RAFAELA MAGALHÃES BRANDÃO

ACTIVE PACKAGING POLY (LACTIC ACID) NANOFIBERS CONTAINING ESSENTIAL OILS FOR TABLE GRAPES:

ANTIFUNGAL AND PHYSICOCHEMICAL PROPERTIES

LAVRAS – MG
2021

RAFAELA MAGALHÃES BRANDÃO

ACTIVE PACKAGING POLY (LACTIC ACID) NANOFIBERS CONTAINING ESSENTIAL OILS FOR TABLE GRAPES: ANTIFUNGAL AND PHYSICOCHEMICAL PROPERTIES

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agroquímica, área de concentração em Química/ Bioquímica, para a obtenção do título de Doutor.

Profa. Dra. Maria das Graças Cardoso
Orientadora

Prof. Dr. Juliano Elvis de Oliveira
Coorientador

Prof. Dr. Luís Roberto Batista
Coorientador

LAVRAS – MG
2021

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

Brandão, Rafaela Magalhães.
Active packaging poly (lactic acid) nanofibers containing essential oils for table grapes: antifungal and physicochemical properties / Rafaela Magalhães Brandão. - 2021.
162 p.: il.

Orientador(a): Maria das Graças Cardoso.
Coorientador(a): Juliano Elvis de Oliveira, Luís Roberto Batista.
Tese (doutorado) - Universidade Federal de Lavras, 2021.
Bibliografia.

1. Produtos naturais. 2. Ocratoxina A. 3. Polímeros. I. Cardoso, Maria das Graças. II. Oliveira, Juliano Elvis de. III. Batista, Luís Roberto. IV. Título.

RAFAELA MAGALHÃES BRANDÃO

EMBALAGENS ATIVAS DE NANOFIBRAS DE POLI (ÁCIDO LÁTICO) CONTENDO ÓLEOS ESSENCIAIS PARA UVAS DE MESA: PROPRIEDADES ANTIFÚNGICA E FÍSICO-QUÍMICA

ACTIVE PACKAGING POLY (LACTIC ACID) NANOFIBERS CONTAINING ESSENTIAL OILS FOR TABLE GRAPES: ANTIFUNGAL AND PHYSICOCHEMICAL PROPERTIES

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agroquímica, área de concentração em Química/ Bioquímica, para a obtenção do título de Doutor.

APROVADA em 25 de novembro de 2021.

Dra. Ana Cristina da Silva Figueiredo	FCUL
Dr. Arie Fitzgerald Blank	UFS
Dr. Eduardo Alves	UFLA
Dr. Juliano Elvis de Oliveira	UFLA

Profa. Dra Maria das Graças Cardoso
Orientadora

Prof. Dr. Juliano Elvis de Oliveira
Coorientador

Prof. Dr Luís Roberto Batista
Coorientador

LAVRAS – MG
2021

Aos meus Pais, Dario e Joseani, pelo amor incondicional, pelo carinho, apoio, por acreditar em mim e por não ter medido nenhum esforço para que mais uma etapa de minha vida se realizasse!!!

A minha irmã, Renata, pelo carinho, amor, amizade, paciência e pelo incentivo!!!

A minha avó, Marlene, por estar sempre me apoiando e ajudando, pelo seu carinho e amor!!!

Á minha orientadora, Profa. Dra. Maria das Graças, pelos ensinamentos, pela amizade e por ser um exemplo de Professora!!!

DEDICO...

AGRADECIMENTOS

Acima de tudo, agradeço a Deus pela vida, pelas oportunidades, pela esperança, pela insistência, pela força, pela paciência, pela fé e por me mostrar os caminhos para eu prosseguir. Agradeço também pelas pessoas que o Senhor colocou em meu caminho. Algumas delas me inspiram, me ajudam, me desafiam e me encorajam a ser cada dia melhor.

À professora Dra. Maria das Graças Cardoso, por ter aberto as portas do laboratório, pela oportunidade, pelos ensinamentos, amizade, pelos puxões de orelha e confiança. Por dedicar o seu tempo, orientando-me não só profissionalmente, mas também para a vida.

Aos meus pais, Dario e Joseani, à minha irmã, Renata, à minha avó Marlene e ao meu namorado, Lucas pelo amor incondicional, pelo carinho, incentivo, apoio, persistência, paciência, respeito e amizade. Por acreditar em mim e tornar realidade meus sonhos.

Aos demais familiares e amigos pela amizade e apoio.

À Universidade Federal de Lavras, especialmente ao Departamento de Química e ao programa de Agroquímica, pela oportunidade e por me permitirem desenvolver esta pesquisa.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (Capes) – Código de Financiamento 001.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (Capes) e à Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig), pelo financiamento concedido à realização deste projeto.

Aos Professores Juliano Elvis de Oliveira, Luís Roberto Batista e Maria Alice Martins, pela dedicação e disponibilidade em contribuir com a nossa pesquisa. Por cederem materiais, equipamentos e o laboratório para que as análises fossem realizadas. Prof. Luís Roberto, obrigada por ceder os fungos da Coleção de Culturas de Microrganismos do Laboratório de Micotoxinas e Micologia de Alimentos da Universidade Federal de Lavras.

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

À Dra. Lidiany Zacaroni, pelas análises cromatográficas dos óleos essenciais e pelas análises no Calorímetro de Digitalização Diferencial das nanofibras que foram realizadas no Centro de Análise e Prospecção e Química - CAPQ.

À Ana Carolina Cortez Lemos e ao Kelvi Miranda, por me ajudarem e ensinarem as análises de produção das nanofibras.

À Professora Dra. Mariana Mansanares, coordenadora do Herbário ESAL e ao funcionário Antônio, pela atenção e pela gentileza na identificação das espécies em estudo.

Aos amigos do Laboratório de Química Orgânica – Óleos Essenciais e de Análise de Qualidade de Aguardente, pela amizade, companheirismo, auxílio na condução das análises.

À Embrapa Instrumentação, pelo fornecimento dos equipamentos para as análises de nanofibras. Ao pessoal do Departamento de Alimentos, em especial aos amigos do Laboratório de Micotoxinas e Micologia de Alimentos

Ao Horto de Plantas Medicinais da UFLA, por cederem as plantas utilizadas neste estudo, e ao pessoal do Horto, pelo auxílio na coleta do material vegetal.

À Central de Análises e Prospecção Química da Universidade Federal de Lavras, pelo fornecimento dos equipamentos para análises.

Aos professores do programa de pós-graduação em Agroquímica, pelos ensinamentos.

Aos meus colegas de doutorado, pela convivência e amizade.

Aos funcionários do Departamento de Química, por toda a atenção e pela ajuda prestada.

Aos membros da banca, Profa. Ana Cristina da Silva Figueiredo, Prof. Arie Fitzgerald Blank, Prof. Eduardo Alves e Prof. Juliano Elvis de Oliveira, por aceitarem contribuir e participar do meu trabalho.

E a todos que, de alguma forma, contribuíram para a realização de mais uma etapa de minha vida!

RESUMO

Os objetivos foram extrair e determinar a composição química dos óleos essenciais (OEs) de *Alpinia speciosa*, *Cymbopogon flexuosus*, *Ocimum basilicum* L e *Ocimum gratissimum* L.; produzir através de fiação por sopro em solução (SBS) nanofibras de poli (ácido lático) (PLA) incorporadas com os OEs e caracterizá-las físico-quimicamente, bem como avaliar os testes *in vitro* antifúngicos e antiocratoxigênicos sobre *Aspergillus carbonarius*, *Aspergillus niger*, *Aspergillus ochraceus* e *Aspergillus westerdjikiae*. Também foram estudados os efeitos antifúngicos *in vivo* (uva) das nanofibras sobre *Aspergillus carbonarius* e *Aspergillus niger* como potencial embalagem ativa a ser aplicada no controle da degradação de uvas de mesa. Os OEs foram extraídos por hidrodestilação e caracterizados por cromatografia gasosa (CG/EM e CG/DIC). As nanofibras foram produzidas pela técnica de SBS e caracterizadas por microscopia eletrônica de varredura (MEV), calorimetria exploratória diferencial (DSC), espectroscopia de infravermelho (FTIR), ângulo de contato e por análise termogravimétrica (TGA). As propriedades antifúngicas e antiocratoxigênica foram avaliadas pelo método de fumigação. O teste *in vivo* foi realizado usando uma embalagem de politereftalato de etileno para uvas contendo as nanofibras, avaliando os parâmetros físico-químicos da uva e a proliferação fúngica após 10 e 20 dias de incubação. Terpinen-4-ol (20,23%), sabineno (20,18%), 1,8-cineol (16,69%), γ-terpineno (11,03%); e citral (97,67%) foram os compostos majoritários presentes nos OEs de *A. speciosa* e *C. flexuosus*, respectivamente. Os principais constituintes de *O. basilicum* foram linalol (26,89%), 1,8-cineol (23,62%) e canfora (15,69%), ao passo que no de *O. gratissimum* o eugenol (79,04%) foi encontrado majoritariamente. As eletromicrografias mostraram que a adição dos OEs ocasionou um aumento do diâmetro das nanofibras. A eficiência da encapsulação dos OEs nas nanofibras foi evidenciada e comprovada pelos resultados de FTIR. As curvas de DSC também indicaram a existência de interações entre os OEs e as macromoléculas poliméricas através de sua ação plastificante, resultando em redução da cristalinidade do PLA. O caráter hidrofóbico das nanofibras foi revelado pela técnica de ângulo de contato. A técnica de TGA conseguiu mostrar a eficiência da nanofibra de PLA em controlar a liberação dos OEs, prolongando o efeito antifúngico. As nanofibras proporcionaram um efeito antifúngico significativo, diminuindo o crescimento micelial de *A carbonarius* (17,86% a 100%), *A. niger* (10,25% a 100%), *A. ochraceus* (2,78 a 100%) e *A. westerdijkiae* (3,64 a 100%). A síntese da ocratoxina A dos fungos *A carbonarius* (12,09% a 100%), *A. niger* (8,93% a 100%), *A. ochraceus* (25,94 a 100%) e *A. westerdijkiae* (56,26 a 100%) foi inibida com a presença das nanofibras. As embalagens ativas conseguiram reduzir o índice da proliferação fúngica de *A. carbonarius* e *A. niger* nas uvas, bem como controlar a perda de peso, amolecimento e as mudanças de cor, manter os parâmetros de acidez, °Brix e preservar a textura das uvas. Os resultados indicaram que as nanofibras em estudo podem ser promissoras e aplicadas como embalagens ativas em alimentos no controle de fungos toxigênicos e da ocratoxina A, conseguindo manter os parâmetros físico-químicos das uvas, preservando a qualidade e contribuindo com a segurança alimentar, bem como aumentando a vida útil das frutas.

Palavras-chave: Produtos naturais. Ocratoxina. Alfavaca. Capim-indiano. Colônia. Manjericão

ABSTRACT

The essential oils (EOs) from *Alpinia speciosa*, *Cymbopogon flexuosus*, *Ocimum basilicum* L and *Ocimum gratissimum* L. were extracted and the chemical compositions were determined. The nanofibers incorporated with EOs were produced through the solution blow spinning (SBS) of poly (lactic acid) (PLA), they were characterized physicochemically, and the *in vitro* antifungal and antiocratoxigenic tests against *Aspergillus carbonarius*, *Aspergillus niger*, *Aspergillus ochraceus* and *Aspergillus westerdjikiae* were performed. The *in vivo* antifungal effect (grape) of EOs encapsulated in PLA nanofibers against *Aspergillus carbonarius* and *Aspergillus niger* as potential active packaging to be applied to control the degradation of table grapes was also studied. The EOs were extracted using the hydrodistillation and characterized by gas chromatography (GC/MS and GC/FID). The nanofibers were produced by the SBS technique and characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), contact angle and thermogravimetric analysis (TGA). The antifungal and antiocratoxigenic properties were evaluated by the fumigation method. The *in vivo* test was achieved using a polyethylene terephthalate package for grapes containing the nanofibers, evaluating the physicochemical parameters of the grapes and fungal proliferation after 10 and 20 days of incubation. Terpinen-4-ol (20.23%), sabinene (20.18%), 1,8-cineole (16.69%), γ-terpinene (11.03%); and citral (97.67%) were the principal compounds present in the EOs from *A. speciosa* and *C. flexuosus*, respectively. The main constituents from *O. basilicum* were linalool (26.89%), 1,8-cineol (23.62%) and camphor (15.69%), whereas eugenol (79.04%) was the principal component from *O. gratissimum*. Electromicrographs showed that the addition of EOs caused an increase in the diameter of the nanofibers. The encapsulation efficiency of essential oils in PLA nanofibers was proven by FTIR results. The DSC curves also indicated the existence of interactions between EOs and polymeric macromolecules through their plasticizing action, resulting in reduced crystallinity of PLA. The hydrophobic character of nanofibers was revealed by the contact angle technique. The efficiency of the PLA nanofiber in controlling the release of essential oils and prolonging the antifungal effect was demonstrated by the TGA technique. The nanofibers provided a significant antifungal effect, decreasing the mycelial growth of *A carbonarius* (17.86% to 100%), *A. niger* (10.25% to 100%), *A. ochraceus* (2.78 to 100%) and *A. westerdjikiae* (3.64 to 100%). The synthesis of ochratoxin A from *A carbonarius* (12.09% to 100%), *A. niger* (8.93% to 100%), *A. ochraceus* (25.94 to 100%) and *A. westerdijkiae* (56.26 to 100%) was inhibited in the presence of nanofibers. The fungal proliferation index of *A. carbonarius* and *A. niger* in the grapes decreased in the active packages, weight loss, softening and color change were controlled, maintaining the acidity and °Brix parameters, as well as preserving the texture of the grapes. The results indicate that the nanofibers under study can be promising, and they can be applied as active packaging in food for the control of toxigenic fungi and the synthesis of ochratoxin A. They can help to maintain the physicochemical parameters of the grapes, preserve quality, contribute to food safety, and increase the shelf life of the fruits.

Keywords: Natural products. Ochratoxin. Alfavaca. Capim indiano. Colônia. Basil

LISTA DE FIGURAS

PRIMEIRA PARTE

ARTIGO 1

ARTIGO 2

LISTA DE TABELAS

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO

A segurança alimentar é um dos assuntos de maior relevância, uma vez que os alimentos são suscetíveis à deterioração e contaminação microbiológica (bactérias patogênicas, parasitas, vírus e fungos toxigênicos) e as indústrias de alimentos e agrícola são desafiadas continuamente a evitar a propagação desses patógenos ao longo da cadeia alimentar, visto que a elevação da atividade metabólica desses microrganismos faz com que o alimento deteriore, proporcionando uma enorme perda econômica, desperdícios de matéria-prima, alteração na qualidade e segurança do produto.

A contaminação fúngica impacta tanto em parâmetros físico-químicos das frutas como peso, textura, cor, sólidos solúveis totais e acidez total, quanto na formação de metabólicos secundários, como as micotoxinas que são prejudiciais à saúde humana. A ocratoxina A é o principal metabólito secundário produzido pelos fungos do gênero *Aspergillus*, como *A. carbonarius*, *A. niger*, *A. ochraceus* e *A. westerdjikiae*. Essa micotoxina é classificada pela Agência Internacional do Câncer como um carcinógeno do grupo 2B, além de apresentar propriedades imunotóxicas, teratogênicas, nefrotóxicas e genotóxicas para humanos.

Os fungos *A. carbonarius* e *A. niger* são os principais em contaminar uvas, induzindo a infecções não controladas que permitem o desenvolvimento de micélio aéreo, que se espalha rapidamente pelas frutas. A contaminação fúngica leva a perdas de qualidade e alta ocorrência de deterioração durante o armazenamento prolongado das uvas de mesa. A uva de mesa, Niagara Rosada (*Vitis labrusca*), considerada a mais atraente ao consumidor brasileiro, especialmente no caso do consumo *in natura*, devido à sua coloração e ao seu sabor característico. Porém, é uma fruta não climatérica e com grandes desafios na manutenção de sua qualidade pós-colheita, devido a contaminações fúngicas durante seu manuseio, armazenamento e comercialização.

O controle do crescimento fúngico em alimentos é convencionalmente feito por processamentos térmicos ou por fungicidas sintéticos. Entretanto, há uma demanda crescente dos consumidores por alimentos mais naturais e com garantia da segurança alimentar, pois pesquisas demonstram que o uso desses produtos sintéticos está relacionado com resíduos de sabor desagradável, toxicidade e danos à saúde humana e ao meio ambiente. Além disso, o uso incorreto e contínuo desses produtos está selecionando microrganismos resistentes, sendo necessário o aumento da dose a cada aplicação. Nesse sentido, vários estudos se atentam em métodos alternativos de controle natural com produtos que sejam menos agressivos e ecologicamente seguros. Dentre os recursos naturais, os óleos essenciais extraídos de plantas,

compostos por uma mistura de substâncias voláteis, líquidas e odoríferas, são particularmente promissores e um importante produto a ser explorado por empresas de vários ramos, como a alimentícia e têm despertado interesse por apresentarem diversas propriedades biológicas. Os óleos essenciais são reconhecidos como seguros (GRAS) pela Food and Drug Administration (FDA). Entretanto, para acentuar o uso desses compostos e diminuir algumas limitações do seu uso direto nos alimentos, torna-se necessária a incorporação dos óleos essenciais em matriz polimérica na forma de nanofibras para a melhoria do seu uso e o aumento da eficiência.

As nanofibras apresentam várias características que proporcionam diversas aplicações e vantagens em relação a outras estruturas nanoméricas. Dentre essas características, destacam-se a biocompatibilidade com a incorporação de substâncias ativas; biodegradabilidade aceitável; área de superfície maior; alta porosidade com tamanho de poros pequenos e a possibilidade de controlar a liberação de princípios ativos. Devido ao potencial tecnológico das nanofibras e a possibilidade de agregar princípios ativos em suas estruturas por meio da incorporação de óleos essenciais, a produção de nanofibras seria considerada adequada para aplicações, como embalagens e conservantes de alimentos, evitando possíveis contaminações. Dentro dessa abordagem, torna-se de grande importância o desenvolvimento de embalagens ativas nas quais empregam produtos naturais antifúngicos, como os óleos essenciais, capazes de estender a vida de prateleira de frutas, como a uva de mesa.

Diante do exposto, objetivou-se neste trabalho extrair e caracterizar os constituintes químicos dos óleos essenciais de *Alpinia speciosa*, *Cymbopogon flexuosus*, *Ocimum basilicum* L e *Ocimum gratissimum* L.; produzir través de fiação por sopro em solução (SBS) nanofibras incorporadas com óleos essenciais e caracterizá-las físico-quimicamente, bem como avaliar os testes *in vitro* antifúngicos e antiocratoxigênico sobre *Aspergillus carbonarius*, *Aspergillus niger*, *Aspergillus ochraceus* e *Aspergillus westerdjikiae*, de forma a agregar técnicas inovadoras aos tratamentos de fungos toxigênicos; e estudar o efeito antifúngico *in vivo* (uva) dos óleos essenciais encapsulados em nanofibras de poli (ácido lático) sobre *Aspergillus carbonarius* e *Aspergillus niger*, como potencial embalagem ativa a ser aplicada no controle da degradação de uvas de mesa Niagara rosada.

2 REFERENCIAL TEÓRICO

2.1 Histórico, importância e uso de plantas medicinais

O uso de plantas pela população, a exploração de suas propriedades medicinais e o uso alternativo em terapias, têm sido práticas comuns desde relatos arqueológicos antes de Cristo. Em busca de alívio ou até mesmo cura de doenças, as civilizações antigas utilizavam folhas, sementes e raízes das ervas por meio da ingestão de chás ou através de compressa, sendo essas consideradas as primeiras formas de utilização desses produtos (VIEGAS JR; BOLZANI; BARREIRO, 2006). O homem primitivo buscava descobrir soluções para as necessidades básicas de sobrevivência e por meio de experiências e observações resultaram em descobertas importantes no tratamento de feridas ou doenças através de plantas, especiarias e ervas (PEREIRA; CARDOSO, 2012).

A utilização de recursos naturais na alimentação, na cura de doenças, e também na agricultura procede desde o desenvolvimento das civilizações Oriental e Ocidental, merecendo destaque as culturas Egípcias, Greco-romana e Chinesa. A população egípcia, grega e romana usava o aroma das plantas como incenso para fins religiosos e também descobriram que alguns sabores apresentavam efeitos revigorantes e refrescantes, enquanto outros proporcionavam efeitos sedativos. Além disso, muitas especiarias eram utilizadas para condimentar os alimentos de reis e nobres, como também eram usadas como itens valiosos de troca e comércio, como uma renda monetária. Em várias regiões da China à Roma Antiga, foram relatados o uso das plantas medicinais como perfumes, cosméticos, incensos, conservantes de embalsamento e para fins medicinal e terapêutico (NASCIMENTO et al., 2020; VIEGAS JR; BOLZANI; BARREIRO, 2006).

Alguns registros importantes do uso de plantas medicinas, que são utilizadas até hoje devido ao seu potencial biológico, têm um marco importante na história. O uso, por exemplo, na China (2.735 a.C.) de genciana-amarela (*Gentia lutea*), ginseng (*Panax ginseng*), canela (*Cinnamomum sp.)* e efedra (*Ephedra sinica* Stapf.). No Antigo Egito, descrito por mais de 4000 anos foi o uso da papoula (*Papaver somnniferum* L.), maconha (*Cannabis sativa* L.), mirra (Commiphora myrrha (T. Nees) Engl.), sena (Sena alexandrina Mill.) e babosa (Aloe vera (L.) Burm. f) (DUTRA et al., 2016; ROCHA et al., 2015; SEWELL; RAFIEIAN-KOPAEI, 2014).

Na América, plantas como ipecacuanha (*Cephaelis ipecacuanha* (Brot.) Tussac), quina (*Chincona sp.*) e coca (*Erythroxylum coca* Lam.), dentre outras, eram utilizadas em tratamentos de doenças em diversas culturas Pré-Colombianas. Os índios, no Brasil, usavam especiarias e

ervas, como pimenta-preta e canela, na alimentação, no tratamento de doenças e para fins cosméticos (NASCIMENTO et al., 2020; ROCHA et al., 2015).

Com o desenvolvimento da medicina tradicional chinesa, muitas espécies vegetais consideradas plantas medicinais são estudadas pela busca do entendimento do seu mecanismo de ação e no isolamento dos princípios ativos (VIEGAS JR; BOLZANI; BARREIRO, 2006). Nesse cenário, no século XIX, com os avanços científicos, desencadeou-se a concepção da primeira droga com características conhecidas atualmente, que levou a uma busca contínua por outros medicamentos derivados de plantas. O pioneiro foi Friedrich Serturner (1806), isolando a morfina, um alcaloide da papoula. Em 1824 e 1848, foram isolados da mesma planta os alcaloides codeína (agente antitussígeno) e a papaverina (agente antiespasmódico). Outras substâncias ativas importantes isoladas de plantas medicinais foram atropina (antagosnista muscarínico, 1831), cafeína (1820), digoxina (digitalis, 1869) e curare (relaxante muscular, 1943). Entretanto, o marco histórico na indústria farmacêutica foi em 1832, com a descoberta da salicina isolada de *Salix alba* (salgueiro) por Rafaele Piria, um composto com propriedades analgésica e antipirética. Em 1839, a salicina foi modificada em ácido salicílico para ser usado em tratamentos de artrites reumáticas e, posteriormente, em 1897, a aspirina foi sintetizada a partir do ácido salicílico, período durante o qual inaugurou a Bayer na Alemanha, bem com a primeira patente na área de medicamentos da indústria farmacêutica (DUTRA et al., 2016).

O uso atualmente de diferentes plantas medicinais em infusões, pomadas, compressas e loções está enraizado no patrimônio cultural, sendo as informações do efeito medicinal ou terapêutico passadas de geração em geração. Em 1978, a Organização Mundial de Saúde (OMS) reconheceu os benefícios das plantas medicinais e definiu como a melhor e maior fonte de medicamentos para a humanidade. Posteriormente, em 2004, o Brasil começou a avaliar os medicamentos fitoterápicos seguindo os mesmos padrões de segurança e qualidade dos medicamentos convencionais. Assim, o Ministério da Saúde do Brasil implementou a Política Nacional de Plantas Medicinais e Fitoterápicas (NETO et al., 2020). Com o progresso na área farmacêutica e química, os estudos das plantas medicinais têm gerado descobertas e desenvolvimento de compostos bioativos desses produtos naturais, com base em seu uso tradicional, desempenhando papel importante no desenvolvimento da evidência científica de suas propriedades e aplicações farmacêuticas, cosméticas e alimentícias.

2.2 Espécies vegetais e uso na medicina popular

A grande extensão territorial e as condições climáticas muito diversas fazem com que a flora brasileira possua inúmeras espécies vegetais, muitas consideradas importantes matérias-primas, outras já incorporadas ao hábito alimentar dos brasileiros e algumas pouco conhecidas e, potencialmente, benéficas. Além disso, o reino vegetal constitui uma reserva de vários constituintes bioativos e de uma grande importância econômica a serem descobertos.

2.2.1 *Alpinia speciosa* (Colônia)

A espécie *Alpinia speciosa* (sinonímia científica, *Alpinia zerumbet*) pertencente à família Zingiberaceae é uma planta aromática, herbácea, perene, atingindo de 1 a 2 metros de altura, conhecida popularmente no Brasil como colônia (Figura 1). É originária da Ásia Oriental, mas naturalizou-se na América do Sul, Ásia e na Oceania, nas regiões tropicais e subtropicais. A espécie está distribuída pela Ásia tropical e ocidental, China, Polinésia, Indonésia, Malásia, Filipinas e Brasil, sendo bastante cultivada no sudeste asiático (BRASIL, 2014; GRANDI, 2014; XUAN et al., 2019).

Figura 1 – Aspecto geral da espécie *Alpinia speciosa*.

Fonte: Do autor (2021).

Geralmente, as partes mais utilizadas dessa planta são as folhas, rizomas e sementes nas formas de infusão, decocto e tinturas. A espécie é comumente utilizada na medicina popular como um agente hipotensivo, diurético, tônico, estomáquico, carminativo, excitante, vermífugo e também usado contra amenorreia e diversas doenças da pele (GRANDI, 2014; SANTOS et al., 2011). Estudos de Pereira et al. (2018), Victório (2011) e Xuan et al. (2019) revelaram que as espécies do gênero *Alpinia* fazem parte da dieta humana, sendo utilizadas no preparo de alimentos, como as folhas de *A. speciosa* empregadas para enrolar bolinhos de arroz, embrulhar peixes para assar e os rizomas no preparo de alimentos como temperos e bebidas. De modo geral, a planta é utilizada como especiarias, alimentos, medicamentos, chás, doces, sorvete, perfume, cosméticos, sabão, produtos desodorizantes e para fins ornamentais, por ter flores em tons de rosa e vermelho (XUAN et al., 2019). No Brasil, essa espécie é frequentemente utilizada na medicina popular. Em algumas regiões, o seu uso é no tratamento de reumatismo e doenças do coração, além do seu chá feito das folhas ser utilizado como anti-hipertensivo, diurético e no tratamento da ansiedade (CRUZ et al., 2020; VICTÓRIO, 2011).

As partes da planta mais utilizadas para obtenção dos óleos essenciais, extratos e também constituintes isolados são as folhas, seguidas pelos rizomas, inflorescência, semesntes, raízes, pseudocolmos e pedúnculo (BRASIL, 2014). O óleo essencial das folhas frescas de *A. speciosa* apresenta como principais constituintes químicos o 1,8-cineol, terpinen-4-ol, sabineno, ρ-cimeno, α-tujeno, α-terpieno, β-terpineno. As atividades testadas *in vitro* comprovaram que a espécie apresenta propriedades antioxidante, antimicrobiana, antibacteriana, atifúngica, antitumoral, larvicida, antiviral, entre outras (BRASIL, 2014; CASTRO et al., 2016).

2.2.2 *Cymbopogon flexuosus* (Capim limão)

O gênero *Cymbopogon* representa cerca de 140 espécies, das quais algumas delas são cultivadas para a produção de óleo essencial, sendo *Cymbopogon flexuosus* a que representa uma das principais espécies amplamente cultivadas em diferentes regiões do mundo pelo alto conteúdo de citral (70-80%) em seu óleo essencial (HAQUE; REMADEVI; NAEBE, 2018; VERMA et al., 2019). Apresenta grande variabilidade em relação à morfologia e quimiotipo. A espécie *C. flexuosus*, pertencente à família Poaceae, conhecida popularmente como capim-limão-indiano, é uma gramínea perene, com folhas estreitas e longas, nativa da Índia (Figura 2) (VERMA et al., 2019). Crescem e florescem praticamente em todos os países tropicais e

subtropicais. No Brasil, a produção dessa espécie ocorre principalmente nas Regiões Sul e Sudeste (GOMES; NEGRELLE, 2015; SARMA et al., 2011).

Figura 2 – Aspecto geral da espécie *Cymbopogon flexuosus*.

Fonte: Do autor (2021).

As folhas frescas, secas ou em pó de *C. flexuosus* são utilizadas na culinária como ervas e traz um leve sabor cítrico. Essa planta é utilizada geralmente como chás verdes, em sopas, em carnes de frango, carneiro, peixes, boi e frutos do mar (UPADHYAY et al.,2019). As folhas também são utilizadas na medicina popular por meio de infusão, por apresentarem propriedades febrífugas, sudoríficas, analgésicas, antiespasmódica, sedativa, calmantes, antidepressivas, diuréticas, antipirética, anti-inflamatória e expectorantes (HAQUE; REMADEVI; NAEBE, 2018; GOMES; NEGRELLE, 2015; SARMA et al., 2011).

Essa espécie tem um papel importante nas indústrias farmacêutica, cosmética e de perfumes, em função do seu óleo essencial apresentar um aroma agradável e atividades biológica, como anticancerígena, analgésica, repelente a insetos, antimicrobiana e antifúngica. Os constituintes químicos presentes no óleo essencial são citral, geraniol, metileugenol, mirceno, citronelal. Geralmente, o constituinte majoritário é o citral, formado por dois isômeros, neral e geranial, que conferem o aroma de limão e as propriedades medicinais da planta (AZEVEDO et al., 2016; DESAI; PARIKH, 2012).

2.2.3 *Ocimum basilicum* L. (Manjericão)

A espécie *Ocimum basilicum* L., pertencente à família Lamiaceae, é uma planta herbácea, anual ou perene, bastante ramificada, aromática e perfumada. Conhecida vulgarmente

como manjericão, essa espécie atinge uma altura de 0,5 a 1 metro. Possui haste reta com muitas folhas carnosas, ovaladas, sem pelos e de cor verde-brilhante; suas flores são brancas ou avermelhadas (Figura 3) (FAVORITO et al., 2011; GRANDI, 2014).

Figura 3 – Aspecto geral da espécie *Ocimum basilicum*.

Fonte: Do autor (2021).

O manjericão é originário da Índia, chegando à Europa e passando pelo Oriente Médio. É subespontâneo em todo o Brasil. Na medicina popular, é utilizado na cura de feridas; tuberculose pulmonar; gripe, tosse e resfriado; infecções de boca e garganta; espasmos e queda de cabelo nas formas de cataplasma, xarope, banho, decocção e infusão, respectivamente. Indicado também na forma de chá para tratamentos de dor de cabeça, disenteria, náusea, flatulência, anticonvulsivante e anti-inflamatório. É considerado poderoso antisséptico, carminativo, digestivo, inseticida e analgésico (BAE et al., 2020; MILITÃO; FURLAN, 2014; NASCIMENTO et al., 2020).

No comércio, na indústria alimentícia e farmacológica, o manjericão é utilizado no preparo de fitoterápicos, em condimentos, molhos, sopas, queijos, vinagre, óleos e temperos, fornecendo aromas nos pratos culinários do dia a dia, além de ser usado na aromaterapia e indústrias de perfumaria. Em combinação com outras especiarias, pode ser usado no preparo de condimentos, alimentos de panificação, sorvetes, entre outros (FAVORITO et al., 2011; MILITÃO; FURLAN, 2014; NASCIMENTO et al., 2020).

O óleo essencial da espécie *Ocimum basilicum* L. pode ser extraído de folhas e ápices que possuem inflorescências, sendo os principais constituintes eugenol, estragol, linalol, lineol, alcanfor, cineol, pineno, carvacrol e timol. O óleo apresenta propriedades antioxidantes,

antimicrobianas, inseticidas e repelentes (FAVORITO et al., 2011; MILITÃO; FURLAN, 2014).

2.2.4 *Ocimum gratissimum* L. (Alfavaca)

A espécie *Ocimum gratissimum* L., pertencente à família Lamiaceae, é conhecida popularmente como alfavaca. *Originária* da Ásia e comumente encontrada em regiões tropicais e quentes, na África, Índia, Europa, América Central e Sul, é subespontâneo em todo o Brasil, principalmente na Região Nordeste (BRASIL, 2015). Morfologicamente, a alfavaca é um subarbusto aromático com até 2 metros de altura, folhas ovais lanceoladas, flores pequenas lilases ou brancas (Figura 4) (GRANDI, 2014).

Figura 4 – Aspecto geral da espécie *Ocimum gratissimum*.

Fonte: Do autor (2021).

Na medicina popular, a planta é utilizada na forma de infusão, decocto, xarope, maceração ou emplasto contra gripes, resfriados e febres. O suco das folhas é usado para aliviar sintomas de dores de cabeça, tontura e tosse. Indicada também como emoliente, expectorante, sudorífero, antisséptico bucal, doenças oftálmicas, infecções de ouvido, contra frieiras e

doenças de pele, entre outros. Essa espécie tem potencial germicida e é bastante usada em cremes dentais e pomadas de uso tópico, bem como gargarejo para dores de garganta e amigdalite. O extrato da planta é usado contra parasitas gastrointestinais e sua propriedade carminativa apresenta um bom aliado para dores de estômago. Além das propriedades farmacológicas, a alfavaca é utilizada na culinária como tempero e considerada um excelente condimento (BHAVANI et al., 2019; GRANDI, 2014; KUMAR et al., 2019; OLIVEIRA et al., 2016).

Esta planta é considerada como importante fonte de óleos essenciais usados principalmente nas indústrias para a produção de fármacos, perfumes e cosméticos, consequência do seu cheiro forte e aromático. Os principais constituintes do óleo essencial da alfavaca são eugenol, 1,8-cineol, timol, estragol, metil-chavecol, linalol e cânfora, conferindo propriedades antioxidante, antifúngica, antibacteriana, antiparasitária, antiproliferativa (BRASIL, 2015; OLIVEIRA et al., 2016). O eugenol é considerado como o composto majoritário e é empregado na indústria farmacêutica em produtos dentários por ser antisséptico local e analgésico, em formulação de xaropes para tratar bronquite e tosse, sendo utilizado também como aromatizantes em creme dentais (CRUZ; BEZERRA, 2017).

2.3 Metabólitos secundários

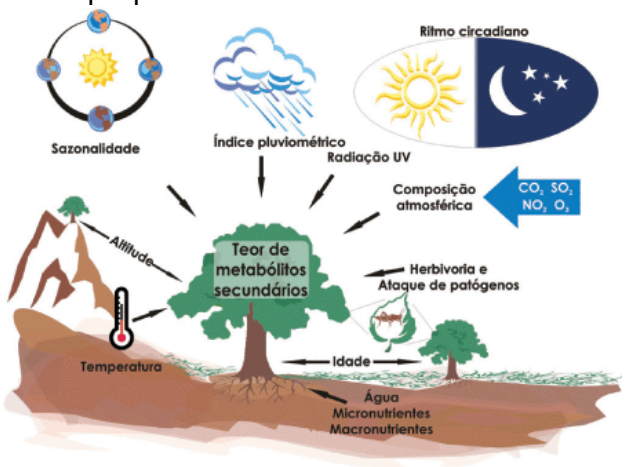
As plantas medicinais são bastante importantes para a química e a medicina moderna, devido às substâncias ativas presentes nessas plantas, que foram isoladas e identificadas com o decorrer dos avanços científicos, permitindo o desenvolvimento de produtos que foram introduzidos na medicina terapêutica, permanecendo até hoje como medicamentos. Essas substâncias são derivadas do metabolismo primário ou secundário das plantas (PEREIRA; CARDOSO, 2012).

O metabolismo é o conjunto de reações que ocorrem nas células vivas e envolve síntese, quebra ou transformações de moléculas orgânicas catalisadas por enzimas. O metabolismo primário é responsável pela formação de proteínas, lipídeos, carboidratos, ácidos nucleicos e outras substâncias importantes na realização das funções vitais dos seres vivos (PEREIRA; CARDOSO, 2012). Já o metabolismo secundário é responsável por sintetizar substâncias que aparentemente não estão envolvidos com a geração de energia ou desenvolvimento do organismo; entretanto, atuam em funções indispensáveis para as interações ecológicas entre as espécies vegetais e o meio ambiente (PEREIRA; CARDOSO, 2012; SIMÕES et al., 2017).

Os metabólitos secundários apresentam-se em baixas concentrações e em determinados grupos de plantas, geralmente são de estrutura complexa, baixo peso molecular e responsável por apresentarem diversas atividades biológicas. A quantidade e a qualidade dos metabólitos secundários estão relacionadas com a genética da planta e também são afetados pelas condições ambientais e edafoclimáticas (DEHSHEIKH et al., 2020). Nutrientes no solo, como por exemplo, nitrogênio e fósforo, são essenciais para o desenvolvimento e crescimento de plantas aromáticas, bem como a formação de metabólitos secundários para defesa contra estresse. Esses nutrientes participam na estrutura precursora dos metabólitos secundários, assim como nas enzimas e estruturas de moléculas transportadoras de energia (ATP e NADPH) necessárias para as reações bioquímicas (DEHSHEIKH et al., 2020). Os metabólitos podem ser sintetizados nas plantas em estágios específicos de crescimento ou em períodos de estresse provocados por algum agente externo, como exposição à radiação UV-B, irrigação deficiente ou por ataque de herbívoros e microrganismos, desempenhando assim um papel protetor contra esses agentes. Esses constituintes despertam grande interesse pelas funções exercidas nas plantas em resposta ao meio em que vivem, como defesa contra predadores, atrativos voláteis ou agentes corantes para atrair polinizadores ou animais dispersores de sementes, bem como a participação em alelopatia e também pela imensa ação na área farmacológica, alimentícia, agronômica, cosmética e perfumaria (SIMÕES et al., 2017).

O teor e a composição dos metabólitos secundários são influenciados pela época em que a planta é coletada, sendo um dos fatores de maior importância, visto que a quantidade e a natureza dos constituintes ativos não são constantes durante todo o período do ano. Dentre alguns fatores que também influenciam o conteúdo e o teor desses constituintes, destacam-se a sazonalidade, clima, idade da planta, temperatura, disponibilidade hídrica, radiação ultravioleta, nutrientes do solo, altitude, poluição atmosférica, bem como a indução de estímulos mecânicos e ataque de patógenos (Figura 5) (GOBBONETO; LOPES, 2007).

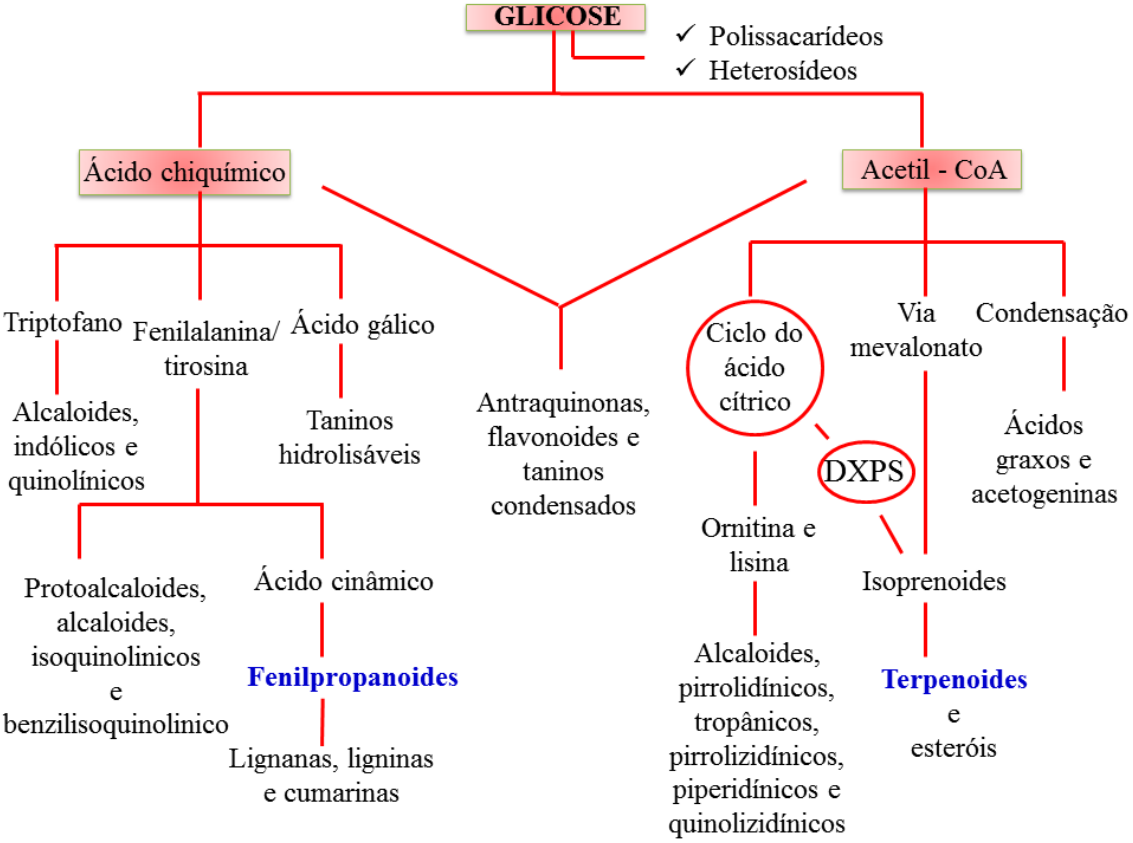
Figura 5 – Principais fatores que podem influenciar o teor de metabólitos secundários.



Fonte: Gobbo-Neto e Lopes (2007).

Entre os metabólitos secundários, citam-se os alcaloides, flavonoides, cumarinas, taninos, quinonas e óleos essenciais. Eles podem ser divididos em três grupos: terpenos, compostos fenólicos e compostos nitrogenados, sendo todos eles originados do metabolismo da glicose, a partir de dois intermediários principais: o ácido chiquímico e o acetil-coA, conforme apresentado na Figura 6.

Figura 6 – Biossíntese dos metabólitos secundários.



Fonte: Simões et al. (2017).

O precursor básico dos metabólitos secundários é a glicose. O catabolismo da glicose pela rota do ácido chiquímico é responsável pela formação de compostos que apresentam em sua estrutura um anel aromático, como os taninos hidrolisáveis, cumarinas, alcaloides derivados de aminoácidos aromáticos e fenilpropanoides. Pela via do acetato (acetil-coenzima A, acetil-CoA), serão formados os terpenos, esteróis, ácidos graxos, triglicerídeos, aminoácidos alifáticos e os alcaloides derivados deles. Alguns metabólitos secundários derivam de ambos os intermediários, como é o caso das antraquinonas, flavonoides e taninos condensados, os quais resultam da combinação de uma unidade de ácido chiquímico e uma ou mais unidades de acetato ou derivados (PEREIRA; CARDOSO, 2012; SIMÕES et al., 2017).

2.4 Óleos essenciais

Os óleos essenciais fazem parte de um grupo de metabólitos secundários bastante importante, formados por uma mistura complexa de substâncias que podem ser chamados de óleos voláteis, etéreos ou essências. Essas denominações provêm das características físico-químicas, como a de serem geralmente líquidos de aparência oleosa, volátil, aroma agradável e intenso na maioria dos óleos e são solúveis em solventes orgânicos. Além dessas características, apresentam um sabor geralmente ácido e picante; incolores ou ligeiramente amarelados; pouco estáveis na presença de luz, calor, umidade e metais; a maioria possui índice de refração e são opticamente ativos (SIMÕES et al., 2017). A ISO (International Standard Organization) define óleo essencial como “produto obtido de matéria-prima de origem vegetal através da destilação a vapor, processos mecânicos de epicarpos de frutos cítricos ou destilação a seco, após separação da fase aquosa, se houver, por processos físicos. A destilação a vapor pode ser com adição de água ao destilador (hidrodestilação) ou sem adição de água (diretamente por vapor)” (INTERNATIONAL STANDARD ORGANIZATION, 2013).

A estrutura química dos constituintes dos óleos essenciais é formada basicamente por carbono, oxigênio e hidrogênio, variando em relação à função orgânica, como hidrocarbonetos terpênicos, álcoois simples e terpênicos, aldeídos, cetonas, fenóis, ésteres, éteres, óxidos, entre outros. Os óleos essenciais podem conter aproximadamente entre 20-60 componentes em diferentes concentrações, sendo geralmente caracterizados por um, dois ou três com concentrações elevadas (compostos majoritários), outros em proporções menores (compostos minoritários) e alguns em concentrações mínimas (traços). Podem ser sintetizados por todos os órgãos das plantas, entre eles, botões florais, flores, folhas, caule, galhos finos, sementes, frutos, raízes e cascas, e são estocados em células secretoras, cavidades, canais, células epidérmicas

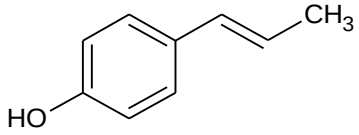
ou tricomas glandulares (BAKKALI et al., 2008; SIMÕES et al., 2017). Atualmente, os óleos essenciais podem ser extraídos por diferentes técnicas (enfloração, arraste por vapor d'água, prensagem ou espressão, hidrodestilação e extração por CO_2 supercrítico), de acordo com a parte da planta que será utilizada e a finalidade de aplicação do óleo. Os métodos de hidrodestilação e destilação por arraste a vapor são os mais empregados, pois os óleos essenciais obtidos apresentam maior pureza por não terem contato com nenhum solvente, apenas com água. Nesse sentido, essas técnicas permitem que os óleos essenciais obtidos sejam aplicados em alimentos, na elaboração de fitoterápicos, cosméticos ou perfumes (SIMÕES et al., 2017).

2.4.1 Biossíntese dos óleos essenciais

Os óleos essenciais são constituídos de diversos compostos derivados de fenilpropanoides e de terpenos, sendo esses últimos predominantes na forma de monoterpenos e sequiterpenos (SIMÕES et al., 2017).

Os fenilpropanoides são compostos derivados da glicose, oriundo da via do ácido chiquímico. São substâncias naturais amplamente encontradas nos vegetais e apresentam em sua estrutura um anel aromático unido a uma cadeia lateral de três átomos de carbonos, com uma dupla ligação, podendo apresentar uma hidroxila na posição *para* (Figura 7). Os fenilpropanoides também podem conter em suas estruturas outros grupos funcionais oxigenados, como aldeído, cetona ou álcoois (BUCHANAN; GRUISSEM; JONES, 2015).

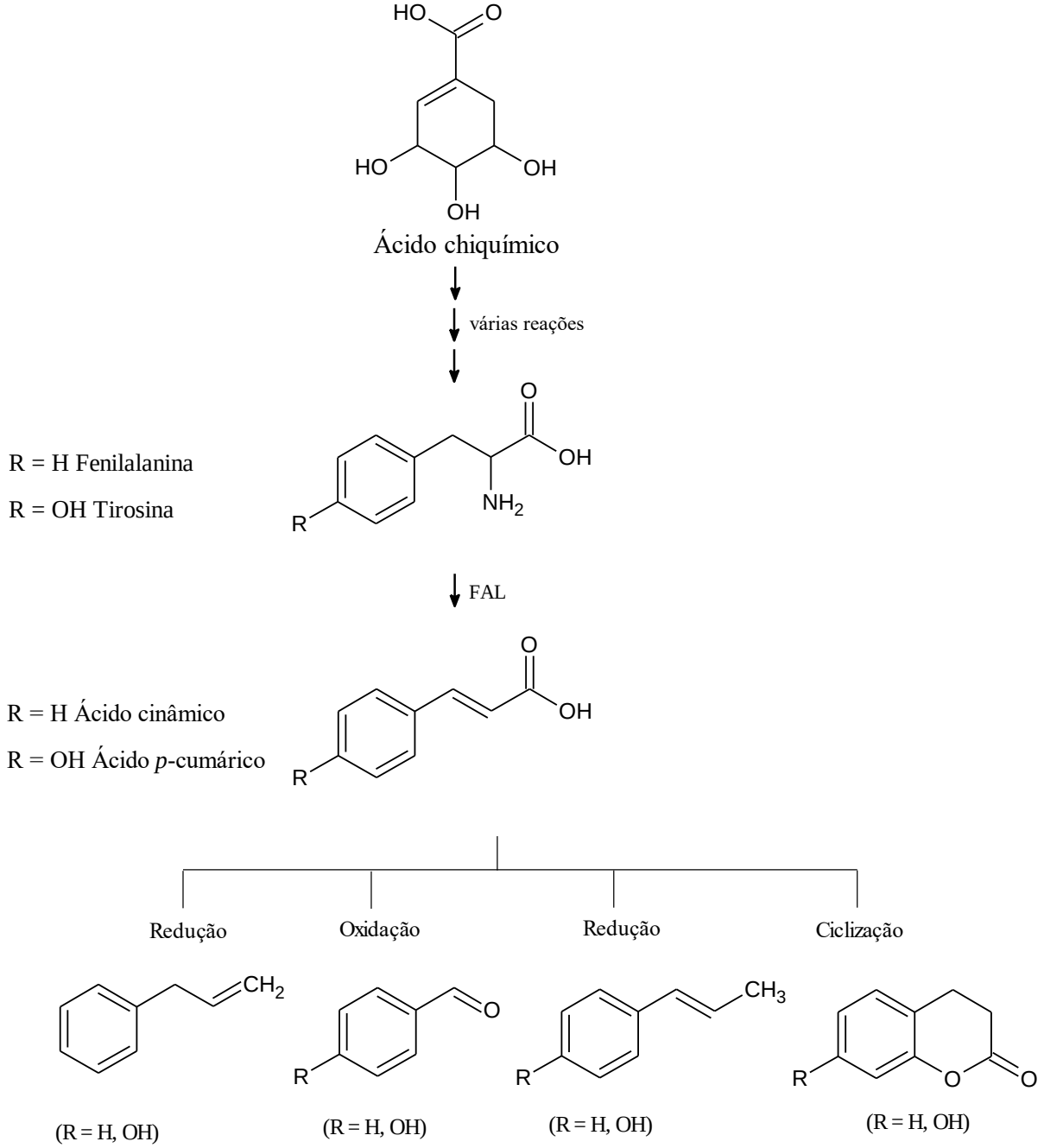
Figura 7 – Estrutura química de um fenilpropanoide.



