural e Compostos Sintéticos), bem como com o índice de retenção linear. Por sua vez, a quantificação dos constituintes do OECB foi obtida após a normalização das áreas de cada constituinte identificado, sendo os resultados expressos como porcentagem da área (%) (BRAGA et al., 2019a)

3.4.2. Preparo da solução de goma arábica, emulsões de óleo essencial de *C. bonariensis* e revestimentos

Para obter a dispersão de GA, foi utilizado 0.1 g de para cada mL de água purificada, os quais foram dissolvidos com agitação (150 rpm) a 40 °C durante 60 minutos, sendo os materiais insolúveis removidos por filtração (ANDRADE et al., 2017).

As emulsões do OECB foram obtidas por dissolução da substância (1,6 µL/mL) em água destilada esterilizada (temperatura de aproximadamente 45 ± 1 ºC) contendo Tween 80 [1%, v/v] como agente de estabilização (BRAGA et al., 2019a), com diluições sucessivas (1:1) no mesmo meio para se obter soluções com diferentes concentrações (0,1; 0,2; 0,4; 0,6; 0,8; 1,0 µL/mL).

Para a aplicação combinada de GA e OECB na formulação dos revestimentos, inicialmente, a GA (0,1 g/mL) foi diluída em água purificada sob agitação (150 rpm) a 40 °C durante 60 minutos. Em seguida, diferentes quantidades do OECB, as quais foram suficientes para alcançar as concentrações escolhidas após obtenção dos resultados de inibição de crescimento micelial fúngico, foram adicionadas, seguindo-se por agitação (150 rpm) durante 18 horas sob temperatura ambiente. Glicerol (2,5 mL/100 mL) foi adicionado a dispersão como agente plastificante imediatamente após a adição do OECB na dispersão de GA (ANDRADE et al., 2017).

3.4.3. Avaliação dos efeitos de goma arábica e do óleo essencial de *C. bonariensis* sobre o crescimento micelial radial fúngico

A inibição provocada pela GA e OECB sobre o crescimento micelial dos isolados de *C. musae* foi determinada utilizando-se a técnica de diluição em meio sólido. Para isso, discos de 5 mm, retirados de culturas dos isolados de *C. musae* em BDA a 25 ± 0,5 °C com sete dias de idade, foram colocados no centro de uma placa de Petri contendo BDA + GA (0,1 g/mL) ou BDA + OECB (OECB: 0,1; 0,2; 0,4; 0,6; 0,8 ou 1 µL/mL). O crescimento micelial radial (mm) foi medido com um paquímetro considerando os diâmetros ortogonais das colônias por meio do uso de paquímetro, sendo a leitura realizada a cada 24 h durante sete dias ou até que as placas de Petri do experimento controle negativo estivessem totalmente cobertas com micélios fúngicos. O meio de crescimento (pH 5,6) sem GA ou OECB foi testado de forma semelhante como experimento controle.

A porcentagem de inibição do crescimento micelial fúngico (ICM%) foi calculada com a equação:

$$ICM\% = [(C - T) / C] \times 100 \qquad \text{(Eq. 1)}$$

onde C é o diâmetro da colônia no experimento controle e T é o diâmetro da colônia no experimento com BDA + GA ou OECB na concentração testada (BRAGA et al., 2019a).

As concentrações de OECB que causaram ICM% superiores a 40% foram selecionadas para uso posterior nos experimentos de avaliação dos efeitos de revestimentos com GA e OECB combinados sobre o desenvolvimento de lesões de antracnose em bananas.

3.4.4. Avaliação dos efeitos do óleo essencial de *C. bonariensis* sobre a integridade da membrana citoplasmática e atividade enzimática de *C. musae*

Para avaliar a resposta fisiológica de conídios de *C. musae* quando expostos ao OECB foi utilizada a técnica de citometria de fluxo multiparamétrica. Para isso, três sistemas foram testados para os isolados *C. musae* RP10 e MM5 isoladamente: (i) controle negativo (conídios suspensos em solução tampão fosfato (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); (ii) sistema teste (conídios expostos ao OECB em solução tampão fosfato); e (iii) controle não-marcado (PBS com solução de conídios sem os marcadores). As suspensões (10 mL) de conídios de *C. musae* (retirados de uma colônia cultivada em PDA durante sete dias a 25 ± 0,5 ºC, aproximadamente 10^6 conídios/mL, contagem de conídios padronizada com uso de hemocitômetro) em solução tampão fosfato foram expostas a concentração de 0,6 µL/mL de OECB por três e cinco dias (25 ± 0,5 °C). Após o período de incubação, os materiais dos diferentes sistemas foram centrifugados (4500 g x 10 min, 4 °C), os *pellets* obtidos foram lavados duas vezes e ressuspensos em solução tampão fosfato, e, imediatamente, marcados com iodeto de propídio (PI, Sigma-Aldrich, St. Louis, EUA) para avaliar a integridade da membrana citoplasmática e diacetato de fluoresceína (FDA; ThermoFisher Scientific, Molecular Probes, F1303) para avaliar alteração na atividade enzimática (KIM; KIM; KANG, 2017; CARRILLO; LABAJO, 2018; ALMEIDA et al., 2019).

3.4.5. Procedimento de coloração

Para avaliar a integridade de membrana, os *pellets* suspensos em solução tampão fosfato (100 µL) foram incubados por 30 minutos na presença de 0,1 µL de PI (1 mg/mL), resultando em concentração final de 1 µg/mL (KIM et al., 2017). Para avaliação da atividade enzimática, os *pellets* suspensos em solução tampão fosfato (200 µL) foram incubados por 30 minutos na presença de 0,1 µL de FDA (5 mg/mL), resultando em concentração final de 2,5 µg/mL (ALMEIDA et al., 2019). Após o período de incubação sob temperatura ambiente e abrigo de

luz, as amostras foram centrifugadas (4500 g x 10 min, 4 °C), lavadas em solução tampão fosfato e os *pellets* ressuspensos novamente em solução tampão fosfato (100 e 200 µL, respectivamente), seguindo-se por análise em citômetro de fluxo.

3.4.6. Análises de citometria de fluxo

As análises de citometria de fluxo foram realizadas com uso de citômetro de fluxo equipado com *laser* de íon argônio com emissão a 488 nm, possuindo dois detectores de dispersão de luz (FSC e SSC) e quatro detectores de fluorescência (BD Accuri C6, Becton Dicknson e Company, Franklin Lakes, New Jersey, EUA). Os sinais de fluorescência verde e vermelha foram coletados nos canais FL1 (533 nm ± 30 nm) e FL3 (> 670 nm). Os sinais de dispersão e fluorescência de conídios individuais que passaram pela zona do laser foram coletados como sinais logarítmicos. Os sinais de fluorescência foram coletados pelo filtro ótico no canal FL1 para FDA e FL3 para PI. O nível dos limiares (*threshold*) para aquisição de dados foi definido para FSC igual a 30000, com o intuito de eliminar ruídos ou partículas consideradas muito menores que os conídios intactos. Os conídios fúngicos foram identificados por parâmetros FSC/SSC. Cada aquisição de amostra foi operada em baixa taxa de fluxo (14 µL/minuto) e um total de 10000 eventos foram analisados. Todos os citogramas de emissões de fluorescência foram registrados usando o Software BD Accuri C6 (Becton Dicknson e Company). A análise de gráfico de pontos de FL1 versus FL3 foi empregada para medir as propriedades de fluorescência dos conídios. O fenótipo PI+ correspondeu a conídios corados com membranas citoplasmáticas danificadas; enquanto o fenótipo PI- correspondeu a conídios não corados com membranas citoplasmáticas intactas. A análise do gráfico de densidade de SSC versus FL1 foi utilizada para determinar as propriedades de fluorescência de FDA. O fenótipo FDA- correspondeu a conídios com atividade enzimática alterada, enquanto o fenótipo FDA+ correspondeu a conídios com atividade enzimática não alterada (ALMEIDA et al., 2019).

3.4.7. Avaliação dos efeitos dos revestimentos contendo goma arábica e óleo essencial de *C. banariensis* sobre o desenvolvimento de lesões de antracnose em bananas

A avaliação dos efeitos dos revestimentos formulados com GA e OECB na inibição do desenvolvimento da infecção (antracnose) causada por *C. musae* foi realizada utilizando-se dois ensaios *in situ* para simular efeitos preventivos e curativos. A superfície de cada banana foi

ferida (duas feridas por fruta) em sua região de extremidade com uma agulha esterilizada (3 mm de profundidade e 2 mm de largura), cuidadosamente imersa em 500 mL de dispersão formadora de revestimento com GA (0,1 g/mL) + OECB (0,4; 0,6; 0,8 ou 1 μL/mL) e a fruta girada suavemente dentro da imersão por 5 minutos com auxílio de bastão de vidro esterilizado. Um grupo de frutas foi revestido antes da contaminação artificial com o isolado testado para o ensaio preventivo, enquanto outro grupo de frutas foi revestido após contaminação artificial com o isolado testado para o ensaio curativo. Para cada isolado de *C. musae*, um plug de ágar (5 mm de diâmetro) com estruturas do patógeno obtido da margem de uma colônia cultivada por sete dias em BDA (25 ± 0,5 °C) foi inoculado no ponto ferido de cada fruta. As frutas foram imersas por um minuto na solução de fungicidas comercial (trifloroxistrobina: 100 g/L + tebuconazol: 200 g/L) como ensaio controle positivo. As frutas revestidas foram submetidas à secagem da superfície durante 2 horas sob temperatura ambiente (25 ± 0,5 °C, aproximadamente 85% de umidade relativa) em câmara de biossegurança.

As frutas revestidas foram cobertas individualmente com um saco plástico de polietileno com o intuito de evitar o contato direto entre diferentes frutas (unidades experimentais), colocadas em recipientes com papel toalha umedecido para gerar umidade relativa satisfatória e armazenadas (25 ± 0,5 °C). Após 24 horas, os sacos plásticos e toalhas de papel foram retirados (LIMA et al., 2015).

Nos dias 2, 3, 4 e 5 de armazenamento, o diâmetro das lesões de antracnose (mm) foram medidos com uso de paquímetro (média de duas medições diametralmente opostas). Os resultados foram expressos como porcentagem de redução do diâmetro da lesão de antracnose (%RDLA) determinada pela diferença do diâmetro da lesão nas frutas revestidas com GA + OECB ou tratadas com a formulação comercial de fungicidas quando comparados ao diâmetro da lesão em frutas não revestidas/não tratadas (controle negativo), o qual foi calculado com a equação:

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

onde N é o diâmetro da lesão de antracnose medido nas frutas relativas ao controle negativo e F é o diâmetro da lesão de antracnose medido nas frutas revestidas com GA + OECB ou tratadas com a solução comercial de fungicidas (OLIVEIRA et al., 2018).

3.4.8. Avaliação dos efeitos dos revestimentos de goma arábica e óleo essencial de *C. bonariensis* sobre parâmetros físico-químicos de bananas ao longo do armazenamento

Bananas que não foram contaminadas artificialmente com *C. musae* revestidas e não revestidas com as dispersões contendo GA e OECB foram avaliadas quanto à perda de peso, sólidos solúveis, acidez titulável, pH e cor logo após a aplicação do revestimento (dia zero) e nos dias 1, 3, 5, 7 e 9 de armazenamento (25 ± 0,5 °C).

Perda de peso: As bananas foram pesadas e, em seguida, armazenadas, de modo que permaneceram separadas para posterior acompanhamento da diferença de peso final durante os diferentes intervalos de tempo pré-estabelecidos. Os resultados foram expressos como porcentagem relativa ao peso inicial da fruta (BRAGA et al., 2020).

Sólidos Solúveis: Amostras da polpa das frutas foram trituradas com uso de processador doméstico (Philips Walita, São Paulo, Brasil) e a polpa foi analisada utilizando refratômetro digital Modelo digital Brix/RI Chek (Reichert Analytical Instruments, NY, EUA). Os resultados foram expressos como ºBrix (BRAGA et al., 2020).

Acidez Titulável (AT): A AT foi determinada em 10 mL da polpa da fruta homogeneizada em água destilada por meio de titulometria com NaOH 0,1 M na presença de fenolftaleína como indicador. A AT foi calculada com a fórmula:

$$AT\ (\%) = (C \times V2 \times K/\ V1) \times (V0/\ W) \times 100 \quad \text{(Eq. 3)}$$

onde C é a concentração padronizada de NaOH (0,1 M), P é o peso total da amostra (g), V2 é o volume de NaOH utilizado (mL), V1 é o volume de amostra utilizado (mL), V0 é o volume total de amostra (mL) e K é o fator de conversão de ácido. Os resultados foram expressos em gramas de ácido málico por 100 g de amostra de fruta (AOAC, 2016).

pH: Os valores de pH foram determinados em 10 mL da polpa da fruta homogeneizada em água destilada utilizando um potenciômetro com eletrodo de vidro combinado (Modelo Q400AS, São Paulo, Brasil), calibrado com solução tampão pH 7,0 e 4,0 (AOAC, 2016).

Cor: A cor da casca das frutas foi medida pelo sistema CIELab utilizando os seguintes parâmetros: L* (Luminosidade 0: escuro, 100: branco), a* (valor negativo: verde, valor positivo: vermelho) e b* (valor negativo: azul, valor positivo: amarelo) de acordo com a Comissão Internacional de Iluminação (CIE, 1986). Os valores de a* foram desconsiderados porque as bananas utilizadas nos experimentos não apresentavam mais tonalidades de cor verde no início do experimento. As medidas foram tomadas a partir de três posições equatoriais das frutas. Estas medidas foram realizadas usando colorímetro portátil Konica Minolta CR400 (Osaka, Japão) após a calibração com placa de porcelana (CR-A43) (BRAGA et al., 2020).

3.4.9. Análises estatísticas

Os ensaios para mensuração do crescimento micelial fúngico foram realizados em triplicata em três experimentos independentes. Os ensaios para avaliar os efeitos dos revestimentos sobre o desenvolvimento da lesão de antracnose foram realizados utilizando delineamento experimental inteiramente randomizado com quatro repetições para cada grupo experimental em dois experimentos independentes.

Foram realizadas análises descritivas (medidas de tendência central e distribuição de frequência) para obter a descrição das variáveis observadas. Posteriormente, foram realizados testes paramétricos (análise de variância – ANOVA seguida por teste de Tukey ou Teste t de *Student*) para determinação de diferenças estatisticamente significativas ($p \leq 0,05$). Para avaliar a severidade do desenvolvimento da antracnose em bananas foi realizada a análise da área abaixo da curva do progresso da doença (MACEDO et al., 2020). Os gráficos foram construídos utilizando o *software* GraphPad Prism versão 8. As análises estatísticas foram realizadas utilizando o *software* SISVAR versão 5.7 - DEX-UFLA (UFLA, Lavras, Minas Gerais, Brasil).

4. RESULTADOS

Os resultados desta tese serão apresentados na forma de artigos. O artigo apresentado no apêndice A intitulado "*An Analysis of the Published Literature on the Effects of Edible Coatings Formed by Polysaccharides and Essential Oils on Postharvest Microbial Control and Overall Quality of Fruit"* (https://doi.org/10.1111/1541-4337.12498) aborda os diversos revestimentos formulados com polissacarídeos e óleos essenciais e utilizados *in vitro* e *in situ* com foco em seus efeitos antimicrobianos quando aplicados em uma variedade de frutas. No total, 30 artigos publicados no período de 2010 e 2019 foram analisados e as informações organizadas considerando os tipos de materiais (utilizados na formulação dos revestimentos e frutas), as concentrações dos materiais utilizados nos revestimentos, os microrganismos alvo, os tipos de ensaio, os principais efeitos antimicrobianos (*in vitro* e *in situ*) e efeitos dos revestimentos sobre parâmetros físico-químicos e sensoriais relacionados à qualidade geral das frutas revestidas. A análise das informações contidas nestes estudos indicou que os revestimentos formulados com polissacarídeos (ou seja, quitosana - o único polissacarídeo usado como antimicrobiano, amido de mandioca, goma de linhaça, goma arábica, hidroxipropilmetilcelulose, goma de alfarroba,

goma de algaroba, pectina, pululano e alginato de sódio) e diferentes óleos essenciais (ou seus constituintes individuais) são eficazes para reduzir as perdas pós-colheita em frutas e, geralmente, não afetam negativamente suas características físico-químicas e sensoriais durante o armazenamento.

O artigo apresentado no apêndice B intitulado *"In vitro antifungal effects of Conyza bonariensis (L.) Cronquist essential oil on Colletotrichum musae and its incorporation in gum Arabic coating to control anthracnose in banana"* aborda o efeito do OECB na inibição do crescimento micelial de diferentes isolados de *C. musae* em meio de cultura laboratorial, as alterações causadas na membrana citoplasmática e atividade enzimática dos conídios fúngicos, bem como o efeito inibitório de revestimentos de GA + OECB sobre o desenvolvimento de lesões de antracnose em bananas contaminadas artificialmente com *C. musae* durante o armazenamento. Ainda, foram avaliados os efeitos da aplicação destes revestimentos sobre alguns aspectos físico-químicos indicadores da qualidade pós-colheita das frutas durante o armazenamento. O OECB (0,4 - 1 μL/mL) inibiu o crescimento micelial dos isolados de *C. musae* testados, enquanto GA (1 mg/mL) não apresentou efeito inibitório. A exposição ao OECB (0,6 μL/mL) resultou em danos na membrana citoplasmática e perda de atividade enzimática em elevado percentual de conídios de *C. musae*. Houve redução no desenvolvimento de lesões de antracnose em bananas artificialmente contaminadas com *C. musae* e revestidas com GA (0,1 mg/mL) e OECB (0,4 - 1 μL/mL) nos ensaios de efeito preventivo e curativo. Na maioria dos casos, os índices de severidade da doença encontrados para bananas revestidas com GA+OECB foram menores ou semelhantes aos encontrados para bananas tratadas com uma formulação comercial de fungicidas. Bananas revestidas com GA + OECB apresentaram menores alterações em parâmetros físico-químicos indicadores de qualidade durante o armazenamento, bem como de retardo na evolução do processo de amadurecimento e possibilidade de armazenamento mais prolongado quando comparadas com as frutas não revestidas. A aplicação de revestimentos formulados com GA + OECB pode ser considerada uma estratégia para retardar o desenvolvimento da antracnose e prolongar o tempo de armazenamento em bananas.

No apêndice C, é apresentado o pedido de depósito de patente junto ao Instituo Nacional de Propriedade Intelectual (INPI) intitulado "Revestimento a base de goma arábica e óleo essencial para proteção de frutos pós-colheita, processo e produto" (Pedido de depósito BRI 1020200131060), o qual foi derivado do desenvolvimento desta tese.

REFERÊNCIAS

AHMED, Z. F. R.; PALTA, J. P. Postharvest Dip Treatment with a Natural Lysophospholipid plus Soy Lecithin Extended the Shelf Life of Banana Fruit. **Postharvest Biology and Technology**, v. 113, p. 58–65, 2016.

ALMEIDA, E. T. da C. et al. *Mentha Piperita* L. Essential Oil Inactivates Spoilage Yeasts in Fruit Juices through the Perturbation of Different Physiological Functions in Yeast Cells. **Food Microbiology**, v. 82, p. 20–29, 2019.

ALVES, E. J. **A cultura da banana aspectos técnicos, socioeconômicos e agroindustriais.** Brasilia: Serviço de Produção de Informação, 1999.

AMORIM, L.; BERGAMIN FILHO, A.; REZENDE, J. A. M. **Manual de Fitopatologia**. 5. ed. [s.l.] Agronômica Ceres, 2018.

ANDRADE, S. C. A. et al. Control of Rhizopus Soft Rot and Quality Responses in Plums (*Prunus Domestica* L.) Coated with Gum Arabic, Oregano and Rosemary Essential Oils. **Journal of Food Processing and Preservation**, v. 41, n. 6, p. e13251, 2017.

AOAC — OFFICIAL METHODS OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. **Association of Official Analytical Chemist**. 20th edn ed. [s.l: s.n.]

ARAUJO, L. et al. Chemical Composition and Biological Activity of *Conyza Bonariensis* Essential Oil Collected in Mérida, Venezuela. **Natural Product Communications**, v. 8, n. 8, p. 1934578X1300800, 2013.

ARNON-RIPS, H.; POVERENOV, E. Improving Food Products' Quality and Storability by Using Layer by Layer Edible Coatings. **Trends in Food Science & Technology**, v. 75, p. 81–92, 2018.

ARX, J. A. von. **Revision der zu Gloeosporium gestellten pilze**. [s.l.] Verhandelingen der Koninklijke Nederlandsche Akademie van Wetenschappen. Afdeeling Natuurkunde. Tweede Sectie, 1957a. v. 51

ARX, J. A. von. Die Arten der Gattung Colletotrichum. **Phytopathologische Zeitschrift**, v. 29, n. 4, p. 413–468, 1957b.

ASSIS, O. B. G.; BRITTO, D. de. Revisão: coberturas comestíveis protetoras em frutas: fundamentos e aplicações. **Brazilian Journal of Food Technology**, v. 17, n. 2, p. 87–97, jun. 2014.

AURORE, G.; PARFAIT, B.; FAHRASMANE, L. Bananas, Raw Materials for Making Processed Food Products. **Trends in Food Science & Technology**, v. 20, n. 2, p. 78–91, fev. 2009.

BALDWIN, E. A. **Edible coatings and films to improve food quality, second edition.** Place of publication not identified: CRC Press, 2016.

BARBOSA, I. de M. et al. Evidence on the Induction of Viable but Non-Culturable State in *Listeria Monocytogenes* by *Origanum vulgare* L. and *Rosmarinus officinalis* L. Essential Oils in a Meat-Based Broth. **Innovative Food Science & Emerging Technologies**, v. 62, p. 102351, 2020.

BARBOSA, L. C. A. et al. Essential Oil Composition from Some Plant Parts of *Conyza Bonariensis* (L.) Cronquist. **Flavour and Fragrance Journal**, v. 20, n. 1, p. 39–41, jan. 2005.

BARROS, A. F. et al. Nematicidal Screening of Essential Oils and Potent Toxicity of *Dysphania Ambrosioides* Essential Oil against *Meloidogyne Incognita* in Vitro and in Vivo. **Journal of Phytopathology**, v. 167, n. 7–8, p. 380–389, 2019.

BAŞER, K. H. C.; BUCHBAUER, G. (ed.). **Handbook of essential oils: science, technology, and applications**. Third edition ed. Boca Raton: CRC Press, 2020.

BAXTER, A.; VAN DER WESTHUIZEN, G. C. A.; EICKER, A. A review of literature on the taxonomy, morphology and biology of the fungal gems Colletotrichum. **Phytophylactica**, v. 17, n. 1, p. 15–18, 1985.

BERKELEY, M. J. Notices of North American fungi (cont.). **Grevillea**, v. 2, n. 24, p. 177–181, 1874.

BHUIYAN, FarhanaR. et al. Characterizing Fruit Ripening in Plantain and Cavendish Bananas: A Proteomics Approach. **Journal of Proteomics**, v. 214, p. 103632, 2020.

BLAINSKI, É. et al. Eficiência do cloransulam-metílico no controle em pós-emergência de *Conyza bonariensis* na cultura da soja RR®. **Revista Brasileira de Herbicidas**, v. 14, n. 3, p. 235, 2015.

BORGES, A. L. **A cultura da banana.** Brasília, DF: Embrapa informação Tecnológica; Cruz das Almas: Embrapa Mandioca e Fruticultura Tropical, 2006.

BORGES, C. V. et al. Characterisation of Metabolic Profile of Banana Genotypes, Aiming at Biofortified Musa Spp. Cultivars. **Food Chemistry**, v. 145, p. 496–504, 2014.

BRAGA, S. dos P. et al. Application of Coatings Formed by Chitosan and Mentha Essential Oils to Control Anthracnose Caused by *Colletotrichum gloesporioides* and *C. brevisporum* in Papaya (*Carica papaya* L.) Fruit. **International Journal of Biological Macromolecules**, v. 139, p. 631–639, 2019a.

BRAGA, S. dos P. et al. Application of Coatings Formed by Chitosan and Mentha Essential Oils to Control Anthracnose Caused by *Colletotrichum Gloesporioides* and *C. Brevisporum* in Papaya (*Carica papaya* L.) Fruit. **International Journal of Biological Macromolecules**, v. 139, p. 631–639, 2019b.

BRAGA, S. dos P. et al. Characterization of Edible Coatings Formulated with Chitosan and Mentha Essential Oils and Their Use to Preserve Papaya (*Carica papaya* L.). **Innovative Food Science & Emerging Technologies**, v. 65, p. 102472, out. 2020.

BUKHARI, I. A. et al. Gut modulator effects of *Conyza bonariensis* explain its traditional use in constipation and diarrhea. **European Review for Medical and Pharmacological Sciences**, v. 17, n. 4, p. 552–558, 2013.

CAMPELO, M. E. da S. et al. Caracteriazação e aceitação sensorial de banana prata (*Musa paradisiaca*) produzida em sistema orgânico e convencional. **Brazilian Journal of Development**, v. 6, n. 9, p. 65623–65640, 2020.

CARRILLO, Martha. H.; LABAJO, V. A Case for teaching: descriptive modeling for supply chain information. In: 2018 IEEE World Engineering Education Conference (EDUNINE), Buenos Aires. **Anais**... In: 2018 IEEE WORLD ENGINEERING EDUCATION CONFERENCE (EDUNINE). Buenos Aires: IEEE, mar. 2018. Disponível em: <https://ieeexplore.ieee.org/document/8450963/>. Acesso em: 22 jan. 2021.

CIE — COMMISSION INTERNATIONALE DE L'ÉCLAIRAGE. **Colourimetry**. Vienna: CIE Publication, 1986. v. 2nd ed

COLTRO, L.; KARASKI, T. U. Environmental Indicators of Banana Production in Brazil: Cavendish and Prata Varieties. **Journal of Cleaner Production**, v. 207, p. 363–378, jan. 2019.

COOKE, M. C. New Australian fungi. **Grevillea**, v. 16, n. 77, p. 1–6, 1887.

CORDEIRO, Z. J. N. (Org.). **Banana: produção e aspectos técnicos.** Brasilia: Embrapa Comunicaco para Transferncia de Tecnologia, 2000.

CORDEIRO, Z. J. N. (Org.); MATOS, A. P. Doenças da banana. **Informe Agropecuário**, v. 26, n. 228, p. 12–16, 2005.

DA SILVA, I. A. et al. Mecanismos de resistência das plantas alimentícias não convencionais (PANC) e benefícios para a saúde humana. **Anais da Academia Pernambucana de Ciência Agronômica**, v. 15, n. 1, p. 77–91, 2018.

DE COSTA, D. M.; GUNAWARDHANA, H. M. D. M. Effects of Sodium Bicarbonate on Pathogenicity of *Colletotrichum Musae* and Potential for Controlling Postharvest Diseases of Banana. **Postharvest Biology and Technology**, v. 68, p. 54–63, jun. 2012.

DE OLIVEIRA, K. Á. R. et al. Synergistic Mixtures of Chitosan and *Mentha Piperita* L. Essential Oil to Inhibit *Colletotrichum Species* and Anthracnose Development in Mango Cultivar Tommy Atkins. **Food Microbiology**, v. 66, p. 96–103, set. 2017.

DE PAULA, C. C. et al. Antimicrobial screening of medicinal plants popularly used in Mato Grosso for treating infections: advances on the evaluation of *Conyza bonariensis* (L.) cronquist in vitro and in vivo antibacterial activities. **Pharmacognosy Journal**, v. 10, n. 6s, p. s152–s166, 12 nov. 2018.

DEAN, R. et al. The Top 10 Fungal Pathogens in Molecular Plant Pathology: Top 10 Fungal Pathogens. **Molecular Plant Pathology**, v. 13, n. 4, p. 414–430, maio 2012.

DELORENZI, J. C. M. O. B. et al. **Avaliação farmacológica e toxicológica de formulações contendo óleo essencial de *Conyza bonariensis***. [s.l: s.n.].

DENHAM, T. P. Origins of Agriculture at Kuk Swamp in the Highlands of New Guinea. **Science**, v. 301, n. 5630, p. 189–193, 2003.

EFSA PANEL ON FOOD ADDITIVES AND NUTRIENT SOURCES ADDED TO FOOD (ANS) et al. Re-evaluation of Acacia Gum (E 414) as a Food Additive. **EFSA Journal**, v. 15, n. 4, abr. 2017. Disponível em: <https://data.europa.eu/doi/10.2903/j.efsa.2017.4741>. Acesso em: 19 jun. 2021.

FAOSTAT – FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS STATISTICS. 2019. Disponível em: <http://faostat.fao.org/> Acesso em: 06 set. 2019.

FASOLIN, L. H. et al. Biscoitos Produzidos Com Farinha de Banana: Avaliações Química, Física e Sensorial. **Ciência e Tecnologia de Alimentos**, v. 27, n. 3, p. 524–529, 2007.

FISHER, M. C. et al. Worldwide Emergence of Resistance to Antifungal Drugs Challenges Human Health and Food Security. **Science**, v. 360, n. 6390, p. 739–742, 2018.

FORSTER, M. et al. Distribution of nutrients in edible banana pulp. v. 41, n. 2, p. 167–171, 2003.

FUNGO, R.; PILLAY, M. β-Carotene content of selected banana genotypes from Uganda. **African Journal of Biotechnology**, v. 10, n. 28, p. 5423–5430, 2013.

GASPAROTTO, L. et al. **Sigatoka-negra da bananeira.** Manaus: Embrapa Amazônia Ocidental, 2006.

GASPAROTTO, L. **A cultura da bananeira na região Norte do Brasil.** Brasília, DF: Embrapa Informação Tecnológica; Manaus: Embrapa Amazônia Ocidental, 2010.

GONZÁLEZ-MONTELONGO, R.; GLORIA LOBO, M.; GONZÁLEZ, M. Antioxidant Activity in Banana Peel Extracts: Testing Extraction Conditions and Related Bioactive Compounds. **Food Chemistry**, v. 119, n. 3, p. 1030–1039, 2010.

GOWEN, S. **Bananas and Plantains.** Dordrecht: Springer Netherlands, 1995.

GUERRA, I. C. D. et al. The Effects of Composite Coatings Containing Chitosan and *Mentha (Piperita* L. or x *Villosa* Huds) Essential Oil on Postharvest Mold Occurrence and Quality of Table Grape Cv. Isabella. **Innovative Food Science & Emerging Technologies**, v. 34, p. 112–121, abr. 2016.

HAILU, M.; WORKNEH, T. S.; BELEW, D. Review on postharvest technology of banana fruit. **African Journal of Biotechnology**, v. 12, n. 7, p. 635–647, 2013.

HASSAN, B. et al. Recent Advances on Polysaccharides, Lipids and Protein Based Edible Films and Coatings: A Review. **International Journal of Biological Macromolecules**, v. 109, p. 1095–1107, 2018.

HAWKINS, N. J.; FRAAIJE, B. A. Fitness Penalties in the Evolution of Fungicide Resistance. **Annual Review of Phytopathology**, v. 56, n. 1, p. 339–360, 2018.

HUANG, H. et al. The Combined Effects of Phenylurea and Gibberellins on Quality Maintenance and Shelf Life Extension of Banana Fruit during Storage. **Scientia Horticulturae**, v. 167, p. 36–42, 2014.

IBGE – INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. 2019. Disponível em: <http://www.ibge.gov.br/> Acesso em: 20 ago. 2019.

INDEX FUNGORUM. **Colletotrichum musae (Berk. & M.A. Curtis) Arx**, 2019. . Disponível em: <http://www.indexfungorum.org/names/NamesRecord.asp?RecordID=295348>. Acesso em: 10 out. 2019.

JONGEN, W. **Fruit and vegetable processing: improving quality**. Boca Raton, Fla: CRC, 2002.

JUGREET, B. S. et al. Chemistry, Bioactivities, Mode of Action and Industrial Applications of Essential Oils. **Trends in Food Science & Technology**, v. 101, p. 89–105, 2020.

KARUNANAYAKE, K. O. L. C. et al. Basil oil incorporated beeswax coating to increase shelf life and reduce anthracnose development in mango cv. Willard. **Ceylon Journal of Science**, v. 49, n. 5, p. 355, 2020.

KEVERS, C. et al. Evolution of Antioxidant Capacity during Storage of Selected Fruits and Vegetables. **Journal of Agricultural and Food Chemistry**, v. 55, n. 21, p. 8596–8603, 2007.

