GABRIELA AGUIAR CAMPOLINA

ELABORAÇÃO DE REVESTIMENTOS COMESTÍVEIS e AVALIAÇÃO DAS ATIVIDADES ANTIFÚNGICA E INSETICIDA DE ÓLEOS ESSENCIAIS E COMPOSTOS-PADRÃO

LAVRAS – MG

2023

GABRIELA AGUIAR CAMPOLINA

ELABORAÇÃO DE REVESTIMENTOS COMESTÍVEIS, AVALIAÇÃO DAS ATIVIDADES ANTIFÚNGICA E INSETICIDA DE ÓLEOS ESSENCIAIS E COMPOSTOS-PADRÃO

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, Ciência dos Alimentos, para a obtenção do título de Doutor.

Profa. Dra. Maria das Graças Cardoso
Orientadora

Prof. Dr. Khalid Haddi
Coorientador

Prof. Dr. Luís Roberto Batista
Coorientador

LAVRAS – MG
2023

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Campolina, Gabriela Aguiar.
Elaboração de revestimentos comestíveis e avaliação das atividades antifúngica e inseticida de óleos essenciais e compostos-padrão / Gabriela Aguiar Campolina. - 2023.
180 p. : il.

Orientador(a): Maria das Graças Cardoso.
Coorientador(a): Khalid Haddi, Luís Roberto Batista.
Tese (doutorado) - Universidade Federal de Lavras, 2023.
Bibliografia.

1. Óleos essenciais. 2. Atividade Antifúngica. 3. Atividade Inseticida. I. Cardoso, Maria das Graças. II. Haddi, Khalid. III. Batista, Luís Roberto. IV. Título.

GABRIELA AGUIAR CAMPOLINA

ELABORAÇÃO DE REVESTIMENTOS COMESTÍVEIS, AVALIAÇÃO DAS ATIVIDADES ANTIFÚNGICA E INSETICIDA DE ÓLEOS ESSENCIAIS E COMPOSTOS-PADRÃO

ELABORATION OF EDIBLE COATINGS, EVALUATION OF THE ANTIFUNGAL AND INSECTICIDE ACTIVITIES OF ESSENTIAL OILS AND STANDARD COMPOUNDS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, Ciência dos Alimentos, para a obtenção do título de Doutor.

APROVADA em 21 de julho de 2023.

Dra. Maria das Graças Cardoso	UFLA
Dra. Samisia Maria Fernandes Machado	UFS
Dra. Maria da Graça Costa Miguel	UAlg
Dr. Luis Roberto Batista	UFLA
Dr. David Lee Nelson	UFVJM

_____

Profa. Dra. Maria das Graças Cardoso
Orientadora

Prof. Dr. Khalid Haddi
Coorientador

Prof. Dr. Luís Roberto Batista
Coorientador

LAVRAS – MG
2023

AGRADECIMENTOS

A Deus, pelo dom da vida, pela capacidade para concluir mais esta etapa e por sempre estar junto a mim em todos os momentos desta jornada.

À Universidade Federal de Lavras (UFLA), pela oportunidade de cursar o Doutorado em Ciência dos Alimentos, ao Departamento de Ciência dos Alimentos por toda a estrutura disponibilizada e ao Departamento de Química por ter me recebido tão bem e ter disponibilizado sua estrutura.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela bolsa concedida, à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) e à Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig), pelo apoio que tornou possível a realização deste estudo.

À minha querida orientadora, Profa. Maria das Graças Cardoso, pela oportunidade de aprender muito além de um curso de doutorado. Por sempre acreditar em meu potencial, pela convivência, amizade, paciência, incentivo e ensinamentos que levarei por toda a minha vida e serei eternamente grata.

Aos meus pais, Eugênio e Solange, e às minhas irmãs, Júlia e Camila, pelos princípios que me passaram, por estarem sempre comigo mesmo que à distância, por todo o amor, apoio, incentivo e por entenderem o caminho que escolhi seguir.

Á minha afilhada, Alice, por todos os sorrisos, carinho e abraços apertados que me fazem esquecer qualquer problema.

Ao meu noivo, Alex, por todo o amor, paciência, ajuda nos experimentos e por sempre me incentivar, principalmente nos momentos mais difíceis.

Aos meus padrinhos, Suzana e Davi, pelas orações e incentivo.

A todos os meus familiares, amigos e às meninas da Home, que tornaram esse caminho mais leve e feliz.

Aos meus coorientadores, Prof. Luis Roberto Batista e Prof. Khalid Haddi, por terem aberto as portas de seus laboratórios e me recebido com tanta disponibilidade.

Ao Prof. Eduardo Alves, pela disponibilidade, por ter aberto as portas do seu laboratório para a realização deste estudo.

Ao Prof. Dr. David Lee Nelson (UFVJM-MG), pela disponibilidade e por sempre contribuir com nossos trabalhos.

A todos os professores, técnicos e funcionários do DQI e DCA/UFLA, pela convivência, ajuda e ensinamentos.

Às minhas coorientadas de Iniciação Científica Anna Beatriz e Mariely, por toda a ajuda, disponibilidade e convivência.

Ao Laboratório de Química Orgânica – Óleos Essenciais (DQI/UFLA).

Aos colegas de trabalho do Laboratório de Química Orgânica – Óleos Essenciais e do Laboratório de Análises e Qualidade de Aguardentes: Alex, Carol, Anna Beatriz, Vanuzia, Gislaine, Maria Augusta, Isadora, Luciano, Lucas, Giovana, Cassia, Sophia, Renata, Sara e à Técnica Maria Luisa, pela convivência diária, ajuda na condução dos experimentos e palavras de apoio.

A todos os colegas do Laboratório de Micologia de Alimentos (DCA/UFLA), pela ajuda e disponilibidade.

À Central de Análises de Prospecção Química da Universidade Federal de Lavras (CAPQ-UFLA), em especial às técnicas Lidyane e Francine.

Ao Laboratório de Microscopia Eletrônica e Análises Ultraestruturais (LME-UFLA), em especial à técnica Aline.

E a todos que, de alguma forma, contribuíram para a concretização deste trabalho.

RESUMO

A aplicação de óleos essenciais em alimentos visando à redução do uso de inseticidas e fungicidas sintéticos tem sido cada vez mais promissora. Neste estudo objetivou-se extrair e caracterizar quimicamente os óleos essenciais de cominho (OEC), louro (OEL) e tangerina (OET), bem como avaliar suas atividades inseticida e antifúngica e de seus compostos majoritários, cuminaldeído, 1,8-cineol e limoneno, por aplicação direta ou inseridos a revestimentos comestíveis. Os compostos-padrão foram adquiridos comercialmente, os óleos essenciais foram extraídos por hidrodestilação e caracterizados por cromatografia gasosa acoplada a um detector de massa. A atividade inseticida foi realizada *in vitro* por meio do teste de inibição da acetilcolinesterase e *in vivo* sobre *D. suzukii*, avaliando a toxicidade do OEC e do seu composto majoritário cuminaldeído incorporados a revestimentos comestíveis aplicados em morangos. Os revestimentos foram elaborados contendo diferentes concentrações do OEC ou cuminaldeído. Um tratamento-controle com a adição do revestimento sem óleo essencial e outro sem adição de revestimento também foi realizado. Os revestimentos foram avaliados quanto à capacidade de prevenir ou reduzir a natalidade das moscas *D. suzukii.* A atividade antifúngica sobre *Rhizopus stolonifer* foi avaliada pelo método de contato, e as amostras utilizadas foram OEC, OEL, OET e seus respectivos compostos-padrão. Avaliou-se também a atividade antifúngica e antiocratoxigênica dos OEC, OEL e seus respectivos compostos-padrão sobre os fungos do gênero *Aspergillus: A.carbonarius, A.niger, A.ochraceus* e *A. westerdijkiae.* Foram realizadas imagens em microscópio eletrônico de varredura (MEV), a fim de sugerir possíveis formas de atuação dos compostos analisados. A atividade antiocratoxigênica foi realizada por HPLC, após o crescimento dos fungos em meios de cultura específicos. Os resultados obtidos para caracterização dos óleos essenciais apresentaram como majoritário o composto cuminaldeído no OEC; o 1,8-cineol no OEL e o limoneno no OET. A inibição da acetilcolinesterase foi maior para a amostra de OEC que para a amostra de cuminaldeído (CPC). Em relação à toxicidade sobre a mosca *D. suzukii*, ambas as amostras avaliadas, OEC e cuminaldeído, apresentaram bons resultados quando aplicadas incorporadas aos revestimentos. Esses revestimentos não impediram a ovoposição em nenhum dos tratamentos avaliados; porém, reduziram a natalidade de moscas *D. suzukii.* Em relação à atividade antifúngica sobre *R. stolonifer*, todas as amostras avaliadas inibiram completamente o crescimento micelial do fungo na maior concentração testada, com destaque para o OEC e o CPC, que apresentaram as menores concentrações mínimas inibitórias. Na inibição do crescimento dos fungos do gênero *Aspergillus,* o OEC também apresentou melhores resultados, bem como na inibição da síntese de ocratoxina. As imagens de MEV mostraram que, após os tratamentos, houve deformações, alterações morfológicas nas estruturas fúngicas e inibição de esporos. Assim, infere-se que os revestimentos comestíveis incorporados com OEC ou CPC podem ser utilizados como uma boa estratégia de manejo de *D. suzukii* e *R. stolonifer* em morangos. Os resultados obtidos sobre fungos do gênero *Aspergillus* comprovaram o efeito antifúngico e antiocratoxigênico dos óleos essenciais e dos compostos-padrão sobre eles e os apontam como promissores constituintes de antifúngicos à base de produtos naturais.

Palavras-chave: *Aspergillus.* Ocratoxina A. *Drosophila suzukii*. *Rhizopus stolonifer*. Óleos essenciais

ABSTRACT

The application of essential oils in food to reduce the use of insecticides and fungicides has been increasingly promising. The aim of this study was to extract and chemically characterize the essential oils of cumin (CEO), laurel (LEO) and tangerine (TEO), as well as to evaluate the insecticidal and antifungal activity of these oils and their principal compounds, cuminaldehyde, 1,8-cineole and limonene, by direct application or by inclusion in edible coatings. The standard compounds were purchased commercially, whereas the essential oils were extracted by hydrodistillation and characterized by gas chromatography coupled to a mass detector. The insecticidal activity was determined in vitro through the acetylcholinesterase inhibition test and in vivo on *D. suzukii*. The toxicity of CEO and its principal compound cuminaldehyde were evaluated by incorporation into edible coatings applied to strawberries. The coatings contained different concentrations of CEO or cuminaldehyde, a control treatment involving the addition of the coating without the essential oil and another without the addition of a coating were also performed. The coatings were evaluated for their ability to prevent or reduce the birth rate of *D. suzukii* flies. The antifungal activity against *Rhizopus stolonifer* was evaluated by the contact method, and the samples used were CEO, LEO, TEO and their respective standard compounds. The antifungal and antiocratoxigenic activity of CEO, LEO and their respective standard compounds against the *Aspergillus* fungi *A.carbonarius, A.niger, A.ochraceus* and *A. westerdijkiae* was also evaluated. Scanning electron microscope (SEM) images were taken to suggest possible modes of action of the compounds analyzed. Antiocratoxigenic activity was determined by HPLC, after the fungi grew in specific culture media. Cuminaldehyde was the principal component in the CEO; 1,8-cineole in the LEO and; limonene in TEO. Acetylcholinesterase inhibition was greater for the CEO sample than for the cuminaldehyde sample. Regarding the toxicity to the *D. suzukii* fly, good results were obtained for both evaluated samples, CEO and cuminaldehyde, when they were incorporated into the coatings. These coatings did not prevent oviposition in any of the treatments evaluated; however, the natality of *D. suzukii* flies decreased. Regarding the antifungal activity against *R. stolonifer*, the mycelial growth of the fungus was completely inhibited by all evaluated samples at the highest concentration tested, with emphasis on the CEO and CPC, for which the lowest minimum inhibitory concentrations were observed. Better results were obtained for CEO for the inhibition of the growth of fungi of the *Aspergillus* genus and for the inhibition of the synthesis of ochratoxin. Deformations, morphological changes in fungal structures and spore inhibition were observed in the SEM images. Thus, it is inferred that edible coatings incorporated with CEO or CPC can be used as a good management strategy for *D. suzukii* and *R. stolonifer* in strawberries. The antifungal and antiocratoxigenic effects of essential oils and standard compounds on fungi of the genus *Aspergillus* were demonstrated, and these results indicate that they can be promising constituents of antifungals based on natural products.

Keywords: *Aspergillus*. Ochratoxin A. *Drosophila suzukii*. *Rhizopus stolonifer*. Essential oils.

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

As frutas vermelhas, em particular os morangos, são amplamente apreciados por sua aparência atraente, sabor agradável e benefícios nutricionais. Além de serem uma fonte rica de vitaminas, minerais e antioxidantes, essas frutas têm sido associadas a diversos efeitos positivos para a saúde, como a redução do risco de doenças crônicas. No entanto, a sua produção e a comercialização não estão isentas de desafios, entre os quais podem ser destacados o ataque de insetos e de espécies fúngicas.

A mosca *Drosophila suzukii*, inseto de origem asiática, espalhou-se rapidamente por várias partes do mundo, tornando-se um inseto-praga preocupante para os produtores de morangos. Sua capacidade de ovoposição em frutas maduras e saudáveis pode causar graves danos e tornar as frutas inadequadas para o consumo humano. Além disso, o ataque dessa mosca facilita também a proliferação de fungos, que já é um grave problema relatado pelos produtores e consumidores desse alimento.

O fungo *Rhizopus stolonifer* é um dos mais preocupantes fungos que deterioram os morangos, devido à sua alta e rápida capacidade destrutiva. *R. stolonifer* se desenvolve rapidamente, contaminando a fruta por inteiro em poucos dias e comprometendo sua qualidade e vida útil. Dessa forma, a busca por soluções para enfrentar esses desafios e controlar essas pragas de forma eficiente e sustentável e, além disso, continuar oferecendo um alimento de qualidade para o consumidor por um período mais longo, tem sido cada vez maior entre os pesquisadores, as indústrias alimentícias e produtores de frutas vermelhas.

Além dos desafios enfrentados para a produção, distribuição e comercialização de frutas como os morangos, outros fungos têm sido preocupantes para a segurança dos alimentos, devido à capacidade de contaminação e produção de micotoxinas, metabólitos tóxicos, que mesmo em baixas concentrações, causam malefícios graves a quem as consomem. Entre os fungos produtores de micotoxinas, os pertencentes ao gênero *Aspergillus* são amplamente distribuídos na natureza e podem contaminar diversos alimentos, incluindo grãos, frutas, vegetais e alimentos processados. As espécies *Aspergilus carbonarius*, *Aspergilus niger*, *Aspergilus ochraceus* e *Aspergilus westerdijkiae* têm a capacidade de produzir ocratoxinas, em especial ocratoxina A, representando um risco para a saúde humana.

A aplicação de produtos naturais extraídos de plantas, como os óleos essenciais, para o controle de insetos-pragas e fungos tem sido considerada uma estratégia promissora para a proteção de alimentos e tem atendido aos pré-requisitos almejados pelos consumidores que

estão cada vez mais em busca de produtos saudáveis e livres de inseticidas e fungicidas sintéticos.

Os óleos essenciais são uma mistura complexa de compostos voláteis que podem ser extraídos de diversas partes das plantas e têm se destacado por suas propriedades biológicas, como seu potencial inseticida, antimicrobiano, antioxidante, antitumoral, antiviral, entre outros. Porém, a aplicação de óleos essenciais em alimentos pode ser dificultada pois, apesar dessas propriedades biológicas de interesse, a alta volatilidade desses compostos pode interferir no odor e sabor dos alimentos. Assim, novas tecnologias tem sido desenvolvidas, a fim de facilitar essas aplicações, como por exemplo os revestimentos comestíveis.

Os revestimentos comestíveis são películas finas aplicadas de forma a estar em contato direto com os alimentos e atuar como uma embalagem ativa. Esses revestimentos podem ser elaborados por diferentes materiais, como polissacarídeos, lipídeos, proteínas ou a sua combinação. Quando os óleos essenciais são incorporados a esses revestimentos, têm se mostrado eficientes para prolongar a vida útil dos produtos alimentícios, preservar sua qualidade e proporcionar uma barreira de proteção que pode auxiliar a evitar a perda de umidade, contaminação externa, ataque de pragas e desenvolvimento de microrganismos. A aplicação de óleos essenciais na forma de revestimentos ajuda a mascarar as característica de odor e sabor dos óleos essenciais, não interferindo, assim, nas características sensoriais dos alimentos.

Assim, neste estudo objetivou-se avaliar a atividade antifúngica e antiocratoxigênica dos óleos essenciais de *Cuminum cyminum* e *Laurus nobilis,* e seus respectivos compostos majoritários, cuminaldeído e 1,8-cineol, sobre os fungos *Aspergillus carbonarius, Aspergillus niger, Aspergillus ochraceus* e *Aspergillus westerdijkiae;* avaliar a toxicidade do óleo essencial de cominho e do composto-padrão cuminaldeído sobre *D. suzukii* e analisar a influência das amostras na taxa de natalidade das moscas, quando incorporados a revestimentos comestíveis à base de amido de mandioca e aplicados a morangos; elaborar e avaliar a atividade antifúngica, *in vitro* e *in vivo,* de revestimentos comestíveis incorporados com os óleos essenciais de *Cuminum cyminum*, *Laurus nobilis*, *Citrus reticulata x sinensis* e seus respectivos compostos majoritários, cuminaldeído, 1,8-cineol e limoneno sobre *Rizopus stolonifer*.

2 REFERENCIAL TEÓRICO

2.1 Frutas vermelhas

As frutas vermelhas, ou *berries* como também são conhecidas, destacam-se das demais frutas principalmente por suas características sensoriais agradáveis e benefícios à saúde, que estão atrelados às suas propriedades antioxidantes. Dentre as frutas pertencentes a esse grupo, popularmente conhecido como "frutas vermelhas", destacam-se a amora, o pseudofruto morango, a framboesa e o mirtilo, frutas avermelhadas ou arroxeadas e de tamanho relativamente pequeno (LAMOUNIER *et al*., 2019; BARBIERI e VIZZOTTO, 2012).

De acordo com Barbieri e Vizzotto (2012), dentre as características das "frutas vermelhas", a alta concentração de vitaminas A e C, substâncias antioxidantes e flavonoides merecem ser ressaltadas. Essas características, além de contribuírem para o sabor, cor e aroma desse alimento, podem proteger células humanas dos radicais livres (intermediários químicos altamente reativos que podem acarretar no surgimento de doenças crônicas) por sua ação antioxidante. Porém, apesar desses aspectos positivos em relação a essas frutas, elas possuem alta perecibilidade, o que torna sua vida pós-colheita reduzida e o fato de não possuírem casca firme as tornam mais frágeis e disponíveis à ação de fatores externos, como microrganismos e até mesmo insetos (PAVINATTO *et al*., 2019; LOEBLER *et al*., 2018).

O pseudofruto morango, a amora, a framboesa e o mirtilo são frutos não climatéricos, assim devem ser colhidos em seu estágio ideal de maturação, pois não ocorre aumento na sua taxa respiratória e na produção de etileno após a colheita, fatos que dificultam o manejo dessas frutas (RODRÍGUEZ *et al*., 2020; FUENTES, FIGUEROA e VALDENEGRO, 2019; OH *et al*., 2017). Apesar de fatores ambientais, forma de manuseio e espécie influenciarem na manutenção das características dos frutos, quando essas são mantidas sob refrigeração (0 a 4°C), suas características são preservadas por um período maior. Os morangos, por exemplo, possuem vida útil média de apenas 5 dias, as amoras de 4 dias, a framboesa de no máximo 14 dias e o mirtilo, por possuir casca menos sensível, se destaca das demais "frutas vermelhas" e é capaz de manter suas características por 20 a 30 dias (ÁVILA *et al*., 2020; GONZÁLES-OROZCO *et al*., 2020; ABUGOCH *et al*., 2016; PERDONES *et al*., 2012).

A produção de "frutas vermelhas" no Brasil tem crescido nos últimos anos, devido principalmente ao alto valor de mercado desses produtos. A estimativa em 2019 era que o cultivo das frutas vermelhas, em geral, ocupassem aproximadamente 4.200 hectares.

Atualmente, os últimos panoramas indicaram uma ocupação territorial de 5.500 hectares apenas para o cultivo de morangos, sendo esse o principal fruto do grupo de “frutas vermelhas” cultivada no país. No ano de 2021, foram produzidas 165.440 toneladas de morangos, totalizando uma movimentação de aproximadamente R$1,7 bilhão. (SOUZA, BATISTA e MENEZES *et al*., 2022; JÚNIOR, OLIVEIRA e RODRIGUES, 2019).

Estima-se que a demanda por morangos (Figura 1) cresça ainda mais, atingindo um aumento de 30% em todo o mundo em 2030. Porém, infelizmente, o crescimento na produção de frutas ainda encontra-se atrelado ao crescimento das perdas mercadológicas. No Brasil, aproximadamente 40% da produção de frutas e hortaliças são descartadas, desde a colheita até a mesa do consumidor, sendo as frutas os produtos que representam maior percentual de perdas. Entre as frutas vermelhas, o morango possui destaque por se situar em terceiro lugar no ranking, de frutas, legumes e verduras de maior perda de valor e quarto lugar no mesmo ranking quando o quesito é quantidade perdida no varejo (SOUZA, BATISTA e MENEZES *et al*., 2022; ABRAS, 2019a; ABRAS, 2019b).

Figura 1 – Aspecto de um morango.

Fonte: Do autor

Com isso, muitos pesquisadores têm buscado a implantação de tecnologias como forma de minimizar esses desperdícios, visando a proporcionar aos consumidores uma alimentação saudável, segura e livre de microrganismos deterioradores. Porém, pouco é estudado em relação ao ataque de insetos em frutos.

Pesquisas que visam ao prolongamento da vida útil de frutos com a combinação do binômio temperatura e atmosfera modificada têm apresentado resultados interessantes. Liu *et al*. (2018), ao aplicarem revestimentos elaborados com quitosana a fim de combater fungos no

período da pós-colheita de mirtilo, obtiveram resultados que confirmaram o potencial antifúngico da quitosana. Vilaplana *et al*. (2020) também aplicaram revestimentos de quitosana, empregando-o em frutos de amora, e conseguiram controlar a ação de fungos na pós-colheita. Silva *et al*. (2016) avaliaram a aplicação de revestimentos de amido de mandioca em morangos e observaram o prolongamento da vida útil desses produtos. Gomes *et al*. (2017) estudaram os efeitos da aplicação de revestimentos de alginato com óleos essenciais de limão e laranja em framboesas, percebendo a melhoria da qualidade pós-colheita dessas frutas.

2.2 Microrganismos e insetos causadores de perdas de qualidade na produção de frutas vermelhas

2.2.1 *Drosophila suzukii*

A mosca *Drosophila suzukii* (Figura 2), conhecida também como mosca-da-asa-manchada devido aos pontos escuros nas asas dos machos dessa espécie, é uma mosca de origem asiática considerada uma praga invasora. Na Europa e América do Norte, seu surgimento ocorreu no ano de 2008 e, atualmente, já se difundiu para outros países, causando danos econômicos em muitas variedades cultivadas (ENRIQUEZ *et al*., 2020; IBOUH *et al*., 2019; POZZA e CUNHA, 2019). No Brasil, a espécie *Drosophila suzukii* foi identificada pela primeira vez no ano de 2013 no Rio Grande do Sul e em Santa Catarina, difundindo-se para outros estados e chegando a Minas Gerais no ano de 2016 (ANDREAZZA *et al*., 2016; NAVA *et al*., 2015).

Figura 2 – a) Machos da espécie *Drosophila suzukii*; b) Fêmeas da espécie *Drosophila suzukii*.

Fonte: Cooper (2014).

Ao contrário de outras pragas que atingem apenas frutos maduros, fermentados e danificados mecanicamente, as moscas da espécie *D. suzukii* se desenvolvem bem em frutos saudáveis. As principais frutas atingidas por essa praga são as frutas vermelhas, como o morango, o mirtilo, a framboesa e a amora, podendo atingir também frutas como nectarina, uvas, entre outras (ZIVKOVIC *et al*., 2019; NAVA *et al*., 2015).

O ataque das moscas *D. suzukii* às frutas ocorre por meio da ovoposição realizada pelas fêmeas dessa espécie. Essas ovopositam em frutos macios e saudáveis que são consumidos pelas larvas, causando o apodrecimento e impedindo a comercialização dessas frutas. Além dos danos causados pelas larvas, a ovoposição e o crescimento das larvas facilitam a proliferação de fungos filamentosos, leveduras e bactérias (ENRIQUEZ *et al*., 2020; IBOUH *et al*., 2019; SCHLESNER *et al*., 2015).

Os adultos da espécie *D. suzukii* medem de 2 a 3 mm de comprimento e os machos são um pouco menores que as fêmeas. Os machos se diferenciam das fêmeas fisicamente pela presença de uma mancha escura nas pontas das asas e as fêmeas possuem ovopositor pontudo e serrilhado, características que permitem a ovoposição nas frutas (Figura 2). Os ovos depositados são caracterizados por medirem 0,6 x 0,2 mm; apresentarem coloração esbranquiçada a translúcidos, possibilitando a visualização das larvas antes de eclodirem e possuírem dois filamentos respiratórios (SCHLESNER *et al*., 2015; CALABRIA *et al*., 2012).

As perdas econômicas causadas pela *D. suzukii* são difíceis de serem mensuradas por existirem perdas diretas e indiretas; porém, podem ser imensas devido à capacidade de infestar diferentes frutos, principalmente os de casca fina. As perdas diretas estão associadas à infestação em si e as indiretas, à proliferação de microrganismos facilitada pela danificação das cascas das frutas. Alguns relatos mostram que as perdas por infestações não controladas podem alcançar 80%; perdas de 40% da produção já foram registradas no Japão, China e EUA (SANTOS, 2014).

2.2.2. *Rhizopus stolonifer*

As possíveis alterações que ocorrem nas características dos alimentos como alterações na aparência, no odor, no sabor ou outras que tornem o alimento impróprio para consumo são definidas como deterioração alimentar. Um dos principais motivos dessa deterioração pode ocorrer em virtude da ação de microrganismos. A deterioração microbiológica de alimentos

frescos como as frutas pode acontecer tanto no período de pré quanto no de pós-colheita (MADIGAN *et al*., 2016).

De forma geral, o fato de as frutas serem produtos frescos e consumidos crus faz com que elas sejam contaminadas facilmente. Na pós-colheita, etapa de interesse neste estudo, as principais formas de contaminação das frutas são pelas mãos de manipuladores, ao entrarem em contato direto com as frutas e pelas contaminações presentes no ambiente do produto, como a água utilizada, o ar e os recipientes. Além disso, os danos físicos precisam ser evitados e, caso ocorram, os produtos não devem ser comercializados, pois muitos nutrientes requeridos pelos microrganismos se encontram no interior das frutas e se tornam disponíveis com a danificação (YOON e LEE, 2017; CARVALHO, 2010).

De acordo com Carvalho (2010), no período de pós-colheita, os principais microrganismos responsáveis pela deterioração das frutas são os fungos dos gêneros *Alternaria, Botrytis, Diplodia, Monilinia, Penicillium, Phomopsis, Rhizopus* e as bactérias dos gêneros *Erwinia* e *Pseudomonas.* Nas frutas vermelhas, os fungos dos gêneros *Botrytis, Alternaria, Cladosporium, Penicillium* e *Rhizopus* são os principais microrganismos deterioradores (BARVORÁKOVÁ *et al*., 2023; GUIMIRE *et al*., 2022).

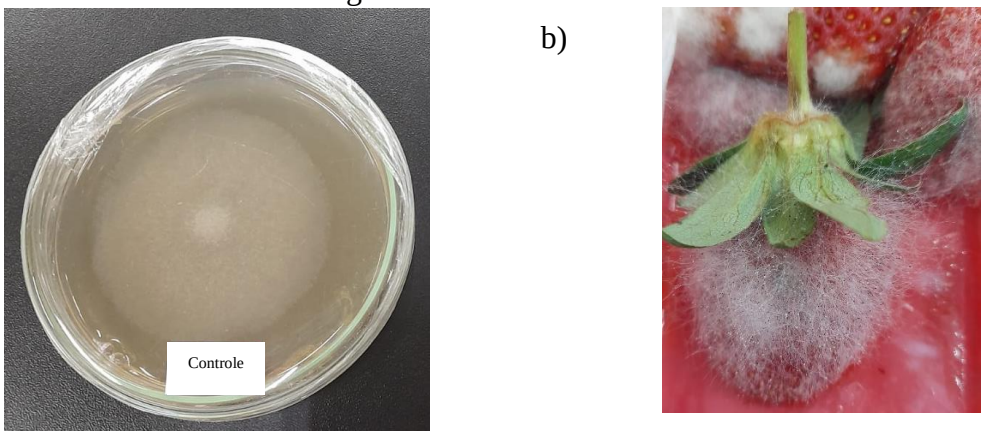
No morango, fruta amplamente conhecida e consumida, geralmente ocorre a contaminação pelos fungos *Botrytis cinerea, Collerotrichum* spp. e *Rhizopus stolonifer,* responsáveis pela podridão-acinzentada, antracnose e podridão-mole, respectivamente (LOEBLER *et al*., 2018). Outras frutas vermelhas, como as amoras, são afetadas principalmente pelos fungos *Botrytis cinerea* e *Rhizopus stolonifer,* sendo esses os causadores do apodrecimento desta fruta (CHÁVEZ-DIAS *et al*., 2014; VILAPLANA *et al*., 2020). As framboesas são deterioradas principalmente pelo fungo *Botrytis cinerea* (PIECHOWIAK *et al*., 2019). Já os mirtilos têm sua qualidade afetada principalmente pelos fungos *Alternaria* spp. (ZHU e XIAO, 2015).

Entre os fungos citados, *Rhizopus stolonifer* (Figura 3) é considerado o fungo com maior potencial destrutivo entre esses fitopatógenos. É um dos principais patógenos que ataca o morangueiro, sendo responsável por perdas econômicas de até 50% de sua produção. Esse fungo, naturalmente presente na atmosfera, pode contaminar frutas rapidamente ao se espalhar em temperaturas superiores a 5ºC. Como já mencionado, *R. stolonifer* provoca a popularmente chamada de prodridão-mole ou aquosa que causa o vazamento de suco e é capaz de recobrir todo o fruto com micélio, entremeado por estruturas de patógenos. Assim, cresce rapidamente

e pode causar a perda total do fruto de 3 a 4 dias após a infecção (VENTURA-AGUILAR *et al*., 2021; SILVA *et al*., 2020; KONG *et al*., 2019; BAUTISTA-BAÑOS *et al*., 2014).

Figura 3 – Aspecto do fungo *Rhizopus stolonifer:* (a) Crescimento em placa em meio MEA; (b) Crescimento em morango.

a) b)



Fonte: Do autor

R. stolonifer pertence ao filo Zygomycota, ordem Mucorales e família Mucoraceae (MASSOLA JUNIOR; KRUGNER, 2011). Apresenta micélio bem desenvolvido, hifas cenocíticas, esporângios escuros sustentados por esporangióforos longos, além de rizoides que fixam a hifa ao substrato. Sua temperatura ótima de crescimento encontra-se próxima a 25ºC e o crescimento de seu micélio ocorre por meio de estolões que se fixam ao substrato. O ciclo da doença se inicia quando os aplanósporos, disseminados pelo vento, caem na superfície do fruto. Estes posteriormente germinam e penetram no fruto por meio de ferimentos (BEDENDO, 2011).

Após a penetração, a ação de *R. stolonifer* nos morangos ocorre a partir da produção de enzimas responsáveis pela degradação da pectina presente nas células vegetais, seguido da deterioração dos frutos maduros após a colheita. Assim, produz odor desagradável e, em alguns casos, até mesmo a fermentação. Durante o processo, são liberados compostos orgânicos voláteis como resultado da degradação no interior das células (VENTURA-AGUILAR *et al*., 2021; OLIVEIRA FILHO *et al*., 2021; BAUTISTA-BAÑOS *et al*., 2014).

De acordo com Silva *et al*. (2020), o controle de patógenos como o *R. stolonifer* ocorre atualmente pela aplicação de fungicidas sintéticos. Esses, por sua vez, são em grande parte das vezes aplicados de forma incorreta e excessiva, o que acarreta problemas como a resistência dos patógenos e contaminação do meio ambiente. Oliveira Filho *et al*. (2021) destacaram que esses aditivos podem ser prejudiciais à saúde humana, à saúde vegetal, além de, a longo prazo, acumularem resíduos no meio ambiente. Dessa forma, os óleos essenciais podem ser uma

alternativa viável e interessante para os produtores e comerciantes de morangos *in natura*. Acredita-se ainda que a substituição de fungicidas sintéticos por fungicidas à base de óleos essenciais possa agregar valor ao produto, aumentando sua qualidade e a sustentabilidade da cadeia produtiva, pelo fato de o produto ser natural, de baixa toxicidade e baixo impacto ambiental.

Alguns óleos essenciais, bem como alguns constituintes isolados, já tiveram sua eficiência sobre *R. stolonifer* comprovada. Os óleos essenciais extraídos de cascas de duas variedades de *Citrus sinensis* (laranja-lima e laranja-baiana) foram capazes de inibir 91,95% e 80,05% do crescimento micelial de *R. stolonifer*, respectivamente, na maior dose avaliada (100 µL) pelo método de disco-difusão (REZENDE *et al*., 2020). Os óleos essenciais de *Zataria multiflora, Cinnamomum zeylanicum* e *Satureja khuzestanica* inibiram o crescimento micelial de *R. stolonifer* completamente nas concentrações de 600 µL L^{-1}, 300 µL L^{-1} e 600 µL L^{-1}, respectivamente (TAHMASEBI *et al*., 2020), e os compostos-padrão de carvacrol, isoeugenol, cuminaldeído e citral também inibiram completamente o crescimento micelial na concentração de 400 µg mL^{-1} (ZHOU *et al*., 2019).

2.3 Fungos do gênero *Aspergillus* produtores de micotoxinas: *A. carbonarius*, *A. niger*. *A. ochraceus* e *A. westerdijkiae*

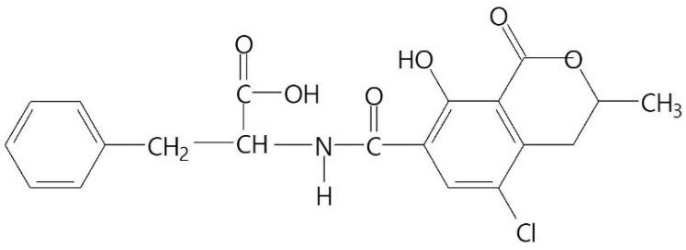
Os fungos produtores de ocratoxinas são conhecidos como fungos ocratoxigênicos, pertencem principalmente aos gêneros *Aspergillus* e *Penicillium*. *Aspergillus*, compõem o gênero dos fungos mais abundantes no mundo, e predominam em ecossistemas de diversos climas e ambientes. Essa capacidade de sobreviver em habitats ambientais e geográficos diferentes pode ser explicada pela diversidade metabólica, alta capacidade reprodutiva e capacidade de competição das cepas na natureza. É considerado o gênero fúngico de maior importância quanto à deterioração de alimentos e produção de micotoxinas (TANIWAKI, PITT e MAGAN, 2018; ABDEL-AZEEM *et al*., 2016).

O gênero *Aspergillus* é formado por aproximadamente 339 espécies, podendo-se destacar o *Aspergillus ochraceus, A. niger, A. carbonarius* e *A. westerdijkiae*, que são os principais fungos produtores de micotoxinas, principalmente no café (ABDEL-AZEEM *et al*., 2016; SARTORI *et al*., 2010). Os fungos *Aspergillus steynii* e *A. sclerotiorum* também já foram isolados do café por alguns pesquisadores; porém, são encontrados com menor frequência nesse alimento (TANIWAKI *et al*., 2014).

Os fungos do gênero *Aspergillus* possuem efetiva dispersão de seus esporos no ar e muitas espécies são produtoras de micotoxinas, como já foi mencionado (KRIJGSHELD *et al*., 2013). As micotoxinas podem ser definidas como metabólitos secundários produzidos por determinados gêneros fúngicos. Essas toxinas podem contaminar alimentos, mesmo quando presentes em concentrações baixas, além de interferir na sua qualidade, causando perdas econômicas e rejeição de lotes alimentícios. Assim, atualmente são vistas como um dos maiores problemas relacionados à segurança alimentar (JIN *et al*., 2021; AGRIOPOULOU, STAMATELOPOULOU E VARZAKAS, 2020).

Os alimentos nos quais se encontram micotoxinas com mais frequência são os cereais, como milho, arroz e trigo; as oleaginosas como amendoim e nozes; o café; o leite; o cacau e os condimentos (PRADO, 2017). Esses metabólitos, quando produzidos por *Aspergillus ochraceus, A. niger*, *A. carbonarius* e *A. westerdijkiae*, são nomeados como ocratoxinas e podem ser de três tipos: ocratoxina A, ocratoxina B e ocratoxina C. A ocratoxina encontrada com maior frequência em alimentos é a do tipo A (Figura 4), presente geralmente em grãos de café, especiarias, uvas, grãos em geral, entre outros alimentos. Essa é também a ocratoxina mais preocupante devido à sua toxicidade e ao fato de não se decompor durante a maioria dos processos de lavagem, cozimento ou fermentação dos alimentos (SARTORI *et al*., 2010).

Figura 4 – Estrutura química da ocratoxina A.



Fonte: Adaptado de Sartori *et al*. (2010).

A ocratoxina A (OTA) demora aproximadamente 35 dias para ser eliminada quando ingerida por humanos, fato que aumenta os riscos de efeitos colaterais. Entre os efeitos causados por OTA, a nefrotoxicidade é o que se manifesta nos mamíferos não ruminantes e pode causar a degeneração tubular, fibrose intersticial, redução da função renal, alteração do tamanho dos rins e do volume da urina, desenvolvimento de adenomas e tumores renais (VIEIRA, CUNHA e CASAL, 2015; NOGUEIRA e OLIVEIRA, 2006). Além disso, OTA é classificada pela Agência Internacional de Pesquisa em Câncer (IARC) como pertencente ao Grupo 2B, que se refere aos agentes possívemente cancerígenos para humanos, estando atrás apenas dos Grupos

1 e 2A, que se referem, respectivamente, aos agentes cancerígenos e aos provavelmente cancerígenos a humanos (IARC, 1993).

De acordo com a legislação brasileira, IN nº 160, de 1º de julho de 2022 da Anvisa, os limites máximos de ocratoxina A em alimentos são: 2 mcg Kg^{-1} para alimentos à base de cereais para alimentação infantil, suco de uva e polpa de uva, vinhos e seus derivados; 5 mcg Kg^{-1} para produtos de cacau e chocolate; 10 mcg Kg^{-1} para grãos de cacau, café torrado ou solúvel, cereais e derivados, feijões secos e outras sementes e frutas secas e secas; 20 mcg Kg^{-1} para cereais para posterior processamento; 30 mcg Kg^{-1} para especiarias (BRASIL, 2022).

A estrutura química básica da OTA consiste em uma isocumarina ligada a uma unidade de L-fenilalanina por uma ligação amida, sendo a OTA a forma clorada da ocratoxina. Para a biossíntese da OTA, são necessárias reações enzimáticas e a relação de alguns genes tem sido estudada. De acordo com Hertweket (2009), as micotoxinas, em sua maioria, consistem em um policetídeo ou peptídeo catalisado por uma policetídeo diidroisocumarina sintase (PKS) ou uma peptídeo sintase não ribossômica (NRPS). A função dos genes de PKS e NRPS já foram identificados em fungos como *A. carbonarius* e *A ochraceus* (LAPPA, KIZIS e PANAGOU, 2017).

A ação tóxica da OTA ainda não é completamente elucidada; porém, acredita-se que o mecanismo esteja envolvido com a inibição da síntese proteica (VIEIRA, CUNHA e CASAL, 2015). De acordo com Nogueira e Oliveira (2006), uma possível atuação de OTA ocorre por meio da ligação da ocratoxina a proteínas plasmáticas, levando essa micotoxina ao sangue, o que a torna tóxica ao organismo.

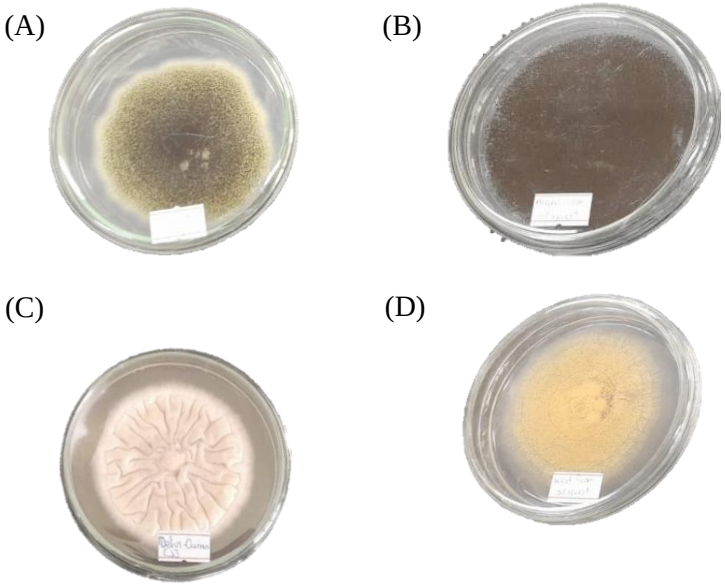
Entre os fungos destacados, *Aspergillus carbonarius* [Figura 5 (A)] apresenta-se como um fungo patogênico e micotoxigênico, produtor de ocratoxina A. Esse fungo pode infectar muitas plantas hospedeiras e deteriorar uma variedade de frutas frescas como uvas, pêssegos, peras, nectarinas, entre outras, além de secretar OTA após a colheita das frutas (BARDA *et al*., 2020). Segundo Pitt (2014), o desenvolvimento de *A.carbonarius in vitro* é rápido em meio de cultivo Czapek Yeast Agar (CYA), 60 mm ou mais, após sete dias de incubação a 25±1ºC. Possui como características visuais a cor preta ou marrom-avermelhada. Além disso, pode produzir conídios de 6 a 7 mm de diâmetro, e, apesar de seu crescimento ótimo ocorrer em temperaturas próximas a 25ºC, esse fungo também pode se desenvolver em temperaturas variáveis de 10 a 40ºC e atividade de água de 0,85.

Aspergillus niger [Figura 5 (B)] possui algumas características semelhantes a *A. carbonarius,* como sua coloração na cor preta ou marrom-avermelhada e sua capacidade de

crescer rapidamente no meio de cultivo CYA. Porém, *A. niger* produz conídios menores de 4 a 5 mm, desenvolve-se em temperaturas que podem variar de 8 a 45ºC e atividade de água de 0,8 ou menos (PITT, 2014).

Aspergillus ochraceus [Figura 5 (C)] foi o primeiro fungo no qual OTA foi descrita e ainda encontra-se entre uma das principais espécies capazes de sintetizar OTA. O fungo *A. ochraceus* é associado a climas tropicais e quentes e se prolifera principalmente em alimentos armazenados (CABAÑAES *et al*., 2002). Possui crescimento ótimo em temperaturas próximas a 25ºC e, em meio de cultivo CYA, são capazes de produzir colônias de 40 a 55 mm de diâmetro após 1 semana de incubação. A cor deste fungo pode variar de amarelo a marrom claro e seus conídios possuem paredes lisas e esféricas (PITT, 2014).

Figura 5 - Aspecto dos fungos do gênero *Aspergillus* obtidos por Crescimento em placa a 25ºC nos meios de cultura Sucrose Yeast Extract Agar (YES) for *A. ochraceus* and Czapek Yeast Extract Agar (CYA,). (A) *A. carbonarius*; (B) *A. niger*; (C) *A. ochraceus*; (D) *A. westerdijkiae.*



Fonte: Do autor.

Aspergillus westerdijkiae [Figura 5 (D)] é conhecido por ter sido confundido com *A. ochraceus* durante muito tempo e ter sua espécie separada apenas nos últimos anos. Assim como *A. ochraceus* é capaz de se desenvolver em temperaturas amenas, atividade de água entre 0,77 e 0,8 e ampla faixa de pH, sua coloração também é similar; porém, possui conídios finos esféricos e rugosos, diferente de *A. ochraceus*, que apresenta conídios lisos. Também está entre as principais espécies produtoras de OTA, sendo o produtor de OTA mais provável e capaz de produzir em maior quantidade, até mesmo que *A. ochraceus* (PARUSSOLO *et al*., 2019; PITT,

2014). De acordo com Vipotnik, Rodríguez e Rodrigues (2017), *A. westerdijkiae* pode ser considerado um risco potencial de contaminação por OTA, pois este fungo é capaz de produzir essa micotoxina em condições diversas de temperatura, atividade de água e matéria-prima.

2.4 Óleos essenciais

Os óleos essenciais são misturas complexas de compostos orgânicos voláteis sintetizados pelas plantas. Essa síntese é realizada como uma resposta da planta ao estresse fisiológico, fatores ecológicos e ataque de patógenos, ou seja, a produção de óleos essenciais como metabólito secundário é uma forma de defesa da planta. Além disso, é uma forma de atrair polinizadores para facilitar a reprodução (HASHEMI; KHANEGHAH; SANT'ANA, 2018).

De acordo com a *International Organization for Standardization* (ISO 9235: 2013) os óleo essenciais são definidos como "o produto obtido a partir de uma matéria-prima natural de origem vegetal por destilação a vapor, por processos mecânicos do epicarpo de frutas cítricas ou por destilação a seco, após separação da fase aquosa, se houver, por processos físicos". A ISO 9235:2021 ressalta ainda, que os óleos essenciais podem ser submetidos à filtração, decantação, centrifugação ou outros tratamentos físicos que não alterem sua composição significativamente e que a destilação a vapor pode ser realizada com adição de água ao destilado, sendo nomeada de hidrodestilação ou sem adição de água, sendo o óleo essencial extraído diretamente pelo vapor (ISO 9235, 2021).

A escolha do processo de extração é de suma importância para a qualidade e rendimento dos óleos essenciais serem satisfatórias, bem como a preservação da bioatividade de interesse (FERRENTINO *et al*., 2020). Atualmente existem vários processos de extração de compostos voláteis, como: extração a vapor, por hidrodestilação, supercrítica, subcrítica, por gás refrigerante, por extrusão ou prensagem, a vácuo, por solvente, por óleo e por enfluerage, entre outras técnicas desenvolvidas recentemente (CONDE-HERNÁNDEZ *et al*., 2017; HASSANEIN *et al*., 2020; WOLFFENBUTTEL, 2011). Porém, os métodos convencionais, mais utilizados e aceitos para a extração de óleos essenciais são a extração por hidrodestilação e a extração por destilação a vapor. Alguns autores incluem nos métodos convencionais a extração por prensagem a frio, utilizada principalmente para extração de óleos essenciais de cítrus e a extração pelo método de enflueragem, utilizado na extração de óleos essenciais de flores (DRINIC *et al*., 2020; FERRENTINO *et al*., 2020; STRATAKOS e KOIDIS, 2016).

Além do processo de extração, a variedade da planta atrelada a variações ambientais, fatores climáticos, índice pluviométrico, utilização de fertilizantes e armazenamento podem influenciar na capacidade da planta em produzir óleo e, consequentemente, na sua qualidade (HASHEMI; KHANEGHAH; SANT'ANA, 2018).