O ácido chiquímico é formado pela condensação aldólica de dois metabólitos da glicose, o fosfoenolpiruvato (via glicolítica) e a eritrose-4-fosfato (via das pentoses). Por meio da reação do ácido chiquímico e de uma molécula de fosfoenolpiruvato catalisada pela enzima ácido 3-fosfato-3-enolpiruvilchiquímico sintase (EPSP) ocorre a formação do ácido corísmico. Esse ácido é responsável por gerar aminoácidos aromáticos, como a fenilalanina e a tirosina que, por sua vez, com a ação da enzima fenilalanina amonialiase (FAL), perde uma molécula de amônia, resultando na formação dos ácidos cinâmicos e p-cumárico (Figura 8). Por meio de redução enzimática, os ácidos cinâmicos e p-cumárico formam propenilbenzenos e/ou alilbenzenos, que

por meio de reações de oxidação com degradação das cadeias laterais e ciclização, geram diversos compostos presentes nos óleos essenciais, entre eles os fenilpropanoides (SIMÕES et al., 2017).

Figura 8 – Biossíntese de fenilpropanoides.

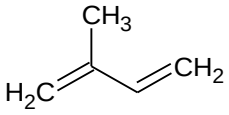


Fonte: Adaptado de Dewick (2009) e Simões et al. (2017).

Os terpenos são constituídos de carbono e hidrogênio a partir de unidades de isopreno (2-metilbuta-1,3-dieno) (Figura 9), podendo apresentar também oxigênio na cadeia carbônica (chamados de terpenoides), como álcoois, aldeídos, cetonas, fenóis, éteres e ésteres. Os

compostos terpênicos ocorrem em uma grande variedade de espécies vegetais e compreendem uma classe de metabólitos secundários com uma grande variedade estrutural (SIMÕES et al., 2017). Os terpenos são formados a partir de combinações de duas ou mais moléculas de isopreno e são classificados pelo número de unidade isoprênica que possuem. Nessa classe, incluem-se os monoterpenos (10 átomos de carbono), sesquiterpenos (15 átomos de carbono), diterpenos (20 átomos de carbono), sesterpenos (25 átomos de carbonos), triterpenos (30 átomos de carbonos), tetraterpenos (40 átomos de carbonos) e polisoprenoides. Os monoterpenos e sesquiterpenos são os compostos encontrados com maior frequência nos óleos essenciais e as unidades de isopreno podem ser ligadas de forma a originar cadeias lineares ou moléculas cíclicas (SIMÕES et al., 2017).

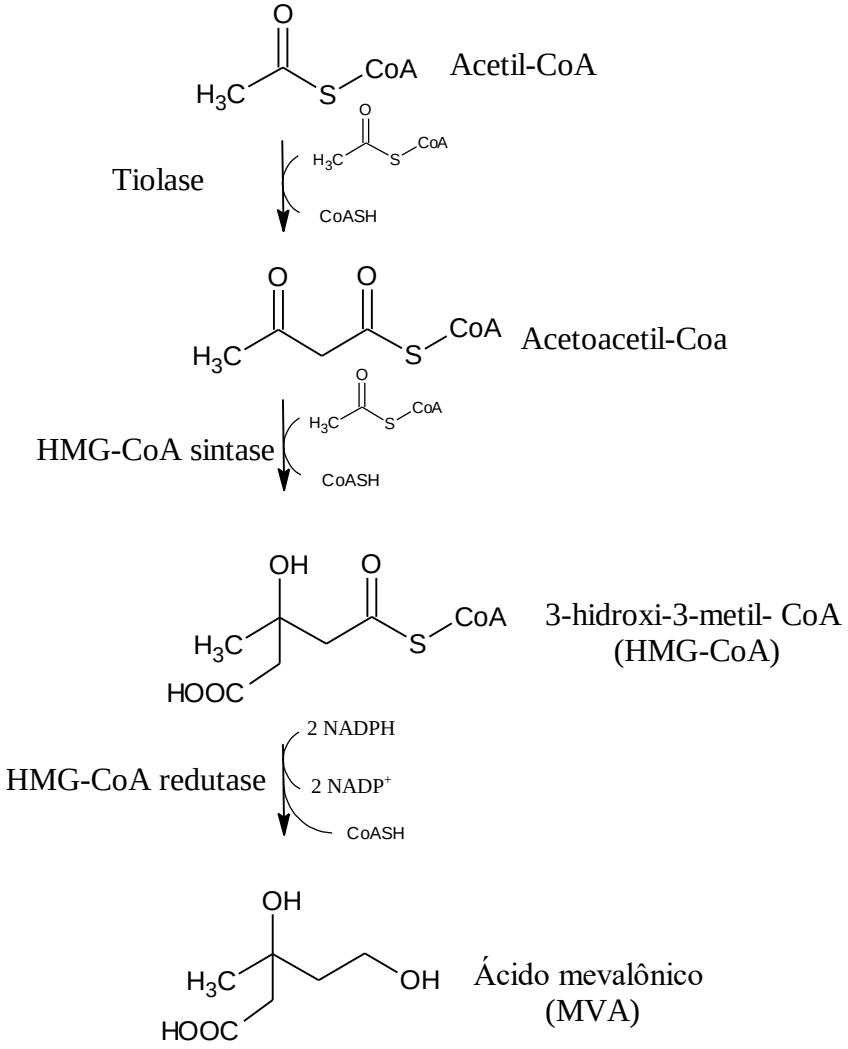
Figura 9 – Estrutura química do isopreno (unidade básica)

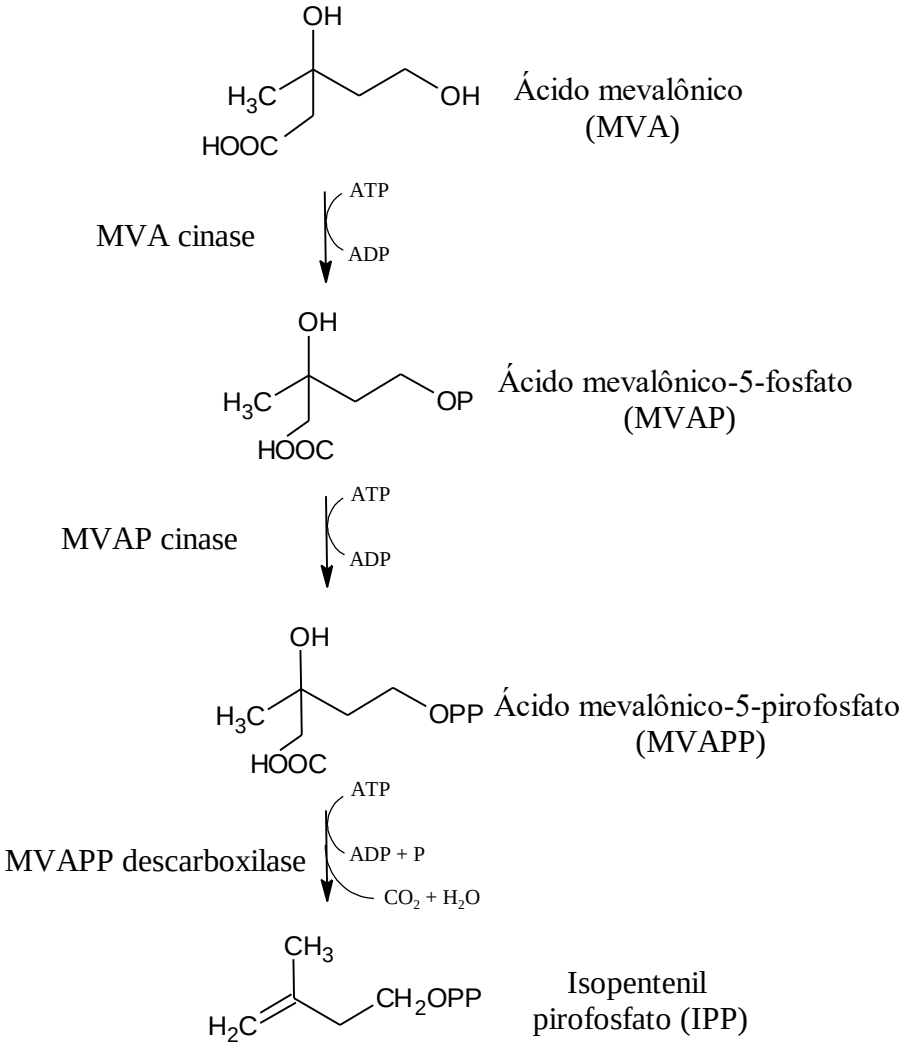


A biossíntese dos terpenos ocorre a partir da glicose. Por meio de uma série de reações do metabolismo primário, a glicose é catabolizada em acetil-coenzima A (acetil-CoA), um tio-éster, que dará origem aos terpenoides a partir de duas vias, a via do mevalonato, sendo ativa no citosol e no retículo endoplasmático, e a via 1-deoxi-D-xilulose-5-fosfato (DXPS), ocorrendo nos plastídios (SIMÕES et al., 2017). A biossíntese dos terpenoides pode ser dividida em 4 etapas: síntese do precursor fundamental, ou isopreno ativo, isopentenil difosfato (IPP); adições repetitivas do IPP para formação de uma série de homólogos prenil difosfato; ação de enzimas específicas na produção dos esqueletos terpênicos; modificações enzimáticas secundárias dos esqueletos para originar funcionalidade e uma grande diversidade de compostos (DEWICK, 2009).

A rota do ácido mevalônico (Figura 10) envolve a condensação de três moléculas de acetil-CoA em dois passos, acetil-CoA catalisada por tiolase e hidroximetilglutaril-CoA sintase, resultando no 3-hidroxi-3-metilglutaril-CoA (HMG-CoA), que é subsequentemente reduzido por HMG-CoA redutase em duas reações acopladas, obtendo o ácido mevalônico. Duas fosforilações sequenciais do ácido mevalônico dependem de ATP e uma subsequente fosforilação/descarboxilação seguida por eliminação, obtém-se então o IPP (BUCHANAN; GRUISSEM; JONES, 2015).

Figura 10 – Via do mevalonato para a formação do isopentenil pirofosfato.

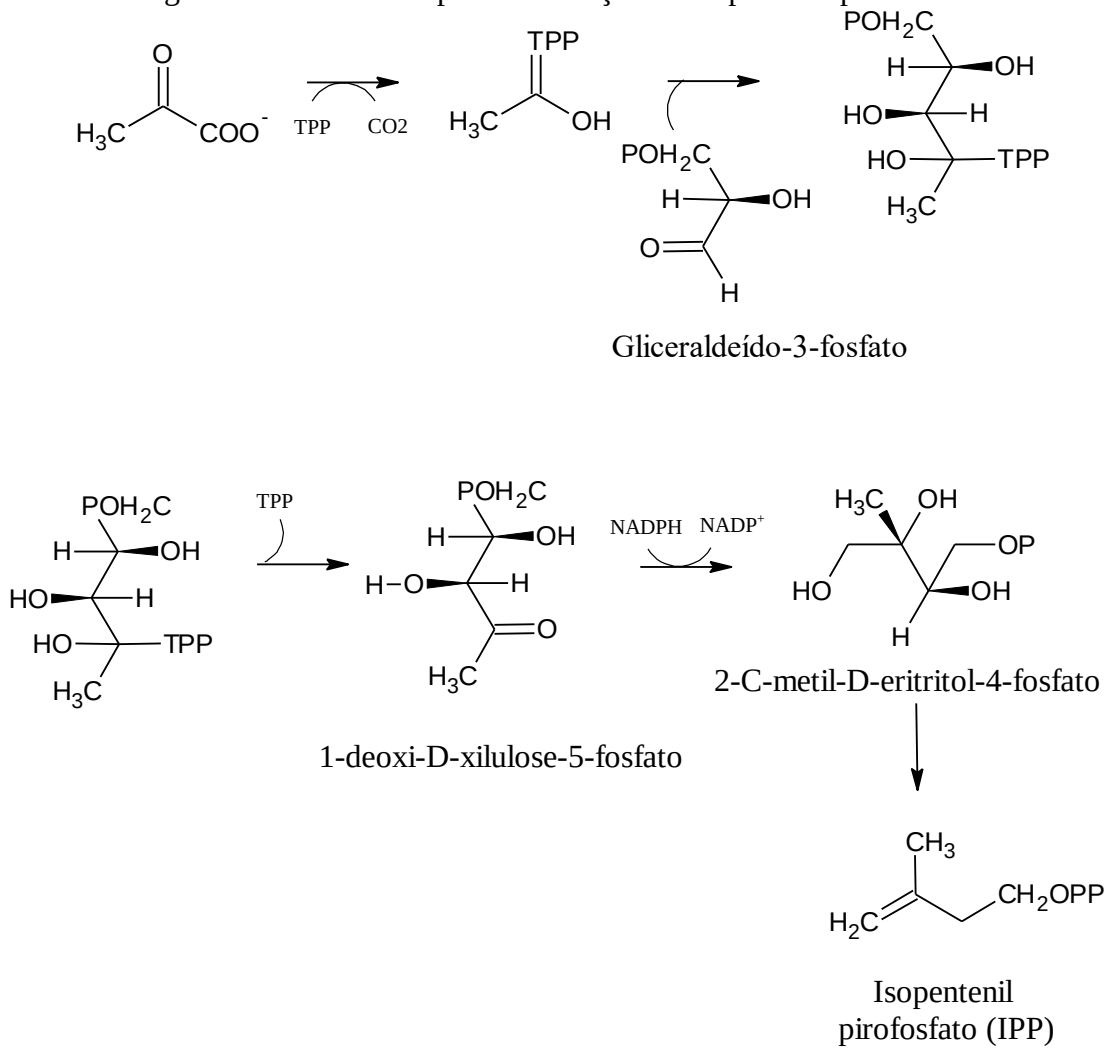




Fonte: Buchanan, Gruissem e Jones (2015).

Na rota biossintética para a formação do IPP pela via 1-deoxi-D-xilulose-5-fosfato (DXPS) (Figura 11), o piruvato que sofreu descarboxilação reage com tiamina pirofosfato (TPP), dando origem a um fragmento de dois carbonos, hidroxietil-TPP, que se condensa com gliceraldeído 3- fosfato por transferências C2 da TPP catalisada pela transcetolase. TPP é liberado para formar um composto de cinco átomos de carbono intermediário, 1-deoxi-D-xilulose-5-fosfato, o qual é rearranjado e reduzido para formar 2-C-metil-D-eritritol-4-fosfato e, subsequentemente, transformado para se obter o IPP (BUCHANAN; GRUISSEM; JONES, 2015).

Figura 11 – Via DXPS para a formação do isopentenil pirofosfato.

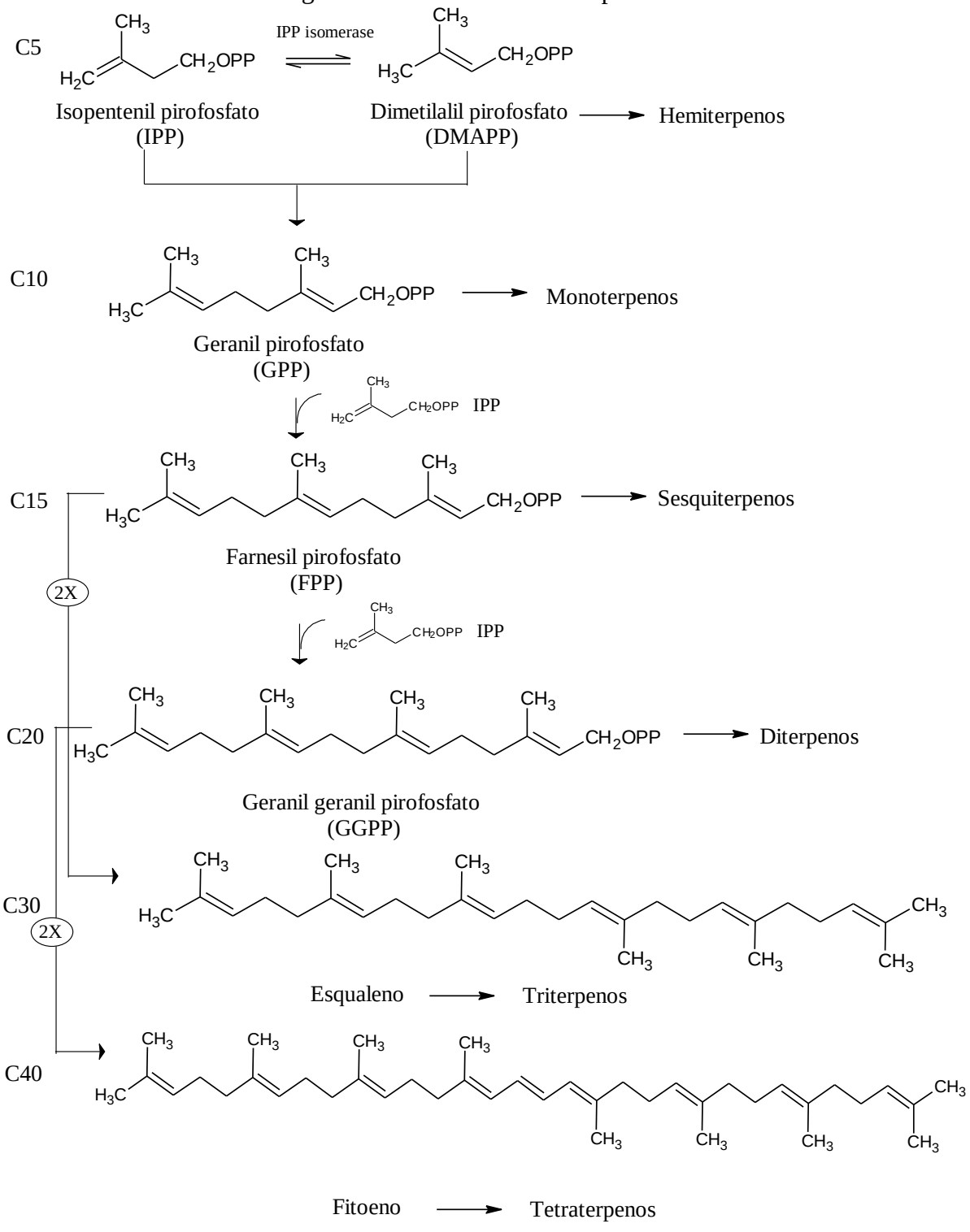


Fonte: Buchanan, Gruissem e Jones (2015).

As diferentes classes de terpenos são formadas por adições repetitivas do isopentenil pirofosfato (Figura 12). O IPP sofre ação de uma isomerase estereoespecífica, interconvertendo-se em dimetilalil pirofosfato (DMAPP), isômero favorecido no equilíbrio (DEWICK, 2009; SIMÕES et al., 2017). O isopentenil pirofosfato e seu isômero são as unidades penta carbonadas ativas na biossíntese dos terpenos que se unem para formar moléculas maiores. Primeiro o IPP e o DMAPP combinam-se em reações do tipo condensação cabeça-cauda, para formar os terpenos a partir dos intermediários *trans*-geranildifosfato (GPP), uma molécula que possui 10 carbonos, na qual são formados os monoterpenos. Esse tipo de condensação se inicia com a perda do grupo difosfato ligado à molécula de DMAPP (cauda), formando um carbocátion primário, que se adiciona à molécula de IPP (cabeça) por uma reação enzimática de adição eletrofílica na dupla ligação (SIMÕES et al., 2017). O GPP pode se ligar a outra molécula de IPP, formando um composto de 15 carbonos, farnesil pirofosfato (FPP),

precursor da maioria dos sesquiterpenos. A adição de outra molécula de IPP forma o geranil geranil pirofosfato (GGPP), composto que contém 20 carbonos precursores dos diterpenos. Assim, as moléculas de FPP e GGPP podem dimerizar para formar triterpenos (C30) e tetraterpenos (C40), respectivamente (BUCHANAN; GRUISSEM; JONES, 2015).

Figura 12 – Biossíntese dos terpenoides



Fonte: Buchanan, Gruissem e Jones (2015).

2.4.2 Óleos essenciais e seu potencial biológico

Os óleos essenciais obtidos a partir de plantas aromáticas vêm sendo usados como agentes terapêuticos devido ao potencial biológico que seus constituintes apresentam e por serem geralmente considerados agentes GRAS (geralmente reconhecidos como seguro), produtos biodegradáveis e com baixo risco na seleção de resistência (PRAKASH et al., 2015). A descoberta e o desenvolvimento de compostos bioativos desses produtos naturais, com base em seus usos tradicionais e no papel importante no sistema de defesa químico das plantas devido às interações com predadores na natureza, desempenham um papel fundamental no desenvolvimento de evidências científicas de suas ações para aplicações em vários ramos industriais (NASCIMENTO et al., 2020). Nesse sentido, destacam-se as indústrias agroquímica, (na elaboração de novos repelentes, pesticidas e herbicidas); alimentícia (na utilização para conservação devido às propriedades antioxidantes e antimicrobianas, além de conferir aroma e sabor aos alimentos); farmacêutica (devido às suas propriedades farmacológicas); de cosméticos (atuando como matéria-prima para sabonetes, cremes e perfumes); de perfumaria (utilizados para fixação e produção de fragrâncias dentre outras aplicações); e na aromaterapia, em massagens como misturas com óleo vegetal ou em banhos, sendo descritos como produtos com grande potencial terapêutico e farmacológico (BAKKALI et al., 2008; CARVALHO; ESTEVINHO; SANTOS, 2016; EL ASBAHANI et al., 2015; MACHADO; FERNANDES JUNIOR, 2011; PAVELA; BENELLI, 2016).

Os constituintes dos óleos essenciais têm sido uma fonte alternativa para o desenvolvimento e descoberta de novos compostos bioativos com ampla aplicação devido às suas propriedades anti-inflamatória, antibacteriana, antifúngica, repelente, inseticida, antioxidante, alelopática, neuroprotetora, antisséptica, analgésica, sedativa, espasmolítica e citotóxica contra linhagens de células cancerígenas. Além disso, os óleos essenciais vêm sendo pesquisado como um aditivo alternativo na indústria de alimentos em contraste com antioxidantes e fungicidas sintéticos, que podem exibir efeitos deletérios e indesejáveis à saúde humana (BAKKALI et al., 2008; NASCIMENTO et al., 2020).

Algumas espécies de plantas que contêm compostos bioativos têm se tornado cada vez mais objeto de estudo, pois essas substâncias, como, por exemplo, os óleos essenciais, possuem alta propriedade biológica e valor terapêutico. As espécies de *Alpinia* são utilizadas como plantas ornamentais e aromatizantes, além de apresentar propriedades terapêuticas e serem utilizadas em medicamentos tradicionais (KERDUDO et al., 2017). Estudando o óleo essencial de *A. zerumbet*, os pesquisadores observaram que ele apresentou atividades antioxidante,

antimicrobiana, antibacteriana, antifúngica, inseticida e herbicida, além de ser considerado promissor para o desenvolvimento de pesticidas ecológicos (KERDUDO et al., 2017; XUAN et al., 2019). Pereira et al. (2018), pesquisando o óleo essencial de *A. speciosa*, observaram que ele foi bastante eficaz contra os parasitas *Trypanosoma cruzi* e *Leishmania brasiliensis*, apresentando valores com relevância clínica e baixa toxicidade.

O gênero *Cymbopogon* compreende várias espécies de gramíneas, sendo algumas espécies aromáticas importantes comercialmente cultivadas para extração de óleos essenciais, utilizando-se principalmente de suas partes aéreas (VERMA et al., 2019). Estudando o óleo essencial de *C. flexuosus*, Gaonkar et al. (2018) observaram que ele suprime significativamente a expressão do gene HSP90, que é responsável pelo dobramento adequado das proteínas do câncer. Efeitos antifúngicos, repelentes, inseticidas, antimicrobianos, anticancerígeno e antibacteriano também são atribuídos aos constituintes presentes no óleo essencial de *C. flexuosus* (BHATT; KALE, 2019; DEVI et al., 2020; GUNDEL et al., 2020; ROSSI et al., 2017).

As espécies do gênero *Ocimum* foram descritas por apresentarem propriedades larvicida, inseticida, repelente, nematicida, antimicrobiana, antibacteriana, antifúngica, antioxidante, anti-inflamatória, antinociceptiva, antipirética, antiúlcera, analgésica e anti-helmíntica, bem como citotoxicidade contra linhagens de células cancerígenas (ATIF et al., 2020; BALASUBRAMANI et al., 2018; COUTO et al., 2021; MELO et al., 2019; NASCIMENTO et al., 2020; PANDEY; SINGH; TRIPATHI, 2014; SOUSA et al., 2021). Entre as espécies desse gênero, a aplicabilidade do óleo essencial de *O. basilicum* está relacionada com a atividade larvicida sobre mosquitos com relevância médica (*Aedes albopictus* e *Anopheles subpictus*), sendo os constituintes linalol e cinamato de metila responsáveis pelas atividades repelente, larvicida e inseticida (NASCIMENTO et al., 2020). Balasubramani et al. (2018), estudando as folhas de *O. basilicum*, relataram que seu óleo essencial foi eficaz no controle de larvas de mosquito, sendo considerado um larvicida ecológico verde contra a febre da malária. Carrasco et al. (2012), pesquisando o óleo essencial de *O. gratissimum*, encontraram como um dos compostos majoritários o eugenol, atribuindo a esse constituinte atividade antifúngica sobre diferentes linhagens fúngicas. Os pesquisadores sugeriram que a atividade do óleo essencial está relacionada com a biossíntese de ergosterol, interferindo na integridade da membrana celular (CARRASCO et al., 2012). Posteriormente, Brandão et al. (2020) e Brandão et al. (2021) observaram que os óleos essenciais de candeia e alecrim-d'angola aplicados em fungos do gênero *Aspergillus* são eficientes na redução significativa da biossíntese de ergosterol, indicando um possível mecanismo de ação dos óleos essenciais. Os pesquisadores

relataram que, por meio de eletromicrografias de varredura, foi possível visualizar alterações morfológicas e danos à integridade da membrana celular do fungo, resultando em perda da integridade e não formação dos conídios, após tratamento com os óleos essenciais.

2.5 Contaminação alimentar

A contaminação e/ou deterioração de alimentos pode ser causada por diversos fatores, como químicos (metais pesados, pesticidas, detergentes, toxinas de plantas e de animais e antibióticos); físicos (partículas metálicas, fragmentos de insetos, pedaços de vidro, poeira e outros particulados sujos) e biológicos (bactérias patogênicas, parasitas, vírus e fungos toxigênicos), além de reações químicas (oxidação) e mudanças físicas, sendo essa última classe considerada a mais preocupante, pois é a principal fonte de contaminação à saúde pública (FRANCO; LANDGRAF, 2008; MOGOŞANU et al., 2017). Os alimentos são facilmente contaminados pelos microrganismos, durante a manipulação e o processamento. Quando contaminados, os alimentos servem como meio de cultura ideal para o crescimento dos microrganismos, uma vez que são fontes de substâncias nutritivas. Assim, a preservação é um processo importante na tecnologia de alimentos para manter a qualidade, estabilidade físico-química, prazo de validade e prevenção da deterioração dos alimentos. Nesse sentido, a conservação dos produtos pode ser classificada em conservantes antimicrobianos, agindo contra microrganismos, na prevenção e limitação do crescimento e ação microbiana; conservantes antioxidantes, para desacelerar a oxidação dos lipídios (gorduras); e inibitórios, que bloqueiam o amadurecimento, escurecimento após a colheita de frutas ou vegetais (MOGOŞANU et al., 2017).

2.5.1 Contaminação de alimentos por fungos

Os fungos são microrganismos eucarióticos, heteretróficos, pertencentes ao Reino Fungi. Possuem parede celular constituída por glicoproteínas e polissacarídeos, principalmente glucano e quitina, além de uma membrana celular, cujo principal componente é o ergosterol. Os fungos unicelulares são considerados leveduras e os multicelulares são fungos filamentosos e formam micélios. Os fungos podem apresentar morfologias diferentes de acordo com as condições de sobrevivência, entre elas, temperatura e nutrientes (BOWMAN, FREE, 2006).

Esses microrganismos podem apresentar grande importância biotecnológica, pois podem ser empregados nas indústrias farmacêuticas, na produção de alimentos e de grande

importância agrícola e ecológica na manutenção do equilíbrio do ambiente. Determinados fungos são substratos altamente desejados e favoráveis na produção de queijos ou no processo de fermentação das cervejas e vinhos, enquanto outros são usados na obtenção de antibióticos, como a penicilina. Em contrapartida, muitos fungos podem causar transformações indesejáveis, tanto na composição química quanto na estrutura e aparência, produzindo sabores e odores desagradáveis, causados por diferentes graus de deterioração (ABREU; ROVIDA, PAMPHILE, 2015). Dessa forma, os produtos alimentícios passam a ser rejeitados pelos consumidores, representando perdas econômicas e/ ou desperdícios de matéria-prima. Outro problema grave associado à contaminação de alimentos por fungos é a capacidade que esses microrganismos têm em produzir toxinas que representam sérios riscos para a saúde humana e animal, podendo provocar doenças que, em casos mais graves, levam ao óbito.

Os fungos são largamente distribuídos no meio ambiente, incluindo água, ar e solo. Assim, os alimentos podem ser contaminados com uma ampla variedade fúngica originárias de fontes ambientais, e que sob condições favoráveis, podem multiplicar-se nos alimentos, provocando sua deterioração. Os fungos conseguem proliferar em substratos e em condições que outros microrganismos não se desenvolvem, como o crescimento em condições de atividade de água (a_w) reduzida dentro do limite de 0,65 até 0,99; pH reduzido; em uma ampla faixa de temperatura, variando de 0 a 40 °C; capacidade de esporulação e disseminação em diferentes condições. Esses atributos tornam os fungos capazes de causar deterioração em alimentos com diferentes níveis de umidade e de condições climáticas (MEDINA et al., 2005).

Entre os vários fungos contaminadores de alimentos e considerados toxigênicos, as espécies pertencentes ao gênero *Aspergillus* são consideradas uma das principais, capazes em produzir micotoxinas. São microrganismos que, geralmente, contaminam os produtos agrícolas no campo, antes da colheita, pós-colheita ou durante o processamento, transporte e no armazenamento. As micotoxinas produzidas pelas espécies do gênero *Aspergillus,* quando ingeridas por meio de alimento contaminado, são responsáveis por doenças crônicas e agudas em animais e humanos (ANDRADE; LANÇAS, 2015; MILICEVIC; SKRINJAR; BALTIC, 2010).

2.5.2 *Aspergillus*

Os fungos do gênero *Aspergillus* pertencem à família Trichocomaceae, ao Reino Fungi, divisão Ascomycota, ordem Eurotiales e existem mais de 300 espécies, mas cerca de 20 têm sido encontrados como causadores de doenças. As colônias apresentam uma superfície de cor

branca, na fase inicial de crescimento e sua cor vai evoluindo para verde, amarelo, castanho ou preto, de acordo com as espécies.

2.5.2.1 *Aspergillus carbonarius*

A espécie *Aspergillus carbonarius* pertencente ao gênero *Aspergillus*, seção Nigri, é caracterizada em sua morfologia por apresentar conídeos pretos e/ou marrom-escuros, paredes rugosas, estipes longas e largas. A germinação dessa espécie é muito rápida e ocorre em um período de 24 horas a uma temperatura entre 10 - 37 °C. A temperatura ótima para o crescimento é de 32 - 35 °C, com temperatura mínima de 10 °C e máxima de 42 °C. A atividade de água mínima e ótima para o crescimento do fungo é de 0,85 e 0,96 - 0,98, respectivamente e a atividade mínima para a produção de micotoxina é de 0,92 a_w (IAMANAKA; OLIVEIRA; TANIWAKI, 2010; IOANNIDS et al., 2015). A espécie *Aspergillus carbonarius* pode ser encontrada em diversos produtos vegetais, como os cereais, café, cacau, especiarias, frutos secos, amendoim e uva. A principal micotoxina relacionada às cepas pertencentes a essa espécie é a ocratoxina A (OTA), produzida na maioria das vezes em altas concentrações. Esse fungo vem sendo considerado a principal fonte de contaminação de OTA em uvas e vinhos (OLIVEIRA; OLIVEIRA; MENEGHELLO, 2013).

2.5.2.2 *Aspergillus niger*

A espécie *Aspergillus niger*, pertencente ao gênero *Aspergillus*, seção Nigri, apresenta como característica conídeos de marrom-escuro a preto. É considerado um fungo xerofílico e cresce a uma temperatura que varia entre 6 a 47 °C, com uma temperatura ótima relativamente alta, entre 35 e 37 °C. Esse fungo é capaz de crescer em uma faixa de pH extremamente ampla entre 1,4 a 9,8. É encontrado principalmente em frutos, como as uvas, alguns vegetais, grãos, cereais e especiarias (JAY, 2005; SCHUSTER et al., 2002). Essa espécie é considerada também principal fonte de OTA em vinhos provenientes de uvas infectadas pelo microrganismo.

2.5.2.3 *Aspergillus ochraceus*

A espécie *Aspergillus ochraceus*, pertencente ao gênero *Aspergillus*, seção Circumdati, apresenta como característica conídeos castanho-claros. Esse fungo cresce a uma temperatura entre 8 a 37 °C, sendo sua temperatura ótima de crescimento de 31 °C. É encontrado em vários

alimentos, entre eles, arroz, trigo, aveia, cevada, vinho, cereais, café e bebida (HASHIMOTO et al., 2012; REZENDE et al., 2013). A principal micotoxina relacionada às cepas pertencentes a essa espécie é a OTA. Esse fungo é considerado a principal fonte de contaminação de OTA em café. A atividade de água mínima e ótima para o crescimento do fungo é de 0,79 e 0,95 - 0,99, respectivamente e para a produção de micotoxina, a atividade de água mínima é de 0,83 e a ótima entre 0,98 a 0,99 (IAMANAKA; OLIVEIRA; TANIWAKI, 2010).

2.5.2.4 *Aspergillus westerdijkiae*

A espécie *Aspergillus westerdijkiae*, pertencente ao gênero *Aspergillus*, seção Circumdati, apresenta como característica conídeos amarelo a ocre e sendo considerada a espécie mais produtora de OTA em sua seção. (SAMSON; HONG; FRISVAD, 2006). Esse fungo cresce em uma temperatura de 15 a 30 °C, sendo o seu crescimento ótimo à temperatura de 30 °C e atividade de água ótima entre 0,93 a 0,97 (VIPOTNIK; RODRIGUEZ; RODRIGUES, 2017). Essa espécie contamina principalmente alimentos de origem vegetal, como cereais, café, frutos secos e bebidas. Podendo também ser encontrados em produtos cárneos curados. O fungo *A. westerdijkiae* é considerado uma das principais espécies responsáveis pela presença de OTA no café. As condições ótimas de produção da OTA para *A. westerdijkiae* é de 0,94 - 0,97 a_w e temperatura entre 20 - 25 °C (VIPOTNIK; RODRIGUEZ; RODRIGUES, 2017).

2.5.3 Micotoxinas

Micotoxinas são metabólitos secundários, aparentemente sem função no metabolismo do fungo e não são essenciais para seu crescimento e sobrevivência. Elas são produzidas quando os fungos atingem a maturidade, sendo consideradas tóxicas ao homem e animais. Os fungos que normalmente produzem toxinas são os pertencentes aos gêneros *Aspergillus*, *Penicilium* e *Fusarium* (ROCHA et al., 2014; RUYCK et al., 2015).

A produção de micotoxinas depende do crescimento fúngico, portanto pode aparecer em qualquer momento no campo antes da colheita, na colheita, durante manuseio pós-colheita, no armazenamento do alimento e em produtos processados. Contudo, o crescimento do fungo não implica que há micotoxina, porque nem todos os fungos são produtores de toxinas. Por outro lado, as micotoxinas podem permanecer no alimento mesmo após a eliminação dos fungos que as produziram. Isso é explicado pela estabilidade térmica que as micotoxinas apresentam,

resistindo aos tratamentos térmicos ou processos de desidratação que são suficientes para destruir o micélio dos fungos que as produziram (ABD-ELSALAM, et al., 2017; IAMANAKA; OLIVEIRA; TANIWAKI, 2010).

A contaminação de alimentos por micotoxinas geram perdas econômicas, dentre elas, perdas diretas de produtos agrícolas e no rendimento da colheita; perdas de animais acompanhadas de diversas taxas de morte; doenças em animais e em humanas e diminuição da produtividade; diminuição da velocidade de crescimento em animais; custos indiretos dos sistemas de controle existentes para algumas micotoxinas; custos para a desintoxicação na recuperação de um produto aceitável e rejeição de produtos pelo mercado importador. A não aceitação de mercadorias por países importadores são prejudiciais à economia do país, principalmente para o Brasil, que exporta grande quantidade de produtos alimentícios, que são altamente susceptíveis à contaminação por micotoxinas. Estima-se que 25 a 50% dos produtos agrícolas, principalmente os alimentos básicos do mundo estejam contaminados por micotoxinas (ABD-ELSALAM, et al., 2017; FREIRE et al., 2017; IAMANAKA; OLIVEIRA; TANIWAKI, 2010).

As micotoxinas podem entrar na cadeia alimentar humana pela contaminação direta ou indireta. A contaminação direta ocorre quando há o consumo de alimento infectado por um fungo toxigênico, com a subsequente formação das micotoxinas, como o consumo de cereais, oleaginosas e derivados contaminados. Os animais se alimentam com rações previamente contaminadas, podendo excretar micotoxinas no leite e seus derivados, carne e ovos e, consequentemente, considerados como fonte de contaminação indireta para os humanos (ROCHA et al., 2014).

A ingestão de alimentos que contêm micotoxinas pode causar graves efeitos, conhecidos como micotoxicose. A gravidade depende da toxicidade da micotoxina, concentração e do grau de exposição, bem como a idade, saúde e estado nutricional do indivíduo exposto. Como todas as síndromes toxicológicas, as micotoxicoses podem ser classificadas como agudas ou crônicas. A toxicidade aguda geralmente tem um início rápido e uma resposta tóxica óbvia, ao passo que a toxicidade crônica é caracterizada por exposição a baixas doses durante um longo período de tempo, resultando em cânceres e outros efeitos geralmente irreversíveis (RUYCK et al., 2015).

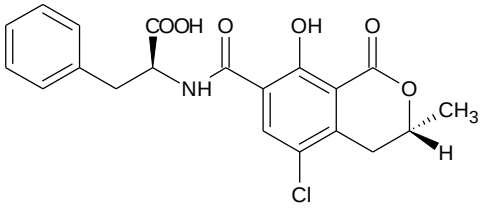
2.5.3.1 Ocratoxina A

A ocratoxina A (OTA) (Figura 13) é uma micotoxina que contamina os produtos alimentícios. Foi descoberta em 1965 como um metabólito secundário da espécie *Aspergillus*

ochraceus, por um estudo que visava à identificação de novas moléculas de micotoxinas (MERWE et al., 1965). É classificada pela Agência Internacional para Pesquisa em Câncer (International Agency for Research on Câncer – IARC) como possível carcinógeno humano do Grupo 2B, causando o desenvolvimento de doenças nos rins, que é considerado um fator contribuinte para uma doença crônica, em que os rins são diminuídos de tamanho e peso, com difusa fibrose e tumores no trato urinário (IARC, 1993).

A OTA está relacionada a propriedades nefrotóxicas em todos os animais estudados até o momento. Além disso, ela comporta-se como hepatóxica, imunussupressora, teratogênica e cancerígena. Kruger et al. (2015) indicaram que essa toxina causa câncer no fígado, rins, glândulas mamárias e testículos em animais, e pode apresentar uma variação no grau de intoxicação (aguda a crônica), dependendo da concentração e duração da exposição à toxina e da idade e estado nutricional do animal.

Figura 13 – Estrutura química da Ocratoxina A.



Essa micotoxina pode ser encontrada em aveia, cevada, centeio, trigo, grãos de café, nozes, amendoim seco, feijão, temperos e frutas secas. Há também a preocupação de contaminação de vinhos por OTA, provenientes de uvas contaminadas por *A. carbonarius* e *A. niger*.

A Agência Nacional de Vigilância Sanitária (Anvisa) atribui limites máximos a serem tolerados para micotoxinas em alimentos. Esse regulamento aplica-se às empresas que importam, produzam, distribuam e comercializam bebidas, alimentos e matéria-prima. Entre elas, citam-se amendoim e seus derivados; alimentos à base de cereais para alimentação infantil; café torrado (moído ou em grão) e solúvel; cereais e produtos de cereais; especiarias; frutas secas e desidratadas; nozes e castanhas; amêndoas de cacau e seus derivados; suco de maçã e polpa de maçã; suco de uva e polpa de uva; vinho e seus derivados; fórmulas infantis para lactentes e fórmulas infantis de seguimento para lactentes e crianças de primeira infância; leite e produtos lácteos; leguminosas e seus derivados. Os níveis de OTA em alimentos determinados pela Resolução RDC n° 7, de 18 de fevereiro de 2011, sendo alguns itens modificados pela

Resolução RDC n° 138, de 8 de fevereiro de 2017, estão descritos no Quadro 1 (BRASIL, 2011 e 2017).

Quadro 1 – Limites máximos (µg Kg^{-1}) de Ocratoxina A em alguns alimentos estabelecidos no Brasil.

Micotoxina	**Alimento**	**LMT (µg Kg^{-1})**
Ocratoxina A	Cereais e produtos de cereais, incluindo cevada malteada	20
	Feijão	10
	Café torrado (moído ou em grão) e café solúvel	10
	Vinho e seus derivados	2
	Suco de uva e polpa de uva	2
	Especiarias*: Capsicum* spp. (o fruto seco, inteiro ou triturado, incluindo pimentas, pimenta em pó, pimenta-de-caiena e pimentão-doce) *Piper* spp. (o fruto, incluindo a pimenta-branca e a pimenta-preta) *Myristica fragrans* (noz-moscada) *Zingiber officinale* (gengibre) *Curcuma longa* (curcuma) Misturas de especiarias que contenham uma ou mais das especiarias acima indicadas	30
	Alimentos a base de cereais para alimentação infantil (lactentes e crianças de primeira infância)	2
	Produtos de cacau e chocolate	5
	Amêndoa de cacau	10
	Frutas secas e desidratadas	10

Legenda: LMT – limites máximos tolerantes. Fonte: Brasil (2011 e 2017).

2.6 Uva de mesa (*Vitis labrusca* 'Niagara Rosada')

A videira é considerada economicamente uma espécie produtora de frutos mais importante no mundo, com diferentes utilidades, desde a produção de vinho, uva de mesa, uvas secas até diversos compostos orgânicos. No Brasil, a produção de uvas de mesa é bastante diversificada devido às condições climáticas das regiões de produção e à matriz heterogênea de cultivares, que inclui uvas americanas e as finas e híbridas com e sem sementes. Entretanto, a preferência do consumidor brasileiro é pela uva Niagara Rosada. A Niagara Rosada substituiu em grande parte Niagara Branca, em virtude de sua coloração rosada mais atraente ao consumidor brasileiro, especialmente no caso do consumo *in natura* (MAIA; RITSCHEL; LAZZAROTTO, 2018). Além da coloração, o sabor característico é muito apreciado pelo consumidor e sua polpa desprende-se facilmente da película, quando a baga é pressionada (DETONI et al., 2005).

As uvas de mesa são predominantemente produzidas nos estados de Pernambuco, Bahia, São Paulo e Minas Gerais. O Brasil encontra-se entre os países que se destacam no crescimento em relação à produção, exportação e ao consumo de uvas de mesa. Metade da produção no Brasil está voltada para atender à demanda de consumo *in natura* (MAIA; RITSCHEL; LAZZAROTTO, 2018). O estado de São Paulo destac-se como maior produtor nacional de uva de mesa e as cultivares de uva comum são representadas pela Niagara Rosada. Essa cultivar é considerada de alta qualidade para consumo *in natura*, apresenta excelente aceitação pelo mercado consumidor, bem como baixo custo de produção do que a uva fina de mesa (CAMARGO; COSTA, 2017; CIA et al., 2010).

O mercado de uvas de mesa vem crescendo nesse aspecto, o manuseio, armazenamento e comercialização da fruta requerem alternativas que concentram em manter a aparência fresca sem alterar o seu sabor. Entretanto, as uvas são suscetíveis à infecção por patógenos devido ao seu pericarpo fino e polpa suculenta. A decomposição fúngica ocorre pós-colheita, durante o armazenamento e comercialização, levando a uma enorme perda econômica na indústria da uva (ABDOLAHI et al., 2010; AN et al., 2019; LI et al., 2017). O período entre colheita e a venda da Niagara Rosada precisa ser o mais curto possível, em razão de problemas como esbagoamento e incidência de podridões (CIA et l., 2010). Nesse sentido, é importante medidas de controle de podridões na pós-colheita e desenvolvimento de tecnologias para melhor conservação dos frutos que sejam eficientes na redução de perdas decorrentes desses problemas. Atualmente, pesquisadores vêm realizando trabalhos para o desenvolvimento de técnicas alternativas no controle de podridões, como uso de tratamentos naturais no controle da qualidade e proteção dos frutos em pós-colheita, que garantam segurança do alimento e não coloque em risco a saúde humana. Entre os produtos naturais que podem ser empregados para o aumento da conservação dos frutos e controle de podridão pós-colheita de uva, destacam-se os óleos essenciais. Esses compostos bioativos podem ser encapsulados em nanofibras poliméricas e ser usadas como embalagens bioativas no controle de microrganismos que causam a podridão e redução de perdas decorrentes a desses problemas e consequentemente redução de antifúngicos sintéticos.

2.6.1 Contaminação de uva

As uvas são contaminadas por fungos do gênero *Aspergillus* da seção *Nigri*, principalmente por *Aspergillus carbonarius* e *Aspergillus niger*. Esses microrganismos parecem ser considerados invasores secundários das uvas, ou seja, contaminam depois de as

uvas terem sido danificadas pelas chuvas durante o período de maturação, por outros fungos, insetos, impactos mecânicos ou mesmo outros fatores (LEONG et al., 2006; MONDANI et al., 2020). O fungo *A. niger* é considerado a principal espécie que contamina as uvas em todas as fases de desenvolvimento, enquanto *A. carbonarius* apresenta uma incidência menor; entretanto, sua frequência aumenta durante a maturação até a colheita das uvas. Mondani et al (2020) e Welke, Hoeltz e Noll (2009) relataram que *A. carbonarius* é considerada uma espécie mais invasiva, capaz de penetrar nos bagos mesmo sem danos na película, sendo susceptíveis à infecção pelo fungo desde os estágios iniciais da maturação.

Esses fungos causam na uva o bolor negro, a principal causa da podridão pós-colheita e doença destrutiva das uvas de mesa, levando subsequentemente à perda das uvas de mesa e a um grande obstáculo para o armazenamento, tornando-as secas e com aspecto enrugado (AN et al., 2019; LAPPA et al., 2018; LI et al., 2017). Essa doença manifesta-se geralmente na fase avançada de maturação, na qual ocorre o aumento do teor de açúcar em frutos com existência de feridas e em períodos de stress hídrico com outros de abundância de água. Outros aspectos que são atribuídos à doença caracterizam-se por alterações típicas e facilmente reconhecidas, observadas pela desagregação da polpa, alteração nos tecidos devido à oxidação, adquirindo uma coloração variável em função do estado de evolução da podridão e da casta em questão. As uvas brancas adquirem uma tonalidade castanha, enquanto as uvas tintas uma coloração castanha-violácea ou avermelhada (LEONG et al., 2006; MONDANI et al., 2020).