KHALIQ, G. et al. Influence of Gum Arabic Coating Enriched with Calcium Chloride on Physiological, Biochemical and Quality Responses of Mango (Mangifera Indica L.) Fruit Stored under Low Temperature Stress. **Postharvest Biology and Technology**, v. 111, p. 362–369, 2016.

KIM, D.-K.; KIM, S.-J.; KANG, D.-H. Bactericidal Effect of 266 to 279 Nm Wavelength UVC-LEDs for Inactivation of Gram Positive and Gram Negative Foodborne Pathogenic Bacteria and Yeasts. **Food Research International**, v. 97, p. 280–287, 2017.

KINUPP, V. F.; LORENZI, H. **Plantas alimentícias não convencionais (PANC) no Brasil: guia de identificação, aspectos nutricionais e receitas ilustradas**. Nova Odessa, SP, Brasil: Instituto Plantarum de Estudos da Flora Ltda, 2014.

LAGROUH, F.; DAKKA, N.; BAKRI, Y. The Antifungal Activity of Moroccan Plants and the Mechanism of Action of Secondary Metabolites from Plants. **Journal de Mycologie Médicale**, v. 27, n. 3, p. 303–311, 2017.

LIMA, N. B. et al. Comparative Epidemiology of Colletotrichum Species from Mango in Northeastern Brazil. **European Journal of Plant Pathology**, v. 141, n. 4, p. 679–688, 2015.

LINS, S. R. de O. et al. Controle alternativo da podridão peduncular em manga. **Summa Phytopathologica**, v. 37, n. 3, p. 121–126, 2011.

LOBO, M. G.; ROJAS, F. J. F. Biology and postharvest physiology of banana. In: SIDDIQ, M.; AHMED, J.; LOBO, M. G. (Ed.). **Handbook of Banana Production, Postharvest Science, Processing Technology, and Nutrition**. 1. ed. [s.l.] Wiley, 2020. p. 19–44.

LOMBARDO, P. et al. Effectiveness of Essential Oils for Postharvest Control of *Phyllosticta Citricarpa* (Citrus Black Spot) on Citrus Fruit. **Postharvest Biology and Technology**, v. 121, p. 1–8, 2016.

LÓPEZ, V. et al. Exploring Pharmacological Mechanisms of Lavender (*Lavandula angustifolia*) Essential Oil on Central Nervous System Targets. **Frontiers in Pharmacology**, v. 8, p. 280, 2017.

LORENZI, H. **Manual de identificação e controle de plantas daninhas: plantio direto e convencional**. Sétima edição [revisada e atualizada] ed. Nova Odessa, SP, Brasil: Instituto Plantarum de Estudos da Flora Ltda, 2014.

LOURA, D. et al. Germination Ecology of Hairy Fleabane (*Conyza bonariensis*) and Its Implications for Weed Management. **Weed Science**, v. 68, n. 4, p. 411–417, 2020.

MABROUK, S. et al. Chemical Composition of Essential Oils from Leaves, Stems, Flower Heads and Roots of *Conyza bonariensis* L. from Tunisia. **Natural Product Research**, v. 25, n. 1, p. 77–84, 2011.

MACEDO, S. A. et al. Combined Chitosan and *Cympobogon Citratus* (D.C. Ex Nees) Stapf. Essential Oil to Inhibit the Fungal Phytopathogen *Paramyrothecium Roridum* and Control Crater Rot in Melon (*Cucumis Melo* L.). **Brazilian Journal of Microbiology: [publication of the Brazilian Society for Microbiology]**, v. 51, n. 4, p. 2057–2065, 2020.

MADUWANTHI, S. D. T.; MARAPANA, R. Biochemical changes during ripening of banana: A review. **International Journal of Food Science and Nutrition ISSN: 2455-**, v. 2, n. 5, p. 166–169, 2017.

MAIA, J. G. S. et al. Composition of the Essential Oils of *Conyza bonariensis* (L.) Cronquist. **Journal of Essential Oil Research**, v. 14, n. 5, p. 325–326, 2002.

MANNOZZI, C. et al. Study on the Efficacy of Edible Coatings on Quality of Blueberry Fruits during Shelf-Life. **LWT - Food Science and Technology**, v. 85, p. 440–444, nov. 2017.

MAPA. **Sistemas de Agrotóxicos Fitossanitários**, 2019. . Disponível em: <http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Acesso em: 10 out. 2019.

MAQBOOL, M. et al. Postharvest Application of Gum Arabic and Essential Oils for Controlling Anthracnose and Quality of Banana and Papaya during Cold Storage. **Postharvest Biology and Technology**, v. 62, n. 1, p. 71–76, 2011.

MATHIAS, T. R. dos S. et al. Avaliação do comportamento reológico de diferentes iogurtes comerciais. **Brazilian Journal of Food Technology**, v. 16, n. 1, p. 12–20, 5 mar. 2013.

MATSUURA, F. C. A. U. **Banana pós-colheita.** Brasília, DF: Embrapa Informação Tecnológica; Cruz das Almas: Embrapa Mandioca e Fruticultura, 2001.

MUSSIN, J. E. et al. Inhibitory Activity of Conyza Bonariensis (L.) Cronquist Tincture against Fungi and Bacteria Causing Superficial Infections. **Revista Cubana de Plantas Medicinales**, v. 22, n. 3, 26 fev. 2017. Disponível em:

<http://revplantasmedicinales.sld.cu/index.php/pla/article/view/560>. Acesso em: 14 jun. 2021.

MUSTAFA, N. A. A.; BORDOH, P. K.; AHMAD, A. Dried Garcinia atroviridis crude extracts incorporated with gum arabic coating controlled postharvest anthracnose diseases in dragon fruits. **Tropical Agricultura Science**, v. 41, n. 4, p. 1643–1658, 2018.

NABI, S. U. et al. Post Harvest Diseases of Temperate Fruits and their Management Strategies-A Review. **International Journal of Pure & Applied Bioscience**, v. 5, n. 3, p. 885–898, 20 2017.

NAWAB, A.; ALAM, F.; HASNAIN, A. Mango Kernel Starch as a Novel Edible Coating for Enhancing Shelf- Life of Tomato (*Solanum lycopersicum*) Fruit. **International Journal of Biological Macromolecules**, v. 103, p. 581–586, 2017.

NEGREIROS, R. J. Z. de et al. Controle da antracnose na pós-colheita de bananas-'prata' com produtos alternativos aos agrotóxicos convencionais. **Revista Brasileira de Fruticultura**, v. 35, n. 1, p. 51–58, 2013.

OLIVEIRA, S. M. A. de. **Patologia pós-colheita frutas, olerícolas e ornamentais tropicais.** Brasilia, DF: Embrapa Informação Tecnológica; Fortaleza: Embrapa Agroindústria Tropical, 2006.

OLIVEIRA, P. D. L. et al. Control of Anthracnose Caused by Colletotrichum Species in Guava, Mango and Papaya Using Synergistic Combinations of Chitosan and *Cymbopogon Citratus* (D.C. Ex Nees) Stapf. Essential Oil. **International Journal of Food Microbiology**, v. 266, p. 87–94, 2018.

PATEL, S.; GOYAL, A. Applications of Natural Polymer Gum Arabic: A Review. **International Journal of Food Properties**, v. 18, n. 5, p. 986–998, 4 maio 2015.

PATRA, J. K.; KIM, S. H.; BAEK, K.-H. Antioxidant and Free Radical-Scavenging Potential of Essential Oil from *E Nteromorpha Linza* L. Prepared by Microwave-Assisted Hydrodistillation: Antioxidant Potential of Essential Oil from *Enteromorpha Linza*. **Journal of Food Biochemistry**, v. 39, n. 1, p. 80–90, 2015.

PAVELA, R.; SEDLÁK, P. Post-Application Temperature as a Factor Influencing the Insecticidal Activity of Essential Oil from Thymus Vulgaris. **Industrial Crops and Products**, v. 113, p. 46–49, 2018.

PLOETZ, R. C. (ed.). **Diseases of tropical fruit crops**. Wallingford, Oxon, UK ; Cambridge, MA, USA: CABI Pub, 2003.

POURGHANBARI, G. et al. Antiviral Activity of the Oseltamivir and *Melissa officinalis* L. Essential Oil against Avian Influenza A Virus (H9N2). **VirusDisease**, v. 27, n. 2, p. 170–178, jun. 2016.

PRILL, M. A. de S. et al. Métodos para a climatização de bananas "Prata-Anã" produzidas na Amazônia Setentrional Brasileira. **Revista Brasileira de Fruticultura**, v. 34, n. 4, p. 1030–1042, 2012.

QAMAR, S.; SHAIKH, A. Therapeutic Potentials and Compositional Changes of Valuable Compounds from Banana - A Review. **Trends in Food Science & Technology**, v. 79, p. 1–9, 2018.

RANASINGHE, L. S.; JAYAWARDENA, B.; ABEYWICKRAMA, K. Use of Waste Generated from Cinnamon Bark Oil (*Cinnamomum zeylanicum* Blume) Extraction as a Post Harvest Treatment for Embul Banana. **Journal of Food, Agriculture and Environment**, v. 1, n. 2, p. 340–344, 2003.

RIVERO, J. et al. In Vitro Evaluation of Oestrogenic/Androgenic Activity of the Serum Organochlorine Pesticide Mixtures Previously Described in a Breast Cancer Case–Control Study. **Science of The Total Environment**, v. 537, p. 197–202, 2015.

SALOMÃO, L. C. C.; SIQUEIRA, D. L. de. **Cultivo da Bananeira**. [s.l.] Editora UFV, 2015.

SHAH, N. Z. et al. Antimicrobial and phytotoxic properties of *Conyza bonariensis*. **Pharmacy and Pharmacology Research**, v. 1, n. 1, p. 8–11, 2013.

SHIRWAIKAR, A. et al. Herbal Excipients in Novel Drug Delivery Systems. **Indian Journal of Pharmaceutical Sciences**, v. 70, n. 4, p. 415, 2008.

SIDHU, J. S.; ZAFAR, T. A. Bioactive Compounds in Banana Fruits and Their Health Benefits. **Food Quality and Safety**, v. 2, n. 4, p. 183–188, 2018.

SILVA, S. de O. e et al. Melhoramento genético da bananeira: estratégias e tecnologias disponíveis. **Revista Brasileira de Fruticultura**, v. 35, n. 3, p. 919–931, 2013.

SILVA, L. M. E. et al. Temporal progress and control of anthracnose on banana in semiarid in the north of Mina Gerais. **Revista Brasileira de Fruticultura**, v. 38, n. 1, p. 81–91, 2016.

SILVA-VERA, W. et al. Study of Spray System Applications of Edible Coating Suspensions Based on Hydrocolloids Containing Cellulose Nanofibers on Grape Surface (Vitis Vinifera L.). **Food and Bioprocess Technology**, v. 11, n. 8, p. 1575–1585, 2018.

SILVESTRE, W. P. et al. Pervaporation in the Separation of Essential Oil Components: A Review. **Trends in Food Science & Technology**, v. 93, p. 42–52, 2019.

SIMÕES, C. M. O.; SCHENKEL, E. P.; MELLO, J. C. P. de. **Farmacognosia: do produto natural ao medicamento**. Porto Alegre, RS: Artmed, 2017.

SINGH, B. et al. Bioactive Compounds in Banana and Their Associated Health Benefits – A Review. **Food Chemistry**, v. 206, p. 1–11, 2016.

SIRIWARDANA, H. et al. Control of Postharvest Crown Rot Disease in Cavendish Banana with Aluminium Sulfate and Vacuum Packaging. **Journal of Agricultural Sciences**, v. 12, n. 3, p. 162, 1 2017.

SIVAKUMAR, D.; BAUTISTA-BAÑOS, S. A Review on the Use of Essential Oils for Postharvest Decay Control and Maintenance of Fruit Quality during Storage. **Crop Protection**, v. 64, p. 27–37, o2014.

SMERIGLIO, A. et al. Essential Oil of *Citrus lumia* Risso: Phytochemical Profile, Antioxidant Properties and Activity on the Central Nervous System. **Food and Chemical Toxicology**, v. 119, p. 407–416, 2018.

SOUZA, E. L. et al. An Analysis of the Published Literature on the Effects of Edible Coatings Formed by Polysaccharides and Essential Oils on Postharvest Microbial Control and Overall Quality of Fruit. **Comprehensive Reviews in Food Science and Food Safety**, v. 18, n. 6, p. 1947–1967, 2019.

SPAGNOL, W. A. et al. Redução de perdas nas cadeias de frutas e hortaliças pela análise da vida útil dinâmica. **Brazilian Journal of Food Technology**, v. 21, n. 0, 5 fev. 2018. Disponível em: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1981-67232018000100303&lng=pt&tlng=pt>. Acesso em: 18 set. 2019.

SU, Y.-Y. et al. Epitypification of *Colletotrichum musae*, the Causative Agent of Banana Anthracnose. **Mycoscience**, v. 52, n. 6, p. 376–382, 2011.

SUTTON, B. C. **The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata**. 1980 [s.l: s.n.]

TACO - **Tabela brasileira de Composição de Alimentos**. NEPA. UNICAMP. 4ª edição. rev. e ampl. NEPA- UNICAMP. 2011.

TAHIR, H. E. et al. Quality and Postharvest-Shelf Life of Cold-Stored Strawberry Fruit as Affected by Gum Arabic (*Acacia senegal*) Edible Coating. **Journal of Food Biochemistry**, v. 42, n. 3, p. e12527, 2018.

TAJKARIMI, M. M.; IBRAHIM, S. A.; CLIVER, D. O. Antimicrobial Herb and Spice Compounds in Food. **Food Control**, v. 21, n. 9, p. 1199–1218, 2010.

THABIT, R. A. S. et al. Antioxidant and *Conyza bonariensis*: A Review. **European Academic Research**, v. 2, n. 6, p. 8454–8474, 2014.

THABIT, R. A. S. et al. Antioxidant and Antibacterial Activities of Extracts from *Conyza Bonariensis* Growing in Yemen. **Pakistan Journal of Pharmaceutical Sciences**, v. 28, n. 1, p. 129–134, 2015.

THAKUR, R. et al. A Starch Edible Surface Coating Delays Banana Fruit Ripening. **LWT**, v. 100, p. 341–347, 2019.

THANGAMANI, P. et al. Morphological and physiological characterization of *Colletotrichum musae* the causal organism of banana anthracnose. **World Journal of Agricultural Sciences**, v. 7, n. 6, p. 743–754, 2011.

TIAN, J. et al. The Mechanism of Antifungal Action of Essential Oil from Dill (*Anethum Graveolens* L.) on *Aspergillus Flavus*. **PLoS ONE**, v. 7, n. 1, p. e30147, 2012.

TUREK, C.; STINTZING, F. C. Stability of Essential Oils: A Review: Stability of Essential Oils…. **Comprehensive Reviews in Food Science and Food Safety**, v. 12, n. 1, p. 40–53, 2013.

TURHAN, K. N. IS EDIBLE COATING AN ALTERNATIVE TO MAP FOR FRESH AND MINIMALLY PROCESSED FRUITS? **Acta Horticulturae**, n. 876, p. 299–305, 2010.

ULLAH, A. et al. Influence of Edible Coatings on Biochemical Fruit Quality and Storage Life of Bell Pepper Cv. “Yolo Wonder”. **Journal of Food Quality**, v. 2017, p. 1–11, 2017.

VENTURA, J. A.; HINZ, R. H. Controle de doenças da bananeira. In: ZAMBOLIM, L. et al. (Ed.). **Controle de doenças de plantas: fruteiras**. 2002 [s.l: s.n.]1p. 839–938.

VIEIRA, W. A. dos S. et al. Thiophanate-Methyl Resistance and Itness Components of *Colletotrichum Musae* Isolates from Banana in Brazil. **Plant Disease**, v. 101, n. 9, p. 1659–1665, 2017.

VIEIRA, W. A. S. et al. Endophytic Species of *Colletotrichum* Associated with Mango in Northeastern Brazil. **Fungal Diversity**, v. 67, n. 1, p. 181–202, 2014.

VIVIANI, L.; LEAL, P. M. Qualidade pós-colheita de banana Prata Anã armazenada sob diferentes condições. **Revista Brasileira de Fruticultura**, v. 29, n. 3, p. 465–470, 2007.

YAHIA, E. M.; CARRILLO-LÓPEZ, A. (ed.). **Postharvest physiology and biochemistry of fruits and vegetables**. Duxford: Woodhead Publishing, 2019.

YANG, Z. et al. Improved Postharvest Quality of Cold Stored Blueberry by Edible Coating Based on Composite Gum Arabic/Roselle Extract. **Food and Bioprocess Technology**, v. 12, n. 9, p. 1537–1547, 2019.

YE, A. et al. Temperature-Dependent Complexation between Sodium Caseinate and Gum Arabic. **Food Hydrocolloids**, v. 26, n. 1, p. 82–88, j2012.

YU, C.; ZHANG, J.; WANG, T. Star Anise Essential Oil : chemical Compounds, Antifungal and Antioxidant Activities: A Review. **Journal of Essential Oil Research**, v. 33, n. 1, p. 1–22, 2021.

ZAPPI, D. C. et al. Growing knowledge: an overview of Seed Plant diversity in Brazil. **Rodriguésia**, v. 66, n. 4, p. 1085–1113, 2015.

ZARITZKY, N. Edible Coatings to Improve Food Quality and Safety. In: AGUILERA, J. M. et al. (Ed.). **Food Engineering Interfaces**. New York, NY: Springer New York, 2010. p. 631–659.

ZHANG, P. et al. Banana Starch: Production, Physicochemical Properties, and Digestibility—a Review. **Carbohydrate Polymers**, v. 59, n. 4, p. 443–458, 2005.

ZHANG, W. et al. Different Molecular Weights Chitosan Coatings Delay the Senescence of Postharvest Nectarine Fruit in Relation to Changes of Redox State and Respiratory Pathway Metabolism. **Food Chemistry**, v. 289, p. 160–168, 2019.

APÊNDICES

4.1. APÊNDICE A – ARTIGO I

AN ANALYSIS OF THE PUBLISHED LITERATURE ON THE EFFECTS OF EDIBLE COATINGS FORMED BY POLYSACCHARIDES AND ESSENTIAL OILS ON POSTHARVEST MICROBIAL CONTROL AND OVERALL QUALITY OF FRUIT

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An Analysis of the Published Literature on the Effects of Edible Coatings Formed by Polysaccharides and Essential Oils on Postharvest Microbial Control and Overall Quality of Fruit

Evandro L. de Souza, Giovanna A. Lundgren, Kataryne Á. R. de Oliveira, Lúcia R. R. Berger, and Marciane Magnani

Abstract: Consumers have shown increased concern about the importance of adopting regular fresh fruit consumption. Because fresh fruit are highly susceptible to postharvest decay, several studies have focused on the development of alternative technologies to extend their market period. The application of polysaccharides in combination with essential oils (EOs) to formulate edible coatings has been considered an innovative strategy to reduce postharvest losses in fruit. However, available studies have used different methodological procedures related to the production and application of these coatings on fruit, which could be potential influential factors on the achievement of the desired effects in coated fruit. This review summarized the studies focusing on the application of edible coatings formed by polysaccharides and EOs to preserve fruit, in addition to examine and discuss possible factors affecting their functionalities. The approach given in this review envisages to contribute to research in edible coatings formed by polysaccharides and EOs and help to their optimized application as a postharvest treatment of fruit. Despite of the different methods selected for use in experimental assays, data of available literature demonstrate that coatings formed by polysaccharides (that is, chitosan—the only polysaccharide used as an antimicrobial, cassava starch, flaxseed gum, gum arabic, hydroxypropylmethylcellulose, locust bean gum, mesquite gum, pectin, pullulan, and sodium alginate) and different EOs (or their individual constituents) are effective to reduce postharvest losses in fruit and generally do not adversely affected their physicochemical and sensory characteristics during storage.

Keywords: coating, fruit decay, plant compounds, polysaccharides, postharvest treatment

Introduction

Consumers have shown increasing concern on the importance of adopting a healthy diet with regular fresh fruit consumption. Fruit are rich sources of a variety of nutrients and bioactive compounds capable of exerting health-promoting effects (Septembre-Malaterre, Remize, & Poucheret, 2018). However, an important problem of fresh fruit is their short shelf life mostly because microbial infection and decay (Poverenov et al., 2014).

Traditional strategies to control postharvest decay in fruit have included the use of chemical fungicides, but fungal strains resistant to these treatments have emerged (Guerra et al., 2015). The use of some chemicals to control fungal infection and decay of fruit (for example, methyl bromide, iprodione, and dichloran) has been banned in many countries because of their potential negative impacts on environment and human health (Aloui et al., 2014). Other postharvest techniques, such as microwave, heating, ozonation, and controlled/modified atmosphere packaging, have been proposed as alternatives to replace the treatment of fruit with chemical fungicides. However, their negative impacts on fruit quality characteristics are considered drawbacks for a practical use (Aloui et al., 2014; Botondi, Moscetti, & Massantini, 2016; Castellanos, Mendoza, Gavara, & Herrera, 2017; Rodriguez & Zoffoli, 2016).

A number of studies have been performed to develop safe, environmentally friendly, and effective technologies to preserve the postharvest quality of fruit. Naturally occurring substances with recognized antimicrobial and antioxidant properties have been considered to use in the formulation of edible coatings capable of extending the market period of fruit (dos Santos et al., 2012; Guerra et al., 2015). Edible coatings are thin layers composed mainly by polysaccharides, proteins, and lipids that are formed directly on the fruit surface (Azarakhsh, Osman, Ghazali, Tan, & Mohd Adzahan, 2014; Guerra et al., 2015). This preservation technique plays a dual function in fruit: (i) it controls the growth

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of spoilage and pathogenic microorganism due to the common presence of antimicrobial substances in edible coatings composition; and (ii) it controls the occurrence of undesirable physical and biochemical alterations in fruit because the reduction in water loss and respiration rate (RR; Guerra et al., 2015; Maqbool et al., 2011; Sánchez-González et al., 2011). Edible coatings can also improve mechanical and textural properties of fruit, as well as prevent flavor loss (Azarakhsh et al., 2014; de Aquino, Blank, & de Aquino Santana, 2015; Maqbool et al., 2011).

Considerable attention has been directed to the use of polysaccharides and essential oils (EOs) in the formulation of edible coatings to be used on fruit (Aloui et al., 2014; Azarakhsh et al., 2014; dos Santos et al., 2012; Sánchez-González et al., 2011; Shao et al., 2015; Xing et al., 2015). EOs are generally recognized as safe and present the feature of low resistance inductive effects in a range of microorganisms (Anderson, 1986; Burt, 2004; Calame, Weseler, Viebke, Flynn, & Siemensma, 2008; Kean & Thanou, 2010).

Polysaccharides ordinarily provide transparent and homogenous edible coatings with moderate mechanical properties, but the application of these materials alone on fruit is commonly limited by their poor water solubility and water vapor permeability. To solve these shortcomings, studies have proposed the use of EOs (as hydrophobic substances) for the formulation of polysaccharides-based coatings (Campos, Gerschenson, & Flores, 2011). EOs in edible coatings are continuously released on fruit surface, maintaining a suitable concentration of the antimicrobial components over time and, consequently, extending the fruit shelf life (Suhr & Nielsen, 2003; Tian et al., 2015; Wilkinson & Cavanagh, 2005; Xing et al., 2015). However, the available studies that assessed the effects of coatings formulated with polysaccharides and EOs on postharvest microbial control and overall quality of fruit have used different methodological approaches for their production and application, which should be potential factors to influence the achievement of the desired effects in fruit.

Considering these aspects, this review summarizes and discusses the studies focusing on the application of edible coatings comprising polysaccharides and EOs to preserve distinct fruit, as well as the possible factors affecting the antimicrobial efficacy of these coatings and their properties to preserve the physicochemical characteristics in fruit. The information presented in this review envisages to contribute to the research in edible coatings comprising polysaccharides and EOs and help to their optimized application as a postharvest treatment of fresh fruit.

Methods

PubMed and Scopus database were searched for English-language articles published during the period 2010 to 2019, using keyword combinations as search strategy. Keywords included "coating," "edible films," "composition," "essential oil," "phytoconstituents," "antimicrobial activity," "antifungal," "antibacterial," "postharvest diseases," "microbiological safety," "effects," "effectiveness," "application," "fruit," "vegetable," "quality standards," "sensory aspects," and "nutritional aspects." A total of 30 articles were selected from this search.

This review was limited to search original articles evaluating the efficacy of edible coatings formed by polysaccharides and EOs to preserve fresh fruit. The researches that used edible coatings formed by polysaccharides and individual constituents (ICs) present in EOs were also evaluated because these compounds typically present antimicrobial properties similar to the EO. Different studies were included approaching (i) the antimicrobial activity and the effects of edible coatings on microbial spoilage and infection when applied on fruit; and (ii) the influence of edible coatings on physicochemical and sensory parameters of coated fruit without antimicrobial analyses. Original articles using only *in vitro* assays against spoilage and pathogenic microorganisms were not included. Studies that used proteins (for example, gelatin, casein, zein, and soy protein) and lipids (for example, carnauba wax and beeswax) as base materials for edible coatings production were also excluded.

Methodological aspects regarding the production of coatings formed by polysaccharides and EOs or ICs, such as (i) type and characteristics of the polysaccharide and EO or IC, and (ii) the concentrations of these substances/compounds and their combined use to formulate the coatings, were described. Additionally, the methodological aspects employed for the production and application of edible coatings on fruit were presented and discussed.

Considering the application of the edible coatings on fruit, the pathogen microorganisms used in the antimicrobial assays *in situ*, the main effects against these microorganisms, and the influence of the edible coatings on overall quality parameters of coated fruit were evaluated, to cite: pH, titratable acidity, total soluble solids (TSS), color, firmness, weight loss, RR, phenolic compounds, ascorbic acid, enzymatic activity, and sensory characteristics.

Results

Considering the established criteria, the present review identified a total of 30 studies (articles) published during the period 2010 to 2019, which examined either the efficacy of edible coatings formed by polysaccharides and EOs or ICs to avoid the development of postharvest infection and microbial decay in fruit, as well as to maintain or improve physicochemical and sensory parameters of fresh fruit.

Thereby, to evaluate each of the retrieved studies, this review organized the information considering: (i) the materials, such as the type of coating materials and fruit, as presented in Table 1; (ii) the concentration of coating materials, the target microorganism, the type of assays performed, and the main antimicrobial effects exerted by the materials used to prepare the coatings (*in vitro* assays) and/or by formulated coatings when applied on fruit (*in situ* assays), as presented in Table 2; and (iii) the effects of the formulated coatings on physicochemical and sensory parameters related to the overall quality of coated fruit, as presented in Table 3.

Edible coatings characteristics

Coating-base material: Polysaccharides. Chitosan (CH) was identified as the polysaccharide most commonly used to formulate edible coatings, being included in 22 studies. Two of these studies evaluated the influence of different concentrations of CH and cassava starch (CS) on the antimicrobial properties of the formulated edible coatings through a response surface methodology (Azevedo et al., 2014; de Aquino et al., 2015). Ten studies used only CH as a coating-base material (Table 1), and two studies compared the application of CH with other polymeric matrices, that is, locust bean gum (Aloui et al., 2014) and hydroxypropylmethylcellulose (Sánchez-González et al., 2011), using *in vitro* and/or *in situ* assays.

Most of the retrieved studies used commercial crustacean CH, with the exception of three studies that used fungal CH from *Mucor circinelloides* and *Cunninghamella elegans* (Mucorales order, Zygomycetes; de Oliveira et al., 2014a, 2014b; de Souza et al., 2015), and two studies that used crustacean CH extracted in laboratory from shells of the shrimp *Litopenaeus vannamei* Boone (Guerra et al., 2015, 2016). CH used in most of these studies had similar

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Table 1–Studies assessing the effects of coatings comprising polysaccharides and essential oils or their individual constituents plant compounds on postharvest microbial control and quality of fruit.

Number of articles	Coating-base materials		Fruit	Reference
	Polysaccharide	Essential oil/individual constituent		
1	Gum arabic (GA)	Cinnamon essential oil	Guava	Etemadipoor et al. (2019)
2	Chitosan (CH) (deacetylation degree [DD] 75% to 85%), medium molecular weight (MW)	Lemongrass (*Cymbopogon citratus* (D.C.) Stapf.)	Guava, mango, and papaya	Oliveira et al. (2018)
3	Gum arabic (density 0.424 g/cm^3)	Oregano (*Origanum vulgare* L.) Rosemary (*Rosmarinus officinalis* L.)	Plum	Andrade et al. (2017)
4	CH (DD 75% to 85%, medium MW)	Peppermint (*Mentha piperita* L.)	Mango	de Oliveira et al. (2017)
5	Flaxseed gum	Lemongrass	Pomegranate arils	Yousuf and Srivastava (2017)
6	CH (DD 75% to 85%, medium MW)	Lemongrass	Tomato	Athayde et al. (2016)
7	CH (DD 75% to 85%, medium MW)	Oregano	Tomato	Barreto et al. (2016)
8	Shrimp CH (DD 83%)	Peppermint, *Mentha* × *villosa* Huds	Table grape cv. Isabella	Guerra et al. (2016)
9	Pectin	Oregano (*Lippia graveolens*)	Tomatoes	Rodriguez-Garcia et al. (2016)
10	CH (DD ≥95%)	Cinnamon (*Cinnamomum zeylanicum* Blume)	"Brooks" sweet cherry	Xing et al. (2016)
11	Shrimp CH (DD 83%)	Peppermint, *M. villosa*	Tomato	Guerra et al. (2015)
12	Pectin Sodium alginate	Citral and eugenol	Raspberry	Guerreiro et al. (2015)
13	CH (DD 85.9%) + Cassava starch (CS)	Lippia gracilis Schauer	Guava	de Aquino et al. (2015)
14	Fungal CH from *Mucor circinelloides* (DD 82%)	Carvacrol	Tomato	de Souza et al. (2015)
15	CH (DD 95%)	Clove (*Syzygium aromaticum* (L.) Merr.)	Satsuma mandarin	Shao et al. (2015)
16	CH (DD ≥95%)	Cinnamon	Lingwu jujube	Xing et al. (2015)
17	CH (DD >75%, MW approximately 150,000 Da) Locust bean gum (MW approximately 310,000 Da)	Bergamot (*Citrus bergamia*), bitter orange (*C. aurantium*), sweet orange (*C. sinensis* (L.) Osbeck), mandarin (*C. reticulata*), and lemon (*C. lemon*)	Tunisian date	Aloui et al. (2014)
18	CH (DD 85.9%) + CS	Lippia gracilis Schauer	Strawberry	Azevedo et al. (2014)
19	Fungal CH from *Cunninghamella elegans* (DD 81%)	Not used	Table grape	de Oliveira et al. (2014a)
20	Fungal CH from *M. circinelloides* (DD 82%)	Not used	Table grape	de Oliveira et al. (2014b)
21	CH (DD 75% to 85%, medium MW)	Carvacrol, cinnamaldehyde, eugenol, *trans*-cinnamaldehyde, and eucalyptol	Blueberry	Sun et al. (2014)
22	CH (DD 82.7%, high MW)	Bergamot, thyme, and tea tree (*Melaleuca alternifolia*)	Orange	Cháfer et al. (2012)
23	CH (DD 75% to 85%, medium MW)	Oregano	Table grape	dos Santos et al. (2012)
24	CH (DD 75.6%, high MW)	Lemon	Strawberry	Perdones et al. (2012)
25	Pullulan	Thymol	Apple and mandarin	Gniewosz and Synowiec (2011)
26	GA	Lemongrass and cinnamon	Banana and papaya	Maqbool et al. (2011)
27	CH (DD 82.7%) and hydroxypropylmethylcellulose (high MW)	Bergamot	Table grape	Sánchez-González et al., 2011
28	CH (DD 85% to 89%)	Oregano, red thyme (*Thymus vulgare* L.), peppermint, lemongrass, and limonene	Strawberry	Vu et al. (2011)
29	CH (DD ≥95%)	Cinnamon	Sweet pepper	Xing et al. (2011)
30	Mesquite gum	Thyme, Mexican lime (*Citrus aurantiifolia* (Christ.) Swingle)	Papaya	Bosquez-Molina et al. (2010)

deacetylation degree (that is, ≥75%). Seven studies used medium molecular weight (MW) CH (Aloui et al., 2014; Athayde et al., 2016; Barreto et al., 2016; de Oliveira, Berger, de Araújo, Câmara, & de Souza, 2017; dos Santos et al., 2012; Oliveira, de Oliveira, Vieira, Câmara, & de Souza, 2018; Sun et al., 2014) and two studies used high MW CH (Cháfer, Sánchez-González, González-Martínez, & Chiralt, 2012; Perdones, Sánchez-González, Chiralt, & Vargas, 2012). Eight studies did not cite the MW of tested CH (Azevedo et al., 2014; de Aquino et al., 2015; Sánchez-González et al., 2011; Shao et al., 2015; Vu, Hollingsworth, Leroux, Salmieri, & Lacroix, 2011; Xing et al., 2011; Xing et al., 2015, 2016). CH was the only polysaccharide used as an antimicrobial ingredient to formulate the edible coatings in retrieved studies.