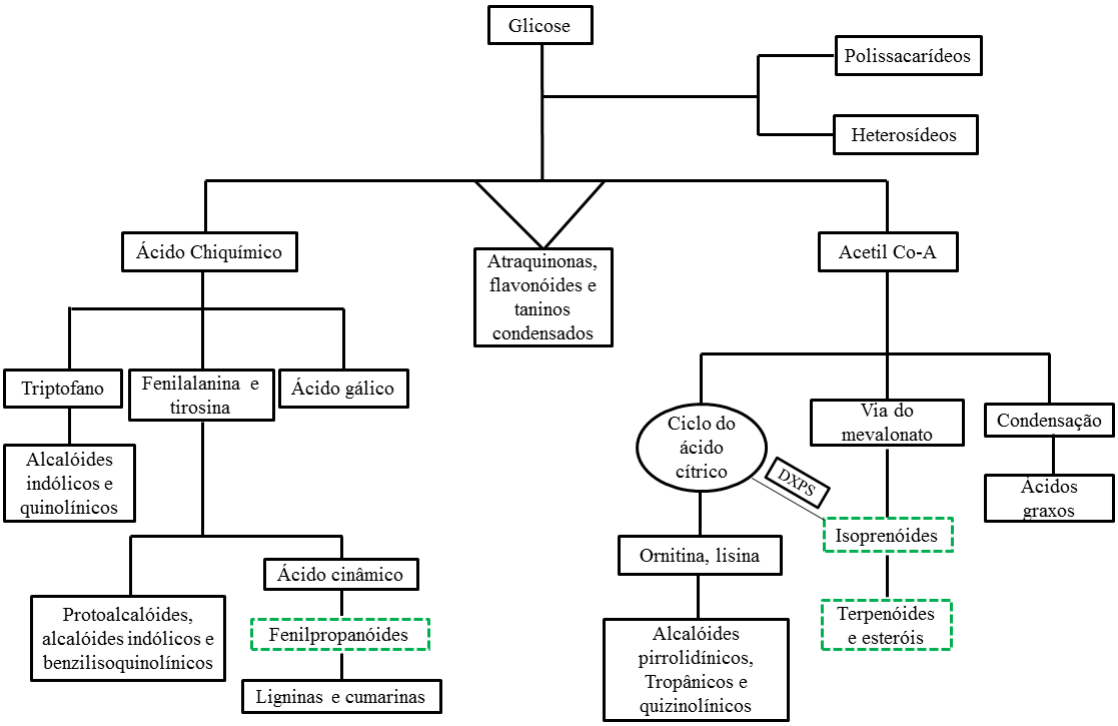
Os óleos essenciais apresentam como principais características sua complexa composição por moléculas de baixo peso molecular e diferentes estruturas químicas, como monoterpenos, sesquiterpenos, álcoois, aldeídos, ésteres, éteres, cetonas, vários derivados de fenilpropanoides e diversos compostos orgânicos voláteis. Além disso, são líquidos à temperatura ambiente, hidrofóbicos e possuem baixa solubilidade em água (SINGH *et al*., 2020; THORMAR *et al*., 2011).

A composição dos óleos essenciais atribuem a eles diferentes atividades biológicas, dentre as quais podem ser citadas as atividades: antimicrobiana (antifúngica, antibacteriana e antiviral) (BRANDÃO *et al*., 2020; CAMARGO *et al*., 2020; TSELIOU *et al*., 2019), inseticida (SALES *et al*., 2017), larvicida (CHUNG, HUONG e OGUNWANDE, 2020), antitumoral (MAGALHÃES *et al*., 2020), antioxidante (FERREIRA *et al*., 2019), anti-inflamatória (LORENÇONI *et al*., 2020), antiparasitária (AZADBAKHT *et al*., 2020), carrapaticida (LUNGUINHO *et al*., 2021), entre outras. Essas propriedades benéficas citadas despertam a atenção de muitas indústrias e também de pesquisadores. Na área alimentícia, o uso de óleos essenciais se mostra ainda mais interessante, por serem compostos naturais e também devido à percepção dos consumidores sobre o uso de aditivos sintéticos em alimentos (ALVES-SILVA *et al*., 2020; HASHEMI; KHANEGHAH; SANT'ANA, 2018). Portanto, a ampla possibilidade de aplicação dos óleos essenciais em alimentos como substituinte de aditivos, de conservantes, de agrotóxicos, de inseticidas, dentre de outros compostos sintéticos utilizados tradicionalmente, têm impulsionado a busca por sua aplicação.

2.4.1 Formação dos óleos essenciais: Terpenos e Fenilpropanoides

Os terpenos e os fenilpropanoides, constituintes dos óleos essenciais, são originados do metabolismo da glicose a partir do acetil-CoA e do ácido chiquímico, respectivamente (Figura 6).

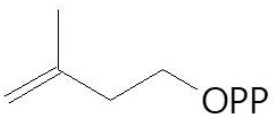
Figura 6 – Rota biossintética dos metabólitos secundários.



Fonte: Adaptado de Simões *et al*. (2007).

A formação dos terpenos pode ocorrer a partir de duas vias distintas, a via do mevalonato e a via DXPS (1-deoxi-D-xilulose-5-fosfato). A via do mevalonato ocorre no citoplasma e a via DXPS, conhecida como via alternativa, ocorre nos plastídios. Em ambas as vias, a formação dos terpenos se inicia com a condensação de dois compostos ativos intermediários, o isopentenilpirofosfato (IPP) e diametilalilpirofosfato (DMAPP) (Figura 7). Já os fenilpropanóides são sintetizados a partir do ácido chiquímico (Figura 8).

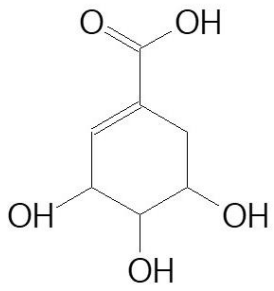
Figura 7 – Estruturas químicas do isopentenilpirofosfato (IPP) (a) e do diametilalilpirofosfato (DMAPP) (b).

a) 

b) 

Fonte: Dewick (2009).

Figura 8 – Estrutura química do ácido chiquímico.



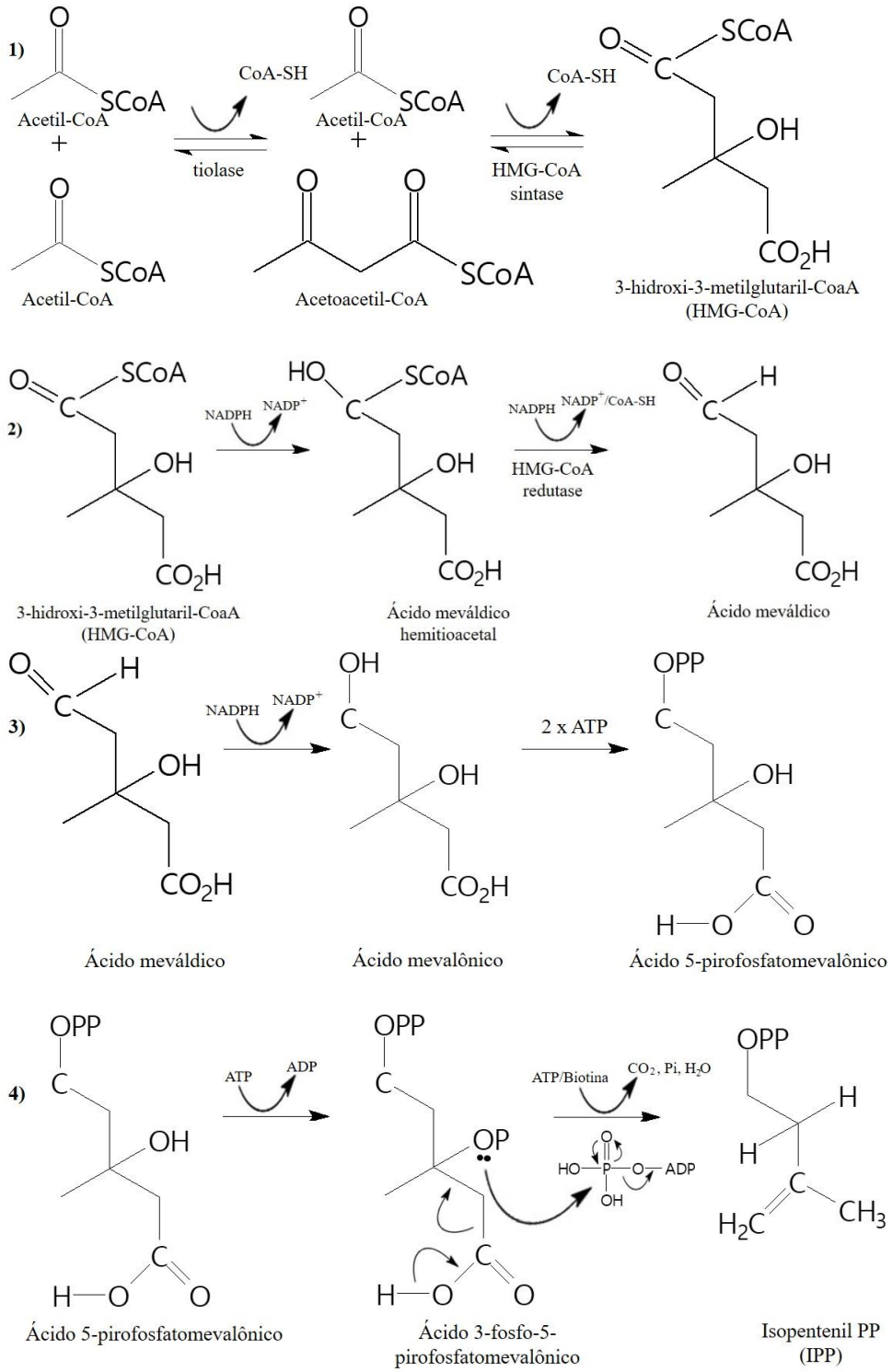
Fonte: Dewick (2009).

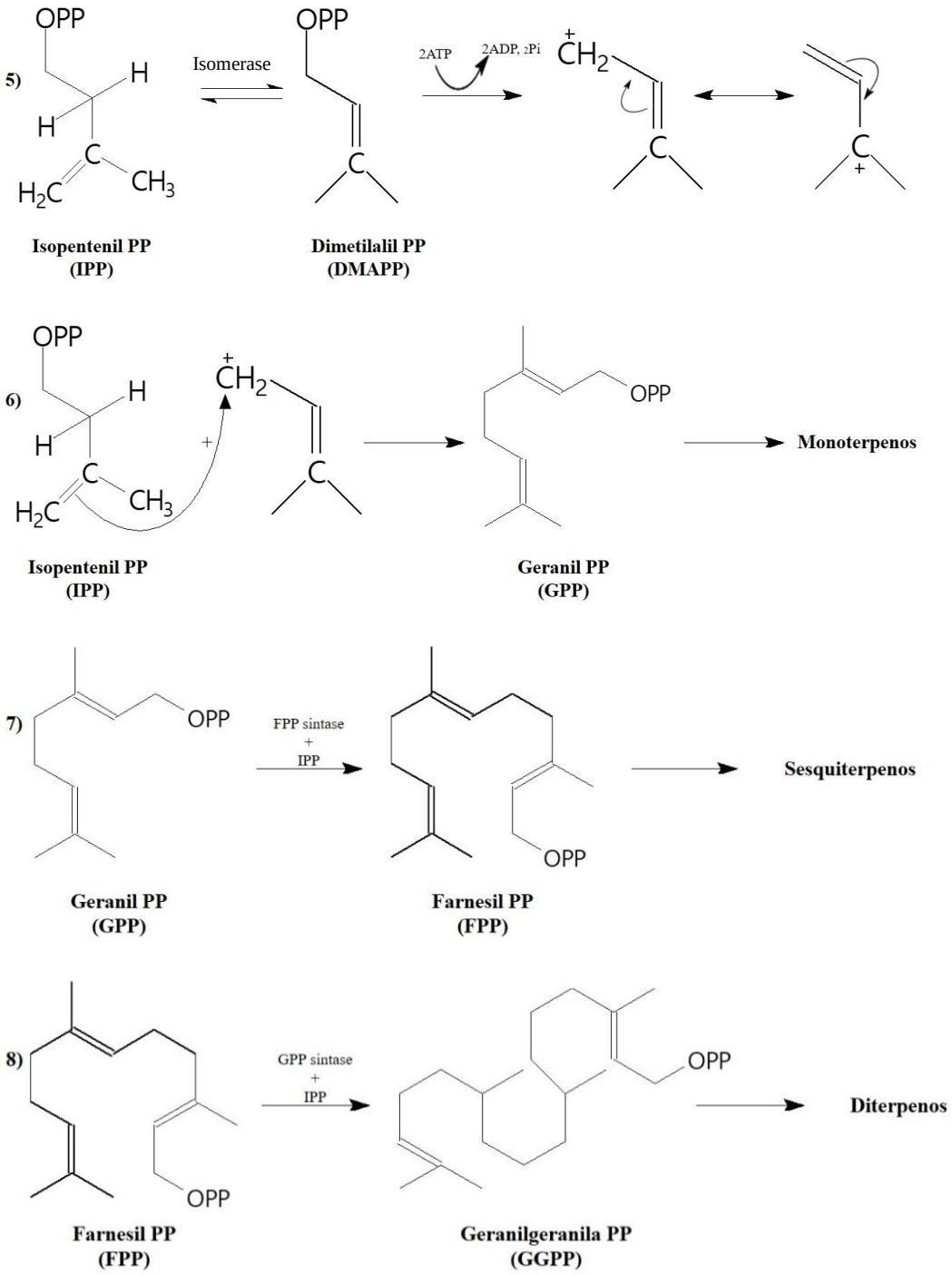
2.4.2 Biossíntese de formação dos terpenos

Os terpenos, como já mencionado, iniciam sua formação com a condensação de dois compostos intermediários, o isopentenilpirofosfato (IPP) e diametilalilpirofosfato (DMAPP). Porém, antes de esses compostos intermediários serem formados, ocorre uma série de reações a partir da glicose.

Na Figura 9, estão detalhadas as etapas de formação dos terpenos pela via do mevalonato. Pode-se perceber que, inicialmente, a glicose é catabolizada em acetil-coenzima A (acetil-CoA). Assim, ocorrerá, em seguida, a condensação de três moléculas de Acetil-CoA. Essa etapa é catalisada por duas enzimas, tiolase e hidroximetilglutaril-CoA sintase, e originará o 3-hidroxi-3-metilglutaril-CoA (HMG-CoA), que será então reduzido a ácido mevalônico pela enzima HMG-CoA redutase utilizando três moléculas de NADPH. Esse ácido formado será fosforilado, com três moléculas de ATP, formando, assim, o ácido 3-fosfo-5-pirofosfatomevalônico que, por sua vez, sofrerá uma descarboxilação para formar o precursor de interesse, isopentenilpirofosfato (IPP). Após a formação do IPP, por meio da atuação da enzima isomerase, será formado o diametilalil pirofosfato (DMAPP). A condensação desses intermediários forma o geranilpirofosfato (GPP), composto precursor dos monoterpenos. A partir da condensação dessa cadeia de 10 carbonos (GPP) com novas unidades de IPP são formados os precursores dos sesquiterpenos (C15), diterpenos (C20), sesterterpenos (C25), (Figura 10) (HASHEMI; KHANEGHAH; SANT'ANA, 2018; SIMÕES *et al*., 2007; DEWICK, 2009).

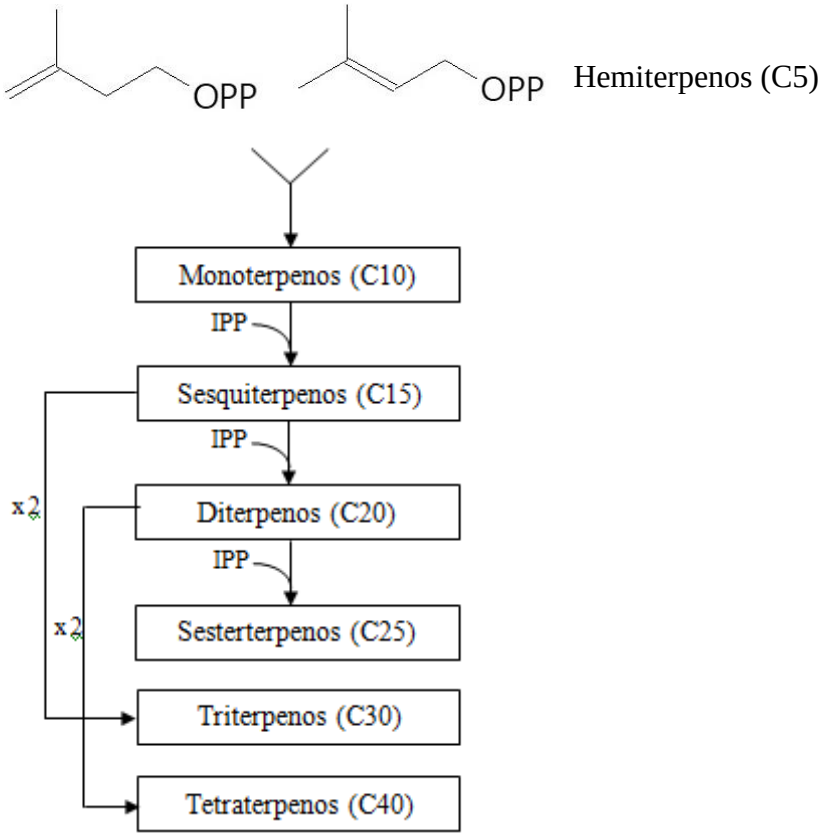
Figura 9 - Rota metabólica para formação de monoterpenos a partir do ácido mevalônico.





Fonte: Adaptada Dewick (2009).

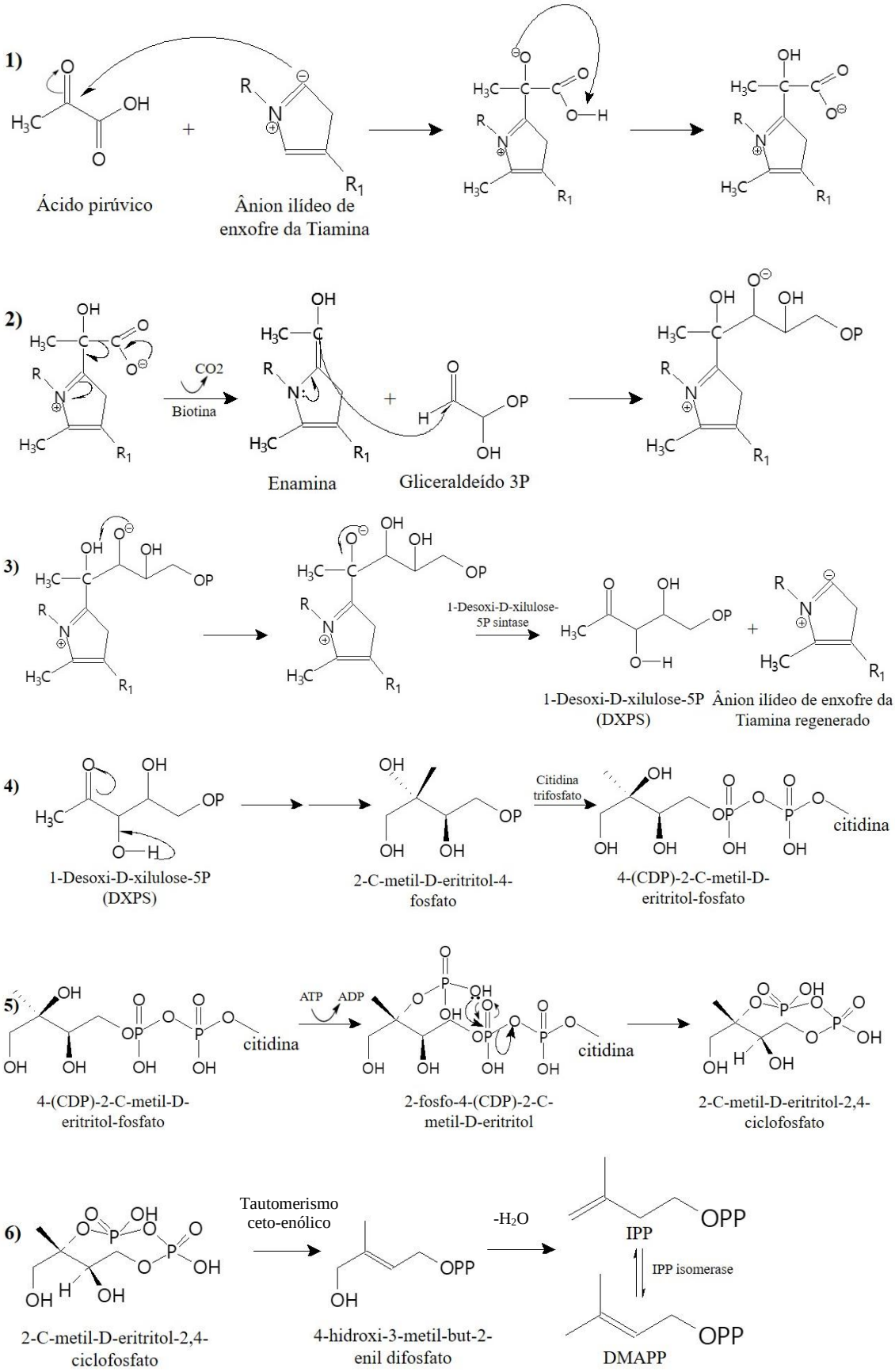
Figura 10 – Classificação dos terpenos.



Fonte: Adaptado de Dewick (2009).

O precursor de interesse, IPP, como já mencionado também pode ser formado por uma segunda via, a via DXPS, que está representada na Figura 11. À formação do IPP por essa via se inicia com a adição do ânion ilideo de enxofre de tiamina ao grupo carbonílico do piruvato. A reação do produto formado é catalisada pela coenzima biotina e, assim, forma-se a enamina, que se rearranja com o auxílio da enzima 1-desoxi-D-xilulose-5P-sintase formando-se a 1-Desoxi-D-xilulose-5P (DXPS). A molécula de DXPS formada também passa por uma etapa de rearranjo em que é reduzido a 2-C-metil-D-eritritol-4P. Em seguida, por meio da adição da citidina e do processo de fosforilação, é formado 2-fosfo-4-(CDP)-2-C-metil-D-eritritol-4P, que se rearranja, liberando citidina fosfato e formando produto 2-C-metil-D-eritritol-2,4-ciclofosfato. Esse composto cíclico, após sofrer tautometismo ceto-enólico, etapas de redução e de remoção de H_2O, forma o precursor de interesse IPP, que segue, a partir deste ponto, as mesmas etapas já mencionadas na via do mevalonato para a formação dos monoterpenos (DEWICK, 2009).

Figura 11 - Rota metabólica para formação de monoterpenos a partir da via DXPS.

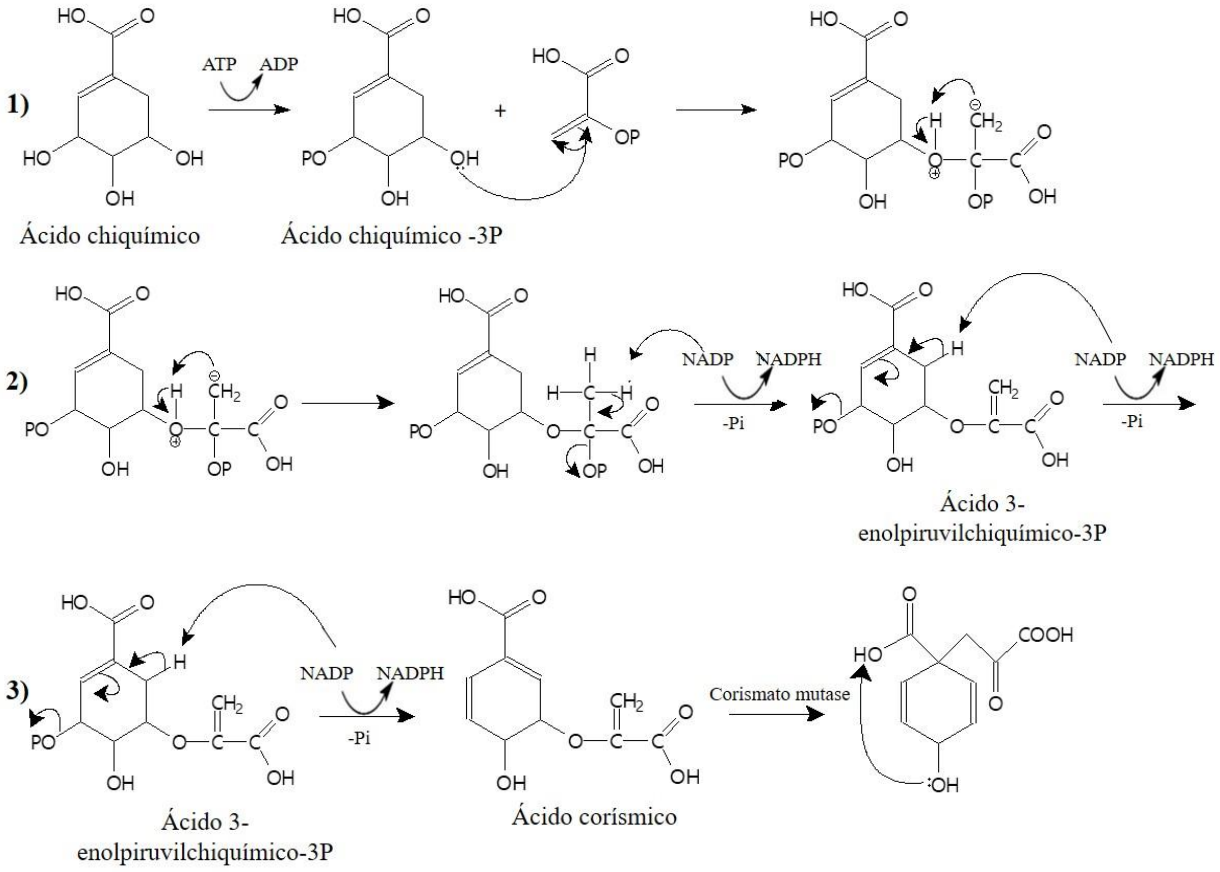


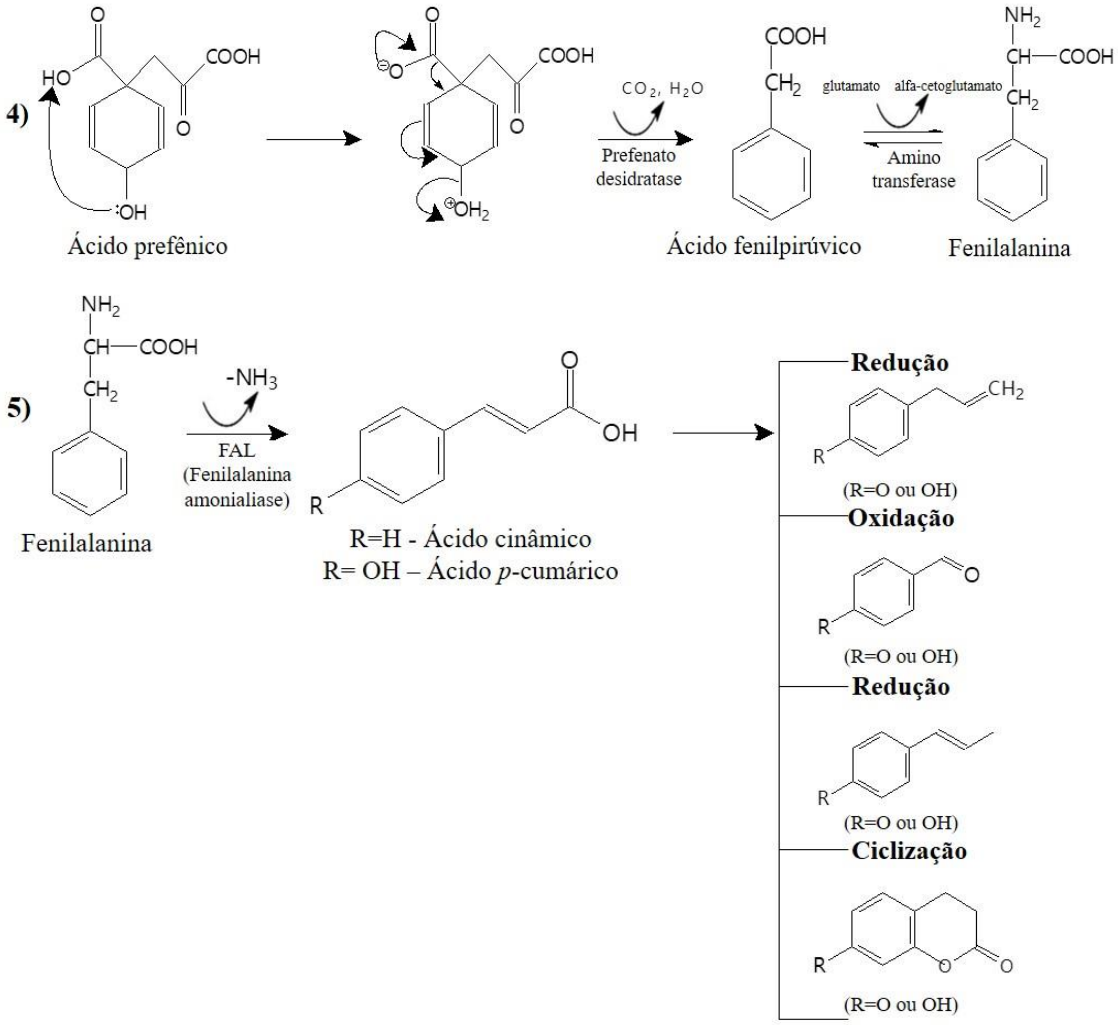
Fonte: Adaptado de Dewick (2009).

2.4.3 Biossíntese dos fenilpropanoides

Os fenilpropanoides são sintetizados a partir do ácido chiquímico (Figura 12), que forma o ácido chiquímico-3P ao ser fosforilado. Esse, ao reagir com uma molécula de fosfoenolpiruvato, seguido de uma oxidação e liberação de Pi, forma o ácido 3-enolpiruvilchiquimico-3P. Esse por sua vez, ao ser reduzido, forma o ácido corísmico. O ácido corísmico é transformado em ácido prefênico pela ação da enzima corismato mutase; a reação desse ácido é catalisada pela enzima prefenato desidratase e forma o ácido fenilpirúvico. Ao reagir com o glutamato, com o auxílio da enzima amino transferase, o ácido fenilpirúvico forma a fenilalanina, que forma as unidades de ácido cinâmico e ácido p-cumárico por meio da ação enzimática. Esses compostos darão origem aos fenilpropanóides (SIMÕES *et al*., 2007).

Figura 12 - Biossíntese de fenilpropanoides.





Fonte: Adaptada de Dwick (2009) e Simões *et al*. (2007).

2.4.4 Óleo essencial de Cominho

O cominho (*Cuminum cyminum* L.) é uma planta herbácea pertencente à família Apiacea (Figura 13). O cultivo do cominho ocorre basicamente na Arábia Saudita, Índia e China, porém é amplamente utilizado na culinária de diversos países, sendo o segundo tempero mais popular do mundo (ZAKY, SHIM e ABD EL-ATY, 2021; THIPPESWAMY e NAIDU, 2005).

Além da utilização na culinária, as sementes de cominho são utilizadas como planta medicinal no auxílio de tratamentos de doenças como diarreia, tosse, dor de dente, dispepsia, epilepsia, icterícia e outras doenças. O óleo essencial extraído das sementes de cominho (OEC), pode ser aplicado na indústria de alimentos e embalagem para os alimentos, por ser considerado

seguro e eficaz na atuação sobre fungos e bactérias (MOUSTAFA, EL-SAYED e YOUSSEF, 2023; MIRI e DJENANE, 2018; NOSTRO *et al*., 2005).

Figura 13 – Aspecto geral de *Cuminum cyminum* L.

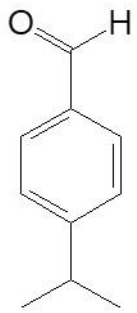
Fonte: Do autor.

Os constituintes presentes no OEC podem variar de acordo com o planta na qual o óleo é extraído, mesmo pertencendo a uma mesma espécie. Fatores como localização geográfica, índice pluvial, temperatura, método de extração do OE, entre outros, afetam diretamente a constituição e rendimento do OE (GOBBO-NETO e LOPES, 2007; HASHEMI, KHANEGHAH e SANT'ANA, 2018). Porém, os principais constituintes desse óleo tendem a ser semelhantes, mesmo que em concentrações variáveis. Moustafa, El-Sayed e Youssef (2023) e Nostro *et al* (2005) afirmaram que os grupos contendo aldeído e álcool podem ser os responsáveis pelas atividades antimicrobianas e antivirais. Ghasemi *et al*. (2022), ao caracterizarem quimicamente o OEC, encontraram cinco constituintes: cuminaldeído (40,1%), o-cimeno (13,765), γ-terpineno (12,38%), β-pineno (9,93%) e α-terpinen-7-al (8,47%); Padilla-Camberos *et al*. (2022) encontraram 30 constituintes, sendo os principais: cuminaldeído (30,85%), γ-terpineno (15,42%), 2-caren-10-al (8,81%), β-pineno (8,76%) e álcool cumínico (8,49%); e Miri e Djenane (2018) obtiveram 16 constituintes como resultado na caracterização química do OEC, sendo os principais: cuminaldeído (65,98%) e o-cimeno (18,39%).

Em todas as caracterizações, o composto majoritário presente no óleo essencial de cominho é o cuminaldeído (Figura 14). Esse aldeído pode ser considerado o principal responsável pelo odor e efeitos biológicos desse óleo essencial. Sua atividade antimicrobiana

está relacionada com a capacidade de aldeídos em alterar a função das proteínas e inibir o crescimento microbiano (LI *et al*., 2023).

Figura 14 – Estrutura química do constituinte cuminaldeído



Fonte: Do autor.

Li *et al*. (2023) estudaram a aplicação do óleo essencial de cominho como conservantes em carne de carneiro refrigerada. Os pesquisadores constataram que o OEC, quando combinado ao óleo essencial de Zanthoxylum, foi um eficiente antimicrobiano sobre *S. aureus* e *E. coli*, mesmo em baixas concentrações. Ghasemi *et al*. (2022) relataram em seu trabalho que o óleo essencial de cominho foi capaz de inibir o crescimento de *S. aureus, E.coli, B.cereus* e *S. entérica*, quando aplicado incorporado a fibras, sendo assim um óleo essencial em potencial para embalagens ativas de produtos alimentícios como queijos e carnes. Miri e Djenane (2018) mostraram que o OEC é capaz de inibir o crescimento de *Aspergillus flavus,* bem como a biossíntese de sua ocratoxina aflatoxina B1, sendo assim uma boa alternativa para a proteção de alimentos.

A atividade inseticida do OEC também já foi estudada por alguns pesquisadores, sendo esse óleo considerado promissor no manejo de vários insetos, como *Sesamia cretica*, inseto praga que ataca culturas como milho e cana-de-açúcar (SADEGHI *et al*., 2019); *Spodoptera littoralis,* verme-do-algodoeiro (EL-SAYED e YOUSEF, 2021); *Sitophilus zeamais,* praga que ataca grãos armazenados (ROSA *et al*., 2020); *Aphis craccivora,* pulgão, que ataca principalmente feijão (ABDELAAL *et al*., 2021), entre outros insetos.

2.4.5 Óleo essencial de Louro

O louro (*Laurus nobilis* L.) é uma planta medicinal pertencente à família Lauraceae (Figura 15), original de países mediterrâneos; porém, já é amplamente cultivada em várias

regiões do mundo (OZDEMIR, TASTAN e GUNEY, 2022; ORDOUDI *et al*., 2022; PAPARELLA *et al*., 2022). As folhas de louro possuem como característica principal odor aromático característico e, se mastigadas, têm sabor amargo (CORRÊA, 1969). De acordo com Ordoudi *et al*. (2022), quando comparado a outras ervas como o orégano, tomilho, sálvia, alecrim, entre outros, o óleo essencial de louro ainda é pouco estudado. Porém, sua aplicação como óleo essencial tem atraído o interesse de pesquisadores e da indústria alimentícia devido às suas atividades biológicas, como antioxidante, antifúngica, antiviral, antibacteriana e inseticida (ORDOUDI *et al*., 2022; REIS *et al*., 2020; RU *et al*., 2022).

Figura 15 – Aspecto geral de *Laurus nobilis.*

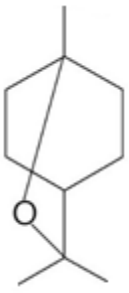
Fonte: Do autor.

O óleo essencial de louro (OEL) possui como constituintes principais os compostos: α-pineno, β-pineno, sabineno, α-terpineno e γ-terpineno, eucaliptol (1,8-cineol), linalol, α-terpineol e γ-terpineol, acetato de α-terpenila, eugenol e metileugenol. Ressalta-se que essa composição pode diferir quantitivamente e qualitativamente dependendo de fatores como localização da planta, estação da coleta, método de secagem e extração, parte da planta, condições ambientais, entre outros fatores (HASHEMI, KHANEGHAH e SANT'ANA, 2018; ORDOUDI *et al*., 2022; PAPARELLA *et al*., 2022). Koc e Kara (2018) analisaram a composição química do OEL e encontraram 39 constituintes, sendo os principais: 1,8-cineol (37,7%), α-terpenilacetato (11,30%), linalol (9,10%), metil eugenol (6,80%), sabineno (6,35%), eugenol (3,80%) e α-pineno (3,21%). Ailli *et al*. (2023) identificaram 35 compostos químicos no OEL, sendo os principais: 1,8-cineol (36,58%), acetato de α-terpenila (15,42%), sabineno (12,08%), metileugenol (5,34%), α-pineno (5,29%), β-pineno (4,06%), linalol (3,45%) e α-

terpineol (3,15%); Para Santos *et al*. (2023), entre os 16 compostos identificados, os principais foram: 1,8-cineol (42,7%), linalol (12,2%), α-terpenil acetato (10,4%), sabineno (5,7%), α-terpineol (5,4%), metil eugenol (4,8%).

Observando os resultados citados, percebe-se que, apesar das variações quantitativas e qualitativas em relação à constituição química do OEL, o seu composto majoritário prevaleceu, sendo o 1,8-cineol (Figura 16). Para Belasli *et al*. (2020), esse composto pode causar alterações morfológicas nas hifas e danificar a membrana plasmática dos fungos. Porém, os outros constituintes não são menos importantes na composição do OEL, pois efeitos sinérgicos podem contribuir para aumentar a eficiência de atividades biológicas de óleos essenciais. Os constituintes linalol e terpineol, por exemplo, já foram relatados como possíveis responsáveis pela atividade antifúngica, visto que esses podem aumentar a permeabilidade da membrana plasmática e inibir a respiração na membrana mitocondrial de fungos (BELASLI *et al*., 2020; DEBA *et al*., 2008; IMELOUANE *et al*., 2009).

Figura 16 – Estrutura química do constituinte 1,8-cineol.



Fonte: Do autor.

A composição química do OEL, em geral, apresenta hidrocarbonetos monoterpênicos, como α-pineno, β-pineno e sabineno; hidrocarbonetos monoterpênicos oxigenados, como 1,8-cineol, acetato de α-terpenila, linalol, α-terpineol; e compostos aromáticos como eugenol e metil eugenol (CHAHAL *et al*., 2017). Chahal *et al*. (2017), Ordoudi *et al*. (2022) e Anzano *et al*. (2022) sugerem que a atividade antifúngica sobre os gêneros *Aspergillus, Fusarium, Erotium* e *Penicillium* ocorra principalmente pela ação dos hidrocarbonetos.

Um dos fatores que torna a aplicação de OE sobre fungos interessante deve-se à sua complexa composição química. Os componentes presentes nos óleos essenciais possuem a capacidade de atuar em alvos diferentes na célula, o que os torna mais eficientes. As características hidrofóbicas dos OEs facilitam a ação na membrana celular, lipídios e mitocôndrias (RU *et al*., 2022; ORDOUDI *et al*., 2022). Assim, atrelado a esses fatores citados,

o interesse por estudos da aplicação do OEL sobre fungos tem aumentado. Dammak *et al*. (2019) avaliaram o efeito do OEL sobre *Aspergillus carbonarius* e comprovaram o efeito atifúngico desse óleo essencial, que foi capaz de inibir 100% do crescimento micelial a partir da concentração de 0,3%. Belasli *et al*. (2020) constataram que o OEL possui atividade antifúngica sobre fungos do gênero *Aspergillus*, como *Aspergillus flavus, Aspergillus carbonarius, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceus, Aspergillus tamarii* e *Aspergillus terreus.*

2.4.6 Óleo essencial de Tangerina

Na fruticultura mundial, a atividade de citricultura possui destaque, sendo os citros as frutas mais produzidas no mundo (COUTO e CANNIATTI-BRAZACA, 2010). Os frutos cítricos apresentam relevante potencial econômico devido à sua aplicação na elaboração de diversos produtos, como sucos, geleias, molhos, doces, polpas, entre outros. Porém, o processamento desses frutos geram uma grande quantidade de resíduos, como cascas e sementes, alcançando globalmente até 20 milhões de toneladas (RAMOS *et al*., 2023).

Um cítrico amplamente conhecido e consumido é a tangerina. No Brasil, a produção de tangerina no ano de 2021 atingiu 1.085.048 toneladas, sendo os estados de São Paulo e Minas Gerais os principais produtores (IBGE, 2021).

Entre as tangerinas, a tangerina murcott é conhecida por ser um híbrido resultado provável do cruzamento entre uma tangerina e uma laranja, *Citrus reticulada* Blanco x *Citrus sinensis.* Acredita-se que tenha surgido a partir de um melhoramento genético de citros realizado na Florida por volta de 1916 (FIKRY *et al*., 2020; SMITH, 1976).

Assim como os demais frutos cítricos, o processamento da tangerina também gera consideráveis quantidades de resíduos, fator que viabiliza a utilização desse coproduto com o intuito de agregar valor, reduzir impactos ambientais e reduzir custos de destinação de resíduos. Entre as alternativas para aproveitamento dos resíduos da tangerina, a extração de óleos essenciais tem apresentado bons resultados.

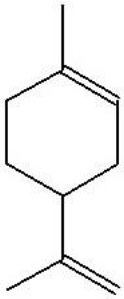
A extração de óleos essenciais utilizando como material vegetal casca de tangerina (Figura 17) possui bom rendimento quando comparado a outros materiais vegetais. Sua constituição química é composta quase que inteiramente pelo monoterpeno limoneno (Figura 18). Azevedo *et al*. (2019), ao extrairem o OE das cascas de tangerina murcott encontraram rendimento de 0,39%, e, ao caracterizar este OE, obteve como constituinte majoritário o

limoneno, representando 92,37% da composição total do OE. Apesar das variações que podem ocorrer na constituição do óleo essencial de tangerinas, de acordo com os estudos realizados por Teixeira, Marques e Pio (2014), que caracterizaram os óleos essenciais extraídos de nove genótipos de tangerina, o constituinte majoritário prevalece sendo o limoneno.

Figura 17 – Aspecto geral das cascas de *Citrus reticulada* Blanco x *Citrus sinensis.*

Fonte: Do autor.

Figura 18 – Estrutura química do constituinte Limoneno.



Fonte: Do autor.

O óleo essencial de tangerina, além de possuir odor agradável e característico do material vegetal, tem sido pesquisado e aplicado de diversas formas conforme suas propriedades biológicas de interesse. Estudos realizados por Denkova-Kostova *et al*. (2020) apresentaram o óleo essencial de tangerina como um potencial antimicrobiano sobre os diversos microrganismos, como *Bacillus subtilis*, *Penicillium chrysogenum*, *Fusarium moniliforme*, *Aspergillus niger*, *Aspergillus flavus*, *Saccharomyces cerevisiae*, *Escherichia coli*, *Salmonella abony*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* e *Candida albicans.*

2.4.7 Potencial inseticida dos óleos essenciais

Nos últimos anos, o uso indevido e excessivo de inseticidas sintéticos tem acarretado a resistência dos insetos, além dos efeitos ambientais e dos custos operacionais que geram. Por esses motivos, a elaboração de novos produtos para controle de insetos é considerado um desafio.

Os metabólitos secundários podem ter efeitos no desenvolvimento, na reprodução e na mortalidade dos insetos, atuando sobre o seu sistema nervoso. Podem agir também como repelentes e atraindo predadores (RODRIGUES *et al*., 2017). Os óleos essenciais têm sido amplamente estudados como pesticidas, devido a suas atividades biológicas e aos benefícios à saúde; possuem diversos mecanismos de ação sobre insetos, como o bloqueio dos receptores de octopamina, inibição do ácido *gama*-aminobutírico (GABA) e inibição da enzima acetilcolinesterase, sendo esse último considerado o principal mecanismo de ação (SOUZA *et al*., 2022). A enzima acetilcolinesterase é responsável pela quebra da acetilcolina em ácido acético e colina. Ao inibir essa enzima, ocorre o acúmulo de acetilcolina, acarretando a morte do inseto devido à interrupção da transmissão neural (MOSSA, 2016; CHAUBEY, 2012).

As características inseticidas dos óleos essenciais vêm despertando interesse de pesquisas há alguns anos. Ribeiro *et al*. (2020) avaliaram o potencial inseticida de óleos essenciais de quatro espécies de *Citrus* spp. frente à mosca-branca (*Bemisia tabaci*), que ataca principalmente hortaliças. Os pesquisadores constataram que os constituintes dos óleos essenciais dos *Citrus* investigados apresentam toxicidade para a mosca-branca e possivelmente poderão ser utilizados como uma forma de manejo dessas moscas na agricultura. Esses efeitos constatados foram atribuídos aos constituintes linalol, α-terpineol, α-pineno, β-pineno, terpinoleno e limoneno, encontrados nos óleos essenciais de citrus.

Albiero *et al*. (2019) estudaram o potencial inseticida dos óleos essenciais de nim e endro sobre o besouro-do-milho (*Sitophilus zeamais*), verificando que ambos os óleos inibiram a atividade do besouro, sendo o óleo essencial de endro o mais eficiente. Essa eficiência foi atribuída ao constituinte dilapiol juntamente com outros monoterpenos.

Outros pesquisadores estudaram a toxicidade de diferentes óleos essenciais sobre espécies de *Drosophila*. Souza *et al*. (2020) avaliaram a toxicidade dos óleos essenciais de cinco espécies do gênero *Piper* sobre *Drosophila suzukii* e obtiveram como resultado 100% de mortalidade dos insetos. Jang *et al*. (2017) observaram que os óleos essenciais de *Eucalyptus*

citriodora e *Melaleuca teretifolia* apresentaram toxicidade sobre a *Drosophila suzukii.* Essa atividade provavelmente relaciona-se com a presença dos constituintes geranial e neral do óleo essencial de *Melaleuca teretifolia*; e citronelal e isopulegol, constituintes do óleo essencial de *Eucalyptus citriodora*. Caetano *et al*. (2022), ao avaliarem a toxicidade do óleo essencial de *Rosmarinus officinalis* incorporado em nanopartículas biodegradáveis sobre *D. suzukii*, verificaram que a atividade inseticida do óleo essencial é prolongada quando encapsulado. Park *et al*. (2017), pesquisando o óleo essencial de *Leptospermum citratum*, encontraram como constituintes majoritários geranial e neral, que apresentaram atividade fumigante sobre *Drosophila suzukii*.