Jiang, Shi e Zhu (2013) descreveram que podridão provoca estragos, prejuízos e alteração das uvas e, concomitantemente, perda de qualidade, aromas desagradáveis e produção de enzimas que dificultam ou impedem o processo de vinificação. Caracteriza-se também em alterações visíveis nos frutos e afetam as características organolépticas das uvas. Além das alterações visíveis provocadas pelos fungos, algumas espécies de fungo do gênero *Aspergillus* podem produzir micotoxinas. Dentre as espécies, *A. carbonarius* e *A. niger* são as produtoras de ocratoxina A, frequentemente detectadas nas uvas. Nesse sentido, os alimentos podem estar contaminados com micotoxinas sem apresentar alterações visíveis de contaminação fúngica. Os fungos podem proliferar produtos alimentícios sem formar estruturas reprodutoras, ou micélio visível a olho nu, e também as características organolépticas das uvas podem não ser afetadas de modo perceptível. Assim, cachos sem visível proliferação de fungos também podem conter ocratoxina A, mas bagas com mofo-preto normalmente mostram níveis mais altos de contaminação por essa micotoxina (WELKE; HOELTZ; NOLL, 2009).

2.7 Antifúngicos sintéticos e resistência fúngica

O controle fúngico pode ser feito por processos físicos e químicos; entre as técnicas adotadas destacam; tratamento com água quente, tratamento térmico, fungicidas químicos e fumigação com dióxido de enxofre (SONKER et al., 2014; SONKER; PANDEY; SINGH, 2015). Além do controle fúngico, é preciso ficar atento à remoção da ocratoxina A, que é um problema na cadeia alimentar. Nesse sentido, várias abordagens têm sido utilizadas para prevenção de fungos produtores de OTA, como também inibição da produção dessa micotoxina (WANG et al., 2018). Fungicidas químicos, como ácidos orgânicos de baixo peso molecular, hidrocarbonetos aromáticos, benzimidazol e inibidores da biossíntese de esterol, são geralmente utilizados no controle dos fungos que produzem essas micotoxinas que contaminam os alimentos (WANG et al., 2018).

Os antifúngicos químicos utilizados são classificados em azóis (imidazol, miconazol, oxiconazol, econazol, cetoconazol, tioconazol e clotrimazol), polienos (anfotericina B (AmpB), natamicina e nistatina), análogos de piridina (5-fluorocitosina (5-FC) e 5-fluorouracil (5-FU)), alilamina, tiocarbamatos e morfolinas, equinocandinas (caspofungina, micafungina e anidulafungina). Os azóis fungistáticos atuam principalmente na biossíntese do ergosterol, visando à enzima 14'-lanesterol desmetilase (ERG11), resultando na inibição da conversão do lanosterol dependente do citocromo P450 em ergosterol. Polienos ligam-se ao ergosterol das membranas das células fúngicas, levando à formação de poros na membrana, resultando na perda de equilíbrio iônico, integridade da membrana e morte. Os análogos da piridina incorporam no DNA e no RNA nos quais inibem o funcionamento celular, bloqueando síntese de proteínas ou inibição da replicação do DNA. Alilaminas e tiocarbamatos inibem o gene ERG1 da biossíntese de ergosterol, enquanto morfolinas inibem os genes ERG24 e ERG2 da biossíntese do ergosterol. As equinocandinas inibem a enzima β-1,3-glucano sintase, necessária para a síntese de β-glucano (PRASAD; SHAH; RAWAL, 2016; VAHEDI-SHAHANDASHTI; LASS-FLÖRL, 2020). Embora o uso de fungicidas sintéticos sejam os meios mais baratos e eficazes para controlar as perdas, sua aplicação contínua e, muitas vezes, imprópria no controle do crescimento fúngico, pode favorecer a seleção de fungos produtores de OTA, resistentes aos fungicidas. Além disso, o uso indiscriminado desses produtos pode provocar a indução indesejada da biossíntese da OTA (SCHMIDT-HEYDT; STOLL; GEISEN, 2013; ZHANG et al., 2016).

Diversos fatores justificam a necessidade de novos agentes antifúngicos. Entre eles, citam-se altas taxas de resistência; os produtos sintéticos utilizados no tratamento convencional,

que estão associados a danos à saúde humana e ao meio ambiente devido ao risco de resíduos tóxicos e também sua aplicação requer um controle rigoroso dos métodos de tratamento por razões de segurança alimentar (SONKER et al., 2015). Nesse sentido, surge a necessidade da substituição por produtos naturais que sejam menos agressivos, ecologicamente seguros e geralmente reconhecidos como seguros (GRAS). GRAS é uma designação da Food and Drug Administration (FDA) para produtos químicos ou substâncias adicionadas aos alimentos que são considerados seguros para agricultores, consumidores e para o ecossistema, com baixíssimo efeito fitotóxico (ABD-ELSALAM, et al., 2017). Produtos naturais geralmente apresentam estruturas químicas complexas importantes para as interações específicas e reconhecimento por alvos macromoleculares em fungos, além de revelar atividades antimicrobianas que variam mecanicamente, dificultando o desenvolvimento de mecanismos de resistência dos microrganismos (ABD-ELSALAM, et al., 2017). Dentre os produtos naturais, destacam-se os óleos essenciais como fonte de moléculas bioativas, incluindo antifúngicos potentes.

2.8 Efeito antifúngico dos óleos essenciais

Os óleos essenciais apresentam uma composição complexa, incluindo compostos fenólicos, aldeídos, cetonas, álcoois, ésteres ou hidrocarbonetos. Essas classes de compostos apresentam notável propriedade antifúngica, podendo ser testados separadamente, ou em conjunto, resultando em várias interações, e podendo ter diversos alvos que dificultam o surgimento de resistência fúngica. Devido à complexa composição e interação entre os constituintes, o óleo essencial apresenta quatro tipos possíveis de efeitos; indiferente, aditivo, antagonista ou sinérgico. A ausência de interação entre os constituintes é denominada efeito indiferente. O efeito aditivo é quando os efeitos combinados entre os constituintes são iguais a soma dos efeitos individuais. Quando um efeito de um ou ambos compostos é menor em relação quando aplicados juntos ou individualmente é chamado de efeito antagonista. O efeito sinérgico é quando o efeito combinado das substâncias é maior que a soma dos efeitos individuais (TARIQ et al., 2019).

Os óleos essenciais extraídos de plantas aromáticas, como manjericão, frutas cítricas, erva-doce, capim-limão, orégano, alho, cravo, alecrim, canela e tomilho, têm proporcionado efeitos antifúngicos significativos (ARORA; KAUR, 1999; FU et al., 2007; JUGLAL; GOVINDEN; ODHAV, 2002). Ultee e Smid (2001) relataram que alguns óleos essenciais que apresentam em sua composição carvacrol e timol como compostos majoritários proporcionam um rompimento da membrana celular fúngica. Os autores sugerem que os constituintes podem

ser adicionados a produtos alimentícios em doses abaixo da concentração mínima inibitória contra a redução do risco de produção de toxina por *Bacillus cereus*, aumentando a segurança dos produtos. Tariq et al (2019), estudando o óleo essencial de cravo, observaram que o constituinte principal do óleo essencial, o eugenol, pode causar danos permanentes em fungos, sendo considerado um antifúngico viável economicamente.

Os óleos essenciais atuam sobre os fungos por meio de vários mecanismos de ação: penetração e rompimento da parede celular e da membrana do protoplasma por meio de um processo de permealização, inibindo a função da cadeia de transporte de elétrons mitocondrial, resultando na desintegração das membranas mitocondriais; inibição das bombas de prótons da cadeia respiratória, interrompendo a formação de ATP, levando a danos na parede celular. Citam-se também a interferência no fluxo de elétrons dentro da via de transporte de elétrons; inibição da síntese de RNA, DNA, proteínas e polissacarídeos; rompimento da membrana celular pela inibição da biossíntese de ergosterol (esterol que desempenha um papel fundamental na integridade e função da membrana celular do fungo); inibição da formação da parede celular através do bloqueio da formação de beta-glucanos; inibição da bomba de efluxo reduzindo a resistência aos ativos, visto que essa bomba de efluxo, presente em todas as células vivas, remove as substâncias tóxicas das células (BRANDÃO et al., 2020; BRANDÃO et al., 2021; FREIESLEBEN; JAGER, 2014; TARIQ et al., 2019). Wang et al. (2018) mostraram que o constituinte de óleo essencial, cinamaldeído, provocou alterações morfológicas e ultraestruturais irreversíveis, como dobramento da célula, perda de integridade da parede celular, rompimento da membrana plasmática, destruição da mitocôndria e ausência de organelas intracelulares. Esses efeitos podem ser atribuídos à capacidade do composto em inibir reações enzimáticas que regulam a síntese da parede celular e consequentemente perturbam a morfogênese e o crescimento do fungo.

Além do controle fúngico, é muito importante o bloqueio da produção de micotoxinas como a ocratoxina A em alimentos, pois são substâncias bastante tóxicas. Nesse sentido, os óleos essenciais vêm sendo estudados e alguns mecanismos são sugeridos no controle dessa toxina. Várias enzimas, como a policetídeo sintase (PKS), peptídeo sintase não ribossômica (NRPS), citocromo p450 monoxigenase e halogenase, estão envolvidas nas etapas principais da biossíntese de OTA (WANG et al., 2015; WANG et al., 2018). E também genes que codificam as proteínas reguladoras (VelB, VeA e LaeA) são responsáveis pela transcrição que podem coordenar o desenvolvimento fúngico e o metabolismo secundário, podendo ativar a produção de OTA (CRESPO-SEMPERE et al., 2013). Estudos mostraram que constituintes de óleos essenciais conseguem regular, negativamente, os níveis transcricionais dos genes

biossintéticos e reguladores da OTA, além da inibição do crescimento fúngico (WANG et al., 2018).

Karpinski (2020) relatou que os compostos químicos mais comumente encontrados nos óleos essenciais das plantas pertencentes à família Lamiaceae são β-cariofileno (41 plantas), linalol (27 plantas), limoneno (26), β-pineno (25), 1,8-cineol (22), carvacrol (21), α-pineno (21), p-cimeno (20), γ-terpineno (20) e timol (20). O sesquiterpeno β-cariofileno e os monoterpenos fenólicos (carvacrol, p-cimeno, timol) apresentam grandes atividades antifúngicas e são componentes antifúngicos particularmente importante na família Lamiaceae. Além dessas substâncias químicas, os monoterpenos como linalol, 1,8-cineol, limoneno, pinenos e terpinenos são também considerados antifúngicos importantes. O autor descreveu que o modo de ação desses óleos essenciais é multidirecional, levando à ruptura da parede celular e da membrana celular por meio de um processo de permeabilização. Os compostos lipofílicos dos óleos essenciais podem atravessar a parede celular e provocar danos aos polissacarídeos, ácidos graxos e fosfolipídios, eventualmente tornando-os permeáveis A mudança da permeabilidade para os cátions H^+ e K^+ afeta o pH celular e o dano às organelas celulares, desintegrando a membrana mitocondrial. Também demostrou que o óleo essencial de *Thymus vulgaris* inibe a síntese de aflatoxinas por *Aspergillus flavus* e proporciona a redução da produção de ergosterol.

Nguefack et al. (2009) estudaram os óleos essenciais de três plantas aromáticas (*Cymbopogon citratus*, *Ocimum gratissimum* e *Thymus vulgaris*) e suas frações contra cepas micotoxigênicas de *Aspergillus ochraceus*, *Penicillium expansum* e *P. verrucosum* e observaram que esses produtos naturais exibiram uma forte atividade antifúngica, sendo considerados um potencial conservante de alimentos. Os autores avaliaram a concentração dos óleos essenciais testados como potencial antimicótico e identificaram as frações ativas para a preservação de um alimento à base de peixe contra o mofo e sua influência nas características sensoriais dos alimentos. Além disso, relataram que a alta atividade antifúngica de óleos essenciais e frações indicaram sua aplicação em agroindústrias como conservantes naturais de alimentos.

Devido às características que os óleos essenciais apresentam, como propriedades biológicas, biocompatibilidade, biodegradabilidade e baixa toxicidade, esses compostos vêm sendo estudados para serem usados na preservação das qualidades organolépticas (cor, cheiro, sabor e frescor) dos alimentos, prevenção da degradação rápida e armazenamento para uma vida útil mais longa dos produtos (MOGOŞANU et al., 2017). Entretanto, a incorporação direta nos alimentos pode ser um pouco limitada, devido à sua alta volatilidade e apresentar aroma e

sabor, podendo causar alteração indesejável no perfil organoléptico dos produtos alimentícios (DEEPIKA et al., 2020). Porém, essas limitações podem ser superadas, encapsulando os óleos essenciais em matriz polimérica na forma de nanofibras para uma liberação sustentada e controlada, e consequentemente melhorar o uso desses produtos naturais e prolongar sua eficiência. Como as nanofibras permitem uma autorregulação dos compostos bioativos, a utilização da nanotecnologia com óleos essenciais torna-se extremamente vantajosa, pois além do controle ecológico correto e inteligente de sistemas de nano distribuição, essa técnica tem como foco a redução de uso de fungicidas por meio do desenvolvimento de uma gama de aplicações nanotecnológicas de baixo custo para a liberação lenta e mais eficiente de compostos bioativos (ABD-ELSALAM, et al., 2017).

2.9 Nanotecnologia

Os avanços da nanotecnologia têm se mostrado de grande interesse para inúmeras aplicações em diversas áreas, como na agricultura, energia, preservação do meio ambiente, saúde pública, na indústria de alimentos, entre outros (DIMER et al., 2013; LI et al., 2011). A nanotecnologia pode ser uma ferramenta fundamental para solucionar um conjunto de desafios científicos e tecnológicos para melhorar a segurança da cadeia alimentar. Essa técnica está relacionada com a caracterização, fabricação e/ou manipulação de estruturas com pelo menos uma dimensão na escala nanométrica. Propriedades físicas, químicas e biológicas das estruturas e dos sistemas nanométricos são significativamente diferentes da micro ou macroescala, oferecendo assim melhor desempenho, características únicas e novas aplicações funcionais em alimentos e agricultura (BRANDELLI; LOPES; BOELTER, 2017). Nos últimos anos, a nanociência e a nanotecnologia abriram novas perspectiva para a indústria de alimentos, e as embalagens derivadas da nanotecnologia vêm sendo consideradas benéficas e eficazes. Assim, há um grande interesse no desenvolvimento de novos materiais de embalagem nanométricas com função antimicrobiana capazes de evitar a contaminação microbiana para estender a vida útil e garantir a segurança alimentar. Para atingir a atividade antimicrobiana de embalagens de alimentos, substâncias biológicas com potencial antimicrobiano podem ser incorporadas diretamente no material de embalagem (DENG; NIKIFOROV; LEYS, 2017).

Na indústria alimentícia, a nanotecnologia é utilizada para o desenvolvimento de novos materiais funcionais; processamento em nanoescala; desenvolvimento de produto; e métodos para a melhoria na segurança alimentar e biossegurança. Em relação à segurança alimentar, o desenvolvimento na área de embalagens para alimentos derivados da nanotecnologia inclui

embalagens ativas de nanopartículas com propriedades antimicrobianas e antioxidantes; embalagens inteligentes de alimentos, incorporando nanosensores para monitorar e relatar as condições dos alimentos capazes de detectar produtos químicos, agentes patogênicos e toxinas em alimentos (ALMEIDA et al., 2015; PEREZ et al., 2012).

A nanotecnologia é uma grande promessa para o desenvolvimento de novos produtos em nanoescala, como nanofibras, emulsões, cápsulas e lipossomas. Os sistemas nanométricos foram desenvolvidos como transportadores eficazes para substâncias bioativas, distribuição e liberação controlada, como as nanofibras poliméricas. Nanofungicidas ecológicos de produtos naturais, incluindo os óleos essenciais (GRAS), são uma boa alternativa aos fungicidas sintéticos como um procedimento para diminuir os efeitos nocivos à saúde humana, animal e ambiental, além de serem considerados eficazes (ABD-ELSALAM, et al., 2017).

2.9.1 Nanofibra

Entre os diferentes materiais, as nanofibras estão entre os de maior interesse científico e tecnológico, sendo produzidos e estudados em relação às suas características que atribuem diferentes aplicações e vantagens (COSTA et al., 2012). Dentre essas características, destacam-se pela biocompatibilidade com substâncias ativas incorporadas; biodegradabilidade aceitável; excelente propriedade mecânica, possuindo uma área de superfície maior; alta porosidade com tamanho de poros pequenos e a possibilidade de controlar a liberação de substâncias ativas. Além disso, as formulações de nanofibras possuem estrutura de poros abertos e interconectados, que permitem interação ótima com moléculas bioativas (MORIE et al., 2014).

Na área de alimentos, as nanofibras podem ser utilizadas como um material de embalagem, considerado um composto verde (ambientalmente amigável) pela sua característica biodegradável. Além disso, podem ser empregadas como conservantes de alimentos quando incorporados em sua estrutura compostos bioativos com propriedades antibacterianas, antifúngicas e antioxidantes, entre outros (WEISS; TAKHISTOV; MCCLEMENTS, 2006). Desse modo, a nanofibra tem enorme potencial para a melhoria da segurança alimentar, como uma ferramenta fundamental para a distribuição e liberação controlada de antimicrobianos naturais, como os óleos essenciais (BRANDELLI; LOPES; BOELTER, 2017).

As nanofibras vêm sendo estudadas na utilização como carreadores de compostos antimicrobianos, antibacterianos ou antifúngicos contra diversos microrganismos para poderem ser aplicadas como embalagens e conservantes de alimentos, garantindo a qualidade e preservação dos produtos e evitando possíveis contaminações (LEIDY; XIMENA, 2019).

Compostos bioativos podem ser facilmente incorporados nas estruturas de nanofibras. Assim, os conservantes químicos que são considerados tóxicos e prejudiciais vêm sendo substituídos pelos produtos naturais que apresentam diversas propriedades biológicas. Em geral, com a crescente busca dos consumidores por alimentos mais saudáveis, a utilização de produtos naturais ganhou ampla aceitação para serem usados em dispositivos de liberação controlada como as nanofibras. Entre esses produtos naturais, destacam-se os óleos essenciais.

Bonan et al. (2017), encapsulando o óleo essencial de copaíba em nanofibras obtidas através de blenda de PVP/PLA pela técnica de SBS, conseguiram observar que elas apresentaram efeito antimicrobiano sobre *Staphylococcus aureus*. Os autores relataram que a combinação dos dois polímeros permitiu a produção de um material fibroso versátil, com a possibilidade de adequar a liberação do fármaco incorporado apresentando propriedades de barreira antimicrobiana no controle de infecções preexistentes e prevenir a penetração de patógenos.

Miranda et al. (2019) estudaram nanofibras de poliestireno (PS) fiado em solução usando tolueno (solvente industrial) e óleo de laranja (solvente verde) e observaram que o óleo de laranja foi usado com sucesso como um solvente verde alternativo para substituir o tolueno para produzir fibras de SBS de PS. Além disso, essas fibras contendo óleo de laranja residual tinham propriedades antimicrobianas que inibiram o crescimento de *Staphylococcus aureus*, *Escherichia coli* e *Alternaria alternata*. Os autores sugerem que os resultados encontrados mostram que as esteiras de nanofibras ecológicas fiadas a partir de óleo de laranja/PS podem ser usadas em aplicações como cicatrização de feridas e embalagem ativa para alimentos.

As nanofibras poliméricas são bastante estudadas, porém para aplicação direta em alimentos não há relatos na literatura e nem estudos de interação direta ou indireta na extensão da vida útil do produto como embalagens alimentícias. Dessa forma, esta pesquisa visa a promover o leque de aplicação das nanofibras poliméricas no setor alimentício.

2.9.1.1 Produção de nanofibras

Uma das técnicas utilizadas para produzir as nanofibras poliméricas é a fiação por sopro de solução (*Solution Blow Spinning*) (SBS) (Figura 14). Essa técnica começou a ser utilizada na década de 1950, com Van A. Wente, o qual obteve fibras na faixa de 300 nm. Van utilizou uma extrusora que promovia o estiramento do polímero fundido que depositava na superfície de um coletor por meio do auxílio de um ar quente em alta velocidade, originando uma manta não tecida capaz de coletar partículas radioativas (ELLISON et al., 2007). Paralelo à técnica de

SBS e com o intuito de contribuir com o desenvolvimento da mesma, nos anos 90, a técnica de eletrofiação (*electrospinning*) começou a ser estudada e surgiu interesse por parte dos pesquisadores devido à obtenção de materiais micro e nanométricos.

A técnica de eletrofiação forma fibras por meio da modificação da superfície do fluido (tensão superficial) submetida a um campo elétrico (DOSHI; RENEKER, 1995). A tensão superficial na solução polimérica é superada por uma força elétrica repulsiva associada ao cone de Taylor (cone formado por finos jatos ou gotículas através de fluidos viscosos condutores quando submetidos à força elétrica e diferença de potencial) e a trajetória das fibras formadas a partir da solução polimérica carregada é direcionada e controlada pelo campo elétrico (CASASOLA; THOMAS; GEORGIADOU, 2016; DOSHI; RENEKER, 1995; TAYLOR, 1969). O solvente é evaporado no percurso entre a distância de trabalho aplicada do início da ejeção da solução e o coletor metálico/condutor, no qual as fibras são obtidas (EFKERE et al., 2018; THOMPSON et al., 2007). Embora seja considerada uma técnica convencional e bastante utilizada, ela apresenta desvantagens devido ao elevado custo (obtenção e manutenção) e alto risco associado, pois há o uso de alta voltagem (quilovolts).

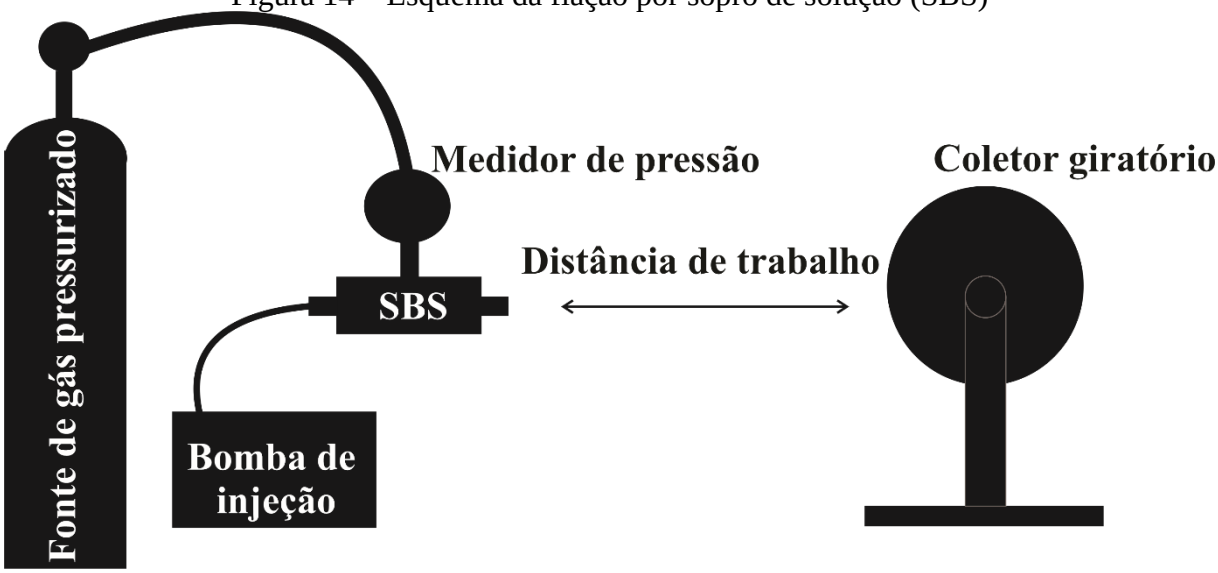
A técnica de SBS forma fibras utilizando bicos concêntricos por meio dos quais uma solução de polímero e um gás pressurizado são simultaneamente injetados. Diferença de pressão e cisalhamento na interface gás/solução ajuda a formar múltiplos filamentos de solução de polímero em direção a um coletor. Durante o voo, os solventes dos filamentos evaporam rapidamente, formando uma teia de micro e/ou nanofibras (MEDEIROS et al., 2009; NEPOMUCENO et al., 2018). De acordo com Daristotle et al. (2016), Medeiros et al. (2009) e Oliveira et al. (2011), a obtenção das fibras pela técnica de SBS são controladas, principalmente, pelos seguintes parâmetros: pressão de arraste (pressão do gás pressurizado); parâmetro reológico da solução polimérica (por exemplo, viscosidade/concentração); vazão da solução polimérica e distância de trabalho (influencia na máxima ou completa evaporação do solvente). Esses parâmetros influenciam diretamente na formação das fibras poliméricas, na morfologia das fibras (por exemplo, diâmetro médio) como também na uniformidade e nos aspectos lisos ou não das fibras. Paralelo a esses parâmetros, a massa molecular do polímero é, também, considerada um importante fator que influencia o processo de obtenção das fibras.

Dentre esses parâmetros, o fator reológico da solução polimérica é considerado o mais importante e de maior influência na alteração morfológica das fibras. Alteração na viscosidade pode contrapor a condução da força de fluxo de ar e dificultar o estiramento do jato da solução polimérica, aumentando a produtividade e espessura das fibras. Quando a viscosidade é baixa, há formação de poucas fibras, fibras mais finas e muitos “beads” (OLIVEIRA et al., 2011;

PARIZE et al., 2016b). Os “beads” são alterações na morfologia linear e uniforme das fibras, com modificações fusiformes em formato de contas, promovendo heterogeneidade nos diâmetros das fibras (SILVA; OLIVEIRA; MEDEIROS, 2015). A vazão da solução polimérica (taxa de ejeção da solução ou taxa de alimentação) é outro fator que influencia na morfologia das fibras, visto que esse parâmetro sofre alterações de acordo com o tipo de interação polímero-solvente durante o preparo das soluções (OLIVEIRA et al., 2011).

A técnica de SBS é considerada simples, segura, barata mostrando-se bastante promissora na produção de micro e nanofibra de uma ampla variedade de polímeros com aplicações potenciais no sistema de liberação controlado de substâncias bioativas. Essa técnica oferece vantagens, como: produção em maior escala, taxas de fiação mais altas, custos reduzidos, eliminação do uso de alta tensão e uma gama mais ampla de coletores que podem ser usados com materiais diferentes em comparação com as técnicas tradicionais, como a eletrofiação (BILBAO-SAINZ et al., 2014; BONAN et al., 2017).

Figura 14 – Esquema da fiação por sopro de solução (SBS)



Fonte: Adaptado de Nepomuceno et al. (2018).

2.9.2 Polímeros

Os materiais utilizados para formulações nanotecnológicas são selecionados de acordo com suas características de biodegradabilidade, biocompatibilidade, capacidade para funcionamento em superfície, conjugação, complexação e encapsulamento (DIMER et al., 2013). Os sistemas de liberação de drogas poliméricas são capazes de melhorar a eficácia terapêutica, reduzir a toxicidade e serem projetados para atingir a localização específica,

controlar e prolongar a liberação do composto ativo. Isso pode acontecer por meio da manipulação da interação entre polímeros e substâncias ativas nas nanofibras. Assim, é possível modificar a cinética de liberação do princípio ativo por um determinado período de tempo usando misturas imiscíveis de polímeros hidrofílicos e hidrofóbicos (FATHI-AZARBAYJAN; CHAN, 2010).

Alguns polímeros utilizados para produzir as nanofibras pertencem à classe de poli (ésteres), considerados biodegradáveis e biocompatíveis. Dentre os poli (ésteres) tem-se os poli (ácido lático) (PLA), poli (ácido glicólico) (PGA) e seus copolímeros, poli (ácido L-lático) (PLLA), poli (ácidoD-lático) (PDLA) e poli (ácido coglicólico) (PLGA), poli (caprolactona) (PCL) e o poli (etilenoglicol) (PEG) também referido como poli (óxido de etileno) (PEO) (UHRICH et al., 1999). Dentre os polímeros mais utilizados para a preparação de nanofibras para o transporte de princípios ativos, destacam-se os poliésteres alifáticos. Entre eles o PLA, PGA, PLGA e PCL. Juntos formam um grupo de polímeros sintéticos não tóxicos e biodegradáveis que, em ambiente aquoso, sofrem degradação (BONAN et al., 2017; NEPOMUCENO et al., 2018).

2.9.2.1 Poli (ácido lático)

O poli (ácido lático) (PLA) (Figura 15) pertencente à família dos poliésteres alifáticos são geralmente formados de α-hidroxiácidos e ácido lático, envolvendo o processamento e a polimerização do monômero de ácido lático. O ácido é uma molécula quiral simples que existe como dois enantiômeros, L e D-ácido lático, diferindo em seu efeito na luz polarizada. Produzido por fermentação bacteriana de açúcares a partir de fontes renováveis (trigo, amido, milho, cana-de-açúcar ou beterraba) ou por processo químico envolvendo matérias-primas petroquímicas por meio da polimerização de condensação do ácido láctico (COSTA et al., 2016; MORI et al., 2015; PARIZE te al., 2016a; PAN; INOUE, 2009).

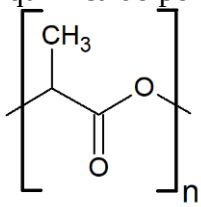
Atualmente, a fermentação é a mais utilizada na obtenção do ácido lático para formação do PLA, na qual o amido de milho é convertido em ácido lático por fermentação bacteriana utilizando-se cepas de *Lactobacillus*. Como todo ácido lático biológico, o ácido lático produzido pela fermentação é exclusivamente (> 99,5%) o isômero L, favorecendo a produção por meio de fontes renováveis em vez da petroquímica (GUPTA; REVAGADE; HILBORN, 2007).

O PLA é um dos polímeros biodegradáveis mais promissores devido às suas características mecânicas, processabilidade termoplástica e propriedades biológicas, além de

biocompatibilidade, biodegradabilidade, não toxicidade e fácil decomposição térmica por hidrólise da ligação éster, não requerendo a presença de enzimas para catalisar essa hidrólise. A taxa de degradação depende do tamanho, da proporção do isômero e da temperatura de hidrólise (GARLOTTA, 2001; GUPTA; REVAGADE; HILBORN, 2007). Além disso, por estar disponível a partir de recursos agrícolas renováveis, ele ajuda a melhorar a economia agrícola. Quando comparado com os plásticos convencionais à base de petróleo, o PLA promove a redução nas emissões de dióxido de carbono, pois ajuda na fixação de quantidades significativas de dióxido de carbono. A habilidade mais importante do PLA é que se pode ajustar suas propriedades físicas por modificações materiais (GUPTA; REVAGADE; HILBORN, 2007).

Por ser um termoplástico de alta resistência e de alto módulo produzido de recursos renováveis anualmente, o PLA tem sido aplicado na produção de plásticos básicos, embalagens industriais, dispositivos biocompatíveis/bioabsorvíveis, produtos agrícolas, materiais descartáveis, bem como materiais médicos e liberação de drogas (CHEN; TSAI; YANG, 2011; GARLOTTA, 2001). É um material de fácil processamento para produção de peças moldadas, filme e fibras. Sua estrutura estereoquímica pode ser modificada pela polimerização de uma mistura controlada dos isômeros L ou D para produção de polímeros amorfos ou cristalinos de alto peso molecular que podem ser usados para materiais de embalagem, filmes, fibras têxteis e produtos farmacêuticos (GARLOTTA, 2001). A Food and Drug Administration (FDA) dos Estados Unidos e as autoridades regulatórias europeias aprovaram o uso de PLA para todas as aplicações de alimentos e algumas aplicações cirúrgicas, como sistemas de liberação de drogas.

Figura 15 – Estrutura química do poli (ácido lático).



REFERÊNCIAS

ABD-ELSALAM, K. A. et al. Nanobiotechnological strategies for toxigenic fungi and mycotoxin control. In **Food Preservation**, Academic Press, p. 337- 364, 2017.

ABDOLAHI, A. et al. Study on the potential use of essential oils for decay control and quality preservation of Tabarzeh table grape. **Journal of Plant Protection Research**, v. 50, n. 1, p. 45-52, 2010.

ABREU, J. A. S.; ROVIDA, A. F. S. PAMPHILE, J. A. Fungos de interesse: aplicações biotecnológicas. **Revista Uningá**, v. 21, n. 1, p. 55-59, 2015.

ALMEIDA, A. C. S. et al. Aplicação de nanotecnologia em embalagens de alimentos. **Polímeros**, v. 25, p. 89-97, 2015.

AN, P. et al. α-Terpineol and terpene-4-ol, the critical components of tea tree oil, exert antifungal activities in vitro and in vivo against *Aspergillus niger* in grapes by inducing morphous damage and metabolic changes of fungus. **Food Control**, v. 98, p. 42-53, 2019.

ANDRADE, M. A.; LANÇAS, F. M. Estado-da-arte na análise cromatográfica de Ocratoxina A em amostras de alimentos. **Scientia Chromatographica**, v. 7, n. 1, p. 31-52, 2015.

ARORA, D. S.; KAUR, J. Antimicrobial activity of spices. **International Journal of Antimicrobial Agents**, v. 12, n. 3, p. 257-262, 1999.

ATIF, M., et al. Essential oils of two medicinal plants and protective properties of jack fruits against the spoilage bacteria and fungi. **Industrial Crops and Products**, v. 147, p. 112239, 2020.

AZEVEDO, I. L. et al. Eficácia *in vitro* do óleo essencial de capim-limão (*Cymbopogon flexuosus* Steud. Wats.) frente as bactérias entéricas de origem avícola. **Acta Veterinária Brasilica**, v.10, n.1, p. 25-31, 2016.

BAE, A. H., et al. Delta-and mu-opioid pathways are involved in the analgesic effect of *Ocimum basilicum* L in mice. **Journal of Ethnopharmacology**, v. 250, p. 112471, 2020.

BAKKALI, F.et al. Biological effects of essential oils. **Food and Chemical Toxicology**, Oxford, v. 46, n. 2, p. 446-475, Feb, 2008.

BALASUBRAMANI, S. et al. Formulation of nanoemulsion from leaves essential oil of *Ocimum basilicum* L. and its antibacterial, antioxidant and larvicidal activities (*Culex quinquefasciatus*). **Microbial Pathogenesis**, v. 125, p. 475-485, 2018.

BHATT, L.; KALE, R. D. Lemongrass (*Cymbopogon flexuosus* Steud.) wats treated textile: A control measure against vector-borne diseases. **Heliyon**, v. 5, n. 12, p. e02842, 2019.

BHAVANI, T. et al. Phytochemical screening & antimicrobial activity of *Ocimum gratissimum* review. **Journal of Pharmacognosy and Phytochemistry**, v. 8, n. 2, p. 76-79, 2019.

BILBAO-SAINZ, C. et al. Solution blow spun poly (lactic acid)/hydroxypropyl methylcellulose nanofibers with antimicrobial properties. **European Polymer Journal**, v. 54, p. 1-10, 2014.

BONAN, R. F. et al. Poly (lactic acid)/poly (vinyl pyrrolidone) membranes produced by solution blow spinning: Structure, thermal, spectroscopic, and microbial barrier properties. **Journal of Applied Polymer Science**, v. 134, n. 19, p. 1-9, 2017.

BOWMAN, S. M.; FREE, S. J. The structure and synthesis of the fungal cell wall. **Bioessays**, v. 28, n. 8, p. 799-808, 2006.

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 l. Antifungal activity and the effect of the essential oil of *Lippia origanoides* Kunth on *Aspergillus* mycotoxins production. **Australian Journal of Crop Science**, v. 15, n. 7, p. 1005-1012, 2021.

BRANDELLI, A.; LOPES, N. A.; BOELTER, J. F. Food applications of nanostructured antimicrobials. In: **Food Preservation**. Academic Press, p. 35-74, 2017.

BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). Diário oficial da união. Resolução - RDC No - 7, de 18 de fevereiro de 2011. **Dispõe sobre limites máximos tolerados (LMT) para micotoxinas em alimentos**, 2011.

BRASIL. Agência Nacional de Vigilância Sanitária (ANVISA). Diário oficial da união. Resolução - RDC No - 138 de 8 de fevereiro de 2017. **Dispõe sobre limites máximos tolerados (LMT) para micotoxinas em alimentos**, 2017.

BRASIL. Ministério da Saúde e Agência Nacional de Vigilância Sanitária. **Monografia das espécies *Alpinia speciosa* e *Alpinia zerumbet* (galanga)**, p. 1-105, 2014.

BRASIL. Ministério da Saúde e Agência Nacional de Vigilância Sanitária. **Monografia da espécie *Ocimum gratissimum* L. (alfavaca)**, p. 1-89, 2015.

BUCHANAN, B.; GRUISSEM, W.; JONES, R. Biochemistry & Molecular Biology of Plants. **American Society of Plant Physiologists**. 2 ed., Editora: John Wiley & Sons, 1280 p, 2015.

CAMARGO, M. P.; COSTA, C. R. Viabilidade econômica do cultivo de videira Niágara Rosada. **Revista iPecege**, v. 3, n. 2, p. 52-85, 2017.

CARRASCO, H. et al. Antifungal activity of eugenol analogues. Influence of different substituents and studies on mechanism of action. **Molecules**, v. 17, n. 1, p. 1002-1024, 2012.

CARVALHO, I. T.; ESTEVINHO, B. N.; SANTOS, L. Application of microencapsulated essential oils in cosmetic and personal healthcare products - a review. **International Journal of Cosmetic Science**, [S.l.], vol. 38, n. 2, p. 109-119, abr. 2016.

CASASOLA, R.; THOMAS, N. L.; GEORGIADOU, S. Electrospinning of poly(lactic acid): Theoretical approach for the solvent selection to produce defect-free nanofibers. **Journal of Polymer Science Part B: Polymer Physics**, v. 54, n. 15, p. 1483–1498, 24 mar. 2016

CASTRO, K. N. C. et al. Composição química e eficácia do óleo essencial e do extrato etanólico de *Alpinia zerumbet* sobre *Staphylococcus aureus*. **Arq. Inst. Biol.**, v.83, 1-7, e0192014, 2016.

CHEN, H.; TSAI, C.; YANG, M. Mechanical properties and biocompatibility of electrospun polylactide/poly (vinylidene fluoride) mats. **Journal of Polymer Research**, v. 18, n. 3, p. 319-327, 2011.

CIA, P. et al. Atmosfera modificada e refrigeração para conservação pós-colheita de uva 'Niagara Rosada'. **Pesquisa Agropecuária Brasileira**, v. 45, n. 10, p. 1058-1065, 2010.

COSTA, R. G. F. et al. Eletrofiação de polímeros em solução. Parte II: aplicações e perspectivas. **Polímeros**, v. 22, n. 2, p. 178-185, 2012.

COSTA, R. G. F. et al. Nanocomposite fibers of poly (lactic acid)/titanium dioxide prepared by solution blow spinning. **Polymer Bulletin**, v. 73, n. 11, p. 2973-2985, 2016.

COUTO, H. G. S. A. et al. Antibacterial activity of *Lippia alba*, *Myrcia lundiana* and *Ocimum basilicum* essential oils against six food-spoiling pathogenic microorganisms. **Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas**, v. 20, n. 3, p. 260-269, 2021.

CRESPO-SEMPERE, A., et al. VeA and LaeA transcriptional factors regulate ochratoxin A biosynthesis in *Aspergillus carbonarius*. **International Journal of Food Microbiology**, v. 166, n. 3, p. 479-486, 2013.

CRUZ, M. J. F.; BEZERRA, S. B. Obtenção do óleo essencial de *Ocimum gratissimum* L para desenvolvimento de cosmético de limpeza facial. **Revista Diálogos Acadêmicos**, Fortaleza, v. 6, n. 2, 2017.

CRUZ, J. D. et al. Chemical standardization, antioxidant activity and phenolic contents of cultivated *Alpinia zerumbet* preparations. **Industrial Crops & Products**, v. 151, p. 112495, 2020.

DARISTOTLE, J. L. et al. A Review of the Fundamental Principles and Applications of Solution Blow Spinning. **ACS Applied Materials & Interfaces**, v. 8, n. 51, p. 34951–34963, 28 dez. 2016

DEEPIKA et al. Nanoencapsulated *Monarda citriodora* Cerv. ex Lag. essential oil as potential antifungal and antiaflatoxigenic agent against deterioration of stored functional foods. **Journal of Food Science and Technology**, v. 57, p. 2863-2876, 2020.

DEHSHEIKH, A. B. et al. Changes in soil microbial activity, essential oil quantity, and quality of Thai basil as response to biofertilizers and humic acid. **Journal of Cleaner Production**, v. 256. p. 120439, 2020.

DENG, X.; NIKIFOROV, A. Y.; LEYS, C. Antimicrobial nanocomposites for food packaging. In: **Food Preservation**. Academic Press, p. 1-34, 2017.

DESAI, M. A.; PARIKH, J. Hydrotropic Extraction of Citral from *Cymbopogon flexuosus* (Steud.) Wats. **Industrial & Engineering Chemistry Research**, v. 51, p. 3750-3757, 2012.

DETONI, A. M., et al. Uva" niágara rosada" cultivada no sistema orgânico e armazenada em diferentes temperaturas. **Food Science and Technology**, v. 25, n.3, p. 546-552, 2005.

DEVI, M. A. et al. Chemical compositions and insecticidal efficacies of four aromatic essential oils on rice weevil *Sitophilus oryzae* L. **International Journal of Tropical Insect Science**, v. 40, p. 549-559, 2020.

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

DIMER, F. A. et al. Impactos da nanotecnologia na saúde: produção de medicamentos. **Química Nova.** São Paulo: Sociedade Brasileira de Química, v. 36, n. 10, p. 1520-1526, 2013.

DOSHI, J.; RENEKER, D. H. Electrospinning process and applications of electrospun fibers. **Journal of Electrostatics**, Selected papers from the special technical session “Electrostatics in Polymer Processing and Charge Monitoring”, 1993 IEEE Industry Applications Society Meeting. v. 35, n. 2, p. 151–160, 1 ago. 1995.

DUTRA, R. C. et al. Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges and perspectives. **Pharmacological Research**, v.112, p. 4–29, 2016.

EFKERE, H. I. et al. The effect of thickness on the optical, structural and electrical properties of ZnO thin film deposited on n-type Si. **Journal of Molecular Structure**, v. 1165, p. 376–380, 5 ago. 2018.

EL ASBAHANI, A. et al. Essential oils: from extraction to encapsulation. **International Journal of Pharmaceutics**, v. 483, n. 1, p. 220-243, 2015.

ELLISON, C. J. et al. Melt blown nanofibers: Fiber diameter distributions and onset of fiber breakup. **Polymer**, v. 48, n. 11, p. 3306–3316, 21 maio 2007.

FATHI-AZARBAYJANI, A.; CHAN, S. Y. Single and multi-layered nanofibers for rapid and controlled drug delivery. **Chemical and Pharmaceutical Bulletin**, v. 58, n. 2, p. 143-146, 2010.

FAVORITO, P. A. et al. Características produtivas do manjericão (*Ocimum basilicum* L.) em função do espaçamento entre plantas e entre linhas. **Revista Brasileira de Plantas Medicinais**, Botucatu, v. 13, especial, p. 582-586, 2011.

FRANCO B. D. G. M.; LANDGRAF, M. **Microbiologia dos Alimentos**. São Paulo: Editora Atheneu, 182 p, 2008.

FREIRE, L. et al. Influence of physical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines. **International Journal of Food Microbiology**, v. 241, p. 181-190, 2017.

FREISESLEBEN, S. H.; JAGER, A. K. Correlation between plant secondary metabolites and their antifungal mechanism—a review. **Medicine and Aromatic Plants**, v. 3, p. 1-6, 2014.

FU, Y. et al. Antimicrobial activity of clove and rosemary essential oils alone and in combination. **Phytotherapy Research**, v. 21, n. 10, p. 989-994, 2007.

GAONKAR, R. et al. Essential oil from *Cymbopogon flexuosus* as the potential inhibitor for HSP90. **Toxicology reports**, v. 5, p. 489-496, 2018.

GARLOTTA, D. A literature review of poly (lactic acid). **Journal of Polymers and the Environment**, v. 9, n. 2, p. 63-84, 2001.

GOBBO-NETO, L.; LOPES, N. P. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. **Química nova**, v. 30, n. 2, p. 374-381, 2007.

GOMES, E. C.; NEGRELLE, R. R. B. Análise da cadeia produtiva do capim limão: estudo de caso. **Revista Brasileira de Plantas Medicinais**, v. 17, n.2, p. 201-209, 2015.

GRANDI, T. S. M. **Tratado das plantas medicinais mineiras, nativas e cultivadas**. 1 ed. Dados eletrônicos. Belo Horizonte: adaequatio estúdio, 1204p, 2014.

GUNDEL, S. da S. et al. *In vivo* antifungal activity of nanoemulsions containing eucalyptus or lemongrass essential oils in murine model of vulvovaginal candidiasis. **Journal of Drug Delivery Science and Technology**, v. 57, p. 101762, 2020.

GUPTA, B.; REVAGADE, N.; HILBORN, J. Poly (lactic acid) fiber: An overview. **Progress in Polymer Science**, v. 32, n. 4, p. 455-482, 2007.

HASHIMOTO, K. et al. Mycotoxin production of some *Aspergillus ochraceus* and *Aspergillus fumigatus* isolated from the air. **Micotoxyns**, v. 62, n. 1, p. 1-6, 2012.

HAQUE, A. N. M. A.; REMADEVI, R.; NAEBE, M. Lemongrass (*Cymbopogon*): a review on its structure, properties, applications and recent developments. **Cellulose**, v. 25, n. 10, p. 5455-5477, 2018.

IAMANAKA, B. T.; OLIVEIRA, I. S.; TANIWAKI, M. H. Micotoxinas em alimentos. **Anais da Academia Pernambucana de Ciência Agronômica**, Recife, v. 7, p. 138-161, 2010.

INTERNACIONAL AGENCY FOR RESEARCH ON CANCER. (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans.Volume 56, 1993. Disponível em: <https://monographs.iarc.fr/ENG/Monographs/vol56/mono56-18.pdf>; <http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf> <http://monographs.iarc.fr/ENG/Classification/index.php> Acesso em: 26 de janeiro de 1993.

INTERNATIONAL STANDARD ORGANIZATION. ISO 9235: **Aromatic natural raw materials - Vocabulary**. 2a ed., 14 p, dez. 2013.

IOANNIDS, A-G et al. Modelling the influence of temperature, water activity and sodium metabisulphite on the growth and OTA production of *Aspergillus carbonarius* isolated from Greek wine grapes. **Food Microbiology**, v. 49, p. 12-22, Aug. 2015.

JAY, J. M. **Microbiologia de alimentos**. 6 ed. Porto Alegre: Atmed, 711 p., 2005.

JIANG, C.; SHI, J.; ZHU, C. Fruit spoilage and ochratoxin a production by *Aspergillus carbonarius* in the berries of different grape cultivars. **Food Control**, v. 30, n. 1, p. 93-100, 2013.

JUGLAL, S.; GOVINDEN, R.; ODHAV, B. Spice oils for the control of co-occurring mycotoxin-producing fungi. **Journal of Food Protection**, v. 65, n. 4, p. 683-687, 2002.

KARPIŃSKI, T. M. Essential oils of Lamiaceae family plants as antifungals. **Biomolecules**, v. 10, n. 1, p. 103, 2020.

KERDUDO, A. et al. Chemical composition, antimicrobial and insecticidal activities of flowers essential oils of *Alpinia zerumbet* (Pers.) BL Burtt & RM Sm. from Martinique Island. **Chemistry & Biodiversity**, v. 14, n. 4, p. e1600344, 2017.

KRUGER, C. D. et al. Níveis séricos de ocratoxina A e lesões em suínos no Rio de Janeiro, Brasil. **Revista Brasileira de Medicina Veterinária**, v. 37, n. 3, p. 198-202, Jul./ Set. 2015.

KUMAR, A. et al. Delineation of *Ocimum gratissimum* L. complex combining morphological, molecular and essential oils analysis. **Industrial Crops and Products**, v. 139, p. 111536, 2019.

LAPPA, I. K. et al. Control of *Aspergillus carbonarius* in grape berries by *Lactobacillus plantarum*: A phenotypic and gene transcription study. **International Journal of Food Microbiology**, v. 275, p. 56-65, 2018.

LEIDY, R.; XIMENA, Q. M. Use of electrospinning technique to produce nanofibres for food industries: A perspective from regulations to characterisations. **Trends in Food Science & Technology**, v. 85, p. 92-106, 2019.

LEONG, S. L. et al. Australian research on ochratoxigenic fungi and ochratoxin A. **International Journal of Food Microbiology**, v. 111, p. S10-S17, 2006.

LI, H. et al. Nano/microscale technologies for drug delivery. **Journal of Mechanics in Medicine and Biology**, v. 11, n. 02, p. 337-367, 2011.

LI, Q. et al. The biocontrol effect of *Sporidiobolus pararoseus* Y16 against postharvest diseases in table grapes caused by *Aspergillus niger* and the possible mechanisms involved. **Biological Control**, v. 113, p. 18-25, 2017.

MACHADO, B. F. M. T.; FERNANDES JUNIOR, A. Óleos Essenciais: Aspectos Gerais e Usos em Terapias Naturais. **Cadernos acadêmicos, Tubarão**, v. 3, n. 2, p. 105-127, 2011.