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Table 2–Studies assessing the *in vitro* and/or *in situ* (in fruit) antimicrobial effects of coatings comprising polysaccharides and essential oils or their individual constituents and main observed effects.

Coating material					
Polysaccharide(s) and tested concentration(s)	Essential oil (EO) or individual constituents and tested concentration(s)	Target microorganism(s)	Assays	Main effects	Reference
Chitosan (CH) (2.5 to 7.5 mg/mL)	Lemongrass (0.15 to 2.5 μL/mL)	*Colletotrichum asianum*, *C. siamense*, *C. fructicola*, *C. karstii*, and *C. tropicale*	*In vitro:* Mycelial growth measurement *In situ:* Lesion development in fruit	*In vitro:* The lowest concentrations achieving synergistic effects against most of the tested *Colletotrichum* species were 5 mg/mL CH + 0.15, 0.3, or 0.6 μL/mL lemongrass EO *In situ:* Coating comprising 5 mg/mL CH + 0.6 μL/mL lemongrass EO caused the largest reductions in anthracnose lesions development in fruit. CH + lemongrass EO coating controlled fungal growth in fruit up to the 6th or 9th day of storage	Oliveira et al. (2018)
Gum arabic (GA) (1 mg/mL)	Oregano (0.25 μL/mL), oregano (0.06 to 0.12 μL/mL) + rosemary (0.25 or 0.5 μL/mL)	*Rhizopus stolonifer*	*In vitro:* Macrodilution in broth and mycelial growth measurement *In situ:* Fungal infection	*In vitro*: GA (1 mg/mL) and oregano EO alone (0.25 μL/mL) or oregano EO (0.12 and 0.6 μL/mL) + rosemary EO (0.5 μL/mL) inhibited mycelial growth *In situ*: Coatings comprising GA + oregano EO alone or GA + oregano EO + rosemary EO controlled Rhizopus soft rot in plums	Andrade et al. (2017)
CH (2.5 to 10 mg/mL)	Peppermint (0.3 to 5 μL/mL)	*C. asianum*, *C. dianesei*, *C. fructicola*, *C. tropicale*, and *C. karstii*	*In vitro:* Mycelial growth *In situ:* Lesion development in fruit	*In vitro*: All tested CH + peppermint EO mixtures presented additive or synergistic effects on tested *Colletotrichum* species *In situ*: All tested CH + peppermint EO mixtures decreased the anthracnose severity in fruit	de Oliveira et al. (2017)
Flaxseed gum (FSG; 0.3% or 0.6%)	Lemongrass (2–8 μL/mL)	Mesophilic and yeasts/molds counts	*In situ:* Yeasts and molds counts	*In situ*: FSG alone decreased yeast and mold counts in fruit. Mesophilic and yeasts/molds counts decreased when lemongrass EO concentration increased in FSG-based coating	Yousuf and Srivastava (2017)
CH (0.25 to 8 mg/mL)	Lemongrass (0.3 to 40 μL/mL)	*R. stolonifer*	*In vitro:* Macrodilution in broth technique and mycelial growth measurement *In situ:* Fungal infection	*In vitro*: CH (4 mg/mL) + lemongrass EO (1.25 μL/mL) alone or in combination caused strong inhibition of *R. stolonifer* growth and spore germination *In situ:* Tomato coated with CH + lemongrass EO presented delayed Rhizopus soft rot development	Athayde et al. (2016)
CH (2 to 16 mg/mL)	Oregano (1.25 to 20 μL/mL)	*R. stolonifer* and *Aspergillus niger*	*In vitro:* Mycelial growth measurement *In situ:* Fungal infection	*In vitro:* CH and oregano EO exhibited MIC of 8 mg/mL and 10 μL/mL, respectively. CH (4 mg/mL) + OVEO (1.25 to 5 μL/mL) strongly inhibited *R. stolonifer* (75% to 84%) and *A. niger* (90% to 100%) spore germination *In situ:* Cherry tomato coated with CH + oregano EO presented visible signs of soft rot only after 8 days of storage at room temperature and after 21 days of storage at cold temperature	Barreto et al. (2016)
Crustacean CH (4 and 8 mg/mL)	Pippermint, *Mentha* × *villosa* (1.25 to 5 μL/mL)	*A. niger*, *Botrytis cinerea*, *Penicillium expansum*, and *R. stolonifer*	*In situ*: Fungal infection	*In situ*: Fruit coated with CH + peppermint EO or CH + *M. villosa* EO presented decreased fungal infection rates. Fruit coated with CH + peppermint EO or CH + *M. villosa* EO presented no mold infections during 24 days of low temperature storage. Fruit coated with 8 or 4 mg/mL CH + 5 μL/mL peppermint EO or *M. villosa* EO presented no mold infection during 12 days of room temperature storage	Guerra et al. (2016)
Pectin (3%)	Oregano EO (0, 15.7, 25.9, and 36.1 g/L)	*Alternaria alternata*	*In vitro:* Agar dilution method *In situ:* Spray the spore suspensions	*In vitro:* Pectin + oregano EO caused inhibition of *A. alternata* mycelial growth *In situ:* Fruit coated with pectin + oregano EO (15.7 g/L) presented no infection caused by *A. alternata* during storage	Rodriguez-Garcia et al. (2016)

(*Continued*)

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Table 2–Continued.

Coating material					
Polysaccharide(s) and tested concentration(s)	Essential oil (EO) or individual constituents and tested concentration(s)	Target microorganism(s)	Assays	Main effects	Reference
CH (1%)	Cinnamon (0.1%)	*A. flavus* and *Penicillium citrinum*	*In vitro*: Disk diffusion method; exosmosis rate of fungal cells (*A. flavus*); morphology of cell at the edge of the inhibition zone (scanning electronic microscopy)	*In vitro:* Cinnamon EO caused *A. flavus* cell exosmosis. The spore growth was completely inhibited by cinnamon EO	Xing et al. (2016)
CH (0.5% to 2%) + cassava starch (CS; 2%)	Lippia gracilis Schauer (1% to 3%)	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *B. subtilis*, *Serratia marcescens*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Enteritidis, mesophllic, yeasts/molds, and thermotolerant coliforms	*In vitro:* Agar diffusion *In situ:* Microbial counts in fruit	*In vitro:* Combinations of 2% CH + 2% CS + 1%, 2%, or 3% *L. gracilis* EO were the most effective to inhibit tested bacteria *In situ*: Coatings formed by 2% CH + 2% CS + 1% or 3% *L. gracilis* EO decreased the counts of mesophilics, molds, and yeasts in fruit	de Aquino et al. (2015)
Fungal CH from *Mucor circinelloides* (3.75 or 7.5 mg/mL)	Carvacrol (Car) (1.25 or 5 μL/mL)	*A. flavus* and autochthonous microflora	*In vitro*: Minimum inhibitory concentration (MIC), mycelial growth, and spore germination *In situ*: Fungal infection (mold appearance) and Autochthonous microflora	*In vitro*: MIC, CH: 7.5 mg/mL, Car: 10 μL/mL. CH (7.5 or 3.75 mg/mL) + CAR (5 or 2.5 μL/) inhibited mycelial growth (77.2% to 100%) and spore germination (86.3 to 100 mL %). 7.5 mg/mL CH + 5 μL/mL Car caused the highest rates of mycelia growth and spore germination inhibition *In situ:* Coating comprising 3.75 mg/mL CH + 2.5 or 1.25 μL/mL Car inhibited *A. flavus* and autochthonous microflora in fruit	de Souza et al. (2015)
CH from shrimp shells of *Litopenaeus vannamei* Boone (0.125 to 8 mg/mL)	Peppermint, *M. villosa* (1.25 or 2.5 μL/mL)	*A. niger*, *B. cinerea*, *P. expansum*, and *R. stolonifer*	*In vitro*: MIC, mycelial growth measurement, and spore germination *In situ*: Fungal infection (mold appearance)	*In vitro*: MIC for all tested fungi: CH: 8 mg/mL, peppermint and *M. villosa* EO: 5 μL/mL. Mixtures of CH (4 mg/mL) + peppermint EO (1.25 or 2.5 μL/mL) or *M. villosa* EO (1.25 or 2.5 μL/mL) caused strong mycelial growth (90.1% to 97.5%) and spore germination inhibition (>75%) *In situ*: Fruit coated with CH + peppermint or *M. villosa* EO had no visible signs of mold infection during low temperature storage. Fruit coated with CH + peppermint or *M. villosa* EO had infection rates <40% after 12 days of room temperature storage	Guerra et al. (2015)
Pectin (1% or 2%) or sodium alginate (1% or 2%)	Citral (0.15% or 0.3%) or eugenol (0.1% or 0.2%) or Citral (0.15%) + Eugenol (0.1%)	Aerobic mesophilic and psychrophilic bacteria and molds and yeasts	*In situ:* Microbial counts in fruit	*In situ:* Pectin or sodium alginate + citral reduced the counts of aerobic mesophilic bacteria and molds and yeasts in fruit up to 14 days of storage.	Guerreiro et al. (2015)
CH (1%)	Clove (0.5 to 2 mL/L)	*P. digitatum*	*In vitro:* Mycelial growth measurement and membrane permeability analysis *In situ:* Mold development in artificially inoculated fruit, disease severity in artificially inoculated fruit	*In vitro*: Combinations of CH (1%) + clove EO (0.5, 1, and 2 mL/L) inhibited mycelial growth and caused cellular material leakage in fungi *In situ*: Coating formed by CH (1%) + clove EO (0.5 or 2 mL/L) delayed mold decay development in fruit up to 7 days of cold storage. CH (1%) + clove EO (0.5 mL/L) decreased lesion severity in fruit over time	Shao et al. (2015)
CH (1%)	Cinnamon (0.1%)	*E. coli*, *S. aureus*, *R. nigricans*, *Penicillium citrinum*, *A. flavus*, and *P. expansum*	*In vitro*: Agar diffusion method	*In vitro:* CH + cinnamon EO was more effective to inhibit bacteria than fungi	Xing et al. (2015)

(*Continued*)

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Table 2–Continued.

Coating material					
Polysaccharide(s) and tested concentration(s)	Essential oil (EO) or individual constituents and tested concentration(s)	Target microorganism(s)	Assays	Main effects	Reference
CH (1%) or Locust bean gum (LBG; 0.5%)	Bergamot, bitter orange, sweet orange, tangerine, and lemon (0.5% to 2%)	*A. flavus*	*In vitro:* Mycelial growth measurement and conidial germination *In situ:* Mold development in fruit	*In vitro*: Bergamot and bitter orange EO (≥2%) were most effective in reducing mycelial growth and conidial germination. Combinations of CH (1%) + bergamot or bitter orange EO (2%) reduced conidial germination. *In situ*: Coatings formed by CH (1%) + bergamot or bitter orange EO (2%) delayed mold decay in fruit up to 12 days under room temperature. Coating comprising LBG (0.5%) + bergamot or bitter orange EO (2%) were less effective to delay mold decay in fruit	Aloui et al. (2014)
CH (0.6% to 3%) + CS (0.4% to 2%)	(0.6% to 3%)	*P. aeruginosa*, *S. aureus*, *B. cereus*, *B. subtilis*, *S. marcescens*, *E. coli*, *Enterococcus faecalis*, *S.* Enteritidis, psychrophilic yeasts/mold, and thermotolerant coliforms	*In vitro:* Disk-diffusion *In situ:* Microbial counts in fruit	*In vitro:* Coating formed by CH (0.6% or 1.5%) + CS (1% or 1.6%) + *L. gracilis* EO (3% or 2.4%) caused the largest growth inhibition zones in tested bacteria *In situ:* Strawberry coated with CH (0.6%) + CS (1.6%) + *L. gracilis* EO (2.4%) maintained psychrophilic aerobic bacteria counts at <10 CFU/g during 7 days at 4° C. Yeast/molds counts in fruit decreased on the 3th day to <10 CFU/g and no recovery was observed up to the 7th day of storage in coated fruit	Azevedo et al. (2014)
CH from *Cunninghamella elegans* (3.75 to 15 mg/mL)	Not used	*B. cinerea* and *P. expansum*	*In vitro:* Mycelial growth measurement and spore germination *In situ:* Fungal infection	*In vitro:* CH inhibited (65.2% to 96.7%) the mycelial growth of tested fungi and spore germination in tested fungi *In situ:* Grapes coated with CH (15, 7.75, or 3.75 mg/mL) presented delayed appearance of infection caused by both fungi over room and cold temperature storage	de Oliveira et al. (2014a)
CH from *Mucor circinelloides* (3.75 to 15 mg/mL)	Not used	*A. niger* and *R. stolonifer*	*In vitro:* Mycelial growth measurement and spore germination *In situ:* Fungal infection	*In vitro:* CH inhibited the mycelial growth of *R. stolonifer* and *A. niger*. Fungal mycelial growth inhibition ranged from 68.3% to 92.8% and 59.2% to 90.2% for *R. stolonifer* and *A. niger*. Rates of spore germination inhibition caused by CH was >73% *In situ*: Fruit coated with CH had decreased visible fungal infection at the end of the storage at room and cold temperature	de Oliveira et al. (2014b)
CH (1%)	Car, cinnamaldehyde (Cin), eugenol, *trans*-cinnamalde hyde (*t*-Cin), and benzaldehyde (0.25% to 2.5%)	*E. coli*, *P. digitatum*, mesophilic, and yeasts/molds	*In vitro:* Disk-diffusion assay *In situ:* Microbial counts in fruit	*In vitro*: Car, Cin, and *t*- Cin at 0.5% and 2.5% caused the largest inhibition zones against *P. digitatum* and *E. coli*, respectively *In situ*: Coatings formed by CH (1%) + Car, Cin, or *t*-Cin (0.5%) decreased total mesophilic and yeasts/mold counts in fruit stored at 5 and 10 °C	Sun et al. (2014)
CH (1%)	Bergamot, tea tree, and thyme (2%)	*P. italicum*	*In situ:* Mold development in fruit	*In situ*: Coating formed by CH (1%) + tea tree EO (2%) or thyme (2%) were more effective to delay mold development in fruit stored under room temperature for 26 days	Cháfer et al. (2012)
CH (5% and 10%)	Oregano (1.25 to 5 μL/mL)	*R. stolonifer*, *A. niger*, and native fungi	*In vitro:* MIC, mycelial growth measurement, and spore germination *In situ:* Mold development in artificially inoculated fruit	*In vitro*: Combinations of CH (10% and 5%) and oregano EO (1.25%, 2.5%, and 5%) inhibited the mycelial growth and fungi spore germination. Combinations of CH (5%) and oregano EO (1.25%, 2.5%, and 5%) caused morphological changes in spores and fungal mycelia *In situ*: Coatings formed by CH (5%) + oregano EO (2.5% and 5%) delayed mold development in fruit stored at 25 and 12 °C	dos Santos et al. (2012)

(*Continued*)

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Table 2–Continued.

Coating material					
Polysaccharide(s) and tested concentration(s)	Essential oil (EO) or individual constituents and tested concentration(s)	Target microorganism(s)	Assays	Main effects	Reference
CH (1%)	Lemon (3%)	*B. cinerea*	*In situ:* Mold development in fruit	Coating comprising CH (1%) and lemon EO (3%) delayed the mold decay in fruit stored at 5 °C for 7 days	Perdones et al. (2012)
Pullulan (5%)	Thymol (Thy) (0.14% to 3%)	*B. subtilis*, *S. aureus*, *S. Enteritidis*, and *E. coli*	*In vitro:* MIC	All bacteria were susceptible to Thy. Stronger inhibitory activity was observed for films prepared with pullulan and >0.9% Thy	Gniewosz and Synowiec (2011)
GA (10%)	Lemongrass (0.05%) and cinnamon (0.4%)	*C. musae* and *C. gloeosporioides*	*In vitro:* Mycelial growth measurement and spore germination *In situ:* Incidence and disease severity in fruit	*In vitro*: Mycelial growth and spore germination decreased by combinations of GA + lemongrass EO or cinnamon EO *In situ*: Disease incidence and disease severity mostly decreased in fruit coated with GA + cinnamon EO	Maqbool et al. (2011)
CH (1%) or hydroxypropyl-methylcellulose (1%)	Bergamot (2%)	Mesophilic and yeasts/molds	*In situ:* Microbial counts in fruit	*In situ*: Coating formed by CH and bergamot EO was effective to reduce total mesophilic bacteria and yeasts/molds counts in fruit stored at cold temperature	Sánchez-González et al. (2011)
CH (2%)	Oregano, red thyme, peppermint, lemongrass, and limonene (0.2%)	Total flora, total molds, *R. stolonifer*, and *B. cinerea*	*In vitro:* Paper disk method *In situ:* Fruit decay and native mold development	*In vitro:* Oregano and red thyme EO caused the highest inhibition of total microflora, as well as of total yeast/molds *In situ:* Red thyme EO, peppermint EO, and limonene were the most effective to delay fungal decay in fruit stored at 4 °C. Coating formed by CH + limonene was the most effective to delay fungal decay in fruit stored at 4 °C	Vu et al. (2011)
Mesquite gum (MG; 10%)	Thyme (0.015% to 0.104%) and Mexican lime EO (0.014% to 0.1%)	*C. gloeosporioides* and *R. stolonifer*	*In vitro:* Agar diffusion *In situ:* Mold development in fruit and incidence and disease severity in fruit	*In vitro*: Thyme (≥0.060%) and Mexican lime EO (0.085% or 0.1%) completely inhibited fungal mycelial growth *In situ*: Thyme (0.15%) and Mexican lime EO (0.14%) reduced mold decay in fruit stored at 20 °C. Coating formed by MG + thyme EO (0.1%) and Mexican lime EO (0.05%) were more effective to reduce disease incidence and severity in fruit stored at 20 °C	Bosquez-Molina et al. (2010)

Twelve studies used other polysaccharides or gums combined with CH, such as CS (de Aquino et al., 2015), hydroxypropyl-methylcellulose (Sánchez-González et al., 2011), and locust bean gum (Aloui et al., 2014), or as the only ingredient to formulate coatings, such as gum arabic (GA; Andrade et al., 2017; Etemadipoor, Ramezanian, Mirzaalian Dastjerdi, & Shamili, 2019; Maqbool et al., 2011), mesquite gum (Bosquez-Molina, Jesús, Bautista-Baños, Verde-Calvo, & Morales-López, 2010), flaxseed gum (Yousuf & Srivastava, 2017), pullulan (Gniewosz & Synowiec, 2011), pectin (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015; Rodriguez-Garcia et al., 2016), and sodium alginate (Guerreiro et al., 2015).

EOs or ICs were primarily used with the purpose of enhancing the antimicrobial effects of the formulated coatings, although their abilities to increase the barrier properties of these coatings have also commonly cited. ICs (for example, carvacrol, thymol, cinnamaldehyde, eugenol, *trans*-cinnamaldehyde, citral, and eucalyptol) used in coating formulation are typically recognized as prevalent constituents in EOs with well-known strong and wide-spectrum antimicrobial properties.

Coating-base material: EOs and ICs. Twenty-four out of the 30 selected studies used EOs for the formulation of edible coatings, such as the EO from cinnamon (Etemadipoor et al., 2019; Maqbool et al., 2011; Xing, Li et al., 2011; Xing et al., 2015, 2016), lemongrass (Athayde et al., 2016; Maqbool et al., 2011; Oliveira et al., 2018; Vu et al., 2011; Yousuf & Srivastava, 2017), peppermint (de Oliveira et al., 2017; Guerra et al., 2015, 2016; Vu et al., 2011), bergamot (Aloui et al., 2014; Cháfer et al., 2012; Sánchez-González et al., 2011), oregano (Andrade et al., 2017; Barreto et al., 2016; dos Santos et al., 2012; Rodriguez-Garcia et al., 2016; Vu et al., 2011), rosemary (Andrade et al., 2017), clove (Shao et al., 2015), thyme (Bosquez-Molina et al., 2010; Cháfer et al., 2012), tea tree (Cháfer et al., 2012), Mexican lime (Bosquez-Molina et al., 2010), and *Lippia gracilis* (Azevedo et al., 2014; de Aquino et al., 2015). Only five studies used ICs to formulate edible coatings, to cite: carvacrol (de Souza et al., 2015; Sun et al., 2014), cinnamaldehyde and *trans*-cinnamaldehyde (Sun et al., 2014), thymol (Gniewosz & Synowiec, 2011), citral and eugenol (Guerreiro et al., 2015), and limonene (Vu et al., 2011).

Twelve studies tested more than one EO or IC to select the concentration most effective to inhibit the target microorganism and/or with improved abilities to preserve the quality parameters of coated fruit (Aloui et al., 2014; Andrade et al., 2017; Azevedo et al., 2014; Bosquez-Molina et al., 2010; Cháfer et al., 2012;

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Table 3–Studies assessing the effects of coatings comprising polysaccharides and essential oils or their individual constituents on physicochemical and/or sensory quality attributes of fruit and mains observed effects.

Coating materials				
Polysaccharide and tested concentration(s)	Essential oil (EO) or individual constituent and tested concentration(s)	Measured quality parameters	Main effects	Reference
Gum arabic (GA; 5% or 10%)	Cinnamon EO (1% or 2%)	Weight loss, firmness, chlorophyll, carotenoid content, total soluble solids (TSS), titrable acidity (TA), pH, and ascorbic acid	GA (10%)–cinnamon EO (1%)-coated fruit showed the lowest weight loss, greater firmness, greater value for b^* in skin color, maximum amount of chlorophyll content, and minimum amount of carotenoid. GA (10%)–cinnamon EO (1%)-coated fruit showed the highest SSC.	Etemadipoor et al. (2019)
GA (10%)	Oregano EO (0.25 μL/mL) or oregano EO (0.06 to 0.12 μL/mL) + rosemary EO (0.25 to 0.5 μL/mL)	Weight loss, firmness, TSS, organic acids, sugars, phenolic compounds, color, and sensory analysis	GA–oregano EO or GA–oregano + rosemary EO coated fruit showed decreased weight loss and improvement in firmness during storage. GA– oregano EO or GA–oregano + rosemary EO coated fruit exhibited decreased TSS and TA; GA–oregano EO or GA–oregano + rosemary EO coatings delayed the loss of phenolic compounds and enhanced the flavor in fruit; lightness/brightness was higher in coated fruit	Andrade et al. (2017)
Flaxseed gum (FSG; 0.3% or 0.6%)	Lemongrass EO (2 – 8 μL/mL)	Weight loss, color, and sensory analysis	FSG–lemongrass EO coated fruit had decreased weight loss during storage, as well as smaller changes in Chroma values. Changes in Hue angle over time were smaller in FSG–lemongrass coated fruit	Yousuf and Srivastava (2017)
Chitosan (CH)	Lemongrass EO (1.25 μL/mL)	Weight loss, firmness, color, soluble solids, titratable acidity, ascorbic acid, pH, and sensory analysis	No difference in most of the evaluated quality parameters between coated and uncoated fruit. Only $b*$ parameter in colorimetry analysis was higher in coated than uncoated fruit. Color was the only sensory parameter that coated fruit had better performance than uncoated fruit; most of the other measured attributes were similar in coated and uncoated fruit	Athayde et al. (2016)
CH (4 mg/mL)	Oregano EO (1.25 μL/mL)	Weight loss, firmness, TSS, TA, lycopene, color, phenolic compounds, and sensory analysis	CH–oregano EO coating decreased weight, firmness and lycopene loss, besides to decrease TA and TSS values in fruit during storage at room or cold temperature. CH–oregano coating increased the amounts of some phenolic compounds in fruit during storage	Barreto et al. (2016)
Crustacean CH (4 mg/mL)	Peppermint EO, *Mentha* × *villosa* Huds EO (2.5 or 5 μL/mL)	Weight loss, color, firmness, TSS, TA, and sensory analysis	CH–peppermint or *M. villosa* EO coated fruit displayed lower weight loss during cold temperature storage. Coated fruit presented improvement in firmness during storage. TA, SS, color $a*$, and color $b*$ did not differ between coated and uncoated fruit. The intent to purchase was the same (possibly purchase) for coated and uncoated fruit	Guerra et al. (2016)
Pectin (3%)	Oregano EO (0, 15.7, 25.9, and 36.1 g/L)	TSS, pH, TA, color, firmness, total phenolics, antioxidant capacity, and sensory analysis	Fruit coated with pectin + oregano EO (25.9 g/L) showed the highest amount of total phenolics at the end of storage period. Fruit coated with pectin + oregano EO (15.7 g/L) presented the better flavor acceptability during storage	Rodriguez-Garcia et al. (2016)
CH (1%)	Cinnamon EO (0.1%)	Respiration rate and polyphenol-oxidase (PPO)	O_2 concentration was reduced and CO_2 production increased within packaging during storage in uncoated and coated fruit. CO_2 production was higher in coated fruit. Coated fruit had decreased PPO activity at the end of the storage period	Xing et al. (2016)
CH (2%) + cassava starch (CS; 2%)	Lippia gracilis Schauer EO (1% or 3%)	pH, TA, TSS, color, and firmness	Fruit coated with CH + CS (2%) + *L. gracilis* EO (1% or 3%) showed lower TA and a^* and b^* values, and higher L^* values during storage No differences were observed for TSS, pH, and firmness between coated and uncoated fruit after 10 days of storage	de Aquino et al. (2015)

(*Continued*)

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Table 3–Continued.

Coating materials				
Polysaccharide and tested concentration(s)	Essential oil (EO) or individual constituent and tested concentration(s)	Measured quality parameters	Main effects	Reference
CH from *Mucor circinelloides* (3.75 or 7.5 mg/mL)	Carvacrol (CAR; 1.25 to 5 μL/mL)	Weight loss, color, firmness, TSS, and TA	CH–CAR-coated fruit showed decreased weight loss and improvement in firmness during storage, as well as decreased TSS and TA and improvements in lightness/brightness	de Souza et al. (2015)
CH from shrimp shells (*Litopenaeus vannamei* Boone (4 mg/mL)	Peppermint EO and *M. villosa* EO (1.25 or 2.5 μL/mL)	Weight loss, color, firmness, TSS, TA, and sensory analysis	CH–peppermint and CH–*M. villosa* EO coated fruit showed decreased weight loss over time. TSS and TA were not different for coated and uncoated fruit. Coated and uncoated fruit received "liked slightly" or "liked moderately" for all sensory parameters. Intention to purchase was "possibly purchase" for coated and uncoated fruit	Guerra et al. (2015)
Pectin (1% or 2%) or sodium alginate (1% or 2%)	Citral (0.15% or 0.3%) or eugenol (0.1% or 0.2%) or citral (0.15%) + eugenol (0.1%)	Color, firmness, TSS, and sensory analysis	Fruit coated with pectin or alginate + eugenol and/or citral presented lower decreases in TSS and alterations in color and firmness during storage. Pectin treatments preserved SSC values. Only fruit coated with alginate or pectin + eugenol or citral presented low acceptance in appearance on the 14th day of storage	Guerreiro et al. (2015)
CH (1%)	Clove EO (0.5 to 2 mL/L)	Phenylalanine ammonia-lyase (PAL), β-1,3-glucanase, and chitinase	CH–clove EO coated fruit had increased PAL and β-1,3-glucanase activity in some monitored storage time points	Shao et al. (2015)
CH (1%)	Cinnamon EO (0.1%)	Weight loss, TA, vitamin C, polyphenol-oxidase (POD), superoxide-dismutase (SOD), TSS, total phenolic compounds (TPC), malondialdehyde (MDA), and sensory acceptance	CH–cinnamon EO coated fruit had lower weight loss and RR and better sensory acceptance. Coated fruit had lower decrease in TA and vitamin C contents over time. PPO activity decreased in coated fruit. Coated fruit had higher TPC and slower increase in MDA contents over time	Xing et al. (2015)
CH (1%) and Locust bean gum (LBG) (0.5%)	Bergamot EO and bitter orange EO (2%)	Sensory profile (off-odors, off-flavors, color, and glossiness)	CH–bergamot EO and CH–bitter orange EO coating fruit had reduced glossiness	Aloui et al. (2014)
CH (1%)	CAR, cinnamaldehyde (Cin) and *trans*-cinnamaldehyde (*t*-Cin) ($\leq$0.5%)	Firmness	CH–CAR or CH–*t*-CIN coated fruit had improvements in firmness during storage	Sun et al. (2014)
CH (1%)	Bergamot EO, thyme EO, and tea tree EO (2%)	Weight loss, respiration rate (RR), TA, TSS, pH, color, ascorbic acid, and texture	CH–bergamot EO coated fruit had reduced weight and water losses during cold storage. CH–bergamot EO or CH–tea tree EO delayed weight loss for 26 days in fruit stored under room temperature. Tested coatings did not affect fruit RR pattern during storage	Cháfer et al. (2012)
CH (5%)	Oregano EO (2.5 to 5 μL/mL)	Weight loss, color, firmness, TSS, TA, anthocyanin, and sensory analysis	No difference in TA, TSS, anthocyanin content, weight loss, and firmness in uncoated and CH–oregano EO coated fruit during cold storage. Coated fruit had lower TSS and anthocyanin content after 12 days of storage at room temperature	dos Santos et al. (2012)
CH (1%)	Lemon EO (3%)	Surface density, water vapor resistance, TA, pH, TSS, maturity index, RR, and sensory analysis	CH–lemon EO coated fruit had decreased RR and lower maturation index and pH during storage	Perdones et al. (2012)
Pullulan (5%)	Thymol (Thy; 0.14% to 3%)	Appearance and aroma	Pullulan–Thy-coated films presented transparent and shiny appearance on fruit. Coated fruit had no change in color. A typical Thy aroma was detectable in coated fruit	Gniewosz and Synowiec (2011)
GA (10%)	Lemongrass EO (0.05%) and cinnamon EO (0.4%)	Weight loss, firmness, TSS, and TA	Coated fruit had lower weight and firmness loss, as well as lower reduction in TA and SSC during storage. GA–cinnamon + lemongrass EO coated fruit had higher scores for overall acceptability	Maqbool et al. (2011)

(*Continued*)

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Table 3–Continued.

Coating materials				
Polysaccharide and tested concentration(s)	Essential oil (EO) or individual constituent and tested concentration(s)	Measured quality parameters	Main effects	Reference
CH (1%) or hydroxypropylmethylcellulose (1%)	Bergamot EO (2%)	TSS, pH, TPC, weight loss, texture, color, and RR	CH–bergamot EO coating was the most effective to delay weight, water, and firmness loss in fruit over time. CH–bergamot EO coated fruit had decreased respiration rate	Sánchez-González et al. (2011)
CH (2%)	Peppermint EO and limonene (0.2%)	Visual appearance	CH–peppermint EO or CH–limonene coated fruit had better visual appearance	Vu et al. (2011)
CH (1%)	Cinnamon EO (0.25%)	Color, vitamin C, MDA, membrane permeability, and sensory acceptability	CH–cinnamon EO coating delayed vitamin and color loss in fruit. Coated fruit presented lower MDA levels and electrolyte leakage	Xing et al. (2011)

de Aquino et al., 2015; Guerra et al., 2015, 2016; Guerreiro et al., 2015; Maqbool et al., 2011; Sun et al., 2014; Vu et al., 2011).

Edible coating formulation. The type and concentration of the polysaccharide as well as of the EO or IC used to formulate the edible coatings used in *in situ* assays were selected overall considering their antimicrobial effects determined firstly in *in vitro* assays, as measured through the determination of the minimum inhibitory concentration (MIC; Aloui et al., 2014; Andrade et al., 2017; Azevedo et al., 2014; Bosquez-Molina et al., 2010; de Aquino et al., 2015; de Souza et al., 2015; dos Santos et al., 2012; Gniewosz & Synowiec, 2011; Guerreiro et al., 2015; Maqbool et al., 2011; Sun et al., 2014; Xing et al., 2016).