2.4.8 Óleos essenciais como agentes antimicrobianos

A atividade antimicrobiana dos óleos essenciais tem sido explorada nos últimos anos devido à necessidade de expansão do espectro de antimicrobianos que sejam seguros para aplicação na indústria alimentícia (PARIS *et al*., 2020).

Nas bactérias, Pombo *et al*. (2018) sugerem que os óleos essenciais são capazes de interferir na bicamada fosfolipídica da parede celular, perturbando a membrana citoplasmática, o que implica no aumento da permeabilidade e perda dos compostos celulares. Essa perturbação altera os sistemas enzimáticos relacionados à produção de energia, síntese de compostos, inativação e destruição do material genético. Nos fungos, o potencial antifúngico dos óleos essenciais também está relacionado ao seu caráter hidrofóbico, que, permite a interação com os lipídios presentes na parede, na membrana celular e na mitocôndria. Dessa forma, os óleos agem alterando a permeabilidade e modificando estas estruturas, podendo danificar membranas e causar o extravasamento do conteúdo celular (BRANDÃO *et al*., 2022; COSTA *et al*., 2011; REZENDE *et al*., 2021).

Assim, a atividade antifúngica dos óleos essenciais podem alterar especificamente as vias metabólicas, causar alterações transcricionais de genes, redução ou aumento da expressão gênica, inibição da síntese de DNA/RNA, disfunção da mitocôndria e alterações estruturais como inibição da formação ou ruptura da parede celular a partir da inibição da síntese dos seus principais constituintes, β-glucanos e quitina. Podem também causar alterações na integridade da membrana celular a partir na inibição do ergosterol, componente de extrema importância na constituição da membrana (KISOVÁ *et al*., 2020; LAGROUH, DAKKA e BAKRI, 2017, SOUZA *et al*., 2021).

Segundo Vieira *et al*. (2014), a atividade antimicrobiana dos grupos funcionais presentes nos óleos essenciais segue a seguinte ordem: fenóis > aldeídos > cetonas > álcoois > éteres > hidrocarbonetos (VIEIRA *et al*., 2014). Souza *et al*. (2021) observaram os efeitos dessa ordem ao avaliarem a atividade antifúngica de compostos-padrão sobre os fungos *Aspergillus ochraceus, A. niger* e *A. carbonarius.* Neste estudo, o composto eugenol, um composto fenólico, apresentou-se mais eficiente que o citral, que apresenta um grupo aldeído que, por sua vez, apresentou-se mais eficiente que o *trans*-farnesol, que é um álcool.

Diversos estudos comprovam os efeitos antifúngicos dos óleos essenciais, Brandão *et al*. (2022), por exemplo, avaliaram o efeito dos óleos essenciais de *Alpinia speciosa* e *Cymbopogon flexuosus* incorporados a nanofibras sobre *Aspergillus ochraceus* e *Aspergillus westerdijkiae.* Os autores obtiveram resultados não apenas de inibição do crescimento micelial, mas também de inibição da biossíntese de ocratoxina A. Rezende *et al*. (2021) comprovaram a atividade antifúngica e antiocratoxigênica dos óleos essenciais de *Satureja montana*, *Myristica fragrans* e *Cymbopogon flexuosus* sobre *Aspergillus flavus e Aspergillus ochraceus*.

Chung, Houng e Ogunwand (2020) relataram o potencial antimicrobiano de óleos essenciais de *Magnolia coco* sobre os microrganismos *Enterococcus faecalis, Staphylococcus aureus, Bacillus cereus* e *Candida albicans.* Encontraram como constituintes majoritários desse óleo, o sabineno e o β-pineno, sendo a atividade antimicrobiana relatada provavelmente relacionada à sua presença. Lee *et al*. (2020) observaram que os óleos essenciais de *Cinnamomum zeylanicum, Origanum vulgare* e *Thymus zygis* apresentam boa atividade antimicrobiana sobre *Leuconostoc citreum*. Estes pesquisadores observaram ainda que a combinação dos óleos essenciais de *Origanum vulgare* e *Thymus zygis* apresentou atividade antimicrobiana sinérgica, atribuída provavelmente aos constituintes majoritários desses óleos, carvacrol e timol, respectivamente. Para os autores, os componentes presentes em menores concentrações também podem ter contribuído para a atividade antimicrobiana.

2.5 Embalagens Ativas na forma de revestimentos comestíveis

As embalagens ativas são definidas por Soares (1998) como aquelas que interagem de maneira intencional com o alimento, visando a melhorar algumas de suas características. Bhardwaj, Alam e Tawar (2019) definem de forma clara que embalagens ativas são aquelas em que há inclusão de um agente ativo, com o intuito de manter a qualidade ou aumentar a vida útil do produto.

Os revestimentos comestíveis atuam como embalagens ativas e são definidos como camadas finas e biodegradáveis constituídas de materiais que podem ser ingeridos. Apesar das similaridades com os filmes, os revestimentos são diferenciados basicamente pela forma de aplicação, pois são aplicados diretamente na superfície dos alimentos enquanto ainda se encontram na forma líquida, tornando-se parte do produto. Usualmente utiliza-se o processo de imersão do alimento na solução formadora do revestimento para aplicação. Já os filmes são moldados como folhas sólidas e, posteriormente, aplicados ao alimento como uma embalagem (BENBETTAIEB, DEBEAUFORT e KARBOWIAK, 2019; PERDONES *et al*., 2012; FALGUERA *et al*., 2011; KROCHTA, 2002).

A tecnologia de revestimento comestível aplicado a frutas têm como principais objetivos melhorar a qualidade e prolongar a sua vida-útil durante seu processamento e armazenamento. Isso ocorre pela modificação da atmosfera na qual essas frutas se encontram. Sendo assim, os revestimentos atuam como uma barreira protetora a gases, umidade, vapor de água, aromas e lipídios, dentre outros compostos. Além dessas funções, outras aplicações dos revestimentos comestíveis têm se destacado: uma delas é a capacidade de aprisionar compostos de interesse e liberá-los de forma controlada, atuando assim em um alvo específico (PERDONES *et al*., 2012; BENBETTAIEB; DEBEAUFORT e KARBOWIAK, 2019; MATTA; TAVERA-QUIROZ e BERTOLA *et al*., 2019; SINGH *et al*., 2019).

Existem algumas particularidades que devem ser consideradas para as aplicações dos revestimentos em alimentos, como custo, atributos funcionais, propriedades mecânicas e ópticas, barreira contra o fluxo de gases, resistência à água e a microrganismos e aceitação sensorial (FALGUERA *et al*., 2011).

Vários compostos têm sido testados para a composição de revestimentos comestíveis. As proteínas e polissacarídeos têm se destacado por serem biopolímeros, derivados de fontes renováveis, biodegradáveis e não tóxicos. Porém, a escolha da composição dos revestimentos deve priorizar a sua integridade, considerando para isso a adesão ao alimento, a tensão superficial e a flexibilidade do revestimento (GALINDO *et al*., 2019; GARCÍA *et al*., 2009).

Os compostos mais utilizados para produção de revestimentos são proteínas (gelatina, proteínas do leite, do ovo e da soja, proteínas miofibrilares), polissacarídeos (amido e derivados, pectina, quitosana, celulose e derivados, carragena, alginato) e lipídeos (ceras e óleos), ou a sua combinação. É importante ressaltar que todos os constituintes de um revestimento comestível devem ser considerados GRAS (Geralmente Reconhecidos Como Seguros). Revestimentos constituídos por proteínas e polissacarídeos possuem como principais características a alta

permeabilidade ao vapor de água e baixa permeabilidade a oxigênio, dióxido de carbono e lipídios. Já os revestimentos lipídicos possuem baixa permeabilidade ao vapor de água devido à sua hidrofobicidade. A combinação de constituintes pode apresentar vantagens devido à possibilidade de atrelar os benefícios de cada um deles ao revestimento desenvolvido (GALUS, 2019; WATKINS, 2019; DURANGO, SOARES e ARTEGA, 2011).

2.5.1 Revestimentos comestíveis à base de Amido

O amido é o principal carboidrato de armazenamento das plantas, podendo ser derivado de vários alimentos, como milho, arroz, mandioca, batata e trigo (PÉREZ e AGAMA-ACEVEDO, 2017).

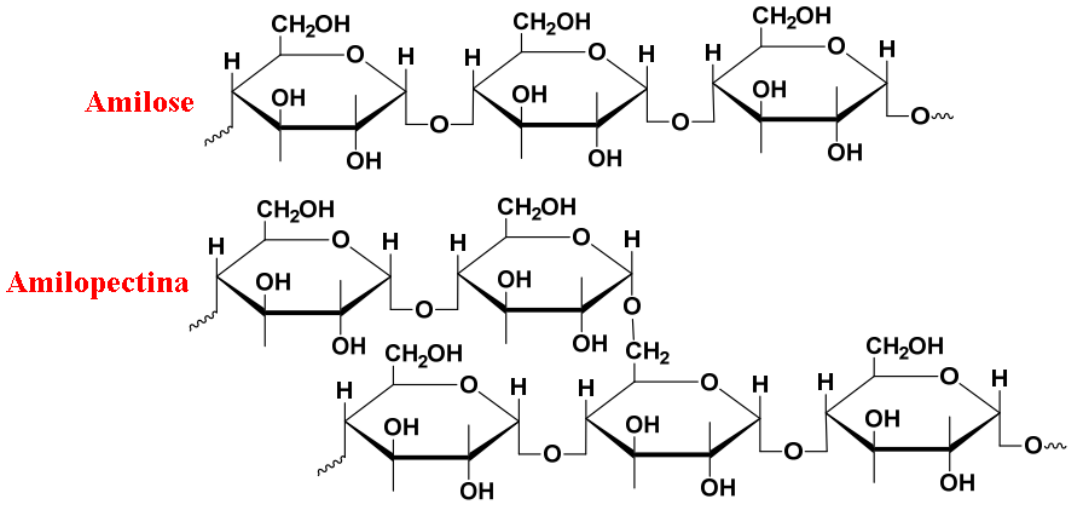
A extração do amido de mandioca torna-se viável e de interesse por ele ser um tubérculo amplamente consumido em mais de 102 países, além de sua fácil obtenção e manejo. Nas lavouras do Brasil, a mandioca encontra-se entre as culturas mais produzidas, sendo o país o quarto maior produtor de mandioca do mundo. No ano de 2021, foram produzidas 18.098.115 toneladas do tubérculo, e aproximadamente a metade do total das raízes produzidas são destinadas para a extração do amido (IBGE, 2021; TAVARES *et al*., 2019; WANG *et al*., 2022).

A obtenção do amido de mandioca ocorre comumente pelo método de moagem úmida, por ser uma técnica mais fácil, de menor custo e melhor qualidade do amido obtido. Na moagem úmida, o amido é obtido após as etapas de lavagem das raízes da mandioca, seguida do descascamento, ralação, adição de água e prensagem da massa, filtração, sedimentação/centrifugação, decantação, secagem, moagem e, por fim, a embalagem. Porém, a extração do amido também pode ser feita por meio da moagem a seco (COSTA *et al*., 2022; WANG *et al*., 2022).

De acordo com Tavares *et al*. (2019), o amido de mandioca, assim como os outros tipos de amido, é constituído de cadeias lineares e ramificadas; são elas amilose e amilopectina, respectivamente (Figura 19), unidas por ligações glicosídicas α-1,4 e ramificações α-1,6, formando grânulos de estrutura semicristalina (WATERSCHOOT *et al*., 2015; ZHU, 2015). Quando aquecido por tempo determinado, juntamente com água, o amido possui a capacidade de gelatinização, formando um gel. Durante a formação de gel, ocorre a ruptura das estruturas cristalinas e da ordem molecular do grânulo por meio do rompimento das ligações de hidrogênio (JACOBS *et al*., 2020).

Na elaboração de revestimentos comestíveis, o amido é um dos biopolímeros mais utilizados devido ao seu baixo custo, alta disponibilidade, facilidade de manuseio, biodegradabilidade, flexibilidade e o fato de ser comestível. Os revestimentos desenvolvidos à base de amido possuem ainda características de interesse, como serem incolores e inodoros, parâmetros vantajosos quando se trata de aplicação em alimentos. Além disso, possuem baixa permeabilidade ao oxigênio, auxiliando assim na redução da taxa de respiração de alimentos frescos. As desvantagens desse tipo de revestimento são a permeabilidade ao vapor de água, possibilidade de retrogradação do amido durante o período de armazenamento do produto e a sua solubilidade em água (SAPPER, BONET e CHIRALT, 2019; SAPPER e CHIRALT, 2018; CANO *et al*., 2014; DURANGO, SOARES e ARTEGA, 2011).

Figura 19 – Estrutura química do amido.



Fonte: Adaptado de Valencia-Llano *et al*. (2022).

As desvantagens apresentadas para os revestimentos à base de amido podem ser corrigidas por meio da combinação desse composto a outros compostos base de revestimentos, plastificantes, surfactantes, lipídios e compostos ativos (SAPPER; CHIRALT, 2018).

A elaboração de revestimentos comestíveis de amido incorporados com óleos essenciais tem mostrado resultados interessantes. Praseptiangga *et al*. (2017) avaliaram os efeitos de revestimentos de amido de mandioca incorporados com óleo essencial de capim-limão aplicados por imersão e pulverização em mamões. Esses autores observaram que a aplicação do revestimento reduziu alterações físico-quimicas, inibiu o crescimento microbiano nos mamões quando comparados ao tratamento-controle e aumentou a firmeza das frutas. Sapper *et al*. (2019) aplicaram revestimentos de amido incorporados com óleo essencial de

tomilho em maçãs e caquis. Os revestimentos impediram a perda de água nos caquis e reduziram o crescimento microbiano nas maçãs.

REFERÊNCIAS

ABDELAAL, K. *et al*. Toxicity of essential oils nanoemulsion against Aphis craccivora and their inhibitory activity on insect enzymes. **Processes**, v. 9, n. 4, p. 624, 2021.

ABDEL-AZEEM, A. M. *et al*. Biodiversity of the genus Aspergillus in different habitats. *In*: **New and future developments in microbial biotechnology and bioengineering**. Elsevier, 2016. p. 3-28.

ABRAS (Associação Brasileira de Supermercados). **19° Avaliação de perdas no varejo brasileiro de supermercados**, p. 1-51 2019a.

ABRAS (Associação Brasileira de Supermercados). **Embrapa e Abras querem reduzir perdas de alimentos no varejo**, 2019b. Disponível em < https://www.abras.com.br/clipping/noticias-abras/68581/embrapa-e-abras-querem-reduzir-perdas-de-alimentos-no-varejo >. Acesso em: 15 mai. 2023.

ABUGOCH, L. *et al*. Shelf-life of fresh blueberries coated with quinoa protein/chitosan/sunflower oil edible film. **Journal of the Science of Food and Agriculture**, v. 96, n. 2, p. 619-626, 2016.

AGRIOPOULOU, S.; STAMATELOPOULOU, E.; VARZAKAS, T. Advances in analysis and detection of major mycotoxins in foods. **Foods**, v. 9, n. 4, p. 518, 2020.

AILLI, A. *et al*. Phytochemistry and Biological Activities of Essential Oils from Six Aromatic Medicinal Plants with Cosmetic Properties. **Antibiotics**, v. 12, n. 4, p. 721, 2023.

ALBIERO, B. *et al*. Potencial inseticida dos óleos essenciais de endro (*Anethum graveolens*) e de nim (*Azadirachta indica*) no controle de *Sitophilus zeamais*/Insecticide potential of essential oils of endro (*Anethum graveolens*) and nim (*Azadirachta indica*) in the control of *Sitophilus zeamais*. **Brazilian Journal of Development**, v. 5, n. 10, p. 21443-21448, 2019.

ALVES-SILVA, J. M. *et al*. Chemical composition of Crithmum maritimum L. essential oil and hydrodistillation residual water by GC-MS and HPLC-DAD-MS/MS, and their biological activities. **Industrial Crops and Products**, v. 149, p. 112329, 2020.

ANDREAZZA, F. *et al*. *Drosophila suzukii* (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil. **Florida Entomologist**, v. 99, n. 4, p. 796-798, 2016.

ANZANO, A. *et al*. *Laurus nobilis* L.: A review of its botany, traditional uses, phytochemistry and pharmacology. **Phytochemistry Review,** v. 21, p. 565-615, 2022.

ÁVILA, J. A. T. *et al*. Shelf life estimation of Blackberry (*Rubus glacus* Benth) with bacterial cellulose film coating from Komagataeibacter xylinus. **Food Science and Nutrition**, v. 8, n. 4, p. 2173-2179, 2020.

AZADBAKHT, M. *et al*. Anti-parasitic activity of some medicinal plants essential oils on Giardia lamblia and Entamoeba histolytica, in vitro. **Research Journal of Pharmacognosy**, v. 7, n. 1, p. 41-47, 2020.

AZEVEDO, M. C. *et al*. Essential oil profiling of six new citrus hybrids from Murcott tangor and Pera sweet orange. **Journal of Essential Oil Research**, v. 31, n. 5, p. 400-408, 2019.

BARBIERI, R. L.; VIZZOTTO, M. Pequenas frutas ou frutas vermelhas. **Informe Agropecuário - Embrapa Clima Temperado**, v. 33, n. 268, p. 7-10, 2012.

BARBORÁKOVÁ, Z. *et al*. Toxin producing micromycetes of the genus *Penicillium* and *Aspergillus* on berries, grapes, and tomato fruits in slovak stores. **Journal of microbiology, biotechnology and food sciences**, p. e9927-e9927, 2023.

BARDA, O. *et al*. The pH-responsive transcription factor PacC governs pathogenicity and ochratoxin A biosynthesis in Aspergillus carbonarius. **Frontiers in Microbiology**, v. 11, p. 210, 2020.

BAUTISTA-BAÑOS, S., BOSQUEZ-MOLINA, E., BARRERA-NECHA, L. *Rizhopus stolonifer* (soft rot). *In*: Bautista-Baños, S. (Ed.), Postharvest Decay: **Control Strategies.** Academic Press, United States of America, 2014, p. 1–44.

BEDENDO, I. Podridões de órgãos de reserva. *In*: AMORIM, L.; REZENDE, J.A.M.; BERGAMIN FILHO, A. (Ed.). **Manual de Fitopatologia: Princípios e Conceitos**. 4 ed. São Paulo: Ceres, 2011, p. 427-434.

BELASLI, A. *et al*. Antifungal, antitoxigenic, and antioxidant activities of the essential oil from laurel (*Laurus nobilis* L.): Potential use as wheat preservative. **Food Science & Nutrition**, v. 8, n. 9, p. 4717-4729, 2020.

BENBETTAIEB, N.; DEBEAUFORT, F.; KARBOWIAK, T. Bioactive edible filmes for food applications: mechanisms of antimicrobial and antioxidant activity. **Critical Reviews in Food Science and Nutrition**, v. 59, n. 21, p. 3431-3455, 2019.

BHARDWAJ, A.; ALAM, T.; TAWAR, N. Recent advances in active packaging of agrid-food products: review. **Journal of Postharvest Technology**, v. 7, n. 1, p. 33-62, 2019.

BRANDÃO, R. M. *et al*. Antifungal and antimycotoxigenic effect of the essential oil of *Eremanthus erythropappus* on three different *Aspergillus* species. **Flavour and Fragrance Journal**, v. 35, n. 5, p. 524-533, 2020.

BRANDÃO, R. M. *et al*. Antifungal and antiocratoxigenic potential of *Alpinia speciosa* and *Cymbopogon flexuosus* essential oils encapsulated in poly (lactic acid) nanofibres against Aspergillus fungi. **Letters in Applied Microbiology**, v. 75, n. 2, p. 281-292, 2022.

BRASIL. Agência Nacional de Vigilância Sanitária (Anvisa). Instrução Normativa IN Nº60 de 1º de Julho de 2022, Estabelece os Limites máximos tolerados de contaminantes em alimentos. 2022.

CABAÑES, F. J. *et al*. What is the source of ochratoxin A in wine?. **International Journal of Food Microbiology**, v. 79, n. 3, p. 213-215, 2002.

CAETANO, A. R. S. *et al*. *Rosmarinus officinalis* essential oil incorporated into nanoparticles as an efficient insecticide against *Drosophila suzukii* (Diptera: Drosophilidae). **Austral Entomology**, v. 61, n. 2, p. 265-272, 2022.

CALABRIA, G. *et al*. First records of the potential pest species *Drosophila suzukii* (Diptera: Drosophilidae) in Europe. **Journal of Applied entomology**, v. 136, n. 1-2, p. 139-147, 2012.

CAMARGO, K. C. *et al*. Antibacterial action of the essential oil from Cantinoa carpinifolia benth. Against *Escherichia coli* and *Staphylococcus aureus* strains. **Flavour and Fragrance Journal**, v. 35, n. 1, p. 99-106, 2020.

CANO, A. *et al*. Effect of amylose: amylopectin ratio and rice bran addition on starch films properties. **Carbohydrate Polymers**, v. 111, p. 543-555, 2014.

CARVALHO, I. T. **Microbiologia dos Alimentos**. Recife: EDUFRPE, 2010, p. 84.

CHAHAL, K. *et al*. A review on chemistry and biological activities of *Laurus nobilis* L. essential oil. **Journal Pharmacognosy and Phytochemistry**, v.6, n. 4, p. 1153–1161, 2017.

CHAUBEY, M. K. Fumigant toxicity of essential oils and pure compounds against *Sitophilus oryzae* L. (Coleoptera: Curculionidae). **Biological Agriculture & Horticulture**, v. 28, n. 2, p. 111-119, 2012.

CHÁVEZ-DÍAZ, I. F. *et al*. Antagonistic bacteria with potential for biocontrol on *Rhizopus stolonifer* obtained from blackberry fruits. **Fruits**, v. 69, n. 1, p. 41-46, 2014.

CHUNG, N. T.; HUONG, L. T.; OGUNWANDE, I. A. Antimicrobial, Larvicidal Activities and Composition of the Leaf Essential Oil of *Magnolia coco* (Lour.) DC. **Records of Natural Products**, v. 14, n. 5, p. 372-377, 2020.

CONDE-HERNÁNDEZ, L. A. *et al*. CO_2-supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (*Rosmarinus officinalis*). **Journal of Food Engineering**, v. 200, p. 81-86, 2017.

COOPER, M. Spotted-wing Drosophila (Drosophila suzukii) male. Flickr, 2014. Disponível em:https://www.flickr.com/photos/m-a-r-t-in/15359228246/in/photolist-p9LTYD-EsvucZ-ppf4s7. Acesso em 10 de Maio de 2020.

CORRÊA, M. P. **Dicionário das plantas úteis do Brasil e das tósicas cultivadas.** Rio de Janeiro, Imprensa Nacional, vol. 4, 1969.

COSTA, A. R. T. *et al*. Ação do óleo essencial de *Syzygium aromaticum* (L.) Merr. & LM Perry sobre as hifas de alguns fungos fitopatogênicos. **Revista brasileira de plantas medicinais**, v. 13, n. 2, p. 240-245, 2011.

COSTA, F. *et al*. Revestimentos comestíveis à base de fécula de mandioca (manihot esculenta) em produtos vegetais: uma revisão. **Research, Society and Development**, v. 11, n. 4, p. e54511427428-e54511427428, 2022.

COUTO, M. A. L.; CANNIATTI-BRAZACA, S. G. Quantificação de vitamina C e capacidade antioxidante de variedades cítricas. **Food Science and Technology**, v. 30, p. 15-19, 2010.

DAMMAK, I. *et al*. Evaluation of antifungal and anti-ochratoxigenic activities of *Salvia officinalis*, *Lavandula dentata* and *Laurus nobilis* essential oils and a major monoterpene constituent 1,8-cineole against *Aspergillus carbonarius*. **Industrial Crops and Products**, v. 128, p. 85-93, 2019.

DEBA, F. *et al*. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. *Radiata*. **Food control**, v. 19, n. 4, p. 346-352, 2008.

DENKOVA-KOSTOVA, R. *et al*. Chemical composition, antioxidant and antimicrobial activity of essential oils from tangerine (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.), lemon (*Citrus lemon* L.) and cinnamon (*Cinnamomum zeylanicum* Blume). **Zeitschrift für Naturforschung C**, v. 76, n. 5-6, p. 175-185, 2020.

DEWICK, P. M. **Medicinal natural products: a biosynthetic approach**. 3rd ed. Chichester: J. Wiley, 2009, p. 539.

DRINIĆ, Z. *et al*. Microwave-assisted extraction of O. vulgare L. spp. hirtum essential oil: Comparison with conventional hydro-distillation. **Food and Bioproducts Processing**, v. 120, p. 158-165, 2020.

DURANGO, A. M.; SOARES, N. F.; ARTEAGA, M. R. Filmes y Revestimientos comestibles como empaques activos biodegradables em la conservación de alimentos. **Biotecnologia em el Sector Agropecuário y Agroindustrial**, v. 9, n. 1, p. 112-118, 2011.

EL-SAYED, Y. A.; YOUSEF, H. Evaluation the insecticidal activity of *Purpureocillium lilacinum* and *Cuminum cyminum* and study their infection impact on some biochemical content in the haemolymph of the cotton leaf worm *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctudiae). **International Journal of Entomology Research**, v. 6, n. 2, p. 22-30, 2021.

ENRIQUEZ, T. *et al*. Effects of fluctuating thermal regimes on cold survival and life history traits of the spotted wing Drosophila (*Drosophila suzukii*). **Insect Science**, v. 27, n. 2, p. 317-335, 2020.

FALGUERA, V. *et al*. Edible films and coatings: Structures, active functions and trends in their use. **Trends in Food Science and Technology**, v. 22, p. 292-303, 2011.

FERREIRA, V. R. F. *et al*. Colorimetric, electoanalytical and theoretical evaluation of the antioxidant activity of *Syzygium aromaticum* L., *Origanum vulgare* L., *Mentha spicata* L. and *Eremanthus erythropappus* M. essential oils, and their major constituents. **New Journal Chemistry**, v. 43, p. 7653-7662, 2019.

FERRENTINO, G. *et al*. Extraction of essential oils from medicinal plants and their utilization as food antioxidants. **Current Pharmaceutical Design**, v. 26, n. 5, p. 519-541, 2020.

FIKRY, A. M. *et al*. Effect of nitrogen fertilization through inorganic, organic and biofertilizers sources on vegetative growth, yield and nutritional status in murcott tangerine trees. **Plant Archives (09725210)**, v. 20, n. 1, 2020.

FUENTES, L.; FIGUEROA, C. R.; VALDENEGRO, M. Recent advances in hormonal regulation and cross-talk during non-climacteric fruit development and ripening. **Horticulturae**, v. 5, n. 2, p. 45, 2019.

GALINDO, M. *et al*. **Microencapsulação de D-limoneno e aplicação em filmes biodegradáveis de quitosana e gelatina**. Ponta Grossa: Atena, 2019.

GALUS, S. Development of edible coatings in the preservation of fruits and vegetables. *In*: Polymers for Agri-Food Applications. **Springer**, Cham, 2019. p. 377-390.

GARCÍA, M. A. *et al*. Characterization of starch and composite edible films and coatings. In: Edible films and coatings for food applications. **Springer**, New York, NY, 2009. p. 169-209.

GHASEMI, M. *et al*. Encapsulation of Cumin essential oil in zein electrospun fibers: Characterization and antibacterial effect. **Journal of Food Measurement and Characterization**, v. 16, n. 2, p. 1613-1624, 2022.

GHİMİRE, D. *et al*. Determination of Mold Diversity of Some Fruits Sold in Eastern Turkey. **Journal of the Institute of Science and Technology**, v. 12, n. 4, p. 2199-2208, 2022.

GOBBO-NETO, L.; LOPES, N. P. Medicinal plants: factors of influence on the content of secondary metabolites. **Química Nova**, v. 30, p. 374-381, 2007.

GOMES, M. S. *et al*. Effect of edible coatings with essential oils on the quality of red raspberries over shelf-life. **Journal of the Science of Food and Agriculture**, v.97, n.3, p.929-938, 2017.

GONZÁLEZ-OROZCO, B. D. *et al*. Effect of short-term controlled atmospheres on the postharvest quality and sensory shelf life of red raspberry (Rubus idaeus L.). **CyTA Journal of Food**, v. 18, n. 1, p. 352-358, 2020.

HASHEMI, S. M. B.; KHANEGHAH, A. M.; SANT'ANA, A. S. **Essential Oils in Food Processing: chemistry, safety and applications**. Hoboken, NJ:**John Wiley e Sons**, 2018, 205p.

HASSANEIN, H. D. *et al*. Profiling of essential oil chemical composition of some Lamiaceae species extracted using conventional and microwave-assisted hydrodistillation extraction methods via chemometrics tools. **Flavour and Fragrance Journal**, v. 35, n. 3, p. 329-340, 2020.

HERTWECK, C. The biosynthetic logic of polyketide diversity. **Angewandte Chemie International Edition**, v. 48, n. 26, p. 4688-4716, 2009.

IBGE. Instituto Brasileiro de Geografia e Estatística**. Produção Agrícola Municipal. 2021.** Disponível em: https://www.cnpmf.embrapa.br/Base_de_Dados/index_pdf/dados/brasil/tangerina/b1_tangerina.pdf. Acesso em: 26 de junho de 2023.

IBGE. Instituto Brasileiro de Geografia e Estatística. **Quantidade produzida em toneladas. 2021**. Disponível em: https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9117-producao-agricola-municipal-culturas-temporarias-e-permanentes.html. Acesso em: 20 de junho de 2023

IBOUH, K. *et al*. Biological control of *Drosophila suzukii*: Efficacy of parasitoids, entomopathogenic fungi, nematodes and deterrents of oviposition in laboratory assays. **Crop Protection**, v. 125, p. 104897, 2019.

IMELOUANE, B. *et al*. Chemical composition and antimicrobial activity of essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. **International Journal of Agriculture & Biology**, v. 11, n. 2, p. 205-208, 2009.

ISO 9235:2021. Aromatic natural raw materials – Vocabulary. Geneva, Switzerland: International Organization for Standardization (ISO); 2021. Available from: https://www. iso.org/obp/ui/#iso:std:iso:9235:ed-3:v1:en.

JACOBS, Vanessa *et al*. Produção e caracterização de biofilmes de amido incorporados com polpa de acerola. **Revista Iberoamericana de Polímeros**, v. 21, n. 3, p. 107-119, 2020.

JANG, M. *et al*. Biological activity of Myrtaceae plant essential oils and their major components against *Drosophila suzukii* (Diptera: Drosophilidae). **Pest management science**, v. 73, n. 2, p. 404-409, 2017.

JIN, J. *et al*. Interaction between food-borne mycotoxins and gut microbiota: A review. **Food Control**, p. 107998, 2021.

JÚNIOR, I. M. R.; OLIVEIRA, A. J. M.; RODRIGUES, F. A. Panorama Nacional da Produção de Frutas Vermelhas. **Campos e Negócios Online**, 2019. Disponível em: <https://revistacampoenegocios.com.br/panorama-nacional-da-producao-de-frutas vermelhas/>. Acesso em: 10 de mai. de 2020.

KISOVÁ, Z. *et al*. Studying the gene expression of *Penicillium rubens* under the effect of eight essential oils. **Antibiotics**, v. 9, n. 6, p. 343, 2020.

KOÇ, F.; KARA, S. Environmental factors affecting efficacy of some essential oils and potassium sorbate to control growth of *Aspergillus flavus*, *Aspergillus parasiticus* on wheat and maize grains. **Journal of Agriculture Science and Technology,** v. 16, p. 1325-1334, 2018.

KONG, J. *et al*. Antifungal effects of thymol and salicylic acid on cell membrane and mitochondria of *Rhizopus stolonifer* and their application in postharvest preservation of tomatoes. **Food Chemistry**, v. 285, p. 380-388, 2019.

KRIJGSHELD, P. *et al*. Development in *Aspergillus*. **Studies in mycology**, v. 74, p. 1-29, 2013.

KROCHTA, J. M. **Proteins as Raw Materials for Films and Coatings: Definitions, Current Status, and Opportunities**. *In*: GENADDIONS, A. Protein-Based Films and Coating. CRC Press, 2002, p. 1-40.

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 medicale**, v. 27, n. 3, p. 303-311, 2017.

LAMOUNIER, M. L. *et al*. Refrigerante de frutas vermelhas: Desenvolvimento, Teste Físico-químico, Microbiológico e Sensorial. **HOLOS**, v. 2, p. 1-24, 2019.

LAPPA, I. K.; KIZIS, D.; PANAGOU, E. Z. Monitoring the temporal expression of genes involved in ochratoxin A production of Aspergillus carbonarius under the influence of temperature and water activity. **Toxins**, v. 9, n. 10, p. 296, 2017.

LEE, S. *et al*. Synergistic antimicrobial activity of oregano and thyme thymol essential oils against *Leuconostoc citreum* in a laboratory medium and tomato juice. **Food Microbiology**, v. 90, p. 103489, 2020.

LI, H. *et al*. *Blended cumin*/*Zanthoxylum* essential oil improve the antibacterial, fresh-keeping performance and flavor of chilled fresh mutton. **Meat Science**, v. 200, p. 109173, 2023.

LIU, Y. *et al*. Selective antifungal activity of chitosan and sulfonated chitosan against postharvest fungus isolated from blueberry. **Journal of Food Biochemistry**, v. 42, n. 6, p. e12658, 2018.

LOEBLER, M. *et al*. Aplicação de extratos de própolis para conservação pós-colheita de morangos. **Vida Rural**, v. 1837, p. 38-40, 2018.

LORENÇONI, M. F. *et al*. Chemical composition and anti-inflammatory activity of essential oil and ethanolic extract of *Campomanesia phaea* (O. Berg.) Landrum leaves. **Journal of Ethnopharmacology**, v. 252, p. 112562, 2020.

LUNGUINHO, A. S. *et al*. Acaricidal and repellent activity of the essential oils of *Backhousia citriodora*, *Callistemon viminalis* and *Cinnamodendron dinisii* against *Rhipicephalus* spp. **Veterinary Parasitology**, v. 300, p. 109594, 2021.

MADIGAN, M. T.; MARTINKO, J. M.; PARKER, J. **Microbiologia de Brock**. 12.ed. São Paulo: Prentice Hall, 2016.

MAGALHÃES, M. L. *et al*. Biological activities of the essential oil from the Moro orange peel (*Citrus sinensis* (L.) Osbeck). **Flavour and Fragrance Journal**, v. 35, n. 3, p. 294-301, 2020.

MASSOLA J. N. S.; KRUGNER, T. L. Fungos Fitopatogênicos. In: AMORIM, L.; REZENDE, J.A.M.; BERGAMIN FILHO, A. (Ed.). **Manual de Fitopatologia: Princípios e Conceitos**. 4 ed. São Paulo: Ceres, 2011. cap. 8, p. 149-206.

MATTA, E.; TAVERA-QUIROZ, M. J.; BERTOLA, N. Active edible films of methylcellulose with extracts of green apple (Granny Smith) skin. **International journal of biological macromolecules**, v. 124, p. 1292-1298, 2019.

MIRI, Y. B.; DJENANE, D. Research Article Evaluation of Protective Impact of Algerian *Cuminum cyminum* L. and *Coriandrum sativum* L. Essential Oils on *Aspergillus flavus* Growth and Aflatoxin B1 Production**. Pakistan Journal of Biological Sciences**, 2018.

MOSSA, A. T. H. Green pesticides: Essential oils as biopesticides in insect-pest management. **Journal of environmental science and technology**, v. 9, n. 5, p. 354, 2016.

MOUSTAFA, H.; EL-SAYED, S. M.; YOUSSEF, A. M. Synergistic impact of cumin essential oil on enhancing of UV-blocking and antibacterial activity of biodegradable poly (butylene adipate-co-terephthalate)/clay platelets nanocomposites. **Journal of Thermoplastic Composite Materials**, v. 36, n. 1, p. 96-117, 2023.

NAVA, D. E. *et al*. **Bioecologia, monitoramento e controle de *Drosophila suzukii* na cultura do morangueiro.** Rio grande do Sul: EMBRAPA. (Documento 398), 2015, p. 25.

NOGUEIRA, S.; OLIVEIRA, M. B. B. P. Prevalência de ocratoxina A em alimentos e consequentes problemas de segurança alimentar. **Revista da Sociedade Portuguesa de Ciências da Nutrição e Alimentação**, v. 12, n. 2, p. 69-75, 2006.

NOSTRO, A. L. *et al*. Antibacterial effect of plant extracts against *Helicobacter pylori*. **Phytotherapy Research,** v. 19, p. 198-202, 2005.

OH, H. D. *et al*. Abscisic acid stimulates anthocyanin accumulation in 'Jersey' highbush blueberry fruits during ripening. **Food chemistry**, v. 244, p. 403-407, 2017.

OLIVEIRA FILHO, J. G. *et al*. Essential oils as natural fungicides to control *Rhizopus stolonifer*-induced spoiled of strawberries. **Biointerface Research in Applied Chemistry**, v. 11, p. 1344-13251, 2021.

ORDOUDI, S. A. *et al*. Bay Laurel (*Laurus nobilis* L.) essential oil as a food preservative source: Chemistry, quality control, activity assessment, and applications to olive industry products. **Foods**, v. 11, n. 5, p. 752, 2022.

ÖZDEMIR, R.C.; TAŞTAN, Y.; GÜNEY, K. Prevention of Saprolegniasis in rainbow trout (Oncorhynchus mykiss) eggs using oregano (Origanum onites) and laurel (*Laurus nobilis*) essential oils. **Journal of Fish Diseases**, v. 45, n. 1, p. 51-58, 2022.

PADILLA-CAMBEROS, E. *et al*. Natural essential oil mix of sweet orange peel, cumin, and allspice elicits anti-inflammatory activity and pharmacological safety similar to non-steroidal anti-inflammatory drugs. **Saudi Journal of Biological Sciences**, v. 29, n. 5, p. 3830-3837, 2022.

PAPARELLA, A. *et al*. A Review of the Botany, Volatile Composition, Biochemical and Molecular Aspects, and Traditional Uses of *Laurus nobilis*. **Plants**, v. 11, n. 9, p. 1209, 2022.

PARIS, M. J. *et al*. Modelling release mechanisms of cinnamon (*Cinnamomum zeylanicum*) essential oil encapsulated in alginate beads during vapor-phase application. **Journal of Food Engineering**, p. 110024, 2020.

PARK, C. G. *et al*. Myrtaceae plant essential oils and their β-triketone components as insecticides against *Drosophila suzukii*. **Molecules**, v. 22, n. 7, p. 1050, 2017.

PARUSSOLO, Gilson *et al*. Ochratoxin A production by *Aspergillus westerdijkiae* in Italian-type salami. **Food microbiology**, v. 83, p. 134-140, 2019.

PAVINATTO, A. *et al*. Coating with chitosan-based edible films for mechanical/biological protection of strawberries. **International journal of biological macromolecules**, v. 151, p. 1004-1011, 2019.

PERDONES, A. *et al*. Effect of chitosan-lemon essential oil coatings on storagekeeping quality of strawberry. **Postharvest Biology and Technology**, v. 70, p. 32-41, 2012.

PÉREZ, L. A. B.; AGAMA-ACEVEDO, E. **Starch**. *In*: VILLAR M. A. *et al*. Starch-Based Materials in Food Packaging: Processing, Characterization and Applications. Elsevier, 2017, p. 313.

PIECHOWIAK, T. *et al*. Impact of ozonation process on the microbiological and antioxidant status of raspberry (Rubus ideaeus L.) fruit during storage at room temperature. **Agricultural and Food Science**, v. 28, n. 1, p. 35-44, 2019.

PITT, J. I. **Mycotoxins: Ochratoxin.** *In:*A. Y. Motarjemi, G. Moy, E. Todd (Eds.), Encyclopedia of food safaty, vol. 2, Amsterdam: Elsevier, 2014, p. 304-309.

POMBO, J. C. P. *et al*. Efeito antimicrobiano e sinergístico de óleos essenciais sobre bactérias contaminantes de alimentos. **Segurança Alimentar e Nutricional**, v. 25, n. 2, p. 108-117, 2018.

POZZA, J. M.; CUNHA, G. F. Estudo preliminar sobre a ocorrência de *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) no município de Farroupilha, Rio Grande do Sul. **Revista Interdisciplinar de Ciência Aplicada**, v. 4, n. 7, p. 6-11, 2019.

PRADO, G. Contaminação de alimentos por micotoxinas no Brasil e no mundo. **Gerais: Revista de Saúde Pública do SUS/MG**, v. 2, n. 2, p. 13-26, 2017.

PRASEPTIANGGA, D. *et al*. Effect of cassava starch-based edible coating incorporated with lemongrass essential oil on the quality of papaya MJ9. **Materials Science and Engineering**, v.176, p.1-6, 2017.

RAMOS, S. A. *et al*. Reaproveitamento de resíduos alimentares: Desenvolvimento e caracterização de farinha de casca de tangerina (*Citrus reticulata*). **Scientia Plena**, v. 19, n. 4, 2023.

REIS P. M. C. L. *et al*. Formation, stability and antimicrobial activity of laurel leaves essential oil (*Laurus nobilis* L.) particles in suspension obtained by SFEE. J. **Supercrit. Fluids**, v. 166, p. 105032, 2020.

REZENDE, D. A. C. S. *et al*. Effect of the essential oils of *Satureja montana* L., *Myristica fragrans* H. and *Cymbopogon flexuosus* S. on mycotoxin-producing *Aspergillus flavus* and *Aspergillus ochraceus* antifungal properties of essential oils. **FEMS Microbiology Letters**, v. 368, n. 19, p. fnab137, 2021.

REZENDE, J. L. *et al*. Antifungal potential of essential oils from two varieties of *Citrus sinensis* (lima orange and bahia navel orange) in postharvest control of *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. **Food Science and Technology**, v. 40, p. 405-409, 2020.

RIBEIRO, N. C. *et al*. Insecticidal potential of citrus and mango essential oils and selected constituents on silverleaf whitefly. **Revista Caatinga**, v. 33, n. 1, p. 90-99, 2020.

RODRIGUES, U. V. *et al*. Tópicos especiais em Ciência Animal VI [e-book]. 1.edAlegre – ES: **UNICOPY**, 2017, p. 411.

RODRÍGUEZ, M. C. *et al*. Effect of a multifunctional edible coating based on cassava starch on the shelf life of Andean blackberry. **Heliyon**, v. 6, n. 5, p. e03974, 2020.

ROSA, J. S. *et al*. Bioactivity of some Apiaceae essential oils and their constituents against *Sitophilus zeamais* (Coleoptera: Curculionidae). **Bulletin of entomological research**, v. 110, n. 3, p. 406-416, 2020.

RU, Q. *et al*. Formulation of *Laurus nobilis* essential oil nanoemulsion system and its application in fresh-cut muskmelons. **Coatings**, v. 12, n. 2, p. 159, 2022.

SADEGHI, R. *et al*. The Effects of the essential oils isolated from four ecotypes of cumin (*Cuminum cyminum* L.) on the blood cells of the pink stem borer, *Sesamia cretica* Ledere (Lepidoptera: Noctuidae). **Journal of the Kansas Entomological Society**, v. 92, n. 1, p. 390-399, 2019.

SALES, T. A. *et al*. Essential oils from the leaves and flowers of Callistemon viminalis: chemical characterization and evaluation of the insecticide and antifungal activities. **American Journal of Plant Sciences**, v. 8, n. 10, p. 2516, 2017.

SANTOS, A. R. *et al*. Evaluation of essential oils and diluents against Chrysomya megacephala, an important mechanical vector. **Journal of Natural Pesticide Research**, v. 3, p. 100024, 2023.

SANTOS, R. S. S. Ocorrência de Drosophila suzukii (Matsumura, 1931),(Diptera: Drosophilidae) atacando frutos de morango no Brasil. **Embrapa Uva e Vinho Comunicado Técnico** (INFOTECA-E), 2014.

SAPPER, M. *et al*. Antifungical starch-gellan edible coatings with Thyme Essential Oil for the Postharvest Preservation of Apple and Persimmon. **Coatings**, v. 9, n. 5, p. 333, 2019.

SAPPER, M.; BONET, M.; CHIRALT, A. Wettability of starch-gellan coatings on fruits, as affected by the incorporation of essential oil and/or surfactants. **LWT – Food Science and Technology**, v. 116, 2019.

SAPPER, M.; CHIRALT, A. Starch-Based Coatings of Preservation of Fruits and Vegetables. **Coatings**, v. 8, n. 5, p. 152, 2018.

SARTORI, D. *et al*. **Molecular diagnosis of ochratoxigenic fungi**. In: Molecular identification of fungi. Berlin: Springer, 2010. p. 195-212.

SCHLESENER, D. C. H. *et al*. *Drosophila suzukii:* nova praga para a fruticultura brasileira. **O Biológico**, v. 77, n. 1, p. 47-54, 2015.

SILVA, B. K. O. *et al*. Películas de amido de mandioca na conservação pós-colheita de morango, maracujá e pimenta doce. **Revista Brasileira de Produtos Agroindustriais**, c. 18, n. 3, p. 283-291. 2016.

SILVA, P. P. M. *et al*. Essential oils from *Eucalyptus staigeriana* F. Muell. ex Bailey and *Eucalyptus urograndis* W. Hill ex Maiden associated to carboxymethylcellulose coating for the control of *Botrytis cinerea* Pers. Fr. and *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. in strawberries. **Industrial Crops and Products**, v. 156, p. 112884, 2020.

SIMÕES, C. M. O. *et al*. **Farmacognosia: da planta ao medicamento**. 6 ed. Porto Alegre: UFSC/ UFRGS, 2007, p. 1104.

SINGH, B. *et al*. Phenolic composition, antioxidant potential and health benefits of citrus peel. **Food Research International**, v. 132, p. 109114, 2020.

SINGH, P. *et al*. Cellulose-based edible films for probiotic entrapment. **Food Hydrocolloids**, v. 88, p. 68-74, 2019.

SMITH, P. F. Collapse of 'Murcott'Tangerine Trees1. **Journal of the American Society for Horticultural Science**, v. 101, n. 1, p. 23-25, 1976.

SOARES, N. F. F. **Bitterness reduction in citrus juice through naringinase immobilized into polymer film.** 1998. Dissertation. Cornell University, New York, f.130.

SOUZA, L. *et al*. Toxicity, Histopathological Alterations and Acetylcholinesterase Inhibition of *Illicium verum* Essential Oil in *Drosophila suzukii*. **Agriculture**, v. 12, n. 10, p. 1667, 2022.

SOUZA, M. A.; BATISTA, E. J.; MENEZES, A. E. T. Panorama Nacional da Produção de morangos. **Campos e Negócios Online,** 2022. Disponível em: <https://revistacampoenegocios. com.br/panorama-nacional-da-producao-de-morangos/>. Acesso em: 22 de mai. de 2023.

SOUZA, M. T. *et al*. Chemical composition of essential oils of selected species of Piper and their insecticidal activity against *Drosophila suzukii* and *Trichopria anastrephae*. **Environmental Science and Pollution Research**, v. 27, p. 13056-13065, 2020.

SOUZA, R. V. *et al*. Potencial antifúngico de constituintes de óleos essenciais. **Research, Society and Development**, v. 10, n. 12, p. e457101220537-e457101220537, 2021.