MAIA, J. D. G.; RITSCHEL, P.; LAZZAROTTO, J. J. A viticultura de mesa do Brasil: produção para o mercado nacional e internacional. **Embrapa Uva e Vinho-Artigo em periódico indexado (ALICE)**, v. 9, p. 1-9, 2018.

MEDEIROS, E. S. et al. Solution blow spinning: A new method to produce micro-and nanofibers from polymer solutions. **Journal of Applied Polymer Science**, v. 113, n. 4, p. 2322-2330, 2009.

MEDINA, A. et al. Study of Spanish grape mycobiota and ochratoxin A production by isolates of Aspergillus tubingensis and other members of *Aspergillus* section Nigri. **Applied and Environmental Microbiology**, v. 71, n. 8, p. 4696-4702, 2005.

MELO, R. S. et al. Chemical composition and antimicrobial effectiveness of *Ocimum gratissimum* L. essential oil against multidrug-resistant isolates of *Staphylococcus aureus* and *Escherichia coli*. **Molecules**, v. 24, n. 21, p. 3864, 2019.

MERWE, K. J. V. et al. Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. **Nature,** London, v. 205, n. 4976, p. 1112-1113, Mar. 1965.

MILICEVIC, D. R.; SKRINJAR, M.; BALTIC, T. Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control. **Toxins**, Basel, v. 2, n. 4, p. 572-592, Apr. 2010.

MILITÃO, F. L.; FURLAN, M. R. Alimento funcional através do uso de *Ocimum basilicum* L. (manjericão) como aromatizante e tempero. **Revista Acadêmica Oswaaldo Cruz**, v.1, n. 4, p. 1-12, 2014.

MIRANDA, K. W. et al. Polystyrene bioactive nanofibers using orange oil as an ecofriendly solvent. **Journal of Applied Polymer Science**, v. 136, n. 15, p. 47337, 2019.

MOGOŞANU, G. D., et al. Natural products used for food preservation. In: **Food Preservation**. Academic Press, p. 365-411, 2017.

MONDANI, L. et al. Pest management and ochratoxin a contamination in grapes: A review. **Toxins**, v. 12, n. 5, p. 303, 2020.

MORI, C. L. S. O. et al. Nanostructured polylactic acid/candeia essential oil mats obtained by electrospinning. **Journal of Nanomaterials**, v. 16, n. 1, p. 1-9, 2015.

MORIE, A. et al. Nanofibers as novel drug carrier–an overview. **Artificial Cells, Nanomedicine, and Biotechnology**, v. 44, n. 1, p. 135-143, 2014.

NASCIMENTO, L. D. do et al. Bioactive natural compounds and antioxidante activity of essential oils from spice plants: new findings and potential applications. **Biomolecules**, v. 10, n. 7, p. 988, 2020.

NEPOMUCENO, N. C. et al. Antimicrobial activity of PLA/PEG nanofibers containing terpinen-4-ol against *Aggregatibacter actinomycetemcomitans*. **Journal of Applied Polymer Science**, v. 135, n. 6, p. 45782, 2018.

NETO, J. A. R. et al. Using the plants of Brazilian Cerrado for wound healing: from traditional use to scientific approach. **Journal of Ethnopharmacology**, v. 260, p. 112547, 2020.

NGUEFACK, J. et al. Food preservative potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus* against mycotoxigenic fungi. **International Journal of Food Microbiology**, v. 131, n. 2-3, p. 151-156, 2009.

OLIVEIRA, J. E. et al. Nano and submicrometric fibers of poly(D,L-lactide) obtained by solution blow spinning: Process and solution variables. **Journal of Applied Polymer Science**, v. 122, n. 5, p. 3396–3405, 5 dez. 2011.

OLIVEIRA, J. N.; OLIVEIRA; A. V.; MENEGHELLO, E. R. Análise molecular de espécies de aspergillus contaminantes de uvas vendidas no comércio de maringá-pr. **Iniciação Científica Cesumar**, v. 15, n. 2, p. 157-163, Jul./ Dez. 2013.

OLIVEIRA, L. B. S et al. Atividade antifúngica e possível mecanismo de ação do óleo essencial de folhas de *Ocimum gratissimum* (Linn.) sobre espécies de Candida. **Revista Brasileira de Plantas Medicinais**, Campinas, v. 18, n. 2, p. 511-523, 2016.

PAN, P.; INOUE, Y. Polymorphism and isomorphism in biodegradable polyesters. **Progress in Polymer Science**, v. 34, n. 7, p. 605-640, 2009.

PANDEY, A. K.; SINGH, P.; TRIPATHI, N. N. Chemistry and bioactivities of essential oils of some *Ocimum* species: an overview. **Asian Pacific Journal of Tropical Biomedicine**, v. 4, n. 9, p. 682-694, 2014.

PARIZE, D. D. S. et al. Poly (lactic acid) fibers obtained by solution blow spinning: Effect of a greener solvent on the fiber diameter. **Journal of Applied Polymer Science**, v. 133, n. 18, p. 43379, 2016a.

PARIZE, D. D. DA S. et al. Solution blow spinning: parameters optimization and effects on the properties of nanofibers from poly(lactic acid)/dimethyl carbonate solutions. **Journal of Materials Science**, v. 51, n. 9, p. 4627–4638, 3 fev. 2016b.

PAVELA, R.; BENELLI, G. Essential oils as ecofriendly biopesticides? Challenges and Constrains. **Trends in Plant Science**, [S.l.], vol. 21, n. 12, p. 1000-1007, dez. 2016.

PEREIRA, R. J.; CARDOSO, M. G. Metabólitos secundários vegetais e benefícios antioxidantes. **Journal of Biotechnology and Biodiversity**, v. 3, n. 4, p. 146-152, 2012.

PEREIRA, P. S. et al. Cytotoxic and anti-kinetoplastid potential of the essential oil of *Alpinia speciosa* K. Schum. **Food and Chemical Toxicology**, v. 119, p. 387-391, 2018.

PEREZ, F. S.et al. Nanotecnologia: aplicações na área de alimentos. **Disciplinarum Scientia**, v. 13, n. 1, p. 97-110, 2012.

PRAKASH, B. et al. Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities–Potentials and challenges. **Food Control**, v. 47, p. 381-391, 2015.

PRASAD, R.; SHAH, A. H.; RAWAL, M. K. Antifungals: mechanism of action and drug resistance. In: **Yeast Membrane Transport**. Springer, Cham. p. 327-349, 2016.

REZENDE, E. F. et al. Potencial enzimático e toxigênico de fungos isolados de grãos de café. **Coffe Science**, Lavras , v. 8, n. 1, p. 69-77, Jan./ Mar. 2013.

ROCHA, F. A. G. et al. O uso terapêutico da flora na história mundial. **Holosphere**, Natal, v. 1, n. 31, p. 49-61, mar. 2015.

ROCHA, M. E. B. et al. Mycotoxins and their effects on human and animal health. **Food Control**, v. 36, n.1, p. 159-165, Feb. 2014.

ROSSI, G. G. et al. Antibiofilm activity of nanoemulsions of *Cymbopogon flexuosus* against rapidly growing mycobacteria. **Microbial Pathogenesis**, v. 113, p. 335-341, 2017.

RUYCK, K. D. et al. Dietary mycotoxins, co-exposure, and carcinogenesis in humans: Short review. **Mutation Research**, v. 766, p. 32-41, Oct./Dec. 2015.

SAMSON, R. A.; HONG, S-B; FRISVAD, J. C. Old and new concepts of species differentiation in *Aspergillus*. **Medical Mycology**, v. 44, n. Supplement_1, p. S133-S148, 2006.

SANTOS, B. A. et al. Cardiodepressive effect elicited by the essential oil of *Alpinia speciosa* is related to L-type Ca2+ current blockade. **Phytomedicine**, v. 18, p. 539–543, 2011.

SARMA, A. et al. Screening of essential oil obtained from inflorescence of lemongrass [*Cymbopogon flexuosus* (Nees ex Steud.) Wats] accessions. **Indian Journal of Natural Products and Resources**, v. 2, n. 2, p. 236-241, 2011.

SCHUSTER, E. et al. On the safety of *Aspergillus niger* – a review. **Applied Microbiology and Biotechnology**, v. 59, p. 426-435, 2002.

SCHMIDT-HEYDT, M.; STOLL, D.; GEISEN, R. Fungicides effectively used for growth inhibition of several fungi could induce mycotoxin biosynthesis in toxigenic species. **International Journal of Food Microbiology**, v. 166, n. 3, p. 407-412, 2013.

SEWELL, R. D. E.; RAFIEIAN-KOPAEI, M. The history and ups and downs of herbal medicine usage. **Journal of HerbMed Pharmacology**, [S.l.], vol. 3, n. 1, p. 1-3, jun. 2014.

SILVA, T. H.; OLIVEIRA, J. E. DE; MEDEIROS, E. S. DE. Obtenção de micro e nanofibras de PVC pela técnica de Fiação por Sopro em Solução. **Polímeros**, v. 25, n. 2, p. 229–235, abr. 2015.

SIMÕES, C. M. O. et al. **Farmacognosia: do produto natural ao medicamento.** 1 ed. Artmed, 502 p. 2017.

SONKER, N. et al. Assessment of *Cymbopogon citratus* (DC.) stapf essential oil as herbal preservatives based on antifungal, antiaflatoxin, and antiochratoxin activities and in vivo efficacy during storage. **Journal of Food Science**, v. 79, n. 4, p. M628-M634, 2014.

SONKER, N.; PANDEY, A. K.; SINGH, P. Efficiency of *Artemisia nilagirica* (Clarke) Pamp. essential oil as a mycotoxicant against postharvest mycobiota of table grapes. **Journal of the Science of Food and Agriculture**, v. 95, n. 9, p. 1932-1939, 2015.

SOUSA, A. I. P. et al. Essential oils from *Ocimum basilicum* cultivars: analysis of their composition and determination of the effect of the major compounds on Haemonchus contortus eggs. **Journal of Helminthology**, v. 95, e17, p. 1-5, 2021.

TARIQ, S. et al. A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. **Microbial Pathogenesis**, v. 134, p. 103580, 2019.

TAYLOR, G. Electrically Driven Jets. **Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences**, v. 313, n. 1515, p. 453–475, 1969.

THOMPSON, C. J. et al. Effects of parameters on nanofiber diameter determined from electrospinning model. **Polymer**, v. 48, n. 23, p. 6913–6922, 2 nov. 2007.

ULTEE, A.; SMID, E. J. Influence of carvacrol on growth and toxin production by *Bacillus cereus*. **International Journal of Food Microbiology**, v. 64, n. 3, p. 373-378, 2001.

UPADHYAY, R. K. et al. Development of postharvest management practices for quality essential oil production of *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson**. Journal of Essential Oil Research**, v. 31, n. 6, p. 556-561,2019.

UHRICH, K. E. et al. Polymeric systems for controlled drug release. Chemical reviews, v. 99, n. 11, p. 3181-3198, 1999.

VAHEDI-SHAHANDASHTI, R.; LASS-FLÖRL, C. Novel antifungal agents and their activity against *Aspergillus* species. **Journal of Fungi**, v. 6, n. 4, p. 213, 2020.

VERMA, R. S. et al. Essential oil composition of the sub-aerial parts of eight species of *Cymbopogon* (Poaceae). **Industrial Crops and Products**, v. 142, p.111839, 2019.

VICTÓRIO, C. P. Therapeutic value of the genus *Alpinia*, Zingiberaceae. **Brazilian Journal of Pharmacognosy**, v. 21, n.1, p. 194-201, Jan./Feb., 2011

VIEGAS JR, C.; BOLZANI, V. S.; BARREIRO, E. J. Os produtos naturais e a química medicinal moderna. **Química Nova**, v. 29, n. 2 p. 326-337, 2006.

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, L. et al. Functional characterization of new polyketide synthase genes involved in ochratoxin A biosynthesis in *Aspergillus ochraceus* fc-1. **Toxins**, v. 7, n. 8, p. 2723-2738, 2015.

WANG, L. et al. Effect of cinnamaldehyde on morphological alterations of *Aspergillus ochraceus* and expression of key genes involved in ochratoxin A biosynthesis. **Toxins**, v. 10, n. 9, p. 340, 2018.

WEISS, J.; TAKHISTOV, P.; MCCLEMENTS, D. J. Functional materials in food nanotechnology. **Journal of Food Science**, v. 71, n. 9, p. R107-R116, 2006.

WELKE, J. E.; HOELTZ, M.; NOLL, I. B. Aspectos relacionados à presença de fungos toxigênicos em uvas e ocratoxina A em vinhos. **Ciência Rural**, v. 39, n.8, p. 2567-2575, 2009.

XUAN, T. D. et al. Allelopathic Plants: 26. *Alpinia zerumbet* (Pers.) B.L.Burtt & R.M.Sm. (Zingiberaceae). **Allelopathy Journal**, v. 48, n. 1, p. 01-14, Sept. 2019.

ZHANG, H. et al. Control of ochratoxin A-producing fungi in grape berry by microbial antagonists: A review. **Trends in Food Science & Technology**, v. 51, p. 88-97, 2016.

SEGUNDA PARTE – ARTIGOS

ARTIGO 1 - Antifungal and antiocratoxigenic potential of *Alpinia speciosa* and *Cymbopogon flexuosus* essential oils encapsulated in poly(lactic acid) nanofibers against *Aspergillus* fungi

Artigo aceito para a publicação, no periódico Letters in Applied Microbiology.

Periódico: Letters in Applied Microbiology

Classificação: B1 nas Ciências Agrárias,

A2 para Ciência dos Alimentos

JCR: 2.858

Significance and impact of the study: The fungi *Aspergillus ochraceus* and *Aspergillus westerdjkiae* contaminate food products with Ochratoxin A and cause deterioration because of their high metabolic activity, causing concerns about human health, food safety and economic losses. The induction of the biosynthesis of this toxic substance and the selection of fungi resistant to synthetic fungicides led to the need to search for new substances with inhibitory efficacy. The ability of the essential oils from *Alpina speciosa* and *Cymbopogon flexuosus* encapsulated in polymeric nanofibers to inhibit fungal proliferation and OTA production indicated that they have an antifungal potential to be explored against toxigenic fungi.

Abstract

Essential oils encapsulated in a polymeric matrix can be used as an alternative method to control fungi and mycotoxins. The essential oils were extracted by hydrodistillation and characterized by gas chromatography. The nanofibers were produced from poly (acid lactic) (PLA) containing essential oils by the Solution Blow Spinning method. The antifungal and antimicotoxygenic properties were evaluated against *Aspergillus ochraceus* and *Aspergillus westerdijkiae* by the fumigation method. Terpinen-4-ol (20.23%), sabinene (20.18%), 1.8-cineole (16.69%), and γ-terpinene (11.03%) were the principal compounds present in the essential oil from *Alpinia speciosa*, whereas citral (97.67%) was dominant from *Cymbopogon flexuosus*. Microscopy images showed that the addition of essential oils caused an increase in the diameter of the nanofibers. The infrared spectroscopy results indicated the presence of essential oils in the PLA nanofibers. Differential scanning calorimetry curves also indicated the existence of interactions between the essential oils and polymeric macromolecules through their plasticizing action. The hydrophobic character of nanofibers was revealed by the contact angle technique. An antifungal effect was observed, the mycelial growths (3.25–100%) and the synthesis of ochratoxin A (25.94–100%) were inhibited by the presence of the nanofibers. The results suggest that bioactive nanofibers hold promise for application to control toxigenic fungi.

Keywords: Solution blow spinning; *Aspergillus ochraceus*; *Aspergillus westerdijkiae*; ochratoxin A; Capim indiano; Colônia

Introduction

Ochratoxin A (OTA) is a secondary metabolite produced by some species of *Aspergillus* and *Penicilium;* the *Aspergillus* genus is considered to be the most important species responsible for contaminating food, beverages and animal feed with this mycotoxin (Zhang et al. 2016). In addition, the increase in the metabolic activity of these species causes the food to deteriorate, resulting in an enormous economic loss (Hua et al. 2014). OTA is classified by the International Cancer Agency as a group 2B carcinogen, in addition to having immunotoxic, teratogenic, nephrotoxic and genotoxic properties for humans (Iarc 1993; Zhang et al. 2016). The control of contamination is usually achieved with synthetic fungicides that inhibit the growth of OTA-producing fungi and reduce the production of this mycotoxin (Farbo et al. 2018). However, the continuous and improper use can favor the selection of resistant OTA-producing fungi and result in the induction of the biosynthesis of this toxic substance (Schmidt-Heydt et al. 2013; Zhang et al. 2016).

Several studies focus on alternative methods of natural control that can reduce contamination and the production of mycotoxins by different fungal strains, both in the field and throughout the entire post-harvest process. Among the natural resources, essential oils extracted from plants are particularly promising, and they represent an important product to be explored by various companies. Essential oils consist of monoterpenes, sesquiterpenes and phenylpropanoids that are synthesized by different parts of the plant through its secondary metabolism (Tariq et al. 2019).

Essential oils possess several proven biological properties such as antifungal activity (Nazzaro et al. 2017; Tariq et al. 2019). However, their direct incorporation in food tends to be limited because they are volatile and lipophilic, and they can alter the organoleptic profile of food products because of their aroma and flavor (Deepika et al. 2020). However, these limitations can be overcome by encapsulating essential oils in polymeric matrices in the form of nanofibers to reduce their volatility and their direct contact with food.

Polymeric nanofibers can be produced using the solution blow spinning technique (SBS). This technology has several advantages such as the ease of scheduling the process, high spinning rates, reduced costs and the possibility of depositing the nanofibers on different surfaces (Bilbao-Sainz et al. 2014; Bonan et al. 2017). Among the various polymers that can be used for the production of nanofibers, lactic polyacid (PLA) is of great interest because it is biodegradable and it can be obtained from renewable sources (Garlotta 2011; Bonan et al. 2017). Therefore, the aim of the present study was to investigate the effect of nanoencapsulation

on the antifungal and antiocratoxigenic action of the essential oils from *Alpinia speciosa* and *Cymbopogon flexuosus* against the *Aspergillus ochraceus* and *Aspergillus westerdijkiae* fungi.

Results and Discussion

Chemical composition of the essential oils

The yield of the essential oils from *A. speciosa* and *C. flexuosus* were 0.73% and 2% (w/w, DWB), respectively. The variability in yield can be explained by the genetic determination of each plant in producing secondary metabolites. In addition, gene expression can change with biochemical, physiological, evolutionary processes and with the ecological conditions where the plants were grown. These factors significantly affect the quantity and composition of essential oils (Gobbo-Neto and Lopes 2007).

The total number of constituents identified in the essential oils from *A. speciosa* and *C. flexuosus* were 19 and 3, respectively (Table 1). The principal constituents of the essential oil from *A. speciosa* were terpinen-4-ol (20.23%), sabinene (20.18%), 1.8-cineole (16.69) and γ-terpinene (11.03%). In the study by Rezende et al. (2011) it was found that the most abundant constituents in the essential oil were 1,8-cineole, sabinene and terpinen-4-ol. Their data corroborate those of the present study, but with different percentages. Differences in the chemical compositions of essential oils of the same species that are grown in different places are already common in the literature because edaphoclimatic factors influence the synthesis of secondary metabolites (Gobbo-Neto and Lopes 2007).

Three constituents classified as oxygenated monoterpenes comprise the essential oil from *C. flexuosus*, of which neral (36.10%) and geranial (61.57%) were the most abundant components (Table 1). *C. flexuosus* is a natural source of citral because more than 90% of its essential oil is composed of this oxygenated monoterpene. Citral is responsible for a strong, pleasant and lemon-like fragrance, and it has several biological properties, which increase the economic value of the plant and its essential oil (Fagodia et al. 2017; Devi et al. 2020). Similar results with a high concentration of citral (81.7 to 86.2%) were also reported by Upadhyay et al. (2019), who assessed the quality of essential oil production with respect to harvest management practices. The authors suggested that the plant material should be distilled fresh and on the same day of harvest to furnish higher yields and an essential oil of better quality (Upadhyay et al. 2019).

A. speciosa and *C. flexuosus* are of great importance for obtaining essential oils, and they can be used in the food, perfumery, cosmetic and pharmaceutical industries. *A. speciosa* is an ornamental plant that belongs to the Zingiberaceae family. It is used as a flavoring agent, with therapeutic properties, and its essential oil has significant antifungal activity (Kerdudo et al., 2017). *C. flexuosus* is a perennial, aromatic, medicinal herb, native to India, and it belongs to the Poaceae family. Studies report that the compounds present in the essential oil of this plant, mainly citral, have antifungal properties and a wide range of inhibitions of fungal growth (Pandey et al., 2003; Kumar et al., 2009; Sahal et al., 2020).

Characterization of nanofibers

The addition of the essential oils resulted in a greaterr average diameter of PLA nanofibers, producing fibers that were thicker. This increase was statistically significant ($p < 0.05$) (Table 2). In the SBS process, several factors can contribute to the morphology of the nanofibers obtained. The most important are the rheological profile of polymeric solutions and the gas pressure (Oliveira et al. 2011; Oliveira et al. 2014). The incorporation of essential oils can also significantly alter the morphology of polymeric nanofibers (Bonan et al. 2015; Souza et al. 2015). Studies by Liu et al. (2018) showed that the viscosity of polymeric solutions increases proportionally with the addition of essential oils. The authors suggest that the change in viscoelastic forces could counteract the force of the air flow and make it difficult to stretch the jet of the polymeric solution. Consequently, it would result in the formation of nanofibers with a larger diameter.

The electromicrographs obtained by SEM are shown in Figure 1A. All the nanofiber samples had a homogeneous morphology with a smooth surface. Smooth, uniform, continuous fibers were formed without the presence of granules, beads and films. The formation of homogeneous fibers is the result of adequate control of solution, process and environmental parameters (Oliveira et al. 2013ab).

FTIR analysis is very useful for characterizing intermolecular interactions and verifying whether the substances have been encapsulated (Figure 1B). Important bands at 2997 and 2943 cm^{-1} that are attributed to the C-H stretching vibration; and the 1452 cm^{-1} band that corresponds to CH_3 characterized by a 1360 cm^{-1} band are presented in the PLA spectrum (Figure 1B). The most intense band was observed at 1751 cm^{-1}, corresponding to the stretching vibration of the carbonyl groups (C = O) of the ester function. The peaks observed at 1184, 1130 and 1086 cm^{-1} represent the C-O-C group, where the absorption at 1184 cm^{-1} is characteristic of the

asymmetric vibrations and that at 1086 cm^{-1} corresponds to the symmetrical vibrations. The 1045 and 872 cm^{-1} bands correspond to C-CH_3 and C-COO stretching vibrations, respectively (Bilbao-Sainz et al. 2014; Bonan et al. 2017; Altan et al. 2018; Nepomuceno et al. 2018).

Peaks characteristic of PLA can also be seen in the spectra of the nanofibers incorporated with the essential oils. However, absorption peaks attributed to volatile constituents also appeared, such as the intense peak at 1672 cm^{-1} corresponding to the essential oil from *C. flexuosus*. In addition, slight changes have occurred in the spectrum of the PLA nanofiber containing the essential oil from *A. speciosa*. These changes in the intensity ratio and the formation of new peaks in the spectrum of PLA containing essential oils compared to the spectrum of nanofibers of PLA confirm the encapsulation of the essential oil. The charged particles overlapped the vibrational frequency of the pure polymer and the essential oil, exposing only hints of these particles, which suggests the presence of molecular interactions between PLA and the encapsulated compounds.

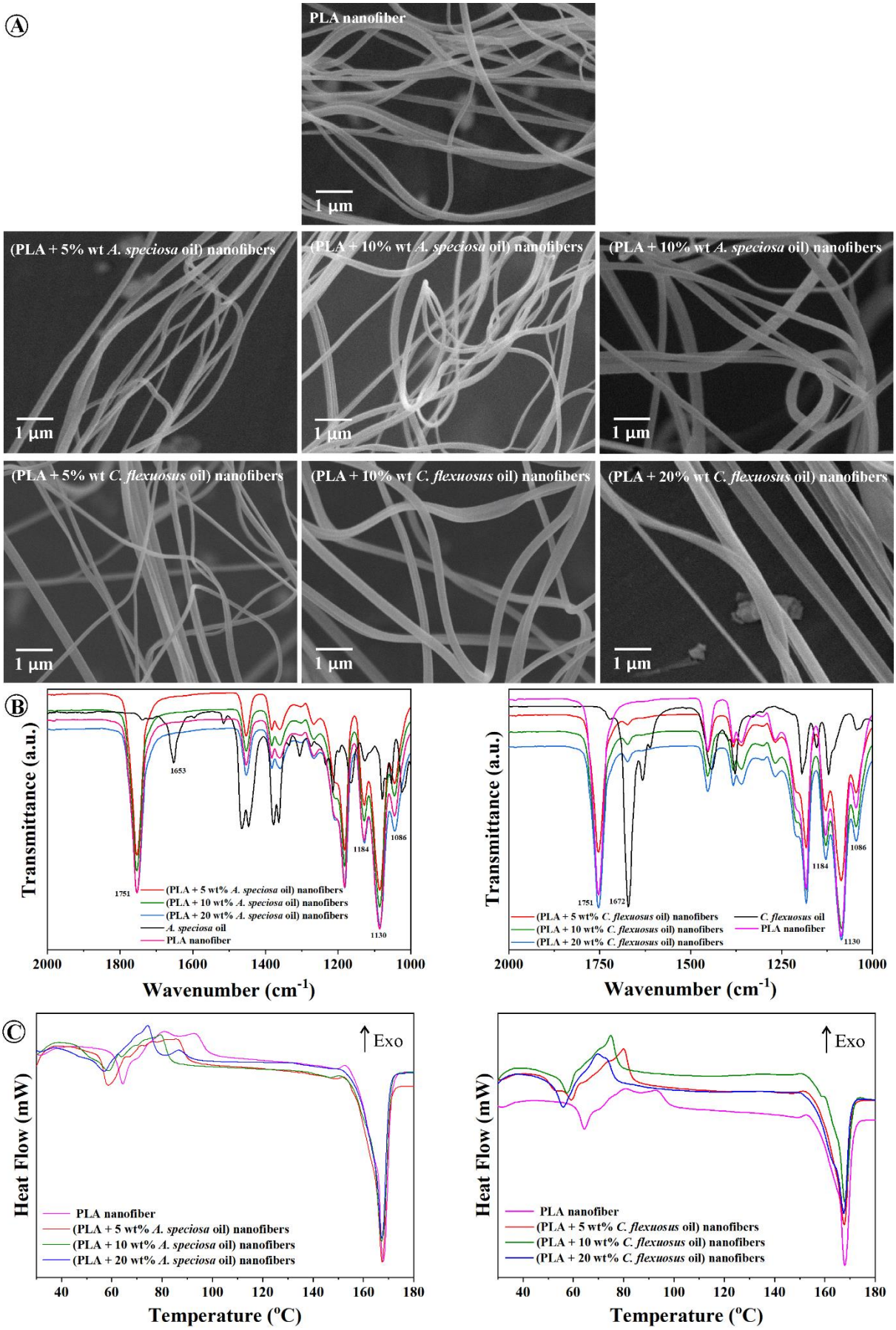


Figure 1 – Electromicrographs (A), FTIR-ATR spectra (B) and DSC curves (C) of nanofiber mats of pure PLA and PLA incorporated with essential oils from *A. speciosa* and *C. flexuosus*.

Differential scanning calorimetry (DSC) analyses were performed to evaluate the effect of essential oils on the thermal properties of PLA nanofibers. The thermal parameters for the first and second temperature cycles are shown in Figure 1C, and the calculated values are shown in Table 2.

As shown in Table 2, the incorporation of essential oils lead to a decrease in the cold crystallization temperature of PLA (T_c); however, the glass transition temperature (T_g) and melting temperature (T_m) did not undergo significant changes. These results are similar to those found in the literature (Mori et al. 2015; Souza et al. 2015; Nepomuceno et al 2018). The decrease in Tc with the incorporation of essential oils indicates that the essential oils acted as nucleating agents and reduced the energy required for the transformation of amorphous regions into crystalline regions in polymeric nanofibers (Bonan et al. 2017; Miranda et al. 2019). In addition, studies report that molecules of low molar mass as constituents of essential oils can act as lubricants to decrease intermolecular interactions and facilitate the formation of crystalline structures (Martínez-Sanz et al. 2015; Bonan et al. 2017). The nucleating effect of the essential oils is also related to a greater macromolecular orientations caused by the SBS process that favors the crystallization. This effect can be explained by the unidirectional force arrangement that is subjected to the jet of the polymer solution (Martínez-Sanz et al. 2015; Bonan et al. 2017; Cam et al. 2019).

The hydrophobic nature of the nanofibers were assessed by measuring the contact angle (Table 2). There was a significant difference in the contact angles between the nanofibers containing essential oil and that of pure PLA.

All the nanofibers studied were hydrophobic, that is, their contact angles were greater than 90° (Table 2). However, the addition of essential oils resulted in a slight reduction in the contact angle when compared to the pure PLA nanofiber. This decrease in the contact angle can be attributed to the small polar fraction of oxygenated monoterpenes and sesquiterpenes present in essential oils that are exposed on the fiber surface and provide a slight interaction with water (Wen et al. 2016). However, this interaction was not sufficient to make the nanofibers hydrophilic because the carbon chain protrudes and prevents water molecules from approaching and interacting by hydrogen bonding. This fact is justified by the hydrophobic nature of PLA and the essential oils that make up the nanofiber, and it is in line with previous studies (Bonan et al. 2015; Rahmani et al. 2020).

Antifungal properties

The growth of the two species of toxigenic fungi of the *Aspergillus* genus was inhibited by nanofibers containing the essential oils in the fumigation test (Figure 2). Although both essential oils have antifungal properties against *A. ochraceus* and *A. westerdijkiae*, the essential oil from *C. flexuosus* inhibited mycelial growth more strongly. A complete inhibition of the growth rate of the fungi was detected in the presence of 20% of essential oil from *C. flexuosus* encapsulated in the nanofibers. However, none of the concentrations tested with the essential oil from *A. speciosa* completely suppressed the growth of the fungi (Table 3).

Terpenoids and phenylpropanoids, constituents of essential oils, are responsible for antifungal activity because their lipophilic nature has great affinity for the lipid portion of the fungus plasma membrane. This interaction causes damage to biological membranes and, consequently, ion leakage (Lyu et al. 2019). In addition, the presence of chemical functions with hydrophilic characteristics is important for the effectiveness of the biological activity of the constituents of essential oils because they play a fundamental role in the mechanisms of interaction with vital metabolites such as proteins, carbohydrates and nucleic acids (Saad et al. 2013; Lyu et al. 2019). Thus, antifungal activity decreases according to the type of functional groups: phenolic compounds, aldehydes, ketones, alcohols, ethers and hydrocarbons. This relationship explains the greatest activity observed for the essential oil from *C. flexuosus*, which is composed of 97.67% citral, an aldehyde with an unsaturated chain. It also explains the low antifungal activity of the essential oil from *A. speciosa* because it is principally composed of compounds with alcohol and hydrocarbon functions.

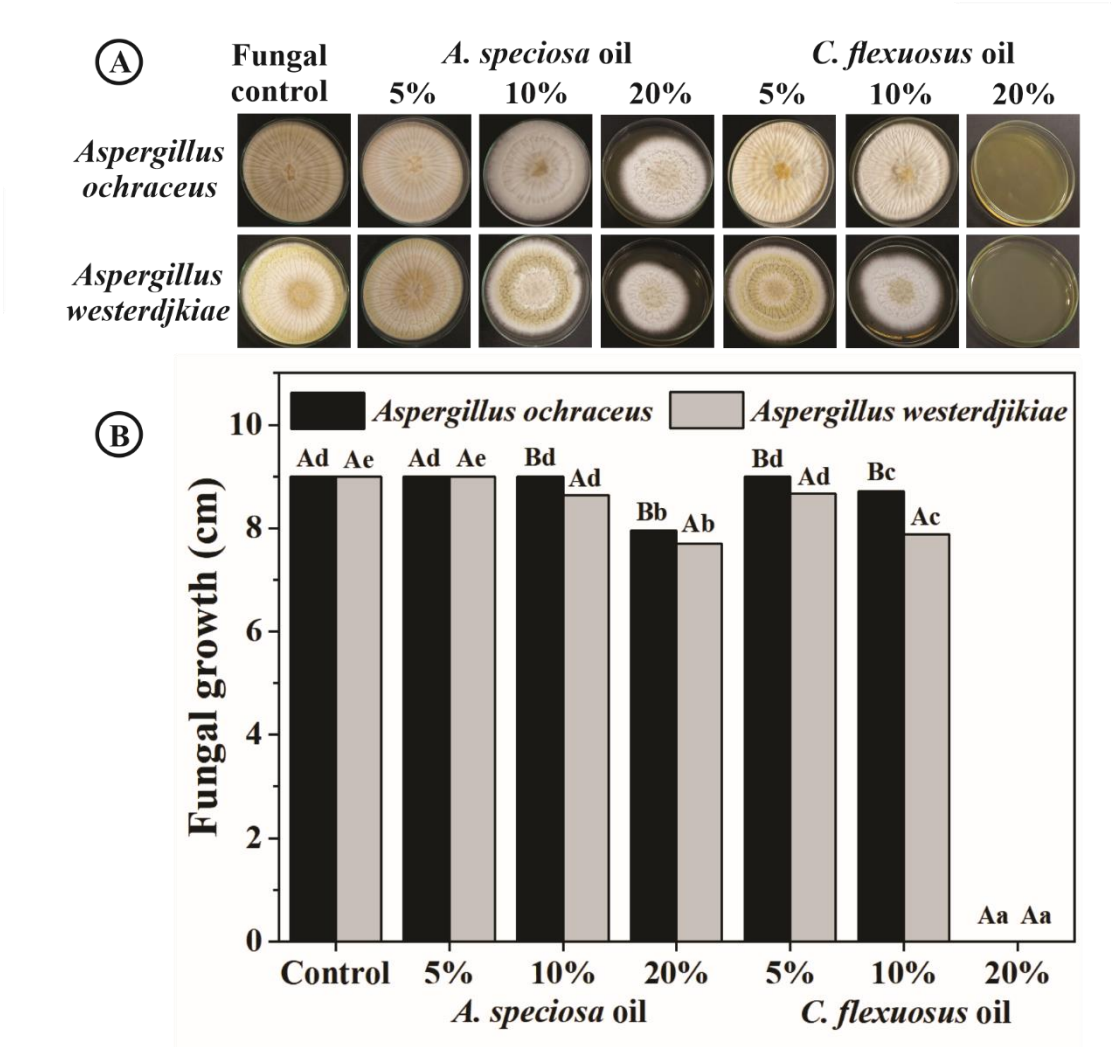


Figure 2 – Antifungal effects of different concentrations of essential oils from *A. speciosa* and *C. flexuosus* incorporated in PLA nanofiber mats on the growth rate of *A. ochraceus* and *A. westerdijikiae*. Images of antifungal action on colonies in Petri dish with culture medium after 10 days of incubation (A) and inhibition of colonies (cm) (B). The different lowercase and uppercase letters indicate significant differences between the concentrations tested in the treatments and between the two fungi, respectively, according to the Tukey test ($p < 0.05$).

Essential oils have several mechanisms of action because the rich and varied chemical compositions can act on several cellular targets, making it difficult to select resistant pathogens. According to Tang et al. (2018), the citral constituent can alter the permeability of the fungal membrane, induce the intracellular accumulation of reactive oxygen species that are capable of exerting oxidative stress and damage to the fungal cell structures and, consequently, induce apoptosis. In addition, the authors observed that this aldehyde interferes with genes related to growth and the formation of secondary metabolites by the fungus (Tang et al., 2018). Some terpenoids with an alcohol function, such as terpinen-4-ol and linalool, are responsible for causing deleterious effects to cyto-membrane permeability, morphological damage and to the metabolic pathways (An et al., 2019).

Antiocratoxigenic properties

The production of OTA significantly decreased and antiocratoxigenic effects were observed in a dose-dependent manner in the presence of both essential oils (Table 3). In general, the percentage of inhibition of the growth rate of fungi by essential oils was low; however, all the treatments resulted in a large reduction in OTA production by the isolates under study, mainly in the mycotoxin synthesized by the fungus *A. westerdjikiae*. An inhibition of 70.71 to 100% was obsered. This fact shows that, even with a limited inhibition of the growth of the fungus, the nanofiber mats containing the essential oils were efficient in controlling the production of OTA.

Several studies report the potential of essential oils to control the production of mycotoxins, but few show the mode of action. Hua et al. (2014) suggested that the inhibition of OTA production is related to the decrease in fungal biomass. Some studies propose that the regulation of gene expression that interferes with the production of OTA may indicate a possible mode of action of the essential oils (Lappa et al. 2016). In addition, the reduction in the expression of genes regulating secondary metabolism and genes encoding the lipase and metallopropase enzymes might be related to the significant inhibition of mycotoxin production. These enzymes are important in the lipid and protein metabolism of fungi (Oliveira et al. 2020).

The PLA nanofibers incorporated with essential oils might be suitable for use in dry food packaging applications, where the nanofiber will not have direct contact with the food. However, the bioactive compounds will be released in a controlled way into the packaging space. The essential oils incorporated into the PLA nanofiber mat will control the fungi and toxins produced by these microorganisms that contaminate the food through the fumigation process. In addition, as nanofibers release bioactive compounds in a controlled manner, they can increase the duration of the control of these microorganisms and increase the shelf life of products to ensure food safety.

Material and Methods

Extraction of essential oils

The leaves of *Alpinia speciosa* and *Cymbopogon flexuosus* (250 g) were added to a round bottom flask containing of distilled water and subjected to the hydrodistillation process for a period of 2 hours using a modified Clevenger apparatus (Anvisa, 2010). The hydrolate was

centrifuged for 15 minutes, and the essential oil was separated and stored in amber bottles at 4 °C. The essential oil yield was determined by refluxing of plant material with cyclohexane in a round-bottom flask connected to a Dean-Stark graduated collector for 2 hours. The yield was expressed as weight of oil per unit weight of plant material on a dry-weight basis (% w/w DWB) (Pimentel et al. 2006).

Chemical characterization

The chemical constituents of the essential oils were characterized on a gas chromatograph coupled to a mass spectrometer (GC-MS, Shimadzu Corporation, model QP2010 Plus, Kyoto, Japan) according to the method of Adams (2017). The following experimental conditions were used: fused silica capillary column containing a DB5 bound phase (30 m x 0.25 mm id, film thickness 0.25 μm); He 5.0 (White Martins, Rio de Janeiro, Brazil) was the carrier gas, and the flow rate was 1 mL min^{-1}. The injector temperature was 220 °C, and the detector temperature was 240 °C. The volume of the sample diluted in hexane (1%) injected was 0.5 μL using a split ratio of 1:100. The temperature was programmed from 60 °C, increasing to 240 °C at a rate of 3 °C min^{-1} and from 240 °C to 300 ° C at 10 °C min^{-1}. The final temperature was held for 7 min. The operational parameters of the MS were the following: ionization potential (70 eV), ion source temperature (200 °C), scan speed of 1000 Da sec^{-1}, scan interval of 0.50 fragments sec^{-1}. The analyses were performed in the full scan mode, ranging from 45 to 500 Da. The data regarding the chemical constituents were acquired using LabSolutions LC/GC Workstation 2.72. Van den Dool and Kratz's (1963) equation was used to calculate the retention index. The standards used were the homologous series of n-alkanes (nC8-nC18). The identification of the compounds was based on the comparison of the retention indices with those of the literature (Adams, 2017), and the mass spectra of the essential oil constituents with a similarity greater than 95% were compared with those of the FFNSC 1.2, NIST 107 and NIST 21 libraries of mass spectra. The essential oil constituents were quantified by gas chromatography using a flame ionization detector (FID) (Shimadzu GC-2010, Kyoto, Japan). The experimental parameters were the same as those used in the GC-MS analysis, with the exception of the detector temperature, which was 300 °C. The percentages of compounds were calculated using the area normalization method.

Production of nanofibers by SBS

The poly (lactic acid) nanofibers were produced by SBS at room temperature (28 – 30 °C) and with a relative humidity of 30 - 42% according to the methodologies of Bonan et al. (2017) and Nepomuceno et al. (2018), with modifications The SBS wiring system consisted of a concentric nozzle with a 4 mm protrusion, polymer injection pump (NE-300; New Era Pump Systems, Syringe Pump, New York, EUA), a source of compressed air at 140 kPa (Chiaperini MC 12 BPV 150 L 2HP, Brazil) and a collector. The polymeric solutions were prepared by solubilizing the poly (lactic acid) (PLA, M_w – 66,000 g mol^{-1}; Nature Works, 3251D, Minneapolis, USA) (12% p/v) in chloroform. The essential oils were added to the PLA solution in proportions of 5, 10 and 20% (v/m) relative to the total polymer weight. The polymer solutions were injected at a rate of 6 mL h^{-1} and the fibers formed were deposited in a rotary collector covered with aluminum foil positioned at a distance of 20 cm.

Scanning Electron Microscopy (SEM)

The nanofibers were collected on aluminum foil, cut and glued on stubs using a double-sided carbon adhesive and sprayed with gold using a gold coater (Bal-tec SCD 050, Balzers AG, Liechtenstein). The samples were examined using a scanning electron microscope (SEM, JSM-6510, Jeol) and diameter of the fibers was evaluated using the Image J software (National Institutes of Health, Bethesda, MD, USA). The average values and the standard deviation of the nanofiber diameters were obtained through the analysis of 100 randomly selected fibers (Miranda et al. 2019).

Fourier Transformed Medium Infrared Spectrometer (FTIR)

The medium-range infrared (IR) spectra of the nanofibers were obtained using the Fourier transform medium infrared spectrometer (FTIR, Vertex 70, Bruker, Germany). Infrared analyses were performed using the attenuated reflectance (ATR) technique. The spectra were recorded in a spectral range from 4000 to 400 cm^{-1}, performing 32 scans with a resolution of 4 cm^{-1} (Miranda et al. 2019).

Differential Scanning Calorimetry (DSC)

The analyses were performed in a differential scanning calorimeter (DSC-60, Shimadzu, Japan). The samples (4.08 ± 0.02 mg) were placed in sealed pans, and the measurements were performed under a nitrogen atmosphere at a flow rate of 50 mL min^{-1} and heated from 25 to 180 °C at a heating rate of 10 °C min^{-1}. All the samples were subjected to two temperature cycles. The values of glass transition (T_g), crystallization (T_c), melting temperature (T_m), heat of fusion (ΔH_m) and degree of crystallinity (%) were determined. The degree of crystallinity (Xc) was measured according to the following equation of Parize et al. (2016).

Angle of contact

The wettability of the nanofibers was investigated with the aid of a goniometer (Kruss Drop Shape Analyzer, DSA25, Hamburg, Germany) equipped with a microchamber. In each measurement, a drop of 10 µL of deionized water was pipetted onto the surface of the nanofibers and images of the drop were immediately collected. The contact angle of the water was measured in digital images using the Advance software (Kruss, Hamburg, Germany) at different locations on the mat surface. At least ten measurements were recorded from each sample and in triplicate. The experiments were performed at room temperature (Lago et al. 2020).

Fungus isolates and preparation of spore suspension

Aspergillus ochraceus (CCDCA10506) and *Aspergillus westerdijkiae* (CCDCA11469) were supplied by the Collection of Microorganism Cultures of the Laboratory of Mycotoxins and Food Mycology of the Federal University of Lavras. The fungi were grown in Petri dishes containing Agar Malt Extract culture medium (MEA, HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated in a BOD for 7 days at 25 °C. After the incubation time, the spores were scraped from the mycelium with a sterile glass rod, and the suspension of each isolate was prepared using a 1% aqueous solution of Tween 80 (Synth, Diadema, SP, Brazil). The spore suspensions were assessed using a Neubauer Chamber (Sigma, São Paulo, Brazil), and the concentrations were adjusted to 10^6 spores mL^{-1} (Brandão et al. 2020).

Antifungal activity

The antifungal activities against *A. ochraceus* and *A. westerdijkiae* of nanofibers containing 5, 10 and 20% of the essential oils were evaluated using the fumigation method described by Guimarães et al. (2011) and Hua et al. (2014), with modifications. Ten-µL aliquots of the spore suspension (10^6 spores mL^{-1}) were added to the Petri dish (9 cm in diameter) containing 20 mL of Yeast Extract Sucrose Agar (YES, HiMedia Laboratories Pvt. Ltd., Mumbai, India) culture medium. Disks (4 cm in diameter) of nanofiber mats were attached to the top of the Petri dish. Nanofiber mats without the addition of essential oil were used as a negative control. The fungal control was prepared with only the spore suspension of the isolate. The plates were incubated for 10 days at 25 °C. The tests were performed in triplicate. After incubation, the diameter of the fungal colony was measured in two perpendicular directions to evaluate the growth of the fungus, and the percentage of inhibition was calculated using the equation of Brandão et al. (2020).

Antiocratoxigenic activity

The effect of nanofibers containing the essential oils on the production of OTA was investigated under the same culture conditions described in the previous section (2.7). OTA extraction was performed with three plugs from the center, middle and edge of the colony and placing them in a test tube containing 1 mL of HPLC grade methanol after 10 days of incubation (Passamani et al., 2014). The tubes were vigorously homogenized for 5 seconds and maintained at 25 °C for 60 minutes. The extracts were filtered through polytetrafluoroethylene membranes (PTFE; 0.22 µm, Millipore) and then analyzed by HPLC (Shimatzu, Kyoto, Japan) with two high-pressure pumps (model SPD-M20A), a DGU 20A3 degasser, a CBM-20A interface, a SIL-10AF automatic injector and a RF-10 AXL fluorescence detector. The column used for the separation was the Agilent-Zorbax Eclipse XDB-C18 (4.6 x 250 mm, 5 µm) connected to an Agilent-Zorbax Eclipse XDB-C18 4-Pack (4.6 x 12, 5 mm, 5 µm) precolumn. The excitation wavelength employed was 332 nm, and the emission wavelength was 476 nm. The flow rate used during the analysis was 0.8 mL min^{-1}, and the injected volume of the samples and the standard was 20 µL. The elution was performed in an isocratic system of methanol:acetronitrile:water:acetic acid (5:35:29:1). The external standardization method was used to quantify OTA after the construction of an analytical curve obtained by linear regression using the commercial standard (Passamani et al., 2014). All the sample and standard OTA

solutions were analyzed in triplicate. The calculation of the percentage of inhibition of OTA production by treatments relative to the fungal control was performed using the equation of Brandão et al (2020).

Statistical analysis

The statistical analysis was performed using the Sisvar program (Ferreira 2011) and a completely randomized design (DIC) was adopted. The results were subjected to analysis of variance, and the averages were compared by the Tukey test at the level of 5% probability.

Acknowledgements

This work was supported by the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brasil (CAPES) - Financial Code 001. The authors thank the funding agencies for the scholarships and financial support, the Central of Analysis and Chemical Prospecting of the Federal University of Lavras and Embrapa Instrumentation for supplying the equipment for analyses and the Medicinal Plants Garden of the Federal University of Lavras, located in the city of Lavras, MG, Brazil.

Conflict of interest

The authors declare that no conflict of interests exists.

Data availability

Study data are available from the corresponding author upon request.

References

Adams, R.P. (2017) *Identification of essential oils componentes by gas chromatography/ mass spectroscopy*, 4.1 th ed. Carol Stream: Allured.

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

http://portal.anvisa.gov.br/documents/33832/260079/5%C2%AA+edi%C3%A7%C3%A3o+-+Volume+1/4c530 f86-fe83-4c4a-b907-6a96b 5c2d2fc.

Altan, A., Aytac, Z. and Uyar, T. (2018) Carvacrol loaded electrospun fibrous films from zein and poly(lactic acid) for active food packaging. *Food Hydrocolloids* **81**, 48-59.

An, P., Yang, X.,Yu, J. Qi, J., Ren, X. and Kong, Q. (2019) α-terpineol and terpene-4-ol, the critical components of tea tree oil, exert antifungal activities in vitro and in vivo against *Aspergillus niger* in grapes by inducing morphous damage and metabolic changes of fungus. *Food Control* **98**, 42-53.

Bilbao-Sainz, C., Chiou, B.S., Valenzuela-Medina, D. Du, W., Gregorski, K.S., Williams, T.G., Wood, D.F., Glenn, G.M. and Orts, W.J. (2014) Solution blow spun poly(lactic acid)/hydroxypropyl methylcellulose nanofibers with antimicrobial properties. *Eur Polym J* **54**, 1-10.

Bonan, R.F., Bonan, P.R., Batista, A.U., Sampaio, F.C., Albuquerque, A.J., Moraes, M.C., Mattoso, L.H.C., Glenn, G.M., Medeiros, E.S. and Oliveira, J.E. (2015) In vitro antimicrobial activity of solution blow spun poly (lactic acid)/polyvinylpyrrolidone nanofibers loaded with Copaiba (Copaifera sp.) oil. *Materials Science and Engineering*: C **48**, 372-377.

Bonan, R.F., Bonan, P.R., Batista, A.U., Perez, D.E., Castellano, L.R., Oliveira, J.E. and Medeiros, E.S. (2017) Poly(lactic acid)/poly(vinyl pyrrolidone) membranes produced by solution blow spinning: Structure, thermal, spectroscopic, and microbial barrier properties. *J Appl Polym Sci* **134**, 44802.