The studies have ordinarily used sub-MICs of these substances/compounds to formulate the coatings (de Oliveira et al., 2017; de Souza et al., 2015; Guerra et al., 2015, 2016; Oliveira et al., 2018). Only five studies did not perform *in vitro* assays to select the concentrations of EOs or ICs used to formulate the coatings (Cháfer et al., 2012; Etemadipoor et al., 2019; Perdones et al., 2012; Xing et al., 2011; Xing et al., 2016) and two studies used the same concentration in both *in vitro* and *in situ* assays (Maqbool et al., 2011; Rodriguez-Garcia et al., 2016), which were selected considering data of previous published studies.

The selection of the amounts of polysaccharides or gums (CS, flaxseed gum, GA, hydroxypropylmethylcellulose, locust bean gum, mesquite gum, pectin, pullulan, and sodium alginate) used to formulate coatings has primarily considered the amounts of these substances capable of providing dispersions with viscosity enough to form coatings when applied on fruit (Andrade et al., 2017; Bosquez-Molina et al., 2010; Gniewosz & Synowiec, 2011; Maqbool et al., 2011; Rodriguez-Garcia et al., 2016; Yousuf & Srivastava, 2017).

CH-coating forming dispersions were usually prepared by dissolving this polymer in an aqueous 0.1 M acetic acid solution under stirring (mostly 150 rpm) for a variable time period, to cite: 1 hr (Xing et al., 2011; Xing et al., 2015, 2016), 6 hr (Guerra et al., 2016), 12 hr (Aloui et al., 2014; Sánchez-González et al., 2011), or 24 hr (Athayde et al., 2016; de Oliveira et al., 2017; de Souza et al., 2015; dos Santos et al., 2012; Oliveira et al., 2018). Some studies have used an overnight stirring (Cháfer et al., 2012; Sun et al., 2014; Vu et al., 2011) or have not reported the stirring time (Azevedo et al., 2014; de Aquino et al., 2015; Perdones et al., 2012; Shao et al., 2015).

The stirring of CH-coating forming dispersions has been mostly performed at room temperature (Athayde et al., 2016; Cháfer et al., 2012; de Oliveira et al., 2017; de Souza et al., 2015; dos Santos et al., 2012; Guerra et al., 2016; Oliveira et al., 2018; Sun et al., 2014; Vu et al., 2011; Xing et al., 2011; Xing et al., 2015, 2016), although two studies have performed this operation at 40 °C (Aloui et al., 2014; Sánchez-González et al., 2011). Four studies have not reported the stirring temperature (Azevedo et al., 2014; de Aquino et al., 2015; Perdones et al., 2012; Shao et al., 2015). Sixteen studies incorporated glycerol or glycol as plasticizer ingredient in CH coating-forming dispersions in concentrations of 0.25% to 2.5% (Azevedo et al., 2014; de Aquino et al., 2015; de Oliveira et al., 2017; de Souza et al., 2015; dos Santos et al., 2012; Gniewosz & Synowiec, 2011; Guerra et al., 2016; Oliveira et al., 2018; Sun et al., 2014; Xing et al., 2011; Xing et al., 2015, 2016), 5% (Bosquez-Molina et al., 2010), or 20% to 30% (v/v) (Cháfer et al., 2012; Perdones et al., 2012; Vu et al., 2011).

CS dispersion (2%, w/v) was prepared with glycerol (0.64%) and heated in a water bath (temperature not exceeding 70 °C) under stirring. After cooling to 25 °C, chitosan was added to the CS dispersion and stirred up to complete dissolution (de Aquino et al., 2015). Flaxseed gum powder was dissolved in distilled water with stirring up to complete dissolution; however, only one study used this polysaccharide to formulate coating (Yousuf & Srivastava, 2017). Hydroxypropylmethylcellulose (1%, w/v) was dissolved in distilled water (80 C for 2 hr) under overnight stirring at room temperature (Sánchez-González et al., 2011). Three studies used GA to formulate coatings and this polysaccharide was firstly dissolved in ultra-purified water and stirred at 40 °C for 60 min (Andrade et al., 2017; Etemadipoor et al., 2019; Maqbool et al., 2011). One study used pullulan or mesquite gum to formulate coatings. Pullulan was dissolved in distilled water with glycerol at 80 °C (Gniewosz & Synowiec, 2011) and mesquite gum was dissolved in distilled water at 70 °C (Bosquez-Molina et al., 2010); the stirring time was not reported for these two polysaccharides forming dispersions. Two studies used pectin and pectin + sodium alginate to formulate coatings. Pectin was dissolved in distilled water with 1% glycerol (v/v) for 15 min (Rodriguez-Garcia et al., 2016); pectin + sodium alginate was dissolved in distilled water with heating at 70 °C, stirred up to the dispersion became clear, followed by addition of 1.5% glycerol (v/v) and 1% ascorbic acid (v/v) as antibrowning agents (Guerreiro et al., 2015).

Tween 20 and 80 are commonly used (17 studies) to homogenize the EOs or ICs into the polysaccharides dispersions (Aloui et al., 2014; Andrade et al., 2017; Athayde et al., 2016; Azevedo et al., 2014; Barreto et al., 2016; Cháfer et al., 2012; de Aquino

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et al., 2015; de Oliveira et al., 2017; Etemadipoor et al., 2019; Maqbool et al., 2011; Oliveira et al., 2018; Shao et al., 2015; Sun et al., 2014; Vu et al., 2011; Xing et al., 2011; Xing et al., 2015, 2016). Three studies used only vigorous stirring for homogenizing EOs or ICs into the polysaccharides dispersions (Bosquez-Molina et al., 2010; Guerra et al., 2016; Yousuf & Srivastava, 2017). Three studies adjusted the pH of the coating-forming dispersion to 5.5 or 5.6, which were referred as suitable pH for fungal growth (Aloui et al., 2014; de Oliveira et al., 2017; Maqbool et al., 2011). Thereby, in these cases, the pH values (5.5 or 5.6) were cited as being not capable of adversely affecting the growth of target fungi.

Methods used in *in vitro* assays

Many of the selected studies initially performed *in vitro* assays to evaluate the antimicrobial effects of the polysaccharide (specifically of CH) and EO or IC alone or in combination using different methods, to cite: disk diffusion, as the most common; dilution in broth (Andrade et al., 2017; de Souza et al., 2015; dos Santos et al., 2012; Gniewosz & Synowiec, 2011); cavity slide (Aloui et al., 2014; Maqbool et al., 2011) and dilution in agar (Aloui et al., 2014; Andrade et al., 2017; de Oliveira et al., 2017; de Souza et al., 2015; dos Santos et al., 2012; Maqbool et al., 2011; Oliveira et al., 2018; Sun et al., 2014).

Two studies assessed the type of interaction among the different combined concentrations of CH and EOs (specifically peppermint and lemongrass EO) through the determination of the Abbot index (de Oliveira et al., 2017; Oliveira et al., 2018).

Sixteen studies used fungi as target organisms in antimicrobial assays *in vitro* (Aloui et al., 2014; Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Bosquez-Molina et al., 2010; de Oliveira et al., 2014a, 2014b; de Oliveira et al., 2017; de Souza et al., 2015; dos Santos et al., 2012; Guerra et al., 2016; Maqbool et al., 2011; Oliveira et al., 2018; Rodriguez-Garcia et al., 2016; Shao et al., 2015; Vu et al., 2011; Xing et al., 2016). Three studies used only bacteria as target organisms (Azevedo et al., 2014; de Aquino et al., 2015; Gniewosz & Synowiec, 2011) and two studies used both fungi and bacteria (Sun et al., 2014; Xing et al., 2015).

Methods used in *in situ* assays

Some studies carried out the microbiological analysis by coating the fruit and inoculating a specific microorganism (preventive effect) or by inoculating a specific microorganism and coating the fruit (curative effect; Aloui et al., 2014; Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Bosquez-Molina et al., 2010; Cháfer et al., 2012; de Oliveira et al., 2017; de Souza et al., 2015; dos Santos et al., 2012; Guerra et al., 2015, 2016; Maqbool et al., 2011; Oliveira et al., 2018; Rodriguez-Garcia et al., 2016; Shao et al., 2015). Other studies performed analysis of the autochthonous microflora on coated fruit (Azevedo et al., 2014; de Aquino et al., 2015; Guerreiro et al., 2015; Perdones et al., 2012; Sánchez-González et al., 2011; Sun et al., 2014; Vu et al., 2011; Yousuf & Srivastava, 2017).

Three studies carried out tests only to measure the antimicrobial effects of the formulated coatings on fruit, considering the autochthonous microflora or an artificial contamination (de Oliveira et al., 2017; Maqbool et al., 2011; Oliveira et al., 2018). Seven studies only measured the effects of the formulated coatings on overall quality parameters of fruit over time, including physicochemical, enzymatic, and/or sensory analyses (Cháfer et al., 2012; Etemadipoor et al., 2019; Gniewosz & Synowiec, 2011; Xing et al., 2011; Xing et al., 2015, 2016). The other studies measured the antimicrobial effects of the formulated coatings on fruit, as well as their influence on physicochemical and/or sensory parameters related to overall postharvest quality over time (Aloui et al., 2014; Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Bosquez-Molina et al., 2010; de Aquino et al., 2015; de Souza et al., 2015; dos Santos et al., 2012; Guerra et al., 2016; Guerreiro et al., 2015; Perdones et al., 2012; Rodriguez-Garcia et al., 2016; Sánchez-González et al., 2011; Shao et al., 2015; Sun et al., 2014; Vu et al., 2011; Yousuf & Srivastava, 2017).

In general, fruit are sanitized after a dipping in the coating-forming dispersions containing polysaccharide and EO or IC for a preselected time interval considering data of available literature or results from preliminary experiments. After dipping, fruit are typically air-dried to form the coating on their surface.

Banana and papaya were dipped for 2 to 3 min in 10% GA, 0.05% lemongrass EO, and 0.4% cinnamon EO alone and in combination, being maintained at room temperature for drying (Maqbool et al., 2011). Plums were immersed for 1 min in a dispersion formed by GA (1 mg/mL), GA (1 mg/mL) + oregano EO (0.25 μL/mL), or GA (1 mg/mL) + oregano EO (0.06 μL/mL) + rosemary EO (0.25 μL/mL) and stored at room and cold temperature (Andrade et al., 2017). Guava was dipped for 2 min in GA (5% or 10%) + cinnamon EO (1% or 2%), kept to dry for 30 min at room temperature, and transferred to cold storage (10 °C) (Etemadipoor et al., 2019). Pomegranate arils were immersed for 3 min in flaxseed gum (0.3% or 0.6%) + lemongrass EO (0, 2, 5 or 8 /mL), kept on a blotting paper for 5 min to allow excessive coating dispersion to drip off, and stored at cold temperature (5 °C; Yousuf & Srivastava, 2017). Raspberries were dipped in sodium alginate or pectin (1% or 2%) + citral (0.15% or 0.3%) or eugenol (0.1% or 0.2%), as well as in alginate or pectin (1% or 2%) + citral (0.15%) + eugenol (0.1%) for 2 min; the excessive coating dispersion was allowed to drip off for 30 s and dipped for a second time in a calcium chloride solution (Guerreiro et al., 2015).

Different fruit (guava, mango, papaya, tomato, grape, strawberry, and blueberry) were immersed in coating-forming dispersions containing CH (3.75 to 20 mg/mL) and EOs or IC in a range of concentrations (alone or in combination) for 30 s (Sun et al., 2014), 1 min (Aloui et al., 2014; Athayde et al., 2016; Barreto et al., 2016; Cháfer et al., 2012; de Oliveira et al., 2014a, 2014b; de Souza et al., 2015; dos Santos et al., 2012; Guerra et al., 2015, 2016; Oliveira et al., 2018; Perdones et al., 2012; Sánchez-González et al., 2011), 2 min (Shao et al., 2015), 3 min (Azevedo et al., 2014; de Aquino et al., 2015), 5 min (de Oliveira et al., 2017; Xing et al., 2015, 2016), or for a not-informed dipping time (Vu et al., 2011). Tomatoes were brushed with 5 mL of pectin (3%) and/or oregano EO (15.7, 25.9, or 36.1 g/L) and allowed to dry at 25 °C (Rodriguez-Garcia et al., 2016).

Antimicrobial effects in *in vitro* assays

Eighteen out of the 30 studies selected performed *in vitro* assays to evaluate the antimicrobial effects of CH and EOs or ICs alone or in combination. Thyme EO inhibited the mycelial growth of *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* and these effects were directly associated with the EO concentrations; in the same study, Mexican lime EO was not as effective as thyme EO to inhibit these fungi (Bosquez-Molina et al., 2010). A study assessed the antimicrobial effects of limonene (0.02%), as well as of lemongrass, oregano, peppermint, and red thyme EO against autochthonous microorganisms in strawberry, being reported medium to strong antimicrobial effects, with the exception of limonene that presented weak inhibitory effects (Vu et al., 2011).

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Formulations of CH (0.5%, 1%, 1.5%, or 2%) + CS (2%) + EOs from two genotypes of *L. gracilis* (0%, 1%, 2% and 3%) were tested for their efficacy to inhibit *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Serratia marcescens*, and *Salmonella* Enteritidis. All the tested bacteria were sensitive to at least one of the examined coating formulations, but *E. coli* and *S. marcescens* were the most sensitive bacteria, being inhibited by three of the tested formulations (de Aquino et al., 2015).

Combinations of oregano and rosemary EO inhibited *R. stolonifer* more than these EOs alone at either MIC or sub-MIC. GA (1 mg/mL) and oregano EO alone (0.25 mL/mL) or oregano EO (0.12 or 0.06 mL/mL) + rosemary EO (0.5 or 0.25 mL/mL) caused strong inhibition of mycelial growth, spore germination, and sporulation of *R. stolonifer* (Andrade et al., 2017). Another study using GA (10% w/v) reported no inhibition of mycelial growth of *Colletotrichum musae* and *C. gloeosporioides*, but the combination of GA + cinnamon EO (0.4%, v/v) caused strong inhibition of these fungi (Maqbool et al., 2011). Another study reported a MIC of 10 mg/mL and 10 µL/mL for CH and oregano EO against *R. stolonifer* and *Aspergillus niger*, respectively; the combination of CH (5 or 10 mg/mL) + oregano EO (1.25, 2.5, or 5 µL/mL) inhibited the mycelial growth (88% to 100%) of *R. stolonifer* and *A. niger* (dos Santos et al., 2012).

Thymol displayed strong inhibitory effects against *S. aureus*, with a MIC of 0.012% (w/v). *B. subtilis*, *E. coli*, and *Salmonella* Enteritidis were less sensitive to thymol, which presented a MIC of 0.024% (w/v) against these bacteria. Pullulan coatings containing 0.14% to 0.6% thymol (2.5 to 10.6 mg/cm^2) did not display any additional inhibitory effect on these bacteria when compared with the film containing only thymol. The highest inhibitory effects were observed for the coatings containing the highest thymol amounts (3%; Gniewosz & Synowiec, 2011).

A study assessed the efficacy of EOs from two genotypes of *L. gracilis* to inhibit eight bacterial species when used in a formulation containing CS and CH. The formulations more effective to inhibit bacterial growth were those containing 1.6% CS + 0.6% CH + 2.4% *L. gracilis* EO or 1% CS + 1.5% *L. gracilis* EO (Azevedo et al., 2014). The efficacy of cinnamon EO (0.1%, v/v) as well as of a coating containing CH (1%, w/v) + cinnamon EO (0.1%, v/v) to inhibit different fungi and bacteria was investigated, being found a higher antimicrobial effect when CH and cinnamon EO were tested in combination (Xing et al., 2015).

CH (0.063%, w/v) and clove EO (1.50 mL/L) caused 89.2% and 99% inhibition of *Penicillium digitatum* mycelial growth, respectively (Shao et al., 2015). A combination of CH (3.75 or 7.5 mg/mL) + carvacrol (2.5 or 5 µL/mL) caused inhibition in mycelial growth of *Aspergillus flavus* in a range of 77.2% to 100% (de Souza et al., 2015). Cinnamon EO (1% to 4%) and *trans*-cinnamaldehyde (1% to 4%) alone were also effective to inhibit the mycelial growth of *A. flavus* and *Penicillium citrinum*, and these inhibitory effects increased when the concentration of cinnamon EO and CH increased (Xing et al., 2016).

Pectin (3%, w/v) + oregano EO (15.7, 25.9, or 36.1 g/L) was tested for inhibitory activity against *Alternaria alternata*, being reported that all tested pectin + oregano EO combinations caused total mycelial growth inhibition of *A. alternata* during 5 days at 28 °C (Rodriguez-Garcia et al., 2016).

Combinations of CH + peppermint EO showed additive effects against *Colletotrichum asianum* and synergistic effects against *Colletotrichum karstii*. Mixtures of 5 mg/mL CH + 0.6 or 1.25 mL/mL peppermint EO and 7.5 mg/mL CH + 0.6 mL/mL peppermint EO showed synergistic effects against *Colletotrichum tropicale* and *Colletotrichum dianesei*. For *Colletotrichum fructicola*, synergistic effects were observed when 5 or 7.5 mg/mL CH was combined with 0.3 or 0.6 mL/mL peppermint EO (de Oliveira et al., 2017). All the tested *Colletotrichum* strains were previously identified as causative agents of anthracnose in different fruit (Lima et al., 2013; Lima, Lima, Tovar-Pedraza, Michereff, & Câmara, 2015). Lemongrass EO (2.5 µL/mL) caused a 100% inhibition of the mycelial growth of five different *Colletotrichum* species. The combination of CH (5 mg/mL) + lemongrass EO (0.15, 0.3, or 0.6 µL/mL) presented synergistic inhibitory effects against the tested *Colletotrichum* species (Oliveira et al., 2018). None of the studies that assessed the type of interaction among different concentrations of CH and EOs reported antagonistic interactions toward the target microorganisms.

Antimicrobial effects in *in situ* assay

The effects of coatings formed by polysaccharides and EOs or ICs have been analyzed concerning disease incidence (percentage of fruit showing characteristic symptoms of a specific disease) and disease severity (lesion development) based on a grade scale. Flaxseed gum ordinarily does not possess any inherent antimicrobial property and the incorporation of lemongrass EO was an effective strategy to retard microbial growth in pomegranate arils. The initial total plate count of uncoated pomegranate arils increased from 1.9 to 8.3 log CFU/g during 12 days of storage (5 °C), whereas counts of 4.3 to 4.5 CFU/g were found when pomegranate arils were coated with flaxseed gum (0.3% or 0.6%, w/v) + lemongrass EO (800 ppm) (Yousuf & Srivastava, 2017).

Coatings containing GA (10%) + lemongrass EO (0.05%) or cinnamon EO (0.4%) were effective to decrease the incidence of Rhizopus soft rot in these fruit during storage (13 °C for banana and 12 °C for papaya; Maqbool et al., 2011). Conversely, a coating containing GA (10%) + oregano EO (0.06 or 0.25 µL/mL) + rosemary EO (0.25 µL/mL) was effective to control Rhizopus soft rot in plums during storage at room temperature (Andrade et al., 2017).

The development of Rhizopus soft rot in tomato during storage (25 °C) was also delayed by a coating containing CH (4 mg/mL) + lemongrass EO (1.25 µL/mL) (Athayde et al., 2016). A coating containing CH from shells of *L. vannamei* Boone shrimp (4 mg/mL) + peppermint EO or *Mentha* × *villosa* EO (2.5 or 1.25 µL/mL) delayed the appearance of visible signs of infection caused by *A. niger*, *Botrytis cinerea*, *Penicillium expansum*, and *R. stolonifer* in tomato during cold and room temperature storage (Guerra et al., 2015). Tomato coated with CH (4 mg/mL) + oregano EO (1.25 µL/mL) presented no visible signs of infection caused by *R. stolonifer* and *A. niger* during 8 and 21 days of storage at room and cold temperature, respectively (Barreto et al., 2016). A coating formed by CH from *Mucor circinelloides* (3.75 mg/mL) + carvacrol (2.5 or 1.25 µL/mL) also inhibited the development of infection caused by *A. flavus* in tomato, as well as controlled the population of the autochthonous microflora in this fruit during 12 and 24 days of room and cold storage, respectively (de Souza et al., 2015).

Carvacrol and *trans*-cinnamaldehyde were tested for use into CH dispersions to control microbial population in blueberries. The concentrations of 0.2% and 0.5% of carvacrol and *trans*-cinnamaldehyde, respectively, were the most effective to inhibit bacteria and fungi populations in blueberries. Carvacrol at concentrations >0.2% was also effective to inhibit mesophilic bacteria (Sun et al., 2014). A coating containing CH (0.6%) + CS (1.6%) +

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EOs from two genotypes of *L. gracilis* (2.4%) decreased the counts of psychrophilic bacteria and fungi to <10 CFU/g in strawberry up to 17 days of cold storage (Azevedo et al., 2014). A coating containing CH (2%) + CS (2%) + EOs from two genotypes of *L. gracilis* (1% to 3%) was more effective to decrease the counts of mesophilic bacteria and fungi in guava than a coating containing only CH during 10 days of room temperature storage (de Aquino et al., 2015).

A study reported that coatings containing CH (1%) + bergamot EO (2%) or bitter orange EO (2%) were more effective to control *A. flavus* in Tunisian dates when compared to coatings containing locust bean gum (0.5%) + bergamot EO (2%) or bitter orange EO (2%) during 12 days of room temperature storage (Aloui et al., 2014). Another study reported that a coating containing CH (1%) + bergamot EO (2%) was more effective in reducing the counts of mesophilic bacteria and fungi in table grapes when compared with a coating containing hydroxypropylmethylcellulose (1%) + bergamot EO (2%) during 22 days of cold storage (Sánchez-González et al., 2011).

The application of coatings containing CH (5 or 7.5 mg/mL) + peppermint EO (0.3, 0.6 or 1.25 μL/mL) in mango (de Oliveira et al., 2017), as well as of coatings containing CH (5 mg/mL) + lemongrass EO (0.6 μL/mL) in guava, mango, and papaya (Oliveira et al., 2018), decreased the anthracnose lesion severity in these fruit during room temperature storage. A coating containing CH (1%, w/v) + clove EO (1 or 2 mL/L) sharply decreased the diameter of the lesions caused by *P. digitatum* in citrus when compared to CH alone during 17 days of cold storage (Shao et al., 2015).

A study reported that table grapes coated with CH (4 or 8 mg/mL) + peppermint EO or *M. villosa* EO (5, 2.5, or 1.25 μL/mL) displayed no visible signs of infection caused by *A. niger*, *B. cinerea*, *P. expansum*, or *R. stolonifer* during 24 days of cold storage. Furthermore, grapes coated with CH (8 or 4 mg/mL) + peppermint EO or *M. villosa* EO (5 μL/mL) displayed no visible signs of mold infection during 12 days of room temperature storage (Guerra et al., 2016). Two studies reported the efficacy of coatings containing CH from *Cunninghamella elegans* or *Mucor circinelloides* (3.75, 7.75, and 15 mg/mL) to control infection caused by *B. cinerea*, *P. expansum*, *A. niger*, and *R. stolonifer* in table grapes during room or cold storage (de Oliveira et al., 2014a). A coating formed by CH (1%) + oregano EO (2.5 and 5 μL/mL) also delayed the development of infection caused by *R. stolonifer* and *A. flavus* in table grapes at room or cold storage (dos Santos et al., 2012).

Coatings formed by pectin or sodium alginate (1% or 2%) + citral (0.15% or 0.3%) or eugenol (0.1% or 0.2%), as well by pectin or sodium alginate (1% or 2%) + citral (0.15%) + eugenol (0.1%), were capable of decreasing the molds and yeasts counts in raspberries at the end of 14 days of storage (0.5 °C; Guerreiro et al., 2015). A coating formed by pectin (3%) + oregano EO (36.1 g/L) was effective to inhibit the development of infection caused by *A. alternata* in tomato during 12 days of storage (28 °C) (Rodriguez-Garcia et al., 2016).

The results of studies that measured the antimicrobial effects of tested coatings on fruit stored under room and cold temperatures have conversely indicated that the inhibitory effects on target microorganisms are enhanced when the fruit are stored under cold temperature (Andrade et al., 2017; Barreto et al., 2016; de Oliveira et al., 2014a, 2014b; dos Santos et al., 2012; Guerra et al., 2015, 2016).

Physicochemical and sensory parameters measured in fruit

Fruit coated with polysaccharides and EOs or ICs have been evaluated concerning a variety of physicochemical parameters related to quality aspects of fruit when stored under room or cold temperature for different storage time intervals, to cite: RR (Cháfer et al., 2012; Perdones et al., 2012; Sánchez-González et al., 2011; Xing et al., 2016; Yousuf & Srivastava, 2017), weight loss (Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Cháfer et al., 2012; de Souza et al., 2015; dos Santos et al., 2012; Etemadipoor et al., 2019; Guerreiro et al., 2015; Sánchez-González et al., 2011; Xing et al., 2015; Yousuf & Srivastava, 2017), firmness (Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Cháfer et al., 2012; de Aquino et al., 2015; de Souza et al., 2015; dos Santos et al., 2012; Etemadipoor et al., 2019; Guerra et al., 2016; Guerreiro et al., 2015; Sun et al., 2014), pH (Athayde et al., 2016; Cháfer et al., 2012; de Aquino et al., 2015; Perdones et al., 2012; Yousuf & Srivastava, 2017), TSS (Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Cháfer et al., 2012; de Aquino et al., 2015; de Souza et al., 2015; dos Santos et al., 2012; Etemadipoor et al., 2019; Guerra et al., 2016; Guerreiro et al., 2015; Perdones et al., 2012; Sánchez-González et al., 2011; Yousuf & Srivastava, 2017), titratable acidity (Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Cháfer et al., 2012; de Souza et al., 2015; dos Santos et al., 2012; Etemadipoor et al., 2019; Guerra et al., 2016; Perdones et al., 2012; Xing et al., 2015; Yousuf & Srivastava, 2017), color (*L* value, chroma and hue angle; Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Cháfer et al., 2012; de Aquino et al., 2015; de Souza et al., 2015; dos Santos et al., 2012; Etemadipoor et al., 2019; Guerra et al., 2016; Guerreiro et al., 2015; Perdones et al., 2012; Sánchez-González et al., 2011; Yousuf & Srivastava, 2017), peroxidase activity (Xing et al., 2015, 2016), total phenolic compounds (Barreto et al., 2016; Rodriguez-Garcia et al., 2016; Sánchez-González et al., 2011; Xing et al., 2015), ascorbic acid (Athayde et al., 2016; Etemadipoor et al., 2019; Xing et al., 2011; Xing et al., 2015), antioxidant activity (Guerreiro et al., 2015; Sánchez-González et al., 2011; Rodriguez-Garcia et al., 2016), and chlorophyll and carotenoid content (Etemadipoor et al., 2019).

Fourteen out of the 30 selected studies compared the effects of the formulated coatings on the physicochemical parameters of fruit stored under room and cold temperatures (Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Cháfer et al., 2012; de Oliveira et al., 2014a, 2014b; de Souza et al., 2015; dos Santos et al., 2012; Gniewosz & Synowiec, 2011; Guerra et al., 2015, 2016; Maqbool et al., 2011; Sánchez-González et al., 2011; Sun et al., 2014). Ten studies measured the physicochemical parameters in coated fruit stored only under cold temperature (Azevedo et al., 2014; Etemadipoor et al., 2019; Guerreiro et al., 2015; Perdones et al., 2012; Vu et al., 2011; Xing et al., 2011; Xing et al., 2015, 2016; Yousuf & Srivastava, 2017), whereas six studies measured these parameters only in fruit stored under room temperature (Aloui et al., 2014; Bosquez-Molina et al., 2010; de Aquino et al., 2015; de Oliveira et al., 2017; Oliveira et al., 2018; Shao et al., 2015).

Respiration rate. RR is an important factor contributing to postharvest losses of fruit. However, only five studies evaluated the influence of the formulated coatings on fruit RR, which is reflected by O_2 and CO_2 concentrations. Coatings formed by CH (1%) + cinnamon EO (0.1%) decreased the O_2 concentration besides to increase the CO_2 concentration in "Brooks" sweet cherry fruit (*Prunus avium* L.), indicating a reduction in fruit RR

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and modification of gas concentration inside coated fruit during storage (Xing et al., 2016). However, RR of orange, measured by O_2 consumption and CO_2 production, was not affected by a coating formed by CH (1%) + bergamot (2%), thyme (2%), or tea tree EO (2%) (Cháfer et al., 2012). The same behavior was induced in pomegranate arils by a coating formed by flaxseed gum (0.3% or 0.6%) + lemongrass EO (200, 500, or 800 ppm; Yousuf & Srivastava, 2017).

Table grapes coated with CH (1%) + bergamot EO (2%) decreased O_2 consumption and CO_2 production during storage, which could be associated with a decreased gas permeability in tested coating (Sánchez-González et al., 2011). Another study found that a CH (1%) coating reduced gas permeability on strawberry surface, affecting gas exchange during fruit respiration. However, the incorporation of lemon EO (3%) in CH coating seemed to affect the metabolism of strawberries through the modification of their respiratory pattern (Perdones et al., 2012).

Weight loss. Fourteen studies measured the weight loss in fruit coated with polysaccharides and EOs or ICs, being overall observed lower weight losses in coated fruit during storage (Athayde et al., 2016; Azarakhsh et al., 2014; Barreto et al., 2016; Cháfer et al., 2012; de Oliveira et al., 2014a, 2014b; de Souza et al., 2015; Etemadipoor et al., 2019; Guerra et al., 2015, 2016; Maqbool et al., 2011; Sánchez-González et al., 2011; Xing et al., 2015; Yousuf & Srivastava, 2017). Only two studies reported no decrease in weight loss of grapes coated with CH (5 mg/mL) + oregano EO (5 and 2.5 μL/mL) (dos Santos et al., 2012) and raspberries coated with alginate or pectin (1% or 2%) + citral (0.15% or 0.3%) + eugenol (0.1% or 0.2%) during storage (Guerreiro et al., 2015).

Firmness. Twelve studies evaluated the firmness of coated fruit using experimental probes (Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; Cháfer et al., 2012; de Aquino et al., 2015; de Oliveira et al., 2014a, 2014b; de Souza et al., 2015; dos Santos et al., 2012; Guerra et al., 2016; Guerreiro et al., 2015). Plums coated with GA (1 mg/mL) + oregano EO (0.06 or 0.25 μL/mL) and/or rosemary EO (0.25 μL/mL) presented improvements in firmness maintenance during storage (Andrade et al., 2017). The same behavior was reported in grapes coated with CH (4 mg/mL) + *M. villosa* EO (2.5 or 5 μL/mL; Guerra et al., 2016). One study reported no improvements in firmness of guava coated with CH (2%) + CS (2%) + *L. gracilis* EO (3%; de Aquino et al., 2015), as well as of raspberries coated with pectin or sodium alginate (1% or 2%) + citral (0.15% or 0.3%) + eugenol (0.1% or 0.2%) during storage (Guerreiro et al., 2015).

pH, titrable acidity, and TSS. Six studies measured the pH values in coated fruit, 12 measured the titrable acidity (TA), and 15 the TSS contents. Five studies observed no effects in pH values over time in fruit coated with polysaccharides and EOs or ICs (Athayde et al., 2016; Cháfer et al., 2012; Etemadipoor et al., 2019; Perdones et al., 2012; Sánchez-González et al., 2011). One study reported a lower decrease in pH values over time in coated fruit when compared to uncoated fruit (de Aquino et al., 2015).

Six out of the 12 studies that measured TA observed reductions in TA values in fruit during storage regardless the coating application (Cháfer et al., 2012; de Souza et al., 2015; Etemadipoor et al., 2019; Guerra et al., 2015, 2016; Xing et al., 2015), whereas three studies observed increases in TA values in fruit during storage regardless the coating application (Barreto et al., 2016; Maqbool et al., 2011; Perdones et al., 2012). Two studies reported decreased TA values over time in coated fruit when compared to uncoated fruit (Athayde et al., 2016; de Aquino et al., 2015).