STRATAKOS, A. C.; KOIDIS, A. **Methods for extracting essential oils**. *In*: Essential Oils in Food Preservation, Flavor and Safety. Academic Press, 2016. p. 31-38.

TAHMASEBI, M. *et al*. Control of nectarine fruits postharvest fungal rots caused by *Botrytis cinerea* and *Rhizopus stolonifer* via some essential oils. **Journal of food science and technology**, v. 57, p. 1647-1655, 2020.

TANIWAKI, M. H. *et al*. Ochratoxigenic fungi and ochratoxin A in defective coffee beans. **Food research international**, v. 61, p. 161-166, 2014.

TANIWAKI, M. H.; PITT, J. I.; MAGAN, N. Aspergillus species and mycotoxins: occurrence and importance in major food commodities. **Current Opinion in Food Science**, v. 23, p. 38-43, 2018.

TAVARES, K. M. *et al*. Corn and cassava starch with carboxymethyl cellulose films and its mechanical and hydrophobic properties. **Carbohydrate polymers**, v. 223, p. 115055, 2019.

TEIXEIRA, J. P. F.; MARQUES, M. O. M.; PIO, R. M. Caracterização dos óleos essenciais em frutos de nove genótipos de tangerina. **Citrus Research & Technology**, v. 35, n. 1, p. 1-10, 2017.

THIPPESWAMY, N. B.; NAIDU, K. A. Antioxidant potency of cumin varieties-cumin, black cumin and bitter cumin-on antioxidant systems. **European Food Research. Technology**, v. 220, p. 472-476, 2005.

THORMAR, H. **Lipids and Essential Oils as Antimicrobial Agents**. United Kingdom: John Wiley e Sons, 2011.

TSELIOU, M. *et al*. Antiviral effect of an essential oil combination derived from three aromatic plants (*Coridothymus capitatus* (L.) Rchb. f., *Origanum dictamnus* L. and *Salvia fruticosa* Mill.) against viruses causing infections of the upper respiratory tract. **Journal of Herbal Medicine**, v. 17, p. 100288, 2019.

VALENCIA-LLANO, C. H. *et al*. Histological Evaluation of Cassava Starch/Chicken Gelatin Membranes. **Polymers**, v. 14, n. 18, p. 3849, 2022.

VENTURA-AGUILAR, R. I. *et al*. Monitoring the infection process of *Rhizopus stolonifer* on strawberry fruit during storage using films based on chitosan/polyvinyl alcohol/polyvinylpyrrolidone and plant extracts. **International Journal of Biological Macromolecules**, v. 182, p. 583-594, 2021.

VIEIRA, P. R. *et al*. Chemical composition and antifungal activity of essential oils from Ocimum species. **Industrial Crops and Products**, v. 55, p. 267-271, 2014.

VIEIRA, T.; CUNHA, S.; CASAL, S. **Mycotoxins in coffee**. In: Coffee in health and disease prevention. Academic Press, 2015. p. 225-233.

VILAPLANA, R. *et al*. Chitosan coatings to control soft mold on fresh blackberries (*Rubus glaucus* Benth.) during postharvest period. **Scientia Horticulturae**, v. 262, p. 109049, 2020.

VIPOTNIK, Z.; RODRÍGUEZ, A.; RODRIGUES, P. *Aspergillus westerdijkiae* as a major ochratoxin A risk in dry-cured ham based-media. **International journal of food microbiology**, v. 241, p. 244-251, 2017.

WANG, Z. *et al*. Cassava starch: Chemical modification and its impact on functional properties and digestibility, a review. **Food Hydrocolloids**, v. 129, p. 107542, 2022.

WATERSCHOOT, J. *et al*. Production, structure, physicochemical and functional properties of maize, cassava, wheat, potato and rice starches. **Starch-Stärke**, v. 67, n. 1-2, p. 14-29, 2015.

WATKINS, C. **Fisiologia pós-colheita de tecidos vegetais comestíveis**. *In:* DAMODARAN, S.; PARKIN, K. L; FENNEMA, O. R. **Química de Alimentos de Fennema**, 5ed., 2019, p. 1015- 1085.

WOLFFENBUTTEL, A. N. **Base da química dos óleos essenciais e aromaterapia: abordagem técnica e científica.** São Paulo: Roca. 2011, p.288.

WORLD HEALTH ORGANIZATION *et al*. **Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins**. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, v. 56, 1993.

YOON, J. H.; LEE, S. Y. Comparison of the effectiveness of decontaminating strategies for fresh fruits and vegetables and related limitations. **Critical reviews in food science and nutrition**, v. 58, n. 18, p. 3189-3208, 2017.

ZAKY, A. A.; SHIM, J. H.; ABD EL-ATY, A. M. A Review on extraction, characterization, and applications of bioactive peptides from pressed black cumin seed cake. **Frontier in Nutrition,** v. 8, p. 743909, 2021.

ZHOU, L. *et al*. Evaluation of the antifungal activity of individual and combined monoterpenes against *Rhizopus stolonifer* and *Absidia coerulea*. **Environmental Science and Pollution Research**, v. 26, p. 7804-7809, 2019.

ZHU, F. Composition, structure, physicochemical properties, and modifications of cassava starch. **Carbohydrate polymers**, v. 122, p. 456-480, 2015.

ZHU, X. Q.; XIAO, C. L. Phylogenetic, morphological, and pathogenic characterization of Alternaria species associated with fruit rot of blueberry in California. **Phytopathology**, v. 105, n. 12, p. 1555-1567, 2015.

ZIVKOVIĆ, I. P. *et al*. The development of drosophilid species (Diptera, Drosophilidae) in different strawberry cultivars. **European journal of horticultural science**, v. 84, n. 1, p. 48-52, 2019.

SEGUNDA PARTE – ARTIGOS

Este trabalho gerou 3 artigos, apresentados de acordo com as normas da revista submetido ou de acordo com as normas da UFLA

ARTIGO 1 - Essential oils from *Cuminum cyminum* and *Laurus nobilis* and their principal constituents: evaluation of antifungal and antimycotoxigenic potential on fungi of the genus *Aspergillus.*

Periódico: FEMS Microbiology Letters.
JCR: 2,1.

Essential oils from *Cuminum cyminum* and *Laurus nobilis* and their principal constituents: evaluation of antifungal and antimycotoxigenic potential in *Aspergillus species*

Effect of essential oils and standard compounds on mycotoxigenic fungi

Gabriela Aguiar Campolina[1], Maria das Graças Cardoso[2], Carolina Salles Freire[2], Alex Rodrigues Silva Caetano[2], Anna Beatriz de Souza Campos[2], Vanuzia Rodrigues Fernandes Ferreira[2], Eduardo Alves[3], David Lee Nelson[4], Luis Roberto Batista[1]

[1]Food Sciences Department, Federal University of Lavras (UFLA), Lavras, MG, Brazil
[2]Chemistry Department, Federal University of Lavras (UFLA), Lavras, MG, Brazil
[3]Phytopathology Department, Federal University of Lavras (UFLA), Lavras, MG, Brazil
[4]Postgraduate Program in Biofuels, Federal University of The Jequitinhonha and Mucuri Valleys, Diamantina, MG, Brazil

Abstract

The use of essential oils as the basis of natural antifungal agents for application in agriculture and the food industry is a good alternative because of its antifungal potential, its biodegradability and the fact that it is generally considered safe. The antifungal and antimycotoxigenic activities of the essential oils (EO) from *Cuminum cyminum* and *Laurus nobilis*, as well as their respective principal compounds, cuminaldehyde and 1,8-cineole, were evaluated against fungi of the genus *Aspergillus*: *A.carbonarius, A.niger*, *A.ochraceus* and *A. westerdijkiae*. The antifungal activity was determined by the contact method and the mycelial growth of the fungi was evaluated. Scanning Electron Microscopic (SEM) images were obtained to suggest possible modes of action of the compounds analyzed. The antimycotoxigenic activity was determined by HPLC. The fungus *A. carbonarius* was completely inhibited by cumin EO (500 µL L^{-1}), by laurel EO and by cuminaldehyde (5000 µL L^{-1}). The concentration of cumin EO applied (500 µL L^{-1}) completely inhibited the growth of *A. niger*, this being the best result observed for this fungus. All the samples inhibited the mycelial growth of *A. ochraceus,* especially cumin EO (250 µL L^{-1}) and cuminaldehyde (250 µL L^{-1}), for which complete inhibition of mycelial growth was observed, together with laurel EO (5000 µL L^{-1}) and 1,8-cineole (10000 µL L^{-1}). The mycelial growth of *A. westerdijkiae* was

completely inhibited by cumin EO (1000 µL L^{-1}), by cuminaldehyde (1000 µL L^{-1}), by laurel EO (10000 µL L^{-1}) and 1,8-cineole (10000 µL L^{-1}). A significant decrease in the production of ochratoxin A (OTA) was observed after the application of treatments, except in *A. ochraceus* where OTA biosynthesis was only inhibited by laurel EO. SEM images showed that there were deformations, morphological changes in fungal structures and spore inhibition after treatments. The results confirmed the antifungal and antimycotoxigenic effect of EO and their principal constituents on fungi of the genus *Aspergillus* and indicate that these substances can be promising components of antifungal agents based on natural products.
Keywords: essential oil, ochratoxin A, fungal control, natural products, cumin, bay leaf.

Introducion

Aspergillus fungi make up the most abundant genus of fungi in the world.These fungi have an effective dispersion of their spores in the air, and many species of fungi produce mycotoxins (Kriijgsheld *et al*., 2013). Among the species of this genus, *A. carbonarius*, *A. niger*, *A. ochraceus* and *A. westerdijkiae* stand out. They are the main mycotoxin-producing fungi. These fungi grow internally and externally on food and secrete toxins (Sartori *et al*., 2010).

Mycotoxins can be defined as secondary fungal metabolites that have the ability to contaminate food even when present in low concentrations. In addition to directly interfering with food quality, they can cause economic losses, batch rejection and serious damage to human and animal health. These mycotoxins can be mutagenic, teratogenic, carcinogenic and immunosuppressive and, therefore, are currently considered to be one of the greatest food safety problems (Puri, Shingh and Tiwari, 2019; Agriopoulou, Stamatelopoulou and Varzakas, 2020; Haque *et al*., 2020; Jin *et al*., 2021).

The most frequently found ochratoxin in food is type A, usually present in coffee beans, spices, grapes, grains in general, cereals, and oilseeds, among other foods. This toxin is also the most worrisome ochratoxin because of its toxicity and the fact that it does not decompose during most processes of washing, cooking or fermenting food (Sartori *et al*., 2010; Prado, 2017).

Ochratoxin A, the mycotoxin produced by fungi of the genus *Aspergillus*, is the microtoxin contaminant of greatest concern The nephrotoxic effects of this mycotoxin have already been proven, and the kidneys are considered to be its target organ (Sartori *et al*., 2010; Paterson, Lima and Taniwaki, 2014; Taniwaki *et al*., 2014). In addition, the International

Agency for Research on Cancer (IARC) has classified ochratoxin A as a possible carcinogenic molecule in humans, and other effects such as neurotoxicity, immunotoxicity, myelotoxicity and reproductive toxicity have already been associated with exposure to ochratoxin A (IARC, 1993; Ostry *et al*., 2017; Viegas *et al*., 2018). Brazilian legislation, IN No. 160 of July 1, 2022 of ANVISA, establishes that the maximum limits of ochratoxin A in foods are: 2 mcg Kg^{-1} for foods based on cereals for infant feeding, grape juice and pulp of grapes, wines and their derivatives; 5 mcg Kg^{-1} for cocoa and chocolate products; 10 mcg Kg^{-1} for cocoa beans, roasted or soluble coffee, cereals and cereal products, dried beans and other seeds, and dried and dried fruits; 20 mcg Kg^{-1} for cereals for further processing; 30 mcg Kg^{-1} for spices (Brasil, 2022)

The simplest solution to control ochratoxin is to prevent the proliferation of fungi. However, this prevention is achieved through the application of synthetic fungicides, which, in turn, cause pathogen resistance and their acceptance by consumers has decreased. Thus, alternatives for the control the proliferation of these ochratoxigenic fungi using antagonistic microorganisms and natural antimicrobial compounds have been investigated (Zhu *et al*., 2015; Schlosser and Prange, 2019; Souza *et al*., 2021).

Among the natural antimicrobial compounds, essential oils, which are complex mixtures of volatile organic compounds synthesized by plants, have stood out (Hashemi; Khaneghah; Sant'ana, 2018). Because of their biological properties and the fact that they are biodegradable and mostly classified as “generally recognized as safe” (GRAS) by the Food and Drug Administration (FDA), essential oils can be applied to several areas, including agriculture and the food industry (Rezende *et al*., 2021).

Several studies have already reported the antifungal activity of essential oils. Brandão *et al*. (2022) evaluated the antifungal and anti-ocratoxigenic properties of essential oils extracted from *Alpina speciosa* and *Cymbopogon flexuosus* encapsulated in polymeric nanofibers against *Aspergillus ochraceus* and *Aspergillus westerdijkiae*. The authors obtained promising results for the application of the nanofibers that they produced, and they observed up to 100% inhibition of mycelial growth and ochratoxin A biosynthesis. Rezende *et al*. (2021) evaluated the antifungal and antimycotoxigenic effect of essential oils from *Satureja montana* L., *Myristica fragrans* H. and *Cymbopogon flexuosus* S. on *Aspergillus ochraceus* and observed up to 100% inhibition of mycelial growth and significant inhibition of ochratoxin A production.

Thus, the importance of research related to fungal control and, consequently, the control of ochratoxin biosynthesis is evident. The present study sought to evaluate the antifungal and antiocratoxigenic activity of the essential oils from *Cuminum cyminum* and

Laurus nobilis, and their respective principal components, cuminaldehyde and 1,8-cineole, against the fungi *Aspergillus carbonarius, Aspergillus niger, Aspergillus ochraceus* and *Aspergillus westerdijkiae.*

Material and methods

Obtaining plant materials and standard compounds

The plant material used for the extraction of essential oils was cumin (*Cuminum cyminum*), purchased at the market in the city of São Paulo, SP, Brazil, (23°32'51"S; 46°38'10"W) and bay leaf (*Laurus nobilis*), purchased at the market in the city of Lavras, MG, Brazil (21º14′43" S, 44º59′50" W). The standard compounds used were cuminaldehyde (98%, Sigma-Aldrich®, São Paulo, Brazil) and 1,8-cineole (Sigma-Aldrich®, São Paulo, Brazil).

Extraction of essential oils

The essential oils were obtained by hydrodistillation during a period of 2 hours using a modified Clevenger apparatus (Brasil, 2010). The hydrosol obtained was centrifuged (Fanem Baby I Model 260 BL, São Paulo, Brazil) at 965*g* for 15 minutes, and the essential oil was removed with the aid of a Pasteur micropipette and stored in an amber bottle under refrigeration.

Chemical composition of essential oils

The chemical characterization of the essential oils was performed at the Chemical Analysis and Prospection Center (CAPQ) of the Federal University of Lavras – UFLA. Chromatographic analysis was performed on a Shimadzu model QP2010 Plus gas chromatograph (Kyoto, Japan) equipped with a fused silica capillary column containing a DB5-bound phase (30 m x 0.25 mm internal diameter; thickness 0.2 μm) coupled to a mass detector under the conditions described by Brandão *et al*., 2022.

The data obtained were processed using the LabSolutions LC/GC Software from workstation 2.72. The retention indices of the compounds were determined using the Van den Dool and Kratz equation (Van Den Dool and Kratz, 1963) and the n-alkane homologous series (nC8-nC18) as standards. The retention indices were compared with those in the literature (Adams, 2017), and the mass spectra of essential oil constituents with greater than 95% similarity were compared with those from the FFNSC 1.2, NIST 107 and NIST 21 mass spectral libraries. Constituents were quantified using the area normalization (%) method based on automatically integrated peak areas of the GC-FID signal.

Preparation of spore solutions of microorganisms

The species of filamentous fungi studied were *Aspergillus ochraceus* (URMICRO 10490), *Aspergillus niger* (URMICRO10443), *Aspergillus carbonarius* (URMICRO 10447) and *Aspergillus westerdijkiae* (URMICRO 11469). All the species used were acquired from the Microorganism Culture Collection of the Department of Food Science, UFLA. The preparation of spore solutions was performed as described by Rezende *et al*. (2021).

The fungi were incubated in plates containing the culture medium: Sucrose Yeast Extract Agar (YES) for *A. ochraceus* and Czapek Yeast Extract Agar (CYA,) for the other fungi. The plates were incubated in a BOD incubator for 7 days at 25 °C. After 7 days, a spore suspension was prepared using a solution containing 1% Tween 80 (Synth, Diadema, SP, Brazil) in distilled water. A Neubauer chamber was used to determine the spore concentration (10^6 spores mL^{-1}).

Effect of essential oils and standard compounds on mycelial growth

Mycelial growth of fungi was evaluated according to the method described by Rezende *et al*. (2021), with modifications. Plating was performed with 10 μL of the spore suspension (10^6 spores mL^{-1}) in the center of the plate containing 20 mL of Yeast Extract Sucrose Agar (YES) medium for *A. ochraceus* or Czapek Yeast Extract Agar (CYA) for the other fungi. Essential oils or standard compounds were diluted and mixed with culture medium containing 1% Tween 80. The concentrations tested were 31, 62.5, 125, 250, 500, 1000, 5000 and 10000 μL L^{-1}. The fungal control was prepared with 10 μL of the spore suspension added to a plate containing only the culture medium for comparison purposes. All the plates were incubated in a BOD oven at 25 °C for 10 days, after which measurements of mycelial growth diameters were performed. The experiments were performed in triplicate, and the percentage of mycelial growth inhibition was calculated on the basis of the growth of the fungal control. The minimum fungicidal concentration (MFC) was considered to be the lowest concentration of essential oil or standard compound that completely inhibited fungal growth.

Effect of essential oils or standard compounds on the biosynthesis of ochratoxin A

The evaluation of the antimycotoxigenic potential was performed considering the mycelial growth assay. The inhibition of OTA by the essential oils from *C. cyminum* and *L.*

nobilis and by the standard compounds cuminaldehyde and 1,8-cineole was tested at a concentration below the MFC of each fungus.

OTA extraction was performed on the tenth day of incubation as per Passamani *et al*. (2014). Three plugs from the center, middle and edge of each colony were used. The extracts were filtered using polytetrafluoroethylene (PTFE) filter units (0.22 µm; Millipore). Twenty µL of filtered extract were injected directly into a high-performance liquid chromatograph (HPLC, Shimadzu, model SPD-M20A, Kyoto, Japan) equipped with two high-pressure pumps, a degasser (DGU 20A3), an interface (CBM-20A), an autosampler (SIL-10AF) and a fluorescence detector (RF-10 AXL). OTA was separated using an Agilent-Zorbax Eclipse XDB-C18 column (4.6 x 250 mm, 5 µm) connected to an Agilent-Zorbax Eclipse XDB-C18 4-Pack precolumn (4.6 x 12.5 mm, 5 µm). Elution was achieved in an isocratic system of 35:35:29:1 methanol:acetonitrile:water:acetic acid. The excitation wavelength used was 332 nm and the emission wavelength was 476 nm. The flow rate was 0.8 mL min^{-1}. The mean OTA retention time was 11 ± 0.1 minutes. The OTA in the samples was quantified by external standardization, with the construction of an analytical curve obtained by linear regression correlating the peak area and the concentration of the standard. The coefficient of determination (R^2) was 0.9999, and the limits of detection (LOD) and quantification (LOQ) were 0.0004 and 0.0016 µg g^{-1}, respectively. All the samples and standard OTA solutions were analyzed in triplicate. To calculate the inhibition (%) of OTA production by essential oils ou standard compounds, the equation I = [(Oc-Ot)/Oc] x 100 was used, where Oc is the ochratoxin produced by the control and Ot is the ochratoxin produced after the treatment with essential oil or with standard compounds.

Scanning electron microscopy (SEM)

SEM analysis was performed with samples obtained from mycelial growth after 10 days of incubation. Plugs (5 mm in diameter) with a concentration below the MFC were taken. These plugs were added to Karnovsky's fixative solution (2.5% glutaraldehyde, 2% formaldehyde, 0.05 M cacodylate buffer, pH 7.2 and 0.001 M CaCl2 buffer; Sigma-Aldrich®, São Paulo, Brazil) where they were left until the time of analysis. Sample preparation was performed by washing with cacodylate buffer solution for 10 minutes. This procedure was performed twice. After washing the samples, they were dehydrated with an acetone gradient series (25, 50, 75 and 100%), dried in a critical point dryer (Bal-Tec CPD 030), mounted on stubs with double-sided tape and coated with gold (Bal-Tec CPD 050), as described by Rezende *et al*., (2021).

The micrographs of the prepared samples were obtained on a scanning electronic microscope (Leo Evo 040).

Statistical analysis

Statistical analyzes were performed using a Completely Randomized Design (CID). The evaluation of the effects of essential oils from *C. cyminum* and *L. nobilis*, as well as their respective major compounds (Cuminalaldehyde and 1,8-cineol standards) on the percentage of inhibition of mycelial growth and OTA biosynthesis were analyzed using the Analysis of Variance (ANOVA) followed by ScottKnott test of means at 5% and Tukey at 5% significance, respectively. The statistical software used was Rstudio® version 4.2.1. (R Core Team, 2016).

Results and Discussion

Chemical composition of the essential oils

The chemical compositions of the essential oils (EO) from cumin and laurel are presented in Table 1. Seven constituents were identified in the EO of cumin, the principal constituent found being cuminaldehyde, representing 60.79%, followed by α-terpinen-7-ol (12.29%), γ-Terpinene (7.51%), myrtenol (7.13%) and other compounds in smaller proportions. In the EO of laurel, 13 constituents were identified; 1,8-cineole was the principal constituent, representing 60.15% of the composition of this EO, followed by terpenyl acetate (16.19%), sabinene (4.30%) and other minor compounds. These results corroborate those obtained by other authors who found the same major compounds, cuminaldehyde and 1,8-cineole, respectively, in cumin and laurel essential oils (Fidan *et al*., 2019; Riabov *et al*. al., 2020; Xu *et al*., 2021; Osanloo *et al*., 2023).

According to Riabov *et al*. (2020), the chemical profile of essential oils can vary because of several factors. These authors studied essential oils extracted from the same species of plant obtained in different geographic regions, and they demonstrated the presence of small differences in the composition of oils from the same plant. The authors also pointed out the influence of climate, plant development stages, nutrient content in the soil, harvest time, drying method and essential oil extraction.

Antifungal activity of essential oils and the standard compounds

The results obtained for the antifungal activity of cumin and laurel essential oils and the standard compounds (SC) cuminaldehyde and 1,8-cineole are shown in Figure 1. A dose-dependent effect on the evaluated fungi was observed for all the samples studied.

The mycelial growth of *A. carbonarius* was 100% inhibited by cumin EO. The minimum fungicidal concentration (MFC) was 500 µL L^{-1}. The MFC observed for laurel EO and standard cuminaldehyde was 5000 µL L^{-1}. The fungus *A. niger* was the least sensitive to the applied treatments with respect to the inhibition of mycelial growth. There was no effect of laurel EO, at concentrations from 31 to 1000 µL L^{-1}, on the growth of *A. niger*, nor was there any effect of the standard 1,8-cineole on the growth of *A. niger* at any of the concentrations evaluated. The best results were obtained for cumin EO, for which the mycelial growth was inhibited at all the concentrations evaluated, and it was able to completely inhibit the growth when concentrations from 500 to 10.000 µL L^{-1} were applied. Unlike *A. niger*, *A. ochraceus* was the fungus with the highest sensitivity to all the treatments with EO and the standard compounds. Some inhibition eof the mycelial growth of *A. ochraceus* was observed for all the EO and SC samples studied, with emphasis on cumin EO and cuminaldehyde, for which complete inhibition of mycelial growth was observed, with an MFC of 250 µL L^{-1}. The mycelial growth of *A. westerdijkiae* was completely inhibited by EO from cumin and cuminaldehyde, with an MFC of 1000µL L^{-1} and by the EO from lourel and 1,8-cineole, with an MFC of 10000µL L^{-1}.

The antifungal activity of the EOs and SC on the four fungi evaluated can probably be attributed to the ability of the EOs to damage the cell wall and cytoplasmic membranes of the fungi. In addition, the lipophilic constituents and the complex constitution are factors that might have facilitated the action in different specific cellular sites (Nazzaro *et al*., 2013; Dammak *et al*., 2019).

Better results with respect to the inhibition of mycelial growth were obtained for the EOs from laurel and cumin than for their principal compounds 1,8-cineole and cuminaldehyde. The correlation of biological activities with a single constituent or a class of these compounds is difficult to affirm because, even in small proportions, some constituents can have considerable effects because of synergism. However, some authors believe that these results can be attributed to the synergistic effect caused by specific constituents (Djenane *et al*., 2018; Belasli *et al*., 2020). For cumin EO, some authors attribute the antifungal activity to the synergism between cuminaldehyde and α-terpinen-7-al and also to compounds present in smaller proportions such as γ-terpinene and β-pinene (Pajohi *et al*., 2011; Zheljazkov *et al*., 2015; Miri and Djenane, 2018; Petretto *et al*., 2018; Tanapichatsakul, Hkruengsai and

Pripdeevech, 2020). For laurel EO, some authors believe that monoterpene alcohols, such as linalool and terpineol, might be responsible for the increase in the permeability of the plasmatic membrane and for inhibiting the respiration of the mitochondrial membrane (Deba *et al*., 2008; Imelouane *et al*., 2009; Belasli *et al*., 2020).

Antimycotoxigenic activity

The biosynthesis of ochratoxin A (OTA) by fungi of the genus Aspergillus was affected by most of the treatments performed, as is shown in Table 2. The fungus *A. ochraceus*, despite having its mycelial growth more easily inhibited by the compounds tested, was the fungus in which ochratoxin biosynthesis was most resistant to the treatments (Table 2). Among the four compounds tested, OTA synthesis by this fungus was only inhibited by laurel EO (1000 µL L^{-1}). The OTA biosynthesis by *A. niger*, which exhibited greater resistance to treatments in relation to mycelial growth, was inhibited in 97.7% by cumin EO (250 µL L^{-1}), 96.8% by 1, 8-cineole (10000 µL L^{-1}), 84% by laurel EO (1000 µL L^{-1}) and 78.6% by cuminaldehyde (1000 µL L^{-1}), the first two being the more efficient treatments with respect to OTA biosynthesis.

Greater than 70% inhibition of the production of OTA by the fungus *A. carbonarius* was observed for all the treatments. In addition, 99.8% and 92.7% inhibitions were obtained for the EO from laurel (1000 µL L^{-1}) and 1,8-cineole (10000 µL L^{-1}), respectively. No inhibition was observed when *A. westerdijkiae* was treated with laurel EO, and the best results observed were for treatments with cumin EO (500 µL L^{-1}) and cuminaldehyde (500 µL L^{-1}), for which 62.3% and 68.4% inhibition were observed, respectively.

According to Dammak *et al*. (2019), the inhibition of mycotoxin synthesis is not necessarily associated with the inhibition of mycelial growth; that is, an essential oil can have different mechanisms of action regarding antifungal activity and OTA inhibition.

According to the results shown in Table 2, the greatest inhibition of OTA production for each fungus, at the evaluated concentrations, was obtained with laurel EO for *A. ochraceus*, cumin EO for *A. niger*, laurel EO for *A. carbonarius* and cumin EO and cuminaldehyde for *A. westerdijkiae*. However, it should be considered that the evaluation of the inhibition of OTA biosynthesis could be determined only in the samples where the fungi grew. That is, the OTA production was not evaluated for the treatments in which the mycelial growth was totally inhibited, thus inferring that it was null. Considering this factor, cumin EO was the most efficient treatment for all the fungi evaluated, followed by cuminaldehyde, when applied to *A. ochraceus* and *A. westerdijkiae*.

Scanning electron microscopy (SEM)

The SEM images of the morphological structures of the fungi *A. carbonarius, A.niger, A. ochraceus* and *A. westerdijkiae* and the possible damage caused by treatments with essential oils and the standard compounds can be seen in Figure 2.

The fungi corresponding to the control treatments exhibited a regular morphology and uniform development of structures, conidia, conidiophores and hyphae [Figure 2 (A, B, C and D)]. However, when comparing the control treatments with the fungi that received the other treatments, structural damage to the fungi that received the experimental treatments can be seen.

All the treatments applied to *A. carbonarius* resulted in inhibition of the formation of spores and the formation of uneven and irregular hyphae [Figure 2 (A1, A2, A3 and A4)]. In *A. niger* treated with cumin EO [Figure 2 (B1)], disorganization of conidiaand irregular hyphae were observed. When treated with cuminaldehyde [Figure 2 (B2)], small changes in the morphology of the conidia were observed, whereas changes were observed in the formation of conidiophores and conidia in treatments with EO laurel and CP 1,8-cineole [Figure 2 (B3) and (B4)]. All the treatments applied to *A. niger* aggressively altered its morphology.

The cumin EO may have acted by breaking the conidiophores of *A. ochraceus* [Figure 2 (C1)]. Cuminaldehyde prevented the formation of conidia [Figure 2 (C2)]. In Figure 2 (C3) and (C4) it can be seen that the EO from laurel and 1,8-cineole, respectively, prevented the formation of conidia. The treatment interfered abruptly in the development of *A. ochraceus*, preventing this fungus from forming vesicles, phialides and conidia [Figure 2 (C3)]. This change in morphology can explain the inhibition of OTA biosynthesis that occurred when treatment with laurel EO was applied (Table 2).

In *A. westerdijkiae*, cumin EO prevented the formation of conidia and affected the development of conidiophores and hyphae [Figure 2 (D1)], Cuminaldehyde, the EO from laurel and 1,8-cineole acted by modifying the conformation of phialides and conidia [Figure 2 (D2), (D3) and (D4), respectively].

As noted, many morphological changes were noted in fungi of the genus *Aspergillus*. Morphological changes in hyphae, deterioration of cellular tissue and other structures can cause membrane damage and cell leakage (Belasli *et al*., 2020; Tanapichatsakul, Khruengsai and Pripdeevech, 2020).

Conclusion

The antifungal and antimycotoxigenic effects of cumin and laurel EO, as well as the respective principal compounds, cuminaldehyde (60.79%) and 1,8-cineole (60.15%), on the *Aspergillus* fungi studied were demonstrated. The treatments performed affected the morphological structures of the fungi, altering their structures and inhibiting the normal development. Among the treatments performed, the cumin EO stood out with the lowest MFC for all the analyzed fungi, in addition to significant OTA inhibition. Despite the prominence of the cumin EO, the results obtained indicate that all the samples used in this study are promising for the constitution of antifungals based on natural products.

Funding

This work was supported by the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG – 02390/18); the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – 311183/2122-0); and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance code 001.

Conflict of interest

The authors declare that no conflict of interests exists.

Acknowledgments

The authors thank the funding agencies for the scholarships and financial support and the Central of Analysis and Chemical Prospecting of the Federal University of Lavras for supplying the equipment for chromatographic analyses.

References

Adams RP. Identification of essential oils componentes by gas chromatography/ mass spectroscopy. 4 ed. Carol Stream: Allured, 2017.

Agriopoulou S, Stamatelopoulou E, Varzakas T. Advances in analysis and detection of major mycotoxins in foods. *Foods*. 2020, DOI: 10.3390/foods9040518

Belasli A, Miri YB, Aboudaou M *et al*. Antifungal, antitoxigenic, and antioxidant activities of the essential oil from laurel (*Laurus nobilis* L.): Potential use as wheat preservative. *Food Sci. Nutr*. 2020, DOI: 10.1002/fsn3.1650

Brandão RM, Cardoso MG, Oliveira JE *et al*. Antifungal and antiocratoxigenic potential of *Alpinia speciosa* and *Cymbopogon flexuosus* essential oils encapsulated in poly (lactic acid) nanofibres against *Aspergillus* fungi. *Lett. Appl. Microbiol*. 2022, DOI: 10.1111/lam.13704

Brasil. Agência Nacional de Vigilância Sanitária (Anvisa). In: Farmacopeia Brasileira, Brasília, 5th ed.; 2010; **1:**198-199.

Brasil. Agência Nacional de Vigilância Sanitária (Anvisa). Instrução Normativa IN Nº60 de 1º de Julho de 2022, Estabelece os Limites máximos tolerados de contaminantes em alimentos. 2022.

Dammak I, Hamdi Z, Euch SKE *et al*. Evaluation of antifungal and anti-ochratoxigenic activities of *Salvia officinalis*, Lavandula dentata and *Laurus nobilis* essential oils and a major monoterpene constituent 1,8-cineole against *Aspergillus carbonarius*. *Ind. Crops Prod*. 2019, DOI: 10.1016/j.indcrop.2018.11.006

Deba F, Xuan TD, Yasuda M *et al*. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. Radiata. *Food control*. 2008, DOI: 10.1016/j.foodcont.2007.04.011

Djenane D, Gómez D, Yanguela J *et al*. Olive leaves extract from algerian oleaster (*Olea europaea* var. Sylvestris) on microbiological safety and shelf-life stability of raw halal minced beef during display. *Foods*. 2018, DOI: 10.3390/foods8010010

Fidan H, Stefanova G, Kostova I *et al*. Chemical composition and antimicrobial activity of *Laurus nobilis* L. essential oils from Bulgaria. *Mol*. 2019, DOI: 10.3390/molecules24040804

Haque MA, Wang Y, Shen Z *et al*. Mycotoxin contamination and control strategy in human, domestic animal and poultry: A review. *Microb. pathog*. 2020, DOI: 10.1016/j.micpath.2020.104095

Hashemi SMB, Khaneghah AM, Sant'ana AS. Essential Oils in Food Processing: chemistry, safety and applications. Hoboken, NJ:John Wiley e Sons, 2018.

Imelouane B, Amhamdi H, Wathelet JP *et al*. Chemical composition and antimicrobial activity of essential oil of thyme (*Thymus vulgaris*) from Eastern Morocco. *Int. J. Agric. Biol*. 2009; **11**: 205-208.

International Agency For Research On Cancer (IARC). Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monographs on the evalution carcinogenic risks to humans. 1993;**56**: 489-421.

Jin J, Beekmann K, Ringo E *et al*. Interaction between food-borne mycotoxins and gut microbiota: A review. *Food Control*. 2021, DOI: 10.1016/j.foodcont.2021.107998

Krijgsheld P, Bleichrodt RV, Van Veluw GJ *et al*. Development in *Aspergillus*. Stud. Mycol. 2013, DOI: 10.3114/sim0006

Miri YD, Djenane D. Evaluation of Protective Impact of Algerian *Cuminum cyminum* L. and *Coriandrum sativum* L. Essential Oils on *Aspergillus flavus* Growth and Aflatoxin B 1 Production. *PJBS*. 2018, DOI: 10.3923/pjbs.2018.67.77

Nazzaro F, Fratianni F, Martino L *et al*. Effect of essential oils on pathogenic bacteria. *Pharm*. 2013, DOI: 10.3390/ph6121451

Osanloo M, Eskandari Z, Zarenezhad E *et al*. Studying the microbial, chemical, and sensory characteristics of shrimp coated with alginate sodium nanoparticles containing *Zataria multiflora* and *Cuminum cyminum* essential oils. *Food Sci. Nutr*. 2023, DOI: 10.1002/fsn3.3261

Ostry V, Malir F, Toman J *et al*. Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Res*. 2017, DOI: 10.1007/s12550-016-0265-7

Pajohi MR, Tajik H, Farshid AA *et al*. Synergistic antibacterial activity of the essential oil of *Cuminum cyminum* L. seed and nisin in a food model. *J. appl. microbiol*. 2011. DOI: 10.1111/j.1365-2672.2011.04946.x

Passamani FR, Hernandes T, Lopes NA *et al*. Effect of temperature, water activity, and pH on growth and production of ochratoxin A by *Aspergillus niger* and *Aspergillus carbonarius* from Brazilian grapes. *J Food Prot.* 2014. DOI: 10.4315/0362- 028X.JFP-13-495.

Paterson RRM, Lima N, Taniwaki MH. Coffee, mycotoxins and climate change. *Food Res. Int*. 2014, DOI: 10.1016/j.foodres.2014.03.037

Petretto GL, Fancello F, Bakhy K *et al*. Chemical composition and antimicrobial activity of essential oils from *Cuminum cyminum* L. collected in different areas of Morocco. *Food biosci*. 2018, DOI: 10.1016/j.fbio.2018.01.004

Prado G. Contaminação de alimentos por micotoxinas no Brasil e no mundo. Gerais: Revista de Saúde Pública do SUS/MG, 2017; **2**:13-26.

Puri S, Shingh S, Tiwari P. Mycotoxins: A Threat to Food Security and Health. *IJASBT*. 2019. DOI: 10.3126/ijasbt.v7i3.24651

R CORE TEAM (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Disponível em: <https://www.R-project.org>.

Rezende DACS, Cardoso MG, Alves E *et al*. Effect of the essential oils of *Satureja montana* L., *Myristica fragrans* H. and *Cymbopogon flexuosus* S. on mycotoxin-producing *Aspergillus flavus* and *Aspergillus ochraceus* antifungal properties of essential oils. *FEMS Microbiol. Lett*. 2021, DOI: 10.1093/femsle/fnab137

Riabov PA, Micic D, Bozovic RB *et al*. The chemical, biological and thermal characteristics and gastronomical perspectives of *Laurus nobilis* essential oil from different geographical origin. *Ind. Crops prod.* 2020, DOI: 10.1016/j.indcrop.2020.112498

Sartori D, Taniwaki MH, Iamanaka B *et al*. Molecular diagnosis of ochratoxigenic fungi. *Molec. Ident. fungi*. 2010, DOI: 10.1007/978-3-642-05042-8_10

Schlösser I, Prange A. Effects of selected natural preservatives on the mycelial growth and ochratoxin A production of the food-related moulds Aspergillus westerdijkiae and Penicillium verrucosum. Food Addit. Contam.: Part A. 2019. DOI: 10.1080/19440049.2019.1640397

Souza ML, Ribeiro LS, Miguel MGCP *et al*. Yeasts prevent ochratoxin A contamination in coffee by displacing *Aspergillus carbonarius*. *Biol. Control*. 2021, DOI: 10.1016/j.biocontrol.2020.104512

Tanapichatsakul C, Khruengsai S, Pripdeevech P. In vitro and in vivo antifungal activity of *Cuminum cyminum* essential oil against *Aspergillus aculeatus* causing bunch rot of postharvest grapes. *PLoS One*. 2020. DOI: 10.1371/journal.pone.0242862

Taniwaki MH, Teixeira AA, Teixeira ARR *et al*. Ochratoxigenic fungi and ochratoxin A in defective coffee beans. *Food Res, Int.* 2014, DOI: 10.1016/j.foodres.2013.12.032

Viegas S, Osteresch B, Almeida A *et al*. Enniatin B and ochratoxin A in the blood serum of workers from the waste management setting. *Mycotoxin Res*. 2018, DOI: 10.1007/s12550-017-0302-1

Van Den Dool, H. and Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. **J Chromatogr A**, v. 11, p. 463–471, 1963.

Xu D, Wei M, Peng S *et al*. Cuminaldehyde in cumin essential oils prevents the growth and aflatoxin B1 biosynthesis of *Aspergillus flavus* in peanuts. *Food Control*. 2021, DOI: 10.1016/j.foodcont.2021.107985

Zheljazkov VD, Gawde A, Cantrell CL *et al*. Distillation time as tool for improved antimalarial activity and differential oil composition of cumin seed oil. *PLoS One*. 2015, DOI: 10.1371/journal.pone.0144120

Zhu C, Shi J, Jiang C *et al*. Inhibition of the growth and ochratoxin A production by *Aspergillus carbonarius* and *Aspergillus ochraceus* in vitro and in vivo through antagonistic yeasts. *Food Control*, 2015. DOI:10.1016/j.foodcont.2014.08.042

Tables

Table 1 - Chemical composition of cumin and laurel essential oils.

Compound	RT (min)	RI_{ref}	RI_{cal}	Área (%)	
				C.cyminum	*L.nobilis*
α-Thujene	6.208	924	925	-	0.32
α-Pinene	6.450	932	933	-	3.78
Sabinene	7.592	969	972	-	4.30
β-Pinene	7.810	974	980	4.48	3.16
p-Cimene	9.352	1020	1025	5.97	1.31
Limonene	9.517	1024	1028	-	1.55
1,8-Cineole	**9.650**	**1026**	**1032**	-	**60.15**
α-Phelandrene	10.592	1002	1057	-	0.43
γ-Terpinene	10.620	1054	1058	7.51	-
Linalool	12.200	1101	1099	-	1.02
Terpinen-4-ol	15.683	1174	1180	-	3.47
α-Terpineol	16.308	1186	1195	-	2.71
Pulegone	16.352	1233	1196	1.83	-
Cuminaldeído	**18.446**	**1238**	**1244**	**60.79**	-
Isobornyl acetate	20.175	1283	1283	-	0.29
Mirtenol	20.339	1195	1195	7.13	-
α-Terpinen-7-al	20.531	1290	1292	12.29	-
α-Terpenyl acetate	22.858	1349	1345	-	16.19
Total identified				100.00	98.95

RI_{cal} is the experimental retention index and RI_{ref} is the reference index tabulated in the literature. The principal constituent is highlighted in bold.

Table 2 - Effect of essential oils and standard compounds on the production of ochratoxin A by *A. ochraceus, A. niger, A. carbonarius* and *A. westerdijkiae*.

Treatment	**Ochratoxin A biosynthesis (mg g^{-1}) and**			
µL L^{-1}	*A. ochraceus*	*A. niger*	*A. carbonarius*	*A. westerdijkiae*
Cumin EO (125)	2644.1±0.02[a]	-	390.021±0.00[b]	-
Cumin EO (250)	-	1.095±0.00[d]	-	-
Cumin EO (500)	-	-	-	2.016±0.00[b]
Cuminaldehyde (125)	56.238±0.00[b]	-	-	-
Cuminaldehyde (500)	-	-	252.651±0.01[c]	1.688±0.00[b]
Cuminaldehyde (1000)	-	10.227±0.00[b]	-	-
Laurel EO (1000)	4.821±0.00[bc]	7.647±0.00[c]	1.909±0.00[e]	-
Laurel EO (5000)	-	-	-	15.277±0.00[a]
1,8-Cineole (5000)	-	-	-	2.439±0.00[b]
1.8-Cineole (10000)	21.132±0.00[c]	1.532±0.00[d]	94.978±0.00[d]	-
Control	9.61±1.0[c]	47.95±2.0[a]	1318.12±0.8[a]	5.34±0.6[b]

The means followed by the same lowercase letter in the columns do not differ from each other by Tukey's test at the 5% probability level.

Figure legends

Figure 1 - The effect of essential oils and standard compounds on the inhibition of mycelial growth of fungi of the genus *Aspergillus*.

Figure 2 - Scanning electron microscopy of *A. carbonarius, A. niger, A. ochraceus* and *A. westerdijkiae* treated with essential oils and standard compounds. (A, A1, A2, A3 and A4) Fungal control and *A. carbonarius* treated with cumin EO, cuminaldehyde, laurel EO and 1,8-cineole, respectively. (B, B1, B2, B3 and B4) Fungal control and *A. niger* treated with cumin EO, cuminaldehyde, laurel EO and 1,8-cineole, respectively. (C, C1, C2, C3 and C4) Fungal control and *A. ochraceus* treated with cumin EO, cuminaldehyde, laurel EO and 1,8-cineole, respectively. (D, D1, D2, D3 and D4) Fungal control and *A. westerdijkiae* treated with cumin EO, cuminaldehyde, laurel EO and 1,8-cineole, respectively.

Fig. 1

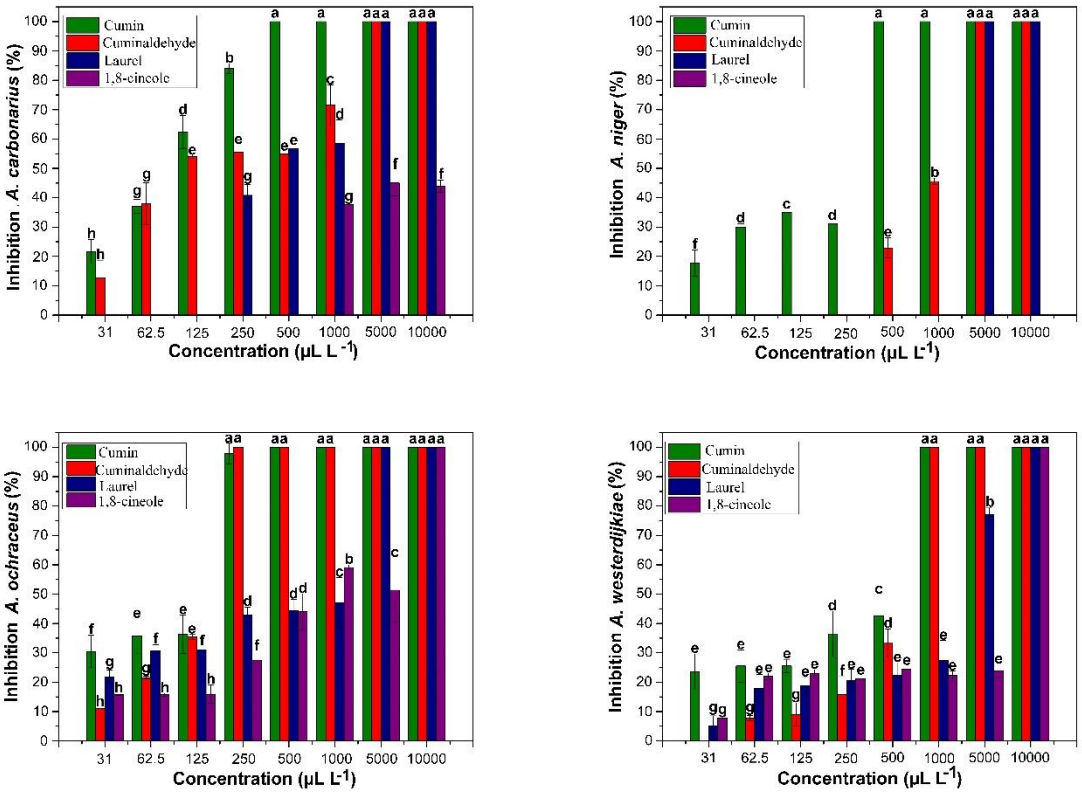
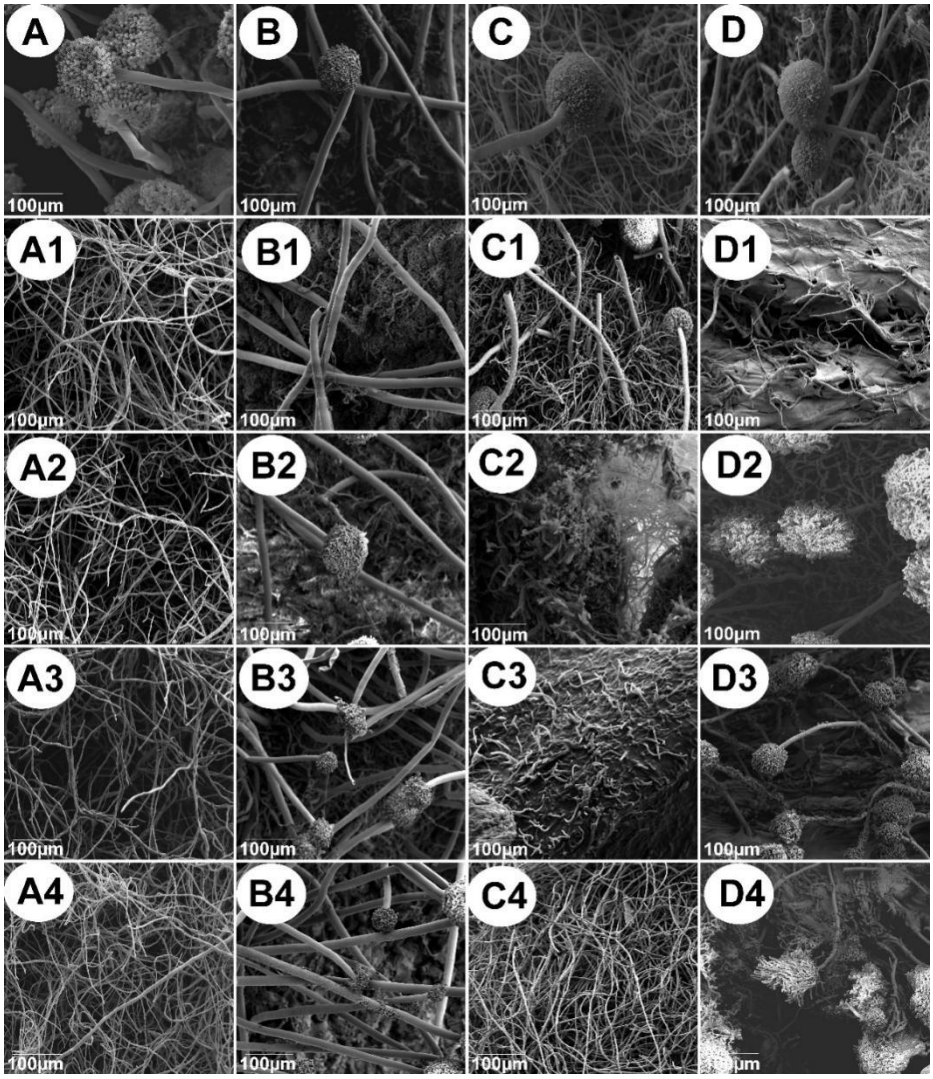

Cumin
Cuminaldehyde
Laurel
1,8-cineole
Inhibition A. carbonarius (%)
Inhibition A. niger (%)
Inhibition A. ochraceus (%)
Inhibition A. westerdijkiae (%)
Concentration (µL L-1)
31
62.5
125
250
500
1000
5000
10000

Fig. 2



ARTIGO 2 – In vitro and in vivo efficacy of edible coatings incorporated with the essential oil from *Cuminum cyminum* and cuminaldehyde against *Drosophila suzukii* in strawberries

In vitro and in vivo efficacy of edible coatings incorporated with the essential oil from *Cuminum cyminum* and cuminaldehyde against *Drosophila suzukii* in strawberries

Gabriela Aguiar Campolina[1], Maria das Graças Cardoso[2], Alex Rodrigues Silva Caetano[2], Luciano de Souza[2], David Lee Nelson[3], Khalid Haddi[4]

[1]Food Sciences Department, Federal University of Lavras (UFLA), Lavras, MG, Brazil
[2]Chemistry Department, Federal University of Lavras (UFLA), Lavras, MG, Brazil
[3]Postgraduate Program in Biofuels, Federal University of The Jequitinhonha and Mucuri Valleys, Diamantina, MG, Brazil
[4]Entomology Department, Federal University of Lavras, Lavras, Minas Gerais, Brazil.