Brandão, R.M., Ferreira, V.R.F., Batista, L.R., Alves, E., Lira, N.D.A., Bellete, B.S., Scolforo, J.R.S. 448 and Cardoso, M.G. (2020) Antifungal and antimycotoxigenic effect of the essential oil of *Eremanthus erythropappus* on three different *Aspergillus* species. *Flavour Frag J* **35**, 524-533.

Cam, M.E., Cesur, S., Taskin, T., Erdemir, G., Kuruca, D.S., Sahin, Y.M., Kabasakal, L. and Gunduz, O. (2019) Fabrication, characterization and fibroblast proliferative activity of electrospun *Achillea lycaonica*-loaded nanofibrous mats. *Eur Polym J* **120**, 109239.

Deepika, S.A., Chaudhari, A.K., Das, S. and Dubey, N. K. (2020) Nanoencapsulated Monarda citriodora Cerv. ex Lag. essential oil as potential antifungal and antiaflatoxigenic agent against deterioration of stored functional foods. *J Food Sci Tech* **57**, 2863.

Devi, M.A., Nameirakpam, B., Devi, T.B., Mayanglambam, S., Singh, K.D., Sougrakpam, S., Shadia, S., Tongbram, M., Singh, S.D., Sahoo, D. and Rajashekar, Y. (2020) Chemical compositions and insecticidal efficacies of four aromatic essential oils on rice weevil *Sitophilus oryzae* L. *Int J Trop Insect Sci* **40**, 549.

Fagodia, S.K., Singh, H.P., Batish, D.R., Kohlia, R. K. (2017) Phytotoxicity and cytotoxicity of Citrus aurantiifolia essential oil and its major constituents: Limonene and citral. *Ind Crops Prod* **108,** 708-715.

Farbo, M.G., Urgeghe, P.P., Fiori, S., Marcello, A., Oggiano, S., Balmas, V., Hassan, Z.U., Jaoua, S. and Migheli, Q. (2018) Effect of yeast volatile organic compounds on ochratoxin A-producing *Aspergillus carbonarius* and *A. ochraceus*. Int J Food Microbiol **284,** 1-10.

Ferreira, D.F. (2011) Sisvar: a computer statistical analysis system. *Cienc Agrotecnol* **35**, 1039 -1042.

Garlotta, D. (2001) A literature review of poly (lactic acid). J Polym Environ **9**, 63-84.

Gobbo-Neto, L. and Lopes, N.P. (2007) Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Quim nova* **30**: 374-381.

Guimarães, L.G.D.L., Cardoso, M.G., Sousa, P.E.D., Andrade, J.D. and Vieira, S.S. (2011) Atividades antioxidante e fungitóxica do óleo essencial de capim limão e do citral. *Rev Cienc Agron* **42,** 464-472.

Hua, H., Xing, F., Selvaraj, J.N., Wang, Y., Zhao, Y., Zhou, L., Liu, X. and Liu, Y. (2014) Inhibitory Effect of Essential Oils on *Aspergillus ochraceus* Growth and Ochratoxin A Production. *Plos one* **9**, e108285.

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

Kerdudo, A., Ellong, E.N., Burger, P., Gonnot, V., Boyer, L., Chandre, F., Adenet, S., Rochefort, K., Michel, T. and Fernandez, X. (2017) Chemical composition, antimicrobial and insecticidal activities of flowers essential oils of *Alpinia zerumbet* (Pers.) BL Burtt & RM Sm. from Martinique Island. *Chem Biodiversity* **14**, e1600344.

Kumar, A., Shukla, R., Singh, P. and Dubey, N.K. (2009) Biodeterioration of some herbal raw materials by storage fungi and aflatoxin and assessment of *Cymbopogon flexuosus* essential oil and its components as antifungal. *Int Biodeterior Biodegrad* **63**, 712-716.

Lago, R.C., Oliveira, A.L.M., Dias, M.C., Carvalho, E.E.N., Tonoli, G.H.D. and Boas, E.V.D.B.V. (2020) Obtaining cellulosic nanofibrils from oat straw for biocomposite reinforcement: Mechanical and barrier properties. *Ind Crops Prod* **148**, 112264.

Lappa, I.K., Simini, E., Nychas, G.E. and Panagou, E.Z. (2016) *In vitro* evaluation of essential oils against *Aspergillus carbonarius* isolates and their effects on Ochratoxin A related gene expression in synthetic grape médium. *Food Control* **73**, 71-80.

Liu, F., Saricaoglu, F.T., Avena-Bustillos, R.J., Bridges, D.F., Takeoka, G.R., Wu, V.C.H., Chiou, B., Wood, D.F., McHugh, T.H. and Zhong, F. (2018) Antimicrobial carvacrol in Solution Blow-Spun fish-skin gelatin nanofibers. *J Food Sci* **83**, 984-991

Lyu, X., Lee, J and Chen, W.N. (2019) Potential Natural Food Preservatives and Their Sustainable Production in Yeast: Terpenoids and Polyphenols. *J Agric Food Chem* **67**, 4397-4417.

Martínez-Sanz, M., Bilbao-Sainz, C., Du, W., Chiou, B., Williams, T.G., Wood, D.F., Imam, S.H., Orts, W.J., Lopez-Rubio, A. and Lagaron, J.M. (2015) Antimicrobial poly(lactic acid)-based nanofibres developed by solution blow spinning. *J Nanosci Nanotechnol* **15**, 616-627.

Miranda, K.W., Mattoso, L.H., Bresolin, J.D., Hubinger, S.Z., Medeiros, E.S. and Oliveira, J.E. (2019) Polystyrene bioactive nanofibers using orange oil as an ecofriendly solvent. *J Appl Polym Sci* **136**, 47337.

Mori, C.L., Passos, N.A., Oliveira, J.E., Altoé, T.F., Mori, F.A., Mattoso, L.H.C., Scolforo, J.R. and Tonoli, G.H.D. (2015) Nanostructured polylactic acid/candeia essential oil mats obtained by electrospinning. *J Nanomater* **2015**, 1-9.

Nazzaro, F., Fratianni, F., Coppola, R. and Feo, V.D. (2017) Essential oils and antifungal activity. *Pharmaceuticals* **10**, 86.

Nepomuceno, N.C., Barbosa, M.A., Bonan, R.F., Oliveira, J.E., Sampaio, F.C. and Medeiros, E.S. (2018). Antimicrobial activity of PLA/PEG nanofibers containing terpinen-4-ol against *Aggregatibacter actinomycetemcomitans***.** *J Appl Polym Sci* **135**, 45782.

Oliveira, J.E., Moraes, E.A., Costa, R.G.F., Afonso, A.S., Mattoso, L.H.C., Orts, W.J. and Medeiros, E.S. (2011) Nano and submicrometric fibers of poly(d,l-lactide) obtained by Solution Blow Spinning: process and solution variables. *J Appl Polym Sci* **122**, 3396-3405.

Oliveira, J.E., Moraes, E.A., Marconcini, J.M., Mattoso, L.H.C., Glenn, G.M., Medeiros, E.S. (2013a) Properties of poly(lactic acid) and poly(ethylene oxide) solvent Polymer mixtures and nanofibers made by Solution Blow Spinning. *J Appl Polym Sci* **129**, 3672-3681.

Oliveira, J.E., Mattoso, L.H.C., Orts, W.J. and Medeiros, E.S. (2013b) Structural and morphological characterization of micro and nanofibers produced by Electrospinning and Solution Blow Spinning: a comparative study. *Adv Mater Sci Eng* **2013**, 1.

Oliveira, J., Brichi, G.S., Marconciniet, J.M., Mattoso, L.H.C., Glenn, G.M. and Medeiros, E.S. (2014) Effect of solvent on the physical and morphological properties of poly(lactic acid) nanofibers obtained by Solution Blow Spinning. *Eng Fibers Fabr* **9**, 117-125.

Oliveira, R.C., Carvajal-Moreno, M., Mercado-Ruaro, P., Rojo-Callejas, F. and Correa, B. (2020) Essential oils trigger an antifungal and anti-aflatoxigenic effect on *Aspergillus flavus* via the induction of apoptosis-like cell death and gene regulation. *Food Control* **110**, 107038.

Pandey, A.K., Rai, M.K. and Acharya, D. (2003) Chemical composition and antimycotic activity of the essential oils of corn mint (*Mentha arvensis*) and lemon grass (*Cymbopogon flexuosus*) against human pathogenic fungi. *Pharm Biol* **41**, 421-425.

Parize, D.D.S., Foschini, M.M., Oliveira, J.E., Klamczynski, A.P., Glenn, G.M., Marconcini, J.M. and Mattoso, L.H.C. (2016) Solution blow spinning: parameters optimization and effects on the properties of nanofibers from poly(lactic acid)/dimethyl carbonate solutions. *J Mater Sci* **51**, 4627.

Passamani, F.R.F., Hernandes, T., Lopes, N.A., Bastos, S.C., Santiago, W.D., Cardoso, M.D.G. and Batista, L.R. (2014) 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 Protect* **77**, 1947.

Pimentel, F.A., Cardoso, M.G., Salgado, A.P.S., Aguiar, P.M., Silva, V.D.F., Morais, A.R.D. and Nelson, D.L. (2006) A convenient method for the determination of moisture in aromatic plants. *Quim Nova* **29**, 373-375.

Rahmani, M., Khani, M.M., Rabbani, S., Mashaghi, A., Noorizadeh, F., Faridi-Majidi, R. and Ghanbari, H. (2020) Development of poly (mannitol sebacate)/poly (lactic acid) nanofibrous scaffolds with potential applications in tissue engineering. *Mater Sci Eng C* **110**, 110626.

Rezende, M.E., Jasmim, J.M., Caprini, G.P., Sousa, E.F., Schripsema, J. and Thiébaut, J.T.L. (2011) Teor e composição química do óleo essencial de alpínia em razão da adubação e da disponibilidade de água no solo. *Rev Ceres* **58**, 208-215.

Saad, N.Y., Muller, C.D. and Lobstein, A. (2013) Major bioactivities and mechanism of action of essential oils and their componentes. *Flavour Frag J* **28**, 269-27.

Sahal, G., Woerdenbag, H.J., Hinrichs, W.L., Visser, A., Tepper, P.G., Quax, W.J., Mei, H.C. and Bilkay, I.S. (2020) Antifungal and biofilm inhibitory effect of *Cymbopogon citratus* (lemongrass) essential oil on biofilm forming by Candida tropicalis isolates; an in vitro study. *J Ethnopharmacol* **246**, 112188.

Schmidt-Heydt, M., Stoll, D. and Geisen, R. (2013) Fungicides effectively used for growth inhibition of several fungi could induce mycotoxin biosynthesis in toxigenic species. I*nt J Food Microbiol* **166**, 407-412.

Souza, M.A., Oliveira, J.E., Medeiros, E.S., Glenn, G.M. and Mattoso, L.H.C. (2015) Controlled release of linalool using nanofibrous membranes of poly(lactic acid) obtained by electrospinning and solution blow spinning: a comparative study. *J Nanosci Nanotechnol* **15**, 5628-5636.

Tang, X., Shao, Y., Tang, Y. and Zhou, W. (2018) Antifungal Activity of Essential Oil Compounds (Geraniol and Citral) and Inhibitory Mechanisms on Grain Pathogens (*Aspergillus flavus* and *Aspergillus ochraceus*). *Molecules* **23**, 2108.

Tariq, S., Wani, S., Rasool, W., Shafi, K., Bhat, M.A., Prabhakar, A., Shalla, A.H. and Rather, M.A. (2019) A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. *Microb Pathogenesis* **134**, 103580.

Upadhyay, R.K., Singh, V.R., Verma, R.S., Padalia, R.C., Kumar, R., Chauhan, A. and Tewari, S.K. (2019) Development of postharvest management practices for quality essential oil production of *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson. *J Essent Oil Res* **31**: 556.

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

Wen, P., Zhu, D., Wu, H., Zong, M., Jing, Y. and Han, S. (2016) Encapsulation of cinnamon essential oil in electrospun nanofibrous film for active food packaging. *Food Control* **59**, 366-376.

Zhang, H., Apaliya, M.T., Mahunu, G.K., Chen, L. and Li, W. (2016) Control of ochratoxin A producing fungi in grape berry by microbial antagonists: A review. *Trends Food Sci Tech* **51**, 88-97.

Table 1 – Chemical composition of the essential oils from *Alpinia speciosa* and *Cymbopogon flexuosus*.

Peak	**Compound**	**RT (min)**	**RI_{ref}**	**RI_{cal}**	**Área (%)**	
					A. speciosa	***C. flexuosus***
1	α-Tujene	5.577	924	926	2.53	-
2	α-Pinene	5.794	932	934	1.57	-
3	Camphene	6.250	946	950	0.22	-
4	**Sabinene**	6.875	969	973	**20.18**	-
5	β-Pinene	7.043	974	979	5.03	-
6	Mircene	7.361	988	991	0.78	-
7	α-Terpinene	8.281	1014	1018	1.80	-
8	ρ-Cymene	8.550	1020	1026	4.32	-
9	Limonene	8.718	1024	1030	1.30	-
10	**1.8-cyneol**	8.799	1026	1032	**16.69**	-
11	**γ-Terpinene**	9.741	1054	1058	**11.03**	-
12	*cis*-Sabinene hydrate	10.234	1065	1071	2.03	-
13	Terpinolene	10.818	1086	1087	0.92	-
14	**Linalool**	11.348	1095	1102	-	1.44
15	*trans*-Sabinene hydrate	11.435	1090	1104	3.30	-
16	*trans*-ρ-Menth-2-en-1-ol	12.406	1136	1127	0.83	-
17	**Terpinen-4-ol**	14.717	1174	1182	**20.23**	-
18	α-Terpineol	15.397	1186	1198	0.95	-
19	**Neral**	17.184	1235	1240	-	**36.10**
20	**Geranial**	18.491	1264	1270	-	**61.57**
21	(E)-β-Caryophylene	24.806	1417	1418	2.51	-
22	Caryophylene oxide	31.350	1582	1581	3.11	-
Composition						
Monoterpene hydrocarbons					49.67	-
Oxygen-bearing monterpenes					44.03	99.11
Sesquiterpene hydrocarbons					2.51	-
Oxygen-bearing sesquiterpenes					3.11	-
Total identified					99.32	99.11

RT: Retention time; RI_{ref}: Reference retention index (Adams 2017); RI_{cal}: Calculated retention index.

Table 2 – Average diameter of nanofibers, contact angle, wetting rate and thermal properties of pure PLA and PLA nanofiber mats incorporated with essential oils from *A. speciosa* and *C. flexuosus*.

Sample	Mean Diameter (nm)	Mean contact angle (°)	Mean wetting rate (°/s)	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_c (J g^{-1})	ΔH_m (J g^{-1})	X_c (%)
PLA nanofibers	136±47^{c}	115.30±0.75^{a}	0.0089±0.0022^{a}	59	89	165	2	49	50
(PLA + 5 wt% *A. speciosa* oil) nanofibers	171±49^{b}	112.95±0.62^{b}	0.0041±0.0027^{a}	57	79	163	3	46	47
(PLA + 10 wt% *A. speciosa* oil) nanofibers	211±68^{ba}	105.18±0.37^{d}	0.0117±0.0025^{a}	57	78	163	2	45	46
(PLA + 20 wt% *A. speciosa* oil) nanofibers	240±93^{a}	109.45±0.29^{c}	0.0063±0.0020^{a}	56	72	164	2	42	42
(PLA + 5 wt% *C. flexuosus* oil) nanofibers	186±62^{b}	108.67±0.36^{c}	0.0086±0.0036^{a}	57	77	161	4	45	44
(PLA + 10 wt% *C. flexuosus* oil) nanofibers	204±75^{ba}	113.04±0.46^{b}	0.0076±0.0020^{a}	57	73	161	4	42	41
(PLA + 20 wt% *C. flexuosus* oil) nanofibers	230±92^{a}	108.79±0.62^{c}	0.0037±0.0010^{a}	56	68	163	3	41	40

Identical letters indicate no significant difference between samples in the same column by the Tukey test ($p < 0.05$). Glass transition temperature (Tg); Crystallization temperature (T_c); Melting temperature (T_m); Crystallization enthalpy (ΔH_c); Melting enthalpy (ΔH_m); Degree of crystallinity (X_c).

Table 3 – Effects of nanofiber mats with essential oils on the growth of fungal species and on the production of ochratoxin A by fungi of the *Aspergillus* genus.

Treatment	**Inhibition of growth (%)**		**Inhibition of the production of ocratoxina A (%)**		**(µg g^{-1})**	
	A. ochraceus	***A. westerdijkiae***	***A. ochraceus***	***A. westerdijkiae***	***A. ochraceus***	***A. westerdijkiae***
Controle fúngico	0.00±0.00^{dA}	0.00±0.00^{eA}	0.00±0.00^{gA}	0.00±0.00^{fA}	0.012	4.35
(PLA + 5 wt% *A. speciosa* oil) nanofibers	0.00±0.00^{dA}	0.00±0.00^{eA}	25.94±0.63^{fB}	79.43±0.63^{dA}	0.0089	0.90
(PLA + 10 wt% *A. speciosa* oil) nanofibers	0.00±0.00^{dB}	4.11±0.50^{dA}	36.03±0.25^{eB}	90.04±0.84^{cA}	0.0077	0.43
(PLA + 20 wt% *A. speciosa* oil) nanofibers	11.53±0.53^{bB}	14.44±0.00^{bA}	49.28±0.66^{cB}	96.80±0.95^{bA}	0.0061	0.14
(PLA + 5 wt% *C. flexuosus* oil) nanofibers	0.00±0.00^{dB}	3.64±0.58^{dA}	40.92±0.66^{dB}	56.26±0.40^{eA}	0.0071	1.90
(PLA + 10 wt% *C. flexuosus* oil) nanofibers	3.25±0.47^{cB}	12.50±0.44^{cA}	53.42±0.92^{bB}	89.39±0.72^{cA}	0.0056	0.46
(PLA + 20 wt% *C. flexuosus* oil) nanofibers	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	100±0.00^{aA}	0	0

Identical lowercase letters in the column do not differ statistically by the Tukey test at the 5% probability level; identical capital letters in the row do not differ statistically by the Tukey test at the 5% probability level.

ARTIGO 2 - Antifungal and physicochemical properties of *Ocimum* essential oil loaded in poly(lactic acid) nanofibers

Artigo aceito para a publicação, no periódico Letters in Applied Microbiology.
Periódico: Letters in Applied Microbiology
Classificação: B1 nas Ciências Agrárias,
A2 para Ciência dos Alimentos
JCR: 2.858

Significance and impact of the study: The continuous and improper application of synthetic fungicides can favor the selection of resistant OTA-producing fungi, and it can result in the induction of the biosynthesis of this toxic substance. In addition, it can cause adverse health effects for consumers. Essential oils are chemically safe and acceptable to consumers. The essential oils from *Ocimum basilicum* and *Ocimum gratissimum* encapsulated in lactic polyacid nanofibers hold promise for the control of fungal contamination and the inhibition of the production of ochratoxin A (OTA).

Abstract

Poly(lactic acid) (PLA) nanofibers containing different proportions of the essential oils from *Ocimum basilicum* L. and *Ocimum gratissimum* L. were prepared by the solution blow spinning method. The essential oils were extracted by hydrodistillation and characterized by gas chromatography. MEV, contact angle, DSC, and FTIR were used to characterize the nanofibers. The effect of bioative nanofibers on the growth of the fungus and on the production of ochratoxin A were evaluated using the fumigation test. Linalool, 1.8-cineole and camphor were the principal components of the essential oil from *O. basilicum*, and eugenol was the principal constituent in the oil from *O. gratissimum*. An increase in the average diameter of the nanofibers was observed with the addition of the essential oils. The essential oils acted as a plasticizer, resulting in a reduction in the crystallinity of the PLA. The encapsulation of essential oils in PLA nanofibers was verified by FTIR. An effective antifungal and antimicotoxygenic activity against *Aspergillus ochraceus* and *Aspergillus westerdjikiae* was observed for the bioative nanofibers. These results confirm the potential of PLA nanofibers containing the essential oils for the control of toxigenic fungi that cause the deterioration of food and are harmful to human health.

Keywords: *Ocimum basilicum*; *Ocimum gratissimum* ochratoxin A; solution blow spinning, *Aspergillus ochraceus, Aspergillus westerdijkiae*.

Introduction

The growth of toxigenic filamentous fungi is favored in countries with hot and humid climates, such as the countries of South America. These microorganisms, in addition to contaminating and deteriorating food in the field and during storage, can also produce mycotoxins that represent a great risk to consumers' health (El Khoury and Atoui 2010; Vipotnik et al. 2017). Ochratoxin A (OTA) is an important mycotoxin produced by the secondary metabolism of several filamentous species of the genus *Aspergillus* and *Penicillium*, and it is considered by the International Cancer Agency to exert a possible carcinogenic activity in humans (Iarc 1993; El Khoury and Atoui 2010). In addition, studies have reported that this substance can cause nephrotoxic, hepatotoxic, neurotoxic, teratogenic, immunotoxic and carcinogenic effects, and it can cause kidney and liver tumors in mice and rats (El Khoury and Atoui 2010; Parussolo et al. 2019). OTA is a common contaminant in food and feed, including cereals, grains, coffee, grapes, beverages, wine, cheese, spices, nuts and olives (Bacha et al. 2009; Wang et al. 2018). OTA was first isolated in 1965 from a culture of *Aspergillus ochraceus* (Van Der Merwe et al. 1965), and this organism is considered to be the main producer of this mycotoxin in the Circumdati section. However, Frisvad et al. (2004) described another important OTA-producing species named *Aspergillus westerdijkiae,* which belongs to the same section. *A. westerdijkiae* generally produces OTA in greater quantty and with more consistency than *A. ochraceus* (Samson et al. 2006).

The usual strategy for controlling toxigenic filamentous fungi and mycotoxins in food is to use synthetic fungicides. However, these fungicides are toxic, and they are not biodegradable. In addition, they can favor the selection of resistant fungi if used incorrectly (Abd-Elsalam et al. 2017). Therefore, techniques that use natural products have been studied as alternative strategies to reduce food contamination (Paranagama et al. 2003; Thipe et al. 2020). Medicinal and aromatic plants are sources of essential oils, complex mixtures of compounds derived from phenylpropanoids and terpenes, which have biological potentials (Bakkali et al. 2008). The antifungal mechanism of essential oils is related to the disruption of the cellular organization of the plasma membrane and to the inhibition of some enzymes involved in the metabolic pathway of carbohydrates and mycotoxins (Hu et al. 2017). The bioactive compounds present in essential oils are generally considered to be GRAS agents (generally recognized as safe). They are biodegradable products with a low risk for the selection of resistant microorganisms (Prakash et al. 2015). However, the use of essential oils in food has some limitations because of their high volatilites. Thus, the encapsulation of these bioactive

compounds in polymeric matrices is a route that can reduce the loss of essential oils and prolong their efficiency against toxigenic filamentous fungi. Polymeric nanofibers are nanostructures with a wide variety of applications. They are used as vehicles for the controlled release of bioactive compounds. They have a high porosity with small pores and a high surface area, and their structures can possibly be controlled (Abd-Elsalam et al. 2017; Nepomuceno et al. 2018). Solution Blow Spinning (SBS) is a technique for producing micro- and nanofibers with several applications for the controlled release of bioactive compounds (Bonan et al. 2015; Nepomuceno et al. 2018). Therefore, the focus of this work was to evaluate the effect of different concentrations of the essential oils from *O. basilicum* and *O. gratissimum* on the physicochemical, antifungal and antimicotoxygenic properties of poly(lactic acid) nanofibers against the fungi *Aspergillus ochraceus* and *Aspergillus westerdjikiae*.

Results and Discussion

Characterization of essential oils

The yield of the essential oil from *O. basilicum* was 2.27%, and that from *O. gratissimum* was 3.42% on a dry-weight basis. According to the chromatographic analyses, a total of 23 and six constituents were identified in the essential oils from *O. basilicum* and *O. gratissimum*, respectively (Table 1).

The essential oil from *O. gratissimum* contained eugenol (79.04%), (Z-β-ocimene (14.60%) and germancrene D (5.04%) as the principal constituents. The principal components of the essential oil from *O. basilicum* were linalool (26.89%), 1.8-cineole (23.62%) and camphor (15.69%). Kumar et al. (2019) studied several varieties of *O. gratissimum* from India and also observed that the essential oil was dominated by the phenylpropanoid eugenol (38.6 - 79.2%), followed by (Z)-β-ocimene (10 - 29.6%) and germanene D (1.6 - 8.1%). Miranda et al. (2015) studied the essential oil from *O. basilicum* and obtained the same principal constituents as were found in this work, but with different percentages (1.8-cineol, 20.86%; linalool, 16.58%; camphor, 14.72%). Mota et al. (2020) observed that the main compounds in the essential oil from *O. basilicum* were eugenol and linalool, and their concentrations varied from 7.1 to 50.85% and 17 to 54.7%, respectively, in all the samples tested. The authors observed that the amount of eugenol increases significantly in the vegetative phase when plants were grown under water stress. The flowering phase is considered to be the ideal stage for obtaining

linalool and 1,8-cineole in a higher percentage, regardless of the growth condition. This fact justifies the results found in this study. The plant was collected in the flowering phase.

Even though the two plants under study belong to the genus *Ocimum*, they were obtained in different yields and their compositions differed. The chemical constitution of the essential oil from *O. basilicum* was more complex, being formed by all classes of compounds. However, oxygenated monoterpenes predominated. The class of phenylpropanoids predominated in the essential oil from *O. gratissimum.* Studies have reported that the genetic composition of each plant and the biotic and abiotic factors can affect the proportions of chemical constituents differently and result in different profiles for the essential oils (Gobbo-Neto and Lopes 2007; Mota et al. 2020).

Characterization of nanofibers

All the nanofibers obtained had regular, smooth, uniform and continuous morphologies (Figure 1A). However, the average diameter of the nanofibers increased significantly ($p < 0.05$) with the increase in the content of essential oil incorporated into the poly(lactic acid) (PLA) solutions (Figure 1B). Mori et al. (2015) showed that the addition of essential oil from candeia to the polymeric solution resulted in an increase in the average diameter of the nanofibers obtained; this fact corroborates the results obtained in this study. Studies suggest that the viscosity and surface tension of the solution are key parameters during the formation of the nanofibers, and the increase in the viscosity of the solutions is the result of the increase in the diameter of the nanofibers (Silva et al. 2015; Liu et al. 2018b). Previous reports have shown that the viscosity of the polymeric solution increases as a result of the incorporation of the constituents of the essential oil, and the fiber diameter is affected (Liu et al. 2018ab), similarly to what was reported in this work.

The hydrophobic nature of the PLA nanofibers was maintained in all the samples because the contact angles were greater than 90° (Figure 1C). The addition of essential oils resulted in a slight increase in the hydrophilicity of the nanofibers, as can be seen by the reduction in the contact angle of the nanofibers containing the essential oil from *O. gratissimum.* On the other hand, there was a slight tendency for the contact angle to increase (Figure 1C) in the PLA nanofibers containing the essential oil from *O. basilicum*. This change in the contact angle can be related to the reorientation of the molecular structure of the nanofibers after the addition of essential oils because the constituents of the essential oils have polar portions in their structures. Thus, the hydrophobic interaction within the PLA system can increase, and the

polar groups are projected onto the surface of the nanofiber mat to provide a slight interaction with water and, consequently, decrease the contact angle (Munhuweyi et al. 2017). Eugenol, the constituent of the essential oil from *O. gratissimum,* has two oxygens in its molecular structure. When undergoing reorientation, it can interact more strongly with water than the principal constituents of *O. basilicum* (linalool, 1,8-cineol and camphor), which have only one oxygen, and the greater interaction with water causes a slight reduction in the contact angle of the nanofibers.

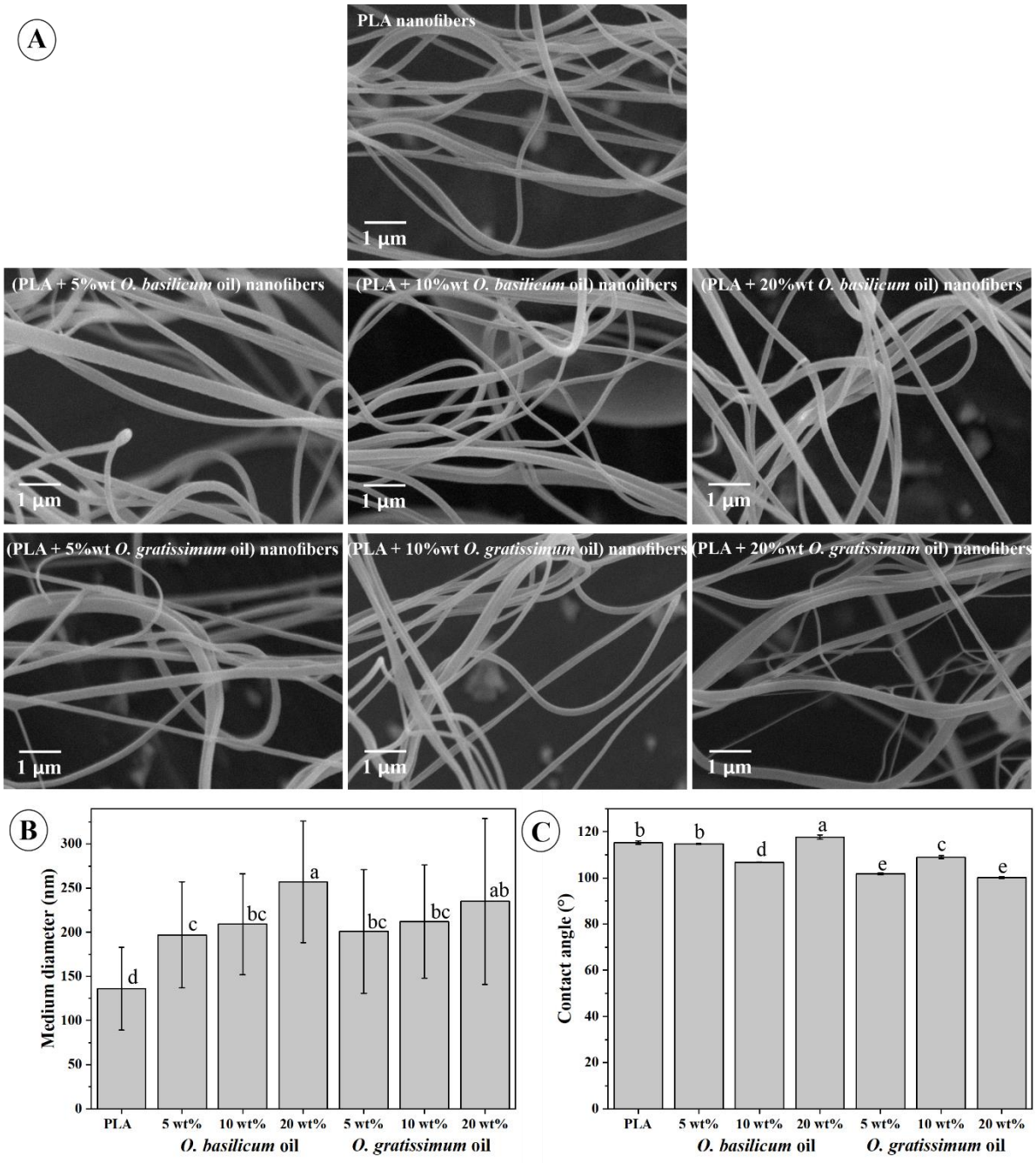


Figure 1 – Electromicrographs (A), medium diameter (B) and contact angle (C) of nanofibers of pure PLA and PLA with essential oils from *O. basilicum* and *O. gratissimum*. Treatments that do not share similar letters differ significantly ($p < 0.05$).

In addition, studies indicate that the hydrophobicity of nanofibers also depends on the surface morphology (diameter and pore size), and the interaction between water and material is related to its surface area. That is, there is a tendency for an increase in the hydrophobicity to occur with the increase in the surface area of PLA nanofibers (Chen et al. 2011; Toncheva et al. 2011; Miranda et al. 2019).

The infrared spectra of essential oils, PLA nanofibers and PLA nanofibers containing the essential oils from *O. basilicum* and *O. gratissimum* are shown in Figure 2A. Bands from 3518 to 3444 cm^{-1} in the infrared spectra (Figure 2A) of the essential oils from *O. basilicum* and *O. gratissimum* represent the OH stretching vibration of phenolic compounds and alcohols; bands at 3076, 3081 and 3004 cm^{-1} are indicative of stretches of =C-H in aromatic compounds and alkenes; and the bands from 2964 to 2840 cm^{-1} refer to the symmetrical and asymmetric stretching of C-H bonds (aromatic and aliphatic). Bands from 1639 to 1512 cm^{-1} are characteristic of the C=C stretching vibration (aromatics and alkenes) (Pereira and Maia 2007; Mahapatra et al. 2009; Sutaphanit and Chitprasert 2014; Ahmed et al. 2017).

Characteristic absorption bands at 2997 and 2943 cm^{-1} in the spectrum of the pure PLA nanofibers refer to the stretching vibrations of CH_3 and C-H, respectively. The intense vibrational peak at 1751 cm^{-1} is characteristic of the carbonyl group (C=O) of the ester function; the symmetrical and asymmetric stretching vibration at 1360 and 1452 cm^{-1} corresponds to CH_3. Bands corresponding to the symmetrical and asymmetrical elongation of C-O can be seen at 1184 and 1086 cm^{-1}, and elongation of C-CH_3 and C-COO bonds are observable at 1045 and 872 cm^{-1}, respectively (Cam et al. 2019; Rahmani et al. 2020). Changes in the spectral regions that represent the OH, C-H and C=C groups of pure PLA nanofibers were observed when the essential oils were incorporated. In addition, the presence of the essential oil from *O. gratissimum* in PLA nanofibers can be attributed to the intense peak at 1512 cm-1 characteristic of the volatile constituents of the oil.

The results observed after the addition of essential oils, such as changes in the intensity of the spectra and the presence of peaks characteristic of the spectrum of essential oils in the spectra of nanofibers of PLA containing the essential oils, indicate a successful formulation. The active compounds were encapsulated, and the existence of interactions between chemical constituents and the PLA nanofiber could be seen (Munhuweyi et al. 2017).

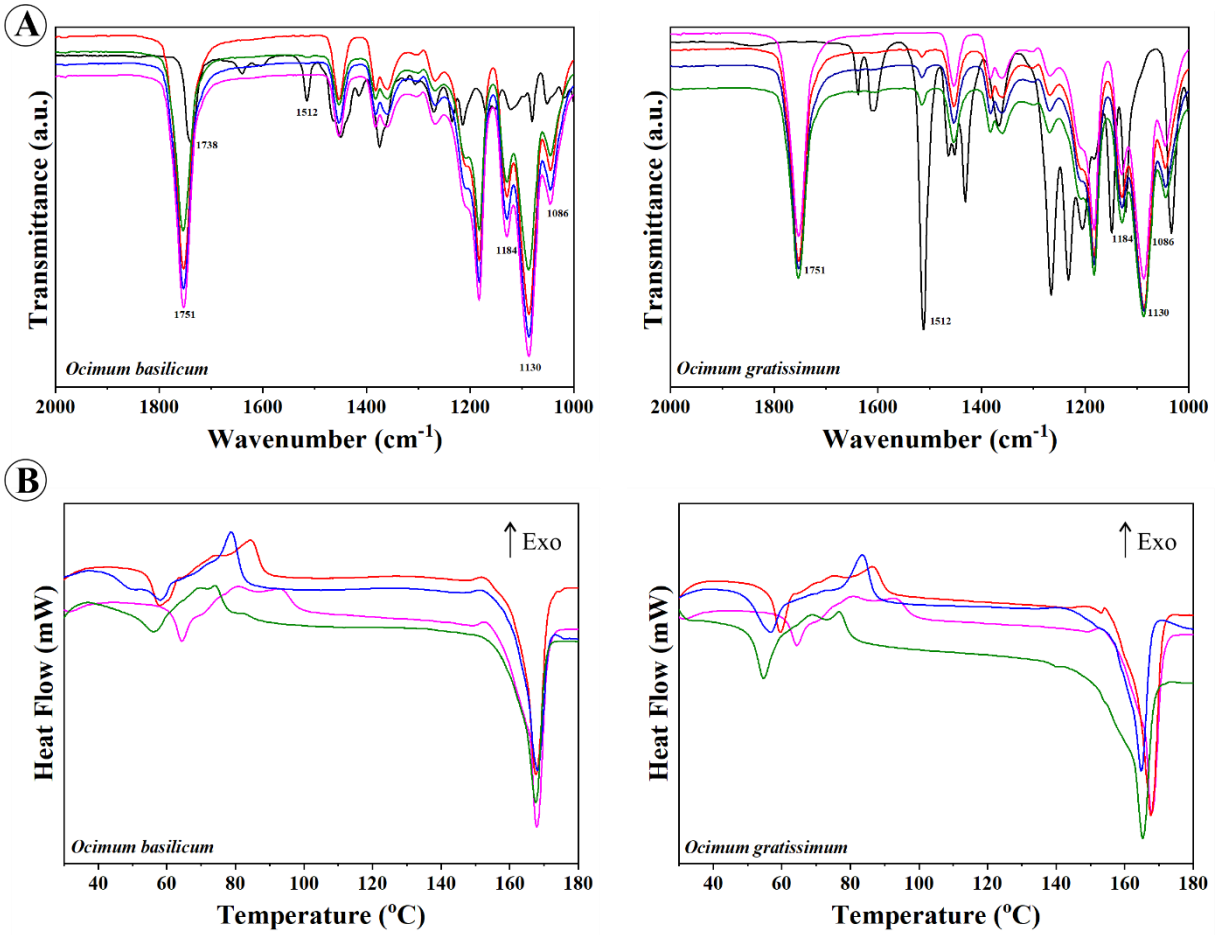


Figure 2 – Fourier Transform Medium Infrared spectra (A) and DSC curves (B) of pure PLA nanofibers and PLA containing essential oils from *O. basilicum* and *O. gratissimum*. PLA nanofibers: (–), (PLA + 5 wt% essential oil); nanofibers: (–), (PLA + 10 wt% essential oil); nanofibers: (–), (PLA + 20 wt% essential oil); nanofibers: (–), essential oil: (–).

The calculated values of the thermal parameters and the differential scanning calorimetry (DSC) curves, respectively, referring to the PLA nanofiber mats and the nanofibers loaded with essential oils are presented in Table 2 and Figure 2B. A slight decrease of approximately 6 °C in the glass transition temperature (T_g) of the PLA nanofibers containing the essential oil from *O. gratissimum* and no significant change in the T_g of the nanofibers containing *O. basilicum* oil were observed. A decrease of up to 10 °C in the temperature of cold crystallization (T_c) of the PLA nanofibers containing essential oils from *O. basilicum* and *O. gratissimum* was observed. The melting temperature (T_m), on the other hand, did not change significantly as a result of the presence of essential oils, and the degree of crystallinity (X_c) of PLA nanofibers containing essential oils was similar to that of pure PLA nanofiber. Thus, a slight decrease was observed for T_m, whereas the values of T_g, T_c and X_c were more notable (Table 2).

The decrease in PLA crystallinity and T_g values can be related to the interaction of essential oil constituents with the PLA ester groups and also to the fact that these compounds

have a low molar mass. This characteristic can allow the constituents of the essential oil of *O. gratissimum* to behave as a chain spacer, making macromolecules more flexible (decrease in T_g). According to Moradkhannejhad et al. (2020), compounds of lower molar mass have a greater plasticizing effect because compounds of lower molar mass provide greater mobility to the functional groups that are at the end of the chain in free rotation.

Antifungal and antiocratoxygenic effect

The growth of *A. ochraceus* and *A. westerdjikiae* and the production of OTA were inhibited by the fumigation test in which the essential oils incorporated in nanofibers had an effect on the fungi under study (Figure 3). Essential oils generally have a short period of antifungal action because they are quite volatile. The incorporation of these active compounds into polymeric nanofibers can be an alternative that permits a controlled release over longer periods to increase the effectiveness against fungi.

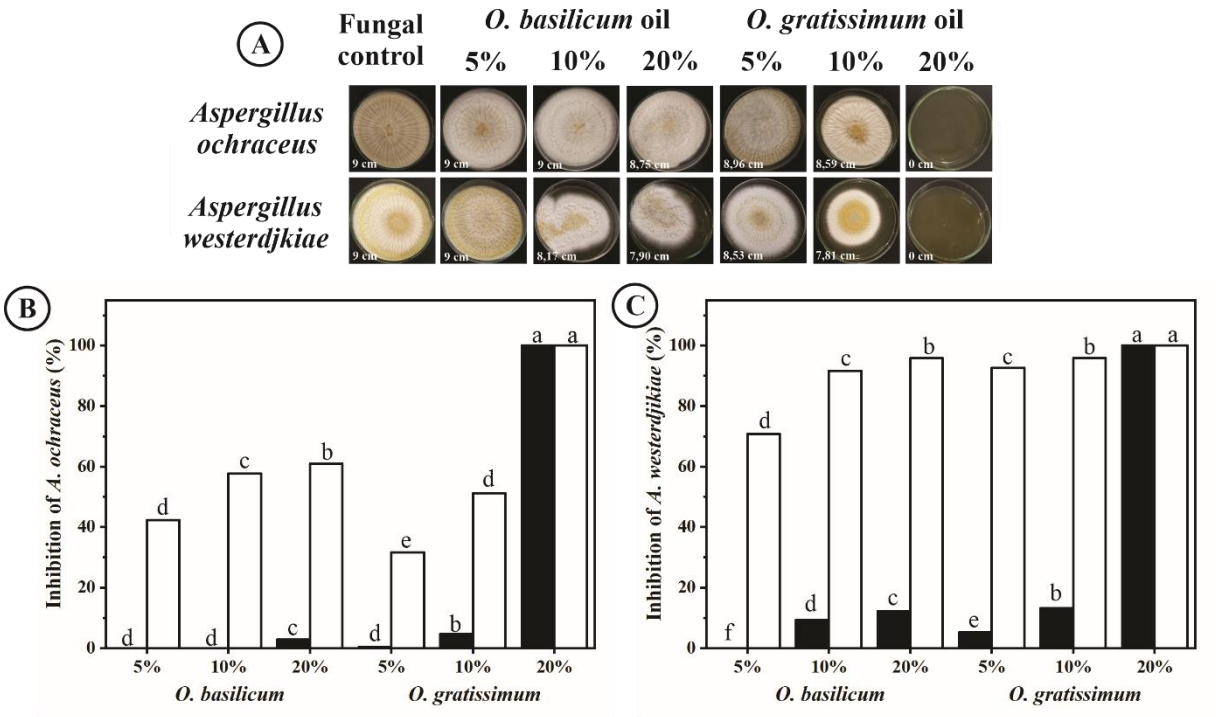


Figure 3 – Antifungal and anti-cratoxigenic activity of PLA nanofibers containing the essential oils from *O. basilicum* and *O. gratissimum* in different proportions. Images of the fungal control in Petri dishes with culture medium after 10 days of incubation (A) and percentage inhibition of mycelial growth and production of ochratoxin A by *A. ochraceus* (B) and *A. westerdjikiae* (C). Treatments for each test that do not share similar letters differ significantly ($p < 0.05$). Mycelial growth: (■), Ochratoxin A: (□).

Even though the inhibition of the fungal growth was weak, the essential oils encapsulated in nanofibers had an impact on the production of OTA by the fungi under study

(Figure 3). The concentration of this mycotoxin decreased significantly in the presence of bioactive compounds because a dose-dependent correlation between the inhibition of OTA production and the concentration of essential oils from *O. basilicum* and *O. gratissimum* was observed. These results are very good because OTA is a troublesome factor in the food industry because it is considered to be a carcinogenic substance. Pure PLA nanofibers acted as a negative control because, without the incorporation of essential oils, they had no effect on filamentous fungi and were unable to inhibit the growth and production of OTA.

The essential oil from *O. gratissimum* was the most effective, both in inhibiting the growth of the fungi under study and in inhibiting the production of OTA (Figures 3B and 3C). This oil completely inhibited the proliferation of fungi at a concentration of 20%. This activity can be explained by the greater proportion of the phenylpropanoid eugenol in the oil. These phenolic compounds interact with plasma membrane proteins and enzymes to cause structural and functional deformation, in addition to increasing the transport of ATP and potassium out of cells (Munhuweyi et al. 2017). The small amount of this compound in the essential oil from *O. basilicum* could explain its relatively small antifungal effect compared to that of the essential oil from *O. gratissimum*. Although the nanofiber containing the essential oil from *O. basilicum* provided a lower percentage of inhibition of fungal growth, it significantly inhibited the production of OTA by *A. ochraceus* (42.37% - 60.86%) and *A. westerdjikiae* (70.71% - 95.93%), making it also a good antifungal agent in the control of toxigenic fungi. The biggest problem of these fungi is their ability to produce OTA, contaminate food with this mycotoxin and be a risk to human health. The antifungal activity of essential oils is related to the lipophilic character of their components, where the principal constituents help to penetrate the cell and mitochondrial membrane. However, because of the synergistic interaction of the principal and secondary compounds and the composition as a whole, the functional groups present add up to a collective and are responsible for the inhibitory efficacy of essential oils (Munhuweyi et al. 2017).

The antifungal activity against toxigenic fungi that cause deterioration in food and risks to human health and the inhibition of the production of OTA by nanofibers incorporated with essential oils can provide parameters for finding alternative and safe packaging of food products. PLA nanofibers with the essential oils can have potential applications as matrices for bioactive compounds to be used in dry food packaging. Such packaging can inhibit the growth of toxigenic fungi, where the release of bioactive compounds to the medium in which the food is found is controlled to increase the useful life of the product.

Material and Methods

Plant material, extraction and chemical characterization of essential oils

The plants *Ocimum basilicum* L. and *Ocimum gratissimum* L. were obtained from the Garden of Medicinal Plants of the Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brazil. The plant material was collected in the morning on a mild day without precipitation. Approximately 250 g of the previously selected and chopped healthy leaves was placed in a round-bottom flask with water. The essential oil was extracted by the hydrodistillation method for a period of 2 hours using a modified Clevenger apparatus (Anvisa 2010). The hydrolate was centrifuged (Fanem Baby I Model 206 BL, São Paulo, Brazil) at 965.36*g* for 15 minutes, and the separated essential oil was stored in amber glass containers under refrigeration. The oil yield was determined according to the method described by Pimentel et al. (2006), and the yield was expressed on a dry-weight basis relative to the weight of plant material (% w/w DWB).

The identification of the constituents present in the essential oil was achieved in a gas chromatograph (Shimadzu Corporation, model QP2010 Plus, Kyoto, Japan) coupled to a mass spectrometer (GC-MS) (Adams 2017). The chromatograph was equipped with a fused silica capillary column containing a DB5 bound phase (30 m X 0.25 mm i.d., film thickness 0.25 μm); the carrier gas was He 5.0 (White Martins, Rio de Janeiro, Brazil) with a flow rate of 1 ml min^{-1}. The injector and detector temperatures were 220 °C and 240 °C, respectively. The temperature program initiated at 60 °C and increased to 240 °C at a rate of 3 °C min^{-1}, followed by an increase at 10 °C min^{-1} to 300 °C, where it was maintained for 7 min. The sample was diluted in 1% hexane (Sigma-Aldrich®, St. Louis, MO, USA); the volume injected into the chromatograph was 0.5 μl; The split ratio was 1:100. The MS was operated with an ionization potential of 70 eV; the ion source temperature was 200 °C; the scanning speed was 1000 Da sec^{-1} and the scanning interval was 0.50 fragments sec^{-1}. The analyses were performed in the full scan mode, ranging from 45 to 500 Da. A LabSolutions LC/GC Workstation was used to obtain the information regarding the constituents of the essential oil. Retention indices were calculated by the equation of Van den Dool and Kratz (1963) using the standards of the homologous series of n-alkanes (nC9-nC18) and compared with those in the literature (Adams 2017) for the identification of the compounds. Mass spectra libraries FFNSC 1.2, NIST 107 and NIST 21 were also used to compare the spectral data of the essential oil constituents (similarity greater than 95%).

Quantitative analysis was performed on a gas chromatograph (Shimadzu - 2010, Kyoto, Japan) equipped with a flame ionization detector (GC-FID). The operating conditions were the same as those used in the qualitative analysis, except for the detector temperature (300 °C). The percentage of essential oil constituents was calculated using the area normalization method.

Solution Blow Spinning (SBS)

The Solution Blow Spinning system was used for the production of nanofiber mats according to the methods of Bonan et al. (2017) and Nepomuceno et al. (2018), with some modifications. The following experimental conditions were adopted: compressed air source (Chiaperini MC 12 BPV 150 L 2HP, Brazil), glass syringe (FLURAN F-5500-A; Ismatec, Wertheim, Germany) coupled to a polymer injection pump (NE -300; New Era Pump Systems, Syringe Pump, New York, USA), a device with a concentric nozzle with a 4 mm protrusion and a rotating collector. The polymeric solution was injected at a rate of 6 ml h^{-1}, and the compressed air pressure was adjusted to 140 kPa. The rotating collector was covered with aluminum foil, and the fibers were collected at a working distance of 20 cm.