Only two out of the 15 studies that measured TSS in fruit reported decreased TSS values in coated fruit when compared to uncoated fruit over time (de Aquino et al., 2015; dos Santos et al., 2012). One study reported no alterations in TSS of raspberry coated with alginate or pectin + eugenol and/or citral during storage, being observed similar results for uncoated fruit (Guerreiro et al., 2015). The other studies reported similar decrease (Andrade et al., 2017; Barreto et al., 2016; Cháfer et al., 2012; Etemadipoor et al., 2019; Guerra et al., 2015, 2016) or increase of TSS (Athayde et al., 2016; de Souza et al., 2015; Maqbool et al., 2011; Perdones et al., 2012; Sánchez-González et al., 2011) over time in coated and uncoated fruit.

Enzymatic activity, phenolics, vitamin C, chlorophyll, and carotenoids. Only three studies investigated the activity of enzymes in fruit coated with polysaccharides and EOs or ICs. Some coatings exerted antioxidant properties in fruit, which were demonstrated through the inhibition of peroxidase enzyme activity in coated fruit during storage. China jujube fruit coated with CH (1%) + cinnamon EO (0.1%) showed lowest activity of polyphenol oxidase (PPO). The superoxide dismutase (SOD) activity was also decreased in fruit coated with CH + cinnamon EO (Xing et al., 2016). A coating formed by CH (1%) + clove EO (0.5 mL/L) increased the activity of phenylalanine ammonia-lyase (PAL) in citrus fruit (Shao et al., 2015).

Four studies measured the ascorbic acid contents in fruit. Only one study showed increases in ascorbic acid content in coated fruit over time (Etemadipoor et al., 2019). Conversely, the other studies reported a decrease in ascorbic acid contents over time in fruit regardless the coating application, although the decreases were always lower in coated than in uncoated fruit (Cháfer et al., 2012; Xing et al., 2011; Xing et al., 2015). The four studies that measured individual phenolic compounds reported increased contents of specific flavonoids and phenolic acids in coated fruit in comparison with uncoated fruit at the end of the measured storage time interval (Andrade et al., 2017; Barreto et al., 2016; Rodriguez-Garcia et al., 2016; Xing et al., 2015). One study measured the chlorophyll and carotenoids contents in coated fruit, being reported decrease and increase in chlorophyll and carotenoid contents in coated fruit, respectively (Etemadipoor et al., 2019).

Instrumental color and sensory parameters. Twelve studies measured the instrumental color of coated fruit using the CIELab system (Andrade et al., 2017; Cháfer et al., 2012; de Aquino et al., 2015; de Oliveira et al., 2014a, 2014b; de Souza et al., 2015; dos Santos et al., 2012; Etemadipoor et al., 2019; Guerra et al., 2016; Perdones et al., 2012; Sánchez-González et al., 2011) and only two studies used the HunterLab Colorimeter system to measure the color in coated fruit (Guerreiro et al., 2015; Yousuf & Srivastava, 2017). Overall, the coating application caused improvements in lightness/brightness ($L*$) of fruit, with no effects on the other measured color attributes. Delayed alterations (green to yellow) in instrumental color observed in coated fruit over time have been associated with lower decrease and increase in chlorophyll and carotenoid contents, respectively (Etemadipoor et al., 2019).

Thirteen studies performed sensory analysis in fruit. Twelve studies used a point-based hedonic scale to categorize the analyzed sensory parameters (Andrade et al., 2017; Athayde et al., 2016; Barreto et al., 2016; dos Santos et al., 2012; Guerra et al., 2015, 2016; Guerreiro et al., 2015; Perdones et al., 2012; Rodriguez-Garcia et al., 2016; Xing et al., 2015; Xing et al., 2011; Yousuf & Srivastava, 2017) and one study used a sensory profile method (Aloui et al., 2014). Overall, most of the studies reported that the coating formed by polysaccharides and EOs or ICs did not

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negatively influence the sensory aspects (for example, appearance, color, odor, taste, texture, and overall acceptance) and intention to purchase of fruit.

One study reported that a coating formed by GA (1 mg/mL) + oregano EO (0.25 μL/mL) affected negatively the aftertaste of plums, while the coating formed by GA + 0.06 μL/mL oregano EO + 0.25 μL/mL rosemary EO enhanced the color and flavor of plums. Another study reported that different coatings formed by pectin (3%) + oregano EO (15.7, 25.9, or 36.1 g/L) caused no negative impacts on odor acceptability of tomato during storage. Specifically, the coating formed by pectin + 15.7 g/L oregano EO increased the flavor acceptability of tomato during storage (Rodriguez-Garcia et al., 2016).

One study observed that raspberries coated with alginate or pectin (1% or 2%) + eugenol (0.1% or 0.2%) or citral (0.15% or 0.3%) presented low appearance acceptance on the 14th day of storage. The authors reported that this low acceptance in coated raspberries could be a consequence of the high weight loss observed in these fruit on the 14th day of storage, because high weight loss should negatively affect the appearance of fresh fruit (Guerreiro et al., 2015).

Discussion

In the last years, the development of edible coatings has been a research focus with a view of increasing the shelf life of fresh fruit through a partial or total replacement of chemical fungicides traditionally used to this end (Azevedo et al., 2014; de Aquino et al., 2015; Elsabee & Abdou, 2013). A number of studies (30 selected in this review) have been published in previous years approaching the formulation of edible coatings formulated with polysaccharides and EOs or ICs, being commonly reported as a strategy to minimize the water insolubility of some film-forming polymers (Oliveira et al., 2018; Xu, Zhao, Wang, Zhao, & Du, 2007; Yousuf & Srivastava, 2017), as well as to potentiate the desired functionalities of these coatings when applied on fruit (Andrade et al., 2017; Barreto et al., 2016; Maqbool et al., 2011).

This study is the first systematic review of research assessing the potential benefits and possible limitations associated with the application of edible coatings formed by polysaccharides and EOs or ICs to preserve fresh fruit, with an additional analysis of possible factors that could influence the properties of these coatings concerning the preservation of quality characteristics in coated fruit. Indeed, the data of the retrieved studies support the efficacy of these coatings to control postharvest losses associated with microbial infection and spoilage in fruit, as well as to maintain the overall quality of these products during storage.

Most of the retrieved studies have used fungi as target organism to assess the efficacy of tested coatings to control postharvest disease in fruit. This fact should be related to the knowledge that fungi (for example, *Penicillium*, *Aspergillus*, *Geotrichum*, *Botrytis*, *Fusarium*, *Alternaria*, *Rhizopus*, *Colletotrichum*, and *Lasiodiplodia*) are the most important causal agents of postharvest diseases in fruit worldwide (Spadaro & Droby, 2016; Usall, Torres, & Teixidó, 2016). Further investigations on the effects of coatings formed by polysaccharides and EOs or ICs on bacteria species characterized as important causal agents of postharvest diseases in fruit should be important to reveal possible additional functionalities and wide the potential applications of these coatings.

CH has been the polysaccharide most frequently studied for the formulation of edible coatings to be used on fruit. CH is a deacetylated derivative of chitin, comprising units of 2-amino-2-desoxy-D-glycopyranose and 2-acetamide-2-desoxy-D-glycopyranose interconnected by glycosidic β-1,4 bonds in variable proportions (Berger et al., 2014; de Oliveira et al., 2014a). The use of CH-based coatings in fruit has been supported by an array of properties presented by this polymer, to cite: capacity to form films, biodegradability, emulsifying properties, aesthetic appearance, wide-spectrum antimicrobial properties, and selective permeability to gases (CO_2 and O_2; Azevedo et al., 2014; Elsabee & Abdou, 2013; Sánchez-González et al., 2011).

The antimicrobial action of CH has been typically associated with its ability to cause extravasation of proteinaceous and other intracellular constituents from target microorganisms, because of the interaction between positively charged CH molecules and negatively charged microbial cell membranes (Jung, Kim, Choi, Lee, & Kim, 1999; Tayel, Gharieb, Zaki, & Elguindy, 2016). The ability to penetrate the nuclei and bind to the DNA, causing the inhibition of the mRNA synthesis in target microorganisms, has been also cited as a potential mechanism underlying the antimicrobial effects of CH (Sudarshan, Hoover, & Knorr, 1992; Tayel et al., 2016).

The MW is one of the factors that may affect the bioactivities of CH. Most of the available studies have used medium MW (MMW) CH to formulate coatings. Although it has been reported that low MW (LMW) CH can exert stronger antimicrobial effects than MMW and high MW (HMW) CH (Jing et al., 2007; Tsai, Zhang, & Shieh, 2004), the use of MMW CH has been commonly justified because of its ability to form dispersions when solubilized in an acidic solution (pH 5.6) with a such viscosity that enable the formation of a thin and translucent self-assembled coating when applied on fruit.

LMW CH at the doses typically found as effective to inhibit phytopathogenic microorganisms commonly fails to provide dispersions with viscosity enough to form a coating when applied on fruit. In turn, HMW CH provides high viscous dispersions that form thick and opaque self-assembled coatings when applied on fruit (Hosseinnejad & Jafari, 2016). Still, the deacetylation degree (DD) of CH used in the retrieved studies has always been >70%. DD is a physicochemical characteristic also associated with the antimicrobial properties of CH, because DD directly affects the solubility and charges (cationic properties) of this polymer (Andres, Giraud, Gerente, & Le Cloirec, 2007; de Oliveira et al., 2014a, 2014b; Tsai et al., 2004). The higher is the DD, the higher is the number of free amino groups ($-NH_2$) in CH molecule. These free amino groups can interact with the negatively charged OH^- groups present on the membranes of target microorganisms, resulting in enhanced antimicrobial effects (Andres et al., 2007; Tsai, Su, Chen, & Pan, 2002).

Only three studies have used fungal CH, particularly from *C. elegans* and *M. circinelloides* (Zygomycetes class members), to formulate coatings. These studies reported that fungal CH should have some advantages in comparison to crustacean CH (the most traditional commercial source of CH), such as the noninfluence of seasonal factors and absence of proteins involved in human allergic reactions to crustaceans, in addition to be amenable to large-scale production (de Oliveira et al., 2014a, 2014b; de Souza et al., 2015). However, the effective doses of fungal CH (3.75 to 7.5 mg/mL) toward target pathogens in fruit have been overall similar to those reported to crustacean CH (1 to 5 mg/mL); these studies have produced fungal CH with MMW and/or DD of 75%. Thus, the origin of the CH *per se* should not be a factor to influence the antimicrobial properties of this polymer.

Although the available studies have used different procedures to prepare the coatings formed by polysaccharides and EOs or ICs,

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specifically considering variable stirring time, exposure to room or warm temperature, use or not of stabilizing agents (Tween 20 or 80) to homogenize EOs or ICs into the polysaccharides dispersions, as well as the use or not of plasticizers agents (glycerol or glycol), there is no clear information about the influence of these agents/conditions on the functionalities of the formulated coatings when applied on fruit.

Only five of the selected studies used ICs (carvacrol, cinnamaldehyde, eugenol, citral, *trans*-cinnamaldehyde, eucalyptol, thymol, and limonene) to formulate coatings to be applied on fruit (de Souza et al., 2015; Gniewosz & Synowiec, 2011; Guerreiro et al., 2015; Sun et al., 2014; Vu et al., 2011), whereas a total of 16 different EOs have been used to formulate coatings in the other 21 studies. These data reveal that EOs have been considered to formulate coatings in combinations with polysaccharides to be used on fruit rather than ICs.

Other polysaccharides, namely, GA (10%), flaxseed gum (0.3% and 0.6%), mesquite gum (10%), locust bean gum (0.5%), hydroxypropylmethylcellulose (1%), pectin (1% to 2%), sodium alginate (1% to 2%), CS (0.4% to 2%) and pullulan (5%), were used in few studies to formulate coatings. These polysaccharides or gums typically have a complex chemical structure being typically formed by residues of D-galacturonic acid, L-rhamnose, L-arabinose, D-galactose, D-xylose, L-fucose, D-mannose, and/or D-glucose (Campos et al., 2011). The use of these polysaccharides or gums has been justified because of their natural origin, previous use in industrial sector (for example, as encapsulating and emulsifying materials), low toxicity, and mostly the abilities to form films. These polysaccharides or gums are commonly reported to exert no antimicrobial effects, being used as film-forming materials to be combined with EOs or ICs during the coating formulation.

The available data have consistently shown that coatings formed by flaxseed gum, GA, hydroxypropylmethylcellulose, locust bean gum, mesquite gum, pectin, sodium alginate, or pullulan in combination with EOs or ICs are effective to inhibit pathogenic microorganisms besides to maintain or improve quality parameters in coated fruit. The antimicrobial effects of these coatings have been clearly attributed to the presence of EOs or ICs (namely, cinnamon EO and oregano EO + rosemary EO) in their composition (Andrade et al., 2017; Maqbool et al., 2011). The selection of the amounts of these polysaccharides or gums used in coatings formulation has mostly considered their amounts capable of providing dispersions with adequate viscosity to form thin and translucent self-assembled coatings on fruit (Andrade et al., 2017; Maqbool et al., 2011).

In addition to improve the functionalities of the polysaccharides -based coatings, many studies have shown that EOs or ICs alone exert antimicrobial effects toward a variety of fruit pathogens. Some mechanisms have been reported to achieve these inhibitory effects on target microorganisms, such as the disorganization of cell membrane structures, inducing depolarization, physical and/or chemical alterations, and disturbance of enzymatic systems and metabolic activities (Atarés & Chiralt, 2016; Shao et al., 2015; Xing et al., 2016). These effects are related not only to the action of the most prevalent ICs in the tested EOs but also to the action of ICs present in minor amounts, which could likely act through synergistic interactions to achieve the observed antimicrobial effects (de Oliveira et al., 2017; Oliveira et al., 2018).

Some studies have reported that the inhibitory effects caused by CH and EOs or ICs on fruit pathogens are higher when these substances are tested in combination rather than when tested alone (de Oliveira et al., 2017; Oliveira et al., 2018). The evaluation of the type of interaction between different concentrations of CH and EOs against pathogenic fungi has been performed through the determination of the Abbott index (Kosman & Cohen, 1996), indicating synergistic or additive interaction. These results overall evidence the possibility of possible reductions in the doses of CH and EOs required in the formulation of the coatings to achieve the desired inhibitory effects on target pathogens in fruit.

The results of these studies indicated that the combination of CH and EOs concentrations that cause high inhibition rates toward the target fungi when acting alone could increase the possibility to reach an additive rather than a synergistic interaction (de Oliveira et al., 2017; Kosman & Cohen, 1996; Oliveira et al., 2018). Most of the retrieved studies have not evaluated the type of interaction achieved by CH and EOs, although commonly is reported the occurrence of a "like-synergistic" interaction from the combination of these substances. Indeed, the use of reliable methods to determine the type of interactions between substances with antimicrobial properties used in the formulation of coatings to control postharvest infections in fruit should be considered to avoid misunderstanding of the obtained data (detailed methods can be seen in Kosman & Cohen, 1996).

The mechanisms underlying the enhanced antimicrobial effects, as occur in additive or synergistic interactions, from the combined use of CH and EOs have been associated with the ability of CH to alter the permeability of microbial membranes and reduce the synthesis of cell wall components, causing decreased ability of target microorganisms to tolerate the disturbing effects on surface characteristics and microbial cell structure caused by EOs (Athayde et al., 2016; de Oliveira et al., 2017; dos Santos et al., 2012; Oliveira et al., 2018). A few studies have proposed that the efficacy of coatings formed by CH and EOs or ICs in inhibiting fruit pathogens could be related to their eliciting properties, which could induce increased production of specific compounds and defense-related enzymes that enhance the resistance of fruit tissues to the action of pathogens (Barreto et al., 2016; Mohammadi, Hashemi, & Hosseini, 2016; Shao et al., 2015; Xing et al., 2011).

Only few studies have also compared the effects exerted by the formulated coatings chemical fungicides commonly used to control postharvest disease on microorganisms of concern in a specific fruit (de Oliveira et al., 2017; Oliveira et al., 2018). The results of these studies have conversely shown that synergistic or additive combined concentrations of CH and EOs caused overall similar or higher inhibition of postharvest disease development (specifically of anthracnose) in coated fruit (mango, guava, and papaya) in comparison with tested chemical fungicides (thiophanate-methyl 10 μg a.i./mL and difenoconazole 0.5 μg a.i./mL). Most of the retrieved studies only use a negative control (distilled sterile water or distilled sterile water with glycerol) to be compared with the tested coating formulations.

Similarly, few studies have used target pathogens (fungi) isolated from fruit presenting characteristic symptoms of a postharvest disease of concern (Bosquez-Molina et al., 2010; de Oliveira et al., 2017; Maqbool et al., 2011; Oliveira et al., 2018; Shao et al., 2015; Vu et al., 2011). Although the other studies have commonly used typed isolates, these isolates were not characterized as being causal agents of postharvest disease in fruit. The use of chemical fungicides as positive controls, as well as of well-characterized target isolates associated with the development of postharvest disease in fruit, should be considered in further studies. Indeed, the use of these isolate types should more appropriately indicate the potential practical use of tested coatings to inhibit fruit colonizing microorganisms and postharvest disease development.

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The retrieved studies have consistently reported that the antimicrobial effects exerted by coatings formulated with polysaccharides and EOs or ICs on fruit are higher when the fruit are stored under cold temperature (Aloui et al., 2014; Azevedo et al., 2014; Barreto et al., 2016; de Oliveira et al., 2014a, 2014b; dos Santos et al., 2012; Guerra et al., 2015, 2016; Shao et al., 2015). Storage at cold temperatures slows physiological processes in fruit and pathogens have weaker pathogenicity at low temperatures, resulting in decreased incidence of infection and decay compared with fruit stored at room temperature, which rapidly decay. The suppression of fruit decay by tested coatings could also partially reflect a delay in senescence of coated fruit, as resistance to infection in fruit might be enhanced through slower senescence (Aloui et al., 2014; Azevedo et al., 2014; Barreto et al., 2016; de Oliveira et al., 2014a, 2014b; dos Santos et al., 2012; Guerra et al., 2015, 2016; Shao et al., 2015).

Still, differences in the results observed for the inhibition of target pathogens in fruit induced by tested coatings could be associated with the influence of fruit structure and composition on fungal–host interaction in each fruit and, consequently, on fungal pathogenicity, affecting the efficacy of antifungal substances to inhibit fruit colonizing fungi (Lima et al., 2015; Oliveira et al., 2018). The information presented in Table 4 summarizes the reported antimicrobial action modes of polysaccharides, EOs, and ICs used in the formulation of edible coatings applied on fruit in retrieved studies.

The studies that evaluated physicochemical parameters reported that fruit coated with polysaccharides and EOs or ICs had overall improvements or maintenance of their overall quality during the measured storage period. Decreased weight and firmness loss are among the most common characteristics verified in coated fruit, which have been primarily associated with decreased water loss as a consequence of lowered transpiration and respiration in fruit (Amarante, Banks, & Ganesh, 2001; Gao, Zhu, & Zhang, 2013).

Additionally, decreased firmness loss in coated fruit during storage has also been related to delayed changes in cell wall strength and cell-to-cell adhesion because reduced pectin degradation (Ali, Maqbool, Ramachandran, & Alderson, 2010; Andrade et al., 2017). Overall, the protective effects of tested coatings regarding decreased weight and firmness losses in fruit have been more remarkable in fruit stored under room temperature rather than under cold temperature (Aloui et al., 2014; Azevedo et al., 2014; Barreto et al., 2016; de Oliveira et al., 2014a, 2014b; dos Santos et al., 2012; Guerra et al., 2015, 2016; Shao et al., 2015).

Tested coatings are reported to form a protective physical barrier on fruit capable of inducing reduced respiration and transpiration, resulting in delayed ripening and senescence (Hong, Xie, Zhang, Sun, & Gong, 2012). Available literature has consistently showed decreased RR and modification of gas concentrations (O_2 consumption and CO_2 production) inside fruit coated with polysaccharides and EOs or ICs, as a consequence of lowered gas permeability in tested coatings (Cháfer et al., 2012; Guerra et al., 2015, 2016; Xing et al., 2016; Yousuf & Srivastava, 2017). These coatings should affect fruit metabolism through the alteration of their respiratory pattern (Perdones et al., 2012).

Results of pH values, TA, and TSS contents in fruit coated with polysaccharides and EOs or ICs have been variable. However, most of these studies have reported that pH, TA, and TSS values decreased in coated fruit over time accompanying alterations in other parameters related to fruit maturation. Decreased pH, TA, and TSS in coated fruit have been also linked to decreased ethylene production and metabolic activity and delayed maturation (Cháfer et al., 2012; de Aquino et al., 2015; dos Santos et al., 2012).

Coatings formed by polysaccharides and EOs or ICs were also able to increase or delay losses of some flavonoids and phenolic acids, as well as of ascorbic acid in fruit during storage, in addition to delay the decrease and increase of chlorophyll and carotenoid contents, respectively. The coating application can generate an internally modified atmosphere that affects directly the synthesis and catabolism of phenolic compounds (as secondary metabolites) and ascorbic acid in fruit during maturation and storage (Andrade et al., 2017; Barreto et al., 2016; Xing et al., 2015). The reported impacts on fruit phenolic profile and ascorbic acid contents in coated fruit should be an additional consequence of the delayed metabolic activity and maturation (Andrade et al., 2017; Barreto et al., 2016). Decreased ascorbic acid loss could be also associated with decreased O_2 contents in coated fruit, causing reduced ascorbic acid oxidation (Xing et al., 2015).

Furthermore, there has been evidence that coatings containing polysaccharides and EOs or ICs can exert antioxidant properties in fruit, which were demonstrated through the inhibition of enzymes (peroxidase, PPO, and SOD) involved in antioxidant defense in fruit (Xing et al., 2016). Coated citrus fruit also presented increased activity of PAL, which exerts a key role in plant defense (Shao et al., 2015). The detection of increased enzymatic (PAL) activity, as well as of greater contents of phenolic compounds and ascorbic acid in coated fruit, was considered indicative of the action of tested coatings as abiotic elicitors, stimulating secondary metabolites pathways and improvements in defense mechanisms of fruit (Andrade et al., 2017; Kim & Hwang, 2014; Xing et al., 2011; Xing et al., 2015).

A remarkable characteristic in fruit coated with polysaccharides and EOs or ICs has been an improvement in lightness/brightness as measured by instrumental techniques, as well as by sensory analysis using panelists. Higher luminosity has been also reported for coated fruit, which could be associated with the characteristic luminosity and high transparency of coatings formed by polysaccharides and EOs or ICs (de Oliveira et al., 2014a; Guerra et al., 2015, 2016). The high lightness/brightness in fruit could be a consequence of the oxygen barrier created by these coatings because oxygen participates actively in biochemical processes involved in fruit darkening (Pastor et al., 2011). This is an important finding of the retrieved studies because color is perceived as one of the most important quality parameters of fresh fruit, affecting the purchase decision of consumers. The information presented in Table 5 summarizes the overall positive effects caused by coatings formed by polysaccharides and EOs or ICs on physicochemical characteristics of fruit reported in retrieved studies.

The application of coatings formed by polysaccharides and EOs or ICs overall did not influence positively or negatively the sensory characteristics of fruit during storage. These findings are noteworthy as the different tested coatings showed strong inhibitory effects against postharvest pathogens and autochthonous microflora in fruit, with no noticeable unsatisfactory effects on most of the sensory properties (for example, appearance, color, odor, taste, texture, and overall acceptance) and intention to purchase of fruit. Furthermore, a concern reported in literature specifically about the use of coatings containing CH is their potential negative impacts on fruit sensory acceptance due to the development of an acidic or even slightly bitter and astringent taste due

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Table 4–Antimicrobial action modes of polysaccharides, essential oils, and individual constituents used in the formulation of edible coatings applied on fruit in retrieved studies.

Substances/compounds	Reported antimicrobial action modes	References
Essential oils and their individual constituents	Inhibition of the biosynthesis phospholipids and sterols Disorganization of cell membrane structures Disturbance of cell membrane permeability Loss of intracellular components Disturbance of enzymatic activity	de Oliveira et al. (2017), Oliveira et al. (2018) Rodriguez-Garcia et al. (2016) de Souza et al. (2015) Shao et al. (2015) Azevedo et al. (2014) dos Santos et al. (2012) Gniewosz and Synowiec (2011)
Chitosan	Disturbance of cell membrane permeability Loss of intracellular components Damage in cell structure Disturbance of DNA/RNA synthesis	Oliveira et al. (2018) de Oliveira et al. (2017) Shao et al. (2015) dos Santos et al. (2012) Xing et al. (2011) Kong, Chen, Xing, and Park (2010)
Gum arabic, pullulan, flaxseed gum, hydroxypropylmethylcellulose, locust bean gum, mesquite gum, pectin, and sodium alginate	None	Etemadipoor et al. (2019) Andrade et al. (2017) Yousuf and Srivastava (2017) Rodriguez-Garcia et al. (2016) Guerreiro et al. (2015) Aloui et al. (2014) Gniewosz and Synowiec (2011) Maqbool et al. (2011) Sánchez-González et al. (2011) Bosquez-Molina et al. (2010)

Table 5–Positive effects caused by coatings formed by polysaccharides, essential oils, or individual constituents on physicochemical characteristics of fruit reported in retrieved studies.

Reported effects on coated fruit	References
Delayed weight loss	Etemadipoor et al. (2019), Andrade et al. (2017), Yousuf and Srivastava (2017), Barreto et al. (2016), Guerra et al. (2016), de Souza et al. (2015), Guerra et al. (2015), Xing et al. (2015), Cháfer et al. (2012), Maqbool et al. (2011), and Sánchez-González et al. (2011)
Delayed firmness loss	Etemadipoor et al. (2019), Andrade et al. (2017), Barreto et al. (2016), Guerra et al. (2016); de Souza et al. (2015), Guerreiro et al. (2015), Sun et al. (2014), Maqbool et al. (2011), and Sánchez-González et al. (2011)
Delayed decrease of titratable acidity	Andrade et al. (2017), Barreto et al. (2016), de Aquino et al. (2015), de Souza et al. (2015);, Xing et al. (2015), and Maqbool et al. (2011)
Increased amounts of phenolic compounds	Andrade et al. (2017), Barreto et al. (2016), and Rodriguez-Garcia et al. (2016)
Decreased polyphenol-oxidase activity	Xing et al. (2016) and Xing et al. (2015)
Delayed decrease in total soluble solid contents	Andrade et al. (2017), Barreto et al. (2016), de Souza et al. (2015), dos Santos et al. (2012), and Maqbool et al. (2011)
Decreased respiration rate	Xing et al. (2015), Perdones et al. (2012), and Sánchez-González et al. (2011)
Delayed decrease in ascorbic acid content	Xing et al. (2015) and Xing et al. (2011)

to an increase in protonated amine groups related to the use of diluted acids to dissolve this polymer (dos Santos et al., 2012; Perdones et al., 2012). However, the retrieved studies have reported overall no negative impacts of coatings containing CH on taste of fruit. No possible negative impacts of the other polysaccharides tested in these studies have been reported in available literature.

Probably, the potential negative effects of the application of EOs or ICs in sensory aspects of foods reported in literature (Bosquez-Molina et al., 2010; Cháfer et al., 2012; Munhuweyi, Caleb, Lennox, van Reenen, & Opara, 2017) were diminished because of the common strategy used in selected studies of combining low doses of EOs or ICs with polysaccharides in coating formulation. This strategy could have minimized possible impacts on coated fruit sensory characteristics, in addition to exert desirable effects on physicochemical and microbiological characteristics of these products.

Although the literature has demonstrated an effort to develop edible coatings formed by polysaccharides and EOs or ICs with a variety of functionalities that could enable their use to control fruit postharvest decay, the commercial application of these coatings could be hindered mainly because the availability of convenient, effective, and relatively inexpensive chemical fungicides (Fagundes, Pérez-Gago, Monteiro, & Palou, 2013). Furthermore, commercial postharvest treatment to control fruit decay ordinarily demands a very high efficacy (>90% disease reduction; Fagundes, Palou, Monteiro, & Pérez-Gago, 2014), which could be difficult to achieve with the use of some edible coatings formulated with polysaccharides and EOs or ICs presenting together low toxicity to fruit and strong and wide spectrum antimicrobial properties.

Considering that the functionalities of these coatings are influenced by the characteristics of ingredients used for their formulation, as well as by characteristics of coated fruit and target organisms, the development of edible coatings composed of polysaccharides and EOs or IC could be directed for a specific fruit pathosystem, which could narrow the spectrum of application of these treatments, but increase the probability of successful achievement of the expected fruit decay control. However, the combined use of these coatings with other postharvest treatments in an integrated strategy to control fruit decay could also widen the possibilities for commercial applications.

Conclusion

The focus of this review considered the research focusing on the use of coatings formulated with polysaccharides and EOs or ICs as strategy for decreasing the postharvest losses of fresh fruit, which are mostly associated with microbial infection that accelerates fruit senescence and decay. Data of available literature demonstrate

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evidence that coatings formed by polysaccharides and EOs or ICs are effective to control or reduce the deleterious effects of postharvest disease in fresh fruit. CH is the most common polysaccharide used to formulate these coatings and the only polysaccharide used also as an antimicrobial ingredient in coating formulation, whereas the other polysaccharides (CS, flaxseed gum, GA, hydroxypropylmethylcellulose, locust bean gum, mesquite gum, pectin, pullulan, and sodium alginate) are used only because of their film forming properties. Various EOs and few ICs have been studied in combination with polysaccharides to formulate the coatings, which have shown effective to inhibit a variety of postharvest pathogens in different fruit stored under room or low temperatures. The protective effects of these coatings have been more remarkable in fruit stored under low temperatures when compared to fruit stored under room temperature. Overall, fruit coated with polysaccharides and low antimicrobial effective doses of EOs or ICs are not adversely affected in their physicochemical and sensory parameters during storage.

Current findings warrant the potential of coatings formed by polysaccharides and EOs or ICs as innovative technologies to prevent microbial infections and reduce postharvest losses in fresh fruit. In addition to the improvements of the functionalities of the coatings already investigated in retrieved studies and development of new effective coating compositions, further studies should be directed to evaluate the duration of these coatings on fruit surface, as well as their effects toward well-characterized isolates of pathogens (fungi and bacteria) associated with postharvest diseases in economically important fruit and compare these effects with those exerted by different synthetic fungicides traditionally used to combat these diseases in such fruit, because only still few studies have been performed using this approach. Finally, studies to assess the ability of coatings comprising polysaccharides and EOs or ICs to induce increased antimicrobial resistance in multiple species of pathogens associated with postharvest loss in agricultural commodities are needed.

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Author Contributions

ELS, LRRB, and MM conceptualized the study and participated in its design. LRRB, KARO, and GAL analyzed the data and prepared the first draft of the manuscript. ELS, GAL, and MM wrote the final manuscript.