ABSTRACT

Drosophila suzukii is a fly known for its ability to infest crops and hinder the production of fruits, especially red fruits. Synthetic insecticides are currently used in an attempt to control the damage caused by these flies, but the search for natural alternatives is necessary. In this study, the efficiency of the application of edible coatings containing cumin essential oil (CEO) or standard cuminaldehyde (SC) was evaluated on *Drosophila suzukii* in strawberries. SC was purchased commercially, CEO was extracted by hydrodistillation and characterized by gas chromatography coupled to a mass detector. The acetylcholinesterase inhibition test was performed with the CEO, with the SC and the toxicity of both samples was studied in vivo against *D. suzukii*, evaluating the mortality of this insect. Edible coatings were prepared containing different concentrations of CEO or SC (0%, 0.25%, 0.5% and 1%) and a control treatment without addition of coating was also performed. The coatings were characterized by FTIR, and the five treatments were tested for their ability to prevent or reduce fly births. The results obtained for the chemical characterization of the CEO showed that most of the oil was composed of cuminaldehyde (60.79%), and the extraction yield was 1.02%. The CEO and its principal component, cuminaldehyde, were to be present in the coatings by FTIR analysis. Inhibition of acetylcholinesterase was greater for the CEO sample (30.35%) than for the SC sample (21.57%) at the highest concentration tested. High toxicities were observed for both of

the samples evaluated, especially the CEO, with an LC_{50} of 2.93±0.6 µL mL^{-1}, where as the LC_{50} of the the SC was 4.3±0.7 µL mL^{-1}. Despite the significant difference between the toxicity of the two samples, similar efficiencies were observed when applied to coatings. The coatings did not prevent oviposition in any of the treatments evaluated, but the natality of *D. suzukii* flies decreased with the coatings containing CEO or SC. It is inferred that edible coatings incorporated with CEO or SC can be used for the management of *D. suzukii* in strawberries.

Keywords: Insects. Natural products. Cumin. Drosophilidae.

1 INTRODUCTION

One of the biggest hurdles faced by producers and traders is the limited shelf life of food. The insect pest *Drosophila suzukii* (Diptera: Drosophilidae), known as the spotted wing fly, is of Asian origin, and it is an invasive pest capable of attacking red fruits, threatening the viability of production, and causing great economic losses (CAETANO *et al*., 2022; ENRIQUEZ *et al*., 2020; SWOBODA-BHATTARAI and BURRACK, 2014). The flies of this species can develop in healthy fruits such as strawberries, blueberries, raspberries and blackberries, and they can also affect fruits such as nectarines, grapes and others (NAVA *et al*., 2015; ZIVKOVIC *et al*., 2019).

Contamination of red fruits by *D. suzukii* occurs through oviposition followed by consumption of the fruits by larvae. Thus, they cause rotting of the fruit, which prevents its consumption and commercialization, in addition to the damage caused by the larvae. The oviposition and growth facilitate the proliferation of filamentous fungi, yeasts and bacteria (ENRIQUEZ *et al*., 2020; IBOUH *et al*., 2019; SCHLESNER *et al*., 2015).

Farmers, in general, use synthetic insecticides to minimize the damage caused by *D. suzukii* and other insects. However, the improper and excessive use of this type of insecticide has led to insect resistance; in addition to being detrimental to health, they generate environmental effects and increase the operating costs. According to Gonzáles *et al*. (2017), the elaboration of new products for insect control has been a challenge because of the effects caused by synthetic insecticides.

Secondary metabolites produced by plants, such as essential oils, can interfere with the development, reproduction and mortality of insects by acting on their nervous systems (RODRIGUES *et al*., 2017). Essential oils are complex mixtures of volatile organic compounds

synthesized by plants (HASHEMI; KHANEGHAH; SANT'ANA, 2018). These compounds have several mechanisms of action on insects, including the inhibition of the enzyme acetylcholinesterase, which is considered to be the main mechanism of action.

Acetylcholinesterase is an enzyme responsible for the cleavage of acetylcholine into acetic acid and choline. By inhibiting this enzyme, the accumulation of acetylcholine occurs, and, consequently, neural transmission is interrupted, which can lead to changes in the development and even death of the insect (CHAUBEY, 2012; MOSSA, 2016).

Because essential oils are mostly considered to be GRAS (Generally Recognized As Safe) by the FDA (Food and Drugs Administration). They represent an option to be incorporated into coatings in a manner that is safe for the consumer. Edible coatings make it possible to increase shelf life, add value and improve the quality of the food they coat. The incorporation of essential oils into edible coatings allows the incorporated essential oil to act in conservation and, consequently, extends the quality of the food through its biological properties, such as an insecticide. These benefits, in addition to the aforementioned benefits, are generated when using this type of coating.

The objectives of this work were to evaluate the toxicity of cumin essential oil and the standard compound cuminaldehyde on *D. suzukii* and to analyze their influence on the birth rate of flies, when cassava starch-based edible coatings were incorporated and applied to strawberries.

2 MATERIAL AND METHODS

2.1 Acquisition of plant material and standard majority compound

The plant material used for extracting the essential oil was cumin (*Cuminum cyminum*), purchased in the market in the city of São Paulo, SP, Brazil (23°32'51"S; 46°38'10"W). The standard used was cuminaldehyde (98%, Sigma-Aldrich®, São Paulo, Brazil).

2.2 Essential oil extraction

The essential oil was obtained by the hydrodistillation technique using a modified Clevenger apparatus for a period of 2 hours (BRASIL, 2010). The hydrolate obtained was centrifuged (Fanem Baby I Model 260 BL, São Paulo, Brazil) at 965.36*g* for 15 minutes, and

the essential oil was removed with a Pasteur micropipette and stored in an amber bottle under refrigeration.

2.3 Determination of the moisture content of plant material

Moisture content was determined according to the method described by Pimentel *et al*. (2008). Five g of plant material and 80 mL of cyclohexane were added to a 250 mL volumetric flask. The volumetric flask was coupled to a Dean Stark graduated volumetric collector and a condenser. The system was heated for 2 hours, and the volume of water present in the plant material was quantified.

2.4 Yield of essential oil

The yield of cumin essential oil extracted was calculated considering the dry weight of the plant calculated according to Equation 1, and the results were expressed in percentage of weight/weight on a Moisture Free Base (BLU).

$$\%R = \frac{100\ x\ Po}{Pat - \frac{Pat\ x\ Moisture}{5}} \quad (1)$$

Where: %R = Yield of essential oil in percentage;
Po = Weight of oil obtained in the extraction;
Pat = Total sample weight.

2.5 Chemical characterization of the essential oil

The chemical characterization of the essential oil was accomplished at the Chemical Analysis and Prospection Center (CAPQ) of the Federal University of Lavras – UFLA, Lavras, Minas Gerais, Brazil. Chromatographic analysis was performed on a Shimadzu model QP2010 Plus gas chromatograph (Kyoto, Japan) equipped with a fused silica capillary column with a DB5 bound phase (30m x 0.25 mm internal diameter; thickness 0.2 μm) coupled to a mass detector, under the conditions described by Brandão *et al*., 2022.

The data were processed using the LabSolutions LC/GC Software from Workstation 2.72. The retention indices of the compounds were determined using the Van den Dool and Kratz equation (Van Den Dool and Kratz, 1963) and the n-alkane homologous series (nC8-nC18) as standards. Retention indices were compared with those in the literature (Adams, 2017), and the mass spectra of essential oil constituents with greater than 95% similarity were compared with those from the FFNSC 1.2, NIST 107 and NIST 21 mass spectral libraries. Constituents were quantified using the area normalization (%) method based on automatically integrated peak areas of the GC-FID signal.

2.6 Evaluation of the acetylcholinesterase inhibitory activity of cumin essential oil and the standard compound cuminaldehyde

The acetylcholinesterase enzyme inhibition test was performed to determine a possible mechanism of action of the essential oil and cuminaldehyde. The method of Ellman *et al*. (1961), modified by Costa *et al*. (2013), was used.

The analysis was performed after the dilution of samples of essential oil or cuminaldehyde in ethanol at concentrations of 0.25, 0.50, 1.00, 5.00, 10.0, 50.0 and 100 µg mL^{-1}. To a test tube, 2970 µL of Tris-HCl buffer, pH 8 (50 mmol L^{-1}), containing NaCl (0.1 mol L^{-1}) and $MgCl_2.6H_2O$ (0.02 mol L^{-1}) was added, followed by 254 µL of 1000 U mL^{-1} acetylcholinesterase solution. After incubating the mixture at 37 °C for 5 minutes, 25 µL of diluted samples, 100 µL of Ellman's reagent solution (10 mmol L^{-1}) and 80 µL of substrate solution were added, and the mixture was again incubated at 37 °C for 15 minutes. The absorbance was measured in a spectrophotometer at a wavelength of 412 nm. For the blank, 3.2 ml Tris-HCl buffer was used. Spontaneous hydrolysis of acetylthiocholine was measured by performing non-enzymatic controls for each oil concentration, replacing the enzyme with Tris-HCl buffer. The negative control contained all the reagents except the essential oil, which was replaced by ethanol. The tests were performed in five repetitions, and the results were expressed in percentage of inhibition (Equation 2).

$$I(\%) = 100 - \left(\frac{A_T - A_C}{A_0}\right) . 100 \qquad (2)$$

Where: I (%) = percentage of inhibition;

A_T = treatment absorbance;

A_C = Non-enzymatic control absorbance;

A_0 = Absorbance of negative control.

2.7 Evaluation of the toxicity of cumin essential oil and the cuminaldehyde standard against *Drosophila suzukii*

2.7.1 Breeding and maintenance of *Drosophila suzukii*

The insects used were acquired from the Department of Entomology, UFLA. All the insects used were reared in plastic cages An artificial diet was also offered to the insects as a substrate for oviposition and food for the larvae. The artificial diet was prepared according to Andreazza *et al* (2016), with modifications. A mixture of 66.75 g of granulated sugar, 23.33 g of brewer's yeast, 41.66 g of fine corn flour and 12 g of bacteriological agar were dissolved in 300 mL of deionized water at room temperature. The solution was added to 933 mL of boiling deionized water, where it was kept under constant stirring for 15 minutes. The mixture was left under a gentle boil for an additional 15 minutes and cooled to approximately 60 °C. Then, 11 mL of 10% Nipagim alcoholic solution and 5.9 mL of propanoic acid were added, which acted as preservatives. The mixture was transferred to Petri dishes in layers with a thickness of approximately 1 cm. In each adult breeding cage, an artificial diet plate was added for oviposition for 48 hours. Subsequently, the oviposited diet was transferred to another plastic cage until the insect's development was complete.

2.7.2 Toxicity of the essential oil and cuminaldehyde against *Drosophila suzukii*

The mortality of *D. suzukii* was evaluated in quadruplicate according to the method described by IRAC (2020), with modifications described by Souza *et al*. (2022). In a glass flask (200 mL), a dental cotton (2 cm) was placed and treated with 2 mL of the solution to be tested, composed of DMSO, 20% aqueous sugar solution and the essential oil or cuminaldehyde in concentrations that varied from 0.75 to 10 µL mL^{-1}. Twenty-five sexless insects of the same age were introduced into the glass flasks, the flasks were closed with foam plugs and kept in a BOD at 23 ± 2 °C and 50% RH, with a photoperiod of 12 L:12 D, to be evaluated after 24 hours. In the evaluation, the flies were classified as (a) unaffected (those that, when gently stimulated by shaking the containers, responded normally with a coordinated movement); or (b) dead or affected, (those that, when stimulated, did not respond or presented abnormal

movement). The results were expressed as percentage of mortality and as LC_{50} and LC_{90}, where LC_{50} is the lethal concentration for 50% of the individuals and LC_{95} is the lethal concentration for 95% of the individuals.

2.8 Elaboration and application of edible coatings

The cassava starch-based edible coatings were prepared according to the method described by Ferreira, Molina and Pelissari (2020) and Tabassum and Khan (2020), with modifications.

Cassava starch suspensions (2%) were prepared at 80 °C, with stirring, until the complete gelatinization of the starch. The suspensions were cooled to room temperature for the addition of glycerol plasticizer (20%), Tween 80 surfactant (1%) and the essential oil or cuminaldehyde in different concentrations (0%, 0.25%, 0.5% and 1 %). The solutions were stirred until complete homogenization, and, after cooling, the coatings formed were applied to the strawberries.

The coatings were applied by immersing each fruit in solutions prepared with different concentrations of essential oil or cuminaldehyde (0; 0.25; 0.5 and 1%). Immersion was performed for 1 minute, and, after complete drying of the coatings at room temperature, the organic strawberries were deposited in cages with a diameter of 12 cm, lined with a layer of cotton, a layer of corrugated cardboard and a layer of filter paper.

2.9 Characterization of edible coatings

2.9.1 Infrared analysis

Mid-range infrared (IR) spectra of the edible coatings were obtained using a Fourier transform mid-infrared spectrometer (FT-IR, Vertex 70, Bruker, Germany). IR analyses were performed using the attenuated reflectance (ATR) technique. The spectra were recorded in a spectral range of 4000 to 400 cm^{-1}, performing 64 scans with a resolution of 4 cm^{-1} (RATHER *et al*., 2022).

2.10 Evaluation of the influence of edible coatings containing cumin essential oil or cuminaldehyde on the birth rate of *Drosophila suzukii* when applied to strawberries

The evaluation of the influence of the edible coatings on the birth rate of *D. suzikii* was performed in quintuplicate by inserting four strawberries in each 12-cm-diameter cage lined with a layer of corrugated cardboard and a layer of filter paper. In each of the cages containing the coated strawberries or the control treatments, 20 unsexed flies were inserted. The flies were left in the cages for 48 hours at 23 ± 2 °C, 50% RH and a 12L:12D photoperiod.

The flies were removed and sacrificed, when alive. The natality of the flies was evaluated daily after 5 days for a period of 12 days, and each fly that was born was removed from the cage, counted and sacrificed. The results obtained were expressed by means of a birth curve of the flies.

2.11 Statistical analyses

The results obtained for the toxicity bioassays were submitted to Probit analysis to estimate the lethal doses (LC_{50} and LC_{90}) and chi-square values (x^2) with a 95% confidence limit. The results obtained for each of the treatments were analyzed using analysis of variance (ANOVA), followed by comparison of means by the Tukey's test ($p<0.05$) using the Rstudio® version 4.0.2 statistical software (R Core Team, 2016).

3 RESULTS AND DISCUSSION

3.1 Chemical composition of the essential oil

The yield of cumin essential oil was 1.02%. It had a yellow color and insufficient moisture for measuring. Some researchers obtained higher yields, such as 2.7% (HAKIMI, GHORBAN and DADMANESH, 2020) and 3.8% (LI and JIANG, 2004). The composition of essential oils and their yield can be influenced by several factors because of the variation in the constitution of plants according to climate, geographic region, variety, age of the plant, form and time of harvest, among other factors. In addition, the extraction method is also a factor that can influence the yield and composition of oils (FATTAHIAN *et al*., 2022).

The results obtained for the chemical composition of cumin essential oil are shown in Table 1. Seven constituents were identified, comprising 100% of the essential oil. The main constituent found was cuminaldehyde, representing 60.79% of the composition of the essential oil, followed by α-terpinen-7-ol (12.29%), γ-terpinene (7.51%) and myrtenol (7.13%). These results corroborate those of other researchers who, when studying the same oil, found these

constituents and cuminaldehyde as the major constituent (CAMPANA *et al*., 2022; NIRMALA *et al*., 2020; TANAPICHATSAKUL, KHRUENGSAI and PRIPDEEVECH, 2020).

Table 1 - Chemical composition of cumin essential oil.

Compound	RT $_{(min)}$	RI_{ref}	RI_{cal}	Área (%)
β-Pinene	7.810	974	980	4.48
p-Cimene	9.352	1020	1025	5.97
γ-Terpinene	10.620	1054	1058	7.51
Pulegone	16.352	1233	1196	1.83
Cuminaldehyde	**18.446**	**1238**	**1244**	**60.79**
Myrtenol	20.339	1195	1195	7.13
α-Terpinen-7-ol	20.531	1290	1292	12.29
Total identified				100.00

RI_{cal} is the calculated experimental retention index and RI_{ref} is the reference index tabulated in the literature. The principal constituent is highlighted in bold.

3.2 Evaluation of the acetylcholinesterase inhibitory activity of cumin essential oil and cuminaldehyde

Acetylcholinesterase inhibitory enzymatic activity was observed for cumin essential oil (CEO) and the standard compound cuminaldehyde (SC). Despite the fact that the IC_{50} for the carvacrol compound, used as a control, was expressively lower than that of the samples analyzed (Table 2), 30.35% and 21.57%, respectively, enzymatic inhibition was observed for the CEO and for the SC. The CEO (Table 1) is mainly composed of cuminaldehyde (60.70%), inferring that this is the main substance responsible for the inhibitory activity of acetylcholinesterase.

According to Gaspar-Pintiliescu *et al*. (2022), acetylcholinesterase is an enzyme of extreme importance in the central nervous system of pests. The inhibition of this enzyme by monoterpenes and sesquiterpenes present in the composition of essential oils and their principal compounds can occur through binding to specific amino acids of the active site of the enzyme or other sites that can allosterically alter the functions of the enzyme. The authors also state that the inhibition of acetylcholinesterase causes hyperactivity of the nervous system and physiological changes that can alter development, reproduction and even lead to death.

Table 2 – Estimated IC_{50} values for acetylcholinesterase inhibition.

Sample	IC_{50} (mg mL^{-1})
Carvacrol	0.058±0.003
Cumin essential oil	> 0.1
Cuminaldehyde standard	> 0.1

3.3 Toxicity of cumin essential oil and free cuminaldehyde to *Drosophila suzukii*

The results obtained for mortality in the toxicity bioassays are shown in Tables 3 and 4. Both tested compounds had a satisfactory lethal effect on *D. suzukii* adults with LC_{50} of 4.28 and 5.22 µL L^{-1}, respectively, for CEO and SC (Table 3). The essential oil was more efficient than the SC, although no significant difference was found between the samples at the high concentrations. A dose-dependent effect can also be observed (Table 4) because, when the concentration of CEO or SC increased, mortality also increased.

According to Perinelli *et al*. (2022), the effectiveness of the insecticidal activity of an essential oil depends on its entire chemical profile and the proportions of its constituents, which can lead to synergistic or antagonistic effects. Despite thefact that good results were obtained with the two compounds evaluated, the essential oil was more efficient than cuminaldehyde, even though it represented about 60% of the oil composition. These results infer that synergism existed between the constituents of the essential oil; that is, the combination of compounds present in the essential oil contributed to its greater toxicity to *D. suzukii*. The process of synergism is very common when it comes to essential oils and has already been reported by several authors, most recently by Santana *et al*. (2022). These results emphasize the usefulness of essential oils in insecticide resistance and how EO mixtures can increase their toxicity. These authors evaluated the combination of several EOs in insects and observed a synergistic effect.

Essential oils, as well as the principal constituent, have biological properties that have been increasingly studied, which makes these natural products viable alternatives for insect control. This fact is mainly due to the safety they provide for the environment and human health, linked, of course, to their efficiency (PINEDA *et al*., 2023). Thus, applications of these natural compounds in foods aimed at inhibiting pest and insect attack can be an option for reducing production losses without the use of synthetic pesticides.

Table 3 - Toxicity of cumin essential oil and cuminaldehyde (SC) on *D. suzukii* after 24 hours of exposure.

Sample	Nº insects	Slope (±EP)	LC_{50} (95% IF) (µL L^{-1})	LC_{90} (95% IF) (µL L^{-1})	X^2	*p*
CEO	600	1.97±0.01	4.28	19.06	5.345	0.930
SC	600	1.93±0.62	5.22	23.98	5.346	0.707

Where CEO is the cumin essential oil, SC is the cuminaldehyde, LC_{50} is the lethal concentration for 50% of the individuals and LC_{90} is the lethal concentration for 90% of the individuals; (95% IF) represents the 95% fiduciary range; X^2 is the chi-square for lack of fit to the probit model; *p* is the probability associated with the chi-square statistic.

Table 4 - Percentage of mortality of *D. suzukii* after 24 hours of exposure to treatments with cumin essential oil and cuminaldehyde in its free forms.

Concentration (µL L^{-1})	Control	0.75	5.0	8.0	9.0	10.0
CEO	$0±0.0^{cA}$	$26±1.2^{bA}$	$58±5.0^{aA}$	$63±3.8^{aA}$	$72±2.7^{aA}$	$83±4.6^{aA}$
SC	$0±0.0^{cA}$	$19±0.9^{bA}$	$51±4.6^{aA}$	$57±3.3^{aA}$	$67±4.3^{aA}$	$76±1.8^{aA}$

Where CEO is the cumin essential oil, and SC is cuminaldehyde. Means followed by the same lowercase letters between rows and uppercase letters between columns do not differ from each other by the Tukey Test at a significance level of 5%.

3.4 Characterization of the edible coating

3.4.1 Infrared analysis

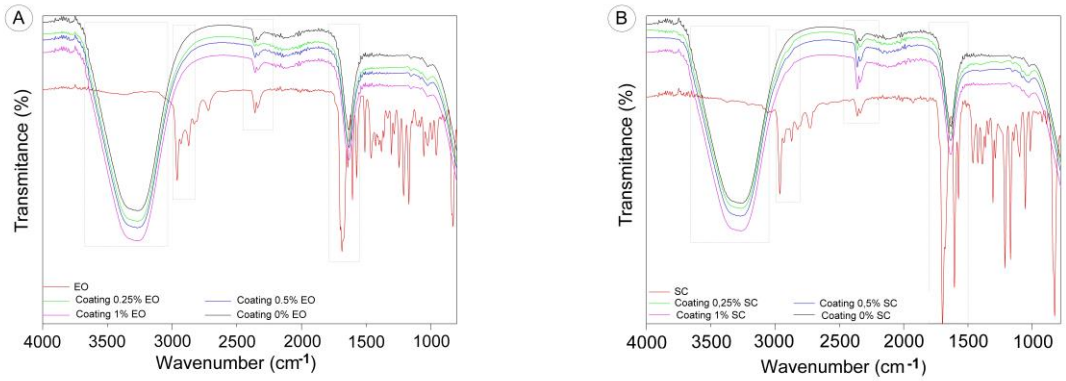
FTIR analysis makes it possible to characterize intermolecular interactions, and it can help confirm the incorporation of substances into the matrix polymer (BRANDÃO *et al*., 2022; CAETANO *et al*., 2022). The FTIR helped confirm the presence of cumin essential oil and cuminaldehyde in the edible coatings, as can be seen in Figures 1A and 1B.

The spectrum obtained for the edible coating prepared using cassava starch as a polymer is in accordance with the spectra found in the literature for this material. In Figures 1A and 1B, broad bands at 3500-3000 cm^{-1} can be observed. These banda are characteristic of the stretching vibration of the hydroxyl group (-OH) of cassava starch and glycerol, except in the samples of pure cumin essential oil and pure cuminaldehyde (standard compound), where this band is not present. For pure cumin essential oil, whose principal compound is cuminaldehyde, peaks close to 2900 cm^{-1} indicate stretching of the -CH bond of the aldehyde and of the aromatic ring, which can also be observed in Figure 1B (HAJJARI, GOLMAKANI and SHARIF, 2021; LUCHESE

et al., 2018). According to Luchese *et al*. (2018), the carbon-hydrogen (-CH) stretching vibration is responsible for the absorption observed in the region close to 2400 cm^{-1}. The peaks in the region between 2000-1500 cm^{-1} are characteristic of the elongation vibration of the double bonds between carbons (-C=C) and between carbon and oxygen (-C=O).

According to Lin *et al*. (2010), when two substances are mixed, the chemical interactions are reflected by changing characteristics in the peaks of the spectrum, which can make data interpretation difficult. Comparing the edible coatings with and without active compounds (EO or standard compound) in both figures (1A and 1B), more intense peaks were observed in the region of 2000-1500 cm^{-1}, the region characteristic of elongation of double bonds (-C= C and -C=O), and in the region close to 2400 cm, referring to hydrogen bonds (-CH) that may be extended, configuring the presence of active compounds in the prepared coatings.

Figure 1 - FTIR spectrum (A) - Cassava starch coatings prepared with the addition of cumin essential oil; (B) Cassava starch coatings prepared with the addition of the cuminaldehyde standard.

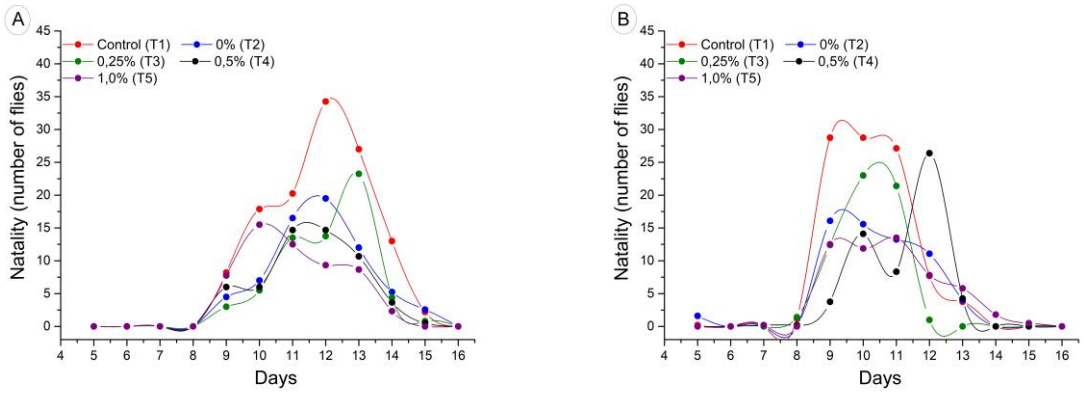


3.5 Evaluation of the influence of edible coatings containing the essential oil from cumin and the cuminaldehyde standard on the natality of *D. suzukii* when applied to strawberries

The natality of the flies after the strawberries were treated with coatings containing CEO or SC (Figure 2) demonstrated that, in all treatments and even in the control treatment, the peak of transformation of larvae into flies occurred between the 8th and 13th day of observation. However, the birth rate of flies exposed to the strawberries treated with both the coating containing CEO (Figure 2A) and SC (Figure 2B) gradually decreased as the concentration of CEO or SC increased. Treatments with 1.0% CEO or SC showed The best results were obtained

on both experiments, Decreases of 54.3% and 44.6% in the natality of flies at the end of the cycle were observed for treatments with 1% of CEO and SC (T5), respectively.

Figure 2 - Influence of treatments with edible coatings applied to strawberries on the birth rate of *D. suzukii* flies.



The results obtained confirm the efficiency of CEO and SC in controlling *D. suzukii*, assuming that this result is due to some possibilities. The first refers to the toxicity that the tested compounds exert on *D. suzukii*, a factor that can have prevented the flies from depositing a large number of eggs as they suffered the effects of exposure to CEO or SC, because both were toxic to the flies. The second possibility is that the coating itself, even without the addition of any active compound, acted as a barrier to oviposition and that the two factors together, that is, the active compound together with the coating, potentiated the control of *D. suzuki*i. The coatings were toxic to the fly and also formed a barrier to oviposition because, even in treatments without CEO or SC, a decrease in the birth rate of flies was observed.

4 CONCLUSION

Cumin essential oil and the cuminaldehyde standard were efficient insecticides against *Drosophila suzukii.* The samples evaluated were efficient both in the toxicological tests performed with pure samples and in those with these samples were incorporated into edible coatings and applied to strawberries. The best results were observed with cumin essential oil. Thus, this essential oil is a potential natural insecticide that could facilitate the management of the pest species *D. suzukii* and reduce the use of synthetic insecticides.

5 ACKNOWLEDGEMENTS

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – 311183/2022-0), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Code 001 and the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG – 02390/18). The authors would like to thank the Chemical Analysis and Prospection Center of the Federal University of Lavras (CAPQ) for providing the equipment for the chemical analyses.

REFERENCES

ADAMS, R. P. **Identification of essential oils componentes by gas chromatography/ mass spectroscopy**. 4 ed. Carol Stream: Allured, 2017. p.804.

ANDREAZZA, F. *et al*. *Drosophila suzukii* (Diptera: Drosophilidae) arrives at Minas Gerais State, a main strawberry production region in Brazil. **Florida Entomologist**, v. 99, n. 4, p. 796-798, 2016.

ANDREAZZA, F. et al. Técnica de Criação de *Drosophila Suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) Em Dieta Artificial. **Boletim de Pesquisa e Desenvolvimento/Embrapa Clima Temperado**. 2016.

BRANDÃO, R. M.s *et al*. Antifungal and antiocratoxigenic potential of *Alpinia speciosa* and *Cymbopogon flexuosus* essential oils encapsulated in poly (lactic acid) nanofibres against *Aspergillus* fungi. **Letters in Applied Microbiology**, v. 75, n. 2, p. 281-292, 2022.

BRASIL. **Agência Nacional de Vigilância Sanitária (Anvisa).** In: Farmacopeia Brasileira, Brasília, 5th ed.; p. 198-199, 2010.

CAETANO, A. R. S. *et al*. Rosmarinus officinalis essential oil incorporated into nanoparticles as an efficient insecticide against *Drosophila suzukii* (Diptera: Drosophilidae). **Austral Entomology**, v. 61, n. 2, p. 265-272, 2022.

CAMPANA, R. *et al*. Comparative analysis of the antimicrobial activity of essential oils and their formulated microemulsions against foodborne pathogens and spoilage bacteria. **Antibiotics**, v. 11, n. 4, p. 447, 2022.

CHAUBEY, M. K. Fumigant toxicity of essential oils and pure compounds against *Sitophilus oryzae* L. (Coleoptera: Curculionidae). **Biological Agriculture & Horticulture**, v. 28, n. 2, p. 111-119, 2012.

COSTA, P. *et al*. Accumulation of phenolic compounds in in vitro cultures and wild plants of Lavandula viridis L'Hér and their antioxidant and anti-cholinesterase potential. Food and **Chemical Toxicology**, v. 57, p. 69-74, 2013.

ELLMAN, G. L. *et al*. A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. **BioChemical Pharmacology,** 1961, 7, 88–95.

ENRIQUEZ, T. *et al*. Effects of fluctuating thermal regimes on cold survival and life history traits of the spotted wing Drosophila (Drosophila suzukii). **Insect Science**, v. 27, n. 2, p. 317-335, 2020.

FATTAHIAN, A. *et al*. The effects of chitosan containing nano-capsulated *Cuminum cyminum* essential oil on the shelf-life of veal in modified atmosphere packaging. **Journal of Food Measurement and Characterization**, v. 16, n. 1, p. 920-933, 2022.

FERREIRA, D. C.; MOLINA, G.; PELISSARI, F. M. Effect of Edible Coating from Cassava Starch and Babassu Flour (Orbignya phalerata) on Brazilian Cerrado Fruits Quality. **Food and Bioprocess Technology**, v.13, p. 172-179, 2020.

GASPAR-PINTILIESCU, A. *et al*. Antioxidant and acetylcholinesterase inhibition capacity of hyrosols from lamiaceae plants for biopesticide use: role of phenolics. **International Journal of Food Properties**, v. 25, n. 1, p. 996-1008, 2022.

GONZÁLEZ, J. O. W. *et al*. Polymer nanoparticles containing essential oils: new options for mosquito control. **Environmental Science and Pollution Research**, v. 24, n. 20, p. 17006-17015, 2017.

HAJJARI, M. M.; GOLMAKANI, M. T.; SHARIF, N. Fabrication and characterization of cuminaldehyde-loaded electrospun gliadin fiber mats. **Lwt**, v. 145, p. 111373, 2021.

HAKIMI, R.; GHORBAN, K.; DADMANESH, M. Combined effects of *Allium sativum* and *Cuminum cyminum* essential oils on planktonic and biofilm forms of *Salmonella typhimurium* isolates. **3 Biotech**, v. 10, n. 7, p. 1-10, 2020.

HASHEMI, S. M. B.; KHANEGHAH, A. M.; SANT'ANA, A. S. **Essential Oils in Food Processing: chemistry, safety and applications.** Hoboken, NJ:John Wiley e Sons, 2018, 205p.

IBOUH, K. *et al*. Biological control of *Drosophila suzukii*: Efficacy of parasitoids, entomopathogenic fungi, nematodes and deterrents of oviposition in laboratory assays. **Crop Protection,** v. 125, p. 104897, 2019.

IRAC (Insecticide Resistance Action Committe). **Susceptibility Test Methods Series**, Method n°26, 2020.

LI, R.; JIANG, Z. Chemical composition of the essential oil of *Cuminum cyminum* L. from China. **Flavour and fragrance journal**, v. 19, n. 4, p. 311-313, 2004.

LIN, B. *et al*. The effect of moist heat treatment on the characteristic of starch-based composite materials coating with chitosan. **Carbohydrate Polymers**, v. 81, n. 3, p. 554-559, 2010.

LUCHESE, C. L. *et al*. Effect of chitosan addition on the properties of films prepared with corn and cassava starches. **Journal of food science and technology**, v. 55, p. 2963-2973, 2018.

MOSSA, A. T. H. Green pesticides: Essential oils as biopesticides in insect-pest management. **Journal of environmental science and technology**, v. 9, n. 5, p. 354, 2016.

NAVA, D. E. *et al*. Bioecologia, monitoramento e controle de *Drosophila suzukii* na cultura do morangueiro. Rio grande do Sul: **EMBRAPA (Documento 398)**, 2015, p. 25.

NIRMALA, M. J. *et al*. Ultrasonic nanoemulsification of *Cuminum cyminum* essential oil and its applications in medicine. **International journal of nanomedicine**, v. 15, p. 795, 2020.

PERINELLI, D.R. *et al*. Development, characterization, insecticidal and sublethal effects of *Bunium persicum* and *Ziziphora clinopodioides*-based essential oil nanoemulsions on *Culex quinquefasciatus.* **Industrial Crops and Products**, v. 186, p. 115249, 2022.

PIMENTEL, F. A. *et al*. Influência da temperatura de secagem sobre o rendimento e a composição química do óleo essencial de *Tanaecium nocturnum* (Barb. Rodr.) BUR. & K. SHUM. **Química Nova**, v.31, n.3, p.523-526, 2008.

PINEDA, M. *et al*. Low Concentrations of Eucalyptus Essential Oil Induce Age, Sex, and Mating Status-Dependent Stimulatory Responses in *Drosophila suzukii*. **Agriculture**, v. 13, n. 2, p. 404, 2023.

R CORE TEAM (2016). R: **A language and environment for statistical computing. R Foundation for Statistical Computing**, Vienna. Disponível em: <https://www.R-project.org>.

RATHER, J. A. *et al*. Recovery of gelatin from poultry waste: Characteristics of the gelatin and lotus starch-based coating material and its application in shelf-life enhancement of fresh cherry tomato. **Food Packaging and Shelf Life**, v. 31, p. 100775, 2022.

RODRIGUES, U. V. *et al*. **Tópicos especiais em Ciência Animal VI** [e-book]. 1.ed. Alegre – ES: UNICOPY, 2017, p. 41

SANTANA, A. S. *et al*. Synergism between essential oils: A promising alternative to control *Sitophilus zeamais* (Coleoptera: Curculionidae). **Crop Protection**, v. 153, p. 105882, 2022.

SCHLESENER, D. C. H. *et al*. *Drosophila suzukii*: nova praga para a fruticultura brasileira. **O Biológico**, v. 77, n. 1, p. 47-54, 2015.

SOUZA, L. *et al*. Toxicity, Histopathological Alterations and Acetylcholinesterase Inhibition of *Illicium verum* Essential Oil in *Drosophila suzukii*. **Agriculture**, v. 12, n. 10, p. 1667, 2022.

SWOBODA-BHATTARAI, K. A.; BURRACK, H. J. Influence of edible fruit coatings on *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) oviposition and development. **International Journal of Pest Management**, v. 60, n. 4, p. 279-286, 2014.

TABASSUM, N.; KHAN, M. A. Modifield atmosphere packaging of fresh-cut papaya using alginate based edible coating: quality evaluation and shelf life study. **Scientia Horticulturae**, v.259, p.1-9, 2020.

TANAPICHATSAKUL, C.; KHRUENGSAI, S.; PRIPDEEVECH, P. In vitro and in vivo antifungal activity of *Cuminum cyminum* essential oil against *Aspergillus aculeatus* causing bunch rot of postharvest grapes. **Plos one**, v. 15, n. 11, p. e0242862, 2020.

VAN DEN DOOL, H. and KRATZ, P.D. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. **J Chromatogr A**, v. 11, p. 463–471, 1963.

ZIVKOVIĆ, I. P. *et al*. The development of drosophilid species (Diptera, Drosophilidae) in different strawberry cultivars. **European journal of horticultural science**, v. 84, n. 1, p. 48-52, 2019.

ARTIGO 3 – Elaboração e avaliação da atividade antifúngica de revestimentos comestíveis incorporados de óleos essenciais de *Cuminum cyminum*, *Laurus nobilis*, *Citrus reticulata x sinensis* e seus respectivos compostos majoritários sobre *Rhizopus stolonifer*

Elaboração e avaliação da atividade antifúngica de revestimentos comestíveis incorporados de óleos essenciais de *Cuminum cyminum, Laurus nobilis, Citrus reticulata x sinensis* e seus respectivos compostos majoritários sobre *Rhizopus stolonifer*

Gabriela Aguiar Campolina[1], Maria das Graças Cardoso[2], Antonia Isadora Fernandes[2], Alex Rodrigues Silva Caetano[2], David Lee Nelson[4], Luis Roberto Batista[1]

[1]Departamento de Ciência dos Alimentos, Universidade Federal de Lavras (UFLA), Lavras, 37200-900 MG, Brasil

[2] Departamento de Química, Universidade Federal de Lavras (UFLA), Lavras, 37200-900 MG, Brasil

[3]Programa de Pós-Graduação em Biocombustíveis, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, 39100-000 MG, Brasil

RESUMO

O morango é um fruto amplamente consumido, de sabor agradável e rico em nutrientes benéficos à saúde humana. No entanto, possui vida útil pós-colheita limitada, principalmente devido ao ataque de insetos e fungos. O fungo *Rhizopus stolonifer* tem sido apontado como o principal microrganismo deteriorador de morangos na pós-colheita. Assim, objetivou-se neste estudo foi avaliar a atividade antifúngica dos óleos essenciais (OE) de *Cuminum cyminum*, *Laurus nobilis*, *Citrus reticulata x sinensis* e seus respectivos compostos majoritários, cuminaldeído, 1,8-cineol e limoneno sobre *R.stolonifer* visando à elaboração de revestimentos comestíveis com atividade antifúngica. Os OE foram caracterizados quimicamente e a atividade antifúngica desses e dos compostos-padrão (CP) foi avaliada *in vitro* sobre *R. stolonifer*. O OE que apresentou maior atividade antifúngica foi incorporado a revestimentos comestíveis à base de amido de mandioca. A atividade antifúngica dos revestimentos foi avaliada *in vitro* e *in vivo.* A caracterização química mostrou predominância do composto cuminaldeído no OE extraído de cominho; 1,8-cineol no OE extraído de louro; e limoneno no OE extraído de tangerina. O OE de cominho e o CP cuminaldeído apresentaram os melhores resultados para a atividade antifúngica *in vitro,* inibindo o crescimento micelial de *R. stolonifer.* Os morangos tratados com os revestimentos incorporados com OE de cominho apresentaram redução na severidade da doença e na perda de peso no segundo dia de armazenamento. Dessa forma, a aplicação de

uma camada fina e biodegradável constituída de materiais que possam ser ingeridos, chamada de revestimento comestível, quando incorporado com óleo essencial de cominho, pode ser uma possível alternativa aos fungicidas sintéticos utilizados para controlar a proliferação de *R. stolonifer* em morangos.

Palavras-chave: Óleos essenciais. *Rhizopus stolonifer.* Revestimentos comestíveis. Morangos.

1 INTRODUÇÃO

A deterioração alimentar ocorre quando as características dos alimentos, como aparência, odor e sabor, são alteradas, tornando-os impróprios para consumo. Uma das principais causas dessa deterioração está relacionada à ação de microrganismos, que pode ocorrer tanto antes como depois da colheita de alimentos frescos, como frutas (MADIGAN *et al*., 2016). A contaminação de frutas é comum devido ao fato de serem consumidas cruas e serem facilmente contaminadas durante a manipulação por mãos humanas ou pelo ambiente, incluindo água, ar e recipientes. Danos físicos também podem afetar a qualidade das frutas, pois fornecem nutrientes necessários para o crescimento dos microrganismos (YOON e LEE, 2017; CARVALHO, 2010).

Em frutas vermelhas, como morangos, amoras, framboesas e mirtilos, os principais microrganismos deterioradores são dos gêneros *Botrytis, Alternaria, Cladosporium, Penicillium, Fusarium* e *Rhizopus* (TOURNAS e KATSOUDAS, 2005). A espécie *Rhizopus stolonifer* é considerada uma das mais destrutivas entre esses patógenos, sendo responsável por perdas significativas na produção de morangos, espalhando-se facilmente em temperaturas acima de 5ºC e causando a chamada podridão-mole ou aquosa nos frutos, resultando em vazamento de suco e crescimento rápido do fungo, o que pode levar à perda total do fruto rapidamente (VENTURA-AGUILAR *et al*., 2021; SILVA *et al*., 2020; KONG *et al*., 2019; BAUTISTA-BAÑOS *et al*., 2014).

O controle de patógenos como *R. stolonifer* é feito principalmente por meio da aplicação de fungicidas sintéticos. No entanto, o uso excessivo e incorreto desses produtos pode levar à resistência dos patógenos, contaminação do meio ambiente e problemas de saúde (SILVA *et al*., 2020). Portanto, o uso de óleos essenciais apresenta-se como uma alternativa viável e interessante para os produtores de morangos, pois são naturais, de baixa toxicidade e baixo impacto ambiental.

Alguns estudos mostraram eficiência de óleos essenciais de diferentes plantas na inibição do crescimento de *R. stolonifer* (REZENDE *et al*., 2020; TAHMASEBI *et al*., 2020; ZHOU *et al*., 2019). Essas pesquisas sugerem que a substituição de fungicidas sintéticos por fungicidas à base de óleos essenciais podem melhorar a qualidade dos morangos, tornando a cadeia produtiva mais sustentável. Porém, apesar de suas propriedades de interesse, os óleos essenciais, bem como seus respectivos compostos-padrão, possuem características de aroma e sabor marcantes, fato que dificulta a sua aplicação. Assim, a incorporação desses compostos ativos em revestimentos comestíveis surgem como uma boa alternativa para facilitar e viabilizar a utilização desses compostos em alimentos (CAMPOLINA *et al*., 2023).

No presente estudo objetivou-se elaborar e avaliar a atividade antifúngica, *in vitro* e *in vivo*, de revestimentos comestíveis incorporados com os óleos essenciais de *Cuminum cyminum, Laurus nobilis, Citrus reticulata x sinensis* e seus respectivos compostos majoritários, cuminaldeído, 1,8-cineol e limoneno sobre *R. stolonifer.*

2 MATERIAL E MÉTODOS

2.1 Obtenção dos materiais vegetais e compostos-padrão

Os materiais vegetais utilizados para a extração dos óleos essenciais foram o cominho (*Cuminum cyminum*), adquirido no mercado da cidade de São Paulo – SP, Brasil (23°32'51"S; 46°38'10"W), o louro (*Laurus nobilis)* e a tangerina (*Citrus reticulata x sinensis*) adquiridos no Mercado da cidade de Lavras – MG, Brasil (21º14′43" S, 44º59′50" W). Os compostos-padrão utilizados foram o cuminaldeído (98%, Sigma-Aldrich®, São Paulo, Brasil), o 1,8-cineol (Eucaliptol) (Sigma-Aldrich®, São Paulo, Brasil) e o limoneno (Sigma-Aldrich®, São Paulo, Brasil).