Characterization of nanofibers

The morphology of the nanofibers was evaluated by scanning electron microscopy (SEM, JSM-6510, Jeol) and electromicrographs were obtained. Before the analysis, the samples were glued to metal stubs with double-sided carbon adhesive tape and coated with gold (Bal-tec SCD 050, Balzers AG, Liechtenstein). The samples (100 fibers) were analyzed by image software (Image J, National Institutes of Health, Bethesda, MD, USA) to determine the average fiber diameter (Miranda et al. 2019).

The infrared spectra of the nanofibers were obtained with a Fourier transform medium infrared spectrometer (FTIR, Vertex 70, Bruker, Germany). The samples were analyzed using the attenuated reflectance (ATR) technique. For each spectrum, 32 consecutive assays were recorded at a resolution of 4 cm^{-1} in the spectral range of 4000 to 400 cm^{-1} (Miranda et al. 2019).

The effect of the essential oils on the thermal properties of PLA nanofibers was investigated with a differential exploratory calorimeter (DSC-60, Shimadzu, Japan). The analyses were performed under a nitrogen atmosphere at a flow rate of 50 ml min^{-1} using 4.07 ± 0.02 mg of sample. The samples were heated from 25 to 180 °C at a rate of 10 °C min^{-1}, and

they were subjected to two temperature cycles. The degree of crystallinity (Xc) was measured according to the equation of Auras et al. (2010).

The hydrophobic nature of the nanofibers was evaluated by measuring the contact angle of water using the sessile drop technique at room temperature. A goniometer (Kruss Drop Shape Analyzer, DSA25, Hamburg, Germany) equipped with a microcamera was used for the analysis, and the contact angle was obtained using the Advance software (Kruss, Hamburg, Germany). The nanofibers were cut and placed in a support, where a drop (10 µl) of deionized water was deposited in different places on the surface of each sample. Ten images of the drop were recorded immediately in triplicate (Lago et al. 2020).

Fungal cultures

The fungus species *Aspergillus ochraceus* (CCDCA10506) and *Aspergillus westerdijkiae* (CCDCA11469) were provided by the Collection of Microorganisms from the Mycotoxins and Food Mycology Laboratory of the Food Science Department at UFLA. These isolates were grown in Agar Malt Extract (MEA, HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated in BOD for 7 days at 25 °C. A 1% solution of Tween 80 (Synth, Diadema, SP, Brazil) in distilled water was added to the Petri dish of each fungal colony to obtain the spore solution. Spores were counted using a Neubauer Chamber (Sigma, São Paulo, Brazil, and the final concentration of the spore solution was adjusted to 10^6 spores ml^{-1} (Brandão et al. 2020).

Antifungal effect

The antifungal effect of nanofiber mats containing the essential oils was evaluated using the fumigation method (Guimarães et al. 2011; Hua et al. 2014). The experiment was accomplished using pure PLA nanofibers (negative control) and fibers incorporated with5, 10 and 20% of the essential oils from *O. basilicum* and *O. gratissimum* (treatments). All the analyses were performed in triplicate. The fumigation method was used to evaluate the growth of the fungus by adding 10 µl of the spore solution (10^6 spores ml^{-1}) to the scepter on the surface of the Yeast Extract-Sucrose-Agar culture medium (YES, HiMedia Laboratories Pvt. Ltd., Mumbai, India). Disks of nanofiber mats (4 cm in diameter) were glued to the Petri dish lid. The fungal control was prepared using only the spore solution of the fungal species under study. The plates were incubated in BOD at 25 °C for 10 days, and the diameter of the colony was

measured perpendicularly. The percentage of inhibition was calculated using the equation proposed by Brandão et al. (2020).

Antimicotoxygen effect

The antimicotoxygenic analysis of the nanofiber mats containing the essential oils was performed according to the method proposed by Passamani et al. (2014). The culture conditions were prepared in the same manner as that described by the fumigation method. After 10 days of incubation, three plugs were removed from the center, middle and edge of the colony and placed in a test tube containing 1 ml of HPLC grade methanol (Merck, Darmstadt, Germany) for the extraction of OTA. The extracts were homogenized on a vortex stirrer for 5 seconds, kept at rest for 60 minutes at room temperature and then filtered through polytetrafluoroethylene (PTFE; 0.22 μm, Millipore) membranes. After being filtered, they were analyzed by HPLC (Shimatzu, Kyoto, Japan). The conditions for the analysis of OTA were the following: two high pressure pumps (model SPD-M20A); degasser (DGU 20A3); interface (CBM-20A); automatic injector (SIL-10AF); fluorescence detector (RF-10 AXL); Agilent-Zorbax Eclipse XDB-C18 column (4.6 x 250 mm, 5 μm); Agilent-Zorbax Eclipse XDB-C18 4-Pack pre-column (4.6 x 12, 5 mm, 5 μm); excitation (332 nm) and emission (476 nm) wavelengths; flow rate of 0.8 ml min^{-1}, injection volumes of the samples and the standard were 20 μl; and elution was performed in an isocratic system of methanol:acetronitrile:water:acetic acid (5:35:29:1). Quantification of OTA was performed according to the procedure described by Passamani et al. (2014) using the external standardization method from the construction of an analytical curve. The analyses were performed in triplicate. The percentage of inhibition was calculated using the equation of Brandão et al. (2020).

Statistical analysis

The data were subjected to analysis of variance and the means were compared significantly according to the Tukey test ($p < 0.05$). A completely randomized design (DIC) was employed for statistical analysis using the Sisvar program (Ferreira, 2011).

Acknowledgements

This work was supported by the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brasil (CAPES) - Financial Code 001. The authors thank the funding agencies for the scholarships and financial support, the Central of Analysis and Chemical Prospecting of the Federal University of Lavras and Embrapa Instrumentation for supplying the equipment for analyses and the Medicinal Plants Garden of the Federal University of Lavras, located in the city of Lavras, MG, Brazil.

Conflict of interest

The authors declare that no conflict of interests exists.

Data availability

Study data are available from the corresponding author upon request.

References

Abd-Elsalam, K.A., Hashim, A.F., Alghuthaymi, M.A. and Said-Galiev, E. (2017) Nanobiotechnological strategies for toxigenic fungi and mycotoxin control. In *Food Preservation* Academic Press, 337-364.

Adams, R.P. (2017) *Identification of essential oils componentes by gas chromatography/ mass spectroscopy*, 4.1th ed. Carol Stream: Allured.

Agência Nacional de Vigilância Sanitária (ANVISA) (2010) *Métodos de Farmacognosia*. In: Mendes JCC, ed. Farmacopeia Brasileira. Brasília, Brasil: Fiocruz, 5th ed.; n. 1, 198-199. http://portal.anvisa.gov.br/documents/33832/260079/5%C2%AA+edi%C3%A7%C3%A3o+-+Volume+1/4c530 f86-fe83-4c4a-b907-6a96b 5c2d2fc.

Ahmed, A.H., Hussein, K. and Alsyari, A.H. (2017) Chemotaxonomy and Spectral Analysis (GC/MS and FT-IR) of Essential Oil Composition of Two *Ocimum basilicum* L. Varietiesv and their Morphological Characterization. *Jordan J Chem* **12**, 147-160.

Auras, R., Lim, L., Selke, S.E.M and Tsuji, H. (2010) *Poly (lactc acid): synthesis, structures, properties, processing and application.* Wiley: New Jersey.

Bacha, N., Atoui, A., Mathieu, F., Liboz, T. and Lebrihi, A. (2009) *Aspergillus westerdijkiae* polyketide synthase gene “aoks1” is involved in the biosynthesis of ochratoxin A. *Fungal Genet Biol* **46**, 77-84.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008) Biological effects of essential oils–a review. *Food Chem Toxicol* **46**, 446-475.

Bonan, R.F., Bonan, P.R., Batista, A.U., Sampaio, F.C., Albuquerque, A.J., Moraes, M.C., Mattoso, L.H.C., Glenn, G.M., Medeiros, E.S. and Oliveira, J.E. (2015) In vitro antimicrobial activity of solution blow spun poly (lactic acid)/polyvinylpyrrolidone nanofibers loaded with Copaiba (*Copaifera sp*.) oil. *Materials Science and Engineering: C* **48**, 372-377.

Bonan, R.F., Bonan, P.R., Batista, A.U., Perez, D.E., Castellano, L.R., Oliveira, J.E. and Medeiros, E.S. (2017) Poly (lactic acid)/poly (vinyl pyrrolidone) membranes produced by solution blow spinning: Structure, thermal, spectroscopic, and microbial barrier properties. *J Appl Polym Sci* **134**, 44802.

Brandão, R.M., Ferreira, V.R.F., Batista, L.R., Alves, E., Lira, N.D.A., Bellete, B.S., Scolforo, J.R.S. and Cardoso, M.G. (2020) Antifungal and antimycotoxigenic effect of the essential oil of *Eremanthus erythropappus* on three different *Aspergillus* species. *Flavour Frag J* **35**, 524-533.

Cam, M.E., Cesur, S., Taskin, T., Erdemir, G., Kuruca, D.S., Sahin, Y.M., Kabasakal, L. and Gunduz, O. (2019) Fabrication, characterization and fibroblast proliferative activity of electrospun Achillea lycaonica-loaded nanofibrous mats. *Eur Polym J* **120**, 109239.

Chen, H-C., Tsai, C-H., Yang, M-C (2011) Mechanical properties and biocompatibility of electrospun polylactide/poly(vinylidene fluoride) mats. *J Polym Res* **18**, 319-327.

El Khoury, A. and Atoui, A. (2010) Ochratoxin A: general overview and actual molecular status. *Toxins* **2**, 461-493.

Ferreira, D.F. (2011) Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia* **35**, 1039-1042.

Frisvad, J.C., Frank, J.M., Houbraken, J.A.M.P., Kuijpers, A.F. and Samson, R.A. (2004) New ochratoxin A producing species of *Aspergillus* section Circumdati. *Stud Mycol* **50**, 23-43.

Gobbo-Neto, L. and Lopes, N.P. (2007) Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Quim Nova* **30**, 374-381.

Guimarães, L.G.D.L., Cardoso, M.G., Sousa, P.E.D., Andrade, J.D. and Vieira, S.S. (2011) Atividades antioxidante e fungitóxica do óleo essencial de capim-limão e do citral. *Revista Ciência Agronômica* **42**, 464-472.

Hu Y., Zhang J., Kong W., Zhao G. and Yang M. (2017) Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*. *Food Chem* **220**, 1-8.

Hua, H., Xing, F., Selvaraj, J.N., Wang, Y., Zhao, Y., Zhou, L., Liu, X. and Liu, Y. (2014) Inhibitory effect of essential oils on Aspergillus ochraceus growth and ochratoxin A production. *PloS one* **9**, e108285.

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

Kumar, A., Mishra, P., Rodrigues, V., Baskaran, K., Verma, R. S., Padalia, R. C. and Sundaresan, V. (2019) Delineation of *Ocimum gratissimum* L. complex combining morphological, molecular and essential oils analysis. *Ind Crop Prod* **139**, 111536.

Lago, R.C., Oliveira, A.L.M., Dias, M.C., Carvalho, E.E.N., Tonoli, G.H.D. and Boas, E.V.D.B.V. (2020) Obtaining cellulosic nanofibrils from oat straw for biocomposite reinforcement: Mechanical and barrier properties. *Ind Crop Prod* **148**, 112264.

Liu, F., Saricaoglu, F.T., Avena-Bustillos, R.J., Bridges, D.F., Takeoka, G.R., Wu, V.C., Chiou, B-S., Wood, D.F., McHugh,, T.H. and Zhong, F. (2018a) Antimicrobial Carvacrol in Solution Blow-Spun Fish-Skin Gelatin Nanofibers. *J Food Sci* **83**, 984-991.

Liu, F., Saricaoglu, F.T., Avena-Bustillos, R.J., Bridges, D.F., Takeoka, G.R., Wu, V.C., Chiou, B-S., Wood, D.F., McHugh, T.H. and Zhong, F. (2018b) Preparation of fish skin gelatin-based nanofibers incorporating cinnamaldehyde by solution blow spinning. *Int J Mol Sci* **19**, 618.

Mahapatra, S.K., Chakraborty, S.P., Majumdar, S., Bag, B.G. and Roy, S. (2009) Eugenol protects nicotine-induced superoxide mediated oxidative damage in murine peritoneal macrophages in vitro. *Eur J Pharmacol* **623**, 132-140.

Miranda, C.A.S.F., Cardoso, M.G., Carvalho, M.L.M., Machado, S.M.F., Gomes, M.S., Andrade, J. and Teixeira, M.L. (2015) Allelopathic activity of medicinal plant essential oils on seed germination and vigor of lettuce achenes. *Semina: Ciências Agrárias* **36**, 1783-1798.

Miranda, K.W., Mattoso, L.H., Bresolin, J.D., Hubinger, S.Z., Medeiros, E.S. and Oliveira, J.E. (2019) Polystyrene bioactive nanofibers using orange oil as an ecofriendly solvent. *J Appl Polym Sci* **136**, 47337.

Moradkhannejhad, L., Abdouss, M., Nikfarjam, N., Shahriari, M.H. and Heidary, V. (2020) The effect of molecular weight and content of PEG on in vitro drug release of electrospun curcumin loaded PLA/PEG nanofibers. *J Drug Deliv Sci Technol* **56**, 101554.

Mori, C.L., Passos, N.A., Oliveira, J.E., Altoé, T.F., Mori, F.A., Mattoso, L.H.C., Scolforo, J.R. and Tonoli, G.H.D. (2015) Nanostructured polylactic acid/candeia essential oil mats obtained by electrospinning. *J Nanomater* **16**, 33.

Mota, I., Sánchez-Sánchez, J., Pedro, L.G. and Sousa, M. J. (2020) Composition variation of the essential oil from *Ocimum basilicum* L. cv. Genovese Gigante in response to Glomus intraradices and mild water stress at different stages of growth. *Biochem Syst Ecol* **90**, 104021.

Munhuweyi, K., Caleb, O.J., Lennox, C.L., Reenen, A.J.V. and Opara, U.L. (2017) In vitro and in vivo antifungal activity of chitosan-essential oils against pomegranate fruit pathogens. *Postharvest Biol Technol* **129**, 9-22.

Nepomuceno, N.C., Barbosa, M.A., Bonan, R.F., Oliveira, J.E., Sampaio, F.C. and Medeiros, E.S. (2018) Antimicrobial activity of PLA/PEG nanofibers containing terpinen-4-ol against *Aggregatibacter actinomycetemcomitans*. *J Appl Polym Sci* **135**, 45782.

Paranagama, P.A., Abeysekera, K.H.T., Abeywickrama, K. and Nugaliyadde, L. (2003) Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf.(lemongrass) against *Aspergillus flavus* Link. isolated from stored rice. *Lett Appl Microbiol* **37**, 86-90.

Parussolo, G., Oliveira, M.S., Garcia, M.V., Bernardi, A.O., Lemos, J.G., Stefanello, A., Mallmann, C. A. and Copetti, M.V. (2019). Ochratoxin A production by Aspergillus westerdijkiae in Italian-type salami. *Food Microbio*l **83**, 134-140.

Passamani, F.R.F., Hernandes, T., Lopes, N.A., Bastos, S.C., Santiago, W.D., Cardoso, M.D.G. and Batista, L.R. (2014) 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 Protect* **77**, 1947-1952.

Pereira, C.A.M. and Maia, J. F. (2007) Study of the antioxidant activity and essential oil from wild basil (*Ocimum gratissimum* L.) leaf. *Ciência e Tecnologia de Alimentos* **27**, 624-632.

Pimentel, F.A., Cardoso, M.G., Salgado, A.P.S., Aguiar, P.M., Silva, V.D.F., Morais, A.R.D. and Nelson, D.L. (2006) A convenient method for the determination of moisture in aromatic plants. *Quim Nova* **29**, 373-375.

Prakash, B., Kedia, A., Mishra, P. K. and Dubey, N. K. (2015) Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities–Potentials and challenges. *Food Control* **47**, 381-391.

Rahmani, M., Khani, M.M., Rabbani, S., Mashaghi, A., Noorizadeh, F., Faridi-Majidi, R. and Ghanbari, H. (2020) Development of poly (mannitol sebacate)/poly (lactic acid) nanofibrous scaffolds with potential applications in tissue engineering. *Mat Sci Eng C* **110**, 110626.

Samson, R.A., Hong, S.B. and Frisvad, J.C. (2006) Old and new concepts of species differentiation in *Aspergillus*. *Med Mycol* **44**, S133-S148.

Silva, T.H., Oliveira, J.E. and Medeiros, E.S. (2015) PVC micro and nanofibers produced via Solution Blow Spinning. *Polímeros* **25**, 229-235.

Sutaphanit, P. and Chitprasert, P. (2014) Optimisation of microencapsulation of holy basil essential oil in gelatina by response surface methodology. *Food Chem* **150**, 313-320.

Thipe, V.C., Bloebaum, P., Khoobchandani, M., Karikachery, A.R., Katti, K. K. and Katti, K.V. (2020) Green nanotechnology: nanoformulations against toxigenic fungi to limit mycotoxin production. In *Food Preservation* Academic Press, 337-364.

Toncheva, A., Paneva, D., Manolova, N. and Rashkov, I. (2011) Electrospun poly (L-lactide) membranes containing a single drug or multiple drug system for antimicrobial wound dressings. *Macromol Res* **19**, 1310-1319.

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

Van der Merwe, K.J., Steyn, P.S., Fourie, L., Scott, D.B. and Theron, J.J. (1965) Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. *Nature* **205**,1112–1113.

Vipotnik, Z., Rodríguez, A. and Rodrigues, P. (2017) *Aspergillus westerdijkiae* as a major ochratoxin A risk in dry-cured ham based-media. *Int J Food Microbiol* **241**, 244-251.

Wang, Y., Wang, L., Wu, F., Liu, F., Wang, Q., Zhang, X., Selvaraj, J.N., Zhao, Y., Xing, F., Yin, W-B. and Liu, Y. (2018) A consensus ochratoxin A biosynthetic pathway: Insights from the genome sequence of *Aspergillus ochraceus* and a comparative genomic analysis. *Appl Environ Microbiol* **84**, e01009-18.

Table 1 – Composition of volatile compounds in essential oils from *O. basilicum* and *O. gratissimum.*

Peak	Compound	RT (min)	*RI_{ref}	RI_{cal}	Area (%)	
					Ocimum basilicum	***Ocimum gratissimum***
1	α-Tujene	5.577	924	926	-	0.27
2	α-Pinene	5.794	932	934	1.03	-
3	Camphene	6.250	946	950	0.79	-
4	Sabinene	6.875	969	973	0.99	0.20
5	β-Pinene	7.043	974	979	1.96	-
6	Myrcene	7.361	988	991	1.49	-
7	Limonene	8.718	1024	1030	1.50	-
8	1.8-cineole	8.799	1026	1032	**23.62**	-
9	(Z) -β-Ocimene	8.883	1032	1035	-	**14.60**
10	(E) -β-Ocimene	9.332	1044	1047	-	0.54
11	*cis*-Sabinene hydrate	10.234	1065	1071	0.22	-
12	Terpinolene	10.818	1086	1087	0.59	-
13	Fenchone	10.915	1083	1090	1.72	-
14	Linalool	11.348	1095	1102	**26.89**	-
15	Camphor	13.270	1141	1147	**15.69**	-
16	Terpinen-4-ol	14.717	1174	1182	0.65	-
17	α-Terpineol	15.397	1186	1198	2.89	-
18	Eugenol	22.188	1356	1356	4.38	**79.04**
19	α-Copaene	22.990	1374	1374	0.16	-
20	β-Borbonene	23.319	1387	1381	0.14	-
21	(E) -β-Caryophylene	24.806	1417	1418	1.58	-
22	*trans*-α-Bergamotene	25.375	1432	1432	1.14	-
23	α-Humulene	26.285	1452	1454	0.52	-
24	Germacrene D	27.338	1484	1480	4.75	**5.04**
25	Bicyclogermacrene	27.937	1500	1495	0.54	-
26	α-Murulol	33.687	1640	1642	4.80	-
Composition						
Monoterpene hydrocarbons					8.35	15.60
Oxygenated monoterpenes					71.69	-
Sesquiterpene hydrocarbons					8.82	5.05
Oxygenated sesquiterpenes					4.80	-
Phenylpropanoids					4.38	79.04
Total identified					98.04	99.69

RT: retention time; RI: retention index. *Mass spectrum retention index library (Adams, 2017)

Table 2 – Thermal properties of pure PLA and PLA with essential oils from *O. basilicum* and *O. gratissimum*.

Sample	**T_g (°C)**	**T_c (°C)**	**T_m (°C)**	**ΔH_c (J g^{-1})**	**ΔH_m (J g^{-1})**	**X_c (%)**
PLA nonofibers	59	89	165	2	49	50
(PLA + 5wt% *O. basilicum* oil) nanofibers	58	80	164	5	44	42
(PLA + 10wt% *O. basilicum* oil) nanofibers	58	76	164	3	41	41
(PLA + 20wt% *O. basilicum* oil) nanofibers	57	72	164	1	39	40
(PLA + 5wt% *O. gratissimum* oil) nanofibers	57	82	161	4	53	53
(PLA + 10wt% *O. gratissimum* oil) nanofibers	53	80	161	4	44	43
(PLA + 20wt% *O. gratissimum* oil) nanofibers	53	74	163	3	40	40

Glass transition temperature (T_g), crystallization temperature (T_c), melting temperature (T_m), crystallization enthalpy (ΔHc), melting enthalpy (ΔHm) and degree of crystallinity (X_c).

ARTIGO 3 - Active packaging of poly(lactic acid) nanofibers and essential oils with antifungal action on table grapes

Artigo escrito de acordo com as normas da NBR 6022 da ABNT.

ABSTRACT

The table grape is a climacteric fruit that is very susceptible to fungal contamination, in addition to exhibiting an accelerated loss of quality during storage. The in vitro and in vivo antifungal and antiocratoxigenic effects of the essential oils from *Alpinia speciosa* and *Cymbopogon flexuosus* against *Aspergillus carbonarius* and *Aspergillus niger* were studied. The oils were encapsulated in poly(lactic acid) (PLA) nanofibers as a potential active packaging to be applied to control the degradation of grapes stored during the post-harvest period. The essential oils were extracted by hydrodistillation and characterized by GC-MS and GC-FID. The nanofibers were produced by the Solution Blow Spinning method from PLA containing the essential oils and characterized by scanning electron microscopy and thermogravimetric analysis. The in vitro antifungal and antimycotoxigenic properties were evaluated by the fumigation method. In the in vivo test, an active nanofiber package was created with the addition of essential oils to preserve the quality and safety of the table grape. Fungal proliferation and ochratoxin A synthesis decreased in the presence of the essential oils encapsulated in nanofibers. In addition, weight loss and color changes were controlled and the parameters of acidity, °Brix, softening and the texture of the grape were maintained. A very small mass loss by the nanofibers containing the essential oils was observed by thermogravimetric analysis, showing that the nanofiber was efficient in enabling the controlled release of volatile compounds present in essential oils. The quality and safety of table grapes were maintained for longer periods of storage in the presence of active packaging, so the incorporation of these oils in nanofibers can be a promising way to increase the shelf life of fruits.

Keywords: *Alpinia speciosa*. *Cymbopogon flexuosus*. solution blow spinning. ochratoxin A. *Aspergillus carbonarius. Aspergillus niger*.

1 INTRODUCTION

Active packaging made from biodegradable polymeric nanofibers has attracted considerable attention in recent years because of its versatility and low production cost (NAZARI et al., 2019). Polymeric nanofibers incorporated with natural products with recognized antimicrobial action are gaining importance as a potential alternative to extend the shelf life of different food products such as cheese, meat and fruits and to reduce the risk of pathogens (LIAKOS et al., 2014). Synthetic fungicides are used to solve the problems caused by fungi. However, the adverse effects of these chemicals on human health, potential residues and toxicity, in addition to the development of resistant strains, has stimulated the search for natural antimicrobial agents as an alternative (TEJESWINI et al., 2014). Essential oils are gaining importance as a sustainable strategy for the post-harvest preservation of fruits, they can show good efficacy and little or no toxicity, they produce small amounts of residues, and they are recognized as GRAS (generally recognized as safe) by the Food and Drug Administration. In addition, they have antibacterial (CAMARGO et al., 2020; NOGUEIRA et al., 2021), antifungal (BRANDÃO et al., 2020; BRANDÃO et al., 2021) and antioxidant (FERREIRA et al., 2019) properties, which indicates that they can be used in the food industry. In this sense, the nanofibers produced by the Solution Blow Spinning (SBS) process have a larger contact area between the active packaging and the food, and the volatilities of the oils are greatly reduced (BONAN et al., 2017).

The Niagara Rosada grape (*Vitis labrusca*) is a non-climacteric fruit (SONKER et al., 2014; MAIA; RITSCHEL; LAZZAROTTO, 2018) with major challenges in maintaining its postharvest quality because of microbiological contamination during its handling, storage and marketing (AN et al., 2019). Microbial contamination leads to quality losses and a frequent occurrence of spoilage during prolonged storage of table grapes.

The microbiological contamination of table grapes can be caused by different microorganisms such as bacteria (*Gluconobacter spp.* and *Acetobacter spp*.) and fungi (*Aspergillus carbonarius* and *Aspergillus niger*). The latter induce uncontrolled infections that lead to the development of aerial mycelium that spreads quickly throughout the fruits.

Fungal contamination impacts both physicochemical parameters of fruits such as weight, texture and color and the formation of secondary metabolites such as mycotoxins that are harmful to human health (FREIRE et al., 2017). Ochratoxin A is the main secondary metabolite produced by these fungi that contaminate grapes and wines. This mycotoxin has

been shown to be neurotoxic, genotoxic, carcinogenic, mutagenic, teratogenic and immunosuppressive (FREIRE et al., 2017; FREIRE et al., 2018).

The use of synthetic fungicides is associated with the development of resistant strains and the population's concern with environmental pollution and human health. On the other hand, the development of active packaging using natural antifungal products capable of extending the shelf life of table grapes is of great importance.

Therefore, the aim of the present study was to investigate antifungal and antiocratoxigenic effect *in vitro* and *in vivo* (grape) of the essential oils from *Alpinia speciosa* and *Cymbopogon flexuosus* encapsulated in poly(lactic acid) nanofibers against *Aspergillus carbonarius* and *Aspergillus niger*, as potential active packaging to be applied in the control of degradation of Niagara Rosada table grapes.

2 MATERIAL AND METHODS

2.1 Extraction and chemical characterization of the essential oils

The essential oils from *A. speciosa* and *C. flexuosus* were extracted by the hydrodistillation process during 2 hours using a modified Clevenger apparatus according to the Anvisa method (2010). Plant material (leaves) was collected at the Medicinal Plants Garden of the Federal University of Lavras, Lavras, Minas Gerais, Brazil (21°13'S, 44°58'W).

The chemical characterization of the essential oils was performed by gas chromatography coupled to a mass spectrometer (CG-MS, Shimadzu Corporation, model QP2010 Plus, Kyoto, Japan) according to the method of Adams (2017). The analysis was performed under the following conditions: fused silica capillary column phase bonded to DB5 (30 m X 0.25 mm i.d., 0.25 µm film thickness); He 5.0 (White Martins, Rio de Janeiro, Brazil) as the carrier gas with a flow rate of 1 mL/min. The oven temperature program began at 60 °C for 1 min, increasing to 240 °C at a rate of 3 °C/min, followed by a temperature gradient from 240 °C to 300 °C at 10 °C min^{-1}. The final temperature was held for 7 min. Injector and detector temperatures were 220 ºC and 240 ºC, respectively. The sample was diluted in hexane (1%) (Sigma-Aldrich®, St. Louis, MO, USA), and the injected volume was 0.5 µL with a split ratio of 1:100. The conditions of the mass spectrometer were as follows: ionization potential, 70 eV; ion source temperature, 200 °C; scan speed, 1000 Da/sec; scan interval 0.50 fragments/sec; and fragments were detected in the range of 45 to 500 Da. Data for chemical constituents were acquired using LabSolutions LC/GC Workstation 2.72. The equation of Van den Dool & Kratz

(1963) was used to calculate the retention index (RI = 100n + 100 [(tR (i) - tR (n))/(tR (n + 1)n), where tR (i), tR (n) and tR (n + 1) correspond to the retention times of the tested compounds and standards, respectively. The standards used were the homologous series of n-alkanes (nC8-nC18). The compounds were identified. by comparing the retention indices with those in the literature (ADAMS, 2017), and the mass spectra of the essential oil constituents with similarity greater than 95% were compared with those of the FFNSC 1.2, NIST 107 and NIST 21 mass spectral libraries. The quantification of the essential oil constituents was calculated by the method of normalization of areas (%) after separation on a gas chromatograph with a flame ionization detector (DIC) (Shimadzu GC - 2010, Kyoto, Japan). The conditions used were the same as those used for GC-MS, with the exception of the detector temperature, which was 300 ºC.

2.2 Rotating Fiber Method

The nanofiber mats were produced according to the method of Bonan et al. (2017) and Nepomuceno et al. (2018) with some modifications. The polylactic acid solution (PLA, Mw – 66,000 g/mol; Nature Works, Minneapolis, MN, USA) (12% w/v) was prepared in chloroform (Synth, Diadema, SP, Brazil). The essential oils from *A. speciosa* and *C. flexuosus* were added to the PLA solution in proportions of 5, 10 and 20% (v/w) relative to the total weight of polymer in solution. The nanofiber mats were obtained using a solution blow spinning apparatus (SBS) consisting of a compressed air source (Chiaperini MC 12 BPV 150 L 2HP, Brazil) at 140 kPa, an injection pump (NE-300; New Era Pump Systems, Syringe Pump, New York, USA) equipped with a glass syringe (FLURAN F-5500-A; Ismatec, Wertheim, Germany) injected the polymer solution at a rate of 6 mL/h through a concentric nozzle with a protrusion length of 4 mm. The fibers were collected 20 cm apart in a rotating collector lined with aluminum foil at room temperature (28 – 30 °C) and at a relative humidity of 30 – 42%. Fibers were produced without the addition of essential oil (negative control).

2.3 Characterization of nanofibers

2.3.1 Morphological study

The micrographs of the nanofibers were obtained with a scanning electron microscope (MEV, JSM-6510, Jeol). The samples were overlaid with gold using a metallizer (Bal-tec SCD

050, Balzers AG, Liechtenstein). Fiber diameters were measured using image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA). The mean values and standard deviations of the diameters were obtained from at least 100 randomly selected fibers (MIRANDA et al., 2019).

2.3.2 Volatility of the essential oils

The rate of release of pure essential oils and incorporated into nanofibers was measured by thermogravimetric analysis (TGA) using a thermogravimetric analyzer (TGA-Q500, TA instruments, New Castle, TE). Approximately 5-10 mg of the samples were kept at 25 °C in a nitrogen atmosphere at a flow rate of 50 mL/min for a period of 180 minutes. The weight loss of each sample was evaluated at a constant temperature and as a function of time (MIRANDA et al., 2019).

2.4 Fungal strains and spore suspension

The toxigenic filamentous fungi *Aspergillus carbonarius* (CCDCA10507) and *Aspergillus niger* (CCDCA10443) were obtained from the Microorganisms Culture Collection of the Laboratory of Mycotoxins and Food Mycology of the Federal University of Lavras. The isolate of each fungus was activated in a Petri dish containing Agar Malt Extract culture medium (MEA, HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated for 7 days at 25 °C. After the incubation, a spore solution was prepared in sterile distilled water with 1% Tween 80 (Synth, Diadema, SP, Brazil). Spores were counted using a Neubauer Chamber (Sigma, São Paulo, Brazil), and the concentration of the suspension was adjusted to 10^6 spores/mL (BRANDÃO et al., 2020).

2.5 *In vitro* antifungal activity

2.5.1 Fungus growth rate

The antifungal property of nanofiber mats containing 5, 10 and 20% essential oils was tested on the growth of *A. carbonarius* and *A. niger* fungi by the fumigation method (GUIMARÃES et al., 2011; HUA et al., 2014). Circular nanofiber mats (4 cm in diameter) were attached to the upper part of the Petri dish (9 cm in diameter). A negative control was prepared

with nanofiber mats without the addition of essential oil, and a culture control was prepared with the inoculum alone. Ten microliters of the spore suspension (10^6 spores/mL) was added to the center of the plate containing 20 mL of Czapeck Yeast Agar culture medium (CYA, HiMedia Laboratories Pvt. Ltd., Mumbai, India). Tests were performed in triplicate. Plates were incubated for 10 days at 25 °C, and fungus growth was evaluated by measuring two perpendicular diameters (in centimeters) of the colony.

2.5.2 Ochratoxin A production

The antimycotoxigenic activity of nanofiber mats containing essential oil against *A. carbonarius* and *A. niger* was evaluated at concentrations of 5, 10 and 20% for each treatment under which there was growth of the fungus on the tenth day of incubation. Culture conditions were prepared as described in the previous section (2.5.1). OTA extraction was performed with 1 mL of HPLC grade methanol (Merck, Darmstadt, Germany) in test tubes containing three plugs removed from the inner area, the middle and the edge of the colony, according to the method of Passsamani et al. (2014). The extract was stirred for 5 seconds and kept at room temperature for 60 minutes. The extracts were filtered through polytetrafluoroethylene membranes (PTFE; 0.22 μm, Millipore). The quantification of OTA was performed according to the method of Passamani et al. (2014). Twenty microliters of the filtered extract were injected into an HPLC (Shimatzu, Kyoto, Japan) equipped with two SPD-M20A high-pressure pumps, a DGU 20A3 degasser, model CBM-20A interface, a SIL-10AF automatic injetor and a RF-10 AXL fluorescence detector. The column used for the separation was the Agilent-Zorbax Eclipse XDB-C18 column (4.6 x 250 mm, 5 μm) connected to an Agilent-Zorbax Eclipse XDB-C18 4-Pack pre-column (4.6 x 12.5 mm, 5 μm). Elution was achieved in an isocratic methanol:acetronitrile:water:acetic acid (5:35:29:1) system. OTA was monitored by the excitation at 332 nm and emission at 476 nm at a flow rate of 0.8 mL/min. The external standardization method was used to quantify OTA through the construction of an analytical curve obtained by linear regression using the commercial standard (Sigma-Aldrich®, São Paulo, Brazil). The coefficient of determination (R^2) was 0.9999, and the limits of detection (LD) and quantification (LQ) were 0.0004 and 0.0016 μg/g, respectively. All the OTA samples and standard solutions were analyzed in triplicate.

2.6 *In vivo* tests

2.6.1 Preparing the grapes

The bunches of grapes (*Vitis labrusca* Niagara) were purchased in the Lavras, Minas Gerais commerce and taken to the Laboratory of Mycotoxins and Food Mycology at the Federal University of Lavras. The grapes were uniform in size and color and had no physical injuries or infections. The grapes were treated with 70% alcohol for 1 minute, 1% sodium hypochlorite for 30 seconds and three washes with sterile distilled water.

2.6.2 Packaging preparation and antifungal activity

The packages were prepared according to the parameters of the previous section (2.2), where the nanofibers were spun directly on the inside of the lid of polyethylene terephthalate packages for grapes. Packages were prepared containing the treatments (PLA nanofibers with 20% essential oils from *C. flexuosus* and *A. speciosa* (v/w) relative to the total polymer weight), negative control packaging (pure PLA nanofibers) and fungal control packaging (containing only inoculum). The bunches of grapes were placed in the packages after disinfection and contaminated with 1000 µL of spore solution (10^6 spores/mL) of *A. carbonarius* and *A. niger* using a spray bottle. Packages were incubated at 25 °C for 10 days and 20 days. The analyses were performed in triplicate, and the results were expressed as percentage of fruits infected by filamentous fungi and calculated by the following equation: percent incidence of fungus = (number of contaminated fruits/total fruits) x 100.

2.6.3 Percentage of weight loss, size and firmness

Weight loss was determined by weighing the bunches of grapes before and after the incubation period and calculated by the equation: Weight loss (%) = (Initial weight – Final weight)/Initial weight x 100. The diameters of 20 grapes were measured in two orthogonal directions with a digital caliper (Electronic Digital Caliper). Fruit firmness was determined in the equatorial region of 20 berries using a texture analyzer (TA.XT Plus Texture Analyzer) with a penetration distance of 6 mm, a probe of 3 mm in diameter, pre-test speed of 1 .5 mm/s, test speed of 2 mm/s and post-test speed of 1 mm/s. The firmness in Newtons is defined as the

maximum force required the probe to penetrate the fruit. Analyses were performed before and after the incubation period (FREIRE et al., 2017; JAHANI; PIRA; AMINIFARD, 2020).

2.6.4 pH, total soluble solids (TSS), total titratable acidity (TTA) and maturity index

The pH was determined in the crushed fruits using a digital potentiometer (Quimis – Q400MT). The total soluble solids content (TSS) was measured in the crushed fruits with a digital refractometer (Atago PAL-1) and expressed in °Brix. The total titratable acidity (TTA) was determined in 5 g of crushed fruits transferred to a 100 mL volumetric flask and completed with distilled water. This solution was titrated with 0.1 mol/L NaOH, and the TTA was expressed in g of tartaric acid per 100 g of grapes. The fruit maturity index was calculated using the ratio of total soluble solids divided by titratable acidity (TSS/TTA). Analyses were performed before and after the incubation period (ZENEBON; PASCUET; PAULO, 2008).

2.6.5 Color

Color parameters (L*, a*, b*, Chroma and Hue) were recorded on a colorimeter (Konica Minolta, CR 10, Osaka, Japan) and determined in 20 berries per repetition (n=60). Analyses of color parameters were performed before and after the incubation period.

2.7 Statistical analysis

Statistical analyses were performed using a completely randomized design (CRD) employing the Sisvar program (FERREIRA, 2011). The results were subjected to analysis of variance, and the means were compared by the Tukey test ($p<0.05$).

3 RESULTS AND DISCUSSION

3.1 Chemical characterization of essential oils and nanofibers

The results of the GC-MS analysis of the essential oils from *A. speciosa* and *C. flexuosus* are shown in Figure 1A.

The essential oils from *Cymbopogon* species contain neral, geranial, geraniol, citronellol, citronellal and geranyl acetate as the most abundant constituents (BHATNAGAR,

2018; DEVI et al., 2020). In this study, the essential oil from *C. flexuosus* contained a larger amount of oxygenated monoterpenes (99.11%). Among the three constituents obtained, citral was the main component, being formed by the two isomers, neral (36.10%) and geranial (61.57%). These results are in agreement with previous data such as those of Bhatnagar (2018) and Haque, Remadevi and Naebe (2018). Oladeji et al. (2019) and Haque, Remadevi and Naebe (2018), studying the chemical composition of various oils of the *Cymbopogon* genus, observed that it varied according to the plant species and also with the geographical location, culture and agricultural treatment, but the citral constituent is usually predominant and of great importance in providing a pleasant lemon fragrance and plant flavor.

Victório (2011) and Xuan et al. (2019) studied the essential oils from species of the genus *Alpinia* and observed the predominance of monoterpenes, highlighting the constituents γ-terpinene, limonene, 1,8-cineol, camphene, sabinene, terpinen-4-ol, α-pinene, (Z)-β-ocimene and α-terpineol. In the present work, 99.32% of the constituents of the essential oil from *A. speciosa* were identified, corresponding to 19 compounds, among which, terpinen-4-ol (20.23%), sabinene (20.18%), 1,8-cineole (16.69) and γ-terpinene (11.03%) predominated. These results corroborate the studies by Victório (2011) and Xuan et al. (2019).

The morphology represented by the electromicrographs and the distribution of diameters of the nanofibers from pure PLA and PLA solution containing different essential oils in different proportions are shown in Figure 1B.

It can be seen from the morphology that the nanofibers presented regularity even with the addition of essential oils. Continuous, smooth, uniform and homogeneous nanofibers were formed in all the tested polymeric compositions. Furthermore, there was no presence of beads and films, indicating that the conditions of the rotation method for formation of nanofibers were adequate. The concentration of the polymer solution and the spinning variables (injection rate and spinning pressure) used were efficient for the spinning process and the formation of nanofibers (OLIVEIRA et al., 2011).

The mean diameter of the nanofibers containing essential oils was greater than that of the pure PLA nanofibers. According to studies by Medeiros et al. (2009) and Parize et al. (2016ab), the formation of fibers in the SBS process can be affected by viscosity and surface tension, as in the case of diameters that underwent changes in the presence of essential oil. Increasing the viscosity of a polymer solution could lead to a smaller elongation of the injected jet, reducing its path and, consequently producing a greater variability in the diameter of nanofibers and larger fibers (ALTAN; AYTAC; UYAR, 2018; OLIVEIRA et al., 2013ab).

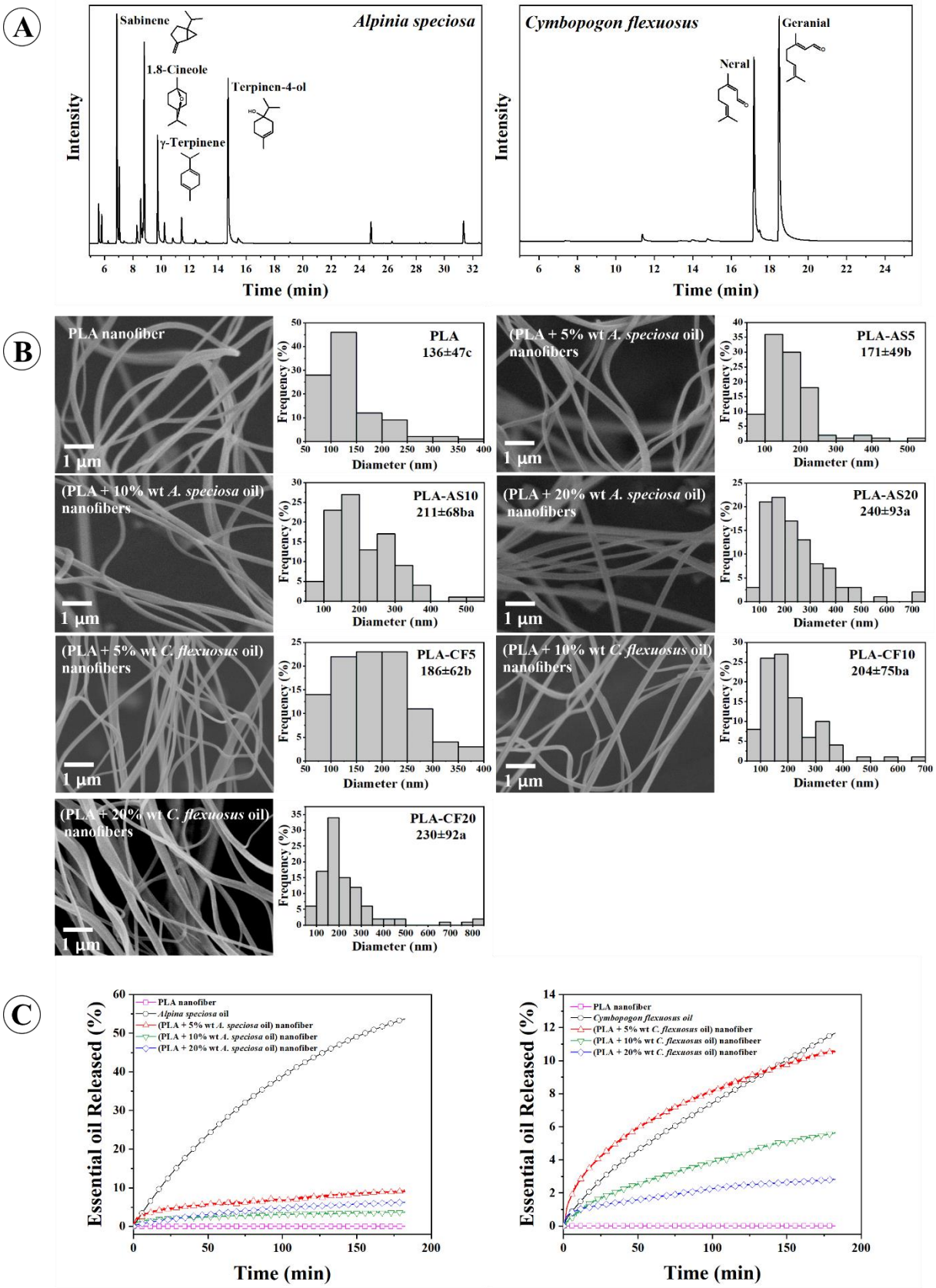


Figure 1 – Chromatograms of essential oils obtained by GC-MS (A), electronmicrographs and diameter distribution (B) of nanofibers prepared from pure PLA and PLA solution with the addition of the essential oils from *A. speciosa* and *C. flexuosus* and evaporation rate of essential oils (C).

The release rate of essential oils obtained by thermogravimetric analysis at 25 °C for a period of 180 minutes is shown in Figure 1C.

The evaporation rate of the essential oil from *A. speciosa* was the highest at 25 °C. On the other hand, the mass loss from *C. flexuosus* was lower. These results can be explained by the composition of the essential oils and by the intermolecular interactions that the molecules have with each other. The oil from *A. speciosa* is made up of more than 50% constituents belonging to the hydrocarbon class, and these nonpolar molecules interact by relatively weak forces of attraction called Van der Waals forces. Consequently, this interaction is more easily ruptured, which explains the higher evaporation rate for the essential oil from *A. speciosa*. The essential oil from *C. flexuosus* is mostly composed of citral (neral and geranial), compounds of the aldehyde class that possess dipole-dipole interactions. Such interactions are stronger than those of Van der Waals, which explains the lower rate of evaporation in relation to the essential oil from *A. speciosa*.

When essential oils were encapsulated in nanofibers, a very small loss of mass occurred, which demonstrated the efficiency of the nanofiber in enabling the controlled release of bioactive compounds such as essential oils. The encapsulation of essential oils in PLA nanofibers can control and prolong the release of active substances and increase the time of action of these bioactive compounds. When pure essential oil is used, it can degrade more easily in the environment and quickly lose its biological effect.

3.2 Effect on fungus growth rate and antiocratoxigenic activity *in vitro*

The antifungal effect of *in vitro* treatments with nanofiber mats containing essential oils from *C. flexuosus* and *A. speciosa* on the growth of *A. carbonarius* and *A. niger* fungi is shown in Figures 2A and 2B. A significant dose-dependent inhibitory activity on the mycelial growth of fungi was observed for the two oils. The antifungal action of the essential oils can be related to the synergism between the constituents because of the complex chemical composition and also to the presence of some organic functions in the compounds. The lipophilic character of the hydrocarbon skeleton and the hydrophilic character of the functional groups are of great importance for the antifungal activity of essential oils.

In general, the essential oil from *C. flexuosus* was the most efficient in inhibiting the growth of the fungi under study, completely inhibiting the growth of *A. carbonarius* at the concentrations of 10 and 20% and the growth of *A. niger* at the 20% concentration. The greater effect can be related to the composition of the oil, because 95% of its composition corresponds

to citral. The presence of a carbonyl function in its chemical structure seems to increase the antifungal property of this terpenoid. The electronegative nature of the aldehyde moiety conjugated to a double bond explains its biological activity, suggesting that greater electronegativity results in an increase in the activity (DORMAN; DEANS, 2004; SAAD; MULLER; LOBSTEIN, 2013).

Functional groups present in essential oils constituents can interfere with biological processes that involve electron transfer and react with vital secondary metabolites such as proteins and nucleic acids. Consequently, this action causes inhibition of the growth of microorganisms (DORMAN; DEANS, 2000). Essential oils can attenuate microbial growth through specific mechanisms such as those mentioned by Nazzaro et al. (2017), who reported the action of therapeutic targets on the main structural elements for the construction of the fungal cell wall, such as glucan and chitin. Later, Brandão et al. (2020) revealed the ability of essential oils to damage fungal membranes and cause deleterious effects on the morphology of fungi of the genus *Aspergillus*. The authors observed changes in the uniformity of hyphae, loss of integrity and no formation of conidia after treatment with essential oil, concluding that essential oils are capable of acting in the ergosterol biosynthetic pathway, leading to a decrease in the quantity of this sterol present in the fungal cell membrane. The reduction of ergosterol in fungal membranes can lead to the osmotic and metabolic instability of the biological cell, compromising fungus development (NAZZARO et al., 2017). In addition to the nhibition of ergosterol synthesis, studies by Hu et al. (2017) suggested that antifungal activity is also related to mitochondrial disruption, inhibition of mitochondrial ATPase, malate dehydrogenase and succinate dehydrogenase enzymes, which disrupts the normal metabolic process and, consequently, inhibits its development.