References

Ali, A., Maqbool, M., Ramachandran, S., & Alderson, P. G. (2010). Gum arabic as a novel edible coating for enhancing shelf-life and improving postharvest quality of tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biology and Technology*, *58*(1), 42–47. https://doi.org/10.1016/j.postharvbio.2010.05.005

Aloui, H., Khwaldia, K., Licciardello, F., Mazzaglia, A., Muratore, G., Hamdi, M., & Restuccia, C. (2014). Efficacy of the combined application of chitosan and locust bean gum with different citrus essential oils to control postharvest spoilage caused by *Aspergillus flavus* in dates. *International Journal of Food Microbiology*, *170*, 21–28. https://doi.org/10.1016/j.ijfoodmicro.2013.10.017

Amarante, C., Banks, N. H., & Ganesh, S. (2001). Relationship between character of skin cover of coated pears and permeance to water vapour and gases. *Postharvest Biology and Technology*, *21*(3), 291–301. https://doi.org/10.1016/S0925-5214(00)00176-9

Anderson, D. M. W. (1986). Evidence for the safety of gum arabic (*Acacia Senegal* (L.) Willd.) as a food additive: A brief review. *Food Additives and Contaminants*, *3*(3), 225–230. https://doi.org/10.1080/02652038609373584

Andrade, S. C. A., Baretto, T. A., Arcanjo, N. M. O., Madruga, M. S., Meireles, B., Cordeiro, Â. M. T., . . . Magnani, M. (2017). Control of Rhizopus soft rot and quality responses in plums (*Prunus domestica* L.) coated with gum arabic, oregano and rosemary essential oils. *Journal of Food Processing and Preservation*, *41*(6), e13251. https://doi.org/10.1111/jfpp.13251

Andres, Y., Giraud, L., Gerente, C., & Le Cloirec, P. (2007). Antibacterial effects of chitosan powder: Mechanisms of action. *Environmental Technology*, *28*(12), 1357–1363. https://doi.org/10.1080/09593332808618893

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in Food Science & Technology*, *48*, 51–62. https://doi.org/10.1016/j.tifs.2015.12.001

Athayde, A. J. A. A., de Oliveira, P. D. L., Guerra, I. C. D., da Conceição, M. L., de Lima, M. A. B., Arcanjo, N. M. O., . . . de Souza, E. L. (2016). A coating composed of chitosan and *Cymbopogon citratus* (Dc. Ex Nees) essential oil to control Rhizopus soft rot and quality in tomato fruit stored at room temperature. *The Journal of Horticultural Science and Biotechnology*, *91*(6), 582–591. https://doi.org/10.1080/14620316.2016.1193428

Azarakhsh, N., Osman, A., Ghazali, H. M., Tan, C. P., & Mohd Adzahan, N. (2014). Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. *Postharvest Biology and Technology*, *88*, 1–7. https://doi.org/10.1016/j.postharvbio.2013.09.004

Azevedo, A. N., Buarque, P. R., Cruz, E. M. O., Blank, A. F., Alves, P. B., Nunes, M. L., & Santana, L. C. L. de A. (2014). Response surface methodology for optimisation of edible chitosan coating formulations incorporating essential oil against several foodborne pathogenic bacteria. *Food Control*, *43*, 1–9. https://doi.org/10.1016/j.foodcont.2014.02.033

Barreto, T. A., Andrade, S. C. A., Maciel, J. F., Arcanjo, N. M. O., Madruga, M. S., Meireles, B., . . . Magnani, M. (2016). A chitosan coating containing essential oil from *Origanum vulgare* L. to control postharvest mold infections and keep the quality of cherry tomato fruit. *Frontiers in Microbiology*, 7, 1724. https://doi.org/10.3389/fmicb.2016.01724

Berger, L., Stamford, T., Stamford-Arnaud, T., de Alcântara, S., da Silva, A., da Silva, A., . . . de Campos-Takaki, G. (2014). Green conversion of agroindustrial wastes into chitin and chitosan by *Rhizopus arrhizus* and *Cunninghamella elegans* strains. *International Journal of Molecular Sciences*, *15*(5), 9082–9102. https://doi.org/10.3390/ijms15059082

Bosquez-Molina, E., Jesús, E. R., Bautista-Baños, S., Verde-Calvo, J. R., & Morales-López, J. (2010). Inhibitory effect of essential oils against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in stored papaya fruit and their possible application in coatings. *Postharvest Biology and Technology*, *57*(2), 132–137. https://doi.org/10.1016/j.postharvbio.2010.03.008

Botondi, R., Moscetti, R., & Massantini, R. (2016). A comparative study on the effectiveness of ozonated water and peracetic acid in the storability of packaged fresh-cut melon. *Journal of Food Science and Technology*, *53*(5), 2352–2360. https://doi.org/10.1007/s13197-016-2207-y

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—A review. *International Journal of Food Microbiology*, *94*(3), 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Calame, W., Weseler, A. R., Viebke, C., Flynn, C., & Siemensma, A. D. (2008). Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. *British Journal of Nutrition*, *100*(6), 1269–1275. https://doi.org/10.1017/S0007114508981447

Campos, C. A., Gerschenson, L. N., & Flores, S. K. (2011). Development of edible films and coatings with antimicrobial activity. *Food and Bioprocess Technology*, *4*(6), 849–875. https://doi.org/10.1007/s11947-010-0434-1

Castellanos, D. A., Mendoza, R., Gavara, R., & Herrera, A. O. (2017). Respiration and ethylene generation modeling of "Hass" avocado and feijoa fruits and application in modified atmosphere packaging. *International Journal of Food Properties*, *20*(2), 333–349. https://doi.org/10.1080/10942912.2016.1160921

Cháfer, M., Sánchez-González, L., González-Martínez, Ch., & Chiralt, A. (2012). Fungal decay and shelf life of oranges coated with chitosan and bergamot, thyme, and tea tree essential oils. *Journal of Food Science*, *77*(8), E182–E187. https://doi.org/10.1111/j.1750-3841.2012.02827.x

de Aquino, A. B., Blank, A. F., & de Aquino Santana, L. C. L. (2015). Impact of edible chitosan–cassava starch coatings enriched with *Lippia gracilis* Schauer genotype mixtures on the shelf life of guavas (*Psidium guajava* L.) during storage at room temperature. *Food Chemistry*, *171*, 108–116. https://doi.org/10.1016/j.foodchem.2014.08.077

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de Oliveira, C. E. V., Magnani, M., de Sales, C. V., de Souza Pontes, A. L., Campos-Takaki, G. M., Stamford, T. C. M., & de Souza, E. L. (2014a). Effects of chitosan from *Cunninghamella elegans* on virulence of post-harvest pathogenic fungi in table grapes (*Vitis labrusca* L.). *International Journal of Food Microbiology*, *171*, 54–61. https://doi.org/10.1016/j.ijfoodmicro.2013.11.006

de Oliveira, C. E. V., Magnani, M., de Sales, C. V., de Souza Pontes, A. L., Campos-Takaki, G. M., Stamford, T. C. M., & de Souza, E. L. (2014b). Effects of post-harvest treatment using chitosan from *Mucor circinelloides* on fungal pathogenicity and quality of table grapes during storage. *Food Microbiology*, *44*, 211–219. https://doi.org/10.1016/j.fm.2014.06.007

de Oliveira, K. Á. R., Berger, L. R. R., de Araújo, S. A., Câmara, M. P. S., & de Souza, E. L. (2017). Synergistic mixtures of chitosan and *Mentha piperita* L. essential oil to inhibit Colletotrichum species and anthracnose development in mango cultivar Tommy Atkins. *Food Microbiology*, *66*, 96–103. https://doi.org/10.1016/j.fm.2017.04.012

de Souza, E. L., Sales, C. V., de Oliveira, C. E. V., Lopes, L. A. A., da Conceição, M. L., Berger, L. R. R., & Stamford, T. C. M. (2015). Efficacy of a coating composed of chitosan from *Mucor circinelloides* and carvacrol to control *Aspergillus flavus* and the quality of cherry tomato fruits. *Frontiers in Microbiology*, *6*, 732. https://doi.org/10.3389/fmicb.2015.00732

dos Santos, N. S. T., Athayde Aguiar, A. J. A., de Oliveira, C. E. V., Veríssimo de Sales, C., de Melo e Silva, S., Sousa da Silva, R., . . . de Souza, E. L. (2012). Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* L. essential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (*Vitis labrusca* L.). *Food Microbiology*, *32*(2), 345–353. https://doi.org/10.1016/j.fm.2012.07.014

Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings: A review. *Materials Science and Engineering: C*, *33*(4), 1819–1841. https://doi.org/10.1016/j.msec.2013.01.010

Etemadipoor, R., Ramezanian, A., Mirzaalian Dastjerdi, A., & Shamili, M. (2019). The potential of gum arabic enriched with cinnamon essential oil for improving the qualitative characteristics and storability of guava (*Psidium guajava* L.) fruit. *Scientia Horticulturae*, *251*, 101–107. https://doi.org/10.1016/j.scienta.2019.03.021

Fagundes, C., Palou, L., Monteiro, A. R., & Pérez-Gago, M. B. (2014). Effect of antifungal hydroxypropyl methylcellulose-beeswax edible coatings on gray mold development and quality attributes of cold-stored cherry tomato fruit. *Postharvest Biology and Technology*, *92*, 1–8. https://doi.org/10.1016/j.postharvbio.2014.01.006

Fagundes, C., Pérez-Gago, M. B., Monteiro, A. R., & Palou, L. (2013). Antifungal activity of food additives in vitro and as ingredients of hydroxypropyl methylcellulose-lipid edible coatings against *Botrytis cinerea* and *Alternaria alternata* on cherry tomato fruit. *International Journal of Food Microbiology*, *166*, 391–398. https://doi.org/10.1016/j.ijfoodmicro.2013.08.001

Gao, P., Zhu, Z., & Zhang, P. (2013). Effects of chitosan–glucose complex coating on postharvest quality and shelf life of table grapes. *Carbohydrate Polymers*, *95*(1), 371–378. https://doi.org/10.1016/j.carbpol.2013.03.029

Gniewosz, M., & Synowiec, A. (2011). Antibacterial activity of pullulan films containing thymol: Antibacterial activity of pullulan films with thymol. *Flavour and Fragrance Journal*, *26*(6), 389–395. https://doi.org/10.1002/ffj.2063

Guerra, I. C. D., de Oliveira, P. D. L., de Souza Pontes, A. L., Lúcio, A. S. S. C., Tavares, J. F., Barbosa-Filho, J. M., . . . de Souza, E. L. (2015). Coatings comprising chitosan and *Mentha piperita* L. or Mentha × villosa Huds essential oils to prevent common postharvest mold infections and maintain the quality of cherry tomato fruit. *International Journal of Food Microbiology*, *214*, 168–178. https://doi.org/10.1016/j.ijfoodmicro.2015.08.009

Guerra, I. C. D., de Oliveira, P. D. L., Santos, M. M. F., Lúcio, A. S. S. C., Tavares, J. F., Barbosa-Filho, J. M., . . . de Souza, E. L. (2016). The effects of composite coatings containing chitosan and *Mentha* (*piperita* L. or x villosa Huds) essential oil on postharvest mold occurrence and quality of table grape cv. Isabella. *Innovative Food Science & Emerging Technologies*, *34*, 112–121. https://doi.org/10.1016/j.ifset.2016.01.008

Guerreiro, A. C., Gago, C. M. L., Faleiro, M. L., Miguel, M. G. C., & Antunes, M. D. C. (2015). Raspberry fresh fruit quality as affected by pectin- and alginate-based edible coatings enriched with essential oils. *Scientia Horticulturae*, *194*, 138–146. https://doi.org/10.1016/j.scienta.2015.08.004

Hong, K., Xie, J., Zhang, L., Sun, D., & Gong, D. (2012). Effects of chitosan coating on postharvest life and quality of guava (*Psidium guajava* L.) fruit during cold storage. *Scientia Horticulturae*, *144*, 172–178. https://doi.org/10.1016/j.scienta.2012.07.002

Hosseinnejad, M., & Jafari, S. M. (2016). Evaluation of different factors affecting antimicrobial properties of chitosan. *International Journal of Biological Macromolecules*, *85*, 467–475. https://doi.org/10.1016/j.ijbiomac.2016.01.022

Jing, Y., Hao, Y., Qu, H., Shan, Y., Li, D., & Du, R. (2007). Studies on the antibacterial activities and mechanisms of chitosan obtained from cuticles of housefly larvae. *Acta Biologica Hungarica*, *58*(1), 75–86. https://doi.org/10.1556/ABiol.57.2007.1.7

Jung, B.-O., Kim, C.-H., Choi, K.-S., Lee, Y. M., & Kim, J.-J. (1999). Preparation of amphiphilic chitosan and their antimicrobial activities. *Journal of Applied Polymer Science*, *72*(13), 1713–1719. https://doi.org/10.1002/(SICI)1097-4628(19990624)72:13<1713::AID-APP7>3.0.CO;2-T

Kean, T., & Thanou, M. (2010). Biodegradation, biodistribution and toxicity of chitosan. *Advanced Drug Delivery Reviews*, *62*(1), 3–11. https://doi.org/10.1016/j.addr.2009.09.004

Kim, D. S., & Hwang, B. K. (2014). An important role of the pepper phenylalanine ammonia-lyase gene (PAL1) in salicylic acid-dependent signalling of the defence response to microbial pathogens. *Journal of Experimental Botany*, *65*(9), 2295–2306. https://doi.org/10.1093/jxb/eru109

Kong, M., Chen, X. G., Xing, K., & Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: A state of the art review. *International Journal of Food Microbiology*, *144*(1), 51–63. https://doi.org/10.1016/j.ijfoodmicro.2010.09.012

Kosman, E., & Cohen, Y. (1996). Procedures for calculating and differentiating synergism and antagonism in action of fungicide mixtures. *Phytopathology*, *86*, 1263–1272.

Lima, N. B., Batista, M. V. A., Junior-Morais, M. A., Barbosa, M. A.G., Michereff, S. J., Hyde, K. D., & Câmara, M. P. S. (2013). Five *Colletotrichum* species are responsible for mango anthracnose in northeastern Brazil. *Fungal Diversity*, *61*, 75–88.

Lima, N. B., Lima, W. G., Tovar-Pedraza, J. M., Michereff, S. J., & Câmara, M. P. S. (2015). Comparative epidemiology of *Colletotrichum* species from mango in northeastern Brazil. *European Journal of Plant Pathology*, *141*(4), 679–688. https://doi.org/10.1007/s10658-014-0570-y

Maqbool, M., Ali, A., Alderson, P. G., Mohamed, M. T. M., Siddiqui, Y., & Zahid, N. (2011). Postharvest application of gum arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biology and Technology*, *62*(1), 71–76. https://doi.org/10.1016/j.postharvbio.2011.04.002

Mohammadi, A., Hashemi, M., & Hosseini, S. M. (2016). Postharvest treatment of nanochitosan-based coating loaded with *Zataria multiflora* essential oil improves antioxidant activity and extends shelf-life of cucumber. *Innovative Food Science & Emerging Technologies*, *33*, 580–588. https://doi.org/10.1016/j.ifset.2015.10.015

Munhuweyi, K., Caleb, O. J., Lennox, C. L., van Reenen, A. J., & Opara, U. L. (2017). *In vitro* and *in vivo* antifungal activity of chitosan-essential oils against pomegranate fruit pathogens. *Postharvest Biology and Technology*, *129*, 9–22. https://doi.org/10.1016/j.postharvbio.2017.03.002

Oliveira, P. D. L., de Oliveira, K. Á. R., Vieira, W. A. dos S., Câmara, M. P. S., & de Souza, E. L. (2018). Control of anthracnose caused by *Colletotrichum* species in guava, mango and papaya using synergistic combinations of chitosan and *Cymbopogon citratus* (D.C. ex Nees) Stapf. essential oil. *International Journal of Food Microbiology*, *266*, 87–94. https://doi.org/10.1016/j.ijfoodmicro.2017.11.018

Pastor, C., Sánchez-González, L., Marcilla, A., Chiralt, A., Cháfer, M., & González-Martínez, C. (2011). Quality and safety of table grapes coated with hydroxypropylmethylcellulose edible coatings containing propolis extract. *Postharvest Biology and Technology*, *60*(1), 64–70. https://doi.org/10.1016/j.postharvbio.2010.11.003

Perdones, A., Sánchez-González, L., Chiralt, A., & Vargas, M. (2012). Effect of chitosan–lemon essential oil coatings on storage-keeping quality of strawberry. *Postharvest Biology and Technology*, *70*, 32–41. https://doi.org/10.1016/j.postharvbio.2012.04.002

Poverenov, E., Zaitsev, Y., Arnon, H., Granit, R., Alkalai-Tuvia, S., Perzelan, Y., . . . Fallik, E. (2014). Effects of a composite chitosan–gelatin edible coating on postharvest quality and storability of red bell peppers. *Postharvest Biology and Technology*, *96*, 106–109. https://doi.org/10.1016/j.postharvbio.2014.05.015

Rodriguez-Garcia, I., Cruz-Valenzuela, M. R., Silva-Espinoza, B. A., Gonzalez-Aguilar, G. A., Moctezuma, E., Gutierrez-Pacheco, M. M., . . . Ayala-Zavala, J. F. (2016). Oregano (*Lippia graveolens*) essential oil added within pectin edible coatings prevents fungal decay and increases the antioxidant capacity of treated tomatoes: Oregano oil to prevent fungal

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decay in tomatoes. *Journal of the Science of Food and Agriculture*, *96*(11), 3772–3778. https://doi.org/10.1002/jsfa.7568

Rodriguez, J., & Zoffoli, J. P. (2016). Effect of sulfur dioxide and modified atmosphere packaging on blueberry postharvest quality. *Postharvest Biology and Technology*, *117*, 230–238. https://doi.org/10.1016/j.postharvbio.2016.03.008

Sánchez-González, L., Pastor, C., Vargas, M., Chiralt, A., González-Martínez, C., & Cháfer, M. (2011). Effect of hydroxypropylmethylcellulose and chitosan coatings with and without bergamot essential oil on quality and safety of cold-stored grapes. *Postharvest Biology and Technology*, *60*(1), 57–63. https://doi.org/10.1016/j.postharvbio.2010.11.004

Septembre-Malaterre, A., Remize, F., & Poucheret, P. (2018). Fruits and vegetables, as a source of nutritional compounds and phytochemicals: Changes in bioactive compounds during lactic fermentation. *Food Research International*, *104*, 86–99. https://doi.org/10.1016/j.foodres.2017.09.031

Shao, X., Cao, B., Xu, F., Xie, S., Yu, D., & Wang, H. (2015). Effect of postharvest application of chitosan combined with clove oil against citrus green mold. *Postharvest Biology and Technology*, *99*, 37–43. https://doi.org/10.1016/j.postharvbio.2014.07.014

Spadaro, D., & Droby, S. (2016). Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. *Trends in Food Science & Technology*, *47*, 39–49. https://doi.org/10.1016/j.tifs.2015.11.003

Sudarshan, N. R., Hoover, D. G., & Knorr, D. (1992). Antibacterial action of chitosan. *Food Biotechnology*, *6*(3), 257–272. https://doi.org/10.1080/08905439209549838

Suhr, K. I., & Nielsen, P. V. (2003). Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. *Journal of Applied Microbiology*, *94*(4), 665–674.

Sun, X., Narciso, J., Wang, Z., Ference, C., Bai, J., & Zhou, K. (2014). Effects of chitosan-essential oil coatings on safety and quality of fresh blueberries. *Journal of Food Science*, *79*(5), M955–M960. https://doi.org/10.1111/1750-3841.12447

Tayel, A. A., Gharieb, M. M., Zaki, H. R., & Elguindy, N. M. (2016). Bio-clarification of water from heavy metals and microbial effluence using fungal chitosan. *International Journal of Biological Macromolecules*, *83*, 277–281. https://doi.org/10.1016/j.ijbiomac.2015.11.072

Tian, J., Zeng, X., Lü, A., Zhu, A., Peng, X., & Wang, Y. (2015). Perillaldehyde, a potential preservative agent in foods: Assessment of antifungal activity against microbial spoilage of cherry tomatoes. *LWT - Food Science and Technology*, *60*(1), 63–70. https://doi.org/10.1016/j.lwt.2014.08.014

Tsai, G.-J., Su, W.-H., Chen, H.-C., & Pan, C.-L. (2002). Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. *Fisheries Science*, *68*(1), 170–177. https://doi.org/10.1046/j.1444-2906.2002.00404.x

Tsai, G.-J., Zhang, S.-L., & Shieh, P.-L. (2004). Antimicrobial activity of a low-molecular-weight chitosan obtained from cellulase digestion of chitosan. *Journal of Food Protection*, *67*(2), 396–398. https://doi.org/10.4315/0362-028X-67.2.396

Usall, J., Torres, R., & Teixidó, N. (2016). Biological control of postharvest diseases on fruit: A suitable alternative? *Current Opinion in Food Science*, *11*, 51–55. https://doi.org/10.1016/j.cofs.2016.09.002

Vu, K. D., Hollingsworth, R. G., Leroux, E., Salmieri, S., & Lacroix, M. (2011). Development of edible bioactive coating based on modified chitosan for increasing the shelf life of strawberries. *Food Research International*, *44*(1), 198–203. https://doi.org/10.1016/j.foodres.2010.10.037

Wilkinson, J. M., & Cavanagh, H. M. A. (2005). Antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. *Journal of Medicinal Food*, *8*(1), 100–103. https://doi.org/10.1089/jmf.2005.8.100

Xing, Y., Li, X., Xu, Q., Yun, J., Lu, Y., & Tang, Y. (2011). Effects of chitosan coating enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annuum* L.). *Food Chemistry*, *124*(4), 1443–1450. https://doi.org/10.1016/j.foodchem.2010.07.105

Xing, Y., Xu, Q., Che, Z., Li, X., & Li, W. (2011). Effects of chitosan-oil coating on blue mold disease and quality attributes of jujube fruits. *Food & Function*, *2*(8), 466–474. https://doi.org/10.1039/c1fo10073d

Xing, Y., Lin, H., Cao, D., Xu, Q., Han, W., Wang, R., . . . Li, X. (2015). Effect of chitosan coating with cinnamon oil on the quality and physiological attributes of china jujube fruits. *BioMed Research International*, *2015*, 1–10. https://doi.org/10.1155/2015/835151

Xing, Y., Xu, Q., Yang, S., Chen, C., Tang, Y., Sun, S., . . . Li, X. (2016). Preservation mechanism of chitosan-based coating with cinnamon oil for fruits storage based on sensor data. *Sensors*, *16*(7), E1111. https://doi.org/10.3390/s16071111

Xu, J., Zhao, X., Wang, X., Zhao, Z., & Du, Y. (2007). Oligochitosan inhibits *Phytophthora capsici* by penetrating the cell membrane and putative binding to intracellular targets. *Pesticide Biochemistry and Physiology*, *88*(2), 167–175. https://doi.org/10.1016/j.pestbp.2006.10.010

Yousuf, B., & Srivastava, A. K. (2017). Flaxseed gum in combination with lemongrass essential oil as an effective edible coating for ready-to-eat pomegranate arils. *International Journal of Biological Macromolecules*, *104*, 1030–1038. https://doi.org/10.1016/j.ijbiomac.2017.07.025

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4.2. APÊNDICE B – ARTIGO II

Antifungal effects of *Conyza bonariensis* (L.) Cronquist essential oil against pathogenic *Colletotrichum musae* and its incorporation in gum Arabic coating to reduce anthracnose development in banana during storage

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Antifungal effects of *Conyza bonariensis* (L.) Cronquist essential oil against pathogenic *Colletotrichum musae* and its incorporation in gum Arabic coating to reduce anthracnose development in banana during storage

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Abstract

Aim: This study evaluated the inhibitory effects on mycelial growth and damage on membrane integrity and enzymatic activity caused by *Conyza bonariensis* essential oil (CBEO) on distinct pathogenic *Colletotrichum musae* isolates, as well as the preventive and curative effects of coatings with gum Arabic (GA) and CBEO to reduce anthracnose development in banana during room temperature storage. The effects of GA-CBEO coatings on some physicochemical parameters of banana were investigated during room temperature storage.

Method and results: CBEO (0.4–1 μl ml^{-1}) inhibited the mycelial growth of *C. musae* isolates in laboratory media. The exposure of *C. musae* conidia to CBEO (0.6 μl ml^{-1}) for 3 and 5 days resulted in high percentages of conidia with damaged cytoplasmic membrane and without enzymatic activity. Coatings with GA (0.1 mg ml^{-1}) and CBEO (0.4–1 μl ml^{-1}) reduced the anthracnose development in banana artificially contaminated with *C. musae* during storage. In most cases, the disease severity indexes found for GA-CBEO-coated banana were lower than or similar to those for banana treated with commercial fungicide. GA-CBEO-coated banana had reduced alterations in physicochemical parameters during storage, indicating more prolonged storability.

Conclusion: The application of GA-CBEO coatings is effective to delay the anthracnose development in banana during storage, which should help to reduce the amount of fungicides used to control postharvest diseases in this fruit.

Significance and Impact of the Study: This is the first study showing the efficacy of coatings formulated with GA and CBEO to delay the development of anthracnose in banana, as well as to decrease alterations in physicochemical parameters indicative of postharvest quality of this fruit during storage. In a practical point of view, GA-CBEO coatings could be innovative strategies to delay the anthracnose development and postharvest losses in banana.

KEYWORDS
antifungal effects, *Colletotrichum*, conidia damage, edible coating, fruit decay, *Musa* spp.

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INTRODUCTION

Banana is a ready-to-eat fruit appreciated worldwide and one of the most accessible fruit for human consumption (Singh et al., 2015). Banana pulp has a good nutritional value, being a rich source of carbohydrates, fibres, vitamins, minerals and bioactive compounds, such as phenolic acids and flavonoids, with antioxidant activities (Borges et al., 2014). However, banana is a climacteric fruit with a fast senescence after harvest, which if not properly controlled is accompanied by fungal infection and fruit decay, affecting negatively the quality, storability and fruit market value (Anyasi et al., 2013; Chillet et al., 2007; Lobo & Rojas, 2020).

Anthracnose is a common disease in banana caused by the fungus *Colletotrichum musae* and characterized by brown-black and sunken lesions after harvesting and during fruit ripening leading to losses as high as 30% of banana production in some producing regions (de Costa & Erabadupitiya, 2005; Siddiqui & Ali, 2014; Silva et al., 2016; Sivakumar & Bautista-Baños, 2014), being necessary to use effective strategies to reduce the remarkable detrimental effects linked to postharvest anthracnose development in banana. The main strategy to control anthracnose in banana has been the application of synthetic fungicides (e.g., trifloroxistrobine, tebuconazole and thiabendazole) in the field and during the postharvest period. However, the excessive use of synthetic fungicides has been associated with induction of antimicrobial resistance in phytopathogenic fungi, besides of a growing consumer concern regarding their harmful effects on human health and environment (Abdel-Rahim & Abo-Elyousr, 2017; Cindi et al., 2015; Farzaneh et al., 2015; Khaliq et al., 2019). Consumers have progressively increased the preference to foods preserved with natural components and refused to consume foods formulated or preserved with chemically synthesized compounds (Souza et al., 2019; Vilaplana et al., 2018). In this context, the development of synthetic fungicide-free strategies with strong antifungal effects on *C. musae* and capability of controlling the anthracnose development in banana should be a research focus.

The use of polymer-based edible coatings has been considered an emerging technology to reduce or even to replace the use of synthetic fungicides for fruit postharvest treatment and preservation (Andrade et al., 2017; Braga et al., 2019; Guerra et al., 2015; Maqbool et al., 2013). Gum Arabic (GA) is a natural polysaccharide extracted from *Acacia* species, being used in foods as an emulsifier, flavour intensifier and film-forming agent (Ali et al., 2010; Andrade et al., 2017). The film-forming properties of GA have been also exploited to coat fruit as an alternative strategy to preserve postharvest fruit quality (Andrade et al., 2017; Maqbool et al., 2010; Saleem et al., 2020). However, GA alone has not been effective to inhibit postharvest pathogens in fruit, being necessary its combination with other effective antimicrobial substances, such as essential oils (EO) (Andrade et al., 2017; Maqbool et al., 2011; Souza et al., 2019). The incorporation of EO should improve the fruit preservation properties of polymer-based coatings because the EO constituents released continuously on fruit surface could inhibit the infection of fruit by pathogenic micro-organisms over time (Braga et al., 2019; Guerra et al., 2016).

Conyza bonariensis (L.) Cronquist (flaxleaf fleabane) is an aromatic and major crop weed native to South America and naturalized in warm areas throughout the world. The infusion of *C. bonariensis* aerial parts has been used in popular medicine as an anti-inflammatory, antidiarrheal, diuretic, vermifuge and antiseptic. Extracts from *C. bonariensis* leaves and roots have shown low acute toxicity in animal models (Araujo et al., 2013; Atta & Mouneir, 2004; Okello et al., 2009; de Paula et al., 2018). *Conyza bonariensis* essential oil (CBEO) has shown effective to inhibit different micro-organisms, such as *Bacillus cereus*, *Staphylococcus epidermidis* and *Candida albicans* (Araujo et al., 2013; Lombardo et al., 2016). These effects have been linked to a variety of constituents commonly found in CBEO, such as limonene, carvone, *trans*-β-farnesene, *trans*-ocimene, β-sesqui-phellandrene and (E)-β-farnecene (Araujo et al., 2013; Barbosa et al., 2005; Mabrouk et al., 2011; Maia et al., 2002). However, investigations on the inhibitory effects of CBEO, alone or when incorporated in edible coatings, on phytopathogenic fungi are still lacking.

This study evaluated the inhibitory effects on mycelial growth and damage on membrane integrity and enzymatic activity caused by CBEO on various isolates of pathogenic *C. musae*, as well as the preventive and curative effects of coatings formulated with GA and different concentrations of CBEO to delay the development of anthracnose in banana during room temperature storage. The effects of these coatings on some physicochemical parameters indicative of overall postharvest quality of banana were also investigated during room temperature storage.

MATERIALS AND METHODS

Materials

Bananas (*Musa acuminata* Cavendish Subgroup, cv. Prata) at commercial maturity stage (yellow peel colour with green tips, maturity index: 5) (Bhuiyan et al., 2020) were purchased from an agricultural farm located at the city of João Pessoa (7°08′29″S, 34°50′48″W, Paraíba, Brazil). Fruit with uniform shape, size and without

physical injuries and visible infection signs were selected and transported into isothermal boxes (25 ± 1°C) to laboratory for use in experiments in the same day. Fruit were washed with domestic detergent and running water, surface disinfected with immersion in sodium hypochlorite (150 ppm, pH 7.2 adjusted with 1 mol l^{-1} NaOH) for 5 min, rinsed with potable water and air-dried at room temperature (25 ± 0.5°C) before use in the experiments.

The GA (CAS 9000-01-5) was purchased from Dinâmica Quím. Contemp. Ltda. (Diadema). CBEO was extracted from branches and leaves through hydrodistillation with a Clevenger apparatus (Guerra et al., 2016). *Conyza bonariensis* branches and leaves were collected from Medicinal Plant Garden, Institute of Research in Drugs and Medicines (Federal University of Paraíba, João Pessoa, Paraíba, Brazil). A voucher specimen of *C. bonariensis* has been deposited at Herbarium Lauro Pires Xavier–JPB (Federal University of Paraíba) under number JPB 26391 and registered with SISGEN ABB39C8.

A commercial fungicide formulation (trifloxystrobine [100 g l^{-1}] + tebuconazole [200 g l^{-1}]) commonly used to control anthracnose development in banana in Brazil was used as a standard fungicide. Fungicide formulation (1 ml) was diluted in sterile distilled water (1 l) to reach a final concentration of 0.1 g l^{-1} trifloroxistrobine and 0.2 g l^{-1} tebuconazole, according to the manufacturer's instructions.

Six isolates of *C. musae*, namely *C. musae* RP3, *C. musae* RP4, *C. musae* RP10, *C. musae* MM5, *C. musae* GM20 and *C. musae* LN2, isolated from banana attacked by anthracnose and previously identified by genetic phylogeny (Vieira et al., 2017) were used as target micro-organisms. These isolates were supplied by Culture Collection of Phytopathogenic Fungi Prof. Maria Menezes (Federal Rural University of Pernambuco, Recife, Pernambuco, Brazil). Before use in the experiments, each isolate was inoculated separately in one banana and after the development of characteristic anthracnose lesion confirming their pathogenicity to this fruit, the isolate was recovered from the lesion and cultured in potato dextrose agar (PDA, HiMedia) at 25 ± 0.5°C for 5 days (Zitter et al., 1996).

Identification of CBEO constituents

The CBEO constituents were identified with gas chromatography using a gas chromatograph coupled to mass spectrometer (GCMS-QP2010 Ultra, Shimadzu). The analytical conditions were as follows: capillary column RTX-5MS (30-m column, internal diameter: 0.25 mm, film thickness: 0.25 μm); temperature programmed from 60 to 240°C at 3°C min^{-1}; injector temperature: 250°C; detector temperature: 220°C; electron impact: 70 eV; carrier gas: helium (0.99 ml min^{-1}); linear velocity: 36.4 cm s^{-1}; pressure: 57 kPa; mass range (m z^{-1}): 40–500. Identification of constituents was done by comparison of their mass spectra with NIST/EPA/NIH Mass Spectral Database (National Institute of Standards Technology) and FFNSC1.3 (Flavor and Fragrance Natural and Synthetic Compounds) libraries and Kovats retention index. Quantification of the constituents was done after normalizing the area of each constituent identified and expressed as a percentage of area (%) (Braga et al., 2019).