2.2 Extração dos óleos essenciais

Os óleos essenciais foram extraídos utilizando a técnica de hidrodestilação, por meio do uso de um aparelho de Clevenger modificado, durante um período de 2 horas (BRASIL, 2010). Os hidrolatos obtidos foram centrifugados (Fanem Baby I Model 260 BL, São Paulo, Brasil) a 965g por 15 minutos, para separação total do óleo essencial, e com o auxílio de uma micropipeta de Pasteur, o óleo essencial foi coletado e armazenado em frasco âmbar sob refrigeração.

2.3 Caracterização química dos óleos essenciais

A caracterização químicas dos óleos essenciais foi realizada na Central de Análise e Prospecção Química (CAPQ) da Universidade Federal de Lavras – UFLA, Minas Gerais.

A análise cromatográfica foi realizada em um cromatógrafo a gás Shimadzu, modelo QP2010 Plus (Kyoto, Japão), equipado com uma coluna capilar de sílica fundida com uma fase ligada a DB5 (30m x 0,25 mm de diâmetro interno; espessura 0,2 μm) acoplado a um detector de massa, nas condições descritas por Brandão *et al*., (2022).

Os dados obtidos foram processados utilizando o Software LabSolutions LC/GC da estação de trabalho 2.72. Os índices de retenção dos compostos foram determinados usando a equação de Van Den Dool e Kratz (1963) e a série homóloga de n-alcanos (nC8-nC18) como padrões. Os índices de retenção foram comparados com os da literatura (Adams, 2017), e os espectros de massa dos constituintes do óleo essencial com similaridade superior a 95% foram comparados com aqueles das bibliotecas de espectros de massa FFNSC 1.2, NIST 107 e NIST 21. Os constituintes foram quantificados usando o método de normalização de área (%) baseado em áreas de pico automaticamente integradas do sinal GC-FID.

2.4 Isolamento do fungo *Rhizopus stolonifer*

A espécie *Rhizopus stolonifer* foi obtida por meio de isolamento direto a partir de morangos naturalmente infectados e identificados morfologicamente. Eles foram isolados de morangos adquiridos no Mercado da cidade de Lavras, MG, Brasil, sendo deixados à temperatura ambiente por 7 dias. Após esse período, os diferentes fungos proliferados nos morangos foram repicados em placas de Petri contendo o meio de cultura Dicloran Rosa Bengala Cloranfenicol Base (DRBC) e incubados em estufa BOD por 5 dias a 25ºC. Os fungos filamentosos identificados foram submetidos ao procedimento de isolamento em meio de cultura Ágar Extrato de Malte (MEA) e incubados em estufa BOD por 5 dias a 25ºC. Esse procedimento foi realizado até alcançar o isolamento completo.

2.4.1 Avaliação da atividade antifúngica *in vitro* dos óleos essenciais e compostos-padrão sobre *Rhizopus stolonifer*

A atividade antifúngica *in vitro* dos OEs e compostos-padrão foi realizada de acordo com a metodologia descrita por Oliveira *et al*. (2019), com modificações. A avaliação da atividade antifúngica foi realizada medindo o crescimento micelial de *R. stolonifer* e avaliando a inibição do crescimento pelo contato direto do fungo com o meio de cultura MEA contendo os OEs ou compostos-padrão nas concentrações de 31; 62,5; 125; 500; 1000; 5000 e 10000 µL L^{-1}. Para homogeneização completa dos OEs e compostos-padrão, foi utilizado o emulsificante Tween 80 (2:1). Um tratamento-controle contendo apenas Tween 80 e meio de cultura também foi utilizado. Após a solidificação do meio MEA, 5 µL de *R. stolonifer* foram transferidos para o centro da placa, a partir de uma suspensão de inóculo contendo 10^5 esporos/mL. As placas foram incubadas em estufa BOD a 25ºC e foram realizadas medições do crescimento micelial após 24 e 48h, em duas direções perpendiculares.

Os resultados da inibição do crescimento fúngico nas diferentes concentrações de OEs e compostos-padrão foram expressos em Porcentagem de Inibição do Crescimento (PI), conforme apresentado pela Equação 1:

$$PI(\%) = \frac{\text{Ccontrole} - \text{Ctratamento}}{\text{Ccontrole}}\ x\ 100 \qquad (1)$$

Onde: PI (%) = Porcentagem de Inibição do Crescimento em porcentagem;
C = Crescimento micelial em centímetros.

2.5 Elaboração do revestimento comestível incorporado com óleo essencial de cominho e avaliação da atividade antifúngica *in vitro* sobre *Rhizopus stolonifer*

A partir dos resultados obtidos na avaliação da atividade antifúngica *in vitro,* a amostra que apresentou resultado mais eficiente sobre *R. stolonifer* foi selecionada para ser incorporada aos revestimentos comestíveis à base de amido de mandioca.

Os revestimentos foram elaborados de acordo com a metodologia descrita por Ferreira, Molina e Pelissari (2020) e Tabassum e Khan (2020), com modificações.

Suspensões de amido de mandioca (2%) foram preparadas a 80°C, sob agitação, até a completa gelatinização do amido. As suspensões foram resfriadas à temperatura ambiente para adição do plastificante glicerol (20%), do surfactante Tween 80 (1%) e do óleo essencial ou composto-padrão em diferentes concentrações (0%; 0,75%; 1,5% e 3% µL L^{-1}). Essas concentrações corresponderam a 2500, 5000 e 10000 µL L^{-1} na aplicação *in vitro.* As soluções formadas foram agitadas até completa homogeneização e, após o resfriamento, os revestimentos

formados foram avaliados quanto à sua atividade antifúngica sobre *R. stolonifer*, da mesma forma descrita anteriormente no item 2.4.

2.6 Avaliação do efeito dos revestimentos aplicados a morangos

A partir dos resultados obtidos para a atividade antifúngica dos revestimentos comestíveis à base de amido incorporados com o óleo essencial, foram preparados quatro tratamentos para serem aplicados aos morangos (Tabela 1).

Os morangos adquiridos no Mercado da cidade de Lavras, MG, Brasil, foram selecionados visualmente quanto à sanidade e aparência, e posteriormente, higienizados em solução de hipoclorito de sódio 2,5% por 15 minutos. Os frutos higienizados e secos foram imersos na solução de revestimento de amido incorporado ou não com OE por 1 minuto. Os frutos revestidos foram submetidos à secagem natural e, em seguida, foram inoculados 30 μL de uma suspensão de esporos de *R. stolonifer* (10^5 esporos/mL). A suspensão de esporos foi adicionada a uma ferida de 3 mm de profundidade (SILVA *et al*., 2020).

Tabela 1 – Tratamentos aplicados aos morangos.

Amostra	Material do revestimento
T1	Controle - Sem revestimento
T2	Revestimento de amido sem OEC
T3	Revestimento de amido com 3% de OEC*
T4	Revestimento de amido com 30% de OEC*

*Ressalta-se que para T3 e T4 a porcentagem de OE foi calculada com base na concentração de amido utilizada para a elaboração dos revestimentos; OEC: óleo essencial de cominho.

Os frutos foram incubados em estufa BOD a 25ºC por quatro dias, até a contaminação completa dos frutos. Foram utilizadas três repetições de cada tratamento, cada uma contendo seis morangos. A atividade antifúngica de cada um dos tratamentos foi avaliada a cada dois dias pela severidade da doença nos frutos, conforme descrito por Lin *et al*. (2021). Os resultados foram obtidos utilizando a Equação 2 e expressos em porcentagem. A escala de escores de severidade utilizada foi composta por cinco graus, sendo 0 = ausência de sintomas; 1 = 1 a 25% da área lesada; 2 = 26 a 50%; 3 = 51 a 75%; 4 = 76 a 100% da área com lesão).

$$Severidade\ (\%) = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4}{4N}\ x\ 100 \qquad (2)$$

Onde: ni é o número de frutos infectados na escala de pontuação correspondente e N é o número total de frutos.

Para avaliar o efeito dos revestimentos nos morangos, foram analisados as alterações na perda de peso e o pH dos frutos a cada dois dias durante o período de armazenamento.

2.6.1 Alterações no pH e perda de peso

Os morangos foram triturados e a polpa obtida foi utilizada para a realização das análises de pH. Os valores de pH das amostras foram mensurados por leitura direta na polpa utilizando um pHmetro de bancada (Quimis – Q400AS). A perda de peso dos morangos foi realizada em balança semianalítica durante o período de armazenamento, calculando-se a perda de peso com o auxílio da Equação 3.

$$Perda\ de\ peso\ (\%) = \frac{Mi - Mf}{Mi} \quad (3)$$

Onde: Mi = Massa inicial (g);
Mf = Massa final (g).

2.8 Análises estatísticas

As análises estatísticas foram realizadas utilizando um Delineamento Inteiramente Casualisado (DIC). A avaliação dos efeitos dos óleos essenciais de *C. cyminum* e *L. nobilis* e *Citrus deliciosa x sinensis*, bem como de seus respectivos compostos majoritários (padrões cuminaldeído, 1,8-cineol e limoneno) na porcentagem de inibição do crescimento micelial, foram analisados por meio na Análise de Variância (ANOVA) seguido pelo teste de médias Tukey a 5% de significância. A avaliação do efeito dos revestimentos na perda de peso, pH e severidade dos morangos foram analisados por meio na Análise de Variância (ANOVA), seguido pelo teste de Scott-Knott a 5% de significância. O software estatístico utilizado foi o Rstudio® versão 4.2.1. (R Core Team, 2016).

3 RESULTADOS E DISCUSSÃO

3.1 Composição química dos óleos essenciais

Os resultados obtidos para a composição química dos óleos essenciais (OE) de cominho, louro e tangerina estão apresentados na Tabela 2. Foram identificados sete constituintes no OE de cominho, que apresentou como composto majoritário o cuminaldeído (60,79%); no OE de louro, foram identificados 13 constituintes, sendo 1,8-cineol (60,15%) o constituinte presente em maior quantidade nesse OE e; no OE de tangerina, foram identificados quatro constituintes, sendo o limoneno (98,39%) o composto majoritário. Esses resultados mostraram-se coerentes com os obtidos por outros autores, que também encontraram cuminaldeído, 1,8-cineol e limoneno, respectivamente, como compostos majoritários para os óleos essenciais de cominho, louro e tangerina (OSANLOO *et al*., 2023; LI *et al*., 2022; UZSÁKOVÁ *et al*., 2022; XU *et al*., 2021; RIABOV *et al*., 2020; FIDAN *et al*., 2019).

Ao comparar os resultados obtidos com outros autores, foi observado que, apesar de os óleos essenciais apresentarem constituição similar, pode haver diferenças na composição, principalmente relacionadas às quantidades de cada constituinte. Gobbo-Neto e Lopes (2007) ressaltam que a expressão gênica pode ser alterada por meio de processos bioquímicos e fisiológicos que ocorrem naturalmente e devido às condições ecológicas às quais a planta foi submetida.

Tabela 2 - Composição química do óleo essencial de cominho, louro e tangerina.

Composto químico	RT (min)	RI_{ref}	RI_{cal}	Área (%)		
				C.cyminum	*L.nobilis*	*C. reticulata x sinensis*
α-Tujeno	6,208	924	925	-	0,32	0,36
α-Pineno	6,450	932	933	-	3,78	-
Sabineno	7,592	969	972	-	4,30	-
β-Pineno	7,810	974	980	4,48	3,16	-
Mirceno	8,047	988	1088	-	-	0,63
p-Cimeno	9,352	1020	1025	5,97	1,31	-
Limoneno	9,517	1024	1028	-	1,55	**98,39**
1,8-Cineol	9,650	1026	1032	-	**60,15**	-
α-Felandreno	10,592	1002	1057	-	0,43	-
γ-Terpineno	10,620	1054	1058	7,51	-	-
Linalol	12,200	1101	1099	-	1,02	0,62
Terpinen-4-ol	15,683	1174	1180	-	3,47	-
α-Terpineol	16,308	1186	1195	-	2,71	-
Pulegona	16,352	1233	1196	1,83	-	-
Cuminaldeído	18,446	1238	1244	**60,79**	-	-
Acetato de Isobornil	20,175	1283	1283	-	0,29	-
Mirtenol	20,339	1195	1195	7,13	-	-
α-Terpinen-7-al	20,531	1290	1292	12,29	-	-
α-Acetato de Terpenil	22,858	1349	1345	-	16,19	-
Total identificado				100,00	98,95	100,00

Onde: RIcal é o índice de retenção experimental calculado e RI_{ref} é o índice de referência tabulado na literatura. Em negrito está destacado o constituinte majoritário.
Fonte: Do autor (2023).

3.2 Atividade antifúngica dos óleos essenciais e compostos-padrão

Os resultados obtidos para a atividade antifúngica, após 24 e 48 horas, dos óleos essenciais de cominho, louro e tangerina; e dos compostos-padrão (CP) cuminaldeído, eucaliptol e limoneno sobre o fungo *R. stolonifer* estão apresentados na Figura 1A e 1B.

Todos os tratamentos avaliados inibiram completamente o crescimento micelial de *R. stolonifer* na maior concentração testada (10000 μL L^{-1}) após 24h e 48h, e apresentaram um comportamento dose-dependente. Porém, em concentrações abaixo de 1000 μL L^{-1}, os OE de

tangerina e louro, assim como seus respectivos CP, limoneno e eucaliptol, não apresentaram nenhuma inibição no crescimento micelial do fungo avaliado, após 48h de aplicação (Figura 1B). Assim, os CP limoneno e eucaliptol apresentaram-se menos eficientes sobre *R. stolonifer*, quando comparado aos demais tratamentos, seguido dos OE de tangerina e louro.

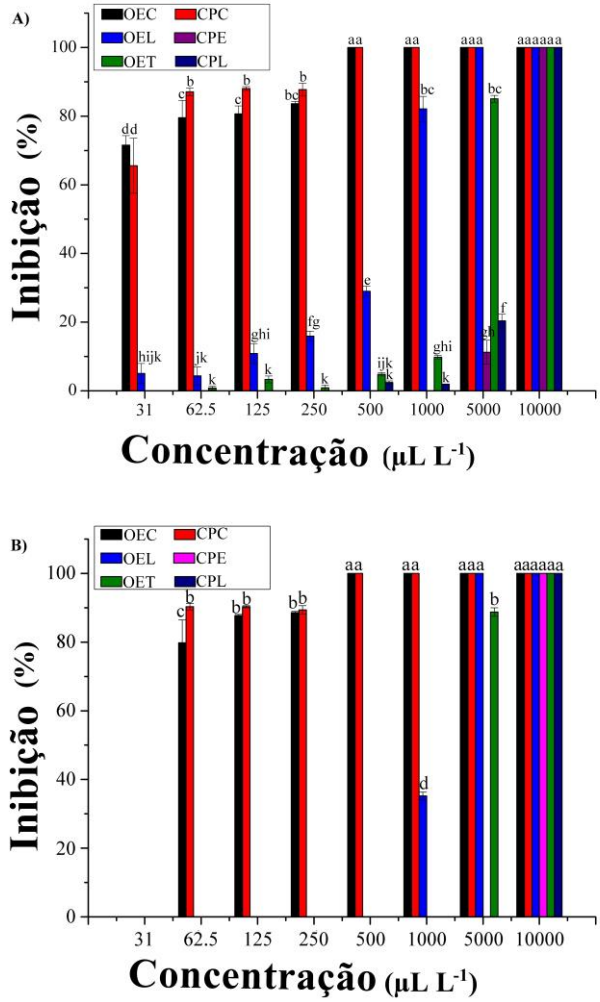
Após 48 horas de aplicação, os CP limoneno e eucaliptol apresentaram atividade antifúngica apenas na maior concentração avaliada (10000 µL L^{-1}). Já o OE de tangerina, após 48 horas, apresentou 88,7% de inibição na concentração de 5000 µL L^{-1} e 100% de inibição na maior concentração avaliada (10000 µL L^{-1}). O OE de louro apresentou 35,2% de inibição na concentração de 1000 µL L^{-1} e 100% de inibição nas maiores concentrações (5000 e 10000 µL L^{-1}).

Os melhores e mais eficientes tratamentos foram aqueles que utilizaram o OE de cominho e o CP cuminaldeído. Esses tratamentos foram capazes de inibir 100% do crescimento micelial a partir da concentração de 500 µL L^{-1}, não se diferiram estatisticamente ($p<0,05$) e mantiveram esses resultados após 48 h de aplicação.

De acordo com Nazarro *et al*. (2017), a ação antifúngica dos óleos essenciais pode ocorrer por meio da alteração da integridade das membranas celulares, assim como alterações da composição, da permeabilidade e até ruptura dessas membranas. Outras formas de atuação são por meio do estresse oxidativo e da inibição de processos intracelulares. Porém, o que determina o potencial antifúngico de um óleo essencial é sua constituição, pois os constituintes químicos que formam o óleo essencial irão facilitar ou dificultar a ação antifúngica dos mesmos.

Em relação aos grupos funcionais, Vieira *et al*. (2014) citam que a atividade antimicrobiana segue a seguinte ordem: fenóis > aldeídos > cetonas > álcoois > éteres > hidrocarbonetos. Correlacionando esses grupos funcionais com aqueles presentes nos óleos essenciais de cominho, louro e tangerina, pode-se inferir que o alto potencial antifúngico do OE de cominho, quando comparado aos demais OE estudados, se deve ao, seu composto majoritário (cuminaldeído) ter um grupo funcional aldólico. No mesmo sentido, a baixa atividade antifúngica do OE de tangerina pode ser atribuída ao fato desse óleo ser constituído de 98,4% de limoneno, um hidrocarboneto que tende a apresentar menor atividade antifúngica.

Figura 1 - Atividade antifúngica dos óleos essenciais de cominho (OEC), louro (OEL), tangerina (OET) e seus respectivos compostos-padrão, cuminaldeído (CPC), eucaliptol (CPE) e limoneno (CPL) sobre *R. stolonifer* após 24 (A) e 48 horas (B).



Letras diferentes entre as colunas indicam diferença significativa entre os tratamentos, pelo teste de Tukey ($p<0,05$).
Fonte: Do autor (2023).

A atividade antifúngica do OE de cominho e de seu composto majoritário, o CP cuminaldeído, que apresentaram as menores concentrações inibitórias entre os tratamentos avaliados foram observadas também por outros autores: Tanapichatsakul, Khruengsai e Pripdeevech (2020) avaliaram o potencial antifúngico do OE de cominho sobre *Aspergillus aculeatus.* Esses autores observaram uma alta taxa de inibição (>80%) a partir de uma concentração de 800 e 1000 µg mL^{-1}. Miri e Djenane (2018), ao estudarem o efeito antifúngico do óleo essencial de cominho sobre *Aspergillus flavus,* também observaram que esse óleo é capaz de inibir o crescimento desse fungo.

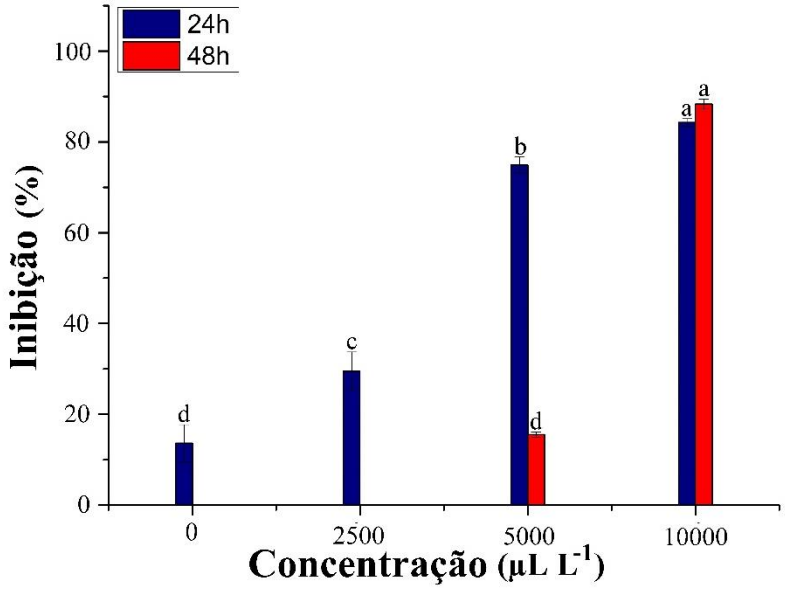
Devido ao resultado promissor apresentado pelo OE de cominho e ao fato desse conter outros compostos além do cuminaldeído, que podem auxiliar na atividade antifúngica, esse OE foi o tratamento selecionado para ser incorporado aos revestimentos e dar continuidade aos testes antifúngicos sobre *R. stolonifer*.

3.3 Atividade antifúngica do revestimento comestível incorporado com óleo essencial de cominho

Os resultados obtidos para a avaliação antifúngica do revestimento elaborado à base de amido de mandioca e incorporado com óleo essencial de cominho nas concentrações de 2500, 5000 e 10000 μL L^{-1}, avaliados 24 e 48 horas após a aplicação, estão apresentados na Figura 2.

Foi observado que, na maior concentração avaliada (10000 μL L^{-1}), o revestimento elaborado foi capaz de manter a inibição do crescimento micelial de *R. stolonifer*, alcançando inibição de 84,2% após 24 horas e 88,3% após 48 horas, validando a atividade antifúngica do revestimento comestível sobre *R. stolonifer*.

Figura 2 – Atividade antifúngica do revestimento comestível à base de amido de mandioca incorporado com óleo essencial de cominho sobre *Rhizopus stolonifer*, 24 e 48 horas após aplicação.



Letras diferentes entre as colunas indicam diferença significativa entre os tratamentos, pelo teste de Tukey ($p<0,05$).
Fonte: Do autor (2023).

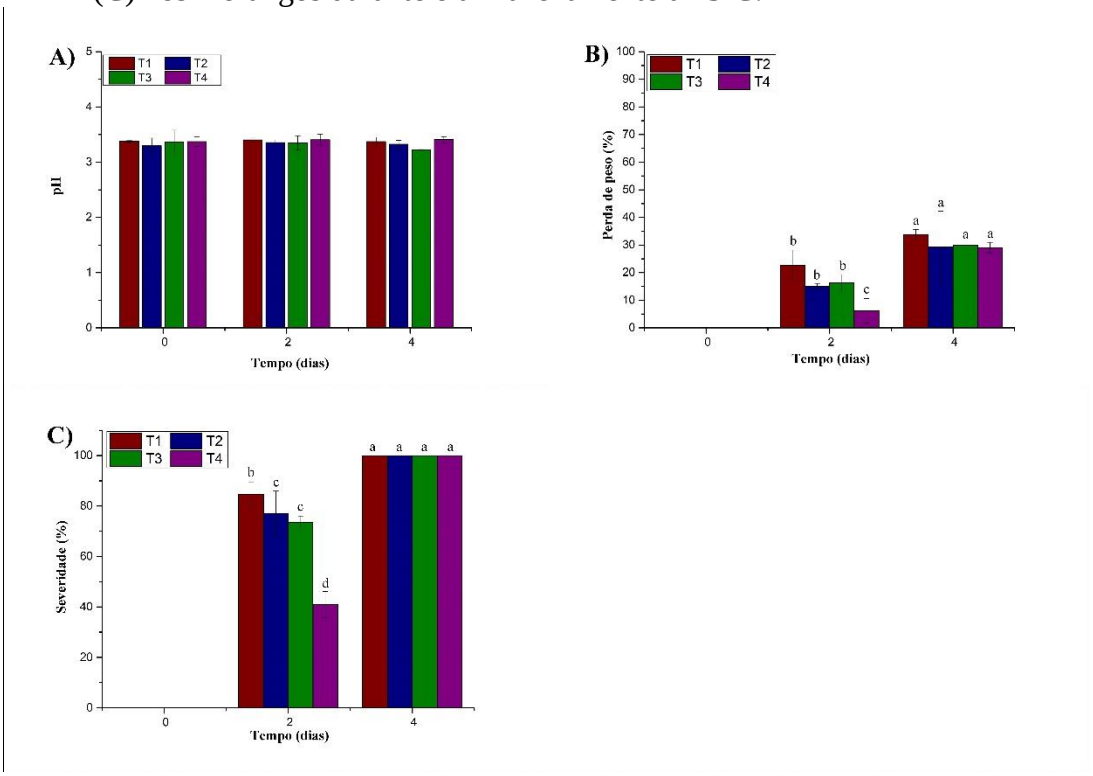
Ao incorporar um composto ativo em biopolímeros, como foi feito no revestimento comestível à base de amido, o composto ativo fica menos disponível e é liberado de forma

controlada, além de mascarar o aroma e o sabor característicos do OE, que podem dificultar a aceitação dos consumidores (MANZOOR *et al*., 2023). Essa liberação de forma controlada pode explicar a inibição do crescimento micelial após 48 horas de aplicação ter sido levemente superior à inibição do crescimento micelial após 24 horas e a concentração mínima inibitória ter sido menor, se comparado ao óleo puro, destacando o potencial do OE de cominho como meio de controle de *R. stolonifer.*

3.4 Avaliação do efeito dos revestimentos aplicados a morangos

Os resultados obtidos para as análises de pH estão apresentados na Figura 3A. As análises realizadas durante os quatro dias de armazenamento mostraram que os tratamentos com revestimentos aplicados aos morangos não influenciaram no pH dos frutos, que variou de 3,15 a 3,48.

Figura 3 – Efeito dos diferentes tratamentos no pH (A), na perda de peso (B) e na severidade (C) nos morangos durante o armazenamento à 25ºC.



Letras diferentes entre as colunas indicam diferença significativa entre os tratamentos, pelo teste de Skott-knott ($p<0,05$).
Fonte: Do autor (2023).

A perda de peso dos morangos durante o período de armazenamento está apresentada na Figura 3B. Pode-se perceber que no tratamento no qual foi aplicado o revestimento incorporado com a maior concentração de óleo essencial de cominho (T4), houve redução

significativamente à perda de peso dos morangos até o segundo dia de armazenamento, com perda de peso de 6,13%, enquanto o tratamento-controle (T1) apresentou perda de peso de 22,6%. Porém, observou-se que, no quarto dia de armazenamento, mesmo os tratamentos com revestimentos incorporados com óleo essencial (T3 e T4) não foram capazes de reduzir a perda de peso quando comparados ao tratamento-controle (T1).

De acordo com Melikoglu *et al*. (2022), a perda de peso durante o armazenamento de morangos é um parâmetro importante e diretamente relacionado a sua qualidade, visto que a perda de peso indica perda de textura e, consequentemente, de qualidade desses frutos. Indica-se que a perda máxima aceitável para evitar alterações na aparência do fruto seja de 6%, valor que se aproxima do obtido neste estudo para o tratamento com 30% de OEC (T4) (CAMPOS, KWIATKOWSKI e CLEMENTE, 2011).

Os resultados apresentados para a perda de peso se correlacionam com os obtidos para a severidade da doença, apresentados na Figura 3C. Os revestimentos contendo OEC conseguiram controlar a proliferação do fungo *R. stolonifer* até o segundo dia de armazenamento e, consequentemente, controlaram a perda de peso. A severidade da doença nos morangos que receberam o revestimento com 30% de OEC (T4) foi de 41% e naqueles que receberam o revestimento com 3% de OEC (T3) foi de 73,6%, enquanto o tratamento-controle (T1) alcançou 84,7% de severidade já no segundo dia de armazenamento, e os morangos que receberam o tratamento com o revestimento sem OEC (T2) alcançaram severidade de 77,08%.

Assim, pode-se perceber que os revestimentos à base de amido aplicado aos morangos influenciaram positivamente no controle de *R. stolonifer,* dificultando sua proliferação, sendo o T4 o tratamento no qual foram observados resultados mais promissores. Porém, após quatro dias, com a proliferação do fungo por todo o morango em todos os tratamentos, a perda de peso, assim como a severidade, foram estatisticamente iguais.

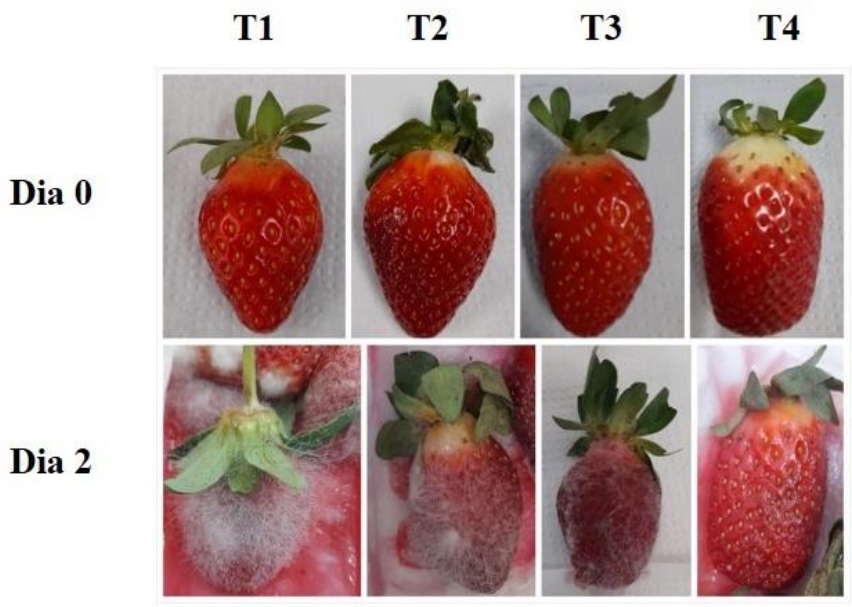
Nos estudos realizados por Oliveira *et al*. (2019), que aplicaram revestimentos de carboximetilcelulose combinado com óleo essencial de *Lippia sidoides* em morangos, os autores observaram que, no segundo dia de armazenamento, houve similaridade entre os tratamentos em relação à incidência da doença, que alcançou 100%. Esses autores ressaltam que *R. stolonifer* é um patógeno muito agressivo e que possui uma rápida taxa de crescimento, fatos que dificultam seu controle.

Na Figura 4, pode ser observada a aparência dos morangos durante o período de armazenamento. No segundo dia de avaliação, as diferenças entre os morangos que receberam o tratamento-controle (T1) e os que receberam tratamentos contendo óleo essencial,

especialmente T4, é facilmente visível, confirmando o potencial antifúngico do revestimento elaborado.

No quarto dia de armazenamento dos morangos a 25ºC, a severidade causada pela proliferação do fungo *R. stolonifer* foi alta em todos os tratamentos. Ressalta-se que isso pode ter acontecido devido à concentração do inóculo aplicada ao fruto e às condições favoráveis para o desenvolvimento do fungo durante o período de armazenamento (SILVA *et al*., 2020). Além disso, é importante considerar que, quando se trata de experimentos *in vivo*, pode ocorrer interações entre os componentes do revestimento, o OE e a complexa matriz alimentar que se apresenta como um meio rico em nutrientes e pode proporcionar um meio de crescimento ideal para a proliferação de fungos (Espitia *et al*., 2012; Feng e Zheng, 2007). Assim, de acordo com Oliveira *et al*. (2019), é esperado que *R. stolonifer* seja menos sensível aos tratamentos, quando cresce na superfície de morangos do que em ágar.

Figura 4 – Aspecto dos morangos tratados e armazenados a 25ºC.



Fonte: Do autor.

4 CONCLUSÃO

Nos resultados obtidos pode-se observar que, dentre os óleos essenciais e compostos-padrão avaliados *in vitro,* o óleo essencial de cominho e seu composto majoritário cuminaldeído apresentaram-se como os tratamentos mais eficientes quanto à inibição do crescimento micelial de *R. stolonifer*. A avaliação da atividade antifúngica *in vitro* de revestimentos comestíveis à base de amido de mandioca incorporados com óleo essencial de cominho também foi eficiente

na inibição do crescimento micelial do fungo em estudo. Os resultados *in vivo* mostraram que o óleo essencial de cominho incorporado ao revestimento comestível é capaz de controlar a proliferação de *R. stolonifer*; porém, não foi capaz de inibir seu crescimento em 48 horas, confirmando o potencial do OE de cominho como meio de controle de *R. stolonifer*.

5 FINANCIAMENTO

Este trabalho contou com o apoio da Fundação de Amparo à Pesquisa de Minas Gerais (Fapemig – 02390/18), do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – 311183/2022-0) e da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (Capes) – Código financeiro 001.

6 CONFLITOS DE INTERESSE

Os autores declaram que não há conflitos de interesse.

7 AGRADECIMENTOS

Às agências financiadoras pelas bolsas e apoio financeiro e à Central de Análises e Prospecção Química da Universidade Federal de Lavras, pelo fornecimento dos equipamentos para análises cromatográficas.

REFERÊNCIAS

ADAMS, R. P. **Identification of essential oils componentes by gas chromatography/ mass spectroscopy**. 4 ed. Carol Stream: Allured, 2017. p.804.

BAUTISTA-BAÑOS, S., BOSQUEZ-MOLINA, E., BARRERA-NECHA, L. *Rizhopus stolonifer* (soft rot). *In*: Bautista-Baños, S. (Ed.), Postharvest Decay: **Control Strategies.** Academic Press, United States of America, 2014, p. 1–44.

BRANDÃO, R. M. *et al*. Antifungal and antiocratoxigenic potential of *Alpinia speciosa* and *Cymbopogon flexuosus* essential oils encapsulated in poly (lactic acid) nanofibres against *Aspergillus* fungi. **Letters in Applied Microbiology**, v. 75, n. 2, p. 281-292, 2022.

BRASIL. **Agência Nacional de Vigilância Sanitária (Anvisa).** In: Farmacopeia Brasileira, Brasília, 5th ed.; p. 198-199, 2010.

CAMPOLINA, G. A. *et al*. Essential Oil and Plant Extracts as Preservatives and Natural Antioxidants Applied to Meat and Meat Products: A Review. **Food Technology & Biotechnology,** v.61, n.2, 2023.

CAMPOS, R. P.; KWIATKOWSKI, A.; CLEMENTE, E. Post-harvest conservation of organic strawberries coated with cassava starch and chitosan. **Revista Ceres**, v. 58, p. 554-560, 2011.

CARVALHO, I. T. **Microbiologia dos Alimentos**. Recife: EDUFRPE, 2010, p. 84.

ESPITIA, P. J. P. *et al*. Assessment of the efficiency of essential oils in the preservation of postharvest papaya in an antimicrobial packaging system. **Brazilian Journal of Food Technology**, v. 15, p. 333-342, 2012.

FENG, W.; ZHENG, X. Essential oils to control *Alternaria alternata in vitro* and *in vivo*. **Food control**, v. 18, n. 9, p. 1126-1130, 2007.

FERREIRA, D. C.; MOLINA, G.; PELISSARI, F. M. Effect of Edible Coating from Cassava Starch and Babassu Flour (Orbignya phalerata) on Brazilian Cerrado Fruits Quality. **Food and Bioprocess Technology**, v.13, p. 172-179, 2020.

FIDAN, H. *et al*. Chemical composition and antimicrobial activity of *Laurus nobilis* L. essential oils from Bulgaria. **Molecules**, v. 24, n. 4, p. 804, 2019.

GOBBO-NETO, L.; LOPES, N. P. Medicinal plants: factors of influence on the content of secondary metabolites. **Química Nova**, v. 30, p. 374-381, 2007.

KONG, J. *et al*. Antifungal effects of thymol and salicylic acid on cell membrane and mitochondria of *Rhizopus stolonifer* and their application in postharvest preservation of tomatoes. **Food Chemistry**, v. 285, p. 380-388, 2019.

LI, C. *et al*. Variation in compositions and biological activities of essential oils from four citrus species: Citrus limon, Citrus sinensis, Citrus paradisi, and Citrus reticulata. **Chemistry & Biodiversity**, v. 19, n. 4, p. e202100910, 2022.

LIN, Y. *et al*. The p-Anisaldehyde/β-cyclodextrin inclusion complexes as fumigation agent for control of postharvest decay and quality of strawberry. **Food Control**, v. 130, p. 108346, 2021.

MADIGAN, M. T.; MARTINKO, J. M.; PARKER, J. **Microbiologia de Brock**. 12.ed. São Paulo: Prentice Hall, 2016.

MANZOOR, A. *et al*. Plant-derived active substances incorporated as antioxidant, antibacterial or antifungal components in coatings/films for food packaging applications. **Food Bioscience**, p. 102717, 2023.

MELIKOĞLU, A. Y. *et al*. Development and characterization of edible films based on carboxymethyl cellulose enriched with pomegranate seed oil and the coating of strawberries. **Journal of Food Processing and Preservation**, v. 46, n. 7, p. e16607, 2022.

MIRI, Y. B.; DJENANE, D. Research Article Evaluation of Protective Impact of Algerian *Cuminum cyminum* L. and *Coriandrum sativum* L. Essential Oils on *Aspergillus flavus* Growth and Aflatoxin B1 Production**. Pakistan Journal of Biological Sciences**, 2018.

NAZZARO, F. *et al*. Essential oils and antifungal activity. **Pharmaceuticals**, v. 10, n. 4, p. 86, 2017.

OLIVEIRA, J. *et al*. Control of *Rhizopus stolonifer* in strawberries by the combination of essential oil with carboxymethylcellulose. **International journal of food microbiology**, v. 292, p. 150-158, 2019.

OSANLOO, M. *et al*. Studying the microbial, chemical, and sensory characteristics of shrimp coated with alginate sodium nanoparticles containing *Zataria multiflora* and *Cuminum cyminum* essential oils. **Food Science & Nutrition**, 2023.

R CORE TEAM (2016). R: **A language and environment for statistical computing. R Foundation for Statistical Computing**, Vienna. Disponível em: <https://www.R-project.org>.

REZENDE, J. L. *et al*. Antifungal potential of essential oils from two varieties of *Citrus sinensis* (lima orange and bahia navel orange) in postharvest control of *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. **Food Science and Technology**, v. 40, p. 405-409, 2020.

RIABOV, P. A. *et al*. The chemical, biological and thermal characteristics and gastronomical perspectives of *Laurus nobilis* essential oil from different geographical origin. **Industrial crops and products**, v. 151, p. 112498, 2020.

SILVA, P. P. M. *et al*. Essential oils from *Eucalyptus staigeriana* F. Muell. ex Bailey and *Eucalyptus urograndis* W. Hill ex Maiden associated to carboxymethylcellulose coating for the control of *Botrytis cinerea* Pers. Fr. and *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. in strawberries. **Industrial Crops and Products**, v. 156, p. 112884, 2020.

TABASSUM, N.; KHAN, M. A. Modifield atmosphere packaging of fresh-cut papaya using alginate based edible coating: quality evaluation and shelf life study. **Scientia Horticulturae**, v.259, p.1-9, 2020.

TAHMASEBI, M. *et al*. Control of nectarine fruits postharvest fungal rots caused by *Botrytis cinerea* and *Rhizopus stolonifer* via some essential oils. **Journal of food science and technology**, v. 57, p. 1647-1655, 2020.

TANAPICHATSAKUL, C.; KHRUENGSAI, S.; PRIPDEEVECH, P. *In vitro* and *in vivo* antifungal activity of *Cuminum cyminum* essential oil against *Aspergillus aculeatus* causing bunch rot of postharvest grapes. **PLoS One**, v. 15, n. 11, p. e0242862, 2020.

TOURNAS, V. H.; KATSOUDAS, E. Mould and yeast flora in fresh berries, grapes and citrus fruits. **International journal of food microbiology**, v. 105, n. 1, p. 11- 17, 2005.

UZSÁKOVÁ, V. *et al*. Antifungal activity of selected essential oils against *Rhizopus stolonifer*. **Journal of microbiology, biotechnology and food sciences**, v. 12, n. 2, p. e9396-e9396, 2022.

VAN DEN DOOL, H. and KRATZ, P.D. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. **J Chromatogr A**, v. 11, p. 463–471, 1963.

VENTURA-AGUILAR, R. I. *et al*. Monitoring the infection process of *Rhizopus stolonifer* on strawberry fruit during storage using films based on chitosan/polyvinyl alcohol/polyvinylpyrrolidone and plant extracts. **International Journal of Biological Macromolecules**, v. 182, p. 583-594, 2021.

VIEIRA, P. R. *et al*. Chemical composition and antifungal activity of essential oils from Ocimum species. **Industrial Crops and Products**, v. 55, p. 267-271, 2014.

XU, D. *et al*. Cuminaldehyde in cumin essential oils prevents the growth and aflatoxin B1 biosynthesis of *Aspergillus flavus* in peanuts. **Food Control**, v. 125, p. 107985, 2021.

YOON, J. H.; LEE, S. Y. Comparison of the effectiveness of decontaminating strategies for fresh fruits and vegetables and related limitations. **Critical reviews in food science and nutrition**, v. 58, n. 18, p. 3189-3208, 2017.

ZHOU, L. *et al*. Evaluation of the antifungal activity of individual and combined monoterpenes against *Rhizopus stolonifer* and *Absidia coerulea*. **Environmental Science and Pollution Research**, v. 26, p. 7804-7809, 2019.

3 CONSIDERAÇÕES FINAIS

Os óleos essenciais extraídos das sementes de cominho e folhas de louro, assim como seus respectivos constituintes majoritários, mostraram-se como potenciais antifúngicos e antiocratoxigênicos sobre *Aspergillus carbonarius, Aspergillus ochraceus, Aspergillus niger e Aspergillus westerdijkiae.*

O óleo essencial de cominho também se apresentou como o mais eficiente quanto à atividade antifúngica sobre *Rhizopus stolonifer* e sobre a mosca *Drosophila suzukii,* quando aplicado puro e incorporado a revestimentos comestíveis à base de amido de mandioca, prolongando a vida últil de morangos.

Infere-se, portanto, que os óleos essenciais estudados são pontenciais antifúngicos e inseticidas, e que, os revestimentos comestíveis podem ser uma opção biodegradável e de baixo custo para minimizar perdas causadas por fungos e insetos na cadeia produtiva de morangos e/ou outras frutas vermelhas e facilitar seu manejo sustentável.

Estima-se que os estudos relacionados à aplicação de óleos essenciais com possíveis substituintes a fungicidas e inseticidas sintéticos continuem sendo realizados e que seus resultados promissores sejam consolidados. Especialmente os estudos que utilizaram revestimentos comestíveis à base de amido de mandioca incorporados com óleos essenciais devem ser aprimorados para uma maior aceitação e consolidação no mercado.

APÊNDICES

APÊNDICE A

APÊNDICE A – Artigo elaborado durante o Doutorado: "Essential Oil and Plant Extracts as Preservatives and Natural Antioxidants Applied to Meat and Meat Products: A Review", volume: 61, páginas: 212 – 225, data de publicação: 07/03/2023.

Periódico: FTB Food Technology & Biotechnology
JCR: 2.4
DOI: 10.17113/ftb.61.02.23.7883

Essential Oil and Plant Extracts as Preservatives and Natural Antioxidants Applied to Meat and Meat Products: A Review

Running title: Natural Preservatives Applied to Meat Products

Gabriela Aguiar Campolina[1], Maria das Graças Cardoso[2]*, Alex Rodrigues Silva Caetano[2], David Lee Nelson[3] and Eduardo Mendes Ramos[1]

[1]Food Sciences Department, Federal University of Lavras (UFLA), Professor Edmir Sá Santos Rotatory Clover, 3037 Lavras, MG, Brazil
[2]Chemistry Department, Federal University of Lavras (UFLA), Professor Edmir Sá Santos Rotatory Clover, 3037 Lavras, MG, Brazil

[3]Postgraduate Program in Biofuels, Federal University of the Jequitinhonha and Mucuri Valleys, MGT 367 Highway Km 583,N/N Diamantina, MG, Brazil

SUMMARY

The meat and meat product industry has evolved according to the needs of the market. Consumers are increasingly seeking quality in food. Thus, the concern regarding the excessive use of additives such as preservatives and antioxidants has driven research towards natural, healthy and safe substitutes. Essential oils and plant extracts have been shown to be a good option for resolving this problem. They are completely natural with biological activity, which mainly includes prevention of oxidation and the proliferation of microorganisms, thus arousing the interest of the industry and consumers. This review will present studies published in the last five years regarding the potential of essential oils and plant extracts to act as preservatives and antioxidants in meat and meat products. The forms of application, innovations in the area, alternatives to the incorporation of essential oils and extracts in meat products, effects caused in food, and limitations of applications will be detailed and discussed.

Keywords: natural compounds; antimicrobial activity; antioxidant activity; meat industry; essential oils; plant extracts

INTRODUCTION

Meat can be defined as animal tissue that is suitable for human consumption. Like meat, its products are complex, highly perishable foods and have in their composition, in addition to proteins, saturated and unsaturated lipids, carbohydrates, vitamins and pigments that can undergo oxidation reactions and microbial deterioration. Thus, the shelf life of meat is influenced by several factors, such as storage temperature, enzyme action, oxygen, humidity, light and microorganisms. The influence of these factors is worrisome because they directly interfere with the quality of food, both nutritionally and in sensory aspects. They can cause changes in attributes such as texture, colour, odour, flavour and aroma (*1,2*).

Oxidation is a process that frequently occurs in meat during storage. The oxidation of lipids, proteins and pigments directly interferes with the sensory and nutritional quality of the product. In addition, toxic compounds can be produced (*1,3*). Oxidation is a factor that must be controlled in meat. However, the proliferation of microorganisms is a factor that deserves even more attention because of the harm they can cause to consumers.

The contaminating and spoilage microorganisms in meat are mostly pathogenic bacteria *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus aureus, Salmonella enterica* and *Escherichia coli*, which are responsible for foodborne outbreaks. The bacterial genera that deserve attention are *Acinetobacter, Alteromonas, Aeromonas, Brochothris, Flavobacterium, Leuconostoc, Pseudomonas, Moraxella,* lactic acid bacteria and those belonging to the *Enterobacteriaceae* family (*4,5*).

The use of food additives, such as preservatives and antioxidants, has been of global concern in recent years. One of the foods that generate greater concern regarding the use of additives is meat and its derived products. Originating from cattle, swine or poultry, meat and meat products are highly perishable, susceptible to the action of various microorganisms and lipid oxidation. Therefore, methods to maintain quality and increase their shelf life are required, one of the methods being the addition of antioxidants and preservatives.

The food industry has constantly sought ways to minimize the loss of quality in meat and its products and increase its shelf life. Conservation methods such as low temperature, specific packaging and adequate storage are frequently used. In addition, the use of additives that act as preservatives and antioxidants is often essential to ensure the quality of meat and its products. However, the use of synthetic additives can be harmful to health, and this fact is increasingly noticeable to consumers, who are searching for healthy foods that are more natural and prefer natural compounds (*6*). According to Lin and Wu (*6*), the fact that plants are the main natural source of antioxidants and, in general, do not pose risks to food safety, makes the use of plant derivatives as antioxidants valuable. Economic growth and the emergence of new technologies have also increased the demand for natural products. Thus, the application

of compounds such as essential oils and plant extracts that act as natural preservatives emerges as an interesting strategy to reduce or replace the use of traditional synthetic additives if they are equally efficient (*6,7*).

This review will address some alternatives researched in the last five years for the application of essential oils and plant extracts in meat and meat products with the aim of preserving and replacing, in whole or in part, synthetic preservatives and antioxidants generally applied to these foods. In addition, the mechanisms of antioxidant and antimicrobial action of the natural products under study will be briefly discussed.

ESSENTIAL OILS AND PLANT EXTRACTS

Essential oils are volatile organic compounds synthesized by plants in response to physiological stress, ecological factors and pathogen attack, as well as acting to attract pollinators to facilitate reproduction (*8*). They can be defined as 'the product obtained from a natural raw material of vegetable origin by steam distillation, from the epicarp of citrus fruits by mechanical processes or by dry distillation, after separation of the aqueous phase, if any, by physical processes'. ISO 9235:2021 (*9*) also emphasizes that steam distillation can be performed with the addition of water to the distillate, a process known as hydrodistillation.