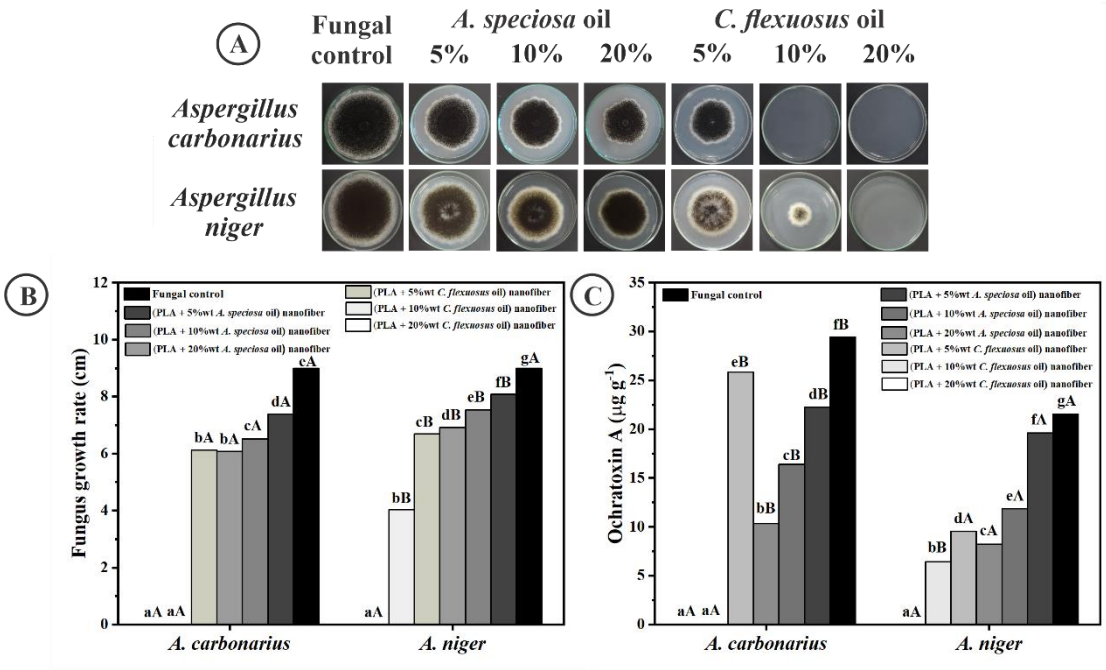


Figure 2 – Effect of nanofiber mats containing different concentrations of the essential oils from *A. speciosa* and *C. flexuosus* on the growth and production of ochratoxin A by the fungi *A. carbonarius* and *A. niger*. Representative images of the antifungal effect in culture medium after 10 days (A), fungal growth inhibition (cm) (B) and ochratoxin A production inhibition (C). Different lowercase and uppercase letters indicate significant differences between the treatments and the two fungi, respectively, according to the Tukey test ($p<0.05$).

The production of ochratoxin A by *A. carbonarius* and *A. niger* treated with nanofiber mats containing the essential oils was significantly ($p<0.05$) lower than that of the fungal control (Figure 2C). The amount of ochratoxin A produced by *A. carbonarius* decreased by 12.09 to 100% and by 24.44 to 64.78% for treatments with different concentrations of essential oils from *C. flexuosus* and *A. speciosa*, respectively. The ochratoxin A index in *A. niger* decreased in a proportion of 55.65 to 100% and from 8.93 to 61.86% in the treatments with the tested concentrations of essential oils from *C. flexuosus* and *A. speciosa*, respectively. Generally, *A. carbonarius* produces a greater quantity of ochratoxin A than *A. niger*, as was observed in this study.

Santiago et al. (2018) explained that the greater production of ochratoxin A by *A. carbonarius* is due to the behavior of each isolate. The authors evaluated the essential oils from *Melaleuca alternifolia, Melaleuca quinquenervia* and *Backousia citriodora* and obtained terpinen-4-ol, α-pinene and citral as the principal constituents. They observed that the synthesis of ochratoxin A decreased in a proportion that varied from 38.66 to 75.93% and from 17.94 to 71.79% for the fungi *A. carbonarius* and *A. niger*, respectively, in the presence of the essential oils. These results partially corroborate those obtained in this work because the majority

composition is similar to that found for the essential oils in the present study. Studies by Dammak et al. (2019) suggested that the principal terpenoids and phenylpropanoids or even the synergism of all the constituents present in essential oils can be considered responsible for the antiocratoxigenic activity by inhibiting the production of toxins by fungi. The authors reported that the decrease in the production of this mycotoxin is related to the inhibition of fungal growth and spore germination.

Some studies highlight hypotheses regarding the mode of action of essential oil constituents in suppressing the proliferation of toxins, such as the research by Lappa et al. (2017) and Wang et al. (2018). The authors suggested that the mechanism of action of essential oils on mycotoxin production is related to changes in the transcriptional level and changes in genes that regulate the biosynthesis of ochratoxin A.

3.3 *In vivo* tests

3.3.1 Antifungal activity

A remarkable efficacy against *A. carbonarius* and *A. niger* was observed for the essential oils from *C. flexuosus* and *A. speciosa* encapsulated in PLA nanofibers as a packaging for grapes (Figure 3). Among the packages tested, the one that contained the essential oil from *C. flexuosus* exhibited a better inhibitory potency than that of the essential oil from *A. speciosa***.**

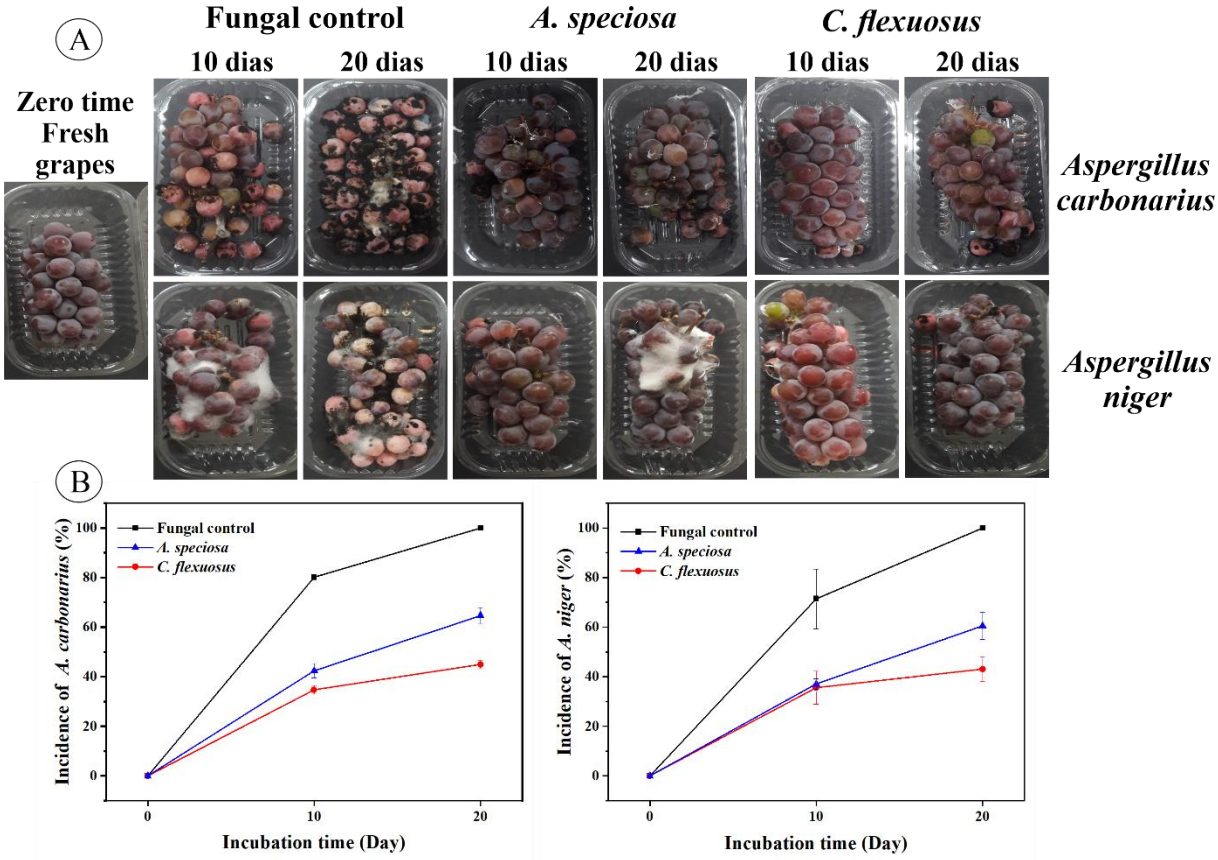


Figure 3 – Control of the incidence of *A. carbonarius* and *A. niger* fungi in grapes by packaging containing PLA nanofibers with essential oils from *A. speciosa* and *C. flexuosus* for periods of 10 and 20 days.

The incidence of contamination by *A. carbonarius* decreased to 45 and 65% and that of *A. niger* decreased to 43 and 61%, respectively, relative to the control after 20 days of incubation in the presence of PLA nanofibers containing the essential oils from *C. flexuosus* and *A. speciosa*. The control grape clusters, on the other hand, contained rot and apparent mycelial biomass in all the fruits. The inhibition of mycelial flora growth in the grapes treated with the active packaging demonstrated the antifungal activity through the fumigation process of the PLA nanofiber packaging containing the bioactive compounds under study.

Sonker et al. (2014) studied essential oils that contain compounds of the aldol class and reported that they possessed strong antifungal activity, as was seen in this study, where the essential oil from *C. flexuosus* had the greatest effect. This antifungal action can be attributed to the high percentage of citral in its composition. For Bang et al. (2000) and Sonker et al. (2014), the hydrophobic portions of these constituents interact with the cytoplasmic membrane through the lipid acyl chains, causing disintegration of the outer membrane and increasing the permeability of the cytoplasmic membrane to adenosine triphosphate of fungal cells, consequently, leading to their death.

3.3.2 Percentage of weight loss, size and firmness

The essential oils encapsulated in PLA nanofibers and their contributions to weight loss, size and firmness of grapes infected with *A. carbonarius* and *A. niger* were significant at the 5% probability level (Table 1).

Table 1 – Effect of packaging containing PLA nanofibers with essential oils on weight loss, size and firmness in grapes contaminated with *A. carbonarius* and *A. niger* fungi.

Fungus	Incubation time	Treatment	Loss of weight (%)	Diameter (mm)	Firmness (Newton)
Aspergillus carbonarius	0 days	(PLA + 20wt% *A. speciosa* oil) nanofibers			
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	0.00±0.00^{a}	17.78±0.27^{a}	4.00±0.21^{a}
		Fungus control			
	10 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	6.14±0.51^{bc}	16.94±0.32^{bc}	2.80±0.78^{bcd}
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	4.00±0.16^{b}	17.43±0.20^{ab}	3.18±0.19^{abc}
		Fungus control	7.23±0.81^{cd}	14.77±0.29^{f}	1.80±0.24^{de}
	20 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	10.84±0.41^{ef}	16.01±0.24^{de}	2.56±0.31^{bcd}
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	8.84±0.17^{de}	16.25±0.09^{cd}	3.07±0.13^{abc}
		Fungus control	14.00±0.19^{h}	10.58±0.32^{g}	1.20±0.13^{ef}
Aspergillus niger	0 days	(PLA + 20wt% *A. speciosa* oil) nanofibers			
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	0.00±0.00^{a}	17.78±0.27^{a}	4.00±0.21^{a}
		Fungus control			
	10 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	5.57±0.93^{bc}	17.07±0.28^{abc}	3.60±0.51^{abc}
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	5.17±0.33^{bc}	17.29±0.15^{ab}	3.71±0.19^{ab}
		Fungus control	10.38±0.41^{ef}	14.71±0.39^{f}	1.71±0.58^{def}
	20 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	13.25±0.78^{gh}	14.75±0.18^{f}	2.45±0.16^{cd}
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	11.20±0.47^{fg}	15.20±0.20^{ef}	3.03±0.15^{abc}
		Fungus control	25.83±1.51^{i}	10.75±0.13^{g}	0.56±0.05^{f}

In each column, different letters indicate a significant difference between treatments at 5% probability levels.

Weight loss relative to the fungal control was controlled in the treatments with essential oils even after 20 days of incubation. The greatest weight loss of the grapes was observed in the fungal control (7.23 and 14.00% with *A. carbonarius* and 10.38 and 25.83% with *A. niger*) after 10 and 20 days of incubation, respectively. The lowest weight loss of the fruits was observed in the treatment with the essential oil from *C. flexuosus* (4.00 and 8.84% with *A. carbonarius* and 5.17% and 11.20% with *A. niger*) after 10 and 20 days of incubation. In general, the weight loss in fruits treated with the essential oils from *C. flexuosus* and *A. speciosa* was considerably lower than that of the fungal control fruits. Research by Jahani, Pira and Aminifard (2020) and by Valverde et al. (2005) indicated that weight loss is one of the essential physiological parameters for assessing the shelf life of grapes, and it is related to the susceptibility of the fruit to fungal decomposition. The authors, using different essential oils, also revealed the benefits of these natural antifungal agents in decreasing the dehydration process and the resulting weight loss in grapes and pomegranates. Parallel to weight loss, there is a decrease in the size of the fruit due to dehydration of the grapes. In the present work, the reduction in size was observed in the fungal control, especially after 20 days of incubation. However, treatments with essential oils encapsulated in PLA nanofibers minimized this problem, keeping the size of the grapes close to that of time zero, that is, before incubation with *A. carbonarius* and *A. niger.*

The grape softening process was greatly accelerated in the presence of *A. carbonarius* and *A. niger* as storage increased, with values of 1.20±0.13 and 0.56±0.05, respectively, after 20 days of incubation. However, the addition of essential oils from *C. flexuosus* and *A. speciosa* in PLA nanofibers significantly delayed the loss of firmness of the grapes. Texture is another important attribute that is demanded by consumers and influences the sale of fresh fruit (JAHANI; PIRA; AMINIFARD, 2020; SUN et al., 2014). The softening of grapes is usually related to dehydration and starch and cell wall degradation in the post-harvest period (SOUZA; LIMA; VIEITEIS, 2010).

Even though the grape is a non-climacteric fruit, fungi were able to significantly reduce firmness during storage, resulting in loss of quality, shorter shelf life and lower market value. *Aspergillus* species are recognized as producers of the pectinase enzyme, which confirms the ability of these filamentous fungi to degrade pectin and cause softening of the fruit (FREIRE et al., 2017). However, the loss of firmness of the berries was significantly lower in the samples with active PLA nanofiber packaging, benefiting and retaining the firmness of the grapes. Deterioration of fruits and, consequently, the loss of texture can be reduced in the post-harvest treatment with essential oils encapsulated in PLA nanofibrs because these compounds have

positive effects on the control of toxigenic fungi. According to Sun et al. (2014), active packaging with essential oils probably prevents the loss of water from the fruit because of the lipophilic property of the constituents of the essential oils, in addition to controlling the proliferation of fungi in the fruit, thereby reducing the pectin degradation caused by the microorganism.

3.3.3 pH, total soluble solids (TSS), total titratable acidity (TTA) and maturity index

Other important parameters that determine the quality of table grapes are pH, SST, TTA and the maturity index, factors that affect the fruit's flavor and, consequently, consumer acceptance (SONEGO et al., 2002; VALVERDE et al., 2005). The fungi *A. carbonarius* and *A. niger* significantly changed these parameters after incubation when compared to time zero, when the grapes were fresh. On the other hand, there was a significant difference ($p<0.05$) in the values of pH, total soluble solids (TSS), total titratable acidity (TTA) and maturity index for the grapes in the presence of the essential oils encapsulated in PLA nanofibers when they were incubated with *A. carbonarius* and *A. niger* (Table 2). The values of these variables remained somewhat close to those at time zero, that is, these treatments had the effect of maintaining the pH, SST, TTA and maturity index close to those observed in the berries before incubation with the fungi. We can say that active packaging managed to preserve the characteristics and, consequently, the quality of the fruits.

Table 2 – Effect of packaging containing PLA nanofibers with essential oils on pH, total soluble solids (TSS), total titratable acidity (TTA) and maturity index in grapes contaminated with *A. carbonarius* and *A. niger* fungi.

Fungus	Incubation time	Treatment	SST (°Brix)	TTA[a]	pH	Maturity index
Aspergillus carbonarius	0 days	(PLA + 20wt% *A. speciosa* oil) nanofibers				
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	14.83±0.62^{a}	0.69±0.02^{a}	3.56±0.11^{a}	21.64±0.61^{a}
		Fungus control				
	10 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	13.57±0.49^{abc}	0.75±0.05^{a}	3.51±0.15^{ab}	18.11±1.11^{b}
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	14.05±0.50^{ab}	0.78±0.00^{a}	3.49±0.07^{abc}	18.07±0.62^{b}
		Fungus control	12.25±0.37^{cd}	0.87±0.05^{b}	3.24±0.03^{abcde}	14.09±1.14^{d}
	20 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	13.07±0.38^{bc}	1.26±0.19^{c}	2.95±0.12^{e}	10.66±1.77^{ef}
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	13.03±0.56^{bc}	1.04±0.12^{bc}	3.04±0.05^{cde}	12.80±1.66^{de}
		Fungus control	10.87±0.42^{de}	1.84±0.01^{d}	2.25±0.02^{f}	5.92±0.21^{g}
Aspergillus niger	0 days	(PLA + 20wt% *A. speciosa* oil) nanofibers				
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	14.83±0.62^{a}	0.69±0.02^{a}	3.56±0.11^{a}	21.64±0.61^{a}
		Fungus control				
	10 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	12.87±0.49^{bc}	0.77±0.02^{a}	3.38±0.04^{abcde}	16.63±0.39^{bc}
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	13.13±0.44^{abc}	0.71±0.01^{a}	3.43±0.10^{abcd}	18.38±0.66^{b}
		Fungus control	10.90±0.27^{de}	0.91±0.04^{b}	3.11±0.05^{abcde}	12.01±0.82^{def}
	20 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	11.93±0.18^{cd}	1.25±0.03^{c}	3.03±0.06^{de}	9.53±0.37^{f}
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	12.10±0.47^{cd}	1.16±0.03^{c}	3.10±0.01^{bcde}	10.44±0.64^{ef}
		Fungus control	9.17±0.91^{e}	1.82±0.03^{d}	2.41±0.40^{f}	5.05±0.57^{g}

[a]grams of tartaric acid per 100 g of grape. In each column, different letters indicate a significant difference between treatments at 5% probability levels.

3.3.4 Color

The color attributes of the fungal control were drastically affected in the presence of *A. carbonarius* and *A. niger*. The atributes increased after 10 and 20 days of storage, corresponding to a pink to white color, which indicated that the berries were rotting (Table 3). However, these

parameters did not chenge significantly with the treatments; that is, the color parameters of the grapes that had been treated with active packages containing essential oils remained close to the values for the berries before incubation with the fungi (healthy grapes).

Table 3 – Effect of packaging containing PLA nanofibers with essential oils on color parameters in grapes contaminated with *A. carbonarius* and *A. niger* fungi.

Fungus	Incubation time	Treatment	L*	a*	b*	Chroma	Hue
Aspergillus carbonarius	**0 days**	(PLA + 20wt% *A. speciosa* oil) nanofibers					
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	29.86±0.29	5.84±0.53	5.66±0.18	23±0.51	43.83±1.69
		Fungus control					
	10 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	30.79±2.54	7.99±2.46	7.91±0.05	11.75±1.89	46.59±6.62
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	27.42±1.72	5.19±0.23	7.85±0.18	9.69±0.17	52.28±2.98
		Fungus control	34.44±0.18	10.22±0.22	12.18±0.20	16.42±0.25	50.03±0.66
	20 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	26.68±1.77	7.49±2.18	10.49±0.60	13.33±0.56	55.75±8.18
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	26.35±0.22	6.71±0.62	10.20±0.76	12.35±0.96	56.82±1.13
		Fungus control	38.87±0.92	18.28±0.15	17.24±0.23	25.40±0.19	43.93±0.30
Aspergillus niger	**0 days**	(PLA + 20wt% *A. speciosa* oil) nanofibers					
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	29.86±0.29	5.84±0.53	5.66±0.18	8.23±0.51	43.83±1.69
		Fungus control					
	10 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	29.56±0.11	6.02±0.34	8.04±0.41	11.17±10.25	53.78±1.85
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	29.46±0.65	7.57±0.53	7.95±0.41	11.05±0.65	46.16±0.77
		Fungus control	34.52±0.36	10.55±0.93	12.58±0.29	16.88±0.76	51.07±2.12
	20 days	(PLA + 20wt% *A. speciosa* oil) nanofibers	30.80±0.49	10.97±0.19	11.93±029	16.46±0.33	47.97±0.21
		(PLA + 20wt% *C. flexuosus* oil) nanofibers	28.01±2.66	7.43±0.80	11.14±0.76	13.66±0.31	55.47±4.03
		Fungus control	41.11±1.19	19.05±2.09	18.21±0.66	26.66±2.05	44.32±2.57

L*: degree of brightness; a*: degree of variation between red and green; b*: degree of variation between blue and yellow.

Valverde et al. (2005) mentioned that color is also a very important attribute required by the consumer, and it is often one of the main parameters for acceptance because it is visible to the naked eye and conveys the sensation of healthy fruit with a beautiful appearance. Although the grape is a climacteric fruit, there was an acceleration of the color change in the present work after incubation with fungi (fungal control). However, there was a delay in the rate of color change in the treatments with nanofibers incorporated with essential oils from *C. flexuosus* and *A. speciosa*, and the grapes maintained a healthy appearance. According to Valverde et al. (2005), the retention of color might be related to the control of weight loss of the berries, which corroborates the results obtained in this work where the treatments controlled both the weight loss and the change in color.

Consumers choose products that do not utilize chemical fungicides as a means of preservation, and they increasingly demand safe food, but the incidence of pathogens and diseases transmitted by them are higher (VALVERDE et al., 2005). In addition, post-harvest losses of agricultural products are caused by fungi and other microorganisms that cause spoilage. In this scenario, several studies have concentrated on the improvement of processing, preservation, distribution and marketing in order to provide consumers with high quality and safe fresh fruit.

No research has reported the control of the growth of *A. carbonarius* and *A. niger* in food using essential oils encapsulated in PLA nanofibers as packaging for grapes with the use of in vivo tests. In the present study, strong inhibitory effects on the incidence of fungi in postharvest grapes, a positive effect on storage time, a decrease in the incidence of decay, maintainance of the physicochemical profiles and quality parameters were observed with the use of active packaging. In addition, the use of the fumigation process is considered ideal for the control of the food deterioration caused by fungi because it does not leave residues of essential oils, as mentioned by Wang et al (2015). Therefore, the use of these active packages in the fumigation process during post-harvest food storage is very promising.

4 CONCLUSION

In both *in vitro* and *in vivo* tests, PLA nanofiber packaging containing essential oils from *C. flexuosus* and *A. speciosa* was efficient in protecting the fruits from contamination by the fungi *A. carbonarius* and *A. niger* during storage, as well as in reducing ochratoxin A, a potential carcinogen. The active packaging maintained the quality properties of the grape longer than the fungal control, controlling weight loss, delaying softening and maintaining texture. It

also kept the pH, SST, TTA, maturity index and color parameters close to those of fresh grapes (zero incubation time); that is, the treatments showed benefits in relation to the parameters by not showing many changes when compared with fresh grapes and preserving the physicochemical properties and quality of the grapes. Consequently, these packaging materials can be potential substitutes for synthetic fungicides in the preservation of bunches of table grapes during storage. They can increase shelf life and preserve fruit quality for a longer period of time through the controlled release of bioactive compounds, in addition to contributing to consumer safety.

ACKNOWLEDGEMENTS

The authors thank the funding agencies for the scholarships and financial support, the Central of Analysis and Chemical Prospecting of the Federal University of Lavras and Embrapa Instrumentation for supplying the equipment for analyses and the Medicinal Plants Garden of the Federal University of Lavras, located in the city of Lavras, MG, Brazil. This work was supported by the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Financial Code 001.

REFERENCES

ADAMS, R. P. **Identification of essential oils componentes by gas chromatography/ mass spectroscopy**. 4.1th ed. Carol Stream: Allured, 804 p. 2017.

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA). **Métodos de Farmacognosia**. In: Mendes JCC, ed. Farmacopeia Brasileira. Brasília, Brasil: Fiocruz, 5th ed.; n. 1, p.198-199, 2010. http://portal.anvisa.gov.br/documents/33832/260079/5%C2%AA+edi%C3%A7%C3%A3o+-+Volume+1/4c530 f86-fe83-4c4a-b907-6a96b 5c2d2fc.

ALTAN, A.; AYTAC, Z.; UYAR, T. Carvacrol loaded electrospun fibrous films from zein and poly(lactic acid) for active food packaging. **Food Hydrocolloids**, v. 81, p. 48-59, 2018.

AN, P. et al. α-terpineol and terpene-4-ol, the critical components of tea tree oil, exert antifungal activities in vitro and in vivo against *Aspergillus niger* in grapes by inducing morphous damage and metabolic changes of fungus. **Food Control**, v. 98, p. 42-53, 2019.

BANG, K. H. et al. Inhibition of fungal cell wall synthesizing enzymes by trans-cinnamaldehyde. **Bioscience, Biotechnology, and Biochemistry**, v. 64, n. 5, p. 1061-1063, 2000.

BHATNAGAR, A. Composition variation of essential oil of *Cymbopogon* spp. growing in Garhwal region of Uttarakhand, India. **Journal of Applied and Natural Science**, v. 10, n. 1, p. 363-366, 2018.

BONAN, R. F. et al. Poly(lactic acid)/poly(vinyl pyrrolidone) membranes produced by solution blow spinning: Structure, thermal, spectroscopic, and microbial barrier properties. **Journal of Applied Polymer Science**, v. 134, n. 19, p. 44802, 2017.

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 activity and the effect of the essential oil of *Lippia origanoides* Kunth on *Aspergillus* mycotoxins production. **Australian Journal of Crop Science**, v. 15, n. 7, p. 1005-1012, 2021.

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.

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.

DEVI, M. A. et al. Chemical compositions and insecticidal efficacies of four aromatic essential oils on rice weevil *Sitophilus oryzae* L. **International Journal of Tropical Insect Science**, v. 40, p. 549-559, 2020.

DORMAN, H. D.; DEANS, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. **Journal of Applied Microbiology**, v. 88, n. 2, p. 308-316, 2000.

DORMAN, H. D.; DEANS, S. G. Chemical composition, antimicrobial and in vitro antioxidant properties of *Monarda citriodora* var. citriodora, *Myristica fragrans*, *Origanum vulgare* ssp. hirtum, *Pelargonium* sp. and *Thymus zygis* oils. **Journal of Essential Oil Research**, v. 16, n. 2, p. 145-150, 2004.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, v. 35, p. 1039-1042, 2011.

FERREIRA, V. R. F. 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 Journal of Chemistry**, v. 43, n. 20, p. 7653-7662, 2019.

FREIRE, L. et al. Influence of physical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines. **International Journal of Food Microbiology**, v. 241, p. 181-190, 2017.

FREIRE, L. et al. A quantitative study on growth variability and production of ochratoxin A and its derivatives by *A. carbonarius* and *A. niger* in grape-based medium. **Scientific Reports**, v. 8, n. 1, p. 14573, 2018.

GUIMARÃES, L. G. D. L. et al. Atividades antioxidante e fungitóxica do óleo essencial de capim-limão e do citral. **Revista Ciência Agronômica**, v. 42, p. 464-472, 2011.

HAQUE, A. N. M. A.; REMADEVI, R.; NAEBE, M. Lemongrass (*Cymbopogon*): a review on its structure, properties, applications and recent developments. **Cellulose**, v. 25, n. 10, p. 5455-5477, 2018.

HU, Y. et al. Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*. **Food Chemistry**, v. 220, p. 1-8, 2017.

HUA, H. et al. Inhibitory effect of essential oils on *Aspergillus ochraceus* growth and ochratoxin A production. **PloS one**, v. 9, n. 9, p. e108285, 2014.

JAHANI, M.; PIRA, M.; AMINIFARD, M. H. Antifungal effects of essential oils against *Aspergillus niger* in vitro and in vivo on pomegranate (*Punica granatum*) fruits. **Scientia Horticulturae**, v. 264, p. 109188, 2020.

LAPPA, I. K. et al. In vitro evaluation of essential oils against *Aspergillus carbonarius* isolates and their effects on Ochratoxin A related gene expression in synthetic grape medium. **Food Control**, v. 73, p. 71-80, 2017.

LIAKOS, I. et al. All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties. **International Journal of Pharmaceutics**, v. 463, n. 2, 137-145, 2014.

MAIA, J. D. G.; RITSCHEL, P.; LAZZAROTTO, J. J. A viticultura de mesa do Brasil: produção para o mercado nacional e internacional. **Embrapa Uva e Vinho-Artigo em periódico indexado (ALICE)**, v. 9, p. 1-9, 2018.

MEDEIROS, E. S. et al. (2009). Solution blow spinning: A new method to produce micro-and nanofibers from polymer solutions. *Journal of Applied Polymer Science*, *113*, 2322-2330.

MIRANDA, K. W. et al. Polystyrene bioactive nanofibers using orange oil as an ecofriendly solvent. **Journal of Applied Polymer Science**, v. 136, n. 15, p. 47337, 2019.

NAZARI, M. et al. Cinnamon nanophytosomes embedded electrospun nanofiber: Its effects on microbial quality and shelf-life of shrimp as a novel packaging. **Food Packaging and Shelf Life**, v. 21, p. 100349, 2019.

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

NEPOMUCENO, N. C. et al. Antimicrobial activity of PLA/PEG nanofibers containing terpinen-4-ol against *Aggregatibacter actinomycetemcomitans*. **Journal of Applied Polymer Science**, v. 135, n. 6, p. 45782, 2018.

NOGUEIRA, J. O. E. et al. Mechanism of action of various terpenes and phenylpropanoids against *Escherichia coli* and *Staphylococcus aureus*. **FEMS Microbiology Letters**, v. 368, n. 9, p. fnab052, 2021.

OLADEJI, O. S. et al. Phytochemistry and pharmacological activities of *Cymbopogon citratus*: A review. **Scientific African**, v. 6, p. e00137, 2019.

OLIVEIRA, J. E. et al. Nano and submicrometric fibers of poly (D, L-lactide) obtained by solution blow spinning: Process and solution variables. **Journal of Applied Polymer Science**, v. 122, n. 5, p. 3396-3405, 2011.

OLIVEIRA, J. E. et al. Properties of poly (lactic acid) and poly (ethylene oxide) solvent polymer mixtures and nanofibers made by solution blow spinning. **Journal of Applied Polymer Science**, v. 129, n. 6, p. 3672-3681, 2013a.

OLIVEIRA, J. E. et al. Structural and morphological characterization of micro and nanofibers produced by electrospinning and solution blow spinning: a comparative study. Advances in Materials Science and Engineering, v. 2013, p. 1-14, 2013b.

PARIZE, D. D. S. et al. Solution blow spinning: parameters optimization and effects on the properties of nanofibers from poly (lactic acid)/dimethyl carbonate solutions. **Journal of Materials Science**, v. 51, n.9, p. 4627-4638, 2016a.

PARIZE, D. D. S. et al. Poly (lactic acid) fibers obtained by solution blow spinning: Effect of a greener solvent on the fiber diameter. **Journal of Applied Polymer Science**, v. 133, p. 1-10, 2016b.

PASSAMANI, F. R. F. 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. **Journal of Food Protection**, v. 77, n. 11, p. 1947-1952, 2014.

SAAD, N. Y.; MULLER, C. D.; LOBSTEIN, A. Major bioactivities and mechanism of action of essential oils and their componentes. **Flavour and Fragance Journal**, v. 28, n. 5, p. 269-279, 2013.

SANTIAGO, J. A. et al. Effect of the essential oils from *Melaleuca alternifolia*, *Melaleuca quinquenervia* and *Backhousia citriodora* on the synthesis of ochratoxin A by *Aspergillus niger* and *Aspergillus carbonarius* isolated from tropical wine grapes. **Journal of Food Science and Technology**, v. 55, n. 1, p. 418-423, 2018.

SONEGO, L. et al. Factors affecting taste scores of early season seedless table grape cv. Mystery and Prime. **Journal of Agricultural and Food Chemistry**, v. 50, n. 3, p. 544-548, 2002.

SONKER, N. et al. Assessment of *Cymbopogon citratus* (DC.) stapf essential oil as herbal preservatives based on antifungal, antiaflatoxin, and antiochratoxin activities and in vivo efficacy during storage. **Journal of Food Science**, v. 79, n. 4, p. M628-M634, 2014.

SOUZA, A. V. D.; LIMA, G. P. P.; VIEITES, R. L. Avaliação nutricional de diferentes variedades de uva (Vitis sp). **Naturalia**, v. 33, p. 100-109, 2010.

SUN, X. et al. Effects of chitosan-essential oil coatings on safety and quality of fresh blueberries. **Journal of Food Science**, v. 79, n. 5, p. M955-M960, 2014.

TEJESWINI, M. G. et al. Antifungal activity of essential oils and their combinations in *in vitro* and *in vivo* conditions. **Archives of Phytopathology and Plant Protection**, v. 47, n. 5, p. 564-570, 2014.

VALVERDE, J. M. et al. Improvement of table grapes quality and safety by the combination of modified atmosphere packaging (MAP) and eugenol, menthol, or thymol. **Journal of Agricultural and Food Chemistry**, v. 53, n. 19, p. 7458-7464, 2005.

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

VICTÓRIO, C. P. Therapeutic value of the genus *Alpinia*, Zingiberaceae. **Revista Brasileira de Farmacognosia**, v. 21, p. 194-201, 2011.

XUAN, T. et al. Allelopathic plants: 26. *Alpinia zerumbet* (Pers.) BL Burtt & RM Sm.(Zingiberaceae). **Allelopathy Journal**, v. 48, p. 1-14, 2019.

WANG, Y. et al. A consensus ochratoxin A biosynthetic pathway: Insights from the genome sequence of *Aspergillus ochraceus* and a comparative genomic analysis. **Applied and Environmental Microbiology**, v. 84, n. 19, p. e01009-18, 2018.

WANG, Y. et al. Inhibitory effect of nerol against *Aspergillus niger* on grapes through a membrane lesion mechanism. **Food Control**, v. 55, p. 54-61, 2015

ZENEBON, O.; PASCUET, N. S.; PAULO, T. **Métodos físico-químicos para análise de alimentos**. (4 th ed.). São Paulo: Instituto Adolfo Lutz, 2008.

ARTIGO 4 - *In vitro* and *in vivo* efficacy of poly(lactic acid) nanofiber packaging containing essential oils from *Ocimum basilicum* L. and *Ocimum gratissimum* L. against *Aspergillus carbonarius* and *Aspergillus niger* in table grapes

Artigo escrito de acordo com as normas da NBR 6022 da ABNT.

ABSTRACT

Apergillus carbonarius and *Aspergillus niger* are the principal fungi that attack table grapes, and they are responsible for producing and contaminating these fruits with ochratoxin A. Active packages of essential oils from *Ocimum gratissimum* L. and *Ocimum basilicum* L. encapsulated in poly(lactic acid) (PLA) nanofibers were produced, the antifungal and antiocratoxigenic activities against *A. carbonarius* and *A. niger* were evaluated *in vitro* and *in vivo*, and the effect of these packages on the quality of table grapes was determined. Essential oils were extracted by hydrodistillation, identified and quantified by GC-MS and GC-FID. The Solution Blow Spinning technique was used to produce PLA nanofibers containing the essential oils. The nanofibers were characterized by Scanning Electron Microscopy and Thermogravimetric Analysis. The fumigation method was used to evaluate the *in vitro* antifungal and antiochratogenic action. An in vivo test was performed to evaluate the effect of active packaging containing the nanofibers on the physicochemical parameters of the grape and on the control of fungal proliferation. Fungal contamination and ochratoxin A production were significantly controlled by PLA nanofibers containing the essential oils. In the in vivo test, the active packaging effectively inhibited the growth of *A. carbonarius* and *A. niger*, and the physicochemical parameters of the grapes were preserved. The active PLA nanofiber packaging containing the essential oils from *Ocimum basilicum* and *Ocimum gratissimum* controlled the proliferation of fungi, as well as maintaining the physicochemical characteristics of the grapes, preserving the quality and the shelf life of the fruit.

Keywords: *Ocimum basilicum*. *Ocimum gratissimum*. ochratoxin A. antifungal activity. antiocratoxigenic activity.

1 INTRODUCTION

One of the food products of great nutritional value and economic importance is the table grape. However, 30-40% of this fruit results in economic losses for the food industry every year as it is very susceptible to fungal contamination as a result of inadequate post-harvest handling and the lack of adequate methods to prevent deterioration and senescence (JIANG et al., 2014; LAPPA et al., 2018). Grapes are hostage to fungal diseases from the Nigri section, which lead to fruit loss and are a major obstacle to storage. The main species responsible for contaminating table grapes are *Apergillus carbonarius* and *Aspergillus niger* (FREIRE et al., 2018ab). These species cause a loss in the quality of fresh grapes because they cause weight loss, fading of the color, acceleration of the softening and reduction of the shelf life of the fruit (KONG et al., 2019). In addition to spoilage, *Aspergillus* species pose a danger by producing a mycotoxin called ochratoxin A (OTA) (FREIRE et al., 2018ab). Exposure to OTA through food consumption poses a risk to human health, as this mycotoxin has been classified as a possible human carcinogen (group 2B) by the International Cancer Agency (IARC, 1993). Therefore, research for safe, effective and economical agents to control the growth of *A. carbonarius* and *A. niger* in postharvest grapes and the production of OTA are needed.

Traditional methods for controlling fungal contamination utilize physical preservation and the use of chemical fungicides. However, there are problems with these treatments because they can harm the grape berries, leave residues with an unpleasant taste and toxicity, present undesirable biological effects on human health, in addition to contributing to the development of fungi resistant to fungicides. Research by Li et al. (2017) focused on alternative means to control post-harvest degradation of table grapes as well as other agricultural commodities to reduce the use of chemical fungicides because of growing consumer concern with environmental and health issues.

Essential oils are considered to be effective antimicrobial agents against different spoilage fungi. They have a broad-spectrum fungitoxicity, they are biodegradable, agreeable to humans and the environment, and recognized as safe (GRAS) by the Food and Drug Administration (FDA) (AN et al., 2019; ZHANG et al., 2019). Some constituents of essential oils, such as linalool, thymol, eugenol, carvone, cinnamaldehyde, carvacrol, citral and limonene, are accepted by the European Commission as food flavorings. Thus, they are considered to be alternative agents for inhibiting fungal growth, for use as food preservatives and for the control of post-harvest degradation of agricultural products stored for long periods of time (AN et al., 2019; MOGOSANU et al., 2017). The encapsulation of essential oils in

biodegradable and biocompatible polymer nanofibers are great alternatives for bioactive packaging in the control of mycotoxin-producing fungi, as well as being a practical approach to increase stability and achieve controlled and sustained release of essential oils.

The Solution Blow Spinning (SBS) technique produces nanofibers loaded with active components at room temperature. Because of their advantages of large surface area, high encapsulation efficiency, large scale production, higher spinning rates, reduced costs and uniform morphology, SBS nanofibers have received substantial attention as food packaging materials (BILBAO-SAINZ et al., 2014; BONAN et al., 2017). Poly(lactic acid) (PLA) is one of the most promising biodegradable polymers because of its mechanical characteristics, thermoplastic processability and biological properties, in addition to its biocompatibility, biodegradability, non-toxicity and facile thermal decomposition by hydrolysis of the ester bond. It does not require enzymes to catalyze this hydrolysis. It can be used to encapsulate hydrophobic compounds, and it has a great potential for the improvement of the effectiveness of controlled distribution of active compounds in food systems (CHEN; TSAI; YANG, 2011; GARLOTTA, 2001; GUPTA; REVAGADE; HILBORN, 2007). In this work, the antifungal and antiocratoxigenic activity of essential oils from *Ocimum gratissimum* L. and *Ocimum basilicum* L. encapsulated in PLA nanofibers against *Aspergillus carbonarius* and *Aspergillus niger* were evaluated *in vitro* and *in vivo*, and the effect of this bioactive packaging on the quality of table grapes was determined.

2 MATERIAL AND METHODS

2.1 Plant material and essential oil extraction

The essential oils from *Ocimum gratissimum* L. and *Ocimum basilicum* L. were extracted from fresh leaves collected in the Medicinal Plants Garden of the Federal University of Lavras (21°13'S, 44°58'W) on a mild day and without precipitation. The essential oil was obtained by hydrodistillation using a modified Clevenger apparatus for a period of 2 hours (ANVISA, 2010). The hydrolate was centrifuged (Fanem Baby I Model 206 BL, São Paulo, Brazil) at 965.36*g* for 15 minutes, and the essential oil was collected with a Pasteur micropipette and stored in amber bottles under refrigeration.

2.2 Analysis of GC-MS and GC-FID

GC-MS analysis of essential oils was performed on a Shimadzu Corporation model QP2010 Plus gas chromatograph (Kyoto, Japan) equipped with a fused silica capillary column with a DB5 bound phase (30 m X 0.25 mm id; film thickness, 0.25 μm) coupled to a mass detector. The sample was diluted in hexane (1%) (Sigma-Aldrich®, St. Louis, MO, USA) and 0.5 μL was injected in 1:100 split mode. Helio 5.0 (White Martins, Rio de Janeiro, Brazil) was used as the carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature was programmed at 60 °C for 1 min, and the temperature was raised to 240 °C at a rate of 3 °C min^{-1}. followed by a temperature gradient from 240 °C to 300 °C at 10 °C min^{-1}, where it was held for 7 min. The injector and detector temperatures were 220 ºC and 240 ºC, respectively. The temperature of the ion source was 200 °C, and the ionization potential was 70 eV. The scan speed was 1000 Da sec+ with a range of 0.50 fragments sec^{-1}, and fragments were detected in the range of 45 to 500 Da. GC-FID analysis (Shimadzu GC - 2010, Kyoto, Japan) was performed under the same conditions as GC-MS, but the detector temperature was 300 °C (ADAMS, 2017). Data processing was performed using LabSolutions LC/GC Workstation 2.72 software. The retention indices of the compounds were determined using the Van den Dool and Kratz equation (1963) and the homologous series of n-alkanes (nC8-nC18) as standards. The retention indices were compared with those of the literature (ADAMS, 2017), and the mass spectra of essential oil constituents with a similarity greater than 95% were compared with those of the FFNSC 1.2, NIST 107 and NIST 21 mass spectra libraries. The constituents were quantified using the area normalization method (%) based on automatically integrated peak areas of the GC-FID signal.

2.3 Polymer solution preparation and nanofiber fabrication

Polylactic acid (PLA, MW 66,000 g mol^{-1}; Nature Works, Cargill Dow LLC, Minneapolis, USA) was dissolved in chloroform (Synth, Diadema, SP, Brazil) at a concentration of 12% (w/v) at room temperature. After the PLA was fully dissolved, the essential oils were added in proportions of 5, 10 and 20% (v/w) relative to the total polymer weight.

The nanofiber fabrication procedure was performed using the Solution Blow Spinning (SBS) technique. The experimental conditions consisted of a needle connected to a concentric nozzle with a protrusion of 4 mm, a source of compressed air (Chiaperini MC 12 BPV 150 L 2HP, Brazil) at 140 kPa, an injection pump (NE-300; New Era Pump Systems, Syringe Pump,

New York, USA) with a glass syringe (FLURAN F-5500-A; Ismatec, Wertheim, Germany) containing the polymer solution. A flow rate of 6 mL h^{-1} was used. The working distance between the tip of the needle and the rotating collector covered with aluminum foil was adjusted to 20 cm. Fibers were also produced without the addition of essential oil (negative control) (BONAN et al., 2017; NEPOMUCENO et al., 2018).

2.4 Scanning electron microscopy

The diameter and morphology of the nanofibers were investigated using a scanning electron microscope (MEV, JSM-6510, Jeol). The surface of the samples was coated with gold using a sprayer (Bal-tec SCD 050, Balzers AG, Liechtenstein). The average fiber diameter was determined using imaging software (Image J, National Institutes of Health, Bethesda, MD, USA). One hundred nanofibers were used to obtain the mean value and the standard deviation (MIRANDA et al., 2020).

2.5 Release rate of essential oils

A thermogravimetric analyzer (TGA-Q500, TA instruments, New Castle, TE) was used to evaluate the evaporation rate of pure essential oils incorporated into PLA nanofibers. Approximately 5-10 mg of sample was used under the following experimental conditions: constant temperature of 25 °C for a period of 180 minutes, and the nitrogen flow rate was 50 mL min^{-1} (MIRANDA et al., 2019).

2.6 *In vitro* antifungal activity

The *in vitro* antifungal test against *A. carbonarius* (CCDCA10507) and *A. niger* (CCDCA10443) using nanofibers containing 5, 10 and 20% of essential oils from *O. basilicum* L. and *O. gratissimum* L. was accomplished by the fumigation method according to the methods of Guimarães et al. (2011) and Hua et al. (2014), with some modifications. The cultures were provided by the Microorganisms Culture Collection of the Mycotoxins and Food Mycology Laboratory at the Federal University of Lavras. Nanofibers without the addition of essential oil were used as a negative control, and a positive control was prepared with the inoculum alone. Nanofibers (4 cm in diameter) and ten microliters of the spore suspension (10^6 spores mL^{-1}) prepared according to the method of Brandão et al. (2020) were added to the center of the plate

containing 20 mL of Czapeck Yeast Agar culture medium (CYA, HiMedia Laboratories Pvt. Ltd., Mumbai, India). Plates were incubated for 10 days at 25 °C, the fungus growth was evaluated, and the percentage of inhibition was calculated according to the method of Brandão et al. (2020). Tests were performed in triplicate.

2.7 *In vitro* ochratoxin A test

The *in vitro* ochratoxin A (OTA) test was performed against A. *carbonarius* and *A. niger* with nanofiber mats containing the essential oils in proportions of 5, 10 and 20%. Culture conditions were prepared as described in the previous section (2.6). On the tenth day of incubation, the antiocratoxigenic effect of the nanofibers was evaluated, and the OTA was extracted with 1 mL of HPLC grade methanol (Merck, Darmstadt, Germany) in test tubes containing three plugs, according to the method of Passsamani et al. (2014). The solution was stirred for 5 seconds and kept at room temperature for 60 minutes. The extracts were filtered through polytetrafluoroethylene membranes (PTFE; 0.22 µm, Millipore). OTA was quantified in an HPLC (Shimatzu, Kyoto, Japan). The analysis was performed under the following conditions: Agilent-Zorbax Eclipse XDB-C18 column (4.6 x 250 mm, 5 µm) connected to an Agilent-Zorbax Eclipse XDB-C18 4-Pack (4.6 x 12) pre-column, 5 mm, 5 µm); two model SPD-M20A high pressure pumps; a DGU 20A3 degasser; a CBM-20A model interface; a SIL-10AF automatic injector and an RF-10 AXL fluorescence detector. Twenty microliters of the filtered sample injected into the chromatographic system was eluted in an isocratic methanol:acetronitrile:water:acetic acid (5:35:29:1) system with a flow rate of 0.8 mL min^{-1}. OTA was monitored from the excitation at 332 nm and emission at 476 nm. The external standardization method was used to quantify OTA, and an analytical curve obtained by linear regression was constructed using the commercial standard (Sigma-Aldrich®, São Paulo, Brazil). The coefficient of determination (R^2) was 0.9999, and the Limits of detection (LD) and quantification (LQ) were 0.0004 and 0.0016 µg g^{-1}, respectively. All OTA samples and standard solutions were analyzed in triplicate. The percentage of OTA inhibition was calculated according to the method of Brandão et al. (2020).

2.8 Niagara Grape Samples

The grapes (*Vitis labrusca* 'Niagara') were collected in the Lavras commerce and transported to the Laboratory of Mycotoxins and Food Mycology of the Federal University of

Lavras. The grapes were uniform in size and color, being free from physical injuries or infections. The bunches of grapes underwent a disinfection process using 70% alcohol for 1 minute and 1% sodium hypochlorite for 30 seconds. They were rinsed three times with sterile distilled water to remove any alcohol and hypochlorite residues.

2.9 Preparation of packages

The nanofibers were spun by the Solution Blow Spinning (SBS) process according to section 2.3 directly onto the lid of the polyethylene terephthalate grape package. PLA nanofibers containing essential oils from *Ocimum gratissimum* L. and *O. basilicum* L. were used in the proportion of 20% (v/w) relative to the total weight of the polymer. Packages were prepared containing only pure PLA nanofibers as a negative control.

2.10 *In vivo* antifungal activity

The bunches of grapes were placed in the packages, and 1000 µl of the spore suspension (10^6 spores mL^{-1}) of *A. carbonarius* and *A. niger* was sprayed onto them. A fungal control package containing only the contaminated grape, and a control package containing uncontaminated grapes were prepared. Packages were incubated in BOD at 25 °C. The experiment was performed in triplicate. After 10 and 20 days of incubation, berries infected by filamentous fungi were expressed as the percentage of contamination according to the equation: % fungal infection = (number of contaminated grapes/total grapes) x 100 and classified by the following score: (0) bunch without rot; (1) 1–5% contaminated berries; (2) 6–10% contaminated berries; (3) 11–25% contaminated berries; (4) 26–50% contaminated berries; (5) 51–75% contaminated berries; (6) greater than 75% contaminated berries (ABDOLAHI et al., 2010).