Preparation of GA dispersions, CBEO emulsions and coatings

The GA was used at the lowest concentration (0.1 g ml^{-1}) needed to produce a dispersion with viscosity enough to form a thin coating when applied on fruit (Maqbool et al., 2011). GA (0.1 g ml^{-1}) was dissolved in ultra-purified water with stirring (150 rpm) at 40°C for 60 min to prepare the dispersions. Emulsions of CBEO at different concentrations (0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 μl ml^{-1}) were obtained after vigorous shaking of CBEO with sterile distilled water (~45°C) + Tween 80 (10 ml l^{-1}, Sigma-Aldrich Corp.) as a stabilizing agent using a Vortex mixer. The range of CBEO concentrations used in this study was selected considering the protocols of previous studies evaluating the effects of distinct EO on various *Colletotrichum* species (Braga et al., 2019; de Oliveira et al., 2017; Oliveira et al., 2018).

For the combined use of GA and CBEO, the GA (0.1 g ml^{-1}) was dissolved in ultra-purified water with stirring (150 rpm) at 40°C for 60 min, the CBEO (final concentrations: 0.1, 0.2, 0.4, 0.6, 0.8 and 1 μl ml^{-1}) was incorporated into the GA dispersion and stirred (150 rpm) for 18 h at room temperature. For the combined use of GA and CBEO to formulate the coatings, glycerol was added (2.5 ml 100 ml^{-1}) as a plasticizer immediately after the addition of CBEO into the GA dispersions.

Evaluation of the effects of GA and CBEO on *C. musae* mycelial growth

The effects of GA and CBEO on radial mycelial growth of *C. musae* isolates were assessed with a solid media dilution procedure (Braga et al., 2019). *Colletotrichum musae* isolates were grown on PDA during 7 days (25 ± 0.5°C), mycelial agar plugs (5 mm diameter) were taken from the margin of the cultures, transferred to the centre of a Petri dish with PDA + GA (0.1 g ml^{-1}) or CBEO (CBEO: 0.1, 0.2, 0.4, 0.6, 0.8 or 1 μl ml^{-1}) and incubated at 25 ± 0.5°C. PDA without GA or CBEO (pH 5.6) was tested as a negative

control. Measurements of orthogonal diameters of fungal colonies were taken daily during 7 days or up to the negative control Petri dishes were fully covered with fungal mycelia.

Percentage of mycelial growth inhibition (MGI%) was calculated with the equation:

$$\text{MGI}\% = \left[\frac{(C-T)}{C}\right] \times 100 \quad (1)$$

where C is the colony diameter in control assay and T is the colony diameter in PDA with GA or CBEO at the examined concentration.

The concentrations of CBEO causing MGI% in the range of 50%–80% were selected for use in experiments to measure the effects of coatings with combined GA and CBEO on anthracnose lesion development in banana.

Evaluation of the effects of CBEO on membrane integrity and enzymatic activity of *C. musae* conidia

The effects of CBEO on membrane integrity and enzymatic activity of *C. musae* RP10 and *C. musae* MM5 conidia were evaluated with flow cytometry. A 10-ml suspension of *C. musae* conidia (taken from a colony grown on PDA during 7 days, 25 ± 0.5°C) in phosphate-buffered saline (PBS; 8 g l^{-1} of NaCl, 0.2 g l^{-1} of KCl, 1.44 g l^{-1} of Na_2HPO_4, 0.24 g l^{-1} of KH_2PO_4, pH 7.4; approximately 10^6 conidia ml^{-1}, conidia count standardized with a haemocytometer) was exposed to 0.6 μl ml^{-1} of CBEO for 3 and 5 days (25 ± 0.5°C), the conidia were harvested with centrifugation (4500 g, 10 min, 4°C), washed two times and resuspended in PBS and labelled with propidium iodide (PI, Sigma-Aldrich) to evaluate cytoplasmatic membrane integrity and fluorescein diacetate (FDA; ThermoFisher Scientific, Molecular Probes F1303) to evaluate enzymatic activity. These fluorochromes were similarly used with a negative control conidia suspension of each examined isolate not exposed to CBEO (Almeida et al., 2019; Kim et al., 2017).

Staining procedure

Conidia suspended in PBS were incubated with PI (1 μg ml^{-1}) and FDA (2.5 μg ml^{-1}) separately for 30 min (37 ± 0.5°C) in the dark. After the staining period, the conidia suspensions were centrifuged (4500 g, 10 min, 4°C) and washed with equal volume of PBS to remove excess dye. Collected conidia were resuspended again in PBS and analysed in flow cytometer (Almeida et al., 2019; Kim et al., 2017).

Flow cytometry analysis

The flow cytometry measurements were done with a flow cytometer equipped with an argon-ion laser emitting at 488 nm (BD Accuri C6, Becton Dickinson and Company). Green and red fluorescences were collected in FL1 (533 nm ±30 nm) and FL3 (>670 nm) channels. Scatter and fluorescence signals of individual conidia passing the laser zone were collected as logarithmic signals. Fluorescence signals (pulse area measurements) were collected by FL1 (FDA) and FL3 (PI) bandpass filters. Thresholds level for data acquisition was set on forward scatter light (FSC; 30,000) to eliminate background and signals from debris considered much smaller than intact conidia. Conidia were gated per FSC/side scatter light (SSC) parameters. Each sample acquisition was operated at a low flow rate setting and a total of 10,000 events were analysed. Density plots indicating FSC vs. side scatter light (SSC) were obtained along measurements. Dot plot analysis of FL1 vs. FL3 was employed to measure fluorescence properties of conidia. PI^+ phenotype corresponded to stained conidia with damaged cytoplasmic membranes; and PI^- phenotype corresponded to unstained conidia with intact cytoplasmic membranes. Density plot analysis of SSC vs. FL1 was used to determine the fluorescence properties of conidia with FDA^+ phenotype corresponding to conidia with altered enzymatic activity, whereas the FDA^- phenotype corresponded to conidia without enzymatic activity (Almeida et al., 2019; Kim et al., 2017).

Evaluation of the effects of GA-CBEO coatings on anthracnose lesion development in banana during storage

The surface of each banana was wounded (two wounds per fruit) in their extremity region with a sterilized needle (3-mm deep and 2-mm wide), carefully immersed in 500 ml of the coating forming dispersion with GA (0.1 g ml^{-1}) + CBEO (0.4, 0.6, 0.8 or 1 μl ml^{-1}), gently rotated for 5 min with a sterilized glass rod and air-dried in a biosafety cabinet. A group of fruit was coated before artificial contamination with tested isolate for preventive assay and another fruit group was coated after artificial contamination with tested isolate for curative assay. For each *C. musae* isolate, an agar plug (5 mm diameter) with mycelia obtained from the margin of a 7-day-old colony grown on PDA (25 ± 0.5°C) was inoculated on the wounded (puncture) point of an individual fruit. Fruit were immersed for 1 min in commercial

fungicide (trifloroxistrobina: 100 g l^{-1} + tebuconazole: 200 g l^{-1}) as a positive control assay.

Fruit were individually kept in a polyethylene plastic bag to avoid direct contact among different fruit (experimental units) and placed in containers with a moistened paper towel to generate satisfactory relative humidity and stored (25 ± 0.5°C, relative humidity of approximately 85%). After 24 h, the plastic bags and paper towels were removed (Lima et al., 2015). On days 2, 3, 4 and 5 of storage, the anthracnose lesion diameters (mm) were measured with a caliper (average of 2 diametrically opposite measurements). Results were expressed as percentage of anthracnose lesion diameter reduction (%ALDR) determined by the difference in lesion diameter in fruit coated with GA + CBEO or treated with commercial fungicide when compared to the lesion diameter in uncoated/untreated fruit (negative control), which was calculated with the equation:

$$\%\,\mathrm{ALDR} = \left[(N - \frac{F}{N}\right] \times 100 \qquad (2)$$

where N is the anthracnose lesion diameter in negative control fruit and F is the anthracnose lesion diameter in fruit coated with GA + CBEO or treated with the commercial fungicide (Oliveira et al., 2018).

Evaluation of the effects of GA-CBEO coatings on physicochemical parameters of banana during storage

Banana uncoated and coated with GA and CBEO but not artificially contaminated with *C. musae* isolates were evaluated for weight loss, colour, soluble solids (SS), titratable acidity (TA) and pH on day zero (just after coating application) and on days 1, 3, 5 and 9 of storage (25 ± 0.5°C). The SS content (in a drop taken from a 5-g macerated banana pulp sample) was measured with a digital refractometer (Model HI 96801, Hanna Instruments) and results were expressed as °Brix. The TA (in a 10-g macerated banana pulp sample previously mixed with 15 ml of boiled distilled water) was measured using phenolphthalein as an indicator with 0.1 mol l^{-1} NaOH and results were expressed as mmol H^+ 100 g^{-1} of fruit (equivalent of malic acid). The pH was measured with a potentiometer with a combined glass electrode (Quimis) (AOAC, 2016, method 981.12). Fruit peel colour was measured at three different equatorial fruit positions with CIELab system (L*a*b*) using a CR-300 colorimeter (Minolta) and a 10-mmquartz cuvette (CIE, 1986). CIELab colour scale (L*a*b*) was used with a D65 illuminant (standard daylight) at a 10° angle. The apparatus was calibrated in reflectance mode with reference plates and specular reflection was excluded. Fruit weight was measured on different selected storage time intervals and fruit weight loss was calculated as a percentage of the initial weight (time zero).

Statistical analysis

The assays to measure the effects of CBEO on fungal mycelial growth and damage in conidia were done in triplicate in three independent experiments. The assays to measure the effects of coatings with GA and CBEO on anthracnose lesion development in banana were done with a completely randomized experimental design with four replicates for each experimental group and eight fruit per replicate in two independent experiments. Results were expressed as average ± standard deviation. Inferential analyses (ANOVA followed by post-hoc Tukey's test or Student's t test) were done to determine differences ($p \leq 0.05$) among results. The area below the disease progress curve was used to measure the disease severity (Macedo et al., 2020). Statistical analyses were done with computational software Sisvar version 5.7—DEX-UFLA (UFLA, Lavras).

RESULTS

Identification of CBEO constituents

Nine different constituents were identified in CBEO in concentration of ≥1%. Sesquicineole (48.46%) was found as the most prevalent constituent in CBEO, followed by sesquisabinene (10.87%), limonene (9.63%), thymol (6.15%) and five other constituents found in concentrations ranging from 1.27 to 1.60% (Table 1).

TABLE 1 Constituents determined in *Conyza bonariensis* (L.) Cronquist essential oil (constituents detected at a concentration of ≥1%)

Linear retention index (LRI)	% (w v^{-1})	Constituents
1024	9.63	Limonene
1290	6.15	Thymol
1416	3.83	Caryophyllene
1451	1.32	Farnesene
1478	1.27	Germacrene D
1493	1.60	Bicyclogermacene
1504	1.40	β-bisabolene
1516	48.46	Sesquicineole
1520	10.87	Sesquisabinene

Effects of GA and CBEO on *C. musae* mycelial growth

The values of MGI% caused by GA and CBEO separately on the six tested *C. musae* isolates are shown in Table 2. The examined GA concentration (0.1 g ml^{-1}) had no inhibitory effect on the mycelial growth of *C. musae* isolates. Concentrations of 0.4, 0.6, 0.8 and 1 μl ml^{-1} of CBEO caused the highest MGI values (44.1%–77.5%) on most of the tested *C. musae* isolates. Concentrations of 0.025 and 0.05 μl ml^{-1} of CBEO caused no mycelial growth inhibition on *C. musae*. Only the concentrations of 0.8 and 1 μl ml^{-1} of CBEO caused MGI values of >60% on tested *C. musae* isolates.

Effects of CBEO on membrane integrity and enzymatic activity of *C. musae*

The effects of 0.6 μl ml^{-1} of CBEO on membrane integrity and enzymatic activity of *C. musae* RP10 and *C. musae* MM5 after 3 and 5 days of exposure are shown in Table 3. The exposure of *C. musae* RP10 and *C. musae* MM5 to CBEO for 3 and 5 days resulted in high percentages of conidia with damaged cytoplasmic membrane (28.8%–43.2%) and without enzymatic activity (59.5%–84.6%). Low percentages of conidia (≤11.2%) with alterations in these physiological functions were found in negative control (conidia not exposed to CBEO). Overall, the exposure

TABLE 2 Percentage of radial mycelial growth inhibition (MGI%, average ± standard deviation) of different *Colletotrichum musae* isolates after 7 days of exposure to gum Arabic (GA) or *Conyza bonariensis* (L.) Cronquist essential oil (CBEO) in solid medium (25 ± 0.5°C)

	MGI %					
Concentrations	*C. musae* RP3	*C. musae* RP4	*C. musae* RP10	*C. musae* GM20	*C. musae* LN2	*C. musae* MM5
GA						
0.1 g ml^{-1}	Zero	Zero	Zero	Zero	Zero	Zero
CBEO						
0.025 μl ml^{-1}	Zero	Zero	Zero	Zero	Zero	Zero
0.05 μl ml^{-1}	Zero	Zero	Zero	Zero	Zero	Zero
0.1 μl ml^{-1}	30.0 (±4.8)^{c}	22.1 (±6.3)^{c}	28.4 (±2.3)^{c}	23.3 (±2.4)^{c}	14.8 (±2.9)^{c}	29.2 (±3.2)^{c}
0.2 μl ml^{-1}	29.2 (±5.3)^{c}	42.1 (±7.0)^{b}	34.6 (±8.0)^{c}	26.6 (±2.9)^{c}	30.4 (±4.7)^{d}	34.6 (±5.0)^{c}
0.4 μl ml^{-1}	63.5 (±4.5)^{b}	63.5 (±4.8)^{a}	70.2 (±2.3)^{a}	44.1 (±3.9)^{b}	49.6 (±2.9)^{c}	55.8 (±6.9)^{b}
0.6 μl ml^{-1}	58.5 (±5.4)^{b}	68.5 (±2.4)^{a}	57.1 (±3.5)^{b}	42.5 (±5.7)^{b}	57.3 (±4.7)^{ab}	59.2 (±8.8)^{b}
0.8 μl ml^{-1}	65.6 (±3.6)^{b}	71.7 (±2.9)^{a}	73.3 (±9.4)^{a}	62.3 (±9.7)^{a}	66.0 (±5.5)^{a}	74.6 (±4.1)^{a}
1 μl ml^{-1}	77.5 (±2.5)^{a}	74.6 (±3.7)^{a}	77.1 (±3.6)^{a}	75.2 (±3.9)^{a}	62.3 (±2.9)^{a}	77.1 (±3.6)^{a}

a–e: Different small letters in a same column for a same isolate and day of exposure denote significant difference, based on Tukey's test ($p \leq 0.05$).

TABLE 3 Percentage of *Colletotrichum musae* conidia (average ± standard deviation) with damaged cytoplasmic membrane and altered respiratory activity after 3 and 5 days of exposure to *Conyza bonariensis* (L.) Cronquist essential oil (CBEO, 0.6 μl ml^{-1}) in PBS (25 ± 0.5°C)

	C. musae RP10		*C. musae* MM5	
Time of exposure	Damaged cytoplasmic membrane	Altered respiratory activity	Damaged cytoplasmic membrane	Altered respiratory activity
CBEO-treated conidia				
3 days	43.2% (±2.4%)^{a}	84.6% (±1.2%)^{a}	28.8% (±0.9%)^{b}	59.5% (±0.7%)^{b}
5 days	41.4% (±1.5%)^{a}	81.9% (±2.5%)^{a}	35.21% (±2.7%)^{a}	72.5% (±1.5%)^{a}
Control				
3 days	10.3% (±0.6%)^{a}	4.9% (±1.4%)^{a}	11.2% (±0.4%)^{a}	8.9% (±0.7%)^{a}
5 days	9.2% (±0.9%)^{b}	6.6% (±0.5%)^{a}	8.5% (±0.7%)^{b}	6.6% (±1.3%)^{b}

Control: Conidia not treated with CBEO; PI-stained conidia: conidia with damaged cytoplasmic membrane; FDA non-stained conidia: conidia with altered enzymatic activity.

a–b: Different small letters in a same column for a same isolate and measured physiological function denote significant difference, based on Student's *t* test ($p \leq 0.05$).

All percentage values found for damaged cytoplasmic membrane and altered enzymatic activity for conidia treated with CBEO differed from those found for control assays at the same exposure time, based on Student's *t* test ($p \leq 0.05$).

of *C. musae* isolates to CBEO resulted in higher percentages of conidia with altered enzymatic activity than with damaged cytoplasmic membrane.

Effects of coatings with GA and CBEO on anthracnose lesion development in banana during storage

The combinations of GA (0.1 g ml^{-1}) with different CBEO concentrations (0.4, 0.6, 0.8 and 1 μl ml^{-1}) were used to formulate coatings evaluated for their efficacy to reduce anthracnose lesion development in banana artificially contaminated with *C. musae* during 5 days of storage under room temperature in preventive and curative assays.

In the preventive assays (Table 4), a total inhibition of anthracnose lesion development (ALDR of 100%) was found up to day 3 of storage in banana coated with different GA+CBEO combinations or treated with commercial fungicide and contaminated with *C. musae* MM5, with the exception of banana coated with GA +0.8 μl ml^{-1} of CBEO. Coatings with different GA + CBEO combinations caused a total inhibition of anthracnose lesion development up to day 2 of storage in banana contaminated with tested *C. musae* isolates, with the exception of banana coated with GA +0.6 μl ml^{-1} of CBEO and contaminated with *C. musae* RP3. Coating with GA + 0.4 μl ml^{-1} of CBEO caused a total inhibition of anthracnose lesion development up to day 3 of storage in banana contaminated with *C. musae* RP4. The commercial fungicide caused a total inhibition of anthracnose lesion development only up to day 2 of storage in banana contaminated with *C. musae* RP3 and *C. musae* LN2 and up to day 3 of storage in banana contaminated with *C. musae* RP10 and *C. musae* MM5. ALDR% values caused by examined GA + CBEO coatings and commercial fungicide on day 5 of storage were in the range of 5.5%–45.8% and 38.8%–53.6%, respectively.

In the curative assays (Table 5), the coatings with different GA + CBEO combinations and commercial fungicide caused a total inhibition of anthracnose lesion development up to day 3 of storage in banana contaminated with *C. musae* RP 10 and *C. musae* MM 5. The examined GA + CBEO coatings also caused a total inhibition of anthracnose lesion development up to day 2 of storage in banana contaminated with *C. musae* RP4 and up to day 3 of storage in banana coated with GA + 0.6 μl ml^{-1} of CBEO. Coatings with GA + 0.6, 0.8 and 1 μl ml^{-1} of CBEO were capable of reducing the anthracnose lesion development in banana contaminated with tested *C. musae* isolates from day 3 of storage onward, with the exception of coating with GA + 0.4 μl ml^{-1} of CBEO in banana contaminated with *C. musae* LN2. ALDR% values caused by commercial fungicide on day 5 of storage were in the range of 39.8%–53.6%. In most cases, ALDR% values caused by GA + CBEO coatings on day 5 of storage were of >20%.

Inhibition rates caused by GA + CBEO coatings on anthracnose lesion development in preventive and curative assays were similar ($p > 0.05$) or higher ($p \leq 0.05$) than those caused by commercial fungicide at some measured storage time periods. Overall, the efficacy of GA + CBEO coatings and commercial fungicide to reduce the anthracnose lesion development decreased as the storage time period increased.

The highest disease severity indexes were found for banana uncoated with GA + CBEO or non-treated with commercial fungicide. In preventive assays, fruit coated with GA + 0.4 μl ml^{-1} of CBEO and contaminated with *C. musae* RP4 and *C. musae* GM20 had the lowest disease severity indexes. For *C. musae* MM5 and *C. musae* LN2, the lowest disease severity indexes were found in fruit coated with GA + 1 μl ml^{-1} of CBEO and GA + 0.6 μl ml^{-1} of CBEO, respectively. For *C. musae* RP3 and *C. musae* RP10, the lowest disease severity indexes were found for fruit treated with commercial fungicide (Figure 1).

In curative assays, similar disease severity indexes were found for banana contaminated with *C. musae* RP4 and coated with examined GA + CBEO combinations or treated with commercial fungicide. For *C. musae* MM5, the lowest disease severity index was found for fruit coated with GA + 0.6 μl ml^{-1} of CBEO. For *C. musae* RP3, *C. musae* RP10, *C. musae* GM20 and *C. musae* LN2, the lowest disease severity indexes were found for fruit treated with commercial fungicide (Figure 2).

Effects of coatings with GA and CBEO on physicochemical parameters of banana during storage

The values of physicochemical parameters related to overall postharvest quality in banana uncoated and coated with GA (1 mg ml^{-1}) + CBEO (0.4, 0.6, 0.8 and 1 μl ml^{-1}) during 9 days of storage under room temperature are shown in Table S1. A decrease ($p \leq 0.05$) in pH values was found up to day 3 of storage, followed by an increase from day 6 of storage onward regardless of GA + CBEO coating application. Coated banana had lower pH values ($p \leq 0.05$) than uncoated banana on day 3 of storage, although coated banana had higher pH values on day 9 of storage. The highest pH value ($p \leq 0.05$) on day 9 of storage was found in banana coated with GA + 1 μl ml^{-1} of CBEO. There was overall a decrease ($p \leq 0.05$) in TA values in uncoated and coated banana from day 3 of storage onward, with the highest values ($p \leq 0.05$) being found in uncoated banana. Coated banana had lower ($p \leq 0.05$)

TABLE 4 Percentage of anthracnose lesion development reduction (%ALDR, average ± standard deviation) in banana cv. Prata coated with combined gum Arabic (GA) and *Conyza bonariensis* (L.) Cronquist essential oil (CBEO) or treated with a commercial fungicide (trifloxystrobina+tebuconazole) and artificially contaminated with different *Colletotrichum musae* isolates during 5 days of storage (25 ± 0.5°C) (preventive assay)

		Days of storage			
Isolates	**Treatments**	**2nd**	**3th**	**4th**	**5th**
C. musae RP3					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	2.5 (±3.0)^{d}	35.4 (±2.4)^{b}	25.7 (±2.5)^{c}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	27.1 (±4.4)^{b}	10.8 (±2.5)^{c}	28.2 (±4.6)^{c}	19.1 (±2.3)^{d}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	14.4 (±5.5)^{c}	29.7 (±2.1)^{c}	14.4 (±3.8)^{e}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	62.4 (±6.6)^{a}	39.9 (±4.7)^{b}	37.1 (±2.0)^{b}
	Commercial fungicide	100 (±0.0)^{a}	41.3 (±3.9)^{b}	57.2 (±2.7)^{a}	52.7 (±8.1)^{a}
C. musae RP4					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	44.4 (±3.8)^{a}	33.5 (±4.8)^{b}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	100 (±0.0)^{a}	59.5 (±6.8)^{c}	34.0 (±4.1)^{b}	16.2 (±3.1)^{c}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	74.1 (±6.7)^{b}	24.8 (±3.0)^{c}	14.7 (±2.1)^{c}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	72.5 (±8.0)^{b}	36.6 (±2.2)^{b}	30.3 (±3.8)^{b}
	Commercial fungicide	12.4 (±5.2)^{b}	30.5 (±7.1)^{d}	50.9 (±4.9)^{a}	46.9 (±3.8)^{a}
C. musae RP10					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	26.2 (±3.7)^{d}	43.6 (±2.9)^{b}	45.8 (±7.6)^{a}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	100 (±0.0)^{a}	71.4 (±8.4)^{c}	40.9 (±7.4)^{b}	28.0 (±5.5)^{b}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	90.0 (±5.8)^{b}	42.2 (±2.4)^{b}	35.2 (±4.2)^{b}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	75.0 (±6.3)^{c}	46.9 (±3.5)^{b}	44.3 (±4.1)^{a}
	Commercial fungicide	100 (±0.0)^{a}	100 (±0.0)^{a}	100 (±0.0)^{a}	49.2 (±4.0)^{a}
C. musae CM20					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	80.0 (±8.3)^{a}	49.5 (±35.7)^{a}	18.2 (±2.7)^{b}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	100 (±0.0)^{a}	5.5 (±3.9)^{c}	12.2 (±3.1)^{c}	13.6 (±2.5)^{c}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	82.0 (±5.3)^{a}	22.7 (±7.8)^{b}	13.0 (±2.6)^{c}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	22.2 (±4.2)^{b}	21.5 (±8.9)^{b}	20.0(±3.7)^{b}
	Commercial fungicide	77.8 (±9.4)^{b}	83.3 (±7.6)^{a}	45.0 (±4.5)^{a}	53.6 (±3.2)^{a}
C. musae LN2					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	36.7 (±6.7)^{c}	11.6 (±3.1)^{c}	5.5 (±0.3)^{c}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	100 (±0.0)^{a}	80.0 (±8.3)^{a}	31.4 (±6.0)^{ab}	27.1 (±2.2)^{b}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	56.7 (±6.9)^{b}	24.3 (±2.7)^{b}	36.8 (±8.9)^{ab}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	81.8 (±5.7)^{a}	43.7 (±5.9)^{a}	32.1 (±6.5)^{b}
	Commercial fungicide	100 (±0.0)^{a}	60.0 (±8.3)^{b}	39.8 (±4.0)^{a}	42.9 (±3.7)^{a}
C. musae MM5					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	37.6 (±7.1)^{c}	17.4 (±3.8)^{b}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	30.3 (±2.1)^{c}	19.1 (±4.3)^{b}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	30.5 (±8.7)^{c}	7.7 (±2.4)^{c}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	72.7 (±9.3)^{a}	38.4 (±8.4)^{a}
	Commercial fungicide	100 (±0.0)^{a}	100 (±0.0)^{a}	55.8 (±3.7)^{b}	37.3 (±7.9)^{a}

a–e: Different small letters in a same column for a same isolate and day of exposure denote significant difference, based on Tukey's test ($p \leq 0.05$).

TSS contents than uncoated banana form day 3 of storage onward.

The colour parameters L* and b* were measured in uncoated and coated banana, with L* being the luminosity and b* the yellow/blue coordinate. The a* values were disregarded because bananas used in experiments had no longer a green colour at the beginning of the experiments. L* values (luminosity) decreased ($p \leq 0.05$) in

TABLE 5 Percentage of anthracnose lesion development reduction (%ALDR, average ± standard deviation) in banana cv. Prata artificially contaminated with different *Colletotrichum musae* isolates and coated with combined gum Arabic (GA) and *Conyza bonariensis* (L.) Cronquist essential oil (CBEO) or treated with a commercial fungicide (trifloroxistrobina +tebuconazole) during 5 days of storage (25 ± 0.5°C) (curative assay)

		Days of storage			
Isolates	**Treatments**	**2nd**	**3th**	**4th**	**5th**
C. musae RP3					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	15.0 (±4.9)^{c}	18.2 (±3.1)^{c}	31.9 (±3.8)^{b}	33.5 (±4.6)^{b}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	100 (±0.0)^{a}	48.1 (±7.7)^{b}	38.4 (±3.1)^{b}	13.8 (±2.6)^{d}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	18.3 (±6.7)^{c}	16.7 (±5.1)^{c}	3.7 (±2.8)^{c}	8.5 (±3.4)^{d}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	78.5 (±12.8)^{b}	83.3 (±23.5)^{a}	31.3 (±5.5)^{b}	21.3 (±4.5)^{c}
	Commercial fungicide	100 (±0.0)^{a}	41.3 (±3.9)^{b}	55.3 (±2.7)^{a}	52.7 (±8.1)^{a}
C. musae RP4					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	20.6 (±4.7)^{d}	26.3 (±5.7)^{b}	34.4 (±2.8)^{c}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	25.1 (±4.2)^{b}	20.8 (±3.7)^{d}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	26.6 (±7.6)^{cd}	27.8 (±3.8)^{b}	30.2 (±2.2)^{c}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	32.8 (±5.1)^{bc}	15.8 (±3.5)^{c}	68.3 (±44.8)^{a}
	Commercial fungicide	100 (±0.0)^{a}	29.5 (±2.9)^{bc}	50.9 (±4.9)^{a}	44.2 (±2.5)^{b}
C. musae RP10					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	45.8 (±4.8)^{bc}	43.9 (±5.0)^{b}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	53.5 (±6.1)^{b}	40.5 (±4.4)^{b}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	50.0 (±4.5)^{b}	46.0 (±3.9)^{ab}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	40.9 (±4.3)^{c}	46.7 (±6.1)^{ab}
	Commercial fungicide	100 (±0.0)^{a}	100 (±0.0)^{a}	100.0 (±0.0)^{a}	52.5 (±3.5)^{a}
C. musae CM20					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	18.7 (±4.2)^{b}	46.8 (±8.9)^{a}	23.0 (±2.7)^{c}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	17.7 (±5.8)^{c}	17.5 (±2.7)^{b}	37.5 (±3.8)^{a}	24.3 (±3.6)^{c}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	25.6 (±8.3)^{b}	36.5 (±8.5)^{a}	37.0 (±8.5)^{b}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	54.2 (±12.8)^{b}	58.3 (±9.6)^{a}	15.1 (±2.4)^{b}	12.5 (±3.3)^{d}
	Commercial fungicide	26.1 (±5.5)^{c}	22.9 (±7.9)^{b}	45.0 (±4.5)^{a}	53.6 (±3.1)^{a}
C. musae LN2					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	Zero	Zero	Zero
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	30.3 (±7.5)^{b}	20.1 (±3.7)^{c}	17.6 (±2.7)^{c}	27.4 (±3.5)^{b}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	32.5 (±4.5)^{b}	15.1 (±3.3)^{c}	16.5 (±2.1)^{c}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	56.8 (±11.6)^{a}	58.8 (±10.2)^{a}	17.5 (±8.8)^{c}
	Commercial fungicide	27.1 (±5.8)^{b}	34.3 (±6.6)^{b}	37.9 (±5.5)^{b}	39.8 (±2.5)^{a}
C. musae MM5					
	0.1 g ml^{-1} GA + 0.4 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	39.7 (±10.3)^{bc}	21.1 (±3.6)^{b}
	0.1 g ml^{-1} GA + 0.6 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	48.2 (±7.7)^{b}	20.6 (±2.8)^{b}
	0.1 g ml^{-1} GA + 0.8 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	35.3 (±7.5)^{c}	14.4 (±2.4)^{c}
	0.1 g ml^{-1} GA + 1 µl ml^{-1} CBEO	100 (±0.0)^{a}	100 (±0.0)^{a}	81.1 (±3.4)^{a}	45.7 (±4.2)^{a}
	Commercial fungicide	100 (±0.0)^{a}	100 (±0.0)^{a}	55.8 (±7.8)^{b}	38.8 (±9.6)^{a}

a–d: Different small letters in a same column for a same isolate and day of exposure denote significant difference, based on Tukey's test ($p \leq 0.05$).

uncoated and coated banana during the measured storage period, with the exception of banana coated with GA + 0.4 µl ml^{-1} of CBEO, which had an increase in L* value up to day 6 of storage, followed by a decrease on day 9 of storage. Uncoated and coated banana had weight loss during the measured storage period. However, coated banana had overall lower weight loss ($p \leq 0.05$) than uncoated banana over time.

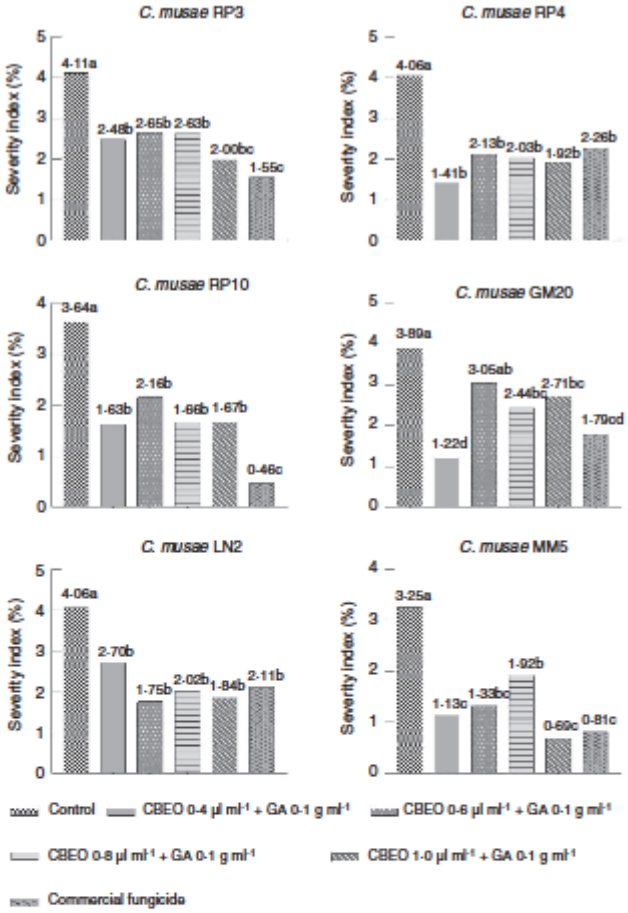

FIGURE 1 Severity index (%) of anthracnose lesion in banana cv. Prata coated with combined gum Arabic (GA) and *Conyza bonariensis* (L.) Cronquist essential oil (CBEO) or treated with a commercial fungicide formulation (trifloroxistrobina+tebuconazole) and further inoculated with different *Colletotrichum musae* isolates after a 5-day storage (25 ± 0.5°C) (preventive assay). a–d: Different small letters denote significant difference, based on Tukey's test ($p \leq 0.05$)

DISCUSSION

The CBEO tested in this study was composed by a variety of constituents, being sesquicineole, sesquisabinene, thymol and limonene found as the most prevalent constituents. Sesquicineole and sesquisabinene are sesquiterpenes and most of the compounds found in CBEO are part of this group, with some of them (e.g. caryophylene and farnesene) with proven antimicrobial properties (Al-Maskri et al., 2011; Celik et al., 2014; Francomano et al., 2019). Sesquicineole and sesquisabinene have been found in EO with reported antimicrobial properties (Benomari et al., 2016; Hanbali et al., 2007; Noriega et al., 2020) and the monoterpenes limonene and thymol have shown strong and wide spectrum antimicrobial activities (Guimarães et al., 2019; Souza et al., 2019).