The main characteristics of the essential oils are their complex compositions of low-molecular-mass molecules with different chemical structures that include monoterpenes, sesquiterpenes, alcohols, aldehydes, esters, ethers, ketones, various phenylpropanoid derivatives and various volatile organic compounds. In addition, they are liquid at room temperature and hydrophobic so they have low water solubility (*10,11*).

Vegetable extracts, unlike essential oils, are preparations obtained by the extraction of the active constituents of vegetables and must contain sapid, aromatic, volatile and fixed principles properties corresponding to the respective natural product. The active ingredients can be extracted using different solvents such as methanol, ethyl acetate, hexane, ethanol, or acetone, and the material used in the extraction can be previously treated by means of enzymatic inactivation, milling or degreasing. After extraction, undesirable compounds can also be eliminated by purifying the extract (*12,13*).

Essential oils and plant extracts, in addition to being natural products extracted from plants, are mostly considered to be GRAS (Generally Recognized as Safe), which allows their use in food products without posing risks to consumers. In addition, different biological activities can be attributed to them, including antioxidant and antimicrobial activity, depending on their compositions. Phenolic compounds, alcohols, aldehydes, phenylpropanoids, terpenes and ketones are the principal constituents responsible for the antioxidant activity of essential oils. They protect against pro-oxidants naturally present in meat, such as free iron ions (*1*). In

terms of antimicrobial activity, the constituents that stand out are those containing aromatic oxygen compounds with carbonyl groups (aldehydes and ketones), phenols, ethers or acids, followed by oxygenated aliphatic terpenes (*14*).

Plant extracts also contain phytochemicals of interest. Those that stand out, such as phenolic compounds, have antioxidant and antimicrobial activities. In particular, there are the tannins and flavonoids that can be subclassified into flavones, flavanones, flavonols, flavanonols, isoflavones, catechins and anthocyanidins (*15,16*).

MECHANISM OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AND PLANT EXTRACTS

Psychrotrophic *Pseudomonas*, lactic acid bacteria, *Enterobacteriaceae* and *Clostridium* spp. are one of the principal spoilage groups in freshly stored and refrigerated meat because they have the ability to be developed at temperatures below 7 °C. The activity of essential oils and plant extracts against microorganisms is directly related to their constituents. However, it is worth noting that the combination of constituents can act synergistically in the antimicrobial mechanism (*17-19*).

The principal mechanism of action of both essential oils and plant extracts involves the interaction with the cell membrane of microorganisms (Fig. 1). These natural compounds can act by increasing membrane permeability, inhibiting the absorption of substrates that are important for microbial growth, and interfering with the cellular metabolism (*20,21*).

Studies report that Gram-positive bacteria are more susceptible to the action of the constituents. This observation can be explained by the involvement of the lipopolysaccharide layer present in the cell wall of Gram-negative bacteria. This layer limits the diffusion of hydrophobic compounds, such as essential oils (*22*).

Thus, the mechanism of action of the essential oils involves the interaction of their constituents with the cell membranes of microorganisms, which are composed of lipids. The cell membrane, when interacting with constituents, can be damaged, leading to an increase in membrane permeability and impairment of functions in the cell such as nutrient uptake, electron transport, nucleic acid synthesis, enzyme activity and can even cause death. In addition, the constituent molecules of essential oils can cross the membrane and reach the cytoplasm, where they can react with other cellular components (*4,23*).

Fig. 1

MECHANISM OF ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS AND PLANT EXTRACTS

The oxidation of lipids present in meat leads to the formation of hydroperoxides, which in turn generate degradation products in meat and compounds such as volatile and

undesirable aldehydes, ketones, acids and alcohols. Protein oxidation causes changes in proteins and amino acids. Thus, the level of digestibility, solubility and bioavailability can be reduced. Pigments, such as myoglobin, which is one of the main pigments responsible for the colour of the product, form brown compounds when oxidized and thus affect the appearance of the meat (*24,25*).

The phenolic compounds present in plant extracts are considered to be the main group responsible for the antioxidant activity of the extracts. In essential oils, phenylpropanoids and terpenoids with phenolic characteristics also have antioxidant activities. These compounds can act in the stabilization of free radicals because their structures bear a hydroxyl group (-OH) on a benzene ring. Thus, they can act by transferring the H atom from the OH group to the free radical, as reducing agents and singlet oxygen inhibitors, as shown in Fig. 2 (21*,26,27*).

Fig. 2

APPLICATIONS OF ESSENTIAL OILS AND PLANT EXTRACTS AS PRESERVATIVES AND ANTIOXIDANTS IN FRESH MEAT AND MEAT PRODUCTS

The proliferation of microorganisms can cause deterioration and contamination of the product, making its commercialization and consumption unfeasible. Food industries, in general, add antioxidants and preservatives to meat products to preserve their microbiological, physicochemical and sensory characteristics. Thus, the application of essential oils and plant extracts to meat products has been shown to be a natural and efficient alternative to preserve these products, preventing the proliferation and action of microorganisms.

The application of natural compounds such as essential oil and plant extracts in meat products basically boils down to the direct application to the meat product, whether diluted or not, and application through nanoemulsions, nanoparticles and active and intelligent packaging, such as films and coatings. In all the forms of application, a combination with other conservation methods, such as refrigeration, freezing and appropriate packaging, is necessary.

Application of essential oils and plant extracts directly on meat and meat products: preservative and antioxidant activity

Some of the main studies of the direct application of essential oil and plant extracts to meat and meat products are presented in Table 1 (*22,28-32*) and Table 2 (*22,33-41*), respectively. Danilović *et al*. (*22*) evaluated the application of essential oils and sage extract to pork to control *E. coli*. Pork pieces were treated separately with essential oil and extract. The results showed that, after 14 days, a significant inhibition of *E. coli* growth was observed

in the treatments involving the addition of the essential oil at all tested concentrations. The treatment with sage extract had a smaller effect against the evaluated microorganism than the treatment with the essential oil, but the proliferation of the bacteria decreased to a concentration of 1.0 µL/g. However, regardless of the treatment used, the number of *E. coli* did not increase in the first eight days of storage, and treatments with essential oil and extract were considered by the authors to be effective methods of controlling this bacterium.

The effect of other essential oils on meat products as also presented relevant studies in the data collection achieved for the elaboration of in this review. Ozaki *et al*. (*29*), seeking to reduce nitrite in 'salaminho', a product made with pork and beef and fermented during processing, used the essential oil from oregano (100 mg/kg) together with radish powder (0.5 and 1 %). The salamis were stored for 30 and 60 days at 4 and 20 °C, and, despite the sensory acceptance and known activity of oregano essential oil, this oil did not inhibit lipid oxidation and did not show antimicrobial activity at the applied mass fraction. This observation was probably due to the added low mass fraction of oregano and a possible decrease in the concentration of bioactive compounds in its commercial oil (*29*). That study is a good example of the impasse between an effective amount of essential oil for antioxidant and antimicrobial activiity and the sensory acceptance of consumers. This fact is one of the reasons why the application of natural compounds through coatings, films and encapsulation is a better option. On the other hand, Fernandes *et al*. (*35*) observed antioxidant activity when they applied oregano extract directly to lamb hamburger as a possible substitute for the synthetic antioxidant sodium erythorbate and stored it for 120 days at -18 °C. In addition, the treated hamburgers did not differ from those produced with a synthetic antioxidant in terms of sensory acceptance.

Harbin sausage, a dry fermented sausage produced in Harbin (PR China), was evaluated by Sun *et al*. (*37*) after adding cinnamon, clove and anise extracts. The application of the extracts reduced the accumulation of biogenic amines, mainly in the treatment containing cinnamon extract, which inhibited the formation of six of the analyzed amines analyzed. The inhibitory effect of the extracts might be related to the inhibition of *Enterobacteriaceae* that can increase the production of biogenic amines such as tyramine, putrescine, cadaverine and histamine. The antimicrobial effect of the extracts is probably due to the synergism of their constituents. In the cinnamon extract, which proved to be the most efficient, the presence of eucalyptol and *trans*-cinnamaldehyde, compounds considered to be antimicrobial, might have cooperated for this effect. Anise extract contains antimicrobial constituents such as carvacrol, linalool, terpineol and eugenol, the last also being present in clove extract. The presence of polyphenols in the spice extracts also contributed to the observed antioxidant activity, which was higher in the anise extract. A major concern when adding extracts to foods is the alteration

of sensory characteristics. In this study, in addition to the improved microbiological characteristics in the presence of the spice extracts, the colour and attributes such as flavour, odour, acidity and acceptance received better scores than the control samples.

The use of natural products to replace, even partially, the nitrite preservative has been widely studied. This additive can favour the formation of N-nitrosamines when it reacts with the secondary amines present in the meat. These N-nitrosamines can lead to gastrointestinal cancer (*42*). Thus, the reduction of the use of nitrite in meat products is a factor of interest to researchers, industry and consumers, and the replacement of this preservative by natural compounds was demonstrated to be a good alternative.

Pinelli *et al*. (*28*) evaluated the partial replacement of nitrite by emulsions and nanoemulsions of the essential oils from oregano, lemon, cinnamon, cardamom and pepper in mortadella. Additive or synergistic actions among the components of these oils can be observed when they are mixed. The biological activity of interest increases because of this synergism, which permits the application in lower concentrations with smaller sensory alterations. Although no significant difference in the mean number of *Clostridium sporogenes* spores was observed between the treated samples and the control, the number of *C. sporogenes* cells was lower in the treated samples than in the control. Nitrite (75 ppm) was added to the control and the samples treated with the nanoemulsion of the essential oil mixture. Thus, treatments with an emulsion or nanoemulsion can be alternatives for the control of this microorganism in products such as mortadella because they were more efficient than nitrite itself. In addition to the microbial control, the treatments influenced the residual nitrite and the thiobarbituric acid reactive substances (TBARS) content. The residual nitrite content is expected to decrease during the storage of products made with cured meat, and this decrease indeed occurred. However, the final mass fraction of residual nitrite in mortadella treated with nanoemulsions was significantly higher than in the control, with values higher than 45 mg/kg. It is likely that there were interactions between the oils and nitrite that increased the antimicrobial activity of these treatments. Regarding the TBARS analysis, the lowest values were observed after treatments with emulsions or nanoemulsions. The presence of constituents that have antioxidant characteristics, such as the phenolic compounds present in the oils, leads to known and scientifically proven antioxidant activities. The emulsions and nanoemulsions have been shown to be a good alternative for reducing nitrite in bologna, but the used amounts must still be evaluated to reduce the sensory interference that was still unsatisfactory.

Yuan and Yuk (*36*) applied *Syzygium antisepticum* extract directly to cooked chicken in an attempt to inhibit the growth of *S. aureus*. The highest concentration used, 32 mg/mL, inhibited the growth of the microorganism, but the colour of the meat was altered, a fact that

would influence the consumer acceptance. The application of plant extracts and essential oils directly to food products, as already mentioned, can interfere with consumer acceptance because of the changes they cause in the food, such as colour, texture and aroma. For this reason, many researchers opt for application through nanoparticles, nanoemulsions, edible coatings or films.

Table 1

Table 2

Incorporation of essential oils and plant extracts in active packaging applied to meat and meat products: preservative and antioxidant activity

The application of essential oils and plant extracts to meat through packaging has been the main focus of many researchers today because it allows the incorporation of active compounds such as antioxidants and antimicrobials and reduces the likelihood of unpleasant sensory changes for the consumer (*43*). The principal research on the application of essential oils and plant extracts as antioxidants and antimicrobial preservatives incorporated into active packaging and used in meat and meat products is presented in Table 3 (*17,44-61*) and Table 4 (*20,47,55,62-67*). Mehdizadeh *et al*. (*47*) evaluated the conservation of beef packaged with cornstarch and chitosan-based films containing the essential oil from *Thymus kotschyanus* and pomegranate (*Punica granatum*) peel extract. A higher antioxidant and antimicrobial activity was observed of the films with combined essential oils and extract. The film containing oil (2 %) and extract (1 %) inhibited the growth of *Listeria monocytogenes* for 12 days. The effect of the films on the other evaluated microorganisms was also more significant when the oil and the extract were present together. This antimicrobial activity might be related to the principal constituents of the oil, thymol and carvacrol, and to the interactions of phenolic compounds in the extract with sulfhydryl groups of proteins found in bacterial structures.

Langroodi *et al*. (*55*) also evaluated the application of a combination of essential oils and extracts to beef. The results of the application of chitosan-based coatings with 1 % essential oil from *Zataria multiflora* and *Rhus coriaria* extract (2 and 4 %) showed that both the extract and the essential oil contributed to the antioxidant activity of the coatings, yielding significantly lower TBARS and peroxide values. The microbial activity was the lowest at the highest concentration of the extract, and the microbiological quality of all the samples was maintained for 20 days. On the other hand, the quality of the control samples was lost after the fifth day of storage. Therefore, an additive or synergistic effect against the evaluated microorganisms was observed when using the combination of the extract with the essential oil.

The ground beef product that undergoes minimal processing can be used for other products such as hamburgers and meatballs. This product has been evaluated in various

studies that applied the oils and extracts to determine the antioxidant and preservative activity of these natural compounds. Almasi *et al*. (*44*) developed films based on sodium alginate containing the essential oil from *Thymus vulgaris* to determine their antimicrobial activity on ground beef. These authors applied 0.05 and 0.04 % of the oil, respectively, using two different techniques, microemulsion and nanoemulsion, and they evaluated the antimicrobial activity of ground beef in contact with the film and under refrigeration. A significant antimicrobial activity against all the tested microorganisms was observed with the films made by the microemulsion technique, with the emphasis on the number of total mesophiles for which a decrease of 2 logarithmic cycles (100 times) relative to the control was found after eight days of storage. This activity is explained by the greater availability of the essential oil that comes into contact with the meat product when it is present in a microemulsion. In addition, the particles diffuse through the films more easily, which makes the oils more readily available to interfere with the cellular activities of microorganisms. The surfactant micelles formed in the films can fuse with the phospholipid bilayers that make up the cell membrane to increase the interaction with bacterial cells. This interaction thereby increases the antimicrobial activity, which can lead to cell death. Work by Akcan *et al.* (*63*) showed that interesting results were also obtained with meat products made from ground beef, such as meatballs and hamburgers. Films based on isolated whey proteins containing extracts of *Laurus nobilis* or *Salvia officinalis* were applied to cooked meatballs. Antioxidant activity throughout storage was observed in the presence of the films, but research to improve the sensory acceptability of the product is necessary. Subsequently, Amiri *et al*. (*46*) investigated the application of cornstarch-based films made by a nanoemulsion containing essential oils from *Zataria multiflora* and applied to hamburger steaks. The increase in pH during storage was lower with the films containing essential oils, and the oxidation of protein and lipid was also lower, especially with the nanoemulsions. The oxidative stability increased with the use of smaller nanoemulsion droplets. The product of this study was sensorially well accepted, but there was a decrease in the acceptability during the days of storage, whereas the control was unacceptable from the tenth day onwards.

Good results were also obtained when the red cabbage extract was incorporated into films based on starch and whey and applied to ground beef. Sanches *et al*. (*62*) observed that the films acted as antioxidants, especially at a amount of 64.15 %, which was sufficient to stabilize oxymyoglobin. This bright red pigment is a derivative of myoglobin, one of the main pigments responsible for meat colour (*2*). Sanches *et al*. (*62*) attributed the high concentration of anthocyanins present in the extract to the antioxidant activity of this film. In addition to helping to preserve the characteristics of the meat, the film possessed the ability to monitor the quality of the product through its colour change due to the change of pH value, and it was thus characterized as a smart packaging. According to the authors, the change in the colour

of the film occurred as a result of the colour change of the present anthocyanins. Anthocyanins are red or purple (due to the flavylium cation) at low pH, but at high pH, they turn blue (formation of quinoidal bases). If the pH continues to increase, the sample becomes colourless (formation of chalcones). High pH values in meat are indicative of microbial spoilage and protein degradation. Therefore, this type of packaging can indicate when the meat is unfit for consumption.

Smart packaging has also been designed for application to lamb meat (*20*). The film obtained from chitosan and methylcellulose nanofiber was incorporated with anthocyanin extract from saffron leaves. The extract was applied to meat that was stored for three days at 25 °C. The anthocyanins present in the extract were responsible for changing the colour of the film by altering the pH of the meat, which indicated the presence of deterioration. In addition to the indication of quality, the films indicated that antimicrobial and antioxidant activity existed, but these biological activities were not evaluated in the meat.

Lamb meat was also evaluated using films embedded with the essential oil from *Rosmarinus officinalis* and coatings embedded with the essential oil from *Satureja khuzestanica* (*45*). The films with rosemary essential oil (2 %) were made from whey proteins and had antioxidant and antimicrobial activities. The addition of rosemary oil was efficient to the point of extending the shelf life of the product from about six days to 12 to 15 days.

The coatings studied by Alizadeh-Sani *et al.* (*45*) were made with chitosan and savory essential oil (1 %) and had sufficient antioxidant and antimicrobial activities to exceed the recommended microbiological limit (7 log CFU/g) only after 20 days in the treated samples, whereas the control exceeded this limit after nine days of storage. Previously, Pabast *et al*. (*56*) studied the application of chitosan-based coatings and concluded that, even without the addition of essential oils, these coatings were able to reduce the pH and act as antimicrobial agents.

The projections of world consumption and production of chicken breast has increased in recent years (*49*). Several studies on the application of natural compounds to chicken meat have been performed. Hosseini *et al*. (*17*) studied the effect of adding the essential oil from *Aloysia citriodora* and *Syzygium aromaticum* to chicken breasts in the form of coatings. Sodium alginate-based coatings were made with each oil and the combination of the oils. Antioxidant and antimicrobial activities were observed of the oils, and the shelf life of the product increased. The use of a modified atmosphere increased the antibacterial effect, and the best effect was observed in the application of the coating containing two oils at 0.5 % of each. No significant difference between the treatments was observed in the sensory analysis. Good results were also observed with other essential oils, such as those of *Cuminum cyminum* (*50*), *Nigella sativa*

(*51*) and *Ziziphora persica* (*52*), which were applied to chicken meat through coatings and films, and preserved the meat stored at 4 °C for 9, 5 and 12 days, respectively.

Satisfactory results were obtained with the essential oil from *Rosmarinus officinalis* when it was incorporated into coatings and applied to chicken breasts (*57*). Because rosemary is a condiment commonly used in meat products in its natural form, consumers tend to recognize the odour and flavour of this plant and do not reject it in coated meat. Thus, the sensory evaluation of the product does not tend to have negative results. Films made from chitosan with rosemary oil were applied to chicken meat, and antioxidant and antimicrobial activities were measured. The total counts of mesophilic aerobic bacteria were lower in the samples treated with the active films. According to the authors, the antimicrobial activity of the films was related to chitosan, and the presence of phenolic compounds derived from rosemary essential oil increased the shelf life of the product. The control sample from the third day onwards was rejected. Thus, that study emerged as a new way to complement the necessary daily consumption of phenolic compounds.

Oregano is also a condiment widely used in food preparation. In addition to presenting biological activities of interest, it is able to improve the quality when applied to meat, mainly because of the action of its principal compounds, thymol and carvacrol, which are efficient inhibitors of bacterial growth. Xiong *et al*. (*67*) applied oregano essential oil incorporated into pectin-based coatings containing a resveratrol nanoemulsion to pork. The meat was stored for 20 days at 4 °C, whereas the total bacterial count in the control sample was considered microbiologically unacceptable from day 15 onwards, exceeding 7 log CFU/g. The treated samples remained below the limit during the 20 days of storage. Furthermore, lipid oxidation was lower with the treatments, whereas the limit of malondialdehye of 0.5 mg/kg was exceeded in the control on the fifth day. The authors concluded that the essential oil from oregano and resveratrol can scavenge free radicals and stop oxidation chain reactions.

The antioxidant activity in pork was reported by Song *et al*. (*68*), who observed lower TBARS values of the treated meat during storage than of the control when films containing green tea extract were applied. It was also observed that the changes in the TBARS values were insignificant in the extract-treated samples during storage.

Table 3

Table 4

LIMITATIONS OF THE APPLICATION OF ESSENTIAL OILS AND PLANT EXTRACTS IN MEAT AND MEAT PRODUCTS

The biological activity of essential oils and plant extracts is increasingly known among researchers, consumers and industries. The existing demand for healthy products can be met using these natural compounds of low toxicity (*4*).

The application of extracts and essential oils to meat and meat products as antioxidant and antimicrobial agents yields excellent results, as was presented in the previous chapters. However, the application of these natural compounds to food still faces some technological challenges. According to Silva *et al*. (*4*), the complexity of the composition of meat-based foods, such as amounts of proteins, lipids and moisture, among others, leads to the interaction of natural compounds with other components of the food, and thus, they are less readily available to act on microorganisms. Other properties, such as water activity and pH, can also influence the performance of natural compounds. Thus, food applications can require concentrations up to 100 times greater than those used in in vitro experiments.

The first point to observe for the application of a compound in food that will be offered to consumers is its safety. Despite being completely natural, some essential oils and plant extracts can be unsuitable for consumption in certain concentrations. Another important point to be observed is the form of application of the compounds. Despite the biological properties already described, the fact that they have a striking characteristic aroma and flavour makes their application in food difficult. To facilitate this application, the incorporation into edible, biodegradable coatings and films made with biopolymers are an alternative for the preservation of food. Thus, it is possible to obtain a material with the activity of interest while improving the value of the food (*43,69*).

The direct use of natural compounds in meat and meat products, as mentioned, can completely change the sensory characteristics of the product, and it might not be very acceptable to consumers, which limits its application (*70*). Danilović *et al*. (*22*) emphasized the fact that the oils and extracts can cause changes in odour and flavour, and therefore, they should be used in the lowest possible concentration. However, the concentration must be sufficient for the action of interest: antioxidant or antimicrobial activity, increase in the shelf life of the product, among others. According to Moraes-Lovison *et al*. (*54*), this challenge can be overcome by using encapsulation and nanoemulsification techniques for the application of natural compounds. These alternative applications of essential oils and plant extracts in meat and meat products can be presented as an economically viable industrial alternative. These, in addition to the advantages already mentioned throughout the text, are low cost, depending on the polymers and plant materials used in the process, easy production and, in general, they do not require high equipment costs (*71,72*).

CONCLUSIONS

Good results were obtained with essential oils and plant extracts when they were applied to beef, pork, goat and poultry. They acted by preserving the products, and consequently, increasing their shelf life. Antimicrobial and antioxidant activities of the extracts and essential oils were observed, and they are possible substitutes for synthetic additives. Many studies have suggested the application methods that have a lower impact on the sensory characteristics of meat products, such as application in films, coatings, emulsions and nanoemulsions. However, studies aimed at alternatives for the application of these natural compounds with the objective of impacting the sensory quality of the products as little as possible must still be explored.

ACKNOWLEDGEMENTS

The authors thank the Federal University of Lavras and funding agencies for the scholarships and financial support.

FUNDING

This work was supported by the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG – 02390/18);, the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – 311183/2022-0) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance code 001.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR´S CONTRIBUTIONS

Gabriela Aguiar Campolina: This author participated of design of the work, data collection, data analysis and interpretation, drafting the article and critical revision.

Maria das Graças Cardoso: This author participated of design of the work, drafting the article and critical revision;

Alex Rodrigues Silva Caetano: This author participated of the drafting the article and critical revision;

Eduardo Mendes Ramos: This author participated of the drafting the article and critical revision;

David Lee Nelson: This author participated of the critical revision.

ORCID ID

G. A. Campolina https://orcid.org/0000-0002-2592-2904
M. G. Cardoso https://orcid.org/0000-0001-8075-1725
A. R. S. Caetano https://orcid.org/0000-0003-2561-9646
D. L. Nelson https://orcid.org/ 0000-0001-7435-3675
E. M. Ramos https://orcid.org/0000-0002-8240-8151

REFERENCES

1. Pateiro M, Barba FJ, Domínguez R, Sant'Ana AS, Khaneghah AM, Gavahian M, *et al*. Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. Food Res Int. 2018;113:156-66.
https://doi.org/10.1016/j.foodres.2018.07.014

2. Gomide LAM, Ramos EM, Fontes PR, editors. Science and meat quality: Fundamentals. Viçosa, MG, Brazil: UFV; 2013.

3. Villalobos-Delgado LH, Mateo J, Caro I, Ramos MYL, Mendez NG, Cansino RG, Mondragón EGG. Natural antioxidants in fresh and processed meat. In: Galanakis CM, editor. Sustainable meat production and processing. London, UK: Academic Press; 2019. pp. 207-36.
https://doi.org/10.1016/B978-0-12-814874-7.00011-0

4. Silva BD, Bernardes PC, Pinheiro PF, Fantuzzi E, Roberto CD. Chemical composition, extraction sources and action mechanisms of essential oils: Natural preservative and limitations of use in meat products. Meat Sci. 2021;176:108463.
https://doi.org/10.1016/j.meatsci.2021.108463

5. Heredia N, García S. Animals as sources of food-borne pathogens: A review. Anim Nutr. 2018;4(3):250-5.
https://doi.org/10.1016/j.aninu.2018.04.006

6. Lin Y, Wu S. Vegetable soybean (*Glycine max* (L.) Merr.) leaf extracts: Functional components and antioxidant and anti-inflammatory activities. J Food Sci. 2021;86(6):2468-80.
https://doi.org/10.1111/1750-3841.15765

7. Nair MS, Nair DVT, Johny AK,. Venkitanarayanan K. Use of food preservatives and additives in meat and their detection techniques. In: Biswas AK, Mandal PK, editors. Meat quality analysis. Advanced methods, techniques, and technologies. London, UK: Academic Press; 2020. pp.187-213.
https://doi.org/10.1016/B978-0-12-819233-7.00012-4

8. Hashemi SMB, Khaneghah AM, Sant'Ana AS, editors. Essential oils in food processing: Chemistry, safety and applications. Hoboken, NJ, USA: John Wiley & Sons; 2018.

9. ISO 9235:2021. Aromatic natural raw materials – Vocabulary. Geneva, Switzerland: International Organization for Standardization (ISO); 2021. Available from: https://www.iso.org/obp/ui/#iso:std:iso:9235:ed-3:v1:en

10. Singh B, Singh JP, Kaur A, Singh N. Phenolic composition, antioxidant potential and health benefits of citrus peel. Food Res. Int. 2020;132:109-14.
https://doi.org/10.1016/j.foodres.2020.109114

11. Thormar H, editor. Lipids and essential oils as antimicrobial agents. Chichester, UK: John Wiley & Sons; 2011.
https://doi.org/10.1002/9780470976623

12. Brazilian Pharmacopoeia, vol. 1. Brasilia, Brazil: National Health Surveillance Agency (ANVISA), Ministry of Health; 2019. Available from: https://www.gov.br/anvisa/pt-br/assuntos/farmacopeia/farmacopeia-brasileira/arquivos/8025json-file-1.

13. Ordinance No. 2, of January 15, 2007: Technical regulation on flavoring additives. Brasília, Brazil: National Health Surveillance Agency (ANVISA), Ministry of Health; 2007.

14. Nogueira JO, Campolina GA, Batista LR, Alves E, Caetano ARS, Brandão RM, *et al*. Mechanism of action of various terpenes and phenylpropanoids against *Escherichia coli* and *Staphylococcus aureus*. FEMS Microbiol Lett. 2021;368(9):fnab052.
https://doi.org/10.1093/femsle/fnab052

15. Difonzo G, Squeo G, Pasqualone A, Summo C, Paradiso VM, Caponio F. The challenge of exploiting polyphenols from olive leaves: addition to foods to improve their shelf-life and nutritional value. J Sci Food Agric. 2021;101(8):3099-116.
https://doi.org/10.1002/jsfa.10986

16. Pereira RJ, Cardoso MG. Vegetable secondary metabolites and antioxidants benefits. J Biotechnol Biodivers. 2012;3(4):146-52.

17. Hosseini M, Jamshidi A, Raeisi M, Azizzadeh M. Effect of sodium alginate coating containing clove (*Syzygium aromaticum*) and lemon verbena (*Aloysia citriodora*) essential oils and different packaging treatments on shelf life extension of refrigerated chicken breast. J Food Process Preserv. 2021;45(3):14946.
https://doi.org/10.1111/jfpp.14946

18. Wicramasinghe NN, Ravensdale J, Coorey R, Chandry SP, Dykes GA. The predominance of psychrotrophic pseudomonads on aerobically stored chilled red meat. Compr Rev Food Sci Food Saf. 2019;18(5):1622-35.
https://doi.org/10.1111/1541-4337.12483

19. Pellissery AJ, Vinayamohan PG, Amalaradjou MAR, Venkitanarayanan K. Spoilage bacteria and meat quality. In: Biswas AK, Mandal PK, editors. Meat quality analysis. Advanced methods, techniques, and technologies. London, UK: Academic Press; 2020. pp. 307-34. https://doi.org/10.1016/B978-0-12-819233-7.00017-3

20. Alizadeh-Sani M, Tavassoli M, Mcclements DJ, Hamishehkar H. Multifunctional halochromic packaging materials: *Saffron petal* anthocyanin loaded-chitosan nanofiber/methyl cellulose matrices. Food Hydrocoll. 2021;111:106237. https://doi.org/10.1016/j.foodhyd.2020.106237

21. Nikmaram N, Budaraju S, Barba FJ, Lorenzo JM, Cox RB, Mallikarjunan K, Roohinejad S. Application of plant extracts to improve the shelf-life, nutritional and health-related properties of ready-to-eat meat products. Meat Sci. 2018;145:245-55. https://doi.org/10.1016/j.meatsci.2018.06.031

22. Danilović B, Đorđević N, Milićević B, Šojić B, Pavlić B, Tomović V, Savić D. Application of sage herbal dust essential oils and supercritical fluid extract for the growth control of *Escherichia coli* in minced pork during storage. LWT - Food Sci Technol. 2021;141:1109355. https://doi.org/10.1016/j.lwt.2021.110935

23. Nazzaro F, Fratianni F, Martino L, Coppola R, Feo VD. Effect of essential oils on pathogenic bacteria. Pharmaceuticals. 2013;6(12):1451-74. https://doi.org/10.3390/ph6121451

24. Domínguez R, Barba FJ, Gómez B, Putnik P, Bursać Kovačević D, Pateiro M, *et al*. Active packaging films with natural antioxidants to be used in meat industry: A review. Food Res Int. 2018;113:93-101. https://doi.org/10.1016/j.foodres.2018.06.073

25. Lorenzo JM, Pateiro M, Domínguez R, Barba FJ, Putnik P, Bursać Kovačević D, *et al*. Berries extracts as natural antioxidants in meat products: A review. Food Res Int. 2018;106:1095-104. https://doi.org/10.1016/j.foodres.2017.12.005

26. Ferreira VRF, Brandão RM, Freitas MP, Sazck AA, Felix FS, Silla JM, *et al*. Colorimetric, electroanalytical and theoretical evaluation of the antioxidant activity of *Syzygium aromaticum* L., *Origanum vulgare* L., *Mentha spicata* L. and *Eremanthus erythropappus* M. essential oils, and their major constituents. New J Chem. 2019;43(20):7653-62. https://doi.org/10.1039/C8NJ05893H

27. Papuc C, Goran GV, Predescu CN, Nicorescu V, Stefan G. Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products:

Classification, structures, sources, and action mechanisms. Compr Rev Food Sci Food Saf. 2018;16(6):1243-68.
https://doi.org/10.1111/1541-4337.12298

28. Pinelli JJ, Martins HHD, Guimaraes AS, Isidoro SR, Gonçalves MC, Moraes TSJ, *et al*. Essential oil nanoemulsions for the control of *Clostridium sporogenes* in cooked meat product: An alternative? LWT - Food Sci Technol. 2021;143:111123.
https://doi.org/10.1016/j.lwt.2021.111123

29. Ozaki MM, Santos M, Ribeiro WO, Ferreira NCD, Picone CSF, Domínguez R, *et al*. Radish powder and oregano essential oil as nitrite substitutes in fermented cooked sausages. Food Res Int. 2021;140:109855.
https://doi.org/10.1016/j.foodres.2020.109855

30. Boskovic M, Djordjevic J, Ivanovic J, Janjic J, Zdravkovic N, Glisic N, *et al*. Inhibition of *Salmonella* by thyme essential oil and its effect on microbiological and sensory properties of minced pork meat packaged under vacuum and modified atmosphere. Int J Food Microbiol. 2017;258:58-67.
https://doi.org/10.1016/j.ijfoodmicro.2017.07.011

31. Sirocchi V, Devlieghere F, Peelman N, Sagratini, G, Maggi F, Vittori S, Ragaert P. Effect of *Rosmarinus officinalis* L. essential oil combined with different packaging conditions to extend the shelf life of refrigerated beef meat. Food Chem. 2017;221:1069-76.
https://doi.org/10.1016/j.foodchem.2016.11.054

32. Bakhtiary F, Sayevand HR, Khaneghah AM, Haslberger AG, Hosseini H. Antibacterial efficacy of essential oils and sodium nitrite in vacuum processed beef fillet. Appl Food Biotechnol. 2018;5(1):1-10.
https://doi.org/10.22037/afb.v5i1.17118

33. Alirezalu K, Hesari J, Yaghoubi M, Khaneghah AM, Alirezalu A, Pateiro M, Lorenzo JM. Combined effects of ε-polylysine and ε-polylysine nanoparticles with plant extracts on the shelf life and quality characteristics of nitrite-free frankfurter-type sausages. Meat Sci. 2021;172:108318.
https://doi.org/10.1016/j.meatsci.2020.108318

34. Firuzi MR, Niakousari M, Eskandari MH, Keramat M, Gahruie HH, Khaneghah AM. Incorporation of pomegranate juice concentrate and pomegranate rind powder extract to improve the oxidative stability of frankfurter during refrigerated storage. LWT - Food Sci Technol. 2019;102:237-45.
https://doi.org/10.1016/j.lwt.2018.12.048

35. Fernandes RPP, Trindade MA, Tonin FG, Pugine SMP, Lima CG, Lorenzo JM, Melo MP. Evaluation of oxidative stability of lamb burger with *Origanum vulgare* extract. Food Chem. 2017;233:101-9.
https://doi.org/10.1016/j.foodchem.2017.04.100

36. Yuan WQ, Yuk HG. Antimicrobial efficacy of *Syzygium antisepticum* plant extract against *Staphylococcus aureus* and methicillin-resistant *S. aureus* and its application potential with cooked chicken. Food Microbiol. 2018;72:176-84.
https://doi.org/10.1016/j.fm.2017.12.002

37. Sun QX, Zhao XX, Chen HS, Zhang C, Kong B. Impact of spice extracts on the formation of biogenic amines and the physicochemical, microbiological and sensory quality of dry sausage. Food Control. 2018;92:190-200.
https://doi.org/10.1016/j.foodcont.2018.05.002

38. Turgut SS, Isikci F, Soyer A. Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. Meat Sci. 2017;129:111-9.
https://doi.org/10.1016/j.meatsci.2017.02.019

39. Fan XJ, Liu SZ, Li HH, He J, Feng JT, Zhang X, Yan H. Effects of *Portulaca oleracea* L. extract on lipid oxidation and color of pork meat during refrigerated storage. Meat Sci. 2019;147:82-90.
https://doi.org/10.1016/j.meatsci.2018.08.022

40. Alirezalu K, Hesari J, Nemati Z, Munekata PES, Barba FJ, Lorenzo JM. Combined effect of natural antioxidants and antimicrobial compounds during refrigerated storage of nitrite-free frankfurter-type sausage. Food Res Int. 2019;120:839-50.
https://doi.org/10.1016/j.foodres.2018.11.048

41. Carvalho FAL, Lorenzo JM, Pateiro M, Bermudez R, Purriños L, Trindade MA. Effect of guarana (*Paullinia cupana*) seed and pitanga (*Eugenia uniflora* L.) leaf extracts on lamb burgers with fat replacement by chia oil emulsion during shelf life storage at 2 °C. Food Res Int. 2019;125:108554.
https://doi.org/10.1016/j.foodres.2019.108554

42. Shpaizer A, Nussinovich A, Kanner J, Tirosh O. S-nitroso-N-acetylcysteine generates less carcinogenic N-nitrosamines in meat products than nitrite. J Agric Food Chem. 2018;66(43):11459-67.
https://doi.org/10.1021/acs.jafc.8b04549

43. Galindo MV, Paglione IS, Alvim ID, Sakanaka LS, Grosso CRF, Shirai MA. Methods of incorporation of D-limonene microparticles in edible films. Semina: Ciências Exatas e Tecnológicas. 2020;41:43-50.

https://doi.org/10.5433/1679-0375.2020v41n1p43

44. Almasi L, Radi M, Amiri S, Mcclements DJ. Fabrication and characterization of antimicrobial biopolymer films containing essential oil-loaded microemulsions or nanoemulsions. Food Hydrocoll. 2021;117:106733.

https://doi.org/10.1016/j.foodhyd.2021.106733

45. Alizadeh-Sani M, Mohammadian E, McClements DJ. Eco-friendly active packaging consisting of nanostructured biopolymer matrix reinforced with TiO_2 and essential oil: Application for preservation of refrigerated meat. Food Chem. 2020;322:126782.

https://doi.org/10.1016/j.foodchem.2020.126782

46. Amiri E, Aminzare M, Azar HH, Mehrasbi MR. Combined antioxidant and sensory effects of corn starch films with nanoemulsion of *Zataria multiflora* essential oil fortified with cinnamaldehyde on fresh ground beef patties. Meat Sci. 2019;153:66-74.

https://doi.org/10.1016/j.meatsci.2019.03.004

47. Mehdizadeh T, Tajik H, Langroodi AM, Molaei R, Mahmoudian A. Chitosan-starch film containing pomegranate peel extract and *Thymus kotschyanus* essential oil can prolong the shelf life of beef. Meat Sci. 2020;163:108073.

https://doi.org/10.1016/j.meatsci.2020.108073

48. Liu Q, Zhang M, Bhandari B, Xu JC, Yang C. Effects of nanoemulsion-based active coatings with composite mixture of star anise essential oil, polylysine, and nisin on the quality and shelf life of ready-to-eat Yao meat products. Food Control. 2020;107:106771.

https://doi.org/10.1016/j.foodcont.2019.106771

49. The production and consumption of chicken meat in Brazil and in the world. Brazil: FPA – Agricultural Parliamentary Front; 2017. Available from: https://agencia.fpagropecuaria.org.br/2017/01/10/producao-e-consumo-de-carne-de-frango-no-brasil-e-no-mundo/.

50. Shahvandari F, Khaniki GJ, Shariatifar N, Mahmoudzadeh M. Chitosan/cumin (*Cuminum cyminum* L.) Essential oil edible biodegradable coating: Its effect on microbial, physical and sensory properties of chicken meat during refrigeration. Carpathian J Food Sci Technol. 2021;13(1):75-89.

https://doi.org/10.1016/j.foodcont.2018.03.047

51. Takma DK, Korel F. Active packaging films as a carrier of black cumin essential oil: Development and effect on quality and shelf-life of chicken breast meat. Food Packag. Shelf Life. 2019;19:210-17.

https://doi.org/10.1016/j.fpsl.2018.11.002

52. Hamedi H, Kargozari M, Shotorbani PM, Mogadam NB, Fahimdanesh. A novel bioactive edible coating based on sodium alginate and galbanum gum incorporated with essential oil of *Ziziphora persica*: The antioxidant and antimicrobial activity, and application in food model. Food Hydrocoll. 2017;72:35-46.
https://doi.org/10.1016/j.foodhyd.2017.05.014

53. Hadian M, Rajaei A, Mohsenifar A, Tabatabaei M. Encapsulation of *Rosmarinus officinalis* essential oils in chitosan-benzoic acid nanogel with enhanced antibacterial activity in beef cutlet against *Salmonella typhimurium* during refrigerated storage. LWT - Food Sci Technol. 2017;84:394-401.
https://doi.org/10.1016/j.lwt.2017.05.075

54. Moraes-Lovison M, Marostegan LFP, Peres MS, Menezes IF, Ghiuraldi M, Rodrigues RAF, *et al*. Nanoemulsions encapsulating oregano essential oil: Production, stability, antibacterial activity and incorporation in chicken pâté. LWT - Food Sci Technol. 2017;77:233-40.
https://doi.org/10.1016/j.lwt.2016.11.061

55. Langroodi AM, Tajik H, Mehdizadeh T, Moradi M, Kia EM, Mahmoudian A. Effects of sumac extract dipping and chitosan coating enriched with *Zataria multiflora* Boiss oil on the shelf-life of meat in modified atmosphere packaging. LWT - Food Sci Technol. 2018;98:372-80.
https://doi.org/10.1016/j.lwt.2018.08.063

56. Pabast M, Shariatifar N, Beikzadeh S, Jahed G. Effects of chitosan coatings incorporating with free or nano-encapsulated Satureja plant essential oil on quality characteristics of lamb meat. Food Control. 2018;91:185-92.
https://doi.org/10.1016/j.foodcont.2018.03.047

57. Souza VGL, Pires JRA, Vieira ET, Coelhoso IM, Duarte MP, Fernando AL. Activity of chitosan-montmorillonite bionanocomposites incorporated with rosemary essential oil: From *in vitro* assays to application in fresh poultry meat. Food Hydrocoll. 2019;89:241-52.
https://doi.org/10.1016/j.foodhyd.2018.10.049

58. Vafania B, Fathi M, Soleimanian-Zad S. Nanoencapsulation of thyme essential oil in chitosan-gelatin nanofibers by nozzle-less electrospinning and their application to reduce nitrite in sausages. Food Bioprod Process. 2019;116:240-48.
https://doi.org/10.1016/j.fbp.2019.06.001

59. Pires JRA, Souza VGL, Fernando AL. Chitosan/montmorillonite bionanocomposites incorporated with rosemary and ginger essential oil as packaging for fresh poultry meat. Food Packag Shelf Life. 2018;17:142-49.
https://doi.org/10.1016/j.fpsl.2018.06.011

60. Ahmed J, Arfat YA, Bher A, Mulla M, Jacob H, Auras R. Active chicken meat packaging based on polylactide films and bimetallic Ag-Cu nanoparticles and essential oil. J Food Sci. 2018;83(5):1299-310.
https://doi.org/10.1111/1750-3841.14121

61. Azarifar M, Ghanbarzadeh B, Khiabani MS, Basti AA, Abdulkhani A. The effects of gelatin-CMC films incorporated with chitin nanofiber and *Trachyspennum ammi* essential oil on the shelf life characteristics of refrigerated raw beef. Int J Food Microbiol. 2020;318:108493.
https://doi.org/10.1016/j.ijfoodmicro.2019.108493

62. Sanches MAR, Camelo-Silva C, Carvalho CD, Mello JR, Barroso NG, Barros ELS, *et al*. Active packaging with starch, red cabbage extract and sweet whey: Characterization and application in meat. LWT - Food Sci. Technol. 2021;135:110275.
https://doi.org/10.1016/j.lwt.2020.110275

63. Akcan T, Estevez M, Serdaroglu M. Antioxidant protection of cooked meatballs during frozen storage by whey protein edible films with phytochemicals from *Laurus nobilis* L. and *Salvia officinalis*. LWT - Food Sci Technol. 2017;77:323-31.
https://doi.org/10.1016/j.lwt.2016.11.051

64. Noor S, Bhat ZF, Kumar S, Mudiyanselage RJ. Preservative effect of *Asparagus racemosus*: A novel additive for bioactive edible films for improved lipid oxidative stability and storage quality of meat products. Meat Sci. 2018;139:207-12.
https://doi.org/10.1016/j.meatsci.2018.02.001

65. Alirezalu K, Movlan HS, Yaghoubi M, Pateiro M, Lorenzo JM. ε-polylysine coating with stinging nettle extract for fresh beef preservation. Meat Sci. 2021;176:108474.
https://doi.org/10.1016/j.meatsci.2021.108474

66. Xiong Y, Chen M, Warner RD, Fang ZX. Incorporating nisin and grape seed extract in chitosan-gelatine edible coating and its effect on cold storage of fresh pork. Food Control. 2020;110:107018.
https://doi.org/10.1016/j.foodcont.2019.107018

67. Xiong Y, Li SM, Warner RD, Fang Z. Effect of oregano essential oil and resveratrol nanoemulsion loaded pectin edible coating on the preservation of pork loin in modified atmosphere packaging. Food Control. 2020;114:107226.
https://doi.org/10.1016/j.foodcont.2020.107226

68. Song XC, Canellas E, Wrona M, Becerril R, Nerin C. Comparison of two antioxidant packaging based on rosemary oleoresin and green tea extract coated on polyethylene terephthalate for extending the shelf life of minced pork meat. Food Packag Shelf Life. 2020;26:100588.

https://doi.org/10.1016/j.fpsl.2020.100588

69. Morales-Olán G, Ríos-Corripio MA, Hernández-Cázares AS, Zaca-Morán P, Luna-Suárez S, Rojas-López M. Effect of chitosan nanoparticles incorporating antioxidants from *Salvia hispanica* L. on the amaranth flour films. Food Technol Biotechnol. 2022;60(1):52-66.

https://doi.org/10.17113/ftb.60.01.22.7144

70. Drosinos EH, Skandamis PN, Mataragas M. Antimicrobials treatment In: Toldrá, F, editor. Safety of meat and processed meat. New York, NY, USA: Springer Science; 2009. pp. 255-96.

https://doi.org/10.1007/978-0-387-89026-5_10

71. Falguera V, Quintero JP, Jiménez A, Muñoz JA, Ibarz A. Edible films and coatings: Structures, active functions and trends in their use. Trends Food SciTechnol. 2011;22:292-303.

https://doi.org/10.1016/j.tifs.2011.02.004

72. Caetano ARS, Cardoso MG, Resende MLV, Chalfuon SM, Martins MA, Gomes HG, *et al*. Antifungal activity of poly(ε-caprolactone) nanoparticles incorporated with Eucalyptus essential oils against *Hemileia vastatrix*. Lett Appl Microbiol. 2022:75(4):1028-41.https://doi.org/10.1111/lam.13782

Table 1. Essential oils as antioxidants and/or preservatives applied directly to meat and meat products

Essential oil/species	Major constituent/%	Form of application	Effect	Dose used	Product	Storage condition Time/day *t*/°C	Reference
Sage (*Salvia officinalis*)	-	Direct	AM (*E. coli*)	0.4 and 0.6 µL/g	Minced pork	14 4	(*22*)
Oregano (*Origanum vulgare*) Cinnamon (*Cinnamomum zeylanicum*) Tahiti lemon (*Citrus aurantifolia*) Cardamom (*Elettaria cardamomo*) Chinese pepper (*Litsea cubeba*)	-	Essential oil, emulsions and nanoemulsions	AM (*C. sporogenes*) NR AO	0.2325 and 0.27 %	Mortadella	20 14	(*28*)
Oregano (*Origanum vulgare*)	Carvacrol 77.19	Direct, combined with 0.5 and 1 % radish powder	NR	100 mg/kg	Fermented cooked sausages (pork/beef meat)	30 and 60 4 and 20	(*29*)
Thyme	Thymol 50.48	Direct	AM (*Salmonella* (*S. enteritidis, S.* Typhimurium*, S.*	0.3, 0.6 and 0.9 %	Pork meat	15 (3±1)	(*30*)

			montevideo and *S. infantis*)				
Rosemary (*Rosmarinus officinalis*)	1,8-cineole 36.2, camphor 16.4	Spraying on packaging	AM (*Pseudomonas* spp., *Brochothrix thermosphacta, Enterobacteriacea*)	4 %	Beef meat	20 4 Extended shelf life up to 15, 4 to 5 more days than the control	(*31*)
Zataria multiflora,*Origanum vulgare* L., *Satureja bachtiarica*	Carvacrol 35.5, thymol 22; Carvacrol 29, γ-terpinene 20;Carvacrol 46, thymol 28.5	Direct	AO (*C. perfringens* and *C. sporogenes*) NR	1- 0.355 and 0.71 % 2- 0.395 and 0.79 % 3- 0.275, 0.55 and 1.1 %	Beef meat	30 room	(*32*)