2.10.1 Physicochemical analyses of grapes before and after incubation

The weight loss, size and firmness were determined according to the methods of Freire et al. (2017) and Jahani et al. (2020) with some modifications. The bunches of grapes were weighed on an analytical balance (Mars AY220), and the weight loss was calculated by the equation: Weight loss (%) = (Initial weight – Final weight)/Initial weight x 100. Size was measured in two perpendicular directions with a digital caliper (Electronic Digital Caliper). The

texture analyzer (TA.XT Plus Texture Analyzer) was used to evaluate the grape firmness with the following parameters: penetration distance of 6 mm; 3-mm diameter probe; pre-test, test and post-test speeds were 1.5 mm s^{-1}, 2 mm s^{-1} and 1 mm s^{-1}, respectively. Firmness was determined in the equatorial region of the grape, and the value obtained in Newtons was defined as the maximum force required for a portion of the probe to penetrate the fruit. Diameter and firmness were evaluated on 20 berries.

The total soluble solids (TSS) was measured with a digital refractometer (Atago PAL-1) and expressed in °Brix; the titratable acidity (TA) was expressed in grams of tartaric acid per 100 g of grapes; the maturity index and the pH measured with a digital potentiometer (Quimis–197 Q400MT) were evaluated according to the method recommended by the Instituto Adolfo Lutz (2008).

A colorimeter (Konica Minolta, CR 10, Osaka, Japan) was used to determine the color of the grapes, analyzing 20 berries per repetition. The parameters L*, a*, b*, Chroma and the Hue angle were obtained.

2.11 Statistical analysis

Data were treated by a completely randomized design (DIC) using the SISVAR program, where values were subjected to analysis of variance and means were compared by the Tukey test ($p<0.005$) (FERREIRA, 2011). The data for the physicochemical parameters of the grape samples (fresh grapes, grapes contaminated with the *A. carbonarius* and *A. niger* fungi and grapes treated with PLA nanofibers containing the essential oils from *O. gratissimum* L. and *O. basilicum* L.) were submitted to Principal Component Analysis (PCA). Data were automatically normalized, and the PCA was performed using CHEMOFACE (NUNES et al., 2012).

3 RESULTS AND DISCUSSION

3.1 Chemical composition of essential oils

The chromatograms showing the chemical compositions of essential oils from *O. basilicum* L. and *O. gratissimum* L. are presented in Figure 1.

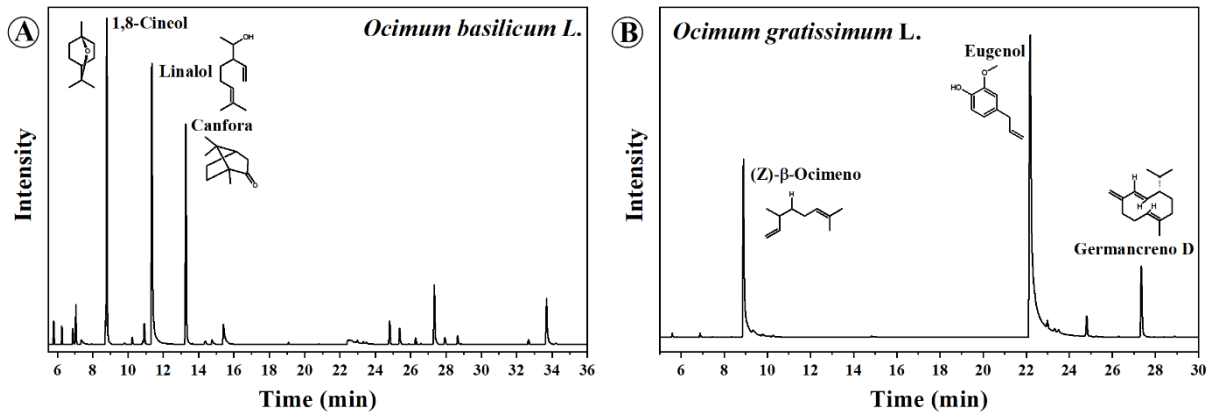


Figure 1 – Chromatograms of essential oils from *O. basilicum* L. (A) and *O. gratissimum* L (B).

Six constituents were identified in the essential oil from *O. gratissimum*, which included 99.69% of the total chemical composition, with eugenol (79.04%) being the principal compound, followed by (Z)-β-ocimene (14.60%) and germancrene D (5.04%) (Figure 1A). These data partially corroborate those found by Lisboa et al. (2020) and Melo et al. (2019), who found eugenol to be the most abundant compound; however, they found other constituents with a lower abundance, such as 1,8-cineole, γ-murolene, and (Z)-caryophyllene. In the essential oil from *O. basilicum* L., a total of 23 compounds (98.04%) were identified. The principal constituents found were linalool (26.89%), 1,8-cineol (23.62%) and camphor (15.69%) (Figure 1B). The chemical composition of the essential oil from *O. basilicum* L. exhibits generalized chemical polymorphism. This species has genetically distinct chemotypes (linalol, eugenol, methyleugenol, methyl cinnamate, methylchavicol) that can be distinguished by the dominant terpene (COSTA et al., 2020; DEHSHEIKH et al., 2020; KIFERLE et al., 2019; STANOJEVIC et al., 2017). Thus, the essential oil from *O. basilicum* L. used in this study contained linalool as the chemotype. Lisbon et al. (2020) and Monfort et al. (2018) suggest that the genotype, nutrients, plant development stage, light intensity, soil, atmospheric CO_2 concentration, temperature, relative humidity and growing region, in parallel with certain precautions, can favor the production of one or another constituent in medicinal plants.

3.2 Morphological characterization of nanofibers

The morphology and diameters of pure PLA and PLA nanofibers containing the essential oils from *O. gratissimum* L. and *O. basilicum* L. are presented in Figure 2.

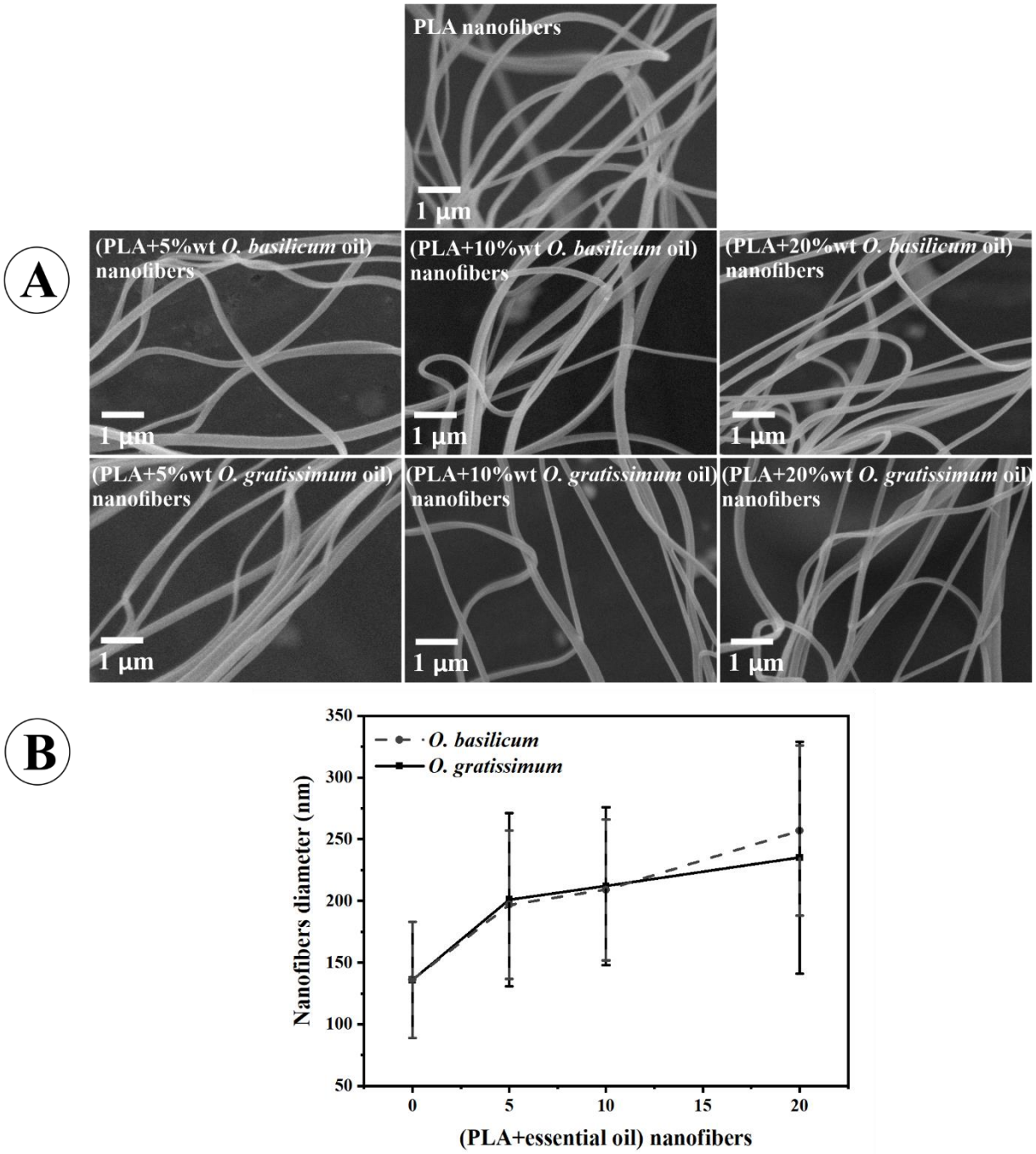


Figure 2 – (A) SEM images of pure PLA and PLA nanofibers containing essential oils from *O. basilicum* L. and *O. gratissimum* L. at concentrations of 5, 10 and 20%. (B) Effect of 5, 10 and 20% proportions of the essential oils on the average diameter of PLA fibers.

The PLA/essential oils solutions that were submitted to the SBS process yielded homogeneous, regular and smooth nanofibers without the formation of granules or fused junctions between the fibers. However, the use of increasing concentrations of the essential oils from *O. gratissimum* L. and *O. basilicum* L. resulted in significantly thicker fibers, with mean diameters of 136 nm for pure PLA nanofibers. The diameters of the PLA nanofibers containing 5, 10 and 20% of the essential oil from *O. gratissimum* L. were 201, 212 and 235 mm. The

respective diameters for the nanofibers containing the essential oil from *O. basilicum* L. were 197, 209 and 257 nm.

Oliveira et al. (2013ab) mentioned that the characteristics that play a fundamental role in obtaining homogeneous nanofibers without granules by the SBS process with a constant injection rate and spinning pressure are the concentrations and mixing ratios of the polymer solution. The parameters used in this study were adequate and efficient in the process of forming the nanofibers, as can be seen in Figure 2. In addition, the viscosity of the polymer solution effects a change in the thickening of the diameter of the fibers. Thus, the increase in the concentration of essential oils under study might have led to an increase in the viscosity of the polymeric solution and, consequently, to a smaller elongation of the injected jet and the production of fibers with greater diameters and variability, which corroborates the data found by Liu et al. (2018ab).

3.3 Release rate of essential oils

The release curves at 25 °C for the essential oils from *O. gratissimum* L. and *O. basilicum* L. are shown in Figure 3. The evaporation rate (Te) was calculated from the mass loss curve, which revealed significant differences when the pure essential oils and the oils encapsulated in PLA nanofibers were compared (Figure 3).

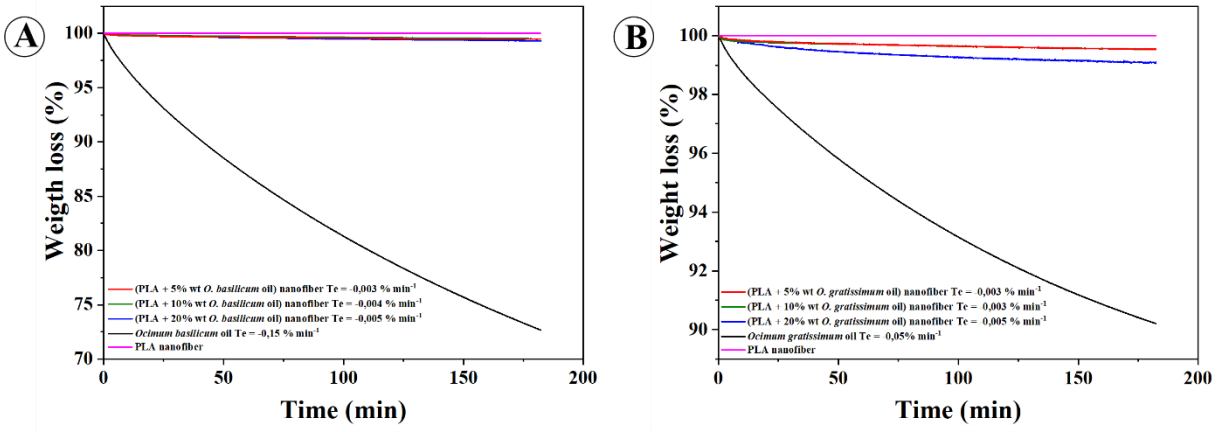


Figure 3 – Release profile of pure *O. basilicum* L. and *O. gratissimum* L. essential oils in PLA nanofibers.

The Te of essential oils encapsulated in PLA nanofibers is lower than that found for pure essential oils (Figure 3), which is probably the result of interactions between the polymer and the oils (MIRANDA et al., 2019). The results were very good. PLA nanofibers are efficient

and provide a controlled release of bioactive compounds, prolong the release of active substances and increase the time for action of the essential oils.

The essential oil from *O. basilicum* L. had a higher release rate than that of *O. gratissimum* L., probably because of the composition of the essential oil. Eugenol is mostly present in the composition of the essential oil from *O. gratissimum* L., and this compound interacts by hydrogen bonding, which is a strong intermolecular interaction and explains the lower loss in mass. The predominance of oxygenated monoterpenes in the essential oil from *O. basilicum* L. explains why its evaporation rate is greater than that of *O. gratissimum* L. because these terpenes contain non-polar portions (hydrocarbons) and polar groups. Three types of interaction should exist; however, the prevailing force is weak (Van der Waals forces).

3.4 *In vitro* antifungal and antiocratoxigenic activity

The antifungal and antiocratoxigenic activities of the essential oils from *O. gratissimum* L. and *O. basilicum* L. encapsulated in PLA nanofibers against *A. carbonarius* and *A. niger* represented by the percentage inhibition of mycelial growth and ochratoxin A production are shown in Figure 4. The effects of essential oils encapsulated in nanofibers against *A. carbonarius* and *A. niger* increased significantly with increasing concentration of the oils. The comparison of the antifungal and antiocratoxigenic effects of the essential oils showed that the essential oil from *O. gratissimum* L. was more efficient. A 100% inhibition of mycelial growth was observed after 10 days of incubation for both fungi.

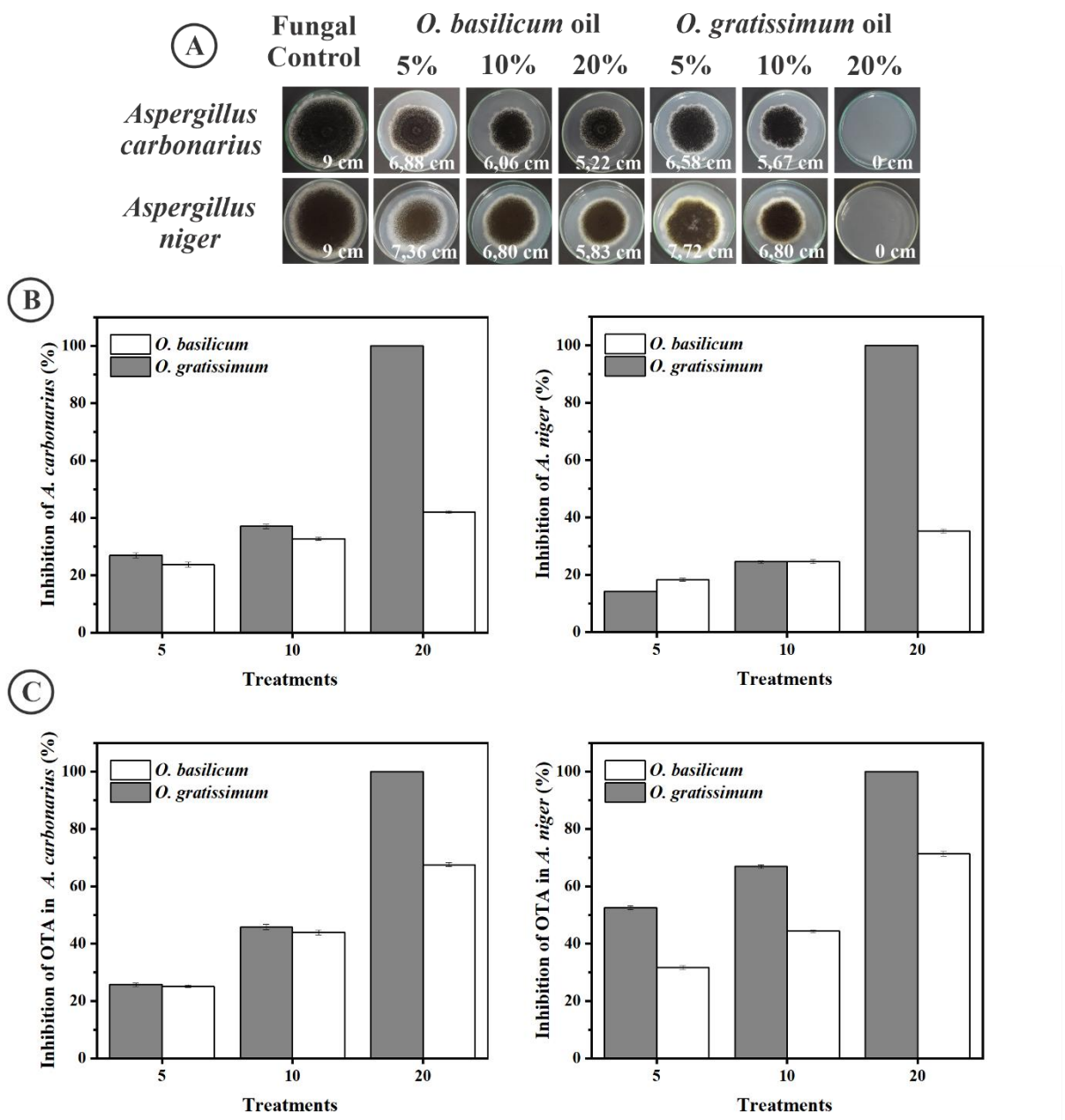


Figure 4 – Inhibitory effects of the essential oils from *O. basilicum* L. and *O. gratissimum* L. encapsulated in PLA nanofibers on the growth and on the production of ochratoxin A by of *A. carbonarius* and *A. niger* after 10 days of incubation. Diameters of fungal growth (A) and percentage of inhibition on the growth (B) and production of ochratoxin A (C) under treatment with the essential oils.

An et al. (2019) and Kong et al. (2019) reported that the fungal cell membrane is a key component for fungal survival. Changes in cytoplasmic membrane permeability result in loss of cell ions and, consequently, loss of function. The antifungal activity of essential oils is attributed to their constituents, which are able to interact with the membrane and have an effect on fungal growth. They cause morphological changes and deformations in hyphae and spores. Other possible mechanisms of action of essential oils can be related to alterations in the

synthesis of the cell wall, cytomembrane, cytoplasm and organelles, which affect the growth and morphology of fungi and spores.

In general, the lower concentrations of essential oils used were sufficient to inhibit the production of ochratoxin A, whereas they were less effective for inhibition of growth. This fact corroborates the data described in the studies by Schlosser and Prange (2019) and Tian et al. (2012). Those authors mention that lower doses are sufficient to inhibit the production of ochratoxin A because of the interference in carbohydrate metabolism. They act on the active sites of specific enzymes responsible for the production of mycotoxins.

The biological activity of essential oils can be related to synergism between constituents, or itt can be attributed to the principal compounds. Phenolic compounds are considered to be highly active because the hydrogen present in the hydroxyl group linked to the aromatic ring can form hydrogen bonds or act as an acid. This fact might explain its strong activity. These constituents can be lethal, or they can just inhibit the growth of microorganisms, depending on the concentration used (MARCHESE et al., 2017; MISHRA et al., 2013; SAAD et al., 2013). Thus, the greater activity of the essential oil from *O. gratissimum* L. compared to that from *O. basilicum* L. in inhibiting the proliferation of fungi and the synthesis of ochratoxin A by *A. carbonarius* and *A. niger* might be due to the fact that it is made up of almost 80% eugenol, a phenylpropanoid. Ahmad et al. (2010) and Mishra et al. (2013) showed that the lipophilic nature of eugenol damages the membrane integrity of toxigenic strains of *A. carbonarius* and *A. flavus* and inhibits the H^+ATPase system by causing intracellular acidification and, consequently, death of the fungus.

Mishra et al. (2013) studied 1,8-cineol and linalool and reported that the antifungal and antimycotoxigenic activity of these constituents is moderate because they are aliphatic, cyclic hydrocarbons with an absence of hydroxyl groups. This fact explains the lower efficiency of the essential oil from *O. basilicum* L. compared to that of *O. gratissimum* L., as these constituents are the principal constituents of the oil from *O. basilicum* L.

3.5 *In vivo* antifungal activity

Functional nanofibers containing essential oils from *O. gratissimum* L. and *O. basilicum* L., used for the application of active packaging, are shown in Figure 5. The deterioration of the grapes was minimized with the treatments, contrasting with the control, in which the grapes were spoiled, rotten and with a very clear fungal growth. After incubation, the percentage of incidence of fungus was significantly smaller ($p<0.05$) with the essential oils encapsulated in

PLA nanofibers than in the control. The active packages provided a protective effect against the mycelial growth of *A. carbonarius* and *A. niger* fungi in grapes.

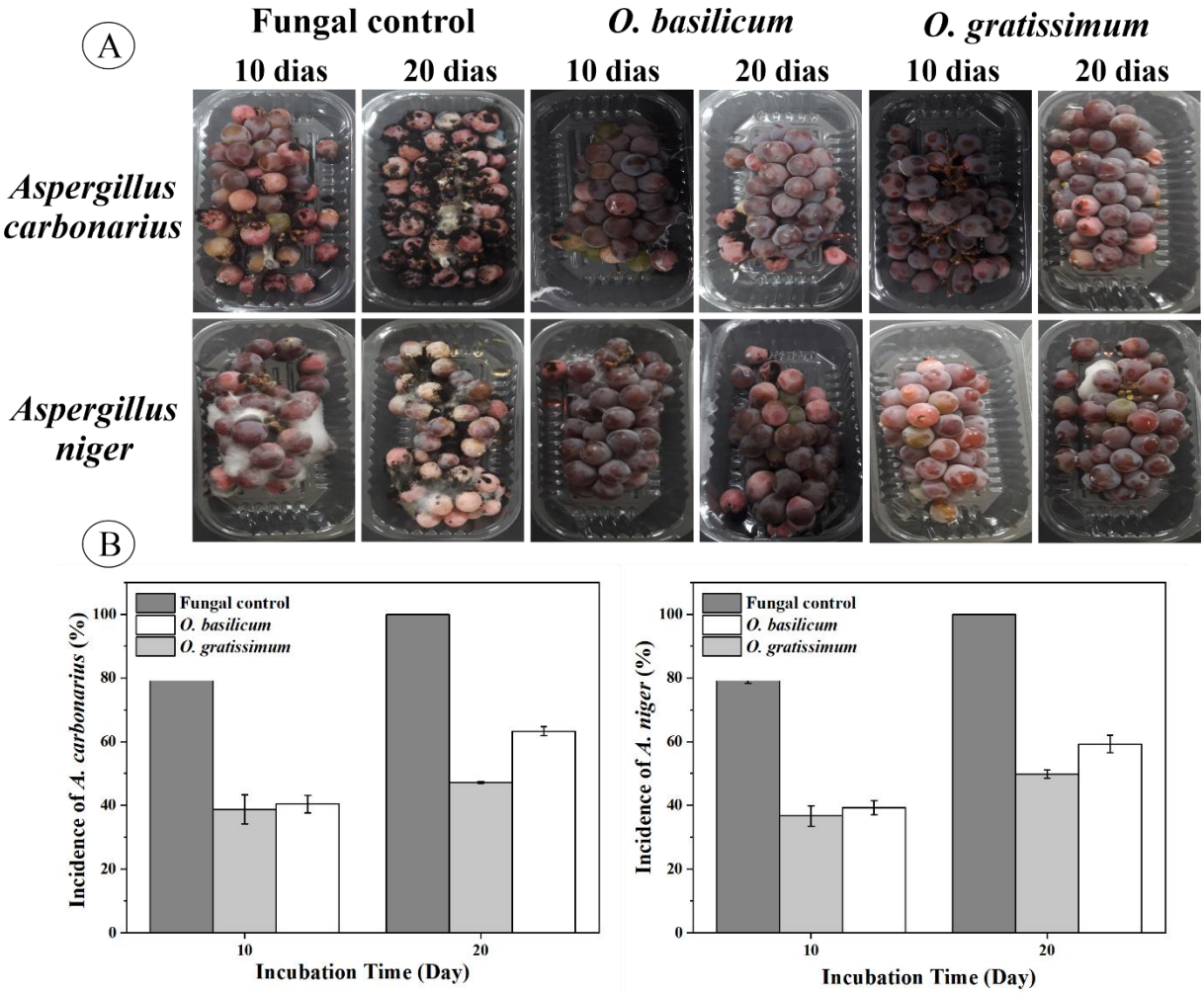


Figure 5 – Control of the incidence of *A. carbonarius* and *A. niger* fungi in grapes by packaging containing the essential oils from *O. gratissimum* L. and *O. basilicum* L. encapsulated in PLA nanofibers during incubation periods of 10 and 20 days.

Softening, rotting and fungal mycelium growth were observed on the control grapes after being inoculated with *A. carbonarius* and *A. niger*. These symptoms were less prominent in the packaging containing essential oils from *O. gratissimum* L. and *O. basilicum* L. encapsulated in PLA nanofibers. Active packaging had a positive effect on the storage time of the grapes, the incidence of the fungus and, consequently, the loss of fruit through contamination and rotting.

Several studies such as those developed by Kong et al. (2019) and Sonker et al. (2015) focused on the application of pure essential oils for the preservation of grapes; however, there are no studies using essential oils encapsulated in nanofibers as packaging for the preservation of grapes by the fumigation process as reported in this study. PLA nanofibers prolonged the

effects of essential oils, releasing them in a controlled manner, as can be seen in Figure 3. Furthermore, according to Wang et al. (2015), the fumigation process does not leave residual essential oils in fruits. Therefore, the use of these active packages through the fumigation process during the storage of grapes or even for other post-harvest foods is considered to be very promising.

2.5.1 Physicochemical characteristics of grapes before and after incubation

The PC1xPC2 biplot of scores and weights of physicochemical parameters (disease severity, weight loss, diameter, firmness, total soluble solids (TSS), total acidity (TA), maturity index, pH, color parameters) in samples of fresh grapes contaminated by *A. carbonarius* and *A. niger* and treated with nanofibers containing essential oils from *O. gratissimum* L. and *O. basilicum* L. after 10 and 20 days of incubation are shown in Figures 6A and 6B, respectively. The PCA showed that it was possible to describe 99.68% (Figure 6A) and 99.32% (Figure 6B) of the data variability with the first two principal components, of which 97.95% (Figure 6A) and 94.60% (Figure 6B) were explained by the first principal component. The grapes treated with nanofibers containing the essential oils possessed similar physicochemical parameters and were similar to the fresh grapes after incubation. Grapes contaminated with *A. carbonarius* and *A. niger* that did not receive treatments were similar to each other; however, their parameters differed from those of fresh grapes and grapes that received treatments. As is demonstrated by the PCA, the results obtained were satisfactory because the parameters of quality characteristic of fresh grapes were maintained in the grapes treated with nanofibers containing the essential oils from *O. gratissimum* L. and *O. basilicum* L. after incubation with the fungi.

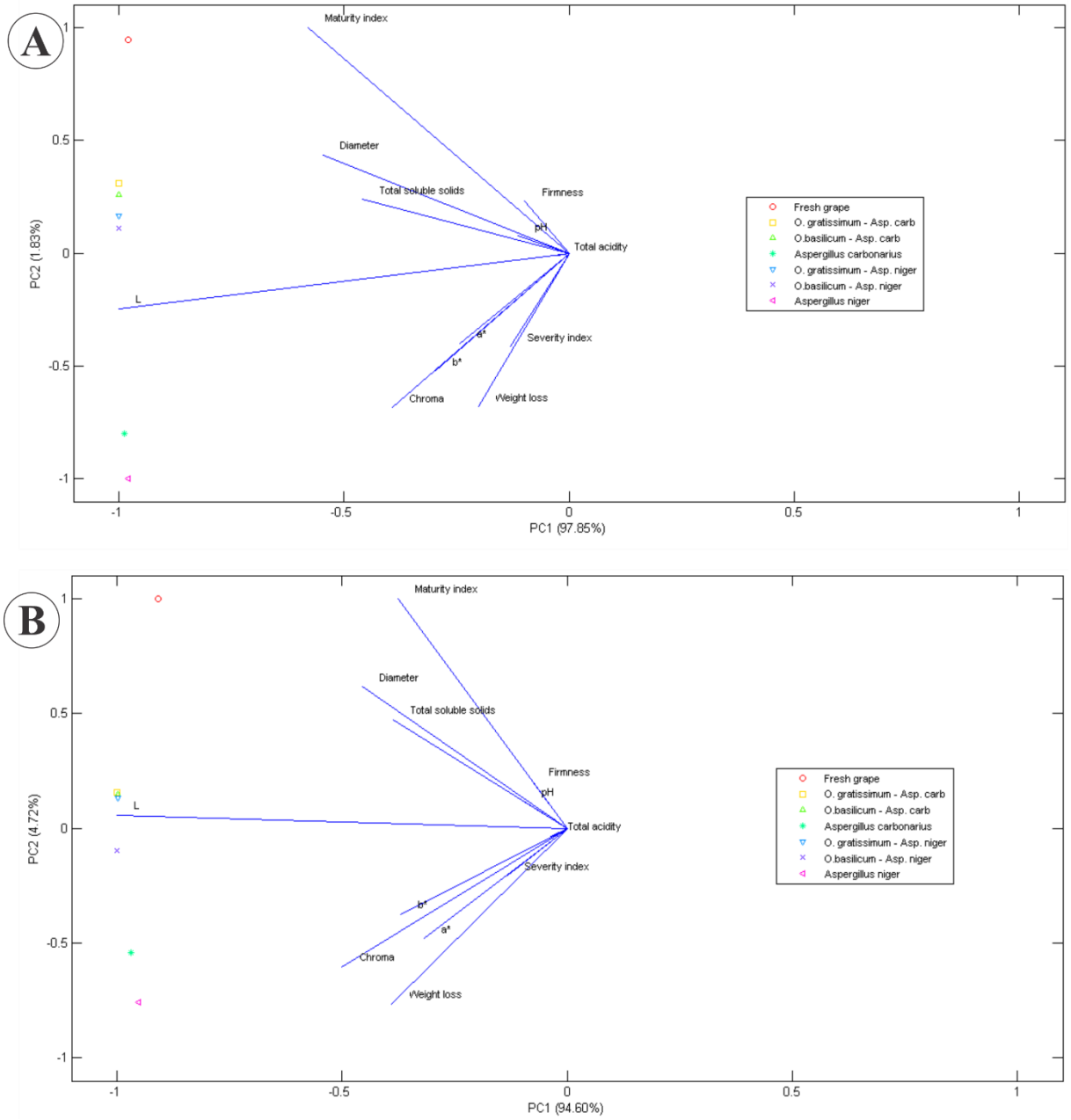


Figure 6 – PC1xPC2 biplot of weights and scores of grape samples treated with the essential oils from *O. gratissimum* L. and *O. basilicum* L. encapsulated in PLA nanofibers after 10 (A) and 20 (B) days of incubation with *A. carbonarius* and *A. niger*.

Weight loss and firmness are essential quality parameters for perishable foods in post-harvest storage systems. These attributes are related to the susceptibility of fruits to fungal decomposition (SONKER et al., 2015; VALVERDE et al., 2005). In this study, weight loss and the severity of the disease were lower in the grapes that received the treatments than in the fungal controls for *A. carbonarius* e *A. niger*, for which weight losses of 14.00 and 25.83%, disease incidences of 100 and 100% and a severity index of 6 and 6, respectively, were observed after 20 days of incubation. Parallel to the weight loss, an accelerated softening and a

decrease in berry size in the fungal controls were observed during storage. However, the diameters and firmness of the grapes stored in the bioactive packaging after incubation with the fungi were similar to that of the fresh grapes. That is, the treatment with PLA nanofibers containing essential oils had a good effect on preserving the firmness and size of the fruit. These results can be attributed to the lipophilic characteristic of essential oils and the biological potential of their constituents, which control the proliferation of fungi, reduce fruit respiration, and, concomitantly, control decomposition and weight loss so that less softening of the grapes occurs (HASSANI et al., 2012; SONKER et al., 2014). Valero et al. (2006) reported that the lack of weight loss caused by essential oil constituents is related to the role of these compounds in reducing the dehydration of fruits. In addition, postharvest softening can also be caused by the loss of moisture from the fruit as a result of reduced turgor and greater change in the cell wall caused by the hydrolysis of starch to sugar and the degradation of pectin by fungi, which has also been reported to be related to a change in firmness (SUN et al., 2014). The PLA nanofiber packaging containing essential oils from *O. gratissimum* L. and *O. basilicum* L. maintained the firmness and avoided weight loss of the grapes by maintaining turbidity, controlling water loss and decreasing the degradation of pectin by reducing fungal contamination. These results are in agreement with previous works that showed that different essential oils, as well as their constituents, had the potential to control decomposition caused by fungi, as well as reducing weight loss and texture loss, parameters considered to be the main contributing factors in fruit quality (ABDOLAHI et al., 2010; GUILLÉN et al., 2007; SONKER et al., 2014; SONKER et al., 2015).

Other important quality factors that determine the acceptability of the grape by consumers are the soluble solids content (SST), the total titratable acidity (TTA), maturity index (SST/TA) and the color of the fruit. Studies have reported that consumers prefer sweet grapes with a touch of acidity (VALERO et al., 2006). After incubation with *A. carbonarius* and *A. niger*, a significant decrease in the total soluble solids content was observed in untreated grapes (fungal control). This decrease can be explained by the fact that *Aspergillus* fungi consume sugars as reported by Freire et al. (2017). These mycotoxigenic fungi have the ability to produce an enzyme complex and degrade specific substrates, in addition to producing secondary metabolites (FREIRE et al., 2017). In addition, pH values, total titratable acidity, maturity index and color of untreated grapes underwent significant changes in the presence of *A. carbonarius* and *A. niger* when compared to fresh grapes before storage. The total acidity increased in the presence of these microorganisms because *Aspergillus* causes oxidation of glucose to producing gluconic acid. However, the total soluble solids, total acidity content, pH, maturity index and

color in the grapes that received the treatments with PLA nanofibers containing essential oils from *O. gratissimum* L. and *O. basilicum* L. continued to be similar to those found in fresh and healthy grapes before incubation. That is, the physicochemical characteristics were preserved and the degradation of the grapes was delayed in the grapes that were stored in the bioactive packaging.

4 CONCLUSION

Loss of quality, due to weight loss; decrease in size; accelerated softening; changes in skin color; changes in pH, total soluble solids, total titratable acidity and maturity index after storage was observed in the fungal control. These changes were also accompanied by a high proliferation of *A. carbonarius* and *A. niger* fungi. All these changes were significantly delayed and controlled in grapes packed with nanofibers containing the essential oils from *O. gratissimum* L. and *O. basilicum* L. Both oils significantly protected the fruits from contamination by *A. carbonarius* and *A. niger* fungi during storage, as well as reducing the production of ochratoxin A, a potential carcinogen, in *in vitro* and *in vivo* tests with PLA nanofiber packaging containing the essential oils. For the first time, essential oils encapsulated in PLA nanofibers as active packaging were used to preserve the overall quality (organoleptic, sensory, nutritious and functional) of the fruit. The essential oils in PLA nanofibers were released in a controlled manner; thus, the shelf life of fruits was extended with active packaging, resulting in greater safety for perishable foods.

ACKNOWLEDGEMENTS

This work was supported by the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brasil (CAPES) - Financial Code 001. The authors thank the funding agencies for the scholarships and financial support, the Central of Analysis and Chemical Prospecting of the Federal University of Lavras and Embrapa Instrumentation for supplying the equipment for analyses and the Medicinal Plants Garden of the Federal University of Lavras, located in the city of Lavras, MG, Brazil.

REFERENCES

ABDOLAHI, A. et al. Study on the potential use of essential oils for decay control and quality preservation of Tabarzeh table grape. **Journal of Plant Protection Research**, v. 50, n. 1, p. 45-52, 2010.

ADAMS, R. P. **Identification of essential oils componentes by gas chromatography/ mass spectroscopy**. 4.1th ed. Carol Stream: Allured, 804p, 2017.

AHMAD, A. et al. Proton translocating ATPase mediated fungicidal activity of eugenol and thymol. **Fitoterapia**, v. 81, n. 8, p. 1157-1162, 2010.

AN, P. et al. α-terpineol and terpene-4-ol, the critical components of tea tree oil, exert antifungal activities in vitro and in vivo against *Aspergillus niger* in grapes by inducing morphous damage and metabolic changes of fungus. **Food Control**, v. 98, n. 98, p. 42-53, 2019.

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA). **Métodos de Farmacognosia**. In: Mendes JCC, ed. Farmacopeia Brasileira. Brasília, Brasil: Fiocruz, 5th ed.; n. 1, p.198-199, 2010. http://portal.anvisa.gov.br/documents/33832/260079/5%C2%AA+edi%C3%A7%C3%A3o+-+Volume+1/4c530 f86-fe83-4c4a-b907-6a96b 5c2d2fc.

BILBAO-SAINZ, C. et al. Solution blow spun poly(lactic acid)/hydroxypropyl methylcellulose nanofibers with antimicrobial properties. **European Polymer Journal**, v. 54, p. 1-10, 2014.

BONAN, R. F. et al. Poly(lactic acid)/poly(vinyl pyrrolidone) membranes produced by solution blow spinning: Structure, thermal, spectroscopic, and microbial barrier properties. **Journal of Applied Polymer Science**, 2017, 134.19.

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.

CHEN, H-C; TSAI, C-H; YANG, M-C. Mechanical properties and biocompatibility of electrospun polylactide/poly (vinylidene fluoride) mats. **Journal of Polymer Research**, v. 18, n. 5, p. 319-327, 2011.

COSTA, A. S. et al. Toxicity of the essential oil of basil cultivars and hybrids and its repellent effect on stored grain pests. **Bioscience Journal**, v. 36, n. 2, p. 341-352, 2020.

DEHSHEIKH, A. B. et al. Changes in soil microbial activity, essential oil quantity, and quality of Thai basil as response to biofertilizers and humic acid. **Journal of Cleaner Production**, v. 256, p. 120439, 2020.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, v. 35, n. 6, p. 1039-1042, 2011.

FREIRE, L. et al. Influence of physical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines. **International Journal of Food Microbiology**, v. 241, p. 181-190, 2017.

FREIRE, L. et al. A quantitative study on growth variability and production of ochratoxin A and its derivatives by *A. carbonarius* and *A. niger* in grape-based medium. **Scientific Reports**, v. 8, n. 1, p. 1-11, 2018a.

FREIRE, L. et al. Influence of maturation stages in different varieties of wine grapes (*Vitis vinifera*) on the production of ochratoxin A and its modified forms by *Aspergillus carbonarius* and *Aspergillus niger*. **Journal of Agricultural and Food Chemistry**, v. 66, n. 33, p. 8824-8831, 2018b.

GARLOTTA, D. A literature review of poly (lactic acid). **Journal of Polymers and the Environment**, v. 9, n. 2, p. 63-84, 2001.

GUPTA, B.; REVAGADE, N.; HILBORN, J. Poly (lactic acid) fiber: An overview. **Progress in Polymer Science**, v. 32, n. 4, p. 455-482, 2007.

GUILLÉN, F. et al. Improvement of the overall quality of table grapes stored under modified atmosphere packaging in combination with natural antimicrobial compounds. **Journal of Food Science**, v. 72, n. 3, p. S185-S190, 2007.

GUIMARÃES, L. G. D. L. et al. Atividades antioxidante e fungitóxica do óleo essencial de capim-limão e do citral. **Revista Ciência Agronômica**, v. 42, n. 2, p. 464-472, 2011.

HASSANI, A. et al. Evaluation 3of plant essential oils for control of postharvest brown and gray mold rots on apricot. **Journal of Food Safety**, v. 32, n. 1, p. 94-101, 2012.

HUA, H. et al. Inhibitory effect of essential oils on *Aspergillus ochraceus* growth and ochratoxin A production. **PloS one**, v. 9, n. 9, p. e108285, 2014.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC). **Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins.** IARC Monographs on the evaluation carcinogenic risks to humans, v. 56, p. 489-491, 1993.

JAHANI, M.; PIRA, M.; AMINIFARD, M. H. Antifungal effects of essential oils against *Aspergillus niger in vitro* and *in vivo* on pomegranate (*Punica granatum*) fruits. **Scientia Horticulturae**, v. 264, p. 109188, 2020.

JIANG, C. et al. Inhibition of *Aspergillus carbonarius* and fungal contamination in table grapes using *Bacillus subtilis*. **Food Control**, v. 35, n. 1, p. 41-48, 2014.

KIFERLE, C. et al., Effect of Iodine treatments on *Ocimum basilicum* L.: Biofortification, phenolics production and essential oil composition. **Plos one**, v. 14, n. 12, p. 0226559, 2019.

KONG, Q., et al. Antifungal mechanisms of α-terpineol and terpene-4-alcohol as the critical components of *Melaleuca alternifolia* oil in the inhibition of rot disease caused by *Aspergillus ochraceus* in postharvest grapes. **Journal of Applied Microbiology**, v. 126, n. 4, p. 1161-1174, 2019.

LAPPA, I. K. et al. Control of *Aspergillus carbonarius* in grape berries by *Lactobacillus plantarum*: A phenotypic and gene transcription study. **International Journal of Food Microbiology**, v. 275, p. 56-65, 2018.

LI, Q., et al. The biocontrol effect of Sporidiobolus pararoseus Y16 against postharvest diseases in table grapes caused by *Aspergillus niger* and the possible mechanisms involved. **Biological Control**, v. 113, p. 18-25, 2017.

LISBOA, C. F. et al. Chemical composition of *Lippia origanoides* Kunt. and *Ocimum gratissimum* L. essential oils stored at −20 °C. **Industrial Crops & Products**, v. 151, p. 112485, 2020.

LIU, F. et al. Antimicrobial carvacrol in Solution Blow-Spun fish-skin gelatin nanofibers. **Journal of Food Science**, v. 83, n. 4, p. 984-991, 2018a.

LIU, F. et al. Preparation of fish skin gelatin-based nanofibers incorporating cinnimaldehyde by solution blow spinning. **International Journal of Molecular Sciences**, v.19, 2018b.

MARCHESE, A. et al. Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. **Critical Reviews in Microbiology**, v. 43, n. 6, p. 668-689, 2017.

MELO, R. S. et al. Chemical composition and antimicrobial effectiveness of *Ocimum gratissimum* L. essential oil against multidrug-resistant isolates of *Staphylococcus aureus* and *Escherichia coli*. **Molecules**, v. 24, n. 21, p3864, 2019.

MIRANDA, K. W. et al. Polystyrene bioactive nanofibers using orange oil as an ecofriendly solvent. **Journal of Applied Polymer Science**, v. 136, n. 15, p. 47337, 2019.

MIRANDA, K. W. et al. Halochromic Polystyrene Nanofibers Obtained by Solution Blow Spinning for Wine pH Sensing. **Sensors**, v. 20, n. 2, p. 417, 2020.

MISHRA, P. K. et al. Assessing essential oil components as plant-based preservatives against fungi that deteriorate herbal raw materials. **International Biodeterioration & Biodegradation**, v. 80, p. 16-21, 2013.

MONFORT, L. E. F. et al. Effects of plant growth regulators, different culture media and strength MS on production of volatile fraction composition in shoot cultures of *Ocimum basilicum*. **Industrial Crops and Products**, v. 116, p. 231-239, 2018.

MOGOSANU, G. D., et al. Natural products used for food preservation. In: **Food Preservation**. Academic Press, p. 365-411, 2017.

NEPOMUCENO, N. C. et al. Antimicrobial activity of PLA/PEG nanofibers containing terpinen-4-ol against *Aggregatibacter actinomycetemcomitans*. **Journal of Applied Polymer Science**, v. 135, n. 6, p. 45782, 2018.

NUNES, C. A. et al. Chemoface: a novel free user-friendly interface for chemometrics. **Journal of the Brazilian Chemical Society**, v. 23, p. 2003-2010, 2012.

OLIVEIRA, J. E. et al. Properties of poly(lactic acid) and poly(ethylene oxide) solvent Polymer mixtures and nanofibers made by Solution Blow Spinning. **Journal of Applied Plymer Science**, v. 129, n. 6, p. 3672-3681, 2013a.

OLIVEIRA, J. E. et al. Structural and morphological characterization of micro and nanofibers produced by Electrospinning and Solution Blow Spinning: a comparative study. **Advances in Materials Science and Engineering**, v. 2013, 2013b.

PASSAMANI, F. R. 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. **Journal of Food Protection**, v. 77, n. 11, p. 1947–1952, 2014.

SAAD, N. Y.; MULLER, C. D.; LOBSTEIN, A. Major bioactivities and mechanism of action of essential oils and their components. **Flavour and Fragrance Journal**, v. 28, n. 5, p. 269-279, 2013.

SCHLOSSER, 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 Additives & Contaminants: Part A**, v. 36, n. 9, p. 1411-1418, 2019.

Sonker, N. et al. Assessment of *Cymbopogon citratus* (DC.) stapf essential oil as herbal preservatives based on antifungal, antiaflatoxin, and antiochratoxin activities and in vivo efficacy during storage. **Journal of Food Science**, v. 79, n. 4, p. M628-M634, 2014.

SONKER, N.; PANDEY, A. K.; SINGH, P. Efficiency of *Artemisia nilagirica* (Clarke) Pamp. essential oil as a mycotoxicant against postharvest mycobiota of table grapes. **Journal of the Science of Food and Agriculture**, v. 95, n. 9, p. 1932-1939, 2015.

STANOJEVIC, L. P. et al. Chemical composition, antioxidant and antimicrobial activity of Basil (*Ocimum basilicum* L.) essential oil. **Journal of Essential Oil Bearing Plants**, v. 20, n. 6, p. 1557-1569, 2017.

SUN, X. et al. Effects of chitosan-essential oil coatings on safety and quality of fresh blueberries. **Journal of Food Science**, v. 79, n. 5, p. M955-M960, 2014.

TIAN, J. et al. The control of *Aspergillus flavus* with *Cinnamomum jensenianum* Hand.-Mazz essential oil and its potential use as a food preservative. **Food Chemistry**, v. 130, n. 3, p. 520-527, 2012.

VALERO, D. et al. The combination of modified atmosphere packaging with eugenol or thymol to maintain quality, safety and functional properties of table grapes. **Postharvest Biology and Technology**, v. 41, n. 3, p. 317-327, 2006.

VALVERDE, J. M. et al. Improvement of table grapes quality and safety by the combination of modified atmosphere packaging (MAP) and eugenol, menthol, or thymol. **Journal of Agricultural and Food Chemistry**, v. 53, n. 19, p. 7458-7464, 2005.

VAN DEN DOOL, H.; KRATZ, P. Dec. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. **Journal of Chromatography A**, v. 11, p. 463-471, 1963.

Wang, Y. et al. Inhibitory effect of nerol against *Aspergillus niger* on grapes through a membrane lesion mechanism. **Food Control**, v. 55, p. 54-61, 2015.

ZENEBON, O.; PASCUET, N. S.; PAULO, T. **Métodos físico-químicos para análise de alimentos**. (4 th ed.). São Paulo: Instituto Adolfo Lutz, 2008.

ZHANG, X., et al. A comparison of electronic nose and gas chromatography–mass spectrometry on discrimination and prediction of ochratoxin A content in *Aspergillus carbonarius* cultured grape-based medium. **Food Chemistry**, v. 297, p. 124850, 2019.

3 CONSIDERAÇÕES FINAIS

Os óleos essenciais encapsulados em nanofibras de poli(ácido lático) (PLA) são bastante promissores para serem usados na forma de embalagens ativas para frutas, visto que a matriz polimérica consegue liberar os ativos de maneira controlada prolongando o efeito biológico dos óleos essenciais sobre a matriz alimentar. As embalagens ativas do estudo proporcionaram um forte efeito inibitório sobre a incidência dos fungos *in vitro* e *in vivo*, e controlaram a produção da ocratoxina A. Dessa forma, exibiram uma ação positiva no tempo de armazenamento em uvas de mesa, reduzindo a incidência de apodrecimento e deterioração, além de preservar os parâmetros físico-químicos das frutas.

O uso de tratamentos pela técnica de fumigação pode ser considerado ideal no controle de processos de deterioração de alimentos causados por fungos, pois não deixa resíduos de óleos essenciais nas frutas; portanto, usar embalagens ativas com a técnica de fumigação pode ser um ponto positivo à segurança alimentar, melhorando a qualidade e estendendo a vida útil das frutas.