The presence of these distinct constituents with reported antimicrobial properties in CBEO should be related to its inhibitory effects on various *C. musae* isolates tested in this study. CBEO (0.1–1 µl ml^{-1}) had inhibitory effects on the mycelial growth of tested *C. musae* isolates, with the highest inhibitory rates being caused by 0.4–1 µl ml^{-1} of CBEO. The inhibitory effects of CBEO on *C. musae* isolates should be linked to the abilities of terpenes found in this essential to cause disturbance in enzymatic reactions and energy metabolism of fungal cells, affecting cell wall synthesis and mycelial growth (Braga et al., 2019; Oliveira et al., 2018). No early investigation reporting the inhibitory effects of CBEO on the mycelial growth of *C. musae* or other *Colletotrichum* species has been found in available literature. GA (1 mg ml^{-1}) was not capable of inhibiting the mycelial growth of any of the tested *C. musae* isolates. Previous investigations have found no in vitro inhibitory effects of GA (1–10 mg ml^{-1}) on *Colletotrichum gloesporioides*, *C. musae* and *Rhizopus stolonifer* (Andrade et al., 2017; Maqbool et al., 2011).

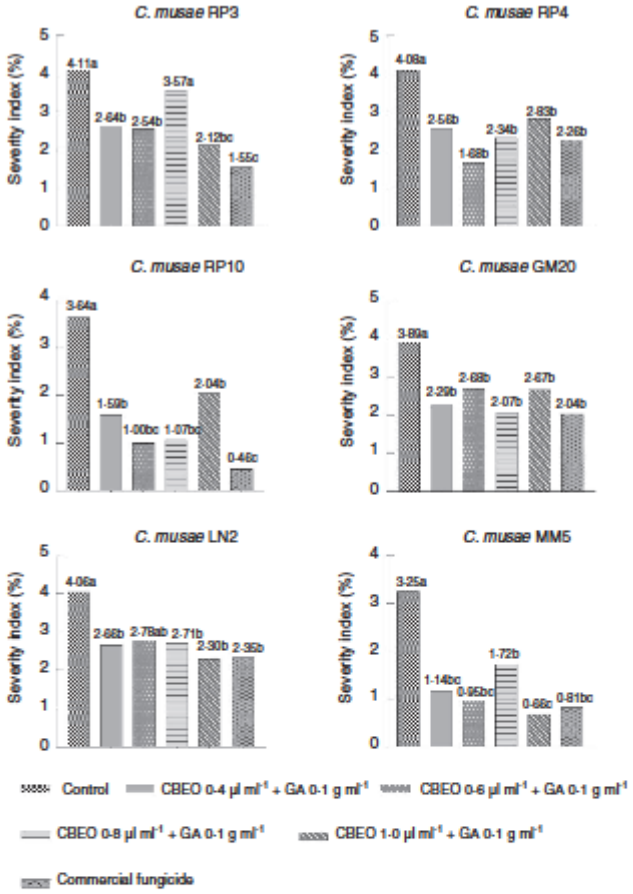

FIGURE 2 Severity index (%) of anthracnose lesion development in banana cv. Prata inoculated with different *Colletotrichum musae* isolates and coated with combined gum Arabic (GA) and *Conyza bonariensis* (L.) Cronquist essential oil (CBEO) or treated with a commercial fungicide formulation (trifloroxistrobina + tebuconazole) after a 5-day storage (25 ± 0.5°C) (curative assay). a–d: Different small letters denote significant difference, based on Tukey's test ($p \leq 0.05$)

Isolated staining with PI and FDA was used to estimate the mortality (non-viability) of conidia treated with CBEO (Xing et al., 2018). PI is able to pass through dead conidia with damaged cell membranes and red-stain them, being the intensity of the fluorescence directly proportional to the number of dead conidia. FDA is a non-fluorescent dye converted metabolically in active conidia by intracellular esterase into a green fluorescent membrane-impermeant compound, being a basis to measure subpopulations of viable and enzymatically/metabolically active conidia (Almeida et al., 2019; Xing et al., 2018). A 3- or 5-day exposure to a fixed amount of CBEO (0.6 µl ml^{-1}) resulted in a high percentage of PI-stained and non-green fluorescent *C. musae* conidia, which is a phenotype indicative of non-viable (dead) conidia (Yun & Lee, 2017). CBEO could be capable of altering severely the plasma membrane structure of *C. musae* conidia from a direct integrity damage, as well as of inducing a metabolic impairment leading to a secondary membrane disturbance (Lee et al., 2018). The primary action mechanism by which CBEO inhibits the *C. musae* mycelial growth could be the disruption of fungal cell plasma membrane leading to cell death, which is in agreement with some reports about the disturbing effects of other EO on fungi cells (Tian et al., 2012; Zeng et al., 2015). These results have shown that CBEO not only inhibited mycelial growth but also directly led to a non-viability state of *C. musae* conidia.

The inhibitory effects of CBEO on *C. musae* continued to be reached when this EO was combined with GA to coat banana. Coatings formulated with GA (1 mg ml^{-1}) and CBEO (0.4–1 µl ml^{-1}) combinations were effective to delay the anthracnose lesion development in banana artificially contaminated with various *C. musae* isolates during a 5-day room temperature storage in either preventive or curative

assays. In general, coatings with GA + 0.6, 08 or 1 μl ml^{-1} CBEO were the most effective to inhibit the anthracnose lesion development in banana during storage, although the inhibition rates had been variable with tested coating formulation and *C. musae* isolate. No study was found in available literature investigating the efficacy of coatings formulated with GA and CBEO to inhibit the anthracnose development in banana. However, early studies have reported the efficacy of coatings formulated with GA (10%) and cinnamon EO (0.4%) to inhibit anthracnose development in banana artificially contaminated with *C. musae* and *C. gloeosporioides* (Maqbool et al., 2011), as well as with GA (1 μl ml^{-1}) and *Origanum vulgare* L. (0.6 and 0.25 μl ml^{-1}) and *Rosmarinus officinalis* L. EO (0.25 μl ml^{-1}) to inhibit soft rot development in plum artificially contaminated with *R. stolonifer* (Andrade et al., 2017).

The disease severity indexes found for banana coated with GA and CBEO were lower than those found to uncoated banana regardless of the inoculated *C. musae* isolate, indicating that a smaller percent area with characteristic anthracnose symptoms was found in GA-CBEO-coated banana in comparison to uncoated banana (Chiang et al., 2017). Furthermore, in most cases, the disease severity indexes found for GA-CBEO-coated banana were lower than or similar to those found for banana treated with commercial fungicide used as positive control. Similar behaviour for disease severity index measurements was found for melon coated with chitosan (5 mg ml^{-1}) and *Cympobogon citratus* EO (0.15 and 0.3 μl ml^{-1}) to control the development of crater rot caused by *Paramyrotecium roridum* during a room temperature storage (Macedo et al., 2020).

For most of the examined coatings with combined GA and CBEO, as well as for the commercial fungicide formulation, the efficacy to reduce the anthracnose lesion development decreased with the increase in storage time. These results could be associated with the typical increase in fruit ripening during storage since the susceptibility of fruit to pathogenic *Colletotrichum* species has been directly related to the ripening stage (Braga et al., 2019; Oliveira et al., 2018). Still, loss of CBEO constituents with antifungal properties from coatings due to volatilization during room temperature storage could have also contributed with a decrease in efficacy of examined coatings to inhibit anthracnose development in banana (Andrade et al., 2017; Guerra et al., 2015).

The application of GA-CBEO coatings caused reduced physicochemical alterations in banana during the measured room temperature storage period, being indicative of a delayed ripening process and more prolonged storability when compared to uncoated fruit. Specifically, GA-CBEO-coated banana had lower pH and higher TA and TSS, besides to smaller weight loss than uncoated banana during storage. These results should be linked to the capability of examined GA-CBEO coatings to form a semi-permeable film on banana surface modifying the internal atmosphere in fruit with reduction of oxygen and/or increase of carbon dioxide levels, decreasing the respiration, metabolic activity and water loss of fruit over time (Al-Juhaimi, 2014; Cosme Silva et al., 2017). These delayed respiratory and metabolic activities in GA-CBEO-coated banana could have directly affected the typical quick and continuous conversion of organic acids to sugars with decrease in acidity, as well as the conversion of complex to simple sugars in coated banana as a consequence of the delayed ripening progress (Al-Juhaimi, 2014). GA-CBEO-coated banana kept the luminosity (L*) for a longer time period than uncoated banana. Still, chroma (b*) had positive values indicative of yellow component in GA-CBEO-coated banana for a more prolonged time period. Delayed alteration in coated fruit colour has been linked to modified atmosphere induced by coatings slowing down the ripening process (Thakur et al., 2019).

As a conclusion, the results showed that CBEO had inhibitory effects on various *C. musae* isolates, while GA was not capable to inhibit them. The action mode of CBEO to cause the antifungal effects on *C. musae* involved damage in cytoplasmic membrane and disturbance of enzymatic activity of conidia, which could be linked to cellular dysfunction, non-viability and death. The application of coatings with GA combined with different concentrations of CBEO was effective to reduce the anthracnose lesion development in banana contaminated with *C. musae* during a 5-day room temperature storage. Additionally, the coatings with different combinations of GA and CBEO delayed the alterations in physicochemical parameters indicative of postharvest quality and more prolonged storability of banana. The application of coatings formulated with selected concentrations of GA and CBEO could be considered an alternative strategy to delay the anthracnose development in banana, as well as to reduce the amount of fungicides used to control postharvest diseases and prolong the storability of this fruit.

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CONFLICT OF INTEREST

Authors confirm no conflict of interest related to the described work.

AUTHOR CONTRIBUTIONS

Conceptualization: GAL, MPSC and ELS; Data curation: GAL and ELS; Formal analysis: GAL, SPB, TMRA

and ELS; Funding acquisition: ELS; Investigation: GAL, SPB, TMRA, KARO and JFT; Methodology: GAL, ACAG, SPB, WASV, MPSC, TMRA, KARO, JFT and ELS; Project administration: ELS; Resources: GAL, JFT and ELS; Supervision: ELS; Writing—original draft: GAL, ELS; Writing—review & editing: ELS.

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REFERENCES

Abdel-Rahim, I.R. & Abo-Elyousr, K.A.M. (2017) Using of endophytic *Saccharomycopsis fibuliger* and thyme oil for management of gray mold rot of guava fruits. *Biological Control*, 110, 124–131. https://doi.org/10.1016/j.biocontrol.2017.04.014

Ali, A., Maqbool, M., Ramachandran, S. & Alderson, P.G. (2010) Gum Arabic as a novel edible coating for enhancing shelf-life and improving postharvest quality of tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biology and Technology*, 58, 42–47. https://doi.org/10.1016/j.postharvbio.2010.05.005.

Al-Juhaimi, F.Y. (2014) Physicochemical and sensory characteristics of Arabic gum-coated tomato (*Solanum lycopersicum* L.) fruits during storage: tomato fruits coated with gum Arabic. *The Journal of Food Processing and Preservation*, 38, 971–979. https://doi.org/10.1111/jfpp.12053.

Al-Maskri, A.Y., Hanif, M.A., Al-Maskari, M.Y., Abraham, A.S., Al-Sabahi, J.N. & Al-Mantheri, O. (2011) Essential oil from *Ocimum basilicum* (Omani Basil): a desert crop. *Natural Products Communications*, 6, 1487–1490.

Almeida, E.T.C., de Souza, G.T., de Sousa Guedes, J.P., Barbosa, L.M., de Sousa, C.P., Castellano, L.R.C. et al. (2019) *Mentha piperita* L. essential oil inactivates spoilage yeasts in fruit juices through the perturbation of different physiological functions in yeast cells. *Food Microbiology*, 82, 20–29. https://doi.org/10.1016/j.fm.2019.01.023.

Andrade, S.C.A., Bareito, T.A., Arcanjo, N.M.O., Madruga, M.S., Meireles, B., Cordeiro, Â.M.T. et al. (2017) Control of Rhizopus soft rot and quality responses in plums (*Prunus domestica* L.) coated with gum Arabic, oregano and rosemary essential oils. *The Journal of Food Processing and Preservation*, 41, e13251. https://doi.org/10.1111/jfpp.13251.

Anyasi, T.A., Jideani, A.I.O. & Mchau, G.R.A. (2013) Functional properties and postharvest utilization of commercial and non-commercial banana cultivars. *Comprehensive Reviews in Food Science and Food Safety*, 12, 509–522. https://doi.org/10.1111/1541-4337.12025.

AOAC—Official Methods of Analysis of the Association of Official Analytical Chemists (2016) *Association of official analytical chemist*, 20th edition. Rockville: AOAC.

Araujo, L., Moujir, L.M., Rojas, J., Rojas, L., Carmona, J. & Rondón, M. (2013) Chemical composition and biological activity of *Conyza bonariensis* essential oil collected in Mérida, Venezuela. *Natural Products Communications*, 8, 1934578X1300800. https://doi.org/10.1177/1934578X1300800838.

Atta, A.H. & Mouneir, S.M. (2004) Antidiarrhoeal activity of some Egyptian medicinal plant extracts. *Journal of Ethnopharmacology*, 92, 303–309. https://doi.org/10.1016/j.jep.2004.03.017.

Barbosa, L.C.A., Paula, V.F., Azevedo, A.S., Silva, E.A.M. & Nascimento, E.A. (2005) Essential oil composition from some plant parts of *Conyza bonariensis* (L.) Cronquist. *Flavour and Fragrance Journal*, 20, 39–41. https://doi.org/10.1002/ffj.1392.

Benomari, F.Z., Djabou, N., Medbouhi, A., Khadir, A., Bendahou, M., Selles, C. et al. (2016) Chemical variability and biological activities of essential oils of *Micromeria inodora* (Desf.) Benth. from Algeria. *Chemistry & Biodiversity*, 13, 1559–1572. https://doi.org/10.1002/cbdv.201600098.

Bhuiyan, FarhanaR, Campos, N.A., Swennen, R. & Carpentier, S. (2020) Characterizing fruit ripening in plantain and cavendish bananas: a proteomics approach. *Journal of Proteomics*, 214, 103632. https://doi.org/10.1016/j.jprot.2019.103632

Borges, C.V., Amorim, V.B.O., Ramlov, F., Ledo, C.A.S., Donato, M., Maraschin, M. et al. (2014) Characterisation of metabolic profile of banana genotypes, aiming at biofortified *Musa* spp. cultivars. *Food Chemistry*, 145, 496–504. https://doi.org/10.1016/j.foodchem.2013.08.041.

Braga, S.P., Lundgren, G.A., Macedo, S.A., Tavares, J.F., dos Santos Vieira, W.A., Câmara, M.P.S. et al. (2019) Application of coatings formed by chitosan and *Mentha* essential oils to control anthracnose caused by *Colletotrichum gloesporioides* and *C. brevisporum* in papaya (*Carica papaya* L.) fruit. *International Journal of Biological Macromolecules*, 139, 631–639. https://doi.org/10.1016/j.ijbiomac.2019.08.010

Celik, K., Togar, B., Türkez, H. & Taspinar, N. (2014) *In vitro* cytotoxic, genotoxic, and oxidative effects of acyclic sesquiterpene farnesene. *Turkish Journal of Biology*, 38, 253–259. https://doi.org/10.3906/biy-1309-55.

Chiang, K.S., Liu, H.I., Tsai, J.W., Tsai, J.R. & Bock, C.H. (2017) A discussion on disease severity index values. Part II: using the disease severity index for null hypothesis testing: hypothesis testing using a disease severity index. *The Annals of Applied Biology*, 171, 490–505. https://doi.org/10.1111/aab.12396.

Chillet, M., Hubert, O. & de Lapeyre de Bellaire, L. (2007) Relationship between physiological age, ripening and susceptibility of banana to wound anthracnose. *Crop Protection*, 26, 1078–1082. https://doi.org/10.1016/j.cropro.2006.09.012.

CIE — Commission Internationale de l'Éclairage (1986) *Colourimetry*. Vienna: CIE Publication.

Cindi, M.D., Shittu, T., Sivakumar, D. & Bautista-Baños, S. (2015) Chitosan boehmite-alumina nanocomposite films and thyme oil vapour control brown rot in peaches (*Prunus persica* L.) during postharvest storage. *Crop Protection*, 72, 127–131. https://doi.org/10.1016/j.cropro.2015.03.011.

Cosme Silva, G.M., Silva, W.B., Medeiros, D.B., Salvador, A.R., Cordeiro, M.H.M., da Silva, N.M. et al. (2017) The chitosan affects severely the carbon metabolism in mango (*Mangifera indica* L. cv. Palmer) fruit during storage. *Food Chemistry*, 237, 372–378. https://doi.org/10.1016/j.foodchem.2017.05.123.

de Costa, D.M. & Erahadupitiya, H.R.U.T. (2005) An integrated method to control postharvest diseases of banana using a member of the *Burkholderia cepacia* complex. *Postharvest Biology and Technology*, 36, 31–39. https://doi.org/10.1016/j.postharvbio.2004.11.007.

de Oliveira, K.Á.R., Berger, L.R.R., de Araújo, S.A., Câmara, M.P.S. & de Souza, E.L. (2017) Synergistic mixtures of chitosan and *Mentha piperita* L. essential oil to inhibit *Colletotrichum* species and anthracnose development in mango cultivar Tommy Atkins. *Food Microbiology*, 66, 96–103. https://doi.org/10.1016/j.fm.2017.04.012.

de Paula, C.C., Martins, D.T.D.O., Arunachalam, K., Balogun, S.O., Borges, Q.I., Picone, M.C. et al. (2018) Antimicrobial screening of medicinal plants popularly used in Mato Grosso for treating infections: advances on the evaluation of *Conyza bonariensis* (L.) cronquist *in vitro* and *in vivo* antibacterial activities. *Pharmacogn J*, 10, s152–s166. https://doi.org/10.5530/pj.2018.6s.28.

Farzaneh, M., Kiani, H., Sharifi, R., Reisi, M. & Hadian, J. (2015) Chemical composition and antifungal effects of three species of Satureja (*S. hortensis*, *S. spicigera*, and *S. khuzistanica*) essential oils on the main pathogens of strawberry fruit. *Postharvest Biology and Technology*, 109, 145–151. https://doi.org/10.1016/j.postharvbio.2015.06.014.

Francomano, F., Caruso, A., Barbarossa, A., Fazio, A., La Torre, C., Ceramella, J. et al. (2019) β-Caryophyllene: a sesquiterpene with countless biological properties. *Applied Sciences*, 9, 5420. https://doi.org/10.3390/app9245420.

Guerra, I.C.D., de Oliveira, P.D.L., de Souza Ponies, A.I., Lúcio, A.S.S.C., Tavares, J.F., Barbosa-Filho, J.M. et al. (2015) Coatings comprising chitosan and *Mentha piperita* L. or *Mentha×villosa* Huds essential oils to prevent common postharvest mold infections and maintain the quality of cherry tomato fruit. *International Journal of Food Microbiology*, 214, 168–178. https://doi.org/10.1016/j.ijfoodmicro.2015.08.009.

Guerra, I.C.D., de Oliveira, P.D.L., Santos, M.M.F., Lúcio, A.S.S.C., Tavares, J.F., Barbosa-Filho, J.M. et al. (2016) The effects of composite coatings containing chitosan and *Mentha* (*piperita* L. or x *villosa* Huds) essential oil on postharvest mold occurrence and quality of table grape cv. Isabella. *Innovative Food Science & Emerging Technologies*, 34, 112–121. https://doi.org/10.1016/j.ifset.2016.01.008.

Guimarães, A.C., Meireles, L.M., Lemos, M.F., Guimarães, M.C.C., Endringer, D.C., Fronza, M. et al. (2019) Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules*, 24, 2471. https://doi.org/10.3390/molecules24132471.

Hanbali, E.F., Mellouki, F., Akssira, M., Boira, H. & Blásquez, M.A. (2007) Composition and antimicrobial activity of essential oil of *Anthemis tenuisecta* ball. *Journal of Essential Oil Bearing Plants*, 10, 499–503. https://doi.org/10.1080/0972060X.2007.10643586.

Khaliq, G., Abbas, H.T., Ali, I. & Waseem, M. (2019) Aloe vera gel enriched with garlic essential oil effectively controls anthracnose disease and maintains postharvest quality of banana fruit during storage. *Horticulture, Environment, and Biotechnology*, 60, 659–669. https://doi.org/10.1007/s13580-019-00159-z

Kim, D.-K., Kim, S.-J. & Kang, D.-H. (2017) Bactericidal effect of 266 to 279 nm wavelength UVC-LEDs for inactivation of Gram positive and Gram negative foodborne pathogenic bacteria and yeasts. *Food Research International*, 97, 280–287. https://doi.org/10.1016/j.foodres.2017.04.009.

Lee, H., Woo, E.-R. & Lee, D.G. (2018) Apigenin induces cell shrinkage in *Candida albicans* by membrane perturbation. *FEMS Yeast Research*, 18. https://doi.org/10.1093/femsyr/foy003.

Lima, N.B., Lima, W.G., Tovar-Pedraza, J.M., Michereff, S.J. & Câmara, M.P.S. (2015) Comparative epidemiology of *Colletotrichum* species from mango in northeastern Brazil. *European Journal of Plant Pathology*, 141, 679–688. https://doi.org/10.1007/s10658-014-0570-y.

Lobo, M.G. & Rojas, F.J.F. (2020). Biology and postharvest physiology of banana. In: Siddiq, M., Ahmed, J. & Lobo, M.G. (Eds.), *Handbook of banana production, postharvest science, processing technology, and nutrition*. Hoboken, NJ: Wiley, pp. 19–44. https://doi.org/10.1002/9781119528265.ch2

Lombardo, P., Guimaraens, A., Franco, J., Dellacassa, E. & Pérez Faggiani, E. (2016) Effectiveness of essential oils for postharvest control of *Phyllosticta citricarpa* (citrus black spot) on citrus fruit. *Postharvest Biology and Technology*, 121, 1–8. https://doi.org/10.1016/j.postharvbio.2016.07.002.

Mabrouk, S., Elaissi, A., Ben Jannet, H. & Harzallah-Skhiri, F. (2011) Chemical composition of essential oils from leaves, stems, flower heads and roots of *Conyza bonariensis* L. from Tunisia. *Natural Product Research*, 25, 77–84. https://doi.org/10.1080/14786419.2010.513685.

Macedo, S.A., Lundgren, C.A., dos Passos Braga, S., de Souza, E.L. & Câmara, M.P.S. (2020) Combined chitosan and *Cympobogon citratus* (D.C. ex Nees) Stapf. essential oil to inhibit the fungal phytopathogen *Paramyrothecium roridum* and control crater rot in melon (*Cucumis melo* L.). *Brazilian Journal of Microbiology*, 51, 2057–2065. https://doi.org/10.1007/s42770-020-00378-y.

Maia, J.G.S., da Silva, M.H.L., Zoghbi, M.G.B. & Andrade, E.H.A. (2002) Composition of the essential oils of *Conyza bonariensis* (L.) Cronquist. *Journal of Essential Oil Research*, 14, 325–326. https://doi.org/10.1080/10412905.2002.9699871.

Maqbool, M., Ali, A. & Alderson, P.R. (2010) A combination of gum arabic and chitosan can control anthracnose caused by *Colletotrichum musae* and enhance the shelf-life of banana fruit. *Journal of Horticultural Science and Biotechnology*, 85, 432–436. https://doi.org/10.1080/14620316.2010.115126.

Maqbool, M., Ali, A., Alderson, P.G., Mohamed, M.T.M., Siddiqui, Y. & Zahid, N. (2011) Postharvest application of gum Arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biology and Technology*, 62, 71–76. https://doi.org/10.1016/j.postharvbio.2011.04.002.

Maqbool, M., Ali, A., Alderson, P.G. & Zahid, N. (2013) Controlling postharvest anthracnose of banana using novel edible composite coatings by stimulating defence-related enzymes. *Acta Horticulture*, 1012, 639–644. https://doi.org/10.17660/ActaHortic.2013.1012.86

Noriega, P., Ballesteros, J., de La Cruz, A. & Veloz, T. (2020) Chemical composition and preliminary antimicrobial activity of the hydroxylated sesquiterpenes in the essential oil from *Piper barbatum* Kunth Leaves. *Plants*, 9, 211. https://doi.org/10.3390/plants9020211.

Okello, S.V., Nyunja, R.O., Netondo, G.W. & Onyango, J.C. (2009) Ethnobotanical study of medicinal plants used by Sabaots of Mt. Elgon Kenya. *African Journal of Traditional, Complementary and Alternative Medicines*, 7, 1–10. https://doi.org/10.4314/ajtcam.v7i1.57223

Oliveira, P.D.L., de Oliveira, K.Á.R., Vieira, W.A.S., Câmara, M.P.S. & de Souza, E.L. (2018) Control of anthracnose caused by Colletotrichum species in guava, mango and papaya using synergistic combinations of chitosan and *Cymbopogon citratus* (D.C. ex Nees) Stapf. essential oil. *International Journal of Food Microbiology*, 266, 87–94. https://doi.org/10.1016/j.ijfoodmicro.2017.11.018.

Saleem, M.S., Ejaz, S., Anjum, M.A., Nawaz, A., Naz, S., Hussain, S. et al. (2020) Postharvest application of gum Arabic edible coating delays ripening and maintains quality of persimmon fruits during storage. *Journal of Food Processing and Preservation*, 44. https://doi.org/10.1111/jfpp.14583.

Siddiqui, Y. & Ali, A. (2014) *Colletotrichum gloeosporioides* (anthracnose) in post harvest decay: control strategies. In: Bautista-Baños, S. (Ed.) *Alternative management of diseases of horticultural produce*. Cambridge: Elsevier Academic Press, pp. 337–371.

Silva, L.M.E., Barbosa, M.G., Fernandes, M.B., Ribeiro, R.C.F. & Mizobutsi, E.H. (2016) Temporal progress and control of anthracnose on banana in semiarid in the north of Mina Gerais. *Revista Brasileira de Fruticultura* 38, 81–91. https://doi.org/10.1590/0100-2945-299/14.

Singh, T.P., Chatli, M.K. & Sahoo, J. (2015) Development of chitosan based edible films: process optimization using response surface methodology. *Journal of Food Science and Technology*, 52, 2530–2543. https://doi.org/10.1007/s13197-014-1318-6.

Sivakumar, D. & Bautista-Baños, S. (2014) A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Protection*, 64, 27–37. https://doi.org/10.1016/j.cropro.2014.05.012.

Souza, E.L., Lundgren, G.A., Oliveira, K.Á.R., Berger, L.R.R. & Magnani, M. (2019) An analysis of the published literature on the effects of edible coatings formed by polysaccharides and essential oils on postharvest microbial control and overall quality of fruit. *Comprehensive Reviews in Food Science and Food Safety*, 18, 1947–1967. https://doi.org/10.1111/1541-4337.12498.

Thakur, R., Pristijono, P., Bowyer, M., Singh, S.P., Scarlett, C.J., Stathopoulos, C.E. et al. (2019) A starch edible surface coating delays banana fruit ripening. *LWT-Food and Science Technology*, 100, 341–347. https://doi.org/10.1016/j.lwt.2018.10.055.

Tian, J., Ban, X., Zeng, H., He, J., Chen, Y. & Wang, Y. (2012) The mechanism of antifungal action of essential oil from Dill (*Anethum graveolens* L.) on *Aspergillus flavus*. *PLoS One*, 7, e30147. https://doi.org/10.1371/journal.pone.0030147.

Vieira, W.A.S., Lima, W.G., Nascimento, E.S., Michereff, S.J., Reis, A., Doyle, V.P. et al. (2017) Thiophanate-methyl resistance and fitness components of *Colletotrichum musae* isolates from banana in Brazil. *Plant Disease*, 101, 1659–1665. https://doi.org/10.1094/PDIS-11-16-1594-RE.

Vilaplana, R., Pazmiño, L. & Valencia-Chamorro, S. (2018) Control of anthracnose, caused by *Colletotrichum musae*, on postharvest organic banana by thyme oil. *Postharvest Biology and Technology*, 138, 56–63. https://doi.org/10.1016/j.postharvbio.2017.12.008.

Xing, K., Li, T.J., Liu, Y.F., Zhang, J., Zhang, Y., Shen, X.Q. et al. (2018) Antifungal and eliciting properties of chitosan against *Ceratocystis fimbriata* in sweet potato. *Food Chemistry*, 268, 188–195. https://doi.org/10.1016/j.foodchem.2018.06.088.

Yun, D.G. & Lee, D.G. (2017) Silymarin exerts antifungal effects via membrane-targeted mode of action by increasing permeability and inducing oxidative stress. *Biochimica et Biophysica Acta (BBA) – Biomembranes*, 1859, 467–474. https://doi.org/10.1016/j.bbamem.2017.01.009.

Zeng, H., Chen, X. & Liang, J. (2015) *In vitro* antifungal activity and mechanism of essential oil from fennel (*Foeniculum vulgare* L.) on dermatophyte species. *Journal of Medical Microbiology*, 64, 93–103. https://doi.org/10.1099/jmm.0.077768-0.

Zitter, T.A., Hopkins, D.L. & Thomas, C.E. (1996) *Compendium of cucurbit diseases. The disease compendium series of the American Phytopathological Society*. St. Paul, MN: APS Press.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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4.3. APÊNDICE C - PEDIDO NACIONAL DE INVENÇÃO, MODELO DE UTILIDADE, CERTIFICADO DE ADIÇÃO DE INVENÇÃO E ENTRADA NA FASE NACIONAL DO PCT.

INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

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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 2020 013106 0

Dados do Depositante (71)

Depositante 1 de 1

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Dados do Pedido

Natureza Patente: 10 - Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade (54): REVESTIMENTO À BASE DE GOMA ARÁBICA E ÓLEO ESSENCIAL PARA PROTEÇÃO DE FRUTOS PÓS-COLHEITA, PROCESSO E PRODUTO

Resumo: A presente invenção tem aplicação na área de tecnologia de preservação de produtos hortifrutícolas e refere-se ao desenvolvimento de revestimento composto da combinação do biopolímero goma arábica e do óleo essencial de Conyza bonariensis (L.) Cronquist. para uso na conservação pós-colheita de frutos in natura. A aplicação do revestimento objetiva promover o controle do desenvolvimento de fungos fitopatógenos pós-colheita nestes produtos, bem como manter as suas caracteristicas de qualidade ao longo do armazenamento em temperatura ambiente, refrigerada ou de resfriamento e apresentar-se como uma alternativa ao uso de fungicidas sintéticos na fase pós-colheita nestes produtos. O produto apresenta ação comprovada frente ao fungo fitopatógeno Colletotrichum musae causador de antracnose, caracterizada como a principal infecção pós-colheita em frutos de banana, com manutenção das características dos parâmetros de qualidade destes produtos, surgindo como tecnologia alternativa de preservação e conservação de frutos, com potencial de atender as demandas correntes do mercado.