AM=antimicrobial, AO=antioxidant, NR=nitrite reduction

Table 2. Plant extracts as antioxidants or preservatives applied directly to meat and meat products

Plant extract/species	Form of application	Effect	Dose used	Product	Storage Condition Time/day *t*/°C	Reference
Sage (*Salvia officinalis*)	Direct	AM (*E. coli*)	0.4, 0.6 and 1.0 µL/g	Pork meat	14 4	(*22*)
Olive leaves, green tea stinging nettle	ε-polylysine nanoparticles	AM (*S. aureus, E. coli* and *C. perfringens*) AO, NR	500 ppm (mixed extract)	Sausage	45 4	(*33*)
Pomegranate (*Punica granatum*) peels	Direct	AO AM (aerobic bactéria)	17.25 mg/kg	Sausage	60 4	(*34*)
Oregano (*Origanum vulgare*)	Direct	AO	13.32, 17.79 and 24.01 mL/kg	Lamb burger	120(-18±1)	(*35*)
Syzygium antisepticum	Direct application by dipping into the solution	AM (*S. aureus*)	2, 8 and 32 mg/mL	Cooked chicken	5 4 and 10	(*36*)
Cinnamon, clove, anise	Direct	Reduction of the accumulation of biogenic amines, AO, AM (total aerobic bacterial counts, *Enterobacteriaceae*)	0.3 g/kg	Harbin dry sausage (pork meat)	9 under fermentation	(*37*)
Pomegranate (*Punica granatum*) peels	Direct	AO	0.5 and 1.0 %	Beef meatball	180 (18±1)	(*38*)
Purslane (*Portulaca oleracea*)	Pulverization	AO and AM (*P. aeruginosa*, *B. subtilis* and *B. cereus*)	0.25, 0.50 and 1.0 %	Pork meat	9 4	(*39*)
Olive leaves,	Direct	Nitrite replacement	500 ppm	Sausage	45 4	(*40*)

green tea, stinging nettle		AO AM (total bacterial count, yeasts and moulds)	(mixed extract)				
Guarana seed, pitanga leaf	Direct	AO	250 mg/kg	Lamb burger	18	(2±1)	(*41*)

AM=antimicrobial, AO=antioxidant, NR=nitrite reduction

Table 3. Essential oil as antioxidants or preservatives applied to meat and meat products in the form of films and coatings

Essential oil/species	Majority constituent/%	Form of application	Effect	Dose used	Product	Storage condition Time/day *t*/°C	Reference
Thyme (*Thymus vulgaris* L.)	Thymol 47	Sodium alginate-based films - Micro and nanoemulsion	ATM (coliforms, *S. aureus*, lactic acid bacteria, moulds and yeasts)	0.05 and 00.04 %	Ground meat	8 (4.0±1)	(*44*)
Lemon verbena (*Aloysia citriodora*) Clove (*Syzygium aromati*)	Eugenol 14.63 D-Limonene 12.41 Eugenol 79.4	Sodium alginate-based coatings with and without modified atmosphere	AM (total bacterial count, *Pseudomonas*, lactic acid bacteria, psychrotrophic bacteria, *Enterobacteriaceae*, moulds and yeasts) AO	0.2 and 0.5 %	Chicken breast	15 in refrigeration	(*17*)
Rosemary (*Rosmarinus officinalis*)	1,8-cineole 27.52 α-pinene 21.15	Whey protein isolate-based film	AM (total count of psychrotrophic bacteria) AO	2 %	Lamb meat	15 (4.0±1)	(*45*)
Zataria multiflora	Thymol 37.94	Corn starch films	AO	6 %	Ground beef patties	20 (4.0±1)	(*46*)
Thymus kotschyanus	Thymol 26.61 Carvacrol 12.60	Films based on corn starch and chitosan	AM (*Pseudomonas*, lactic acid bacteria and *L. monocytogenes*) AO	1 and 2 %	Beef	21 (4.0±1)	(*47*)
Star anise (*Illicium verum*)	-	Coating based on soy protein isolate and lectin with nisin and polylysin	AM (viable aerobic bacteria and *E. coli*)	0.4 and 0.6 %	Yao meat	20 (4.0±1)	(*48*)
Cumin (*Cuminum cyminum*)	-	Chitosan-based coating	AM (total count of bacteria, *Enterobacteriaceae*, *S. aureus*, *E. coli*, mold and	0.2, 0.4 and 0.6 %	Chicken meat	9 days, 4.0 °C	(*49*)

			yeasts), AO				
Black cumin (*Nigella sativa*)	-	Multilayer film based on chitosan and alginate	ATM (*S. aureus* and *E. coli*) ANT	1 %	Chicken meat	5 4.0	(*50*)
Ziziphora pérsica	Pulegone 31.42, Neomenthol 18.58	Alginate based coating	AM (*E. coli, S.* Typhimurium*, P. aeruginosa, L. monocytogenes, B. cereus, S. aureus*) AO	0.5 and 1 %	Chicken meat	12 4.0	(*51*)
Rosemary (*Rosmarinus officinalis*)	-	Chitosan-based film	AM (mesophilic aerobic bacteria, *B. cereus, S. aureus, L. monocytogenes, S. enterica, E. coli, C.albicans*) AO	0.5, 1.0 and 2.0 %	Chicken meat	15 (5.0±2)	(*52*)
Rosemary (*Rosmarinus officinalis*)	-	Nanogel encapsulation of benzoic acid and chitosan applied as coating	AM (*S. typhimurium*)	0.5, 1.0 and 2.0 mg of nanoencapsulated oil per g of meat	Beef cutlet	12 4.0	(*53*)
Oregano (*Origanum vulgare*)	-	Direct and nanoemulsion encapsulation	AM (*S. aureus* and *E. coli*)	5 %	Chicken pate	8 (4.0±2)	(*54*)
Zataria multiflora	-	Chitosan-based coating with Sumac extract	AO and AM (total mesophilic bacteria, lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas*, fungi and yeasts)	1 %	Meat	20 4.0	(*55*)
Satureja (*Satureja khuzestanica*)	-	Chitosan-based coating	AO and AM (*Pseudomonas*, total count of bacteria and lactic acid bacteria)	1 %	Lamb meat	20 4.0	(*56*)
Oregano	-	Pectin-based coating with	AO, AM (total bacterial count)	0.5 %	Pork loin	20 4.0	(*57*)

		resveratrol nanoemulsion						
Z. multiflora	-	Chitosan and gelatin-based nanofibers	NR AM (*C. perfringens*)	20 and 40 %	Sausage	20	(4.0±1)	(*58*)
Rosemary (*Rosmarinus officinalis*) Ginger (*Zingiber officinale*)	-	Chitosan-based films	AO	2 %	Chicken meat	15	(5.0±2)	(*59*)
Cinnamon	-	Polylactide films plasticized with Ag-Cu nanoparticles	AM (*S.* Typhimurium*, C. jejuni* and *L. monocytogenes*)	25 and 50 %	Chicken meat	21	4.0	(*60*)
Ajowan (*Trachyspermum ammi*)	Thymol 70.95	Films based on gelatin and carboxymethylcellulose with chitin nanofiber	ATM (total viable count, psychotrophic count, *Pseudomonas* spp., *S. aureus*, lactic acid bacteria, moulds and yeasts)	0.24, 0.64 and 1 %	Beef	15	4.0	(*61*)

AM=antimicrobial, AO=antioxidant, NR=nitrite reduction

Table 4. Plant extracts as antioxidants or preservatives applied to meat and meat products in the form of films and coatings

Plant extract/species	Form of application	Effect	Dose used/	Product	Storage condition Time/day *t*/°C	Reference
Pomegranate (*Punica granatum*) peel	Films based on corn starch and chitosan	AO AM (*Pseudomonas* spp., lactic acid bacteria and *L. monocytogenes*)	0.5 and 1	Beef	21 , (4.0±1)	(*47*)
Red cabbage	Films based on starch and whey	AO SP	64.18 and 50	Ground beef	4 4.0	(*62*)
Laurel (*Laurus nobilis* L.), sage (*Salvia officinalis*)	Whey protein isolate based films	AO	2 and 4	Cooked meatball	60 , -18.0	(*63*)
Sumac (*Rhus coriaria*)	Chitosan-based coating with essential oil from *Zataria multiflora*	AO and AM (total mesophilic bacteria, lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas*, fungi and yeasts)	2 and 4	Beef	20 4.0	(*55*)
Shatavari (*Asparagus racemosus*)	Edible film based on calcium alginate and maltodextrin	AO and AM (total bacterial count, and yeast and mould counts)	1 and 2	Salsage	21 (4.0±1)	(*64*)
Stinging nettle (*Urtica dioica*)	ε-polylysine coating	AO, AM (moulds and yeasts, total bacterial and coliform counts)	3, 6 and 9	Beef	12 4.0	(*65*)
Grape seed	Chitosan/gelatin-based coating	AO	0.5	Pork	20 4.0	(*66*)
Green tea	Organic film	AO	6 and 8	Pork	14 4.0	(*67*)
Saffron leaves	Films based on chitosan and methylcellulose nanofiber	AM (*E. coli* and *S. aureus*) AO SP	3	Lamb meat	3 25.0	(*20*)

AM=antimicrobial, AO=antioxidant, SP=smart packaging

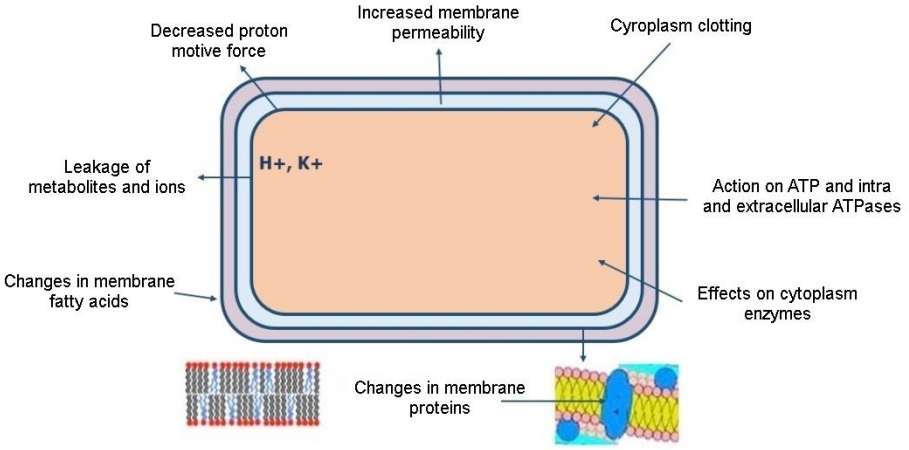


Fig. 1. Possible cellular targets of antibacterial action by natural compounds

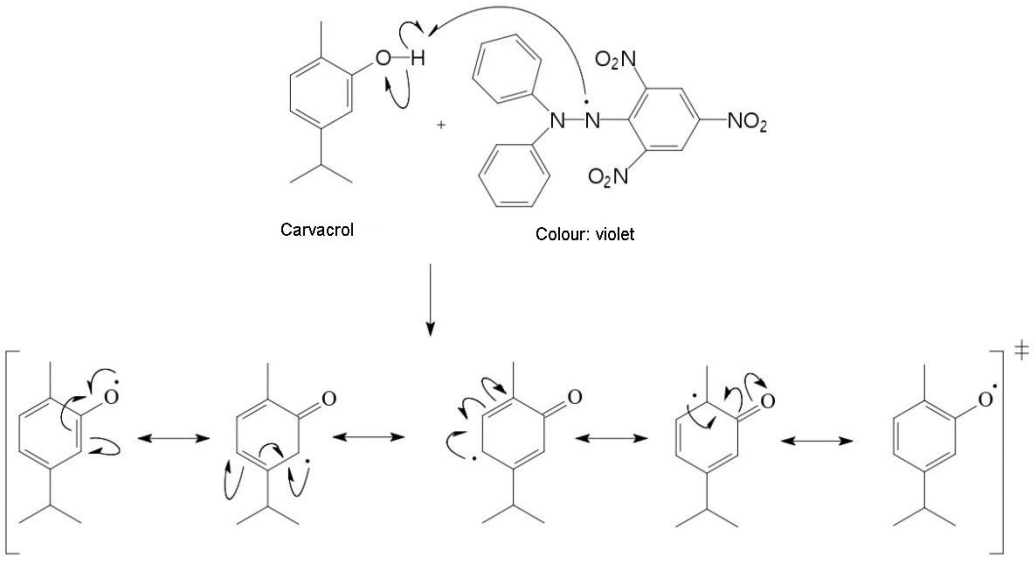


Fig. 2. Antioxidant action of carvacrol by transfering a hydrogen atom from the OH group

APÊNDICE B

APÊNDICE B – Artigo elaborado durante o Doutorado: "Phytochemical screening, antioxidant activity and application of Rosemary extract in chicken luncheon meat", submetido.

Periódico: Natural Product
JCR: 2.2

Phytochemical screening, antioxidant activity and application of Rosemary extract in chicken luncheon meat

Gabriela Aguiar Campolina[a], Alcineia de Lemos Souza Ramos[a], Eduardo Mendes Ramos[a], Gabriela Fontes Alvarenga[a], Elídio Zaidine Maurício Zitha[a], Alex Rodrigues Silva Caetano[b], David Lee Nelson[c], Maria das Graças Cardoso[b*]

[a]*Food Sciences Department, Federal University of Lavras (UFLA), Lavras, Brazil*

[b]*Chemistry Department, Federal University of Lavras (UFLA), Lavras, Brazil*

[c]*Postgraduate Program in Biofuels, Federal University of The Jequitinhonha and Mucuri Valleys, Diamantina, Brazil*

*Maria das Graças Cardoso, PhD, Professor, Chemistry Department, Federal University of Lavras, Lavras, MG, Brazil. Phone: +55 3538261202. E-mail: mcardoso@ufla.br.

Phytochemical screening, antioxidant activity and application of Rosemary extract in chicken luncheon meat

Abstract

In an attempt to discover healthier products/"clean label", the classes of compounds present in rosemary extract were determined, the antioxidant activity was analyzed by different methods and the extract was used to the preparation of chicken luncheon meats as a partial and total substitute for the traditional curing salt. The classes identified in the extract were reducing sugars; tannins; flavonoids; steroids; triterpenoids; depsides; depsidones; and coumarin derivatives. Analyses of antioxidante furnished results, with IC_{50} 201.89; 133.28 and 123.36 µg mL^{-1}, respectively, for the ABTS, DPPH and β-carotene methods. In the reducing power, the activity of the extract was greater than that of the positive control at the highest concentration evaluated. Regarding the application in chicken luncheon meats, in addition to the extract being rich in antioxidant compounds, the replacement of the salt did not interfere with the principal parameters analyzed. Therefor, is a good alternative for the preparation of hams.

Keywords: *Rosmarinus officinalis*; meat product; natural antioxidant.

Introduction

Brazil is one of the main producers of beef, pork and poultry in the world. In addition to fresh and frozen raw meat, the country is also one of the largest processors fof meats and develops several meat products, such as ham, sausages, hamburger, and salamis, among others (CAVALHEIRO et al., 2020). Among these products, chicken-based meats are a good option to meet the market demand for practical foods. They have a lower fat content and high quality protein, in addition to being economically accessible to consumers who, for whatever reason, whether religious or otherwise, do not consume pork products (PRESTES et al., 2013).

Meat processing for the production of derivatives, in general, is a means to increase the value of the product, reduce losses and take advantage of the carcass as a whole. However, a major problem faced by the meat product industries is related to the ease with which these products deteriorate, either by the action of microorganisms or by oxidation.

Lipid oxidation in foods is considered to be the second greatest cause of spoilage, second only to spoilage by microorganisms (MIRA-SANCHES et al., 2020). The presence of compounds capable of acting as pro-oxidants in meat products facilitates their deterioration.

Substances such as polyunsaturated fatty acids, cholesterol, proteins and certain pigments are easily oxidized and act as pro-oxidants (RAMLI et al., 2020). Several factors affect lipid oxidation, among which the constitution of the meat, temperature, light and the presence of oxygen can be mentioned (KUMAR et al., 2015; BHUYAR et al., 2020).

During the oxidation process, compounds containing both aldol and ketone functional groups are formed. These substances interfere with the quality and acceptance of food and can change parameters such as color, texture, flavor and other sensory attributes that directly interfere with the quality and shelf life of meat products (KUMAR et al., 2015; BHUYAR et al., 2020). Therefore, the use of antioxidants in food is very important (MIRA-SANCHES et al., 2020).

The use of additives, such as antioxidants, in processed meat products can be considered to represent a marketing barrier. The main antioxidants used in meat products are nitrites, which, in addition to acting as antioxidants, also act as antimicrobials. The efficient action of nitrites makes it difficult to replace them and, consequently, makes it difficult to prepare meat products that can be considered “clean label”. However, the change in the behavior of consumers, who are increasingly aware of food safety and demand products with more natural characteristics, makes the search for alternatives even more important (DELGADO-PANDO et al., 2021; MIRA-SANCHES et al., 2020; MENEZES et al., 2018).

A possible alternative for the use of synthetic antioxidants is the use of plant extracts as natural additives. These extracts can be used to reduce or replace additives, such as curing salts (nitrate) or other preservatives and antioxidants, yielding a final product with the profile of the “clean label” concept, that is, with a lower content of synthetic additives, few ingredients and containing known ingredients (DELGADO-PANDO et al., 2021; MIRA-SANCHES et al., 2020; MENEZES et al., 2018).

The extract obtained from rosemary (*Rosmarinus officinalis* L.), a plant widely used in cooking and belonging to the Lamiaceae family, has antioxidant and antimicrobial properties. These properties mean that it can represent a possible alternative for the development of “clean label” meat products, contributing to the stability and conservation of these products. The main compounds in rosemary extract are tannins and flavonoids, which can be responsible for the antioxidant and antimicrobial activities of the extract (ABANDANSARIE; ARIAII; LANGERODI, 2019).

The addition of new components to meat product formulations can cause changes in sensory characteristics and physical-chemical parameters, making it necessary to evaluate these

products after developing new formulations and identify the effects of these new components on the final product (KALSCHNE et al., 2014). The objectives of this study were to qualitatively evaluate the phytochemical composition of rosemary extract, its antioxidant activity and the effect of its application as a partial and total substitute for nitrites in luncheon meats prepared from chicken breast.

Results and Discussion

Phytochemical screening

Phytochemical screening of rosemary extract was performed for 16 classes of chemical compounds (Table 1). Among these classes of compounds, 10 were not detected and six were present in the extract. The classes identified were reducing sugars, tannins, flavonoids, steroids, triterpenoids, depsides, depsidones and coumarin derivatives.

The important role that reducing sugars play in plants makes their presence indispensable and already foreseen in this type of plant extract. These sugars, in general, act in nutrition and also in the signaling of regulatory molecules related to growth, development, metabolism, resistance and response to stress (KHATRI and CHHETRI, 2020).

The presence of phenolic compounds in the phytochemical screening of rosemary extract is important. They possess known antioxidant activities and are of extensive interest for the present study. Tannins and flavonoids, natural polyphenols present in the extract, have several properties that are known to act positively on health. They possess important antioxidant activities because their structures are rich in hydroxyl groups. Furthermore, tannins are reported to be possible inhibitors of the lipid peroxidation process, and they have the ability to precipitate proteins. However, their applicability is limited due to low stability and bioavailability, as well as their sensory characteristics (GRASEL and FERRÃO, 2016; ABANDANSARIE; ARIAII; LANGERODI, 2019; KAMLI et al., 2022; ZENG et al., 2022).

Coumarins are secondary metabolites that can be found in various parts of plants, and they are commonly present in plant extracts. Their main functions in plants are related to protection against infections, enzymatic inhibition, control of respiration, photosynthesis and regulation of growth hormones (LONCAR et al., 2020). In addition to the attributes related to their performances in plants, coumarins can perform other functions of human interest. Studies show that coumarins and their derivatives have, in addition to the already widespread antioxidant activity, contributed to the ability to eliminate free radicals, and they possess considerable antitumor activity and other biological activities such as antimicrobial,

anticoagulant, and anti-inflammatory activities, among others (KHALIL and MUSTAFA, 2020; ONDER, 2020).

Table 1

Antioxidant activity

The results obtained for the antioxidant activity of rosemary extract and the BHT positive control, evaluated by the ABTS and DPPH methods, are shown in Figure 1. Although BHT has a higher antioxidant activity than rosemary extract, the latter still proved to be a good natural antioxidant, greater than those of other plant extracts of relevant antioxidant activity, such as grape pulp extract (SRIDHARA and CHARLESA, 2019). Rosemary is considered by some authors to be a natural source of phenolic compounds, which are mainly responsible for its antioxidant activity (CEDEÑO-PINUS et al., 2020).

The two methods presented are capable of evaluating the ability of the sample to stabilize the ABTS and DPPH radicals through the direct donation of a hydrogen atom or electrons. The DPPH radical is more sterically hindered than the ABTS radical (TEIXEIRA et al., 2022). Thus, the steric hindrance of the DPPH radical explains the fact that the results obtained by the ABTS method were closer to the BHT control than the results obtained by the DPPH method.

Figure 1

The IC_{50} values were obtained using the respective regression equations of each test performed, and they are shown in Table 4. The IC_{50} was greater for the rosemary extract than for the BHT control in both methods used, as expected. The values obtained approximate those obtained by other authors who evaluated the antioxidant activity of rosemary extract by the same methods (KAMLI et al., 2022).

The ability of rosemary extract to protect lipid substrates from oxidation was evaluated by the β-carotene method by Alves et al. (2010). For the authors, free radicals formed in the loss of a hydrogen atom from linoleic acid, which react with the unsaturations of the β-carotene molecule and degrade it, are the causes of the loss of the initial color in the method. Greater protective activity was observed for the antioxidant activity by the β-carotene method (Table 2) than for the ABTS and DPPH methods. Thus, rosemary extract is a good option for application in meat products because lipid oxidation is one of the main problems faced by this type of product.

Table 2

Good results were obtained with antioxidant methods that evaluate the ability of metals to complex with the samples, such as phosphomolybdenum and reducing power assays. For the phosphomolybdenum complexation test, higher absorbance values were observed for the BHT sample and, like the rosemary extract, a dose-dependent increase in absorbance was observed (Figure 2).

In the antioxidant reducing power test, lower levels were obtained in the BHT positive control than with the rosemary extract at the highest concentration tested (500 µg mL^{-1}). These results (Figure 2) infer that the absorbance of the positive control was constant at concentrations greater than 150 µg mL^{-1}, whereas a dose-dependant behavior was observed for the rosemary extract sample.

Figure 2

The antioxidant activity of rosemary extract can be verified from the results presented in Figures 1 and 2 and in Table 2. This activity is probably due to the presence of phenolic compounds such as tannins, flavonoids, coumarins and their derivatives. The hydroxyl groups of the phenols are responsible for radical stabilization and metal complexation or reduction (TEIXEIRA et al., 2022).

Some studies mentioned by Mira-Sanches et al. (2020) report that the antioxidant activity of rosemary might be superior or equivalent to synthetic antioxidants, such as BHT, which is used in the food industry. This fact can be verified in Figures 1 and 2, in which the results obtained in the evaluation of the antioxidant activity of the extract by the ABTS and Reducing Power methods, respectively, are presented.

Physicochemical analysis

The results obtained for the physicochemical analyses of the prepared chicken "luncheon meats", are shown in Table 3.

Table 3

The different treatments to which the luncheon meat was submitted did not significantly interfere with the pH. It was close to the normal pH range of meat products (5.8 to 6). The results obtained in this study were similar to those reported by De Souto et al. (2021) for chicken luncheon meat formulations (pH between 6.24 and 6.37) and by Sousa (2019) for chicken sausages (pH between 6.1 and 6.4).

The pH is an important intrinsic factor to be evaluated in meat and meat products because, in addition to influencing the organoleptic characteristics of the product, this parameter is linked to the development of deteriorating microorganisms. Luong et al. (2022) pointed out that lactic acid bacteria, for example, are capable of altering the pH because they produce lactic acid and, consequently, acidify the product. Thus, the pH can be an indicator of the conservation status of the product.

There was a small variation among the elaborated formulations in the results obtained for the moisture and water activity analyses (Table 3). Samples containing extract contained less moisture than the control treatment (F1). The water activities for the samples were between 0.96 and 0.97 and did not differ significantly.

The parameters referring to the colors of the manufactured products are of extreme importance for the quality of the meat and interest in the consumer's willingness to purchase. They are presented in Table 4, along with the illustrated color of each analyzed sample.
The values for the luminosity parameter (L*) ranged from 70.58 to 72.45. Thus, all the samples can be described as clear and luminous. The L* values found in this study corroborate those obtained by Souto et al (2021) in luncheon meats made from free-range chicken, where the authors found a value of 70.2±0.24 for the luminosity parameter.

The values obtained for parameters a* and b* were positive for all the samples, which indicates, in the case of parameter a*, that the red component is stronger than the green one, and in the case of parameter b*, the yellow component is stronger than the blue one. There is a greater proportion of yellow color than red in all the samples because the chicken meat has a naturally light color. Another important fact to be observed is that the partial or total replacement of the curing salt by rosemary extract did not significantly interfere with the color of the samples and, therefore, there was no difference between the treatments containing rosemary extract (F2, F3 and F4) and the control treatment (F1).

Table 4

The results obtained for the texture profile are presented in Table 5. The partial or total replacement of the curing salt by rosemary extract did not influence any of the parameters. The samples containing extract were statistically equal to the control sample. The texture of a food is one of the main attributes in consumer acceptance. Each food or food product has well-defined characteristics, which are usually perceived in the first instance as texture characteristics. Thus, it is of paramount importance that the rheological characteristics be

studied in the development of food products because they demonstrate the textural characteristics that will be perceived by consumers (RAHEEM et al., 2021; SMEWING, 2001).

Table 5

Experimental

Obtaining the extract and characterization by phytochemical screening

The rosemary extract was purchased commercially with the objective of using a standardized extract, suitable for application in meat products and, consequently, for human consumption.

Phytochemical screening to determine the main groups of substances (organic acids, reducing sugars, polysaccharides, proteins, amino acids, tannins, catechins, flavonoids, cardiac glycosides, sesquiterpenolactones and other lactones, azulenes, carotenoids, steroids and triterpenoids, depsides, depsidones, coumarin derivatives, foaming saponins and alkaloids) present in rosemary extract was performed as described by Matos (1988) and cited by Carvalho et al. (2019).

In vitro antioxidant activity of rosemary extract

Stabilization of the ABTS• [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical

The assessment of the stability of the ABTS radical [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] was performed according to the method of Rezende et al. (2017).

Stabilization of the radical DPPH• (2,2-diphenyl-1-picrylhydrazyl)

The evaluation of the antioxidant potential by the DPPH test was performed according to the method described by Teixeira et al. (2022).

Complexation of Phosphomolybdenum

The method that evaluates the antioxidant activity on the basis of phosphomolybdenum complexation was performed according to the method described by Teixeira et al. (2022).

Reducing power

The evaluation of the antioxidant activity using the method of reducing power was performed according to the method described by Rezende at al. (2021).

Oxidation of the β-carotene/linoleic acid system

The evaluation by this method was performed according to a modification of the method described by Ferreira et al. (2019).

Application of rosemary extract in the preparation of chicken luncheon meats

The preparation of the luncheon meats from chicken was performed at the Laboratory of Meat and Derivatives Technology, located at the Federal University of Lavras (UFLA). The formulations were developed on the basis of the usual formulation (Table 1). The cooled chicken breasts were weighed and ground (-1 to 0 °C), with 90% of this meat being ground in a kidney disk and 10% in a 6 mm disk.

Table 6

The weighed dry ingredients were mixed in plastic bags and distributed over the chicken dough, followed by the liquid ingredients, water and extract. The combined mass of meat was homogenized and transferred to synthetic artificial casing. The product obtained were stored in a cold chamber (4 °C) for 20 hours for the curing process.

The meat products obtained were cooked after the curing process using the staggered cooking method. The products were cooked at 60 °C for 1 hour, at 70 °C for 1 hour, or at 85 °C for 30 minutes. The cooked products were cooled in cold water, followed by storage in a cold chamber (4 °C) until it was time for the physical-chemical analyzes.

Physicochemical analysis

Hydrogenionic Potential (pH)

The pH was determined using a portable digital pH meter, with direct penetration of the electrode into the meat samples.

Moisture

Moisture was determined according to the method of Instituto Adolfo Lutz, (2008).

Water activity (Aw)

The activity of water was determined using the AQUALAB equipment.

Instrumental color

Instrumental color assessments (L*, a* and b*) were performed at five different points on the surface of each sample using a digital spectrophotometer (Konica Minolta, CM700d). The parameters used were L (luminosity) L=0 is black and L=100 is White;-a green and +a red;

-b blue and +b yellow. The intensity data obtained from each sample was analyzed using an online color converter (http://colormine.org). This online tool from http://colormine.org/ has been widely used by researchers for color conversions (SRUTHI et al., 2021; ZHANG et al., 2019).

Texture

Texture profile analyses (TPA) of the samples were performed using a TA.XT2i Texture Analysis texturometer (Stable Micro System Inc.). To calculate the parameters of hardness, cohesiveness, flexibility, adhesiveness and chewability, the Texture Expert® software was used. All the measurements were performed in triplicate and at room temperature (25 °C).

Statistical analysis

Data were statistically analyzed using a completely randomized design (CRD) with a factorial scheme of 2 x 9 (extract/positive control x concentrations), with three replications. The statistical program used was Rstudio® version 4.0.2, and the data were submitted to analysis of variance and means compared by the Tukey test at a 5% probability level. In addition to the qualitative test, regression equations were adjusted. They were used to calculate the IC_{50} (concentration that presents 50% antioxidant activity) of the extract and of the positive control using the Origin Pro® version 9.0 program.

Conclusion

The rosemary extract was rich in antioxidant compounds, and the activity was proven by the methods used. In addition to acting as an antioxidant, the extract did not interfere with the evaluated parameters of pH, Aw, instrumental color and texture. Detailed sensory studies must be performed to ensure consumer acceptance, as well as to confirm that there was no interference in incorporating the extract in the prepared product, which is a good option for replacing conventional curing salt in luncheon meats.

Acknowledgments

The authors thank the Federal University of Lavras and funding agencies for the scholarships and financial support.

Funding

This work was supported by the **[**Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) #1] under Grant [number 02390/18]; [Conselho Nacional de Desenvolvimento

Científico e Tecnológico (CNPq) #2] under Grant [number 311183/2122-0]; and [Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) #3] under Grant [number 001].

Conflict of interest

The authors declare no conflict of interest.

References

ABANDANSARIE SSR, ARIAII P, LANGERODI MC. 2019. Effects of encapsulated rosemary extract on oxidative and microbiological stability of beef meat during refrigerated storage. Food Sci Nutri.7(12):3969-3978.

ALVES CQ, DAVID JM, DAVID JP, BAHIA MV, AGUIAR RM. 2010. Métodos para determinação de atividade antioxidante *in vitro* em substratos orgânicos. Quim Nova. 33(10):2202-2210.

BHUYAR P, RAHIM MH, SUNDARARAJU S, MANIAM GP, GOVINDAN N. 2020. Antioxidant and antibacterial activity of red seaweed *Kappaphycus alvarezii* against pathogenic bacteria. Glob J Environ Sci Manag. 6(1):47-58.

CARVALHO MSS, ANDRADE-VIEIRA LF, DOS SANTOS FE, CORREA FF, CARDOSO MG, VILELA LR. 2019. Allelopathic potential and phytochemical screening of ethanolic extracts from five species of *Amaranthus* spp. in the plant model *Lactuca sativa*. Sci Hortic. 245:90-98.

CAVALHEIRO C P, SILVA MCA, LEITE JSF, FÉLIX SKRS, HERRERO AM, RUIZ-CAPILLAS C. 2020. Physical hazards in meat products: Consumers' complaints found on a Brazilian website. Food Control.108:106892.

CEDEÑO-PINOS C, MARTÍNEZ-TOMÉ M, MURCIA MA, JORDÁN MJ, BAÑÓN S. 2020. Assessment of rosemary (Rosmarinus officinalis L.) extract as antioxidant in jelly candies made with fructan fibres and stevia. Antioxidants.9(12):1289.

DELGADO-PANDO G, EKONOMOU SI, STRATAKOS AC, PINTADO T. 2021. Clean label alternatives in meat products. Foods. 10(7):1615.

FERREIRA VRF, BRANDÃO RM, FREITAS MP, SACZK AA, FELIZ FS, SILLA JM, TEIXEIRA ML, CARDOSO MG. 2019. Colorimetric, electroanalytical and theoretical evaluation of the antioxidant activity of *Syzygium aromaticum* L., *Origanum vulgare* L., *Mentha spicata* L. and *Eremanthus erythropappus* M. essential oils, and their major constituents. New J Chem. 43(20):7653-7662.

GRASEL, F. S., FERRÃO, M. F. 2016. A rapid and non-invasive method for the classification of natural tannin extracts by near-infrared spectroscopy and PLS-DA. Anal Methods. 8(3):644-649.

INSTITUTO ADOLFO LUTZ. 2008. Normas Analíticas do Instituto Adolfo Lutz: Métodos físico-químicos para análise de alimentos. São Paulo (SP): IAL.

KALSCHNE DL, GEITENES S, VEIT MR, SARMENTO CMP, COLLA E. 2014. Growth inhibition of lactic acid bacteria in ham by nisin: A model approach. Meat Sci. 98(4):744-752.

KAMLI MR, SHARAF AAM, SABIR JS, RATHER IA. 2022. Phytochemical Screening of *Rosmarinus officinalis* L. as a Potential Anticholinesterase and Antioxidant–Medicinal Plant for Cognitive Decline Disorders. Plants. 11(4):514.

KHALIL RR, MUSTAFA YF. 2020. Phytochemical, antioxidant and antitumor studies of coumarins extracted from Granny Smith apple seeds by different methods. Sys Rev Pharm. 11(2):57-63.

KHATRI D, CHHETRI SBB. 2020. Reducing sugar, total phenolic content, and antioxidant potential of nepalese plants. BioMed Res Int. 2020:1-7.

KUMAR Y, YADAV DN, AHMAD T, NARSAUAH K. 2015. Recent trends in the use of natural antioxidants for meat and meat products. Compr Ver Food Sci Food Saf.14(6):796-812.

LONČAR M, JAKOVLJEVIĆ M, ŠUBARIĆ D, PAVLIĆ M, BUZJAK SLUŽEK V, CINDRIĆ I, MOLNAR M. 2020. Coumarins in food and methods of their determination. Foods. 9(5):645.

LUONG NDM, COROLLER L, ZAGOREC M, MORICEAU N, ANTHOINE V, GUILLOU S, MEMBRÉ JM. 2022.A Bayesian Approach to Describe and Simulate the pH Evolution of Fresh Meat Products Depending on the Preservation Conditions. Foods.11(8):1114.

MATOS FJ. 1988. Introdução a Fitoquímica Experimental. Fortaleza (CE):Universidade Federal do Ceará.

MENEZES NMC, MARTINS WF, LONGHI DA, ARAGÃO GMF. 2018. Modeling the effect of oregano essential oil on shelf-life extension of vacuum-packed cooked sliced ham. Meat Sci. 139:113-119.

MIRA-SÁNCHEZ MD, CASTILLO-SÁNCHEZ J, MORILLAS-RUIZ JM. 2020. Comparative study of rosemary extracts and several synthetic and natural food antioxidants. Relevance of carnosic acid/carnosol ratio. Food Chem. 309:125688.

ÖNDER, A. (2020). Anticancer activity of natural coumarins for biological targets. Stud Nat Prod Chem, 64:85-109.

PRESTES RC, GRABOSKI A, ROMAN SS, KEMPKA AP, TONIAZZO G, DEMIATI, IV, DI LUCCIO M. 2013. Effects of the addition of collagen and degree of comminution in the quality of chicken ham. J Appl Poult Res. 22(4):885-903.

RAHEEM D, CARRASCOSA C, RAMOS F, SARAIVA A, RAPOSO A. 2021. Texture-modified food for dysphagic patients: a comprehensive review. Int J Environ Res Public Health. 18(10):5125.

RAMLI ANM, BADRULZAMAN SZS, HAMID HM, BHUYAR P. 2021.Antibacterial and antioxidative activity of the essential oil and seed extracts of Artocarpus heterophyllus for effective shelf-life enhancement of stored meat. J Food Process and Preserv. 45(1):e14993.

REZENDE, DACS, SANTOS VA, PASQUAL M, BELLETE BS, NELSON DL, ALVARENGA GF, FERREIRA VRF, OLIVEIRA CD, ALVES MVP, CAETANO ARS, et al. 2021. Pulp oil from four avocado varieties: extraction, yield, characterization and evaluation of antioxidant activity. Res, Soc Dev. 10(12): e401101220541-e401101220541.

REZENDE DACS, SOUZA RV, MAGALHÃES ML, CAETANO ARS, CARVALHO MSS, SOUZA EC, GUIMARÃES LGL, NELSON DL, BATISTA LR, et al. 2017. Characterization of the biological potential of the essential oils from five species of medicinal plants. Am J Plant Sci. 8(2):154-170.

SMEWING J. 2001. Hidrocoloides In: ROSENTHAL: A. Textura de los alimentos. Zaragoza: Ed. Acribia.

SOUSA LTF. Características Físico-Químicas de salsichas e mortadelas de frango comercializadas na cidade de João Pessoa – PB. 2019. Pernambico: Universidade Federal Rural de Pernambuco.

SOUTO VO, SANTOS MMF, LIMA DAS, FLORENTINO GIB, GALVÃO MS, BEZERRA TKA, SILVA FAP. 2021. Olive oil-in-water emulsion as a source of desirable fatty acids in free-range "Caipira" chicken ham. LWT. 144:111216.

SRIDHAR K, CHARLES AL. In vitro antioxidant activity of Kyoho grape extracts in DPPH and ABTS assays: Estimation methods for EC50 using advanced statistical programs. 2019. Food Chem. 275:41-49.

SRUTHI OS, BALASUBRAMANIAN S, KUMAR OS, KAPOOR A, PONNUCHAMY M, JACOB MM, PRABHAKAR S. 2021. Eco-friendly pH detecting paper-based analytical device: towards process intensification. Anal Chim Acta. 1182:338953.

TEIXEIRA ML, CARDOSO MG, FERREIRA VRF, CAMPOLINA GA, CAETANO ARS, OLIVEIRA CD, ALVES MVP, NELSON DL. 2022. Evaluation of the antioxidant activity of the essential oils from Cantinoa carpinifolia (Benth.) and Lippia origanoides (Kunth.) by various methods. J Essent Oil Res, p. 1-11.

ZENG X, JIANG W, DU Z, KOKINI JL. 2022. Encapsulation of tannins and tannin-rich plant extracts by complex coacervation to improve their physicochemical properties and biological activities: A review. Crit Rev Food Sci Nutr, 1-14.

ZHANG Q, MEJIA EG, LUNA-VITAL D, TAO T, CHANDRASEKARAN S, CHATHAM L, JUVIK J, SINGH V, DEEPAK K. 2019. Relationship of phenolic composition of selected purple maize (Zea mays L.) genotypes with their anti-inflammatory, anti-adipogenic and anti-diabetic potential. Food Chem. 289:739-750.

Table 1. Phytochemical screening of rosemary extract

Test	Result
Organic acids	Negative
Reducing sugars	Positive
Polysaccharides	Negative
Proteins	Negative
Tannins	Positive
Catechins	Negative
Flavonoids	Positive
Glycosides	Negative
Sesquiterpenlactones and other lactones	Negative
Azulenos	Negative
Carotenoids	Negative
Steroids and triterpenoids	Positive
Depsides and depsidones	Positive
Coumarin derivatives	Positive
Saponin	Negative
Alkaloids	Negative

Table 2. Antioxidant activity (IC_{50}) of rosemary extract and BHT positive control

	Antioxidant Method		
	ABTS	**DPPH**	**β-carotene**
Sample	**IC_{50} (µg mL^{-1})**	**IC_{50} (µg mL^{-1})**	**IC_{50} (µg mL^{-1})**
BHT	3.52 ± 0.03^{b}	9.62 ± 0.06^{b}	$<5^{b}$
Extract	201.89 ± 2.15^{a}	133.28 ± 4.3^{a}	123.36 ± 2.93^{a}

*The means followed by the same lowercase letter in the columns do not differ from each other by the Tukey Test at the 5% probability level.

Table 3. Determination of pH, water activity (Aw) and moisture content of luncheon meat formulations

	Analysis		
Formulation	**pH**	**Aw**	**Moisture (%)**
F1	6.24±0.03[a]	0.968±0.00[a]	73.34±0.27[a]
F2	6.30±0.11[a]	0.969±0.00[a]	72.18±0.14[b]
F3	6.26±0.02[a]	0.969±0.00[a]	72.31±0.21[b]
F4	6.28±0.03[a]	0.70±0.00[a]	72.10±0.27[b]

*Means followed by the same letter in the column do not differ significantly by the Tukey test.

Table 4. Evaluation of the color of chicken luncheon meat formulations prepared with the addition of rosemary extract

Formulation	L*	a*	b*	Color
F1	72.45±1.86[a]	8.39±0.93[a]	15.77±1.67[ab]	
F2	70.58±1.62[a]	8.05±0.30[a]	16.93±0.95[a]	
F3	71.11±0.96[a]	8.58±0.16[a]	15.87±0.53[ab]	
F4	70.63±2.50[a]	8.02±0.89[a]	14.40±1.45[b]	

Table 5. Evaluation of the texture of chicken luncheon meat formulations prepared with the addition of rosemary extract

Formulation	Hardness (N)	Cohesiveness	Flexibility (mm)	Adhesiveness (N. mm)	Chewiness (N. mm)
F1	40.24±4.32[a]	0.65±0.05[a]	4.28±0.09[a]	0.11±0.11[a]	112.47±21.33[a]
F2	40.87±2.62[a]	0.61±0.08[a]	4.22±0.19[a]	0.06±0.01[a]	104.74±15.67[a]
F3	40.49±1.33[a]	0.60±0.05[a]	4.15±0.16[a]	0.05±0.04[a]	101.28±6.67[a]
F4	42.96±3.11[a]	0.58±0.04[a]	4.1±0.11[a]	0.24±0.18[a]	102.35±11.76[a]

Table 6. Formulations used in the preparation of luncheon meat from chicken breast

Ingredients	**F1**	**F2**	**F3**	**F4**
Chicken breast	68.6%	68.6%	68.6%	68.6%
Water/ice	24.0%	24.0%	24.0%	24.0%
Refined salt	1.7%	1.7%	1.7%	1.7%
Cassava starch	2.1%	2.1%	2.1%	2.1%
Conc. Soy protein	1.0%\	1.0%	1.0%	1.0%
Sugar	0.5%	0.5%	0.5%	0.5%
Phosphate	0.3%	0.3%	0.3%	0.3%
Carrageenan	0.3%	0.3%	0.3%	0.3%
Monosodium glutamate	0.3%	0.3%	0.3%	0.3%
Color fixer	0.4%	0.2%	0.3%	0.0%
Quick cure salt	0.0%	0.2%	0.1%	0.4%
Rosemary extract	0.3%	0.3%	0.3%	0.3%

Fig.1

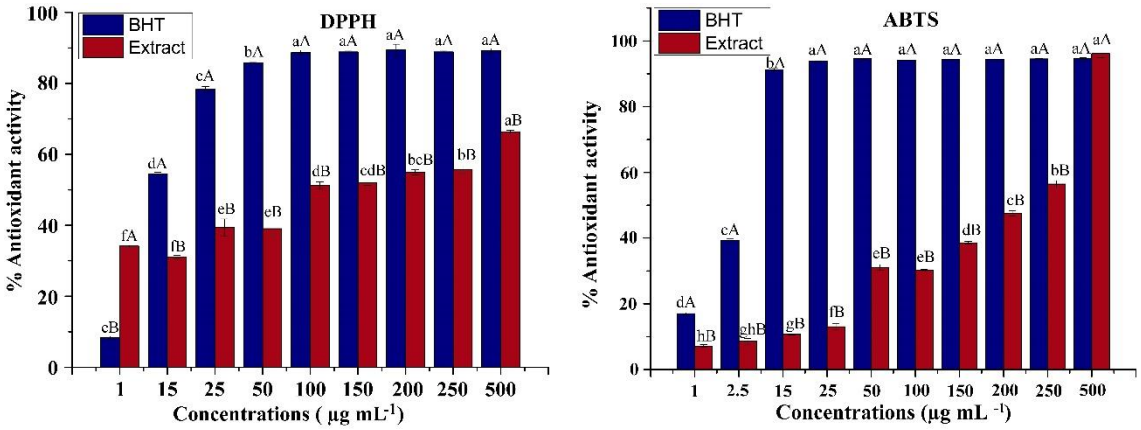


Fig.2

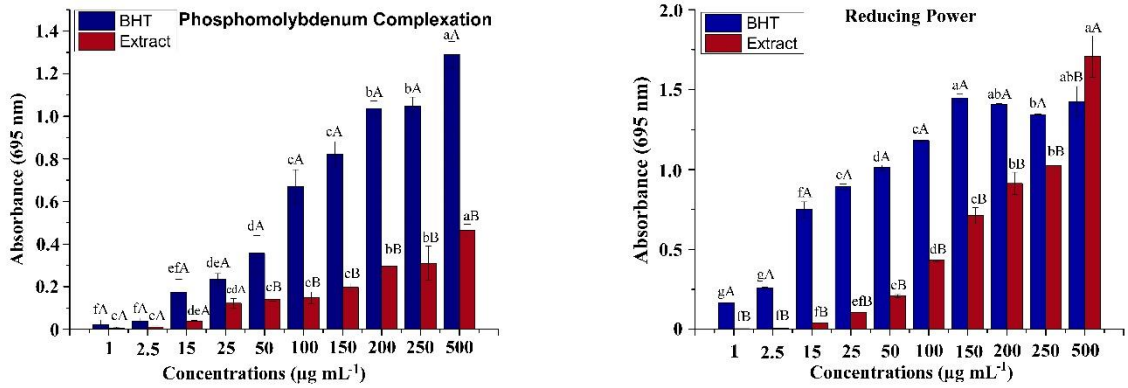


Figure 1. Results obtained for antioxidant activity of rosemary extract by the ABTS and DPPH methods. Means followed by the same lowercase letter, comparing the concentrations within each sample, and the same capital letter, comparing the samples to each other within each concentration, do not differ from one another by the Tukey Test at the 5% probability level.

Figure 2. Results obtained for antioxidant activity of rosemary extract by the Phosphomolybdenum Complexation and Reducing Power methods. Means followed by the same lowercase letter, comparing the concentrations within each sample, and the same capital letter, comparing the samples to each other within each concentration, do not differ from one another by the Tukey test at the 5% probability level.